A word from the editor

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Otto Kinne

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MARINE ECOLOGY

A Comprehensive, Integrated Treatise on Life in Oceans and Coastal Waters

Volume I ENVIRONMENTAL FACTORS

Volume II PHYSIOLOGICAL MECHANISMS

Volume III CULTIVATION

Volume IV DYNAMICS

Volume V OCEAN MANAGEMENT

MARINE ECOLOGY

A Comprehensive, Integrated Treatise on Life in Oceans and Coastal Waters

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VOLUME II
Physiological Mechanisms
Part 1

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FOREWORD
to
VOLUME II: PHYSIOLOGICAL MECHANISMS

'Physiological Mechanisms' deals with synthesis and transversion of organic material, regulation, evolution and population genetics, and orientation to environmental components in space and time. It is subdivided into 2 parts which contain the following chapters:

Part 1

Chapter 1 : Introduction to Volume II
Chapter 2 : Mechanisms of Chemo-autotrophy
Chapter 3 : Mechanisms of Heterotrophy
Chapter 4 : Mechanisms of Thermoregulation
Chapter 5 : Mechanisms of Ion and Osmoregulation
Chapter 6 : Mechanisms of Evolution and Population Genetics

Part 2

Chapter 7 : Orientation in Space: Plants
Chapter 8 : Orientation in Space: Animals
8.0 General Introduction
8.1 Invertebrates
8.2 Fishes
8.3 Mammals
Chapter 9 : Orientation in Time: Endogenous Clocks

Why a volume on Physiological Mechanisms in a treatise on Marine Ecology? Because ecology comprises more than the description of organismic assemblages and food-chain interrelationships. Because studies on functions and structures of living systems and their relation to the environment are based on knowledge of the essential mechanisms effective at the individual level. Because proper synthesis requires pertinent analytical detail. We cannot assess, comprehend and forecast the ecological dynamics of life in oceans and coastal waters without taking into account the physiological mechanisms involved in biotransformation of energy and matter, in metabolic regulation, in population genetics and in orientational behaviour.

The term 'ecology' has been coined by the German biologist E. HAECKEL, and defined as the science investigating the natural relationships of organisms, both to their inorganic and organic environment. HAECKEL's original definition has subsequently been enlarged to encompass life as a whole and practically all earth sciences ('Gesamthaushalt der Natur'), and it has been reduced, e.g. to population dynamics, community structure, biogeography, or to flow charts of energy and matter through living systems. Between these extremes, a variety of definitions has been offered, based on prevailing concepts and perspectives in different

countries, schools of thought and individual investigators. We adhere to the original definition: ecology comprises studies of organisms in relation to their environment, abiotic and biotic.

Based on many disciplines, ecology is a primarily synthetical science. Ultimately, ecology attempts nothing less than to provide the key for comprehending history, present and future of life–environment correlations. Present-day organisms and ecosystems are the result of thousands or millions of years of interaction between living systems and their environment. There is every reason to assume that comparable interrelations will also control and determine the future of such systems. Can there be anything more important on this earth than to know and to apply the principles which support and maintain healthy communities of life? Can there be a better rationale for man's existence than to preserve and to sustain, rather than to misuse and degrade life on earth?

The difficulties involved in organizing and completing the Treatise on Marine Ecology are formidable—more so than we have anticipated. The seemingly inexhaustible body of information available increases exponentially, sometimes competing acutely with the reviewer's 'digestion rate'. There is progressive specialization in the three basic areas of marine ecology—microbiology, botany and zoology—and new subfields with narrow perspectives and specific terminology develop like grain in the summer sun after a warm rain. While such specialization affords advantages, it also leads to fragmentation and loss of synthetic potential. Insufficient integration and lack of comprehensive, critical assessments of the 'state of the art' begin to hinder effective scientific progress and often lead to endless repetitions of studies, ultimately insufficient for comprehending the functions and structures of complex living systems. We have experienced these difficulties in numerous discussions concerning volume scope and detailed chapter outlines.

Fortunately, encouraging support has come from many sources: all contributors, numerous colleagues and several institutions. Most of the work concerning organization and editorial preparation of Volumes II and III was completed while on a 'Senior Foreign Scientist Fellowship'—supported by the National Science Foundation of the United States of America—at the Department of Zoology of Arizona State University, Tempe, Arizona. It is with great pleasure that I gratefully acknowledge this assistance, and the help and kindness received from the Chairman Dr. S. D. GERKING and the members of the Department of Zoology, as well as from my assistants Valerie CLARK and Helga WITT. Among the colleagues who offered advice and criticism regarding Volume II, the following deserve special mention: Drs. H.-P. BULNHEIM, M. S. GORDON, K. H. LÜNING, M. M. MULLIN, D. SIEBERS. Assistance regarding individual chapters is acknowledged at the end of the respective chapter.

O. K.

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PHYSIOLOGICAL MECHANISMS

1. INTRODUCTION TO VOLUME II

O. Kinne

(1) Organization and Scope

How should a volume on Physiological Mechanisms be organized? The first problem was to avoid letting the volume grow into another treatise! The information available is so vast and the disciplines that qualify for inclusion so numerous that rigid thematic restriction had to dominate other organizational considerations. The second problem was to select topics that would extend and deepen the material presented in the preceding volume on *Environmental Factors*, and that would provide a basis for the subsequent volumes: *Cultivation*, *Dynamics*, and *Ocean Management*.

We are aware of the incompleteness and shortcomings of this volume. Originally, it was intended to include a subchapter on mechanisms of photo-autotrophy and a chapter on mechanisms of communication. It turned out that there are sufficient adequate and pertinent reviews available on mechanisms of photo-autotrophy. In addition, none of the essential mechanisms known qualify as being specific to the marine environment. The chapter on communication was deleted because (i) exhaustive treatment of this field would have required a separate volume in its own right; (ii) pertinent research activities are in the midst of an explosive development; (iii) it is still difficult to determine the borderlines of this modern field of research. However, certain aspects of communication between marine organisms have been covered, especially in Part 2 of this volume. Mechanisms of respiration and of reproduction are mentioned under various headings in Volumes I, II, III and IV of the Treatise. Specific reviews would have led to considerable expansion of Volume II and, frequently, to an undesirable amount of overlap.

We have concentrated our attention on topics which we believe contribute significantly to the fundament of marine ecology: Mechanisms of chemo-autotrophy; heterotrophy; thermoregulation; osmoregulation; evolution and population genetics; orientation in space and time. It is sincerely hoped that inclusion of these topics in a Treatise on Marine Ecology will help to bridge the deplorable separation between the fields of ecology and physiology.

Insufficient knowledge on physiological mechanisms among ecologists—just as insufficient knowledge on ecological principles among physiologists—has created a critical gap in the continuum of biological sciences. This gap must be narrowed lest we fail to respond adequately to the greatest challenge mankind has ever faced: the challenge to comprehend and maintain intact ecosystems and to re-integrate our technical and industrial activities into our environment. Healthy ecological systems have produced man, they support man and they remain the fundamental prerequisite for man's continued existence.

Some ecologists have been criticized for the readiness and ease with which they have offered concepts, theories and interpretations. Some have considered organism–environment and organism–organism relationships with much genius, but little

solid fundament. Their discussions, suggestions and conclusions have suffered, among other things, from lack of pertinent information on organismic functions and structures at the individual level: from neglecting information regarding the physiological mechanisms which determine thresholds, types and magnitudes of responses of individuals and populations.

On the other hand, some physiologists have produced information which cannot be used for ecological considerations: they have worked on unstabilized, injured, starving, sick, parasitized, or microbial-infected organisms, maintained under inadequate environmental conditions. In many cases, the test organisms have been offered nutrients they would not normally accept in their natural habitat. Hence, the physiological information at hand requires critical screening. We need more studies on healthy, stabilized biological systems, and more data that can be used with good faith for evaluating, measuring and interpreting life processes in the unrestrained natural marine environment.

Certainly, even 'wholly unnatural' conditions may provide insights into the physiological mechanisms employed by the test organism. The information obtained under unnatural conditions cannot, however, be extrapolated to the situation in the marine environment without further qualification. It is at this point that serious misinterpretations have been introduced time and again, both by ecologists and by physiologists.

Of course, our selection of physiological mechanisms invites criticism. Not only from those who are disappointed that their field has not been included, but also from those who insist that their field has nothing to do with marine ecology. In bio-acoustics, for example, a few investigators visualize their work as belonging to the fields of technology, biophysics or sensory physiology, and sometimes consider it primarily from the aspect of man's interest in underwater object finding. However, acoustic energy provides major cues for orientation of marine animals. In a number of species, underwater sounds are of basic importance for obtaining food, securing environmental conditions essential for reproduction, maintaining social structures and avoiding or escaping from life-endangering situations. Numerous invertebrates, fishes and marine mammals have been shown to employ acoustic energy for relating to their environment, and for organism–organism relationships. Consequently, orientation of marine organisms by sound and vibration constitutes an important aspect of marine ecology.

(2) Part 1: Comments on Chapters 2 to 6

Chapter 2: Mechanisms of Chemo-autotrophy

Mechanisms of autotrophy facilitate synthesis of all organismic constituents from environmental carbon dioxide. They provide the energetic basis for heterotrophs and hence play a central role in the flow of energy and matter through marine ecosystems. There are two basic types of autotrophy: photo-autotrophy or photolitho-autrophy and chemo-autotrophy or chemolitho-autotrophy.

Photolitho-autotrophy comprises the ability of sulphur purple bacteria and green plants to absorb solar radiation and to convert it into chemical free energy usable

for synthesizing organic matter (photosynthesis; Volume IV). The mechanisms of photosynthesis have received detailed attention in numerous extensive symposia, monographs and reviews. They are not covered here.

Chemolitho-autotrophy comprises the capacity of micro-organisms to gain metabolic energy from oxidizing reduced or incompletely oxidized inorganic substances such as ammonia, nitrite, sulphur, hydrogen, hydrogen sulphide, thiosulphate, carbon monoxide and ferrous iron. Chapter 2 is limited to chemolitho-autotrophic bacteria which have been studied in detail.

Ecologically, chemolitho-autrophic bacteria are of considerable importance. As a basic link in the marine energy cycle they participate in the conversion of energy and matter often occupying special ecological niches. Their ability to obtain energy not only from the oxidation of organic carbon sources, but also from reduced inorganic compounds, constitutes a selective advantage especially in habitats with limiting amounts of organic substances.

Chapter 2 summarizes and critically evaluates our present knowledge on the species involved and their mechanisms for gaining biologically usable energy. It considers specialized regulatory phenomena as well as evolutionary and ecological aspects of chemolitho-autotrophy. Major gaps in our knowledge exist in regard to the enzymes concerned with inorganic hydrogen donors, membrane-bound enzymes, regulation of CO_2 fixation, electron transport and metabolic shifts between auto- and heterotrophy. (For trophic role of marine bacteria consult Volume IV.)

Chapter 3: Mechanisms of Heterotrophy

Heterotrophic organisms depend on ambient organic substances both for making biologically useful energy available and for synthesizing their cellular constituents. There are four basic types of heterotrophy: photolithoheterotrophy (some non-sulphur purple bacteria), photo-organoheterotrophy (most non-sulphur purple bacteria), chemolithoheterotrophy (species of the bacterial genus *Desulfovibrio*), and chemo-organoheterotrophy (all organisms depending on organic substrates, i.e. most bacteria, all fungi and all animals).

Chapter 3 is mainly concerned with animals. It represents the first attempt to review and to evaluate all essential processes and mechanisms employed by heterotrophs inhabiting marine, brackish and limnic habitats (see also Volume IV).

Subdivided into 3 major sections—intake of energy and matter, transformation into body functions and structures, output of non-utilized energy and matter—the chapter goes into considerable detail regarding feeding mechanisms, digestion, absorption, conversion and excretion. A special subsection is devoted to the nutritive role of animal faeces—a rather neglected, but nevertheless important, aspect in the recycling of energy and matter in marine ecosystems.

Chapter 4: Mechanisms of Thermoregulation

This brief assessment concentrates on recent information produced on non-mammalian marine, brackish and limnic animals. In contrast to mammals and with the exception of a few large, fast-swimming fishes, mechanisms of thermo-

regulation are poorly developed. The aquatic animals concerned must rely on thermal tolerance, behavioural responses and adaptation.

Mechanisms of non-genetic temperature adaptation depend on activity modifications of key enzymes, i.e. changes in kinetic enzyme characteristics and in intracellular enzyme concentration. The information at hand is insufficient to suggest a model of the molecular basis of thermal adaptation in non-mammalian marine animals.

Chapter 5: Mechanisms of Ion and Osmoregulation

The coupling between many aquatic organisms and their external medium is about as intimate as that between extra- and intracellular fluids. Hence water and salt exchanges are primary denominators of life in oceans and coastal waters. Most of the water contained in a living system is in a free state, providing a vehicle for molecules involved in biochemical interactions. In habitats with fluctuating or non-oceanic salinities, mechanisms of ion and osmoregulation play a decisive role in distribution and performance, and hence in determining the ecological potential of the organism concerned.

The chapter extends and deepens the information presented in Volume I (Chapter 4) on responses of marine organisms (bacteria, fungi and blue-green algae; plants; invertebrates; fishes) to salinity variations. It considers primarily sub-individual aspects and concentrates on the physiological mechanisms which facilitate water and salt regulation in multicellular aquatic animals.

Two major types of regulatory mechanisms are distinguished: mechanisms controlling extracellular fluids (extracellular-fluid anisosmotic regulation) and mechanisms controlling intracellular fluids (intracellular-fluid isosmotic regulation). The first type is important for successful establishment in habitats with fluctuating or non-oceanic salinities; it is available only to a few animals. The second type is a basic property of all living systems studied thus far; it controls the ionic composition of cells, the concentration of intracellular inorganic and organic osmotic effectors, the maintenance of an isosmotic equilibrium between cells and their ambient fluids, and it assists in avoiding excessive cellular volume changes.

Chapter 6: Mechanisms of Evolution and Population Genetics

While there is no reason to postulate a basic difference in the genetic systems of marine and terrestrial organisms, gradients and barriers of gene exchange may be more pronounced and more effective in the sea than previously expected. Inhomogeneous distributions of temperature, salinity, water movement, substrate types, organic substances, nutrients and other environmental entities, as well as the gigantic oceanic dimensions are assumed to cause significant discontinuities and heterogeneities of gene transmission.

The long-term tendency of living matter to assume every possible form compatible with its environment, the inhomogeneity of marine habitats and the resulting heterogeneity of gene flow patterns constitute—against the background of competition among different forms of life and vast periods of time—the principal forces

which participate in producing and maintaining the diversity of marine species and ecosystems that we witness today. Past, present and future of life in oceans and coastal waters can hardly be explored without taking into consideration the mechanisms which determine the properties of living marine systems as a function of geological time.

Discussing and critically evaluating a portion of the literature at hand, Chapter 6 considers genetic aspects of evolution in the marine environment—a topic never thoroughly reviewed before—and relates the principles of evolutionary genetics to marine biota. Genetic variability and genetic differentiation receive particular attention. Studies on population genetics and species diversity, as well as attempts toward environmental predictability have produced the first hypotheses regarding the genetic strategies employed by marine organisms; these hypotheses are briefly outlined. The field of marine genetics is presently entering a phase of rapid development and progress due to concerted scientific efforts, increasing sophistication of cultivation methods, and new techniques of electrophoresis.

(3) Part 2: Comments on Chapters 7 to 9

Part 2 is devoted to mechanisms of orientation. Organismic orientation comprises active relating to spatial and temporal components of the environment, abiotic and biotic. It is based on the endogenous functions and structures available to the responding organism. Consequently, three fundamental aspects can be distinguished: space, time, and endogenous properties. Chapters 7 and 8 concentrate on orientation in space, Chapter 9 considers orientation in time. All three chapters refer to endogenous aspects involved in stimulus perception and stimulus evaluation; they consider the resulting orientational response both in terms of the possible underlying physiological mechanism and its presumptive ecological significance.

The common denominators of orientation are environmental cues, their perception and evaluation, and the resulting response. Traditionally, cues and resulting response belong to the fields of ecology and behaviour, perception and evaluation to the domain of physiology. Such fragmentation has impeded the comprehension of orientation as a general evolutionary and ecological phenomenon which contributes essentially to the successful establishment of a species in a given set of abiotic and biotic environmental conditions.

Chapter 7: Orientation in Space: Plants

Plants orient in response to their environment by (i) locomotion, (ii) changes in growth direction, (iii) polarity induction, and (iv) intracellular chloroplast movements. Locomotion towards or away from a directional environmental stimulus occurs in freely moving plants (or life-cycle stages), e.g. in planktonic flagellates or gliding algae. Changes in growth direction are induced by such environmental factors as light or gravity. Similarly, induction of polarity in originally apolar, non-moving germinating cells, depends upon directional environmental stimuli. Intracellular orientation movement of chloroplasts is affected by prevailing light conditions; it controls the degree of light exposure and thus the rate of photo-

synthetic processes in these organelles. All four basic mechanisms of spatial orientation in plants may be present in one and the same species. However, their apparent significance changes as a function of prevailing habitat conditions, life-cycle stage, and plant group considered.

The sequential steps involved in orientation are: perception of the environmental stimulus and its directive components, primary molecular and secondary physiological processes induced by the stimulus, and the resulting orientation response itself. In addition, tonic processes can control magnitude and direction of the response.

The overwhelming amount of information on plant orientation pertains to light. Little is known as yet regarding the physiological mechanisms facilitating orientation to other environmental stimuli. Hopefully, Chapter 7 will help to stimulate interest among ecologists and physiologists in orientation of plants inhabiting the marine environment.

Chapter 8: Orientation in Space: Animals

The General Introduction (Chapter 8.0) evaluates the theoretical background of animal orientation and presents a comprehensive conceptual framework for present and future studies. Describing and defining different categories of orientation, the early terminology is critically discussed and new terminological concepts are introduced. The physiology of orientation and the importance of learning (experience) are considered in detail.

The early terminology based on the concepts of tropism, taxis and kinesis (pp. 526–530)—still widely used by many marine botanists and zoologists—is criticized because (i) it concentrates on reflexes and does not sufficiently take into account the central disposition which is a prerequisite of the orientation response, and the complex central processes which are part of the physiological mechanisms involved; (ii) it can describe adequately only 'simple' responses and characterize only part of the underlying mechanism.

The new localization principles presented (object orientation, rotation, translation, etc.; pp. 500–503) allow a more differentiated characterization of the orientation processes and mechanisms. The new concept and terms are offered as a contribution towards conceptual clarification. Since most publications on invertebrates, fishes and marine mammals have been written in other conceptual contexts, we could not adhere to a definite terminology in Chapters 8.1, 8.2 and 8.3.

Wherever possible, the sequence of environmental stimuli (light, temperature, salinity, water movement, etc.) has been maintained in Chapters 8.1, 8.2 and 8.3 parallel to that adopted in Volume I. It is hoped that this procedure will facilitate cross-background information on the respective environmental factor, and cross-group comparison between different taxa.

Most papers devoted to mechanisms of orientation in invertebrates, fishes and marine mammals indicate a deplorable lack of knowledge regarding the behaviour of the 'other group'—not to mention the different emphasis and perspectives prevailing in botany. The insufficient exchange of experiences and views among botanists, invertebrate zoologists, ichthyologists and marine mammalogists has prevented the development of a broad solid basis for assessing and evaluating the

principles underlying organismic orientation. Of course, orientation mechanisms differ in accordance with the ecological niche occupied and with the anatomy and physiology of the organism concerned. But comparative studies, carefully analyzing the similarities and differences, can be expected to provide essential new insights and new vigour to this important field of organism–environment relations.

Chapter 8.1 considers mechanisms and processes of orientation in space employed by marine and brackish-water invertebrates. Among the environmental stimuli, light, salinity, organic substances (chemically mediated responses), and gravity have received most attention by investigators.

The variety of mechanisms and processes employed by aquatic invertebrates in active relating to spatial components of their environment is extraordinarily diverse, much more so than in fishes or mammals. This diversity parallels the larger range of different habitats occupied and of body plans represented.

In view of the restricted amount of information available on marine fishes, Chapter 8.2 includes significant detail on orientation in space of limnic species. In the open ocean, investigations on fish migrations are extremely difficult. Tagging has been restricted largely to shore-inhabiting commercial fishes and has produced predominantly descriptive, indirect evidence. Telemetric tracking is possible only with individuals of sufficient size (excluding larvae, small juveniles, as well as adults of small-sized species). Laboratory experiments are confined to a few marine fishes due to lack of appropriate cultivation techniques. A major analytical tool is provided by *in situ* deportation and homing experiments. The stress caused by deportation seems to act as artificial releaser of orientational activities which lead to homing.

Under natural conditions, migratory activities of fishes are initiated and maintained by internal or environmental releasing factors (e.g. physiological state or season); directionality is provided by environmental stimuli such as light, temperature, salinity, water movement, organic substances and, apparently, electrical and magnetic fields. The subchapter focuses on short-distance orientation involved, for example, in food search, social contact, and avoidance responses.

Chapter 8.3 summarizes and evaluates the information at hand on marine mammals. It stresses the multifactorial character of orientation in space: a variety of different cues are perceived and interpreted by several different physiological mechanisms (e.g. visual, auditory, tactile, chemical). While one cue type may be primarily relied on in a given situation, additional input modalities are computed and act in concert, providing key signals for navigational manoeuvres or migratory activities. Adjustments to specific environmental circumstances are facilitated by changes in central disposition and by personal experience. This imparts to the mammal concerned a considerable degree of both plasticity and specificity in its response.

The members of the two amphibious orders Carnivora and Pinnipedia seem to rely to a large extent on visual, and to a lesser degree on auditory, tactile and chemical stimuli; in the holo-aquatic Sirenia, Mysticeti and Odontoceti, however, vision tends to be progressively assisted (or even replaced as the predominant orientation mechanism) by hearing. Several odontocetes possess an active biosonar. As in bats, this sophisticated acoustic orientation mechanism uses self-generated sounds to obtain, via echoes, information on object direction, distance and pro-

perties. Acoustic orientation has received unparalleled attention from investigators. The resulting imbalance in the space allotted in this review to the different environmental cues does not necessarily reflect their ecological importance.

The ecological significance of the physiological mechanisms involved in spatial orientation of marine mammals include (i) location and uptake of food, (ii) securing of conditions essential for reproduction, (iii) establishment and maintenance of social structures, (iv) avoidance of, or escape from, life-endangering situations. The mechanisms employed for navigation and migration, and the hierarchical sequence of cues relied on under *in situ* conditions are still a matter of dispute. We cannot automatically assume parallelism between orientation potentials established in 'trained-seal experiments' and in field performances. The mechanisms studied have evolved under specific ecological and social circumstances, and they may function normally only under such circumstances. Hence there is considerable need for orientation experiments conducted under adequate environmental conditions.

Chapter 9: Orientation in Time: Endogenous Clocks

Not intended to provide exhaustive treatment of the extensive literature on organismic orientation in time, Chapter 9 focuses attention on those aspects of endogenous timing processes which are of interest to the marine ecologist. Whether 'biological clocks' under constant light and temperature conditions are endogenous or exogenous is considered a sterile discussion: the rhythms behave as though they were endogenous. Under field conditions, environmental cycles serve to time the free-running rhythms seen under constant conditions. In addition, the environment provides stimuli to which the organism responds on a moment-to-moment basis. Intensity and nature of the resulting response may be a function of the time within the endogenous cycle at which the stimuli occur.

Ecological phenomena involving endogenous rhythms include daily rhythms of zoo- and phytoplankton, and of benthic organisms. Tidal timing is of importance for organisms inhabiting the intertidal zone. Animals occupying this zone often exhibit endogenous timing processes which are synchronized by the tidal regime. Lunar, semilunar and annual rhythms receive particular attention.

The exact physiological mechanism of biological clocks is unknown. Internal rhythms are unusually immune to chemical treatment. Several authors suspect protein and DNA and/or RNA synthesis to be involved. While heavy-water effects provide little specific information about the mechanism, alcohol effects may implicate membrane processes as rate-limiting factors in endogenous clocks.

2. MECHANISMS OF CHEMO-AUTOTROPHY

H. G. SCHLEGEL

(1) General Characteristic of Chemolitho-autotrophy

Chemolitho-autotrophic micro-organisms (litho-autotrophs) gain metabolic energy from the oxidation of inorganic compounds such as reduced or incompletely oxidized forms of sulphur, nitrogen, and ferrous iron or of hydrogen. The common hydrogen acceptor is oxygen. A few members of the group, however, are able to use nitrate, nitrite or nitrous oxide instead. The litho-autotrophs are able to use carbon dioxide as the sole carbon source. Many litho-autotrophs are facultative, i.e. they are able to perform a purely organoheterotrophic type of metabolism or to co-metabolize organic materials.

Some bacteria are obligate litho-autotrophs. Obligate litho-autotrophy does not mean that organic compounds cannot be incorporated into cellular substances; this rather implies the bacteria do not grow in a non-modified batch-culture containing only organic material as carbon and energy source. Only prokaryotic micro-organisms are known to be obligately bound to this type of metabolism. This chapter is, therefore, limited to bacteria. The trophic role of bacteria receives attention in Volume IV.

(2) Discovery of the Concept

The discovery of chemolitho-autotrophic bacteria and the formulation of the concept of chemo-autotrophy originated with the work of S. N. WINOGRADSKY (1887). When studying the colourless filamentous bacterium *Beggiatoa* sp., which like all sulphur bacteria is always associated with the occurrence of hydrogen sulphide in nature, he observed that this bacterium oxidizes hydrogen sulphide to sulphuric acid, transiently accumulating globules of elemental sulphur intracellularly. The results of his studies have been summarized as follows (p. 608):

> 'Es bilden also die Schwefelbacterien eine scharf charakterisierte physiologische Gruppe, einen physiologischen Typus, der wesentlich von dem allgemeinen abweicht. Ihre Lebensprocesse spielen sich nach einem viel einfacheren Schema ab; durch einen rein anorganischen chemischen Process, den der Schwefeloxydation, werden alle ihre Lebensbewegungen im Gange erhalten. Darum habe ich diese Organismen—Schwefelorganismen oder Schwefelbacterien genannt.'

A year later, WINOGRADSKY (1888) reported on his observations on the iron bacteria (*Gallionella ferruginea* and *Leptothrix ochracea*) and showed that these grow in the absence of organic carbon and obtain energy for growth by the oxidation of ferrous salts to ferric oxide. He already emphasized the low energy yield of this process and realized the relation of the large amounts of iron oxide compared to the low number of cells found in natural habitats. He concluded that both types of bacteria

were able to use carbon dioxide as their source of carbon. Studying the nitrifying bacteria, WINOGRADSKY (1890) confirmed his conclusions by quantitative determinations of the ammonia oxidized and of the organic carbon of the medium.

The new type of metabolism has been called 'Anorgoxydation' by WINOGRADSKY and the principle has been summarized (WINOGRADSKY, 1922, p. 1):

> 'Diese Studien haben gezeigt, daß die Anorgoxydanten sich durch eine Summe untereinander eng verbundener physiologischer Eigenschaften auszeichnen, die sie von der übrigen Bakterienwelt sehr scharf abgrenzen, nämlich:
> 1. Ihr Gedeihen in der Natur findet nur in elektiven, fast rein mineralischen Medien statt, die spezifische oxydable anorganische Substanzen enthalten müssen.—2. Ihre Existenz ist an das Vorhandensein dieser Substanz gebunden, die durch ihren Lebensprozeß eine Oxydation erleidet.—3. Dieser Oxydationsprozeß ist ihre einzige Energiequelle.—4. Sie bedürfen keiner organischen Nährstoffe, weder als plastisches Material, noch als Energiequelle.—5. Sie besitzen so gut wie keine Fähigkeit, organische Substanzen abzubauen, werden vielmehr von denselben in ihrer Entwicklung gehemmt.—6. Als Kohlenstoffquelle brauchen sie ausschließlich Kohlensäure, die durch Chemosynthese assimiliert wird.'

The term 'chemosynthesis' was coined by W. PFEFFER (1897). In his paper '*Studien zur Energetik der Pflanze*' (1893) he had already concerned himself with the problems of carbon-dioxide assimilation using light energy (green plants) or chemical energy (nitrifying bacteria); but he first used the terms photosynthesis and chemosynthesis in his textbook of plant physiology in 1897 (p. 273):

> 'With respect to the sources of driving energy one could generally talk about photosynthesis, chemosynthesis, electrosynthesis etc., depending on the prevailing conditions.'

The term 'autotrophs' was used as it is understood today; that is, organisms which are able to synthesize their cell substances from inorganic carbonate as the main source of carbon (WOODS and LASCELLES, 1954). In this definition the phrase 'which are able' must be emphasized, since the ability to utilize carbon dioxide as the main carbon source is the determining characteristic; it is of minor importance that several autotrophs are facultatives and can occasionally grow with organic substrates.

The term 'chemosynthesis' no longer means energy production by oxidation of inorganic substances (LWOFF and co-authors, 1946). The terms photo- and chemotrophy, as well as the derived terms litho- and organotrophy, emphasize the aspects of energy source (light or chemical process) and hydrogen donor (inorganic or organic material). The derivation of cell carbon is designated by the prefixes 'auto' and 'hetero'.

The biochemical pathway of carbon-dioxide fixation in the autotrophic bacteria thus far investigated has been found to be identical with that responsible for carbon-dioxide fixation in green plants. This pathway, the reductive pentose phosphate cycle or Calvin cycle, has become an integral part of the definition of carbon-dioxide autotrophy (ELSDEN, 1962). If new biochemical pathways are discovered, this definition might prove to be too narrow. In fact, the Hatch–Slack cycle operating

in tropical grasses (HATCH and SLACK, 1970) contributes appreciable amounts of carbon to the assimilation of green plants, especially tropical grasses. We are inclined, therefore, to retain the definition of WOODS and LASCELLES (1954) based on the ability to use carbon dioxide as the main carbon source.

On the basis of this definition, a further group of bacteria may be considered to belong to the litho-autotrophic bacteria *sensu lato*. These are the anaerobic hydrogen bacteria producing either methane or acetic acid from hydrogen and carbon dioxide. *Methanobacterium thermoautotrophicum* is able to grow in a mineral medium with hydrogen and carbon dioxide as energy and carbon source (ZEIKUS and WOLFE, 1972). It closely resembles *M. omelianskii* strain M.O.H., which is able to grow slowly under the same conditions, but whose growth is strongly stimulated by vitamins and other organic compounds (BRYANT and co-authors, 1968).

Enrichment cultures with mineral media and an oxygen-free hydrogen–carbon dioxide atmosphere are highly selective for the growth of *Clostridium aceticum* (EL GHAZZAWI, 1967) and *C. formicoaceticum* (ANDREESEN and co-authors, 1970). These clostridia grow extremely well when performing a homo-acetate fermentation on fructose; acetate synthesis from hydrogen and carbon dioxide, however, has not yet been proved unequivocally.

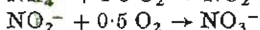
With respect to the mode of carbon-dioxide fixation, to the exclusiveness (fastidiousness) of substrate utilization, the obligate methylotrophs, represented by species like *Methanococcus capsulatus*, *Pseudomonas methanica*, and *Methanomonas methanooxidans*, resemble the litho-autotrophs. More strains, including new types of methane-utilizing bacteria, have been isolated recently (WHITTENBURY and co-authors, 1970). These bacteria are able to grow only on methane, methanol or dimethyl ether in an inorganic medium (FOSTER and DAVIS, 1966; PATEL and HOARE, 1971); they obtain energy from the oxidation of these compounds to carbon dioxide. Most of their cell carbon is obtained from the fixation of formaldehyde by condensation with a pentose phosphate (KEMP and QUAYLE, 1967; LAWRENCE and QUAYLE, 1970; LAWRENCE and co-authors, 1970; QUAYLE, 1972). Although the sub-

Table 2-1

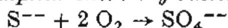
Energy yielding reactions of chemolitho-autotrophic bacteria (Original)

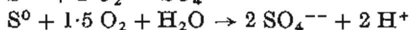
Nitrifying bacteria

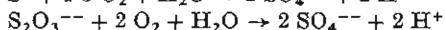
$NH_4^+ + 1{\cdot}5\ O_2 \rightarrow NO_2^- + 2\ H^+ + H_2O$

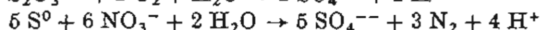

$NO_2^- + 0{\cdot}5\ O_2 \rightarrow NO_3^-$

Sulphur-oxidizing bacteria

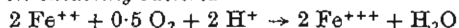

$S^{--} + 2\ O_2 \rightarrow SO_4^{--}$


$S^0 + 1{\cdot}5\ O_2 + H_2O \rightarrow 2\ SO_4^{--} + 2\ H^+$

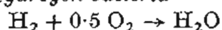

$S_2O_3^{--} + 2\ O_2 + H_2O \rightarrow 2\ SO_4^{--} + 2\ H^+$

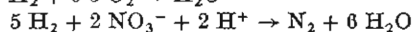

$5\ S^0 + 6\ NO_3^- + 2\ H_2O \rightarrow 5\ SO_4^{--} + 3\ N_2 + 4\ H^+$

Iron-oxidizing bacteria


$2\ Fe^{++} + 0{\cdot}5\ O_2 + 2\ H^+ \rightarrow 2\ Fe^{+++} + H_2O$

Hydrogen bacteria


$H_2 + 0{\cdot}5\ O_2 \rightarrow H_2O$


$5\ H_2 + 2\ NO_3^- + 2\ H^+ \rightarrow N_2 + 6\ H_2O$

strates methane and methanol are not considered to be inorganic compounds, the methylotrophs may be regarded as members of a mixed group of 'autotrophic' organisms dependent on C_1-compounds (RIBBONS and co-authors, 1970; ECCLESTON and KELLY, 1972).

This survey on autotrophic mechanisms will consider only those chemo-autotrophic bacteria which have been studied in great detail. The energy-yielding reactions performed by these bacteria are shown in Table 2-1. Reactions in which intermediates and derivatives (NH_2OH, NO, NO_2, N_2O, CNS^-, SO_3^{--}, $S_3O_6^{--}$, and others) are involved have been omitted from the table.

(3) The Species Described So Far

The taxonomy of chemolithotrophic bacteria is a rather neglected field. These bacteria are held in disrepute as slow growers, and physiological and biochemical research has been concentrated on one strain of each group only. Even biologically oriented microbiologists were reluctant to search for new strains or at least to attempt to grow formerly described species in the laboratory. The majority of the well-characterized chemolithotrophic bacteria are Gram-negative and can be easily classified as pseudomonads or related genera. It was not until recently that new forms were discovered, some of which are of a completely different shape or are Gram-positive.

This chapter considers only species which have recently been grown in pure culture and adequately described. The techniques for enrichment, isolation, cultivation, and sustenance (= maintenance) of autotrophs are well documented (SCHLEGEL, 1966; COLLINS, 1969; VELDKAMP, 1970; see also Volume III).

(a) Nitrifying Bacteria

The nitrifying bacteria were discovered by WINOGRADSKY (1890). Among the ammonia-oxidizing bacteria (Table 2-2), *Nitrosomonas europaea* (WINOGRADSKY,

Table 2-2

Ammonia-oxidizing bacteria (Compiled from the sources indicated)

Species	Number and/or insertion of flagellae	Size (μm) and shape	Author
Nitrosomonas europaea	2 polar	0·8–1·0 × 1·0–1·8	WINOGRADSKY (1890)
Nitrosocystis javanensis	1 polar		WINOGRADSKY (1892, 1931)
Nitrosocystis oceanus (=*Nitrosococcus oceanus*)	polar or peritrichous	2 (diameter); spherical	WATSON (1963)
Nitrosospira briensis	1 to 6 peritrichous	1·0 × 5·0 tight spiral	WINOGRADSKY (1931) WATSON (1971a)
Nitrosolobus multiformis	peritrichous	1·5 × 2·5 lobular	WATSON and co-authors (1971)

All ammonia-oxidizing bacteria are obligate autotrophs.

1890) has been repeatedly investigated. The small oval or rod-like cells, about 0·8 to 0·9 by 1·0 to 2·0 μm are motile due to one or two polar flagella.

The genus *Nitrosocystis* comprises ammonia-oxidizing bacteria which form aggregates surrounded by a common membrane. Two species have been described: *Nitrosocystis javanensis* (WINOGRADSKY, 1892) and *N. oceanus* (WATSON, 1963)

Nitrosospira briensis is a spiral-shaped bacterium and has been originally described by S. N. and H. WINOGRADSKY (1931, 1933). It has been re-isolated and adequately described by WATSON (1971a). When examined with the phase-contrast microscope, the cells appear as rods or cylinders, 1·5 to 2·5 μm long and 0·8 to 1·0 μm wide; electron microscopical inspection reveals tightly coiled spirals and 1 to 6 peritrichously inserted flagella. The cells are very motile. The organism has been grown in a pH-stat fermentor operated on a semi-continuous flow basis. A lobular shaped ammonia-oxidizing bacterium, *Nitrosolobus multiformis*, has recently been isolated from different soils and grown in Erlenmeyer flasks or in a pH-stat fermentor (WATSON and co-authors, 1971). During exponential growth the cells were irregularly shaped or appeared lobular; they measured 1·0 to 1·5 × 1·0 to 2·5 μm, and were motile due to 1 to 20 peritrichously located flagella. Ultrathin sections revealed an unusual compartmentalization, a central part being surrounded by small compartments.

Table 2-3

Nitrite-oxidizing bacteria (Compiled from the sources indicated)

Species	Number of flagellae	Size (μm)	Author
Nitrobacter winogradskyi	—	0·6–0·8 × 1·0–1·2	WINOGRADSKY (1892)
Nitrobacter agilis	1	0·6 × 0·8	NELSON (1931)
Nitrococcus mobilis	2	1·5–1·7; spherical	WATSON and WATERBURY (1971)
Nitrospina gracilis	—	0·3–0·4 × 2·7–6·5	WATSON AND WATERBURY (1971)

All nitrite-oxidizing bacteria are obligate autotrophs except *Nitrobacter agilis* which is a facultative autotroph.

The nitrite oxidizing bacteria comprise four species so far (Table 2-3). *Nitrobacter winogradskyi* (WINOGRADSKY, 1892) has been repeatedly isolated from soil. The cells are ellipsoidal short rods (0·8 by 1·0 – 2·0 μm) and non-motile, while *N. agilis* is a motile bacterium (NELSON, 1931; PAN, 1971). Recently two new species have been isolated from the marine environment (WATSON and WATERBURY, 1971): *Nitrococcus mobilis* is a spherical bacterium, 1·5 μm diameter, motile by means of 1 or 2 flagella inserted subpolarly in elongated cells. Ultrathin sections revealed tubular intracytoplasmic membranes. *Nitrospina gracilis* is a long slender rod, 0·3 to 0·4 μm wide and 2·7 to 6·5 μm long, non-motile and halophilic. Both species are obligate marine organisms; they are obligate chemo-autotrophs. On the basis of studies on the morphology and deoxyribonucleic acid composition (WATSON and MANDEL, 1971), a revision of the family Nitrobacteraceae has been proposed (WATSON, 1971b). Thus *Nitrosocystis* will be transferred to *Nitrosococcus*; *Nitrobacter*

agilis will be included in the species *Nitrobacter winogradskyi*. *Nitrosocystis javanensis* and other names, which have not been mentioned here, are to be regarded as *nomina dubia*.

(b) Sulphur Oxidizers

The sulphur bacteria which oxidize inorganic sulphur compounds and deposit sulphur as globules within the cell have been described by WINOGRADSKY (1887). This group includes the filamentous genera *Beggiatoa*, *Thioploca*, *Thiothrix* and *Thiospirillopsis*, and the non-filamentous genera *Achromatium*, *Thiophysa*, and *Thiovolum*. The early literature on these bacteria has been compiled by BAVENDAMM (1924) and BISSET and GRACE (1954); LA RIVIÈRE (1964) and PRINGSHEIM (1970) have reviewed the more recent literature. These bacteria, which are the source of the concept of chemolitho-autotrophy, deserve greater attention. Their growth physiology has not been adequately investigated, and reproducible methods to grow these bacteria are not yet known; the information on the biochemistry, therefore, is scarce and will not be considered here.

Bacteria which oxidize inorganic-sulphur compounds and deposit sulphur or oxidized sulphur compounds outside the cells have been known since 1902, when NATHANSOHN isolated *Thiobacillus thioparus* from a hydrogen sulphide spring in the Gulf of Naples (Italy). Since then, several species have been isolated and repeatedly investigated (Table 2-4). All species are more or less motile, polarly flagel-

Table 2-4

Sulphur-oxidizing bacteria. (o) obligate; (f) facultative (Compiled from the sources indicated)

Species	Growth at pH	Electron donor	Electron acceptor	Author
Thiobacillus thiooxidans (o)	2–5	S^{--}, $S_2O_3^{--}$, S^0	O_2	WAKSMAN and JOFFE (1922)
Thiobacillus ferrooxidans (o, f)	2–6	Fe^{++}, $S_2O_3^{--}$, S^0	O_2	COLMER and co-authors (1950)
Thiobacillus thioparus =*thiocyanoxidans* (o)	6–8	CNS^-, $S_2O_3^{--}$, S^0	O_2	NATHANSOHN (1902)
Thiobacillus denitrificans (o)	6–8	CNS^-, $S_2O_3^{--}$, S^0	O_2, NO_3^-	BEIJERINCK (1904)
Thiobacillus neapolitanus (o)		$S_2O_3^{--}$, S^0	O_2	PARKER and PRISK (1953)
Thiobacillus intermedius (f)	2–6	$S_2O_3^{--}$, S^0 glucose, glutamate YE	O_2	LONDON (1963)
Thiobacillus novellus (f)	6–8	$S_2O_3^{--}$, (S^0) glutamate, YE, NB	O_2	STARKEY (1935)
Thiobacillus perometabolis (f)		$S_2O_3^{--}$ and succinate, sucrose	O_2	LONDON and RITTENBERG (1967)
Thiobacillus thermophilica (o)		$S_2O_3^{--}$, S^0	O_2	EGOROVA and DERYUGINA (1963)

lated aerobic rods belonging to the one genus, *Thiobacillus*. Some species are obligate chemolitho-autotrophs, a few species can also grow heterotrophically. While the thiobacilli are not bacilli in the sense of spore-forming bacteria and the name coined by BEIJERINCK (1904) is misleading, some sulphur oxidizers have been discovered which form thermoresistant spores and are thermophilic (*T. thermophilica;* EGOROVA and DERYUGINA, 1963). A new thermophilic and acid-tolerant *Thiobacillus* species has been isolated recently (WILLIAMS and HOARE, 1970). Some sulphur oxidizers are able to grow on the basis of the oxidation of ferrous to ferric iron; these iron-oxidizing strains are included in the species *T. ferrooxidans*.

In Table 2-4 only those species have been considered which, on the basis of a numerical analysis, are 'good species' (HUTCHINSON and co-authors, 1969) or have been described since then. The first five strains listed are obligate chemo-autotrophs. *Thiobacillus thiooxidans* (WAKSMAN and JOFFE, 1922) oxidizes sulphur, hydrogen sulphide, and thiosulphate and lowers the pH value below 2·0. Initiation of growth in sulphur-containing media occurs at pH values from 3·0 to 6·4; viable bacteria have even been recovered from media with pH values below 1·0.

With respect to the final pH value reached during growth in sulphur and thiosulphate-containing media, *Thiobacillus ferrooxidans* resembles *T. thiooxidans*. However, it differs from *T. thiooxidans* with respect to its ability to use ferrous iron as the sole energy source (COLMER and co-authors, 1950; TEMPLE and COLMER, 1951). H_2S is not metabolized. Minor differences in substrate utilization of the strains named *Ferrobacillus ferrooxidans* and *F. sulfooxidans* are not considered to justify different species names (UNZ and LUNDGREN, 1961; KELLY and TUOVINEN, 1972).

Thiobacillus thioparus (BEIJERINCK, 1904) is regarded as the type species of this genus. This species oxidizes sulphur, thiosulphate, and thiocyanate under aerobic conditions. The initial pH value is 6 to 8, the final pH value is always below 5·0, however, not less than 3·5. Normal growth occurs under aerobic conditions; anaerobic growth on thiosulphate-nitrate agar is modest and ceases during prolonged anaerobic cultivation. *T. denitrificans* (BEIJERINCK, 1904) is able to oxidize sulphur, thiosulphate and thiocyanate under aerobic conditions as well as anaerobically in the presence of nitrate. Nitrate is reduced to molecular nitrogen. *T. denitrificans* is sensitive to low pH values and grows faster anaerobically than aerobically, since the denitrification reaction compensates for the acid produced by sulphur or thiosulphate oxidation. *T. neapolitanus* (PARKER and PRISK, 1953) differs from *T. thioparus* by the inability to grow anaerobically and to oxidize thiocyanate. Acid production becomes inhibitory at values below pH 2·8. The strains of this species are very resistant to inhibitors and high concentrations of thiosulphate, phosphate, chloride, or even phenol.

Thiobacillus intermedius (LONDON, 1963) is nearly as acid-tolerant as is *T. thiooxidans* (pH 2·0); it is, however, a facultative heterotroph and is able to grow with glucose, glutamate, yeast extract, or citrate as energy and carbon sources. *T. novellus* (STARKEY, 1935) oxidizes thiosulphate slowly during growth and does not decrease the pH value below 5·0. It is a facultative heterotroph and grows on nutrient broth, glutamate, and a few other substrates. *T. perometabolis* (LONDON and RITTENBERG, 1967) is a chemolithoheterotrophic bacterium. Morphologically and with respect to the range of carbon compounds utilized, it resembles the

pseudomonads. It grows in thiosulphate-mineral base media supplemented with fructose or other sugars and can utilize the energy obtained from sulphur oxidation to assimilate the organic substrate. The reader is referred to reviews by VISHNIAC and SANTER (1957), POSTGATE (1969) and RITTENBERG (1969) for further information.

Recently a new acidophilic, thermoresistant, facultative lithotrophic sulphur bacterium has been isolated from acid thermal areas: *Sulfolobus acidocaldarius* (BROCK and co-authors, 1972).

(c) Hydrogen Bacteria

Bacteria which are able to grow in a mineral medium under a hydrogen–oxygen atmosphere and with carbon dioxide as the only carbon source have been discovered by KASERER (1906). However, the strain isolated by him, *Bacillus pantotrophus*, and those isolated by his contemporaries (NABOKICH and LEBEDEFF, 1907; NIKLEWSKI, 1908, 1914; LEBEDEFF, 1909), *Hydrogenomonas flava*, *H. vitrea*, and *H. agilis*, have been inadequately described and are no longer available. Several strains isolated by RUHLAND (1922, 1924; GROHMANN, 1924) were described as spore formers. Among them, *B. pycnoticus* was intensively studied, but the strain died off while the experiments were still in progress. The older literature has been reviewed by EBERHARDT (1965) and SCHLEGEL (1966).

Hydrogen bacteria so far studied are aerobic facultative chemolitho-autotrophs. They can also grow under completely heterotrophic conditions and can perform a mixotrophic type of metabolism. They are defined as a physiological group and comprise Gram-negative as well as Gram-positive bacteria. The Gram-negative hydrogen oxidizers have recently been studied under taxonomical aspects and the formerly described species were attributed to new genera (DAVIS and co-authors, 1969, 1970; RALSTON and co-authors, 1972). The Gram-positive hydrogen bacteria have not been studied from taxonomical viewpoints. In Table 2-5 the well-known strains of hydrogen bacteria are listed.

Pseudomonas saccharophila (DOUDOROFF, 1940) is a polarly flagellated rod, remarkable for its ability to utilize a number of sugars (D-glucose, D-fructose, D-mannose, D-galactose, sucrose, cellobiose, melibiose, maltose, trehalose, D-ribose, D-xylose, L-arabinose) and even to hydrolyze starch (DAVIS and co-authors, 1970). *P. facilis* (SCHATZ and BOVELL, 1952), which morphologically resembles the foregoing species, is able to utilize only a few sugars (D-fructose, D-mannose, D-galactose, D-glucose, D-ribose, D-xylose, L-arabinose) and is characterized by the property to hydrolyze gelatine and exogenous poly-ß-hydroxybutyrate (DAVIS and co-authors, 1970). *P. ruhlandii* (PACKER and VISHNIAC, 1955) prefers organic acids and even alcohols as organic substrates (DAVIS and co-authors, 1970). *P. palleronii* and *P. flava* are characterized by carotenoid pigments (DAVIS and co-authors, 1970).

Hydrogenomonas eutropha (WITTENBERGER and REPASKE, 1958) belongs to those hydrogen bacteria which are most often encountered in enrichment cultures. The type strain resembles *Hydrogenomonas H 1*, *H 20*, and *H 16* (WILDE, 1962), strain *Z 1* (SAVEL'EVA and ZHILINA, 1968; VEDENINA, 1968). Fourteen representative strains have been investigated and reclassified by DAVIS and co-authors (1969) as *Alcaligenes eutrophus*. The Gram-negative rods are motile by 1 to 4 or more peritrichous

Table 2-5

Hydrogen bacteria. −: Gram-negative, +: Gram-positive bacteria (Compiled from the sources indicated)

Species	Gram strain	Number and insertion of flagellae	Denitrification	Author
Pseudomonas saccharophila	−	1 polar	−	Doudoroff (1940)
Pseudomonas facilis	−	1 polar	−	Schatz and Bovell (1952)
Pseudomonas ruhlandii	−	1 polar	−	Packer and Vishniac (1955)
Pseudomonas palleronii	−	1 polar	−	Davis and co-authors (1969)
Hydrogenomonas eutropha = *Alcaligenes eutrophus*	−	2–6 peritrichous	+	Wilde (1962), Repaske (1962)
Alcaligenes paradoxus	−	2–6 peritrichous	−	Davis and co-authors (1969)
Paracoccus denitrificans	−	−	+	Beijerinck and Minkman (1910), Kluyver and Verhoeven (1954)
Nocardia autotrophica	+	−	−	Takamiya and Tubaki (1956)
Nocardia petroleophila	+	−	−	Hirsch (1961)
Nocardia opaca strain *1 b*	+	−	−	Siebert (1969), Aggag and Schlegel (1973)
Brevibacterium 12-60-x	+	−	−	Eberhardt (1969)
Arthrobacter strain *7 C*	+	−	−	Siebert (1969), Tunail and Schlegel (1972)

flagella. Fructose is the only sugar used; a wide variety of organic compounds is metabolized. Strain *H 16* is characterized by organotrophic and autotrophic denitrification. *A. paradoxus*, biotype I (DAVIS and co-authors, 1969) resembles *A. eutrophus* by motility and degenerately peritrichous flagellation; however, it is characterized by yellow pigmentation caused by carotenoids, inability to effect denitrification, hydrolysis of extracellular poly-β-hydroxybutyrate or Tween 80, and utilization of several sugars (glucose, fructose, mannose, galactose, L-arabinose, pentoses).

Micrococcus denitrificans (BEIJERINCK and MINKMAN, 1910; KLUYVER and VERHOEVEN, 1954) has been isolated as a denitrifying bacterium, but proved to be able to grow as a hydrogen bacterium autotrophically (KORNBERG and co-authors, 1960; VOGT, 1965). It is able to use either oxygen or nitrate as a H-acceptor and hydrogen or a wide variety of organic compounds as energy source. This Gram-negative, facultative autotroph has been reclassified as *Paracoccus denitrificans* (DAVIS and co-authors, 1969).

Gram-positive hydrogen bacteria of the genera *Mycobacterium*, *Nocardia*, *Streptomyces*, and *Streptosporangium* have been repeatedly isolated. Only a few of the *Nocardia* strains have been investigated; however, since the strains (*Nocardia saturnea*, *N. petroleophila*) grew very slowly, and *N. autotrophica* not at all, in submerged culture (HIRSCH, 1961) the experiments were discontinued.

A new strain isolated as a propane-oxidizing bacterium (SIEBERT, 1969) exhibited excellent growth under autotrophic conditions and was tentatively identified as *Nocardia opaca* (strain *1 b*). It is able to grow heterotrophically on many compounds, hydrocarbons (C_{11}–C_{18}) included (AGGAG and SCHLEGEL, 1973). A Gram-positive, short, rod-shaped yellow bacterium has been described and tentatively named *Brevibacterium* strain *12-60-x* (EBERHARDT, 1969). A deep yellow coryneform bacterium, *Arthrobacter* strain *7 C*, has been described recently (TUNAIL and SCHLEGEL, 1972; TUNAIL, 1973).

The hydrogen-oxidizing bacteria described above are aerobic bacteria; they cannot ferment organic substrates. Only *Hydrogenomonas eutropha* and *Micrococcus denitrificans* can grow anaerobically as denitrifiers, either organotrophically or autotrophically. In this context, at least a few other hydrogen-oxidizing organisms should be mentioned. The phototrophic sulphur and non-sulphur purple bacteria as well as green bacteria are able to use hydrogen as external hydrogen donor, e.g. *Chromatium*, *Rhodospirillum*, *Rhodopseudomonas*, *Chlorobium*. Some green algae (*Chlorella*, *Ankistrodesmus*), when adapted to hydrogen or anaerobic conditions, are able to photo-oxidize hydrogen or to perform the oxyhydrogen reaction. Sulphate-reducing bacteria (*Desulfovibrio*) are able to use hydrogen and derive energy from anaerobic respiration. Furthermore, hydrogen is the common source for methane production from carbon dioxide by several methane-producing bacteria which have already been mentioned (p. 11). Since the processes have not yet been studied in detail, they will not be further considered here.

The metabolism of carbon monoxide is apparently closely related to that of hydrogen. Under anaerobic conditions *Methanosarcina barkeri* metabolizes CO with the formation of methane and carbon dioxide. Hydrogen has been shown to be an intermediary product of this reaction (KLUYVER and SCHNELLEN, 1947). The

aerobic utilization of carbon monoxide has been studied with *Hydrogenomonas carboxydovorans* (KISTNER, 1954). This bacterium grows either as a hydrogen bacterium or with CO as the hydrogen donor in a mineral medium supplemented by yeast extract. The CO-oxidizing system is strictly adaptive. Recent investigators are surprised to find that a potting-soil mixture and several top soils removed CO from a test atmosphere quickly and completely while sterilized soil failed (INMAN and co-authors, 1971). The study of carbon-monoxide metabolism certainly needs revival.

(d) Iron Bacteria

In neutral or alkaline media, ferrous iron is unstable and in the presence of atmospheric oxygen is oxidized to ferric iron. Final proof of biological energy generation from the oxidation of ferrous to ferric iron is due to the discovery of an acid-tolerant rod, *Thiobacillus ferrooxidans*, able to grow either on ferrous iron or thiosulphate as inorganic hydrogen donors (COLMER and co-authors, 1950).

Our assumption that the classical iron bacteria are chemo-autotrophs is based mainly on old observations in nature and a few experiments. The promising attempts to grow *Gallionella ferruginea* on iron sulphide as an energy source (KUCERA and WOLFE, 1957) were discontinued too soon. Some progress has been made using the material from the natural habitat (HANERT, 1968). Further work should be devoted to the question, whether *Leptothrix ochracea*, *Cladothrix dichotoma*, and *Crenothrix polyspora* profit from the oxidation of iron, possibly mixotrophically.

(4) Peculiarities of Chemolitho-autotrophy

With respect to their respiratory metabolism and to the highly developed metabolic sequences, the chemo-autotrophic bacteria are not different from other aerobic bacteria. They gain their energy from an electron-transport process, in which flavins, quinones, and cytochromes are involved, and synthesize all cellular components from sugars which are internally provided by the reductive assimilation of carbon dioxide. Biologically unique is only the combination of the ability to use inorganic compounds in order to gain energy and reducing power and to fix carbon dioxide in an efficient way. However, chemo-autotrophic bacteria differ from heterotrophic bacteria in a number of properties. These differences deserve more attention than they have received until now; they are outlined in the following.

Since the energy relationship between the inorganic substrates oxidized and the cell material synthesized appeared to be simple and accessible to thermodynamic calculations, the chemo-autotrophic micro-organisms attracted considerable attention; the overall energy changes have been considered by BAAS-BECKING and PARKS (1927) and GIBBS and SCHIFF (1960). The considerations culminated in the calculation of energy efficiencies. These commendable studies failed, however, to assess the relations to intermediary metabolism, carbon-dioxide fixation, and electron transport. The peculiarities of chemo-autotrophy reside in these basic metabolic processes.

(a) Generation of Reducing Power

As far as investigated, all autotrophic bacteria fix carbon dioxide via the Calvin reductive pentosephosphate cycle. Reducing power for CO_2 fixation and many biosynthetic reactions is mediated by reduced pyridine nucleotides. Only the hydrogen bacteria have at their disposal a hydrogen donor, molecular hydrogen, which —by means of the enzyme hydrogenase (hydrogen dehydrogenase)—reduces NAD directly. Even among the hydrogen bacteria, several species have been found, in which this enzyme could not be detected. Except for the hydrogen bacteria, all chemo-autotrophs use electron donors which have too high a potential to be utilized directly for the reduction of pyridine nucleotides.

The involvement of a special mechanism of NAD-reduction becomes obvious, when considering the thermodynamics of the energy-yielding reactions and the redox potentials of the primary hydrogen donors (Table 2-6). No donor redox

Table 2-6

Standard reduction potentials for systems involved in chemo-autotrophy (Original)

System	E_0' (volts)
H^+/H_2	−0·42
NH_4^+/NH_2OH	+0·899
Fe^{+++}/Fe^{++}	+0·77
NO_3^-/NO_2^-	+0·42
NO_2^-/NH_2OH	+0·066

couple has a potential (E_0') lower than −0·200 volts: E_0' for NH_2OH/NH_4^+ is +0·899 V, for NO_2^-/NH_2OH +0·066 V, for NO_3^-/NO_2^- +0·42 V, and for Fe^{+++}/Fe^{++} +0·77 V. Electrons originating from these oxidations cannot be utilized directly for NAD-reduction ($NAD^+/NADH$ = −0·32 V). Hence for theoretical reasons, an indirect way of NAD-reduction had to be postulated (LEES, 1962; ALEEM and LEES, 1963).

(b) Generation of Energy

With respect to oxidative phosphorylation, the chemolithotrophs also find themselves in a bad position. The standard potentials of certain oxidation reactions are so high that the electron transfer to oxygen eventually results only in the phosphorylation of one ADP to yield one ATP. On the other hand, the expenditure of energy is much higher than in heterotrophic organisms. Not only is the indirect way of generating reducing power ($NADH_2$) from the primary electron donors dependent on energy, but also for the conversion of the carbon source to cell material a much higher amount of energy is needed than in heterotrophs. As has been calculated, only 2·9 mole of ATP are required to convert 1 mole of carbon from carbohydrate to cell material, whereas 7·9 mole of ATP are needed to synthesize cell material from carbon dioxide (HEMPFLING and VISHNIAC, 1967; VISHNIAC, 1971).

The consequences of these thermodynamic considerations are easy to comprehend. Compared to the cell yield, the amount of primary electron donor consumed is rather high. The rate of oxygen uptake, compared to growth rate, is also high. Since energy generation via electron-transport processes plays a predominant role in autotrophic bacteria, the occurrence of large internal membrane systems would not be surprising. Catabolic pathways and the tricarboxylic-acid cycle are partially dispensable, as far as these routes are involved in energy production; only those enzymes can be expected to be present in normal amounts which participate also in biosynthetic reactions. Further predictions may be derived with respect to metabolic regulation mechanisms. Since CO_2 fixation plays a predominant role in these cells and consumes the major part of the ATP generated, special regulation mechanisms ought to be involved to guarantee the energy harmony in the cell and to protect endergonic biosynthetic reactions from the ATP pulling action of CO_2-fixation. Finally, carbon dioxide as the substrate of these energy-consuming reactions may effectively control the rate of electron transport and stimulate oxidative phosphorylation. The adaptation to these special requirements (imposed on the cell by the necessity to synthesize all cell material from carbon dioxide) may have reached a point where the autotrophic cells are no longer able to use organic substrates as energy sources and have become obligately bound to the autotrophic mode of life. Although the basic metabolic machinery is similar or almost identical to that of heterotrophic cells, the autotrophs possess several peculiarities. Since we are still far from having explored these special capabilities and properties, a brief survey on the present status of research appears to be necessary. The literature related to energy-coupling mechanisms has recently been reviewed by PECK (1968) and KELLY (1971).

(c) The Electron Transport Process

Electron transport in bacteria seems to be similar to that in mitochondria. The electrons supplied by the substrates are transferred to co-enzymes and reach oxygen via a set of protein-bound electron carriers. The respiratory chain is associated with

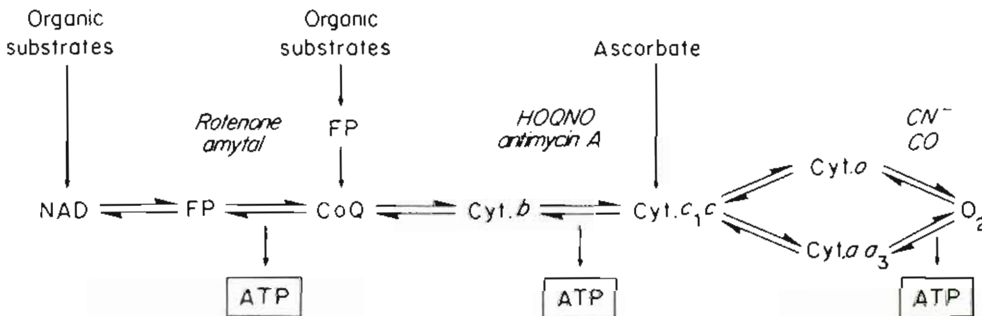


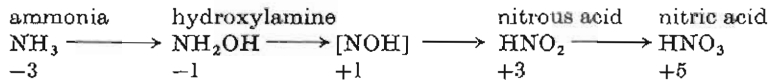
Fig. 2-1: Respiratory chain and entry points of electrons from various substrates. Some hydrogen donors (malate, isocitrate, glutamate, 3-hydroxyacyl-CoA) deliver their electrons to NAD, other substrates (succinate, fatty acyl-CoA, glycerophosphate) transfer reductive equivalents directly to co-enzyme A. Probable sites of energy conservation leading to ATP formation and sites of inhibition by representative inhibitors are indicated. (Original.)

the cell membrane, which in the bacterial cell may either be the cytoplasmic membrane or an intracytoplasmic membrane system. A simple model of a complete respiratory chain is presented in Fig. 2-1.

Compared to the components of the electron-transport chain in mitochondria, there is a high degree of variation in the components found in different bacteria, especially with respect to the cytochromes (HAROLD, 1972). In some bacteria, cytochrome *o* replaces the cytochrome *a*. Cytochromes *c* have been found in all micro-organisms which contain electron-transport chains. Cytochromes *c* vary considerably with respect to their chain length and amino-acid sequence of the apoprotein to which the haem group is covalently bound. Present information has been recently reviewed (HORIO and KAMEN, 1970; KAMEN and HORIO, 1970). Very little is known about structural components, enzymes supplying electrons from inorganic hydrogen donors, and coupling factors for oxidative phosphorylation (HAROLD, 1972).

Nitrifying Bacteria

Oxidation of ammonia to nitrate is accomplished in two steps, each step being catalyzed by a separate, highly specialized group of bacteria; among the chemolithotrophs no organism is known which is able to oxidize ammonia to nitrate directly. The change of the oxidation state of the nitrogen atom indicates that a minimum of three intermediates should be involved:



$$\underset{-3}{\overset{\text{ammonia}}{NH_3}} \longrightarrow \underset{-1}{\overset{\text{hydroxylamine}}{NH_2OH}} \longrightarrow \underset{+1}{[NOH]} \longrightarrow \underset{+3}{\overset{\text{nitrous acid}}{HNO_2}} \longrightarrow \underset{+5}{\overset{\text{nitric acid}}{HNO_3}}$$

One of these intermediates is nitrite; this is the end-product of ammonia oxidation by the nitrite-forming bacteria (*Nitroso-*) and the substrate of nitrate formation by the nitrite-oxidizing bacteria (*Nitro-*).

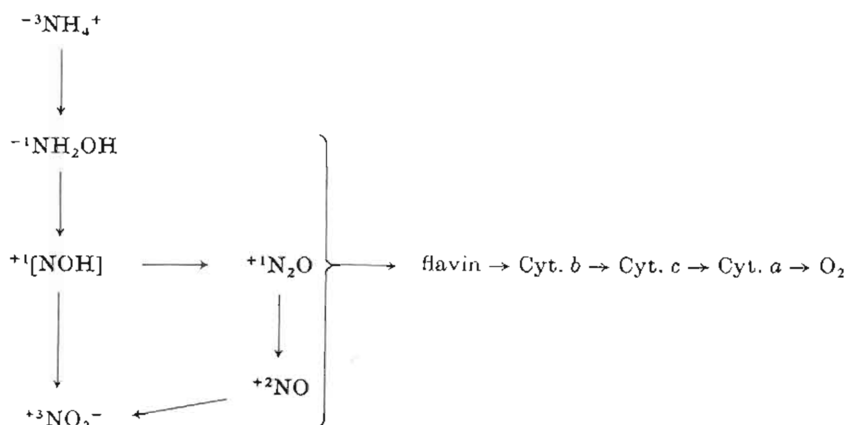
Nitrification is apparently not restricted to the lithotrophs; heterotrophic bacteria and even fungi contribute to nitrification in nature, among them *Aspergillus flavus*, strains of *Corynebacterium*, *Arthrobacter*, and *Nocardia* (EYLAR and SCHMIDT, 1959; DOXTADER and ALEXANDER, 1966; VERSTRAETE and ALEXANDER, 1972). Not only ammonia or nitrite are oxidized, but also a number of organic nitrogenous substances with the transient accumulation of hydroxylamine, hydroxamic acids, 1-nitrosoethanol and other compounds. Heterotrophic nitrifiers are apparently able to oxidize ammonia to nitrate within the same cell.

Hydroxylamine has been shown to be a potential intermediate of ammonia oxidation by *Nitrosomonas europaea* (HOFMAN and LEES, 1953). Although hydroxylamine is toxic in concentrations exceeding 1·7 mM, it is oxidized by suspensions of whole cells if added in still smaller concentrations (0·1 mM). During the oxidation of ammonia in the presence of hydrazine, whole cells accumulated a small amount of hydroxylamine. Furthermore, in contrast to ammonia, both hydroxylamine and hydrazine were oxidized by cell-free preparations of *N. europaea* and *Nitrosocystis oceanus* (HOOPER and NASON, 1965). Nitrite was formed under these conditions. Anaerobically, in the presence of an electron acceptor, hydroxylamine was oxidized

to nitrous oxide (N_2O) and nitric oxide (NO). These observations suggest molecular oxygen to be directly involved in the oxidation of hydroxylamine.

The oxidation of ammonia to hydroxylamine is thermodynamically unfavourable. The system NH_4^+/NH_2OH has a high potential ($E_0' = +0{\cdot}899$ volts); ammonia cannot be oxidized by dehydrogenation by one of the known electron carriers of the respiratory chain. Under standard conditions the reaction is endergonic ($\Delta G_0' = +3{\cdot}7$ kcal mole^{-1}) and may require an energy-dependent activation of ammonia (NICHOLAS, 1963). Since the enzyme is probably localized in the cell-wall region, gentle methods for preparing cell-free homogenates have been used; however, no oxidation of ammonia to hydroxylamine or nitrite has been found (WALLACE and NICHOLAS, 1969a), and it is questionable whether an oxygenase-type reaction is involved.

As components of the electron transfer chain flavins, cytochromes *b*, *c*, *a*, and cytochrome oxidase have been found in particles of *Nitrosomonas europaea*. The experimental evidence so far obtained is compatible with the following scheme:



Since a direct reduction of pyridine nucleotides by intermediates of this pathway could not be observed, energy generation may occur only at the cytochrome level.

During the oxidation of nitrite by *Nitrobacter winogradskyi* the electrons are transferred to cytochrome *c*. The nitrite-oxidizing enzyme ('nitrite oxidase') associated with particles contains cytochromes *c*, *a*, and a_1; cytochrome *b* is apparently not involved in nitrite oxidation. The involvement of a flavin is suggested by inhibition with quinacrine. Older experiments have more recently been confirmed (O'KELLEY and co-authors, 1970).

It is generally agreed that the chemolithotrophic bacteria derive their primary energy from the oxidation of inorganic substrates by oxidative phosphorylation. First evidence has been obtained from experiments using extracts of *Nitrobacter agilis* (ALEEM and NASON, 1960); esterification of inorganic phosphate as well as ATP-formation could be demonstrated. The yield of ATP-formation, expressed as P/O ratio, was rather low in cell-free systems of *Nitrosomonas europaea* or *N. agilis*. Values as high as 1 for nitrite oxidation and 2 for NADH oxidation by particles from *N. agilis* have been reported recently (ALEEM, 1968). Since the electron transport particles are very sensitive and oxidative phosphorylation is a complex process,

these values do not permit conclusions with respect to the number of coupling sites. Two sites may eventually be involved in ammonia oxidation and one site in nitrite oxidation (ALEEM, 1970).

Since chemo-autotrophic bacteria depend on carbon-dioxide fixation and since this process involves endergonic reduction reactions, in addition to ATP, reduced pyridine nucleotides have to be formed. As mentioned above, the electrons originating from nitrite and other intermediates have a much higher potential than NAD; therefore, one has to assume that a reverse electron flow operates in these bacteria. The concept of an energy-dependent reverse electron flow has been developed, observing the reduction of NAD by succinate in mitochondria (CHANCE and HOLLUNGER, 1960; KLINGENBERG, 1963). In extracts of *Nitrosomonas europaea* and *Nitrobacter winogradskyi*, an electron transfer from cytochrome *c* to NAD dependent on ATP was first demonstrated by ALEEM and co-authors (1963). For *N. winogradskyi*, these results have been confirmed by KIESOW (1963, 1964) and extended by SEWELL and ALEEM (1969); 5 mole of ATP were required to reduce 1 mole NAD by nitrite. Although the observations are based mainly on spectrophotometric data and experiments were made employing crude extracts, they appear to be convincing. Since experiments with purified enzyme preparations are lacking, it seems premature to indicate the entry sites of the electrons delivered from various inorganic hydrogen donors into the electron-transport chain.

Electron-microscopy studies have revealed remarkable structures in the nitrifying bacteria (MURRAY and WATSON, 1965; REMSEN and co-authors, 1967). Stacks of lamellae similar to those of some phototrophic bacteria (e.g. *Ectothiorhodospira mobilis*) have been discovered in *Nitrosocystis oceanus*. These membraneous organelles consist of about 20 flattened vesicles and almost traverse the cell. In *Nitrosomonas europaea*, intracytoplasmic membranes intrude from the cytoplasmic membrane and remain almost parallel and close to it, thus resembling the membrane system in *Rhodospirillum molischianum* or *Rhodomicrobium vannielii*. In *Nitrobacter agilis*, multilayers of membranes are located in the polar regions of the cells (MURRAY and WATSON, 1965). A similar multilayered membrane system has been described for *Nitrobacter winogradskyi* (VAN GOOL and co-authors, 1969). In *Nitrosolobus multiformis*, intraplasmic membranes are arranged in a quite different manner. The cells are segmented by the membranes, a frequent arrangement being 1 large central compartment surrounded by 5 to 20 membrane-bound areas (WATSON and co-authors, 1971). A tubular cytomembrane system similar to that in the phototrophic sulphur bacterium *Thiocapsa pfennigii* is characteristic for *Nitrococcus mobilis*; the tubes are distributed randomly throughout the cell (WATSON and WATERBURY, 1971). Exceptions with regard to the extensive intracellular membrane system in nitrifying bacteria are *Nitrospina gracilis* (WATSON and WATERBURY, 1971) and *Nitrosospira briensis* (WATSON, 1971a). Both these bacteria are very thin (0·3 μm diameter) and possibly have solved the problem of the enlargement of the membrane surface area by a high cell surface/volume ratio. The problems mentioned have not yet been satisfactorily investigated.

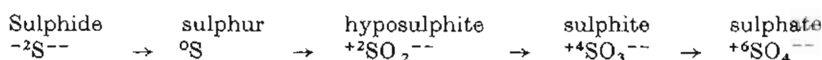
'The electron transfer chain which can oxidize such toxic hydrogen donors as hydroxylamine (*Nitrosomonas* and *Nitrosocystis*) and nitrous acid (*Nitrobacter*) far more effectively than NADH or succinate certainly arouse our curiosity.

> Indeed, more detailed work is necessary to identify the components of the respiratory chain; flavines, quinones, numerous cytochromes—especially cytochromes of the *a* and *o* types reported to be terminal oxidases in these bacteria' (WALLACE and NICHOLAS, 1969b, p. 382).

For more detailed reviews on nitrifying organisms the reader is referred to PECK (1968), WALLACE and NICHOLAS (1969b), and ALEEM (1970).

Sulphur-Oxidizing Bacteria

Sulphur-oxidizing bacteria are able to grow on either hydrogen sulphide, sulphur or thiosulphate as hydrogen donors. Many of them appear to catalyze the oxidation of hydrogen sulphide to sulphate without transiently accumulating an intermediate. The change of the oxidation state of sulphur suggests that four steps are involved in the oxidation of hydrogen sulphide:

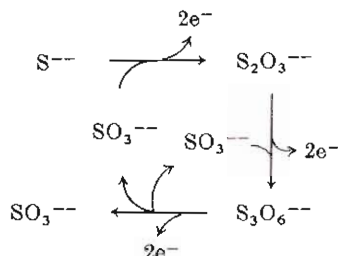


Other intermediates may play a role in sulphur oxidation, e.g. polysulphides or polythionates ($S_2O_3^{--}$, $S_3O_6^{--}$, $S_4O_6^{--}$). Polythionates are linear chains of S-atoms (oxidation state −2), called sulphanes, with terminal groups of sulphonic acid (oxidation state +6). Elemental sulphur is used or produced by many organisms. Elemental sulphur exists in a large number of allotropic modifications; only orthorhombic sulphur is stable under normal conditions of temperature and pressure, and all other allotropes convert to it. It is an eight membered ring (S_8). The refractile sulphur droplets, which are intracellularly accumulated in several phototrophic sulphur bacteria and filamentous non-photosynthetic sulphur bacteria, are amorphous; however, they are quickly converted to orthorhombic sulphur outside the cell (HAGEAGE and co-authors, 1970). For metabolic experiments, hydrophilic elemental (colloidal) sulphur is prepared by acidifying thiosulphate solutions. POSTGATE (1963) has warned in regard to the instability of sulphur compounds.

Although a wealth of information has accumulated during the last year, neither the path of sulphur during sulphur oxidation by thiobacilli nor the electron transport nor the reduction of NAD are completely understood. Presumably, different pathways operate in different organisms. Hence only a brief survey follows, mainly based on the competent reviews by PECK (1968), TRUDINGER (1969), and ROY and TRUDINGER (1970).

Sulphide is oxidized by most thiobacilli. However, during sulphide oxidation by growing cells or bacterial extracts, sulphur, thiosulphate, and polythionate accumulate. Since hydrogen sulphide is auto-oxidizable under acidic conditions in the presence of air, and elemental sulphur reacts non-enzymatically with sulphides to form hydropolysulphides, it is difficult to draw conclusions from the corresponding experiments. However, experiments using membrane fragments of *Thiobacillus thiooxidans* (*concretivorus*) indicated that sulphide oxidation is an enzymic process and that the electrons are transferred via cytochrome *c* finally to oxygen (MORIARTY and NICHOLAS, 1969, 1970).

On the basis of experimental evidence available for the reverse process—the reduction of sulphite in *Desulfovibrio gigas* (SUH and AKAGI, 1969; POLEE and PECK, 1971)—the following cycle may be considered for sulphide oxidation: The first step would be a sulphide oxidation in the presence of sulphite. The thiosulphate formed would be further oxidized (in the presence of sulphite) to trithionate, which can undergo an oxidative cleavage to three molecules of sulphite:

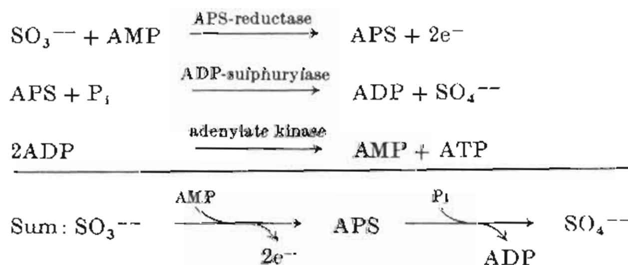


With the methods of enzymology and the knowledge of sulphur chemistry available, the pathways of sulphur oxidation ought to be elucidated shortly. The question whether elemental sulphur is oxidized by thiobacilli via thiosulphate or directly to sulphate is still controversial. Experiments with $^{18}O_2$ suggested that a soluble sulphur-oxidizing enzyme of *Thiobacillus thiooxidans* is an oxygenase. *T. denitrificans* oxidizes sulphur in the absence of oxygen. It appears to be premature to consider details on the electron transfer during sulphur oxidation.

The uptake of elemental sulphur by thiobacilli has been studied by ROY and TRUDINGER (1970). Preparations of colloidal sulphur are oxidized by washed suspensions of *Thiobacillus thioparus* nearly as fast as soluble compounds such as thiosulphate. Flowers of sulphur are oxidized at a slow rate, which is increased by the addition of wetting agents. The superiority of colloidal sulphur compared to flowers of sulphur is, therefore, probably at least partially due to the hydrophilic nature of the sulphur particles. The cells of *T. thiooxidans* excrete phospholipids into the medium, the main components are phosphatidyl N-methylethanolamine, phosphatidyl glycerol and diphosphatidyl glycerol (SHIVELY and BENSON, 1967; BARRIDGE and SHIVELY, 1968). The mechanism of sulphur uptake has not yet been elucidated.

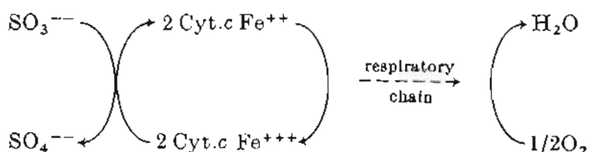
The oxidation of sulphite to sulphate is the terminal step in the oxidation of more reduced sulphur compounds and has been explored in some detail. There are at least two pathways in thiobacilli.

As shown by PECK (1960; 1962), in *Thiobacillus thioparus* and *T. denitrificans* the enzymes necessary for the 'Adenosine-phosphosulphate(APS)-pathway' of sulphite oxidation are present:



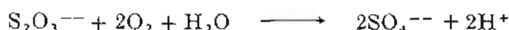
In the reaction ferricyanide serves as electron acceptor. In this pathway the energy is conserved by a unique process of substrate phosphorylation.

An AMP-independent pathway has been found in *Thiobacillus novellus* (CHARLES and SUZUKI, 1965). A partly purified enzyme from *T. novellus* oxidizes sulphite with cytochrome *c* or ferricyanide as electron acceptors. This oxidation is specific for sulphite and independent of AMP. The sulphite, cytochrome *c* oxidoreductase, is probably coupled to the respiratory chain:



There are many controversial results on the function of these two pathways in different thiobacilli, and the relative importance of the APS-pathway and the 'direct' pathway of sulphite oxidation still requires clarification.

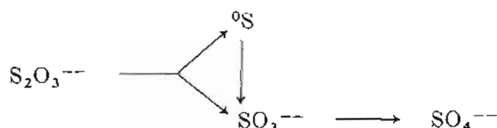
Thiosulphate is oxidized to sulphate by all species and strains of thiobacilli; the complete oxidation is not always found:



$$S_2O_3^{--} + 2O_2 + H_2O \longrightarrow 2SO_4^{--} + 2H^+$$

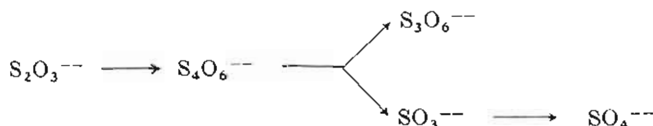
Thiobacillus thioparus owes its name to the incomplete oxidation of thiosulphate in weakly buffered cultures, resulting in the accumulation of elemental sulphur in the neighbourhood of the colony. Several pathways of thiosulphate oxidation are presently being discussed (TRUDINGER, 1969; CHARLES, 1969), and two will be mentioned here.

The simplest pathway has been proposed by CHARLES and SUZUKI (1966); it is based on the observation that extracts of *Thiobacillus novellus* oxidize thiosulphate to sulphate, but are unable to metabolize tetrathionate:



The cleavage of thiosulphate to give sulphur and sulphite is the most direct route.

During oxidation of thiosulphate by whole cells or cell homogenates polythionates are almost invariably formed; hence cyclic pathways may be involved with polythionates as intermediates:



In our context, coupling of the oxidation of sulphur compounds to electron transport is important. Among the components of the electron-transport particles mainly cytochrome *c* appears to be involved. According to present experimental

evidence, electrons of the sulphur compounds enter the respiratory chain on the level of cytochrome *c*, and the cytochromes a, a_3 or *o* mediate terminal oxidation.

Phosphorylation in thiobacilli has been investigated for a long time, and *Thiobacillus thiooxidans* is the first autotrophic organism, in which phosphorylation has been studied (VOGLER and UMBREIT, 1942). Substrate-level as well as electron-transport phosphorylation may occur. The involvement of the latter has been concluded from yield measurements (HEMPFLING and VISHNIAC, 1967) and from the sensitivity of CO_2 fixation to uncoupling agents. Finally, cell-free extracts have been obtained which catalyzed phosphorylation and permitted the determination of P/O-ratios (ROSS and co-authors, 1968; COLE and ALEEM, 1970).

During the oxidation of sulphur compounds electrons enter the electron-transport chain probably at the level of cytochrome *c*; hence a reverse electron flow has to be assumed in order to satisfy the requirement of CO_2 fixation for NADH. An energy-linked reduction of pyridine nucleotide has been demonstrated in extracts from *Thiobacillus novellus* (ALEEM, 1966a, b) and *T. neapolitanus* (SAXENA and ALEEM, 1972). Although the mechanism of ATP formation, entry of electrons, possible activation of sulphur-compounds, and the localization of pertinent activities in membrane components have still to be explored, the involvement of a reverse energy-dependent electron flow appears to be compelling.

Fine structural investigations in the thiobacilli are not consistent with the suggestion that the predominant role of energy generation in autotrophic bacteria is reflected by a well-developed intraplasmic membrane system. Lamellar bodies are seen in *Thiobacillus novellus* and *T. intermedius* and mesosomes in *T. denitrificans* (SHIVELY and co-authors, 1970). However, the differences between autotrophically (thiosulphate) and heterotrophically (glucose) grown cells of *T. novellus* were restricted to the envelope structure and to inclusions (VAN CAESEELE and LEES, 1969).

Iron-Oxidizing Bacteria

The oxidation of ferrous iron to ferric iron by *Gallionella ferruginea* provided one of the arguments for the concept of chemolitho-autotrophic metabolism (WINOGRADSKY, 1888). Although many observations support these early conclusions, iron oxidation and strict autotrophy of the classic iron bacteria have never been confirmed by modern methods. Unlike *G. ferruginea*, the iron-oxidizing thiobacilli prefer and tolerate low pH values, at which ferrous iron is not auto-oxidized by air. With several strains of *Thiobacillus ferrooxidans* it has been proved that energy for autotrophic growth can be obtained from the oxidation of ferrous iron:

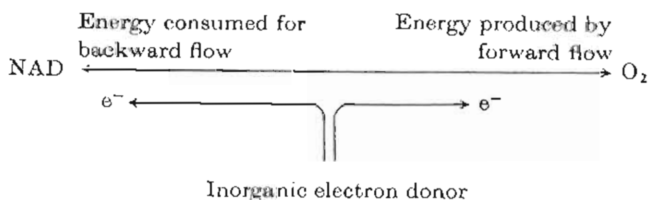
$$4\,FeSO_4 + O_2 + 2\,H_2SO_4 \rightarrow 2\,Fe_2(SO_4)_3 + 2\,H_2O$$

Iron oxidation and CO_2 fixation have been well demonstrated in *T. ferrooxidans* (LANDESMAN and co-authors, 1966; GALE and BECK, 1967). Cytochrome *c* has been purified and cytochrome a_1 has been identified (BECK, 1960). One would expect ferrous iron to be the electron donor for an iron-cytochrome *c* reductase which mediates electron transfer in the terminal respiratory chain. Enzymological studies, however, suffer from the non-enzymatic reduction of cytochrome *c* by ferrous iron

and its stimulation by organic compounds. These difficulties have been discussed in detail by PECK (1968).

Since the standard potential of the iron couple is high, NAD cannot directly be reduced with electrons from this oxidation. An ATP-dependent reverse electron flow in cell-free preparations of a strain of *Thiobacillus ferrooxidans* was reported some time ago (ALEEM and co-authors, 1963). The conclusion is compelling that both the sulphur as well as the iron-oxidizing bacteria depend on reverse electron flow.

Thus, the chemolitho-autotrophic bacteria can be envisaged to be experts in handling the electron transport chain using it partially for energy generation and partially for NAD reduction:

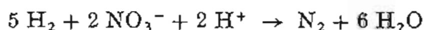


Hydrogen-Oxidizing Bacteria

Molecular hydrogen is oxidized by hydrogen bacteria with the production of water:

$$H_2 + 1/2\ O_2 \rightarrow H_2O$$

This oxyhydrogen reaction is also known as Knallgasreaction, and the hydrogen bacteria are also referred to as Knallgasbacteria. A couple of hydrogen bacteria (*Micrococcus denitrificans, Hydrogenomonas eutropha*) have the potential to use nitrate as a H-acceptor and to perform anaerobic respiration:



$$5\ H_2 + 2\ NO_3^- + 2\ H^+ \rightarrow N_2 + 6\ H_2O$$

Among the H-donors used by chemo-autotrophic bacteria, hydrogen has the most negative standard potential ($E_0' = -0{\cdot}42$ V). The electrons derived from gaseous hydrogen can easily reduce pyridine nucleotides ($E_0' = -0{\cdot}32$ V), provided the corresponding enzyme is present.

The enzymes catalyzing the formation or oxidation of molecular hydrogen are called hydrogenases. These enzymes are present in a large number of micro-organisms and fulfil quite different functions. In phototrophic bacteria they mediate the utilization of hydrogen as H-donor for photosynthetic reactions. In many anaerobes, they serve as 'hydrogen valves' to dispose of excess reducing power (GRAY and GEST, 1965). In N_2- fixing bacteria the hydrogenase activity is apparently due to a minor function of the nitrogenase. Only in the sulphate-reducing bacteria, the methane producers and the hydrogen bacteria do hydrogenases serve essentially the purpose of gaining reducing power for exergonic oxidative reactions. The enzymes, collectively called hydrogenases, differ not only with respect to their role in the metab-

olism of the organisms mentioned but also with regard to their tolerance to oxygen and enzyme inhibitors, to the nature of the hydrogen acceptor, and to their localization inside the cell. Even the hydrogenases of the hydrogen bacteria are not alike. Only the hydrogenases of the aerobic autotrophic hydrogen bacteria will be discussed here. The older literature has been considered by GEST (1954) and SCHLEGEL (1954, 1960, 1966).

A soluble hydrogenase has been described in preparations from *Pseudomonas saccharophila* (PACKER and VISHNIAC, 1955; BERNSTEIN and VISHNIAC, 1959; BONE and co-authors, 1963; BONE, 1963) and from *P. ruhlandii*. The enzyme catalyzes the β-stereospecific reduction of NAD and has been termed hydrogen dehydrogenase. The 300-fold purified enzyme does not reduce methylene blue; however, it reduces benzylviologen.

A particle-bound hydrogenase has been described in cell-free extracts of *Pseudomonas facilis* (ATKINSON and MCFADDEN, 1954). This enzyme reduces methylene blue or ferricyanide; however, it fails to reduce nicotinamide nucleotides.

Both a soluble NAD-reducing hydrogen dehydrogenase and a particulate hydrogenase have been found in extracts of *Hydrogenomonas eutropha* type strain (WITTENBERGER and REPASKE, 1961) and strain *H 16* (EBERHARDT, 1966a) as well as in *P. saccharophila* (BONE, 1960). The hydrogen dehydrogenase in crude extracts of the type strain of *H. eutropha* (REPASKE, 1962) is very sensitive to oxygen; its activity is increased by reducing agents, and the rate of NAD reduction is augmented by FMN (REPASKE and DANS, 1968). The soluble hydrogen dehydrogenase from *H. eutropha H 16* differs markedly from that of the type strain (REPASKE, 1962) and resembles that of *P. ruhlandii*. As reported by PFITZNER and co-authors (1970), the reduction of NAD by the 45-fold purified enzyme does not require cofactors. The enzyme is free from flavins and pyridine nucleotides; it reacts with NAD but not with NADP, FMN, FAD, methylene blue or oxygen, and is rather insensitive to oxygen.

Crude extracts and soluble fractions of *Pseudomonas facilis* catalyze the reduction of methylene blue by H_2, but do not reduce NAD. This type of behaviour is shared by many other strains, Gram-positive bacteria included (EBERHARDT, 1969). Whether in these strains the primary hydrogen acceptor is different from NAD or whether the hydrogenase loses its activity to NAD during the preparation procedure of the extracts remains to be studied.

Since in *Pseudomonas ruhlandii*, *Hydrogenomonas eutropha* and strain *H 16* two systems for the activation of hydrogen are present, it can be assumed that the hydrogen dehydrogenase supplies $NADH_2$ for biosynthesis and the particulate enzyme serves the generation of ATP.

The components of the respiratory chain correspond to those found in other bacteria. Flavoproteins and cytochromes *b* and *c* have been spectrophotometrically demonstrated in whole cells of *Pseudomonas ruhlandii* (PACKER, 1958), cytochromes *b* and *c* in *P. saccharophila* (BONE, 1963; YAMANAKA and co-authors, 1963). There are further reports on the presence of co-enzyme Q (Q_8) (LESTER and CRANE, 1959), a $NADH_2$-specific menadione reductase (REPASKE and LIZOTTE, 1965), cytochromes *b* and *c* in *Hydrogenomonas eutropha* strain *H 20* (BONGERS, 1967) and cytochromes *b*, *c*, *a*, and *o* as well as ubiquinone Q_8 and vitamin K_2 in strain *H 16* (PFITZNER, 1972).

The components of the respiratory chain are contained in the particle fraction, prepared from the crude cell homogenate by fractional centrifugation. The particles of *Hydrogenomonas eutropha H 16* have been studied in some detail (PFITZNER, 1972). They catalyze the oxyhydrogen reaction. By repeated washings the particles lose their catalytic activity almost completely; the ability to oxidize hydrogen could be partially restored by the addition of a heat-stable factor concentrated from the washing water; since it did not stimulate the particle-catalyzed reduction of methylene blue by hydrogen, the factor is not involved in the first step of hydrogen transport.

Employing inhibitors of the electron-transport chain, evidence has been obtained that different carriers are involved in the oxidation of hydrogen and of NADH. While the oxidation of NADH is very sensitive to atebrine, rotenone, piericidine A, and antimycine A, the oxidation of H_2 is not inhibited by atebrine and rotenone and has only a low sensitivity to piericidine and antimycine.

Oxidative phosphorylation associated with the oxyhydrogen reaction has been studied in crude extracts of *Hydrogenomonas eutropha* strain *H 20* (BONGERS, 1967). P/O ratios up to 0·6 have been observed, and pyridine nucleotides were apparently not involved in the electron-transfer system. These measurements, yield determinations in continuous culture, as well as the absence of cytochrome *a*, led BONGERS (1970b) to assume two phosphorylation sites in *H. eutropha*: one between H_2 and cytochrome *b*, the other between cytochrome *b* and oxygen. The assumption of only one coupling site between cytochrome *b* and O_2 has been supported by yield determinations with succinate or fumarate as growth substrates (BONGERS, 1970a). Contrary to these reports, using crude extracts of *H. eutropha* and the luciferin-luciferase assay for ATP-determination, experimental results have been obtained suggesting 'that all of the three conventional sites of energy conservation were functional . . .' (ISHAQUE and ALEEM, 1970, p. 390). Even a succinate- and ascorbate-linked ATP-dependent reverse electron transport from cytochrome *c* to NAD has been found. Further experimental substantiation of these conclusions is urgently needed.

The absence of NAD-reducing hydrogenase in several hydrogen bacteria suggests these strains to depend on a reversed electron flow from cytochrome *b*/UQ to NAD, too.

Growth on the basis of nitrate respiration has been well proven for *Micrococcus denitrificans* (KLUYVER and VERHOEVEN, 1954; VERHOEVEN and co-authors, 1954; KORNBERG and co-authors, 1960; VOGT, 1965) and all strains of *Hydrogenomonas eutropha* (DAVIS and co-authors, 1969). Growth of *H. eutropha* is rapid and abundant with organic substrates, slow and modest with hydrogen and carbon dioxide (PFITZNER, personal communication). *M. denitrificans* needs yeast extract for anaerobic autotrophic growth (BANERJEE and SCHLEGEL, 1966). The presence of the components of the conventional electron-transport chain as well as oxidative phosphorylation by stable membrane fragments of *M. denitrificans* has been reported (IMAI and co-authors, 1967).

Neither the hydrogenases, nor the components of the electron-transport chain, nor oxidative phosphorylation have been studied in great detail in the different species of hydrogen bacteria. Comparative studies are urgently needed on hydrogenases (both hydrogen dehydrogenase and particle-bound enzymes), on cytochromes in-

volved in autotrophic and heterotrophic oxidations as well as in denitrification, on the composition of particles catalyzing the oxyhydrogen reaction, and on oxidative phosphorylation and coupling factors.

Concluding this section on the electron-transport process in chemolithotrophic bacteria, it may be remarked that the generalizations made previously are not yet justified if one employs strict experimental standards. Neither the oxidative phosphorylation nor the reverse energy-dependent electron transport processes have been proved by applying purified enzyme systems. Therefore, the introductory remarks have to be regarded as heuristic principles rather than proven facts.

(d) Carbon-Dioxide Fixation

The chemolitho-autotrophic bacteria fix carbon dioxide via the Calvin reductive pentose phosphate cycle (Fig. 2-2). The statement 'the mechanism of carbon dioxide

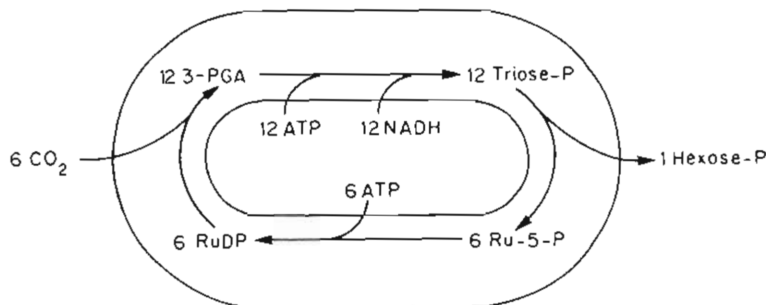


Fig. 2-2: Calvin reductive pentose-phosphate cycle. 3-PGA: 3-phosphoglyceric acid; Ru-5-P: ribulose-5-phosphate; RuDP: ribulose-1,5-diphosphate. (Original.)

fixation in the bacteria is the same as that found in the green plant' (ELSDEN, 1962, p. 11) is still valid. The primary CO_2-acceptor is ribulose-1,5-diphosphate, and phosphoribulokinase serves for its synthesis from ribulose-5-phosphate. The carboxylation is catalyzed by an enzyme alternatively called carboxydismutase or ribulose-diphosphate carboxylase. Both phosphoribulokinase and carboxydismutase are peculiar for autotrophic carbon-dioxide fixation. Among heterotrophic bacteria, these enzymes are only formed by *Pseudomonas oxalaticus* and exclusively with formate as a growth substrate (BLACKMORE and co-authors, 1968). In bacteria, reducing power is supplied in the form of $NADH_2$ rather than $NADPH_2$ as in green plants.

Experimental evidence for the conclusion that carbon dioxide is mainly fixed by the Calvin cycle is based on several methods: (i) After short-time $^{14}CO_2$-fixation the radio-activity is present in the intermediates of the cycle; (ii) intermediates (phosphoglyceric acid, ribose, fructose) isolated from the cells after short-time exposition to $^{14}CO_2$ contain the labelled carbon atom in the expected position; (iii) the presence of the specific enzymes in cell-free extracts; (iv) failure of autotrophic growth of mutants lacking one of the specific enzymes.

All chemolithotrophic bacteria have been tested for the occurrence of the Calvin cycle by one or several of these methods. Although complete analyses are scarce, the conclusion appears to be justified that the ribulose diphosphate cycle is the major pathway of CO_2 fixation in chemolithotrophic bacteria; other pathways contribute to a lesser degree. Since this topic has been extensively reviewed (ELSDEN, 1962; SCHLEGEL, 1966; PECK, 1968), the experimental evidence will not be considered here.

(e) Mixotrophic Growth of Facultative Chemolitho-autotrophs

A few thiobacilli and all hydrogen-oxidizing bacteria are able to grow either in a mineral medium with their specific inorganic H-donors and carbon dioxide or with organic substrates as carbon and energy sources. In several species the enzymes involved in both kinds of metabolism are inducible; they are formed when the cells grow on the corresponding substrate. However, the basal level of some of the enzymes may be rather high. *Hydrogenomonas eutropha H 16*, for example, degrades fructose via the Entner–Doudoroff-pathway, and during growth in a fructose medium under air the enzymes involved are present in normal amounts (100–300 units g^{-1} protein). During autotrophic growth, the specific activity of these enzymes in the crude cell extract is negligible (less than 1 unit g^{-1} protein). However, the enzymes involved in hydrogen activation and CO_2 fixation are rather constitutive and during heterotrophic growth decrease only to 20 or 10% of their maximal specific activity. The enzyme level is influenced by the kind of organic substrate, oxygen pressure, and even nitrogen sources. Furthermore, enzyme patterns may vary from strain to strain.

If the cells have been successively grown under heterotrophic and autotrophic conditions, both enzyme systems will be present. There is no reason why these enzymes should not function when the corresponding substrates are supplied. For example, acetate was consumed and used for lipid (poly-β-hydroxybutyrate) synthesis, when *Hydrogenomonas eutropha H 16* was incubated under a hydrogen–oxygen atmosphere (WILDE, 1962); 96% of the radioactivity of the 2-^{14}C-acetate added had been incorporated into the polymer and was still found in carbon atoms 2 and 4 of the PHB-monomer. Obviously, the oxidation of acetate and other organic acids via the tricarboxylic acid cycle is completely suppressed by the hydrogen–oxygen mixture, otherwise the ^{14}C-isotope would have been more widely distributed throughout the PHB molecule by autotrophic refixation of the ^{14}C-carbon dioxide released via the TCA-cycle. The energy necessary for assimilation of organic materials is derived from hydrogen oxidation. Numerous examples can be given for this type of metabolism—the concomitant utilization of organic and inorganic substrates, called mixotrophy.

(5) Specialized Regulatory Phenomena

As indicated above, the autotrophic cell is faced with regulatory problems different from, and additional to, those of heterotrophic cells. Special regulatory mechanisms, which may be encountered in all autotrophic organisms, are concerned

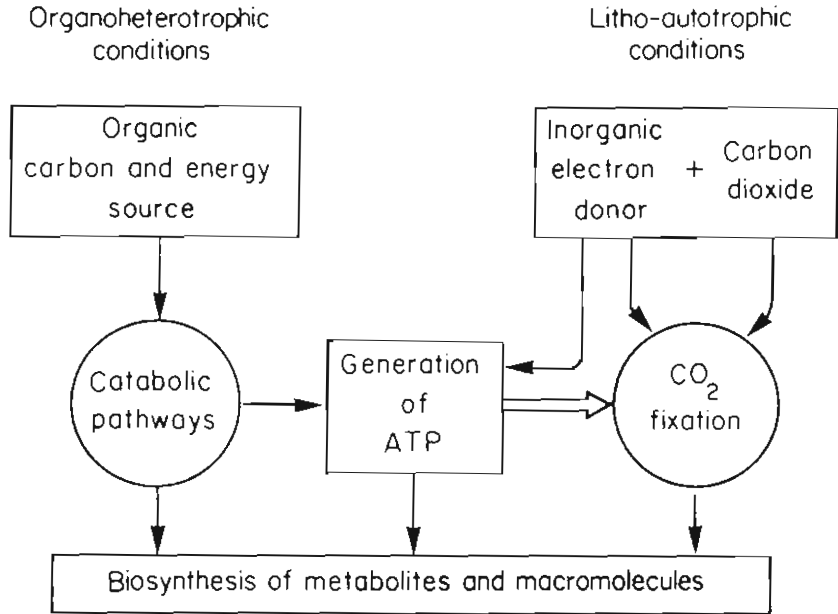


Fig. 2-3: Facultative litho-autotrophs can use organic or inorganic compounds as carbon and energy sources. The organic compound supplies both energy and carbon skeletons. The inorganic substrate consists of two separate compounds: inorganic electron donor and carbon dioxide. If both organic substrate and inorganic electron donor ($\pm CO_2$) are offered concomitantly, the growing cell faces regulatory problems at the levels of enzyme formation (induction and repression) and enzyme function. Depending on organisms and environmental conditions alternative decisions or compromises are made. (Original.)

with CO_2 fixation and the electron-transport chain. Facultative autotrophs are confronted with additional problems of substrate utilization: induction and repression of the formation of cata- and anabolic enzymes as well as control of enzyme function (Fig. 2-3). Regulatory phenomena in chemolithotrophs have been reviewed and discussed recently by RITTENBERG (1969) and by SCHLEGEL and EBERHARDT (1972).

(a) CO_2-Fixation

The enzymes involved in carbon-dioxide fixation are subject to induction and repression as well as to metabolite control (feedback) mechanisms.

In several facultative autotrophs, the enzymes unique to the ribulose-diphosphate cycle are repressed during heterotrophic growth. The basal levels of the enzymes vary, however, with the organism and with the organic growth substrate. When cells of *Hydrogenomonas eutropha H 16* are transferred to a fructose-containing medium under air, the ribulose-diphosphate carboxylase (RDPC) is diluted out until a basal level, 8 to 12% of autotrophic cells, is reached (GOTTSCHALK and coauthors, 1964; EBERHARDT, 1966b). These results were confirmed by using *H.*

eutropha type strain; however, the type strain cells did not contain the enzymes after growth on acetate, lactate (RITTENBERG, 1969) or pyruvate (STUKUS and DECICCO, 1970). Furthermore, glutamate grown cells contained 36% of maximal activity. The repression of RDPC formation by acetate, pyruvate, and glutamate occurred both in the presence and absence of hydrogen (STUKUS and DECICCO, 1970). Cells grown under mixotrophic conditions contained intermediate enzyme concentrations (RITTENBERG and GOODMAN, 1969).

The formation of RDPC in *Hydrogenomonas facilis* appears to be less sensitive to repression. Glucose- and fructose-grown cells have 76% of the autotrophic value (MCFADDEN and TU, 1967); acetate, succinate and lactate grown cells are almost devoid of the enzyme. Secondary factors such as oxygen tension or growth phase influence enzyme formation (KUEHN and MCFADDEN, 1968a) and may be the reason for slight variations in experimental results.

A repression of RDPC formation by organic substrates has also been reported for *Thiobacillus intermedius*; yeast extract was the most repressing substrate, followed by casein hydrolysate and glucose (LONDON and RITTENBERG, 1966). In *T. intermedius*, enzyme activities have only been measured after growth on a mixture of thiosulphate and organic substrates (mixotrophic conditions), since this species does not grow without the inorganic H-donor.

Metabolic control mechanisms at the level of enzyme activity regulation have been discovered by JOHNSON and PECK (1965). AMP inhibited almost completely the ATP-dependent CO_2 fixation in crude extracts of *Thiobacillus thioparus*. This inhibition was tentatively attributed to an allosteric response of phosphoribulokinase, which is the first enzyme specific for the ribulose-diphosphate cycle. This suggestion has been substantiated by work on the partly purified phosphoribulokinase from *T. thioparus*, *T. ferrooxidans*, and *T. neapolitanus* (GALE and BECK, 1966; MACELROY and co-authors, 1968a, b, 1972). These observations have been extended to extracts of *Chromatium* strain *D* and of spinach (JOHNSON, 1966; HART and GIBSON, 1971), *T. novellus* (ALEEM and HUANG, 1965) *Rhodopseudomonas spheroides* and *Rhodospirillum rubrum* (RINDT and OHMANN, 1969).

AMP and ADP were found to inhibit effectively (by about 75%) also the phosphoribulokinase of *Pseudomonas facilis* (MCFADDEN and TU, 1967). In addition, these authors observed that carbon-dioxide fixation by crude extracts was stimulated by $NADH_2$; they assumed that $NADH_2$ would reduce intermediates and that the products of CO_2 fixation would recycle during the experiment. Although the interpretation is questionable, the observation has been confirmed; however, evidence has been provided that phosphoribulokinase of *P. facilis* is a regulatory enzyme with $NADH_2$ acting as a positive effector (MACELROY and co-authors, 1968b). Recently, phosphoenolpyruvate has been reported to act as a non-competitive inhibitor (with respect to ATP and Ru-5-P) of phosphoribulokinase of *P. facilis* (BALLARD and MACELROY, 1971).

Although only a few autotrophs have been tested, and a systematic investigation on allosteric regulation using purified preparations of phosphoribulokinase and RDPC is still lacking, it is tempting to generalize on the basis of the results at hand: CO_2 fixation is the most expensive process in autotrophic metabolism with respect to consumption of energy and reducing power. Phosphoribulokinase is the first enzyme in the specific pathway; it may be regulated by $NADH_2$ and ATP as positive effec-

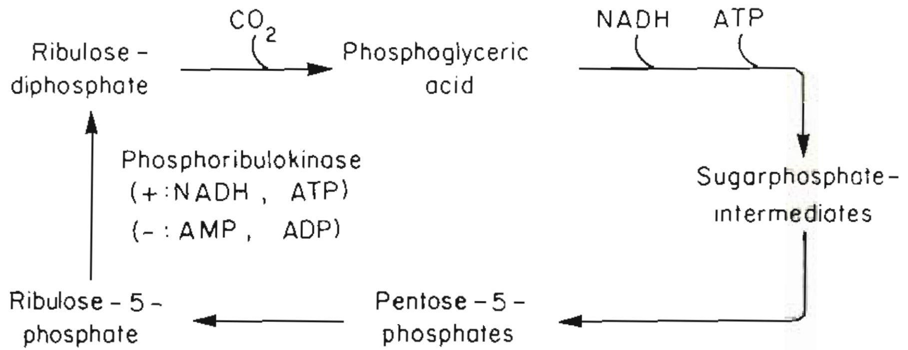


Fig. 2-4: Regulation of the reductive pentose-phosphate cycle by metabolic control of ribulose-5-phosphate kinase. This enzyme is the first catalyst specific for the cycle. In several bacteria this enzyme is subject to inhibition by AMP and ADP and to stimulation by NADH and ATP. Probably, this control mechanism protects fundamental biosynthesis reactions from the ATP and the reducing-power pulling action of carbon dioxide fixation. (Original.)

tors and AMP (ADP) as negative effectors (Fig. 2-4). Whether carbon dioxide also is involved as a positive effector has not yet been tested.

(b) Electron Transport

Electron transport in mitochondria is controlled by ADP and inorganic phosphate. If the energy obtained from substrate oxidation is not utilized the respiratory rate decreases. In bacteria, evidence for respiratory control is said not to exist (WHITE and co-authors, 1964).

Since the hydrogen donor and carbon source can be offered separately to chemo-litho-autotrophic bacteria, these organisms are incomparably advantageous for the study of electron transport and connected reactions. Studies employing intact cells can supply valuable information about the coupling of electron transport and the reactions of energy production and utilization, while with other objects of biochemical research, homogenization and fractionation of the cells is unavoidable.

A control of electron transport by carbon dioxide was first described for intact cells of *Hydrogenomonas eutropha H 16* (SCHLEGEL and VON BARTHA, 1961a, b). When washed cells were incubated in phosphate-buffer (in the absence of a nitrogen source) under an atmosphere of hydrogen and oxygen (9:1), the rate of hydrogen oxidation in the presence of carbon dioxide was nearly three times as high as in its absence. This effect was originally explained by the hypothesis that the electron transport is controlled by the availability of ADP, which is continually regenerated from ATP during CO_2-fixation. Under these conditions, CO_2-fixation is accompanied by the synthesis of PHB. Mutants unable to accumulate PHB and, therefore, unable to fix carbon dioxide in the absence of a nitrogen source were expected not to respond to the addition of carbon dioxide. However, CO_2 also turned out to stimulate hydrogen oxidation in these mutants, although no net carbon-dioxide fixation was possible (SCHLEGEL and co-authors, 1970). This observation does not

agree with the hypothesis mentioned; it indicates a more direct effect of carbon dioxide on the electron transport process. This suggestion has been verified using the particulate fraction of a cell-free extract (PFITZNER, unpublished); the rate of H_2-oxidation is increased four-fold by the addition of carbon dioxide to the hydrogen–oxygen mixture. The effect is strongly pH-dependent and deserves further investigation.

(c) Induction, Repression and Control of Enzyme Function

Enzymes Activating the Inorganic Hydrogen Donor

Regulation of the enzymes activating the inorganic hydrogen donor has been studied in the facultative autotrophic sulphur bacteria as well as in their hydrogen-oxidizing counterparts.

When *Thiobacillus intermedius* was grown on a glucose-yeast extract medium, the capacity for thiosulphate oxidation was diminished compared to glucose-thiosulphate grown cells (MATIN and RITTENBERG, 1970a, b). If grown in a thiosulphate-yeast extract or thiosulphate-glucose medium, ^{14}C-bicarbonate provided less than 10% of the cell carbon (LONDON and RITTENBERG, 1966). Growth yield measurements at varied concentrations of thiosulphate and yeast extract showed that thiosulphate provides energy, and yeast extract supplies both energy and carbon (RITTENBERG, 1969).

Thiobacillus novellus is different from *T. intermedius* in that it can grow on organic substrates in the absence of thiosulphate. Glutamate-grown cells contain virtually no activity of thiosulphate oxidizing enzymes. If both organic substrates (glucose or lactate) and thiosulphate are supplied concomitantly, the formation of the thiosulphite-oxidizing enzyme system is strongly repressed (LE JOHN and co-authors, 1967). The thiosulphate system in the organism is apparently coordinately induced by thiosulphate and repressed by glucose and several other substrates.

The response of hydrogenase formation to environmental factors among the hydrogen bacteria varies from species to species. Complete analyses are still lacking. The behaviour of both the soluble and particulate enzyme should be considered; and it should be elucidated whether hydrogen is an inducer of hydrogenase, whether the formation of hydrogenase depends on the concomitant presence of carbon dioxide, and to what extent hydrogenase formation is repressed by different organic substrates in the absence and presence of carbon dioxide.

In *Hydrogenomonas eutropha H 16*, both hydrogenases—the soluble hydrogen dehydrogenase and the particle-bound hydrogenase—are formed during autotrophic and during heterotrophic growth with certain organic substrates, under the latter condition at a diminished rate (EBERHARDT, 1966b; FRINGS, 1969). The type strain contains considerable amounts of hydrogenase also after heterotrophic growth (RITTENBERG, 1969; STUKUS and DECICCO, 1970). Using intact cells of *H. eutropha* and methylene blue as H-acceptor for measurement of total hydrogenase activity, it was shown that the enzyme varies from one substrate to another (in the ratio 1:6:12 for lactate, glutamate, and fructose) and reaches a still higher value

(20) in the stationary phase (LASCELLES and RITTENBERG *in*: RITTENBERG, 1969). The oxygen partial pressure also exerts influence, and *H. facilis* formed hydrogenase only when the oxygen concentration was less than 5% (WILSON and co-authors, 1953; LINDAY and SYRETT, 1958). Studies with strain *H 16* using the chemostat and electrolytically produced hydrogen and oxygen showed that under hydrogen-limitation the specific activity of hydrogenase increases and is controlled by the growth rate (SCHUSTER and SCHLEGEL, 1967). A similar dependence of hydrogenase activity on the growth rate, as well as on the oxygen concentration of the gas mixture applied, was observed with *Nocardia opaca 1 b*; oxygen limitation of growth increased the total hydrogenase activity 5-fold (AGGAG and SCHLEGEL, 1973).

Enzymes Involved in the Utilization of Organic Substrates

The facultative chemolitho-autotrophs are able to use a variety of organic compounds as growth substrates, and with some substrates the growth rate is much higher than during autotrophic growth. Most of the catabolic enzymes are inducible, some are constitutive. Hexoses are preferentially degraded via the Entner–Doudoroff pathway as has been shown for *Pseudomonas saccharophila* (ENTNER and DOUDOROFF, 1952; MACGEE and DOUDOROFF, 1954), *Hydrogenomonas eutropha H 16* (GOTTSCHALK and co-authors, 1964; SCHLEGEL and GOTTSCHALK, 1965) and type strain (COOK and co-authors, 1967; KUEHN and MCFADDEN, 1968b), *P. facilis* (CROUCH and RAMSEY, 1962; RAMSEY, 1968), *Thiobacillus intermedius* (MATIN and RITTENBERG, 1970a, b), *T. ferrooxidans* (TABITA and LUNDGREN, 1971b, c), *T. perometabolis* (MATIN and RITTENBERG, 1971).

In autotrophically grown cells of *Hydrogenomonas eutropha H 16*, the activity of the enzymes involved in the Entner–Doudoroff pathway is negligibly low or zero. The enzymes are induced by fructose or gluconate (GOTTSCHALK and co-authors, 1964; SCHLEGEL and BLACKKOLB, 1967; BLACKKOLB and SCHLEGEL, 1968a). The induction pattern is similar to that in *Pseudomonas facilis*. In the latter strain, however, gluconate induces all the enzymes of fructose degradation while in *H 16* gluconate only induces gluconokinase and the enzymes of the ED system (BOWIEN and SCHLEGEL, 1972).

Acetate is a good growth substrate also for strain *H 16*; transfer of chemolitho-autotrophically grown cells to an acetate medium is followed by rapid increase of acetate-metabolizing enzymes (acetyl-CoA-kinase, isocitrate lyase, malate synthase). The results obtained for *H 16* (SCHINDLER, 1964; SCHLEGEL and TRÜPER, 1966) are in accordance with those found for *Pseudomonas facilis* (MCFADDEN and HOWES, 1962).

Hydrogenomonas eutropha H 16 and *Pseudomonas facilis* are able to use purine derivatives as carbon and nitrogen sources (KALTWASSER and KRÄMER, 1968). The purines are converted to allantoin via uric acid and degraded with the formation of ammonia, urea, and glyoxylic acid. From glyoxylate, tartronic semialdehyde and D-glyceric acid are formed by the action of glyoxylate carboligase and D-glycerate dehydrogenase (KALTWASSER, 1968, 1969). If the cells grow on uric acid, all enzymes involved are formed. Their formation is not repressed by the addition of fructose to the medium.

While the enzymes of fructose degradation are rapidly formed when chemolithotrophically grown cells of *Hydrogenomonas eutropha H 16* are stirred in a fructose nutrient medium in air, formation of these enzymes is completely suppressed in a hydrogen–oxygen atmosphere (GOTTSCHALK, 1965; SCHLEGEL and TRÜPER, 1966). A similar response to hydrogen has been observed in all of the 58 strains tested so far, the type strain of *H. eutropha, Pseudomonas ruhlandii, Nocardia opaca* strain 1 *b* included (BLACKKOLB and SCHLEGEL, 1968a; TUNAIL and SCHLEGEL, 1972; AGGAG and SCHLEGEL, 1973; PROBST and SCHLEGEL, 1973). The 'hydrogen effect' becomes effective even when fully induced fructose-grown cells are exposed to hydrogen; in a fructose-containing medium under a hydrogen–oxygen mixture (in the absence of carbon dioxide) the cells grow arithmetically rather than exponentially, and the specific activity of the Entner–Doudoroff enzymes in the cells decreases as a result of dilution. With many other substrates, the response of the cells to hydrogen was similar or less pronounced (BLACKKOLB and SCHLEGEL, 1968a). With acetate, the enzymes of the glyoxylate cycle remained repressed (SCHLEGEL and TRÜPER, 1966). The 'hydrogen effect' is similar to the 'glucose effect' (GALE, 1943) and has been interpreted as a 'catabolite repression effect' (MAGASANIK, 1964). For the substrates which are subject to the hydrogen effect, an order or preferential utilization can be established; hydrogen is the most repressive energy source, followed by substrates in the order fructose, glucose, and acetate (SCHLEGEL and TRÜPER, 1966).

A similar repression mechanism has been described in *Thiobacillus intermedius* (MATIN and RITTENBERG, 1970b). If the cells are grown in a glucose–thiosulphate medium, the activity of the Entner–Doudoroff enzymes is low compared to glucose–yeast extract grown cells. In *T. novellus*, glucose and some other compounds appear to be the dominating substrates, which repress the formation of the thiosulphate-oxidizing enzyme system even in the presence of thiosulphate (LE JOHN and co-authors, 1967). A thiobacillus able to grow heterotrophically at faster rates and on a greater range of organic compounds has recently been isolated and designated *Thiobacillus A 2* (TAYLOR and HOARE, 1969). After growth on acetate or formate, the rate of thiosulphate oxidation was rather low. Their excellent growth capabilities and their versatility render facultative chemolithotrophic thiobacilli suitable objects for more intensive studies on the regulation of enzyme synthesis.

The extreme suppressing effect of hydrogen on growth of *Hydrogenomonas eutropha H 16* in a fructose medium is due to a second effect of hydrogen: Hydrogen not only represses the formation of the fructose degrading enzymes, but also inhibits the function of these enzymes already present (SCHLEGEL and BLACKKOLB, 1967). The inhibition results in a decrease of growth rates or of formation of storage material (PHB) by 80%. This observation indicated an inhibitory effect of a metabolite affecting one of the enzymes involved in fructose degradation. The site of inhibition affected by hydrogen had to be located prior to 6-phosphogluconate since the utilization of gluconate by gluconate-grown cells is not impaired by hydrogen. Finally, glucose-6-phosphate dehydrogenase proved to be sensitive to ATP and $NADH_2$ (BLACKKOLB and SCHLEGEL, 1968b). The enzyme of strain *H 16* differs from the enzymes isolated from yeast or higher organisms; it has a lower substrate affinity and can be linked to NADP as well as to NAD. Most pronounced is the shape of the substrate (G-6-P)-saturation curve; while that of the yeast enzyme is hyper-

bolic, the enzyme of *H 16* exhibits a sigmoidal shape; the sigmoidicity is increased by ATP. From the kinetic data, the conclusion could be drawn that glucose-6-phosphate dehydrogenase is an allosteric enzyme and ATP is a negative effector. In addition, the enzyme is inhibited in a competitive fashion by $NADH_2$.

The inhibition of glucose-6-phosphate dehydrogenase by ATP apparently is of general importance and can be regarded as analogous to the inhibition of phosphofructokinase in the Embden–Meyerhof pathway. In both cases the catabolic pathway is regulated by the energy charge of the cell; adenosine nucleotides and pyridine nucleotides act as signals and effectors. Glucose-6-phosphate dehydrogenase from *Pseudomonas aeruginosa* (LESSIE and NEIDHARDT, 1967), *P. fluorescens* (SCHINDLER and SCHLEGEL, 1969), and *Rhodopseudomonas spheroides* (OHMANN and co-authors, 1970) also exhibit sigmoidal substrate (G-6-P)-saturation curves and are inhibited by ATP in an allosteric fashion. The results support the idea that the enzymes of these organisms are subject to adenylate control, in which the enzyme functions only in a degradative pathway and is not involved in biosynthetic reactions. However, the enzymes of *P. facilis* (SCHINDLER and SCHLEGEL, 1969), and *Nocardia opaca 1 b* (PROBST and SCHLEGEL, 1973) did not exhibit sigmoidal substrate saturation curves, even in the presence of unphysiologically high concentrations of ATP.

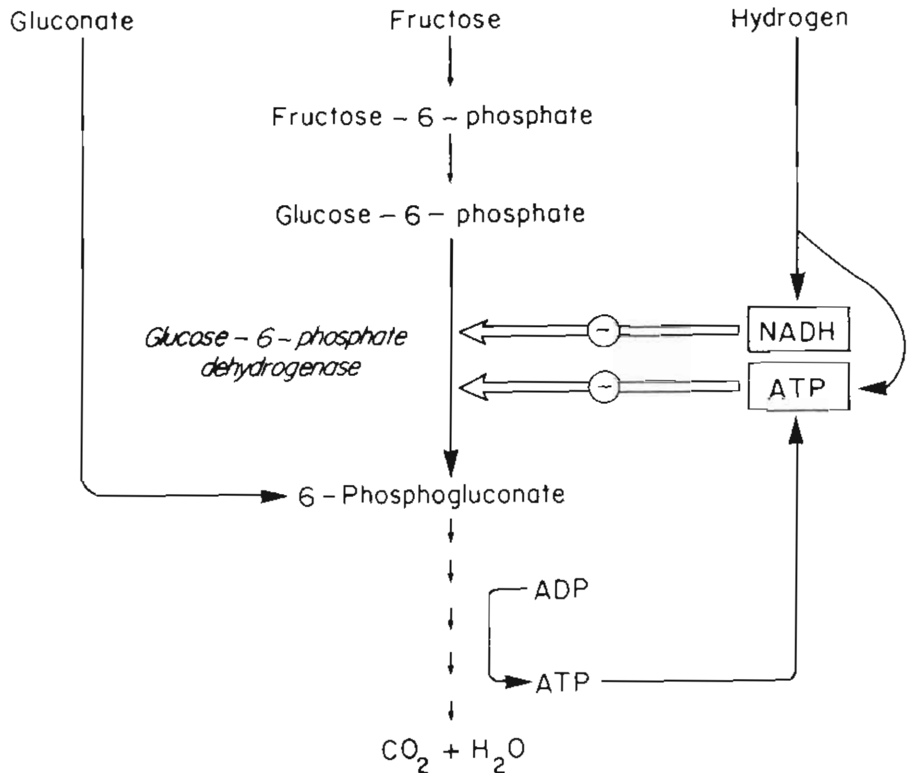


Fig. 2-5: Regulation of fructose degradation via Entner–Doudoroff pathway by inhibition due to adenosine triphosphate and reduced nicotinamide adenine dinucleotide of the enzyme glucose-6-phosphate dehydrogenase. (Original.)

Similar results were obtained with the enzyme from the hydrogen-oxidizing *Arthrobacter* strain *7 C*; surprisingly, recent experiments revealed a high sensitivity of the glucose-6-phosphate dehydrogenase of this Gram-positive bacterium to phosphoenolpyruvate; while ATP exerts only a minor effect, even low concentrations of phosphoenolpyruvate (100 μM) have a strong effect and result in sigmoidal substrate saturation curves (TUNAIL and SCHLEGEL, 1972).

At the present time, the suppression of hexose utilization in facultative autotrophs by the concomitant oxidation of their inorganic substrate can be explained by the following hypothesis: Oxidation of the inorganic substrate results in the generation of energy and reducing power. The corresponding metabolites (nucleoside triphosphates, phosphoenolpyruvate, reduced pyridine dinucleotides) act on the first enzyme specific for the Entner–Doudoroff pathway, glucose-6-phosphate dehydrogenase, and, thereby, inhibit hexose degradation (Fig. 2-5). Further comparative investigations employing these and other metabolites of potential signal properties must be undertaken to substantiate or modify these generalizations.

Enzymes of the Tricarboxylic-Acid Cycle

The tricarboxylic-acid cycle serves two main functions: (i) the terminal oxidation of organic compounds with carbon dioxide and pyridine nucleotide-bound hydrogen as products; (ii) the synthesis of metabolites used for further biosynthetic processes. Hence enzymes of the tricarboxylic-acid cycle serve catabolic as well as anabolic reactions and are considered to be formed partly constitutively. When the catabolic functions of the cycle are not needed, i.e. in facultative anaerobes under anaerobic conditions and in facultative autotrophic bacteria during photo- or chemolithotrophic metabolism, theoretically, at least one enzyme—α-ketoglutarate dehydrogenase—is dispensable. Research on the induction and repression of TCA-cycle enzymes concentrated mainly on the comparison of enzyme activities after lithotrophic and organotrophic growth.

In facultative autotrophic bacteria, the specific activity of some enzymes of the tricarboxylic-acid cycle in cells grown under organotrophic conditions is higher than in those grown under litho-autotrophic conditions; this has been shown for *Hydrogenomonas eutropha H 16* by TRÜPER (1965) and GLAESER and SCHLEGEL (1972a), and for *Thiobacillus novellus* by CHARLES (1971). Special emphasis was paid to α-ketoglutarate dehydrogenase.

After autotrophic growth, the α-ketoglutarate dehydrogenase is either not detectable at all (*Thiobacillus thiooxidans*, *T. thioparus*, SMITH and co-authors, 1967; *T. neapolitanus*, KELLY, 1970; *T. novellus*, TAYLOR and HOARE, 1969; *T.* sp., WILLIAMS and HOARE, 1972; *T. ferrooxidans*, TABITA and LUNDGREN, 1971b) or is present only in minute amounts (*T. intermedius*, *Hydrogenomonas eutropha*, SMITH and co-authors, 1967; *T. ferrooxidans*, ANDERSON and LUNDGREN, 1969; *Nitrobacter agilis*, SMITH and HOARE, 1968a, b; *Nitrosocystis oceanus*, WILLIAMS and WATSON, 1968; *Nitrosomonas europaea*, HOOPER, 1969; *T. thiooxidans*, BUTLER and UMBREIT, 1969). If facultative autotrophic bacteria are grown on acetate, the specific activity of enzymes of the tricarboxylic-acid cycle is high, and enzymes of the glyoxylate cycle are also formed. Levels of some TCA-cycle dehydrogenase enzymes in some lithotrophs have been listed by KELLY (1971).

If facultative autotrophs are adapted to inorganic and organic substrate (grown autotrophically with successive growth for about one generation on the organic substrate) and are then exposed to both substrates concomitantly, one should expect the function of the TCA cycle to be suppressed. Some results indicate a complete suppression of the hydrogen cycle. When 2-^{14}C-acetate was incorporated into *Hydrogenomonas H 16* cells under a hydrogen–oxygen atmosphere in the absence of a N-source, as much as 96% of the radio-activity was found in C-2 and C-4 of the β-hydroxybutyric-acid monomer, derived (for analysis) from the PHB formed during incubation (GOTTSCHALK, 1964a, b; GOTTSCHALK and SCHLEGEL, 1965). If a significant proportion of the acetate had been oxidized through the cycle, the ^{14}C-carbon dioxide would have been re-incorporated by autotrophic fixation and would have been more widely distributed throughout the entire PHB molecule. The inhibition of the TCA cycle exerted by hydrogen may be caused by a high $NADH_2/NAD$ ratio and the inhibition of α-ketoglutarate dehydrogenase by $NADH_2$ (SCHLEGEL, unpublished), which is similar to that of pyruvate dehydrogenase in *Escherichia coli K-12* (HANSEN and HENNING, 1966).

The first enzyme specific for the TCA cycle is citrate synthase. During aerobic metabolism, when the cycle has mainly a catabolic function, citrate synthase should be under the control of the 'end-products' of the cycle, NADH and ATP (or more generally energy charge). In accordance with this postulate the enzyme from many eukaryots and strictly aerobic prokaryots is sensitive to ATP as a negative effector (Fig. 2-6). In some aerobic Gram-negative bacteria the enzyme is inhibited by NADH, and the inhibition is relieved by AMP. In facultative anaerobes, which degrade hexoses mainly via the Embden–Meyerhof pathway, the sensitivity of citrate synthase to AMP is almost nil. A strict regulation by adenylates occurs already during the glycolytic sequence.

In enterobacteria, which are facultative anaerobes, in addition to $NADH_2$, α-ketoglutarate has a function as a negative allosteric effector of citrate synthase. Under anaerobic conditions, when the cycle has only an anabolic function, α-ketoglutarate can be considered to be the end-product of this metabolic sequence, since

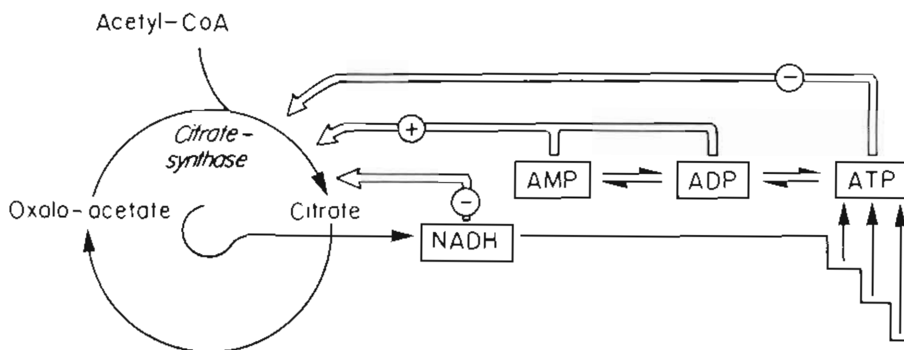


Fig. 2-6: Regulation of citrate synthase in many aerobic micro-organisms. While 'end-products' of the TCA cycle act as negative effectors, AMP (and/or ADP) antagonizes this inhibition. (Original.)

the formation of α-ketoglutarate dehydrogenase is completely repressed under anaerobic conditions.

Analogous to the situation in facultative anaerobes, one may expect a feedback control of citrate synthase by α-ketoglutarate in facultative chemolithotrophs. However, no relevant data are available. Citrate synthase from *Hydrogenomonas eutropha H 16*, *Thiobacillus neapolitanus*, *T. denitrificans*, and *T. novellus* are not inhibited by $NADH_2$ (GLAESER, 1971; TAYLOR, 1970), whereas the enzyme from *Thiobacillus A 2* is inhibited (TAYLOR and HOARE, 1969). The question whether α-ketoglutarate has a regulatory function awaits further experiments.

Isocitrate dehydrogenase is another enzyme eventually underlying regulatory control. In *Thiobacillus thiooxidans* its activity is reported to be inhibited by ADP and ATP (HAMPTON and HANSON, 1969); in *Hydrogenomonas eutropha H 16* both a NAD- and a NADP-linked isocitrate dehydrogenase is inhibited by ATP, and the NADP-specific enzyme is stimulated by AMP and ADP (GLAESER and SCHLEGEL, 1972a, b).

Although it is highly probable that the function of the TCA-cycle is suppressed in facultative autotrophs during the utilization of the inorganic electron donors, this has not been well proven so far. New results obtained with intact cells as well as with isolated enzymes will be appreciated.

Enzymes Involved in Storage Material Synthesis

Storage materials are accumulated in many autotrophic bacteria. Polyphosphates act as a reservoir of phosphate and have been studied in hydrogen bacteria (KALTWASSER and SCHLEGEL, 1959; SCHLEGEL and KALTWASSER, 1961; KALTWASSER, 1962). In *Hydrogenomonas* strain *H 20* polyphosphates were accumulated as long as inorganic orthophosphate was present in the nutrient medium. After external orthophosphate had been consumed by the cells, polyphosphates were used for the continuing synthesis of organic phosphorus compounds. Furthermore, polyphosphate appears to have a function in controlling the level of orthophosphate in the cell (KALTWASSER, 1962; HAROLD, 1966).

Poly-β-hydroxybutyrate is stored by many aerobic chemotrophic and by phototrophic bacteria. This lipid is accumulated when energy and carbon sources are present in excess and growth is limited by another factor or nutrient. Poly-β-hydroxybutyrate has been found in nitrifying bacteria (SMITH and HOARE, 1968b; VAN GOOL and co-authors, 1971) thiobacilli (TABITA and LUNDGREN, 1971a), and hydrogen bacteria (SCHLEGEL and co-authors, 1961a; HEPTINSTALL and co-authors, 1972).

A detailed study on the synthesis of poly-β-hydroxybutyrate has been performed using *Hydrogenomonas eutropha* strain *H 16*. If organic substrates or carbon dioxide are assimilated under conditions which do not allow growth, the polymer is formed and deposited as intracellular granules, up to 65% of the dry weight of the cells (SCHLEGEL and co-authors, 1961b). In the absence of a nitrogen source this process is accompanied by an increase in weight which is entirely due to PHB accumulation. Following the addition of various ^{14}C-labelled organic acids, the synthesis pathway was retraced from the pattern of ^{14}C distribution in the stored PHB (GOTTSCHALK, 1964a, b). From $^{14}CO_2$ uniformly labelled PHB was formed (GOTTSCHALK, 1964c).

Some of the enzymes involved in the synthesis of PHB from organic acids and acetyl co-enzyme A have been demonstrated by SCHINDLER (1964). The pathway of PHB synthesis commences with acetyl-CoA. The intermediates involved are acetoacetyl-CoA and β-hydroxybutyryl-CoA and the conversions are catalyzed by β-ketothiolase and acetoacetyl-CoA reductase. The polymerization is bound to membranes surrounding the PHB granules (Fig. 2-7).

In strain *H 16* the accumulation starts when growth is limited by the exhaustion of the nitrogen source (SCHLEGEL and co-authors, 1961b) or by limiting concentrations of oxygen during chemostat culture (SCHUSTER and SCHLEGEL, 1967). A

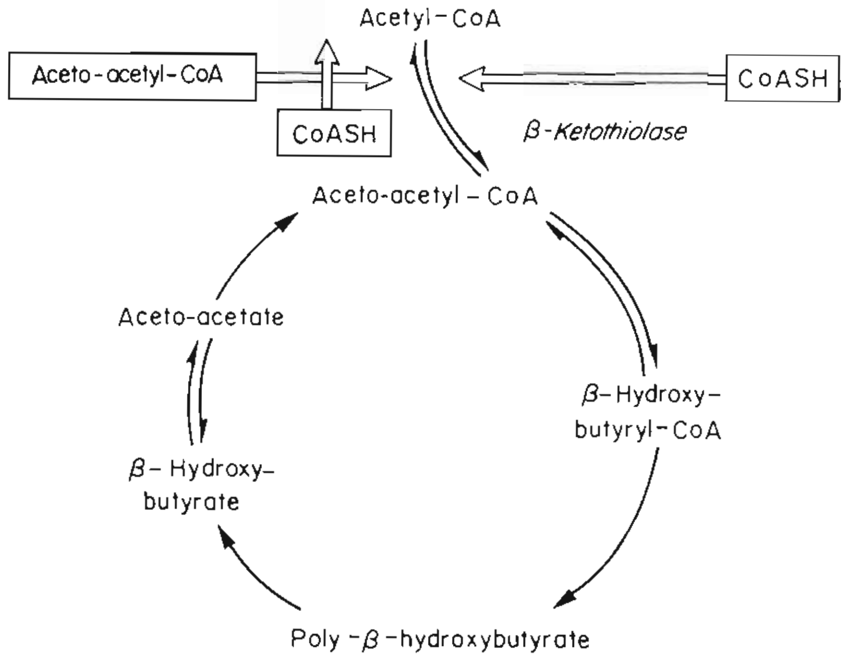


Fig. 2-7: Pathway of synthesis of poly-β-hydroxybutyrate and its regulation by product inhibition due to free co-enzyme A in *Hydrogenomonas eutropha* strain *H 16*. (After OEDING and SCHLEGEL, 1973; reproduced by permission of *Biochemical Journal*.)

detailed analysis of the kinetics and of potential effectors of partially purified β-ketothiolase resulted in the discovery of a strong product inhibition exerted by free co-enzyme A. While the substrate (acetyl-CoA)-saturation curve for the condensation reaction is hyperbolic, in the presence of co-enzyme A sigmoidal curves are obtained with an increasing sigmoidity from 0·03 to 0·30 mM co-enzyme A (OEDING and SCHLEGEL, 1973). Similar data have been obtained with respect to the kinetics of the enzyme from *Azotobacter beijerinckii* (SENIOR and DAWES, 1973). The sensitivity of β-ketothiolase to free co-enzyme A is in accordance with the observations made with intact cells and explains them. It may be assumed that the intracellular concentration of free co-enzyme A varies in an inverse manner with the concentration of acetyl-CoA. The concentration of free co-enzyme A will be low

when the effectiveness of the tricarboxylic-acid cycle is decreased (O_2-limitation of growth) or when the intermediates of this cycle cannot be used for synthetic processes (in the absence of a nitrogen-source). Obviously, the determination of intracellular concentrations of pyruvate, acetyl co-enzyme A, and free co-enzyme A is required for further evaluation of these conclusions.

(d) Obligate Autotrophy

Obligate chemolithotrophic bacteria are unable to grow on organic nutrients in the absence of their inorganic energy sources, i.e. they do not grow in a non-modified batch culture containing only organic material as carbon and energy source. This definition has so far not been contradicted and is the criterium for obligate autotroph in Tables 2-2 to 2-4. The reason for this curious abstinency of autotrophic bacteria is still rather obscure although quite a large amount of work has been invested in order to clarify the relation of obligate autotrophs to organic substrates.

On the basis of his experiments, WINOGRADSKY (1890) first proposed that organic matter is toxic to nitrifying bacteria. This proposal could not be verified for the majority of obligate autotrophs and could not be explained by causal reasons.

According to a hypothesis which prevailed for a long time, obligate autotrophs were considered not to be permeable to organic substrates. With radio-isotopic tracers this supposition was shown to be incorrect. Various organic materials were found to penetrate the cells and to be utilized. The incorporation of one or more of a series of compounds (aspartate, acetate, glutamate, glutamine, serine, glucose) has been demonstrated in *Nitrosomonas europaea* (CLARK and SCHMIDT, 1967), *Thiobacillus neapolitanus* (KELLY, 1967, 1968), *Nitrobacter agilis* (DELWICHE and FINSTEIN, 1965; SMITH and HOARE, 1968a), *Thiobacillus thiooxidans* (BUTLER and UMBREIT, 1966), *T. thioparus*, *T. neapolitanus* (JOHNSON and ABRAHAM, 1969), and others. In all instances, the rate of incorporation of the organic carbon into cell material was more or less dependent on the concomitant availability of the specific inorganic hydrogen donor; organic carbon could not replace carbon dioxide.

The experience that organic compounds could partially act as a carbon source—but were unable to replace the inorganic hydrogen or energy source—prompted conclusions with respect to a restricted function of intermediary metabolism and electron transport. A survey was made on some chemo- and photolithotrophic bacteria and on blue-green algae. These organisms, incapable of growth on glucose in the absence of their specific energy source, lack two enzymes: α-ketoglutarate dehydrogenase and NADH oxidase (SMITH and co-authors, 1967). Theoretically, obligate chemolithotrophs should get along without α-ketoglutarate dehydrogenase (Fig. 2-8). Since for oxidation of ammonia, nitrite, sulphur, and sulphite apparently only the cytochrome segment of the electron transport chain is used, the obligates ought to be able to grow without an active NADH-oxidase. The paper by SMITH and co-authors (1967) had a provocative effect and was followed by a multitude of studies, resulting in the statement that some, but not all, obligate autotrophs are deficient in one of the enzymes mentioned: *Nitrobacter agilis* (SMITH and HOARE, 1968b), *Nitrosocystis oceanus* (WILLIAMS and WATSON, 1968), *Nitrosomonas europaea* (HOOPER, 1969), *Thiobacillus neapolitanus* and *Thiobacillus* strain *C*

Fig. 2-8: The 'open tricarboxylic-acid cycle' as a route for the formation of precursors (oxalo-acetate, succinate, α-ketoglutarate) necessary for the synthesis of a multitude of metabolites. (Original.)

(TRUDINGER and KELLY, 1968), *T. neapolitanus* (SAXENA and VISHNIAC, 1970), *T. thiooxidans* (BUTLER and UMBREIT, 1969), *T. denitrificans* (TAYLOR and co-authors, 1971; TAYLOR and HOARE, 1971), *T. denitrificans* and *Thiobacillus* strain *A 2* (PEETERS and co-authors, 1970).

The toxic effect of organic materials on some obligate chemolithotrophs may be caused partially by regulatory interference with the metabolism. The inhibition of growth by exogenous amino acids, for example, has recently been shown to result from the inhibitory effects of these amino acids on branched biosynthetic pathways for amino acids (KELLY, 1969, 1971; JOHNSON and VISHNIAC, 1970; LU and co-authors, 1971).

An observation made with the obligate lithotroph *Thiobacillus thiooxidans* gave rise to a new experimental approach. This bacterium was reported to grow on glucose, provided toxic products of metabolism were removed by dialysis (BORICHEWSKI and UMBREIT, 1966). During growth on glucose, *T. thiooxidans* excreted pyruvate and oxalo-acetate. Both keto acids were toxic to the cells and inhibited growth on sulphur at a concentration of only $2 \cdot 10^{-5}$ mole (BORICHEWSKI, 1967).

Employing a dialysis apparatus, the growth experiments were extended to other autotrophs (PAN and UMBREIT, 1972); 0·2% glucose was added to the media instead of the normal inorganic energy source. *Nitrosomonas europaea* grew up to 12 mg protein l^{-1} and did not grow without dialysis; the toxic products have not yet been identified. The growth of *Nitrobacter agilis* on nitrite is enhanced by dialysis culture;

growth on glucose continued up to 12 mg l^{-1}; the toxic product appears to be pyruvate. *Thiobacillus denitrificans* proved capable of growing aerobically as well as anaerobically with nitrate in the glucose medium when dialysis was employed. *T. thioparus* and *T. neapolitanus* grew as well as *T. thiooxidans* on glucose. Pyruvic acid has been found in the medium. Pyruvate exerts an inhibitory effect on many bacteria; in some arthrobacters, isocitrate lyase is the sensitive enzyme (WOLFSON and KRULWICH, 1972). Since the excretion of metabolites is frequently the result of imbalanced metabolism or defective control mechanisms, the observations made in dialysis cultures may be indicative of regulatory defects. This line of research still awaits confirmation; however, it appears to be rewarding and should be pursued further.

Some bacteria considered to be obligate chemolithotrophs have recently been grown even on organic substrates without dialysis. *Nitrobacter agilis* is reported to grow on acetate in a complex medium containing casein hydrolysate (SMITH and HOARE, 1968b; POPE and co-authors, 1969). *Thiobacillus ferrooxidans* could utilize and grow on glucose (REMSEN and LUNDGREN, 1963). The strain *TM* grew even faster on glucose (generation time 4·5 hrs) or fructose (3·4 hrs) than on iron (8 hrs), or sulphur (22·3 hrs). The utilization of glucose repressed the ability to oxidize iron and the formation of ribulose diphosphate carboxylase (TABITA and LUNDGREN, 1971a), and induced the enzymes of the Entner–Doudoroff pathway, the α-ketoglutarate dehydrogenase and the NADH oxidase; the two last-mentioned enzymes were extremely low in autotrophically grown cells (TABITA and LUNDGREN, 1971b).

A unifying concept to explain obligate autotrophy does not appear to be attainable; a variety of enzymic properties, specific regulatory patterns included, may preclude organotrophic growth in some autotrophs. The reasons for obligate autotrophy are certainly different in different organisms.

(6) Evolutionary Aspects and Ecological Significance of Chemolithotrophy

On the basis of their aerobic mode of life, the chemolitho-autotrophic bacteria must be considered a fairly recent step on the scale of metabolic evolution. This statement contradicts the opinion held at the beginning of this century, which regarded them as primitive, first forms of life on earth. With respect to their respiratory metabolism, and to their highly developed metabolic sequences, these bacteria do not differ from other aerobic bacteria. The earliest organisms were probably anaerobes fermenting organic compounds in an oxygen-free atmosphere. The ability to use light as an energy source led to photoheterotrophy and photolithotrophy with inorganic sulphur as hydrogen donor and finally to the plant-type, oxygen-producing photosynthesis. The ability to fix carbon dioxide was possibly acquired when the photolithotrophic bacteria of the *Chromatium*-type developed from the photoheterotrophic bacteria of the *Rhodospirillum*-type. At this level, energy generation by oxidative phosphorylation may have arisen; this type of metabolism is represented today by *Desulfovibrio*, which shares with the obligate chemolithotrophs the incomplete TCA-cycle. Aerobic chemolithotrophic metabolism could appear only after oxygen was available.

The chemolithotrophs share with the photolithotrophs the ability to use inorganic compounds as hydrogen donors. The derivation of reducing power from ammonia,

nitrite, or ferrous iron appears to be an independent achievement of aerobic bacteria; it has no analogous process among the phototrophs. Hydrogen oxidation belongs to the fundamental capabilities of both groups of phototrophic bacteria, of sulphate-reducing bacteria, and of blue-green algae; photo-oxidation of hydrogen is a hidden ability of many unicellular green algae.

As stated above, chemolitho-autotrophic bacteria depend upon energy-driven reverse electron flow, except the hydrogen bacteria. BOSE and GEST (1962) suggested that photosynthetic phosphorylation is the primary event in bacterial photosynthesis and that reducing power is generated by an ATP-driven electron flow. Considering phototrophic bacteria as ancestors of the aerobic bacteria, the reverse electron flow in chemolithotrophs loses its spectacular character. From the viewpoint of evolution, reverse electron flow may have been the original function of the electron-transport chain and may have acquired importance for respiratory energy generation at the level of sulphate respiration; for heterotrophic pro- and eukaryotes it is apparently meaningless and represents a relic. Chemolithotrophic bacteria are the only aerobic micro-organisms for which reverse electron transport is still a necessity.

From the ecological point of view, chemolithotrophs are of great interest and importance. They occupy special ecological niches and possess monopolies in the processes of the conversion of energy and matter. Their ability to obtain energy from reduced inorganic compounds, in addition to that from organic sources, represents a selective advantage for the organisms, especially in habitats which are low in organic material.

For facultative autotrophs, lithotrophy can be considered as a bypath of normal organotrophic energy generation, advantageous during transient lack of organic nutrients. Obligate autotrophy is apparently the end of a line of extreme evolutionary specialization. Recent findings suggest that several obligate autotrophs are extremely specialized forms; the fact that they are so frequently isolated is due to the highly selective enrichment conditions employed: the more strains are isolated the more facultative autotrophic strains are found.

As has been mentioned above, all chemolithotrophic bacteria are Gram-negative except a few hydrogen bacteria. According to the present view of systematic bacteriology, the Gram-stain is still of great importance. Its result is due to the composition of the bacterial cell wall. With respect to the Gram-stain reaction, the eubacteria can be divided into two large groups. Groups with polar flagellation are Gram-negative. Several immotile groups are Gram-positive. The hydrogen bacteria, which are most frequently encountered in enrichment culture, belong to the Gram-negative genera *Pseudomonas* or *Alcaligenes*. It was a great surprise, therefore, to see species of the Gram-positive genera *Nocardia*, *Mycobacterium*, *Arthrobacter*, and *Corynebacterium* grow autotrophically, since not one single photo- or chemolithotrophic bacterium is known to be Gram-positive or to share characters which are specific for Gram-positive eubacteria. No close resemblances indicating a phylogenetic relationship can be detected, e.g., between pseudomonads and nocardias.

If the biochemical apparatus and metabolic patterns characteristic for chemolithotrophy have been derived from photosynthetic bacteria, the question arises: how were these properties acquired by the purely Gram-positive bacteria, *Nocardia* and *Arthrobacter*? With regard to recent findings on viral transduction and genetic

transfer by R-factors, one is tempted to speculate that these mechanisms have been involved in transporting segments of DNA across taxonomic barriers. The evolutionary significance of virus infection has recently been discussed by ANDERSON (1970). His considerations have been questioned as far as eukaryotes are concerned (WEST, 1971). The speculation is intriguing that plasmids have transferred genetic information for autotrophic carbon dioxide fixation and independently for using hydrogen as an H-donor for oxidative phosphorylation from one genus to the other. The hypothesis gained probability since transfer of nitrogenase genes from *Klebsiella pneumoniae* to *Escherichia coli* has been reported (DIXON and POSTGATE, 1972).

Research on chemolitho-autotrophy is a young and virgin field; it still harbours many unsolved problems. The gaps in our knowledge comprise the enzymes dealing with inorganic hydrogen donors, membrane-bound enzymes, regulation of CO_2 fixation, electron transport and metabolic alterations connected with shifts from auto- to heterotrophy and vice versa. The story is still fragmentary. Much well-planned research is needed to make the story on mechanisms of chemolitho-autotrophy more complete.

Literature Cited (Chapter 2)

AGGAG, M. and SCHLEGEL, H. G. (1973). Studies on a gram-positive hydrogen bacterium, *Nocardia opaca* strain *1 b*. I. Description and physiological characterization. *Arch. Mikrobiol.*, **88**, 299–318.

ALEEM, M. I. H. (1966a). Generation of reducing power in chemosynthesis, III. Energy-linked reduction of pyridine nucleotides in *Thiobacillus novellus*. *J. Bact.*, **91**, 729–736.

ALEEM, M. I. H. (1966b). Generation of reducing power in chemosynthesis, IV. Energy-linked reduction of pyridine nucleotides by succinate in *Thiobacillus novellus*. *Biochim. biophys. Acta*, **128**, 1–12.

ALEEM, M. I. H. (1968). Mechanism of oxidative phosphorylation in the chemoautotroph *Nitrobacter agilis*. *Biochim. biophys. Acta*, **162**, 338–347.

ALEEM, M. I. H. (1970). Oxidation of inorganic nitrogen compounds. *A. Rev. Pl. Physiol.*, **21**, 67–90.

ALEEM, M. I. H. and HUANG, E. (1965). Carbon dioxide fixation and carboxydismutase. *Biochem. biophys. Res. Commun.*, **20**, 515–520.

ALEEM, M. I. H. and LEES, H. (1963). Autotrophic enzyme systems, I. Electron transport systems concerned with hydroxylamine oxidation in *Nitrosomonas*. *Can. J. Biochem. Physiol.*, **41**, 763–778.

ALEEM, M. I. H., LEES, H. and NICHOLAS, D. J. D. (1963). Adenosine triphosphate-dependent reduction of nicotinamide adenine dinucleotide by ferro-cytochrome c in chemoautotrophic bacteria. *Nature, Lond.*, **200**, 759–761.

ALEEM, M. I. H. and NASON, A. (1960). Phosphorylation coupled to nitrite oxidation by particles from the chemoautotroph, *Nitrobacter agilis*. *Proc. natn. Acad. Sci. U.S.A.*, **46**, 763–769.

ANDERSON, K. J. and LUNDGREN, D. G. (1969). Enzymatic studies of the iron oxidizing bacterium *Ferrobacillus ferrooxidans*: evidence for a glycolytic pathway and a Krebs cycle. *Can. J. Microbiol.*, **15**, 73–79.

ANDERSON, N. G. (1970). Evolutionary significance of virus infection. *Nature, Lond.*, **227**, 1346–1347.

ANDREESEN, J. R., GOTTSCHALK, G. and SCHLEGEL, H. G. (1970). *Clostridium formicoaceticum* nov. spec. isolation, description and distinction from *C. aceticum* and *C. thermoaceticum*. *Arch. Mikrobiol.*, **72**, 154–174.

Atkinson, D. E. and McFadden, B. A. (1954). The biochemistry of *Hydrogenomonas*, I. The hydrogenase of *Hydrogenomonas facilis* in cell-free preparations. *J. biol. Chem.*, **210**, 885–893.

Baas-Becking, L. G. M. and Parks, G. S. (1927): Energy relations in the metabolism of autotrophic bacteria. *Physiol. Rev.*, **7**, 85–106.

Ballard, R. W. and MacElroy, R. D. (1971). Phosphoenolpyruvate, a new inhibitor of phosphoribulokinase in *Pseudomonas facilis*. *Biochem. biophys. Res. Commun.*, **44**, 614–618.

Banerjee, A. K. and Schlegel, H. G. (1966). Zur Rolle des Hefeextraktes während des chemolithotrophen Wachstums von *Micrococcus denitrificans*. *Arch. Mikrobiol.*, **53**, 132–153.

Barridge, J. K. and Shively, J. M. (1968). Phospholipids of the thiobacilli. *J. Bact.*, **95**, 2182–2185.

Bavendamm, W. (1924). Die farblosen und roten Schwefelbakterien des Süß- und Salzwassers. In R. Kolkwitz (Ed.), *Pflanzenforschung*. G. Fischer, Jena. pp. 1–156.

Beck, J. V. (1960). A ferrous-ion-oxidizing bacterium. *J. Bact.*, **79**, 502–509.

Beijerinck, M. W. (1904). Über die Bakterien, welche sich im Dunkeln mit Kohlensäure als Kohlenstoffquelle ernähren können. *Zentbl. Bakt. ParasitKde, II*, **11**, 593–599.

Beijerinck, M. and Minkman, D. C. J. (1910). Bildung und Verbrauch von Stickoxydul durch Bakterien. *Zentbl. Bakt. ParasitKde, II*, **25**, 30–63.

Bernstein, S. and Vishniac, W. (1959). Purification and properties of a DPN-linked hydrogenase. *Fedn Proc. Fedn Am. Socs exp. Biol.*, **18**, 192.

Bisset, K. A. and Grace, J. B. (1954). The nature and relationship of autotrophic bacteria. *Symp. Soc. gen. Microbiol.*, **4**, 28–53.

Blackkolb, F. and Schlegel, H. G. (1968a). Katabolische Repression und Enzymhemmung durch molekularen Wasserstoff bei *Hydrogenomonas*. *Arch. Mikrobiol.*, **62**, 129–143.

Blackkolb, F. and Schlegel, H. G. (1968b). Regulation der Glucose-6-phosphat-Dehydrogenase aus *Hydrogenomonas H 16* durch ATP und $NADH_2$. *Arch. Mikrobiol.*, **63**, 177–196.

Blackmore, M. A., Quayle, J. R. and Walker, J. O. (1968). Choice between autotrophy and heterotrophy in *Pseudomonas oxalaticus*. *Biochem. J.*, **107**, 699–704.

Bone, D. H. (1960). Localization of hydrogen activating enzymes in *Pseudomonas saccharophila*. *Biochem. biophys. Res. Commun.*, **3**, 211–214.

Bone, D. H. (1963). Cytochromes of *Pseudomonas saccharophila*. *Nature, Lond.*, **197**, 517.

Bone, D. H., Bernstein, S. and Vishniac, W. (1963). Purification and some properties of different forms of hydrogen dehydrogenase. *Biochim. biophys. Acta*, **67**, 581–588.

Bongers, L. (1967). Phosphorylation in hydrogen bacteria. *J. Bact.*, **93**, 1615–1623.

Bongers, L. (1970a). Yields of *Hydrogenomonas eutropha* from growth on succinate and fumarate. *J. Bact.*, **102**, 598–599.

Bongers, L. (1970b). Energy generation and utilization in hydrogen bacteria. *J. Bact.*, **104**, 145–151.

Borichewski, R. M. (1967). Keto acids as growth-limiting factors in autotrophic growth of *Thiobacillus thiooxidans*, *J. Bact.*, **93**, 597–599.

Borichewski, R. M. and Umbreit, W. W. (1966). Growth of *Thiobacillus thiooxidans* on glucose. *Archs Biochem. Biophys.*, **116**, 97–102.

Bose, S. K. and Gest, H. (1962). Electron transport systems in purple bacteria. *Nature, Lond.*, **195**, 1168–1172.

Bowien, B. and Schlegel, H. G. (1972). Der Biosyntheseweg der RNS-Ribose in *Hydrogenomonas eutropha* Stamm *H 16* und *Pseudomonas facilis*. *Arch. Mikrobiol.*, **85**, 95–112.

Brock, T. D., Brock, K. M., Belly, R. T. and Weiss, R. L. (1972). *Sulfolobus*: A new genus of sulfur-oxidizing bacteria living at low pH and high temperature. *Arch. Mikrobiol.*, **84**, 54–68.

Bryant, M. P., McBride, B. C. and Wolfe, R. S. (1968). Hydrogen-oxidizing methane bacteria. *J. Bact.*, **95**, 1118–1123.

Butler, R. G. and Umbreit, W. W. (1966). Absorption and utilization of organic matter by the strict autotroph, *Thiobacillus thiooxidans*, with special reference to aspartic acid. *J. Bact.*, **91**, 661–666.

Butler, R. G. and Umbreit, W. W. (1969). Reduced nicotinamide adenine dinucleotide oxi-

dase and α-ketoglutaric dehydrogenase activity by *Thiobacillus thiooxidans*, *J. Bact.*, **97**, 966–967.

CAESEELE, L. VAN and LEES, H. (1969). The ultrastructures of autotrophically and heterotrophically grown *Thiobacillus novellus*. *Can. J. Microbiol.*, **15**, 651–654.

CHANCE, B. and HOLLUNGER, G. (1960). Energy-linked reduction of mitochondrial pyridine nucleotides. *Nature, Lond.*, **185**, 666–672.

CHARLES, A. M. (1969). Mechanism of thiosulfate oxidation by *Thiobacillus intermedius*. *Archs Biochem. Biophys.*, **129**, 124–130.

CHARLES, A. M. (1971). Effect of growth substrate on enzymes of the citric and glyoxylic acid cycles in *Thiobacillus novellus*. *Can. J. Microbiol.*, **17**, 617–624.

CHARLES, A. M. and SUZUKI, I. (1965). Sulfite oxidase of a facultative autotroph, *Thiobacillus novellus*. *Biochem. biophys. Res. Commun.*, **19**, 686–690.

CHARLES, A. M. and SUZUKI, I. (1966). Mechanism of thiosulfate oxidation by *Thiobacillus novellus*. *Biochim. biophys. Acta*, **128**, 510–521.

CLARK, C. and SCHMIDT, E. L. (1967) Growth response of *Nitrosomonas europaea* to amino acids. *J. Bact.*, **93**, 1302–1308.

COLE, J. S. and ALEEM, M. I. H. (1970). Oxidative phosphorylation in *Thiobacillus novellus*. *Biochem. biophys. Res. Comm.*, **38**, 736–743.

COLLINS, V. G. (1969). Isolation, cultivation and maintenance of autotrophs. In J. R. Norris and D. W. Ribbons (Eds), *Methods in Microbiology*, Vol. IIIB. Academic Press, London. pp. 1–52.

COLMER, A. R., TEMPLE, K. L. and HINKLE, M. E. (1950). An iron-oxidizing bacterium from the drainage of some bituminous coal mines. *J. Bact.*, **59**, 317–328.

COOK, D. W., TISCHER, R. G. and BROWN, L. R. (1967). Carbohydrate metabolism in *Hydrogenomonas eutropha*. *Can. J. Microbiol.*, **13**, 701–709.

CROUCH, D. J. and RAMSEY, H. H. (1962). Oxidation of glucose by *Hydrogenomonas facilis*. *J. Bact.*, **84**, 1340–1341.

DAVIS, D. H., DOUDOROFF, M. and STANIER, R. Y. (1969). Proposal to reject the genus *Hydrogenomonas*: Taxonomic implications. *Int. J. Syst. Bact.*, **19**, 375–390.

DAVIS, D. H., STANIER, R. Y., DOUDOROFF, M. and MANDEL, M. (1970). Taxonomic studies on some gram negative polarly flagellated 'hydrogen bacteria' and related species. *Arch. Microbiol.*, **70**, 1–13.

DELWICHE, C. C. and FINSTEIN, M. S. (1965). Carbon and energy sources for the nitrifying autotroph *Nitrobacter*. *J. Bact.*, **90**, 102–107.

DIXON, R. A. and POSTGATE, J. R. (1972). Genetic transfer of nitrogen fixation from *Klebsiella pneumoniae* to *Escherichia coli*. *Nature, Lond.*, **237**, 102–103.

DOUDOROFF, M. (1940). The oxidative assimilation of sugars and related substances by *Pseudomonas saccharophila*. *Enzymologia*, **9**, 59–72.

DOXTADER, K. G. and ALEXANDER, M. (1966). Nitrification by heterotrophic soil microorganisms. *Proc. Soil Sci. Soc. Am.*, **30**, 351–355.

EBERHARDT, U. (1965). Die Anreicherung von Knallgasbakterien. *Zentbl. Bakt. ParasitKde, I*, **1** (Suppl.), 155–169.

EBERHARDT, U. (1966a). Über das Wasserstoff aktivierende System von *Hydrogenomonas H 16*. I. Verteilung der Hydrogenase-Aktivität auf zwei Zellfraktionen. *Arch. Mikrobiol.*, **53**, 288–302.

EBERHARDT, U. (1966b). Über das Wasserstoff aktivierende System von *Hydrogenomonas H 16*. II. Abnahme der Aktivität bei heterotrophem Wachstum. *Arch. Mikrobiol.*, **54**, 115–124.

EBERHARDT, U. (1969). On chemolithotrophy and hydrogenase of a gram-positive knallgas bacterium. *Arch. Mikrobiol.*, **66**, 91–104.

ECCLESTON, M. and KELLY, D. P. (1972). Assimilation and toxicity of exogenous amino acids in the methane-oxidizing bacterium *Methylococcus capsulatus*. *J. gen. Microbiol.*, **71**, 541–554.

EGOROVA, A. A. and DERYUGINA, Z. P. (1963). On the sporogenic thermophil thiobacterium *Thiobacillus thermophilus* IMSHENETSKII nov. spec. *Mikrobiologiya*, **32**, 439–446.

EL GHAZZAWI, E. (1967). Neuisolierung von *Clostridium aceticum* WIERINGA und stoffwechselphysiologische Untersuchungen. *Arch. Mikrobiol.*, **57**, 1–19.

ELSDEN, S. R. (1962). Photosynthesis and lithotrophic carbon dioxide fixation. In I. C. Gunsalus and R. Y. Stanier (Eds), *The Bacteria*, Vol. III. Academic Press, New York. pp. 1–40.

ENTNER, N. and DOUDOROFF, M. (1952). Glucose and gluconic acid oxidation of *Pseudomonas saccharophila*, *J. biol. Chem.*, **196**, 853–862.

EYLAR, O. R. and SCHMIDT, E. L. (1959). A survey of heterotrophic microorganisms from soil for ability to form nitrite and nitrate. *J. gen. Microbiol.*, **20**, 473–481.

FOSTER, J. W. and DAVIS, R. H. (1966). A methane-dependent coccus, with notes on classification and nomenclature of obligate methane-utilizing bacteria. *J. Bact.* **91**, 1924–1931.

FRINGS, W. (1969). An der heterotrophen CO_2-Fixierung von *Hydrogenomonas H 16* beteiligte Reaktionen. PhD. thesis, University of Göttingen.

GALE, E. F. (1943). Factors influencing the enzymic activities of bacteria. *Bact. Rev.*, **7**, 139–173.

GALE, N. L. and BECK, J. V. (1966). Competitive inhibition of phosphoribulokinase by AMP. *Biochem. biophys. Res. Commun.*, **24**, 792–796.

GALE, N. L. and BECK, J. V. (1967). Evidence for the Calvin cycle and hexose monophosphate pathway in *Thiobacillus ferrooxidans*. *J. Bact.*, **94**, 1052–1059.

GEST, H. (1954). Oxidation and evolution of molecular hydrogen by microorganisms. *Bact. Rev.*, **18**, 43–73.

GIBBS, M. and SCHIFF, J. A. (1960). Chemosynthesis: The energy relations of chemoautotrophic organisms. In F. G. Steward (Ed.), *Plant Physiology*, Vol. IB. Academic Press, New York. pp. 279–319.

GLAESER, H. (1971). Regulation des Tricarbonsäure-Cyclus bei *Hydrogenomonas eutropha* Stamm *H 16*. PhD. thesis, University of Göttingen.

GLAESER, H. and SCHLEGEL, H. G. (1972a). Synthese der Enzyme des Tricarbonsäure-Cyclus in *Hydrogenomonas eutropha* Stamm *H 16*. *Arch. Mikrobiol.*, **86**, 315–325.

GLAESER, H. and SCHLEGEL, H. G. (1972b). NADP- und NAD-spezifische Isocitrat-Dehydrogenase in *Hydrogenomonas eutropha* Stamm *H 16*. *Arch. Mikrobiol.*, **86**, 327–337.

GOOL, A. P. VAN, LAMBERT, R. and LAUDELOUT, H. (1969). The fine structure of frozen etched *Nitrobacter* cells. *Arch. Mikrobiol.*, **69**, 281–293.

GOOL, A. P. VAN, TOBBACK, P. P. and FISCHER, I. (1971). Autotrophic growth and synthesis of reserve polymers in *Nitrobacter winogradskyi*. *Arch. Mikrobiol.*, **76**, 252–264.

GOTTSCHALK, G. (1964a). Die Biosynthese der Poly-β-hydroxybuttersäure durch Knallgasbakterien. I. Ermittlung der ^{14}C-Verteilung in Poly-β-hydroxybuttersäure. *Arch. Mikrobiol.*, **47**, 225–229.

GOTTSCHALK, G. (1964b). Die Biosynthese der Poly-β-hydroxybuttersäure durch Knallgasbakterien. II. Verwertung organischer Säuren. *Arch. Mikrobiol.*, **47**, 230–235.

GOTTSCHALK, G. (1964c). Die Biosynthese der Poly-β-hydroxybuttersäure durch Knallgasbakterien. III. Synthese aus Kohlendioxyd. *Arch. Mikrobiol.*, **47**, 236–250.

GOTTSCHALK, G. (1965). Die Verwertung organischer Substrate durch *Hydrogenomonas* in Gegenwart von molekularem Wasserstoff. *Biochem. Z.*, **341**, 260–270.

GOTTSCHALK, G., EBERHARDT, U. and SCHLEGEL, H. G. (1964). Verwertung von Fructose durch *Hydrogenomonas H 16* (L.). *Arch. Mikrobiol.*, **48**, 95–108.

GOTTSCHALK, G. and SCHLEGEL, H. G. (1965). Preparation of ^{14}C-D(−)-β-hydroxybutyric acid from $^{14}CO_2$ using "Knallgas" bacteria (*Hydrogenomonas*). *Nature, Lond.*, **205**, 308.

GRAY, C. T. and GEST, H. (1965). Biological formation of molecular hydrogen. A 'hydrogen valve' facilitates regulation of anaerobic energy metabolism in many microorganisms. *Science, N.Y.*, **148**, 186–192.

GROHMANN, G. (1924). Zur Kenntnis wasserstoffoxydierender Baktorien. *Zentbl. Bakt. ParasitKde, II*, **61**, 256–271.

HAGEAGE, G. J., JR., EANES, E. D. and GHERNA, R. L. (1970). X-ray diffraction studies of the sulfur globules accumulated by *Chromatium* species. *J. Bact.*, **101**, 464–469.

HAMPTON, M. L. and HANSON, R. S. (1969). Regulation of isocitrate dehydrogenase from *Thiobacillus thiooxidans* and *Pseudomonas fluorescens*. *Biochem. biophys. Res. Commun.*, **36**, 296–305.

HANERT, H. (1968). Untersuchungen zur Isolierung, Stoffwechselphysiologie und Morphologie von *Gallionella ferruginea* EHRENBERG, *Arch. Mikrobiol.*, **60**, 348–376.

HANSEN, R. G. and HENNING, U. (1966). Regulation of pyruvate dehydrogenase activity in *Escherichia coli K 12*. *Biochem. biophys. Acta*, **122**, 355–358.

HAROLD, F. M. (1966). Inorganic polyphosphates in biology: structure, metabolism, and function. *Bact. Rev.*, **30**, 772–794.

HAROLD, F. M. (1972). Conservation and transformation of energy by bacterial membranes. *Bact. Rev.*, **36**, 172–230.

HART, B. A. and GIBSON, J. (1971). Ribulose-5-phosphate kinase from *Chromatium* sp. strain *D*. *Archs Biochim. Biophys.*, **144**, 308–321.

HATCH, M. D. and SLACK, C. R. (1970). Photosynthetic CO_2-fixation pathways. *A. Rev. Pl. Physiol.*, **21**, 141–162.

HEMPFLING, W. P. and VISHNIAC, W. (1967). Yield coefficients of *Thiobacillus neapolitanus* in continuous culture. *J. Bact.*, **93**, 874–878.

HEPTINSTALL, J., RITTENHOUSE, H. G., MCFADDEN, B. A. and SHUMWAY, L. K. (1972). Effect of growth conditions on morphology of *Hydrogenomonas facilis* and on yield of a phospholipoprotein. *J. Bact.*, **110**, 363–367.

HIRSCH, P. (1961). Wasserstoffaktivierung und Chemoautotrophie bei Actinomyceten. *Arch. Mikrobiol.*, **39**, 360–373.

HOFMAN, T. and LEES, H. (1953). The biochemistry of nitrifying organisms. *Biochem. J.*, **54**, 579.

HOOPER, A. B. (1969). Biochemical basis of obligate autotrophy in *Nitrosomonas europaea*. *J. Bact.*, **97**, 776–779.

HOOPER, A. B. and NASON, A. (1965). Characterization of hydroxylaminecytochrome *c* reductase from the chemoautotrophs *Nitrosomonas europaea* and *Nitrosocystis oceanus*. *J. biol. Chem.*, **240**, 4044–4057.

HORIO, T. and KAMEN, M. D. (1970). Bacterial cytochromes: II. Functional aspects. *A. Rev. Microbiol.*, **24**, 399–428.

HUTCHINSON, M., JOHNSTONE, K. I. and WHITE, D. (1969). Taxonomy of the genus *Thiobacillus*: the outcome of numerical taxonomy applied to the group as a whole. *J. gen. Microbiol.*, **57**, 397–410.

IMAI, K., ASANO, A. and SATO, R. (1967). Oxidative phosphorylation in *Micrococcus denitrificans*. I. Preparation and properties of phosphorylating membrane fragments. *Biochim. biophys. Acta*, **143**, 462–476.

INMAN, R. E., INGERSOLL, R. B. and LEVY, E. A. (1971). Soil: a natural sink for carbon monoxide. *Science, N.Y.*, **172**, 1229–1231.

ISHAQUE, M. and ALEEM, M. I. H. (1970). Energy coupling in *Hydrogenomonas eutropha*. *Biochim. biophys. Acta*, **223**, 388–397.

JOHNSON, C. L. and VISHNIAC, W. (1970). Growth inhibition in *Thiobacillus neapolitanus* by histidine, methionine, phenylalanine, and threonine. *J. Bact.*, **104**, 1145–1150.

JOHNSON, E. J. (1966). Occurrence of the adenosine monophosphate inhibition of carbon dioxide fixation in photosynthetic and chemosynthetic autotrophs. *Archs Biochem. Biophys.* **114**, 178–183.

JOHNSON, E. J. and ABRAHAM, S. (1969). Assimilation and metabolism of exogenous organic compounds by the strict autotrophs *Thiobacillus thioparus* and *Thiobacillus neapolitanus*. *J. Bact.*, **97**, 1198–1208.

JOHNSON, E. J. and PECK, H. D., JR., (1965). Coupling of phosphorylation and carbon dioxide fixation in extracts of *Thiobacillus thioparus*. *J. Bact.*, **89**, 1041–1050.

KALTWASSER, H. (1962). Die Rolle der Polyphosphate im Phosphatstoffwechsel eines Knallgasbakteriums (*Hydrogenomonas* Stamm *20*). *Arch. Mikrobiol.*, **41**, 282–306.

KALTWASSER, H. (1968). Harnsäureabbau und Biosynthese der Enzyme Uricase, Glyoxylatcarboligase und Urease bei *Hydrogenomonas H 16*. I. Bildung von Glyoxylatcarboligase und D-Glycerat-3-Dehydrogenase. *Arch. Mikrobiol.*, **64**, 71–84.

KALTWASSER, H. (1969). Harnsäureabbau und Biosynthese der Enzyme Uricase, Glyoxylatcarboligase und Urease bei *Hydrogenomonas H 16*. II. Einfluß von Harnsäure, Fructose und Stickstoffmangel. *Arch. Mikrobiol.*, **65**, 288–302.

KALTWASSER, H. and KRÄMER, J. (1968). Verwertung von Cytosin und Uracil durch *Hydrogenomonas facilis* und *Hydrogenomonas H 16*. *Arch. Mikrobiol.*, **60**, 172–181.

KALTWASSER, H. and SCHLEGEL, H. G. (1959). Nachweis und quantitative Bestimmung der Polyphosphate in wasserstoffoxydierenden Bakterien. *Arch. Mikrobiol.*, **34**, 76–92.

KAMEN, M. D. and HORIO, T. (1970). Bacterial cytochromes: I. Structural aspects. *A. Rev. Biochem.*, **39**, 673–700.

KASERER, H. (1906). Die Oxydation des Wasserstoffs durch Mikroorganismen. *Zentbl. Bakt. ParasitKde, II*, **16**, 681–696.

KELLY, D. P. (1967). The incorporation of acetate by the chemoautotroph *Thiobacillus neapolitanus* strain *C. Arch. Mikrobiol.*, **58**, 99–116.

KELLY, D. P. (1968). Fluoroacetate toxicity in *Thiobacillus neapolitanus* and its relevance to the problem of obligate chemoautotrophy. *Arch. Mikrobiol.*, **61**, 59–76.

KELLY, D. P. (1969). Regulation of chemoautotrophic metabolism. I. Toxicity of phenylalanine to Thiobacilli. *Arch. Mikrobiol.*, **69**, 330–342.

KELLY, D. P. (1970). Metabolism of organic acids by *Thiobacillus neapolitanus*. *Arch. Mikrobiol.*, **73**, 177–192.

KELLY, D. P. (1971). Autotrophy: Concepts of lithotrophic bacteria and their organic metabolism. *A. Rev. Microbiol.*, **25**, 177–210.

KELLY, D. P. and TUOVINEN, O. H. (1972). Recommendation that the names *Ferrobacillus ferrooxidans* LEATHEN and BRALEY and *Ferrobacillus sulfooxidans* KINSEL be recognized as synonyms of *Thiobacillus ferrooxidans* TEMPLE and COLMER. *Int. J. Syst. Bact.*, **22**, 170–172.

KEMP, M. B. and QUAYLE, J. R. (1967). Microbial growth on C_1 compounds. Uptake of (^{14}C) formaldehyde and (^{14}C) formate by methane-grown *Pseudomonas methanica* and determination of the hexose labelling pattern after brief incubation with (^{14}C) methanol. *Biochem. J.*, **102**, 94–102.

KIESOW, L. (1963). Über die Reduktion von Diphosphopyridinnucleotid bei der Chemosynthese. *Biochem. Z.*, **338**, 400–406.

KIESOW, L. (1964). On the assimilation of energy from inorganic sources in autotrophic forms of life. *Proc. natn. Acad. Sci.*, **52**, 980–988.

KISTNER, A. (1954). Conditions determining the oxidation of carbon monoxide and of hydrogen by *Hydrogenomonas carboxydovorans*. *Proc. K. ned. Akad. Wet. (Ser. C)*, **57**, 186–195.

KLINGENBERG, M. (1963). Reversibilität der Energieumwandlungen in der Atmungskette. *Angew. Chem.*, **75**, 900–906.

KLUYVER, A. J. and SCHNELLEN, C. G. T. P. (1947). Fermentation of carbon monoxide by pure cultures of methane bacteria. *Archs Biochem.*, **14**, 57–70.

KLUYVER, A. J. and VERHOEVEN, W. (1954). Studies on the dissimilatory nitrate reduction. IV. On adaptation in *Micrococcus denitrificans*. *Antonie van Leeuwenhoek*, **20**, 337.

KORNBERG, H. L., COLLINS, J. F., and BIGLEY, D. (1960). The influence of growth substrates on metabolic pathways in *Micrococcus denitrificans*. *Biochim. biophys. Acta*, **39**, 9–24.

KUCERA, S. and WOLFE, R. S. (1957). A selective enrichment method for *Gallionella ferruginea*. *J. Bact.*, **74**, 344–349.

KUEHN, G. D. and MCFADDEN, B. A. (1968a). Factors affecting the synthesis and degradation of ribulose-1,5-diphosphate carboxylase in *Hydrogenomonas facilis* and *Hydrogenomonas eutropha*. *J. Bact.*, **95**, 937–946.

KUEHN, G. D. and MCFADDEN, B. A. (1968b). Enzymes of the Entner–Doudoroff path in fructose-grown *Hydrogenomonas eutropha*. *Can. J. Microbiol.*, **14**, 1259–1260.

LANDESMAN, J., DUNCAN, D. W. and WALDEN, C. C. (1966). Iron oxidation by washed cell suspensions of the chemoautotroph, *Thiobacillus ferrooxidans*. *Can. J. Microbiol.*, **12**, 25–33.

LA RIVIÈRE, J. W. M. (1964). Enrichment of colorless sulfur bacteria. *Zentbl. Bakt. ParasitKde I*. **1** (Suppl.), 17–27.

LAWRENCE, A. J., KEMP, M. B. and QUAYLE, J. R. (1970). Synthesis of cell constituents by methane-grown *Methylococcus capsulatus* and *Methanomonas methanooxidans*. *Biochem. J.*, **116**, 631–639.

LAWRENCE, A. J. and QUAYLE, J. R. (1970). Alternative carbon assimilation pathways in methane-utilizing bacteria. *J. gen. Microbiol.*, **63**, 371–374.

LEBEDEFF, A. F. (1909). Über die Assimilation des Kohlenstoffs bei wasserstoffoxydierenden Bakterien. *Ber. dt. bot. Ges.*, **27**, 598–602.

LEES, H. (1962). Symposium on autotrophy. IV. Some thoughts on the energetics of chemosynthesis. *Bact. Rev.*, **26**, 165–167.

LEJOHN, H. B., CAESEELE, L. VAN and LEES, H. (1967). Catabolite repression in the facultative chemoautotroph *Thiobacillus novellus*. *J. Bact.*, **94**, 1484–1491.

LESSIE, T. and NEIDHARDT, F. C. (1967). Adenosine triphosphate-linked control of *Pseudomonas aeruginosa* glucose-6-phosphate dehydrogenase. *J. Bact.*, **93**, 1337–1345.

LESTER, R. J. and CRANE, F. L. (1959). The natural occurrence of coenzyme A and related compounds. *J. biol. Chem.*, **234**, 2169–2175.

LINDAY, E. M. and SYRETT, P. J. (1958). The induced synthesis of hydrogenase by *Hydrogenomonas facilis*. *J. gen. Microbiol.*, **19**, 223–227.

LONDON, J. (1963). *Thiobacillus intermedius* nov. sp. A novel type of facultative autotroph. *Arch. Mikrobiol.*, **46**, 329–337.

LONDON, J. and RITTENBERG, S. C. (1966). Effects of organic matter on the growth of *Thiobacillus intermedius*. *J. Bact.*, **91**, 1062–1069.

LONDON, J. and RITTENBERG, S. C. (1967). *Thiobacillus perometabolis* nov. sp., a non-autotrophic *Thiobacillus*. *Arch. Mikrobiol.*, **59**, 218–225.

LU, M. C., MATIN, A. and RITTENBERG, S. C. (1971). Inhibition of growth of obligately chemolithotrophic thiobacilli by amino acids. *Arch. Mikrobiol.*, **79**, 354–366.

LWOFF, A., NIEL, C. B. VAN, RYAN, F. J. and TATUM, E. L. (1946). Nomenclature of nutritional types of microorganisms. *Cold Spring Harb. Symp. quant. Biol.*, **11**, 302–303.

MACELROY, R. D., JOHNSON, E. J. and JOHNSON, M. K. (1968a). Characterization of ribulose diphosphate carboxylase and phosphoribulokinase from *Thiobacillus thioparus* and *Thiobacillus neapolitanus*. *Archs Biochem. Biophys.*, **127**, 310–316.

MACELROY, R. D., JOHNSON, E. J. and JOHNSON, M. K. (1968b). Allosteric regulation of phosphoribulokinase activity. *Biochem. biophys. Res. Commun.*, **30**, 678–682.

MACELROY, R. D., MACK, H. M. and JOHNSON, E. J. (1972). Properties of phosphoribulokinase from *Thiobacillus neapolitanus*. *J. Bact.*, **112**, 532–538.

MACGEE, J. and DOUDOROFF, M. (1954). A new phosphorylated intermediate in glucose oxidation. *J. biol. Chem.*, **210**, 617–626.

MCFADDEN, B. A. and HOWES, W. V. (1962). Isocitrate lyase and malate synthase in *Hydrogenomonas facilis*. *J. biol. Chem.*, **237**, 1410–1412.

MCFADDEN, B. A. and TU, C. L. (1967). Regulation of autotrophic and heterotrophic carbon dioxide fixation in *Hydrogenomonas facilis*. *J. Bact.*, **93**, 886–893.

MAGASANIK, B. (1964). Enzyme induction and catabolite repression. *Abstr. 6th Int. Congr. Biochem.*, **9**, 680–681.

MATIN, A. and RITTENBERG, S. C. (1970a). Utilization of glucose in heterotrophic media by *Thiobacillus intermedius*. *J. Bact.*, **104**, 234–238.

MATIN, A. and RITTENBERG, S. C. (1970b). Regulation of glucose metabolism in *Thiobacillus intermedius*. *J. Bact.*, **104**, 239–246.

MATIN, A. and RITTENBERG, S. C. (1971). Enzymes of carbohydrate metabolism in *Thiobacillus* species. *J. Bact.*, **107**, 179–186.

MORIARTY, D. J. W. and NICHOLAS, D. J. D. (1969). Enzymic sulphide oxidation by *Thiobacillus concretivorus*. *Biochim. biophys. Acta*, **184**, 114–123.

MORIARTY, D. J. W. and NICHOLAS, D. J. D. (1970). Products of sulphide oxidation in extracts of *Thiobacillus concretivorus*. *Biochim. biophys. Acta*, **197**, 143–151.

MURRAY, R. G. E. and WATSON, S. W. (1965). Structure of *Nitrosocystis oceanus* and comparison with *Nitrosomonas* and *Nitrobacter*. *J. Bact.*, **89**, 1594–1609.

NABOKICH, A. J. and LEBEDEFF, A. F. (1907). Über die Oxydation des Wasserstoffs durch Bakterien. *Zentbl. Bakt. ParasitKde, II*, **17**, 350–355.

NATHANSOHN, A. (1902). Über eine neue Gruppe von Schwefelbakterien und ihren Stoffwechsel. *Mitt. zool. Stn Neapel*, **15**, 655.

NELSON, D. H. (1931). Isolation and characterization of *Nitrosomonas* and *Nitrobacter*. *Zentbl. Bakt. ParasitKde, II*, **83**, 280–311.

NICHOLAS, D. J. D. (1963). The metabolism of inorganic nitrogen and its compounds in microorganisms. *Biol. Rev.*, **38**, 530–568.

NIKLEWSKI, B. (1908). Ein Beitrag zur Kenntnis wasserstoffoxydierender Mikroorganismen

II. *Zentbl. Bakt. ParasitKde, II*, **20**, 469–473.

NIKLEWSKI, B. (1914). Über die Wasserstoffaktivierung durch Bakterien unter besonderer Berücksichtigung der neuen Gattung *Hydrogenomonas agilis*. *Zentbl. Bakt. ParasitKde, II*, **40**, 430–433.

OEDING, V. and SCHLEGEL, H. G. (1973). β-Ketothiolase from *Hydrogenomonas eutropha H 16* and its significance in the regulation of poly-β-hydroxybutyrate metabolism. *Biochem. J.*, **134**, 239–248.

OHMANN, E., BORRISS, R. and RINDT, K. P. (1970). Glucose-6-phosphat-Dehydrogenase in autotrophen Mikroorganismen. II. Die Regulation der Aktivität der Glucose-6-phosphat-Dehydrogenase in *Euglena gracilis* und *Rhodopseudomonas spheroides*. *Z. allg. Mikrobiol.*, **10**, 37–53.

O'KELLY, J. C., BECKER, G. E. and NASON, A. (1970). Characterization of the particulate nitrite oxidase and its component activities from the chemoautotroph *Nitrobacter agilis*. *Biochim. biophys. Acta*, **205**, 409–425.

PACKER, L. (1958). Respiratory carriers involved in the oxidation of hydrogen and lactate in a facultative autotroph. *Archs Biochem. Biophys.*, **78**, 54–65.

PACKER, L. and VISHNIAC, W. (1955). Chemosynthetic fixation of carbon dioxide and characteristics of hydrogenase in resting cell suspensions of *Hydrogenomonas ruhlandii* nov. spec. *J. Bact.*, **70**, 216–223.

PAN, P. H. (1971). Lack of distinction between *Nitrobacter agilis* and *Nitrobacter winogradskyi*. *J. Bact.*, **108**, 1416–1418.

PAN, P. and UMBREIT, W. W. (1972). Growth of obligate autotrophic bacteria on glucose in a continuous flow-through apparatus. *J. Bact.*, **109**, 1149–1155.

PARKER, C. D. and PRISK, J. (1953). The oxidation of inorganic compounds of sulphur by various sulphur bacteria. *J. gen. Microbiol.*, **8**, 344–364.

PATEL, R. N. and HOARE, D. S. (1971). Physiological studies of methane and methanol-oxidizing bacteria: oxidation of C-1 compounds by *Methylococcus capsulatus*. *J. Bact.*, **107**, 187–192.

PECK, H. D., JR. (1960). Adenosine 5′-phosphosulfate as an intermediate in the oxidation of thiosulfate by *Thiobacillus thioparus*. *Proc. natn. Acad. Sci. U.S.A.*, **46**, 1053–1057.

PECK, H. D., JR. (1962). Symposium on metabolism of inorganic compounds V. Comparative metabolism of inorganic sulfur compounds in microorganisms. *Bact. Rev.*, **26**, 67–94.

PECK, H. D., JR. (1968). Energy-coupling mechanisms in chemolithotrophic bacteria. *A. Rev. Microbiol.*, **22**, 489–518.

PEETERS, T. L., LIU, M. S. and ALEEM, M. I. H. (1970). The tricarboxylic acid cycle in *Thiobacillus denitrificans* and *Thiobacillus A2*. *J. gen. Microbiol.*, **64**, 29–35.

PFEFFER, W. (1893). Studien zur Energetik der Pflanze. *Abh. sächs. Akad. Wiss. Math.-Phys. Kl.*, **18**, 149–278.

PFEFFER, W. (1897). *Pflanzenphysiologie*, Vol. I, W. Engelmann, Leipzig.

PFITZNER, J. (1972). Über das Elektronentransportsystem bei *Hydrogenomononas eutropha* Stamm *H 16*. *Zentbl. Bakt. ParasitKde, I*, **A220**, 396–401.

PFITZNER, J., LINKE, H. A. B. and SCHLEGEL, H. G. (1970). Eigenschaften der NAD-spezifischen Hydrogenase aus *Hydrogenomonas H 16*. *Arch. Mikrobiol.*, **71**, 67–78.

POLEE, J. and PECK, H. D., JR. (1971). Purification of the enzyme reducing biosulfite to trithionate from *Desulfovibrio gigas* and its identification as desulfoviridin. *Biochem. biophys. Res. Commun.*, **45**, 583–589.

POPE, L. M., HOARE, D. S. and SMITH, A. J. (1969). Ultrastructure of *Nitrobacter agilis* grown under autotrophic and heterotrophic conditions. *J. Bact.*, **97**, 936–939.

POSTGATE, J. R. (1963). The examination of sulphur autotrophs: a warning. *J. gen. Microbiol.*, **30**, 481–484.

POSTGATE, J. R. (1969). The sulphur cycle. In G. Nickless (Ed.), *Inorganic Sulphur Chemistry*. Elsevier, Amsterdam. pp. 259–279.

PRINGSHEIM, E. G. (1970). Die Lebensbedingungen des farblosen Schwefelorganismus *Beggiatoa*. *Beitr. Biol. Pfl.*, **46**, 323–336.

PROBST, I. and SCHLEGEL, H. G. (1973). Studies on a gram-positive hydrogen bacterium. *Nocardia opaca* strain *1 b*. II. Enzyme formation and regulation under the influence of hydrogen or fructose as growth substrates. *Arch. Mikrobiol.*, **88**, 319–330.

QUAYLE, J. R. (1972). The metabolism of one-carbon compounds by micro-organisms. *Adv. microb. Physiol.*, **7**, 119–203.

RALSTON, E., PALLERONI, N. J. and DOUDOROFF, M. (1972). Deoxyribonucleic acid homologies of some so-called '*Hydrogenomonas*' species. *J. Bact.*, **109**, 465–466.

RAMSEY, H. H. (1968). Autotrophic and heterotrophic metabolism in *Hydrogenomonas facilis*. *Antonie van Leeuwenhoek*, **34**, 71–80.

REMSEN, C. C. and LUNDGREN, D. G. (1963). The heterotrophic growth of the chemoautotroph *Ferrobacillus ferrooxidans*. *Bact. Proc.*, **1963**, 33.

REMSEN, C. C., VALOIS, F. W. and WATSON, S. W. (1967). Fine structure of the cytomembranes of *Nitrosocystis oceanus*. *J. Bact.*, **94**, 422–433.

REPASKE, R. (1962). Nutritional requirements for *Hydrogenomonas eutropha*. *J. Bact.*, **83**, 418–422.

REPASKE, R. and DANS, C. L. (1968). A factor for coupling NAD to hydrogenase in *Hydrogenomonas eutropha*. *Biochem. biophys. Res. Commun.*, **30**, 136–141.

REPASKE, R. and LIZOTTE, C. I. (1965). The electron transport system of *Hydrogenomonas eutropha*. *J. biol. Chem.*, **240**, 4774–4779.

RIBBONS, D. W., HARRISON, J. E. and WADZINSKI, A. M. (1970). Metabolism of single carbon compounds. *A. Rev. Microbiol.*, **24**, 135–158.

RINDT, K.-P. and OHMANN, E. (1969). NADH and AMP as allosteric effectors of ribulose-5-phosphate kinase in *Rhodopseudomonas spheroides*. *Biochem. biophys. Res. Commun.*, **36**, 357–364.

RITTENBERG, S. C. (1969). The roles of exogenous organic matter in the physiology of chemolithotrophic bacteria. *Adv. microb. Physiol.*, **3**, 151–196.

RITTENBERG, S. C. and GOODMAN, N. S. (1969). Mixotrophic growth of *Hydrogenomonas eutropha*. *J. Bact.*, **98**, 617–622.

ROSS, A. J., SCHOENHOFF, R. L. and ALEEM, M. I. H. (1968). Electron transport and coupled phosphorylation in the chemoautotroph *Thiobacillus neapolitanus*. *Biochem. biophys. Res. Commun.*, **32**, 301–306.

ROY, A. B. and TRUDINGER, P. A. (1970). *The Biochemistry of Inorganic Compounds of Sulphur*, Cambridge University Press, London.

RUHLAND, W. (1922). Aktivierung von Wasserstoff und CO_2-Assimilation durch Bakterien. *Ber. dt. bot. Ges.*, **40**, 180–184.

RUHLAND, W. (1924). Beiträge zur Physiologie der Knallgasbakterien. *Jb. wiss. Bot.*, **63**, 321–389.

SAVEL'EVA, N. D. and ZHILINA, T. N. (1968). Taxonomy of hydrogen bacteria. *Mikrobiologiya*, **37**, 84–91.

SAXENA, J. and ALEEM, M. I. H. (1972). Generation of reducing power in chemosynthesis. VII. Mechanism of pyridine nucleotide reduction by thiosulfate in the chemoautotroph *Thiobacillus neapolitanus*. *Arch. Mikrobiol.*, **84**, 317–326.

SAXENA, J. and VISHNIAC, W. (1970). Influence of organic acids on the growth of *Thiobacillus neapolitanus*. *Antonie van Leeuwenhoek*, **36**, 109–118.

SCHATZ, A. and BOVELL, C. (1952). Growth and hydrogenase activity of a new bacterium *Hydrogenomonas facilis*. *J. Bact.*, **63**, 87–98.

SCHINDLER, J. (1964). Die Synthese von Poly-β-hydroxybuttersäure durch *Hydrogenomonas H 16*: Die zu β-Hydroxybutyryl-Coenzym A führenden Reaktionsschritte. *Arch. Mikrobiol.*, **49**, 236–255.

SCHINDLER, J. and SCHLEGEL, H. G. (1969). Regulation der Glucose-6-phosphat-Dehydrogenase aus verschiedenen Bakterienarten durch ATP. *Arch. Mikrobiol.*, **66**, 69–78.

SCHLEGEL, H. G. (1954). Die Rolle des molekularen Wasserstoffs im Stoffwechsel der Mikroorganismen. *Arch. Mikrobiol.*, **20**, 293–322.

SCHLEGEL, H. G. (1960). Die wasserstoffoxydierenden Bakterien. *Handb. PflPhysiol.*, **5**, 715–734.

SCHLEGEL, H. G. (1966). Physiology and biochemistry of Knallgasbacteria. *Adv. Comp. Physiol. Biochem.*, **2**, 185–236.

SCHLEGEL, H. G. and BARTHA, R. VON (1961a). 'Leerlauf'-H_2-Oxydation und 'Rückkoppelung' bei Knallgasbakterien. *Naturwissenschaften*, **48**, 414–415.

SCHLEGEL, H. G. and BARTHA, R. VON (1961b). Hemmungsanalytische Versuche zum Rückkoppelungseffekt bei *Hydrogenomonas*. *Z. Naturf.*, **16b**, 777–780.

SCHLEGEL, H. G. and BLACKKOLB, F. (1967). Regulation des Hexose-Abbaus über den Entner-Doudoroff-Weg durch Enzymhemmung. *Nachr. Akad. Wiss. Göttingen* (*II. Math.-Physik. Kl*), **1967**, 51–60.

SCHLEGEL, H. G. and EBERHARDT, U. (1972). Regulatory phenomena in the metabolism of Knallgasbacteria. *Adv. microb. Physiol.*, **7**, 205–242.

SCHLEGEL, H. G. and GOTTSCHALK, G. (1965). Verwertung von Glucose durch eine Mutante von *Hydrogenomonas H 16*. *Biochem. Z.*, **341**, 249–259.

SCHLEGEL, H. G., GOTTSCHALK, G. and BARTHA, R. VON (1961a). Formation and utilization of poly-β-hydroxybutyric acid by Knallgasbacteria (*Hydrogenomonas*). *Nature, Lond.*, **191**, 463–465.

SCHLEGEL, H. G. and KALTWASSER, H. (1961). Veränderungen des Polyphosphatgehalts während des Wachstums von Knallgasbakterien unter Phosphatmangel. *Flora, Jena*, **150**, 259–273.

SCHLEGEL, H. G., KALTWASSER, H. and GOTTSCHALK, G. (1961b). Ein Submersverfahren zur Kultur wasserstoffoxydierender Bakterien: Wachstumsphysiologische Untersuchungen. *Arch. Mikrobiol.*, **38**, 209–222.

SCHLEGEL, H. G., LAFFERTY, R. and KRAUSS, I. (1970). The isolation of mutants not accumulating poly-β-hydroxybutyric acid. *Arch. Mikrobiol.*, **71**, 283–294.

SCHLEGEL, H. G. and TRÜPER, H. G. (1966). Repression of enzyme formation in *Hydrogenomonas* strain *H 16* G^+ by molecular hydrogen and by fructose. *Antonie van Leeuwenhoek*, **32**, 277–292.

SCHUSTER, E. and SCHLEGEL, H. G. (1967). Chemolithotrophes Wachstum von *Hydrogenomonas H 16* im Chemostaten mit elektrolytischer Knallgaserzeugung. *Arch. Mikrobiol.*, **58**, 380–409.

SENIOR, P. and DAWES, E. A. (1973). The regulation of poly-β-hydroxybutyrate metabolism. *Biochem. J.*, **134**, 225–238.

SEWELL, D. L. and ALEEM, M. I. H. (1969). Generation of reducing power in chemosynthesis. V. The mechanism of pyridine nucleotide reduction by nitrite in the chemoautotroph *Nitrobacter agilis*. *Biochim. biophys. Acta*, **172**, 467–475.

SHIVELY, J. M. and BENSON, A. A. (1967). Phospholipids of *Thiobacillus thiooxidans*. *J. Bact.*, **94**, 1679–1683.

SHIVELY, J. M., DECKER, G. L. and GREENAWALT, J. W. (1970). Comparative ultrastructure of the thiobacilli. *J. Bact.*, **101**, 618–627.

SIEBERT, D. (1969). Über propanverwertende wasserstoffoxydierende Bakterien und die Charakterisierung eines Förderungsfaktors. Ph.D. thesis, University of Göttingen.

SMITH, A. J. and HOARE, D. S. (1968a). *Nitrobacter agilis*—an obligate or a facultative autotroph? *Biochem. J.*, **106**, 40P.

SMITH, A. J. and HOARE, D. S. (1968b). Acetate assimilation by *Nitrobacter agilis* in relation to its 'obligate autotrophy'. *J. Bact.*, **95**, 844–855.

SMITH, A. J., LONDON, J. and STANIER, R. Y. (1967). Biochemical basis of obligate autotrophy in blue-green algae and thiobacilli. *J. Bact.*, **94**, 972–983.

STARKEY, R. L. (1935). Isolation of some bacteria which oxidize thiosulfate. *J. Bact.*, **39**, 197.

STUKUS, P. E. and DECICCO, B. T. (1970). Autotrophic and heterotrophic metabolism of *Hydrogenomonas*: Regulation of autotrophic growth by organic substrates. *J. Bact.*, **101**, 339–345.

SUH, B. and AKAGI, J. M. (1969). Formation of thiosulfate from sulfite by *Desulfovibrio vulgaris*. *J. Bact.*, **99**, 210–215.

TABITA, R. and LUNDGREN, D. G. (1971a). Utilization of glucose and the effect of organic compounds on the chemolithotroph *Thiobacillus ferrooxidans*. *J. Bact.*, **108**, 328–333.

TABITA, R. and LUNDGREN, D. G. (1971b). Heterotrophic metabolism of the chemolithotroph *Thiobacillus ferrooxidans*. *J. Bact.*, **108**, 334–342.

TABITA, R. and LUNDGREN, D. G. (1971c). Glucose-6-phosphate dehydrogenase from the chemolithotroph *Thiobacillus ferrooxidans*. *J. Bact.*, **108**, 343–352.

TAKAMIYA, A. and TUBAKI, K. (1956). A new form of streptomyces capable of growing autotrophically. *Arch. Mikrobiol.*, **25**, 58–64.

TAYLOR, B. F. (1970). Regulation of citrate synthase activity in strict and facultatively autotrophic thiobacilli. *Biochem. biophys. Res. Commun.*, **40**, 957–963.

TAYLOR, B. F. and HOARE, D. S. (1969). New facultative *Thiobacillus* and reevaluation of the heterotrophic potential of *Thiobacillus novellus*. *J. Bact.*, **100**, 487–497.

TAYLOR, B. F. and HOARE, D. S. (1971). *Thiobacillus denitrificans* as an obligate chemolithotroph. II. Cell suspension and enzymic studies. *Arch. Mikrobiol.*, **80**, 262–276.

TAYLOR, B. F., HOARE, D. S. and HOARE, S. L. (1971). *Thiobacillus denitrificans* as an obligate chemolithotroph. Isolation and growth studies. *Arch. Mikrobiol.*, **78**, 193–204.

TEMPLE, K. L. and COLMER, A. R. (1951). The autotrophic oxidation of iron by a new bacterium, *Thiobacillus ferrooxidans*. *J. Bact.*, **62**, 605–611.

TRUDINGER, P. A. (1969). Assimilatory and dissimilatory metabolism of inorganic sulphur compounds by micro-organisms. *Adv. microb. Physiol.*, **3**, 111–158.

TRUDINGER, P. A. and KELLY, D. P. (1968). Reduced nicotinamide adenine dinucleotide oxidation by *Thiobacillus neapolitanus* and *Thiobacillus* strain *C*. *J. Bact.*, **95**, 1962–1963.

TRÜPER, H. G. (1965). Tricarboxylic acid cycle and related enzymes in *Hydrogenomonas* strain *H 16* G^+ grown on various carbon sources. *Biochim. biophys. Acta*, **111**, 565–568.

TUNAIL, N. (1973). Charakterisierung des Wasserstoffbacteriums *Arthrobacter 7C* und der Regulation des Kohlenhydratstoffwechsels. Ph.D. thesis, University of Göttingen.

TUNAIL, N. and SCHLEGEL, H. G. (1972). Phosphoenolpyruvate, a new inhibitor of glucose-6-phosphate dehydrogenase. *Biochem. biophys. Res. Commun.*, **49**, 1554–1560.

UNZ, R. F. and LUNDGREN, D. G. (1961). A comparative nutritional study of three chemoautotrophic bacteria: *Ferrobacillus ferrooxidans*, *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*. *Soil Sci.*, **92**, 302–313.

VEDENINA, I. Y. (1968). On the content of main cell components during autotrophic growth of *Hydrogenomonas Z-1*. *Mikrobiologiya*, **37**, 8–9.

VELDKAMP, H. (1970). Enrichment cultures of prokaryotic organisms. In J. R. Norris and D. W. Ribbons (Eds), *Methods in Microbiology*, Vol. IIIA. Academic Press, London. pp. 305–361.

VERHOEVEN, W., KOSTER, A. L. and NIEVELT, M. G. A. VAN (1954). Studies on true dissimilatory nitrate reduction. III. *Micrococcus denitrificans* BEIJERINCK, a bacterium capable of using molecular hydrogen in denitrification. *Antonie van Leeuwenhoek*, **20**, 273–284.

VERSTRAETE, W. and ALEXANDER, M. (1972). Heterotrophic nitrification by *Arthrobacter* sp. *J. Bact.*, **110**, 955–961.

VISHNIAC, W. (1971). Limits of microbial productivity in the ocean. *Symp. Soc. gen. Microbiol.*, **21**, 355–366.

VISHNIAC, W. and SANTER, M. (1957). The thiobacilli. *Bact. Rev.*, **21**, 195–213.

VOGLER, K. G. and UMBREIT, W. W. (1942). Studies on the metabolism of the autotrophic bacteria. III. The nature of the energy storage material active in the chemosynthetic process. *J. gen. Physiol.*, **26**, 157–167.

VOGT, M. (1965). Wachstumsphysiologische Untersuchungen an *Micrococcus denitrificans* BEIJ. *Arch. Mikrobiol.*, **50**, 256–281.

WAKSMAN, S. A. and JOFFE, J. S. (1922). Microorganisms concerned in the oxidation of sulfur in the soil. II. *Thiobacillus thiooxidans*, a new sulfur-oxidizing organism isolated from the soil. *J. Bact.*, **7**, 239–256.

WALLACE, W. and NICHOLAS, D. J. D. (1969a). Glutamate dehydrogenase in *Nitrosomonas europaea* and the effect of hydroxylamine, oximes and related compounds on its activity. *Biochim. biophys. Acta*, **171**, 229–237.

WALLACE, W. and NICHOLAS, D. J. D. (1969b). The biochemistry of nitrifying microorganisms. *Biol. Rev.*, **44**, 359–391.

WATSON, S. W. (1963). Autotrophic nitrification in the ocean. In C. H. Oppenheimer (Ed.), *Marine Microbiology*. C. C. Thomas, Springfield, Ill. pp. 73–84.

WATSON, S. W. (1971a). Reisolation of *Nitrosospira briensis* S. WINOGRADSKY and H. WINOGRADSKY 1933. *Arch. Mikrobiol.*, **75**, 179–188.

WATSON, S. W. (1971b). Taxonomic considesations of the family Nitrobacteraceae BUCHANAN. *Int. J. Syst. Bact.*, **21**, 254–270.

WATSON, S. W., GRAHAM, L. B., REMSEN, C. C. and VALOIS, F. W. (1971). A lobular, ammonia-oxidizing bacterium, *Nitrosolobus multiformis*, nov. gen. nov. sp. *Arch. Mikrobiol.*, **76**, 183–203.

WATSON, S. W. and MANDEL, M. (1971). Comparison of the morphology and deoxyribonucleic acid composition of 27 strains of nitrifying bacteria. *J. Bact.*, **107**, 563–569.

WATSON, S. W. and WATERBURY, J. B. (1971). Characteristics of two marine nitrite oxidizing bacteria. *Nitrospina gracilis* nov. gen. sp. and *Nitrococcus mobilis* nov. gen. nov. sp. *Arch. Mikrobiol.*, **77**, 203–230.

WEST, D. A. (1971). Does virus infection have evolutionary significance? *Nature, Lond.*, **229**, 637.

WHITE, A., HANDLER, P. and SMITH, E. L. (1964). *Principles of Biochemistry*, 3rd ed., McGraw-Hill, New York.

WHITTENBURY, R., PHILLIPS, K. C. and WILKINSON, J. F. (1970). Enrichment, isolation, and some properties of methane-utilizing bacteria. *J. gen. Microbiol.*, **61**, 205–218.

WILDE, E. (1962). Untersuchungen über Wachstum und Speicherstoffsynthese von *Hydrogenomonas*. *Arch. Mikrobiol.*, **43**, 109–137.

WILLIAMS, P. J. LEB. and WATSON, S. W. (1968). Autotrophy in *Nitrosocystis oceanus*. *J. Bact.*, **96**, 1640–1648.

WILLIAMS, R. A. D. and HOARE, D. S. (1970). A new thermophilic *Thiobacillus*. *J. gen. Microbiol.*, **61**, i-ii.

WILLIAMS, R. A. D. and HOARE, D. S. (1972). Physiology of a new facultatively autotrophic thermophilic *Thiobacillus*. *J. gen. Microbiol.*, **70**, 555–566.

WILSON, E., STOUT, H. A., POWELSON, D. and KOFFLER, H. (1953). Comparative biochemistry of the hydrogen bacteria. I. The simultaneous oxidation of hydrogen and lactate. *J. Bact.*, **65**, 283–287.

WINOGRADSKY, H. (1933). Nouvelles recherches sur les organismes de la nitrification. *Annls Inst. Pasteur, Paris*, **50**, 350–432.

WINOGRADSKY, M. S. (1890). Sur les organismes de la nitrification. *Annls Inst. Pasteur, Paris*, **4**, 213–231.

WINOGRADSKY, S. (1887). Über Schwefelbacterien. *Bot. Ztg.*, **45**, 489–600, 606–616.

WINOGRADSKY, S. (1888). Über Eisenbacterien. *Bot. Ztg.*, **46**, 261–276.

WINOGRADSKY, S. (1892). Contribution à la morphologie des organismes de la nitrification. *Archs Sci. biol. St Pétersb.* (*Arkh. biol. Nauk*), **1**, 88–137.

WINOGRADSKY, S. (1922). Eisenbakterien als Anorgoxydanten. *Zentbl. Bakt. ParasitKde, II*, **57**, 1–21.

WINOGRADSKY, S. (1931). Nouvelles recherches sur les microbes de la nitrification. *C.r. hebd. Séanc. Acad. Sci., Paris*, **192**, 1000–1004.

WITTENBERGER, C. L. and REPASKE, R. (1958). Studies on the electron transport system in *Hydrogenomonas eutropha*. *Bact. Proc.*, **1958**, 106.

WITTENBERGER, C. L. and REPASKE, R. (1961). Studies on hydrogen oxidation in cell-free extracts of *Hydrogenomonas eutropha*. *Biochim. biophys. Acta*, **47**, 542–552.

WOLFSON, P. J. and KRULWICH, T. A. (1972). Inhibition of isocitrate lyase: the basis for inhibition of growth of two *Arthrobacter* species by pyruvate. *J. Bact.*, **112**, 356–364.

WOODS, D. D. and LASCELLES, J. (1954). The no-man's land between the autotrophic and heterotrophic ways of life. Autotrophic microorganisms. *Symp. Soc. gen. Microbiol.*, **4**, 1–27.

YAMANAKA, T., MIKI, K. and OKUNUKI, K. (1963). Purification and some properties of a c-type cytochrome from *Pseudomonas saccharophila*. *Biochim. biophys. Acta*, **77**, 654–656.

ZEIKUS, J. G. and WOLFE, R. S. (1972). *Methanobacterium thermoautotrophicus* sp. n., an anaerobic, autotrophic, extreme thermophile. *J. Bact.*, **109**, 707–713.

3. MECHANISMS OF HETEROTROPHY

T. J. PANDIAN

(1) Introduction

Nutritionally, organisms are transformers of energy and matter (e.g. KINNE, 1960). They are subdivided into two major groups: **autotrophs** and **heterotrophs**. These terms were coined by PFEFFER (1897/1904) and are still used in their original meaning. Autotrophic organisms are able to synthesize all their cellular constituents from carbon dioxide, usually reducing oxidized inorganic compounds. Heterotrophic organisms depend on preformed organic material both for synthesis of cell constituents and for liberation of biologically useful energy. Unless referring to carbon-dioxide utilization, the terms autotrophy and heterotrophy require specification. Some scientists specify the independence of essential substances as vitamin autotrophy or as auxo-autotrophy, and the dependence on essential substances as vitamin heterotrophy or auxo-heterotrophy.

Mesotrophic organisms constitute a minor group, characterized by their need for one or more reduced inorganic nutrient(s). Bacteriophages and other viruses are considered not to be organisms because (i) they contain only one type of nucleo-acid, either DNA or RNA and only one nucleic acid is necessary for their reproduction; (ii) they are not able to grow and to multiply by cellular fission; (iii) they are not independent, but require living cells (host cells) for their multiplication.

Regarding the modes of making biologically useful energy available and of gaining cellular carbon, SCHLEGEL (1970) and STANIER and co-authors (1971) differentiate between energy source, hydrogen donor and carbon source:

Metabolic type	Examples of groups of organisms
photolitho-autotrophic	sulphur purple bacteria, green plants
photolithoheterotrophic	some non-sulphur purple bacteria
photo-organoheterotrophic	most non-sulphur purple bacteria
chemolitho-autotrophic	all bacteria oxidizing NH_4^+, NO_2^-, H_2, H_2S, S^{--}, S_2O_3, Fe^{++}
chemolithoheterotrophic	*Desulfovibrio*
chemo-organoheterotrophic	all organisms dependent on organic substrates (most bacteria, all fungi and all animals included)

All animals are chemo-organoheterotrophs, i.e., they obtain their energy by chemical processes; hydrogen donor and carbon are acquired from organic substances.

Based on the type of nutrition and the energy source used, WHITTAKER (1969) distinguishes three functional kingdoms: (i) **autotrophs**, (ii) **saprotrophs**, and (iii) **phagotrophs**. All three nutritional types are found in the Monera and Protista, while fungi, plants, or animals specialize as 'absorbers', 'producers', or 'ingesters'

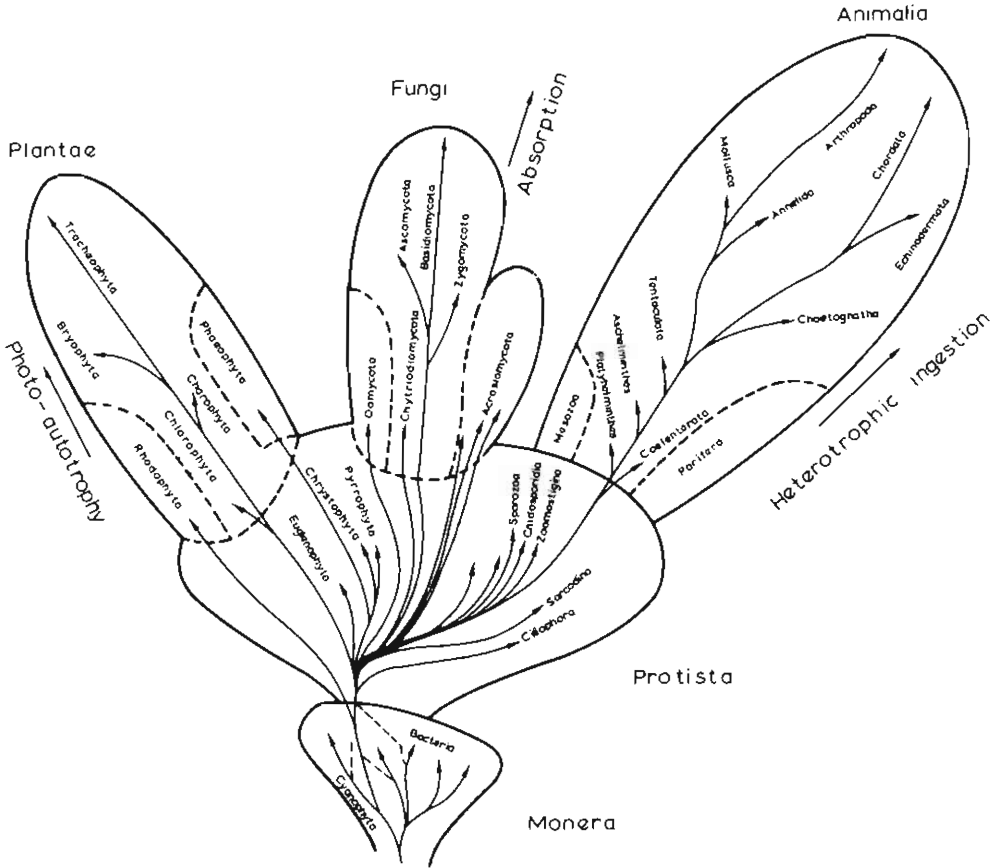


Fig. 3-1: Kingdoms of nature: Monera (procaryotic), Protista (eucaryotic unicellular), Plantae, Fungi, Animalia (eucaryotic multicellular). (After WHITTAKER, 1969; reproduced by permission of *Science*. Copyright 1969 by the American Association for the Advancement of Science.)

(Fig. 3-1). Bacteria and fungi exhibit high rates of metabolism and turnover. Their specializations are more evident biochemically than morphologically. Animals obtain their energy by ingestion of organic matter. The term 'saprotroph' designates organisms living in dead organisms or deteriorating organic matter. 'Phagotroph' is not strictly parallel to the other terms.

Of particular ecological significance is the fact that several unicellular autotrophic algae are capable of assuming heterotrophic abilities when needed, i.e. at very low irradiances. The chrysomonads *Chrysochromulina kappa* and *Dicrateria inornata* survive over 65 days in darkness; when exposed to light again, cultures in glycerol are the first to show visible population growth, indicating that more cells survive in the presence of a favourable carbon source (PINTNER and PROVASOLI, 1968; see also Volume I: FOGG, 1972; Volume III: UKELES, in press). Glycerol is reported to serve as a suitable carbon source in a number of chrysomonads (e.g. PINTNER and PROVASOLI, 1963; RAHAT and JAHN, 1965). Better growth of *C. strobilus* and *C. brevefilum* at 15 ft.c is obtained if the carbon source is supplemented by amino acids, nucleic

acids and vitamins (PINTNER and PROVASOLI, 1968). A similar effect has been reported by LEWIN (1963) in some heterotrophic littoral diatoms grown in darkness on glucose and caesin-digest. During evolutionary development, flagellates and ciliates have collected many enzyme lesions relating to amino-acid synthesis (KIDDER, 1967) and have become mesotrophic or heterotrophic. Therefore, one has to be cautious in classifying lower organisms; it is easier and more convenient to classify higher organisms.

Autotrophic producers and heterotrophic decomposers form a food chain of organisms through which the nutrient matter flows to and from the environment; therefore, they are considered essential biotic components of an ecosystem (E. P. ODUM, 1971). In most ecosystems, photo-autotrophic plants are the major producers. But in certain ecosystems, such as marine sediments below the redox-potential discontinuity layer, chemo-autotrophic bacteria (Chapter 2)—belonging to the genera *Thiobacillus*, *Thiovolum*, *Macromonas*, *Beggiatoa*, etc.—are the only producers (FENCHEL and RIEDL, 1970). In the absence of plants, the vertical shower of particulate organic matter, along with adsorbing bacteria, serves as the main food source for heterotrophic organisms inhabiting the aphotic zone. Horizontal transportation of organic matter by global circulation to warm tropical waters may also be an important food source for animals in the photic zone. For instance, in tropical Pacific waters bacterial production has been recorded ranging between 0·5 and 1·0 g C m^{-2} day^{-1}; this exceeds the primary production of phytoplankton (0·2 to 0·3 g C m^{-2} day^{-1}). About 30% of the cells of the bacterio-plankton populations compose aggregates larger than 4 μ. These aggregates serve as main food source for the coarse suspension feeders such as calanoids (e.g. *Eucalanus attenuatus*), oysters (e.g. *Crassostrea* sp.), tunicates, gastropod veligers, polychaetes (e.g. *Megalomma* sp.), and sponges (e.g. *Toxadocca violacea*) (SOROKIN, 1971; Volume IV: CONOVER, in press; SOROKIN, in press). Saprotrophic micro-organisms, especially bacteria and fungi are considered as decomposers but in some ecosystems animals are more important than bacteria and fungi in the decomposition of organic matter (JOHANNES, 1968; see, however, Volume IV: SOROKIN, in press).

In line with Volume I (KINNE, 1970, p. 466) the processes involved in the transformation of essential organic metabolites into the heterotroph's body-own substances are considered under three headings: (i) Intake of energy and matter (e.g. of ultra-violet irradiation used in vitamin D synthesis and of essential organic substances); (ii) transformation into body functions and structures; (iii) output of non-utilized energy and matter.

In heterotrophs, intake of energy and matter comprises the obtaining of essential organic substances either orally in form of large polymers, or via the body surface in form of low-molecular substances from the immediate environment. In osmotrophic heterotrophs, absorbed low-molecular substances probably enter the ground cytoplasm directly and engage in intermediary metabolism. Osmotrophs do not have elaborate morphological structures associated with feeding and digestion (e.g. most micro-organisms, cestodes, pogonophores). Certain other organisms (some micro-organisms as well as 'skin-digesting' animals) release enzymes into their immediate environment in order to digest the large polymers contained therein; the end-products of such external digestion, low-molecular substances, are subsequently absorbed via the body surface. Hence, these heterotrophs have a functional diges-

tive system. In sea-stars and turbellarians, partial prey digestion occurs while the stomach is everted externally, prior to ingestion. Several heterotrophic animals obtain essential organic substances orally as large polymers in the form of food organisms, and low-molecular substances via the body surface from their environment (e.g. certain molluscs and worms), or from symbiotic algae which live inside the cells of their body (e.g. certain coelenterates, molluscs and planarians).

Transformation of energy and matter obtained in form of food organisms (large polymers) consists of the sequential steps **digestion, absorption** and **conversion** (= assimilation). In most animals, food (large polymers) must be broken down in the digestive system into low-molecular substances, which alone can pass through biological membranes. Part of the food taken in is digested, and part of the digested food is subsequently absorbed; the rest is removed in the form of faeces (Volume I: KINNE, 1970, p. 466). The absorbed food—minus urine excretions—is converted into body functions (internal and external work) and body structures (body growth and reproductive materials). Of the food converted, a fraction is ultimately lost as heat (measurable as respiration or heat loss). The low-molecular substances (absorbed from the alimentary canal or from the immediate environment) are dissimilated in definite pathways to liberate biologically usable energy for body functions. The other fraction is converted into body growth and reproductive material and is temporarily stored. The reproductive material (gametes, spores, buds, etc.) is released when the transforming individual reproduces.

Substances removed or released due to the heterotroph's metabolic activities comprise: (i) **Nitrogenous waste products**; (ii) **undigested and unabsorbed excrements** (faeces); (iii) mucus (e.g. certain coelenterates, polychaetes and most molluscs and fishes; the snail *Tegula funebralis* releases mucus equivalent to 7% of the absorbed food, PAINE, 1971a); (iv) **exuvia** (in *Euphausia pacifica*, for example, exuvia constitute as much as 15% of the absorbed food; LASKER, 1966); (v) **low-molecular substances**, like amino acids, monosaccharides, fatty acids, etc. (e.g. JOHANNES, 1968); organic matter released by the sea-urchin *Strongylocentrotus dröbachiensis* is about twice (4 cal g^{-1} day^{-1}), as much as the energy metabolized (2 cal g^{-1} day^{-1}) at 10° to 12° C and is about 70% (1 cal g^{-1} day^{-1}) of the respiration value (1·6 cal g^{-1} day^{-1}) at 4° to 7° C (FIELD, 1973); (vi) **ectocrines**. Though excreted in trace quantities, ectocrines are known to have inhibitory (antibiotic) or acceleratory (probiotic) effects on growth of other organisms (Volume III, Chapter 2). In this chapter, mechanisms of nitrogen excretion and the nutritive role of faeces are reviewed in the section *Output of non-utilized energy and matter*.

(2) Intake of Energy and Matter

(a) Feeding Responses

In all heterotrophs, intake of matter (feeding) involves a series of behavioural responses. While these responses are related, and perhaps interdependent, they are separate phenomena, each under the control of a particular set of physical and/or chemical conditions (LINDSTEDT, 1971a). The chemical stimuli which govern feeding behaviour have been classified in four successive responses by BECK (1965): (i)

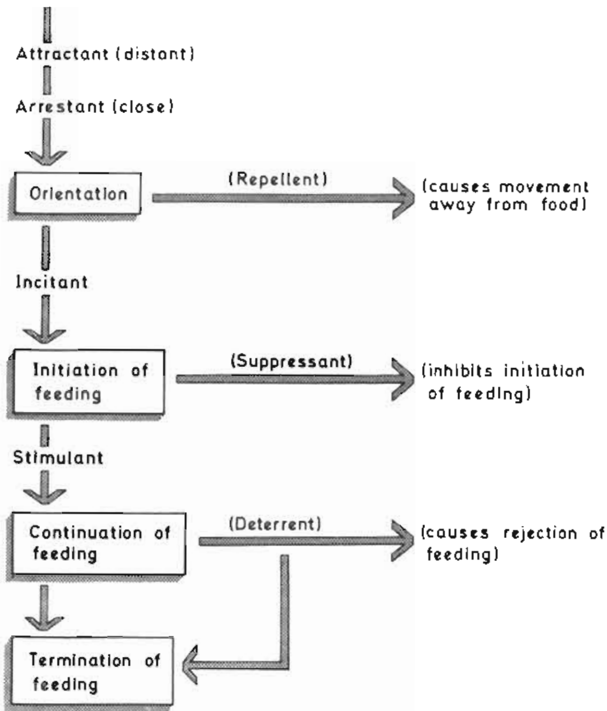


Fig. 3-2: Feeding responses and associated stimuli. Generalized model. (After LINDSTEDT, 1971a: modified; reproduced by permission of Microforms International Marketing Corp.)

Orientation (Chapters 7, 8, 9), involving locomotion in active predators or movement in sessile heterotrophs (e.g. expansion of contracted coral polyp); (ii) initiation of feeding (tasting); (iii) continuation of feeding (ingestion); and (iv) termination of feeding. These responses and the positive or negative stimuli evoking them are illustrated in Fig. 3-2.

LINDSTEDT (1971a) summarized the information available on chemical control of feeding in heterotroph consumer species, and defined the stimuli involved (chemical effectors) as follows: **Attractant**: a stimulus to which an animal responds by orienting towards, or becoming receptive to, the apparent source. It may operate over long distances. **Arrestant**: a stimulus that causes an animal to cease locomotion when in contact with the apparent source. **Repellent**: a stimulus that causes the animal to orient away from, or become non-receptive to, the apparent source. **Incitant**: a stimulus that evokes initiation of feeding (tasting). **Suppressant**: a stimulus that inhibits or prevents initiation of feeding. **Stimulant**: a stimulus that promotes ingestion and continuation of feeding. **Deterrent**: a stimulus that prevents continuation of feeding or hastens termination of feeding.

These terms, originally coined for chemical stimuli by DETHIER and co-authors (1960) and LINDSTEDT (1971a), may now be extended to refer also to other environmental stimuli. Such extension is necessary, as in many cases both physical and

chemical stimuli may be involved in evoking successive responses of feeding in a predator. For instance, the lamprey *Petromyzon marinus* responds by vigorous swimming and orients its movements towards the source of the chemical (amine) stimulus. However, this stimulus alone is not sufficient to enable the lamprey to localize its prey. The final localization is effected by means of an electrical field produced by the lamprey in the water surrounding its head (Chapter 8.2). The field becomes essential at distances between 5 and 10 cm from the prey. The chemical stimulus, e.g. the presence of amines in the prey, acts as attractant to the lamprey, while the physical stimulus of electrical shielding acts as arrestant (KLEEREKOPER and SIBAKIN, 1956a, b; KLEEREKOPER, 1958; KLEEREKOPER and MOGENSEN, 1963). The sharks *Negaprion brevirostris* and *Ginglynstoma cirratum* exhibit a characteristic food-orientation behaviour stimulated by a mixture of glutamic acid and trimethyl amine. Initial responses of both species during chemical stimulation are stereotyped and similar. Subsequently, *G. cirratum* orients towards the chemical source by true-searching of klinotaxis, whereas the orientation of *N. brevirostris* under similar test conditions is characterized by rheotaxis, which normally would bring the shark near the food or prey (MATHEWSON and HODGSON, 1972; see also Chapter 8.2). A physical stimulus such as sound may alone serve as attractant for orienting predators toward the prey. Certain low-frequency sounds have been considered to attract the sharks and, in fact, may be normally useful as attractants for orientation toward the food source (BANNER, 1968; RICHARD, 1968; see also NELSON and JOHNSON, 1972, Chapter 8.2). Delphinids can locate prey animals by active biosonar (Chapter 8.3).

Most investigators have assumed that active substances, capable of evoking positive or negative feeding responses, are small in molecular size, readily diffusible, low in density, and active in fairly low concentrations; such concentration may be expected to occur in nature (MANGUM and COX, 1971). Carnivorous marine animals respond to amino acids, amines and related compounds of small size (molecular weight less than 500), which are widely distributed in animal tissues (LINDSTEDT, 1971a). More than 80% of the tested animals—cnidarians, gastropods and fishes—exhibit feeding responses in the presence of their respective feeding incitants at a concentration of 10^{-6} M (Table 3-1); only *Fungia scutaria* requires a higher concentration (10^{-3} M) of the incitant (MARISCAL and LENHOFF, 1968).

In terrestrial herbivores molecular size does not seem to affect the response to chemical effectors; the distribution of the compound within the plant kingdom appears to be of prime importance (LINDSTEDT, 1971a). Very few marine herbivores have thus far been examined in regard to the chemical control of their feeding behaviour. Sulphuric-acid formation and, particularly, the production of a carbonate matrix by the brown alga *Desmarestia intermedia* can significantly deter feeding in a number of marine herbivores (WIRTH and RIGG, 1937; MEEUSE, 1956; see also PAINE, 1963). The presence of $MgCl_2$ deters feeding in the herbivorous larvae of a number of echinoderms, such as *Parastichopus* sp., *Pisaster* sp., *Ophiopholis* sp., and *Strongylocentrotus dröbachiensis* (STRATHMANN, 1971). As in terrestrial insects, other substances (e.g. lipids, carbohydrates, nucleic acids and their respective compounds) may also serve as stimuli in evoking positive or negative feeding behaviour in marine herbivores (LINDSTEDT, 1971a); but critical tests have yet to be undertaken.

An enormous amount of literature demonstrates the role of chemical stimuli in food-organism recognition by several molluscs (OWEN, 1966a) and fishes (TEICHMANN, 1959; HASLER, 1960; ZAFIROU and co-authors, 1972). A number of inver-

Table 3-1

Chemical stimuli affecting feeding behaviour in aquatic heterotrophs (After LINDSTEDT, 1971a; modified and extended; reproduced by permission of Microforms International Marketing Corp.)

Compound	Attractant	Repellent	Incitant	Stimulant	Conc. (M)	Species	Author
Amine (unidentified)	×					*Petromyzon marinus*	KLEEREKOPER (1967)
Amino-acid mixture			×			*Asterias rubens*	CARTHY (1958)
				×		*Mnemiopsis* sp.	CASE and GWILLIAM (1963)
				×		*Cancer antennuarus*	CASE and GWILLIAM (1963)
				×		*Carcinides* sp.	CASE and GWILLIAM (1963)
Arginine			×			*Pachycerianthus fimbriatus*	ARAI and WALDER (1973)
Betaine			×			*Cambarus* sp.	HODGSON (1958)
			×			*Panulirus argus*	LAVERACK (1963)
			×			*Homarus vulgaris*	LAVERACK (1963)
			×			*Portunus puber*	LAVERACK (1963)
			×			*Carcinus maenas*	LAVERACK (1963)
Buterobetaine		×				*Bullia digitalis*	BROWN (1961)
Crotonbetaine		×				*Bullia digitalis*	BROWN (1961)
Cysteine	×		×		10^{-5}	*Ictalurus natalis*	BARDACH and co-authors (1967)
			×			*Zoanthus danae*	REIMER (1971a)
			×			*Anthopleura midorii*	NAGAI and NAGAI (1973)
Cysteine hydrochloride	×		×			*Ictalurus natalis*	BARDACH and co-authors (1967)
Glutamic acid			×			*Cambarus* sp.	HODGSON (1958)
			×			*Panulirus argus*	LEVANDOWSKY and HODGSON (1965)
			×			*Cancer antennuarus*	CASE (1964)
Reduced glutathione			×		10^{-6}	*Hydra littoralis*	LOOMIS (1955)
			×		10^{-6}	*Physalia physalis*	LENHOFF and SCHNEIDERMAN (1959)
			×			*Anthopleura midorii*	NAGAI and NAGAI (1973)
			×		10^{-6}	*Nanomia cara*	MACKIE and BOAG (1963)
			×		10^{-5}	*Palythoa townsleyi*	REIMER (1971a, b, c)
			×		10^{-5}	*Zoanthus danae*	REIMER (1971a)
			×		10^{-6}	*Zoanthus pacifica*	REIMER (1971a)
			×?	×		*Anthopleura elegantissima*	LINDSTEDT (1971b)
			×		10^{-3}	*Fungia scutaria*	MARISCAL and LENHOFF (1968)
			×		10^{-5}	*Cyphastrea ocellina*	MARISCAL and LENHOFF (1968)
			×		10^{-6}	*Pocillopora damicornis*	MARISCAL and LENHOFF (1968)
Methyl glucothione			×		10^{-5}	*Cyphastrea ocellina*	MARISCAL and LENHOFF (1968)
Glycine			×			*Cambarus* sp.	HODGSON (1958)
			×			*Zoanthus pacifica*	REIMER (1971a)
Glycoprotein (unidentified)			×			*Diopatra cuprea*	GRAHAM and MANGUM (1969)
Leucine			×			*Haliplanella luciae**	LINDSTEDT (unpublished)
Phenylalanine			×			*Diopatra cuprea*	GRAHAM and MANGUM (1969)
Proline			×		10^{-5}	*Cordylophora lacustris*	FULTON (1963a)
			×		10^{-5}	*Pennaria tiarella*	PARDY and LENHOFF (1968)
			×		10^{-5}	*Cyphastrea ocellina*	MARISCAL and LENHOFF (1968)
			×		10^{-3}	*Pocillopora damicornis*	MARISCAL and LENHOFF (1968)
			×		10^{-3}	*Fungia scutaria*	MARISCAL and LENHOFF (1968)
			×			*Palythoa townsleyi*	REIMER (1971a, b, c)
L-iminoacid proline			×		10^{-7}	*Calliactis polypus*	REIMER (1973)
Pipecolic acid			×		10^{-6}	*Pennaria tiarella*	PARDY and LENHOFF (1968)
			×		10^{-3}	*Fungia scutaria*	MARISCAL and LENHOFF (1968)
			×		10^{-7}	*Cyphastrea ocellina*	MARISCAL and LENHOFF (1968)
Hydroxyproline			×		10^{-3}	*Cyphastrea ocellina*	MARISCAL and LENHOFF (1968)
Protein mixture			×			*Patiria miniata*	ARAKI (1965)
Serine			×			*Diopatra cuprea*	GRAHAM and MANGUM (1969)
Serine	×				10^{-7}	*Escherichia coli*	ADLER (1966)
Trimethyl amine oxide			×			*Cambarus* sp.	HODGSON (1958)
			×			*Panulirus argus*	LAVERACK (1963)
			×			*Homarus vulgaris*	LAVERACK (1963)
			×			*Portunus puber*	LAVERACK (1963)
			×			*Carcinus maenas*	LAVERACK (1963)
Trimethylamine	×		×			*Bullia digitalis*	BROWN (1961)
Tetramethyl ammonium hydroxide		×				*Bullia digitalis*	BROWN (1961)
Tyrosine			×			*Diopatra cuprea*	GRAHAM and MANGUM (1969)
			×			*Boloceroides* sp.	LINDSTEDT and co-authors (1968)
Valine			×			*Diopatra cuprea*	GRAHAM and MANGUM (1969)
			×		10^{-5}	*Boloceroides* sp.	LINDSTEDT and co-authors (1968)
Oxalo-acetic acid	×				10^{-9}	*Urosalpinx cinerea*	BLAKE (1960)
Quinone hydrochloride			×?		50^{-6}	*Hydra* sp.	FORREST (1962)

* From preliminary experiments, LINDESTEDT (1971a) has indicated that leucine incites feeding in the Californian *Haliplanella* (= *Diadumene*) *luciae*. However, leucine has been found to be ineffective in Norfolk specimens (WILLIAMS, 1972).

tebrates are noxious or toxic and deter predatory activities of fishes (BAKUS, 1969); in sponges, noxious chemical substances, mineralized sclerites and tough fibrous components, are highly effective in discouraging predation (RANDALL and HARTMAN, 1968). In fact, chemical defence mechanisms seem widespread among invertebrates (RUSSELL, 1966). However, the specific chemical substances concerned have not yet been isolated or identified.

Motile bacteria are known to exhibit chemotaxis in concentration gradients of organic material (e.g. WEIBULL, 1960; ADLER, 1969). Ecological aspects of chemotaxis in organotrophic marine bacteria have not yet been studied in detail; but highly species-specific responses to certain carbohydrates, amino acids and nucleotide bases have been observed (FOGEL and co-authors, 1971), and certain predatory micro-organisms have been shown to exhibit chemotaxis in regard to their prey. At concentrations of 10^{-6} to 10^{-5} M, arginine, sucrose, valine and lysine are the best attractants for the bacterial clones 8712, 8714, 8715 or 8716 (BELL and MITCHELL, 1972). In almost all tested bacteria, the minimum concentrations of the most efficient attractants lie between 10^{-5} and 10^{-6} M (e.g. ADLER, 1969).

Nearshore waters and estuaries usually average 10^{-6} to 10^{-5} M for carbohydrate and 10^{-8} to 10^{-7} M for specific amino acids (WAGNER, 1969). The threshold concentrations for some of the compounds, especially amino acids, eliciting attraction lie generally in the range of 10^{-6} to 10^{-5} M for bacteria and animals. Therefore, ecological studies on the specific compounds eliciting positive response of heterotrophs are needed and may prove to be of great economic importance, especially in fisheries.

A number of other environmental factors—such as light, temperature, salinity, water movement (Volume I)—and prey properties (e.g. size and texture) may also act as stimuli. In the intertidal area, the onset of high tide may stimulate initiation of feeding in animals like barnacles, while high tide may suppress feeding in terrestrial animals visiting the intertidal area for predation. The winkle *Littorina littorea* is incited to feed due to moistening by the returning tide, while air exposure acts as suppressant (NEWELL and co-authors, 1971). Speed and direction of water movement can influence orientation (Chapter 8) and nutrition. Sea fans (e.g. *Muricea californica*) arrange their major body plane at right angles to the dominant water current; this response maximizes the contact between feeding surfaces and suspended prey organisms (Volume I: RIEDL, 1971; Chapter 8.1). Body positioning of these gorgonians seems at least in part to be associated with nutritional advantages (GRIGG, 1972; see also KANWISHER and WAINWRIGHT, 1967). MOORE and MCPHERSON (1965) placed *Lytechinus variegatus* in aquaria with running sea water and observed that increasing the flow from 2·7 to 5·0 l min^{-1} more than tripled their feeding rate. These investigators were further able to show that this effect was due solely to the water current and not to nutrients dissolved in the water.

Biotic environmental factors, like the presence of 'co-eaters' can also evoke feeding responses. When one gastropod *Melongena carona* begins to eat in an aquarium, numerous other individuals tend to 'gang up' on the same prey, ignoring other nearby food (TURNER, 1959). TURNER assumes that a primary attractant stimulus is derived from the first *M. carona* which commences feeding. Comparable biotic environmental factors may play a vital role in feeding of schooling fishes and marine mammals, in which members of the school are known to 'obey' the leader

(Chapter 8). A dominant individual may suppress subordinate individuals (e.g. GERKING, 1952, 1971). In larvae of *Crassostrea virginica*, trichocysts of dinoflagellates as well as certain bacteria can deter feeding at critical food concentrations (UKELES and SWEENEY, 1969). Cell-free filtrates of *Chlorella stigmatophora* culture media have been shown to depress feeding activities in *Pseudocalanus elongatus* (URRY, 1965). Physical properties, such as high chitin content (PANDIAN, 1967b; WINDELL, 1967), thickness of calcium deposition (in animals: RICKER, 1946; in algae: PAINE, 1963), or density and sharpness of spines (e.g. in sea-urchins and in fishes like *Tetrodon* sp.) may evoke repelling, suppressing or deterring, depending upon the intensity of these stimuli as well as upon the physiological state of the predator. On the other hand, smooth, soft, juicy or fleshy prey organisms may attract, incite or stimulate feeding (e.g. PAINE, 1963). Prey colour is another important factor which may act as attractant or repellent stimulus, especially in perceptive feeders.

Different levels of irradiance (PAVLOV, 1962; Volume I, Chapter 2), temperature (KINNE, 1960, 1962; BRETT and co-authors, 1969; PANDIAN, 1970a; Volume I, Chapter 3), salinity (KINNE, 1960, 1962; RAGHURAMAN, 1973; Volume I, Chapter 4), and food concentration (WINTER, 1969, 1970; PAFFENHÖFER, 1970, 1971; STRATHMANN, 1971; WARE, 1972) may govern quantitative aspects of food intake. Extreme levels of these environmental factors can evoke inciting or suppressing effects on feeding. Thus, the echinoid *Diadema setosum* feeds only at low irradiance (LAWRENCE and HUGHES-GAMES, 1972), while the salmon *Oncorhynchus nerka* feeds during dawn and dusk (BRETT, 1971c); a temperature of 3° C suppresses feeding in the flatfish *Limanda limanda* (PANDIAN, 1970a); and a salinity of 13‰ suppresses feeding in the freshwater fish *Mystus gulio* (PANDIAN, 1970e). Such relationships are usually complicated by endogenous timing processes (Chapter 9).

A minimum concentration of planktonic food organisms appears to be necessary to incite feeding in a number of suspension and particle feeders. Using data of SAVAGE (1931, 1937) and BATTLE and co-authors (1936) on stomach-content analyses and digestion rates of herring *Clupea harengus*, CUSHING (1964) showed that copepod concentration levels equivalent to 100 $mm^3\ m^{-3}$ or below fail to evoke predatory attacks. Phytoplankton concentrations equivalent to 70 $\mu g\ C\ l^{-1}$ or below do not incite the copepods *Calanus finmarchicus* and *C. helgolandicus* (ADAMS and STEELE, 1966); the corresponding value reported for *C. plumchrus* and *C. pacificus* is 50 $\mu g\ C\ l^{-1}$ (PARSONS and co-authors, 1969). For *C. helgolandicus*, the critical minimum concentration can be as low as 20 $\mu g\ C\ l^{-1}$, provided the water volume is large enough (PAFFENHÖFER, 1970, see also CONOVER, 1970; Volume IV: CONOVER, in press). REDDY and PANDIAN (1973) reported that different volumes of water containing equal numbers of prey organisms not only altered the feeding response but also the predatory efficiency. Conversely, very high concentrations can evoke suppression or deterring responses in suspension feeders (JØRGENSEN, 1966). The bivalve *Hiatella arctica*, for instance, deters filtration at food concentrations of 11×10^6 *Phaeodactylum tricornutum* cells ml^{-1} and 3×10^6 *Isochrysis galbana* cells ml^{-1} (ALI, 1970). The presence of certain micro-organisms (LOOSANOFF and ENGLE, 1947) or silt (JØRGENSEN, 1966, p. 107) in the water exerts suppressing or deterring of feeding in suspension feeders. According to LOOSANOFF and ENGLE (1947), filtrate of a culture of *Chlorella* sp. depresses feeding rate in the oyster *Crassostrea*

virginica; pumping rate or filtration efficiency of the oyster *C. virginica* is depressed by the green alga *Dunaliella tertiolecta* (TENORE and DUNSTAN, 1973a); and food concentration of less than 300 μg C per l depresses feeding in *Mytilus edulis*, *Crassostrea virginica* and *Mercenaria mercenaria* (TENORE and DUNSTAN, 1973b). Food-organism composition (plankton) has been found to affect the number of successful prey-catching manoeuvres in larvae of the herring *Clupea harengus* (ROSENTHAL, 1969).

(b) Feeding Mechanisms

The food resources available for exploitation by heterotrophs may be broadly subdivided into liquid, particulate and massive. Accordingly the heterotrophs con-

Table 3-2

Mechanisms employed in feeding by marine heterotrophs (After JØRGENSEN, 1968; modified; reproduced by permission of Macmillan Publishing Co., Inc., New York)

Mechanisms	Examples	Feeding type
Absorption of dissolved food through external surfaces; parenteral food uptake	Micro-organisms; many small marine animals, parasites, poganophores	Liquid feeders
Sucking of fluids	Nematodes; trematodes; leeches; parasitic copepods; young mammals	Liquid feeders
Pseudopods	Radiolarians, foraminiferans	Microphages
Cilia	Ciliates, sponges, bivalves; larvae of several marine animals; many worms, acnidarians, brachiopods	Microphages
Production of mucus, especially in the form of a filtering sheet	Tunicates; *Amphioxus*, Ammocoetus larvae; several gastropods	Microphages
Setae and similar structures	Copepods and other crustaceans; basking sharks; some teleosts; baleen whales	Microphages
Swallowing of ambient substrate (e.g. sediment, timber)	Many burrowing and digging forms (e.g. sediment: *Arenicola marina*; timber: *Teredo* sp.)	Microphages
Seizing of prey	Most cnidarians; turbellarians; many polychaetes; sharks, teleosts; marine turtles; snakes	Macrophages
Seizing and masticating of prey, and biting, rasping, grazing, etc; often combined with mastication of food	Many gastropods, cephalopods; crustaceans; some mammals	Macrophages

cerned can be subdivided into (i) liquid feeders, (ii) microphages feeding on small particles, and (iii) macrophages feeding on massive food. Within these principal groups, subdivisions are made according to the mechanisms which handle the food. The various mechanisms involved in feeding, and their distribution among heterotrophs are summarized in Table 3-2. A few feeding mechanisms are described in some more detail in the following sections.

Liquid Feeding

Liquid feeders have no mechanical means of separating their food from the medium. The food is very finely dispersed or in a dissolved state. Whereas osmotrophs absorb food via the outer surface, phagotrophic liquid feeders suck it into their gut.

Osmotrophs

Micro-organisms. Heterotrophic protozoans, fungi and bacteria rely on organic substances for maintenance and multiplication. Inorganic nitrogen compounds can serve as nitrogen source for many phytoflagellates; but organic nitrogen compounds are required by all other unicells (KIDDER, 1967; see, however, FRANZISKET, 1974). In osmotrophic micro-organisms, the dissolved nutrients of small molecular weight are absorbed through the cell membrane or parts thereof. Uptake of dissolved organic nutrients such as amino acids involves active transport; this is indicated by the finding that rate of uptake is markedly reduced by 2,4-dinitrophenol (STEPHENS and KERR, 1962). The nutrients probably enter directly into the compartments of the ground cytoplasm and engage in intermediary metabolism. Thus (almost) no intracellular hydrolytic breakdown of nutrients is necessary. Certain micro-organisms are known to secrete hydrolytic enzymes into the medium, possibly contributing sometimes to the production of nutrients taken up in this way. This may be regarded as a special case of extracellular digestion (BROCK, 1966; MÜLLER, 1967). An extracellular amylase has been isolated from axenic cultures of the free-living amoeba *Mayorella palestinensis* under conditions which excluded death among the amoebae tested (PARNAS and co-authors, 1964). Media separated from *Tetrahymena pyriformis* cultures after short cultivation periods, when practically no cell death occurs, were found to contain a spectrum of enzymes including amylase and proteinases (see also Volume III, Chapters 4, 5). No similar detailed studies have been performed on marine forms. Fungi and bacteria excrete extracellular enzymes which attack a wide variety of organic polymers: Cellulose, starch, pectin, alginic acid, chitin, hyaluronic acid, chondroitin sulphate, various capsular polysaccharides, and proteins (BROCK, 1966).

A series of important field measurements on oxidation rates and concentrations of amino acids and glucose in sea water led ANDREWS and WILLIAMS (1971) to assess the heterotrophic utilization of dissolved organic matter to be in the order of 29 g total free amino acids m^{-2} $year^{-1}$ and 2·6 g glucose m^{-2} $year^{-1}$. From further calculations, these authors suggested that heterotrophic processes resulting in the uptake of organic compounds may consume organic material equivalent to 50% of that measured for phytoplankton production. While the value is only a gross assess-

ment, it does indicate the importance of heterotrophic utilization of dissolved organic matter. Further studies on this aspect are needed; they are likely to advance our understanding of the energy flow via food chains in the aquatic environment (Volume IV).

Metazoan osmotrophs without digestive system. The absence of any form of alimentary system in the parasitic Cestoda, Acanthocephala (Volume III: LAUCKNER, in press) and the free-living Pogonophora implies that these forms obtain their nourishment by active absorption through the body surface. Phosphatase systems, normally associated with absorption phenomena, are present in the cuticle of a number of cestodes (JENNINGS, 1968). Electron microscopic studies reveal that the cuticle contains mitochondria; the outer layers of the cuticle are thrown up into submicroscopic structures, facilitating absorption by increasing the external surface area (ROTHMAN, 1959). The fact that absorption of glucose is inhibited in the presence of the glycoside phlorizin indicates that carbohydrates can actively be absorbed by the cestode against a concentration gradient (PHIFER, 1960a, b, c).

Metazoan osmotrophs with digestive system. PÜTTER (1909) was the first author to claim that dissolved organic substances may be absorbed through the body surface and used as energy source. However, the methods used by PÜTTER led to considerable overestimation in regard to the amounts of dissolved organic material involved (KROGH, 1931). Renewed interest in PÜTTER's hypothesis has been aroused by a series of publications (STEPHENS, 1960, 1962, 1963, 1964, 1967, 1968, 1972; STEPHENS and SCHINSKE, 1961; KERR and STEPHENS, 1962; STEPHENS and VIRKAR, 1966; FERGUSON, 1967a, b, 1970, 1971; NORTH and STEPHENS, 1967, 1969; CHAPMAN and TAYLOR, 1968; LITTLE and GUPTA, 1968; PEQUIGNAT and PUJOL, 1968; SOUTHWARD and SOUTHWARD, 1968; ANDERSON, 1969; ANDERSON and STEPHENS, 1969; PRESTON and STEPHENS, 1969; REISH and STEPHENS, 1969; TAYLOR, 1969; WONG, 1969; GORKIN, 1970; PEQUIGNAT, 1970, 1972, 1973; PRESTON, 1970; WONG and STEPHENS, 1970; NORTH and co-authors, 1972; SCHLICHTER, 1973).

In surface waters of the oceans total dissolved organic carbon amounts to roughly 1 mg l^{-1} and decreases to perhaps half that value in deep waters (DUURSMA, 1965; MENZEL, 1967; DEGENS, 1968; Volume I, Chapter 10; Volume IV: WANGERSKY, in press). Free amino acids (FAA), comprising 5% of the total dissolved organic matter (DOM), occur in the free water in concentrations of 5×10^{-7} M l^{-1}, and in sediments at levels exceeding 10^{-2} to 10^{-4} M l^{-1}. STEPHENS (1963) reported concentrations of 6×10^{-5} to $1{\cdot}1 \times 10^{-4}$ for the interstitial water of sand–mud sediments. The sources of dissolved organic material in oceans and coastal waters have been discussed in several papers (e.g. FOGG, 1966; PARSONS and SEKI, 1970; Volume IV: WANGERSKY, in press).

STEPHENS and SCHINSKE (1961) reported influx of amino acids from ambient sea water into representatives of 35 genera of marine invertebrates representing 11 phyla. The information available may be summarized as follows:

(i) Labelled amino acids disappear from ambient solutions of low concentration (5×10^{-9} M l^{-1}) and can be recovered quantitatively from the test organism.

(ii) Amino acids rapidly enter all soft-bodied animals but not the bodies of arthropods (ANDERSON, 1969; ANDERSON and STEPHENS, 1969; see, however,

McWHINNIE and JOHANNECK, 1966) and vertebrates other than hagfishes (GORKIN, 1970).

(iii) Amino acids enter across the body surface (STEPHENS, 1963, 1964; REISH and STEPHENS, 1969) or across specialized structures such as the ctenidia of *Mytilus edulis* (PEQUIGNAT, 1973) and the apical membrane of the ectoderm of *Anemonia sulcata* (SCHLICHTER, 1973). Small amounts enter via the gut. The rate of amino acid uptake (2 to 10 μg g^{-1} live weight hr^{-1}) via the body surface depends on the type of the amino acid and its concentration.

(iv) In marine invertebrates the influx continues unabated for long periods (STEPHENS, 1968).

(v) Accumulated amino acids are assimilated; they participate in the synthetic and respiratory metabolism of the animals tested (e.g. *Ophiactis arenosa;* STEPHENS and VIRKAR, 1966).

(vi) Amino acids thus assimilated can supplement, either fully or partly, the energy requirements of the animals examined. Certain amino acids, such as glycine and serine, are used in oxidative processes in *Anemonia sulcata* (SCHLICHTER, 1973). According to STEPHENS (1963), oxygen consumption of the bamboo worm *Clymenella torquata* is equivalent to the complete oxidation of about 90 μg amino acid g^{-1} hr^{-1}; the animal could acquire 135 μg amino acid g^{-1} hr^{-1} from the amino-acid mixture found in the interstitial water, where it was collected. However, these estimations are subject to a number of possible errors, as indicated by STEPHENS (1972). STEPHENS (1962) also reported that the anemone *Fungia scutaria* concentrates sufficient ^{14}C-labelled glucose from the ambient medium to account for its standard metabolism. The anemone *A. sulcata* consumes 0·013 ml O_2 g^{-1} live weight hr^{-1} (FLOREY, 1970), which is equivalent to 0·064 cal g^{-1} live weight hr^{-1} (as 1 ml oxygen is equal to 4·8 cal; BROWN and BRENGELMANN, 1966). Glycine uptake of the anemone exposed to the glycine concentration found in its natural habitat is 10 μg g^{-1} live weight hr^{-1} (SCHLICHTER, 1973); 1 g glycine yields 3·12 kcal. With appropriate correction for the energy expended on the absorption process, SCHLICHTER concluded that *A. sulcata* gains 0·027 cal g^{-1} hr^{-1}, which accounts for 40% of the total metabolic energy of the anemone.

(vii) The euryhaline annelids *Nereis limnicola* and *N. succinea* continue to accumulate glycine even from very diluted sea water (10‰ S: STEPHENS, 1960). At about 15‰ S, the rate of amino-acid uptake drops to half of the control value, but assimilation of amino acid into alcohol-insoluble material increases 10-fold (STEPHENS and VIRKAR, 1966).

A number of scientists have drawn attention to the fact that influx of amino acids over a significant concentration gradient may not be possible energetically. CORNER and COWEY (1964) emphasized that amino-acid uptake by zooplankton from sea water would have to be accomplished against steep gradients and, therefore, involve considerable metabolic work. For instance, the concentration of free amino acid N present in tissue fluids of *Calanus finmarchicus* is about 3 mg ml^{-1} (CORNER and DAVIES, 1971, p. 131; see also JEFFRIES, 1969). Considering ranges of FAA of 2 to 16 μg l^{-1} (CHAU and RILEY, 1966), the FAA would have to be transported against a concentration gradient of 10^{-6} to 10^{-7} (CORNER and DAVIES, 1971). However, STEPHENS (1968) calculated that energy expended for this active transport of glycine represents only a small fraction (3 to 4%) of the energy contained in the absorbed

glycine; the process is, therefore, energetically possible. The maximum amount of energy expenditure involved in the absorption process can be only 15% of that contained in the absorbed glycine in the anemone *Anemonia sulcata* exposed to glycine concentrations found in the anemone's habitat (SCHLICHTER, 1973). In his recent review, STEPHENS (1972) indicated that arthropods do not accumulate amino acids as readily as soft-bodied aquatic animals. Based on the results obtained by ANDERSON (1969), STEPHENS (1972) now appears to consider arthropods as an exception to the rule that aquatic invertebrates accumulate FAA from the ambient water. It is not clear, however, why gills of arthropods do not lend themselves for FAA influx, as do the ctenidia of molluscs (see also Chapter 5).

Many papers report efflux of amino acids from marine animals, e.g. POTTS (1967) and HAMMEN (1968) for molluscs; WEBB and JOHANNES (1967) for zooplankton; JOHANNES and co-authors (1969) for flatworms; WONG and STEPHENS (1970) for annelids. JOHANNES and co-authors (1969) stressed that removal of radio-active labelled organic compounds from solution by marine invertebrates does not constitute proof of net uptake of these substances, for no previous study included measurements on total release rates of these compounds. According to JOHANNES and co-authors (1969), there is a net loss of organic substances, such as amino acids, in the flatworm *Bdelloura candida*. FERGUSON (1971) measured both influx and efflux of free amino acids in 10 species of sea-stars and found that the net flux of amino acids was overwhelmingly inward, with a single exception for glycine.

Phagotrophs

Nematodes are, in general, microphagous and/or saprophagous. Food particles, small enough to pass through their buccal cavity, are drawn into the lumen of the

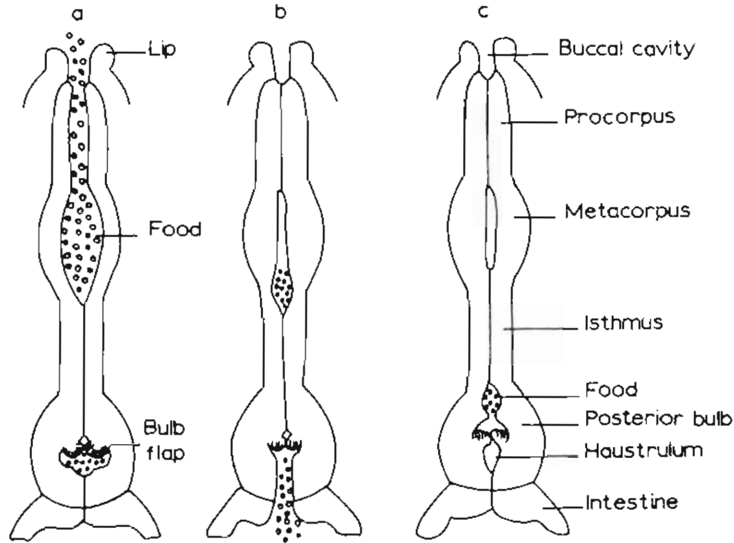


Fig. 3-3: Feeding mechanism of nematode. Generalized diagram. (After LEE, 1965; modified; reproduced by permission of the author.)

metacorpus by sudden dilation of the pro- and metacorpus (Fig. 3-3a); closure of the lumen of the pharynx in these regions expels excess water (Fig. 3-3b), and the mass of the food particles is passed backward along the isthmus by a wave of contraction of the radial isthmus muscles (Fig. 3-3b and c). Food is then drawn between the flaps of the posterior bulb by dilation of the haustrulum, which inverts the bulb flaps (Fig. 3-3a), and is passed to the intestine by closure of the haustrulum and by dilation, followed by closure of the pharyngeal-intestinal valve (Fig. 3-3b). The bulb flaps contribute to the closure of the valve in the posterior bulb and also crush food particles when they invert (Fig. 3-3a; DONCASTER, 1962). Phytophagous and carnivorous nematodes usually possess a stylet with which they pierce the prey and withdraw its liquid contents (LEE, 1965).

Microphages

Pseudopod feeding

Pseudopods are concerned primarily with feeding in foraminiferans, heliozoans and radiolarians. They are capable of extension, flection and retraction. Amoebae form food cups consisting of lateral and dorsal pseudopods flowing around a food mass, which may itself be motile. The pseudopods of the cup meet distally and the food mass is incorporated. The surfaces of reticulopods and axopods are sticky, and food tends to adhere to their surface (e.g. PROSSER and BROWN, 1961).

The acoelous turbellarian *Convoluta convoluta* has 2 distinct feeding methods (JENNINGS, 1965, 1968): (i) It gathers microscopic food by protruding a portion of its digestive syncytium through the mouth and by using this as a large pseudopodium engulfing minute organisms or organic particles; (ii) it grasps suitable-sized prey organisms by its curved and sticky anterior body region and then, rolling up upon itself, brings the prey to the midventral mouth. It then protrudes the digestive syncytium and engulfs the food in a large food vacuole.

Suspension feeding

Suspension feeding may be defined as the concentration and separation of suspended micro-organisms and detritus from the surrounding sea water (JØRGENSEN, 1966). The total measurable particulate organic matter consists of (i) living plankton (about 10%; RILEY and co-authors, 1964; MULLIN, 1965a, b), and (ii) discrete particulate organic aggregates (=detritus). A major fraction of detritus exists in the form of colloidal material (e.g. in the Jamaican Sea); the suspended colloidal material is considered to be concentrated and separated from the medium by the sponges (REISWIG, 1972). Therefore, the term 'detritus' is used to convey both the discrete particulate organic aggregates and colloidal materials. Suspension feeding may be regarded as an inverted system of grazing; owing to the production of water current by suspension feeders, suspended food in water is brought to specific organ(s), where the food is concentrated and separated from the medium. While energy relationships are essentially similar to those in classical systems of grazing, the inability of most suspension feeders to move in search of food imposes certain constraints on the system (CRISP, 1964).

Cilia: Particle selection based on size. Sponges filter particulate organic matter from the surrounding water which is forced to enter their body cavities through minute pores. They constitute a simple but effective device that permits only the smallest particle to pass. Ascon sponges presumably possess the smallest dermal pores; their incurrent pores measure about 9 mμ (HYMAN, 1940, p. 303). The long sieve-like collars of the choanocytes (RASMONT and co-authors, 1957; RASMONT, 1959; FJERDINGSTAD, 1961) protrude into the chambers in such a way that most of the water propelled by the flagellae has to flow through them. The mesh of these sieves, i.e. the distance between 2 adjacent flagellae, is 0·1 μ; the prosopyles of flagellated chambers have a width of 5 μ. The chambers are thus suited for straining particles in the 0·1 to 5·0 μ range (KILIAN, 1952; RASMONT, 1968). REISWIG (1971) suggested that there are two functionally independent capture systems operating in certain desmosponges: particles of 5 to 50 μ are phagocytized directly, those between 0·3 and 1·0 μ are first taken up by the choanocyte collar. Since the previously unrecognized and unresolvable fraction of particulate organic matter in the oceans represents a carbon source 7 times that of all resolvable planktonic material in

Table 3-3

Leuconia aspera. Hydraulics of filter-feeding system (After NICOL, 1967; reproduced by permission of author)

Structure	Estimated number of chambers	Aggregate area of epithelium (cm^2)	Current velocity ($cm\ sec^{-1}$)
Afferent canals	81,000	4·2	0·1
Surface of choanocytes	—	200·0	0·001
Flagellated chambers	2,250,000	52·0	0·01
Efferent canals	5,200	2·5	0·2
Paragaster	1	0·21	2·0
Osculum	1	0·03	8·5

the waters (e.g. Jamaican Sea), the ability of sponges to capture this material via their choanocyte system is of great importance for the dominance of suspension-feeding sponges on coral reefs (REISWIG, 1971).

The water current produced by sponges depends upon the unco-ordinated beating of the choanocyte flagellae; the beat of a flagellum moves the water at about 0·01 mm sec^{-1} (PARKER, 1910). Choanocytes are restricted to small chambers, and thus a large area of ingestive epithelium is achieved. In *Leuconia aspera*, a specimen 10 cm high and 1 cm in diameter is estimated to possess 2,250,000 flagellated chambers (Table 3-3) and to pass 22·5 l sea water day^{-1} (HYMAN, 1940, p. 302). Feeding on low concentrations of food, sponges may have increased the efficiency of their feeding mechanism at the expense of other anatomical advantages in order to maintain greater ingestion surfaces.

The efficiency of filtering is enhanced in higher sponges by (i) changing flow rates at different points of the canal system due to differences in the diameters of incurrent and excurrent canals (Table 3-3), and/or (ii) increasing the angle of supply. In leuco-

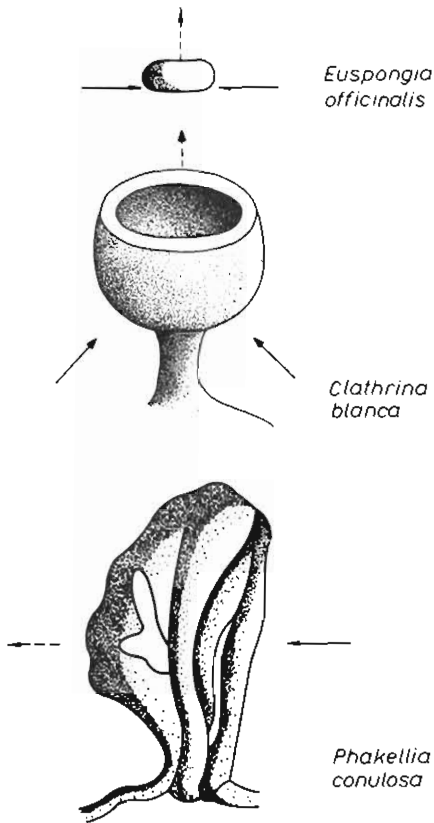


Fig. 3-4: Relationship between body form of sponges and direction of feeding currents. The bath sponge *Euspongia officinalis* exhibits an angle between in- and outflow currents of 90°; Neptune's goblet *Clathrina blanca*: a stalk increases the 'angle of supply', so that mixing of the 2 currents is largely avoided; *Phakellia conulosa*: a flattened fan with an inflow (→) and an outflow (-->) face. (After BIDDER, 1923; modified; reproduced by permission of Company of Biologists Ltd.)

noid sponges, the water flow is much slowed via narrower and narrower channels when it reaches the flagellated chambers, thus permitting sufficient time for food capture. After leaving the chambers and entering larger channels the water is emitted from the osculum with considerable force because the osculum has a smaller diameter than the final canals.

The angle between the in- and outflow is referred to as the 'angle of supply' (BIDDER, 1923). Between the 2 currents, a re-entrant vortex forms, the diameter of which is the 'diameter of supply'. This must be large enough so that the drift will carry away the outgoing water. In non-stalked sponges like *Euspongia officinalis*

the angle of supply is 90° (Fig. 3-4). The presence of a stalk in the Neptune's goblet *Clathrina blanca* increases the angle and thereby reduces the risk of contamination of the intake current. In flattened forms like *Phakellia conulosa*, the angle of supply is 180°, permitting a maximum flow of water through the body and affording improved food securing (BIDDER, 1923; see also BARRINGTON, 1969, p. 167).

The food collection mechanisms in the polychaete *Sabella pavonina* involve interaction of 4 ciliary tracts comprising 1 abfrontal, 1 frontal and 2 latero-frontal rows of cilia, the final suitability of food material being assessed on the basis of particle size (Fig. 3-5; NICOL, 1930). The sorting structure in the longitudinal food groove is such that it leads smaller (lighter) particles to the bottom, while larger

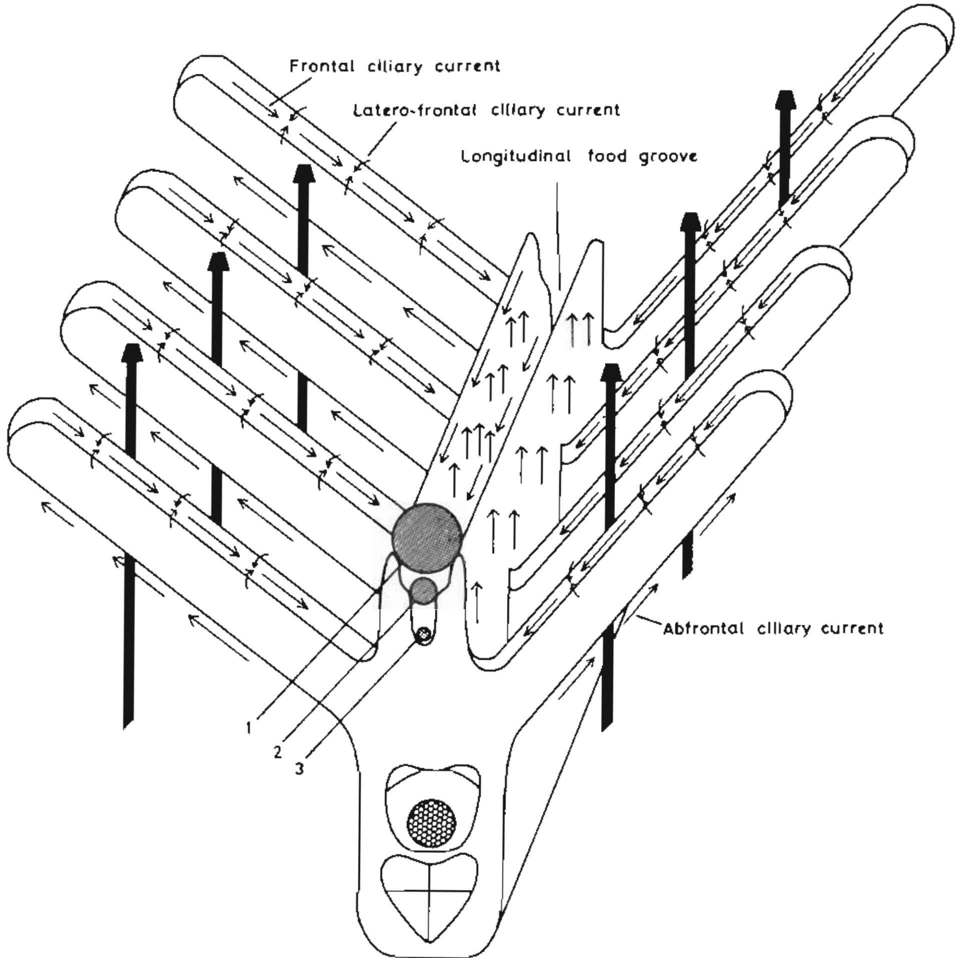


Fig. 3-5: *Sabella pavonina*. Diagrammatic section through a branchial filament demonstrating ciliary currents on pinnules and transportation properties of the longitudinal food groove. 1, 2, 3: particle sizes sorted by the structure of the food grooves. Large arrows indicate water currents; small arrows, feeding currents. (After NICOL, 1930; modified; reproduced by permission of the Royal Society of Edinburgh.)

(heavier) particles are retained in the uppermost tract. This is exactly the opposite to the situation observed in the labial-palp sorting mechanism of bivalves. Whereas selection is primarily based on food density in the labial palps of bivalves, it is based solely on particle size in the longitudinal food groove of sabellid and serpulid polychaetes.

STRATHMANN (1971) and STRATHMANN and co-authors (1972) have recently investigated feeding and rejection mechanisms of particles in a number of planktotrophic larvae. Echinoderm larvae and the tornaria of hemichordates retain particles, employing local cilia-beat reversal, whereas the trochopores of annelids and echiuroids, the veligers of molluscs and entoproct larvae appear to employ a system with 2 opposed bands of cilia. High-speed cinefilm of an echinopluteus reveals that a particle passing through a band of cilia triggers a localized change of beat, which appears to be reversal, and which retains the particles on the upstream side of the cilia band. Retention of particles by induced local reversal of cilia beating implies that the stimulus occurs during the forward effective stroke of a cilium and that the reversed effective stroke of this cilium or its neighbours begin(s) before the forward stroke is completed. In this system, retention and transport of particles, rejection of particles and swimming can be accomplished by a single band of cilia. In bdelloid rotifers and in serpulid trochopores, the opposed action of parallel pre-oral and post-oral bands of cilia apparently causes the longer pre-oral cilia to transport particles during the latter part of effective stroke. In this system, the pre-oral band of cilia effects retention and swimming, the post oral band retention and rejection, and the food groove cilia transportation of particles.

In suspension-feeding larvae of *Adalaria proxima* and *Archidoris pseudoargus*, food selection may involve cessation of the feeding current on encountering unpalatable material, or acceptance only of particles within certain ranges of mass or volume (THOMPSON, 1964); selection is based primarily on size and shape, but not on other properties like texture, etc. THORSON (1946) estimated the maximum diameter of particles eaten by many marine larvae to amount to about half the diameter of the esophagus; for echinoderm larvae he found the maximum diameter to be 40 to 50 μ. The maximum diameters observed for the echinoderm larvae by STRATHMANN (1971) are somewhat greater than THORSON's estimates. Probably most of these larvae can eat chain or rod-shaped algae shorter than 100 to 200 μ, or disks or spheres less than 65 to 85 μ in diameter. Larvae of the nudibranch *Coryphella lineata* reject carmine particles of 20 to 30 μ in diameter, whereas those between 10 and 15 μ are ingested (THOMPSON, 1959). When offered a mixed suspension of the algae *Criscosphaera* sp. (10·0 to 12·5 μ diameter) or *Phaeodactylum* sp. (about 2·5 μ wide and 30·0 μ long) and carmine or calcium-carbonate crystals (about 5 to 15 μ wide), echinoderm larvae indiscriminately filtered and transported both algae and the other particles to the stomach; however, carmine and calcium-carbonate crystals were frequently passed to the intestine and defecated, while the algae were selectively retained in the stomach (STRATHMANN, 1971). Continuous inflow of inorganic particles of no food value does not deter feeding in veliger larvae of prosobranchs; the particles, however, are not retained in the stomach but passed rapidly to the intestine for defecation (FRETTER and MONTGOMERY, 1968).

In several echinoderm larvae tested, particles are sorted in the gut; unsuitable (unwanted) particles can be rejected from the esophagus (STRATHMANN, 1971). The

larvae can (i) stop or reduce feeding by passing particles over the ciliated band with the water; (ii) reject particles from the buccal cavity and upper esophagus by ciliary reversal; or (iii) reject particles from the esophagus by contraction of esophageal muscles. All 3 rejection mechanisms are often aided by contractions of other muscles in varying combinations and are accompanied by activities peculiar to each taxonomic group.

Approximate lengths of ciliated bands, estimated from drawings or photographs of different echinoderm larval types, indicate a direct relationship to the volume of water filtered; all species cleared at least 0·3 to 0·6 μl mm^{-1} of ciliated band min^{-1}. Pluteus larvae may produce more efficient feeding and swimming currents than auricularia or bipinnaria; but the bipinnaria can ingest larger particles than plutei and may obtain more food from the same volumes of water filtered (STRATHMANN, 1971).

Cilia: Particle selection based on density and other properties. The ciliary feeding mechanism of lamellibranchs is most complex and represents an evolutionary culmination of efficiency; food particles are selected on the basis of size, density, and probably other properties. In some protobranchs, the ctenidium is primarily con-

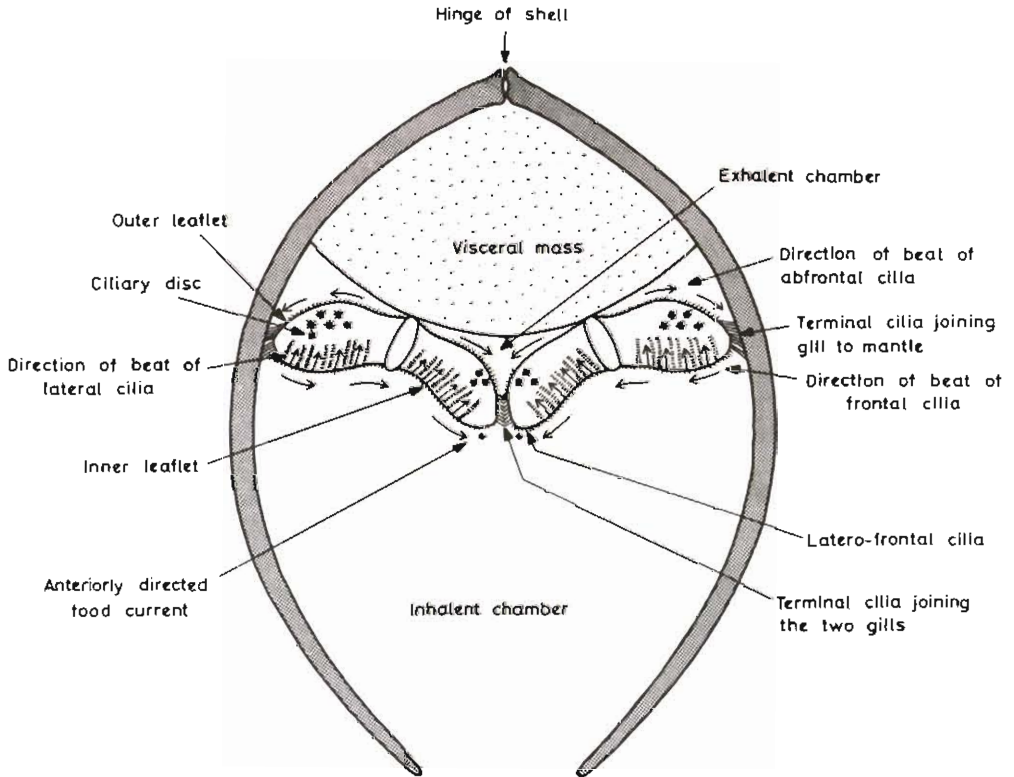


Fig. 3-6: *Nucula nucleus* (Protobranchia). Diagrammatic transverse section showing direction of ciliary currents (small arrows) of the gill. (After ATKINS, 1936; modified; reproduced by permission of Company of Biologists Ltd.)

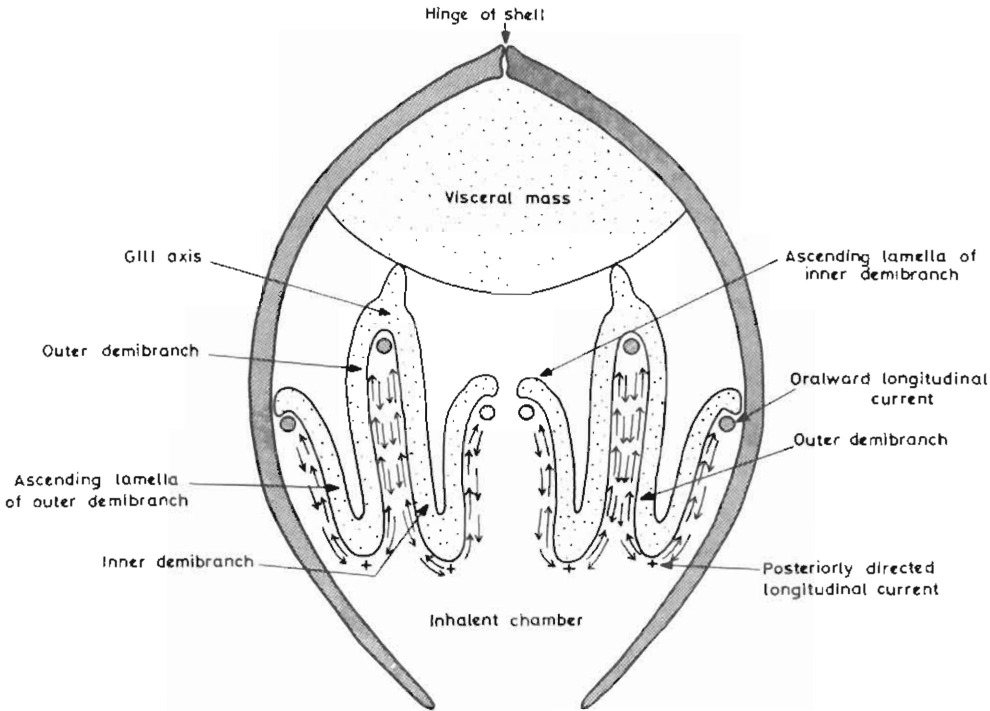


Fig. 3-7: *Arca tetragona* (Filibranchia). Diagrammatic transverse section revealing gill form and direction of ciliary currents (small arrows); circles: oralward longitudinal currents; crosses: posteriorly directed longitudinal currents. (After ATKINS, 1936; modified; reproduced by permission of Company of Biologists Ltd.)

cerned with the production of water currents; palp proboscides—tentacle-like extensions of the labial palps—are used for particle collection (YONGE, 1928). However, in the majority of bivalves ciliary tracts associated with pallial organs create the water current, collect, sort and transport particulate material to the mouth, and carry rejected material (pseudofaeces, p. 202) to a region, where it can be expelled from the mantle cavity. Each ctenidium consists of a long axis, which bears 2 demibranchs, both composed of a parallel row of filaments or lamellae; in the Protobranchia, the filaments are unfolded, and consist of a series of flat leaflets, alternating on each side of a central axis (Fig. 3-6). In the Filibranchia and Eulamellibranchia, the ctenidium has the form of a W (in section), each V of the W being composed of greatly extended filaments, borne on the ctenidial axis and forming a demibranch with descending and ascending lamellae enclosing interlamellar space; the adjacent filaments are joined by ciliary junctions in the Filibranchia (Fig. 3-7), or by vascular tissue junctions in the Eulamellibranchia (Fig. 3-8).

The cilia associated with the ctendidal filaments are differentiated into (i) lateral current-producing cilia (Fig. 3-9), and (ii) frontal cilia transporting the particles. Between lateral and frontal cilia exists another set of cilia, peculiar to bivalves—the latero-frontal cilia, specialized for catching food particles (TAMMES and DRAL, 1955); in some bivalves cilia are present also on the abfrontal surface of the filaments (e.g.

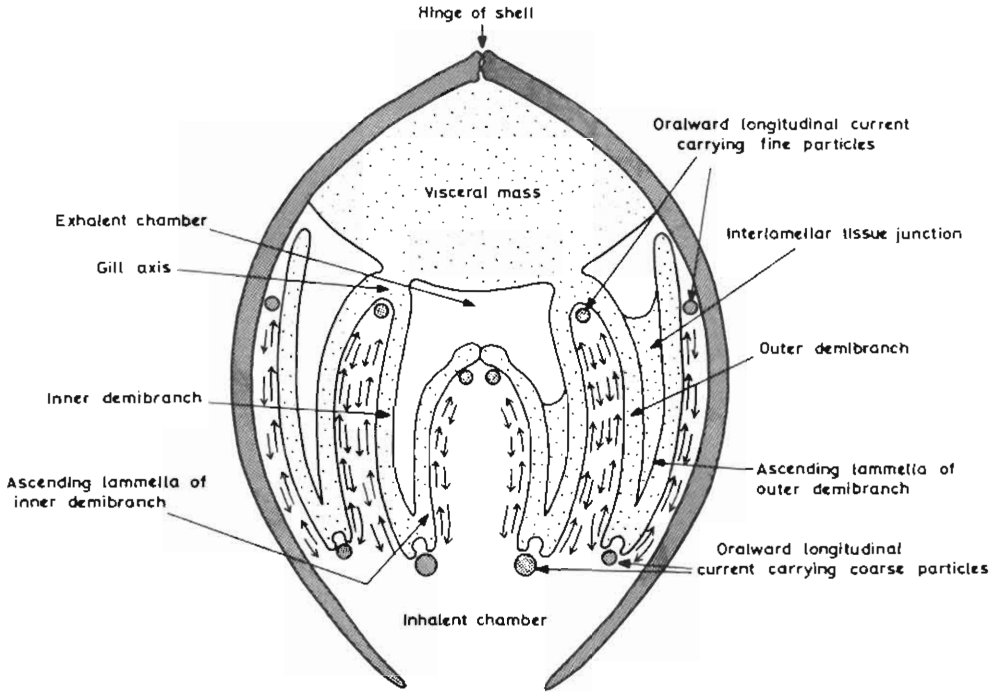


Fig. 3-8: *Solen marginatus* (Eulamellibranchia). Diagrammatic transverse section showing gill form and direction of ciliary currents. Oralward currents carry fine particles (small circles) in dorsal longitudinal groove. Coarse particles (large circles) are carried orally along ventral gill margins. Interlamellar tissue junctions are shown only on the right principal gill filament. (After ATKINS, 1936; modified; reproduced by permission of Company of Biologists Ltd.)

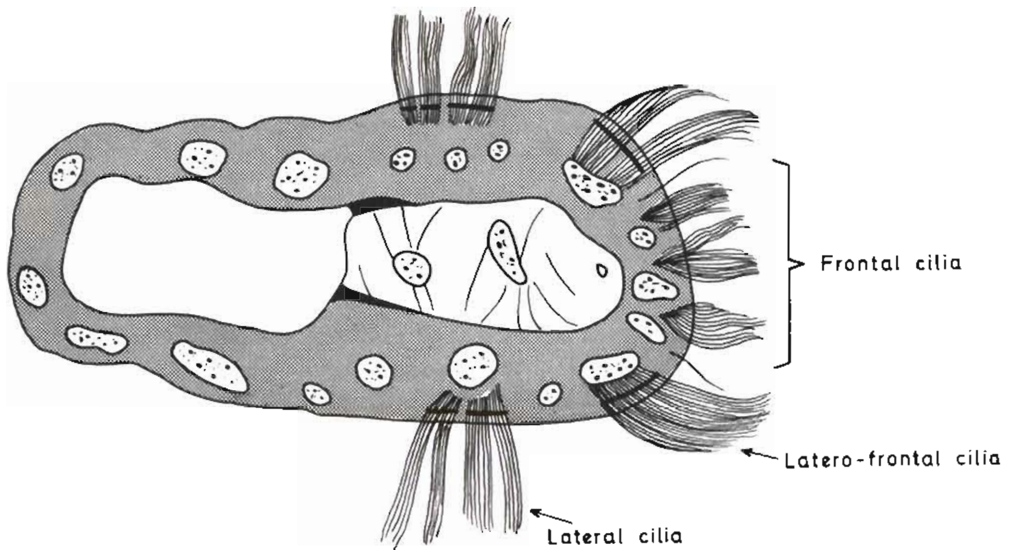


Fig. 3-9: Typical lamellibranch gill filament. Semi-diagrammatic transverse section showing ciliary tracts. (After OWEN, 1966a; reproduced by permission of Academic Press, Inc.)

Nucula nucleus). In some bivalves large cirrus-like frontal cilia (e.g. *Mactra corallina*; Fig. 3-12) or guarding cilia (e.g. *Musculus marmoratus;* Fig. 3-11) are used for sorting coarse from fine particles.

Hydrodynamic studies on ciliary mechanisms (BLAKE, 1971; WILSON and SCHREINER, 1971) indicate that the metachronal wave generates the water flow, not the single cilium. Most of the energy seems to be transferred to the water during the 'recovery stroke'. Furthermore, what has been called the 'effective stroke' does not necessarily dissipate energy (SCHREINER, 1971, 1972). Accordingly, the cilium does not work as an oar. Therefore, the latero-frontal cilia, which have their 'effective stroke' toward the frontal side of the filament on which they are located, probably cannot actively 'wipe off' particles on to the frontal cilia as described by TAMMES and DRAL (1955). The frontal, pro-latero-frontal, latero-frontal and lateral cilia exert influence on each other due to the dimensions involved in the system, resulting in a viscous flow of water; hence their movement must be co-ordinated in some way. Accordingly, the porosity of the gill probably cannot be inferred from observations on cilia made so far. To understand the particle-retaining function of the gill, it is necessary to study the various ciliary tracts simultaneously in normally functioning bivalves (VAHL, 1972).

Many filibranchs and eulamellibranchs have not only anteriorly oriented food grooves, but also rejection tracts of gills. Separation involves the use of sorting devices on the gills, many of which have been described by ATKINS (1936, 1937a, b; for review consult JØRGENSEN, 1955, 1966). Six different types of mechanisms, by

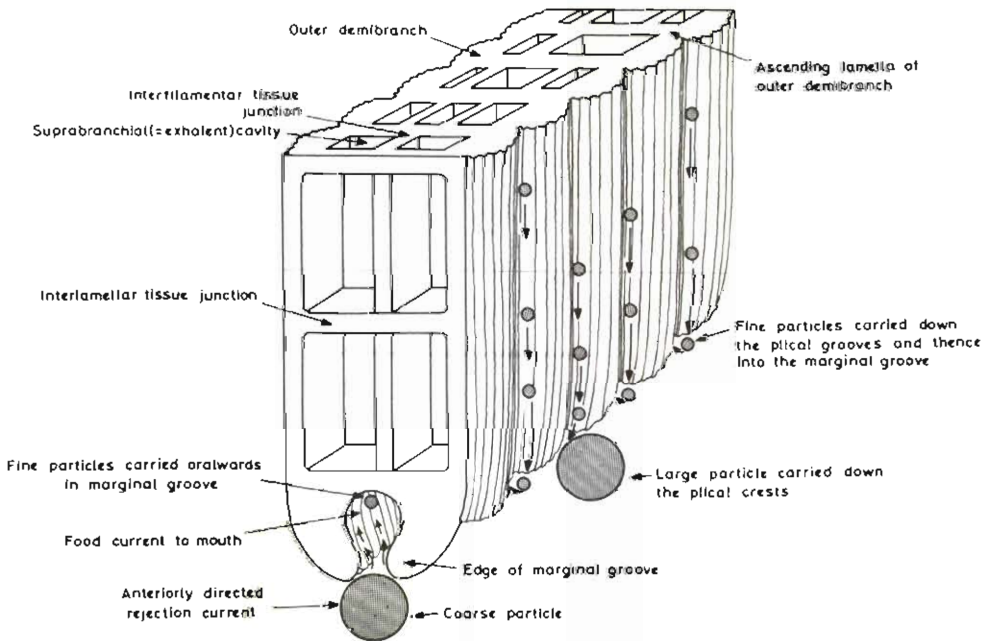


Fig. 3-10: *Pinna fragilis* (Eulamellibranchia). Block diagram of gill part. Plicae and marginal groove sort coarse particles from fine ones. (After ATKINS, 1937a; modified; reproduced by permission of Company of Biologists Ltd.)

which particles of different density are separated on the gills of lamellibranchs may be recognized. Some of them are illustrated in Figs 3-7, 3-8 and 3-10 to 3-12.

In the filibranch *Arca tetragona*, there are 3 tracts of frontal cilia on each filament; of these 3 tracts, the median, ventrally beating course cilia become active only when stimulated by heavy particles on the gills. On each side of this mid-course tract of the frontal cilia is a tract of fine, dorsally beating cilia, which transport food into orally directed current along the dorsal edges of the gill lamellae (Fig. 3-7). Posteriorly directed rejection currents run along the ventral edges of the demibranchs and transport large particles away from the mouth.

In eulamellibranchs like *Solen marginatus*, the surface of the gill lamellae is thrown into a series of folds so that a corresponding series of dorso-ventral ridges and grooves is formed. Only fine particles can enter the grooves; the dorsally beating cilia in the grooves transport the fallen fine particles into anteriorly directed tracts between the bases of the demibranchs on each side of the body and at the dorsal edges of the ascending gill lamellae. Coarse particles are transported to the ventral margins of the demibranch and thence towards the mouth (Fig. 3-8).

In certain eulamellibranchs the ctenidium is plicate (e.g. in *Pinna fragilis*). The frontal cilia on the plical crests and in the grooves beat towards the free ventral edge of the demibranchs. While coarse particles are carried by the frontal cilia on the crests of the plicae, fine particles pass down the troughs. A marginal groove, whose edges can be opposed, runs along the ventral edge of the gills (Fig. 3-10). Coarse particles travelling down the crest of the plicae are excluded from the food current in the marginal groove. The plical grooves lead into the depths of the marginal groove, which is lined in this region with fine anteriorly beating cilia.

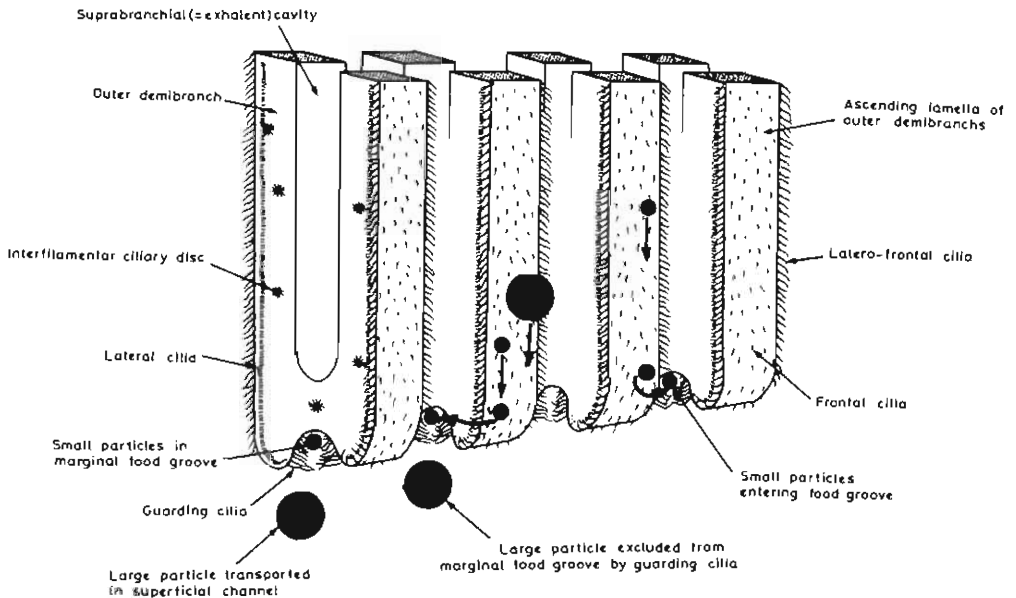


Fig. 3-11: *Musculus marmoratus* (Filibranchia). Block diagram of gill part. Guarding cilia sort coarse particles (large circles) from fine ones (small circles). (After ATKINS, 1937a; modified; reproduced by permission of Company of Biologists Ltd.)

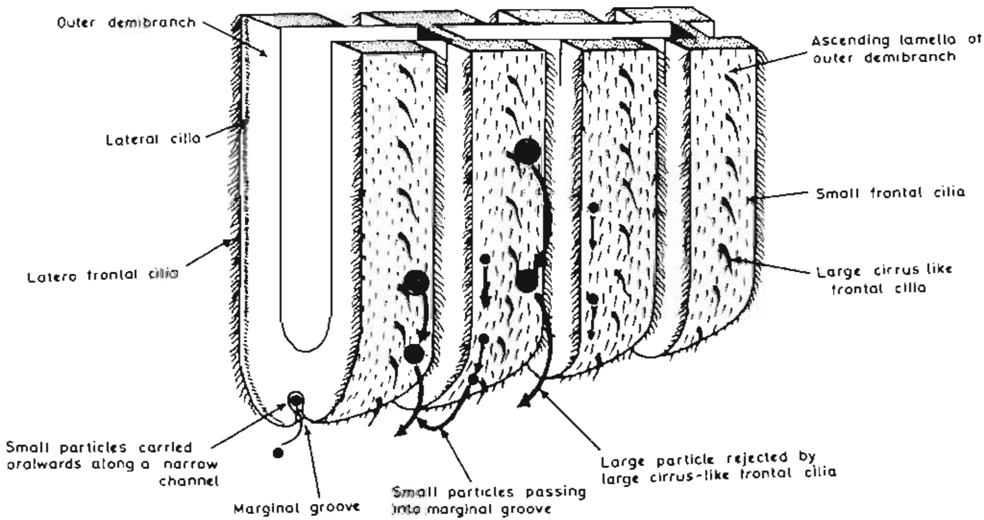


Fig. 3-12: *Mactra corallina* (Eulamellibranchia). Block diagram of gill part. Cirrus-like frontal cilia sort coarse particles (large circles) from fine ones (black dots). (After ATKINS, 1937a; modified; reproduced by permission of Company of Biologists Ltd.)

Several species belonging to the genera *Musculus* and *Modiolus* live in a muddy habitat. Fans of relatively stiff and long cilia arch over the marginal groove, and are active only intermittently. Fine particles pass round the sides of the tips of the filaments, along tracts of fine frontal cilia and slip under these guarding fans of long cilia and enter the marginal groove (Fig. 3-11). Larger particles pass medially along the filaments to their tips under the direction of a tract of coarse frontal cilia; they are then barred and excluded by the fans of guarding cilia.

In the sand-dwelling eulamellibranch *Mactra corallina*, a series of cirrus-like frontal cilia are developed on the flat gill lamellae. When stimulated by the presence of coarse particles, the cirrus-like frontal cilia beat towards the free margins of the demibranchs, moving the particles off the gill and onto the mantle, where they are rejected. Small particles, transported by the fine frontal cilia, enter the wide marginal groove to travel oralward along the narrow channel (Fig. 3-12).

A feeding current is drawn through the interfilamentary slits by the action of lateral cilia, and the particles are forced onto the frontal filament surfaces by latero-frontal cilia. When the particles touch the frontal filament surface, mucus is secreted in proportion to number and size of particles (NELSON, 1960). The entrapped material is then sorted; fine particles are transported to the mouth along protected food grooves, coarser particles passing along more superficial routes (ATKINS, 1936, 1937a, b).

Food particles collected by ctenidia are transported to the mouth via labial palps; during this transfer, the particles may undergo sorting and selection. This is effected by the ciliary tracts on the ridged, inner, opposed palp faces (Fig. 3-13). As many as 8 different ciliary tracts occur over a single ridge and groove; although functional details vary in different species (ALLEN, 1958), there are general similarities (OWEN, 1966a), and the tracts can be considered under 3 headings (PURCHON, 1955): (i)

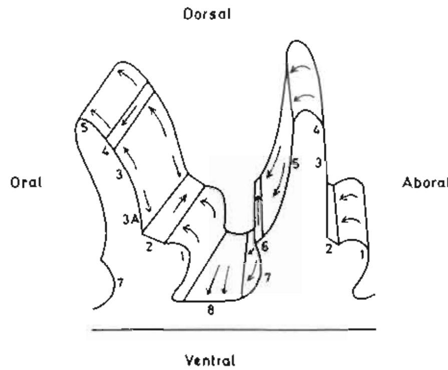


Fig. 3-13: *Yoldia ensifera* (nuculanid protobranch). Diagrammatic representation of 2 folds in the inner faces of the labial palp. (After STASEK, 1965; modified; reproduced by permission of Institute of Malacology Inc.)

Rejection tracts with the main tract situated in the depth of the grooves, where the cilia beat ventralward (Fig. 3-13); (ii) acceptance tracts occurring on the remaining ridges and serving for particle transport oralward across the folds; (iii) resorting tracts which redistribute particles, which fall into the grooves. The net effect of these ciliary sorting mechanisms is that lighter particles are carried from crest to crest across the ridges, toward the oral groove, while heavier particles are transported ventrally in the grooves to the rejection tract along the unridged ventral border of the palps (Fig. 3-14). Thus particle selection is primarily based on particle density. As in the sorting mechanisms of the gill, muscular activity plays a vital part in sorting of palps (ANSELL, 1961). Bivalves have excellent sorting mechanisms both on gills and labial palps; in general, in species with large gills capable of sorting material, the palps are small and vice versa (YONGE, 1949).

Different opinions exist in regard to the mechanism of particle collection and particle sorting of the ctenidium. A number of workers have reported a good correlation between particle size and filter efficiency in several bivalves, e.g. *Crassostrea virginica* (SMITH, 1958), and *Mytilus edulis* (TAMMES and DRAL, 1955). These findings support the view of ATKINS (1936, 1937a, b) that particles are selectively collected by the 'sticky' latero-frontal cilia of the ctenidium. However, filter efficiencies have been found to vary considerably (CHIPMAN and HOPKINS, 1954), even in one and the same individual (TAMMES and DRAL, 1955), although the propulsion rate remained unchanged. LOOSANOFF and ENGLE (1947) found little relation between particle size and particle retention by the gill in *C. virginica*. According to JØRGENSEN and GOLDBERG (1953), *M. edulis* can completely retain 1 to 2 μ particles of colloidal graphite; but DAVIDS (1964) reported that up to 80% of 5 μ *Chlorella* sp. passed through the gill. These differing results seem to indicate that the filter efficiency of the lamellibranch gill is subject to alteration.

MACGINITIE (1941) suggested that a thin sheet of mucus, secreted partly by filaments and partly by glands at the base of the ctenidium, entirely covers the gill sur-

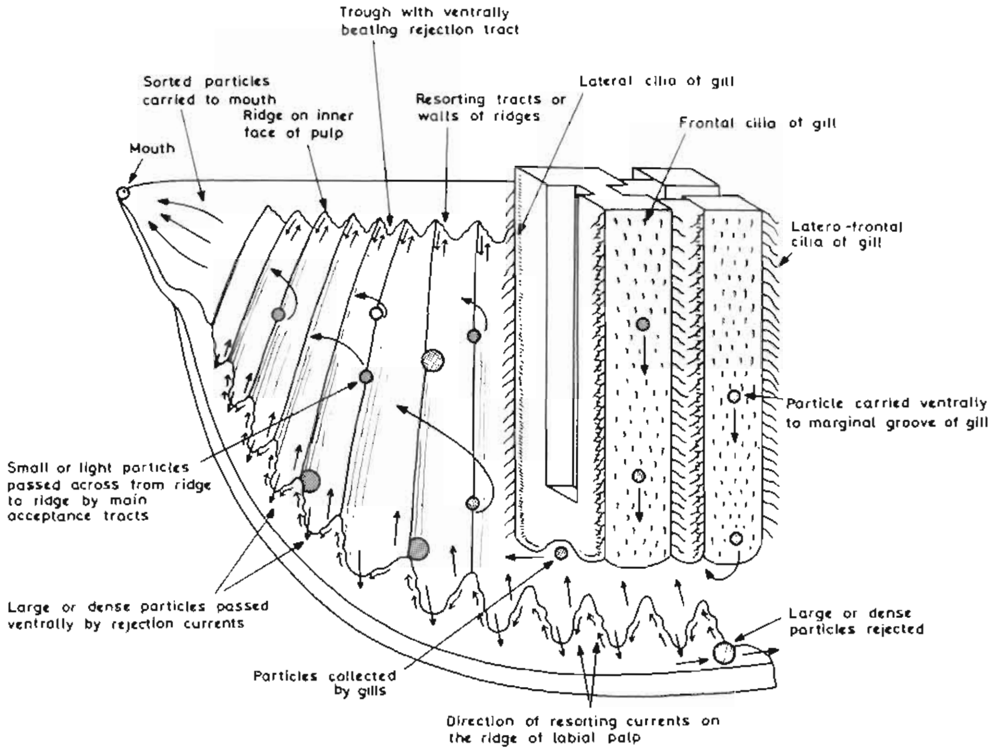


Fig. 3-14: Eulamellibranch gill. Diagram of gill part illustrating relationship between anterior gill and labial palp. Only inner ridged surface of one palp shown. Small arrows: course of main resorting tracts at the dorsal and ventral palp margins; large curved arrows: acceptance and rejection tracts. (After NEWELL, 1970; reproduced by permission of Paul Elek Limited.)

face during active feeding. In *Crepidula fornicata* this mucous sheet is conveyed over the frontal surface of the lamellae by the frontal cilia and acts as a sieve, both intercepting and transporting particulate material (WERNER, 1953). According to MACGINITIE, formation of the mucous sheet can easily be disturbed, and many workers (JØRGENSEN, 1949, 1955, 1960; KORRINGA, 1952; CHIPMAN and HOPKINS, 1954; SMITH, 1958) have interpreted changes in gill filter efficiency, as resulting from the presence or absence of the mucous sheet (OWEN, 1966a). Clearly, sorting of entangled particles as described by ATKINS (1936, 1937a, b) does not occur; particles of all sizes are transported to the sorting organ, the labial palp.

Many authors have raised objections to applying MACGINITIE's mucous-net theory to bivalves. JØRGENSEN (1955, 1966) pointed out that the presence of elaborate ciliary sorting mechanisms on the gills would be surprising, if no sorting took place in that region. OWEN (1966a) asked: What is the function of latero-frontal cilia, and how do they function? These cilia are peculiar to lamellibranchs and are not present in filter-feeding gastropods, which utilize a mucous sheet for feeding (WERNER, 1953, 1959). OWEN (1966a) assumed that the distances between filaments, and between latero-frontal cilia determine primarily the filter efficiency of the gill

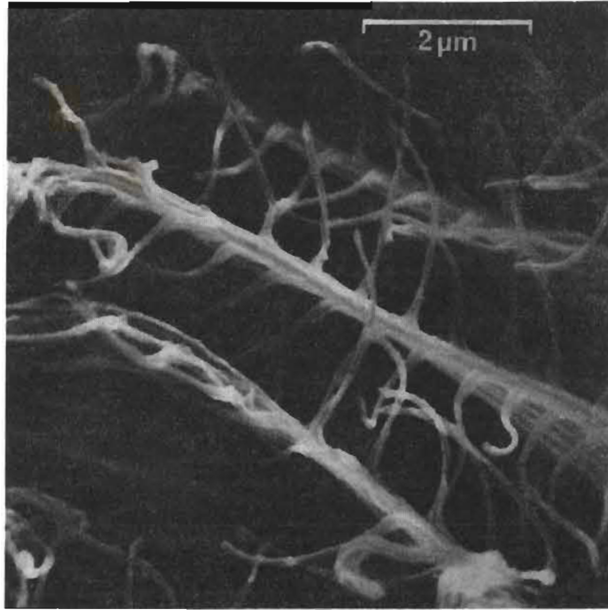


Fig. 3-15: *Mytilus edulis*. Latero-frontal cilia. (After MOORE, 1971; reproduced by permission of Springer-Verlag, Berlin.)

rather than the size of the ostia. The distance between ctenidial filaments is presumably such that the space between them is guarded by the latero-frontal cilia, and they both may interdigitate (TAMMES and DRAL, 1955). The distance between successive latero-frontal cilia is about 2 μ in *Ensis siliqua*, 1·5 to 3·7 μ in *Cras-*

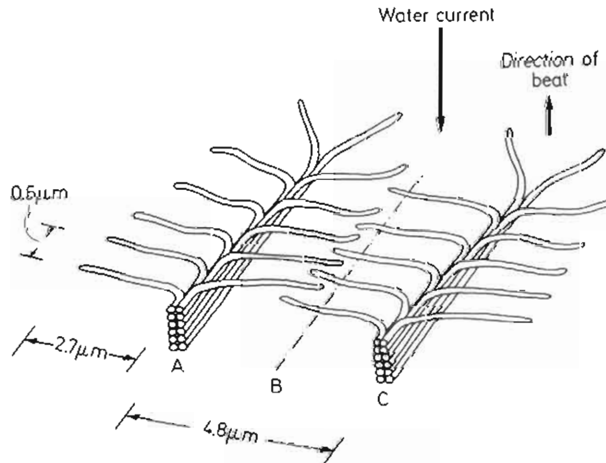


Fig. 3-16: *Mytilus edulis*. Composite diagram of the ends of two latero-frontal cilia at the start of their effective beat. (After MOORE, 1971; reproduced by permission of Springer-Verlag, Berlin.)

sostrea virginica and *Ostrea edulis* (ATKINS, 1938), and 2 to 3 μ or 5 to 6 μ in *Mytilus edulis* (DRAL, 1967); it thus corresponds closely to the minimum particle size reported to be retained by lamellibranch gills (JØRGENSEN, 1955, 1960). An increase in the distance between the ctenidial filaments would result in an increasing percentage of particles, up to the size of the ostia, passing through the gill, and this may account for the conflicting results obtained by different workers (OWEN, 1966a).

On the basis of scanning electron microscopic studies, MOORE (1971) brought supporting evidence to OWEN's explanation. Fig. 3-15 shows three adjacent latero-frontal cilia of *Mytilus edulis*; the cilium is not a simple structure as previously assumed, but bears branches along its axial length; the free lateral part of a branch measures 2·7 μm in length and the branches are arranged along each side of the cilium 0·6 μm apart. The ends of 2 latero-frontal cilia A and C at the beginning of their effective stroke are diagrammatically shown in Fig. 3-16. Cilium B (not shown) is out of phase with the adjacent two by half a beat (DRAL, 1967). The axis at B, at the start of its effective beat, would be in the position marked by the broken line. Since opposite latero-frontal cilia meet, or slightly overlap, in the middle of the ostium, a complex mesh of lateral branches can be formed during the normal beating of the latero-frontal cilia. The resulting filter system with a mesh size of 0·6 × 2·7 μm is present whatever the pumping rate, and explains the high particle retention efficiency recorded by JØRGENSEN and GOLDBERG (1953). The mesh is much smaller than that previously proposed by DRAL (1967), and can effectively retain particles of a few microns in diameter (MOORE, 1971).

On the basis of OWEN's (1966a) mechanical analysis of the ciliary beat, SCHREINER (1971, 1972) suggested that the latero-frontal cilia probably cannot actively 'wipe off' particles on the frontal cilia, as described by TAMMES and DRAL (1955). It is not clear, therefore, how the particles filtered or trapped by the latero-frontal cilia could effectively be passed on to the frontal cilia, which transport them further on. Further experiments are needed to analyse the complex mechanisms of particle collection and sorting in the gills of lamellibranchs.

In addition to particle size and density, selection also appears to be based on the quality of the particle, at least in the oyster *Crassostrea virginica*. When fed a mixture of diatoms and purple bacteria *Cromatium* sp., *C. virginica* rejected the bacteria via pseudofaeces but consumed the diatoms (LOOSANOFF, 1949). Selection of particle quality in the labial palps has been indicated by MENZEL (1955), who observed accumulation of organic plankton in the palps of *C. virginica*, which was offered a bolus of mixed carmine, charcoal and plankton, accumulating on the palps. In a similar situation, echinoderm larvae, in which particle selection is based probably on size only, consumed algae as well as carmine and calcium carbonate crystals (STRATHMANN, 1971).

Mucus-filtering sheet. In ascidians particles are strained from the passing water through the pharynx by ostia; hence ostia size determines the size of the particles retained. The particles are then entrapped in mucus and transported dorsally towards the lamina and thence posteriorly, to be engulfed (Fig. 3-17).

Whereas ostia can retain only larger phytoplankton organisms (WERNER and WERNER, 1954), ascidians have been reported to retain graphite particles of only 1 μ in diameter from the inhalent water current (JØRGENSEN, 1949, 1952, 1966),

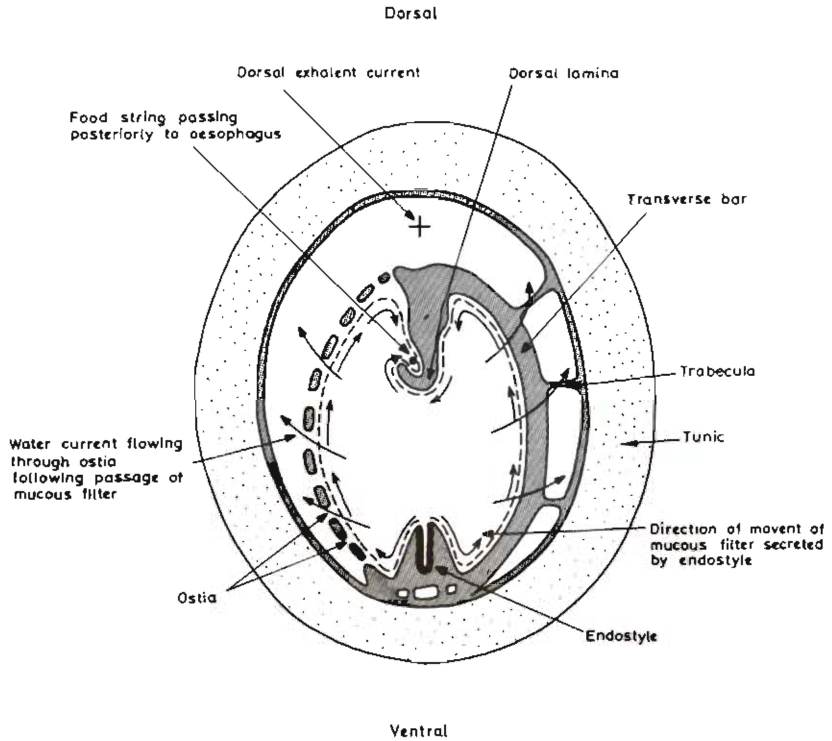


Fig. 3-17: *Clavelina lepadiformis*. Diagrammatic transverse section. Right part of figure: section at level of a ciliary tract which transports the mucus sheet; left part: section at level of a row of ostia which allow water passage. (After WERNER and WERNER, 1954; reproduced by permission of Biologische Anstalt Helgoland.)

since they cover their filtering system with a sheet of mucus (MACGINITIE, 1939; JØRGENSEN and GOLDBERG, 1953, WERNER and WERNER, 1954; WERNER, 1959). Ascidians are thus non-selective suspension feeders.

Feeding by setae. In barnacles, feeding is carried out by setose cirri which differ in form and functions. In balanoids, the first 3 pairs of legs are short and stout with the 2 rami of each limb having different lengths; cirri 4 to 6 are long and thin with rami of almost equal length; the long cirri are used for collection of coarse particles, the short ones for fine particles. While feeding on coarse particles, the long cirri are extended rather slowly, forcing body fluid into them by muscular contraction. The cirri are then retracted and rolled up by means of flexor muscles. Entrapped particles are scraped off by a series of special setae on the short cirri and then passed to the mouth. Selection of particles can occur at or near the mouth, or on the cirri (e.g. in *Balanus balanoides*; CRISP and SOUTHWARD, 1961). In *B. balanoides*, the extended cirral net is rotated through 90° to 180° and the local water currents are allowed to sweep away the accumulated unwanted particles. The smallest particles retained by *B. balanoides* measure 30 μ in diameter (CRISP and SOUTHWARD, 1961). Still smaller

particles of 1 to 2 μ diameter may be filtered by the small cirri, whose setae can be packed so closely that the spaces between the bristles are reduced to 1 or 2 μ.

Deposit feeding

Among deposit feeders too, considerable selection of food particles based on size (e.g. *Cucumaria elongata*) or organic content (e.g. *Mugil cephalus*, *Arenicola marina*) is made. The mullet *Mugil cephalus* scoops and blows the sediments, suspending the lighter organic particles, which are then consumed. The polychaete *Arenicola marina* appears first to select a substratum containing large amounts of nutrients and then indiscriminately feed upon that substratum.

Several species of dendrochirote holothurians are suspension feeders. They capture floating organisms by retractile adhesive papillae at the tip of each tentacle branch, and pass them to the mouth by bending the tentacles inward (MacGinitie and MacGinitie, 1949). In most holothurians, tentacles are used from more or less opposite sides successively, but in *Cucumaria elongata* this is done without definite sequence. Once a long tentacle is in the pharynx, the mouth rim closes. As the large tentacle is withdrawn from the mouth, the food adhering to the tentacle is removed by one of the small fork-shaped ventral tentacles; the fork shape of the ventral tentacles makes them well suited to wipe off adhering prey (Fig. 3-18).

Fish (1967) could not demonstrate histochemically the presence of mucous glands in the tentacles of *Cucumaria elongata*; however, these glands are abundantly present in pharynx and esophagus. Fish assumed that the mucus produced in the pharyngeal glands is picked up by tentacles, each time they are introduced into the pharyngeal lumen in the course of feeding.

Comparing the dimensions of different particles in gut contents of *Cucumaria elongata* with those of bottom sediments, Fish (1967) found that mud taken from the gut consists mainly of silt (0·1 mm diameter), whereas the sediment comprises predominantly fine sand. Obviously, the silt in the digestive tract has been obtained

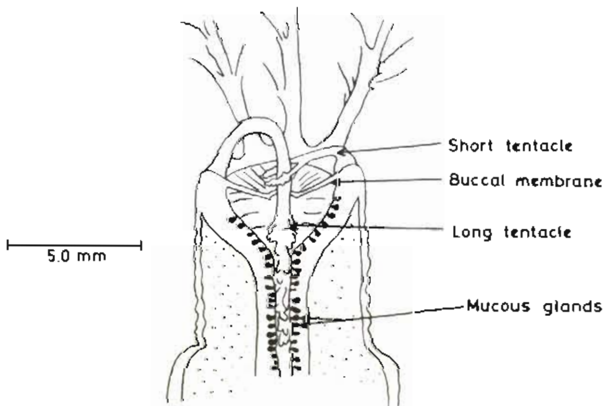


Fig. 3-18: *Cucumaria elongata*. Schematic section illustrating tentacle action during feeding. (After Fish, 1967; reproduced by permission of Marine Biological Association of the United Kingdom.)

from the suspended load above the sediment surface, indicating the presence of a sorting mechanism associated with feeding. Marine intertidal sediment deposits in which particle size is in the range of 0·1 mm in diameter, are known to contain about 0·02% organic nitrogen and 0·2% carbon, and hence the sediments support micro-organisms, probably mostly bacteria (NEWELL, 1970, pp. 252- 256). The nutritional values of such deposits have been studied in a variety of marine animals (e.g. *Hydrobia ulvae*, *Macoma balthica*; NEWELL, 1965, 1970), but not in holothurians.

KRÜGER (1959) suggested that the polychaete lugworm *Arenicola marina* be regarded as a suspension feeder; this suggestion is based in part on the finding that the organic N content of the esophagus is 5 to 10 times higher than that of the surrounding sediment. Equally high N values obtained for both faeces and esophagus contents of *A. marina*, fed sterilized sand, led JACOBSEN (1967) to assume that the high N value reported by KRÜGER (1959) for the esophagus content is due to mucus secreted by the worm. A solution to this problem may be achieved, if the protein N content of esophagus and faeces is estimated by the biuret method; this method (developed by KREY, 1951; KREY and co-authors, 1957; BOJE, 1965, 1966) yields a negative biuret reaction (WINTER, 1969, 1970) with mucus. A close correlation between the total biomass of lugworm populations and the organic N in habitat deposits has been reported by LONGBOTTOM (1968), indicating the nutritive importance of the organic matter for *A. marina*. The organic nitrogen content of intertidal deposits increases logarithmically as the particle size becomes finer and it represents the protein contained in a population of heterotrophic micro-organisms (NEWELL, 1970). *A. marina* appears to have selected substrata which contain small sand grains, where a higher percentage of organic matter can be obtained, and to feed upon it indiscriminately. There is evidence that annelids like *Protodrilus rubropharyngeus* prefer to inhabit sediments containing sand-grain sizes of less than 1 mm diameter (GRAY, 1967).

The difference between the amount of chlorophyll *a* in the contents of the cardiac stomach of the mullet *Mugil cephalus* and the amount present in the sediments of the feeding ground was used by ODUM (1970) to make rough estimates of the proportion 'mud filtered' to 'mud ingested'. In the Sapelo marshes (Georgia, USA), where all substances ingested by the mullet originate from the upper $\frac{1}{2}$ cm of the sediment, the mullets filter about 100 g sediment to obtain 1 g of gut content.

Macrophages

Macrophages sense and seize their food elements individually. Food is often triturated before it is swallowed.

Mechanisms for seizing prey

During feeding, most cnidarians capture and pierce their prey by discharging nematocysts. A substance present in the prey's body fluids (oozing from the resulting wounds) causes the tentacles to bend towards the mouth, and the mouth to open. When the food finally contacts the mouth rim, it is ingested (LENHOFF, 1968b; WILLIAMS, 1972; NAGAI and NAGAI, 1973). The feeding responses of most cnidarians therefore consist of 3 successive phases (Fig. 3-19): (i) snatch (Fig. 3-19 B to C), adherence of one or more tentacles which first come in contact with prey; (ii)

Fig. 3-19: *Diadumene luciae*. Relationships between factors involved in feeding. (After WILLIAMS, 1972; modified; reproduced by permission of Pergamon Press Ltd.)

tentacular response (C to D), movements of tentacles toward the prey to contract and pull it towards the mouth, and simultaneous expansion of oral disc; (iii) oral response, swelling of the circum-oral area and opening of mouth (D to E) and ingestion of the prey (E to F).

In the great majority of predators, speed of movement or locomotion exceeds that of their prey. Sessile and drifting cnidarians represent exceptions to this rule. Given comparable opportunities to predate on suitable food organisms in the laboratory, the free-moving cladoceran *Daphnia pulex* exhibits 13% intake (predatory) efficiency (i.e. 13% of the flagellates *Chlamydomonas reinhardi* available are consumed), whereas the efficiency of sessile *Hydra oligactis* is only 7% (consuming 7% of the *Artemia salina* larvae present; SLOBODKIN, 1959, 1962a, b, 1964). While such comparisons are open to criticism, especially if taken 'too literally', they may be of some use for assessing general trends.

The sessile or drifting mode of life and the poorly integrated nervous system appear to have imposed certain constraints on the predatory efficiency of the cnidarians; as in sessile suspension feeders, prey organisms must be transported or must actively move to the 'waiting' predator. On the other hand, their mechanisms of prey catching assume central importance and apparently considerable complexity and specificity. The presence of tissue juice from *Daphnia magna* does not evoke

nematocyst discharge in *Hydra littoralis* (ZICK, 1929–1932), and filtered extract of fish skin initiates only mild discharge in the anemones (PARKER, 1919). Apparently, mechanical stimuli are also involved (LENHOFF, 1965). While extracts of prey organisms alone are not sufficient to initiate nematocyst discharge, they lower the threshold of mechanical stimulation. In the presence of molluscan mantle extract or human saliva, even light mechanical stimulation is sufficient to evoke nematocyst discharge. Virtually no nematocysts discharge when the tentacles are stimulated mechanically with a glass rod in the absence of chemical reagents (LENTZ, 1966, p. 119).

In the anemones *Metridium senile* and *Anemonia sulcata*, nematocysts may discharge in response to strong mechanical stimulation or to electrical stimulation (PANTIN, 1942); however, discharge is strictly local, i.e. limited to the area directly stimulated, and not transmitted to other body regions. That nematocysts of completely anaesthetized anemones discharge as usual (PARKER, 1919) indicates complete independence of nervous control. The nematocysts of *Hydra littoralis* are not stimulated by commensal ciliates *Kerona* sp. and *Trichodnia* sp. which 'run about' over the cnidarian's body surface; however, they discharge due to contact with other ciliates of similar or even smaller size (ZICK, 1929–1932). The nematocysts of sea anemones belonging to genus *Radianthus* are not discharged on contact with the symbiont fish *Amphiprion xanthurus*, which moves freely about between the tentacles (MARISCAL, 1970).

Anemones such as *Antheopsis* sp., *A. crispa* and *Condylactis aurantiaca* produce specific (protein) substances, which influence the discharge of their nematocysts. These species-specific substances provide protection against self-nettling and prevent the discharge of nematocytes into the objects, which the tentacles continuously contact. The reef fishes (*Amphiprion polymnus*, *A. ephippium* and *Dascyllus trimaculatus*) simply exploit a mechanism existing independently in the anemones. Adapted anemone fishes, neighbouring anemones of the same species and other 'adapted objects' are coated with the inhibitory substances of the anemone and thus do not induce nematocyte discharge (SCHLICHTER, 1972).

Apparently, the nematocysts react primarily to the general mechanical features of objects as texture and shape and secondarily to their chemical emanations. Neither assumption, however, explains the fact that fully fed or contracted *Hydra littoralis* fail to explode as many nematocysts against usual food animals, as do unfed individuals (HYMAN, 1940, p. 389; see also LENHOFF, 1965). Evidence of nerve element associations with cnidoblasts exists in species of *Hydra* (SPANGENBERG and HAM, 1960; LENTZ and BARRNETT, 1961a, b), *Metridium* and *Sagartia* (LENTZ and WOOD, 1964). In *Calliactis parasitica*, the threshold to nematocyst discharge is raised when the pedal disc is fastened to a whelk shell (DAVENPORT and co-authors, 1961). Presumably, these influences are mediated neurally.

Studying the effects of enzyme substances and inhibitors, as well as of neuropharmacological agents on nematocyst discharge, LENTZ and BARRNETT (1962) showed that addition of organic phosphates enhances discharge, provided mechanical stimulation is also offered, whilst enzymatic inhibitors eliminate the discharge potential produced in this way. Acetylcholine produces massive discharge, augmented by eserine but inhibited by hexamethonium and tubocurare. Based on these and other results, LENTZ and BARRNETT concluded that mature nematocysts

contain enzymes responding to chemicals, which serve as substrates for the nematocyst enzymes and, therefore, act as effector substances—provided mechanical stimulation occurs. There is additional evidence available which suggests that, although nematocysts may be regarded as independent effectors, the magnitude of discharge and the type of the nematocysts involved may be influenced by a variety of factors, including satiation level (BURNETT and co-authors, 1960) and season (BOUCHET, 1961).

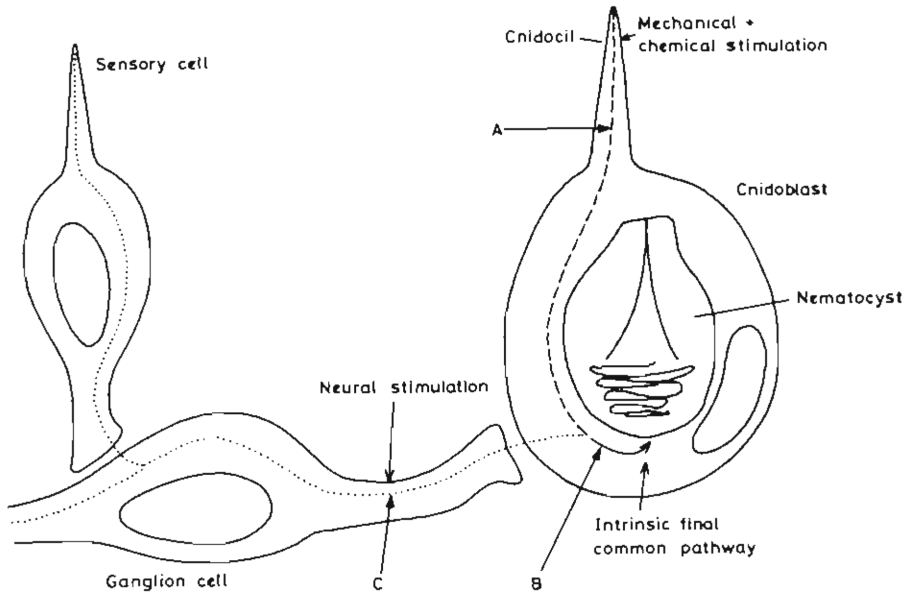


Fig. 3-20: Nematocyst discharge: proposed mechanism of control. Discharge is initiated by combined mechanical and chemical stimulation of the cnidocil. Nervous participation is not required for discharge but modifies the threshold for mechanico–chemical stimulation; neural influences (dotted lines) act via ganglion or sensory cells. Enzymatic inhibitors block chemical stimulation, acting via enzymatic events (A), or nervous stimulation (B); hence the nervous system appears to act through enzyme systems representing the intrinsic final common pathway. Nervous-system inhibition (C) does not block chemico–mechanical stimulation. (After LENTZ, 1966; modified; reproduced by permission of North-Holland Publishing Company.)

A proposed mechanism for the control of nematocyst discharge is illustrated in Fig. 3-20. A combination of mechanical and chemical stimulation, or of mechanical and nervous stimulation results in discharge. This suggests that either chemical or nervous stimulation provides or enlarges the potential for mechanical stimulation. Each stimulus alone does not cause discharge and is, therefore, subliminal. A combination of two stimuli, provided one is mechanical, attains functional threshold properties and effects the response. All stimuli appear to act through a chain of enzymatic events, because enzymatic inhibitors can block all types of stimulation, including the nervous type. Nervous-system inhibition, however, does not block the combined efficiency of chemical and mechanical stimulation.

Mechanisms for seizing and rasping prey

Gastropods provide excellent examples of mechanisms for seizing and rasping. The most important structure in the exploitation of a wide range of food sources by gastropods is the radula-buccal complex. Shape and arrangement of radular teeth are directly related to the type of food taken up and the way in which it is manipulated. There are at least 5 different, and associated, radula mechanisms.

NISBET (1953) describes the feeding of *Monodonta lineata*, an archaeogastropod possessing a rhipidoglossan type of radula (radula formula = α marginals: 5 laterals: 1 rachidian: 5 laterals: α marginals) as follows: (i) the mouth opens, exposing the paired chitinous jaws (Fig. 3-21a). (ii) The buccal mass, containing the radula, protracts forwards and downwards establishing contact with the substratum; the radula sac is obliterated. This is effected partly by action of the tensor muscle, and partly via protraction of the buccal mass on the subradular membrane and hydrostatic-pressure changes in blood spaces of the buccal mass (Fig. 3-21b). (iii) The subradular membrane, along with the radula, moves backwards and downwards over the anterior tip of the cartilage; the teeth on the radula are now erected and come to stand vertically on the surface of the buccal mass; the lateral teeth also move forward and form a radiating series on each side of the central teeth. The region at which such movement occurs, is the 'bending plane' (ANKEL, 1936; FRETTER and GRAHAM, 1962; Fig. 3-21c). (iv) The radula retracts due to downward and backward movement of the cartilage beneath the stationary cartilage. This causes movement of the bending plane of the radula from an antero-dorsal to a postero-ventral position, effecting the return of radular teeth to their original position. The lateral teeth collapse carrying with them scrapped particles into the midline (Fig. 3-21d). (v) The buccal mass withdraws together with the rasped material. (vi) The lips close.

Two other types of prosobranch radulae, the taenioglossan (0 : 3 : 1 : 3 : 0) and rachiglossan (0 : 1 : 1 : 1 : 0), depend on the presence of a bending plane. In both, marginal teeth are reduced or lost; hence crushing and combing of these radulae are unimportant or non-existent, while the rasping effect is increased. In taenioglossan radulae, the mobility of the cartilage, used in retraction and erection of the radula teeth, is increased; the resultant backward movement of the bending plane causes the erected lateral and central teeth to collapse inward and forward on to the radular membrane; as they do so, they grasp the food material and carry it on to the buccal cavity as the radula withdraws (EIGENBRODT, 1941; FRETTER and GRAHAM, 1962; MÄRKEL, 1965). In many taenioglossan mesogastropods, erection and collapse of teeth at the bending plane is utilized for raking, scooping or grasping the food (OWEN, 1966a). In rachiglossan neogastropods, on the other hand, the to and fro movement of the radular membrane over the stationary cartilage increases the rasping action of the radula; the sharp pointed teeth tear and grip bits of food (generally animal flesh) during the retracting stroke (BROCK, 1936; CARRIKER, 1943); the same holds for boring the shells of other molluscs. In *Buccinium undatum* and *Neptunea antiqua*, entry into the prey and feeding on its flesh may be aided by salivary secretions which either kill the victim or assist its ingestion (COLTON, 1908; MAGALHAES, 1948; FÄNGE, 1957).

In all three radula types, the working place of the radula is at the bending plane; hence only one row of teeth is effective at a time. In the docoglossan radula (3 : 3 : 0 : 3 : 3), however, many rows of teeth may be in action simultaneously,

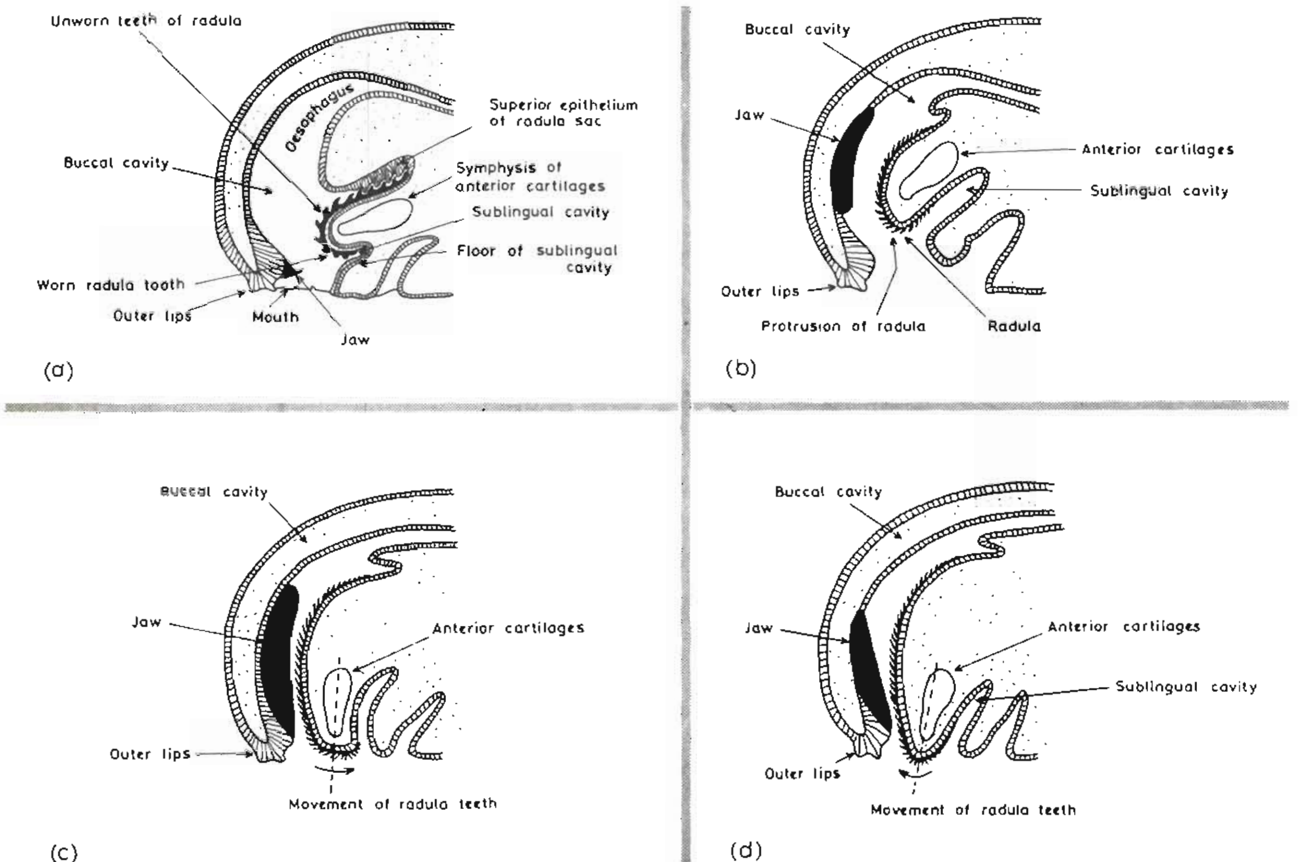


Fig. 3-21: Gastropod feeding mechanisms: (a) *Patella vulgata;* (b) to (d): *Monodonta lineata.* (a) Initial stage of mouth opening; (b) protrusion of buccal mass and radula; (c) downward movement of subradular membrane causing rotation of radular teeth and bending plane (broken line); (d) posterior movement of anterior cartilage and bending plane, causing return of radular teeth to original position. (Prepared from different sources by NEWELL, 1970; modified; reproduced by permission of Paul Elek Limited.)

making the radula a powerful tool for rasping and scraping surface area. Both, radula and underlying cartilage, are moved forward together across the substratum surface employing all rows of teeth on the underside of the protruded cartilage. In sponge-eating opisthobranchs, which possess the docoglossan type of radula, the food is liberally coated with mucus as soon as feeding begins; this serves both lubrication and protection of the gut lining against the sharply pointed sponge spicules (FORREST, 1953).

For obtaining food, various gastropods are known to bore into (or to dissolve) the calcareous exoskeletons of their prey. These borers include members of the families Cassididae, Doliidae, and Tritonidae—thought to secrete acid which may serve in boring or dissolving echinoderm prey—and members of the Cymatridae, Thaididae, Muricidae, Naticidae, Helicidae and Oleacinidae, which feed mainly on other molluscs, drilling their shells to find their way to the soft bodies.

Studies on boring mechanisms are largely restricted to boring species of the families Naticidae, Thaididae, and Muricidae, which are of economic importance. Drilling may result from mechanical processes alone or from a combination of mechanical and chemical activities. Evidence that drilling is achieved purely by mechanical means largely concerns the Naticidae (JENSEN, 1951a, b; ZIEGELMEIER, 1954). Muricids like *Urosalpinx cinerea* alternately use radula (mechanical) and accessory boring organ (ABO; chemical) for drilling (CARRIKER, 1955, 1958, 1959, 1961). While unoperated controls continued boring, operated test individuals of *U. cinerea*, from which either the proboscides or the ABO had been extirpated, did not resume boring until both proboscis and ABO had begun to regenerate (CARRIKER, 1961). However, ZIEGELMEIER (1954) reported that ABO secretion of *Natica alderi* had no effect on calcareous material. Re-investigating the problem with more sophisticated techniques, CARRIKER (1959) suggested that the ABO might secrete an enzyme which digests the concholin matrix of the shell and that this, or other secretions from the organ, may release chelating agents which form water-soluble compounds with the shell calcium. Further work is required to elucidate the role of the ABO in drilling procedures.

(c) External Digestion

Skin Digestion and Absorption

A number of workers have recently reported that there are certain specialized cells or organs in the skin aiding in feeding, digestion and absorption—at least in some sponges, planarians, echinoderms and molluscs. RASMONT (1968) suggested that particles too large to penetrate through the dermal pores of sponges can be phagocytized by dermal cells.

Evidence has been accumulated which indicates that the epidermis of echinoderms is to a large extent functionally isolated from internal reservoirs of nutrients obtained through normal feeding activity and must sustain itself primarily from organic compounds of the ambient sea water (FERGUSON, 1971). The pedicellariae found in many species of echinoderms probably assist in capturing live food (HYMAN,

1955, p. 559); captured prey either autolyzes or is digested by epidermal gland secretions (FERGUSON, 1969). Extracts of all 4 types of pedicellariae of echinoids are toxic to small animals such as young crabs and fishes; but other echinoderms are more or less immune to the extracts (PÉRÈS, 1950).

Migratory amoebocytes may also be important in epidermal digestion. According to PEQUIGNAT (1966a, b) and PEQUIGNAT and TIFFON (1967), amoebocytes together with epidermal secretions can 'predigest' a variety of materials on the body surface of a number of echinoderms. The end-products of digestion are directly absorbed by the epidermis or carried to the gut. In *Psammechinus miliaris*, a large number of spherulocytes have been observed by PEQUIGNAT (1966a, b) to move out via the branchial tufts into the 'digestive' pouch and to digest organic matter on the substrate, permitting the partially solubilized products to be absorbed in the gut. PEQUIGNAT (1972) supplied new information on skin-digestion and absorption in urchins and sea-stars: (i) Extracts from mucus, small spines and dorsal podia from the anterior furrow of *Echinocardium cordatum* produce conspicuous digestion of Remazol-brilliant blue hide-powder *in vitro*. Several *in vivo* tests with artificial substrates suggest the presence of trypsin-like enzyme (not chymotrypsin) in the mucus. (ii) Histochemical studies revealed the presence of chymotrypsin-positive cells and their products in the connective tissue of *Asterias rubens*; strains of red granules issued from them found their way to the surface disc between the columnar epithelial cells. (iii) Positive evidence has been obtained from scintillation counting and autoradiographic studies that radio-active diatoms or ^{14}C-labelled amino acids in sea water, selectively offered to 1 or 2 arms, are digested and absorbed in the ambulacra of *Henricia sanguinolenta*. (iv) The absorbed nutrients reach the muscle layer—not only the epiderm (FERGUSON, 1967b).

Carmine particles introduced into the pallial space of the calico clam *Macrocallista maculata* have been detected histochemically in the epithelium of the outer mantle fold (BEVELANDER and NAKAHARA, 1966). Strong acid phosphatase activity, indicative of digestive capacity for lipid and muco-polysaccharide, has been found in the golgian dictyosomes and lysosomes of epithelial cells in the mantle fold of *M. maculata* (BEVELANDER and NAKAHARA, 1966) and *Mytilus edulis* (PASTEELS, 1967, 1969, 1971a, b). Electron photomicrographs of the microvillous surface of the hypertrophied siphons of the clam *Tridacna maxima* reveal extensive pinocytosis of liquid and particulate material from the ambient sea water. FANKBONER (1971) suggested *a priori* that this endocytosed material contributes to the nutrition of the clam. The very active ciliated epithelia of the mantle-gill system in *Mytilus edulis* play an important role in the direct absorption of amino acids and glucose dissolved in sea water. An important α-amylase, capable of hydrolysing 0·2% starch within 10 hrs and a chymotrypsin-like enzyme have been found in the gill. It is suggested that digestion of small particles as well as the absorption of dissolved food might be initiated on the surface of the pallial-gill areas, and completed later in the 'hepatic' caeca (PEQUIGNAT, 1973).

Evidence for the role of transepidermal absorption of nutrients in regenerating planaria *Dugesia tigrina* has been presented by OSBORNE and MILLER (1962). They employed the peroxidase technique and found that pharyngectomized planarians, exposed to a medium containing protein, absorb protein via their epidermis and form typical spherules in phagocytic cells.

Digestion in External Stomachs

A number of marine animals partially or fully digest their food by everting stomach or pharynx, thus reducing the digestive working load in their intestine (which is usually small or absent).

Most turbellarians capture their prey by wrapping themselves around it; they protrude the powerful pharynx by muscular elongation and press it against the prey's body wall. Small animals are ingested intact; larger ones are held until their body wall ruptures due to the pressure and suction applied by the pharynx. The pharynx is then thrust into every part of the body cavity and sucks out body fluids, tissues and organs; when feeding is completed, generally only the crumpled and collapsed body wall or exoskeleton remains (JENNINGS, 1968). Ingestion of intact prey implies the presence of extracellular digestion; sources of extracellular enzymes have been located histochemically in a number of freshwater rhabdocoels belonging to the genera, *Microstomum*, *Macrostomum* and *Stenostomum* (HYMAN, 1951; JENNINGS, 1957, 1968) and *Temnocephala brenesi* (JENNINGS, 1968); in the marine triclads *Polycelis sabussoui* (ARNOLD, 1909), *P. cornuta*, *Orthodemus terrestris* (JENNINGS, 1962b), *Bdelloura candida* (JENNINGS, unpublished); and in the acotyl polyclad *Lentoplana tremellaris* (JENNINGS, 1957).

Most of the asteriids are carnivorous predators preferring bivalves as food. The sea-star *Asterias rubens* grasps the bivalve with arms and tube feet and moves it toward the mouth; it then humps itself over the prey bunching the basal parts of its arms around it, while the distal parts maintain a firm hold on the shell. The bivalve is then manipulated until the shell edges are brought in contact with the sea-star's mouth, continuing the pull upon the 2 valves (HYMAN, 1955, p. 371). According to the most current view, the sea-star can exert sufficient force with its tube feet to overcome the prey's muscle power; actually, this is rarely necessary, since the very thin cardiac stomach can penetrate through minor openings between the supposedly tightly closed valves. Once a small portion of the stomach has entered the bivalve's mantle cavity, enzymes are released, quickly reducing further resistance (FERGUSON, 1969). Opening the mouth, the sea-star now everts its stomach relaxing the muscles in the stomach wall and retractor harness. Body-wall muscles contract and, by hydrostatic pressure, force the stomach completely out of the mouth (ANDERSON, 1954). Enzymes, secreted by the digestive glands, are released via grooves in the stomach wall. Finally, the digested products are taken up into the body by flagellary currents maintained over the remaining surface of the stomach (ANDERSON, 1953, 1954, 1960). Stomach retraction occurs mainly through contraction of stomach muscles, with the retractor harness possibly providing the final tug (FERGUSON, 1969).

(d) Energy Content of Food and Food Selection

Most of the heterotrophs select their nutrients from a spectrum of food sources. Food selection by copepods (MULLIN, 1963), an opisthobranch (PAINE, 1963; MENGE, 1972) and certain fishes (IVLEV, 1961) can be related either to protective devices of the prey (BAKUS, 1969), or to the feeding behaviour of the predator which

offers the maximum food energy per unit of energy expended by the predator (PAINE and VADAS, 1969).

With a view to testing whether food selection depends upon the calorific content of the prey, PAINE and VADAS (1969) estimated calorific values of a large number of marine algae. With regard to the availability of energy per g dry organic substance, the algae were arranged in this order: green algae (4·92 kcal), red algae (4·75 kcal), brown algae (4·45 kcal). Relating food preference data reported by FUJI (1962), LEIGHTON and BOOLOOTIAN (1963) and LEIGHTON (1966), PAINE and VADAS (1969) revealed that (i) fleshy algae with intermediary calorific values (e.g. species of *Laminaria* and *Gigartina*) constitute the preferred item; (ii) food availability rather than its calorific value has been influential in evolution—a relationship expected in the feeding model of EMLEN (1966). Working on food preference of the isopod *Ligia pallasii*, CAREFOOT (1973) also concluded that there is no significant correlation between food preference and calorific content of 7 potential seaweed foods, and suggested that feeding preference in *L. pallasii* was related to factors other than energy content.

Calorific values of eggs of marine fishes (FLÜCHTER and PANDIAN, 1968), crustaceans (PANDIAN, 1967c, 1970b, c, d, 1974; PANDIAN and SCHUMANN, 1967) and molluscs (PANDIAN, 1969a, 1970d) range from 5·4 to 6·0 kcal g^{-1} dry weight, while those obtained for the sponge (1·2 kcal g^{-1} dry weight) and the coral (e.g. *Alcyonaria* sp.: 2·9 kcal g^{-1} dry weight; CUMMINS and WUYCHECK, 1971, pp. 92–93) are much lower. As may be expected, very few species are known to eat only eggs. For instance, the nudibranch *Calma glaucoides* specializes in feeding on eggs and embryos of shore fishes, and seems specifically adapted to such highly digestible food: its stomach ends blindly and there is neither intestine nor anus (EVANS, 1922; ROWETT, 1946). On the other hand, corals provide relatively small amounts of energy per unit body weight; but there are over 800 species of coral fishes in the Seychelles, Marshall and Marianas Islands (STARCK, 1968 *in:* BAKUS, 1969, pp. 326–327) which feed on corals. Not only are these fish species amazingly diversified, but they are also present in high population densities (185 g m^{-2} in the Hawaii corals; BROCK, 1954).

The size of the daily food ration, however, depends upon the calorific content of the food, since the animals must eat adequately to satisfy their energy demands (MAYR, 1955; ROZIN and MAYR, 1961). The maximum daily ration consumed by the sockeye salmon *Oncorhynchus nerka* is about 115 mg (= 11·5% body weight) dry sockeye mash g^{-1} fish day^{-1} (= 590 cal g^{-1} fish day^{-1}) or 145 mg (14·5% body weight) dry zooplankton g^{-1} fish day^{-1} (= 690 cal g^{-1} fish day^{-1}); zooplankton is known to contain about 10% chitin, which is not digestible and hence the digestive food consumed contained about 590 kcal g^{-1} fish day^{-1}. In order to satisfy the energy requirement of 590 kcal, *O. nerka* consumes ration sizes of 115 mg when fed calorifically rich sockeye mash (5·14 kcal g^{-1} dry sockeye), but 145 mg when fed calorifically poor zooplankton (4·49 kcal g^{-1} dry zooplankton; BRETT, 1971b).

(e) Satiation Time

A heterotroph is considered satiated when it no longer accepts available food after a period of active feeding; the period of time elapsing from the start of feeding

to such voluntary cessation is the 'satiation time' (BRETT, 1971a). The organic carbon content in the marine intertidal deposits is maximal 0·2% (LONGBOTTOM, 1968); consequently, deposit feeders subsisting on organic matter adsorbed to the sand, must eat continuously (e.g. *Mugil*; ODUM, 1970). Sediment uptake, therefore, can hardly be controlled by endogenous factors such as appetite, and food uptake efficiency can only be disturbed when environmental factors change critically. This nutritionally poor substratum and the need for continuous feeding result in amazing quantities of sand being passed through these deposit feeders: according to Fox and co-authors (1948), an opheliid polycheate *Thoracephala mueronata* of 40 mg ingests 57·6% of its own body weight per day; a single individual would thus ingest 84 g sand per year. For the sea cucumber *Paracaudina chilensis*, YAMANOUCHI (1926) and TAO (1930) give values of 144 or 192 g per day and 53 or 70 kg per year. CROZIER (1918) concluded that the *Stichopus* sp. inhabiting an area of 1·7 km^2 in the Bermuda region would pass 500 to 1000 tons of sand through their bodies each year. For a *Mugil cephalus* of 20 cm standard length, ODUM (1970) has estimated turnover rates of 1500 g (dry) sand per day, and 548 kg (dry) sand per year.

Similarly, suspension feeders living in nutritionally dilute media must filter large volumes of water and feed more or less continuously, e.g. sponges and tunicates (JØRGENSEN, 1966). However, pumping rates are indicative not only of the filtering activity, but also of the respiratory exchange of gases. The pumping rates reported are 0·4 to 0·8 ml per day for bivalve larvae (WALNE, 1956; BAYNE, 1965), 4 to 25 ml per day for echinoderm larvae (STRATHMANN, 1971), 9 to 25 ml per day for bryozoans (BULLIVANT, 1968) and 23 ml per day for the sponge *Leuconia aspera* (HYMAN, 1940). Filtration rate varies considerably as function of temperature and particle concentration. For example, in the mussel *Arctica islandica*, filtration rates are 4·8, 9·6 or 10·3 l per day at 4°, 12° or 20° C at a constant *Chlamydomonas* sp. concentration of 20 × 10^6 cells l^{-1} and 14·2, 9·6 or 3·8 l per day at the *Chlamydomonas* sp. concentrations of 10 × 10^6, 20 × 10^6 or 40 × 10^6 cells l^{-1} (12° C; WINTER, 1969, 1970). However, that the amount of food ingested per unit time (food ration) does not increase proportionately with particle concentration indicates a progressive reduction in filter efficiency (LUCAS, 1936; RICHMAN, 1958; SUSHCHENYA, 1958, 1962, 1966, 1970; PETIPA, 1959; MONAKOV and SOROKIN, 1960; IVLEV, 1961). Similarly, the foraging behaviour of macrophages like the rainbow trout *Salmo gairdnerii* also clearly reinforces this tenet that the increase in predation rate is not proportional to prey density (WARE, 1972). One of the reasons adduced to explain this phenomenon is handling time: on empirical grounds, food consumption rate must eventually reach an asymptote because predators have to devote some time to handling each food element they attack (RASHEVSKY, 1959; HOLLING, 1966).

Increase in particle concentration not only tends to reduce the volume of water filtered per unit time (WINTER, 1969, 1970; STRATHMANN, 1971), but also shortens the duration of filter feeding (satiation time). According to LEONG and O'CONNELL (1969), a 4-g anchovy *Engraulis mordax* requires 20·5, 15·3 or 9·7 hrs per day to filter all the available plankters at particle concentrations of 38, 48 or 78 mg m^{-3}, respectively, and at these concentration levels, the fish would need to filter water volumes equal to 1·6, 1·2 or 0·8 m^3. There are sufficient indications that increase in particle size may shorten satiation time (LEONG and O'CONNELL, 1969; BRETT,

1971a); for instance, *E. mordax* consumed *Artemia salina* nauplii equivalent to about 275 mg in about 60 mins, but when offered adults of *A. salina* (each of which is 400 times larger than the nauplius), the fish satiated in 15 mins consuming an equal weight of food.

Food organisms of different sizes can cause certain predators to switch from 'filtering' to 'seizing'. Adult *Artemia salina* (5–10 mm long, 4·4 mg), for example, evoke 'seizing' in some planarians, molluscs and echinoderms, but *A. salina* nauplii (0·6 mm long, 11 μg) cause a 'filtering' reaction in the fish *Engraulis mordax* (LEONG and O'CONNELL, 1969). Similar examples are not uncommon among other animals, e.g. species of *Calanus* (CONOVER, 1956) or *Branchiostoma* (WEBB, 1969). A comparison of the rate curves obtained for filter and predatory feeding in *E. mordax* (Fig. 3-22) indicates that, at the beginning, much more food is obtained by seizing than by filtering; the seizing rate is initially higher (18 *A. salina* min^{-1}) but diminishes long before satiation is reached. By contrast, filtering rate remains relatively uniform over 1 hr and is limited by the straining capacity of the fish (LEONG and O'CONNELL, 1969).

Among macrophages, satiation time varies from a few minutes (e.g. in the fish *Megalops cyprinoides*, PANDIAN, 1967a) to a few hours (e.g. the sea-star *Asterias rubens*, 10 hrs, HYMAN, 1955, p. 372). Laboratory experiments show that satiation time is 65, 13 or 6 mins for the mackerel *Trachurus japonicus*, the puffer fish *Fugi vermicularis* or the file fish *Stephanolepsis cirrhifer* (ISHIWATA, 1968), and 54 or 37

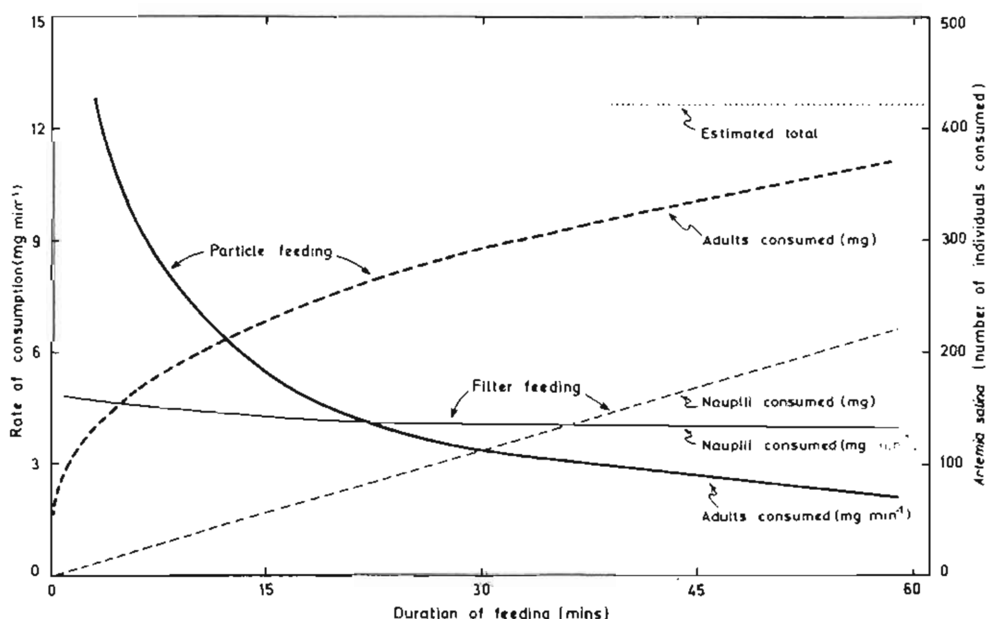


Fig. 3-22: *Engraulis mordax*. Particle and filter-feeding in young anchovy. Filter-feeding (thin continuous and thin broken lines) at a concentration of 4·4 mg of *Artemia salina* nauplii per litre. Particle-feeding (thick continuous and thick broken lines), at 2 to 50 mg of adult *A. salina* per litre. The estimated total feeding capacity is indicated by a dotted horizontal line. (After LEONG and O'CONNELL, 1969; modified; reproduced by permission of *Journal of the Fisheries Board of Canada*.)

mins for 2 or 26 g sockeye salmon *Oncorhynchus nerka* (BRETT, 1971a). Macrophagous predators, which feed on scarce, potentially limiting amounts of food, exhibit adaptations to facilitate food storage; examples are enlargements of stomach (shark), caeca (leech), crop (*Octopus vulgaris*) or temporary 'neck formation' (*Hydra viridissima*; BLANQUET and LENHOFF, 1968). When the required daily ration cannot be accommodated in the storage organ, or when the food available is not sufficient for satiation, the animal feeds more than once daily and may get repeatedly satiated, i.e. the frequency of feeding and satiation periods increase.

(f) Feeding Periodicity

Endogenous Factor: Appetite

Voluntary food intake, a measure of appetite, permits an objective assessment of hunger in relation to deprivation time. When excess food is periodically provided, repeated satiation occurs (BEUKEMA, 1968). Our knowledge on the mechanisms controlling appetite is based on mammals; among marine animals, only fishes have so far received attention. There are many mechanisms controlling appetite in fishes; and all are obviously interrelated: (i) Stomach evacuation has been shown to play a major role in creating appetite (PANDIAN, 1967b; WINDELL, 1967; BRETT and HIGGS, 1970); the same holds for emptying of the intestine (MAYR, 1955). (ii) Stretch receptors in the stomach wall constitute one of the mechanisms controlling the appetite of vertebrates (LEPKOVSKY, 1948; PAINTAL, 1954). The size of individual food particles is of importance, as it will have some effect on the point at which further distension is declined. (iii) Blood glucose level plays an important role in returning appetite (RUITNER, 1963). (iv) There is also evidence that physiological changes, e.g. in the sex-hormone (steroid ?) blood level related to sexual maturity, may affect the response to food and, hence, appetite in the lamprey *Petromyzon marinus* (KLEEREKOPER and MOGENSEN, 1963).

Maximum stomach capacity of the skipjack tuna *Katsuwonus pelamis* is about 7% of its weight; as the skipjack feeds frequently, its daily consumption amounts to 15% of its body weight (MAGNUSON, 1969). *K. pelamis* begins to eat within 2 to 4 hrs after more or less complete satiation; this indicates that even partial stomach clearance is sufficient to return appetite. Within 4 hrs after a full meal, the tuna consumes 75% of the amount eaten at the previous meal, whereas only 50% of the earlier meal was emptied from the stomach. Therefore, the stretch receptors, which are known to control the appetite level by limiting the stomach capacity, appear not to control appetite of the skipjack. MAGNUSON (1969) also reported that blood glucose of the skipjack, which remained at high level of 132 mg 100 ml^{-1} until the 7th hr after feeding, decreased to less than 81 mg 100 ml^{-1} 10 to 24 hrs after a meal. Possibly, the blood glucose level controls appetite in *K. pelamis*.

In the euryhaline fish *Tilapia mossambica*, blood sugar level does not appear to regulate the return of appetite directly. In fresh water, 7‰ and 14‰S, blood-sugar level is around 110 mg 100 ml^{-1} and decreases to 100 and 40 mg 100 ml^{-1} on the 3rd and 50th day of starvation, respectively. When these test individuals are exposed to an unlimited supply of algae, those starved for only 3 days consume more food

and fully restore their blood-sugar level, while those starved for 40 days feed far less and restore their blood-sugar level to only 45 mg 100 ml^{-1} (VIVEKANANDAN, personal communication).

Exogenous Factor: Tidal Cycle

In the intertidal region, the most important phenomenon which profoundly affects timing and duration of feeding is the recurring tides (Chapter 9). During submersion, suspension and deposit feeders become active. The suspension and deposit feeders resume feeding as soon as they are submerged. During air exposure, a variety of other intertidal animals begin to predate, browse or scavenge in the intertidal zone. Only suspension feeders, which remain immersed in pools left by the receding tide are able to maintain continuous feeding throughout the tidal cycle (NEWELL, 1970).

Individuals belonging to the same species, may have longer or shorter feeding periods. In some species, the individuals at upper shore level fail to compensate the short duration of feeding period with faster feeding rate and hence exhibit retarded growth; those in other species are able to compensate the short feeding periods by faster feeding, and presumably display normal growth. For example, SOUTHWARD (1964) found that the duration of suppression of the cirral activity of the high-level populations of the barnacle *Chthamalus dalli* was longer than that of the low-level populations. He suggested that such differences are to be correlated with the low growth rates of upper and lower shore-level populations of barnacles. Presumably *Mytilus californianus*, whose intraspecific differences in pumping rates have been studied by SEGAL and co-authors (1953), also belongs to this category. On the other hand, the individuals of the small intertidal crevice-dwelling bivalve *Lasaea rubra* at upper shore level increase their feeding rate in order to compensate for the reduced time of immersion. During spring tides, upper shore-level *L. rubra* are immersed for only 1 out of 12 hrs; during neap tides they are exposed to air for 12 consecutive days. Individuals from the upper shore-level responded more quickly to wetting and their filtration rate is initially higher than that of the lower shore-level individuals. Moreover, *L. rubra* is small enough to filter the thin water film

Table 3-4

Littorina littorea. Effect of shore-level height on feeding activity during the tidal cycle. Average values obtained from 50 individuals (about 400 mg body weight) at 18°C (After NEWELL and co-authors, 1971; modified; reproduced by permission of Springer-Verlag, Berlin)

Tidal level	Immersion time (hrs)	Number of radular movements per tidal cycle
1 hr from low water of spring tide	10·0	3677
2·5 hrs from low water of spring tide	7·5	6391
Approximately mid-tide level	5·5	6494
2 hrs from high water of spring tide	4·0	6811

covering the rocks due to wave splash. These facts may play an important part in extending the feeding period of the bivalve (MORTON and co-authors, 1957).

The upper-shore winkle *Littorina littorea* is able to compensate for the reduced feeding period by increased radular activity during immersion (NEWELL and co-authors, 1971). The rate of radular activity is mainly modified by the duration of exposure to air, which directly controls the rate of radular activity on immersion (Table 3-4). Thus the radula moves as much as 6811 times per tidal cycle in an upper shore individual, normally exposed to air for over 8 hrs per tidal cycle, against 3677 times per tidal cycle in a lower shore individual, experiencing air exposure for 2 hrs per tidal cycle or less.

(g) Nutrition from Symbiotic Autotrophs

Symbiosis between an autotroph and a heterotroph provides a shortcut in the cycles of oxygen, carbon dioxide, phosphorous and nitrogen from which both partners can benefit. It is not surprising, therefore, that more than 150 genera of invertebrate heterotrophs, representing 8 phyla, possess autotrophic endosymbionts. A nearly complete list has been published by DROOP (1963). A more formal classification is presented in Table 3-5; examples furnishing information on the modes of infestation and the harbouring sites of autotrophs are given in Table 3-6.

Table 3-5

Symbiotic associations between marine algae and invertebrate heterotrophs (After SMITH and co-authors, 1969; modified and extended; reproduced by permission of Biological Review)

Autotroph algae (Class, Order)	Symbiont form	Heterotroph host	Author
		Zoochlorellae	
Prasionophyceae Pyramimonadales	Irregular	Acoellous turbellarians (*Convoluta roscoffensis*)	DOREY (1965), OSCHMAN (1966)
Chlorophyceae Siphonocladiales	Chloroplasts	Sacoglossan opisthobranch (*Elysia viridis*)	KAWAGUTI (1941), KAWAGUTI and co-authors (1965), TAYLOR (1968)
		Zooxanthellae	
Dinophyceae Peridiniales	Coccoid	Protozoans, sponges, coelenterates, molluscs	DROOP (1963), MCLAUGHLIN and ZAHL (1966)
Basillariophyceae Basillariales	Pennate, coccoid	Turbellarian (*Convoluta convoluta*)	AX and APELT (1965)
		Zoocyanellae	
Cyanophyceae Chrooccocales	Coccoid	Demosponges (*Ircinia variabilis*)	SARA (1966), SARA and LIACI (1964)

Table 3-6

Examples of symbiotic associations between algae (autotroph) and invertebrates (heterotroph) (Compiled from the sources indicated)

Autotroph	Heterotroph	Harbouring site	Mode of infection	Author
Platymonas convolutae	*Convoluta roscoffensis*	Intracellular, subepidermal	Ingestion	PARKE and MANTON (1967), OSCHMAN (1966), PROVASOLI and co-authors (1968)
Chloroplast of *Codium tomentosum*	*Elysia viridis*	Cells of hepatic tubules	Ingestion	TAYLOR (1968)
Zooxanthellae	Corals, anemones	Intracellular, gastrodermal		KAWAGUTI (1964)
(a) *Symbiodinium adriaticum*	*Cassipeia* sp.	Mesoglea, interstitial		FREUDENTHAL (1962)
(b) Zooxanthella (Species not mentioned)	Planula larva	Ectodermal		ATODA (1953)
(c) Zooxanthella (Species not mentioned)	Tridacnids	Blood sinus		YONGE (1936)
Licmophora hyalina	*Convoluta convoluta*	Extracellular, parenchymatous	Ingestion	AX and APELT (1965), APELT (1969)
Aphapanocapsa raspaigellae	*Ircinia variabilis*	Extracellular (in mesohyl)	Ingestion or hereditary	SARA (1971)

Most symbiotic algae are functional autotrophs, fixing CO_2 in the light and selectively releasing substantial soluble carbohydrate, which is metabolized by the host. Movement of substances between the component organisms has been regarded as a primary feature of symbiosis (SMITH and co-authors, 1969). Dissection of the host and assay of algal tissue (e.g. MUSCATINE and LENHOFF, 1963), and radio-autographic studies (e.g. MUSCATINE and HAND, 1958; GOREAU and GOREAU, 1960) have revealed that the major product released to the heterotroph is glycerol (see also MUSCATINE, 1967; MUSCATINE and co-authors, 1972; TRENCH, unpublished). The substances known to be released are summarized in Table 3-7. It is further suggested that insoluble carbohydrates are acquired by the host as a result of (i) degeneration and digestion of whole algal cells or their parts (YONGE, 1936; OSCHMAN and GRAY, 1964), or (ii) sloughing of unsuitable material from healthy algae (KAWAGUTI, 1965; OSCHMAN, 1966). Electron microscopic and histochemical studies by FANKBONER (1971) have revealed that older senescent zooxanthellae are selectively culled from the algal population of the mantle edge of the clam *Tridacna maxima* by amoebocytes, and are intracellularly digested via amoebocyte lysosomes.

Table 3-7

Form of carbohydrate and other compounds released by algal symbionts to their hosts (heterotrophs) (After SMITH and co-authors, 1969; modified and extended; reproduced by permission of Biological Review)

Heterotroph	Compound released by autotroph to heterotroph	Author
	Zoochlorellae	
Protozoa		
Paramecium bursaria	Maltose	MUSCATINE and co-authors (1967)
Porifera		
Spongilla lacustris	Glucose	MUSCATINE and co-authors (1967)
Coelenterata		
Chlorohydra viridissima (wild type)	Maltose	MUSCATINE (1965)
Chlorohydra viridissima (mutant)	Glucose	MUSCATINE and co-authors (1967)
Mollusca		
Placobranchus ianthobapsus	Galactose, glucose	TRENCH and co-authors (1970)
	Zooxanthellae	
Coelenterata		
Pocillopora damicornis	Glycerol	MUSCATINE (1967)
Anthopleura elegantissima	Glycerol	TRENCH (unpublished)
Zoanthus confertus	Glycerol	TRENCH (unpublished)
Fungia scutaria	Glycerol	TRENCH (unpublished)
Millepora alciornis	Glycerol	MUSCATINE and co-authors (1972)
Agaricia agaricites	Glycerol	MUSCATINE and co-authors (1972)
Mollusca		
Tridacna crocea	Glycerol	MUSCATINE (1967)

A number of workers have shown that within a short period (1 to 48 hrs in different species) 10 to 55% of the total ^{14}C photosynthate was translocated from the autotroph to the host (MUSCATINE and LENHOFF, 1963; VON HOLT and VON HOLT, 1968a, b; TRENCH and co-authors, 1970; MUSCATINE and co-authors, 1972; GREENE, unpublished). The pertinent findings regarding the selective release of substances by the symbiotic algae can be summarized as follows: (i) Host factor, pH, and nutritional state of the host (STIVEN, 1965) have profound effects on the rate of release of photosynthate from the symbionts. Zooxanthellae of *Tridacna crocea* and *Pocillopora damicornis* exhibit a 16-fold increase in the presence of the host homogenate (MUSCATINE, 1967). At pH 4·5, zoochlorellae released as much as 85% of their total fixed ^{14}C in 30 mins, but only 7% at pH 7·5 (MUSCATINE, 1965). However, release of glucose by zoochlorellae from a sponge was not pH dependent (MUSCATINE and co-authors, 1967); detailed studies on the pH effect on excretion by zooxanthellae are lacking. (ii) Much of the fixed carbon released by most symbiotic algae is in the form of a single, simple carbohydrate; only a relatively small proportion comprises other compounds (SMITH and co-authors, 1969). The major product released and trans-

located to the host is glycerol (MUSCATINE, 1965). (iii) The carbohydrate released by the symbionts is of a 'special' kind; thus, zoochlorellae from *Hydra viridis* produce sucrose intracellularly, but release maltose which only occurs in small amounts within the cells (CERNICHIARI and co-authors, 1969); zooxanthellae from *P. damicornis* excrete glycerol, while glucose is its major intracellular product (MUSCATINE, 1967).

In both zoochlorellae and zooxanthellae associations, evidence exists that the translocated material is converted in the host to different substances. Fractionation of the coral *Pocillopora damicornis* labelled on the reef for 24 hrs revealed that 35 to 50% of the total ^{14}C fixed appears in the animal tissue lipid (as ^{14}C glycerol) and protein (MUSCATINE and CERNICHIARI, 1969). Conversion of the translocated material may thus enhance the one-way flow of carbohydrate into the host. The data obtained by YOUNG and co-authors (1971) on the incorporation of ^{14}C into protein, chitin and lipid of *P. damicornis* suggest that metabolic paths from glycerol to serine and to acetyl-Co-A are important in the utilization of algal photosynthate by the coral. Translocated algal products may also enhance calcification rates in corals (e.g. *Acropora cervicornis*) by serving either as specific substrate in the organic matrix or as general energy source (PEARSE and MUSCATINE, 1971).

The direct inflow of food energy appears to have reduced external food uptake of heterotrophs. Thus APELT (1969) observed reduced food intake by *Convoluta convoluta* harbouring the symbionts *Licmophora hyalina* and *L. communis*. Adult *C. roscoffensis* (KEEBLE and GAMBLE, 1907) and soft xeniid corals (GOHAR, 1940, 1948; see also MUSCATINE, 1961) are believed not to feed and to derive all their nutrients from their respective symbionts; however, critical evidence is lacking. A series of publications by GOREAU (1959, 1961, 1963), McLAUGHLIN and ZAHL (1966), and PEARSE and MUSCATINE (1971) indicates the significant role of zooxanthellae in calcification of corals. For further information on symbiosis between invertebrates and unicellular algae, consult TAYLOR (1973).

(3) Transformation into Body Functions and Structures

(a) Digestion

Digestion involves breaking down complex food substances into monosaccharides, amino acids, fatty acids, glycerol and other less complex constituents which can be used as source for energy and matter by the cells, and incorporated into the living protoplasm.

Mechanical Aspects of Digestion

Mechanical trituration

In protozoans and sponges, digestion is effected exclusively by enzymatic activities. In metazoans, chemical trituration is usually preceded by mechanical commutation (e.g. gastropods) or, less frequently, it may occur simultaneously with mechanical trituration (e.g. malacostracans; VAN WEEL, 1970). In some coelen-

terates, rhythmic contractions assist food commutation and ultimate distribution of food particles (STREHLER, 1961; FULTON, 1963b). Numerous worms possess strong, sharp jaws and use them to pierce or cut their prey (e.g. species of *Eurythae*). In malacostracans, the stomach often has a 'gastric mill' in its anterior part, whereas the posterior pyloric part contains a press and filter apparatus. Crustaceans possessing these gastric structures do not masticate their food and mechanical trituration occurs in the gastric mill. Press and filters allow only the smallest particles to enter the midgut gland; larger particles are pushed back into the mill or directly into the midgut. Non-malacostracan crustaceans triturate their food mechanically by means of their mouth parts before ingesting it (VAN WEEL, 1970); they tear pieces from the food and knead them until they can pass into the esophagus (VONK, 1960). Gastropod radulae have been described on p. 96; they serve to triturate the food in Amphineura (FRETTER, 1937), Scaphopoda (MORTON, 1959) and Cephalopoda (BIDDER, 1966). In the veliger larvae of prosobranchs, rotation of algae against the gastric shield either weakens (e.g. *Cricosphaera carterae*) or fragments (e.g. *Monochrysis lutheri*) the cell wall, enabling gastric juice to penetrate it (FRETTER and MONTGOMERY, 1968). In septibranchs, the muscular, gizzard-like stomach crushes large food into small particles (YONGE, 1928). The teeth of echinoids are incorporated into a complex masticating apparatus, the Aristotle lantern; the lantern consists of 40 calcareous plates and 6 sets of muscles; it is used for rasping and biting the food (FERGUSON, 1969). The oral teeth of many fishes are used to hold prey; mechanical trituration is effected by esophageal teeth (LAGLER and co-authors, 1962, p. 146). Coral-eating fishes possess a powerful masticating beak and oral teeth.

Propulsion of food in the digestive tract

Ingested food is propelled along the digestive tract by ciliary action or muscular activity or by a combination of both. The speed at which food is forced through the digestive tract has great impact on the extent of digestion and the efficiency of absorption (PANDIAN, 1967b, d). Movements of the gut considerably sustain the action of the enzymes. It has been found from *in vitro* experiments that even very moderate stirring (50 to 75 rpm) of fibrin grains in a solution of trypsin increases digestion by 100 to 150% (VONK, 1964, p. 356).

In ciliary feeders, the ingested particles are bound into a more or less continuous mucous string, which is passed through the gut at a regulated pace, slow enough for thorough digestion (OWEN, 1966b). One of the compensating mechanisms for the lack of peristaltic movement (see below) in the gut is rotation of the food string by ergatula (MORTON, 1960). In the ciliary feeders belonging to a number of phyla (except sedentary polychaetes), the cilia rotate the food string in a modified region of the gut; thus, other areas are free to perform different functions such as the sorting of different sized food particles or digestion. This rotating style is most highly developed in molluscs.

The rotating style performs four functions: (i) Formation of a faecal rod. (ii) Continuous stirring of stomach contents; looked at from the esophageal end of the stomach, the crystalline style rotates clockwise (OWEN, 1966b), 5 to 36 times min^{-1} depending on life stage and temperature (YONGE, 1926b; DINAMANI, 1957); rotation effects thorough mixing of stomach contents and extracellular enzymes.

(iii) Regulation of the rate at which the mucous food string passes through the stomach (progress of an individual particle is retarded long enough to facilitate sorting and digestion). (iv) Assistance to entry of material from esophagous into the stomach by the capstan-like action of the rotating style (MORTON, 1952).

Macrophagous higher metazoans depend on well-developed visceral muscles for the movement of gut contents. The arrangement of gut musculature is extremely variable. The muscular activity most characteristic of hollow viscera is referred to as peristalsis—a wave of muscular contraction, preceded by a wave of relaxation. Strong peristaltic motion has been observed in the intestine of sea-urchins and holothurians (STOTT, 1955; D'AGOSTINO and FARMANFARMAIAN, 1960; FUJI, 1961; PROSSER and co-authors, 1965). Peristaltic waves in decapods like *Nephrops norvegicus* are weaker (3 to 5 times min^{-1}) than in vertebrates (YONGE, 1924). An extensive nerve plexus, resembling the Auerbach plexus of vertebrates, has been demonstrated in mid and hind gut of a number of decapods. The plexus is assumed to control normal peristaltic movements (VONK, 1960). Gut motility is controlled by an intrinsic neural plexus in gastropod (e.g. *Arion ater*; ROACH, 1968) and pelecypod (e.g. *Spisula solidissima*; SMUCKER and NYSTROM, 1972). In vertebrates, peristalsis is under the (extrinsic) control of the nervous system. Elasmobranchs possess a well-developed autonomic system containing parasympathetic and sympathetic elements. Abdominal organs are sympathetically innervated by the anterior, median and posterior splanchnic nerves (YOUNG, 1933). The stomach is the only abdominal organ with double autonomic innervation (NICOL, 1952). NILSSON and FÄNGE (1969a), who studied drug effects on tonus changes of longitudinal smooth stomach muscles in cod *Gadus morhua*, concluded that contraction and relaxation are due to activation of α and β receptors, respectively. In the vagus network adrenergic fibres are present; stimulation or inhibition of vagus contraction is mediated via cholinergic neurons. From studies on the digestive response of trout *Salmo gairdnerii* to pellet diet, WINDELL and co-authors (1969) inferred that a dense collagenous layer found in the gut wall of salmonid fishes greatly reduces the extensibility of the gut wall (BURNSTOCK, 1959) and affects the ability of the stretch receptors to initiate peristalsis. As long as sufficient food is present in the stomach to stimulate the stretch receptors, peristalsis occurs at a fixed rate; peristaltic initiation is related to a combination of distension of the gut wall with chemical or tactile stimulation of the mucosa. When a small amount of food is present, the gut wall is not stretched and peristalsis is not or only weakly initiated—an adaptive mechanism for processing small amounts of food slowly and thereby allowing sufficient time for efficient absorption.

Movements of somatic musculature associated with locomotion can also serve as an effective force in the propulsion of food, supplementing the action of poorly developed visceral musculature as in nematode and polychaete worms, and holothurians and other echinoderms (ANDERSON, 1954; PROSSER and BROWN, 1961). Some coelenterates produce rhythmic, peristalsis-like movements to assist in the commutation of food and in the ultimate distribution of digested nutrients over relatively great distances, particularly in some sessile colonial hydroids via the stolon to non-feeding members of the colony (REES and co-authors, 1970). Such peristaltic contractions have been demonstrated in *Cordylophora caspia* by FULTON (1963b). Following food ingestion, contraction rate increases and then declines to

resting after several hours. Electrophysiological studies on *Tubularia larynx* by MACKIE and JOSEPHSON (1965) and JOSEPHSON (1965) have revealed pacemaker co-ordination of a 'concert' of activities associated with digestive movements (see also PASSANO and MCCULLOUGH, 1964, 1965; MCCULLOUGH, 1965). These activities involve contraction of 'neck musculature', tentacle flexion, closing and opening of valves and fluid movements.

Chemical Aspects of Digestion

Chemical trituration

Enzyme kinetics. Enzymes associate themselves reversibly with materials on which they act (substrate), in a relationship (enzyme-substance complex) which is intimate and specific, involving both a protein and a prosthetic group of the enzyme. In this association, the substrate is activated, subjected to molecular strain or otherwise altered, so that the reaction path is smoothed and end-products formed rapidly; however, the enzyme does not contribute energy. Reactions of this type are strictly limited by the specific amount of enzyme surface or reactive centres. When the reactive surfaces are tied up with substrates, reaction velocity declines. When the reactive surfaces are tied up by compounds similar in structure to a substrate but incapable of forming end-products, the essential reactions can be blocked; this blocking phenomenon is called competitive enzyme inhibition.

Since the enzyme–substrate combination is essential for enzyme activity, relative amounts of the 2 substances forming the complex are of major significance in reaction kinetics. When the quantity of enzyme is constant, activity increases steadily with additional amounts of substrate up to a maximum, where all enzyme is associated with the substrate. When the amount of enzyme varies in the presence of excess substrate, activity rises in direct relation to the available enzyme, again in accordance with the enzyme-substrate concept. Hydrolytic activity can also be modified by the concentration of reaction products, which in some way combine with the enzyme or inhibit its further activity. Hence reaction products, in one way or another, can serve as a feedback system to regulate enzyme processes (e.g. BROWN and WHITEGIVER, 1971). Many enzyme reactions are reversible and the equilibrium between reacting compounds and end-products depends upon the law of mass action; e.g. when triolein is incubated with lipase, fatty acids and glycerol are formed; after some time the reaction rate declines and an equilibrium is attained. This equilibrium is the same when fatty acids and glycerol (plus enzymes) are used for the synthesis of triolein (HOAR, 1966).

Temperature, pH and salts are known to modify considerably the degree of hydrolytic activity. Enzyme reactions depend on molecular agitation, which is temperature-dependent. Usually there is a well-marked optimum. In natural habitats, the optimum has an ecological meaning mainly as a function of time. In *in vitro* tests, run for 3 hrs, the optimum temperature for maximum production of amino-acid N for proteolytic activity of the ascidian *Tethyum pyriforme americanum* is 45° C; if, however, the tests were run for 50 hrs, maximum amino-acid N is produced at 25° C (Fig. 3-23). The biological optimum for digestion is 25° C, as the food

takes about 50 hrs to pass the gut of *T. pyriforme americanum* (BERRILL, 1929). Enzymes are proteins, and because of their amino and carboxyl groups, proteins are amphoteric electrolytes, which dissociate either as acids or bases, depending on the pH of the solution. Consequently, the chemical properties of the enzyme and its ability to form reactive enzyme-substrate complexes vary as a function of pH resulting in a well-marked optimum (Fig. 3-24). In enzymatic measurements, the location of the pH optimum depends on nature and concentration of the substrate, concentration of enzyme and electrolytes, temperature, and incubation period (SMIT, 1968). By activating pancreatic lipase and facilitating emulsification of fats to droplets—as small as 0·5 μ in diameter (BAKER, 1951)—bile salts increase the total area of the oil–water interface, at which lipase can act.

Rate of enzymatic activity depends on variable amounts of enzymes, substrates, activators, inhibitors and by-products, reacting under the modifying influence of temperature and pH. No one factor can be singled out as being of prime importance; but in living systems the regulation very often seems to depend on quantitative changes in enzyme concentrations (HOAR, 1966). In this connection, reserves of inactive pro-enzymes awaiting activation are important; pepsinogen is activated (unmasking) in the presence of hydrochloric acid, involving removal of a polypeptide and releasing of the active protease pepsin (HIRSCHOWITZ, 1957; MERRETT

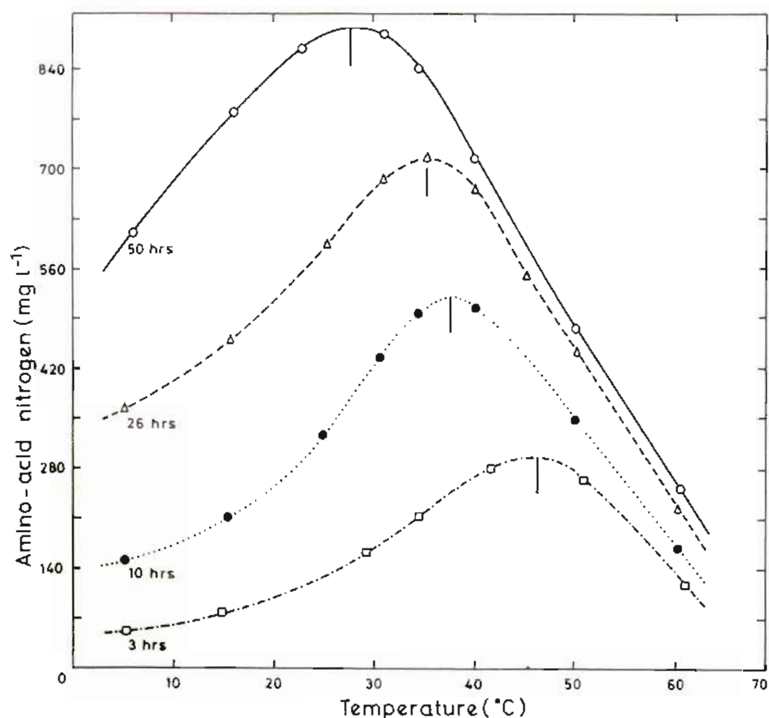


Fig. 3-23: *Tethyum pyriforme americanum. In vitro* effect of temperature on digestion of gelatin by proteinases. Vertical lines indicate optimum temperatures. (After BERRILL, 1929; modified; reproduced by permission of Company of Biologists Ltd.)

and co-authors, 1969). Reserve enzymes may be brought into action via removal of inhibitor(s), presence of specific co-enzyme mechanisms, or specific activating ions.

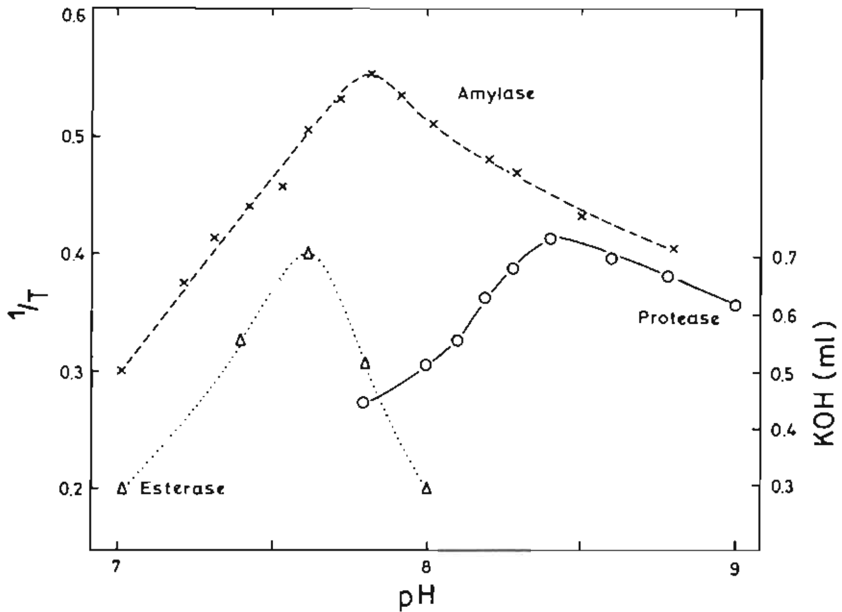


Fig. 3-24: *Ochetostoma erythrogrammon. In vitro* effects of pH on digestive enzymes in the midgut. 1/T: reciprocal of time taken by the amylase–starch mixture to become colourless with iodine; KOH (ml): titer of 0·01 n KOH per 0·1 ml digest of protease–gelatin mixture; per 1 ml digest of esterase-benzyl *n*-butyrate. (After CHUANG, 1963; modified; reproduced by permission of *Biological Bulletin*.)

Classification of digestive enzymes. Digestion mostly involves enzymic hydrolysis of organic macro-molecules, especially carbohydrates, proteins and fats. Accordingly, all digestive enzymes are hydrolases classified under the following categories: (i) Glycosidases (carbohydrases) acting on the glycosidic bonds in carbohydrates; (ii) peptide hydrolases (proteinases, proteases and peptidases) acting on the peptide bonds of protein and their degradation products; (iii) carboxylic ester hydrolases (lipases or esterases) acting on ester bonds of lipids, mostly triglycerides of fatty acids (DIXON and WEBB, 1964).

Glycosidases are characterized by their great specificity towards their substrates. The ability of a particular glycosidase to split carbohydrate appears to depend upon carbohydrate properties, such as glycosidic linkage, type of ring isomerization, and size and structure of the molecule. In animals, complete digestion of the long α-1,4 glucosidic chains of starch (amylose) to glucose is carried out by enzymes (α-glucosidases) in 2 stages: (i) the intact molecule and its larger fractions are digested by a specific glycosidase, α-1,4-glucan-4-glucanohydrolases (α-amylase), producing primarily maltose, some oligosaccharides and small amounts of glucose; (ii) the larger fragments are broken down to free glucose by α-D-glucoside glucohydrolase (maltase). Some carbohydrates and their respective products, formed due to the corresponding enzymes, are indicated in Table 3-8.

Table 3-8

Some carbohydrates of interest in digestive physiology (After HOAR, 1966; modified; reproduced by permission of Prentice-Hall, Inc., Englewood Cliffs, N.J.)

Polysaccharides ($C_6H_{10}O_5$)		Trisaccharides ($C_{18}H_{32}O_{16}$)	Disaccharides ($C_{12}H_{22}O_{11}$)	Monosaccharides ($C_nH_{2n}O_n$)	
				Hexoses ($C_6H_{12}O_6$)	Pentoses ($C_5H_{10}O_5$) e.g. Ribose
Glycogen (Animals) →			Maltose →	Glucose	
Amylases					
Starch (Plants) →	Dextrins →		Maltose		
Cellulose (Plants and animals) →	Cellulodextrins →		Maltases →	Glucose	
Cellulases			Cellobiose		
			Trehalase		
			Trehalose (insects and some plants) →	Glucose	
			Lactose (mammals) → *Lactase*	Galactose + Glucose	
			Sucrose (plants) *Sucrase*	Fructose	
Inulin (Plants) *Inulase* →				Fructose	
		Raffinose (plants) →	Sucrose +		
		Galactosidases (insects) →	Melibiose + *Melibase* →	Galactose + Glucose	
				Fructose	

Peptide hydrolases split protein and peptide molecules by hydrolysis of peptide bonds. Digestion of protein follows a uniform pattern in animals; three main phases of degradation can be recognized. (i) Peptide-peptidohydrolases (endopeptidases) hydrolyse peptide bonds at various places along peptide chains, but do not attack terminal bonds, and exhibit optimum activity in neutral or slightly alkaline media. Examples are trypsin and chymotrypsin; the former hydrolyses peptide bonds, in which the carboxyl group belongs to a basic amino acid, especially lysine or arginine, the latter hydrolyses peptide bonds in which the carboxyl group belongs to an aromatic amino acid. Vertebrates possess acid-producing stomachs* that provide the necessary medium for the activity of pepsin; exceptions are: prochordates, ammocoetus larva and stomachless fishes (BARRINGTON, 1957; CHAO, 1972), the hagfish *Myxine glutinosa* (NILSSON and FÄNGE, 1970) and the holocephalian fish *Chimaera monstrosa* (NILSSON and FÄNGE, 1969b); pepsin attacks peptide bonds with an adjacent aromatic amino acid. Several different cathepsins occur as intracellular proteases in mammalian liver, kidney, and spleen, and extracellularly in many invertebrates, e.g., *Calanus finmarchicus* (BOND, 1934) and *Maja* sp. (MANSOUR BEK, 1934). According to the classification of mammalian cathepsins by FRUTON and MYCEK (1956), cathepsin *A* resembles pepsin in its substrate requirements acting at pH 4, cathepsin *B* resembles trypsin with a pH optimum at 5, and cathepsin *C* (or *C*-like cathepsins) is similar to pancreatic chymotrypsin, exhibiting maximum activities in the pH range of 5·0 to 5·5, with an optimum at pH 6. Cathepsin *C* has been located histochemically in the rhynchocoel *Lineus ruber* (JENNINGS, 1962a), and in triclad turbellarians (JENNINGS, 1962b). (ii) Peptide hydrolases (exopeptidases) specifically hydrolyse terminal bonds in peptide chains. There are two types of peptide hydrolases: one attacks the peptide bond at carboxyl-bearing ends (α-carboxypeptide amino-acidohydrolases), the other at amino terminal ends (α-aminopeptide amino-acidohydrolases) of peptide chains. (iii) Dipeptide hydrolases (dipeptidases and tripeptidases; JØRGENSEN, 1968). The proteins and their respective products formed due to activities of the corresponding peptide hydrolases, along with the respective activating agents, are shown in Table 3-9.

Carboxylic ester hydrolases can be subdivided into three groups: (i) Lipases which hydrolyse esters of higher fatty acids; (ii) esterase hydrolysing esters of shorter acids; the distinction between these two is not sharp, since neither shows marked specificity of substrate and both have fairly broad pH optima (PROSSER and BROWN, 1961); (iii) emulsifiers which are not enzymes and hence are incapable of digesting fatty substances. Emulsifiers play a distinctive and important role in fat digestion and resorption. They reduce the particle size of fatty substances, and thus enlarge considerably the total surface area exposed to lipolytic and esterolytic attack. Consequently they increase digestion rate (VAN WEEL, 1970). In vertebrates, emulsification of fat by bile salts from the liver is extremely important (HOAR, 1966). Surface-tension lowering compounds—like acylsarcosyltaurine in *Cancer*

* Carbonic anhydrase activity, regarded as an indication of the production of hydrochloric acid for digestive purposes, has been observed using histochemical techniques in the acidophil gland cells in the buccal cavity of the rhynchocoel *Lineus ruber* (JENNINGS, 1962a), in the pharynx of the triclad turbellids (JENNINGS, 1962b), and in the intestine of the nematode *Pontonema vulgaris* (JENNINGS and COLAM, 1970).

Table 3-9

Protein-digesting enzymes of animals (After HOAR, 1966; modified and extended; reproduced by permission of Prentice-Hall, Inc., Englewood Cliffs, N.J.)

	'Zymogen'	'Activator Autocatalyst' →	Enzyme	Preferred peptide link
Peptide-peptido-hydrolases	(i) Pepsinogen	HCl / pepsin →	Pepsin	Carboxyl group of dicarboxylic amino acid to amino group of aromatic amino acids
	Trypsinogen	Enterokinase / Trypsin →	Trypsin	Carboxylic groups of arginine or lysine
	Chymotrypsinogen	Trypsin →	Chymotrypsin	Carboxyl group of aromatic amino acids
	Cathepsins			See text
Peptide hydrolases	(ii) Aminopeptidase (Mn, Mg, Zn)			Terminal amino acid with free amino group
	Carboxy peptidase (Zn)			Terminal amino acid with free carboxyl group
Dipeptide hydrolases	(iii) Tripeptidase			Tripeptides
	Dipeptidase (Mn, Mg, Zn)			Dipeptides

pagurus (VAN DEN OORD and co-authors, 1964) and taurochenodeoxycholic acid in *Procambarus clarkii* (YAMASAKI and co-authors, 1965)—in all probability produced by the midgut gland (VAN DEN OORD, 1966), exert an emulsifying action. Taurine conjugated with bile acids is used as intracellular emulsifier in vertebrates (exception: selachians; see JACOBSEN and SMITH, 1968). Perhaps, finely emulsified fats are directly absorbed by many animals (BALDWIN, 1963).

Digestive 'tools'

Certain enzymes and other fluids may act as 'tools' to make the hydrolysable tissue readily accessible to the hydrolytic action of the enzymes (HYLLEBERG KRISTENSEN, 1972). For instance, hydrochloric acid decalcifies the hard crustacean cuticle and the shells of molluscs and echinoderms, speeding penetration of enzymes into the soft tissues; cellulase digests the (cellulose) cell wall of plants and makes the cell sap accessible to enzyme action; chitinase digests the chitin-containing skin of arthropods. Enzymes with tool function may thus be very important, even if present in small quantities only.

Acids. A number of acids are known to occur in the digestive tract of animals. They aid decalcification of crustacean exoskeletons (PANDIAN, 1967b), of molluscan and echinoderm shells (PROSSER and BROWN, 1961), and of calcified algae (e.g. in *Strongylocentrotus purpuratus*: STOTT, 1955; FUJI, 1961; HOLLAND and NIMITZ, 1964; in *Diadema antillarum*: LEWIS, 1964), thus facilitating enzyme penetration into the soft parts of the food organisms.

Hydrochloric acid, a component of the gastric juice of fishes, performs two important tool functions: (i) It lowers the pH to the acidic optimum required for pepsin action: (ii) it aids decalcification of hard external parts of food organisms. In the fish *Ictalurus nebulosus*, gastric secretion was evoked by distending the stomach, employing a plastic sponge; and the volume of gastric secretion, output and concentration of gastric acid, pepsin, and buffering substances were determined (SMIT, 1967). Output and composition of gastric juice as a function of tissue at 20° C are listed in Table 3-10. As time proceeds, total juice output increases; since

Table 3-10

Ictalurus nebulosus. Secretory output and composition of gastric juice, sampled after different secretion periods at 20° C. meq.: milli-equivalents; PU: Pepsin unit; mN: millinormal (After SMIT, 1967; modified; reproduced by permission of Microforms International Marketing Corp.)

Component	Secretion period (mins)				
	30	75	120	180	270
Volume (ml)	1·9	2·9	4·7	6·4	7·7
Acid (meq.)	0·00	0·14	0·52	1·66	2·33
Pepsin (PU)	22	39	52	79	86
Acidity (mN)	2	42	99	231	282
Pepsin concentration ($PU\ ml^{-1}$)	28	16	13	16	14

acid output increases much more than that of pepsin, acidity increases and pepsin concentration decreases. Pepsinogen-secreting cells discharge all accumulated zymogen granules upon gastric stimulation (HIRSCHOWITZ, 1957); hence pepsin concentration remains rather constant except during the first 30 mins; the parietal cells appear to begin secretion and to release acid only after receiving the stimulus; they continue to release the acid during the 270-min test period. Thus, course and speed of release of the different components differ during the secretion period. Acidity increases from 10° to 25° C and drops at 30° C (Table 3-11); but pepsin content is rather independent of temperature and remains between 11·4 and 16·4

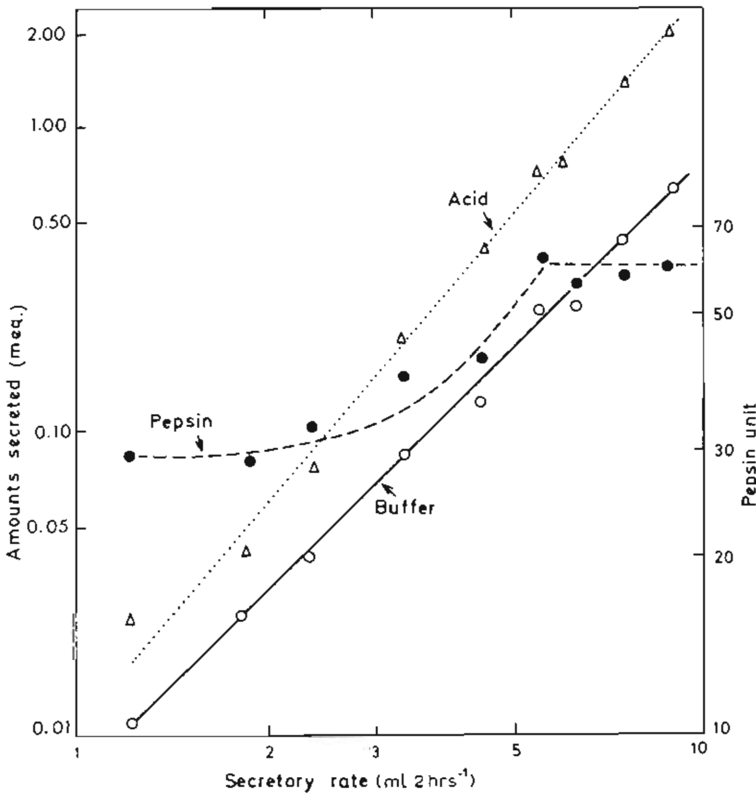


Fig. 3-25: *Ictalurus nebulosus*. Output of gastric acid, pepsin and buffer as a function of secretory rate, measured during a 2-hr period. (After SMIT, 1967; modified; reproduced by permission of Microforms International Marketing Corp.)

pepsin units per ml over the whole range of test temperatures (10° to 30° C). The relationship between gastric secretory rate and output of acid or buffer is illustrated in Fig. 3-25. The composition of the gastric juice changes significantly with secretion rate. The amounts of acid and pepsin in the gastric juice are influenced by temperature and possibly by other environmental factors. Nevertheless, the digestive power of the gastric juice may be enhanced by increased tool function of the acid rather than by the increased pepsin content.

Table 3-11

Ictalurus nebulosus. Acidity, pepsin concentration and buffer concentration in gastric juice secreted at different acclimation temperatures during a period of 2 hrs. PU: Pepsin unit; mN: millinormal (After SMIT, 1967; modified; reproduced by permission of Microforms International Marketing Corp.)

Acclimation temperature (°C)	Acidity (mN)	Pepsin concentration (PU ml^{-1})	Buffer concentration (mN)
10	12 ± 3	16·4 ± 4·1	14 ± 1
15	42 ± 8	13·0 ± 2·3	16 ± 2
20	99 ± 14	12·6 ± 2·5	26 ± 3
25	249 ± 19	11·4 ± 1·6	105 ± 6
30	97 ± 17	11·9 ± 1·9	27 ± 2

In the brook trout *Salvelinus fontinalis*, temperature acclimation does not affect the pH characteristics of pepsin (Fig. 3-26). However, temperature shifts the pH optimum: pH optima were 2·8 at 1° C, 3·6 at 10° C, and 3·0 and 3·6 at 25° C (OWEN and WIGGS, 1971). Such environmental dependence of digestive efficiency must be taken into consideration by ecologists who study organismic performance in different environments. Multiple forms of pepsinogen have been recorded in *Mustelus canis* (MERRETT and co-authors, 1969) and *S. fontinalis* (WIGGS, unpublished). It is not clear whether different pepsinogens are released under different temperature conditions.

Cellulase. Cellulose is a β glucoside and is digested in 2 stages: the intact cellulose molecule is broken into cellobiose and glucose by cellulase; subsequently, the cellobiose is broken by trehelase into glucose (Table 3-8). Until recently, it was questionable whether animals can secrete cellulase and chitinase; these enzymes were thought to have been acquired from symbiotic micro-organisms harboured in the gut of the host. Numerous recent investigations strongly suggest that cellulase can be produced by polychaetes, e.g. *Arenicola marina* (JEUNIAUX, 1963), *Liomia medusa* and *Sabellarstarte indica* (YOKOE and YASUMASU, 1964); by the wood-boring isopod *Limnoria lignorum* (RAY and JULIAN, 1952; RAY, 1959a, b; ARVY, 1969) and other crustaceans, like *Palinurus japonicus*, *Calappa lophus*, *Pachygrapsus crassipes* (YOKOE and YASUMASU, 1964) and *Procambarus clarkii* (YASUMASU and YOKOE, 1965), *Idotea balthica*, *Balanus crenatus*, *Corophium volutator*, *Carcinus maenas* (HYLLEBERG KRISTENSEN, 1972); by gastropods, e.g. *Strombus gigas* and *Pterocerca* sp. (YONGE, 1932), *Dolabella scapula* (HASHIMOTO and ONOMA, 1949), *Melanoides tuberculatus* (FISH, 1955), *Patella vulgata* and *Littorina littorea* and *Aplysia punctata* (STONE and MORTON, 1958), *Oncomelania* sp. (WINKLER and WAGNER, 1959), *Tegula funebralis* (GALLI and GIESE, 1959), *Strombus gigas* (HORIUCHI and LANE, 1965, 1966), *Hydrobia ventrosa*, *H. ulvae*, *Nassarius recticulatus*, *Littorina littorea* (HYLLEBERG KRISTENSEN, 1972); and by bivalves, e.g. *Teredo* (YONGE, 1938; GREENFIELD, 1959), *Xylophaga dorsalis* (PURCHON, 1941),

Mytilus edulis and *Ostrea edulis* (NEWELL, 1953), *Bankia indica* (NAIR, 1955a, b, 1956a, b, 1957), *Scrobicularia plana*, *Cardium edule*, *Mya arenaria* and *Anodonta cygnea* (STONE and MORTON, 1958), *Aplysia vaccaria* (KONINGSOR and HUNSAKER, 1971; KONINGSOR and co-authors, 1972), *Yoldia ensifera*, *Chlamys hericius*, *Mytilus californianus*, *Crassostrea gigas*, *Macoma secta*, *M. nasuta*, *M. lipara*, *Bankia setacea*

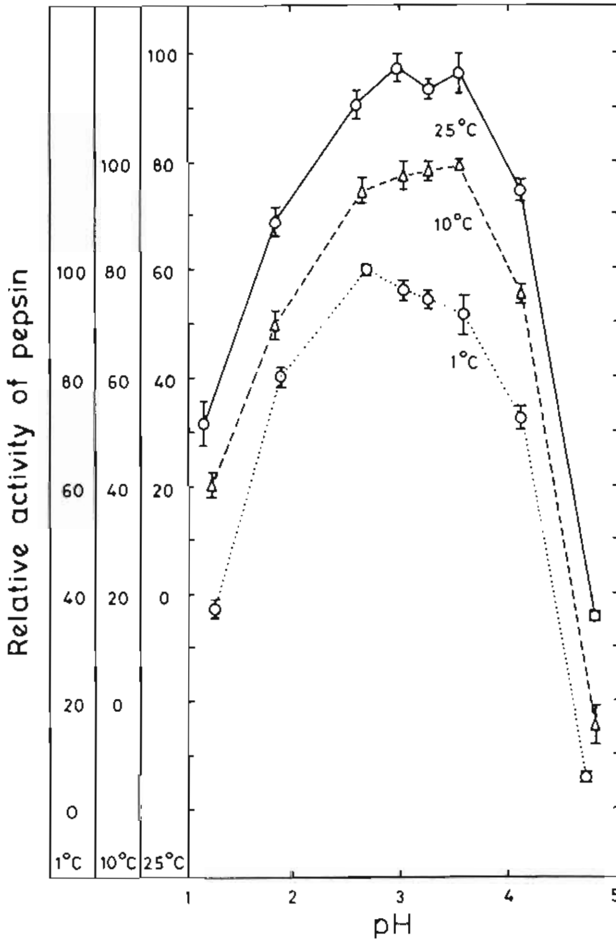


Fig. 3-26: *Salvelinus fontinalis*. Effect of pH on hydrolysis of methylated haemoglobin by pepsin at 25°, 10° and 1° C. (After OWEN and WIGGS, 1971; reproduced by permission of Microforms International Marketing Corp.)

(CROSBY and REID, 1971), *Macoma balthica*, *Mya arenaria* and *Mytilus edulis* (HYLLEBERG KRISTENSEN, 1972). A survey for cellulase in 37 species of marine invertebrates from different phyla and habitats revealed that several molluscs and crustaceans exhibit high cellulase activity (ELYAKOVA, 1972; see also ARAKI and GIESE, 1970 for echinoderms).

Only a few of the above-mentioned workers have conclusively demonstrated that the animals tested possess cellulase and that no cellulolytic micro-organisms are present in the gut (e.g. RAY and JULIAN, 1952). In *Tegula funebralis*, the contribution made by cellulolytic gut bacteria is relatively unimportant (GALLI and GIESE, 1959); in *Levantina hierosolyma*, possible contamination by microbial enzyme has been eliminated experimentally using animals whose gut had been sterilized (PARNAS, 1961). The procedure used by a number of investigators (e.g. HYLLEBERG KRISTENSEN, 1972) does not permit one to decide whether the occurrence of cellulase is due to its production in the animal concerned and/or due to micro-organisms harboured in that animal. Furthermore, much work on degradation of carbohydrates is based on viscosimetry (e.g. HORIUCHI and LANE, 1965; ELYAKOVA, 1972) or turbidity (NAIR, 1955b; NAIR, 1956b, subsequently proved chromatographically the production of cellobiose and glucose) readings, that it is difficult to rely on the results reported. These difficulties have been demonstrated, for example, by EPPLEY and LASKER (1959), who found viscosimetric evidence of alginase in *Strongylocentrotus purpuratus* but failed to show reducing sugar as a result of the alginolysis. Similarly, turbidimetric tests indicate a high level of cellulolytic activity in *Crassostrea gigas*, while calorimetric tests failed to show glucose production. This may also testify to the multiple nature of the enzyme system (CROSBY and REID, 1971). Results by PARNAS (1961) provide good evidence that at least some gastropods possess the ability to secrete cellulase. PARNAS fed *L. hierosolyma* on a mixture of sterile flour and antibiotics until the day on which faecal pellets were completely free from bacteria. He prepared enzyme extracts from individuals with a completely sterilized gut. In normal snails, extracts from salivary gland, crop and digestive diverticula indicated cellulolytic activity, which was high in the extract of diverticula and low in that of salivary gland; in snails treated with antibiotics, the activity persisted only in the diverticula. From this and other results, PARNAS concluded that the cellulase in the digestive diverticula of *L. hierosolyma* is produced by the snail, whereas the cellulolytic activities of crop and salivary glands result either from bacteria or from passage of enzymes produced by the digestive diverticula. Confirming PARNAS' results, HORIUCHI and LANE (1965) reported that the cellulolytic activity of bacteria-free crystalline-style extract of *Strombus gigas* is as active as an unsterile preparation, and that the cellulolytic activities of the crystalline style—together with certain other amylolytic activities—are of molluscan rather than bacterial origin. Although VAN WEEL (1960) categorically denies the secretion of cellulases by molluscs, the foregoing account indicates that it does occur.

A similar ambiguous situation exists with regard to the cellulase of the crystalline style of bivalves. The style of many bivalves has a rich flora of large spirochaetes of unknown enzyme capacity (BERKELEY, 1959). Both DESCHAMPS (1953) and HIDAKA (1955) claim to have isolated cellulose-digesting bacteria from the digestive tract of *Teredo navalis*. On the other hand, DEAN (1958) has found that the style of *Crassostrea virginica* acts as a powerful microbicide. GREENFIELD and LANE (1953) have also failed to demonstrate cellulose-digesting bacteria or protozoans in the gut of *Teredo* sp.; they considered that the enzyme is produced by the animal. Moreover, the differences in pH optima for cellulolytic activity in pre-caecal and post-caecal regions of the gut led them to suggest that more than one enzyme might

be involved (see MYERS and NORTHCOTE, 1959 for a similar report on a gastropod).

Chitinase. Exoskeletal chitin cannot be digested in the absence of chitinase; hence the inner soft tissues are protected from enzyme action (PANDIAN, 1967b; WINDELL, 1967). While PROSSER and BROWN (1961) state that chitinase very rarely occurs in animals, more recent investigations suggest that chitinase is secreted, for example, by the anthozoans *Adamsia palliata*, *Anemonia sulcata*, *Anthopleura balli* and *Edwardsia callimorpha*; the polychaetes *Arenicola marina*, *Spirographis spallanzanii* (JEUNIAUX, 1963); *Sipunculus nudus* (ARVY and GABE, 1952); the crustaceans *Eriocheir sinensis*, *Homarus gammarus* and *Maja squinado* (JEUNIAUX, 1960, 1963); the gastropods *Oxychilus cellarius* (TERCAFS and JEUNIAUX, 1961), *Haliotis tuberculata*, *Gibbula umbilicalis*, *Patella vulgata*, *Nassa reticulata*, *Purpura lapillus* and *Doris* sp. (JEUNIAUX, 1963); the amphineuran *Acanthochites discrepans*, the cephalopod *Sepia officinalis* and the echinoderms *Ophiocoma nigra* and *Anseropoda membranacea* (JEUNIAUX, 1963). The chitinase of *Helix pomatia* and other snails was thought to be solely of bacterial origin (JEUNIAUX, 1954; FLORKIN, 1957 *in:* OWEN, 1966b). Re-investigations by JEUNIAUX (1961, 1963) revealed, however, that a comparison of the chitinolytic activity of gastric juice, intestinal juice and digestive diverticula extract indicates no significant differences between normal and partly sterilized animals. The contribution of chitinolytic bacteria to the production of intestinal chitinase is negligible; the digestive diverticula are the actual site of chitinase secretion (for a survey of chitinases in marine invertebrates consult ELYAKOVA, 1972).

Symbiotic Digestion

FENCHEL (1970) reported that bacteria and other micro-organisms are removed by amphipods from detritus (dead organic matter) particles and digested during passage through the gut, while the detritus itself remains undigested. The findings of GEORGE (1964), NEWELL (1965), HARGRAVE (1970), KISSELEVA and VITYUK (1970) and PAFFENHÖFER and STRICKLAND (1970) reveal that detritus plays only a minor role, whereas the bacteria adsorbed to the detritus are of vital importance in the nutrition of detritus feeders (see also ODUM, 1970). Death of these bacteria and subsequent release of their cytoplasm in the gut of the detritus feeders should alter 'the digestive climate' (NEWELL, 1965).

Apart from such possible nutritive roles, micro-organisms are reported to occur consistently in the gut of a number of marine animals; for instance, in the leech *Platybdella anarrhicae* (JENNINGS and VAN DER LANDE, 1967), the molluscs *Hydrobia ulvae* and *Macoma balthica* (NEWELL, 1965), and the echinoderm *Strongylocentrotus purpuratus* (LASKER and GIESE, 1954; FARMANFARMAIAN and PHILLIPS, 1962).

Micro-organisms can aid in the digestion of cellulose (GALLI and GIESE, 1959; PARNAS, 1961) and chitin (JEUNIAUX, 1961, 1963). Since more than 2 types of cellulases are suggested to occur (GREENFIELD and LANE, 1953; MYERS and NORTHCOTE, 1959; CROSBY and REID, 1971), the microbial cellulolytic activity may hydrolyse another kind of cellulose not processed by the animal's own cellulase, and this may enhance the digestive power of their respective hosts.

Timber-eating isopods, like *Limnoria lignorum*, depend on the fungus *Lulworthia floridina* for a number of amino acids; infesting the timber, *L. floridina* renders it edible to *L. lignorum*. On sterile timber, *L. lignorum* makes no attempt to feed and dies (LANE, 1959; RAY, 1959b). The Indian sphaeromid isopod *Sphaeroma walkeri* depends on fungi (Ascomycetes and Basidomycetes) not only (probably) for amino acids, but also for 'predigestion' of timber. The fungi convert infested timber (cellulose) into cellobiose after 10 weeks; it is only on such 'predigested' timber, that *S. walkeri* feeds (GEORGE, 1965). Many wood-boring bivalves and isopods are also known to depend on fungi for amino acids and/or 'predigestion' (RAY, 1959a). Nutritive and/or predigesting fungi are, therefore, 'external symbionts' of the wood borers.

The abundance of bacteria in the sea-urchin *Strongylocentrotus purpuratus* prompted LASKER and GIESE (1954) to postulate that they digest agar and thus aid the urchin to digest algae (see also PROSSER and BROWN, 1961, p. 117). But the bacteria are hardly essential, since their numbers can be drastically reduced without ill effects to the urchin (FARMANFARMAIAN and PHILLIPS, 1962). Whether some micronutrients are provided to the host has yet to be investigated.

AUTRUM and GRAETZ (1934) and GRAETZ and AUTRUM (1935) reported that the enzymes (endopeptidases) initiating proteolysis are absent in the freshwater leech *Haemopis sanguisuga*. BÜSING (1951) and BÜSING and co-authors (1953) reported the consistent presence of a single species of bacterium *Pseudomonas liquefaciens* (BULLOCK, 1961) in the gut of the leech *Hirudo officinalis*; inclusion of antibiotics in the food *in vivo* inhibited digestion and *in vitro* studies showed that *P. liquefaciens* is capable of slow digestion. Extending the studies to several freshwater and marine leeches, JENNINGS and VAN DER LANDE (1967) established that the gastrodermis of these leeches is not differentiated morphologically into secretory and absorptive structures; exopeptidases are produced in the intestinal gastrodermis,* but endopeptidases which initiate digestion in most animals, as well as lipases and amylases are also not produced; many other symbiotic bacteria regularly occur in the gut of leeches, e.g. species of *Aeromonas* (in *Hirudo*), *Pseudomonas* (in *Hemiclepsis* and *Piscicola*), *Xanthomonas* (in *Theromyzon*), and *Pseudomonas*, *Aeromonas* and *Klebsiella* (in *Herpobdella*); all the cultures (100%) of gut contents from freshwater leeches, e.g. *Hirudo medicinalis* and *Herpobdella octoculata* contained proteases, lipases and amylases. In such cultures of the marine leech *Platybdella anarrhicae*, only 31% of the cultures contained proteases, 41% contained lipases, and 0% contained amylases. Thus, there is evidence that bacteria participate, to a lesser but significant degree, also in the digestion of blood in marine leeches. In view of their fairly general occurrence in leech intestines (REICHENOW, 1922; JASCHKE, 1933; HOTZ, 1938), bacteria may also contribute vitamins (probably of the B group, since these will be scarce in the diet of sanguivorous leeches). A comparable situation exists in sanguivorous arthropods, where symbionts contribute significant

* Occurrence of exopeptidases in the gastrodermis at all times in an active form—independent of the nutritive state of the leech—is quite different from the situation in other invertebrates, such as flatworms and nemerteans, where an amino-peptidase reaction can be demonstrated only at specific times after feeding (ROSENBAUM and ROLON, 1960; JENNINGS, 1962a, b). JENNINGS and VAN DER LANDE (1967) considered that these may be concerned with intracellular as well as extracellular digestion, since haem compounds were found in the gastrodermis apparently undergoing slow digestion.

quantities of vitamins (WIGGLESWORTH, 1965). Comparative studies on the digestive physiology of marine predatory and sanguivorous leeches are required for a more complete assessment.

Enzyme spectrum and food of heterotrophs

A definite correlation exists between the food of an animal and the nature and relative strength of its digestive enzymes (YONGE, 1937). Evidence supporting this statement has been provided by BARRINGTON (1957), VAN WEEL (1961), PROSSER and BROWN (1961), JØRGENSEN (1968) and CROSBY and REID (1971). A number of publications concern changes in the diet during different life stages (e.g. of crustaceans and fishes) and their ecological significance in regard to increasing exploitation of different food organisms (DARNELL, 1958; PANDIAN, 1969b). In the prawn *Palaemon serratus*, amylase activity rapidly reaches a high level during the second zoea II stage, whereas increase in protease activity occurs only at the fifth zoea or mysis stage. All these changes are related to modifications of the shrimp's diet (VAN WORMHOUDT, 1973). AL-HUSSAINI (1949) reported that the concentration of carbohydrases is highest in the predominantly herbivorous *Cyprinus carpio* and lowest in the predominantly carnivorous *Gobius gobio*, while the relationship for protease is the exact opposite. Reports of similar observations on other fishes (FISH, 1960; COCKSON and BOURNE, 1972) suggest a definite correlation between food and spectrum and concentration of enzymes.

HYLLEBERG KRISTENSEN (1972) studied the carbohydrase spectrum of several marine invertebrates and found a poor correlation between the type of diet and carbohydrases present in the gut. The carbohydrase spectrum of the carnivorous snail *Nassarius reticulatus* proved to be very similar to that of the detritus feeding *Hydrobia ventrosa*, indicating that the spectra of carbohydrases cannot be predicted from information of ingested food. BROWN (1969), however, showed that though *Nassarius obsoletus* possesses all structural features of a typical carnivorous rachiglossan, it produces hydrolytic enzymes necessary to break down the principal constituents of algae, and enzymatically is a facultative herbivore/carnivore. REID (1968) surveyed digestive enzymes in a variety of suspension-feeding bivalves and found no significant relationship between gut morphology and enzyme distribution. KOZLOVSKAYA and VASKOVSKY (1970) concluded from their extensive survey on proteolytic activities (on globulin, casein and gelatin) of over 50 marine invertebrates that proteolytic activity is connected with the respective systematic position rather than with other factors.

Poor correlation between food and carbohydrase spectrum may be explained by 2 facts: (i) A number of carbohydrases are of universal occurrence in plants and probably also in animals; alginases occur not only in phytophagous species, but also in carnivorous species, such as *Purpura lapillus* and *Nassa reticulata*; there is no correlation between dietary habits and amount of alginase present in the digestive system (FRANSSEN and JEUNIAUX, 1963 *in:* OWEN, 1966b). Demonstration of trehelase activity in crude extracts cannot indicate whether the tested animals possess trehelase to digest plants or other animals, or whether the enzyme is used elsewhere in the metabolism. Leucine amino-peptidase activity—diagnostic of the presence of several peptidases—is consistently present in an active form in the blood

system of rhynchocoelan worms, irrespective of the nutritive state of the animal (GIBSON and JENNINGS, 1967; JENNINGS and GIBSON, 1969). (ii) According to earlier theories (VEIBEL, 1958), only 5 different enzymes are necessary to hydrolyse all glycosides. Neither this theory, nor the view that hydrolysis of each glycoside demands a specific enzyme, are accepted today. Many records exist of highly purified enzymes attacking a number of substrates with identical linkage between the molecules (BRUNI and co-authors, 1969; HASEGAWA and NORDIN, 1969). Studies on esterase zymograms of the gastric fluid and digestive diverticula of 8 species of *Macoma* revealed no definite relationship between diet and distribution of esterases (REID and DUNNILL, 1969). Further work is needed to determine the degree of correlation between enzyme spectrum of a particular species and its food constituents.

Digestive adaptations

Quality of food. To what extent can enzyme spectrum and enzyme concentration be adjusted to changes in diet? Individuals of the fish *Brevoortia* sp., which consume much fatty food, have a lipase concentration no greater than individuals consuming less fatty food (CHESLEY, 1934). The predominantly herbivorous-omnivorous fish *Tilapia mossambica* (after 15 days acclimation to animal diet) was exposed to an unrestricted supply of *Tubifex tubifex*, *Chironomus* larva or *Gambusia affinis* and consumed 60 cal g^{-1} fish day^{-1} (PANDIAN and RAGHURAMAN, 1972; RAGHURAMAN, 1973); this consumption amounts to only 1/3 of what the fish would eat if offered algae and detritus. Separate groups of *T. mossambica* were fed rabbit muscle (protein diet), bread (carbohydrate diet) and fatty beef (fat diet); subsequently the production of enzymes was measured. Pepsin and lipase activities were not affected by diet but trypsin and amylase showed adaptive changes (NAGASE, 1964). More critical studies are required before we can present ecologically meaningful details on digestive adaptations to changes in food quality in marine animals.

Quantity of food. Fishes consuming different food rations do not exhibit significant variation in the efficiency of (digestion and) absorption (GERKING, 1955; DAVIES, 1963, 1964; PANDIAN, 1967d). However, when fed nearly 3 times more food, the fish *Lepisosteus platyrhincus* doubled its rate of digestion (HUNT, 1960). More recently, WINDELL (1966), KITCHELL and WINDELL (1968), WINDELL and co-authors (1969) and TYLER (1970) have also reported that increased meal size augments digestion rate. PANDIAN (1967d) concluded from his data that there exists a range of feeding levels over which efficiency of digestion and absorption remain unchanged: rates of enzyme secretion and/or activity increase to deal with increased food intake over a certain range of feeding levels. Enhanced peristalsis can considerably increase the digestive power of the enzymes present (VONK, 1964, p. 356), and increased meal size can elicit extended periods of augmented peristalsis. In the cephalopod *Octopus cyanea* digestion rate is so regulated in response to different ration levels that a constant percentage of food is digested per unit time (BOUCHER-RODONI, 1973).

Intracellular Digestion

General aspects

Intracellular digestion is regarded as a primitive type of digestion derived from protozoans. It constitutes a process in which semi-digested food is absorbed and subsequently digested within the cell. Intracellular digestion occurs in various invertebrates: (i) In gastrodermal (phagocytosis) cells, e.g. of coelenterates *Companularia* sp. (LUNGER, 1963), *Aiptasia* sp. (LENHOFF and co-authors, 1968); platyhelminthes *Syndesmis franciscana* (JENNINGS and METTRICK, 1968; METTRICK and JENNINGS, 1969), *Polystoma integerrimum* (JENNINGS and HALTON, 1965); rhynchocoel *Lineus ruber* (JENNINGS, 1962a, 1969; JENNINGS and GIBSON, 1969); arthropod *Limulus polyphemus* (BROWN and WHITEGIVER, 1971); and molluscs (OWEN, 1966b). (ii) In mobile cells, e.g. digestive archaeocytes of sponges (RASMONT, 1968); amoebocytes of annelids (KERMACK, 1955) and molluscs (YONGE, 1926a, b; OWEN, 1966b); and coelomocytes of echinoderms (FERGUSON, 1969).

Among the metazoans, extracellular digestion appears to have evolved first for reducing large particle sizes which would allow intracellular digestion to take over (PROSSER and BROWN, 1961, p. 111). However, macrophagy need not necessarily result in pronounced extracellular digestion: coelenterates, platyhelminthes, septibranchs (YONGE, 1928; PURCHON, 1956), the isopod *Eurydice pulchra* (JONES and co-authors, 1969) and the king crab *Limulus polyphemus* (BROWN and WHITEGIVER, 1971) are all macrophagous carnivores which possess intracellular digestion. Nor is intracellular digestion typical of all microphagous feeders: there are conspicuous exceptions among microphagous animals and fluid feeders, in which only extracellular digestion takes place, e.g. in nuculids (OWEN, 1956); tunicates (MORTON, 1960); archiannelids (JENNINGS and GELDER, 1969); hirudineans such as *Pontobdella muricata, Platybdella anarrhicae* (JENNINGS and VAN DER LANDE, 1967); and nematodes such as *Pontonema vulgaris* (JENNINGS and COLAM, 1970).

Thus intracellular digestion is not directly associated with taxonomic status or size of food. Intracellular digestion seems to have evolved as adaptive process among metazoans to deal at least with 2 different trophic situations: (i) To accomplish incessant digestion at a slow rate, in pace with the rate at which minute food particles are continuously ingested (e.g. in bivalves); and (ii) to survive during periods of low food supply by slowing down the digestion rate (e.g. in coelenterates and turbellarians). Gastrodermic cells, in which digestion proceeds very slowly, serve as storage organs. That microphagy is associated with intracellular digestion (e.g. in bivalves) has been repeatedly documented (YONGE, 1937). To appreciate the second point, it is necessary to consider quantitative aspects of intracellular digestion.

Quantitative aspects have been investigated in coelenterates. Measuring radioactivity in the lumen of the coelenteron and certain tissues of animals fed on ^{35}S-labelled protein, LENHOFF (1961) and LENHOFF and co-authors (1968) were able to determine rate and efficiency of phagocytosis, and site and extent of protein digestion. Following ingestion, the food is phagocytized somewhat faster during the first 3 hrs than in the latter $3\frac{1}{2}$ hrs; as much as 80 or 90% of the phagocytized food is retained by the anemone *Aiptasia* sp. (MURDOCK and LENHOFF, 1968) or by

symbiotic and aposymbiotic *Chlorohydra virridissima* (MUSCATINE and LENHOFF, 1965). The gastrodermal cells retained most of the phagocytized labelled food in an incompletely digested state within their food vacuoles for about 1 day before transferring a significant portion to the epidermis. On the second day following ingestion of a single meal of naupli, the body substance of *Hydra* sp. increased by 30%; this slow conversion of ingested protein into hydra protein was partly due to the gradual digestion occurring within food vacuoles (LENHOFF, 1961). Histochemical studies indicate that extracellular digestion lasts 12 hrs, and intracellular digestion lasts more than 48 hrs in turbellarians (JENNINGS, 1962b) as well as monogeneans (HALTON and JENNINGS, 1965); hence the slow digestion rate appears to be characteristic of macrophagous carnivores with intracellular digestion. The slow rate of intracellular digestion, as compared to extracellular digestion, may confer benefits by performing a storage function. In many sessile coelenterates, feeding in nature is a fortuitous and often discontinuous process, and one meal may have to last for several days. Predatory efficiency of colenterates, such as species of *Hydra*, is only 7%, i.e. half of that (13%) observed for other animals (p. 93), hence it would be inefficient to break down the meal in a single process and to maintain large amino-acid pools. Gradual nutrient supply for cellular needs seems more beneficial (LENHOFF, 1968b).

In leeches, one meal may have to last for several days, weeks or months. Paradoxically, extracellular digestion prevails in leeches. Endopeptidases are not produced by the digestive system of the leeches *Pontobdella muricata* and *Platybdella anarrhicae*, and proteins are digested by exopeptidases of various groups and bond specificities, which progressively remove terminal amino residues from protein chains (JENNINGS and VAN DER LANDE, 1967). *In vitro* tests reveal that pure leucine amino-peptidase can remove stepwise 109 of the 185 amino-acid residues of papain, and completely hydrolyze the polypeptide glucagon (HILL and SMITH, 1958, 1959, 1960). Comparable activity in the animal digestive system would be slow and inefficient, without initial intervention of endopeptidases to provide a greater number of terminal units for exopeptidase attack. The leeches are, however, tolerant to retarded and extended digestion (JENNINGS and VAN DER LANDE, 1967). In freshwater leeches like *Hirudo medicinalis* and *Herpobdella octoculata*, symbiotic micro-organisms are known to produce a certain amount of endopeptidases; but microbial contribution of endopeptidases is very limited in marine leeches, e.g. *Pontobdella muricata* and *Platybdella anarrhicae* (p. 124). For the ecologist it seems important to realize that animals which cannot easily procure sufficient food in time, compensate for this by switching over either to intracellular digestion or to the extracellular digestion without initial intervention of endopeptidases.

Gastrodermic intracellular digestion

Gastrodermal phagocytic food uptake has been described in some detail for the planarian *Dugesia tigrina* by OSBORNE and MILLER (1962) and for the rhynchocoel *Lineus ruber* by JENNINGS (1960, 1969). Fifteen minutes after ingestion of the test meal (quail erythrocytes), haemolysis, rupture of nuclear membranes and release of nuclear contents occur extremely rapidly in the intestine of *L. ruber*. The plasma membrane between cilia (phagocytosis by ciliated cells is rare in animals and its

biological significance in rhynchocoels remains uncertain; JENNINGS, 1969; JENNINGS and GIBSON, 1969) and microvilli shows bud-like protuberances, which rapidly extend outwards into the lumen and develop into pseudopodia-like lamellae; eventually, the lamellae tips meet and fuse, trapping semi-digested food between themselves and the cell surface. In this way, a vacuole is formed which is bounded by a single membrane containing semi-digested food. As the vacuole moves away from the cell surface, more lamellae develop, and the entire process may be repeated several times (JENNINGS, 1969). Material ingested by phagocytosis remains, however, separated from the cell proper by the enclosing membrane (PROSSER and BROWN, 1961; OWEN, 1966b).

In the vacuoles, digestion is completed in 1 or 2 phases: (i) The supposed intracellular digestion in the digestive diverticula of bivalves (OWEN, 1966b) appears to take place in the acidic phase with an average pH of 5·7 (YONGE, 1925, 1962); optimum pH for amylase in the diverticula of the clam *Venerapsis philippinarum* is 5·5 (HORIUCHI, 1963). Several intracellular enzymes of *Limulus polyphemus* exhibit optimum activity in the acid range, e.g. acid phosphatase at pH 4·5; acid ribonuclease at pH 4·3; aryl sulphatase at pH 6·0; and 3 glucuronidase at pH 4·3 (BROWN and WHITEGIVER, 1971). (ii) In rhynchocoelans and flatworms intracellular digestion requires 2 phases. The contents of the food vacuoles are first attacked by endopeptidases similar to those secreted into the gut lumen in acidic medium (pH 5·0), and then by carbohydrases, true lipases (homologous with mammalian pancreatic) and exopeptidases (such as leucine amino peptidase)—all acting in concert in an alkaline medium of pH 7·2 (JENNINGS, 1962b, 1969; JENNINGS and METTRICK, 1968; METTRICK and JENNINGS, 1969). Acidic and alkaline phosphatases are associated with the appropriate digestive stage (JENNINGS and GIBSON, 1969). Acid phosphatase, closely linked with the first acidic phase, is suggested to be concerned with food vacuole formation (ROSENBAUM and ROLON, 1960), and alkaline phosphatase facilitates the release of energy needed for secretion of various enzymes and absorption of digestive products from the vacuole (JENNINGS, 1962a). According to current views, intracellular digestion of phagocytized material takes place within the phagosome/lysosome system of subcellular organelles; hydrolases are responsible for hydrolysis of the sequestered material having been synthesized by the digesting cell (for a review, consult DINGLE and FELL, 1969; for papers on marine animals, BEVELANDER and NAKAHARA, 1966; PASTEELS, 1969, 1971; BROWN and WHITEGIVER, 1971).

In the Monopisthocotylea, digestion (of gill epidermis of fishes)—even though completed intracellularly—causes no problem regarding the elimination of unwanted end-products. In the Polyopisthocotylea, intracellular digestion (blood of host) results in intracellular production of haematin. This insoluble substance is eliminated via the deciduous gastrodermis, involving considerable wastage of cellular material; thus the polyopisthocotylids seem incompletely adapted to blood diet (HALTON and JENNINGS, 1965; JENNINGS, 1968). Trematodes form haematin extracellularly, or degrade haemoglobin via other pathways, which permit elimination of unwanted iron in soluble form (HALTON, unpublished). Disintegration of gastrodermal cells in the Polyopisthocotylea leads to release of intracellular enzymes which unite with gut contents and initiate hydrolysis of the next meal (HALTON and JENNINGS, 1965). Such a possibility may exist also in free-living

echinoderms, in which coelomocytic digestion occurs (FERGUSON, 1969), since mortality of coelomocytes can considerably alter the 'digestive climate' via autolysis and enzymes. The digestive gland of the lamellibranch *Lasaea rubra* discharges spheres, which rupture to release residues of intracellular digestion, and may also release enzymes responsible for intraluminar digestion (MORTON, 1956).

Phagocytosis

Amoebocytes or coelomocytes engulf particles from the gut and digest, transport and release nutrient materials within the tissues; they play a dual role of hydrolysis and transportation. Their importance has been widely recognized (e.g. WAGGE, 1955; OWEN, 1966b; RASMONT, 1968; FERGUSON, 1969; JEUNIAUX, 1969). In deposit-feeding polychaetes like *Arenicola marina* (KERMACK, 1955) and in holothurians, it seems more beneficial to digest and transport nutrient matter via phagocytes than to secrete the required amount of enzymes into the large quantity of indigestible matter (see, however, REID, 1968). Phagocytic amoebocytes (VONK, 1924; YONGE, 1926b) are known to 'pick up' selectively the more inert particles and to break them down or to carry them off (FERGUSON, 1969). MARDSON (1966) described the invasion of the anterior intestine of the polychaete *Hermodice carenculata* by coelomocytes after 2 to 4 hrs of feeding; after 4 to 8 hrs, they occur in the coelom carrying a load of nutrient material; their occurrence is periodic, like the one observed for calcium-transporting amoebocytes in *Helix pomatia* (WAGGE, 1955). Amoebocytes of lamellibranchs contain intracellular amylase, protease and lipase (YONGE, 1926b; TAKATSUKI, 1934; GEORGE, 1952; ZACKS and WELSH, 1953). No detailed data are available regarding the contribution of amoebocytes to digestion; OWEN (1966b), however, has postulated that digestion efficiency of amoebocytes varies in different animal taxa.

Pinocytosis

Pinocytosis involves discontinuous fluid uptake in form of droplets; these are engulfed or sucked in by cells; the process is thus different from the well-known uptake of fluid by diffusion through the cell surface (HOLTER, 1959). Electron microscopic studies have indicated that the difference between phagocytosis, where the particle ingested is visible, and pinocytosis, where the particle is invisible, is not sharp; the vesicle formed in the latter process may be as small as 0·01 μ (OWEN, 1966b). Evidence for occurrence of pinocytosis in marine animals has been provided by several authors (e.g. in the isopod *Eurydice pulchra* by JONES and co-authors, 1969, and in bivalves by BEVELANDER and NAKAHARA, 1966; OWEN, 1966b; FANKBONER, 1971).

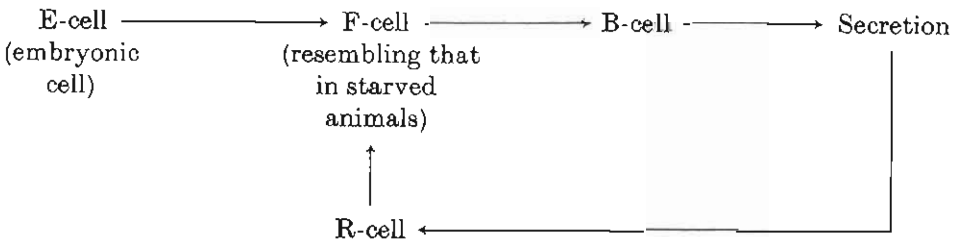
Pinocytosis can be induced by specific salts and proteins. Cations such as sodium and potassium induce different morphological types of pinocytosis, particularly with regard to the width of the channels and the way they are formed. For every salt or protein there exists an optimum range (about 0·1 M) of concentration, where pinocytosis is very intense. The upper limit of concentration, however, is set by the toxic action of salt upon the cytoplasm and lies between 0·25 and 1·0 M. There appears to be no simple correlation between osmotic pressure of the solutions and their pinocytosis-inducing effect (CHAPMAN-ANDRESEN and PRESCOTT, 1956; CHAPMAN-ANDRESEN, 1958).

Enzyme Secretion

Synthesis and extrusion

Enzymes are considered to be products of lysosomal activity. The primary lysosomal particles contain hydrolases capable of splitting almost all types of biological macromolecules and some small molecules. The surrounding membrane of the particles does not allow the passage of hydrolases out of the lysosomal system or entry of substrates into it. This behaviour of the particles is referred to as structural latency of the enzymes (MÜLLER, 1967). Enzyme extrusion may take place with intermittent release of lysosomal contents of the cell in form of droplets, spheres or granules; in the majority of marine animals, enzyme extrusion is apocrine, involving practically no loss of the gland cell itself (e.g. *Nassarius obsoletus*, BROWN, 1969, p. 460), or merocrine, i.e. involving loss of parts of the gland cell (e.g. midgut caecum of the isopod *Eurydice pulchra*, JONES and co-authors, 1969); the gland cell is not destroyed and regenerates its contents. In other marine animals (e.g. in the hepatopancreas of the lobster *Homarus* sp., HIRSCH, 1937), enzyme extrusion is holocrine, involving loss of the entire gland cell; spent holocrine cells are replaced by new cells via cell division in the distal end of the tubules. Endopeptidases—like pepsin, trypsin and chymotrypsin—are synthesized in their respective inactive forms (pepsinogen, trypsinogen and chymotrypsinogen); upon extrusion into the gut lumen, they are activated by hydrochloric acid, enterokinase and trypsin, respectively. This mechanism of functional latency may be interpreted as a safety device to prevent lysis of gland cells.

Enzyme-secreting cells in digestive tract and associated organs exhibit fluctuations (maxima and minima) of secretory activities. Histological analyses performed at different times after feeding have revealed a succession (waves) of various cell types. The cell types may represent (i) basically different types, each with its own specific properties (resorption, storage, extrusion) and genesis; or (ii) one cell type which undergoes cyclic changes of restitution, storage of 'ripe' secretion and extrusion at different times after feeding. This is still a point of dispute. Investigators supporting the first possibility have recognized 2 different cell types (VAN WEEL, 1950, assumes 4 different types): (i) B-cells ('Blasenzellen'), the true secreting cells; (ii) R-cells ('Restzellen'), the storage and resorption cells (e.g. KRIJGSMAN, 1928; ABOLINS-KROGIS, 1960). The different cell types in the crustacean midgut caecum have been reported to represent different stages in the secretion–absorption cycle of a single cell type (VAN WEEL, 1955; BEECHER-MOORE, 1959; STANIER and co-authors, 1968). On the basis of electron micrograph profiles of midgut caeca of starved or fed isopods *Eurydice pulchra*, JONES and co-authors (1969) proposed the following cyclic changes:



Summarizing results obtained by several authors on gastropods and bivalves, OWEN (1966b) supported the concept of cyclic changes; however, he did not rule out the possibility that certain cells are specialized for secretion or phagocytosis in some molluscs.

Digestive periodicity

Endogenous factors. Continuous feeders are characterized by the continuity of food inflow, secretion of hydrolases, digestion and absorption. Periodic feeders (carnivores, intertidal animals) obtain a large amount of food during a restricted period; the meal proceeds along the digestive tract, involving sequential muscular action or glandular secretion, each process occurring at a precise time and only over a limited period. At least 4 kinds of stimuli can affect glandular secretion: (i) 'Neurogenic' stimuli originating from sensory perceptors associated with feeding; (ii) local mechanical stimuli; (iii) vascular transport of a secretion-stimulating agent to the gastric glands (e.g. gastro-intestinal hormones); and (iv) local action of secretagogues or chemical substances, present in the food, or formed during its digestion (GORBMAN and BERN, 1962).

'Neurogenic' stimuli. There is evidence that secretion in fishes is under nervous and hormonal control (SMIT, 1968). In the bullhead *Ictalurus nebulosus*, swallowing is accompanied by a copious flow of gastric juice, and this secretion is evoked by an unconditioned reflex (KRAYUKHIN, 1958, 1959). In the burbot *Lota lota*, digestion rate is greatly reduced by vagotomy, indicating that the vagus nerve plays a vital role in the regulation of secretion of gastric juice (GOMAZKOV and KRAYUKHIN, 1963). Secretion rate also depends on the kind of food ingested. These observations allow to distinguish a cephalic phase and a chemical phase—the first mediated by the vagus nerve, the latter possibly by hormones (see also HOAR, 1966).

In the crayfish *Procambarus clarkii*, neuro-endocrine secretion appears to regulate synthesis and flow of amylase of the midgut gland. Removal of eyestalks from starved individuals results in reduced total-nitrogen and amylolytic activity as well as in a marked pH increase and in a decrease of gastric-juice production. At the same time, the midgut gland suffers a loss of RNA. Starvation of normal crayfish results in an increase in amylolytic activity in the midgut gland. Since a neuro-endocrine substance of the eyestalk stimulates RNA synthesis in the midgut gland, FINGERMAN and co-authors (1967) concluded that RNA is responsible for amylase synthesis. Studies of digestive juice and enzyme extracts in a moulting *P. clarkii*, however, yield different results. More pertinent information is needed before we can assess the dynamics of digestive-juice extrusion and its relation to pH and environmental factors (e.g. VAN WEEL, 1970) in more detail.

Local mechanical stimuli. In fishes, digestion of voluminous meals is more intensive than that of small ones (KRAYUKHIN, 1958), suggesting increase in secretory rate due to local mechanical stimulation of the stomach wall. There is sufficient evidence to show that fishes which consumed an increased ration exhibit faster digestion (PANDIAN, 1967b; KITCHELL and WINDELL, 1968; WINDELL and co-authors, 1969). According to TYLER (1970), digestive activity of enzymes is not simply surface-dependent, but also volume-dependent; for the different intensities

of local mechanical stimuli—which may, in turn, alter peristalsis rate—depend to a greater extent upon the volume of meal (see also p. 126).

Gastro-intestinal hormones. Vertebrate physiologists have described a series of gastro-intestinal hormones associated with the regulation of digestive-gland secretion (Table 3-12). There is evidence that such gastro-intestinal hormones occur in fishes; secretin has been detected in the anterior intestine of trout and hagfish (BAYLISS and STARLING, 1903). Results obtained by NILSSON and FÄNGE (1969b) indicate the presence of secretin and pancreozymin in the holocephalian *Chimaera monstrosa*, suggesting that digestive functions are under hormonal control as in higher vertebrates. In the cyclostome *Myxine glutinosa*, NILSSON and FÄNGE (1970)

Table 3-12

Site of origin and function of gastro-intestinal hormones of vertebrates (Based on data of GORBMAN and BERN, 1962; reproduced by permission of Wiley, New York)

Hormone	Site of origin	Function	Remarks
Gastrin	Pyloric stomach	Balances production of HCl in stomach	Release is under nervous control
Enterogastrone	Duodenum	Inhibits gastric secretion (HCl) and motility	Does not affect pepsin secretion
Urogastrone	Extract from human or dog urine	Inhibits gastric secretion (HCl) in birds and mammals	Does not affect gastric secretion in frogs
Secretin	Anterior intestinal mucosa	Stimulates flow of pancreatic juice and bile formation in liver	Release is not under nervous control; secretion inactivated in blood. Mammalian secretin stimulates the same functions in species of *Raja* and *Octopus*
Pancreozymin	Anterior intestinal mucosa	Stimulates production of pancreatic enzymes	Not under nervous control; inactivated in blood and tissues
Cholecystokinin	Duodenum	Empties gall bladder	Inactivated in blood plasma
Villikinin	Villi	Controls mobility of absorptive villi	—

obtained evidence for the presence of secretin but not of cholecystokinin. Injection of mammalian secretin is known to 'gear up' the secretion of pancreatic juice in the skate *Raja* sp. and of hepatopancreatic juice in the cephalopod *Octopus* sp. (KOSCHTOJANZ and IWANOFF, *in*: GORBMAN and BERN, 1962).

Local action of secretagogues. In marine invertebrates, digestion is either completely intracellular (protozoans, sponges) or extracellular (annelids, crustaceans, cephalopods), or both extracellular and intracellular (coelenterates, flatworms, bivalves). In most higher invertebrates, digestion, secretion and storage take place side by side, and a nervous control of gut motility (with some direct response of the enzyme-secreting cells to the presence of secretagogues) appears to be adequate

(HOAR, 1966). For example, in a number of coelenterates, the presence of reduced glutathione, released from injured prey, induces an elaborate series of feeding and digestion activities (LENHOFF, 1968a, b, 1969). The physiological mechanism by which coelenterate gastrodermal cells recognize the presence of (foreign) food-protein has been studied in the anemone *Anthopleura elegantissima* by PHILLIPS and co-authors (1966 *in*: LENHOFF, 1968b). ^{14}C-labelled albumin stimulates the anemone cells (in culture) to synthesize a macromolecule (molecular weight ca. 200,000), called 'complexor', which binds and hydrolyzes the labelled protein. It is not clear whether the relationship involved between protein and 'complexor' is similar to the one of classical antigen and antibody, and whether the same or a similar mechanism is involved in recognition of protein and release of corresponding protease among invertebrates.

Exogenous factor: Tidal cycle. For the marine ecologist, the effects of exogenous factors on digestion are of particular importance. In the upper-shore bivalve *Lasaea rubra*, feeding is restricted to less than 2 hrs per tidal cycle (BALLANTINE and MORTON, 1956). Histochemical examinations of *L. rubra* at known times after feeding and tide exposure revealed the following 4 phases in the activity of digestive diverticula (MORTON, 1956): (i) **Absorption**: the tubules are widely distended and the digestive cells are flattened (Fig. 3-27); (ii) **digestion**: the digestive cells are loaded with ingested material and bulged into the lumen of the tubules; (iii) **excretion**: the contents of each bulging cell are nipped off into the lumen of the tubules to form a small sphere filled with yellow vacuoles; and (iv) **final phase**: the whole diverticula are pale and colourless and the tubules contain mostly 'empty' cells. At no single period were all cells at a particular stage; however, MORTON recognized a well-marked predominance of activity, which could be correlated to feeding time and tidal exposure. Possibly, the net activity of the digestive system of *L. rubra* is under tidal control (MORTON, 1956). Feeding has been shown or suggested to be related to the tidal rhythm in several other intertidal animals; for instance, in the isopods *Marinogammarus obtusatus* and *M. pirloti* (MARTIN, 1966), in the cirripede *Balanus balanoides* (RITZ and CRISP, 1970), in the winkle *Littorina littorea* (NEWELL and co-authors, 1971), and in the bivalves *Dreissena polymorpha* (MORTON, 1969) and *Cardium edule* (MORTON, 1970). Tidal rhythmicity of feeding and digestion may well prove to be much more widespread in other animals than is at present appreciated (see also Chapter 9).

(b) Absorption and Related Processes

After completion of extracellular digestion, part of the gut content is transferred into the animal system. Three main processes are involved in the transfer of partially or fully digested food into the gastrodermal cells or phagocytic amoebocytes for ultimate circulation: **phagocytosis**, **pinocytosis**, and **absorption**, which may be due to passive diffusion or active transport.

Phagocytic amoebocytes or gastrodermal cells may pick up particles larger than colloids. The intake of particles of colloidal size (100 mμ diameter) in a way that can be made visible microscopically is called pinocytosis. Transfer of particles below a diameter of 100 mμ is referred to as absorption (VONK, 1964, p. 378).

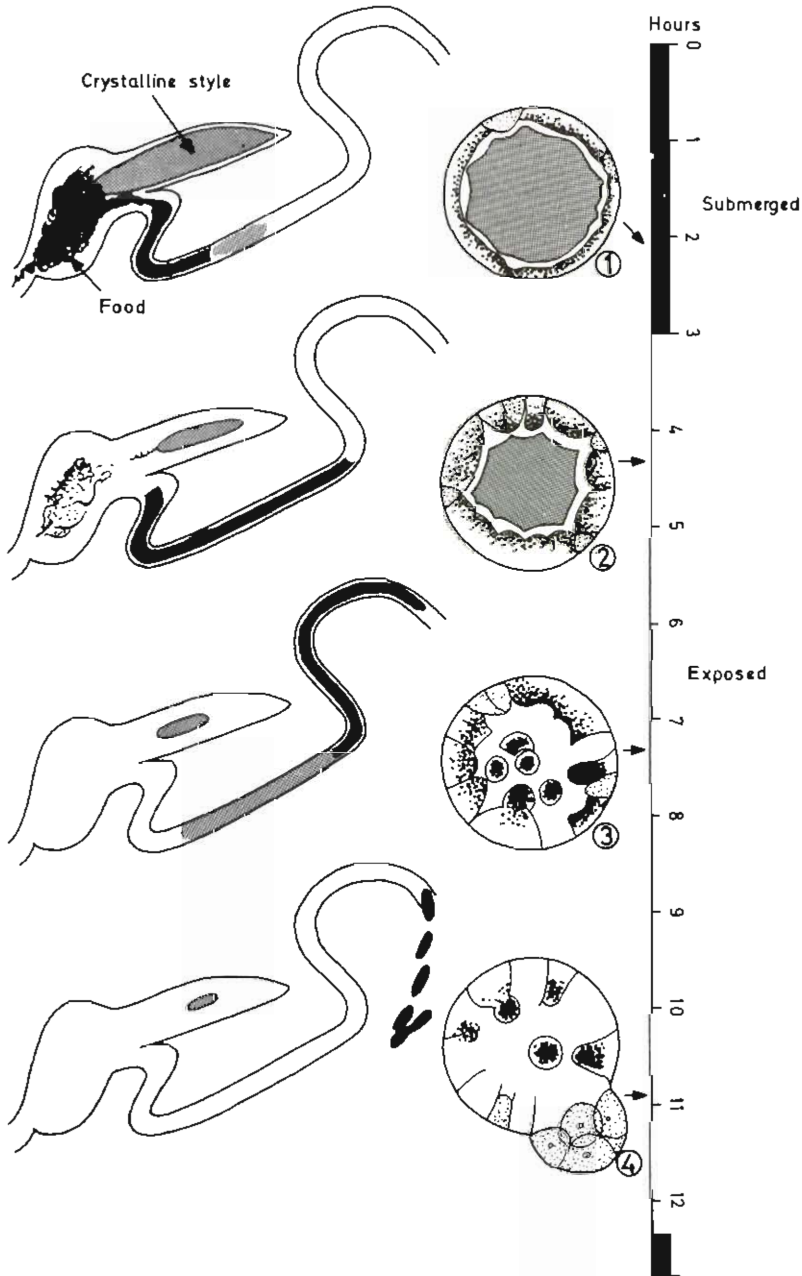


Fig. 3-27: *Lasaea rubra.* Relationship between digestive periodicity and tidal rhythm. Vertical: tidal regime; black column indicates submersion. Outlines of alimentary canal demonstrate size of crystalline style and position of food in the gut. Cross-sections of digestive tubules (right) reveal 4 different stages: (1) absorption, (2) fully loaded cells, (3) fragmentation, (4) discharged epithelium. (After MORTON, 1956; modified; reproduced by permission of Marine Biological Association of the United Kingdom.)

These processes occur in most metazoans, including marine forms; phagocytosis and absorption occur in bivalves, e.g. *Ostrea edulis* (TAKATSUKI, 1934), *Tridacna elongata* (MANSOUR-BEK, 1946), *Kellia suborbicularis* (OLDFIELD, 1961) and echinoderms (e.g. *Echinus esculentus*; STOTT, 1955); pinocytosis and absorption occur in isopods, e.g. *Eurydice pulchra* (JONES and co-authors, 1969). Phagocytosis has neither been observed in the hepatopancreas or midgut of crustaceans (YONGE, 1937) nor in the intestine of vertebrates.

As mentioned in the previous section, digestion may be surface-dependent (when meal size is too small to elicit peristalsis) or volume-dependent (when meal size is sufficiently large to initiate and maintain peristalsis). Unlike digestion, absorption is surface-dependent. Absorption is facilitated by increasing the mucosal surface, for instance, mesenteries of anthozoans, digestive diverticula of bivalves, spiral valves of elasmobranchs and chimaeras, or pyloric caecae of teleosts. In mammals, absorbing mucosal folds increase the surface area by a factor of 3 (Fig. 3-28); the densely spaced, 1 mm long villi cause a further 10-fold increase (VERZAR and

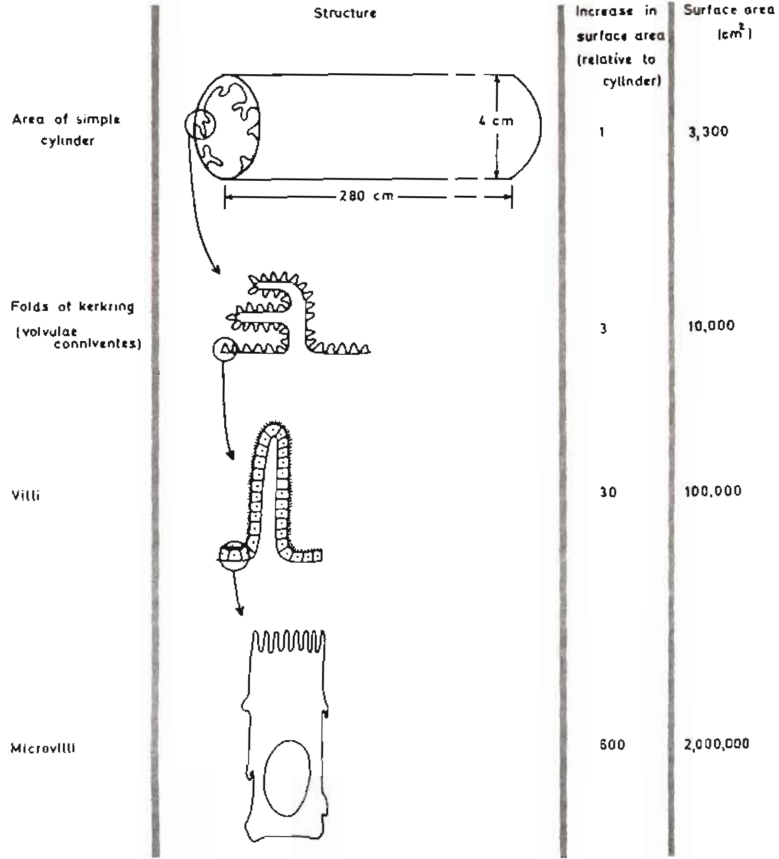


Fig. 3-28: Increase in surface area of the small intestine of a mammal. (After WILSON, 1962; modified; reproduced by permission of W. B. Saunders Company.)

McDougall, 1936). The laminal border of the epithelial cell is lined with about 600 finger-like projections (or 50,000,000 mm^{-2} of intestinal surface) called microvilli (about 1 μ long and 0·1 μ wide). Microvilli increase the absorbing surface area about 20 times (Zetterqvist, 1956; Palay and Karlin, 1959).

Similar structural adaptations occur among marine metazoans. Anthozoan mesenteries are comparable to the intestinal mucosal folds of vertebrates. The alveoli-like digestive tubules of the digestive diverticula of bivalves (Owen, 1966b) may be comparable to those of the intestinal villi. Microvilli, similar to those in mammals, have been reported from several rhynchocoels (e.g. *Lineus ruber*, Jennings, 1969), arthropods (isopod *Eurydice pulchra*, Jones and co-authors, 1969) and fishes (e.g. *Opsanus tau*, Farmanfarmaian and co-authors, 1972). In *L. ruber*, the cell surface between the gastrodermal cilia forms irregularly distributed microvilli; each microvilli is 0·5 to 0·6 μ long and 0·1 μ in diameter; in a few cases, the microvilli are dichotomous, splitting into 2 equal-sized structures 0·1 μ from the base (Jennings, 1969).

Mechanisms of Transport

Diffusion

In **passive transport** materials move through cells, membranes and intercellular spaces due to concentration gradients. Simple passive diffusion appears to be the mechanism of absorption of such important substances as some water soluble vitamins, some nucleic-acid derivatives and many lipid soluble substances (Wilson, 1962).

Active transport involves movement of a substance across a membrane against a concentration gradient; it requires energy from cellular metabolism. Some of the phenomena associated with active transport are: (i) Inhibition of transport due to inhibiting of energy-yielding reactions, e.g. phlorizin and iodoacetate inhibit the active transport of D-glucose in the intestinal segments of the turtle *Chrysemys picta* (Fox, 1965). (ii) Saturation kinetics, e.g. *in vitro* intestinal absorption of sugar in the toadfish *Opsanus tau* exhibits saturation kinetics conforming to the Michaelis–Menten equation (Farmanfarmaian and co-authors, 1972). (iii) Competitive inhibition with similar compounds that have a common carrier protein (p. 144). (iv) Inhibition at very low temperature, e.g. at 0° to 2° C active absorption of D-glucose in the isolated intestine of *C. picta* is inhibited (Fox, 1965).

Three different **mechanisms of transport** have been suggested:

(i) **Trapping reaction**. To increase the rate of movement of a substance across a membrane, its concentration on the opposite side of that membrane is decreased by converting it into a second substance. Many nucleosides diffuse into the corresponding free base and presumably ribose (or deoxyribose)-1-phosphate. The non-diffusible ribose-1-phosphate is metabolized within the cell. The reduced concentration of nucleoside within the cell presumably facilitates further entrance of the substance from the intestinal lumen.

(ii) Differential **permeability**. This mechanism is based on the assumption that the intestinal epithelial cells are more permeable on the side facing the lumen than on the opposite side. There is experimental evidence for such differential permeabil-

ity of membranes of a cell accounting for the preferential movement of lactic acid from the epithelial cell of the toad bladder to the serosal side; the serosal border of the cell is 14 times as permeable as the cell membrane on the opposite pole of the cell (LEAF, 1959). *In vitro* studies on the intestinal segments of the marine fish *Stenotomus versicolor* indicate that L-leucine is actively absorbed and the oxygen-dependent process is unidirectional from mucosa to serosa (NEFF and MUSACCHIA, 1967).

(iii) Membrane carrier. A substance approaches the outer surface of the cell membrane and reacts with a cell-wall component. The substrate-carrier complex then moves across a permeability barrier and releases the compound on the inner surface of the membrane. Of the number of speculations about the specific chemical reaction, the one supported by experimental data is the phosphatidic-acid hypothesis for sodium ion transport, proposed by HOKIN and HOKIN (1960). These authors have amassed an imposing array of data which support their view that sodium ion reacts with phosphatidic acid at one side of the membrane to produce its sodium salt, which in turn moves through a lipid area of the membrane to the opposite border of the membrane, where the ion is released into the ambient aqueous medium. Two specific enzymes are involved in breakdown and resynthesis of the carrier molecule (Fig. 3-29).

Facilitated diffusion (DANIELLI, 1954) is a carrier-mediated transport phenomenon, in which the rate of attainment of diffusion equilibrium is greatly accel-

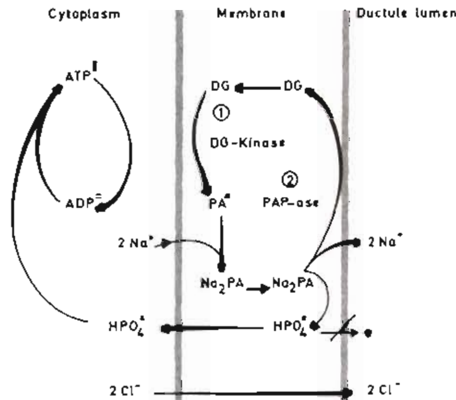


Fig. 3-29: Phosphatidic acid as carrier for active sodium-ion transport across the membrane of a salt-secreting cell. Schematic representation. ATP: adenosine triphosphate; DG: diglyceride; DG-kinase: diglyceride kinase; PA: phosphatidic acid; PAP-ase: phosphatidic acid phosphatase. $HPO_4^{=}$ does not leave the external surface of the membrane. (After HOKIN and HOKIN, 1960; modified; reproduced by permission of Rockefeller University Press.)

erated, and requires no direct energy expenditure. The fundamental distinction between active transport and this process is the lack of movement against a concentration gradient in the latter. For instance, the sugar 6-deoxy-1,5-anhydro-*d*-glucitol is not actively transported into the intestinal epithelial cells; however, its entrance into the cells is inhibited by phlorizin and stimulated by sodium ions (CRANE and co-authors, 1961).

The mechanisms of uptake of the various components of the digested food thus far described have been studied especially in mammals. From what is known about gastro-intestinal absorption in other animals, especially in fishes (e.g. toadfish *Opsanus tau*; FARMANFARMAIAN and co-authors, 1972), echinoderms (e.g. echinoids *Echinus esculentus*, *Paracentrotus lividus*; BAMFORD and JAMES, 1972; BAMFORD and co-authors, 1972), and molluscs (e.g. *Cryptochiton stelleri*; GREER and LAWRENCE, 1967; LAWRENCE and LAWRENCE, 1967), it may be suggested that the mechanisms are strikingly similar in marine animals.

Carbohydrate absorption

The intestinal epithelium is not permeable to polysaccharides; measurable amounts of disaccharides pass into the intestinal epithelial cells, where they are split by disaccharases. The greatest amount of carbohydrate is absorbed after being split into monosaccharides. The effect of molecular weight on the rate of intestinal absorption of some monosaccharides is documented in Table 3-13.

Passive diffusion is perhaps the only mechanism of uptake of several monosaccharides normally occurring in the food, such as fructose, mannose and most

Table 3-13

Effect of molecular weight on absorption rate of sugars (After WILSON, 1962; modified; reproduced by permission of W. B. Saunders Company)

Carbohydrate	Molecular weight	Rate of movement from mucosal to serosal sides (μM 100 mg^{-1} wet weight hr^{-1})
Polysaccharide		
Starch	50,000	0
Inilun	5,000	0
Disaccharide		
Lactose	342	0·5
Monosaccharide		
(a) Hexose		
Mannose, Sorbose	180	1·9
(b) Pentose		
Ribose, Arabinose	150	2·2
(c) Triose		
Glyceraldehyde	90	4·5

pentoses. The uptake of hexose, glucose and galactose is significantly aided by active transport. An active-transport system for glucose has been identified for molluscs (e.g. *Cryptochiton stelleri*, LAWRENCE and LAWRENCE, 1967); cucumbers (e.g. *Thyone* sp., FARMANFARMAIAN, 1969a, b); echinoids (e.g. *Paracentrotus lividus* and *Echinus esculentus*, WEST and JEAL, 1971; BAMFORD and JAMES, 1972; BAMFORD and co-authors, 1972; JEAL and WEST, 1974); elasmobranch (CARLISKY and HUANG, 1962); teleosts (e.g. *Opsanus tau*, FARMANFARMAIAN and co-authors, 1972); and turtle (e.g. *Chrysemys picta*, FOX, 1965); active absorption galactose has, however, been demonstrated only in a few animals (e.g. *P. lividus*, BAMFORD and co-authors, 1972). D-Mannose and D-fructose are not actively absorbed in the intestine of *C. stelleri* (LAWRENCE and LAWRENCE, 1967). FARMANFARMAIAN (1969a, b) reported that glucose is directly absorbed by the intestinal epithelium of the holothurian *Thyone* sp.; its transport rate is modified by a number of physiological parameters such as the pH, the net flux of water and nutritional state (starvation in captivity).

Transport studies with compounds which may be considered as modifications of the glucose molecule at carbon atoms 1–6, have clearly shown that the important feature for active transport is a D-pyranose ring structure with a hydroxyl group of the glucose configuration at carbon 2 (WILSON and LANDAU, 1960; WILSON and co-authors, 1960). This feature is only common to D-glucose and its epimer galactose, which has an inversion of the hydroxyl at carbon 4 and is, therefore, a suitable substrate. Fructose and other hexose and pentose monosaccharides lack the essential configuration and are absorbed by simple diffusion or in the case of D-xylose by facilitative diffusion (BAMFORD and JAMES, 1972). Therefore, absorption of the monosaccharides such as mannose, xylose, arabinose by passive diffusion show a linear relationship between concentration and absorption rate, while those actively transported (like glucose and galactose) exhibit saturation kinetics. Some sugars (e.g. glucose) can be passively absorbed (at a concentration-dependent rate) as well as actively transported; absorption rates of these sugars are higher than those (e.g., mannose, fructose) which are only passively absorbed (Table 3-14).

Results obtained for the uptake of D-glucose and D-galactose by intestinal segments of *Paracentrotus lividus* strongly suggest the presence of an active carrier-mediated process for these molecules. D-Glucose is also absorbed by an active

Table 3-14

Selective absorption of sugars by fishes (After WILSON, 1962; modified; reproduced by permission of W. B. Saunders Company)

Species	Rate of sugar absorption (glucose taken as 100)					Author
	Galactose	Glucose	Fructose	Xylose	Arabinose	
Scorpaena porcus	102	100	45	78	80	CORDIER and CHANEL (1953)
Tinca tinca	99	100	65	43	57	CORDIER and co-authors (1954)

process in the stomach of *P. lividus* and *Echinus esculentus*, as evidenced by its accumulation against a concentration gradient; but in neither species was evidence obtained for 'uphill' movement of D-galactose in the stomach. This implies that an active carrier-mediated transport system operates for glucose in this region which is not shared by its epimer galactose. The regional variation in the absorption of glucose and galactose appears to show that two receptor sites are available to the glucose molecule, only one of which is available to galactose (BAMFORD and co-authors, 1972).

Protein absorption

Uptake of protein via the gut epithelium is negligible. Dipeptides may be absorbed to some extent and hydrolysed intracellularly in the intestinal epithelium (e.g. in the dogfish; VAN SLYKE and WHITE, 1911). By far the greatest amount of ingested protein is degraded to amino acids before absorption. Though amino acids can diffuse passively through the gut wall, uptake of most amino acids is supplemented by active transport mechanisms.

Only a few papers pertaining to amino-acid absorption in marine animals have been published thus far. Examples are the cucumber *Stichopus parvimensis* (LAWRENCE and co-authors, 1967), the echinoids *Paracentrotus lividus* (JEAL and WEST, 1974) and *Echinus esculentus* (BAMFORD and JAMES, 1972), and the fish *Fundulus heteroclitus* (HUANG and CHEN, 1971). A preliminary study (JEAL and WEST, 1974) indicated active absorption of ^{14}C-labelled L-alanine in the *E. esculentus* stomach. Active absorption of ^{14}C L-alanine and L-leucine, observed by BAMFORD and JAMES (1972), provides strong evidence for the presence of a neutral carrier mechanism in the stomach of *E. esculentus*, which also efficiently adsorbs

Table 3-15

Echinus esculentus. Absorption rates of L-alanine by stomach segments at different initial concentrations (After BAMFORD and JAMES, 1972; modified; reproduced by permission of Pergamon Press Ltd, Oxford)

Initial concentration of alanine (mM)	Concentration ratio	Absorption rate (μM g^{-1} dry stomach tissue hr^{-1})
0·1	11·6	5
1·0	6·1	33
3·0	7·6	119
5·0	3·0	151
7·5	3·0	180
10·0	2·5	235
15·0	1·5	218
20·0	1·4	241

L-alanine at concentrations up to 20 mM (Table 3-15). The presence of a neutral carrier in the intestine has also been demonstrated in other marine invertebrates, e.g. *Cryptochiton stelleri* (LAWRENCE and co-authors, 1967) and *Stichopus parvimensis* (GREER and LAWRENCE, 1967). A comparison of K_t—a measure of the affinity of the substrate for a carrier molecule, obtained for L-alanine of *E. esculentus* (5·5) and rat (5·0; FINCH and HIRD, 1960)—suggests a very similar affinity of the amino acid for the neutral carriers in both animals. The mechanism involved in the active absorption of the basic amino acid L-lysine in the *E. esculentus* stomach shows a similar characteristic in that movement occurs against a concentration gradient of 1 mM (BAMFORD and JAMES, 1972). The acidic amino acid L-aspartic is not absorbed against a gradient in the mammal intestine (WISEMAN, 1953), nor does this occur in the *E. esculentus* stomach at any of the concentrations (from 1 to 5 mM) used (BAMFORD and JAMES, 1972). GREER and LAWRENCE (1967) reported similar active-transport mechanisms for neutral and basic amino acids in the gut of a primitive mollusc *Cryptochiton stelleri*. L-Alanine and glycine have also been found to be actively absorbed in the intestine of the cucumber *S. parvimensis* (LAWRENCE and co-authors, 1967).

Early studies on absorption of basic amino acids suggested that a charge in the side chain (R–) prevents intestinal absorption of these amino acids by an active process. Studies on gut absorption of charged amino acids in the echinoid *Echinus esculentus* revealed a net transport of the basic amino acid L-lysine against a concentration gradient in the stomach segments incubated with a low concentration (1 mM). This observation indicates the presence of an active carrier mechanism for the basic amino acid (BAMFORD and JAMES, 1972). BAMFORD and JAMES (1972) found that concentration of the acidic amino acid aspartate in stomach segments of *E. esculentus*, incubated at 10° C for 90 mins with 1 to 5 mM concentrations of aspartate, did not rise significantly more than in the incubated media; they considered that this evidence argues strongly against the presence of an active carrier mechanism for aspartate, but for a process mediated by simple or facilitated diffusion.

According to subsequent publications (BAMFORD and STEWART, 1973a, b), charged amino acids are actively absorbed against concentration gradients in the intestine of the polychaete *Arenicola marina*, suggesting two separate transport loci for basic and acidic amino acids. Comparisons of K_t and V_{max} values obtained for the neutral amino acid L-lysine (0·63 mM and 6·12 μM g^{-1} dry weight per 2 mins; BAMFORD and STEWART, 1973a) with those known for the basic amino acid L-lysine (0·43 mM and 1·18 μM g^{-1} dry weight per 2 mins; BAMFORD and STEWART, 1973b) reveal that the affinity of lysine for its carrier is marginally higher than that of alanine for the neutral carrier; the uptake rate of lysine, indicated by its V_{max}, is less than a third of that obtained for alanine. Since substrate affinity of each site is a constant property of the transport molecule, the greater V_{max} recorded for alanine implies that the number of functioning sites available for neutral amino acids is greater than that available for basic amino acids (BAMFORD and STEWART, 1973b).

Transport of the acidic amino acid L-aspartate in the intestine of *Arenicola marina* is perhaps active and energy dependent (BAMFORD and STEWART, 1973b): (i) kinetic studies on aspartate uptake clearly suggest the presence of a carrier-mediated system for the absorption of this molecule; (ii) susceptibility of 'uphill'

aspartate transport to metabolic inhibitors proves that this process is energy dependent.

A useful phenomenon associated with active transport is the reduction in absorption rate when energy yielding reactions are inhibited. In the intestine of *Arenicola marina*, effects of 3 substances—sodium iodoacetate (NaIoA), 2:4 dinitrophenol (DNP) and sodium cyanide (NaCN)—known to act as inhibitors on the uptake of L-alanine in mammalian intestine, were examined by BAMFORD and STEWART (1973a). Uptake of L-alanine from 0·2 mM solution during 60 mins incubation was examined in the presence of 2 mM of each inhibitor. Incubations were carried out in 2 ways: (i) direct incubation in substrate + inhibitor; (ii) pre-incubation (for 30 mins) in inhibitor preceding incubation in substrate + inhibitor. Direct incubation with DNP affects uptake by only 31%; but pre-incubation in NaCN and DNP

Table 3-16

Arenicola marina. Effects of metabolic inhibitors on the intestinal uptake of L-alanine from 0·02 mM solution (After BAMFORD and STEWART, 1973a; modified; reproduced by permission of Springer-Verlag, Berlin)

Inhibitor	Concentration ratio	Uptake (μM g^{-1} ethanol extracted dry weight)	Inhibition of uptake (%)
	Incubated directly		
Control	11·0	3·5	—
NaIoA	10·3	3·3	6·1
Control	13·3	3·8	—
DNP	9·2	2·7	31·2
Control	13·0	3·4	—
NaCN	11·6	3·1	10·3
	Pre-incubated in inhibitor for 30 mins		
Control	14·1	4·5	—
NaCN	6·4	2·1	52·5
Control	14·5	2·7	—
DNP	2·7	0·55	81·2

greatly reduces uptake by 53 and 81%, respectively (Table 3-16). Inhibition of L-alanine uptake by DNP and NaCN substantiates active absorption.

In all these cases, however, reduction was less than the expected level of inhibition, compared with mammalian intestine. In order to explain this, BAMFORD and STEWART (1973a) proposed 3 alternative suggestions: (i) A pathway other than glycolysis functions during glycogen breakdown in *Arenicola marina* (DALES, 1969). Investigations on terminal cellular respiration in *A. marina* have shown the presence of cytochrome a and a_3 (MATTISSON, 1959), and KRISHNASWAMY and co-authors (1967) have postulated the presence of a Krebs cycle after demonstrating succinic dehydrogenase activity. Since DNP and NaCN cause significant inhibition of uptake, energy liberation is possibly blocked at the cytochrome level. Pre-incubation

in these two inhibitors lowers the uptake to a much greater extent (Table 3-16). (ii) The energy reserves in the intestine have been used up and are not regenerated, as cellular oxidation is inhibited. (iii) The permeability of the intestine to these inhibitors is initially low and pre-incubation allows the build up of effective concentrations.

Several intertidal animals are facultative anaerobes and continue to produce ATP under extended (days, weeks) anoxia at a level exceeding 60% that achieved during aerobiosis (Zs.-NAGY and ERMINI, 1972b); the end-product of glycolytic anaerobiosis is succinic acid (HOCHACHKA and co-authors, 1973a). It seems possible, therefore, that *Arenicola marina* is able to liberate the energy required for active amino-acid transport in its intestine. The reason for the greater reduction in alanine uptake may be that the permeability of the intestine to these inhibitors is low and that an effective concentration of the inhibitor is built up during the pre-incubation.

The possibility of competition for a transport carrier common to two or more amino acids has been studied in some detail in the intestine of *Arenicola marina* by BAMFORD and STEWART (1973a, b). To examine the intensity of the inhibition,

Table 3-17

Arenicola marina. Effects of competing amino acids on the intestinal absorption of L-alanine and L-lysine. Concentration of alanine or lysine was 0·02 mM. Concentration of competing amino acids was 0·2 mM in all cases. Incubation: 60 mins at 10° C (After BAMFORD and STEWART, 1973a, b; modified; reproduced by permission of Springer-Verlag, Berlin and Pergamon Press Ltd, Oxford)

Amino-acid combination	Mean concentration ratio	Uptake rate (μM g^{-1} dry weight hr^{-1})	Inhibition of uptake (%)
Alanine control	11·4	2·3	—
Alanine + L-Methionine	4·05	0·95	57·6
Alanine + L-Leucine	4·1	0·8	64·7
Alanine control	12·15	2·5	—
Alanine + L-Valine	5·6	1·0	47·0
Alanine + L-Histidine	6·3	1·1	45·5
Alanine control	13·8	2·5	—
Alanine + L-Phenylalanine	5·9	1·1	56·4
Alanine + L-Proline	10·6	2·1	18·6
Alanine control	9·3	1·8	—
Alanine + L-Lysine	7·1	1·3	29·5
Alanine control	11·3	2·4	—
Alanine + L-Aspartic acid	10·0	2·1	12·1
Lysine control	8·1	1·1	—
Lysine + L-Arginine	1·9	0·25	76·7
Lysine control	7·8	1·0	—
Lysine + L-Histidine	2·6	0·5	56·3
Lysine control	7·9	1·0	—
Lysine + L-Alanine	6·1	0·85	18·45
Lysine control	8·1	1·3	—
Lysine + L-Aspartic acid	7·3	1·1	17·4

which is a measure of such competition, the uptake rate of the neutral amino acid L-alanine, the basic amino acid L-lysine, or the acidic amino acid L-aspartate was measured in the presence of another neutral or charged amino acid. All amino acids tested produce a significant reduction in alanine uptake; but reduction is most pronounced with neutral amino acids (methionine, leucine, valine, histidine and phenylalanine) except proline (Table 3-17). The competitive inhibitor methionine reduces uptake by 58%, the other neutral amino acids by 10%, about the value obtained for methionine. Inhibition by these neutral amino acids is due to competition for a common step in the carrier system. The low level of inhibition by charged amino acids (basic lysine: 29% and acidic aspartate: 12%) and the neutral amino acid proline (19%) may be attributable to their absorption by separate pathways.

To characterize the general specificity of the transport locus for L-lysine, the effect on lysine uptake was measured in the presence of neutral or charged amino acids. Maximum inhibition (77%) occurred in the presence of the basic amino acid arginine (Table 3-17). Histidine, a neutral amino acid with basic tendencies, inhibited the uptake by 56%. The levels of inhibition by the neutral amino acid alanine and the acidic amino acid aspartate were below 19%. The uptake of 0·02 mM (acidic) L-aspartate was also measured in the presence of 2·0 mM L-alanine, L-lysine, or L-glutamic acid. The mean uptake of aspartic acid after 1-hr incubation at 10° C was reduced by 23% in the presence of alanine. Lysine had no significant effect on aspartate-acid absorption, while the acidic glutamate inhibited the acidic aspartate by 55% (BAMFORD and STEWART, 1973b).

Inhibition between amino acids of different classes approximates 20%. It may imply separate carrier mechanisms for the transport of the classes of amino acids, and that interclass inhibition results from lack of specificity of the 3 loci, or from inhibition of a non-competitive type. Alanine inhibits lysine absorption by only 18%. Histidine, however, inhibits uptake of lysine (56%) as well as of alanine (46%). Histidine, a neutral amino acid, exhibits basic tendencies and this may explain its powerful inhibitive effect on uptake of both neutral and basic amino acids.

In the vertebrate intestine, the carrier system for neutral amino acids is known to show a preference for the L-stereo isomer. The D-isomer may share the same carrier, but invariably has a much poorer affinity and is not absorbed against a concentration gradient (exceptions: D-methionine: JERVIS and SMYTH, 1960;

Table 3-18

Echinus esculentus. Absorption rates of the enantiomorphs of alanine by stomach segments (After BAMFORD and JAMES, 1972; modified; reproduced by permission of Pergamon Press Ltd, Oxford)

Amino acid	Concentration (mM)	Concentration ratio	Absorption rate (μM g^{-1} dry stomach tissue hr^{-1})
L-Alanine	1	6	33
D-Alanine	1	4	22
L-Alanine	5	3	151
D-Alanine	5	2	73

D-serine: RANDALL and EVERED, 1964). Absorption rate of the two enantiomorphs of alanine, presented separately to stomach segments of *Echinus esculentus* in 1 or 5 mM concentrations, indicates preferential absorption of the L-isomer at both concentrations (Table 3-18). Possibly, D- and L-alanine share a common stereo-specific carrier, or a separate carrier is involved in the transport of each isomer. Testing these two possibilities, BAMFORD and JAMES (1972) found that 10 mM D-alanine does not significantly inhibit L-alanine uptake from 1·0 mM or 0·1 mM solutions, but that 1 mM D-alanine is strongly inhibited by 10 mM L-alanine; this result implies that D-alanine shares a common transport locus with L-alanine for which it has much poorer affinity.

A similar experiment on intestinal segments of *Arenicola marina* (BAMFORD and STEWART, 1973a) revealed that, though absorbed at a slower rate than the L-form, D-alanine is transported against its concentration gradient (Table 3-19), indicating an active carrier-mediated process. A reciprocal inhibition test, where uptake of 0·02 mM L-alanine was determined in the presence of a 10-fold D-alanine concentration and vice versa, revealed a small increase in L-alanine uptake in the presence

Table 3-19

Arenicola marina. Interference of each enantiomorph of alanine on the transport of the other. Intestinal segments were incubated for 60 mins at 10° C (After BAMFORD and STEWART, 1973a; modified; reproduced by permission of Springer-Verlag, Berlin)

L-Alanine (mM)	D-Alanine (mM)	Concentration ratio	Uptake (μM g^{-1} ethanol extracted dry weight)	Change in uptake (%)
0·02	—	10·7	2·8	—
0·02	0·20	10·65	3·0	(+) 6·3
—	0·02	7·1	2·2	—
0·20	0·02	5·9	1·95	(−)10·0

of the D-form, while in the presence of the L-isomer, the uptake of D-alanine is only reduced by 10% (Table 3-19). This result suggests the possibility of separate carriers for each enantiomorph (BAMFORD and STEWART, 1973a).

Analysis of food and faeces formed during an extended study enabled CAREFOOT (1967a, b) to estimate the percentage absorption of 17 common amino acids in 3 species of opisthobranchs. The major error introduced with such an approach is the metabolism of the ingested amino acids by the gut microflora, which was not controlled in the experiment. However, faeces of *Aplysia punctata* fed on *Plocamium coccineum* left in sea water for 0, 12, 24 or 48 hrs were analyzed for their N-content; the total N-loss over 48 hrs was found to be only 3% of the original concentration (CAREFOOT, 1967b), a value well within the error range of the method used. Another source of error could have been the addition of N via epithelial cells abraded from the alimentary canal while food is passing through it (see PANDIAN, 1967a). Yet CAREFOOT's experiments are ecologically more revealing than short-term experiments on intestine preparations. The efficiency of amino-acid absorption is fairly

high; it ranges from 56% for glycine in *Dendronotus frondosus* fed on the hydroid *Tubularia larynx* to 100% for histidine in the same opisthobranch (Table 3-20). Taurine absorption efficiency is 100% in all 3 species tested. Amino-acid absorption efficiency ranges mostly between 83 and 94% for the carnivorous species *Archidoris panicea* and *D. frondosus*, while most values for the efficiency are around 70% in the herbivorous species *Aplysia punctata* (CAREFOOT, 1967a, b).

Table 3-20

Efficiency of amino-acid absorption in some opisthobranchs (After CAREFOOT, 1967b; modified; reproduced by permission of Microforms International Marketing Corp.)

Amino acid	Absorption efficiency (%)		
	Aplysia punctata fed on *Plocamium coccineum*	*Archidoris pseudo-argus* fed on *Halichondria panicea*	*Dendronotus frondosus* fed on *Tubularia larynx*
Alanine	80	90	83
Arginine	67	90	94
Aspartic acid	75	90	86
Glutamic acid	82	91	91
Glycine	73	90	56
Histidine	68	94	100
Isoleucine	75	92	92
Leucine	76	93	91
Lysine	65	93	98
Methionine	82	95	87
Phenylalanine	71	94	65
Proline	73	88	71
Serine	77	89	87
Threonine	71	90	87
Tyrosine	77	94	90
Valine	75	92	93
Taurine	100	100	100

Fat absorption

Until recently, it was uncertain whether unhydrolysed fat can be absorbed in the gut or whether complete or partial hydrolysis is necessary. Feeding experiments with fats in which the glycerol residues were labelled with ^{14}C have revealed that 25 to 60% of the ingested fat is fully hydrolysed to free fatty acids before absorption. The greater part of the remaining fat may be absorbed as monoglyceride, whereas only a small amount of the fat is absorbed as di- and triglycerides (WILSON, 1962). Diffusion of molecularly dispersed glycerides or free fatty acids into the epithelial cells is the widely accepted mechanism of uptake, although the mechanism of fat absorption is still a matter of discussion. The dispersion of the fats and their digestive products is aided by (bile-like) salts, which form water-soluble complexes with the fatty acids. Uptake by diffusion may be facilitated by the solubility of the free acids in the cell membranes. Electron microscopy and other methods have indicated

that ingested fat may be taken up by the intestinal epithelial cells in the form of microdroplets by way of pinocytosis or phagocytosis; however, the quantitative importance of this mechanism is not clear (see JØRGENSEN, 1968).

Water

The mechanisms regulating water absorption in heterotrophs may also control the uptake of water-soluble substances like vitamins (DAVENPORT, 1969). Therefore, a brief account of water transport is necessary here; this aspect has been documented in detail in Volume I, Chapter 4 and in Chapter 5 of the present volume.

Simultaneous intestinal absorption of water and sugars has been studied in few animals other than mammals. There is no net movement of water, Na^+ or Cl^- across the gut of *Stichopus parvimensis* during incubation with different sugars and amino-acid solutions (LAWRENCE and co-authors, 1967), and there is no net water transport even though glucose is transported against the gradient in the intestines of *Cryptochiton stelleri* (LAWRENCE and LAWRENCE, 1967) and *Thyone* sp. (FARMANFARMAIAN, 1969b). Active transfer of glucose across the mammalian intestinal wall creates an osmotic gradient which results in net water transfer (WILSON, 1962; SMYTH, 1965). The three marine species studied do not normally face any water-balance problems.

The digestive tract of vertebrates is highly permeable to water, somewhat permeable to monovalent ions, but rather impermeable to di- and trivalent ions (JØRGENSEN, 1968). Net absorption of water is affected by the osmotic pressure and electrolyte composition of the fluid in the lumen. Marine fishes drink considerable quantities of water, and intestinal absorption of that water plays an important

Table 3-21

Ingestion rate of water by some marine fishes (After CONTE, 1969; modified and extended; reproduced by permission of Academic Press, Inc., New York)

Species	Ingestion rate (μl g^{-1} day^{-1})	Author
Lampetra fluviatilis	150	MORRIS (1960)
Myxine glutinosa	65	MORRIS (1965)
Anguilla rostrata	66	SMITH (1930)
Anguilla anguilla	78	MAETZ and SKADHAUGE (1968)
Anguilla japonica	80	OIDE and UTIDA (1968)
Paralichthys lethostigma	110	HICKMAN (1968)
Serranus scriba	120	MOTAIS and MAETZ (1965)
Salmo gairdnerii	129	SHEHADEH and GORDON (1969)
Xiphister atropurpureus	195	EVANS (1967)
Platichthys flesus	240	MOTAIS and MAETZ (1965)
Fundulus heteroclitus	461	POTTS and EVANS (1967)
Gasterosteus aculeatus	960	MULLINS (1950)
Blennius pholis	1440	MULLINS (1950)
Tilapia mossambica	5616	EVANS (1968)
Pholius gunnellus	6384	POTTS and co-authors (1967)

role in osmoregulation (Volume I: HOLLIDAY, 1971; Chapter 5 of present volume); for example, the euryhaline *Salmo gairdnerii* drinks 47, 97 or 129 μl water g^{-1} per day when exposed to 11‰, 16‰ or 32‰ S; 66, 78 and 80% respectively of these amounts were absorbed from the intestine. In fresh water, *S. gairdnerii* does not drink water (SHEHADEH and GORDON, 1969). To document the importance of intestinal water absorption, water-ingestion rates of some euryhaline fishes are presented in Table 3-21.

Water ingestion rates are 144 μl g^{-1} hr^{-1} for *Uca pulicator*, 415 μl g^{-1} hr^{-1} for *Penaeus duorarum* and 175 μl g^{-1} hr^{-1} for the horseshoe crab *Limulus polyphemus*. *U. pulicator* exposed to dilute sea water (of about 1·6‰ S) reduced the water ingestion rate to 60 μl g^{-1} hr^{-1} (HANNAN and EVANS, 1973). These amounts are less than those reported by some authors for fishes, and ingestion of water in the marine crustaceans mentioned contributes less than 3% of the total influx (HANNAN and EVANS, 1973), as against 80% in *Salmo gairdnerii*.

Salt absorption

Marine animals obtain the minerals required from the surrounding sea water via gills or other epithelia (e.g. copper: see ZUCKERKANDL, 1960; KERKUT and co-authors, 1961). Food may constitute a source of essential ions and contribute to ion and osmoregulation in suboptimal salinities (KINNE, 1952: *Gammarus duebeni*; MCLUSKY, 1970: *Corophium volutator*). Food also seems to be the main source of copper in molluscs (GHIRETTI and VIOLANTE, 1964) and in intertidal crustaceans (WIESER, 1967). Copper can be absorbed by the isopod *Ligia oceanica* and the amphipod *Orchestia gammarella* from the food alga *Fucus vesiculosus* which contains less than 1·5 to 2·0 μg Cu mg^{-1} dry weight; the absorption efficiency of copper by these intertidal crustaceans is about 50%, while that of the terrestrial isopod *Porcellio scaber* can be as high as 95%. Micro-organisms are considered to liberate bound copper (BLACK and MITCHELL, 1952) from decomposing faeces; re-ingesting such faeces, *P. scaber* can readily absorb copper (WIESER, 1967).

Some marine gastropods obtain salts via their gut. CAREFOOT (1967a, 1970) reported the amount of food consumed and faeces defecated as well as the percentage of ash contained in food and faeces. His data permit recalculation of the salts absorbed from food. Rates of dietary salt absorption via the gut range from 0·2 mg g^{-1} live snail per day in *Aplysia dactylomela* fed on the red alga *Galaxaura oblongata* to 24·3 mg g^{-1} per day in *A. juliana* eating the green alga *Enteromorpha* sp. (Table 3-22). There appears to be no simple correlation between dietary salt-absorption rate and quantity percentage of ash contained in the algae consumed. For instance, *Cladophora* sp. contains 84% ash; neither *A. dactylomela* nor *A. juliana* show the highest absorption rate when fed on this alga. The red alga *Ulva fasciata* contains only 28% ash but both species of *Aplysia* exhibit dietary salt absorption rates of over 4 mg g^{-1} per day; however, *A. dactylomela* feeding on *Galaxura oblongata* (64% ash content) displays a dietary salt-absorption rate of only 0·2 mg g^{-1} per day. *A. dactylomela* consumes dry matter of *U. fasciata* equivalent to 11·9 g and defecates 5·8 g during a 15-day experiment; it absorbs 4·4 mg salts g^{-1} per day. When fed *Enteromorpha* sp., *A. dactylomela* consumes as much as 24·8 g and defecates 5·9 g but displays an absorption rate of only 1·5 mg g^{-1} per day.

Hence the quantity of food consumed also appears to bear no correlation to the dietary salt-absorption rate. There are indications in the literature that also other marine animals obtain salts from food.

However, small animals with large surface areas per unit body volume may possibly secure practically all salts required via their body-surface or gill epithelia. Describing a new method for estimating absorption efficiency of organic matter by zooplankton, CONOVER (1966a) assumes that only the organic fraction of the ingested food is affected in the digestive process of zooplankters (Volume IV: CONOVER, in press). Diatoms, an important food source of zooplankton, have a

Table 3-22

Estimation of salts (ash) absorbed via the gut in some opisthobranchs fed different algae and animals. All values calculated from original data reported by CAREFOOT (1967a, b, 1970). C: Chlorophyceae, P: Phaeophyceae, R: Rhodophyceae

Snail species	Food species	Salts absorbed per day
Archidoris pseudoargus	*Halichondria panicea* (Sponge)	8·0 mg/snail
Dendronotus frondosus	*Tubularia larynx* (Hydrozoa)	2·0 mg/snail
Aplysia punctata	*Plocamium coccineum* (R)	23·4 mg/snail
Aplysia punctata	*Enteromorpha intestinalis* (C)	14·0 mg/snail
Aplysia punctata	*Ulva lactuca* (C)	6·1 mg/snail
Aplysia punctata	*Heterosiphonia plumosa* (R)	10·3 mg/snail
Aplysia punctata	*Cryptopleura ramosa* (R)	5·8 mg/snail
Aplysia punctata	*Delesseria sanguinea* (R)	0·0 mg/snail
Aplysia punctata	*Laminaria aculeata* (P)	0·2 mg/snail
Aplysia dactylomela	*Cladophora* sp. (C)	2·7 mg g^{-1}
Aplysia dactylomela	*Enteromorpha* sp. (C)	1·5 mg g^{-1}
Aplysia dactylomela	*Ulva fasciata* (C)	4·4 mg g^{-1}
Aplysia dactylomela	*Galaxaura oblongata* (R)	0·2 mg g^{-1}
Aplysia dactylomela	*Laurencia papillosa* (R)	6·1 mg g^{-1}
Aplysia juliana	*Cladophora* sp. (C)	8·8 mg g^{-1}
Aplysia juliana	*Enteromorpha* sp. (C)	24·3 mg g^{-1}
Aplysia juliana	*Ulva fasciata* (C)	4·1 mg g^{-1}

high ash content, composed chiefly of silica (hydrated SO_2; PARSONS and co-authors, 1961). However, dissolution of silicate from *Thalassiosira fluviatalis* shells crushed by feeding *Calanus hyperboreus* is not significant (CONOVER, 1966a). The assumption of CONOVER that a significant change in diatom ash weight may not occur during the relatively brief passage through the gut of a planktonic herbivore remains to be proved experimentally. Differences between absorption-efficiency values based on weight and ratio methods are less than 4% in 5 of CONOVER's experiments, over 10% (but less than 16%) in 4 experiments, and over 20% in 3 experiments (CONOVER, 1966a; his Table 1). Consequently, it is perhaps necessary to reconsider the assumption that ash content of the ingested alga is not affected by the digestive processes in the zooplankter.

Table 3-23

Percentage of ash content of algae and absorption efficiency of the copepod *Calanus hyperboreus* (After CONOVER, 1966b; modified; reproduced by permission of the American Society of Limnology and Oceanography)

Alga	Ash content of alga (%)		Absorption efficiency (%)	
	Mean	Range	Mean	Range
Bacillariophyceae				
Ditylum brightwelli	57	44–62	51	40–66
Coscinodiscus sp.	56	37–70	51	33–63
Skeletonema costatum	44	42–48	40	37–45
Rhizosolenia setigera	42	30–59	57	19–73
Cyclotella nana	30	27–32	59	43–77
Thalassiosira fluviatilis	27	11–43	67	37–88
Dinophyceae				
Exuviaella sp.	11	10–16	72	56–83
Chlorophyceae				
Dunaliella sp.	8	4–14	87	75–90

Table 3-24

Percentage of ash content of algae and absorption efficiency of 3 species of *Aplysia* (After CAREFOOT, 1967a, 1970; modified; reproduced by permission of the Marine Biological Association of the UK and North-Holland Publishing Company)

Aplysia species	Alga	Ash content of alga (%)	Absorption efficiency (%)
A. punctata	*Enteromorpha intestinalis*	33	59
	Heterosiphonia plumosa	27	71
	Cryptopleura ramosa	25	71
	Ulva lactuca	22	75
	Laminaria digitata	19	53
	Delesseria sanguinea	17	45
A. dactylomela	*Cladophora* sp.	84	35
	Galaxura oblongata	64	24
	Laurencia papillosa	54	67
	Enteromorpha sp.	54	68
	Ulva fasciata	28	62
A. juliana	*Cladophora* sp.	84	15
	Enteromorpha sp.	54	69
	Ulva fasciata	28	84

The percentage of ash in food organisms appears to affect digestion as well as absorption, and hence absorption efficiency. Tables 3-23 and 3-24 indicate that, in general, there is a negative correlation between percentage of absorption and percentage of ash content in microphyte-feeding species of *Calanus* (CONOVER, 1966b), and macrophyte-feeding species of *Aplysia* (CAREFOOT, 1967a, 1970; see also PANDIAN and DELVI, 1973). Comparable data on carnivorous gastropods reveal a variation in absorption efficiencies from 52% for *Archidoris pseudoargus* (CAREFOOT, 1967b) feeding exclusively on the sponge *Halichondria panicea* (ash content over 50%), to 62% for *Navanax inermis* (PAINE, 1965) feeding on a tectibranch *Haminoea virescens* (ash content: 40%; PAINE, 1965), and to 86% for *Dendronotus frondosus* (CAREFOOT, 1967b) eating the hydroid *Tubularia larynx* (ash content about 25%). Plants and animals with a high ash content tend to have a thicker skeleton, affording additional protection against mastication and digestion (PAINE and VADAS, 1969); or, possibly, the inert ash material competes with the organic matter for enzymes through some adsorptive mechanism (CONOVER, 1966b); or, probably, the release of mono- or divalent ions from these skeletons in the gut during digestion may alter considerably the 'digestive climate' by affecting enzyme-pH relationships (SMIT, 1968).

(c) Conversion

The absorbed low-molecular substances, except excreted urine, are converted into body functions and body structures. The converted low-molecular substances are in a dynamic state; they are interchangeable from one monomer into another; i.e. amino acids can be converted into organic acids, organic acids into monosaccharides, and monosaccharides into fatty acids (Table 3-25); they may also enter the intermediary metabolism, when the temporarily stored polymers are hydrolysed. These low-molecular substances are dissimilated in definite pathways to liberate biologically usable energy required for body functions. When converted low-molecular substances are available in excess concentrations, they are synthesized into the respective polymers, which are temporarily stored in form of body structures. Therefore, these aspects of conversion are dealt with under two subheadings: (i)

Table 3-25

Metabolism of absorbed nutrient substances (Original)

Polymers		Monomers		End products	
Nucleic acids	⇌	Purines and pyrimidine bases	⟶	$CO_2 + H_2O + NH_3$	NH_3 excreted
Proteins	⇌	Amino acids	⟶	$CO_2 + H_2O + NH_3$	
		⇅			
Lipids	⇌	Fatty acids	⟶	$CO_2 + H_2O$	
		⇅			
Polysaccharides	⇌	Monosaccharides	⟶	$CO_2 + H_2O$	

Liberation of biologically usable energy; (ii) temporary storage in form of body structures.

Liberation of Biologically Usable Energy

It is a necessary prelude to summarize briefly the fundamental pathways through which biologically usable energy is liberated and made available for body functions, in order to enable ecologists to appreciate the variations and many shortcuts in the biochemical machinery involved in the intermediary metabolism of heterotrophs.

Complete oxidation of glucose into carbon dioxide and water releases 686 kcal M^{-1}. However, oxidation of glucose or an equivalent 6-carbon sugar releases energy in a number of steps; each step is catalyzed by a specific enzyme. The advantage of stepwise reactions is that they can be coupled energetically by a reactant; in certain steps the energy liberated is transferred via such coupled reactions to high energy phosphate bonds (e.g. adenosine triphosphate, ATP) in which it is stored. The energy of such a phosphate bond is about 7 kcal M^{-1} at standard conditions (GIESE, 1968). High-energy compounds are 'the currency of the metabolic economy of the cell' (PROSSER and BROWN, 1961, p. 179).

Anaerobic dissimilation

The first phase in the oxidation of glucose is anaerobic glycolysis (Embden-Meyerhoff–Parnas (EMP)-pathway) in which each molecule of the 6-carbon sugar glucose is degraded to 2 molecules of pyruvic acid (Fig. 3-30). In animal tissues, glycogen rather than glucose is the starting point of glycolysis. Glycogenolysis liberates a phosphorylated glucose, which already has one high-energy phosphate bond. On the whole, during anaerobic glycolysis in animal tissues, 4 molecules of ATP are synthesized at an expenditure of 1 molecule of ATP (Fig. 3-29), i.e., there is a net gain of 3 ATP molecules for each 6-carbon unit of glycogen metabolized, in contrast to the net gain of only 2 ATP molecules in the fermentation processes of microbes, in which glucose is the starting point. In terms of energy, this means that the anaerobic oxidation of glucose nets about 21 kcal M^{-1} starting with glycogen in animals or about 14 kcal M^{-1} starting with glucose in micro-organisms. The chemical potential energy in the 6 carbon compound liberated during glycolysis ending with formation of lactate is only 52 kcal M^{-1}. Hence,

$$\frac{686-52}{686} \times 100 = 92{\cdot}4\%$$

of the glucose energy contained in the form of lactic acid could not be decomposed in the anaerobic glycolysis. Energy-extraction efficiency by anaerobic cells and micro-organisms from the original 6-carbon compound in the absence of oxygen is very low (7·6%). It is not surprising to find, therefore, that anaerobic cells or micro-organisms use relatively vast amounts of metabolic fuel to achieve an amount of work comparable to that carried out by more efficient energy-extraction processes. However, the energy-utilization efficiency of anaerobic glycolysis, calculated on the basis of the chemical potential of ATP produced at the expense of free energy released in producing lactate from glycogen ($\frac{21}{52} \times 100 = 40\%$) is 40% in the

glycolytic process of animals, or glucose ($\frac{14}{52} \times 100 = 27\%$) is 27% in the fermentation process of micro-organisms.

A second pathway of glucose catabolism is the hexose monophosphate shunt (HMP); it diverges from the EMP pathway at the level of glucose 6 phosphate (G6P; Fig. 3-30). Since any cell, in theory, can oxidize the NADH produced by the first 2 steps of the shunt, and can thus regenerate the NADP, the shunt provides for the

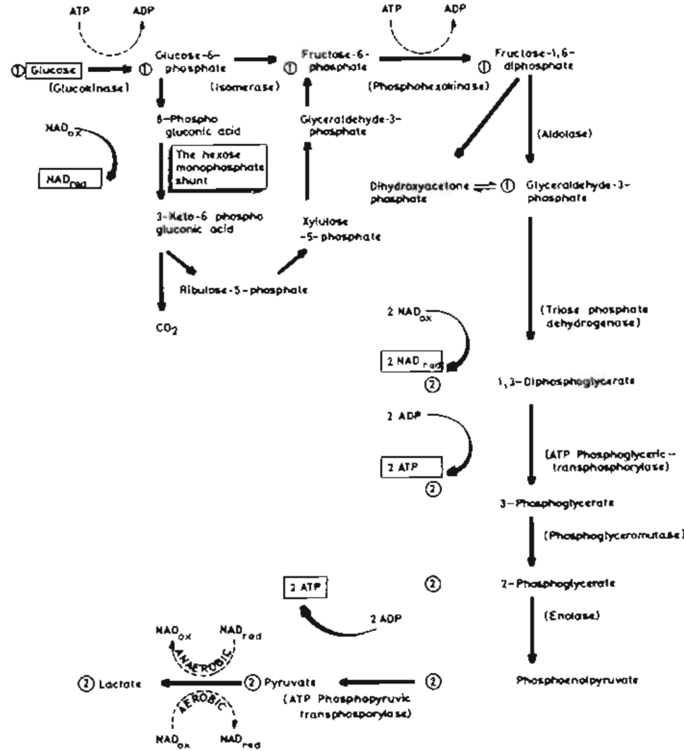
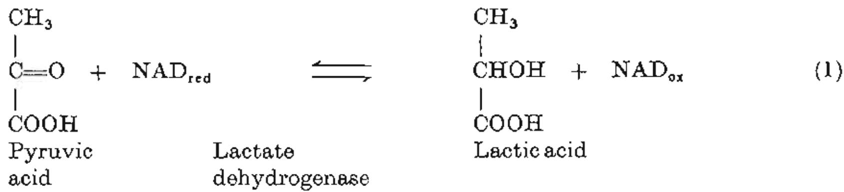


Fig. 3-30: Important steps in glycolytic sequence. Enzymes responsible for catalysing the steps are indicated in brackets; circles signify number of molecules formed from the original glucose. Steps resulting in high-energy products are characterized by heavy tapering arrows. (After NEWELL, 1970; modified; reproduced by permission of Paul Elek Limited.)

complete oxidation of G6P to CO_2 and H_2O. This entails the catalytic intervention of 5 additional molecules of G6P and a cyclic set of reaction intermediates (HOCHACHKA, 1969). The glucose dissimilation occurs primarily by the EMP pathway with only a minor contribution of the HMP shunt, for instance in fishes (HOSKIN, 1959; WILLIAMSON and co-authors, 1967). In the fish *Cichlasoma bimaculatum*, as much as 66% of glucose dissimilation occurs via the EMP pathway, whereas that via the HMP is only 19% (LIU and co-authors, 1970).

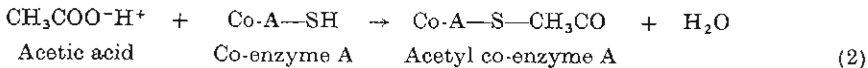
Pyruvic acid is converted to L-lactic acid* in the glycolysis of muscle tissue of animals (e.g. barnacles: BARNES and co-authors, 1963) or ethyl alcohol in the fermentation process in micro-organisms. In animals, this reaction is reversible and is catalyzed by lactate dehydrogenase (LDH):



In this process, the NAD (nicotinamide–adenine–dinucleotide) acts as an electron carrier; it donates its electrons to the pyruvate to form lactate and hence becomes oxidized. The lactic acid is oxidized back to pyruvic acid under favourable aerobic conditions and the NAD is reduced again. Under unfavourable conditions, pyruvic acid may, however, be excreted by animals to prevent a lethal reduction of the pH of the body fluids.

Aerobic dissimilation

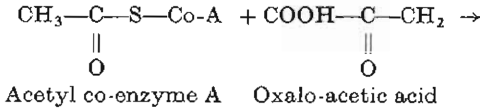
The aerobic phase of respiration consists of the Krebs tricarboxylic acid (or citric acid) cycle followed by a process of oxidative phosphorylation. The products of glycolysis or the other nutrient substances such as fatty acids and protein must be converted to acetyl co-enzyme A directly or via pyruvate, or to intermediary compounds of the Krebs cycle. For instance, glutamic acid, on deamination, forms α-ketoglutaric acid, aspartic acid forms oxalo-acetic acid, alanine forms pyruvic acid, and tyrosine forms acetyl co-enzyme A (KREBS and KORNBERG, 1957). Fatty acids are oxidized by β oxidation in the course of which a 2-carbon acid (acetyl) is split from the long chain of the fatty acid. Like these compounds entering the Krebs cycle, pyruvate is enzymatically oxidized into acetic acid, which is not in a free form but attached to co-enzyme A (Co-A) forming the compound acetyl co-enzyme A. The acetylation of Co-A occurring within the mitochondria may be expressed as follows:

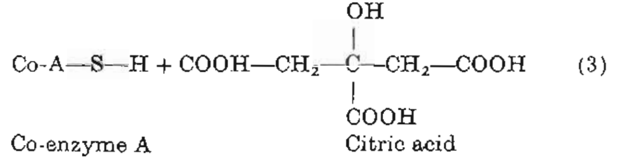


The oxidation of 3-carbon pyruvate to 2-carbon acetic acid involves the transfer of a pair of electrons to the NAD.

* The generalization that only L-lactic acid is involved in animal metabolism is perhaps no longer tenable. LONG and KAPLAN (1968) discovered that the king crab *Limulus polyphemus* has lactate dehydrogenase specific for D-lactate. The oyster *Crassostrea virginica* and a few representative molluscs were reported to oxidize only D-lactate (HAMMEN, 1969a, 1971). In their survey of 18 species of invertebrates, LONG and KAPLAN (1968) found that, in each animal, the lactate dehydrogenases are specific for only one isomer of lactate (see also GLEASON, and co-authors, 1971; SCHEID and AWAPARA, 1972). However, HAMMEN and LUM (1972) have shown that the sea urchin *Arbacia lixula* has enzymes for the oxidation of both D-lactate and L-lactate.

The first stage of the Krebs cycle is enzymatic transfer of the acetyl group of acetyl co-enzyme A to oxalo-acetic acid to form the 6-carbon tricarboxylic acid citric acid:





The co-enzyme A is thus regenerated and becomes available to accept further acetyl groups.

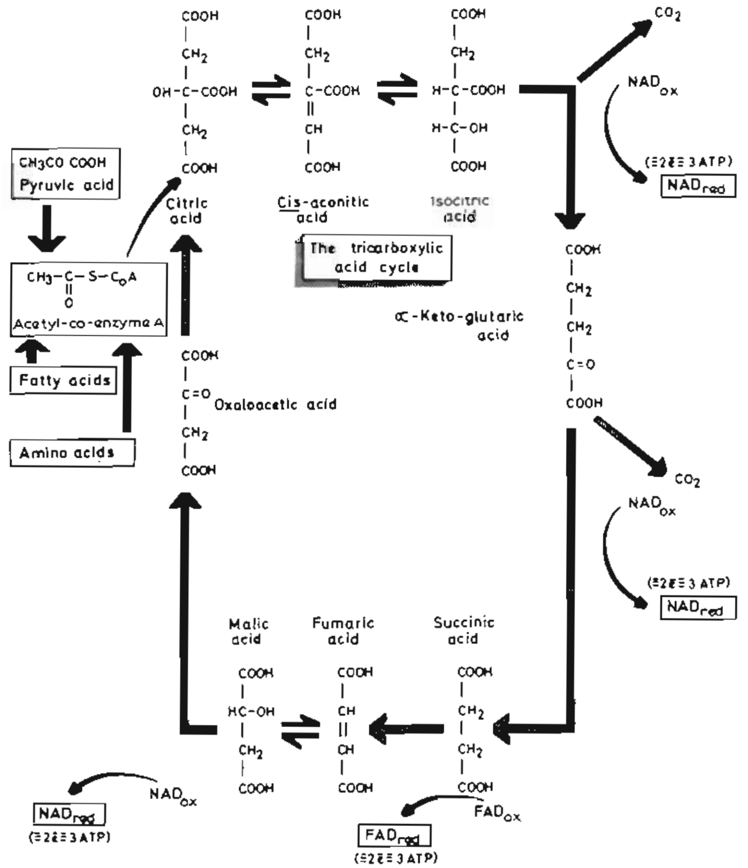


Fig. 3-31: Krebs tricarboxylic acid cycle. Energy-yielding steps are indicated together with approximate values of ATP produced by oxidative phosphorylation. (After NEWELL, 1970; modified; reproduced by permission of Paul Elek Limited.)

The 4 important energy-yielding steps in the Krebs tricarboxylic acid cycle (Fig. 3-31) are:

(i) Oxidation of the 6-carbon isocitric acid to the 5-carbon α-ketoglutaric acid plus CO_2 in the presence of the isocitrate dehydrogenase. Two electrons are removed from isocitric acid by the electron carrier NAD (Fig. 3-31).

(ii) Oxidation of the 5-carbon α-ketoglutaric acid into the 4-carbon succinic acid by the enzymic action of α-ketoglutarate dehydrogenase. A molecule of CO_2 is produced and a pair of electrons is accepted by NAD.

(iii) Removal of a pair of hydrogen atoms (dehydrogenation) from succinic acid in presence of flavoprotein (FAD_{ox}), which itself transports a pair of electrons and catalyzes the formation of fumaric acid.

(iv) Dehydrogenation of malic acid in presence of malate dehydrogenase; acceptance of a pair of electrons by the NAD, and formation of oxalo-acetic acid.

The citric-acid cycle thus performs 2 important functions: (i) Formation of C_4, C_5, C_6 skeletons for synthesis of many different cell constituents (KREBS and KORNBERG, 1957; see also HAMMEN, 1969a, p. 317; SCHOFFENIELS and GILLES, 1970, p. 206), and (ii) donation of electrons from each energy-yielding step (KREBS and KORNBERG, 1957); these electrons, along with those produced in glycolysis, are transferred to the site of oxidative phosphorylation, where reaction with ADP occurs and ATP is formed.

In the oxidative phosphorylation process, the electrons contained in each of the 3 NAD_{red} molecules derived from each revolution of the Krebs cycle are donated to a series of mitochondrial enzymes called cytochromes. Electrons pass via cytochromes either from the NAD_{red} to a flavoprotein, or directly from the Krebs cycle, where the step from succinic acid to fumaric acid is associated with the formation of reduced flavoprotein (FAD_{red}). From these hydrogen-accepting flavoproteins, electrons are transferred to a series of cytochromes as indicated in Fig. 3-32. Subsequently, the electrons pass to the terminal link, cytochrome oxidase, from which oxygen accepts the electron to form water.

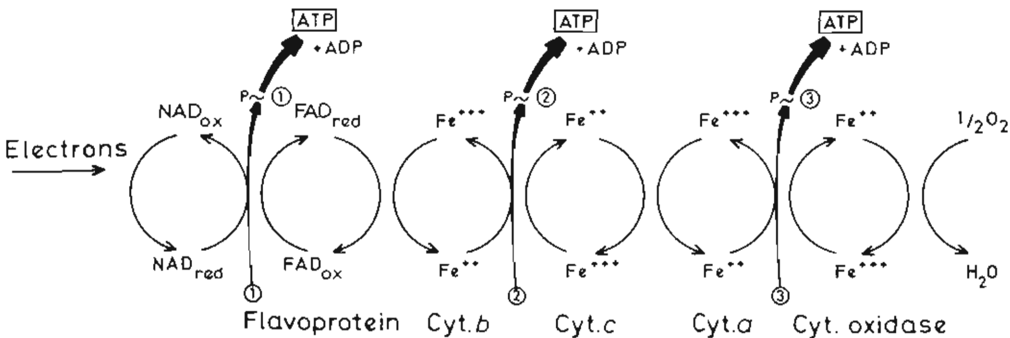
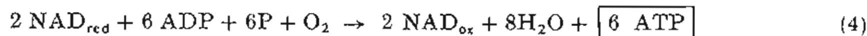


Fig. 3-32: Sites of ATP formation during passage of one electron pair along the cytochrome system. Compounds 1, 2 and 3 combine with NAD, cytochrome *b*, or cytochrome *a* to form the high-energy intermediates P ~ 1, P ~ 2 and P ~ 3 during electron transfer. High-energy intermediates then donate their high-energy phosphate groups to ADP to form ATP. (After NEWELL, 1970; modified; reproduced by permission of Paul Elek Limited.)

In this sequence of electron transfer, there is a relatively large fall in energy at three points: (i) from NAD_{red} to flavoprotein, (ii) from cytochrome *b* to cytochrome *c*, and (iii) from cytochrome *a* to cytochrome oxidase (Fig. 3-32). The liberated energy at these points is stored in the phosphate groups of high energy intermediates, which in turn donate a phosphate group to ADP to form ATP. For the passage of 2 electrons down the respiratory chain, 3 ATP molecules are formed (NEWELL, 1970):



$$2\ NAD_{red} + 6\ ADP + 6P + O_2 \rightarrow 2\ NAD_{ox} + 8H_2O + \boxed{6\ ATP} \quad (4)$$

Since 4 pairs of electrons are contributed for each molecule of acetyl co-enzyme A in the Krebs cycle, and since there are 2 molecules of acetyl co-enzyme A for each molecule of the 6-carbon sugar, the total number of ATP molecules produced during the process of oxidative phosphorylation is 24 for each 6-carbon sugar. The 2 molecules of NAD_{red} produced during glycolysis, and again 2 molecules of NAD_{red} formed during the conversion of each of the 2 molecules of pyruvate to 2 molecules of lactate produce in total 12 ATP. To these, the net gain of 3 ATP molecules formed during the anaerobic glycolysis (of animal tissues) may be added. The complete oxidation of the 6-carbon sugar produces 39 ATP molecules, which is equivalent to $(39 \times 7) = 273$ kcal. The total energy utilization efficiency amounts to

$$\frac{273 \times 100}{686} = 40\%$$

Special cases

Several intestinal parasites (SCHWABE and KILEJIAN, 1968), and intertidal organisms (VON BRAND, 1946; NEWELL, 1970) are obligatory or facultative anaerobes. Since supply of nutrient substances is not a limiting factor in intestinal parasites, they can afford to use vast amounts of metabolic fuels. When exposed to adverse conditions of reduced availability of O_2 or lack of O_2, the intertidal animals may (i) switch over to aerial respiration, e.g. gastropods *Monodonta lineata* (MICALLEF, 1966), *M. turbinata* (MICALLEF and BANNISTER, 1967), several species of *Littorina* (SANDISON, 1966, 1967); crustaceans *Ligia oceanica* (EDNEY, 1960), *Balanus balanoides* (GRAINGER and NEWELL, 1965); several species of fishes, *Anguilla anguilla* (BERG and STEEN, 1965), *Gillichthys mirabilis* (TODD and EBERLING, 1966), for a review consult SAXENA (1963); (ii) reduce their metabolic rate so that the demand for O_2 is kept to a minimum, e.g. coelenterates (BRAFIELD and CHAPMAN, 1965; SASSAMAN and MANGUM, 1972); annelids (VON BRAND, 1946); the barnacle *Chthamalus depressus* (HAMMEN, 1972); or (iii) become anaerobic. Anaerobiosis may permit intertidal animals to survive adverse conditions such as desiccation or reduced salinity (Volume I: HOLLIDAY, 1971; KINNE, 1971); the process is accomplished at the expense of relatively large amounts of metabolic reserves.

In some animals (iiia), duration of anaerobiosis is too short to permit accumulation of lactic acid without excess reduction in pH of tissue fluids; when favourable aerobic conditions return, a considerable fraction of the accumulated lactic acid is converted back to pyruvate and oxidized to water and carbon dioxide. Such

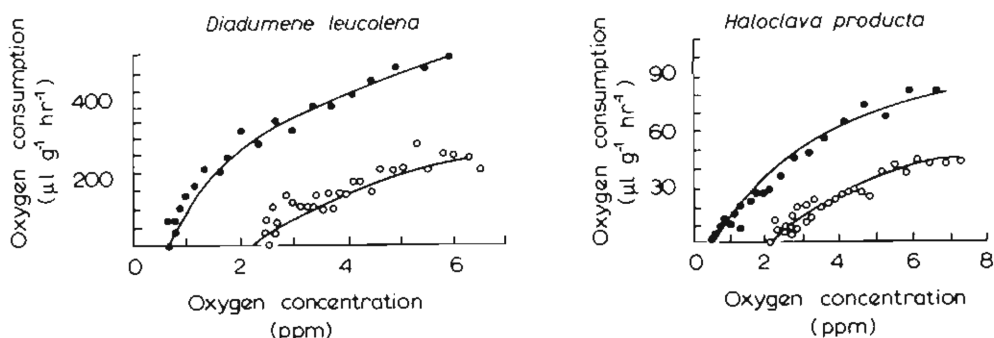


Fig. 3-33: *Diadumene leucolena* and *Haloclava producta*. Relationship between oxygen consumption rate and oxygen concentration before (○) and after (●) 9 or 24-hr exposure to anoxia. (After SASSAMAN and MANGUM, 1973; modified; reproduced by permission of Pergamon Press Ltd.)

oxidation results in an increased oxygen demand of tissues over normal metabolic requirements, constituting the repayment of an 'oxygen debt', e.g. anemones *Calliactis parasitica* (BRAFIELD and CHAPMAN, 1965), *Diadumene leucolena*, *Haloclava producta* (SASSAMAN and MANGUM, 1973; Fig. 3-33). The responses of the anemone *Metridium senile* restored from anoxic and hypoxic conditions considerably differ and are of great interest. Exposure to anoxic conditions induces an oxygen debt implying oxidation of accumulated products of anaerobiosis (Fig. 3-34). However, immediate return of the anemone, whose aerobic metabolism was reduced owing to hypoxy (less than 33% air saturation) to air saturation is not accompanied by increased oxygen uptake at any tested oxygen concentration (Fig. 3-34). *M. senile* does not incur any measurable oxygen debt until after it completely switches

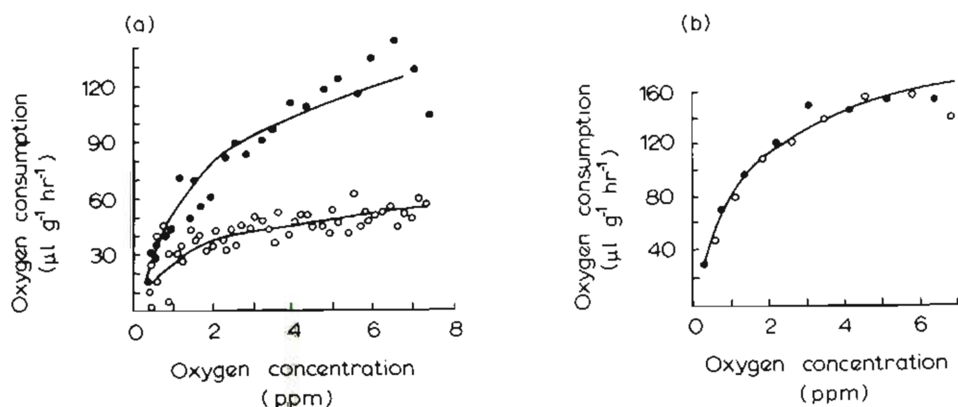


Fig. 3-34: *Metridium senile*. Relationship between oxygen consumption rate and oxygen concentration. (a) Oxygen uptake before (○) and after (●) 24-hr exposure to anoxia. (b) Oxygen uptake before (○) and immediately after (●) drastic reduction of aerobic metabolism. (After SASSAMAN and MANGUM, 1973; modified; reproduced by permission of Pergamon Press Ltd.)

off aerobic metabolism (SASSAMAN and MANGUM, 1973). The bivalves *Gelonia ceylonica* and *Anadara granosa*, which can regulate their oxygen consumption at reduced tension in sea water, fail to regulate O_2 uptake to declining oxygen tension at reduced salinity (BAYNE, 1973).

In other animals (iiib), the anaerobic decomposition of 6-carbon compounds ends with substances other than lactic acid. Examples are available for annelids (DALES, 1969); for crustaceans (HOHNKE and SCHEER, 1970); for molluscs (SIMPSON and AWAPARA, 1966; HAMMEN, 1969b; DE ZWAAN and ZANDEE, 1972b; DE ZWAAN and co-authors, 1973; see also NEWELL, 1964; GOUDSMIT, 1972) a similar anaerobic dissimilation of 6 carbon compounds is also known to occur in certain bacteria (LYNCH and CALVIN, 1952) and in intestinal parasites (SAZ and BUEDING, 1966; SAZ, 1971).

Intertidal animals. During anaerobiosis, a number of molluscs either produce lactic acid in amounts much smaller than those dictated by the stoichiometry of glycolysis, or produce no lactate at all; they accumulate succinate (HUMPHREY, 1944; HAMMEN and OSBORNE, 1959; SIMPSON and AWAPARA, 1965; AWAPARA and SIMPSON, 1967; DE ZWAAN and ZANDEE, 1972b). This aspect of carbohydrate catabolism during anoxia in intertidal animals has been investigated (i) using radio-active precursors (e.g. SIMPSON and AWAPARA, 1966), (ii) studying the presence and levels of enzymes (e.g. BENNETT and NAKADA, 1968; O'DOHERTY and FELTHAM, 1971; DE ZWAAN, 1971), or (iii) estimating the effects of glycogen dissimilation on the levels of D-lactate, alanine and succinate in test individuals before and after the induced anaerobiosis (e.g. DE ZWAAN and ZANDEE, 1972b). STOKES and AWAPARA (1968) incubated under anoxia (nitrogen) the mantle of the brackish-water clam *Rangia cuneata* with randomly labelled ^{14}C glucose and found that succinate and alanine in equimolar amounts constituted the major portion of the end-products. In their scheme for glucose dissimilation, the reduction of pyruvate to lactate for the re-oxidation of glycolytic NADH is replaced by two other steps namely oxalo-acetate (OXA) → malate, and fumarate → succinate, and phosphoenolpyruvate (PEP) is carboxylated to OXA. The second half undergoes reactions not involving oxidoreductions, namely transamination into alanine. According to this scheme, the NADH/NAD redox pair is kept in the same state necessary to maintain glycolysis.

In the European oyster *Mytilus edulis*, the production of succinate, alanine and D-lactate were measured in individuals before and after induced (by leaving them to dry) anaerobiosis for 48 hrs (DE ZWAAN and ZANDEE, 1972b). Although a relatively unimportant amount of D-lactate was produced, its presence is remarkable, as (i) the enzyme D-lactate dehydrogenase (LDH) is present in the bivalve *Mercenaria mercenaria* (MASSARO, 1970; see also p. 155), and (ii) D-lactate and D-LDH have not received attention by the specialists in this field. Succinate and alanine production in *M. edulis* during anoxia amounted to 635 $\mu M\ g^{-1}$ live weight in 48 hrs; this amount is equivalent to 5·4 mg glucose per individual (mean body weight 9·6 g) and to about 6 mg glycogen (mg glycogen = mg glucose × 1·111; MORRIS, 1948). Direct estimates of glycogen content of test individuals before and after 48 hrs of anaerobiosis indicated a loss of 10 to 15 mg glycogen per 48 hrs. Therefore, alanine and succinate (plus D-lactate) account for about 50% of the degraded glycogen,

Table 3-26

Activities of lactic dehydrogenase (LDH), pyruvate kinase, malic dehydrogenase (MDH) and phosphoenolpyruvic (PEP) carboxykinase in some marine invertebrates, intestinal parasites and terrestrial mammals (After SIMPSON and AWAPARA, 1966; modified and extended; reproduced by permission of Microforms International Marketing Corp.)

Species	LDH	Pyruvate kinase	MDH	PEP carboxy-kinase	Author
	(μm NADH oxidized mg^{-1} protein min^{-1})				
Rangia cuneata	0·038	0·041	3·98	0·045	SIMPSON and AWAPARA (1966)
Crassostrea virginica	0·004	0·055	1·06	0·002	
Volsella demissus	0·010	0·053	0·80	0·008	
Littorina irrorata	0·0085	0·007	2·13	0·001	
Thais haemastoma	0·031	0·024	1·22	0·001	
Bunodosoma cavernata	0·002	0·189	0·34	0·004	
Rat	1·290	0·025	3·33	0·007	
Chicken	2·400	0·027	3·96	0·016*	
Placopecten magellanicus	0·022	0·031	2·91	0·037	O'DOHERTY and FELTHAM (1971)
Rat	1·320	0·230	3·23	0·008	
Ascaris lumbricoides	0·143	0·007	5·28	0·168	SAZ (1971)

* μM $^{14}CO_2$ exchanged mg^{-1} protein min^{-1} at 37° C (SIMPSON and AWAPARA, 1964). Tissue used for assay: marine invertebrates, mantle; *A. lumbricoides*, muscle; rat and chicken, liver.

and succinate and alanine were formed in almost equimolar amounts, resembling the findings of STOKES and AWAPARA (1968).

Based on enzyme-activity ratios in the American oyster *Crassostrea virginica*, HAMMEN (1966, 1969b) postulated 'the malate route' through which succinate is produced. It begins with the carboxylation of pyruvate yielding OXA, which is converted to malate, then to fumarate and finally to succinate. The scheme proposed for *C. virginica* is essentially similar to that proposed by SAZ and WEIL (1962) for *Ascaris lumbricoides*. These authors suggested that glucose becomes degraded to pyruvate, which is converted into OXA by a NAD-dependent 'malic enzyme'. Recently, the scheme for *A. lumbricoides* underwent modification, because pyruvate kinase (PK) activity appeared to be absent (SAZ and LESCURE, 1969; Table 3-26). Thus it seems that in most animals producing succinate, PEP rather than pyruvate becomes carboxylated. Taking this into account, HAMMEN (1966) could detect only 'malic enzyme' activity by using malate as substrate and from the observations that PK activity becomes strongly inhibited by decreasing pH within the physiological range (MUSTAFA and HOCHACHKA, 1971; DE ZWAAN and HOLWERDA, 1972), it seems possible that in *C. virginica* PEP is carboxylated and that 'malic enzyme' plays an active role in the overall pathway in providing NADH in the mitochondrion, which becomes re-oxidized by the reduction of fumarate (DE ZWAAN and VAN MARREWIJK, 1973).

Pyruvate kinase activity in fresh extracts from the posterior adductor muscle of *Mytilus edulis* shows allosteric properties in contrast to skeletal muscle PK of mammals. These properties of PK explain why a part of PEP is available for succinate production during anaerobic glycolysis (DE ZWAAN, 1972). In a survey of several marine molluscs, SIMPSON and AWAPARA (1964) found that the most widely distributed carboxylating enzyme was PEP-carboxykinase. High activity of PEP carboxykinase diverts a large amount of PEP from the main EMP pathway into the dicarboxylic acids with ultimate accumulation of succinate, Many invertebrates tested (Table 3-26) have very active PEP carboxykinases which permit a rapid conversion of PEP to OXA and also have active PKs that would catalyze the breakdown of PEP to pyruvate (SIMPSON and AWAPARA, 1966). Therefore, PEP must be divided equally between pyruvate and OXA (DE ZWAAN, 1972). However, recent investigations on *M. edulis* have revealed that alanine is the initial major end-product of anaerobic glycolysis and as the concentration of alanine increases PK is reduced to 70% after 48 hrs of shell closure and an increasing part of PEP is carboxylated to OXA. The latter is converted to succinate via malate and fumarate. Accumulation of organic acids resulting in lowering of the pH is the second factor which decreases PK activity (DE ZWAAN, 1972; DE ZWAAN and HOLWERDA, 1972; DE ZWAAN and VAN MARREWIJK, 1973; HOLWERDA and co-authors, 1973). After a period of 48-hr shell closure (anoxia), however, alanine and succinate are accumulated in almost equimolar amounts.

PEP reacts with CO_2 to form OXA; a survey of carbon dioxide fixation in 20 species of marine invertebrates belonging to 14 phyla (Table 3-27) reveals fixation of CO_2 into organic acids in all species tested (HULTIN and WESSEL, 1952; HAMMEN and OSBORNE, 1959; CHENG and WAELSCH, 1962; HAMMEN and LUM, 1962, 1964; HAMMEN, 1964). Malate, formed from OXA in at least 2 different pathways (Fig. 3-35), has 2 alternative metabolic fates: one route of metabolism ends in production

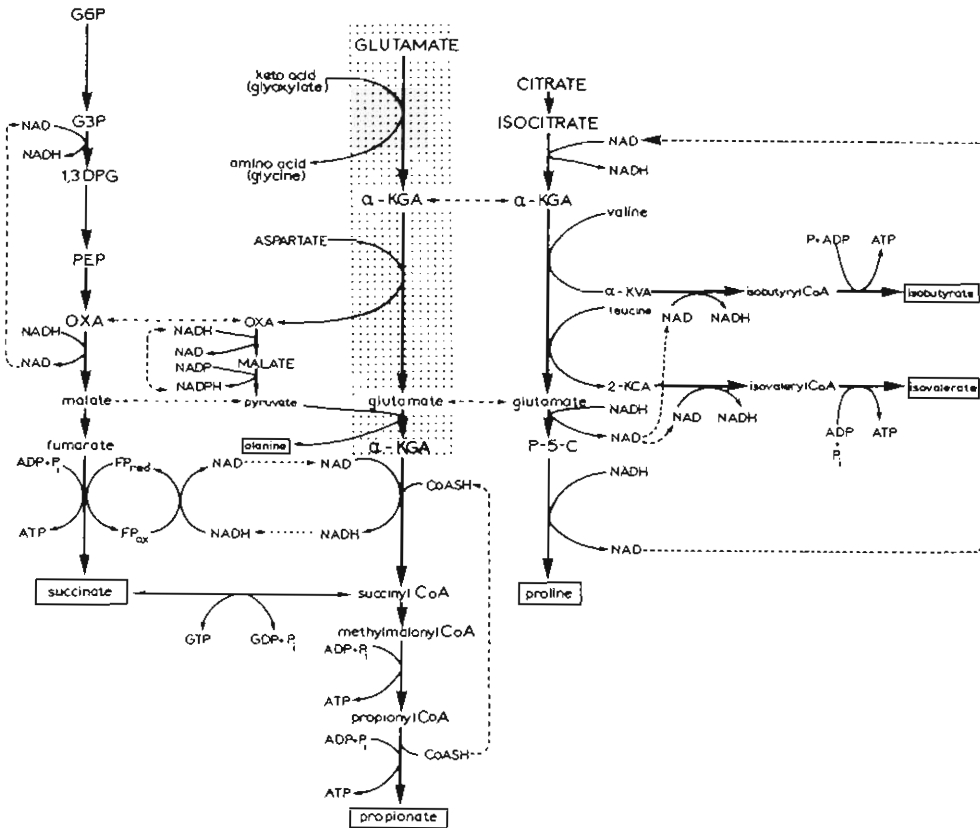


Fig. 3-35: A metabolic map accounting for currently known end-products of anaerobic carbohydrate and amino-acid catabolism in facultatively anaerobic animals. Most of the data are from parasitic helminths and from intertidal bivalves. G6P: glucose-6-phosphate; G3P: glyceraldehyde-3-phosphate; 1,3 DPG: 1,3 diphosphoglycerate; PEP: phosphoenol-pyruvate; OXA: oxalo-acetate; α-KGA, α-ketoglutarate; NAD and NADH: oxidized and reduced nicotinamide adenine dinucleotide; NADP and NADPH, oxidized and reduced nicotinaminde adenine dinucleotide phosphate; FP_{ox} and FP_{red}: oxidized and reduced flavoprotein; ADP and ATP; adenosine di- and triphosphate; GDP and GTP: guanosine di- and triphosphate; P_i: inorganic phosphate; α-KVA: α-keto-isovalerate; 2-KCA: 2-ketoisocaproate; P-5-C: pyrroline-5-carboylate. (After HOCHACHKA and co-authors, 1973a; reproduced by permission of American Society of Zoologists.)

of succinate; in the other, malate, catalyzed by malic enzyme is decarboxylated to pyruvate, which upon transamination accumulates as alanine (Fig. 3-35; HOCHACHKA and co-authors, 1973a). This part of glycolysis, i.e. up to the formation of malate occurs in cytoplasm. Malate migrates into the mitochondrion and becomes partly transformed into succinate via reverse direction of the Krebs cycle and partly into glutamate via α-ketoglutamate (KGA) in the Krebs cycle. This route involves one reduction (fumarate → succinate) and three oxidations (malate → oxalo-acetate, pyruvate → acetyl-Co-A, isocitric → 2-KGA; DE ZWAAN and VAN MARREWIJK, 1973; DE ZWAAN and co-authors, 1973). Perhaps this may explain the

observations of WEGENER and co-authors (1969), who found 55% of the radioactivity in succinate, about 10% in malate or OXA and 2% in KGA or citrate, when the mantle of the oyster *Crassostrea virginica* was anaerobically incubated with labelled fumarate.

Current information on this subject has been summarized by HOCHACHKA and co-authors (1973a) in an overall scheme through which glucose is converted to succinate and alanine, aspartate to alanine and glutamate to succinate. The dissimilation of glucose to the level of PEP occurs by the classical EMP pathway; in the presence of O_2, PEP is converted to pyruvate which is completely oxidized

Table 3-27

Marine invertebrates known to fix CO_2 into organic acids (After HAMMEN, 1964)

Phylum	Class	Species
Porifera	Demospongia	*Hymeniacidon heliophila*
Platyhelminthes	Turbellaria	*Bdelloura candida*
		Stylochus zebra
	Trematoda	*Entobdella bumpusi*
Cnidaria	Anthozoa	*Aiptasia pallida*
Ctenophora	Tentaculata	*Mnemiopsis leidyi*
Nemertina	Anopla	*Cerebratulus lacteus*
Ectoprocta	Gymnolaemata	*Bugula neritina*
Brachiopoda	Inarticulata	*Lingula reevi*
Hemichordata	Enteropneusta	*Saccoglossus kowaleuskii*
Echinodermata	Holothuroidea	*Leptosynapta inhaerens*
	Echinoidea	*Psammechinus miliaris*
Annelida	Polychaeta	*Chaetopterus variopedatus*
Echiuroidea		*Thalassema mellita*
Mollusca	Pelecypoda	*Crassostrea virginica*
		Mercenaria mercenaria
Arthropoda	Crustacea	*Callinectes sapidus*
		Homarus americanus
	Arachnida	*Limulus polyphemus*
Urochordata	Ascidiacea	*Styela plicata*

into CO_2 and H_2O. During anoxia, however, PEP is carboxylated to OXA by PEP carboxykinase (PEPCK) and OXA is quickly reduced to malate by malate dehydrogenase (MDH) (CHEN and AWAPARA, 1969a; HOCHACHKA and MUSTAFA, 1973; MUSTAFA and HOCHACHKA, 1973a, b). That is, PK and PEPCK function at a critical branch-point and their activities must be tightly controlled during aerobic–anaerobic transition.

From Fig. 3-35 it is also evident that the formation of 2 M from each mole of G6P involves simultaneous mobilization of 2 M of aspartate and 2 M of KGA and sustains a constant NAD/NADH ratio. The thus sustained redox balance further assures simultaneous mobilization of carbohydrate and amino acids; the NADH scheme is comparable to that of glycolysis in that there is no depletion or accumu-

lation of NADH in either process. This is important, for the central problem of anaerobiosis is to prevent the cell from passing into a reduced state (HOCHACHKA and co-authors, 1973a). On energetic considerations, the above scheme displays surprising advantages over the classical glycolysis. If, for the purposes of redox balance, the stoichiometry implied in Fig. 3-35 is assumed, it is evident that for each mole of G6P, 3 M of ATP are gained in the production of fumarate and 2 M of ATP are gained at the fumarate reduction; the net yield is 7M ATP M^{-1} glucose + 2 M aspartate + 2 M α-KGA. The advantages over the classical HMP pathway are: (i) Two- to three-fold increase in ATP production. (ii) Succinate, a weaker acid than lactate, causes, during its accumulation in the extrapallial fluid, less hydrolization of $CaCO_3$ from the shell. (iii) Upon return of aerobic conditions, succinate, an intermediate of the Krebs cycle, can be easily oxidized. In animals, biosynthesis of such compounds as purines, pyrimidines and amino acids results in constant egress of the intermediates from the Krebs cycle (MUNDAY and POAT, 1971); the same is true for bacteria (WOOD and STJERNHOLM, 1962). Since carbon-dioxide fixation serves to synthesize 4-carbon dicarboxylic acids of the Krebs cycle from 3-carbon precursors, CO_2 fixation would be needed to replace the intermediates drawn off for biosynthesis of amino acids, etc. (iv) Formation of alanine includes detoxication of NH_3 by fixation of pyruvate. Ammonia is the main end-product of protein catabolism and is excreted under aerobic conditions. (v) With reduction of fumarate to succinate an extra step is introduced for regenerating ATP from glucose (HAMMEN, 1964; HOCHACHKA and co-authors, 1973a; DE ZWAAN and co-authors, 1973).*

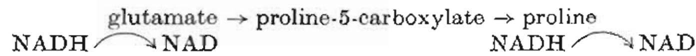
Intestinal parasites. In parasitic worms (mostly non-marine species) intermediary metabolism has received considerable attention (for reviews consult FAIRBRAIN, 1957; READ, 1961, 1968; BUEDING, 1962; SCHWABE and KILEJIAN, 1968; SAZ, 1969). The information available for the intestinal nematode *Ascaris lumbricoides* is presented here as a model system for the elucidation of an anaerobic energy-yielding pathway of carbohydrate dissimilation, which appears to be operating in a number of other intestinal parasites (SAZ, 1971). Glucose is converted to succinate and alanine by the intestinal parasites through the pathways described for intertidal molluscs (SAZ, 1971; Fig. 3-35). Succinate may either accumulate, or be converted to propionate. A number of nematodes, trematodes, cestodes (VON BRAND, 1966; FAIRBRAIN, 1970, 1971) and annelids (COLES, 1970) are known to produce propionate as one of the major end-products of anaerobic metabolism. Succinyl Co-A, formed from succinate and α-KGA (see Fig. 3-35) is converted into propionate; for each mole of succinyl Co-A converted to propionate, 2 M of ATP can be produced, i.e. the production of propionate from succinyl Co-A is energetically more advantageous than the succinate thiokinase reaction. The scheme can yield about 11 M of ATP

* A special mechanism of endogenous oxidation is activated in certain molluscs (e.g. *Mytilus galloprovincialis*) by gradual environmental decrease in oxygen tension (ZS.-NAGY and ERMINI, 1972a, b). The anoxic tolerance of the molluscs tested is connected with the presence of specific pigment granules in nervous and other tissues (ZS.-NAGY, 1971a). The granules—'cytosomes' (NOLTE and co-authors, 1965)—contain respiratory enzyme activities (ZS.-NAGY, 1967; ZS.-NAGY and KEREL-FRONIUS, 1970). The amount of this oxidative agent decreased significantly during prolonged anoxia. The electron-acceptor capacity is most probably bound to the yellow lipochrome pigment of the cytosomes (ZS.-NAGY, 1971b; ZS.-NAGY and ERMINI, 1972b).

M^{-1} of G6P + 2 M asparate + 2 M of glutamate (HOCHACHKA and co-authors, 1973a).

In addition to propionate, other volatile fatty acids, often produced by nematodes, cestodes (FAIRBRAIN, 1970; WARREN and POOLE, 1970) and annelids (COLES, 1970), are isovalerate, isobutyrate and methylbutyrate. These end-products appear to be derived from the anaerobic catabolism of leucine, valine or isoleucine (Fig. 3-35).

Proline is one of the major end-products of energy metabolism in trematodes (ISSERHOFF and co-authors, 1972). Though the glutamate → proline conversion is reversible, both of the following reduction steps proceed with large free energy drops:



Therefore, the production of proline from glutamate is highly favourable thermodynamically. HOCHACHKA and co-authors (1973a) consider that, in terms of redox balance, this is an unusually efficient reaction scheme, as 2 M NADH are oxidized per mole of proline formed, and that the regenerated NAD could be utilized by the KCDH (α-ketoisovalerate dehydrogenase) reactions in isovaleryl Co-A formation, as well as regeneration of α-KGA for the initial transamination.

Diving vertebrates. Diving marine mammals supplement body oxygen stores to be used during the dive by increasing the O_2 storage capacity of their lung (e.g. the sea otter *Enhydra lutris*; KOOYMAN, 1973) or the blood (e.g. the seal *Phoca vitulina*; KOOYMAN, 1973). Increasing the O_2 storage capacity of the lung results in concomitant increase in N_2 level, which could have adverse buoyancy effects during deep dives. In contrast to this, the O_2 storing capacity of the blood is increased significantly without changing the total blood volume simply by increasing the volume of red blood cells and their haemoglobin concentrations (e.g. the Weddell seal *Leptonychotes weddelli*; KOOYMAN and co-authors, 1971).

The seal *Cystophora cristata* easily endures prolonged apnoea; on recovery, the excess of O_2 uptake covers only a quarter of the debt which could have been incurred had the metabolism remained at the pre-dive level (SCHOLANDER, 1940). *C. cristata* also suffers a steady temperature loss during apnoea, even though it is suspended in a normally thermoneutral medium and the loss of temperature during apnoea must be caused by a reduction in heat production (SCHOLANDER and co-authors, 1942). SCHOLANDER (1964) concluded that the aerobic energy metabolism is strongly reduced during the dive because of the limited O_2 store and that lactate production as one facet of the anaerobic metabolism is much too low to make up the deficit.

Studies on lactate dehydrogenases in the seal *Cystophora cristata* and the eider *Somateria mollissima* (BLIX and FROM, 1971), the penguin *Pygoscelis adeliae* (MARKERT and MASUI, 1969) and the (freshwater) turtle *Pseudomys scripta elegans* (ALTMAN and ROBIN, 1969) reveal that, as a response to hypoxic conditions induced during prolonged diving, biochemical adaptations take place at the enzyme level in these animals. Summarizing the pertinent literature, HOCHACHKA and co-authors

(1973a) predicted that in the diving vertebrate animals, the classical glycolytic production of ATP is supplanted by (i) fumarate reduction coupled to the electron transfer system; (ii) glutamate mobilization via the α-KGA $\rightarrow$ succinyl Co-A $\rightarrow$ succinate pathway; or (iii) both mechanisms in a manner directly comparable to that of invertebrate facultative anaerobes. Confirming the third possibility, HOCHACHKA and co-authors (1973b) reported that when the oxygen supply to working muscles is limited by peripheral vasoconstriction, the muscles ferment glycogen and amino acids. Multiple anaerobic end-products are accumulated. Succinate is the chief end-product of anaerobic amino-acid catabolism, alanine is a minor end-product, while pyruvate and lactate are produced by glycolysis. During recovery following diving, increased blood concentrations of all four metabolites can be readily measured in the green turtle (*Chelonia mydas mydas*), the harbour seal (*Phoca vitulina*), the sea lion (*Zalophus californianus*) and the porpoise (*Phocoena phocoena*).

Temporary Storage in Form of Body Structures

The efficiency food conversion into body structures may be expressed as percentage of food consumed or as percentage of food absorbed. In the former case, it is called gross efficiency (K_1) and in the latter net efficiency (K_2) (PANDIAN, 1967a).

Heterotrophic animals use D-sugars and L-amino acids and thus have narrowed the common field of exploitation; certain micro-organisms utilize more unusual constituents (NEEDHAM, 1964). Gross conversion efficiency ranges from 3% in certain nitrogen-fixing bacteria, which must synthesize protoplasm all the way from simple inorganic substances (NEEDHAM, 1964), to as high as 50% in *Nereis virens* (GOERKE, 1971) and over 60% in the embryo of some animals (e.g. fish: FLÜCHTER and PANDIAN, 1968; crustaceans: PANDIAN, 1967c, 1970b, c; molluscs: PANDIAN, 1969a). Quantitative aspects of rate and efficiency of food conversion are dealt with in Volume I (KINNE, 1970, pp. 466–474; KINNE, 1971, pp. 927–928; ALDERDICE, 1972, pp. 1704–1709, and in Volume IV: CONOVER, in press). For additional information, the reader may consult PAFFENHÖFER (1968): hydroids; HARLEY (1956), IVLEVA (1970), GOERKE (1971), KAY and BRAFIELD (1973): polychaetes; LASKER (1966): euphausiid crustaceans; CAREFOOT (1697a, 1970), PAINE (1971a), CONOVER and LALLI (1972): gastropods; KUENZLER (1961), HUGHES (1970): bivalves; FUJI (1967): echinoids; MENZEL (1959, 1960), KINNE (1960, 1962), PANDIAN (1967a, b, 1970a), BRETT and co-authors (1969): fishes. Data on specific aspects of conversion regarding anthozoans, cephalopods or asteroids are still lacking. There are over 100 publications concerned with conversion in fishes fed animal food, but to the reviewer's knowledge, MENZEL (1959) is the only author who reports data on conversion in the herbivorous angelfish *Holocanthus bermudensis*. No quantitative data are available on herbivorous fishes and on sediment/detrivorous invertebrates. Methods for estimating retention and absorption of organic matter by detritivores have been developed by JOHANNES and SATOMI (1967) and HUGHES (1969).

Storage organs

Converted substances may be stored in special organs. Examples of storage organs are the integument: sea cucumbers (GIESE, 1966a), many polychaetes (see

SCHEER, 1969); muscles: fishes (LOVE, 1970); gut: sea-urchins (LAWRENCE and co-authors, 1966; LAWRENCE, 1967); digestive glands: sea-stars (GIESE, 1966a); hepatopancreas: decapods and molluscs; foot: gastropods and amphineurans (GIESE, 1966a); and gonads: all higher sexually reproducing animals.

Proteins

Organisms store reserve energy in the form of fat, carbohydrate or protein. Synthesis and accumulation of proteins have been regarded as main denominators of true growth by several scientists (e.g. GERKING, 1952). Nutrient substances accumulated in the form of protein may also serve as energy source during starvation (e.g. echinoderms: GIESE, 1966a; fishes: LOVE, 1970; RAGHURAMAN, 1973).

Carbohydrates

Some bivalves store large amounts of glycogen (11 to 30%, Table 3-28). In *Martesia fragilis*, an unusually high value of 52% has been reported for glycogen (SRINIVASAN, 1963; SRINIVASAN and KRISHNASWAMY, 1964); values of glycogen ranging from 10 to 35% for soft parts of the mussel *Mytilus edulis* are common (DE

Table 3-28

Percentages of glycogen and lipid in some lamellibranchs (After GIESE, 1966a; modified; reproduced by permission of American Physiological Society)

Species	Glycogen (%)	Lipid (%)	Author
Ostrea gigas	11	8	MASUMOTO and HIBINO (1932)
Mytilus edulis	13	8	MOLINS and BESADA RIAL (1959)
Crassostrea virginica	26	8	PEASE (1932)
Teredo pedicellata	30	5	GREENFIELD (1953)

ZWAAN and ZANDEE, 1972a), with extremes around 60% (in *M. edulis*, DE ZWAAN and ZANDEE, 1972a; in *Ostrea edulis*, WALNE, 1970). GIESE (1966a) states that, unlike echinoderms and other molluscs, bivalves have a prominent glycogen economy with correspondingly lesser storage of lipid. However, turnover rate as well as the level of a particular nutrient substance may have to be considered before a final assessment of economy can be made.

Table 3-29 shows that carbohydrates are stored in varying quantities in different annelids. Carnivorous polychaetes (e.g. species belonging to the genus *Nereis*) have low glycogen contents (less than 10% dry weight), as do herbivores and detritivores (e.g. *Arenicola marina*). VON BRAND (1927 *in:* SCHEER, 1969) concluded that the carbohydrate content is related more to the mode of life of the species than specifically to its nutrition.

High glycogen content is also characteristic of endo-parasitic helminths (SMYTH, 1962). Glycogen content ranges from 19% in the turbellarians *Syndesmis francis-*

Table 3-29

Total carbohydrate content of annelids (After SCHEER, 1969; modified; reproduced by permission of Academic Press, New York)

Species	Carbohydrate content (%)
Polychaeta	
Hermione hystrix	1·4
Glycera siphonostoma	3·2*
Dasybranchus caduceus	3·8*
Myxicola infundibulum	4·4
Nereis pelagica	4·0
N. fuliginosa	4·5*
N. virens	6·0
N. diversicolor	16·8
Marphysa sanguinea	4·6
Arenicola marina	4·8
Nephthys scolopendroides	8·5*
Aricia foetida	9·2
Protula protula	11·2
Lanice conchilega	16·5
Amphitrite rubra	20·4
Andouinia tentaculata	21·5*
Owenia fusiformis	23·4
Terebella lapidaria	24·0
Branchiomma vesiculosum	24·5
Hirudinea	
Pontobdella muricata	5·0*

* Mean values.

cana, an entocommensal in the coelom and intestine of the sea-urchin *Strongylocentrotus purpuratus* (METTRICK and JENNINGS, 1969) to over 30% in several cestodes (CLEGG and SMYTH, 1968). In cestodes, glycogen content seems to fluctuate with the amount of carbohydrate ingested by the host (READ, 1961). But it is likely that the high glycogen content is related more to the anaerobic respiratory metabolism (see p. 165). In these anaerobic parasites, fatty acids are never oxidized (FAIRBRAIN, 1930 *in:* SAZ, 1971).

Fat is an ideal storage material: (i) It liberates twice as much energy as an equal weight unit of carbohydrate or of protein (1 g carbohydrate, protein, or fat yields 4150, 5650 or 9400 cal g^{-1}; MAYNARD and LOOSLI, 1962). (ii) Fat contains less water than carbohydrate or protein (only 0·1 g water is associated with 1 g fat, but as much as 3 g water with 1 g protein; BRODY, 1968, p. 52). (iii) Fat has a lower specific gravity than water and hence serves to buoy floating animals. For total fat contents of marine organisms consult VINOGRADOV (1953) and GIESE (1966a).

Eggs of marine invertebrates often accumulate considerable amounts of lipids, ranging from 14% in *Loligo opalescens* egg (GIESE, 1966a) to 44% in *Homarus gammarus* egg (PANDIAN, 1970b) and to 46% in ovary of *Cucumaria frondosa* (GIESE, 1966b). Sometimes, body components store surprisingly high amounts of

lipids; for example, the digestive glands store 17% in *Oreaster reticulatus* and 41% in *Palmites membranaceous* (GIESE, 1966a). Lipids of marine invertebrates have lower melting points than lipids of terrestrial vertebrates and are, therefore, fluid not solid at room temperature. Some values for total lipid contents in body fluids are given in Table 3-30. In bivalves, the lipid level is generally considered to be lower (Table 3-28). The female oyster *Ostrea gigas* stores twice as much lipid as the male at the time of gametogenesis (MASUMOTO and HIBINO, 1932). Pregravid oysters are rich in glycogen, less so in lipids. When a hermaphroditic oyster functions as a female, it is found to contain more lipid than when it is a male (TIMON-DAVID and CERESOLA, 1935).

Table 3-30

Total amount of lipids in body fluids of some echinoderms, molluscs and crustaceans (After GIESE, 1966a; modified; reproduced by permission of American Physiological Society)

Species	Lipid (mg 100 ml^{-1})	Species	Lipid (mg 100 ml^{-1})
Echinodermata		Mollusca	
Asteroidea		Amphineura	
Patiria miniata	11	*Cryptochiton stelleri*	15
Pycnopodia helianthoides	15	*Katharina tunicata*	67
P. giganteus	13	*Mopalia mucosa*	72
P. brevispinus	15	*M. lignosa*	77
P. ochraceus	20	*M. ciliata*	101
Evasterias troschelii	22		
Echinoidea		Gastropoda	
Allocentrotus fragilis	8	*Haliotis rufescens*	12
Strongylocentrotus franciscanus	26	*H. cracherodii*	17
S. purpuratus	34	*Megathura crenulata*	18
S. dröbachiensis	44	*Lottia gigantea*	58
Holothuroidea			
Stichopus californicus	9	Crustacea	
Echiura		*Maja squinado*	458
*Urechis canpo**	930	*Pagurus prideauxii*	520

*After LAWRENCE and co-authors (1971).

Energy

In general, calorific values vary between a lower limit of 3740 cal g^{-1} dry organic substances (hereafter expressed as cal g^{-1}) in glucose and an upper limit of 9370 cal g^{-1} in oils and fatty acids. Because all organisms contain carbohydrates, proteins and fats, intermediate calorific values must be anticipated (PAINE, 1971b). Some of the lowest calorific values recorded are: 2686 cal g^{-1} for the triclad *Dugesia tigrina* and 3500 cal g^{-1} for the razor clam *Ensis minor*; 11 values reported for 8 species belonging to the genus *Oncorhynchus* average 3736 ± 193 cal g^{-1} (CUMMINS and WUYCHECK, 1971). These low values may be due to the inclusion of sclerotized protein and polysaccharide shells, skin and scales (SLOBODKIN, 1962b). The highest values have been recorded (SLOBODKIN, 1962b) for *Calanus hyperboreus* (7432 cal

g^{-1}) and *C. finmarchicus* (7380 cal g^{-1}). Grand mean calorific values (producers: 4681 cal g^{-1}, sample size $N = 342$; detritus consumers: 4885 cal g^{-1}, $N = 44$; macro-consumers: 5821 cal g^{-1}, $N = 357$) indicate that macro-consumers concentrate more energy than producers or detritivores (CUMMINS and WUYCHECK, 1971). If macro-consumers are subdivided into aquatic and terrestrial forms, aquatic macro-consumers contain less energy per unit weight (5645 cal g^{-1}, $N = 155$) than their terrestrial counterparts (6099 cal g^{-1}, $N = 202$). Large differences abound in these data, even when calculated for major groups of organisms. There is a difference of 200 cal g^{-1} between fishes, characterized by relatively low calorific values, and birds—many with premigratory fat deposits and hence with seemingly inflated values.

The maximum range of variations in calorific value for a single species, the polychaete *Aphrodite hastata*, is estimated to be up to 1570 cal g^{-1} dry weight (1914 to 3407 cal g^{-1}; TYLER, 1973). Feeding and starvation experiments reveal a maximum variation range of 1332 cal g^{-1} dry weight in the fish *Tilapia mossambica* (RAGHURAMAN, 1973). The maximum variation in calorific content of about 1500 cal g^{-1} dry weight may be due to the nutritional history of the animal examined.

The energy-storing capacity of animals varies during different ontogenetic stages. In eggs, high calorific values have been reported. Eggs of crustaceans, for example, contain 5915 to 6636 cal g^{-1} dry weight (PANDIAN, 1970c); of gastropods 6209 cal g^{-1} dry weight (PANDIAN, 1969a); of fishes: 6093 to 6238 cal g^{-1} dry weight in marine demersal eggs of teleosts; 5386 to 5768 cal g^{-1} dry weight in marine

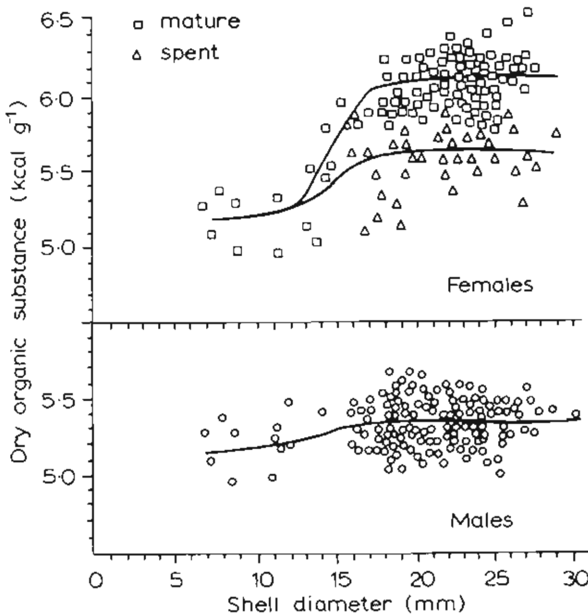


Fig. 3-36: *Tegula funebralis*. Changes in calorific values of males and females. (After PAINE, 1971a; modified; reproduced by permission of American Society of Limnology and Oceanography.)

planktonic eggs of teleosts (FLÜCHTER and PANDIAN, 1968); 5600 cal g^{-1} in *Raja erinacea* eggs (SLOBODKIN, 1962b). In freshly hatched larvae or juveniles, calorific values are low, for example, 4175 to 4780 cal g^{-1} dry weight in crustaceans (PANDIAN, 1970c); 5298 cal g^{-1} dry weight in gastropods (PANDIAN, 1969a). Low values prevail also in adults, e.g. 4569 to 5923 cal g^{-1} dry weight in decapod crustaceans (CUMMINS and WUYCHECK, 1971). Fig. 3-36 illustrates changes in calorific content of male and female *Tegula funebralis* during ontogenesis. Juveniles (<12 mm shell diameter) contain on average 5200 cal g^{-1}. Sexually mature males (>14 mm) contain 5400 cal g^{-1}; fully mature and spent females, 5800 and 6100 cal g^{-1}, respectively (PAINE, 1971a).

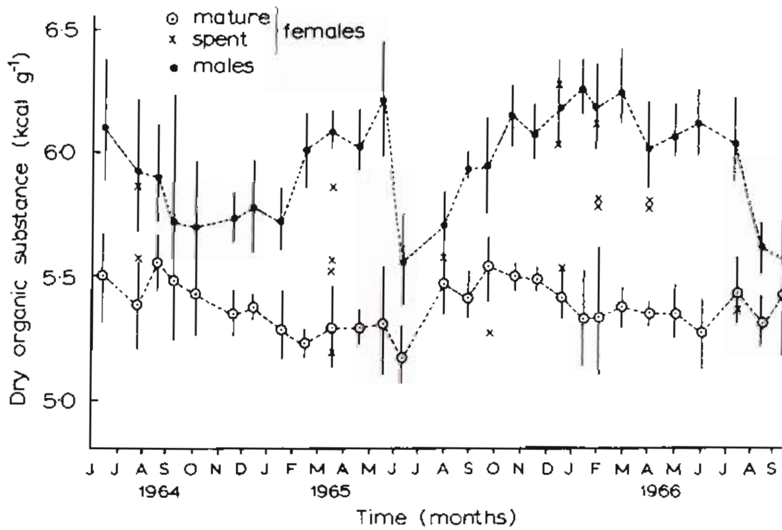


Fig. 3-37: *Tegula funebralis*. Seasonal changes in calorific values of large (>20 mm diameter) females and males. (After PAINE, 1971a; modified; reproduced by permission of American Society of Limnology and Oceanography.)

Seasonal variations in calorific content of several animals have been related to their reproductive cycle. For instance, the calorific content of males of *Tegula funebralis* have been shown to oscillate closely around 5400 cal g^{-1} throughout the year (Fig. 3-37; PAINE, 1971a). This value is about 12% below the approximate peak of 6100 cal g^{-1} attained by females. In spawning *T. funebralis* females, the calorific value decreased from 6100 cal g^{-1} to 5700 cal g^{-1} in 1964, and to 5500 cal g^{-1} in 1965 and 1966 (PAINE, 1971a). In the shrimp *Pandalus montagui*, the August peak (4815 cal g^{-1} dry weight) and the late summer decline (about 4400 cal g^{-1} dry weight) have been related to the reproductive cycle (TYLER, 1973). Energy accumulated in form of fats, carbohydrates and proteins in one or the other of the storage organs is translocated to gonads during maturation, or metabolized as energy source when the animals do not find sufficient suitable food. For details consult GIESE (1959, 1966a, b) and LOVE (1970).

Factors Affecting Conversion

Feeding, digestion, absorption and conversion may be affected by environmental and/or endogenous factors. The combined impact of these factors is reflected in the rate and efficiency of conversion.

Temperature

In the temperate fish *Oncorhynchus nerka*, experiments on combined effects of temperature and ration revealed a small area (25%) of optimum conversion efficiency with a graphically determined centre at 11·5° C at a ration of 4% body

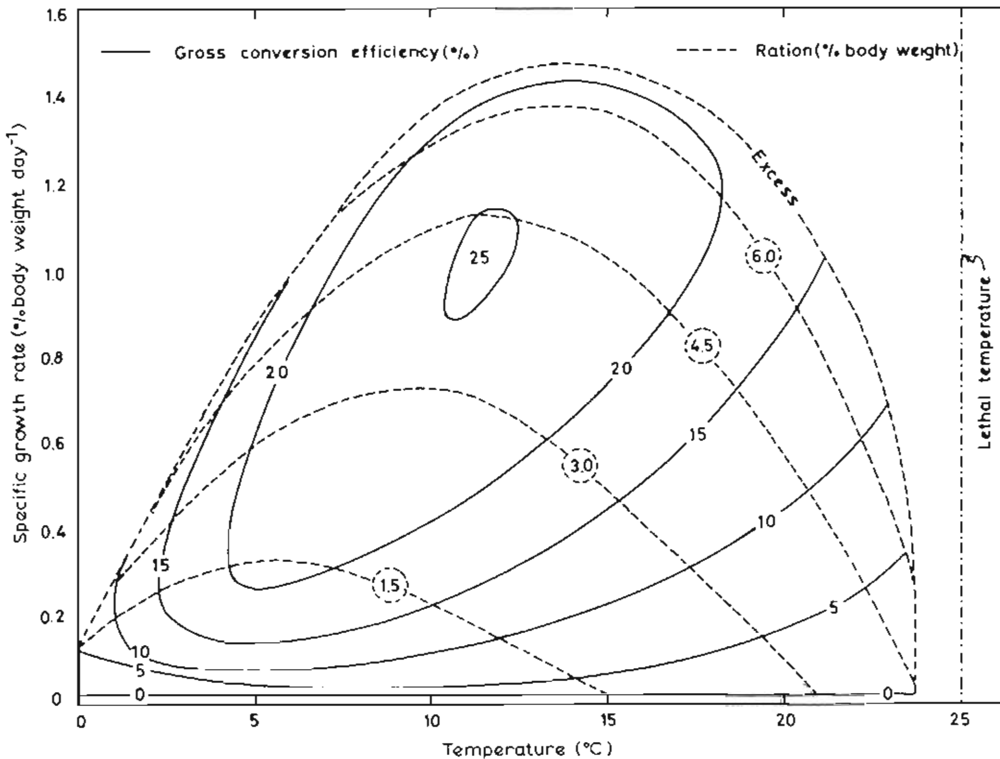


Fig. 3-38: *Oncorhynchus nerka*. Gross conversion efficiency as a function of temperature and ration. (After BRETT and co-authors, 1969; modified; reproduced by permission of Fisheries Research Board of Canada.)

weight day^{-1} (Fig. 3-38). At lower rations, the fish converts more efficiently at lower temperatures than at higher temperatures (BRETT and co-authors, 1969). The tropical fish *Ophiocephalus striatus* receiving reduced rations converts more efficiently at ca 30° C than at ca 20° C (VICTY MERCY, personal communication). It appears that the higher efficiencies are obtained at reduced feeding levels at temperatures prevailing in the natural habitats. PALOHEIMO and DICKIE (1966, 1970) and KERR (1971a, b) re-analyzed data reported by previous workers and discussed theoretical aspects of conversion.

Salinity

The euryhaline fish *Tilapia mossambica* consumes a maximum of 85–90 cal of food g^{-1} fish day^{-1} (75·2 mg *Gambusia affinis* g^{-1} day^{-1}) at 28‰ S. It converts only 6·2 cal g^{-1} day^{-1}, since it loses most energy in faeces, for maintenance (8·2 cal g^{-1} day^{-1}), and during specific dynamic action (SDA) and other metabolic activities (60·5 cal g^{-1} day^{-1}; Fig. 3-39). *T. mossambica* feeds less (74·4 cal g^{-1} day^{-1}) at 7‰ S, but converts a maximum of 24·5 cal g^{-1} day^{-1}, as it expends less energy on main-

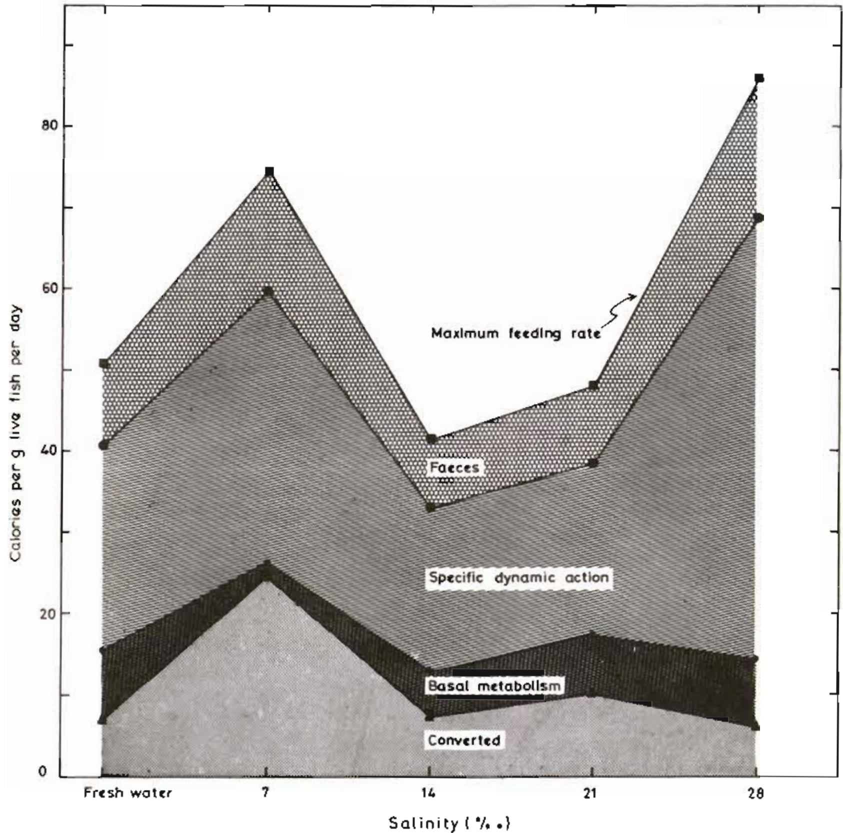


Fig. 3-39: *Tilapia mossambica*. Salinity effects on scopes for growth (converted), metabolism (basal metabolism and specific dynamic action) and faeces output in young fish receiving an unrestricted supply of *Gambusia affinis* for 60 days. (After RAGHURAMAN, 1973.)

tenance (1·6 cal g^{-1} day^{-1}) and on SDA and other metabolic activities (33·3 cal g^{-1} day^{-1}). Food intake is lowest (41·4 cal g^{-1} day^{-1}) at 14‰ S, but conversion (7·4 cal g^{-1} day^{-1}) is higher than that at 28‰ S (RAGHURAMAN, 1973).

Parasites

Parasitic infestation can considerably alter feeding rate and conversion efficiency (see also Volume III: LAUCKNER, in press). HISCOX and BROCKSEN (1973) have

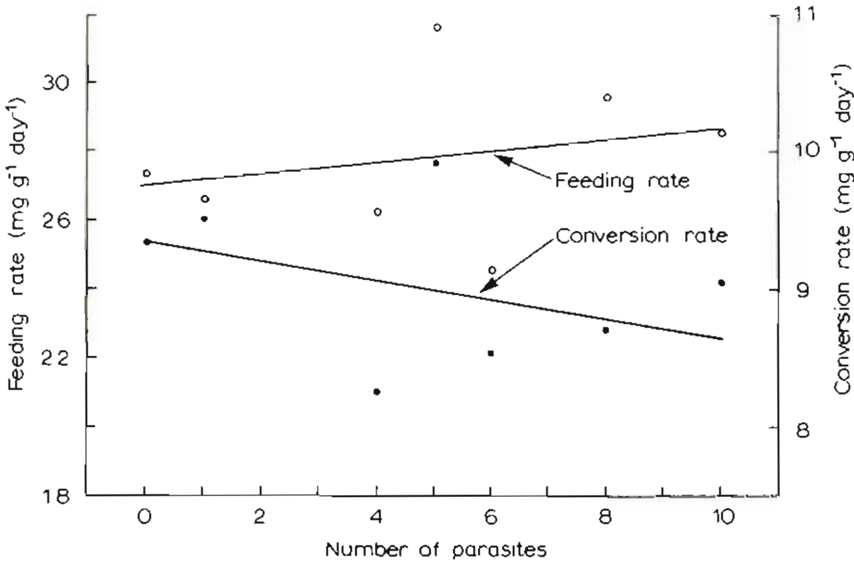


Fig. 3-40: *Salmo gairdnerii*. Rates of feeding and conversion as a function of parasitic load. A known number of parasitic gut nematodes *Bulbodacnitis ampullastoma* was permitted to infect the fish prior to the feeding experiment. All feeding and conversion values are expressed as dry weight. (After HISCOX and BROCKSEN, 1973; modified; reproduced by permission of Fisheries Research Board of Canada.)

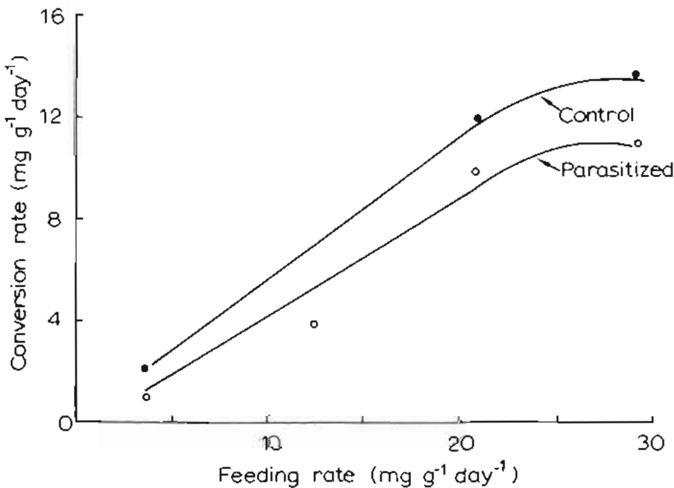


Fig. 3-41: *Salmo gairdnerii*. Conversion rate as a function of feeding rate in control and parasitized fish. The fish were parasitized with the gut nematode *Bulbodacnitis ampullastoma* prior to the feeding experiment. (After HISCOX and BROCKSEN, 1973; modified; reproduced by permission of Fisheries Research Board of Canada.)

successfully infected the trout *Salmo gairdnerii* with a known number of the nematode parasites *Bulbodacnitis ampullastoma*. With increasing parasite density in the gut, the infected trout consume more but convert less food (Fig. 3-40). In comparison to the control trout, the parasitized trout convert significantly less at the feeding levels tested (Fig. 3-41). In addition to enzymatic functions, the pyloric caecae are considered as primary sites of fat absorption in the trout. The caecae are also known to constitute attachment sites of parasitic gut nematodes (MAGGENTI, 1971). Parasitic interference may be primarily mechanical (with masses of parasites reducing the absorptive surfaces of the caecae), chemical or hormonal. Rate of weight loss and survival capacity of starving trout depend upon parasite density: heavily infected trout lose almost double the amount of body substance and their survival capacity is reduced to half that of the controls (Table 3-31). In starving fish, the presence of *B. ampullastoma* induces increased mobilization of energy stores. This increased mobilization, in turn, leads to premature death.

Table 3-31

Salmo gairdnerii. Rate of weight loss and survival (days) in starving juveniles artificially infected with different numbers of gut parasites *Bulbodacnitis ampullastoma* (After HISCOX and BROCKSEN, 1973; modified; reproduced by permission of Fisheries Research Board of Canada)

Parasite density (No./fish)	Survival period after infection (days)	Rate of weight loss (mg g^{-1} dry weight day^{-1})
0	12	38
1	8	36
2	8	36
3	7	43
4	7	67
6	7	55
7	6	60

Chemical composition

The gross chemical composition of consumer animals may be altered considerably by the chemical composition of their food. Water, ash and calorific contents of the flatfish *Limanda limanda* were estimated before and after feeding; the food—cod flesh (1137 cal g^{-1} wet weight) and herring flesh (200 cal g^{-1} wet weight)—was chosen so that per unit weight of food taken in, the energy input would be almost twice as great in herring-fed *L. limanda* as in those receiving cod flesh. After 30 days of feeding, net increase in energy content per gram dry weight of *L. limanda* was 394 cal in the former case, but only 223 cal in the latter (Table 3-32). Net decrease in ash content was roughly 3 times greater in flatfish feeding on herring flesh (2·9% decrease) than in those receiving cod flesh (1·1%; PANDIAN, 1968).

Table 3-32

Limanda limanda. Effect of water, ash and calorific content of food (frozen flesh of cod *Gadus morhua* and herring *Clupea harengus*) on composition and calorific content of test individuals fed for 30 days at 13° C. Water expressed as percentage of live weight, ash as percentage of dry weight (After PANDIAN, 1968)

Food and test fish		Water (%)	Ash (%)	Energy (cal g^{-1} dry weight)
Herring flesh		68·8	4·2	6411
L. limanda	before feeding	76·8	15·0	5181
	after feeding	74·8	12·1	5575
Cod flesh		78·7	4·4	5339
L. limanda	before feeding	78·0	15·9	4983
	after feeding	77·9	14·8	5206

Likewise, regimens or rates of feeding can considerably alter gross chemical composition—especially fat and its quality (LOVE, 1970). Wax esters and triglycerides serve as reserve energy in animals such as *Calanus helgolandicus* and *Gaussia princeps* (LEE and co-authors, 1970a, b). In *C. helgolandicus*, total lipid drops from 37 to 9% when the concentration of *Skeletonema costatum* is decreased from 800 to 100 μg C l^{-1} (Table 3-33). Wax esters of chain length 42 and 44, detected in wild adults, are absent in all the copepodites reared on *S. costatum* and 36, 38 and 40 are lacking in those reared at lower concentrations of 100 and 200 μg C l^{-1} (LEE

Table 3-33

Calanus helgolandicus. Effect of different feeding regimens on wax esters and total lipid content in individuals reared from egg to copepodite stages over 3 to 4 weeks. Food: *Skeletonema costatum* N.d.: not detected (After LEE and co-authors, 1970a; modified; reproduced by permission of American Association for the Advancement of Science)

Chain length of wax esters	Feeding regimens (μg C l^{-1})			
	800	400	200	100
30	21	12	81	90
31			7	5
32	32	31	9	4
34	12	16·5	4	N.d.
36	18	24	N.d.	
38	18	13		
40	4	3		
42	N.d.	N.d.		
Total lipid (% dry weight)	37	18	15	9

and co-authors, 1970a). However, the phospholipid fatty acids were not affected by changes in the amount and type of food and had a fatty acid composition quite different from that of the wax esters (LEE and co-authors, 1971).

Lower temperatures result in greater fat unsaturation (more double bonds) in the body of *Anguilla anguilla* (LOVERN, 1938). HOAR and COTTLE (1952) found that the resistance of *Carassius auratus* to both low and high temperatures can be modified

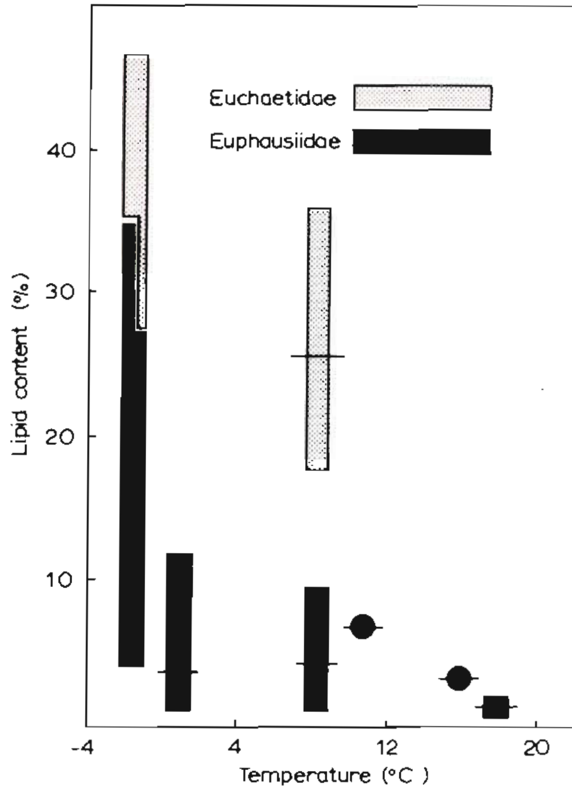


Fig. 3-42: *Euchaeta antarctica* and *Euphausia crystallophobia*. Relation between lipid content and temperature. Horizontal lines: means; circles: single determinations. (After LITTLEPAGE, 1964; modified; reproduced by permission of Hermann, Paris.)

by feeding high concentrations of certain fatty acids. Working on marine and freshwater fishes, REISER and co-authors (1963) observed that lauric and mysteric acids (saturated) are absorbed from the diet at 23° C but not at 13° C. Whether or not temperature affects the lipid composition of fish is still open to debate. The relation between temperature and lipid content of the Antarctic euphausiid *Euphausia crystallophobia* and calanoid copepod *Euchaeta antarctica* suggests that the capacity to store fat is of a very high order (36% in *E. crystallophobia* and 46% in *E. antarctica*); maximum lipid reserve is reached when the environmental tem-

perature is the lowest (−1·9° C; Fig. 3-42). The lipid is presumably a store permitting survival and preparation for reproductive activity during long periods of cold weather when no or few nutrients are available (LITTLEPAGE, 1964).

Endogenous Factors

Age and body weight are known to affect growth and conversion efficiency. In fact age and body weight are so closely correlated with conversion efficiency in most cases that either of these parameters may be used to predict efficiency values. Recent studies revealed that both feeding rate and body weight affect conversion efficiency (e.g. GERKING, 1971). In the freshwater gastropod *Pila globosa*, feeding rate (as well as radula weight, in mg g^{-1} snail) decreases as a function of body weight; presumably, feeding rate reduction is, at least in part, due to the decreased radula weight (HANIFFA and PANDIAN, 1973).

There are indications that, with increasing age, growth declines or ceases in many marine animals (e.g. male *Octopus cyanea*; VAN HEUKELEM, 1973). Age-dependent growth cessation indicates that the individual concerned has entered the maintenance phase (see also Volume I).

(4) Output of Non-utilized Energy and Matter

(a) Excretion

The intermediary metabolism produces a number of by-products: water and carbon dioxide are formed from all classes of food, and nitrogenous wastes (ammonia) are produced when proteins and nucleic acids are metabolized. In a very general way, excretion may be defined as separation and ejection of metabolic wastes (HOAR, 1966). Urea, which is an excretory product in many aquatic animals including cetaceans such as the blowhead whale *Balaena mysticetus* (Table 3-34), is indispensable for osmoregulation in elasmobranchs (e.g. SMITH, 1953; Chapter 5). The excretory system eliminates excess deleterious nitrogenous metabolites and conserves useful substances. Whereas carbon dioxide and water are relatively innocuous and can often be removed in form of gases, ammonia is highly toxic and is usually removed in solution, frequently in some detoxified form (HOAR, 1966; Volume III, Chapter 2).

Terminal Nitrogenous Wastes

Products of protein degradation

Ammonia. The prime source of ammonia is deamination of amino acids. The toxic ammonia must be excreted rapidly, or must be converted to a less toxic substance. Ammonia tolerance varies among animals, but the upper critical concentration is always low. Examples of recorded values (as mg NH_3-N 100 ml^{-1} or 100 g^{-1} blood) are: worms, 0·1 to 1·3; crustaceans, 0·4 to 2·5; molluscs, 0·1 to 4·8; echinoderms, 0·1 to 0·4; elasmobranchs, 1·4 to 2·5; teleosts, 0·3 to 5·5 (Table 3-35; for more data

Table 3-34

Nitrogenous excretion in marine animals. All values expressed as percentage of total non-protein nitrogen for the compounds listed (After NICOL, 1967; modified and extended; reproduced by permission of the author)

Animal	NH_3	Urea	Uric acid	Amino acids	Purines	Creatine	Creatinine	Trimethyl-amine oxide	Other
Worms									
Aphrodite aculeata	80	0·2	1	—	—	—	—	—	—
Sipunculus nudus	50	10	0·0	17	4	—	—	—	19
Molluscs									
Aplysia fasciata	37	7	9·2	13	17	—	—	—	17
Aplysia fasciata	30	10	trace	—	15	—	—	—	45
Littorina littorea, summer	40	13	1	7	29	—	—	—	—
Littorina littorea, winter	61	2	1	10	16	—	—	—	—
Mya arenaria	21·5	4·5	trace	18	5	—	—	—	51
Mytilus edulis	11	trace	0	35·5	16	—	—	—	38
*Crassostrea virginica**	65	13	—	5	—	—	—	—	17
Sepia officinalis	64	2	2	8	5	—	—	—	18
Octopus vulgaris	33	—	1	12·5	25	—	—	—	28
Octopus vulgaris	12·5	5	—	—	—	—	—	—	—
Crustaceans									
Gammarus locusta	80	1	0	7	—	—	—	—	non-dialysable 3; unaccounted 9
Gammarus zaddachi	83	1	0	3	—	—	—	—	,, 3; ,, 9
Marinogammarus marinus	87	0	—	4	—	—	—	—	,, 7; ,, 2
Marinogammarus pirloti	87	0	0	2	—	—	—	—	,, 2; ,, 9
Orchestia sp.	70	1	0	11	—	—	—	—	,, 8; ,, 10
Ligia oceanica	83	0	0	6	—	—	—	—	,, 4; ,, 9
Carcinus maenas†	71	2	1	8	4	—	—	—	14
Maja squinado†	48	5	3	22	5·5	—	—	—	—
Cancer pagurus	43	13	3	20	10	—	—	—	11
Echinoderms									
*Asterias rubens**	36	13·5	trace	22·5	6·8	—	—	—	—
Paracentrotus lividus†	28	7·6	1	27	11	—	—	—	—
Holothuria tubulosa	39·0	6·0	0	18	12	—	—	—	25

Table 3-34—*Continued*

Animal	NH_3	Urea	Uric acid	Amino acids	Purines	Creatine	Creatinine	Trimethyl-amine oxide	Other
Fishes									
Mustelus canis†	4·5	85	0	—	—	—	—	—	—
Torpedo sp.	2	85	—	2	—	—	—	—	—
Gadus callarias	0	11	2	15	—	51	2	—	19
Pseudopleuronectes americanus	3	13·5	1	8	—	—	26	—	48
Pseudopleuronectes americanus	2	21	1	10	—	—	15	—	51
Spheroides maculatus	5	2·5	1	—	—	56	2	—	33
Spheroides maculatus	6	19	1	20·5	—	39	5	—	9
Lophius piscatorius†	2	28	—	5	—	—	—	—	65
Lophius piscatorius†	0·4	0·4	0·3	10	—	45	3	32	—
Lophius piscatorius	3	2	2	18	—	38	0	—	—
Lophius piscatorius	13	1	0	3	—	34	3	28	—
Lophius piscatorius	3	14	0	—	—	17	—	—	—
Myoxocephalus octodecimspinosus	2	14	1	4	—	—	25	23	31
Myoxocephalus octodecimspinosus	1	15	1	4	—	—	22		33
Reptiles									
Chelonia mydas	18	45	19	—	—	10	2	—	—
Chelonia mydas	16	38	16·5	—	—	3	1	—	—
Chelonia mydas	43	0	2	7·5	—	6	1·5	—	allantoin 13·6; hippuric acid 13·85; undetermined 12
Chelonia mydas	14·5	31	14	—	—	3	1	—	—
Mammals									
Balaena mysticetus	1·5	90	3	—	—	—	—	—	—
Phoca vitulina	10	73	—	—	—	5	8	—	—
Phoca vitulina	15	73	—	—	—	5	8	—	—

*From HAMMEN and co-authors (1966).
†Averaged.

Table 3-35

Distribution of non-protein-N in body fluids of marine invertebrates. All values are given in mgN 100 ml^{-1} body fluid (After NICOL, 1967; modified; reproduced by permission of the author)

Animal	Non-protein-N	NH_3-N	Urea-N	Uric acid-N	Amino acid-N	Purine-N	Undetermined and other
Annelids, Sipunculoids							
Arenicola marina	—	—	1·4	trace	—	—	—
Aphrodite aculeata	4·5	0·1	0·5	0.1	1·6	—	1·8
Chaetopterus variopedatus	—	—	—	0·2	—	—	—
Amphitrite ornata	—	—	—	0	—	—	—
Nereis pelagica	—	—	—	0	—	—	—
Sipunculus nudus	11·2	1·3	0·6	0	1·3	—	7·2
Golfingia gouldii	—	—	—	0	—	—	—
Crustaceans, Xiphosurans							
Palinurus vulgaris	26·5	2·5	5·5	0·3	8	2·1	8·1
Homarus	13	—	—	0·8	—	—	—
Cancer productus	18	1·05	5·15	1·6	—	—	creatine 0·1 creatinine 0·2
Cancer pagurus	15	0·80	0·90	0	7	0·3	6·2
Carcinus maenas	37·5	2·10	2·70	0·4	12	—	20
Maja squinado	10·4	1·4	0·9	0·2	5	0·3	—
Molluscs							
Cryptochiton stelleri	25	0·5	3·5	0·0	13	—	creatinine 0·3
Cryptochiton stelleri	6·5	0·0	1·3	0·0	—	—	—
Aplysia fasciata	5·2	0·7	0·2	0·1	1·6	0·2	—
Aplysia fasciata	2·2	0·2	0·05	0·1	0·7	0·1	—
Haliotis rufescens	21	0·4	5·6	—	—	—	—
Haliotis rufescens	23·3	0·1	2·3	—	—	—	—
Haliotis rufescens	15	0·3	1·1	trace	—	—	—
Mya arenaria (mantle fluid)	10	0·5	0·5	0·1	3·0	—	—
Crassostrea angulata	6·2	0·45	0·2	0·01	0·8	—	—
Saxidomus nuttalli	13	1·0	0·05	—	—	—	—

Table 3-35—*Continued*

Animal	Non-protein-N	NH_3-N	Urea-N	Uric acid-N	Amino acid-N	Purine-N	Undetermined and other
Schizothaerus nuttalli	10	—	—	—	—	—	NH_3 + urea 3
Schizothaerus nuttalli	14	0·7	2·9	trace	8·0	—	—
Sepia officinalis	26	4·8	1·9	0·4	9	1·8	8
Sepia officinalis	14·8	2·8	1·2	0·1	7	—	3·6
Octopus vulgaris	14	1·6	1·2	trace	—	—	—
	10	1·4	0·8	—	—	—	—
Echinoderms							
Asterias rubens	2	0·4	0·1	—	0·8	—	0·7
Asterias forbesii	—	—	—	0·1	—	—	—
Pisaster ochraceus	4	—	—	—	—	—	creatinine 0·3 NH_3 + urea 1
Paracentrotus lividus	3·7	0·2	0·1	0·1	2·4	0·2	0·8
Strongylocentrotus franciscanus	8·6	0·1	0·9	trace	—	—	creatinine 0·3
Holothuria tubulosa	1·1	0·1	0·1	0·0	0·4	0·04	0·1
Thyone briareus	—	—	—	0·1	—	—	—

consult DELAUNAY, 1931; 1934; FLORKIN and DUCHÂTEAU, 1943; NICOL, 1967; Volume III: KINNE, in press).

Marine invertebrates readily lose the freely soluble ammonia by diffusion. The small molecule size and the highly lipid-soluble nature of the free base of ammonia permit easy elimination without body-water loss. At the normal pH of body fluids, only 1% of the compound is in the form of free base, but the conversion of $NH_4^+ \rightarrow NH_3$ is instantaneous and hence probably not a rate-limiting step in its elimination (FORSTER and GOLDSTEIN, 1969). Except for its relative toxity, ammonia has another advantage over urea and uric acid as the chief end-product of nitrogen metabolism: Whereas synthesis of urea and uric acid requires expenditure of energy, some of the reactions involved in the production of ammonia, such as deamination of glutamate, ultimately lead to the production and capture of free energy. Coupling of the glutamic-acid dehydrogenase reaction with the cytochrome system is of considerable importance in regenerating ATP (Fig. 3-43). Glutamic-acid dehydrogenase, the key enzyme in amino-acid metabolism in aquatic animals, requires NAD^+ or $NADP^+$ which in the reduced form enters the chain of oxidative

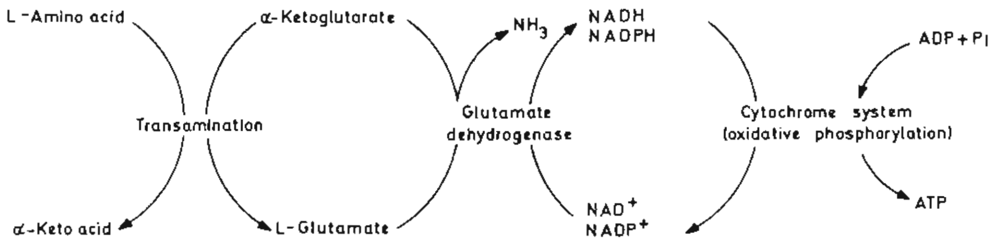


Fig. 3-43: Deamination of amino acids and production of ATP via coupled transamination and glutamate dehydrogenase. (After FORSTER and GOLDSTEIN, 1969; modified; reproduced by permission of Academic Press, Inc.)

phosphorylation (BRAUNSTEIN, 1951; KREBS and KORNBERG, 1957; COHEN and BROWN, 1960).

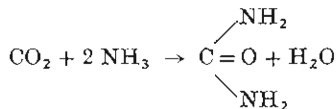
Animals which excrete ammonia as the main end-product of amino-acid metabolism are said to be ammonotelic (Table 3-34). In some non-ammonotelic marine animals, ammonia excretion counteracts blood acidosis and conserves alkaline reserves (N^+) (RECTOR and co-authors, 1954, 1955). MAETZ and GARCIA ROMEU (1964) have shown that NH_4^+ in the blood of the goldfish *Carassius auratus* can be excreted in exchange for Na^+ in the water. Thus there may be two mechanisms for excreting ammonia, simple diffusion as well as exchange at the gills. The euryhaline fish *Tilapia mossambica*, acclimated by KUTTY (1972) to fresh water at 30° C and tested under the same conditions, maintained a respiratory quotient (RQ) of about unity and an ammonia quotient (AQ = volume of NH_3 excreted/volume of O_2 uptake) of about 0·2 at high ambient oxygen concentration (6 ppm). When the fish was forced to exercise continuously, it derived some anaerobic energy throughout the exercise (RQ: 1·2), utilizing more protein the longer the exercise. At low oxygen concentrations (below 2 ppm), RQ and AQ increased sharply to 8 and 1 respectively (at 0·6 ppm), indicating a close relationship of increase in anaerobic energy utilization and increase in protein metabolism at inadequate oxygen concentrations.

KUTTY (1972) suggests that this coupling of increased protein metabolism and anaerobic energy utilization prevents acidosis and conserves sodium (Na^+).

Urea. Like ammonia, urea is very soluble and diffusible but much less toxic. The urea molecule is a dipole, which in aqueous solution behaves in many ways as do water molecules themselves. It has a low oil–water partition coefficient and penetrates through aqueous pores more easily than the lipid-protein component of cell membranes (FORSTER and GOLDSTEIN, 1969). Despite the similarity in diffusion coefficients of ammonia and urea, the former passes through most biological membranes faster than urea, probably because of the high lipid solubility of the NH_3 form of ammonia and the low oil–water partition coefficient of urea. Blood samples collected simultaneously from afferent and efferent gill arteries of the sculpin *Myoxocephalus scorpius* reveal that more than 2/3 of the blood ammonia is lost during branchial blood circulation; but there is no detectable drop in urea concentration (GOLDSTEIN and co-authors, 1964), and hence urea is likely to be excreted in the kidney.

In marine invertebrates, urea forms a much smaller proportion of the total nitrogen excreted than ammonia (Table 3-34). Marine elasmobranchs and mammals, in which the principal excretory end-product is urea, are termed ureotelic. The highest amounts of urea recorded are 5·5 mg urea N 100 ml^{-1} body fluid for the crustacean *Palinurus vulgaris*; 9·1 mg urea N 100 ml^{-1} for the mollusc *Saxidomus nutalli* (Table 3-35); 21 mg % for the teleost *Lophius piscatorius* (DELAUNAY, 1927 *in:* NICOL, 1967); and 344 mg % for the elasmobranch *Squalus acanthias* (SMITH, 1931; for additional data consult BLACK, 1957).

Chemically, urea consists of 2 ammonia molecules united to 1 carbon dioxide molecule:



$$CO_2 + 2\,NH_3 \rightarrow C(=O)(NH_2)_2 + H_2O$$

During the ornithine cycle carbamyl phosphate, a key compound in biochemical evolution, is synthesized in 2 steps from NH_3 and CO_2 and requires 2 molecules of ATP along with the enzyme carbamyl phosphate synthetase and certain co-factors, including Mg^{++} in animals; bacteria can make the combination with 1 molecule of ATP (HOAR, 1966). Aspartic acid couples with citrulline at the cost of 1 unit of ATP to form argino-succinic acid. Thus, the cost of detoxifying ammonia is 3 ATP molecules per molecule of urea formed.

Previous workers considered that the ornithine cycle (Fig. 3-43) is not present in invertebrates (BALDWIN, 1964) and fishes (MANDERSCHEID, 1933). According to BALDWIN (1960), although 4 of the 5 enzymes of the cycle were present in the liver of elasmobranchs, the enzyme carbamyl phosphate synthetase (CPS) was not detectable. Arginase, reported from the liver of several elasmobranchs (BALDWIN, 1958, 1960), was considered to act on dietary arginine to form urea; this may account for the small amounts of urea excreted by these fishes (HOAR, 1966). However, recent studies using improved assay conditions (BROWN, 1964; WATTS and WATTS, 1966), and radio-isotopic techniques (GOLDSTEIN and FORSTER, 1971a) have shown

that low levels of CPS are present in the liver of elasmobranchs (see also READ, 1967) and that the ornithine cycle is functional (SCHOOLER and co-authors, 1966). In the dogfish *Squalus acanthias*, SCHOOLER and co-authors (1966) compared the rate of urea synthesis via the purine pathway (p. 189) with that synthesized via the ornithine-urea cycle. The activity of the purine pathway *in vivo* was 1/10, and *in vitro* 1/100, that of the ornithine-urea cycle. Hence the ornithine-urea cycle appears to be the main pathway of urea formation in elasmobranchs (GOLDSTEIN, 1972). Furthermore, the enzymes identified in the liver of the marine coelacanth *Latimeria chalumnae* also suggest that urea is synthesized mainly via the ornithine-urea cycle (BROWN and BROWN, 1967). Conclusive evidence for the presence of the entire sequence of ornithine-urea cycle enzymes in *L. chalumnae* was supplied recently by GOLDSTEIN and co-authors (1973); the levels of activity of the enzymes (including carbamyl phosphate synthetase) were comparable to those in the southern sting ray *Dasyatis americana*.

BROWN and COHEN (1960) have shown that the enzymes carbamyl phosphate synthetase and ornithine transcarbamylase (Fig. 3-44) are present in teleost livers. Since arginase has been reported from teleost livers (HUNTER, 1929), it was thought that dietary arginine could potentially be a source of urea. Working on a number of teleosts, HUGGINS and co-authors (1969) reported that the activities of all 5

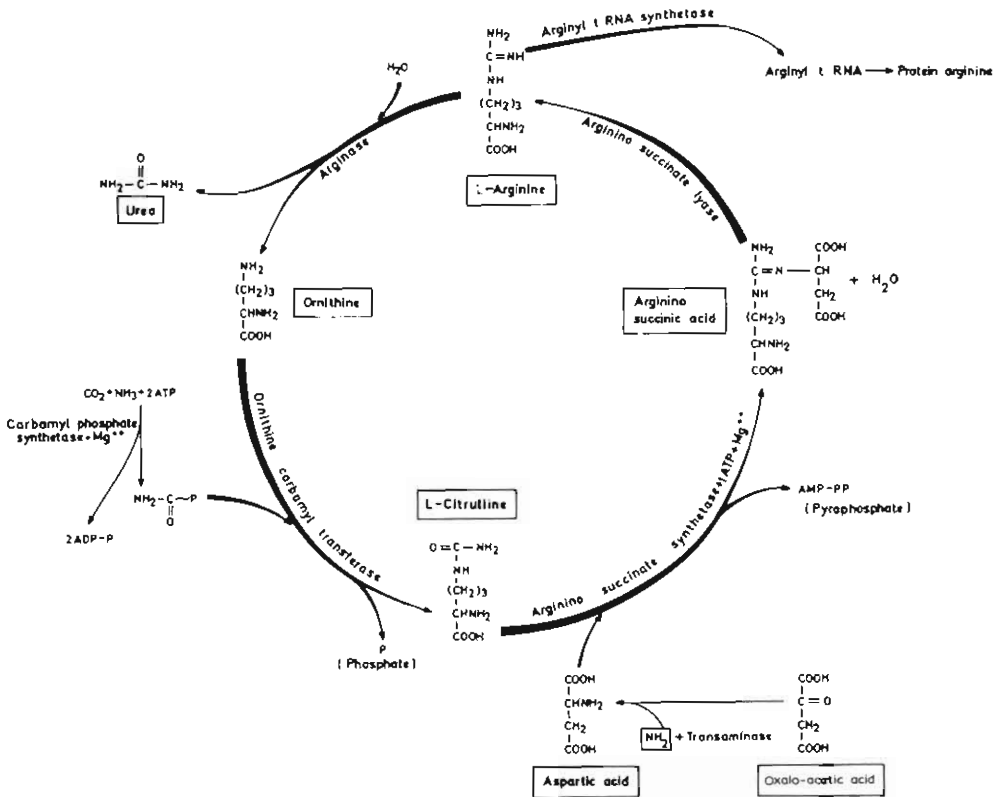


Fig. 3-44: Urea biosynthesis in the ornithine cycle. (Original.)

enzymes of the ornithine-urea cycle are significantly high. The level of CPS activity (0·36 μM g^{-1} liver hr^{-1}), for example, is comparable to that of the freshwater elasmobranch *Potamotrygon* sp. (GOLDSTEIN and FORSTER, 1971a).

The presence of ornithine transcarbamylase, argininosuccinate lyase, arginase and citrulline in the tissues of the polychaete *Cirriformia spirabranchia* strongly suggests a functional ornithine-urea cycle (HULT, 1969). HORNE and BOONKOOM (1970) presented evidence for the occurrence of the ornithine cycle in several gastropods. Similarly, the presence of the enzymes ornithine transcarbamylase, arginosuccinate synthetase, arginosuccinate lyase, and arginase in the digestive diverticula of the bivalves *Mytilus californianus*, *Saxidomus giganteus* and *Compsomyax subdiaphana* indicates the existence of a functional ornithine cycle

Table 3-36

Excretion rates of nitrogenous end-products in some molluscs (After POTTS, 1967; DUERR, 1968; modified; reproduced by permission of Biological Review and Microforms International Marketing Corp.)

Species	Excretion rate	Author
Ammonia excretion rate (μg ammonia N g^{-1} day^{-1})		
Gastropods		
Thais lima	4	DUERR (1968)
T. lamellosa	7	
Fusitriton oregonensis	21	
Acmaea digitalis	4	
A. scutum	43	
Calliostoma ligatum	85	
Littorina sitkana	60	
L. littorea	(summer) 220	POTTS (1967)
	(winter) 145	
Arion rufus	20	
Bivalves		
Mytilus edulis	4	
Unio pictorum	(summer) 14	
	(winter) 6	
Modiolus demissus	43	
Urea excretion rate (μg urea N g^{-1} day^{-1})		
Gastropods		
Littorina littorea	(summer) 72	POTTS (1967)
	(winter) 64	
Arion rufus	6	
Uric acid excretion rate (μg uric acid N g^{-1} day^{-1})		
Gastropods		
Littorina littorea	(summer) 5	POTTS (1967)
	(winter) 3	
Arion rufus	4 to 16	

(ANDREWS and REID, 1972). It is possible that carbamylphosphate synthetase activity is below the level of detection in these cases, as an activity of less than 4 μM of citrulline, produced per g tissue per hr, would escape detection with the method (BROWN and COHEN, 1959) employed (LINTON and CAMPBELL, 1962). Arginase occurs in soluble and particulate fractions of the hepatopancreas, abdominal muscle and thoracic wall of the crayfish *Cambarus bartoni* (HARTENSTEIN, 1971). Probably, with improved assay conditions and radio-isotope methods, it may be possible to detect all 5 enzymes of the ornithine-urea cycle in marine teleosts and invertebrates.

Uric acid is highly insoluble and easily precipitated from a supersaturated colloidal solution. It can be removed in solid form without involving water loss. It is a member of the purines (for biosynthesis consult KARLSON, 1963). Non-chelonian reptiles and birds (uricotelic animals) inhabiting marine and estuarine environments apparently excrete uric acid. The chief non-chelonian marine reptiles are the Galapagos iguana *Amblyrhynchus cristatus*, the oviparous (Laticaudinae) and viviparous (e.g. *Pelamis platurus*; Hydrophiinae) sea snakes belonging to the family Hydrophiidae, and the estuarine crocodile *Crocodilus porosus* (NICOL, 1967). A fair amount of comparative data are available on the role of electrolyte elimination by the salt glands in these animals (e.g. for reptiles: DUNSON, 1970; for birds: SCHMIDT-NIELSEN, 1960); however, almost nothing is known about quantitative and qualitative aspects of their nitrogenous wastes.

Table 3-36 presents data on rate of ammonia, urea and uric acid excretion in some molluscs. Seasonal changes in the rates of excretion of ammonia, urea and uric acid are considerable.

Excretion products of nucleic acids

Approximately 5% of the excretory nitrogen originates from nucleic-acid metabolism. The bases are pyrimidines (cytosine or uracil [RNA], or thymine [DNA]) and purines (adenine or guanine). During metabolism, the pyrimidine molecule is completely degraded into its building blocks CO_2, H_2O and NH_3, and ammonia is excreted. The purines, on the other hand, are excreted as such only in a few cases (some flatworms, annelids and the cyclostome *Lampetra fluviatalis*; PROSSER and BROWN, 1961).

Uric acid. The purines are first deaminated and oxidized to uric acid, as shown in Fig. 3-45. Uric acid thus produced may be excreted, or stored, in ascidians such as *Ascidia nigra*, *Phallusia mammillata* (GOODBODY, 1965), and *Molgula manhattensis* (NOLFI, 1970). The general scheme of the functions of stored uric acid suggested by NOLFI indicates that uric acid may be involved in pigment formation and nucleic-acid synthesis.

Allantoin. The pyrimidine ring of the uric acid is opened by uricase to form allantoin. Uricase has been detected in several bivalves studied (for references see FLORKIN and BRICTEUX-GRÉGOIRE, 1972).

Allantoic acid. Allantoin is oxidized in the presence of allantoinase to form allantoic acid.

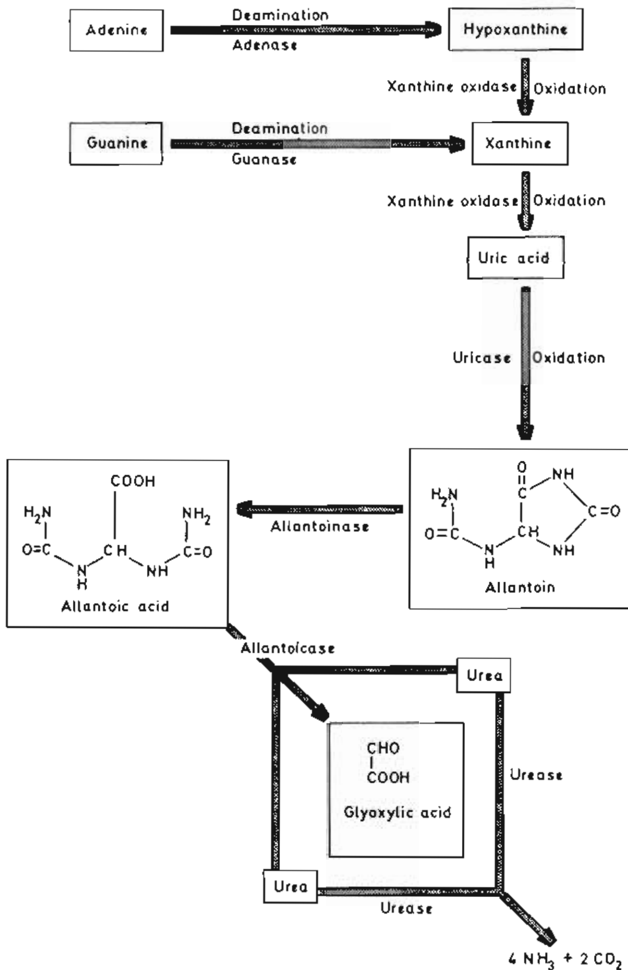


Fig. 3-45: Purine catabolism, based on FLORKIN (1949) and BALDWIN (1963). (After HOAR, 1966; modified; reproduced by permission of Prentice-Hall, Inc., Englewood Cliffs, N.J.).

Urea. Allantoic acid is broken by allantoicase to glyoxylic acid and urea. The blood of teleosts has been shown to contain a significant quantity of urea, and the urea may account for 20% of the total nitrogen excreted (WOOD, 1958). BRUNEL (1937a, b) found that all teleosts examined by him possess uricase and allantoinase, whereas only certain families have allantoicase. Using a refined procedure, GOLDSTEIN and FORSTER (1965) detected allantoicase in 18 teleost fishes, including a number of species used by BRUNEL. The fact that urate, allantoin and allantoicate are all readily converted to urea by liver slices of the fish *Myoxocephalus octdecimspinosus* confirms the pathway proposed by BRUNEL (1937a) for the conversion of urate to urea:

urate → allantoin → allantoicate → urea (GOLDSTEIN and FORSTER, 1965).

Ammonia. Purines may be completely degraded to 4 molecules of ammonia and 2 molecules of CO_2 in the presence of 6 or 7 enzymes in the sequence indicated in Fig. 3-45. The complete chain of purinolytic enzymes has been found only in the worms *Sipunculus nudus* (FLORKIN and DUCHÂTEAU, 1943) and *Cirriformia spirabranchia* (HULT, 1969); the molluscs *Mya* sp. (PRZYLECKI, 1926), *Mytilus edulis* (BRUNEL, 1938; FLORKIN and DUCHÂTEAU, 1943), *Meretrix meretrix* (ISHIDA, 1955; see also ISHIDA, 1954; AIKAWA, 1959, 1966), *Mytilus californianus*, *Saxidomus giganteus* and *Compsomyax subdiaphana* (ANDREWS and REID, 1972); and the crustaceans *Homarus gammarus* (FLORKIN and DUCHÂTEAU, 1943) and *Balanus balanoides* (BOULESTEIX, 1964, 1965).

Miscellaneous nitrogenous compounds

Amino acids. During degradation, proteins are hydrolyzed to their constituent amino acids. Except for a few species (e.g. the polychaete *Cirriformia spirabranchia*, HULT, 1969), most marine animals release some free amino acids. The amino acids may comprise up to 35·5% of the total nitrogenous waste (Table 3-34). It is questionable, however, whether release of amino acids represents true excretion of waste material or whether it involves unavoidable leakage of relatively small molecules (HOAR, 1966; see also p. 74).

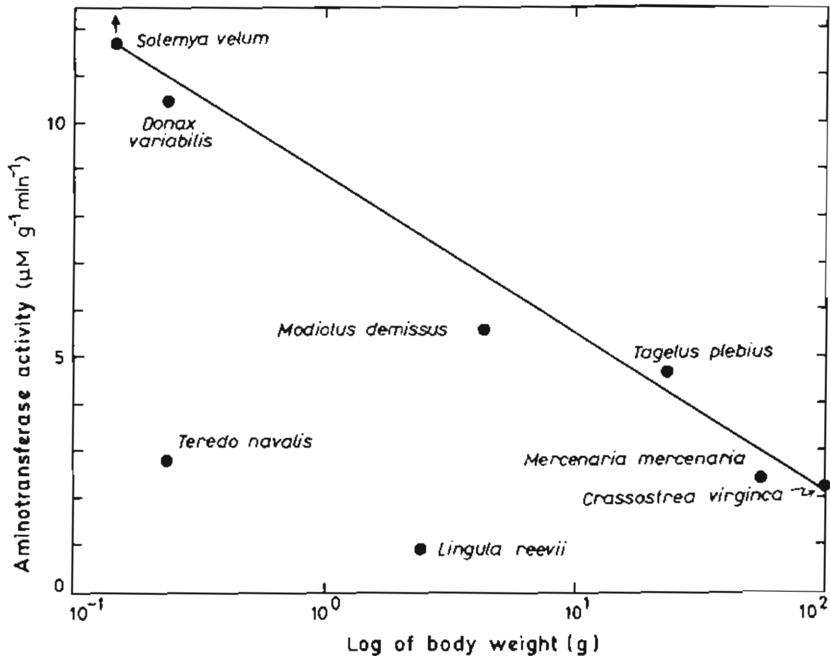


Fig. 3-46: Total aminotransferase activities of tissue homogenates as a function of body weight in various bivalves and the brachiopod *Lingula reevii*. (After HAMMEN, 1968; modified; reproduced by permission of Microforms International Marketing Corp.)

The most important pathway in the metabolism of many amino acids is transamination, in which pairs of keto acids and amino acids are reciprocally aminated and deaminated. Aminotransferase (AT) activities are related to their amino-acid excretion rates (Figs 3-46 and 3-47). HAMMEN (1968) found that total AT activities and rates of amino-acid loss in 7 species of bivalves and the brachiopod *Lingula reevii* were directly proportional and both were inversely proportional to the (logarithm of) total body weight. It appears that amino-acid loss is a form of leakage rather than true excretion (POTTS, 1967); because of their greater surface per unit volume, smaller bivalves exhibit higher loss rates. The shipworm *Teredo navalis* departs markedly from the general pattern and resembles larger animals in both low AT activities and very low amino-acid excretion (Figs 3-46 and 3-47). It is not clear whether this is due to the low-protein diet (wood) of this highly specialized bivalve (HAMMEN, 1968).

Trimethylamine. Occurrence of trimethylamine (TMA) and its oxide (TMAO) have been reported from a number of invertebrates, elasmobranchs and marine teleosts (PROSSER and BROWN, 1961; NICOL, 1967). Marine teleosts such as the goosefish *Lophius piscatorius* may excrete up to 50% of their total nitrogen in the form of TMAO (GROLLMAN, 1929). TMAO is a soluble but non-toxic substance. There appear to be two different sources of TMAO in marine fishes: exogenous and endogenous. Marine invertebrates rich in TMA are a good exogenous dietary source (BENOIT and

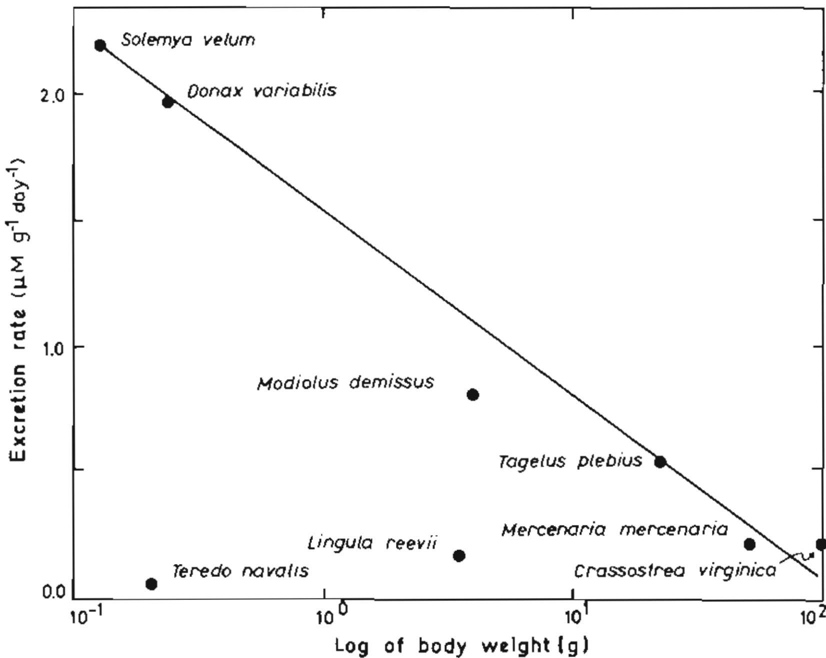
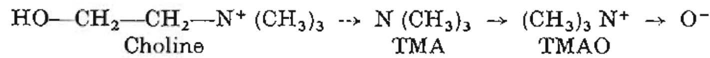


Fig. 3-47: Excretion rate of amino acids as a function of body weight in various bivalves and the brachiopod *Lingula reevii*. (After HAMMEN, 1968; modified; reproduced by permission of Microforms International Marketing Corp.)

NORRIS, 1945); unequivocal evidence for the endogenous origin or biosynthesis of TMAO has recently been provided by L. J. READ (1968b) and GOLDSTEIN and FUNKHOUSER (1972). BILINSKI's (1960, 1964) studies on TMAO biosynthesis in the marine teleosts *Parophrys vetulus* and *Platichthys stellatus* and the lobster *Homarus americanus* revealed that both choline and TMA are the precursors of TMAO. Working on the nurse shark *Ginglymostoma cirratum*, GOLDSTEIN and FUNKHOUSER (1972) suggested the following biochemical pathway for TMAO synthesis:



$$\underset{\text{Choline}}{HO{-}CH_2{-}CH_2{-}N^+(CH_3)_3} \rightarrow \underset{\text{TMA}}{N(CH_3)_3} \rightarrow \underset{\text{TMAO}}{(CH_3)_3N^+ \rightarrow O^-}$$

However, the pathway for choline conversion to TMAO via TMA is hypothetical; the mechanism for conversion of choline to TMA is not clear. Bacteria can produce TMA from choline (BILINSKI, 1960); therefore, the possibility of TMAO synthesis by microbial activity in the digestive tract of animals has not been ruled out. The mechanism for the conversion of TMA to TMAO is fairly well understood (GOLDSTEIN and FUNKHOUSER, 1972). L. J. READ (1968b) showed that the total TMAO content in the egg of the oviparous skate *Raja binoculata* increases as the embryo grows. This finding suggests strongly that the *R. binoculata* embryo synthesizes TMAO endogenously, since the embryo is, apparently, a closed system with regard to organic substrates.

Taurine. This sulphonic amino acid (FLORKIN and BRICTEUX-GRÉGOIRE, 1972) has been reported from several marine and estuarine animals. The information

Table 3-37

Concentrations of 5 amino acids and taurine in tissues of marine invertebrates. Values (μM amino acid g^{-1} tissue) estimated by ALLEN and GARRETT (1971b) from histograms presented by SIMPSON and co-authors (1959) (Reproduced by permission of Academic Press, New York)

Species	Alanine	Arginine	Glycine	Glutamic	Aspartic	Taurine
Bunodosoma cavernata	1·0	1·0	3·0	1·0	0·8	10·0
Penaeus aztecus	5·2	6·5	56·0	2·0	0·8	23·0
Clibanarius vittatus	4·0	4·0	6·0	2·8	0·5	2·4
Pagurus pollicaris	7·0	4·5	23·0	2·4	0·3	36·0
Siphonaria lineolata	5·5	1·4	2·4	3·0	1·2	4·3
Fasciolaria distans	2·0	2·0	5·0	2·3	1·5	12·0
Busycon perversum	4·0	3·0	2·5	1·2	2·0	15·0
Thais haemastoma	7·4	4·5	20·0	1·0	3·0	16·0
Polynices duplicata	18·0	3·0	8·0	1·5	2·3	60·0
Oliva sayana	2·3	1·0	1·0	1·5	0·5	5·6
Lithophaga bisulcata	0·8	1·0	14·0	—	1·4	11·0
Crassostrea virginica	7·0	1·0	6·3	5·5	1·5	6·0
Arca umbonata	5·0	1·5	6·0	5·0	2·5	70·0
Volsella demissus	12·0	1·0	11·5	2·2	1·2	10·0
Loliguncula brevis	15·0	1·4	20·0	2·0	0·6	55·0
Thyone sp.	—	0·5	—	14·0	2·0	20·0
Luidia clathrata	5·0	0·4	34·0	2·5	2·6	11·0

available on the occurrence of taurine and related compounds in marine invertebrates has been summarized by ALLEN and GARRETT (1971b) and SEVERIN and co-authors (1972). Taurine contents of different (marine) animal tissues vary, but taurine may attain concentrations higher than those of other amino acids (KERMACK and co-authors, 1955; SIMPSON and co-authors, 1959; LANGE, 1963; Table 3-37). ALLEN and AWAPARA (1960) reported that taurine is formed in the freshwater bivalve *Rangia cuneata*, and in the marine *Mytilus edulis*. Taurine is excreted by *R. cuneata* as soon as it is formed; but in *M. edulis* it is retained and plays a role in intracellular osmotic pressure. However, marine invertebrates may also excrete taurine in smaller quantities (e.g. the polychaete *Cirriformia spirabranchia*; HULT, 1969). Individuals of *Mya arenaria* that were starved at low salinities and subsequently subjected to stepwise salinity increase, showed no increase in taurine content. This suggests that any increase in taurine content in higher salinities occurs via the food (ALLEN and GARRETT, 1972).

Discharge of Urine

In higher marine animals, the 3 well-defined physiological processes involved in urine production are: ultra-filtration, re-absorption and secretion. During ultra-filtration, non-colloidal solutions are removed by hydrostatic differences via a semi-permeable membrane from the body fluids into a space connected with the outside (HOAR, 1966). The filtered material then passes through a tube or other space lined with cells capable of actively transporting molecules back into the body fluids (re-absorption), or capable of secreting additional substances into the filtered fluids. Obviously, the cellular level involves only 2 processes: diffusion and active transport (HOAR, 1966).

In general, marine invertebrates and teleost fishes excrete their nitrogenous wastes principally in the form of ammonia with some urea and amino acid as well as a very small amount of uric acid (Table 3-34). The greater part of the nitrogenous wastes is lost directly by diffusion, mostly via the thin gills and other body surfaces (e.g. tentacles of the polychaete *Cirriformia spirabranchia*; HULT, 1969). Calculations of data reported by NEEDHAM (1955) and BINNS (1969a, b) reveal that in the crab *Carcinus maenas* the elimination of nitrogen as ammonia, urea, uric acid and L-amino nitrogen in the urine accounts for 3·2% of the total nitrogen excreted. In fishes, branchial excretion consists of highly soluble substances such as ammonia, urea, amines and amine oxide derivatives; the less diffusible nitrogenous end-products creatine, creatinine, and uric acid are excreted solely by the kidneys. Fish gills (e.g. those of the sculpin *Myoxocephalus scorpius*) contain active glutaminase and glutamic-acid dehydrogenase; hence most of the NH_3 excreted is formed in the gills from blood glutamine (GOLDSTEIN and FORSTER, 1960, 1961; see also LUND and GOLDSTEIN, 1969). In *Salmo gairdnerii*, nitrogen excreted by the kidney is only 3% of the total nitrogen loss (FROMM, 1963). Uric acid is sometimes stored in 'nephrocytes', or utilized in the white chromatophores (e.g. *Eriocheir sinensis*; MOLLITOR, 1937). Thus, the renal contribution to nitrogenous end-product excretion, though present, is probably of relatively little importance (fishes: HICKMAN and TRUMP, 1969), except in some animals such as *Octopus dofleini*. Aspects of water and electrolyte balances in marine animals have been dealt with in

Volume I (HOLLIDAY, 1971; KINNE, 1971) and in Chapter 5 of this volume. General accounts of the mechanisms of filtration, re-absorption and secretion have been published by RIEGEL (1971) for crustaceans and by CONTE (1969) and HICKMAN and TRUMP (1969) for fishes. Hence ultra-filtration in marine animals is exemplified here only briefly on the basis of pertinent information obtained on molluscs.

Measurements of blood pressures, inulin concentration and filtration rate in the cephalopod *Octopus dofleini* have shown conclusively that filtration occurs in the branchial heart appendages (HARRISON and MARTIN, 1955, 1965; JOHANSEN and MARTIN, 1962). Since cephalopod blood pressures are much higher (systolic pressure in *O. dofleini*: 25 to 20 cm H_2O; JOHANSEN and MARTIN, 1962) than those of bivalves (5 to 7·5 cm H_2O in 4 species; TIFFANY, 1972; see also SMITH and DAVIS, 1965) or gastropods (JONES, 1970), the feasibility of filtration in these non-cephalopods is doubtful.

However, inulin-concentration studies on the marine gastropod *Haliotis rufescens* led HARRISON (1962) to conclude that the pericardial fluid (for details on anatomy consult MARTIN and HARRISON, 1966; KIRSCHNER, 1967) is an ultra-filtrate of the blood, and to propose that the walls of the arteria are filtration sites. Working on the freshwater snail *Viviparus viviparus*, LITTLE (1965) came to the same conclusion. Ligating the reno-pericardial canal in the freshwater giant snail *Achatina fulica*, MARTIN and co-authors (1965) found that urine production does not decrease, although urine to blood ratios (U/B) of inulin suggest that filtration is, indeed, taking place. They concluded that the filtration site is probably the kidney itself.

In bivalves, ultra-filtration across the ventricular wall into the pericardial cavity can take place only if the hydrostatic pressure in the heart exceeds the osmotic-pressure difference between blood and pericardial fluid (POTTS, 1968). In 4 species of marine bivalves, TIFFANY (1972) measured the ventricular hydrostatic pressure and the osmotic pressure. In every case, the osmotic-pressure difference between blood and pericardial fluid (π) is at least 20 times greater than the positive ventricular hydrostatic pressure added to the absolute pericardial-cavity pressure (=the effective filtration pressure or EEP; see GUYTON and co-authors, 1960; LANDIS and PAPPENHEIMER, 1963; Table 3-38). Therefore, in bivalves, ultra-filtration cannot take place across the heart ventricle into the pericardial cavity. TIFFANY (1972) indicated that non-cephalopod molluscan auricular pressures (SMITH and DAVIS,

Table 3-38

Osmotic-pressure difference between blood and pericardial fluid (π), effective filtration pressure (EFP), and π/EFP ratios in various bivalves (After TIFFANY, 1972; modified; reproduced by Pergamon Press Ltd., Oxford)

Species	π (atm)	EFP (atm)	π/EFP
Dinocardium robustum	$1{\cdot}71 \times 10^{-1}$	$8{\cdot}77 \times 10^{-3}$	20
Macrocallista nimbosa	$2{\cdot}20 \times 10^{-1}$	$5{\cdot}65 \times 10^{-3}$	29
Mercenaria campechiensis	$2{\cdot}45 \times 10^{-1}$	$6{\cdot}53 \times 10^{-3}$	38
Mercenaria mercenaria	$2{\cdot}20 \times 10^{-1}$	$5{\cdot}65 \times 10^{-3}$	39

1965; JONES, 1970) are even lower than those of ventricular pressures; thus in these animals no part of the heart can be the ultra-filtration site. While the ultra-filtration site is not clearly known, there is no doubt about the occurrence of ultra-filtration in these molluscs; Table 3-39 presents data on the ultra-filtration rate in some representatives.

Table 3-39

Filtration rates in the kidney of some molluscs (After KIRSCHNER, 1967; modified; reproduced by permission of Annual Reviews Inc.)

Species	Filtration rate ($\mu l\ g^{-1}\ hr^{-1}$)
Gastropods	
Haliotis rufescens	12
Aplysia californicus	13
Amphineurans	
Cryptochiton stelleri	35
Bivalves	
Mytilus californicus	23
Cephalopods	
Octopus dofleini	3

Ecological Significance of Nitrogen Excretion

Composition and quantity of urine depend very much upon the osmotic concentration of the surrounding medium and the physiological availability of water to the animal. While retention of amino acids and taurine increase the intracellular osmotic pressure of marine invertebrates, urea and TMAO-retention in the blood allows marine elasmobranchs to maintain osmotic equilibrium with the ambient sea water. Differences in the availability of water in intertidal or estuarine environments alter considerably the composition and quantity of nitrogenous wastes excreted. If water is readily available, bivalves or elasmobranchs may excrete a greater quantity of taurine or urea and TMAO. Exposed to dry conditions in the supratidal zone, marine animals may considerably alter the composition of urine, excreting their nitrogenous wastes in form of uric acid. The information available on these aspects is briefly summarized in the following paragraphs.

DUCHÂTEAU and FLORKIN (1955) observed that the free amino-acid component in the muscle of the euryhaline crab *Eriocheir sinensis* was smaller in individuals kept in fresh water than in crabs adapted to sea water. In marine invertebrates, the intracellular amino-acid component is present at relatively higher concentrations and compensates for the much less concentrated inorganic effectors in the cells (FLORKIN, 1966, 1969; SCHOFFENIELS and GILLES, 1970; FLORKIN and BRICTEUX-GRÉGOIRE, 1972; Chapter 5).

SIMPSON and co-authors (1959) have investigated the distribution of taurine in invertebrates and have found it in high concentrations in all marine species examined, but not in freshwater and terrestrial animals. This finding is correlated with the role of taurine as one of the effectors of intracellular osmolar concentration; intracellular taurine is reduced in marine bivalves transferred to brackish water; adaptation to fresh water—if tolerated—is accompanied by an even more pronounced taurine decrease (FLORKIN and BRICTEUX-GRÉGOIRE, 1972). Absence of taurine in freshwater animals like *Rangia cuneata* is not due to their inability to synthesize taurine, but to rapid disposal of it. For more details consult Chapter 5.

As has been pointed out, elasmobranchs maintain osmotic gradients to the ambient water by regulating the levels of urea and trimethylamine oxide in their body fluids (GOLDSTEIN and co-authors, 1968; see also Volume I: HOLLIDAY, 1971, and Chapter 5 of the present Volume). Blood urea concentration in sharks averages about 350 mM l^{-1} (SCHMIDT-NIELSEN and RABINOWITZ, 1964; BURGER, 1967) and constitutes more than one-third of their osmolarity. In the smooth dogfish *Mustelus canis*, blood-urea concentration varies over a surprisingly wide range (e.g. from 260 to 764 mM l^{-1}; KEMPTON, 1953). TMAO occurs in relatively high concentrations (~70 mM) in the extracellular fluids of all elasmobranchs examined (GRONINGER, 1959). The base constitutes 7% of the total osmotic pressure or about 16% of the total organic solutes. Both urea and TMAO are always re-absorbed against their concentration gradient, but neither is completely removed from the urine. Urine/plasma ratios for urea average about 0·3, but may range from 0·07 to 0·89 in different species or in the same species at different times (SMITH, 1931; KEMPTON, 1953). In the dogfish *Squalus acanthias*, 90 to 95% of the filtered urea and 95 to 98% of the filtered TMAO are re-absorbed by the tubules (FORSTER, 1967).

Following salinity reduction, *Squalus acanthias* exhibits a progressive increase in glomerular filtration rate and urine flow (BURGER, 1965) leading to decreased urea concentrations in plasma and urine (SMITH, 1936). Similar results have been reported for the skate *Raja eglanteria* (PRICE, 1967; PRICE and CREASER, 1967). In lemon shark *Negaprion brevirostris*, exposed to 50% sea water, urea and TMAO concentrations are reduced to 45 and 40%, respectively, of their levels in 100% sea water (GOLDSTEIN and co-authors, 1968). According to GOLDSTEIN and FORSTER (1971a, b), the rate of ammonia excretion does not change with a dilution of sea water to 50%; reduction in plasma-urea concentration results from increased renal clearance and decreased biosynthesis of urea.

In the coelacanth *Latimeria chalumnae*, levels of urea and trimethylamine oxide in blood and body fluids are observed to be comparable to those in marine elasmobranchs (PICKFORD and GRANT, 1967; LUTZ and ROBERTSON, 1971). The entire sequences of orinithine-urea and trimethylamine oxidase are found to have activity levels comparable to those previously ascertained in marine elasmobranchs (GOLDSTEIN and co-authors, 1973). Retention of urea and trimethylamine oxide is suggested to participate in osmoregulation.

The presence of urea-synthesizing enzymes in early embryos of dogfish *Squalus suckleyi* and skate *Raja binoculata* (L. J. READ, 1968a) can be correlated with the need to detoxicate ammonia during the intra-uterine period, or while encapsulated in the characteristically tough keratinized egg cases (FORSTER and GOLDSTEIN, 1969). There is a net increase in urea and TMAO contents during ontogenetic

development of *R. binoculata*. Urea is lost from the developing, but not from the undeveloped egg; however, TMAO does not appear to be lost from the developing egg. Since there is no apparent route for TMAO to enter the egg, this base is presumably synthesized endogenously. The maintenance of relatively constant urea and TMAO concentrations implies that regulation occurs throughout embryonic development, and that these organic solutes play a similar role in embryos and in adults (L. J. READ, 1968b). Even the earliest ontogenetic stages of the oviparous *R. binoculata* exhibit the ability to retain a relatively constant urea level. This finding would seem to diminish the suggestion that the need for urea retention represented a selective pressure forcing the elasmobranchs toward intra-uterine development (PRICE and DAIBER, 1967).

The classification of animals as ammonotelic, ureotelic or urecotelic according to the major end-product of protein catabolism is somewhat arbitrary; the body fluids and excreta of most animals contain a mixture of ammonia, urea and uric acid in varying proportions. The ratios of one to the other vary with environmental conditions (GOLDSTEIN and FORSTER, 1965; FORSTER and GOLDSTEIN, 1969; Volume III: KINNE, in press). As discussed above, decrease in the plasma urea and TMAO concentrations following dilutions of salinity results in increased renal clearance of urea and TMAO, and reduced biosynthesis of urea. GORDON and coauthors (1965) found that the amphibious goboid fish *Perioptalmus sobrinus* excretes, in 100% sea water, almost as much urea (0·36 mM kg^{-1} hr^{-1}) as ammonia (0·49 mM kg^{-1} hr^{-1}); in lower salinities, the fish exhibits a shift toward ammonotelism; in 40% sea water, urea excretion amounts to only 0·06 mM kg^{-1} hr^{-1}, while ammonia production is 0·77 mM kg^{-1} hr^{-1}.

When the freshwater crayfish *Orconectes rusticus* is transferred to 50% sea water, urea excretion increases from 4 μg urea N g^{-1} hr^{-1} in fresh water to 17 μg urea N g^{-1} hr^{-1} in 50% sea water. This increase occurs not at the expense of ammonia excretion, which remains at about 15 μg ammonia N g^{-1} hr^{-1} (SHARMA, 1966). The increased urea excretion is caused by an increase in the concentration of ions in blood and tissues of *O. rusticus* (SHARMA, 1969). To localize the source of this excess urea excretion, SHARMA (1968) estimated protein and non-protein nitrogen content of blood and abdominal muscles of *O. rusticus* in fresh water and 50% sea water and found that excess urea excretion does not originate from blood protein. The excess urea is produced neither via the ornithine cycle nor by purine catabolism (SHARMA and NEVEU, 1971). Further studies must be awaited to reveal source and mechanism of excess urea production in *O. rusticus* acclimated to dilute sea water.

In a number of euryhaline invertebrates, the influence of salinity on ammonia excretion has been studied as a function of time and body weight (EMERSON, 1969). There is a consistent decrease in ammonia excretion rate with time (beginning with collection of experimentals) in all species, except *Strongylocentrotus dröbachiensis* (Table 3-40, Fig. 3-48). EMERSON also noted that ammonia-nitrogen excretion rate decreased with increasing body weight in the bivalve *Mya arenaria* (Fig. 3-49) and requested that body weight and time course be noted in future reports on ammonia-nitrogen excretion rates. Ammonia-N excretion rates decrease as a function of time also in the crayfish *Orconectes limosus* (SIEBERS, 1970). With increasing salinity, ammonia-nitrogen excretion rate decreases in all the species tested (EMERSON, 1969): e.g. from 115 μg ammonia-N g^{-1} dry weight hr^{-1} in 50% sea water to 67 μg

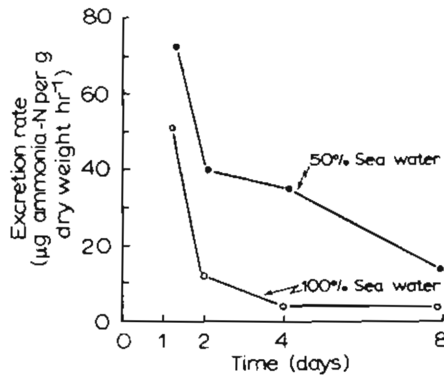


Fig. 3-48: *Nephtys caeca.* Ammonia-excretion rate as function of time and salinity. All values are based on dry weights of whole animals. (After EMERSON, 1969; modified; reproduced by permission of Microforms International Marketing Corp.)

ammonia-N g^{-1} dry weight hr^{-1} in 100% sea water on the first day of the test in the polychaete *Nephtys caeca* (Table 3-40); corresponding values for the gastropod *Littorina sitchana* are 7·5, 4 and 2 μg ammonia-N g^{-1} dry weight hr^{-1} in 50, 100 and

Table 3-40

Ammonia-excretion rate as function of salinity and time in some invertebrates. Salinities (100% = 35‰) were obtained by adding salts to fresh water. Time is expressed in days after collection. All values are given as μg ammonia excreted g^{-1} dry weight hr^{-1}; dry weight is based on soft parts for molluscs and on whole body for the other species (After EMERSON, 1969; modified; reproduced by permission of Microforms International Marketing Corp.)

Species	50% Sea water		100% Sea water		150% Sea water	
	1st day	7th day	1st day	7th day	1st day	7th day
Echinoderms						
Strongylocentrotus dröbachiensis	6	13	3	9	—	—
Eupentacta quinquesemita	12	8·5	11·5	5	—	—
Polychaete						
Nephtys caeca	115	14	67	14	—	—
Crustacean						
Pagurus sp.	79	51	43	34	—	—
Molluscs						
Macoma inconspicua	24·5	13	0·5	0·4	—	—
Thais emarginata	67	19	32	7	—	—
T. lamellosa	13	3	14	10	10	2
Littorina sitkana	7·5	2	4	1	2	1
Acmaea scutum	38	36	36	38	13	5

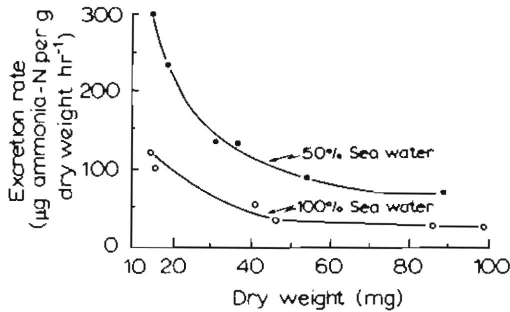


Fig. 3-49: *Mya arenaria*. Ammonia-excretion rate as function of body size and salinity. Values are based on dry weights of soft parts. (After EMERSON, 1969; modified; reproduced by permission of Microforms International Marketing Corp.)

150% sea water, respectively. The reason for the increased ammonia-N excretion in low salinities appears to be: The level of glutamic acid dehydrogenase (GDH) activity controls ammonia excretion rate; at reduced salinities, GDH activity decreases and 3-glycerophosphate dehydrogenase activity increases, and hence more reducing equivalents are directed toward the cytochrome chain; this results in increased ammonia excretion and oxygen uptake, and in decreased amino-acid synthesis (SCHOFFENIELS, 1968). In the bivalve *Mya arenaria* (starving test individuals of 5·5 to 6·3 cm total shell length), ammonia-N excretion rapidly increases from 3·2 mg NH_3 N day^{-1} in sea water (34‰S) to a maximum of 64·4 mg NH_3 N day^{-1} on the 3rd test day (ALLEN and GARRETT, 1971a). An important point is that between the 4th and 6th test days, ammonia-N excretion of *M. arenaria* levels off around 3 mg NH_3 N day^{-1} and that urea-N excretion remains around 0·22 mg urea-N day^{-1} throughout the 6-day experiment. In *Acmacea scutum*, ammonia-N excretion rate remains about 37 μg NH_3 N g^{-1} day^{-1} at 50 and 100% sea water on the 1st and 7th days of the experiment, while those exposed to 150% sea water excrete 13 or 5 μg NH_3 N g^{-1} day^{-1} (EMERSON, 1969; Table 3-40). More information is likely to enable definite patterns of responses to be recognized in ammonia and urea excretion as functions of salinity and time.

Starvation profoundly affects excretion rates of nitrogenous waste products (e.g. MAYZAUD, 1973). However, pertinent information on marine animals is limited. Progressive starvation leads to decreased ammonia-excretion rates in various invertebrates (EMERSON, 1969). However, the spiny dogfish *Squalus acanthias* maintains a constant level of trimethylamine oxide in its blood during starvation. This is attributed to release from the large muscle pool (GOLDSTEIN and co-authors, 1967). During 70 days of starvation, the snail *Littorina planaxis* oxidizes fat as major energy source; apparently, the snail excretes neither amino acid nor ammonia during this period, and the end-product of the reduced protein metabolism is stored as uric acid (EMERSON and DUERR, 1967).

According to NEEDHAM (1935), high-level intertidal snails, such as *Littorina neritoides*, excrete more uric acid than deeper water species like *Buccinum undatum*

(Table 3-41). This correlation does not seem to hold for all intertidal species (DUERR, 1968). Nevertheless, pertinent biochemical adaptations are certainly of ecological significance and have allowed many intertidal species to inhabit high shore levels (GILLES, 1972).

In marine and semi-terrestrial amphipods and isopods, the main nitrogenous end-product is still ammonia; it accounts for 83% of the total non-protein nitrogen (NPN) excreted by *Ligia oceanica* (Table 3-34), and for some 50 to 60% of that excreted by *Oniscus asellus*, *Porcellio laevis* and *Armadellium vulgare* (DRESEL and MOYLE, 1950). However, the total nitrogenous excretion in these 3 terrestrial crustaceans was much lower (approximately 0·03 mg N g^{-1} day^{-1}) than that in the semi-terrestrial *L. oceanica* (0·13 mg N g^{-1} day^{-1}) or *Orchestia* sp. (0·20 mg N g^{-1}

Table 3-41

Uric-acid content of kidneys in prosobranch molluscs from different habitats (After NEEDHAM, 1935, 1938; modified; reproduced by permission of Biochemical Journal)

Habitat and species	Uric acid content (mg g^{-1} kidney dry weight)
Sea water (permanently submerged)	
Buccinum undatum	4·1
Nucella lapillus	4·4
Osilinus lineatus	1·6
Gibbula umbilicalis	2·3
Turritella communis	6·0
Intertidal (temporarily air exposed)	
Littorina littorea	1·4
Littorina rudis	5·1
Littorina saxatilis	5·0
Littorina neritoides	25·0

day^{-1}), the estuarine *Gammarus zaddachi* (0·60 mg N g^{-1} day^{-1}), or the marine *G. locusta* (0·49 mg N g^{-1} day^{-1}; Table 3-42). DRESEL and MOYLE (1950) suggested that terrestrial isopods and amphipods have adjusted to land life by suppressing their protein metabolism.

During embryonic development, many marine invertebrates—especially the semi-terrestrial isopod *Ligia oceanica*—considerably suppress their protein metabolism. In order to compare the data available, the quantity of protein metabolized during conversion of yolk (equivalent to 1 cal) into the embryonic substance was calculated; the data thus obtained are presented in Table 3-43. Non-cleidoic eggs of freshwater animals, e.g. of the salmon *Oncorhynchus* sp., oxidize as much as 122 μg protein in order to convert yolk substance equivalent to 1 cal; cleidoic terrestrial eggs, e.g. of *Gallus domesticus* and *Bombyx mori*, oxidize only about 20 μg protein;

Table 3-42

Nitrogen excretion in isopods and amphipods. Mean values for total non-protein nitrogen (NPN) (After DRESEL and MOYLE, 1950; modified; reproduced by permission of Company of Biologists Ltd.)

Species	Habitat	NPN (mg N g^{-1} day)
Gammarus locusta	Marine littoral	0·49
Marinogammarus pirloti	Marine littoral	0·29
Marinogammarus marinus	Marine littoral and estuarine	0·11
Gammarus zaddachi	Estuarine	0·60
Orchestia sp.	Semi-terrestrial	0·20
Ligia oceanica	Semi-terrestrial	0·13
Oniscus asellus	Terrestrial	0·03
Gammarus pulex	Freshwater	0·23
Asellus aquaticus	Freshwater	0·26

demersal eggs of marine animals oxidize about 30 μg protein, and those of the semi-terrestrial *L. oceanica* only 9 μg (PANDIAN, 1972). In other words, compared to the eggs of non-cleidoic freshwater animals, the suppression of protein metabolism amounts to one-fourth in marine demersal eggs, one-sixth in terrestrial cleidoic eggs, and one-thirteenth in eggs of the semi-terrestrial *L. oceanica*. Moreover, suppression of protein metabolism is progressively intensified as the animal's habitat shifts closer to the coast. PANDIAN (1972) suggested that developing eggs of *L. oceanica* have adapted to semi-terrestrial life primarily through suppression of protein metabolism, rather than through converting the end-product of protein metabolism into urea or uric acid.

Table 3-43

Protein metabolized during conversion of yolk substance equivalent to 1 cal in developing eggs of the species (After PANDIAN, 1972; modified; reproduced by permission of Indian National Scientific Academy)

Species	Habitat	Amount of protein metabolized (μg)	Author
Gallus domesticus	Terrestrial	18·4	NEEDHAM (1931)
Bombyx mori	Terrestrial	22·0	NEEDHAM (1931)
Salmon (species name not given)	Freshwater	122·0	NEEDHAM (1931)
Eupagurus bernhardus	Marine	40·6	PANDIAN and SCHUMANN (1967)
Crepidula fornicata	Marine	33·3	PANDIAN (1969)
Crangon crangon	Marine	31·3	PANDIAN (1967c)
Homarus gammarus	Marine	15·7	PANDIAN (1970b)
Ligia oceanica	Marine, supratidal	9·3	PANDIAN (1972)

(b) Nutritive Role of Animal Faeces

Availability and Production of Faeces

Faecal material is often abundant in benthic marine habitats (e.g. MOORE, 1931; MANNING and KUMPF, 1959; HAVEN and MORALES-ALAMO, 1966). FRANKENBERG and co-authors (1967) estimated that an intertidal population of the ghost shrimp *Callianassa major* produces faecal pellets at a rate sufficient to provide 0·06 g organic carbon m^{-2} day^{-1} for coprophagous organisms. Table 3-44 indicates that organic carbon from *C. major* faecal pellets is made available at rates ranging from 2% (SCHELSKE and ODUM, 1961) to 28% (RAGOTZKIE, 1959) of the rate due to local (Sapelo Island, Georgia, USA) estuarine phytoplankton populations. Carbon made available as *C. major* faecal pellets amounts to 10% of that obtained from benthic algae in the salt marches (Georgia, USA; POMEROY, 1959) to 100% of that obtained

Table 3-44

Organic carbon production by various ecosystem components (After FRANKENBERG and co-authors, 1967; modified; reproduced by permission of American Society of Limnology and Oceanography)

Producing unit	Time	Production (g C m^{-2} day^{-1})
Callianassa major faeces	August	0·06
Phytoplankton (estuary)	August	0·2
Phytoplankton (estuary)	August	2·5
Phytoplankton (6·4 km offshore)	August	2·07
Marsh		
Spartina alterniflora	Annual average	ca. 4·5
S. alterniflora detritus		
Exported from marsh	Summer	0·3
Benthic algae	Summer	0·6
Benthic diatoms	During 'warm weather'	0·1
Phytoplankton-offshore waters		
Tropical	Annual average	0·05
Temperate	Annual average	0·33

from offshore tropical phytoplankton (RYTHER, 1963). These values suggest that faecal material constitutes an important link in the transfer of energy and matter in marine ecosystems.

In the Clyde Sea, *Calanus* sp. and euphausiids deposit 4·8 g faeces m^{-2} day^{-1} in spring (MOORE, 1931). The total of faecal pellets deposited by a Japanese oyster population is in the range of 0·01 metric ton m^{-2} day^{-1} (ITO and IMAI, 1955). An American oyster (*Crassostrea virginica*) population voided 1·5 metric tons of faeces ha^{-1} day^{-1} (LUND, 1957). The bivalve *Cardium* sp. population in the Wadden Sea produces 100,000 metric tons of faeces per year (VERWEY, 1952).

The suspended particles filtered by the bivalves are subjected to selection in the gills and/or labial palps; non-accepted particles are accumulated and voided as

pseudofaeces. Faeces and pseudofaeces settled on the bottom are collectively referred to as biodeposit (HAVEN and MORALES-ALAMO, 1966). A considerable quantity of pseudofaeces is deposited by bivalves (e.g. *Crassostrea virginica* of 15 g body weight deposit up to 140 mg pseudofaeces g^{-1} day^{-1}); the pseudofaeces contain 12·3 to 15·7% (dry weight) of organic matter and 5·4 to 5·9% organic carbon. Hence they must be considered an important nutrient source for cophrophages. For more details on pseudofaeces and biodeposition consult HAVEN and MORALES-ALAMO (1966), HUGHES (1969, 1970, 1971).

Composition and Types of Faeces

Organic matter contained in freshly voided faeces ranges from 2% in *Littorina planaxis* to 20% carbon in *Palaemonetes pugio* (JOHANNES and SATOMI, 1966); nitrogen content ranges from 0·02% in *Hydrobia ulvae* (NEWELL, 1965) to 4·48% in *P. pugio* (JOHANNES and SATOMI, 1966; Table 3-45). The calorific content of faeces of aquatic animals has received attention only recently (PANDIAN and DELVI, 1973).

Table 3-45

Composition of faecal pellets obtained from various marine invertebrates (After FRANKENBERG and co-authors, 1967; modified; reproduced by permission of American Society of Limnology and Oceanography)

Species	Carbon content (% dry weight)	Nitrogen content (% dry weight)
Littorina planaxis	2·0	
Cirriformia tentaculata	2·1	
Hydrobia ulvae		
fresh pellets	10·75	0·02
pellets aged 3 days	9·68	1·7
Macoma balthica		
fresh pellets	8	0·03
pellets aged 3 days	7	1·2
Palaemonetes pugio		
fresh pellets	20	4·48
pellets aged 4 days	9	1·6
Crassostrea virginica		
fresh pellets	4·6–6·1	
Balanus eburneus		
fresh pellets	5·5–6·8	
Mya arenaria		
fresh pellets	5·3	
Modiolus demissus		
fresh pellets	4·4–5·6	
Molgula manhattensis		
fresh pellots	5·4	
Callianassa major		
fresh pellets	2·9	0·28
pellets aged 2 days	2·9	0·15

Table 3-46

Ash and calorific contents of food and faeces in marine invertebrates and a fish (Compiled from the sources indicated)

Species	Food species	Food		Faeces		Author
		Ash (%)	Energy (kcal g^{-1} organic substance)	Ash (%)	Energy (kcal g^{-1} organic substance)	
Ligia pallasii	*Ulva* sp.	20	5628	—	2642*	CAREFOOT (1973)
	Nereocystis luetkeana	40	5048	—	2787*	
Aplysia dactylomela	*Cladophora* sp.	84	4033	76	3220	CAREFOOT (1970)
	Galaxaura oblongata	64	3566	58	2445	
	Enteromorpha sp.	54	3228	47	3823	
	Laurencia papillosa	54	4908	45	3982	
	Ulva fasciata	28	4029	47	4233	
Littorina littorea	*Ulva lactuca*	18	3924	66	1216	GRAHAME (1973)
Belone belone	*Daphnia* sp.	23	5478	64	4300	ROSENTHAL and
	Tetramin	12	5539	33	6020	PAFFENHÖFER (1972)

*kcal g^{-1} dry weight; ash content values not reported.

Ash and calorific contents of food and faeces of a few marine animals are listed in Table 3-46. Within a given species, carbon content of faecal pellets may vary considerably, e.g. from 4·6 to 6·1% in *Crassostrea virginica* (HAVEN and MORALES-ALAMO, 1966).

Changes with ageing of faecal pellets follow 3 patterns: (i) Faecal pellets decrease slightly in organic content and increase significantly in nitrogen content within 3 days. While fresh faecal pellets are unacceptable as food, increase in nitrogen content due to microbial activities renders them acceptable after some time. Examples are *Hydrobia ulvae* and *Macoma balthica* (NEWELL, 1965). (ii) Faecal pellets decrease in nitrogen content, but neither change appreciably in their carbon content nor in their apparent attractiveness as food when held for 2 days. An example is *Callianassa major* (FRANKENBERG and co-authors, 1967). (iii) Faecal pellets lose both organic carbon and nitrogen fairly rapidly when held for 3 to 7 days. While freshly voided faecal pellets are accepted as food, the pellets become less nutritious when aged. This pattern is exemplified by *Palaemonetes pugio* (JOHANNES and SATOMI, 1966).

In the chitin-lined crustacean hind gut, no digestion occurs and bacterial activity is fairly high (ZHUKOVA, 1963). According to JOHANNES and SATOMI (1966), freshly voided faecal pellets of *Palaemonetes pugio* were densely packed with bacteria and undigestible food residues; presumably, they had been processed by bacteria in the posterior gut; hence the freshly voided faecal pellets are ready to serve as food for coprophagous animals.

Nutritive Importance of Faeces

The potential importance of faecal pellets as food for marine animals has been discussed by several workers (HARVEY and co-authors, 1935; ZENKEVICH and BIRSTEIN, 1956; KINNE, 1960; JØRGENSEN, 1962; BEKLEMISHEV, 1962; CONOVER, 1964; PAFFENHÖFER and STRICKLAND, 1970). NEWELL (1965) was probably the first author who demonstrated that the deposit-feeding gastropod *Hydrobia ulvae* ingests and transforms its own faeces, when live diatoms are not available. The shrimp *Palaemonetes pugio* re-ingests its own faeces, when other food is not available (JOHANNES and SATOMI, 1966). Analyses of the resulting 'double-digested' faeces revealed reductions in carbon (from 20 to 12%), protein (from 28 to 14%), and phosphorus (from 1·7 to 0·9%). Since *P. pugio* evacuates only 0·35 mg faeces for every 1 mg faeces ingested, protein absorption efficiency was 82% (JOHANNES and SATOMI, 1966); this compares favourably with a similar value (96%) reported for *Hydrobia ulvae* re-ingesting its own aged faeces (NEWELL, 1965). 'Double-digested' faeces were initially unacceptable to *P. pugio*, but subsequently were re-ingested several times (JOHANNES and SATOMI, 1966).

Faecal pellets of *Palaemonetes pugio*, fed on *Nitzschia closterium*, were observed to be consumed by other species such as the teleost *Fundulus heteroclitus*, the gastropod *Nassa obsoleta* and the crustaceans *Uca pugnax* and *Pagurus longicarpus* (JOHANNES and SATOMI, 1966). Similar studies revealed that the decapods *Callianassa major* and *Pagurus* sp. ingest *C. major* faecal pellets extensively; the bivalve *Mulina lateralis*, the amphipods *Parahaustorius longimerus* and *Neohaustorius schmitzi*, and the isopod *Chiridotea stenops* ingest them occasionally (FRANKENBERG and

Table 3-47

Examples of faecal-pellet ingesters and producers, and of rates of faecal-pellet ingestion (After FRANKENBERG and SMITH, 1967; modified; reproduced by permission of American Society of Limnology and Oceanography)

Species ingesting faecal pellets	Species producing faecal pellets	Ingestion rate (mg g^{-1} day^{-1})
Palaemonetes pugio	*Penaeus setiferus*	415
Pagurus longicarpus	*Penaeus setiferus*	350
Fundulus heteroclitus	hydrobiid spp.	265
Pagurus annulipes	*Crassostrea virginica*	165
Pagurus longicarpus	*Onuphis microcephala*	115
Hydrobiid spp.	hydrobiid spp.	105
Capitella sp.	hydrobiid spp.	95
Palaemonetes pugio	hydrobiid spp.	10
Fundulus majalis	*Penaeus setiferus*	55
Pagurus longicarpus	*Mugil cephalus*	55
Fundulus majalis	*Crassostrea virginica*	48
Glycera dibranchiata	*Mugil cephalus*	46
Palaemonetes pugio	*Onuphis microcephala*	41
Pagurus longicarpus	*Callianassa major*	34
Fundulus majalis	*Callianassa major*	34
Callinectes sapidus	*Mugil cephalus*	28
Nereis (Neanthes) succinea	*Crassostrea virginica*	28
Nassa (Nassarius) obsoletus	*Mugil cephalus*	19
Mugil cephalus	*Penaeus setiferus*	19
Glycera dibranchiata	*Penaeus setiferus*	18
Glycera dibranchiata	*Onuphis microcephala*	17
Panopeus herbstii	*Crassostrea virginica*	16
Ovalipes ocellatus	*Penaeus setiferus*	14
Penaeus setiferus	*Penaeus setiferus*	12
Penaeus setiferus	*Mugil cephalus*	12
Mugil cephalus	*Onuphis microcephala*	9
Callinectes sapidus	*Callianassa major*	8
Urosalpinx cinerea	*Crassostrea virginica*	1
Glycera dibranchiata	*Callianassa major*	6
Callinectes sapidus	*Onuphis microcephala*	6
Nassa (Nassarius) obsoletus	*Penaeus setiferus*	5
Penaeus setiferus	*Callianassa major*	4
Brachidontes exustus	*Crassostrea virginica*	2
Penaeus setiferus	*Crassostrea virginica*	1
Urosalpinx cinerea	*Onuphis microcephala*	1
Penaeus setiferus	*Onuphis microcephala*	0·5
Mulinia lateralis	*Penaeus setiferus*	0
Mulinia lateralis	*Onuphis microcephala*	0

co-authors, 1967). Table 3-47 lists the rates of faecal-pellet ingestion by several polychaetes, gastropods, bivalves, crustaceans and teleosts. Faecal pellets of the polychaete *Onuphis microcephala*, the bivalve *Crassostrea virginica*, the crustaceans *Callianassa major* and *Penaeus setiferus*, the teleost *Mugil cephalus*, and a gastropod served as food. The animals consumed from 0 to 415 mg faeces g^{-1} day^{-1}. There exists a direct correlation between the rate of faecal-pellet ingestion and the carbon and nitrogen contents of the faeces (Table 3-48). The correlation between mean ingestion rate and chemical content of the faeces is 0·83 for carbon and 0·80 for nitrogen (FRANKENBERG and SMITH, 1967). These correlations suggest that faeces containing large quantities of organic matter are eaten more readily than those with small quantities.

Coprophagy and Metabolism

The potential role of coprophagy in meeting the energy demands of faeces-ingesting animals has been assessed by FRANKENBERG and SMITH (1967). Their gross estimates (Table 3-49) suggest that marine animals may obtain a substantial fraction of the organic material required for their (standard) metabolism by ingesting faeces. For instance, *Palaemonetes pugio, Pagurus longicarpus* and *Glycera dibranchiata* can, in this way, obtain sufficient energy to meet a substantial portion of their metabolic needs.

Coprophagy and Salt Distribution

Coprophagy appears to play an important role in short-circuiting salt distribution. Table 3-46 shows the salt (ash) content of food plant and of faeces of some opisthobranchs; it indicates that heterotrophic animals concentrate considerable amounts of salt in their faeces. Trace elements may also be concentrated greatly in the faecal material of primary consumers (e.g. in the crab *Pugettia producta* feeding on the brown alga *Macrocystis pyrifera*; BOOTHE and KNAUER, 1972). If all faecal pellets were to sink quantitatively out of the euphotic zone, this would result in rapid removal of large quantities of important salts, especially the limiting ones like phosphorus. Sinking rates of natural faecal pellets from the euphausiids can account for the rapid descent of radionucleids (FOWLER and SMALL, 1972). Perhaps, such loss is minimized by coprophagous animals; through ingestion and transformation of faeces, considerable amounts of salt are retained in the surface waters. Calculations based on values reported by JOHANNES and SATOMI (1966) indicate that *Palaemonetes pugio* absorbs 84% of the phosphorus contained in the faeces ingested by it. That zooplankters like *Calanus helgolandicus* frequently ingest their own faecal pellets (PAFFENHÖFER and STRICKLAND, 1970) may be an adaptive mechanism for retaining biogenic salts in surface waters, instead of losing the faecal pellets to deep waters.

Natural coprophagy may have negative effects by expediting transfer of parasites (Volume III: JOHNSON, in press; LAUCKNER, in press), toxic substances, and radio-isotopes through food chains. Such aspects of coprophagy remain to be studied.

Table 3-48

Mean rate of faeces ingestion and chemical composition of faeces (After FRANKENBERG and SMITH, 1967; modified; reproduced by permission of American Society of Limnology and Oceanography)

Species ingesting faecal pellets	Species producing faecal pellets	Mean ingestion rate (mg g^{-1} day^{-1})	Carbon content (% dry weight)	Nitrogen content (% dry weight)
Fundulus heteroclitus Hydrobiid sp. *Capitella* sp. *Palaemonetes pugio*	Hydrobiid sp.	135	29	6·2
Palaemonetes pugio *Pagurus longicarpus* *Fundulus majalis* *Mugil cephalus* *Glycera dibranchiata* *Ovalipes ocellatus* *Penaeus setiferus* *Nassarius obsoletus* *Mulinia lateralis*	*Penaeus setiferus*	93	12	1·5
Pagurus annulipes *Fundulus majalis* *Neanthes succinea* *Panopeus herbstii* *Urosalpinx cinerea* *Brachiodontes exustus* *Penaeus setiferus*	*Crassostrea virginica*	38	18	3·4
Pagurus longicarpus *Glycera dibranchiata* *Callinectes sapidus* *Nassarius obsoletus* *Penaeus setiferus*	*Mugil cephalus*	32	9	1·2

Table 3-48—*Continued*

Species ingesting faecal pellets	Species producing faecal pellets	Mean ingestion rate (mg g^{-1} day^{-1})	Carbon content (% dry weight)	Nitrogen content (% dry weight)
Pagurus longicarpus *Palaemonetes pugio* *Glycera dibranchiata* *Mugil cephalus* *Callinectes sapidus* *Urosalpinx cinerea* *Penaeus setiferus* *Mulinia lateralis*	*Onuphis microcephala*	21	5	0·5
Pagurus longicarpus *Fundulus majalis* *Callinectes sapidus* *Glycera dibranchiata* *Penaeus setiferus*	*Callianassa major*	17	4·5	0·3

Table 3-49

Metabolic rates supportable by ingested organic faecal matter (After FRANKENBERG and SMITH, 1967; modified; reproduced by permission of American Society of Limnology and Oceanography)

Nutritional relationships	Live animal weight (mg)	Estimated metabolic rate of test animals (ml O_2 hr^{-1})	Calculated metabolic rate supportable by metabolism of ingested faecal organic matter (ml O_2 hr^{-1})	Calculated metabolic energy percentage supported by ingested faeces
Palaemonetes pugio ingesting	130	0·065	0·10	154
Penaeus setiferus faeces	120	0·060	0·20	333
Palaemonetes pugio ingesting	90	0·047	0·04	85
hydrobiid spp. faeces	60	0·033	0·03	91
Palaemonetes pugio ingesting	150	0·075	0·01	13
Onuphis microcephala faeces	100	0·052	0·01	19
Pagurus longicarpus ingesting	300	0·15	0·32	213
Penaeus setiferus faeces	400	0·20	0·37	185
Pagurus longicarpus ingesting	580	0·28	0·07	25
Onuphis microcephala faeces	480	0·23	0·07	30
Pagurus longicarpus ingesting	1020	0·36	0·11	31
Mugil cephalus faeces	750	0·32	0·09	28
Pagurus longicarpus ingesting				
Callianassa major faeces	460	0·22	0·02	9
Pagurus annulipes ingesting	750	0·32	0·53	166
Crassostrea virginica faeces	910	0·34	0·61	179
Fundulus majalis ingesting	2050	1·58	0·49	31
Crassostrea virginica faeces	2410	1·81	0·39	22
Fundulus majalis ingesting	1110	0·88	0·17	19
Penaeus setiferus faeces	1410	1·11	0·23	21
Glycera dibranchiata ingesting	700	0·11	0·28	256
Mugil cephalus faeces	550	0·07	0·08	114
Glycera dibranchiata ingesting	500	0·07	0·06	86
Penaeus setiferus faeces	210	0·04	0·02	50
Nereis succinea ingesting	290	0·05	0·008	16
Crassostrea virginica faeces	90	0·02	0·02	100

Table 3-49—*Continued*

Nutritional relationships	Live animal weight (mg)	Estimated metabolic rate of test animals (ml O_2 hr^{-1})	Calculated metabolic rate supportable by metabolism of ingested faecal organic matter (ml O_2 hr^{-1})	Calculated metabolic energy percentage supported by ingested faeces
Hydrobiid spp. ingesting	12	0·007	0·003	43
hydrobiid spp. faeces	8	0·004	0·008	20
Callinectes sapidus ingesting *Mugil cephalus* faeces	6020	1·35	0·35	26

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Literature Cited (Chapter 3)

ABOLINS-KROGIS, A. (1960). The histochemistry of the hepatopancreas of *Helix pomatia* (L) in relation to regeneration of the shell. *Ark. Zool.*, **13**, 159–201.

ADAMS, J. A. and STEELE, J. H. (1966). Shipboard experiments on feeding of *Calanus finmarchicus* (GUNNERUS). In H. Barnes (Ed.), *Some Contemporary Studies in Marine Science.* Allen and Unwin, London. pp. 19–35.

ADIYODI, K. G. and ADIYODI, R. G. (1974). Comparative physiology of reproduction in arthropods. In O. Lowenstein (Ed.), *Advances in Comparative Physiology and Biochemistry*, Vol. V. Academic Press, London. (In press.)

ADLER, J. (1966). Chemotaxis in bacteria. *Science, N.Y.*, **153**, 709–716.

ADLER, J. (1969). Chemoreceptors in bacteria. *Science, N.Y.*, **166**, 1588–1597.

AIKAWA, T. (1959). Deamination of adenosine and adenylic acids in the clam *Meretrix meretrix lusoria*. *Sci. Rep. Tôhoku Univ.* (*Ser.* 4), **25**, 73–80.

AIKAWA, T. (1966). Adenosine aminohydrolase from the clam, *Meretrix meretrix lusoria* (GMELIN). *Comp. Biochem. Physiol.*, **17**, 271–284.

ALDERDICE, D. F. (1972). Factor combinations: Responses of marine poikilotherms to environmental factors acting in concert. In O. Kinne (Ed.), *Marine Ecology*, Vol. I Environmental Factors, Part 3. Wiley, London. pp. 1659–1722.

AL-HUSSAINI, A. H. (1949). On the functional morphology of alimentary tract of some fish in relation to differences in their feeding habits: Cytology and physiology. *Q. Jl microsc. Sci.*, **90**, 323–354.

ALI, A. M. (1970). The influence of suspension density and temperature on the filtration rate of *Hiatella arctica*. *Mar. Biol.*, **6**, 291–302.

ALLEN, J. A. (1958). On the basis, forms and adaptations to habitat in the *Lucinaces* (Eulamellibranchia). *Phil. Trans. R. Soc.*, **241**, 421–484.

ALLEN, J. A. and GARRETT, M. R. (1971a). The excretion of ammonia and urea by *Mya arenaria* L. (Mollusca: Bivalvia). *Comp. Biochem. Physiol.*, **39A**, 633–642.

ALLEN, J. A. and GARRETT, M. R. (1971b). Taurine in marine invertebrates. *Adv. mar. Biol.*, **9**, 205–253.

ALLEN, J. A. and GARRETT, M. R. (1972). Studies on taurine in the euryhaline bivalve *Mya arenaria*. *Comp. Biochem. Physiol.*, **41A**, 307–316.

ALLEN, K. and AWAPARA, J. (1960). Metabolism of sulphur amino acids in *Mytilus edulis* and *Rangia cuneata*. *Biol. Bull. mar. biol. Lab., Woods Hole*, **118**, 173–182.

ALTMAN, M. and ROBIN, E. D. (1969). Survival during prolonged anaerobiosis as a function of an unusual adaptation involving lactate dehydrogenase subunits. *Comp. Biochem. Physiol.*, **30**, 1179–1187.

ANDERSON, J. M. (1953). Structure and function in the pyloric caeca of *Asterias forbesi*. *Biol. Bull. mar. biol. Lab., Woods Hole*, **105**, 47–67.

ANDERSON, J. M. (1954). Studies on the cardiac stomach of the starfish *Asterias forbesi*. *Biol. Bull. mar. biol. Lab., Woods Hole*, **107**, 157–173.

ANDERSON, J. M. (1960). Histological studies on the digestive system of a starfish *Henricia*, with notes on Tiedemann's pouches in starfishes. *Biol. Bull. mar. biol. Lab., Woods Hole*, **119**, 371–398.

ANDERSON, J. W. (1969). The analysis of the apparent uptake of glycine by crustaceans. Ph.D. thesis, University of California, Irvine.

ANDERSON, J. W. and STEPHENS, G. C. (1969). Uptake of organic material by aquatic invertebrates. VI. Role of epiflora in apparent uptake of glycine by marine crustaceans. *Mar. Biol.*, **4**, 243–249.

ANDREWS, P. and WILLIAMS, P. J. L. (1971). Heterotrophic utilization of dissolved organic compounds in the sea. III. Measurement of the oxidation rates and concentrations of glucose and amino acids in sea water. *J. mar. biol. Ass. U.K.*, **51**, 111–125.

ANDREWS, T. R. and REID, R. G. B. (1972). Ornithine cycle and uricolytic enzymes in four bivalve molluscs. *Comp. Biochem. Physiol.*, **42B**, 475–491.

ANKEL, W. E. (1936). Die Fraßspuren von *Helcion* und *Littorina* und die Funktion der Radula. *Zool. Anz.*, **9** (Suppl.), 174–182.

ANSELL, A. D. (1961). The functional morphology of the British species of *Veneracea* (Eulamellibranchia). *J. mar. biol. Ass. U.K.*, **41**, 489–515.

APELT, G. (1969). Die Symbiose zwischen dem acoelen Turbellar *Convoluta convoluta* und Diatomeen der Gattung *Licmophora*. *Mar. Biol.*, **3**, 165–187.

ARAI, M. N. and WALDER, G. L. (1973). The feeding response of *Pachycerianthus fimbriatus* (Ceriantharia). *Comp. Biochem. Physiol.*, **44A**, 1085–1092.

ARAKI, G. S. (1965). On the physiology of feeding and digestion in the sea star *Patiria miniata*. *Diss. Abstr.*, **23**, 4306.

ARAKI, G. S. and GIESE, A. C. (1970). Carbohydrases in sea stars. *Physiol. Zoöl.*, **43**, 298–305.

ARNOLD, G. (1909). Intracellular and general digestive processes in Planaria. *Q. Jl microsc. Sci.*, **54**, 207–220.

ARVY, L. (1969). Les enzymes chez les crustaces. *Année Biol.*, **8**, 505–580.

ARVY, L. and GABE, M. (1952). Particularités histochimiques du tube digestif de *Phascolion strombi* MONTAGU. *Bull. Lab. marit. Dinard*, **36**, 24–31.

ATKINS, D. (1936). On the ciliary mechanisms and interrelationships of lamellibranchs. I. Some new observations on sorting mechanisms in certain lamellibranchs. *Q. Jl microsc. Sci.*, **79**, 181–308.

ATKINS, D. (1937a). On the ciliary mechanisms and interrelationships of lamellibranchs. II. Sorting devices on the gills. *Q. Jl microsc. Sci.*, **79**, 339–373.

ATKINS, D. (1937b). On the ciliary mechanisms and interrelationships of lamellibranchs. III. Types of lamellibranch gills and their food currents. *Q. Jl microsc. Sci.*, **79**, 375–421.

ATKINS, D. (1938). On the ciliary mechanisms and interrelationships of lamellibranchs. VII. Laterofrontal cilia of the gill filaments and their phylogenetic value. *Q. Jl microsc. Sci.*, **80**, 345–436.

ATODA, K. (1953). The larval and post larval development of the reef building coral. *Sci. Rep. Tohoku Univ. (4th Ser.)*, **20**, 105–121.

AUTRUM, H. and GRAETZ, E. (1934). Vergleichende Untersuchungen zur Verdauungsphysiologie der Egel. I. Die lipatischen Fermente von *Hirudo* und *Haemopis*. *Z. vergl. Physiol.*, **21**, 429–439.

AWAPARA, J. and SIMPSON, J. W. (1967). Comparative physiology: metabolism. *A. Rev. Physiol.*, **29**, 87–112.

AX, P. and APELT, G. (1965). Die 'Zooxanthellae' von *Convoluta convoluta* (Turbellaria: Acoela) entstehen aus Diatomeen. Erster Nachweis einer Endosymbiose zwischen Tieren und Kieselalgen. *Naturwissenschaften*, **15**, 444–446.

BAKER, J. R. (1951). Fat absorption by intestinal epithelium of mouse. *J. Micr. Sci.*, **92**, 79–86.

BAKUS, G. J. (1969). Energetics and feeding in shallow marine waters. *Int. Rev. gen. exp. Zool.*, **4**, 275–369.

BALDWIN, E. (1958). Ureogenesis in elasmobranchs. *Nature, Lond.*, **181**, 1591–1592.

BALDWIN, E. (1960). Ureogenesis in elasmobranch fishes. *Comp. Biochem. Physiol.*, **1**, 24–37.

BALDWIN, E. (1963). *Dynamic Aspects of Biochemistry*, 4th ed., Cambridge University Press, London.

BALDWIN, E. (1964). *An Introduction to Biochemistry*, 4th ed., Cambridge University Press, London.

BALLANTINE, D. and MORTON, J. E. (1956). Filtering, feeding, digestion in the lamellibranch *Lasaea rubra*. *J. mar. biol. Ass. U.K.*, **35**, 241–274.

BAMFORD, D. R. and JAMES, D. (1972). An *in vitro* study of amino acid and sugar absorption in the gut of *Echinus esculentus*. *Comp. Biochem. Physiol.*, **42A**, 579–590.

BAMFORD, D. R. and STEWART, M. (1973a). Absorption of neutral amino acids by the gut of *Arenicola marina*. *J. comp. Physiol.*, **82**, 291–304.

BAMFORD, D. R. and STEWART, M. (1973b). Absorption of charged amino acids by the intestine of *Arenicola marina*. *Comp. Biochem. Physiol.*, **46A**, 537–547.

BAMFORD, D. R., WEST, B. and JEAL, F. (1972). An *in vitro* study of monosaccharide absorption in echinoid gut. *Comp. Biochem. Physiol.*, **42A**, 591–600.

BANNER, A. (1968). Attraction of young lemon sharks *Negaprion brevirostris* by sound. *Copeia*, **1968**, 871–872.

BARDACH, J. E., TODD, J. H. and CRICKMER, R. (1967). Orientation by taste in fish of the genus *Ictalurus*. *Science, N.Y.*, **155**, 1276–1278.

BARNES, H., FINLAYSON, D. M. and PIATIGORSKY, J. (1963). The effect of desiccation and anaerobic conditions on the behaviour, survival and general metabolism of three common cirripedes. *J. Anim. Ecol.*, **32**, 233–252.

BARRINGTON, E. J. W. (1957). The alimentary canal and digestion. In M. E. Brown (Ed.), *The Physiology of Fishes*, Vol. I. Academic Press, New York. pp. 109–161.

BARRINGTON, E. J. W. (1969). *Invertebrate Structure and Function*, The English Language Book Society and Nelson, Crawley, Sussex.

BATTLE, H. T., HUNTSMAN, A. G., JEFFERS, A. M., JEFFERS, G. W., JOHNSON, W. H. and MCNAIRN, N. A. (1936). Fatness, digestion and food of passamquoddy young herring. *J. biol. Bd Can.*, **2**, 401–429.

BAYLISS, W. M. and STARLING, E. H. (1903). On the uniformity of the pancreatic mechanism in vertebrates. *J. Physiol., Lond.*, **29**, 174–180.

BAYNE, B. L. (1965). Growth and the delay of metamorphosis of larvae of *Mytilus edulis* (L.). *Ophelia*, **2**, 1–47.

BAYNE, B. (1973). The responses of three species of bivalve mollusc to declining oxygen tension at reduced salinity. *Comp. Biochem. Physiol.*, **45A**, 793–806.

BECK, S. D. (1965). Resistance of plants to insects. *A. Rev. Ent.*, **10**, 207–232.

BEECHER-MOORE, B. M. (1959). The functional morphology of the alimentary canal of *Idotea emarginata*, with some references to its affinities. Ph.D. Thesis, University of London.

BEKLEMISHEV, C. W. (1962). Superfluous feeding of marine herbivorous zooplankton. *J. Cons. perm. int. Explor. Mer*, **26**, 94–116.

BELL, W. and MITCHELL, R. (1972). Chemotactic and growth responses of marine bacteria to algal extracellular products. *Biol. Bull. mar. biol. Lab., Woods Hole*, **143**, 265–277.

BENNETT, R. and NAKADA, H. I. (1968). Comparative carbohydrate metabolism of marine molluscs. I. The intermediary metabolism of *Mytilus californianus* and *Haliotis rufescens*. *Comp. Biochem. Physiol.*, **24**, 787–797.

BENOIT, G. J. and NORRIS, R. P. (1945). Studies on trimethylamine oxide. II. The origin of trimethylamine oxide in young salmon. *J. biol. Chem.*, **158**, 439–442.

BERG, T. and STEEN, J. B. (1965). Physiological mechanisms for aerial respiration in the eel. *Comp. Biochem. Physiol.*, **15**, 469–484.

BERKELEY, C. (1959). Some observations on *Cristispira* in the crystalline style of *Saxidomus giganteus* DESHAYES and in that of some other Lamellibranchiata. *Can. J. Zool.*, **37**, 53–58.

BERRILL, N. J. (1929). Digestion in ascidians and the influence of temperature. *J. exp. Biol.*, **6**, 275–292.

BEUKEMA, J. J. (1968). Predation by three-spined stickleback (*Gasterosteus aculeatus* L.). The influence of hunger and experience. *Behaviour*, **31**, 1–126.

BEVELANDER, G. and NAKAHARA, H. (1966). Correlation of lysosomal activity and ingestion by the mantle epithelium. *Biol. Bull. mar. biol. Lab., Woods Hole*, **131**, 76–82.

BIDDER, A. M. (1966). Feeding and digestion in cephalopods. In K. M. Wilbur and C. M. Yonge (Eds), *Physiology of Mollusca*, Vol. II. Academic Press, New York. pp. 97–124.

BIDDER, G. P. (1923). The relation of the form of a sponge to its currents. *Q. Jl microsc. Sci.*, **67**, 293–323.

BILINSKI, E. (1960). Biosynthesis of trimethylammonium compounds in aquatic animals. I. Formation of trimethylamine oxide and betaine from ^{14}C-labelled compounds by lobster (*Homarus americanus*). *J. Fish. Res. Bd Can.*, **17**, 895–902.

BILINSKI, E. (1964). Biosynthesis of trimethylamine compounds in aquatic animals. IV. Precursors of trimethylamine oxide and betaine in marine teleosts. *J. Fish. Res. Bd Can.*, **21**, 765–771.

BINNS, R. (1969a). The physiology of antennal gland of *Carcinus maenas* (L.). II. Urine

production rates. *J. exp. Biol.*, **51**, 11–16.

BINNS, R. (1969b). The physiology of antennal gland of *Carcinus maenas*. V. Some nitrogenous constituents in the blood and urine. *J. exp. Biol.*, **51**, 41–45.

BLACK, V. (1957). Excretion and osmoregulation, In M. E. Brown (Ed.), *The Physiology of Fishes*, Vol. I. Academic Press, New York. pp. 163–205.

BLACK, W. A. P. and MITCHELL, R. L. (1952). Trace elements in the common brown algae and in sea water. *J. mar. biol. Ass. U.K.*, **30**, 575–584.

BLAKE, J. R. (1971). A spherical envelope approach to ciliary propulsion. *J. Fluid Mech.*, **46**, 199–208.

BLAKE, J. W. (1960). Oxygen consumption of bivalve prey and their attractiveness to the gastropod *Urosalpinx cinerea*. *Limnol. Oceanogr.*, **5**, 273–280.

BLANQUET, R. S. and LENHOFF, H. M. (1968). Tryosine enteroreceptor of *Hydra*: Its function in eliciting a behaviour modification. *Science, N.Y.*, **159**, 633–634.

BLIX, A. S. and FROM, S. H. J. (1971). Lactate dehydrogenase in diving animals—a comparative study with special reference to the eider. *Comp. Biochem. Physiol.*, **40B**, 579–584.

BOJE, R. (1965). Die Bedeutung von Nahrungsfaktoren für das Wachstum von *Mytilus edulis L.* in der Kieler Förde und im Nord-Ostsee-Kanal. *Kieler Meeresforsch.*, **21**, 81–100.

BOJE, R. (1966). Protein. *Limnologica*, **4**, 383–386.

BOND, R. M. (1934). Digestive enzymes of the pelagic copepod *Calanus finmarchicus*. *Biol. Bull. mar. biol. Lab., Woods Hole*, **67**, 461–465.

BONOTTO, S. (in press). Cultivation of plants: Multicellular plants. In O. Kinne (Ed.), *Marine Ecology*, Vol. III, Cultivation. Wiley, London.

BOOTHE, P. N. and KNAUER, G. A. (1972). The possible importance of fecal material in the biological amplification of trace and heavy metals. *Limnol. Oceanogr.*, **17**, 270–274.

BOUCHER-RODONI, R. (1973). Vitesse de digestion d'*Octopus cyanea* (Cephalopoda: Octopoda). *Mar. Biol.*, **18**, 237–242.

BOUCHET, C. (1961). Le contrôle de la discharge nematocystique chez phydre. *C.r. hebd. Séanc. Acad. Sci., Paris*, **252**, 327–328.

BOULESTEIX, R. (1964). Sur la presence de l'uricase chez les crustaces. *C.r. hebd. Séanc. Acad. Sci., Paris*, **258**, 3098–3100.

BOULESTEIX, R. (1965). Sur la presence de l'allantoise de l'allantoicase et de l'urease chez les crustaces. *C.r. hebd. Séanc. Acad. Sci., Paris*, **260**, 1767–1768.

BRAFIELD, A. E. and CHAPMAN, G. (1965). The oxygen consumption of *Pennatula rubra* ELLIS and some other anthozoans. *Z. vergl. Physiol.*, **50**, 363–370.

BRAND, T. VON (1946). Anaerobiosis in invertebrates. *Biodynamica*, **4**, 1–328.

BRAND, T. VON (1966). *Biochemistry of Parasites*, Academic Press, New York.

BRAUNSTEIN, A. E. (1951). Transamination and the integrative functions of the dicarboxylic acids in nitrogen metabolism. *Adv. Protein Chem.*, **3**, 1–526.

BRETT, J. R. (1971a). Satiation time, appetite, and maximum food intake of sockeye salmon (*Oncorhynchus nerka*). *J. Fish. Res. Bd Can.*, **28**, 409–425.

BRETT, J. R. (1971b). Growth responses of young sockeye salmon (*Oncorhynchus nerka*) to different diets and planes of nutrition. *J. Fish. Res. Bd Can.*, **28**, 1635–1643.

BRETT, J. R. (1971c). Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerka*). *Am. Zool.*, **11**, 99–113.

BRETT, J. R. and HIGGS, D. A. (1970). Effect of temperature on the rate of gastric digestion in fingerling sockeye salmon, *Oncorhynchus nerka*. *J. Fish. Res. Bd Can.*, **27**, 1767—1779.

BRETT, J. R., SHELBOURN, J. E. and SHOOP, C. T. (1969). Growth rate and body composition of fingerling sockeye salmon, *Oncorhynchus nerka* in relation to temperature and ration size. *J. Fish. Res. Bd Can.*, **26**, 2363–2394.

BROCK, F. (1936). Suche, Aufnahme und enzymatische Spaltung der Nahrung durch die Wallhornschnecke *Buccinum undatum* L. *Zoologica, Stuttg.*, **34**, 1–136.

BROCK, T. D. (1966). *Principles of Microbial Ecology*, Prentice Hall Inc., Englewood Cliffs, New Jersey.

BROCK, V. (1954). A preliminary report on a method of establishing reef fish production. *J. Wildl. Mgmt.*, **18**, 297–308.

BRODY, S. (1968). *Bioenergetics and Growth*, Hafner Publishing Company, New York.

BROWN, A. C. (1961). Chemoreception in the sandy beach snail *Bullia*. *S. Afr. J. Lab. clin. Med.*, **7**, 160.

BROWN, A. C. and BRENGELMANN, G. (1966). Energy metabolism. In T. C. Ruch and M. D. Patton (Eds), *Physiology and Biophysics*. W. B. Saunders, Philadelphia. pp. 1030–1047.

BROWN, G. W., JR. (1964). Urea synthesis in elasmobranchs. In C. A. Leone (Ed.), *Taxonomic Biochemistry and Serology*. Ronald Press, New York. pp. 467.

BROWN, G. W., JR. and BROWN, S. G. (1967). Urea and its formation in coelacanth liver. *Science, N.Y.*, **155**, 570–573.

BROWN, G. W., JR. and COHEN, P. P. (1959). Comparative biochemistry of urea synthesis. I. Methods for the quantitative assay for urea cycle enzymes in liver. *J. biol. Chem.*, **234**, 1769–1777.

BROWN, G. W., JR. and COHEN, P. P. (1960). Activities of urea cycle enzymes in various higher and lower vertebrates. *Biochem. J.*, **75**, 82–91.

BROWN, S. C. (1969). The structure and function of the digestive system of the mud snail *Nassarius obsoletus* (SAY). *Malacologia*, **9**, 447–500.

BROWN, S. C. and WHITEGIVER, E. R. (1971). Cell fractionation studies on the digestive gland of *Limulus polyphemus* L. *Comp. Biochem. Physiol.*, **40B**, 637–650.

BRUNEL, A. (1937a). Catabolisme de l'azote d'origine purique chez les Selaciens. *Bull. Soc. Chim. biol.*, **19**, 805–826.

BRUNEL, A. (1937b). Catabolisme de l'azote d'origine purique chez Teleostéens. *Bull. Soc. Chim. biol.*, **19**, 1027–1036.

BRUNEL, A. (1938). Sur la degradation des substances d'origine purique chez les Mollusques Lamellibranchs. *C.r. hebd. Séanc. Acad. Sci., Paris*, **206**, 858–860.

BRUNI, C. B., AURICCHIO, F. and COVELLI, I. (1969). Acid α-D-glucosidase glucohydrolase from cattle liver. *J. biol. Chem.*, **244**, 4735–4742.

BUEDING, E. (1962). Comparative aspects of carbohydrate metabolism. *Fedn Proc. Fedn Am. Socs exp. Biol.*, **21**, 1039–1046.

BÜSING, K. H. (1951). *Pseudomonas hirudinis*, ein bakterieller Darmsymbiont des Blutegels (*Hirudo officinalis*). *Zentbl. Bakt. Parasitkde*, **157**, 478–484.

BÜSING, K. H., DOLL, W. and FREYTAG, K. (1953). Die Bakterienflora der medizinischen Blutegel. *Arch. Microbiol.*, **19**, 52–86.

BULLIVANT, J. S. (1968). The rate of feeding of the bryozoan *Zoobotryon verticillatum*. *N.Z. Jl mar. Freshwat. Res.*, **2**, 111–134.

BULLOCK, G. L. (1961). The identification and separation of *Aeromonas liquefaciens* from *Pseudomonas fluorescens* and related organisms occurring in diseased fish. *Appl. Microbiol.*, **9**, 587–590.

BURGER, J. W. (1965). Roles of rectal gland and the kidneys in salt and water excretion in the spiny dogfish. *Physiol. Zool.*, **38**, 191–196.

BURGER, J. W. (1967). Problems in the electrolyte economy of the spiny dogfish *Squalus acanthias*. In P. W. Gilbert, R. F. Mathewson, and D. P. Rall (Eds), *Sharks, Skates and Rays*. John Hopkins Press, Baltimore. pp. 177–185.

BURNETT, A. L., LENTZ, T. and WARREN, M. (1960). The nematocysts of *Hydra*. I. The question of control of the nematocyst discharge reaction by fully-fed *Hydra*. *Annls Soc. r. zool. malacol. Belg.*, **90**, 247–267.

BURNSTOCK, G. (1959). The morphology of the gut of the brown trout (*Salmo trutta*). *Q. Jl microsc. Sci.*, **100**, 183–198.

CAREFOOT, T. H. (1967a). Growth and nutrition of *Aplysia punctata* feeding on a variety of marine algae. *J. mar. biol. Ass. U.K.*, **47**, 565–589.

CAREFOOT, T. H. (1967b). Growth and nutrition of three species of opisthobranch molluscs. *Comp. Biochem. Physiol.*, **21**, 627–652.

CAREFOOT, T. H. (1970). A comparison of absorption and utilization of food energy in two species of tropical *Aplysia*. *J. exp. mar. Biol. Ecol.*, **5**, 47–62.

CAREFOOT, T. H. (1973). Feeding, food preference and the uptake of food energy by the supratidal isopod *Ligia pallasii*. *Mar. Biol.*, **18**, 228–236.

CARLISKY, N. J. and HUANG, K. C. (1962). Glucose transport by the intestinal mucosa of the dogfish. *Proc. Soc. exp. Biol. Med.*, **109**, 405–408.

CARRIKER, M. R. (1943). On the structure and function of the proboscis in the common oyster drill, *Urosalpinx cinerea*. *J. Morph.*, **73**, 441–506.

CARRIKER, M. R. (1955). Critical review of biology and control of oysters drill *Urosalpinx* and *Eupleura*. *Spec. Scient. Rep., U.S. Fish Wild. Serv., Fisheries*, **148**, 1–150.

CARRIKER, M. R. (1958). Additional information on the mechanical-chemical nature of drilling mechanism in *Urosalpinx* and *Eupleura* (muricid gastropods). *Bull. Ass. S-East. Biol.*, **5**, 5.

CARRIKER, M. R. (1959). Comparative functional morphology of the drilling mechanism in *Urosalpinx* and *Eupleura* (muricid gastropods). *Int. Congr. Zool.*, **15**, 373–376.

CARRIKER, M. R. (1961). Comparative functional morphology and boring mechanism in gastropods. *Am. Zool.*, **1**, 263–266.

CARTHY, J. D. (1958). *An Introduction to the Behaviour of Invertebrates*, Allen and Unwin, London.

CASE, J. (1964). Properties of the dactyl chemoreceptors of *Cancer antennuarus* STIMPSON and *C. productus* RANDALL. *Biol. Bull. mar. biol. Lab., Woods Hole*, **127**, 428–446.

CASE, J. and GWILLIAM, G. F. (1963). Amino acid detection by marine invertebrates. *Int. Congr. Zool.*, **16**, 75.

CERNICHIARI, E., MUSCATINE, L. and SMITH, D. C. (1969). Maltose excretion by the symbiotic algae of *Hydra viridis*. *Proc. R. Soc.*, **173**, 557–576.

CHAO, L. N. (1972). A study of the digestive system and feeding habits of a stomachless fish *Tautagolabrus adspersus*. M.S. thesis, Northeastern University, Boston.

CHAPMAN, G. and TAYLOR, A. G. (1968). Uptake of organic solutes by *Nereis virens*. *Nature, Lond.*, **217**, 763–764.

CHAPMAN-ANDRESEN, C. (1958). Pinocytosis of inorganic salts by *Amoeba proteus* (*Chaos diffluens*). *C.r. Trav. Lab., Carlsberg* (*Ser. chim.*), **31**, 77–92.

CHAPMAN-ANDRESEN, C. and PRESCOTT, D. M. (1956). Studies on pinocytosis in the amoebae *Chaos chaos* and *Amoeba proteus*. *C.r. Trav. Lab., Carlsberg* (*Ser. chim.*), **30**, 57–58.

CHAU, Y. K. and RILEY, J. P. (1966). The determination of amino acids in sea water. *Deep Sea Res.*, **13**, 1115–1124.

CHEN, C. and AWAPARA, J. (1969a). Intracellular distribution of enzymes catalyzing succinate production from glucose in *Rangia* mantle. *Comp. Biochem. Physiol.*, **30**, 727–737.

CHEN, C. and AWAPARA, J. (1969b). Effect of oxygen on the end-products of glycolysis in *Rangia cuneata*. *Comp. Biochem. Physiol.*, **31**, 395–401.

CHENG, S. and WAELSCH, H. (1962). Carbon dioxide fixation in lobster nerve. *Science, N.Y.*, **136**, 782–783.

CHESLEY, L. C. (1934). The concentration of proteases, amylase and lipase in certain marine fishes. *Biol. Bull. mar. biol. Lab., Woods Hole*, **66**, 133–144.

CHIPMAN, W. A. and HOPKINS, J. G. (1954). Water filtration by the bay scallop, *Pecten irradians*, as observed with the use of radioactive plankton. *Biol. Bull. mar. biol. Lab., Woods Hole*, **107**, 80–91.

CHUANG, S. H. (1963). Digestive enzymes of the echiuroid *Ochetostoma erythrogrammon*. *Biol. Bull. mar. biol. Lab., Woods Hole*, **125**, 464–469.

CLEGG, J. A. and SMYTH, S. D. (1968). Growth, development and culture methods: Parasitic platyhelminths. In M. Florkin and B. T. Scheer (Eds), *Chemical Zoology*, Vol. II. Academic Press, London. pp. 395–466.

COCKSON, A. and BOURNE, D. (1972). Enzymes in the digestive tract of two species of euryhaline fish. *Comp. Biochem. Physiol.*, **41A**, 715–718.

COHEN, P. P. and BROWN, G. W., JR. (1960). Ammonia metabolism and urea biosynthesis. *Comp. Biochem. Physiol.*, **2**, 161–244.

COLES, G. C. (1970). Some biochemical adaptations of the swamp worm *Alona emini* to low oxygen levels in tropical swamps. *Comp. Biochem. Physiol.*, **34**, 481–489.

COLTON, H. S. (1908). How *Fulgur* and *Sycotypus* eat oysters, mussels and clams. *Proc. Acad. nat. Sci. Philad.*, **60**, 3–10.

CONOVER, R. J. (1956). Oceanography of Long Island Sound 1952–54. V. Biology of *Acartia clausi* and *A. lonsa*. *Bull. Bingham oceanogr. Coll.*, **15**, 156–233.

CONOVER, R. J. (1964). Food relations and nutrition of zooplankton. *Occ. Publ. Narragansett mar. Lab.*, **2**, 81–91.

CONOVER, R. J. (1966a). Assimilation of organic matter by zooplankton. *Limnol. Oceanogr.*, **11**, 338–345.

CONOVER, R. J. (1966b). Factors affecting the assimilation of organic matter by zooplankton and the question of superfluous feeding. *Limnol. Oceanogr.*, **11**, 346–354.

CONOVER, R. J. (1970). Cultivation of plankton populations. (Convener's report.) *Helgoländer wiss. Meeresunters.*, **21**, 401–444.

CONOVER, R. J. (in press). Transformation of organic matter. In O. Kinne (Ed.), *Marine Ecology*, Vol. IV, Dynamics. Wiley, London.

CONOVER, R. J. and LALLI, C. M. (1972). Feeding and growth in *Clione limacina* (PHIPPS), a pteropod mollusc. *J. exp. mar. Biol. Ecol.*, **9**, 279–302.

CONTE, F. P. (1969). Salt secretion. In W. S. Hoar and D. J. Randall (Eds), *Fish Physiology*, Vol. I. Academic Press, New York. pp. 241–292.

CORDIER, D. and CHANEL, J. (1953). Influence de la tension d'oxygene sur l'absorption intestinale des solutions de pentoses et de pentoses et d'hexoses chez la Rascasse (*Scorpäena porcus* L.). *J. Physiol., Paris.*, **45**, 91–93.

CORDIER, D., MAURICE, A. and CHANEL, J. (1954). Influence de la temperature sur l'absorption intestinale des oses chez la Tanche (*Tinca vulgaris*). *C.r. Séanc. Soc. Biol.*, **148**, 1417–1418.

CORNER, E. D. S. and COWEY, C. B. (1964). Some nitrogenous constituents of the plankton. *Oceanogr. mar. Biol. A. Rev.*, **2**, 147–167.

CORNER, E. D. S. and DAVIES, A. G. (1971). Plankton as a factor in the nitrogen and phosphorus cycles in the sea. *Adv. mar. Biol.*, **9**, 101–204.

CRANE, R. K., MILLER, D. and BIHLER, I. (1961). The restrictions on possible mechanisms of intestinal active transport of sugars. In A. Kleinzeller and A. Kotyk (Eds), *Symposium on Membrane Transport and Metabolism*. Czechoslovak Academy of Science, Prague. pp. 439–449.

CRISP, D. J. (1964). An assessment of plankton grazing by barnacles. In D. J. Crisp (Ed.), *Grazing in Terrestrial and Marine Environments*, Blackwell Scientific Publications, Oxford. pp. 251–264.

CRISP, D. J. and SOUTHWARD, A. J. (1961). Different types of cirral activity in barnacles. *Phil. Trans. R. Soc.*, **243**, 271–318.

CROSBY, N. D. and REID, R. G. B. (1971). Relationships between food phylogeny, and cellulose digestion in the Bivalvia. *Can. J. Zool.*, **49**, 617–622.

CROZIER, W. J. (1918). The orientation of a holothurian by night. *Am. J. Physiol.*, **36**, 8–20.

CUMMINS, K. W. and WUYCHECK, J. C. (1971). Caloric equivalents for investigations in ecological energetics. *Mitt. int. Verein. theor. angew. Limnol.*, **18**, 1–158.

CUSHING, D. H. (1964). The work of grazing in the sea. In D. J. Crisp (Ed.), *Grazing in Terrestrial and Marine Environments*. Blackwell Scientific Publications, Oxford. pp. 207–225.

D'AGOSTINO, A. S. and FARMANFARMAIAN, A. (1960). Transport of nutrients in the holothurian *Leptosynapta inhaerens*. *Biol. Bull. mar. biol. Lab., Woods Hole*, **119**, 301.

DALES, R. P. (1969). Respiration and energy metabolism in annelids. In M. Florkin and B. T. Scheer (Eds), *Chemical Zoology*, Vol. IV. Academic Press, New York. pp. 93–109.

DANIELLI, J. F. (1954). The present position in the field of facilitated diffusion and selective active transport. In J. A. Kitching (Ed.), *Recent Developments in Cell Physiology*. Butterworth, London.

DARNELL, R. M. (1958). Food habits of fishes and larger invertebrates of Lake Pontchartrain, Louisiana, an estuarine community. *Publs Inst. mar. Sci. Univ. Texas*, **5**, 353–416.

DAVENPORT, D., ROSS, D. M. and SUTTON, L. (1961). The remote control of nematocyst discharge in the attachment of *Calliactis parasitica* to the shells of hermit crabs. *Vie Milieu*, **12**, 197–209.

DAVENPORT, W. W. (1969). *Physiology of the Digestive Tract*, Yearbook Medical Publishers, Chicago.

DAVIDS, C. (1964). The influence of suspensions of microorganisms of different concentrations on the pumping and retention of food by the mussel (*Mytilus eludis* L.). *Neth. J. Sea Res.*, **2**, 233–249.

DAVIES, P. M. C. (1963). Food input and energy extraction efficiency in *Carassius auratus*. *Nature, Lond.*, **198**, 707.

DAVIES, P. M. C. (1964). The energy relations of *Carassius auratus* L. I. Food input and

extraction efficiency at two experimental temperatures. *Comp. Biochem. Physiol.*, **12**, 67–80.

DEAN, D. (1958). New property of the crystalline style of *Crassostrea virginica*. *Science, N.Y.*, **128**, 837.

DEGENS, E. T. (1968). Molecular composition of nitrogenous compounds in sea water and recent marine sediments. *Woods Hole, Oceanogr Inst. Ref.* 6–52, *Woods Hole, Mass.*, 27 pp.

DELAUNAY, H. (1927). Recherches biochimiques sur l'excrétion azotée des Invertébrés. *Bull. Stn biol. Arcachon*, **24**, 95–214.

DELAUNAY, H. (1931). L'excretion azotée des invertebrés. *Biol. Rev.*, **6**, 265–301.

DELAUNAY, H. (1934). Le metabolisme de l'ammoniaque d'après les recherches relatives aux invertebrés. *Annls Physiol. Physicochim. biol.*, **10**, 695–724.

DESCHAMPS, P. (1953). Recherche de la cellulase chez *Teredo navalis* au moyen de la chromatographie de partage. *Bull. Soc. zool. Fr.*, **78**, 174 177.

DETHIER, V. G., BROWNE, L. and SMITH, C. N. (1960). The designation of chemicals in terms of responses they elicit from insects. *J. econ. Ent.*, **53**, 134–136.

DINAMANI, P. (1957). On the stomach and associated structures in the backwater clam *Villorita cyprinoides* (GRAY) var. *Cochinensis* (HANLEY). *Bull. cent. Res. Inst. Univ. Kerala*, **5**, 123–148.

DINGLE, J. and FELL, H. (1969). *Lysosomes in Biology and Pathology*, Vols I and II, Elsevier, New York.

DIXON, M. and WEBB, E. C. (1964). *Enzymes*, 2nd ed., Longmans, Green & Co., London.

DONCASTER, C. C. (1962). Nematode feeding mechanisms. I. Observations on *Rhabditis* and *Pelodera*. *Nematologica*, **8**, 313–320.

DOREY, A. E. (1965). The organization and replacement of the epidermis in acoelus turbellarians. *Q. Jl microsc. Sci.*, **106**, 147–172.

DRAL, A. D. G. (1967). The movement of the latero-frontal cilia and mechanisms of particle retention in the mussel (*Mytilus edulis* L.). *Neth. J. Sea Res.*, **3**, 391–422.

DRESEL, E. I. B. and MOYLE, V. (1950). Nitrogenous excretion in amphipods and isopods. *J. exp. Biol.*, **27**, 210–225.

DROOP, M. (1963). Algae and invertebrates in symbiosis. *Sym. Soc. gen. Microbiol.*, **13**, 171–199.

DUCHÂTEAU, G. and FLORKIN, M. (1955). Concentration du milieu extérieur et état stationarie du pool des acides aminés non protéiques des muscles d'*Eriocheir sinensis*, MILNE EDWARDS. *Archs int. Physiol. Biochim.*, **63**, 249–251.

DUERR, F. G. (1968). Excretion of ammonia and urea in seven species of marine prosobranch snails. *Comp. Biochem. Physiol.*, **26**, 1051–1059.

DUNSON, W. A. (1970). Some aspects of electrolyte and water balance in three estuarine reptiles, the diamondback terrapin, American and 'saltwater' crocodiles. *Comp. Biochem Physiol.*, **32**, 161–174.

DUURSMA, E. K. (1965). The dissolved organic constituents of sea water. In J. P. Riley and G. Skirrow (Eds), *Chemical Oceanography*, Vol. I. Academic Press, New York. pp. 433–475.

EDNEY, E. B. (1960). Terrestrial adaptations. In T. H. Waterman (Ed.), *The Physiology of Crustacea*, Vol. I. Academic Press, New York. pp. 367–393.

EIGENBRODT, H. (1941). Untersuchungen über die Funktion der Radula einiger Schnecken. *Z. Morph. Ökol. Tiere*, **37**, 735–791.

ELYAKOVA, L. A. (1972). Distribution of cellulases and chitinases in marine invertebrates. *Comp. Biochem. Physiol.*, **43B**, 67–70.

EMERSON, D. N. (1969). Influence of salinity on ammonia excretion rates and tissue constituents of euryhaline invertebrates. *Comp. Biochem. Physiol.*, **29**, 1115–1133.

EMERSON, D. N. and DUERR, F. G. (1967). Some physiological effects of starvation in the intertidal prosobranch *Littorina planaxis* (PHILLIPPI, 1847). *Comp. Biochem. Physiol.*, **20**, 45–53.

EMLEN, J. M. (1966). The role of time and energy in food preference. *Am. Nat.*, **100**, 611–617.

EPPLEY, R. W. and LASKER, R. (1959). Alginase in the sea urchin *Strongylocentrotus purpuratus*. *Science, N.Y.*, **129**, 214–215.

EVANS, D. (1967). Sodium, chloride and water balance of the intertidal teleost, *Xiphister atropurpureus*. II. The role of the kidney and the gut. *J. exp. Biol.*, **47**, 519–542.

EVANS, D. (1968). Measurement of drinking rates in fish. *Comp. Biochem. Physiol.*, **25**, 751–753.

EVANS, T. J. (1922). *Calma glaucoides*: a study in adaptation. *Q. Jl microsc. Sci.*, **66**, 439–455.

FÄNGE, R. (1957). An acetylcholine-like salivary poison in the marine gastropod *Neptunea antiqua*. *Nature, Lond.*, **180**, 196–197.

FAIRBRAIN, D. (1957). The biochemistry of *Ascaris*. *Expl Parasitol.*, **6**, 491–554.

FAIRBRAIN, D. (1970). Adaptation and loss of genetic capacity in helminth parasites. *Biol. Rev.*, **45**, 29–64.

FANKBONER, P. V. (1971). Intracellular digestion of symbiotic zooxanthellae by host amoebocytes in giant clams (Bivalvia: Tridacnidae), with a note on the nutritional role of the hypertrophied siphonal epidermis. *Biol. Bull. mar. biol. Lab., Woods Hole*, **141**, 222–234.

FARMANFARMAIAN, A. (1969a). Intestinal absorption and transport in *Thyone*. I. Biological aspects. *Biol. Bull. mar. biol. Lab., Woods Hole*, **137**, 118–131.

FARMANFARMAIAN, A. (1969b). Intestinal absorption and transport in *Thyone*. II. Observations on sugar transport. *Biol. Bull. mar. biol. Lab., Woods Hole*, **137**, 132–145.

FARMANFARMAIAN, A. and PHILLIPS, J. H. (1962). Digestion, storage and translocation of nutrients in the purple sea urchin *Strongylocentrotus purpuratus*. *Biol. Bull. mar. biol. Lab., Woods Hole*, **123**, 105–120.

FARMANFARMAIAN, A., ROSS, A. and MAZAL, D. (1972). *In vivo* intestinal absorption of sugar in the toad fish (marine teleost *Opsanus tau*). *Biol. Bull. mar. biol. Lab., Woods Hole*, **142**, 427–445.

FENCHEL, T. M. (1970). Studies on the decomposition of organic detritus derived from the turtle grass *Thalassia testudinum*. *Limnol. Oceanogr.*, **15**, 14–20.

FENCHEL, T. M. and RIEDL, R. J. (1970). The sulphide system: a new biotic community underneath the oxidized layer of marine sand bottoms. *Mar. Biol.*, **7**, 255–268.

FERGUSON, J. C. (1967a). Utilization of dissolved exogenous nutrients by the starfishes *Asterias forbesi* and *Henricia sanguinolenta*. *Biol. Bull. mar. biol. Lab., Woods Hole*, **132**, 161–173.

FERGUSON, J. C. (1967b). An autoradiographic study of the utilization of free exogenous amino acids by starfishes. *Biol. Bull. mar. biol. Lab., Woods Hole*, **133**, 317–329.

FERGUSON, J. C. (1969). Feeding, digestion, and nutrition in Echinodermata. In M. Florkin and B. T. Scheer (Eds), *Chemical Zoology*, Vol. III. Academic Press, New York, pp. 71–100.

FERGUSON, J. C. (1970). An autoradiographic study of the translocation and utilization of amino acids by starfish. *Biol. Bull. mar. biol. Lab., Woods Hole*, **138**, 14–25.

FERGUSON, J. C. (1971). Uptake and release of free amino acids by starfishes. *Biol. Bull. mar. biol. Lab., Woods Hole*, **141**, 122–129.

FIELD, J. G. (1973). Some observations on the release of dissolved organic carbon by the sea urchin *Strongylocentrotus dröbachiensis*. *Limnol. Oceanogr.*, **17**, 759–761.

FINCH, L. R. and HIRD, F. J. R. (1960). The uptake of amino acids by isolated segments of rat intestine. II. A survey of affinity for uptake from rates of uptake and competition for uptake. *Biochem. biophys. Acta*, **43**, 278–287.

FINGERMAN, M., DOMINICZAK, T., MIYAWAKI, M., OGURO, C. and YAMAMOTO, Y. (1967). Neuroendocrine control of hepatopancreas in the crayfish *Procambarus clarkii*. *Physiol. Zool.*, **40**, 23–30.

FISH, G. R. (1955). Digestion and the production of sulphuric acid by Mollusca. *Nature, Lond.*, **175**, 733–734.

FISH, G. R. (1960). The comparative activity of some digestive enzymes in the alimentary canal of *Tilapia* and perch. *Hydrobiologia*, **15**, 161–178.

FISH, J. D. (1967). The biology of *Cucumaria elongata* (Echinodermata: Holothuroidea). *J. mar. biol. Ass. U.K.*, **47**, 129–143.

FJERDINGSTAD, E. J. (1961). The ultrastructure of choanocyte collars in *Spongilla lacustris* (L.). *Z. Zellforsch. mikrosk. Anat.*, **53**, 645–657.

FLOREY, E. (1970). *Lehrbuch der Tierphysiologie*, Thieme, Stuttgart.

FLORKIN, M. (1966). Nitrogen metabolism. In K. M. Wilbur and C. M. Yonge (Eds), *Physiology of Mollusca*, Vol. II. Academic Press, New York. pp. 309–351.

FLORKIN, M. (1969). Nitrogen metabolism. In M. Florkin and B. T. Scheer (Eds), *Chemical Zoology*, Vol. IV. Academic Press, New York. pp. 147–162.

FLORKIN, M. and BRICTEUX-GRÉGOIRE, S. (1972). Nitrogen metabolism in molluscs. In M. Florkin and B. T. Scheer (Eds), *Chemical Zoology*, Vol. VII. Academic Press, New York.

pp. 301–348.

FLORKIN, M. and DUCHÂTEAU, G. (1943). Les formes du système enzymatique de l'uricolyse et l'evolution du catabolisme chez les animaux. *Archs int. Physiol.*, **53**, 267–307.

FLÜCHTER, J. and PANDIAN, T. J. (1968). Rate and efficiency of yolk utilization in the sole *Solea solea*. *Helgoländer wiss. Meeresunters.*, **18**, 53–60.

FOGEL, S., CHET, I. and MITCHELL, R. (1971). The ecological significance of bacterial chemotaxis. *Bact. Proc.*, **1971**, 28 (G31).

FOGG, G. E. (1966). The extracellular products of algae. *Oceanogr. mar. Biol. A. Rev.*, **4**, 195–212.

FOGG, G. E. (1972). Organic substances: Plants. In O. Kinne (Ed.), *Marine Ecology*, Vol. I, Environmental Factors, Part 3. Wiley, London. pp. 1551–1563.

FORREST, H. (1962). Lack of dependence of the feeding reactions in hydra on reduced glutathione. *Biol. Bull. mar. biol. Lab., Woods Hole*, **122**, 343–361.

FORREST, J. E. (1953). On the feeding habits and the morphology and mode of functioning of the alimentary canal in some littoral dorid nudibranchiate Mollusca. *Proc. Linn. Soc. Lond.*, **164**, 225–236.

FORSTER, R. P. (1967). Osmoregulatory role of the kidney in cartilagenous fishes (Chondrichthyes). In P. W. Gilbert, R. F. Mathewson and D. P. Rall (Eds), *Sharks, Skates and Rays*. John Hopkins Press, Baltimore. pp. 187–195.

FORSTER, R. P. and GOLDSTEIN, L. (1969). Formation of excretory products. In W. S. Hoar and D. J. Randall (Eds), *Fish Physiology*, Vol. I. Academic Press, New York. pp. 293–350.

FOWLER, S. W. and SMALL, L. F. (1972). Sinking rates of euphausid pellets. *Limnol. Oceanogr.*, **17**, 293–296.

FOX, A. M. (1965). Effect of inhibitors on active transport by turtle intestinal segments. *Biol. Bull. mar. biol. Lab., Woods Hole*, **129**, 490–494.

FOX, D. L., CRANE, S. E. and MCCONNAUGHEY, B. A. (1948). A biochemical study of the marine annelid worm *Thoracephala mucronata*. *J. mar. Res.*, **7**, 567–585.

FRANKENBERG, D., COLES, S. L. and JOHANNES, R. E. (1967). The potential trophic significance of *Callianassa major* fecal pellets. *Limnol. Oceanogr.*, **12**, 113–120.

FRANKENBERG, D. and SMITH, K. L., JR. (1967). Coprophagy in marine animals. *Limnol. Oceanogr.*, **12**, 443–450.

FRANZISKET, L. (1974). Uptake and accumulation of nitrate and nitrite by reef corals. *Naturwissenschaften* (in press).

FRETTER, V. (1937). The structure and function of the alimentary canal of some species of Polyplacophora (Mollusca). *Trans. R. Soc. Edinb.*, **59**, 119–164.

FRETTER, V. and GRAHAM, A. (1962). *British Prosobranch Molluscs*, Ray Society, London.

FRETTER, V. and MONTGOMERY, M. C. (1968). The treatment of food by prosobranch veligers. *J. mar. biol. Ass. U.K.*, **48**, 499–520.

FREUDENTHAL, H. (1962). *Symbiodinium*, gen. nov. and *Symbiodinium microadriaticum* sp. nov., a Zooxanthella: taxonomy, life cycle and morphology. *J. Protozool.*, **9**, 45–52.

FROMM, P. O. (1963). Studies on renal and extra-renal excretion in a fresh water teleost *Salmo gairdneri*. *Comp. Biochem. Physiol.*, **10**, 121–128.

FRUTON, J. S. and MYCEK, M. J. (1956). Proteolytic enzymes. *A. Rev. Biochem.*, **25**, 57–78.

FUJI, A. (1961). Studies on the biology of the sea urchin. IV. Histochemical observation of the food canal of *Strongylocentrotus intermedius*. *Bull. Fac. Fish. Hokkaido Univ.*, **11**, 195–202.

FUJI, A. (1962). Studies on the biology of the sea urchin. V. Food consumption of *Strongylocentrotus intermedius*. *Jap. J. Ecol.*, **12**, 181–186.

FUJI, A. (1967). Ecological studies on the growth and food consumption of Japanese common littoral sea urchin *Strongylocentrotus intermedius* (A. AGASSIZ). *Mem. Fac. Fish. Hokkaido Univ.*, **15**, 83–160.

FULTON, C. (1963a). Proline control of the feeding reactions of *Cordylophora*. *J. gen. Physiol.*, **46**, 823–837.

FULTON, C. (1963b). Rhythmic movements in *Cordylophora*. *J. cell. comp. Physiol.*, **61**, 39–51.

GALLI, D. R. and GIESE, A. C. (1959). Carbohydrate digestion in a herbivorous snail *Tegula funebralis*. *J. exp. Zool.*, **140**, 415–440.

GEORGE, D. (1964). Organic matter available to the polychaete *Cirriformia tentaculata*

(MONTAGU) living in an intertidal mud flat. *Limnol. Oceanogr.*, **9**, 453–455.

GEORGE, R. Y. (1965). The significance of fungi in the distribution of timber by sphaeromids. *Preprint Proc. Symp. mar. Paints*, *New Delhi* (1964). pp. 110–114.

GEORGE, W. C. (1952). The digestion and absorption of fat in lamellibranchs. *Biol. Bull. mar. biol. Lab., Woods Hole*, **102**, 118–127.

GERKING, S. D. (1952). The protein metabolism of sunfishes of different ages. *Physiol. Zoöl.*, **25**, 358–373.

GERKING, S. D. (1955). Influence of rate of feeding on body composition and protein metabolism of bluegill sunfish. *Physiol. Zoöl.*, **28**, 267–282.

GERKING, S. D. (1971). Influence of rate of feeding and body weight on protein metabolism of bluegill sunfish. *Physiol. Zoöl.*, **44**, 9–19.

GHIRETTI, F. and VIOLANTE, U. (1964). Ricerche sul metabolismo del rame in *Octopus vulgaris*. *Boll. Zool.*, **31**, 1081–1092.

GIBSON, R. and JENNINGS, J. B. (1967). 'Leucine aminopeptidase' activity in the blood system of rhynchocoelan worms. *Comp. Biochem. Physiol.*, **23**, 645–651.

GIESE, A. C. (1959). Comparative physiology: Annual reproductive cycles of marine invertebrates. *A. Rev. Physiol.*, **21**, 547–576.

GIESE, A. C. (1966a). Lipids in the economy of marine invertebrates. *Physiol. Rev.* **46**, 244–298.

GIESE, A. C. (1966b). On the biochemical constitution of some echinoderms. In R. A. Boolootian (Ed.), *Physiology of Echinodermata*, Wiley, New York. pp. 757–789.

GIESE, A. C. (1968). *Cell Physiology*, W. B. Saunders, Philadelphia.

GILLES, R. (1972). Biochemical ecology of Mollusca. In M. Florkin and B. T. Scheer (Eds), *Chemical Zoology*, Vol. VII. Academic Press, New York. pp. 467–499.

GLEASON, F. H., PRICE, J. S., MANN, R. A. and STUART, T. D. (1971). Lactate dehydrogenases from crustaceans and arachnids. *Comp. Biochem. Physiol.*, **40B**, 387–394.

GOERKE, H. (1971). Nahrungsaufnahme, Nahrungsausnutzung und Wachstum von *Nereis virens* (Polychaeta, Nereidae). *Veröff. Inst. Meeresforsch. Bremerh.*, **13**, 51–78.

GOHAR, H. A. F. (1940). Studies on the Red Sea. *Publs mar. biol. Stn Ghardaqa*, **2**, 25–118.

GOHAR, H. A. F. (1948). A description of some biological studies on a new alcyonarian species *Clavularia hamra* GOHAR. *Publs mar. biol. Stn Ghardaqa*, **6**, 1–33.

GOLDSTEIN, L. (1972). Urea metabolism in aquatic vertebrates. In J. W. Campbell and L. Goldstein (Eds), *Nitrogen Metabolism and the Environment*. Academic Press, New York. pp. 55–77.

GOLDSTEIN, L. and FORSTER, R. P. (1960). Ammonia excretion from gills of sculpin *Myoxocephalus*. *Fedn Proc. Fedn Am. Socs exp. Biol.*, **19**, 359.

GOLDSTEIN, L. and FORSTER, R. P. (1961). Source of ammonia excreted by the gills of the marine teleost *Myoxocephalus scorpius*. *Am. J. Physiol.*, **200**, 1116–1118.

GOLDSTEIN, L. and FORSTER, R. P. (1965). The role of uricolysis in the production of urea by fishes and other aquatic vertebrates. *Comp. Biochem. Physiol.*, **14**, 567–576.

GOLDSTEIN, L. and FORSTER, R. P. (1971a). Urea biosynthesis and excretion in freshwater and marine elasmobranchs. *Comp. Biochem. Physiol.*, **39B**, 415–421.

GOLDSTEIN, L. and FORSTER, R. P. (1971b). Osmoregulation and urea metabolism in the little skate *Raja erinacea*. *Am. J. Physiol.*, **220**, 742–746.

GOLDSTEIN, L., FORSTER, R. P. and FANELLI, G. M., JR. (1964). Gill blood flow and ammonia excretion in the marine teleost *Myoxocephalus scorpius*. *Comp. Biochem. Physiol.*, **12**, 489–499.

GOLDSTEIN, L. and FUNKHOUSER, D. (1972). Biosynthesis of trimethylamine oxide in the nurse shark *Ginglymostoma cirratum*. *Comp. Biochem. Physiol.*, **42A**, 51–57.

GOLDSTEIN, L., HARTMAN, S. C. and FORSTER, R. P. (1967). On the origin of trimethylamine oxide in the spiny dogfish *Squalus acanthias*. *Comp. Biochem. Physiol.*, **21**, 719–722.

GOLDSTEIN, L., OPPELT, W. W. and MAREN, T. H. (1968). Osmotic regulation and urea metabolism in the lemon shark *Negaprion brevirostris*. *Am. J. Physiol.*, **215**, 1493–1497.

GOLDSTEIN, L., WITT, S. H. DE and FORSTER, R. P. (1973). Activities of ornithine urea cycle enzymes and trimethylamine oxidase in the coelacanth *Latimeria chalumnae*. *Comp. Biochem. Physiol.*, **44B**, 357–362.

GOMAZKOV, O. A. and KRAYUKHIN, B. V. (1963). The role of the vagus nerve in the regulation

of digestion in fish. (Russ.) *Byul. Inst. Biol. Vodokhran.*, **8**, 124–131.

GOODBODY, I. (1965). Nitrogen excretion in Ascidiacea. II. Storage, excretion and the uricolylic enzyme system. *J. exp. Biol.*, **42**, 299–305.

GORBMAN, A. and BERN, H. A. (1962). *A Textbook of Comparative Endocrinology*, Wiley, New York.

GORDON, M. S., BOETIUS, I., EVANS, D. H., McCARTHY, R. and OGLESBY, L. C. (1965). Salinity adaptation in the mudskipper fish *Periophthalmus sobrinus*. *Hvalrad. Skr.*, **48**, 85–93.

GOREAU, T. F. (1959). The physiology of skeleton formation in corals. I. A method for measuring the rate of calcium deposition by corals under different conditions. *Biol. Bull. mar. biol. Lab., Woods Hole*, **116**, 59–75.

GOREAU, T. F. (1961). Problems of growth and calcium deposition in reef corals. *Endeavour*, **20**, 32–39.

GOREAU, T. F. (1963). Calcium carbonate deposition by coralline algae and corals in relation to their roles as reef builders. *Ann. N.Y. Acad. Sci.*, **109**, 127–167.

GOREAU, T. F. and GOREAU, N. I. (1960). Distribution of labelled carbon in reef building corals with and without zooxanthellae. *Science, N.Y.*, **131**, 668–669.

GORKIN, R. A. (1970). *In vitro* uptake of amino acids by erythrocytes of the hagfish *Eptatretus stouti*. *Physiologist, Wash.*, **13**, 210.

GOUDSMIT, E. M. (1972). Carbohydrates and carbohydrate metabolism in Mollusca. In M. Florkin and B. T. Scheer (Eds), *Chemical Zoology*, Vol. VII. Academic Press, New York. pp. 219–243.

GRAETZ, E. and AUTRUM, H. (1935). Vergleichende Untersuchungen zur Verdauungsphysiologie der Egel. II. Die Fermente der Eiweißverdauung bei *Hirudo* und *Haemopis*. *Z. vergl. Physiol.*, **22**, 273–283.

GRAHAM, R. A. and MANGUM, C. P. (1969). Further studies of the feeding response in the onuphid polychaete *Diopatra cuprea*. *Va J. Sci.*, **20**, 20.

GRAHAME, J. (1973). Assimilation efficiency of *Littorina littorea* (L.) (Gastropoda: Prosobranchiata). *J. Anim. Ecol.*, **42**, 383–389.

GRAINGER, F. and NEWELL, G. E. (1965). Aerial respiration in *Balanus balanoides*. *J. mar. biol. Ass. U.K.*, **45**, 469–479.

GRAY, J. S. (1967). Substrate selection by the archiannelid *Protodrilus rubropharyngeus*. *Helgoländer wiss. Meeresunters.*, **15**, 253–269.

GREENFIELD, L. J. (1953). Observations on the nitrogen and glycogen content of *Teredo (Lyrodus) pedicellata* DE QUATREFAGES at Miami, Florida. *Bull. mar. Sci. Gulf Caribb.*, **2**, 486–496.

GREENFIELD, L. J. (1959). Cellulolytic activity in *Teredo*. In D. L. Ray (Ed.), *Marine Boring and Fouling Organisms*. University of Washington Press, Seattle. pp. 363–371.

GREENFIELD, L. J. and LANE, C. E. (1953). Cellulose digestion in *Teredo*. *J. biol. Chem.*, **204**, 669–672.

GREER, M. L. and LAWRENCE, A. L. (1967). The active transport of selected amino acids across the gut of the chiton (*Cryptochiton stelleri*). I. Mapping determinations and effects of anaerobic conditions. *Comp. Biochem. Physiol.*, **22**, 665–674.

GRIGG, R. W. (1972). Orientation and growth form of sea fans. *Limnol. Oceanogr.*, **17**, 185–192.

GROLLMAN, A. (1929). The urine of the goosefish (*Lophius piscatorius*). Its nitrogenous constituents with special reference to the presence in it of trimethylamine oxide. *J. biol. Chem.*, **81**, 267–278.

GRONINGER, H. S. (1959). The occurrence and significance of trimethylamine oxide in marine animals. *Spec. scient. Rep. U.S. Fish. Wildl. Serv., Fisheries*, **333**, 1–22.

GUYTON, A. C., ARMSTRONG, G. C. and CROWELL, J. W. (1960). Negative pressure in the interstitial spaces. *Physiologist, Wash.*, **3**, 70.

HALTON, D. W. and JENNINGS, J. B. (1965). Observations on the nutrition of monogenetic trematodes. *Biol. Bull. mar. biol. Lab., Woods Hole*, **129**, 257–272.

HAMMEN, C. S. (1964). Carbon dioxide fixation in marine invertebrates: a review. *Proc. Symp. exp. mar. Ecol. (Univ. Rhode Island)*, **2**, 48–50.

HAMMEN, C. S. (1966). Carbon dioxide fixation in marine invertebrates. Rate and pathway in the oyster. *Comp. Biochem. Physiol.*, **17**, 289–296.

HAMMEN, C. S. (1968). Aminotransferase activities and amino acid excretion of bivalve molluscs and brachiopods. *Comp. Biochem. Physiol.*, **26**, 697–705.

HAMMEN, C. S. (1969a). Lactate and oxidoreductase in marine invertebrates. *Mar. Biol.*, **4**, 233–238.

HAMMEN, C. S. (1969b). Metabolism of the oyster *Crassostrea virginica*. *Am. Zool.*, **9**, 309–318.

HAMMEN, C. S. (1971). Metabolism of brachiopods and bivalve molluscs. In R. Alvarado, E. Gadea and A. Haro (Eds), *Actas del Symposio Internacional de Zoofilogenia*. Universidad de Salamanca. pp. 471–478.

HAMMEN, C. S. (1972). Lactate oxidation in the upper shore barnacle, *Chthamalus depressus* (POLI). *Comp. Biochem. Physiol.*, **43A**, 435–442.

HAMMEN, C. S. and LUM, S. C. (1962). Carbon dioxide fixation in marine invertobrates. III. The main pathway in flatworms. *J. biol. Chem.*, **237**, 2419–2422.

HAMMEN, C. S. and LUM, S. C. (1964). Carbon dioxide fixation in marine invertebrates: qualitative relations. *Nature, Lond.*, **201**, 416–417.

HAMMEN, C. S. and LUM, S. C. (1972). D-Lactate oxidation in a marine gastropod and a sea urchin. *Comp. Biochem. Physiol.*, **42B**, 123–129.

HAMMEN, C. S., MILLER, H. F., JR. and GEER, W. H. (1966). Nitrogen excretion of *Crassostrea virginica*. *Comp. Biochem. Physiol.*, **17**, 1199–1200.

HAMMEN, C. S. and OSBORNE, P. J. (1959). Carbon dioxide fixation in marine invertebrates. A survey of major phyla. *Science, N.Y.*, **130**, 1409–1410.

HANIFFA, M. A., and PANDIAN, T. J. (1973). Effects of body weight on feeding rate and radula size in the freshwater snail *Pila globosa*. *Veliger* (in press).

HANNAN, J. V. and EVANS, D. H. (1973). Water permeability in some euryhaline decapods and *Limulus polyphemus*. *Comp. Biochem. Physiol.*, **44A**, 1199–1214.

HARGRAVE, B. T. (1970). The effect of deposit-feeding amphipod on the metabolism of benthic microflora. *Limnol. Oceanogr.*, **15**, 21–30.

HARLEY, M. B. (1956). The feeding habits of *Nereis diversicolor* and related polycheates. Ph.D. thesis, University of London.

HARRISON, F. M. (1962). Some excretory processes in the abalone *Haliotis rufescens*. *J. exp. Biol.*, **39**, 179–192.

HARRISON, F. M. and MARTIN, A. W. (1955). Kidney function in a cephalopod. *Fedn Proc. Fedn Am. Socs exp. Biol.*, **14**, Abstr. No. 224.

HARRISON, F. M. and MARTIN, A. W. (1965). Excretion in the cephalopod *Octopus dofleini*. *J. exp. Biol.*, **42**, 71–98.

HARTENSTEIN, R. (1971). Characteristics of arginase from the freshwater crayfish *Cambarus bartoni*. *Comp. Biochem. Physiol.*, **40B**, 781–795.

HARVEY, H. W., COOPER, L. H. N., and RUSSELL, F. S. (1935). Plankton production and its control. *J. mar. biol. Ass. U.K.*, **20**, 407–441.

HASEGAWA, S. and NORDIN, J. H. (1969). Enzymes that hydrolyze fungal cell wall polysaccharides. I. Purification and properties of an endo-α-D (1,3)-glucanase from *Trichoderma viride*. *J. biol. Chem.*, **244**, 5460–5470.

HASHIMOTO, Y. and ONOMA, K. (1949). Digestion of higher carbohydrates by Mollusca (*Dolabella scapula* and *Teredo* sp.). *Bull. Jap. Soc. scient. Fish.*, **15**, 253–258.

HASLER, A. D. (1960). Guidepost of migrating fishes. *Science, N.Y.*, **132**, 785.

HAVEN, D. S. and MORALES-ALAMO, R. (1966). Aspects of bio-deposition by oysters and other invertebrate filter feeders. *Limnol. Oceanogr.*, **11**, 487–498.

HEUKELEM, F. VAN (1973). Growth and life-span of *Octopus cyanea* (Mollusca: Cephalopoda). *J. Zool., Lond.*, **169**, 299–315.

HICKMAN, C. (1968). Ingestion, intestinal absorption and elimination of sea water and salts in the southern flounder *Paralichthys lethostigma*. *Can. J. Zool.*, **46**, 457–466.

HICKMAN, C. P., JR. and TRUMP, B. F. (1969). The kidney. In W. S. Hoar and D. J. Randall (Eds), *Fish Physiology*, Vol. I. Academic Press, New York. pp. 91–240.

HIDAKA, T. (1955). On the cellulose decomposing bacteria found in the digestive organs of teredo (*Teredo navalis* sp.). *Biol. Abstr.*, **29**, 19169.

HILL, R. L. and SMITH, E. L. (1958). Hydrolysis of mercuripapain by leucine aminopeptidase without loss of enzymatic activity. *J. biol. Chem.*, **231**, 117–134.

HILL, R. L. and SMITH, E. L. (1959). Complete hydrolysis of glucagon by leucine aminopeptidase. *Biochem. biophys. Acta*, **31**, 257–258.

HILL, R. L. and SMITH, E. L. (1960). Isolation and characterization of an enzymically active fragment of papain. *J. biol. Chem.*, **235**, 2332–2339.

HIRSCH, G. C. (1937). Probleme der Restitution in Drüsen. *Arch. exp. Zellforsch.*, **19**, 385–404.

HIRSCHOWITZ, B. I. (1957). Pepsinogen: its origins, secretion and excretion. *Physiol. Rev.*, **37**, 475–511.

HISCOX, J. I. and BROCKSEN, R. W. (1973). Effects of parasitic gut nematode on consumption and growth of juvenile rainbow trout (*Salmo gairdneri*). *J. Fish. Res. Bd Can.*, **30**, 443–450.

HOAR, W. S. (1966). *General and Comparative Physiology*, Prentice-Hall, Englewood Cliffs, New Jersey.

HOAR, W. S. and COTTLE, M. C. (1952). Dietary fat and temperature tolerance of goldfish. *Can. J. Zool.*, **30**, 41–48.

HOCHACHKA, P. W. (1969). Intermediary metabolism in fishes. In W. S. Hoar and D. J. Randall (Eds), *Fish Physiology*, Vol. I. Academic Press, New York. pp. 351–389.

HOCHACHKA, P. W., FIELDS, J. and MUSTAFA, T. (1973a). Animal life without oxygen: Basic biochemical mechanisms. *Am. Zool.*, **13**, 543–555.

HOCHACHKA, P. W. and MUSTAFA, T. (1973). Enzymes in facultative anaerobiosis of molluscs: malic enzyme of oyster adductor muscle. *Comp. Biochem. Physiol.* **45B**, 625–637.

HOCHACHKA, P. W., OWEN, T. G., ALLEN, J. F. and WHITTOW, G. C. (1973b). Anaerobic energy sources in diving vertebrates: the role of succinate production. *Proc. natn. Acad. Sci. U.S.A.* (in press).

HODGSON, E. S. (1958). Electrophysiological studies of arthropod chemoreception. III. Chemoreceptors of terrestrial and freshwater arthropods. *Biol. Bull. mar. biol. Lab., Woods Hole*, **115**, 114–125.

HOHNKE, L. and SCHEER, B. T. (1970). Carbohydrate metabolism in crustaceans. In M. Florkin and B. T. Scheer (Eds), *Chemical Zoology*, Vol. V. Academic Press, New York. pp. 147–166.

HOKIN, L. E. and HOKIN, M. R. (1960). Studies on the carrier function of phosphatidic acid in sodium transport. I. The turnover of phosphatidic acid and phosphoinositide in the avian salt gland on stimulation of secretion. *J. gen. Physiol.*, **44**, 61–85.

HOLLAND, N. D. and NIMITZ, A. (1964). An autoradiographic and histochemical investigation of the gut mucopolysaccharides of the purple sea urchin (*Strongylocentrotus purpuratus*). *Biol. Bull. mar. biol. Lab., Woods Hole*, **127**, 280–293.

HOLLIDAY, F. G. T. (1971). Salinity: Animals—Fishes. In O. Kinne (Ed.), *Marine Ecology*, Vol. I, Environmental Factors, Part 2, Wiley, London. pp. 997–1033.

HOLLING, C. S. (1966). The functional responses of invertebrate predators to prey density. *Mem. ent. Soc. Can.*, **48**, 1–86.

HOLT, C. VON and HOLT, M. VON (1968a). Transfer of photosynthetic products from zooxanthellae to coelenterate hosts. *Comp. Biochem. Physiol.*, **24**, 73–81.

HOLT, C. VON and HOLT, M. VON (1968b). The secretion of organic compounds by zooxanthellae isolated from various types of *Zoanthus*. *Comp. Biochem. Physiol.*, **24**, 83–92.

HOLTER, H. (1959). Pinocytosis. *Int. Rev. Cytol.*, **8**, 481–504.

HOLWERDA, D. A., ZWAAN, A. DE and MARREWIJK, W. J. A. VAN (1973). The regulatory role of pyruvate kinase in the sea mussel *Mytilus edulis* L. *Neth. J. Zool.*, **23**, 232–235.

HORIUCHI, S. (1963).On the nature of carbohydrates of digestive diverticula in the marine lamellibranch *Venerupis philippinarum* ADAMS et REEVE. *Sci. Rep. Tokyo Kyoiku Daigaku (B)*, **11**, 133–152.

HORIUCHI, S. and LANE, C. E. (1965). Digestive enzymes of crystalline style of *Strombus gigas* LINNAEUS. I. Cellulase and some other carbohydrases. *Biol. Bull. mar. biol. Lab., Woods Hole*, **129**, 273–281.

HORIUCHI, S. and LANE, C. E. (1966). Carbohydrases of the crystalline style and hepatopancreas of *Strombus gigas* L. *Comp. Biochem. Physiol.*, **17**, 1189–1197.

HORNE, F. and BOONKOOM, V. (1970). Evidence for ornithine cycle in several gastropods. *Comp. Biochem. Physiol.*, **32**, 141–153.

HOSKIN, F. C. G. (1959). Intermediate metabolism of electric tissue in relation to function. III. Oxidation of substrates by tissues in *Electrophorus electricus* as compared to other verte-

brates. *Archs Biochem. Biophys.*, **85**, 141–148.

Hotz, H. (1938). *Protoclepsis tesselata* (O. F. Müller). Ein Beitrag zur Kenntnis von Bau- und Lebensweise der Hirudineen. *Revue suisse Zool.*, **45**, 1–380.

Huang, J. C. and Chen, T. S. T. (1971). Ion transport across intestinal mucosa of winter flounder, *Pseudopleuronectes americanus*. *Am. J. Physiol.*, **220**, 1734–1738.

Huggins, A. K., Skutsch, G. and Baldwin, E. (1969). Ornithine-urea cycle enzymes in teleostean fish. *Comp. Biochem. Physiol.*, **28**, 587–602.

Hughes, R. N. (1969). Appraisal of the iodate–sulphuric acid wet oxidation procedure for the estimation of the calorific content of marine sediments. *J. Fish. Res. Bd Can.*, **26**, 1956–1964.

Hughes, R. N. (1970). An energy budget for a tidal-flat population of the bivalve *Scrobicularia plana* (Da Costa). *J. Anim. Ecol.*, **39**, 357–381.

Hughes, R. N. (1971). Ecological energetics of *Nerita* (Archaeogastropoda, Neritacea) on Barbados, West Indies. *Mar. Biol.*, **11**, 12–22.

Hult, J. E. (1969). Nitrogenous waste products and excretory enzymes in the marine polychaete *Cirriformia spirabranchia* (Moore 1904). *Comp. Biochem. Physiol.*, **31**, 15–24.

Hultin, T. and Wessel, G. (1952). Incorporation of ^{14}C labeled carbon dioxide into the proteins of developing sea urchin eggs. *Expl Cell Res.*, **3**, 613–616.

Humphrey, G. F. (1944). Intermediary metabolism of molluscs. *J. exp. biol. med. Sci.*, **27**, 353–359.

Hunt, B. P. (1960). Digestion rate and food consumption of Florida gar, warmouth and large-mouth bass. *Trans. Am. Fish. Soc.*, **89**, 206–210.

Hunter, A. (1929). Further observations on the distribution of arginase in fishes. *J. biol. Chem.*, **81**, 505–511.

Hylleberg Kristensen, J. (1972). Carbohydrases of some marine invertebrates with notes on their food and on the natural occurrence of the carbohydrates studied. *Mar. Biol.*, **14**, 130–142.

Hyman, L. E. (1940). *The Invertebrates: Protozoa through Ctenophora*, McGraw-Hill, New York.

Hyman, L. E. (1951). *The Invertebrates: Platyhelminthes and Rhynchocoela*, McGraw-Hill, New York.

Hyman, L. E. (1955). *The Invertebrates: Echinodermata*, McGraw-Hill, New York.

Ishida, S. (1954). Metabolic patterns in bivalves. I. Distribution of the activity to deaminate guanine in soft parts of the clam *Meretrix meretrix lusoria* Gmelin. II. Distribution of the activity to deaminate urea in the soft parts of the clam *Meretrix meretrix lusoria* Gmelin. *Sci. Rep. Tôhoku Univ. (Ser. 4)*, **20**, 249–259.

Ishida, S. (1955). Metabolic patterns in bivalves. VII. Some aspects of the purine metabolism in the clam *Meretrix meretrix lusoria* Gmelin. *J. Coll. Arts Sci. Chiba Univ.*, **1**, 270–272.

Ishiwata, N. (1968). Ecological studies on feeding of fishes. IV. Satiation curve. *Bull. Jap. Soc. scient. Fish.*, **34**, 691–693.

Isserhoff, H., Tunis, M. and Read, C. P. (1972). Changes in amino acids of bile in *Fasciola hepatica* infections. *Comp. Biochem. Physiol.*, **41B**, 157–163.

Ito, S. and Imai, T. (1955). Ecology of oyster beds. I. On the decline of productivity due to repeated culture. *Tohoku J. agric. Res.*, **5**, 251–268.

Ivlev, V. S. (1961). *Experimental Ecology of the Feeding of Fishes*, Yale University Press, New Haven.

Ivleva, U. S. (1970). The influence of temperature on the transformation of matter in marine invertebrates. In J. H. Steele (Ed.), *Marine Food Chains*. Oliver and Boyd, Edinburgh. pp. 96–112.

Jacobsen, J. G. and Smith, L. H., Jr. (1968). Biochemistry and physiology of taurine and taurine derivatives. *Physiol. Rev.*, **48**, 424–511.

Jacobsen, V. H. (1967). The feeding of lugworm *Arenicola marina* (L.). Quantitative studies. *Ophelia*, **4**, 91–109.

Jaschke, W. (1933). Beiträge zur Kenntnis der symbiontischen Einrichtungen bei Hirudineen und Ixodiden. *Z. ParasitKde*, **5**, 515–541.

Jeal, F. and West, B. (1974). *Proc. Challenger Soc.* (in press).

Jeferies, H. P. (1969). Seasonal composition of temperate plankton communities: free amino

acids. *Limnol. Oceanogr.*, **14**, 41–52.

JENNINGS, J. B. (1957). Studies on feeding, digestion and food storage in free-living flatworms (Platyhelminthes: Turbellaria). *Biol. Bull. mar. biol. Lab., Woods Hole*, **112**, 63–80.

JENNINGS, J. B. (1960). Observations on the nutrition of the rhynchocoelan *Lineus ruber* (O. F. MÜLLER). *Biol. Bull. mar. biol. Lab., Woods Hole*, **119**, 189–196.

JENNINGS, J. B. (1962a). A histochemical study of digestion and digestive enzymes in the rhynchocoelan *Lineus ruber* (O. F. MÜLLER). *Biol. Bull. mar. biol. Lab., Woods Hole*, **122**, 63–82.

JENNINGS, J. B. (1962b). Further studies on feeding and digestion in triclad Turbellaria. *Biol. Bull. mar. biol. Lab., Woods Hole*, **123**, 571–581.

JENNINGS, J. B. (1965). *Feeding, Digestion and Assimilation in Animals*, Pergamon Press, London.

JENNINGS, J. B. (1968). Nutrition and digestion. In M. Florkin and B. T. Scheer (Eds), *Chemical Zoology*, Vol. II. Academic Press, New York. pp. 303–326.

JENNINGS, J. B. (1969). Ultrastructural observations on the phagocytic uptake of food materials by the ciliated cells of the rhynchocoelan intestine. *Biol. Bull. mar. biol. Lab., Woods Hole*, **137**, 476–485.

JENNINGS, J. B. and COLAM, J. B. (1970). Gut structure, digestive physiology and food storage in *Pontonema vulgaris* (Nematoda: Enoplida). *J. Zool., Lond.*, **161**, 211–221.

JENNINGS, J. B. and GELDER, S. R. (1969). Feeding and digestion in *Dinophilus gyrociliatus*. *J. Zool., Lond.*, **158**, 441–451.

JENNINGS, J. B. and GIBSON, R. (1969). Observations on the nutrition of seven species of rhynchocoelan worms. *Biol. Bull. mar. biol. Lab., Woods Hole*, **136**, 405–433.

JENNINGS, J. B. and HALTON, D. W. (1965). Observations on the nutrition of monogenetic trematodes. *Biol. Bull. mar. biol. Lab., Woods Hole*, **129**, 257–272.

JENNINGS, J. B. and LANDE, V. M. VAN DER (1967). Histochemical and bacteriological studies on digestion in nine species of leeches (Annelida: Hirudinea). *Biol. Bull. mar. biol. Lab., Woods Hole*, **133**, 166–183.

JENNINGS, J. B. and METTRICK, D. F. (1968). Observations on the ecology, morphology and nutrition of the rhabdocoel *Syndesmis franciscana* (LEHMAN, 1946) in Jamaica. *Caribb. J. Sci.*, **8**, 57–69.

JENSEN, A. S. (1951a). Do the Naticidae drill by mechanical or by chemical means? *Nature, Lond.*, **167**, 901.

JENSEN, A. S. (1951b). Do the Naticidae (Gastropoda, Prosobranchia) drill by chemical or by mechanical means? *Dansk. Naturhist. Foren. Videnskab. Medd.*, **113**, 251–261.

JERVIS, E. L. and SMYTH, D. H. (1960). The active transfer of D-methionine by the rat small intestine *in vitro*. *J. Physiol., Lond.*, **151**, 51–58.

JEUNIAUX, C. (1954). Sur la chitinase et la flore bacterienne intestinale des mollusques gastropodes. *Mem. Acad. r. Belg. Cl. Sci.*, **28**, 1–45.

JEUNIAUX, C. (1960). Sur la liberation d'acetylglucosamine à partir de chitine pulverisée, sous l'action des chitobiases. *Archs int. Physiol. Biochim.*, **68**, 685–686.

JEUNIAUX, C. (1961). Evolution des enzymes chitinotytiques dans le regne animal. *Int. Congr. Biochem.*, **5**, 143.

JEUNIAUX, C. (1963). *Chitine et Chitinolyse un Chapitre de la Biologie Moleculaire*, Masson, Paris.

JEUNIAUX, C. (1969). Nutrition and digestion. In M. Florkin and B. T. Scheer (Eds), *Chemical Zoology*, Vol. IV. Academic Press, New York. pp. 69–91.

JOHANNES, R. E. (1968). Nutrient regeneration in lakes and oceans. In M. Droop and E. J. Ferguson (Eds), *Advances in Microbiology of the Sea*, Vol. I. Academic Press, New York. pp. 203–213.

JOHANNES, R. E., COWARD, S. J. and WEBB, K. L. (1969). Are dissolved amino acids an energy source for marine invertebrates? *Comp. Biochem. Physiol.*, **29**, 283–288.

JOHANNES, R. E. and SATOMI, M. (1967). Measuring organic matter retained by aquatic invertebrates. *J. Fish. Res. Bd Can.*, **24**, 2467–2471.

JOHANSEN, K. and MARTIN, A. W. (1962). Circulation in the cephalopod *Octopus dofleini*. *Comp. Biochem. Physiol.*, **5**, 161–176.

JOHNSON, T. W., JR. (in press). Diseases of plants. In O. Kinne (Ed.), *Marine Ecology*, Vol. III,

Cultivation. Wiley, London.
JONES, D. A., BABBAGE, P. C. and KING, P. E. (1969). Studies on digestion and the fine structure of digestive caeca in *Eurydice pulchra* (Crustacea: Isopoda). *Mar. Biol.*, **2**, 311–320.
JONES, H. D. (1970). Hydrostatic pressures within the hearts and pericardium of *Patella vulgata* L. *Comp. Biochem. Physiol.*, **34**, 263–272.
JØRGENSEN, C. B. (1949). The rate of feeding by *Mytilus* in different kinds of suspension. *J. mar. biol. Ass. U.K.*, **28**, 333–344.
JØRGENSEN, C. B. (1952). On the relation between water transport and food requirements in some marine filter feeding invertebrates. *Biol. Bull. mar. biol. Lab., Woods Hole*, **103**, 356–363.
JØRGENSEN, C. B. (1955). Quantitative aspects of filter feeding in invertebrates. *Biol. Rev.*, **30**, 391–454.
JØRGENSEN, C. B. (1960). Efficiency of particle retention and rate of water transport in undisturbed lamellibranch. *J. Cons. perm. int. Explor. Mer*, **26**, 94–116.
JØRGENSEN, C. B. (1962). The food of filter feeding organisms. *Rapp. P.-v. Cons. perm. int. Explor. Mer*, **153**, 99–107.
JØRGENSEN, C. B. (1966). *Biology of Suspension Feeding*, Pergamon Press, New York.
JØRGENSEN, C. B. (1968). Nutrition. In M. S. Gordon (Ed.), *Animal Functions: Principles and Adaptations*. Amarint Publishing Co, New Delhi. pp. 12–47.
JØRGENSEN, C. B. and GOLDBERG, E. D. (1953). Particle filtration in some ascidians and lamellibranchs. *Biol. Bull. mar. biol. Lab., Woods Hole*, **105**, 477–489.
JOSEPHSON, R. K. (1965). The coordination of potential pacemakers in the hydroid *Tubularia*. *Am. Zool.*, **5**, 483–490.
KANWISHER, J. W. and WAINWRIGHT, S. A. (1967). Oxygen balance in some coral reefs. *Publs mar. biol. Stn Ghardaqa*, **2**, 3–22.
KARLSON, P. (1963). *Introduction to Modern Biochemistry*, Academic Press, New York.
KAWAGUTI, S. (1941). Study on the invertebrates associating with unicellular algae. I. *Placobranchus ocellatus* VON HASSELT, a nudibranch. *Palao trop. biol. Stn Stud.*, **2**, 307–308.
KAWAGUTI, S. (1964). Zooxanthellae in the corals are intracellular symbionts. *Proc. Japan. Acad.*, **40**, 545–548.
KAWAGUTI, S. (1965). An electron microscopic proof for a path of nutritive substances from zooxanthellae to reef coral tissue. *Proc. Japan Acad.*, **40**, 832–835.
KAWAGUTI, S., YAMAMOTO, M. and KAMISHIMA, Y. (1965). Electron microscopy on the symbiosis between blue green algae and an opisthobranch, *Placobranchus*. *Proc. Japan Acad.*, **41**, 614–617.
KAY, J. G. and BRAFIELD, A. E. (1973). The energy relationships of the polychaete *Neanthes* (=*Nereis*) *virens* (SARS). *J. Anim. Ecol.*, **42**, 673–692.
KEEBLE, F. and GAMBLE, F. W. (1907). The origin and nature of the green cells of *Convoluta roscoffensis*. *Q. Jl microsc. Sci.*, **51**, 167–219.
KEMPTON, R. T. (1953). Studies on elasmobranchs kidney. II. Reabsorption of urea by the smooth dogfish *Mustelus canis*. *Biol. Bull. mar. biol. Lab., Woods Hole*, **104**, 45–56.
KERKUT, G. A., MORITZ, P. M. and MUNDAY, K. A. (1961). Variations of copper concentrations in *Carcinus maenas*. *Cah. Biol. mar.* **2**, 399–408.
KERMACK, D. M. (1955). Anatomy and physiology of gut of *Arenicola*. *Proc. zool. Soc. Lond.*, **125**, 347–381.
KERMACK, W. O., LEES, H. and WOOD, J. D. (1955). Some non protein constituents of tissue of lobster. *Biochem. J.*, **60**, 424–428.
KERR, N. and STEPHENS, G. C. (1962). The uptake of amino acids by *Tetrahymena*. *Am. Zool.*, **2**, 450.
KERR, S. R. (1971a). Analysis of laboratory experiments on growth efficiency of fishes. *J. Fish. Res. Bd Can.*, **28**, 801–808.
KERR, S. R. (1971b). Prediction of fish growth efficiency in nature. *J. Fish. Res. Bd Can.*, **28**, 809–814.
KIDDER, G. W. (1967). Nitrogen: Distribution, nutrition and metabolism. In M. Florkin and B. T. Scheer (Eds), *Chemical Zoology*, Vol. I. Academic Press, New York. pp. 93–151.
KILIAN, E. F. (1952). Wasserströmung und Nahrungsaufnahme beim Süsswasserschwamm

Ephydatia fluviatilis. *Z. vergl. Physiol.*, **34**, 407–447.

KINNE, O. (1952). Zur Biologie und Physiologie von *Gammarus duebeni* LILLJ. V: Untersuchungen über Blutkonzentration, Herzfrequenz und Atmung. *Kieler Meeresforsch.*, **9**, 134–150.

KINNE, O. (1960). Growth, food intake and food conversion in a euryplastic fish exposed to different temperatures and salinities. *Physiol. Zoöl.*, **28**, 288–317.

KINNE, O. (1962). Irreversible nongenetic adaptation. *Comp. Biochem. Physiol.*, **5**, 265–282.

KINNE, O. (1970). Temperature: Animals—Invertebrates. In O. Kinne (Ed.), *Marine Ecology*, Vol. I, Environmental Factors, Part 1. Wiley, London. pp. 407–514.

KINNE, O. (1971). Salinity: Animals—Invertebrates. In O. Kinne (Ed.), *Marine Ecology*, Vol. I, Environmental Factors, Part 2. Wiley, London. pp. 821–996.

KINNE, O. (in press). Concepts, management and technology. In O. Kinne (Ed.), *Marine Ecology*, Vol. III. Cultivation. Wiley, London.

KIRSCHNER, L. B. (1967). Comparative physiology: invertebrate excretory organs. *A. Rev. Physiol.*, **29**, 169–196.

KISSELEVA, M. I. and VITYUK, D. M. (1970). The feeding of *Arenicola grubii* (Arenicolidae) in the Black Sea. *Zool. Zh.*, **49**, 219–222.

KITCHELL, J. E. and WINDELL, J. T. (1968). Rate of gastric digestion in pumpkinseed sunfish *Lepomis gibbosus*. *Trans. Am. Fish. Soc.*, **97**, 489–492.

KLEEREKOPER, H. (1958). The localization of prey by the sea lamprey *Petromyzon marinus* L. *Anat. Rec.*, **132**, 464.

KLEEREKOPER, H. (1967). Some effects of olfactory simulation on locomotor pattern in fish. In I. Hayashi (Ed.), *Olfaction and Taste—II. Int. Symp. Ser.*, **8**, 625–645.

KLEEREKOPER, H. and MOGENSEN, J. (1963). Role of olfaction in the orientation of *Petromyzon marinus*. I. Response to a single amine in prey's body odor. *Physiol. Zoöl.*, **36**, 347–360.

KLEEREKOPER, H. and SIBAKIN, K. (1956a). Spike potentials produced by the sea lamprey (*Petromyzon marinus*) in the water surrounding the head region. *Nature, Lond.*, **178**, 490–491.

KLEEREKOPER, H. and SIBAKIN, K. (1956b). An investigation of the electrical 'spike' potentials produced by the sea lamprey (*Petromyzon marinus*) in the water surrounding the head region. *J. Fish. Res. Bd Can.*, **13**, 375–383.

KONINGSOR, R. L., JR. and HUNSAKER, D. (1971). Cellulase from the crop of *Aplysia vaccaria* WINKLER. *Veliger*, **13**, 285–289.

KONINGSOR, R. L., JR., MCLEAN, N. and HUNSAKER, D. (1972). Radio-autographic evidence for a digestive cellulase in the sea hare *Aplysia vaccaria* (Gastropoda: Opisthobranchia). *Comp. Biochem. Physiol.*, **43B**, 237–240.

KOOYMAN, G. L. (1973). Respiratory adaptations in marine mammals. *Am. Zool.*, **13**, 457–468.

KOOYMAN, G. L., KEREM, D. H., CAMPBELL, W. B. and WRIGHT, J. J. (1971). Pulmonary function in freely diving Weddell seal *Leptonychotes weddelli*. *Resp. Physiol.*, **12**, 271–282.

KORRINGA, P. (1952). Recent advances in oyster biology. *Q. Rev. Biol.*, **27**, 266–308; 339–365.

KOZLOVSKAYA, E. P. and VASKOVSKY, V. E. (1970). A comparative study of protinases of marine invertebrates. *Comp. Biochem. Physiol.*, **34**, 137–142.

KRAYUKHIN, B. V. (1958). The regulatory mechanism of secretion of digestive juices in fishes. (Russ.). *Trudy̆ Soveshch. ikhtiol. Kom.*, **8**, 179–185.

KRAYUKHIN, B. V. (1959). Effect of food swallowing on the intensity of digestive processes in fishes. (Russ.) *Vop. Ikhtiol.*, **12**, 133–137.

KREBS, H. A. and KORNBERG, H. L. (1957). A survey of the energy transformation in living matter. *Ergebn. Physiol.*, **49**, 212–298.

KREY, J. (1951). Quantitative Bestimmung von Eiweiß im Plankton mittels der Biuretreaktion. *Kieler Meeresforsch.*, **8**, 16–29.

KREY, J., BANSE, K. and HAGMEIER, E. (1957). Über die Bestimmung von Eiweiß im Plankton mittels der Biuretreaktion. *Kieler Meeresforsch.*, **13**, 35–40.

KRIJGSMAN, B. J. (1928). Arbeitsrhythmus der Verdauungsdrüsen bei *Helix pomatia*. I. Sekretion, Resorption und Phagocytose. *Z. vergl. Physiol.*, **2**, 264–296.

KRISHNASWAMY, S., RAYMONT, J. E. G. and TUNDISI, J. (1967). Succinic dehydrogenase activity in marine animals. *Int. Revue ges. Hydrobiol.*, **52**, 447–451.

KROGH, A. (1931). Dissolved substances as food of aquatic organisms. *Biol. Rev.*, **6**, 412–442.

KRÜGER, F. (1959). Zur Ernährungsphysiologie von *Arenicola marina* (L.). *Zool. Anz.*, **22** (Suppl.), 115–120.

KUENZLER, E. J. (1961). Structure and energy flow of a mussel population in a Georgia salt marsh. *Limnol. Oceanogr.*, **6**, 191–204.

KUTTY, M. N. (1972). Respiratory quotient and ammonia excretion in *Tilapia mossambica*. *Mar. Biol.*, **16**, 126–133.

LAGLER, K. F., BARDACH, J. E. and R. R. MILLER (1962). *Ichthyology: The Study of Fishes*, Wiley, New York.

LANDIS, E. M. and PAPPENHEIMER, J. R. (1963). Exchange of substances through the capillary walls. In W. F. Hamilton (Ed.), *Handbook of Physiology*, Sect 2: *Circulation*, Vol. II. American Physiological Society, Washington. pp. 961–1034.

LANE, C. E. (1959). General histology and nutrition of *Limnoria*. In D. L. Ray (Ed.), *Marine Boring and Fouling Organisms*. University of Washington Press, Seattle. pp. 34–45.

LANGE, R. (1963). Osmotic function of amino acids and taurine in the mussel *Mytilus edulis*. *Comp. Biochem. Physiol.*, **10**, 173–179.

LASKER, R. (1966). Feeding, growth, respiration and carbon utilization of a euphausiid crustacean. *J. Fish. Res. Bd Can.*, **23**, 1291–1317.

LASKER, R. and GIESE, A. C. (1954). Nutrition of sea urchin *Strongylocentrotus*. *Biol. Bull. mar. biol. Lab., Woods Hole*, **106**, 328–340.

LAUCKNER, G. (in press). Diseases of animals. In O. Kinne (Ed.), *Marine Ecology*, Vol. III, Cultivation. Wiley, London.

LAVERACK, M. S. (1963). Aspects of chemoreception in Crustacea. *Int. Congr. Zool.*, **16**, 72–73.

LAWRENCE, A. L. and LAWRENCE, D. C. (1967). Sugar absorption in the intestine of the chiton *Cryptochiton stelleri*. *Comp. Biochem. Physiol.*, **22**, 341–357.

LAWRENCE, A. L., LAWRENCE, J. M. and GIESE, A. C. (1971). Carbohydrate and lipid levels in the blood of *Urechis canpo* (Echiura). *Comp. Biochem. Physiol.*, **38B**, 463–465.

LAWRENCE, D. C., LAWRENCE, A. L., GREER, M. L. and MAILMAN, D. (1967). Intestinal absorption in the sea cucumber, *Stichopus parvimensis*. *Comp. Biochem. Physiol.*, **20**, 619–627.

LAWRENCE, J. M. (1967). Lipid reserves in the gut of three species of tropical sea urchins. *Caribb. J. Sci.*, **7**, 65–68.

LAWRENCE, J. M. and HUGHES-GAMES, L. (1972). The diurnal rhythm of feeding and passage of food through the gut of *Diadema setosum* (Echinoderma: Echinoidea). *Israel J. Zool.*, **21**, 13–16.

LAWRENCE, J. M., LAWRENCE, A. L. and GIESE, A. C. (1966). Role of the gut as a nutrient-storage organ in the purple sea urchin (*Strongylocentrotus purpuratus*). *Physiol. Zoöl.*, **39**, 281–290.

LEAF, A. (1959). The mechanism of the asymmetrical distribution of endogenous lactate about the isolated toad bladder. *J. cell. comp. Physiol.*, **54**, 103–108.

LEE, D. L. (1965). *The Physiology of Nematodes*, Oliver and Boyd, Edinburgh.

LEE, R. F., NEVENZEL, J. C. and PAFFENHÖFER, G.-A. (1970a). Wax esters in marine copepods. *Science, N.Y.*, **167**, 1510–1511.

LEE, R. F., NEVENZEL, J. C. and PAFFENHÖFER, G.-A. (1971). Importance of wax esters and other lipids in the marine food chain: Phytoplankton and copepods. *Mar. Biol.*, **9**, 99–108.

LEE, R. F., NEVENZEL, J. C., PAFFENHÖFER, G.-A. and BENSON, A. A. (1970b). The metabolism of wax esters and other lipids by the marine copepod *Calanus helgolandicus*. *J. Lipid Res.*, **11**, 237–239.

LEIGHTON, D. L. (1966). Studies on food preference in algivorous invertebrates of Southern California kelp beds. *Pacif. Sci.*, **20**, 104–113.

LEIGHTON, D. L. and BOOLOOTIAN, R. A. (1963). Diet and growth in the black abalone *Haliotis cracherodii*. *Ecology*, **44**, 227–238.

LENHOFF, H. M. (1961). Digestion of protein in *Hydra* as studied using radioautography and fractionation by differential solubilities. *Expl Cell Res.*, **23**, 335–353.

LENHOFF, H. M. (1965). Mechanical stimulation of feeding in *Epiactis prolifera*. *Nature Lond.*, **207**, 1003.

LENHOFF, H. M. (1968a). Behaviour, hormones and *Hydra*. *Science, N.Y.*, **161**, 434–442.

LENHOFF, H. M. (1968b). Chemical perspectives on the feeding response, digestion, and nutrition of selected coelenterates. In M. Florkin and B. T. Scheer (Eds), *Chemical Zoology*, Vol. II. Academic Press, New York. pp. 158–221.

LENHOFF, H. M. (1969). pH profile of a peptide receptor. *Comp. Biochem. Physiol.*, **28**, 571–586.

LENHOFF, H. M., MUSCATINE, L. and DAVIS, L. (1968). *Experimental Coelenterate Biology*, University of Hawaii Press, Honolulu.

LENHOFF, H. M. and SCHNEIDERMAN, H. A. (1959). The chemical control of feeding in the Portuguese man-of-war *Physalia physalis* L. and its bearing on the evolution of the Cnidaria. *Biol. Bull. mar. biol. Lab., Woods Hole.*, **116**, 452–460.

LENTZ, T. L. (1966). *The Cell Biology of Hydra*, North-Holland Publishing Co., Amsterdam.

LENTZ, T. L. and BARRNETT, R. J. (1961a). Enzyme histochemistry of *Hydra*. *J. exp. Zool.*, **147**, 125–150.

LENTZ, T. L. and BARRNETT, R. J. (1961b). Enzyme histochemistry of *Hydra* nematocysts. *Anat. Rec.*, **139**, 312.

LENTZ, T. L. and BARRNETT, R. J. (1962). The effects of enzyme substrates and pharmacological agents on nematocyst discharge. *J. exp. Zool.*, **149**, 33–38.

LENTZ, T. L. and WOOD, J. G. (1964). Amines in the nervous system of coelenterates. *J. Histochem. Cytochem.*, **12**, 37.

LEONG, R. J. H. and O'CONNELL, C. P. (1969). A laboratory study of particulate and filter feeding of the northern anchovy (*Engraulis mordax*). *J. Fish. Res. Bd Can.*, **26**, 557–582.

LEPKOVSKY, S. (1948). The physiological basis of voluntary food intake (appetite?). *Adv. Fd Res.*, **1**, 105–148.

LEVANDOWSKY, M. and HODGSON, E. S. (1965). Amino acid and amine receptors of lobsters. *Comp. Biochem. Physiol.*, **16**, 159–161.

LEWIN, J. (1963). Heterotrophy in marine diatoms. In C. H. Oppenheimer (Ed.), *Marine Microbiology*. C. C. Thomas, Springfield, Ill. pp. 229–235.

LEWIS, J. B. (1964). Feeding and digestion in the tropical sea urchin *Diadema antillarum* PHILIPPI. *Can. J. Zool.*, **42**, 549–557.

LINDSTEDT, K. J. (1971a). Chemical control of feeding behaviour. *Comp. Biochem. Physiol.*, **39A**, 553–581.

LINDSTEDT, K. J. (1971b). Biphasic feeding response in a sea anemone: Control by asparagine and glutathione. *Science, N.Y.*, **173**, 333–334.

LINDSTEDT, K. J., MUSCATINE, L. and LENHOFF, H. M. (1968). Valine activation of feeding in the sea anemone *Boloceroides*. *Comp. Biochem. Physiol.*, **26**, 567–572.

LINTON, S. N. and CAMPBELL, J. W. (1962). Studies of urea cycle enzymes in the terrestrial snail *Otala bactea*. *Archs Biochem. Biophys.*, **97**, 360–369.

LITTLE, C. (1965). The formation of urine by the prosobranch gastropod mollusc *Viviparus viviparus*. *Linn. J. exp. Biol.*, **35**, 260–279.

LITTLE, C. and GUPTA, B. J. (1968). Pogonophora: Uptake of dissolved nutrients. *Nature, Lond.*, **218**, 873–874.

LITTLEPAGE, J. L. (1964). Seasonal variations in lipid content of two antarctic marine Crustacea. In *SCAR Symposium on Antarctic Biology*, 1962. *Antarctique Actual. scient. ind.*, **1312**, 463–470.

LIU, D. W. H., KRUEGER, H. and WANG, C. (1970). Catabolic pathways for glucose in the cichlid fish *Cichlasoma bimaculatum*. *Comp. Biochem. Physiol.*, **36**, 173–181.

LONG, G. L. and KAPLAN, N. O. (1968). D-Lactate specific pyridine nucleotide lactate dehydrogenase in animals. *Science, N.Y.*, **162**, 685–686.

LONGBOTTOM, M. R. (1968). Nutritional factors affecting the distribution of *Arenicola marina* L. Ph.D. thesis, University of London.

LOOMIS, W. F. (1955). Glutathione control of specific feeding reactions of *Hydra*. *Ann. N.Y. Acad. Sci.*, **62**, 209–228.

LOOSANOFF, V. L. (1949). On the food selectivity of oysters. *Science, N.Y.*, **110**, 122.

LOOSANOFF, V. L. and ENGLE, J. B. (1947). Effect of different concentrations of microorganisms on the feeding of oysters (*C. virginica*). *Fish. Bull. Fish. Wildl. Serv. U.S.*, **51**, 31–57.

LOVE, R. M. (1970). *The Chemical Biology of Fishes*, Academic Press, New York.

LOVERN, J. A. (1938). Fat metabolism in fishes. XIII. Factors influencing the composition of the fat of fishes. *Biochem. J.*, **32**, 1214 1224.

LUCAS, C. E. (1936). On certain interrelations between phytoplankton and zooplankton under experimental conditions. *J. Cons. perm. int. Explor. Mer*, **11**, 343–362.

LUND, E. J. (1957). A quantitative study of clearance of a turbid medium and feeding by the oyster. *Publs Inst. mar. Sci. Univ. Texas*, **4**, 296–312.

LUND, P. and GOLDSTEIN, L. (1969). Glutamine synthetase activity in tissue of lower vertebrates. *Comp. Biochem. Physiol.*, **31**, 205–210.

LUNGER, P. D. (1963). Fine structural aspects of digestion in a colonial hydroid. *J. Ultrastruct. Res.*, **9**, 362–380.

LUTZ, P. L. and ROBERTSON, J. D. (1971). Osmotic constituents of the coelacanth *Latimeria chalumnae* SMITH. *Biol. Bull. mar. biol. Lab., Woods Hole*, **141**, 553–560.

LYNCH, V. H. and CALVIN, M. (1952). Carbon dioxide fixation by micro-organisms. *J. Bact.*, **63**, 525–531.

McCULLOUGH, C. B. (1965). Pace maker interaction in *Hydra*. *Am. Zool.*, **5**, 499–504.

MACGINITIE, G. (1939). The method of feeding of tunicates. *Biol. Bull. mar. biol. Lab., Woods Hole*, **77**, 443–447.

MACGINITIE, G. E. (1941). On the method of feeding of four pelecypods. *Biol. Bull. mar. biol. Lab., Woods Hole*, **80**, 18–25.

MACGINITIE, G. E. and MACGINITIE, N. (1949). *Natural History of Marine Animals*, McGraw-Hill, New York.

MACKIE, G. O. and BOAG, D. A. (1963). Fishing, feeding, and digestion in siphonophores. *Pubbl. Staz. zool. Napoli*, **33**, 178–196.

MACKIE, G. O. and JOSEPHSON, R. K. (1965). Multiple pace makers and the behaviour of the hydroid *Tubularia*. *J. exp. Biol.*, **43**, 293.

McLAUGHLIN, J. A. and ZAHL, P. (1966). Endozoic algae. In S. Henry (Ed.), *Symbiosis*, Vol. I. Academic Press, New York, pp. 257–297.

McLUSKY, D. S. (1970). Osmoregulation in *Corophium volutator*. The effect of starvation. *Comp. Biochem. Physiol.*, **35**, 303–306.

McWHINNIE, M. A. and JOHANNECK, R. (1966). Utilization of inorganic and organic carbon compounds by Antarctic zooplankton. *Antarct. Jl U.S.*, 1–210.

MÄRKEL, K. (1965). Modell-Untersuchungen zur Klärung der Arbeitsweise der Gastropodenradula. *Zool. Anz.*, **28** (Suppl.), 232–243.

MAETZ, J. and GARCIA ROMEU, F. (1964). The mechanism of sodium and chloride uptake by the gills of a fresh water fish *Carassius auratus*. III. Evidence for NH^+/Na^+ and HCO_3^-/Cl^- exchanges. *J. gen. Physiol.*, **47**, 1209–1227.

MAETZ, J. and SKADHAUGE, E. (1968). Drinking rates and gill ionic turnover in relation to external salinities in the eel. *Nature, Lond.*, **217**, 371–373.

MAGALHAES, H. (1948). An ecological study of the genus *Busycon* at Beaufort, North Carolina. *Ecol. Monogr.*, **18**, 379–409.

MAGGENTI, A. R. (1971). A review of the family Cucullanidae (COBBOLD, 1864) and the genus *Bulbodacnitis* (LANE, 1916) with a description of *Bulbodacnitis ampullastoma* sp. (Nematoda: Cucullanidae) from *Salmo gairdneri* (RICHARDSON). *Proc. helminth. Soc. Wash.*, **38**, 80–85.

MAGNUSON, J. J. (1969). Digestion and food consumption by the skipjack tuna (*Katsuwonus pelamis*). *Trans. Am. Fish. Soc.*, **98**, 379–392.

MANDERSCHEID, H. (1933). Uber die Harnstoffbildung bei den Wirbeltieren. *Biochem. Z.*, **263**, 245–249.

MANGUM, C. P. and COX, D. O. (1971). Analysis of feeding response in the onuphid polychaete *Diopatra cuprea* (BOSC). *Biol. Bull. mar. biol. Lab., Woods Hole*, **140**, 215–229.

MANNING, R. B. and KUMPF, H. E. (1959). Preliminary investigation of the fecal pellets of certain invertebrates of the South Florida area. *Bull. mar. Sci. Gulf Caribb.*, **9**, 291–309.

MANSOUR-BEK, J. J. (1934). Protein digestion in *Murex*. *Z. vergl. Physiol.*, **20**, 343–369.

MANSOUR-BEK, J. J. (1946). The digestive enzymes of *Tridacna elongata* LAM. and *Pinctada vulgaris* L. *Proc. Egypt. Acad. Sci.*, **1**, 13–20.

MARDSON, J. T. (1966). The coelomocytes of *Hermodice carunculata* (Polychaeta: Amphino-

midae) in relation to digestion and excretion. *Can. J. Zool.*, **44**, 377–389.

MARISCAL, R. N. (1970). The nature of the symbiosis between Indo-Pacific anemone fishes and sea anemones. *Mar. Biol.*, **6**, 58–65.

MARISCAL, R. N. and LENHOFF, H. M. (1968). The chemical control of feeding behaviour in *Cyphastrea ocellina* and some other Hawaiian corals. *J. exp. Biol.*, **49**, 689–699.

MARKERT, C. L. and MASUI, Y. (1969). Lactate dehydrogenase isoenzymes of the penguin (*Pygoscelis adeliae*). *J. exp. Zool.*, **172**, 121–146.

MARTIN, A. L. (1966). Feeding and digestion in two intertidal gammarids *Marinogammarus obtusatus* and *M. pirloti*. *J. Zool., Lond.*, **148**, 515–525.

MARTIN, A. W. and HARRISON, F. M. (1966). Excretion. In K. M. Wilbur and C. M. Yong (Eds), *Physiology of Mollusca*, Vol. II. Academic Press, New York. pp. 353–386.

MARTIN, A. W., STEWART, D. M. and HARRISON, F. M. (1965). Urine formation in the pulmonate land snail *Achatina fulica*. *J. exp. Biol.*, **42**, 99–123.

MASSARO, E. J. (1970). Horseshoe crab lactate dehydrogenase. *Science, N.Y.*, **167**, 994–996.

MASUMOTO, S. and HIBINO, H. (1932). The difference according to sex in the chemical composition of *Ostrea gigas*. *J. Sci. Hiroshima Univ.*, **A2**, 155–158.

MATHEWSON, R. F. and HODGSON, E. S. (1972). Klinotaxis and rheotaxis in orientation of sharks toward chemical stimuli. *Comp. Biochem. Physiol.*, **42A**, 79–84.

MATTISON, A. G. M. (1959). *Ark. Zool.*, **12**, 149.

MAYNARD, A. L. and LOOSLI, K. J. (1962). *Animal Nutrition*, 6th ed., McGraw-Hill, New York.

MAYR, J. (1955). Regulation of energy intake and the body weight: the glucostatic theory and the lipostatic hypothesis. *Ann. N.Y. Acad. Sci.*, **63**, 15–43.

MAYZAUD, P. (1973). Respiration and nitrogen excretion of zooplankton. II. Studies of the metabolic characteristics of starved animals. *Mar. Biol.*, **21**, 19–28.

MEEUSE, B. J. D. (1956). Free sulphuric acid in the brown alga *Desmarestia*. *Biochem. biophys. Acta*, **19**, 372–374.

MENGE, B. A. (1972). Foraging strategy of a starfish in relation to actual prey availability and environmental predictability. *Ecol. Monogr.*, **42**, 25–50.

MENZEL, D. W. (1959). Utilization of algae for growth by the angel fish. *J. Cons. perm. int. Explor. Mer*, **24**, 308–313.

MENZEL, D. W. (1960). Utilization of food by a Bermuda reef fish, *Epinephelus guttatus*. *J. Cons. perm. int. Explor. Mer*, **25**, 216–222.

MENZEL, D. W. (1967). Particulate organic carbon in the deep sea. *Deep Sea Res.*, **14**, 229–238.

MENZEL, R. W. (1955). Some phases of biology of *Ostrea equistris* SAY and a comparison with *Crassostrea virginica* (GMELIN). *Publs Inst. mar. Sci. Univ. Texas*, **3**, 69–153.

MERRETT, T. G., BAR-ELI, E. and VUNAKIS, H. VAN (1969). Pepsinogen A, C and D from the smooth dogfish. *Biochemistry*, **8**, 3696–3702.

METTRICK, D. F. and JENNINGS, J. B. (1969). Nutrition and chemical composition of rhabdocoel turbellarian *Syndesmis franciscana*, with notes on the taxonomy of *S. antillarum*. *J. Fish. Res. Bd Can.*, **26**, 2669–2679.

MICALLEF, H. (1966). The ecology and behaviour of selected intertidal gastropods. Ph.D. thesis, University of London.

MICALLEF, H. and BANNISTER, W. H. (1967). Aerial and aquatic oxygen consumption of *Monodonta turbinata* (Mollusca, Gastropoda). *J. Zool.*, **151**, 479–482.

MOLINS, L. R. and BESADA RIAL, J. R. (1959). Chemical studies on *Mytilus edulis* of the Vigo estuary. *Boln Inst. esp. Oceanogr.*, **87**, 29.

MOLLITOR, A. (1937). Beiträge zur Untersuchung des Exkretesstoffwechsels und der Exkretion von *Eriocheir sinensis*. *Zool. Jb.* (*Abt. allg. Zool. Physiol. Tiere*), **57**, 323–354.

MONAKOV, A. V. and SOROKIN, YU. I. (1960). Experimental study of feeding of *Daphnia* by means of the method ^{14}C. (Russ.) *Dokl. Akad. Nauk SSSR.*, **135**, 1516–1518. (English Transl. in *Dokl.* (*Proc.*) *Acad. Sci. USSR.*, **135**, 925–926, 1961.)

MOORE, H. B. (1931). The muds of the Clyde Sea area. III. Chemical and physical conditions, rate and nature of sedimentation, and fauna. *J. mar. biol. Ass. U.K.*, **17**, 325–358.

MOORE, H. B. and MCPHERSON, B. F. (1965). A contribution to the study of the productivity of the urchins *Tripneustes esculentus* and *Lytechinus variegatus*. *Bull. mar. Sci.*, **15**, 855–871.

MOORE, H. J. (1971). The structure of the latero-frontal cirri on gills of certain lamellibranch

molluscs and their role in suspension feeding. *Mar. Biol.*, **11**, 23–27.

MORRIS, D. L. (1948). Quantitative determination of carbohydrates with Dreywood's anthrone reagent. *Science, N.Y.*, **107**, 254–255.

MORRIS, R. (1960). General problems of osmoregulation with special reference to cyclostomes. *Symp. Zool. Soc. Lond.*, **1**, 1–16.

MORRIS, R. (1965). Studies on salt and water balance in *Myxine glutinosa* L. *J. exp. Biol.*, **42**, 359–371.

MORTON, B. S. (1969). Studies on the biology of *Dreissena polymorpha* PALL. II. *Proc. malac. Soc. Lond.*, **38**, 401–414.

MORTON, B. S. (1970). The tidal rhythm and rhythm of feeding and digestion in *Cardium edule*. *J. mar. biol. Ass. U.K.*, **50**, 499–512.

MORTON, J. E. (1952). The role of crystalline style. *Proc. malac. Soc. Lond.*, **29**, 85–92.

MORTON, J. E. (1956). The tidal rhythm and action of the digestive system of the lamellibranch, *Lasaea rubra*. *J. mar. biol. Ass. U.K.*, **35**, 563–586.

MORTON, J. E. (1959). The habits and feeding organs of *Dentalium entalis*. *J. mar. biol. Ass. U.K.*, **38**, 225–238.

MORTON, J. E. (1960). The functions of the gut in ciliary feeders. *Biol. Rev.*, **35**, 92–140.

MORTON, J. E., BONEY, A. D. and CORNER, E. D. S. (1957). The adaptations of *Lasaea rubra* (MONTAGU), a small intertidal lamellibranch. *J. mar. biol. Ass. U.K.*, **36**, 383–405.

MOTAIS, R. and MAETZ, J. (1965). Comparaison des échanges de sodium chez un téléostéen euryhalin (le flet) et un téléostéen stenohalin (le serran) en eau de mer. Importance relative du tube digestif et de la branchie dans ces échanges. *C.r. hebd. Séanc. Acad. Sci., Paris*, **261**, 532–535.

MÜLLER, M. (1967). Digestion. In M. Florkin and B. T. Scheer (Eds), *Chemical Zoology*, Vol. I. Academic Press, New York. pp. 351–380.

MULLIN, M. M. (1963). Some factors affecting the feeding of marine copepods of the genus *Calanus*. *Limnol. Oceanogr.*, **10**, 239–250.

MULLIN, M. M. (1965a). Size fractionation of particulate organic carbon in the surface waters of the western Indian Ocean. *Limnol. Oceanogr.*, **10**, 459–462.

MULLIN, M. M. (1965b). Size fractionation of particulate organic carbon in the surface waters of the western Indian Ocean: Addenendum. *Limnol. Oceanogr.*, **10**, 610–611.

MULLINS, L. J. (1950). Osmotic regulation in fish as studied with radioisotopes. *Acta physiol. scand.*, **21**, 303–314.

MUNDAY, K. A. and POAT, P. C. (1971). Respiration and energy metabolism in Crustacea. In M. Florkin and B. T. Scheer (Eds), *Chemical Zoology*, Vol. VI. Academic Press, New York. pp. 191–211.

MURDOCK, G. R. and LENHOFF, H. M. (1968). Alcohol soluble proteins: their formation and assimilation during intracellular digestion in *Hydra littoralis* and *Aiptasia* sp. *Comp. Biochem. Physiol.*, **26**, 963–970.

MUSCATINE, L. (1961). Symbiosis in marine and fresh water coelenterates. In H. M. Lenhoff and W. F. Loomis (Eds), *Biology of Hydra*. University of Miami Press, Coral Cables. pp. 255–268.

MUSCATINE, L. (1965). Symbiosis of *Hydra* and algae. III. Extracellular products of algae. *Comp. Biochem. Physiol.*, **16**, 77–92.

MUSCATINE, L. (1967). Glycerol excretion by symbiotic algae from corals and *Tridacna* and its control by the host. *Science, N.Y.*, **156**, 519.

MUSCATINE, L. and CERNICHIARI, E. (1969). Assimilation of photosynthetic products of zooxanthellae by a reef coral. *Biol. Bull. mar. biol. Lab., Woods Hole*, **137**, 506–523.

MUSCATINE, L. and HAND, C. (1958). Direct evidence for transfer of materials from symbiotic algae to the tissues of a coelenterate. *Proc. natn. Acad. Sci. U.S.A.*, **44**, 1259–1263.

MUSCATINE, L., KARAKASHIAN, S. J., and KARAKASHIAN, M. W. (1967). Soluble extracellular products of algae symbiotic with a ciliate, a sponge and mutant *Hydra*. *Comp. Biochem. Physiol.*, **20**, 1–12.

MUSCATINE, L. and LENHOFF, H. M. (1963). Symbiosis: on the role of algae symbiotic with *Hydra*. *Science, N.Y.*, **142**, 956–958.

MUSCATINE, L. and LENHOFF, H. M. (1965). Symbiosis of *Hydra* and algae. II. Effects of

limited food and starvation of symbiotic and aposymbiotic *Hydra*. *Biol. Bull. mar. biol. Lab., Woods Hole*, **129**, 316–328.

MUSCATINE, L., POOL, R. R. and CERNICHIARI, E. (1972). Some factors influencing selective release of soluble organic material by zooxanthellae from reef corals. *Mar. Biol.*, **13**, 298–308.

MUSTAFA, T. and HOCHACHKA, P. W. (1971). Catalytic and regulatory properties of pyruvate kinases in tissues of a marine bivalve. *J. biol. Chem.*, **246**, 3196–3203.

MUSTAFA, T. and HOCHACHKA, P. W. (1973a). Enzymes in facultative anaerobiosis of molluscs: II. Basic catalytic properties of phosphoenol-pyruvate carboxykinase in oyster adductor muscle. *Comp. Biochem. Physiol.*, **45B**, 639–656.

MUSTAFA, T. and HOCHACHKA, P. W. (1973b). Enzymes in facultative anaerobiosis of molluscs: III. Phosphoenol-pyruvate carboxykinase and its role in aerobic-anaerobic transition. *Comp. Biochem. Physiol.*, **45B**, 657–668.

MYERS, F. L. and NORTHCOTE, D. H. (1959). The partial purification and some properties of a cellulase from *Helix pomatia*. *Biochem. J.*, **71**, 749–756.

NAGAI, Y. and NAGAI, S. (1973). Feeding factors for the sea anemone *Anthopleura midorii*. *Mar. Biol.*, **18**, 55–60.

NAGASE, G. (1964). Contribution to the physiology of digestion in *Tilapia mossambica* PETERS: digestive enzymes and the effects of diets on their activity. *Z. vergl. Physiol.*, **49**, 270–284.

NAIR, N. B. (1955a). The digestive enzymes of *Bankia indica* NAIR. *Curr. Sci.*, **24**, 126–127.

NAIR, N. B. (1955b). Cellulase activity of the crystalline style of the wood-boring pelecypod *Bankia indica* NAIR. *Curr. Sci.*, **24**, 201.

NAIR, N. B. (1956a). Physiology of digestion in *Bankia indica*. The enzymatic activity of the digestive diverticula. *J. Madras Univ.*, **24**, 599–627.

NAIR, N. B. (1956b). The path of enzymic hydrolysis of cellulose in the wood-boring pelecypod *Bankia indica* NAIR. *J. scient. ind. Res.* (*Sect. B*), **15**, 155–156.

NAIR, N. B. (1957). Physiology of digestion in *Bankia indica*. The enzyme activity of the crystalline style. *J. scient. ind. Res.* (*Sect. C*), **16**, 39–41.

NEEDHAM, A. E. (1955). Factors affecting nitrogenous excretion in Crustacea. *Physiologia comp. Oecol.*, **4**, 209–239.

NEEDHAM, A. E. (1964). *The Growth Process in Animals*, Pitman, London.

NEEDHAM, J. (1931). *Chemical Embryology*, Vol. II, Cambridge University Press, London.

NEEDHAM, J. (1935). Problems of nitrogen catabolism in invertebrates. II. Correlation between uricotelic metabolism and habitat of the phylum Mollusca. *Biochem. J.*, **29**, 238–251.

NEEDHAM, J. (1938). Contributions of chemical physiology to the problem of reversibility in evolution. *Biol. Rev.*, **13**, 225–251.

NEFF, S. S. and MUSACCHIA, X. J. (1967). Intestinal absorption of L-leucine *in vitro* in fish. *Comp. Biochem. Physiol.*, **21**, 337–343.

NELSON, D. R. and JOHNSON, R. H. (1972). Acoustic attraction of Pacific reef sharks: effect of pulse intermittency and variability. *Comp. Biochem. Physiol.*, **42A**, 85–95.

NELSON, T. C. (1960). The feeding mechanism of the oyster. II. On the gills and palps of *Ostrea edulis*, *Crassostrea virginica* and *C. angulata*. *J. Morph.*, **107**, 163–191.

NEWELL, B. S. (1953). Cellulolytic activity in the lamellibranch crystalline style. *J. mar. biol. Ass. U.K.*, **32**, 491–495.

NEWELL, G. E. (1964). Physiological aspects of the ecology of intertidal molluscs. In K. M. Wilbur and C. M. Yonge (Eds), *Physiology of Mollusca*, Vol. I. Academic Press, New York. pp. 59–82.

NEWELL, R. C. (1965). The role of detritus in the nutrition of two marine deposit feeders, the prosobranch *Hydrobia ulvae* and the bivalve *Macoma balthica*. *Proc. zool. Soc. Lond.*, **143**, 25–45.

NEWELL, R. C. (1970). *Biology of Intertidal Animals*, Lagos Press, London.

NEWELL, R. C., PYE, V. I. and AHSANULLAH, M. (1971). Factors affecting the feeding rate of the winkle *Littorina littorea*. *Mar. Biol.*, **9**, 138–144.

NICOL, E. A. T. (1930). The feeding mechanism, formation of the tube, and physiology of digestion in *Sabella pavonina*. *Trans. R. Soc. Edinb.*, **56**, 537–596.

NICOL, J. A. C. (1952). Autonomic nervous systems in lower chordates. *Biol. Rev.*, **27**, 1–48.

NICOL, J. A. C. (1967). *The Biology of Marine Animals*, Pitman, London.

NILSSON, A. and FÄNGE, R. (1969a). Adreninergic and cholinergic vagal effects of on the stomach of a teleost (*Gadus morhua*). *Comp. Biochem. Physiol.*, **30**, 691–694.

NILSSON, A. and FÄNGE, R. (1969b). Digestive proteases in the holocephalian fish *Chimaera monstrosa* (L.). *Comp. Biochem. Physiol.*, **31**, 147–165.

NILSSON, A. and FÄNGE, R. (1970). Digestive properties in the cyclostome *Myxine glutinosa* L. *Comp. Biochem. Physiol.*, **32**, 237–250.

NISBET, R. H. (1953). The structure and function of the buccal mass of some gastropod molluscs. I. *Monodonta lineata* (DE COSTA). Ph.D. thesis, University of London.

NOLFI, J. R. (1970). Biosynthesis of uric acid in the tunicate *Molgula manhattensis* with general scheme for the function of stored purines in animals. *Comp. Biochem. Physiol.*, **35**, 827–842.

NOLTE, A., BREUCKER, H. and KUHLMANN, D. (1965). Cytosomale Einschlüsse und Neurosekret im Nervengewebe von Gastropoden. *Z. Zellforsch.*, **68**, 1–27.

NORTH, B. B. and STEPHENS, G. C. (1967). Uptake and assimilation of amino acids by *Platymonas*. *Biol. Bull. mar. biol. Lab., Woods Hole*, **133**, 391–400.

NORTH, B. B. and STEPHENS, G. C. (1969). Dissolved amino acids and *Platymonas* nutrition. *Int. Seaweed Symp.*, **6**, 263–273.

NORTH, W. J., STEPHENS, G. C. and NORTH, B. B. (1972). Marine algae and their relations to pollution problems. In M. Ruivo (Ed.), *Marine Pollution and Sea Life*. Fishing News (Books) Ltd., London. pp. 330–340.

O'DOHERTY, P. J. A. and FELTHAM, L. A. W. (1971). Glycolysis and gluconeogenesis in the giant scallop *Placopecten magellanicus* (GMELLIN). *Comp. Physiol. Biochem.*, **38B**, 543–551.

ODUM, E. P. (1971). *Fundamentals of Ecology*, W. B. Saunders, Philadelphia.

ODUM, W. E. (1970). Utilization of the direct grazing and plant detritus food chains by the striped mullet *Mugil cephalus*. In J. H. Steele (Ed.), *Marine Food Chains*. University of California Press, Berkeley. pp. 222–240.

OIDE, M. and UTIDA, S. (1968). Changes in intestinal absorption and renal excretion of water during adaptation to sea water in the Japanese eel. *Mar. Biol.*, **1**, 102–106.

OLDFIELD, E. (1961). The functional morphology of *Kellia suborbicularis* (MONTAGU), *Montaguta ferrugionosa* (MONTAGU) and *M. substriata* (MONTAGU) (Mollusca, Lamellibranchiata). *Proc. malac. Soc. Lond.*, **34**, 255–295.

OORD, A. VAN DEN (1966). The biosynthesis of the emulsifiers of the crab *Cancer pagurus* L. *Comp. Biochem. Physiol.*, **17**, 715–718.

OORD, A. VAN DEN, DANIELSON, H. and RYHAGE, R. (1964). Absence of bile salts in the gastric juice of a crab. *Nature, Lond.*, **203**, 301.

OSBORNE, P. J. and MILLER, A. T., JR. (1962). Uptake and intracellular digestion of protein (peroxidase) in planarians. *Biol. Bull. mar. biol. Lab., Woods Hole*, **123**, 589–596.

OSCHMAN, J. L. (1966). Development of the symbiosis of *Convoluta roscoffensis* GRAFF and *Platymonas* sp. *J. Phycol.*, **2**, 105–111.

OSCHMAN, J. L. and GRAY, P. (1964). An electron microscope study of *Convoluta roscoffensis* and its endosymbiotic algae. *Am. Zool.*, **4**, 291.

OWEN, G. (1956). Observations on the stomach and digestive diverticula of the Lamellibranchia. II. The Nuclidae. *Q. Jl microsc. Sci.*, **97**, 541–567.

OWEN, G. (1966a). Feeding. In K. M. Wilbur and C. M. Yonge (Eds), *Physiology of Mollusca*, Vol. II. Academic Press, New York. pp. 1–51.

OWEN, G. (1966b). Digestion. In K. M. Wilbur and C. M. Yonge (Eds), *Physiology of Mollusca*, Vol. II. Academic Press, New York. pp. 52–96.

OWEN, T. G. and WIGGS, A. J. (1971). Thermal compensation in the stomach of the brooktrout (*Salvelinus fontinalis* MITCHELL). *Comp. Biochem. Physiol.*, **40B**, 465–473.

PAFFENHÖFER, G.-A. (1968). Nahrungsaufnahme, Stoffumsatz und Energiehaushalt des marinen Hydroidpolypen *Clava multicornis*. *Helgoländer wiss. Meeresunters.*, **18**, 1–44.

PAFFENHÖFER, G.-A. (1970). Cultivation of *Calanus helgolandicus* under controlled conditions. *Helgoländer wiss. Meeresunters.*, **20**, 346–359.

PAFFENHÖFER, G.-A. (1971). Grazing and ingestion rates of nauplii, copepodids and adults of the marine planktonic copepod *Calanus helgolandicus*. *Mar. Biol.*, **11**, 286–298.

Paffenhöfer, G.-A. and Strickland, J. D. H. (1970). A note on feeding of *Calanus helgolandicus* on detritus. *Mar. Biol.*, **5**, 97–99.

Paine, R. T. (1963). Food recognition and predation on opisthobranchs by *Navanax inermis*. *Veliger.*, **6**, 1–8.

Paine, R. T. (1965). Natural history, limiting factors and energetics of the opisthobranch *Navanax inermis*. *Ecology*, **46**, 603–619.

Paine, R. T. (1971a). Energy flow in a natural population of the herbivorous gastropod *Tegula funebralis*. *Limnol. Oceanogr.*, **16**, 86–98.

Paine, R. T. (1971b). The measurement and application of the calorie to ecological problems. *A. Rev. Ecology Syst.*, **2**, 145–164.

Paine, R. T. and Vadas, R. L. (1969). Calorific values of benthic marine algae and their postulated relation to invertebrate food preference. *Mar. Biol.*, **4**, 79–86.

Paintal, A. S. (1954). A study of gastric stretch receptors. Their role in the peripheral mechanism of satiation of hunger and thirst. *J. Physiol., Lond.*, **126**, 271–285.

Palay, S. L. and Karlin, L. J. (1959). An electron microscopic study of the intestinal villus. I. The fasting animal. *J. biophys. biochem. Cytol.*, **5**, 363–372.

Paloheimo, J. E. and Dickie, L. M. (1966). Food and growth of fishes. II. Effects of food and temperature on the relation between metabolism and body weight. *J. Fish. Res. Bd Can.*, **23**, 869–908.

Paloheimo, J. E. and Dickie, L. M. (1970). Production and food supply. In J. H. Steele (Ed.), *Marine Food Chains*. University of California Press, Berkeley and Oliver and Boyd, Edinburgh. pp. 499–527.

Pandian, T. J. (1967a). Intake, digestion, absorption, and conversion of food in the fishes *Megalops cyprinoides* and *Ophiocephalus striatus*. *Mar. Biol.*, **1**, 16–32.

Pandian, T. J. (1967b). Transformation of food in the fish *Megalops cyprinoides*. I. Influence of quality of food. *Mar. Biol.*, **1**, 60–64.

Pandian, T. J. (1967c). Changes in chemical composition and caloric content of developing eggs of the shrimp *Crangon crangon*. *Helgoländer wiss. Meeresunters.*, **16**, 216–224.

Pandian, T. J. (1967d). Transformation of food in the fish *Megalops cyprinoides*. II. Influence of quantity of food. *Mar. Biol.*, **1**, 107–109.

Pandian, T. J. (1968). Nahrungsaufnahme und Nahrungskonversion bei der Kliesche *Limanda limanda*. Inaugural Dissertation, University of Kiel.

Pandian, T. J. (1969a). Yolk utilization in the gastropod *Crepidula fornicata*. *Mar. Biol.*, **3**, 117–121.

Pandian, T. J. (1969b). Feeding habits of the fish *Megalops cyprinoides* Broussonet in the Cooum backwaters, Madras. *J. Bombay nat. Hist. Soc.*, **65**, 569–580.

Pandian, T. J. (1970a). Intake, and conversion of food in the fish *Limanda limanda* exposed to different temperatures. *Mar. Biol.*, **5**, 1–17.

Pandian, T. J. (1970b). Ecophysiological studies on the developing eggs and embryos of the European lobster *Homarus gammarus*. *Mar. Biol.*, **5**, 154–167.

Pandian, T. J. (1970c). Yolk utilization and hatching time in the Canadian lobster *Homarus americanus*. *Mar. Biol.*, **7**, 249–254.

Pandian, T. J. (1970d). Cleidoic properties of marine demersal eggs. *Indian J. expl Biol.*, **8**, 340–342.

Pandian, T. J. (1970e). Feeding and reproductive cycles of the fish *Mystus gulio* in the Cooum backwaters Madras. *Indian. J. Fish.*, **13**, 322–333.

Pandian, T. J. (1972). Egg incubation and yolk utilization in the isopod *Ligia oceanica*. *Proc. natn. Inst. Sci. India*, **38B**, 430–441.

Pandian, T. J. and Delvi, M. R. (1973). Energy extraction efficiency in some chosen insects. *Oecologia (Berl.)*, **13**, 397–401.

Pandian, T. J. and Raghuraman, T. (1972). Effects of feeding rate on conversion efficiency and chemical composition of the fish *Tilapia mossambica*. *Mar. Biol.*, **12**, 129–136.

Pandian, T. J. and Schumann, K. H. (1967). Chemical composition and calorific content of the egg and zoea of the hermit crab *Eupagurus bernhardus*. *Helgoländer wiss. Meeresunters.*, **16**, 225–230.

Pantin, C. F. A. (1942). The excitation of nematocysts. *J. exp. Biol.*, **19**, 294–310.

PARDY, R. L. and LENHOFF, H. M. (1968). The feeding biology of the gymnoblastic hydroid *Pennaria tiarella*. *J. exp. Zool.*, **168**, 197–202.

PARKE, M. and MANTON, I. (1967). The specific identity of algal symbiont in *Convoluta roscoffensis*. *J. mar. biol. Ass. U.K.*, **47**, 445–464.

PARKER, G. H. (1910). Reactions of sponges. *J. exp. Zool.*, **8**.

PARKER, G. H. (1919). *The Elementary Nervous System*, Lippincott, London.

PARNAS, I. (1961). The cellulolytic activity in the snail *Levantina hierosolyma* BOISS. *J. cell. comp. Physiol.*, **58**, 195–201.

PARNAS, I., HERSHKOVITZ, G. and REICH, K. (1964). Amylolytic activity in axenic cultures of the amoeba *Mayorella palestinensis* REICH. *Physiol. Zoöl.*, **37**, 65–76.

PARSONS, T. R., LEBRASSEUR, R. J., FULTON, J. D. and KENNEDY, O. D. (1969). Production studies in the strait of Georgia. II. Secondary production under the Fraser River plume, February to May 1967. *J. exp. mar. Biol. Ecol.*, **3**, 39–50.

PARSONS, T. R. and SEKI, H. (1970). Importance and general implications of organic matter in aquatic environments. *Occ. Publ. Inst. mar. Sci. Univ. Alaska*, **1**, 1–27.

PARSONS, T. R., STEPHENS, K. and STRICKLAND, J. D. H. (1961). On the chemical composition of eleven species of marine phytoplankton. *J. Fish. Res. Bd Can.*, **18**, 1001–1016.

PASSANO, L. M. and MCCULLOUGH, C. B. (1964). Coordinating systems and behaviour in *Hydra*. I. Pacemaker system of the periodic contractions. *J. exp. Biol.*, **41**, 643–664.

PASSANO, L. M. and MCCULLOUGH, C. B. (1965). Coordinating systems and behaviour in *Hydra*. II. The rhythmic potential system. *J. exp. Biol.*, **42**, 205–231.

PASTEELS, J. J. (1967). Absorption et arthrocytose par l'epithelium branchial de *Mytilus edulis*. *C. hebd. Séanc. Acad. Sci., Paris*, **264**, 2505–2507.

PASTEELS, J. J. (1969). Excretion de phosphatase acide par les cellules mucipares de la branchie de *Mytilus edulis* L.: Etudean microscope eletronique. *Z. Zellforsch. mikrosk. Anat.*, **102**, 594–600.

PASTEELS, J. J. (1971a). Phosphatase acide et polarité golgienne dans les cellules absorbantes de la branchie de *Mytilus edulis*. *Histochemie*, **28**, 296–304.

PASTEELS, J. J. (1971b). Activité phosphatasique acide dans les cloisons intercellulaires de l'epithelium branchial de *Mytilus edulis* (Mollusque Lamellibranche). *Arch. Biol., Liége*, **82**, 567–570.

PAVLOV, D. S. (1962). On the availability of the young of *Atherina mochonpontica* EICHW. for *Samaris samaris* L. under different conditions of illumination. (Russ; Engl. summary.) *Zool. Zh.*, **41**, 948–950.

PEARSE, V. B. and MUSCATINE, L. (1971). Role of symbiotic algae (Zooxanthellae) in coral calcification. *Biol. Bull. mar. biol. Lab., Woods Hole*, **141**, 350–363.

PEASE, H. D. (1932). The oyster: modern science comes to the support of an ancient food. *J. chem. Educ.*, **9**, 1673–1712.

PEQUIGNAT, E. (1966a). 'Skin digestion' and epidermal absorption in irregular and regular urchins and their probable relation to the outflow of spherule-coelomocytes. *Nature, Lond.*, **210**, 397–399.

PEQUIGNAT, E. (1966b). Observations sur les cellules mobiles d'*Echinocardium* et de *Psammechinus*. *Bull. Soc. linn. Normandie* (*10e Ser.*), **7**, 22–236.

PEQUIGNAT, E. (1970). On the biology of *Echinocardium cordatum* (PENNANT) of the Seine Estuary. *Forma Functio*, **2**, 121–168.

PEQUIGNAT, E. (1972). Some new data on skin-digestion and absorption in urchins and sea stars (*Asterias* and *Henricia*). *Mar. Biol.*, **12**, 28–41.

PEQUIGNAT, E. (1973). A kinetic and autoradiographic study of the direct assimilation of amino acids and glucose by organs of the mussel *Mytilus edulis*. *Mar. Biol.*, **19**, 227–244.

PEQUIGNAT, E. and PUJOL, J. P. (1968). Absorption de proline tritiée par *Psammechinus* et sa fixation sur le collagene. *Bull. Soc. linn. Normandie* (*10e Ser.*), **9**, 209–219.

PEQUIGNAT, E. and TIFFON, Y. (1967). Mise en evidence d'un activité amylolytique cutanée chez les Echinides. *C.r. hebd. Séanc. Acad. Sci., Paris*, **264**, 3014–3115.

PÉRÈS, J. (1950). Recherches sur les pedicellaires glandulaires de *Sphaerechinus*. *Archs Zool. exp. gén.*, **86** (Notes Revue **3**).

PETIPA, T. S. (1959). Feeding of the copepod *Acartia clausi*. (Russ.) *Trudӯ sevastopol'. biol. Sta.*,

11, 72–100.

Pfeffer, W. (1897/1904). *Pflanzenphysiologie*, Vols I and II, 2nd ed., Fischer, Leipzig.

Phifer, K. (1960a). Permeation and membrane transport in animal parasites. The absorption of glucose by *Hymenolepis diminuta*. *J. Parasit.*, **46**, 51–62.

Phifer, K. (1960b). Permeation and membrane transport in animal parasites. Further observations on the uptake of glucose by *H. diminuta*. *J. Parasit.*, **46**, 137–144.

Phifer, K. (1960c). Permeation and membrane transport in animal parasites. On the mechanism of glucose uptake by *Hymenolepis diminuta*. *J. Parasit.*, **46**, 145–152.

Pickford, G. E. and Grant, F. B. (1967). Serum osmolarity in the coelacanth *Latimeria chalumnae*: urea retention and ion regulation. *Science, N.Y.*, **155**, 568–570.

Pintner, I. J. and Provasoli, L. (1963). Nutritional characteristics of some chrysomonads. In C. H. Oppenheimer (Ed.), *Marine Microbiology*. C. C. Thomas, Springfield, Ill. pp. 114–121.

Pintner, I. J. and Provasoli, L. (1968). Heterotrophy in subdued light of 3 *Chrysochromulina* species. *Bull. Misaki mar. biol. Inst.*, **12**, 25–31.

Pomeroy, L. R. (1959). Algal productivity in salt marshes of Georgia. *Limnol. Oceanogr.*, **4**, 386–397.

Potts, W. T. W. (1967). Excretion in the molluscs. *Biol. Rev.*, **42**, 1–41.

Potts, W. T. W. (1968). Aspects of excretion in molluscs. In V. Fretter (Ed.), *Studies in the Structure, Physiology and Ecology of Molluscs*. Academic Press, New York. pp. 187–192.

Potts, W. T. W. and Evans, D. (1967). Sodium and chloride balance in the killifish *Fundulus heteroclitus*. *Biol. Bull. mar. biol. Lab., Woods Hole*, **133**, 411–424.

Potts, W. T. W., Foster, M. A., Rudy, P. P. and Parry Howells, G. (1967). Sodium and water balance of the ichlid teleost *Tilapia mossambica*. *J. exp. Biol.*, **47**, 461–470.

Preston, R. L. (1970). The accumulation of amino acids by the coelomocytes of the bloodworm, *Glycera dibranchiata*. Ph.D. thesis, University of California, Irvine.

Preston, R. L. and Stephens, G. C. (1969). The accumulation of amino acids by coelomocytes of *Glycera dibranchiata*. *Am. Zool.*, **9**, 1116.

Price, K. S., Jr. (1967). Fluctuations in two osmoregulatory components, urea and sodium chloride, of the clearnose skate *Raja eglanteria* Bosc 1802. II. Upon natural variation of the salinity of the external medium. *Comp. Biochem. Physiol.*, **23**, 77–82.

Price, K. S., Jr. and Creaser, E. P., Jr. (1967). Fluctuations in two osmoregulatory components, urea and sodium chloride, of the clearnose skate *Raja eglanteria* Bosc 1802. I. Upon laboratory modification of external salinities. *Comp. Biochem. Physiol.*, **23**, 65–76.

Price, K. S., Jr. and Daiber, F. C. (1967). Osmotic environments during fetal development of dogfish *Mustelus canis* (Mitchill) and *Squalus acanthias* (Linnaeus) and some comparisons with skates and rays. *Physiol. Zoöl.*, **40**, 248–260.

Prosser, C. L. and Brown, F. A., Jr. (1961). *Comparative Animal Physiology*, W. B. Saunders, Philadelphia.

Prosser, C. L., Nystrom, R. A. and Nagae, T. (1965). Electrical and mechanical activity in intestinal muscles of several invertebrate animals. *Comp. Biochem. Physiol.*, **14**, 53–70.

Provasoli, L., Yasumasu, T. and Manton, I. (1968). Experiments on the resynthesis of symbiosis in *Convoluta roscoffensis* with different flagellate cultures. *J. mar. biol. Ass. U.K.*, **48**, 465–479.

Przylecki, St. J. (1926). La reparation et la degradation de l'acide urique chez les Invertebrates. *Archs int. Physiol.*, **27**, 159–202.

Pütter, A. (1909). *Die Ernährung der Wassertiere und der Stoffhaushalt der Gewässer*, G. Fischer, Jena.

Purchon, R. D. (1941). On the biology and relationships of the lamellibranch *Xylophagha dorsalis* (Turton). *J. mar. biol. Ass. U.K.*, **25**, 1–39.

Purchon, R. D. (1955). The structure and function of the British Pholadidae (rock-boring Lamellibranchia). *Proc. zool. Soc. Lond.*, **124**, 859–911.

Purchon, R. D. (1956). The stomach in the Protobranchia and Septibranchia (Lamellibranchia). *Proc. zool. Soc. Lond.*, **127**, 511–525.

Raghuraman, R. (1973). Studies on food intake and utilization in some fishes. Ph.D. thesis, Bangalore University.

Ragotzkie, R. A. (1959). Plankton productivity in estuarine waters of Georgia. *Publs Inst. mar. Sci. Univ. Texas*, **6**, 146–158.

Rahat, M. and Jahn, T. L. (1965). Growth of *Prymnesium parvum* in the dark; note on ichthyotoxin formation. *J. Protozool.*, **12**, 246–250.

Randall, H. G. and Evered, D. F. (1964). Amino acid uptake by living cells. I. Amino group requirement by small intestine of the rat *in vitro*. *Biochim. Biophys. Acta*, **93**, 98–105.

Randall, J. E. and Hartman, W. D. (1968). Sponge-feeding fishes of the West Indies. *Mar. Biol.*, **1**, 216–225.

Rashevsky, N. (1959). Some remarks on the mathematical theory of nutrition of fishes. *Bull. math. Biophys.*, **21**, 161–183.

Rasmont, R. (1968). Nutrition and digestion. In M. Florkin and B. T. Scheer (Eds), *Chemical Zoology*, Vol. II. Academic Press, New York. pp. 43–51.

Rasmont, R., Bouillon, J., Castiaux, P. and Vandemeersche, (1957). Structure submicroscopique de la collerette des choanocytes d'Eponges. *C.r. hebd. Séanc. Acad. Sci., Paris*, **245**, 1571–1574.

Ray, D. L. (Ed.) (1959a). *Marine Boring and Fouling Organisms*, University of Washington Press, Seattle.

Ray, D. L. (1959b). Nutritional physiology of *Limnoria*. In D. L. Ray (Ed.), *Marine Boring and Fouling Organisms*. University of Washington Press, Seattle. pp. 46–61.

Ray, D. L. and Julian, J. R. (1952). Occurrence of cellulase in *Limnoria*. *Nature, Lond.*, **169**, 32–33.

Read, C. P. (1961). The carbohydrate metabolism in animals. In A. W. Martin (Ed.), *Comparative Physiology of Carbohydrate Metabolism in Heterothermic Animals*. Washington University Press, Seattle.

Read, C. P. (1968). Intermediary metabolism. In M. Florkin and B. T. Scheer (Eds), *Chemical Zoology*, Vol. II. Academic Press, New York. pp. 327–357.

Read, L. J. (1967). Enzymes in the ornithine-urea cycle in the chimaera *Hydrolagus colliei*. *Nature, Lond.*, **215**, 1412–1413.

Read, L. J. (1968a). Ornithine-urea cycle enzymes in early embryos of the dogfish *Squalus suckleyi* and the skate *Raja binoculata*. *Comp. Biochem. Physiol.*, **24**, 669–674.

Read, L. J. (1968b). Urea and trimethylamine levels in elasmobranch embryos. *Biol. Bull. mar. biol. Lab., Woods Hole*, **135**, 537–547.

Rector, F. C., Seldin, D. W. and Copenhaver, J. H. (1955). The mechanism of ammonia excretion during ammonium chloride acidosis. *J. clin. Invest.*, **34**, 20–26.

Rector, F. C., Seldin, D. W., Roberts, A. D. and Copenhaver, J. H. (1954). Ammonia excretion and urine acidification. *Am. J. Physiol.*, **192**, 392–400.

Reddy, S. R. and Pandian, T. J. (1973). Effects of volume and shape of aquaria on the predatory efficiency of the fish *Gambusia affinis*. *Curr. Sci*, **42**, 644–645.

Rees, J., Davis, L. V. and Lenhoff, H. M. (1970). Paths and rates of food distribution in the colonial hydroid *Pennaria*. *Comp. Biochem. Physiol.*, **34**, 309–316.

Reichenow, E. (1922). Intracellular Symbioten bei blutsaugenden Milben und Egeln. *Arch. Protistenk.*, **45**, 95–116.

Reid, R. G. B. (1968). The distribution of digestive tract enzymes in lamellibranchiate bivalves. *Comp. Biochem. Physiol.*, **27**, 417–433.

Reid, R. G. B. and Dunnill, R. M. (1969). Specific and individual differences in the esterases of members of the genus *Macoma* (Mollusca: Bivalvia). *Comp. Biochem. Physiol.*, **29**, 601–610.

Reimer, A. A. (1971a). Feeding behaviour in the Hawaiian zoanthids *Palythoa* and *Zoanthus*. *Pacif. Sci.*, **25**, 512–520.

Reimer, A. A. (1971b). Chemical control of feeding behaviour in *Palythoa* (Zoanthidea, Coelenterata). *Comp. Biochem. Physiol.*, **40A**, 19–38.

Reimer, A. A. (1971c). Specificity of feeding chemoreceptors in *Palythoa* (Zoanthidea, Coellenterata). *Comp. gen. Pharm.*, **2**, 383–396.

Reimer, A. A. (1973). Feeding behaviour in the sea anemone *Calliactis polypus* (Forskäl, 1775). *Comp. Biochem. Physiol.*, **44A**, 1289–1301.

Reiser, R., Stevenson, B., Kayama, M., Choudhury, R. B. R. and Hood, D. W. (1963). The influence of dietary fatty acids and environmental temperature on the fatty acid

composition of teleost fish. *J. Am. Oil Chem. Soc.*, **40**, 507–513.

REISH, D. J. and STEPHENS, G. C. (1969). Uptake of organic material by aquatic invertebrates. V. The influence of age on the uptake of glycine-^{14}C by the polychaete *Neanthes arenaceodentata*. *Mar. Biol.*, **3**, 352–355.

REISWIG, H. M. (1971). Particle feeding in natural populations of three marine desmosponges. *Biol. Bull. mar. biol. Lab., Woods Hole*, **141**, 568–591.

REISWIG, H. M. (1972). The spectrum of particulate organic matter of shallow-bottom boundary waters of Jamaica. *Limnol. Oceanogr.*, **17**, 341–348.

RICHARD, J. D. (1968). Fish attraction with pulsed low frequency sound. *J. Fish. Res. Bd Can.*, **25**, 1441–1452.

RICHMAN, S. (1958). The transformation of energy by *Daphnia pulex*. *Ecol. Monogr.*, **28**, 273–291.

RICKER, W. E. (1946). Production and utilization of fish populations. *Ecol. Monogr.*, **16**, 373–391.

RIEDL, R. (1971). Water movement—Animals. In O. Kinne (Ed.), *Marine Ecology*, Vol. I, Environmental Factors, Part 2. Wiley, London. pp. 1123–1149.

RIEGEL, J. A. (1971). Excretion–Arthropoda. In M. Florkin and B. T. Scheer (Eds), *Chemical Zoology*, Vol. VI. Academic Press, New York. pp. 249–277.

RILEY, G. A., WANGERSKY, P. J. and HEMMERT, P. J. VAN (1964). Organic aggregates in tropical and subtropical surface waters of the North Atlantic Ocean. *Limnol. Oceanogr.*, **9**, 546–550.

RITZ, D. A. and CRISP, D. J. (1970). Seasonal changes in feeding rate of *Balanus balanoides*. *J. mar. biol. Ass. U.K.*, **50**, 223–240.

ROACH, S. K. (1968). Rhythmic muscular activity in the alimentary tract of *Arion ater* (L.). *Comp. Biochem. Physiol.*, **24**, 865–878.

ROSENBAUM, R. M. and ROLON, C. I. (1960). Intracellular digestion and hydrolytic enzymes in the phagocytes of planarians. *Biol. Bull. mar. biol. Lab., Woods Hole*, **118**, 315–323.

ROSENTHAL, H. (1969). Untersuchungen über das Beutefangverhalten bei Larven des Herings *Clupea harengus*. *Mar. Biol.*, **3**, 208–221.

ROSENTHAL, H. and PAFFENHÖFER, G.-A. (1972). On the digestion rate and calorific content of food and feces in young gar fish. *Naturwissenschaften*, **59**, 274–275.

ROTHMAN, A. H. (1959). The role of bile salts in the biology of tapeworm. II. Further observations on the effects of bile salts on metabolism. *J. Parasit.*, **45** (Suppl.), 28.

ROWETT, H. G. Q. (1946). A comparison of the feeding mechanisms of *Calma glaucoides* and *Nebaliopsis typica*. *J. mar. biol. Ass. U.K.*, **26**, 352–357.

ROZIN, P. and MAYR, J. (1961). Regulation of food intake in the gold fish. *Am. J. Physiol.*, **201**, 968–974.

RUITNER, L. DE (1963). The physiology of vertebrate behaviour towards a synthesis of the ethological and physiological approaches to problems of behaviour. *Z. Tierphysiol. Tierernähr. Futtermittelk.*, **20**, 498–516.

RUSSELL, E. (1966). An investigation of the palatability of some marine invertebrates to four species of fish. *Pacif. Sci.*, **20**, 452–460.

RYTHER, J. H. (1963). Geographic variations in productivity. In M. N. Hill (Ed.), *The Sea*, Vol. II. Wiley, New York. pp. 347–380.

SANDISON, E. E. (1966). The oxygen consumption of some intertidal gastropods in relation to zonation. *J. Zool.*, **149**, 163–173.

SANDISON, E. E. (1967). Respiratory response to temperature and temperature tolerance of some intertidal gastropods. *J. exp. mar. Biol. Ecol.*, **1**, 271–281.

SARA, M. (1966). Associazioni tra Poriferi e alghe in acque superficiali del litorale marino. *Ricerca scient.*, **36**, 277–282.

SARA, M. (1971). Ultrastructural aspects of the symbiosis between two species of the genus *Aphanocapsa* (Cyanophyceae) and *Ircinia variabilis* (Demospongiae). *Mar. Biol.*, **11**, 214–221.

SARA, M. and LIACI, L. (1964). Associazioni fra la Cianoficicea *Aphanocapsa feldmanni* e alcune Demospongie marine. *Boll. Zool.*, **31**, 55–65.

SASSAMAN, C. and MANGUM, C. P. (1972). Adaptations to environmental oxygen levels in

infaunal and epifaunal sea anemones. *Biol. Bull. mar. biol. Lab., Woods Hole*, **143**, 657–678.

SASSAMAN, C. and MANGUM, C. P. (1973). Relationship between aerobic and anaerobic metabolism in estuarine anemones. *Comp. Biochem. Physiol.*, **44A**, 1313–1321.

SAVAGE, R. E. (1931). The relation between the feeding of the herring off the East Coast of England and the plankton of the surrounding waters. *Fishery Invest., Lond.* (*Ser.* 2), **12** (3).

SAVAGE, R. E. (1937). The food of the North Sea herring 1930–1934. *Fishery Invest., Lond.* (*Ser.* 2), **15** (5).

SAXENA, D. B. (1963). A review on ecological studies and their importance in the physiology of air-breathing fishes. *Ichthyologica*, **11**, 116–128.

SAZ, H. J. (1969). Carbohydrate and energy metabolism of nematodes and Acanthocephala. In M. Florkin and B. T. Scheer (Eds), *Chemical Zoology*, Vol. III. Academic Press, New York. pp. 329–360.

SAZ, H. J. (1971). Facultative anaerobiosis in the invertebrates: pathways and control systems. *Am. Zool.*, **11**, 125–135.

SAZ, H. J. and BUEDING, E. (1966). Relationship between anthelmintic effects and biochemical and physiological mechanisms. *Pharmac. Rev.*, **18**, 871–894.

SAZ, H. J. and LESCURE, O. L. (1969). The functions of phosphoenolpyruvate carboxykinase activity and malic enzyme in the anaerobic formation of succinate by *Ascaris lumbricoides*. *Comp. Biochem. Physiol.*, **30**, 49–60.

SAZ, H. J. and WEIL, A. (1962). Pathway of formation of α-methylvalerate by *Ascaris lumbricoides*. *J. biol. Chem.*, **237**, 2053–2056.

SCHEER, B. T. (1969). Carbohydrates and carbohydrate metabolism: Annelida, Sipunculida, Echiurida. In M. Florkin and B. T. Scheer (Eds), *Chemical Zoology*, Vol, IV. Academic Press, New York. pp. 135–144.

SCHEID, M. J. and AWAPARA, J. (1972). Stereospecificity of some invertebrate lactic dehydrogenases. *Comp. Biochem. Physiol.*, **43B**, 619–626.

SCHELSKE, C. L. and ODUM, E. P. (1961). Mechanisms maintaining high productivity in Georgia estuaries. *Proc. Gulf Caribb. Fish. Inst.*, **14**, 75–80.

SCHLEGEL, H. (1970). *Allgemeine Mikrobiologie*. Thieme-Verlag, Stuttgart.

SCHLICHTER, D. (1972). Chemische Tarnung. Die stoffliche Grundlage der Anpassung von Anemonenfischen an Riffanemonen. *Mar. Biol.*, **12**, 137–150.

SCHLICHTER, D. (1973). Ernährungsphysiologische und ökologische Aspekte der Aufnahme in Meerwasser gelöster Aminosäuren durch *Anemonia sulcata* (Coelenterate, Anthozoa). *Oecologia* (*Berl.*), **11**, 315–350.

SCHMIDT-NIELSEN, B. and RABINOWITZ, L. (1964). Methylurea and acetamide: Active reabsorption by elasmobranch renal tubules. *Science, N.Y.*, **146**, 1587–1588.

SCHMIDT-NIELSEN, K. (1960). The salt secreting gland in the marine birds. *Circulation*, **21**, 1027–1037.

SCHOFFENIELS, E. (1968). The control of intracellular hydrogen transport by inorganic ions. *Archs int. Physiol. Biochim.*, **76**, 319–343.

SCHOFFENIELS, E. and GILLES, R. (1970). Nitrogenous constituents and nitrogen metabolism in arthropods. In M. Florkin and B. T. Scheer (Eds), *Chemical Zoology*, Vol. V. Academic Press, New York. pp. 199–228.

SCHOLANDER, P. F. (1940). Experimental investigations on the respiratory function in diving mammals and birds. *Hvalråd. Skr.*, **22**, 1–31.

SCHOLANDER, P. F. (1964). Animals in aquatic environments: diving mammals. In D. B. Dill, E. F. Adolph and C. G. Wilber (Eds), *Handbook of Physiology*, Sect. 4. Adaptation to the environment. American Physiological Society, Washington, D.C. pp. 729–739.

SCHOLANDER, P. F., IRVING, L. and GRINNELL, S. W. (1942). On the temperature and metabolism of the seal during diving. *J. cell. comp. Physiol.*, **19**, 67–78.

SCHOOLER, J. M., GOLDSTEIN, L., HARTMAN, S. C. and FORSTER, R. P. (1966). Pathways of urea synthesis in the elasmobranch *Squalus acanthias*. *Comp. Biochem. Physiol.*, **18**, 271–281.

SCHREINER, K. E. (1971). A mechanical analysis of the ciliary beat. *Prepr. Ser. Inst. Math. Univ. Oslo*, **1**, 1–19 (mimeographed).

SCHREINER, K. E. (1972). Sliding filaments in cilia and flagella. *Prepr. Ser. Inst. Math. Univ. Oslo*, **1**, 1–23 (mimeographed).

SCHWABE, C. W. and KILEJIAN, A. (1968). Chemical aspects of the ecology of platyhelminthes. In M. Florkin and B. T. Scheer (Eds), *Chemical Zoology*, Vol. II. Academic Press, New York. pp. 467–549.

SEGAL, E., RAO, K. P. and JAMES, T. W. (1953). Rate of activity as a function of intertidal height within populations of some littoral molluscs. *Nature, Lond.*, **172**, 1108–1109.

SEVERIN, S. E., BOLDYREV, A. A. and BEDEV, A. V. LE (1972). Nitrogenous extractive compounds of muscle tissue of invertebrates. *Comp. Biochem. Physiol.*, **43B**, 369–381.

SHARMA, M. L. (1966). Studies on the changes in the pattern of nitrogenous excretion of *Orconectes rusticus*. *Comp. Biochem. Physiol.*, **19**, 681–690.

SHARMA, M. L. (1968). Studies on the sources and mechanisms of increased urea production by *Orconectes rusticus*. *Comp. Biochem. Physiol.*, **24**, 55–60.

SHARMA, M. L. (1969). Trigger mechanism of increased urea production by the crayfish *Orconectes rusticus* under osmotic stress. *Comp. Biochem. Physiol.*, **30**, 309–321.

SHARMA, M. L. and NEVEU, M. C. (1971). Studies on the role of ornithine cycle and purine catabolism in urea biosynthesis in the fresh-water crayfish, *Orconectes rusticus*. *Comp. Biochem. Physiol.*, **40B**, 863–870.

SHEHADEH, Z. H. and GORDON, M. S. (1969). The role of the intestine in salinity adaptation of the rainbow trout *Salmo gairdneri*. *Comp. Biochem. Physiol.*, **30**, 397–418.

SIEBERS, D. (1970). Mechanismen der intrazellulären isosmotischen Regulation der Aminosäurenkonzentration bei den Flußkrebs *Orconectes limosus*. Ph.D. thesis, Berlin University.

SIMPSON, J. W., ALLEN, K. and AWAPARA, J. (1959). Free amino acids in some aquatic invertebrates. *Biol. Bull. mar. biol. Lab., Woods Hole*, **117**, 371–381.

SIMPSON, J. W. and AWAPARA, J. (1964). Phosphoenolpyruvate carboxykinase in invertebrates. *Comp. Biochem. Physiol.*, **12**, 457–464.

SIMPSON, J. W. and AWAPARA, J. (1965). Biosynthesis of glucose from pyruvate-2-^{14}C and aspartate-3-^{14}C in a mollusc. *Comp. Biochem. Physiol.*, **15**, 1–6.

SIMPSON, J. W. and AWAPARA, J. (1966). The pathway of glucose degradation in some invertebrates. *Comp. Biochem. Physiol.*, **18**, 537–548.

SLOBODKIN, L. B. (1959). Energetics in *Daphnia pulex* populations. *Ecology*, **40**, 232–243.

SLOBODKIN, L. B. (1962a). *Growth and Regulation of Animal Populations*, Holt, Rinehart and Winston, New York.

SLOBODKIN, L. B. (1962b). Energy in animal ecology. *Adv. Ecol. Res.*, **1**, 69–101.

SLOBODKIN, L. B. (1964). The strategy of evolution. *Am. Scient.*, **52**, 342–357.

SLYKE, D. D. VAN and WHITE, G. F. (1911). Digestion of protein in the stomach and intestine of the dogfish. *J. biol. Chem.*, **9**, 209–217.

SMIT, H. (1967). Influence of temperature on the rate of gastric juice secretion in the brown bullhead *Ictalurus nebulosus*. *Comp. Biochem. Physiol.*, **21**, 125–132.

SMIT, H. (1968). Gastric secretion in the lower vertebrates and birds. In *Handbook of Physiology*. Sect. 6: Alimentary canal, Vol. V. American Physiological Society, Washington, D.C. pp. 2791–2805.

SMITH, A. U. (1958). The resistance of animals to cooling and freezing. *Biol. Rev.*, **33**, 197–253.

SMITH, D., MUSCATINE, L. and LEWIS, D. (1969). Carbohydrate movement from autotrophs to heterotrophs in parasitic and mutualistic symbioses. *Biol. Rev.*, **44**, 17–90.

SMITH, H. W. (1930). The absorption and excretion of water and salts by marine teleosts. *Am. J. Physiol.*, **93**, 480–505.

SMITH, H. W. (1931). The absorption and excretion of water and salts by the elasmobranch fishes. 1. Freshwater elasmobranchs. *Am. J. Physiol.*, **98**, 279–295.

SMITH, H. W. (1936). The retention and physiological role of urea in the Elasmobranchii. *Biol. Rev.*, **11**, 49–82.

SMITH, H. W. (1953). *From Fish to Philosopher*, Little, Boston.

SMITH, L. S. and DAVIS, J. C. (1965). Haemodynamics in *Tressus nutallii* and certain other bivalves. *J. exp. Biol.*, **43**, 171–180.

SMUCKER, L. A. and NYSTROM, R. A. (1972). Control of gut motility by an intrinsic neural plexus in the pelecypod *Spisula*. *Comp. Biochem. Physiol.*, **41A**, 731–748.

SMYTH, D. H. (1965). Water movement across the mammalian gut. *Symp. Soc. exp. Biol.*, **19**, 307–328.

SMYTH, S. D. (1962). *Introduction to Animal Parasitology*, English University Press, London.

SOROKIN, YU. I. (1971). Bacterial populations as components of oceanic ecosystems. *Mar. Biol.*, **11**, 101–105.

SOROKIN, YU. I. (in press). Decomposition of organic matter and nutrient regeneration. In O. Kinne (Ed.), *Marine Ecology*, Vol. IV, Dynamics. Wiley, London.

SOUTHWARD, A. J. (1964). The relationship between temperature and rhythmic cirral activity in some Cirripedia considered in connection with their geographical distribution. *Helgoländer wiss. Meeresunters.*, **10**, 391–403.

SOUTHWARD, A. J. and SOUTHWARD, E. C. (1968). Uptake and incorporation of labelled glycine by pogonophores. *Nature, Lond.*, **218**, 875–876.

SPANGENBERG, D. B. and HAM, R. G. (1960). The epidermal nerve net of *Hydra*. *J. exp. Zool.*, **143**, 195–201.

SRINIVASAN, V. V. (1963). The distribution of glycogen in *Martesia fragilis*, a common wood-borer of Madras. *Curr. Sci.*, **32**, 211.

SRINIVASAN, V. V. and KRISHNASWAMY, S. (1964). The chemical composition of *Martesia fragilis*, a wood-boring pholad of Madras. *Zool. Jb.* (*Abt. allgem. Zool. Physiol. Tiere*), **70**, 534–546.

STANIER, J. E., WOODHOUSE, M. A. and GRIFFIN, R. L. (1968). The fine structure of the hepatopancrease of *Carcinus maenas* (L.) (Decapoda: Brachyura). *Crustaceana*, **14**, 56–66.

STANIER, R. Y., DOUDOROFF, M. and ADELBERG, E. A. (1971). *General Microbiology*, 3rd ed., Macmillan, New York.

STEPHENS, G. C. (1960). Uptake of glucose from solution by the solitary coral *Fungia scutaria*. *Science, N.Y.*, **131**, 1532.

STEPHENS, G. C. (1962), Uptake of organic material by aquatic invertebrates. I. Uptake of glucose by the solitary coral, *Fungia scutaria*. *Biol. Bull. mar. biol. Lab., Woods Hole*, **123**, 648–659.

STEPHENS, G. C. (1963). Uptake of organic material by aquatic invertebrates. II. Accumulation of amino acids by the bamboo worm *Clymenella torquata*. *Comp. Biochem. Physiol.*, **10**, 191–202.

STEPHENS, G. C. (1964). Uptake of organic material by aquatic invertebrates. III. Uptake of glycine by brackish-water annelids. *Biol. Bull. mar. biol. Lab., Woods Hole*, **126**, 150–162.

STEPHENS, G. C. (1967). Dissolved organic material as a nutritional source for marine and estuarine invertebrates. In G. H. Lauff (Ed.), *Estuaries*. A.A.A.S., Washington, D.C. pp. 367–373. (*Publs Am. Ass. Advmt Sci.*, **83**.)

STEPHENS, G. C. (1968). Dissolved organic matter as a potential source of nutrition for marine organisms. *Am. Zool.*, **8**, 95–106.

STEPHENS, G. C. (1972). Amino acid accumulation and assimilation in marine organisms. In J. W. Campbell and L. Goldstein (Eds), *Nitrogen Metabolism and the Environment*. Academic Press, New York. pp. 155–184.

STEPHENS, G. C. and KERR, N. S. (1962). Uptake of phenylalamine by *Tetrahymena pyriformis*. *Nature, Lond.*, **194**, 1094–1095.

STEPHENS, G. C. and SCHINSKE, R. A. (1961). Uptake of amino acids by marine invertebrates. *Limnol. Oceanogr.*, **6**, 175–181.

STEPHENS, G. C. and VIRKAR, R. A. (1966). Uptake of organic material by aquatic invertebrates. IV. The influence of salinity on the uptake of amino acids by the brittle star *Ophiactis arenosa*. *Biol. Bull. mar. biol. Lab., Woods Hole*, **131**, 172–185.

STIVEN, A. E. (1965). The relationship between size, budding rate and growth efficiency in three species of *Hydra*. *Res. Pop. Ecol.*, **7**, 1–15.

STOKES, T. and AWAPARA, J. (1968). Alanine and succinate as end products of glucose degradation in the clam *Rangia cuneata*. *Comp. Biochem. Physiol.*, **25**, 883–892.

STONE, B. A. and MORTON, J. E. (1958). The distribution of cellulases and related enzymes in Mollusca. *Proc. malac. Soc. Lond.*, **33**, 127–141.

STOTT, F. C. (1955). The food canal of the sea urchin *Echinus esculentus* L. and its functions. *Proc. zool. Soc. Lond.*, **125**, 63–86.

STRATHMANN, R. R. (1971). The feeding behaviour of planktotrophic echinoderm larvae: Mechanisms, regulation and rates of suspension feeding. *J. exp. mar. Biol. Ecol.*, **6**, 109–160.

STRATHMANN, R. R., JAHN, T. L. and FONSECA, J. R. C. (1972). Suspension feeding by marine invertebrate larvae: clearance of particles by ciliated bands of a rotifer, pluteus, and trochophore. *Biol. Bull. mar. biol. Lab., Woods Hole*, **142**, 505–519.

STREHLER, B. L. (1961). Aging in coelenterates. In H. M. Lenhoff and W. F. Loomis (Eds), *Biology of Hydra*. University of Miami Press, Coral Gables. pp. 373–398.

SUSHCHENYA, L. M. (1958). Quantitative data on the filtration feeding of plankton Crustacea. (Russ.) *Nauch. Dokl. vyssh. Shk.*, **1**, 16–20.

SUSHCHENYA, L. M. (1962). Quantitative data on the feeding and energy balance of *Artemia salina* (L.). (Russ.) *Dokl. Akad. Nauk SSSR.*, **143**, 1205–1207. (Engl. transl. in *Dokl. (Proc.) Acad. Sci. U.S.S.R.*, **143**, 329–330, 1962.)

SUSHCHENYA, L. M. (1966). Quantitative regularities of feeding and its connection with energy balance of the edible crab *Memippe mercenaria* (SAY). (Russ.) *Trudy̆ sov. Kub. morsk. Eksped.*, **1**.

SUSHCHENYA, L. M. (1970). Food rations, metabolism and growth of crustaceans. In J. H. Steele (Ed.), *Marine Food Chains*. Oliver and Boyd, Edinburgh. pp. 127–141.

TAKATSUKI, S. (1934). On the nature and functions of the amoebocytes of *Ostrea edulis*. *Q. Jl microsc. Sci.*, **76**, 379–431.

TAMMES, P. M. L. and DRAL, A. D. G. (1955). Observations on the straining of suspensions of mussels. *Archs néerl. Zool.*, **11**, 87–112.

TAO, L. (1930). Notes on the ecology and the physiology of *Caudina chilensis* (J. MÜLLER) in Mutsu Bay. *Proc. Pacif. Sci. Congr.*, **3**, 7–11.

TAYLOR, A. G. (1969). The direct uptake of amino acids and other small molecules from sea-water by *Nereis virens* SARS. *Comp. Biochem. Physiol.*, **29**, 243–250.

TAYLOR, D. L. (1968). Chloroplasts as symbiotic organelles in the digestive gland of *Elysia viridis*. *J. mar. biol. Ass. U.K.*, **48**, 1–16.

TAYLOR, D. L. (1973). The cellular interactions of algal-invertebrate symbiosis. *Adv. mar. Biol.*, **11**, 1–56.

TEICHMANN, H. (1959). Über die Leistung des Geruchssinnes beim Aal *Anguilla anguilla* (L.), *Z. vergl. Physiol.*, **42**, 206–254.

TENORE, K. R. and DUNSTAN, W. M. (1973a). Comparison of rates of feeding and biodeposition of the American oyster, *Crassostrea virginica* GMELIN, fed on different species of phyto-plankton. *J. exp. mar. Biol. Ecol.*, **12**, 19–26.

TENORE, K. R. and DUNSTAN, W. M. (1973b). Comparison of feeding and biodeposition of three bivalves at different food levels. *Mar. Biol.*, **21**, 190–195.

TERCAFS, R. R. and JEUNIAUX, C. (1961). Comparaison entre les individus épigés et cavernicoles de l'espèce *Oxychilus cellarius* au point de vue de la teneur en chitinase du tube digestif et de l'hépatopancréas. *Archs int. Physiol. Biochim.*, **69**, 364–368.

THOMPSON, T. E. (1959). Feeding in nudibranch larvae. *J. mar. biol. Ass. U.K.*, **38**, 239–248.

THOMPSON, T. E. (1964). Grazing and life cycles of British nudibranch. In D. J. Crisp (Ed.), *Grazing in Terrestrial and Marine Environments*. Blackwell Scientific Publications, Oxford. pp. 275–297.

THORSON, G. (1946). Reproduction and larval development of Danish marine bottom invertebrates. *Meddr. Kommn Danm. Fisk.-og Havunders. (Ser. Plankton)*, **4**, 1–525.

TIFFANY, W. J., III. (1972). Aspects of excretory ultrafiltration in bivalve molluscs. *Comp. Biochem. Physiol.*, **43A**, 527–536.

TIMON-DAVID, J., and CERESOLA, G. (1935). Influence of sex on the lipids of marine molluscs. *C.r. hebd. Séanc. Acad. Sci., Paris*, **201**, 853.

TODD, E. S. and EBERLING, A. W. (1966). Aerial respiration in the longjaw mudsucker *Gillichthys mirabilis* (Teleostei: Gobiidae). *Biol. Bull. mar. biol. Lab., Woods Hole*, **130**, 265–288.

TRENCH, E. M., TRENCH, R. K. and MUSCATINE, L. (1970). Utilization of photosynthetic products of symbiotic chloroplasts in mucus synthesis by *Placobranchus ianthobapsus* (GOULD), Opisthobranchia, Sacoglossa. *Comp. Biochem. Physiol.*, **37**, 113–117.

TURNER, R. D. (1959). Notes on the feeding of *Melongena carona*. *Nautilus*, **73**, 11–13.

TYLER, A. V. (1970). Rate of gastric emptying in young cod. *J. Fish. Res. Bd Can.*, **27**, 1177–1189.

TYLER, A. V. (1973). Caloric values of some North Atlantic invertebrates. *Mar. Biol.*, **19**, 258–261.

UKELES, R. (in press). Cultivation of plants: Unicellular plants. In O. Kinne (Ed.), *Marine Ecology*, Vol. III, Cultivation. Wiley, London.

UKELES, R. and SWEENEY, B. M. (1969). Influence of dinoflagellate trichocysts and other factors on the feeding of *Crassostrea virginica* larvae on *Monochrysis lutheri*. *Limnol. Oceanogr.*, **14**, 403–410.

URRY, D. L. (1965). Observations on the relationship between the food and survival of *Pseudocalanus stigmatophora* in the laboratory. *J. mar. biol. Ass. U.K.*, **45**, 49–58.

VAHL, O. (1972). Effect of particle retention in *Mytilus edulis* L. *Ophelia*, **10** (in press).

VEIBEL, S. (1958). Die Fermente des Auf- und Abbaus (besonders die speziellen Heterosidasen). In W. Ruhland (Ed.), *Encyclopedia of Plant Physiology*, Vol. VI. Springer Verlag, Berlin. pp. 780–798.

VERWEY, J. (1952). On the ecology of distribution of cockle and mussel in the Dutch Waddensea, their role in sedimentation and the source of their food supply, with a short review of the feeding behaviour of bivalve molluscs. *Archs néerl. Zool.*, **10**, 172–239.

VERZAR, F. and McDOUGALL, E. J. (1936). *Absorption from the Intestine*, Longmans Green & Co., London.

VINOGRADOV, A. P. (1953). *The Elementary Composition of Marine Organisms* (Transl. from the Russ.), Yale University Press, New Haven. (*Mem. Sears Fdn Mar. Res.*, **2**).

VONK, H. J. (1924). Verdauungsphagocytose bei den Austern. *Z. vergl. Physiol.*, **1**, 607–623.

VONK, H. J. (1960). Digestion and metabolism. In T. H. Waterman (Ed.), *The Physiology of Crustacea*, Vol. I. Academic Press, New York. pp. 291–316.

VONK, H. J. (1964). Comparative biochemistry of digestive mechanisms. In M. Florkin and H. S. Mason (Eds), *Comparative Biochemistry*, Vol. VI. Academic Press, New York. pp. 347–401.

WAGGE, L. E. (1955). Amoebocytes. *Int. Rev. Cytol.*, **4**, 31–78.

WAGNER, F. S., JR. (1969). Composition of the dissolved organic compounds in sea water: a review. *Contrib. mar. Sci.*, **14**, 115–153.

WALNE, P. R. (1956). Experimental rearing of the larvae of *Ostrea edulis* L. in the laboratory. *Fishery Invest., Lond.* (*Ser. 2*), **20**, 1–23.

WALNE, P. R. (1970). The seasonal variation of meat and glycogen content of seven populations of oyster *Ostrea edulis* L. *Fishery Invest., Lond.* (*Ser. 2*), **26**(3), 1–35.

WANGERSKY, P. (in press). Production of dissolved organic matter. In O. Kinne (Ed.), *Marine Ecology*, Vol. IV, Dynamics. Wiley, London.

WARE, D. M. (1972). Predation by rainbow trout (*Salmo gairdneri*), the influence of hunger, prey density and prey size. *J. Fish. Res. Bd Can.*, **29**, 1193–1201.

WARREN, L. G. and POOLE, W. J. (1970). Biochemistry of the dog hookworm. II. Nature and origin of the excreted fatty acids. *Expl Parasitol.*, **27**, 408–416.

WATTS, D. C. and WATTS, R. L. (1966). Carbamoyl phosphate synthetase in the Elasmobranchii: osmoregulatory function and evolutionary implications. *Comp. Biochem. Physiol.*, **17**, 785–798.

WEBB, J. E. (1969). On the feeding behaviour of *Branchiostoma lanceolatum*. *Mar. Biol.*, **3**, 58–72.

WEBB, K. L. and JOHANNES, R. E. (1967). Studies of the release of dissolved free amino acids by marine zooplankton. *Limnol. Oceanogr.*, **12**, 376–382.

WEEL, P. B. VAN (1950). Contribution to physiology of the glandula media intestine of the African giant snail *Achatina fulica* BOWDICH. *Z. vergl. Physiol.*, **42**, 333–448.

WEEL, P. B. VAN (1955). Processes of secretion, restitution and resorption in gland of midgut (glandula media intestinini) of *Atya spinipes* NEWPORT (Decapoda-Brachyura) *Physiol. Zoöl.*, **28**, 40–54.

WEEL, P. B. VAN (1960). Comparative physiology of digestion in molluscs. *Z. vergl. Physiol.*, **43**, 567–577.

WEEL, P. B. VAN (1961). The comparative physiology of digestion in molluscs. *Am. Zool.*, **1**, 245–252.

WEEL, P. B. VAN (1970). Digestion in Crustacea. In M. Florkin and B. T. Scheer (Eds), *Chemical Zoology*, Vol. V. Academic Press, New York. pp. 97–115.

WEGENER, B., BARNITT, A. E. and HAMMEN, C. S. (1969). Reduction of fumarate and oxidation of succinate in *Crassostrea virginica* (GMELIN). *Life Sci.*, **8** (II), 335–343.

WEIBULL, C. (1960). Movement. In I. C. Gunsalus and R. Y. Stanier (Eds), *The Bacteria*, Vol. I. Academic Press, New York. pp. 153–205.

WERNER, B. (1953). Über den Nahrungserwerb der Calyptraeidae (Gastropoda, Prosobranchia). Morphologie, Histologie und Funktion der am Nahrungserwerb beteiligten Organe. *Helgoländer wiss. Meeresunters.*, **4**, 260–315.

WERNER, B. (1959). Das Prinzip des endlosen Schleimfilters beim Nahrungserwerb wirbelloser Meerestiere. *Int. Revue ges. Hydrobiol. Hydrogr.*, **44**, 181–216.

WERNER, E. and WERNER, B. (1954). Über den Mechanismus des Nahrungserwerbs der Tunicaten, speziell der Ascidien. *Helgoländer wiss. Meeresunters.*, **5**, 57–92.

WEST, B. and JEAL, F. (1974). Absorption of nutrients by the urchin *Paracentrotus lividus*. *Proc. Challenger Soc.* (In press).

WHITTAKER, R. H. (1969). New concepts of kingdoms of organisms. *Science, N.Y.*, **163**, 150–160.

WIESER, W. (1967). Conquering terra firma: The copper problem from the isopods point of view. *Helgoländer wiss. Meeresunters.*, **15**, 282–293.

WIGGLESWORTH, V. B. (1965). *The Principles of Insect Physiology*, Methuen, London.

WILLIAMS, R. B. (1972). Chemical control of feeding in the sea anemone *Diadumene luciae* (VERRILL). *Comp. Biochem. Physiol.*, **41A**, 361–371.

WILLIAMSON, J. R., CHEONG, W. Y., COLES, H. S. and HERCZEG, B. E. (1967). Glycolytic control mechanisms. IV. Kinetics of glycolytic intermediate changes during electrical discharge and recovery in the main organ of *Electrophorus electricus*. *J. biol. Chem.*, **242**, 5112–5118.

WILSON, T. A. and SCHREINER, K. E. (1971). Flow due to the motion of cilia. *Prepr. Ser. Inst. Univ. Oslo*, **2**, 1–13 (mimeographed).

WILSON, T. H. (1962). *Intestinal Absorption*, W. B. Saunders, Philadelphia.

WILSON, T. H. and LANDAU, B. R. (1960). Specificity of sugar transport by the intestine of the hamster. *Am. J. Physiol.*, **198**, 99–102.

WILSON, T. H., LIN, E. C. C., LANDAU, B. R. and JØRGENSEN, C. B. (1960). Intestinal transport of sugars and amino acids. *Fedn Proc. Fedn Am. Socs exp. Biol.*, **19**, 870–875.

WINDELL, J. T. (1966). Rate of digestion in the bluegill sunfish. *Invest. Indiana Lakes Streams*, **7**, 185–214.

WINDELL, J. T. (1967). Rates of digestion in fishes. In S. D. Gerking (Ed.), *Biological Basis of Freshwater Fish Production*. Blackwell Scientific Publications, Oxford. pp. 151–173.

WINDELL, J. T., NORRIS, D. O., KITCHELL, J. F. and NORRIS, J. S. (1969). Digestive response of rainbow trout, *Salmo gairdneri*, to pellet diets. *J. Fish. Res. Bd Can.*, **26**, 1801–1812.

WINKLER, R. L. and WAGNER, E. D. (1959). Filter paper digestion by the crystalline style in *Oncomelania*. *Trans. Am. microsc. Soc.*, **78**, 262–268.

WINTER, J. E. (1969). Über den Einfluß der Nahrungskonzentration und anderer Faktoren auf Filtrierleistung und Nahrungsausnutzung der Muscheln *Arctica islandica* und *Modiolus modiolus*. *Mar. Biol.*, **4**, 87–135.

WINTER, J. E. (1970). Filter feeding and food utilization in *Arctica islandica* L. und *Modiolus modiolus* L. at different concentrations. In J. H. Steele (Ed.), *Marine Food Chains*. Oliver and Boyd. Edinburgh. pp. 196–206.

WIRTH, H. E. and RIGG, G. B. (1937). The acidity of the juice of *Desmarestia*. *Am. J. Bot.*, **24**, 68–74.

WISEMAN, G. (1953). Absorption of amino acids using an *in vitro* technique. *J. Physiol., Lond.*, **120**, 63–72.

WONG, L. (1969). A study of the accumulation and loss of free amino acids by the polychaete *Dorvillea articulata*. M.A. thesis, University of California, Irvine.

WONG, L. and STEPHENS, G. C. (1970). Leakage rates of amino acid in the annelid, *Stauronereis rudolphi* (DELLA CHIAJE). *Am. Zool.*, **10**, 312.

WOOD, H. G. and STJERNHOLM, R. L. (1962). Assimilation of carbon dioxide by heterotrophic organisms. In I. C. Gunsalus and R. Y. Stanier (Eds), *The Bacteria*, Vol. III, Biosynthesis. Academic Press, New York. pp. 41–117.

WOOD, J. D. (1958). Nitrogen excretion in some marine teleosts. *Can. J. Biochem. Physiol.*, **36**, 1237–1242.

WORMHOUDT, A. VAN (1973). Variation des protéases des amylases et des proteines solubles au cours du développement larvaire chez *Palaemon serratus*. *Mar. Biol.*, **19**, 245–248.

YAMANAOUCHI, T. (1926). Some preliminary notes on the behaviour of the holothurian *Caudina chilensis* (J. MÜLLER). *Sci. Rep. Tohoku Univ.* (*Ser. 4*), **2**, 85–91.

YAMASAKI, K., USUI, T., IWATA, T., NAKASONE, S., HOZUMI, M. and TAKATSUKI, S. (1965). Absence of bile acids in the digestive juice of the swamp crayfish (*Procambarus clarkii*). *Nature, Lond.*, **205**, 1326–1327.

YASUMASU, I. and YOKOE, Y. (1965). Release of cellulase activity from microsome fraction of crayfish hepatopancreas by the treatment with adenosine triphosphate (*Procambarus clarkii*). *Sci. Pap. Coll. gen. Educ. Tokyo*, **15**, 95–98.

YOKOE, Y. and YASUMASU, I. (1964). The distribution of cellulase in invertebrates. *Comp. Biochem. Physiol.*, **13**, 323–609.

YONGE, C. M. (1924). The mechanism of feeding, digestion and assimilation in *Nephrops norvegicus*. *Br. J. exp. Biol.*, **1**, 343–389.

YONGE, C. M. (1925). The hydrogen ion concentration in the gut of certain lamellibranchs and gastropods. *J. mar. biol. Ass. U.K.*, **13**, 938–952.

YONGE, C. M. (1926a). The digestive diverticula in the lamellibranchs. *Trans. R. Soc. Edinb.*, **54**, 703–718.

YONGE, C. M. (1926b). Structure and physiology of the organs of feeding and digestion in *Ostrea edulis*. *J. mar. biol. Ass. U.K.*, **14**, 295–386.

YONGE, C. M. (1928). Structure and function of the organs of feeding and digestion in the septibranchs *Cuspidaria* and *Poromya*. *Phil. Trans. R. Soc.* (*B*), **216**, 212–263.

YONGE, C. M. (1932). Notes on feeding and digestion in *Pterocera* and *Vermetus*, with a discussion on the occurrence of the crystalline style in the Gastropoda. *Scient. Rep. Gt Barrier Reef Exped.*, **1**, 259–281.

YONGE, C. M. (1936). Mode of life, feeding, digestion and symbiosis with zooxanthellae in the Tridacnidae. *Scient. Rep. Gt Barrier Reef Exped.*, **1**, 283–321.

YONGE, C. M. (1937). Evolution and adaptation in the digestive system of the Metazoa. *Biol. Rev.*, **12**, 87–115.

YONGE, C. M. (1938). The digestion of cellulose and chitin. *Sci. Progr. Lond.*, **32**, 638–647.

YONGE, C. M. (1949). On the structure and adaptations of *Tellinacea* deposit feeding Eulamellibranchia. *Phil. Trans. R. Soc.*, **234**, 29–76.

YONGE, C. M. (1962). On *Etheria elliptica* LAM. and the course of evolution, including assumption of monomyarianism, in the family Etheriidae (Bivalvia: Unionacea). *Phil. Trans. R. Soc.* (*B*), **244**, 423–458.

YOUNG, J. Z. (1933). The autonomic nervous system of selachians. *Q. Jl microsc. Sci.*, **75**, 571–624.

YOUNG, S. D., O'CONNOR, J. D. and MUSCATINE, L. (1971). Organic material from scleractinian coral skeletons. II. Incorporation of ^{14}C into protein, chitin and lipid. *Comp. Biochem. Physiol.*, **40B**, 945–958.

ZACKS, S. I. and WELSH, J. H. (1953). Cholinesterase and lipase in the amoebocytes, intestinal epithelium and heart muscle of the quahog *Venus mercenaria*. *Biol. Bull. mar. biol. Lab., Woods Hole*, **105**, 200–211.

ZAFIRIOU, O., WHITTLE, K. J. and BLUMER, M. (1972). Response of *Asterias vulgaris* to bivalves and bivalve tissue extracts. *Mar. Biol.*, **13**, 127–136.

ZENKEVICH, L. A. and BIRSTEIN, J. A. (1956). Studies on the deep water fauna and related problems. *Deep Sea Res.*, **4**, 54–64.

ZETTERQVIST, H. (1956). The ultrastructural organization of the columnar absorbing cells of the mouse jejunum. *Karolinska Institutet, Aktiebolaget Godvil, Stockholm.*

ZHUKOVA, A. I. (1963). On the quantitative significance of microorganisms in the nutrition of aquatic invertebrates. In C. H. Oppenheimer (Ed.), *Marine Microbiology*. C. C. Thomas,

Springfield, Ill. pp. 699–710.

ZICK, C. (1929–1932). Wirkung der Nasselkapselen auf Protozoen. *Zool. Anz.*, **83**, 98.

ZIEGELMEIER, E. (1954). Beobachtungen über den Nahrungserwerb bei der Naticidae *Lunatia nitida* DONOVAN (Gastropoda, Prosobranchia). *Helgoländer wiss. Meeresunters.*, **5**, 1–33.

ZS.-NAGY, I. (1967). Histological, histochemical and electron microscopical studies on the cytosomes of the nerve cells in *Anodonta cygnea* L. (Mollusca, Lamellibranchiata). *Annls Inst. biol. Tihany*, **34**, 25–39.

ZS.-NAGY, I. (1971a). Pigmentation and energy dependent Sr^{2+}-accumulation of molluscan neurons under anaerobic conditions. *Annls Inst. biol. Tihany*, **38**, 117–129.

ZS.-NAGY, I. (1971b). The lipochrome pigment of molluscan neurons as a specific electron acceptor. *Comp. Biochem. Physiol.*, **40A**, 595–602.

ZS.-NAGY, I. and ERMINI, M. (1972a). Oxidation of $NADH_2$ by the lipochrome pigment of the tissues of the bivalve *Mytilus galloprovincialis* (Mollusca, Pelecypoda). *Comp. Biochem. Physiol.*, **43B**, 39–46.

ZS.-NAGY, I. and ERMINI, M. (1972b). ATP production in the tissues of the bivalve *Mytilus galloprovincialis* (Pelecypoda) under normal and anoxic conditions. *Comp. Biochem. Physiol.*, **43B**, 593–600.

ZS.-NAGY, I. and KEREL-FRONIUS, S. (1970). Electron microscopic histochemical investigations on the energy production in the neurones of Pelecypoda (Mollusca). *Septieme Congres International de Microscopie Electronique, Grenoble*, Vol. III, 155–156.

ZUCKERKANDL, E. (1960). Hemocyanine et cuivre chez un crustace decapode, dans leurs rapports avec le cycle d'enternue. *Annls Inst. océanogr. Monaco*, **38**, 1–122.

ZWAAN, A. DE (1971). The anaerobic carbohydrate metabolism in the sea mussel *Mytilus edulis* L. Ph.D. thesis, Utrecht University.

ZWAAN, A. DE (1972). Pyruvate kinase in muscle extracts of the sea mussel *Mytilus edulis* L. *Comp. Biochem. Physiol.*, **42B**, 7–14.

ZWAAN, A. DE and HOLWERDA, D. A. (1972). The effect of phosphoenolpyruvate, fructose 1,6-diphosphate and pH on allosteric pyruvate kinase in muscle tissue of the bivalve *Mytilus edulis* L. *Biochim. Biophys. Acta*, **276**, 430–433.

ZWAAN, A. DE and MARREWIJK, W. J. A. VAN (1973). Intracellular localization of pyruvate carboxylase, phosphoenolpyruvate carboxykinase and 'malic enzyme' and the absence of glyoxylate cycle enzymes in the sea mussel (*Mytilus edulis* L.). *Comp. Biochem. Physiol.*, **44B**, 1057–1066.

ZWAAN, A. DE, MARREWIJK, W. J. A. VAN and HOLWERDA, D. A. (1973). Anaerobic carbohydrate metabolism in the sea mussel *Mytilus edulis* L. *Neth. J. Zool.*, **23**, 225–228.

ZWAAN, A. DE and ZANDEE, D. I. (1972a). Body distribution and seasonal changes in the glycogen content of the common sea mussel *Mytilus edulis*. *Comp. Biochem. Physiol.*, **43A**, 53–58.

ZWAAN, A. DE and ZANDEE, D. I. (1972b). The utilization of glycogen and accumulation of some intermediates during anaerobiosis in *Mytilus edulis* L. *Comp. Biochem. Physiol.*, **43B**, 47–54.

4. MECHANISMS OF THERMOREGULATION

R. GILLES

(1) Introduction

Thermal energy is a primary physical property of the environment (Volume I, Chapter 3). Animals have developed a variety of mechanisms to cope with changes in external temperature. Endogenous homeostatic mechanisms for thermoregulation confer on the animal an increased degree of independence towards its external environment.

With the exception of a few mammalian species, oceans and coastal waters are populated by animals which have either no or only very limited capacities for thermoregulation. While the mechanisms by which homeotherm species regulate their body temperature are well known and have been the main subject of numerous reviews (e.g. WHITTOW, 1971), until recently, not much was known about the biochemical processes occurring in poikilotherm animals during temperature acclimation. This chapter comprises a brief account of recent information on physiological mechanisms of thermoregulation in non-mammalian marine, brackish and freshwater animals. For references concerning reviews on thermoregulation in marine mammals consult Chapter 8.3.

(2) Behaviour

In most poikilotherm animals, body-fluid temperature remains within a few degrees parallel to ambient temperature. In aquatic invertebrates, body temperature follows closely that of the environment (Volume I: KINNE, 1970); however, a limited degree of thermoregulation may be achieved by behavioural means. Thus a number of intertidal species employ evaporative cooling (LEWIS, 1963). In addition modifications in pigmentation may support thermal control. WILKENS and FINGERMAN (1965), for instance, assume that blanching at high temperatures has a thermoregulatory function in *Uca pugnax*. Under conditions of extreme high temperatures, fiddler crabs (genus *Uca*) return periodically to their burrows, which are several degrees cooler than the substrate surface and thus transitorily lower their body temperature.

Many species avoid places with too high or too low temperatures. Such avoidance can also be considered a behavioural mechanism of thermoregulation. In thermally stratified lakes, young sockeye salmon *Oncorhynchus nerka* gather in areas providing their physiological optimum temperature (BRETT, 1971). The mechanism involved seems by no means simple; the sockeye has indeed evolved behavioural thermoregulation which favourably balances daily metabolic expenditures, with the result that energy is conserved when the food supply is limited. Response of aquatic animals to environmental temperature may be modulated with regard to variations in the intensity pattern of other simultaneously effective environmental

factors such as light, salinity, water movement, pressure or food availability (Volume I, Chapters 2, 4, 5 and 8).

(3) Metabolic Adaptation

With the exception of mammals, and perhaps a few large fast-swimming fishes (CAREY and TEAL, 1969a, b; CAREY and co-authors, 1971), aquatic animals possess no or only weak ability for regulating their body temperature. The main mechanism used by these animals to compensate for changes in ambient temperature is metabolic adaptation (non-genetic adaptation: KINNE, 1967; Volume I, pp. 435, 474). Since the energy metabolism of aquatic animals involves oxygen as electron acceptor, most studies in this field have employed oxygen consumption as indicator of the animals' metabolic state in a given set of experimental conditions. Various quantitative relationships between oxygen consumption and environmental temperature have been described (e.g. PRECHT, 1958; PROSSER, 1958; KINNE, 1964, 1970; Volume I, Chapter 3). These schemes are of value to the ecologist since they provide a fundament for classifying and comparing responses to temperature of different organisms or their constituent parts; however, they consider only overall net results; analysis of the mechanisms involved requires studies and interpretations at the molecular level.

Only a few cases have come to the reviewer's attention, where a more direct approach has been attempted for assessing and analysing temperature effects on metabolism. Using various labelled substrates of intermediary metabolism, HOCHACHKA and his colleagues have investigated metabolic thermocompensation in fishes. The results obtained have been documented extensively (HOCHACHKA, 1962, 1967; HOCHACHKA and HAYES, 1962; FRY and HOCHACHKA, 1970). The main conclusions drawn from these studies carried out on cold and warm-acclimated trout *Salmo gairdnerii* are the following:

In tissues of cold-acclimated *Salmo gairdnerii*—compared to the same tissues of warm-acclimated ones—glycolysis rate increases significantly, and the participation of the hexose monophosphate pathway may increase from negligible contributions to activities accounting for about 10% of the glucose metabolism. Lipogenesis, glycogen synthesis, protein and RNA metabolism appear to be higher in cold-acclimated fishes. In contrast, Krebs-cycle activity may decrease, remain unchanged or increase slightly, while respiratory-chain functions characteristically increase. All these processes may not necessarily occur in a single tissue. For instance, in the epaxial muscle of the cold-acclimated trout, there is an important increase in glycolysis activity while Krebs-cycle activity remains unchanged. In the muscle, the pentose pathway appears not to be very important, while there is a clear rise in pentose shunt activity in the liver of cold-compensating fish (HOCHACHKA and HAYES, 1962). Already in 1958, EKBERG had obtained evidence that the increased oxygen demand in cold-compensating goldfish *Carassius auratus* results, at least in part, from an increased activity of the pentose shunt.

These results demonstrate that metabolic adjustments during thermal acclimation are not based simply on rate changes in overall intermediary metabolism, but on metabolic re-organization, some pathways being activated, others inhibited. The biological significance of these results is still not clear; it cannot be accounted for

by a simple biochemical relationship between the various metabolic pathways. For instance, synchronous increase of glycolysis, gluconeogenesis and lipogenesis, and no change or even a decrease in Krebs-cycle activity parallel to an increase in electron transport, cannot be interpreted in terms of a simple relationship between metabolic pathways (such as those considered when dealing with metabolic regulation processes). Nevertheless, these results clearly show that the molecular basis of metabolic temperature acclimation is more complex than previously assumed.

What are the mechanisms triggering such substantial metabolic re-organization? According to general theory, modulation of enzyme activity is an important control mechanism of metabolic activity. Numerous studies on thermal properties of enzymes in poikilotherms have thus been concerned with heat denaturation, thermal activity optimum, and activation energy; in the last few years, also with the influence of temperature on allosteric modification of enzyme activity.

It is sometimes held that enzymes of poikilotherms have low activation energy characteristics so that they can perform efficaciously despite the sometimes low thermal energy available. SOMERO and HOCHACHKA (1968) showed that the activation energy of pyruvate kinase extracted from the muscle of the Antarctic fish *Trematomus bernacchii* is substantially lower than that of the trout *Salmo gairdnerii* enzyme. This may be an adaptative feature. However, the same authors noticed that the activation energy of rat muscle enzyme is of the same order of magnitude as that of *T. bernacchii*. This clearly demonstrates that activation energy is not consistently correlated with the environmental temperature in which the enzyme is normally performing.

A correlation between the thermal optimum for enzyme maximum velocity (V_{max}) and habitat temperature has been demonstrated in some species. However, such correlation appears to be exceptional. Usually, Arrhenius plots for V_{max} are linear up to temperatures well above the lethal level (FRY and HOCHACHKA, 1970). Hence, thermal optimum does not appear to be a meaningful parameter in temperature acclimation. The influence of temperature on the allosteric control or on the co-operative effect of substrate on enzyme activity appears to be more important.

The kinetics of various enzymatic systems display co-operative substrate effects. These effects are indicated by sigmoidal, rather than by hyperbolical, increase in enzyme activity with increasing substrate concentration. Several authors have considered the temperature effect on the apparent affinity for the substrate (K_m) of various enzymes exhibiting co-operative effects. K_m values given by the substrate concentration for which the reaction velocity is half maximum are lower at low temperature than at high temperature. Such higher substrate affinity of the enzyme at low temperature has been shown for lungfish *Lepidosiren paradoxa* hexosediphosphatase (BEHRISCH and HOCHACHKA, 1969) and lactate dehydrogenase (HOCHACHKA and SOMERO, 1968), king crab *Paralithodes camtschatica* phosphofructokinase (FREED, 1965), and lactate dehydrogenase (SOMERO and HOCHACHKA, 1969). As shown in Fig. 4-1, in the case of acetylcholinesterase, the relationship between temperature and K_m may be more complex. Over the upper temperature range, the K_m value for acetylcholine (ACh) of *Salmo gairdnerii* brain decreases with decreasing temperatures. However, at the lower thermal extremes, the effect may be reversed, that is, K_m values increase with decreasing temperatures. For some species, plots of K_m against temperature thus yield V-shaped curves,

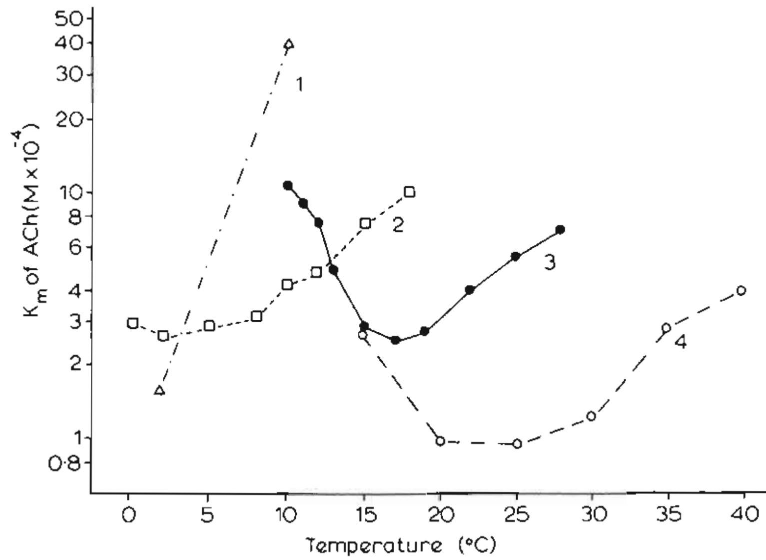

Fig. 4-1: Effect of temperature on the apparent affinity for the substrate (K_m) of brain acetylcholinesterase (ACh) of *Trematomus bernachii* (1), rainbow trout *Salmo gairdnerii* acclimated to 2° C (2) or 17° C (3), and the electric eel *Electrophorus electricus* (4). (After FRY and HOCHACHKA, 1970; modified; reproduced by permission of Academic Press.)

with minimum K_m values (highest affinity of the enzyme for the substrate) near temperatures normally encountered in nature.

Another possible mechanism of metabolic adjustment to thermal stress may be found in the temperature-dependent modulation by allosteric effectors of the activity of some key enzymes implicated in the intermediary metabolism. An example is the effect of temperature on the negative modulation (inhibition) of hexosediphosphatase by adenosine-5′-monophosphate (AMP). Hexosediphosphatase, together with phosphofructokinase, is an important enzyme in the control of glycolysis-gluconeogenesis activity at the level of interconversion between fructose-6-phosphate and fructose 1-6 diphosphate. AMP acts as a positive modulator (activator) of phosphofructokinase and as a negative modulator (inhibitor) of hexosediphosphatase. This is part of a control mechanism by which the carbon flow in the Embden-Meyerhof pathway can be directed towards glucose oxidation to pyruvate (glycolysis) or towards glucose synthesis from intermediary metabolism compounds (gluconeogenesis), depending on the level of high energy phosphate compounds. The effect of temperature on the AMP negative modulation of hexosediphosphatase from *Salmo gairdnerii* liver is shown in Fig. 4-2. The effect of AMP on enzyme activity is much larger at 5° C than at 37° C.

Such a control mechanism may be of ecological significance. It must be pointed out, however, that positive modulation by fructose diphosphate as well as negative modulation by ATP of the activity of pyruvate kinase are not affected by temperature (SOMERO and HOCHACHKA, 1968), although this enzyme is very important

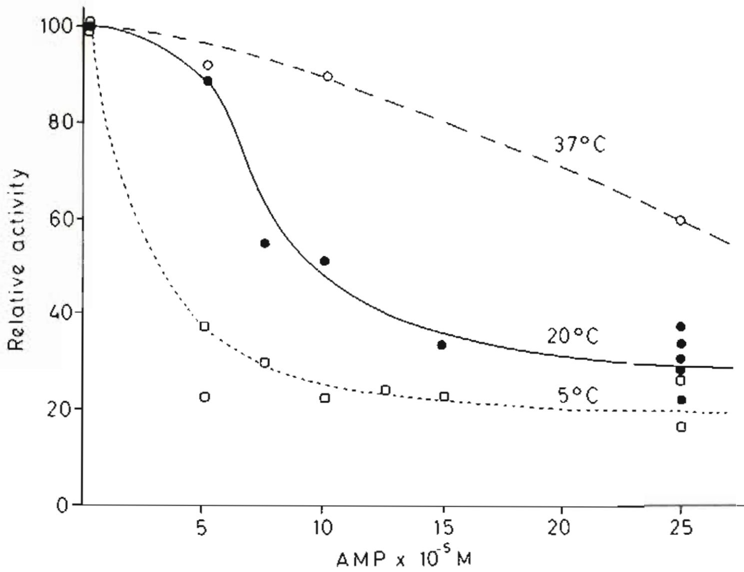

Fig. 4-2: Effect of temperature on the negative modulation by adenosine-5'-monophosphate (AMP) of the activity of *Salmo gairdnerii* liver hexosediphosphatase. (After HOCHACHKA, 1967; modified; reproduced by permission of American Association for the Advancement of Science.)

in regulating the type of activity of the Embden–Meyerhof pathway. Pyruvate kinase of the Alaskan king crab *Paralithodes camtschatica* exists under two different functional forms (SOMERO, 1969): one with maximum affinity for the substrate (phosphoenol–pyruvate), near 5° C, the other with a minimum K_m value near 12° C. These temperatures represent approximately the lower and upper extremes of the habitat temperature. Acting together, the two enzyme forms promote the temperature-independent activity which can be observed during kinetical studies. SOMERO (1969) showed that the two isozymes are formed through a temperature-dependent interconversion of one single protein molecule into two molecules of different structure. As the temperature is raised, the 'cold' form of pyruvate kinase is converted into the 'warm' form of the enzyme. Such a mechanism provides the animal with two kinetically distinct variants of the enzyme, functioning maximally either at low or high temperatures. This allows the tissues to convert phosphoenol pyruvate optimally at every intermediate temperature. Obviously, this is more advantageous than if the king crab would have to rely either on the 'cold' or the 'warm' form of the enzyme. On the other hand, it has been pointed out previously that acetylcholinesterase of rainbow trout acclimated to 2° C displays kinetical properties completely different from the enzyme from fishes acclimated to 17° C, although both enzyme preparations were assayed in the same temperature range (Fig. 4-1). This indicates structural modification of the enzyme molecule during temperature acclimation. Such structural modification can hardly be accounted for by a mechanism such as that described for pyruvate kinase, involving

rapid changes in molecule structure dependent upon the temperature of the incubation medium. If this were the case, there would be no reason for acetylcholinesterase to display different kinetic characteristics when assayed at the same temperature.

It must be concluded, therefore, that thermal acclimation induces synthesis of a 'modified type' of acetylcholinesterase which is more effective under a specific set of temperature conditions. In fact, BALDWIN (1970, *in*: FRY and HOCHACHKA, 1970) has identified two isozymes of acetylcholinesterase in the *Salmo gairdnerii* brain. One of these occurs in warm-acclimated individuals, the other in cold-acclimated fishes. Both isozymes are present simultaneously when the trout are acclimated to an intermediate temperature. In the goldfish *Carassius auratus*, choline acetyltransferase also appears to occur in two 'thermo' isozymic forms (HEBB and co-authors, 1969). In the same way, new isozymes of lactic dehydrogenase are induced during cold acclimation of *C. auratus* (HOCHACHKA, 1965) and the trout *Salvelinus fontinalis* and *Salmo namaycush* (HOCHACHKA, 1967). These 'thermo-isozymes' have kinetic characteristics different from those of the non-inducible isozymes of lactic dehydrogenase: they have lower K_m values at lower temperatures (HOCHACHKA and SOMERO, 1968). Essentially identical results have been obtained with *S. gairdnerii* muscle pyruvate kinase (SOMERO, 1969).

These recent findings point to what could be a main mechanism of metabolic temperature acclimation. The process involves adaptive synthesis of thermo-isozymes with maximum performance at a specific temperature. Such qualitative adjustments in thermal enzyme specificity are obviously of greater biological significance than quantitative changes in enzyme concentration, although various studies suggest that changes also occur during non-genetic adaptation to temperature (EKBERG, 1962; FREED, 1965). Increase in enzyme concentration can be of ecological value, provided the kinetic properties of the enzyme concerned are temperature insensitive or display the interconversion phenomena described for king crab *Paralithodes camtschatica* pyruvate kinase. In most cases where an increase in enzyme activity has been ascribed to an increase in enzyme concentration, the enzyme forms responsible for the activity in different acclimation groups of animals have, however, never been tested.

Thermocompensation of acetylcholinesterase activity by formation of thermo-isozyme is of importance, particularly in view of the limiting role of the nervous system in survival of animals under thermal stress (FRY, 1947; BRETT, 1956; FISHER, 1958). This mechanism, together with others—such as changes in the central nervous system amino-acid concentration (BASLOW, 1967)—can be considered as an integrant part of the process allowing the central nervous system to continue to function properly when the animal must withstand thermal stress. In this context, it is significant that rainbow trout *Salmo gairdnerii* become immobile following transfer to 2° C (BALDWIN, *in*: FRY and HOCHACHKA, 1970). Normal activity appears to return only with the appearance of the 'cold' thermo-isozyme form of acetylcholinesterase.

(4) Conclusions

With the exception of mammals and a few large, fast-swimming fishes, the capacities for thermoregulation of marine organisms are quite restricted. Thermo-

regulation in these species, if present, appears to be largely behavioural. Under thermal stress, most aquatic animals rely on metabolic adaptation. Little is known about the mechanisms of metabolic temperature adaptation. This brief review presents evidence that mechanisms of non-genetic temperature adaptation must be looked for in activity modifications of key enzymes implicated in different metabolic sequences. Changes in enzyme activity can be achieved by affecting (i) the kinetic characteristics of the enzyme, (ii) its intracellular concentration, (iii) both aspects.

Modifications in enzyme kinetic characteristics may be due to a direct effect of temperature on the enzyme structure with resulting changes either in affinity of the enzyme for its substrate or in the allosteric control that a modulator exerts on enzyme activity. Another mechanism which appears to be at work in modifying enzyme kinetic characteristics is the temperature-dependent induction of thermo-isozyme forms of an enzyme. Thermo-isozymes have kinetic characteristics suited to perform efficient catalysis under different temperature conditions; they bring about a compensatory adjustment in metabolic rate which tends to increase the degree of freedom of the organism from the stringencies of environmental temperature.

The information at hand is insufficient to suggest even a tentative model of the molecular basis of metabolic temperature adaptation. The considerations given above, however, indicate lines of research which will probably prove to be rewarding in the near future. In addition to changes in enzyme kinetic characteristics, other processes are probably implicated in metabolic re-organization during the process of acclimation. Alterations in the degree of unsaturation of fatty-acid chains with temperature is a well-known example. Similarly, specific phospholipids occur in direct relation to the state of acclimation (ROOTS, 1968). These changes in lipid biochemistry may be related to effects on membrane functions, such as transport processes, which might represent yet another aspect of metabolic temperature acclimation. In such a view, induction of thermo-isozymes would not constitute the main feature of non-genetic adaptation to temperature but would be part of a more complex cellular re-organization allowing maintenance of proper biological functions.

Literature Cited (Chapter 4)

BASLOW, M. H. (1967). Temperature adaptation and the central nervous system of fish. In C. L. Prosser (Ed.), *Molecular Mechanisms of Temperature Adaptation*. American Association for the Advancement of Science, Washington, D.C. pp. 205–226.

BEHRISCH, H. W. and HOCHACHKA, P. W. (1969). Temperature and the regulation of enzyme activity in poikilotherms. *Biochem. J.*, **111**, 287–295.

BRETT, J. R. (1956). Some principles in the thermal requirements of fishes. *Q. Rev. Biol.*, **31**, 75–87.

BRETT, J. R. (1971). Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerka*). *Am. Zool.*, **11**, 99–113.

CAREY, F. G. and TEAL, J. M. (1969a). Regulation of body temperature by the bluefin tuna. *Comp. Biochem. Physiol.*, **28**, 205–213.

CAREY, F. G. and TEAL, J. M. (1969b). Mako and porbeagle: warm-bodied sharks. *Comp. Biochem. Physiol.*, **28**, 199–204.

CAREY, F. G., TEAL, J. M., KANWISHER, J. W. and LAWSON, K. D. (1971). Warm-bodied fish. *Am. Zool.*, **11**, 137–145.

Ekberg, D. R. (1958). Respiration in tissues of goldfish adapted to high and low temperatures. *Biol. Bull. mar. biol. Lab., Woods Hole*, **114**, 308–316.

Ekberg, D. R. (1962). Anaerobic and aerobic metabolism in gills of the crucian carp adapted to high and low temperatures. *Comp. Biochem. Physiol.*, **5**, 123–128.

Fisher, K. C. (1958). An approach to the organ and cellular physiology of adaptation to temperature in fish and small mammals. In C. L. Prosser (Ed.), *Physiological Adaptation*, American Physiological Society, Washington, D.C. pp. 3–49.

Freed, J. (1965). Changes in activity of cytochrome oxidase during adaptation of goldfish to different temperatures. *Comp. Biochem. Physiol.*, **14**, 651–659.

Fry, F. E. J. (1947). Effects of environment on animal activity. *Publs Ont. Fish. Res. Lab.*, **55**, 1–62.

Fry, F. E. J. and Hochachka, P. W. (1970). Fish. In G. C. Whittow (Ed.), *Comparative Physiology of Thermoregulation*, Vol. I. Academic Press, New York. pp. 79–134.

Hebb, C., Morris, D. and Smith, M. W. (1969). Choline acetyl-transferase activity in the brain of goldfish acclimated to different temperatures. *Comp. Biochem. Physiol.*, **28**, 29–36.

Hochachka, P. W. (1962). Thyroidal effects on pathways for carbohydrate metabolism in a teleost. *Gen. comp. Endocrinol.*, **2**, 499–505.

Hochachka, P. W. (1965). Isoenzymes in metabolic adaptation in a poikilotherm: subunit relationships in lactic dehydrogenases of goldfish. *Archs Biochem. Biophys.*, **111**, 96–103.

Hochachka, P. W. (1967). Organization of metabolism during temperature compensation. In C. L. Prosser (Ed.), *Molecular Mechanisms of Temperature Adaptation*. A.A.A.S., Washington, D.C. pp. 177–203.

Hochachka, P. W. and Hayes, F. R. (1962). The effect of temperature acclimation on pathways of glucose metabolism in the trout. *Can. J. Zool.*, **40**, 261–270.

Hochachka, P. W. and Somero, G. N. (1968). The adaptation of enzymes to temperature. *Comp. Biochem. Physiol.*, **27**, 659–668.

Kinne, O. (1964). Non-genetic adaptation to temperature and salinity. *Helgoländer wiss. Meeresunters.*, **9**, 433–458.

Kinne, O. (1967). Physiology of estuarine organisms with special reference to salinity and temperature. In G. H. Lauff (Ed.), *Estuaries*. A.A.A.S., Washington, D.C. pp. 525–540. (*Publs Am. Ass. Advmt Sci.*, **83**.)

Kinne, O. (1970). Temperature: Animals–Invertebrates. In O. Kinne (Ed.), *Marine Ecology*, Vol. I, Environmental Factors, Part 1. Wiley, London. pp. 407–514.

Lewis, J. B. (1963). Environmental and tissue temperatures of some tropical intertidal marine animals. *Biol. Bull. mar. biol. Lab., Woods Hole*, **124**, 277–284.

Precht, H. (1958). Concepts of the temperature adaptation of unchanging reaction systems of cold-blooded animals. In C. L. Prosser (Ed.), *Physiological Adaptation*. American Physiological Society, Washington, D.C. pp. 50–78.

Prosser, C. L. (1958). The nature of physiological adaptation. In C. L. Prosser (Ed.), *Physiological Adaptation*. American Physiological Society, Washington, D.C. pp. 167–180.

Roots, B. I. (1968). Phospholipids of goldfish (*Carassius auratus* L.) brain: the influence of environmental temperature. *Comp. Biochem. Physiol.*, **25**, 457–466.

Somero, G. N. (1969). Pyruvate kinase variants of the Alaskan king-crab. *Biochem. J.*, **114**, 237–241.

Somero, G. N. and Hochachka, P. W. (1968). The effect of temperature on catalytic and regulatory functions of pyruvate kinases of the rainbow trout and the antarctic fish *Trematomus bernacchii*. *Biochem. J.*, **110**, 395–400.

Somero, G. N. and Hochachka, P. W. (1969). Isoenzymes and short term temperature compensation in poikilotherms: activation of lactate dehydrogenase isoenzymes by temperature decreases. *Nature, Lond.*, **223**, 194–195.

Whittow, G. C. (Ed.) (1971). *Comparative Physiology of Thermoregulation*, Vol. II, Mammals, Academic Press, New York.

Wilkens, J. L. and Fingerman, M. (1965). Heat tolerance and temperature relationship of the fiddler crab *Uca pugilator* with reference to body coloration. *Biol. Bull. mar. biol. Lab., Woods Hole*, **128**, 133–141.

5. MECHANISMS OF ION AND OSMOREGULATION

R. GILLES

(1) Introduction

Life on earth organized itself around the properties of water. Water provides the principal physical framework in which the chemical reactions of organisms take place. All living systems contain a high proportion of water. Most of this water is in a free, uncombined state and provides a vehicle for molecules involved in the chemical interactions characteristic of life. Among the solutes encountered in living organisms, inorganic ions are very important. They participate as co-factors in many enzyme reactions. They provide chemical gradients which act as stores of potential energy, and they influence the permeability of biological membranes to other solutes.

Many enzymatic systems are located in the cell on highly organized supports. This points to the importance of the cellular volume in reactions which involve these enzymes as catalysts. Solutes influence the osmotic mobility of water and play an important part in cell volume regulation.

Organisms occupy a large variety of habitats. A basic prerequisite for successful conquest of aquatic habitats with different salinity regimes is the capacity to regulate internal water and inorganic solutes, keeping an internal medium suitable for life-supporting molecular interactions.

Aquatic organisms have developed various mechanisms for regulating their cellular volume and ionic composition. We can distinguish between two major systems: regulation can be achieved at the intracellular level or by controlling the composition and osmotic pressure of extracellular fluid.

In this review we shall restrict ourselves to the mechanisms of ion and osmoregulation occurring in aquatic multicellular animals. Ion-transport mechanisms in plants as well as ion regulation related to buoyancy will not be considered here. These topics have been reviewed recently (GUTKNECHT and DAINTY, 1968; MACROBBIE, 1970; DENTON, 1971). Quantitative aspects of ion and osmoregulation have been covered in Volume I, Chapter 4.

In multicellular animals, the extracellular fluid osmotic pressure can evolve in different ways as a function of the osmotic pressure of the surrounding medium. Four different types of extracellular fluid regulation are illustrated in Fig. 5-1. Species able to maintain their blood osmotic pressure at a more or less constant level independently of the osmotic pressure of the external medium are called homeosmotic; examples are the prawn *Palaemonetes varians* and the eel *Anguilla anguilla*. Only a few species exhibit such an effective regulatory mechanism (Volume I: KINNE, 1971). Even in species usually considered homeosmotic—such as euryhaline teleosts—changes of 20 to 30% in plasma osmotic pressure have been

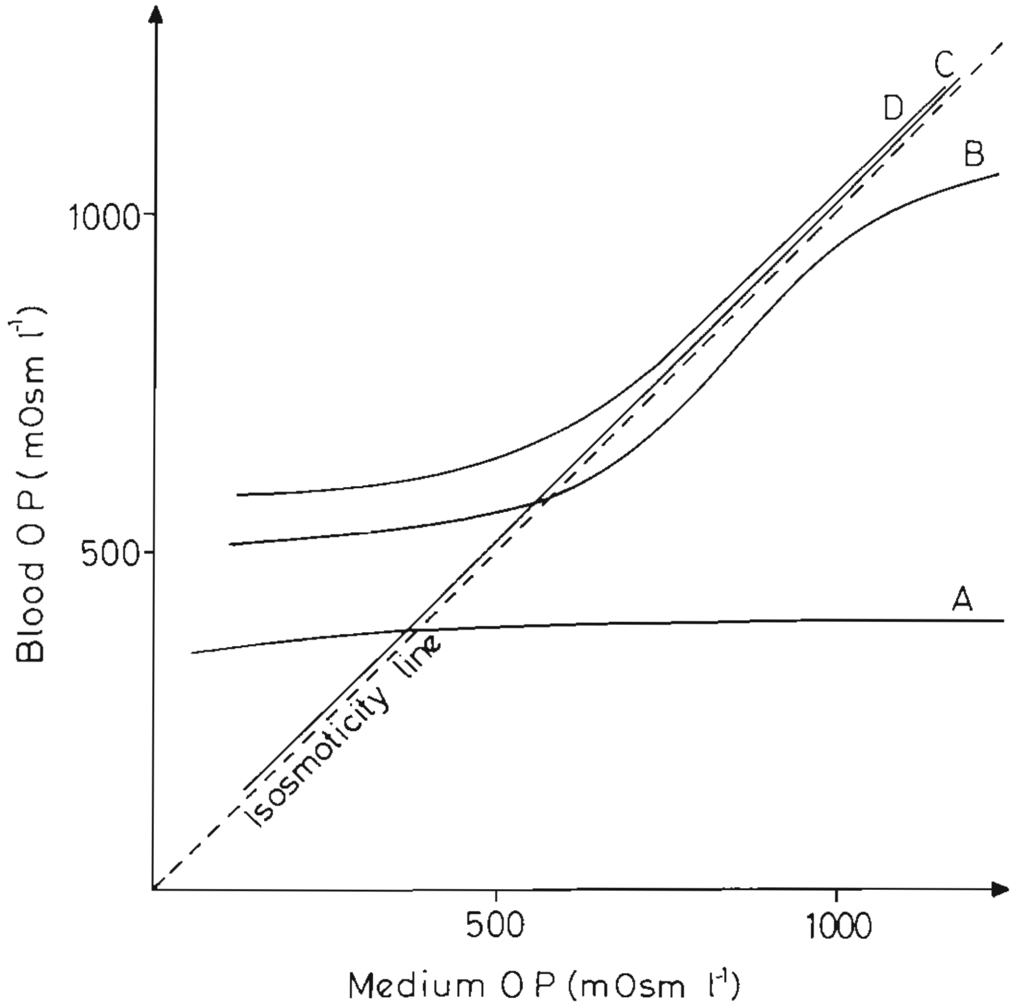

Fig. 5-1: Schematical representation of four different types of regulation of blood osmotic pressure (OP) as a function of osmotic pressure of the ambient medium. A: homeosmotic animals; B, C, D: poikilosmotic animals. (Original.)

reported after transfer from sea water to fresh water (HUGGINS and COLLEY, 1971; LASSERRE and GILLES, 1971). Many species are unable to regulate their blood osmotic pressure effectively when exposed to osmotic stress. These forms are called poikilosmotic. Some of these, e.g. marine molluscs, are not able to regulate their blood osmotic concentration. Others regulate in diluted or highly concentrated media. These species with only a limited capacity for blood-osmotic pressure regulation constitute the most important group. They are hypo-osmotic, hyper-osmotic or hypo-hyperosmotic regulators (Volume I: KINNE, 1971). The cells of these poikilosmotic forms must cope with osmotic stress every time the animal is confronted with salinity change.

Two general types of mechanism are thus used by multicellular animals to maintain their cellular volume and inorganic ion concentration. In accordance with FLORKIN (1962), we shall consider (i) an **extracellular-fluid anisosmotic regulation** which is involved in the control of the blood osmotic pressure; (ii) a regulation of the osmotic pressure of the cell: the **intracellular-fluid isosmotic regulation.** This terminology, which is now widely accepted, should of course be restricted to aquatic multicellular animals. Isosmoticity between cell fluid and environmental medium is not found in limnic unicellulars. Besides, the vacuolar fluid, which can be strongly hyperosmotic, cannot properly be considered an extracellular fluid.

(2) Extracellular-Fluid Anisosmotic Regulation

When dealing with extracellular-fluid anisosmotic regulation, we are concerned with the mechanisms which allow an organism to maintain an osmotic gradient between its blood and the environmental medium. Most of the time this is achieved by regulation of the amount of inorganic ions in extracellular fluids. Extracellular-fluid anisosmotic regulation cannot, however, be considered as synonymous with blood ion regulation since, as we shall see, various species although effecting some degree of ion regulation are unable to achieve an osmotic gradient between their blood and the surrounding medium. Moreover, in some chondrichthyeans, urea participates in the blood-hyperosmotic state that the animal is maintaining when in diluted media (p. 291).

Thousands of references are available on ionic composition of the blood of living organisms. Unless otherwise specified, the data presented in Tables 5-1 and 5-2 have been collected from review articles (worms: OGLESBY, 1969; molluscs: SCHOFFENIELS and GILLES, 1972; crustaceans: FLORKIN, 1960; fishes: HOLMES and DONALDSON, 1969; see also the papers by ROBERTSON, 1949, 1953, 1954 and ANDREWS, 1967).

Table 5-1 gives the ionic composition and the osmotic pressure of the blood of several aquatic animals. The blood of most marine invertebrates tested is close to isosmoticity with the surrounding medium. This is also the case for the blood of vertebrates such as the hagfish and the elasmobranchs. However, a passive equilibrium between sea water and blood plasma cannot account for this observation. As a matter of fact, blood concentration of several inorganic ions is maintained at a level different from that of the surroundings. These species are capable of ionic regulation (although this mechanism is not directly involved in an extracellular-fluid anisosmotic regulation). In some of these species, which are euryhaline, inorganic-ion regulation becomes a means of regulation of the blood osmotic pressure in diluted media. As shown in Table 5-2, the Chinese crab *Eriocheir sinensis* is capable of blood hyperosmotic regulation in diluted media although its blood remains close to isosmoticity with the surrounding medium in sea water. This behaviour, however, is not a general rule among euryhaline marine invertebrates, since many of them, for instance molluscs, are not capable of extracellular-fluid anisosmotic regulation. On the other hand, some euryhaline invertebrates, such as species of *Artemia* or *Palaemonetes* behave similarly to euryhaline teleosts, maintaining a blood hypo-osmotic when in concentrated media and hyperosmotic when in diluted media. Extracellular-fluid anisosmotic regulation is also observed

Table 5-1

Ionic composition and osmotic pressure of blood (mesogleal fluid) of aquatic animals, and of sea water (SW) and fresh water (FW) (Compiled from the sources mentioned in the text)

Species	Na	K	Ca	Mg	Cl	SO_4	Urea	Osmotic pressure	Habitat[a] water
Invertebrates									
Coelenterates									
Aurelia sp. (mesogleal fluid)	474	10·72	10·03	53·0	580	15·77	—	1143	SW
Worms									
Aphrodite aculeata	477·3	12·81	10·42	54·05	561·7	28·81	—	1145	SW
Eunice sebastiani	486·9	20·37	13·07	74·13	560·0	35·85	—	1190	SW
Arenicola marina	480·7	10·52	10·40	54·84	558·3	26·59	—	1141	SW
Golfingia vulgaris	498·4	11·16	10·87	37·53	551·6	26·31	—	1135	SW
Echiurus echiurus	507·6	14·09	10·16	46·50	557·0	28·67	—	1164	SW
Molluscs									
Mytilus edulis	472	13·5	10·3	53·8	556·4	27·8	—	1133	SW
Mya arenaria	476·7	10·7	11·0	53·3	550·9	28·6	—	1131	SW
Buccinum undatum	457·8	14·2	10·7	55·4	550·9	25·5	—	1114	SW
Archidoris pseudoargus	476·3	12·8	13·6	57·6	550·9	27·2	—	1138	SW
Sepia officinalis	439	20·6	9·4	52·7	578·4	6·2	—	1106	SW
Eledone cirrhosa	457·8	15·2	11·0	55·4	501·9	24·8	—	1066	SW
Anodonta cygnea	15·6	0·49	4·2	0·1	11·7	—	—	32	FW
Limnea stagnalis	47·4	2·8	1·5	2·4	42·6	—	—	96	FW
Crustaceans									
Callinectes sapidus	454·7	13·3	19·65	9·55	480·2	—	—	977	SW
Maja squinado	488	12·37	13·56	44·1	554	14·50	—	1126	SW
Carcinus maenas	531	12·26	13·32	19·5	557	16·46	—	1149	SW
Nephrops norvegicus	541	7·81	11·95	9·28	552	19·80	—	1141	SW
Uca pugnax	328	11	16	23	537	42	—	957	SW
Palaemon serratus	394	7·7	—	25·2	430	5·2	—	862	SW
Artemia salina	207	8	—	2	179	—	—	386	SW
Astacus fluviatilis	212	4·1	15·8	1·5	199	—	—	432	FW
Potamon niloticus	259	8·4	12·7	—	242	—	—	522	FW

Table 5-1—*Continued*

Species	Na	K	Ca	Mg	Cl	SO_4	Urea	Osmotic pressure	Habitat[a] water
				Vertebrates					
				Fishes					
Myxine glutinosa	529	10·4	6·4	25·6	534	18·3	—	1038*	SW
Polistotrema stouti	522	10·9	3·9	13·8	501	—	—	1029*	SW
Lampetra fluviatilis	119·6	3·2	2·0	2·1	95·9	2·7	—	225	FW
Raja stabuloforis	255	4·9	3·8	2·8	241	—	453	960	SW
Squalus acanthias	263	4·1	6·6	3·1	249	—	357	1007*	SW
Acterodontus triseriata	235	10·0	5·0	3·0	230	0·5	338	821	SW
Carcharhinus leucas	245	6·4	4·5	1·6	219	0·7	180	657	FW
Hydrolagus colliei	268	6·9	4·8	1·5	272	0·6	303	856	SW
Latimeria chalumnae	181	51·3	3·5	14·4	199	—	355	804	SW
Acipenser transmontanus	130	2·5	1·7	2·1	115·1	0·4	1·0	252	SW
Mureana helena	211·8	1·95	3·87	2·43	188·4	5·7	—	414	SW
Paralabrax clathratus	180	5·0	3·0	1·5	147	0·5	0·1	337	SW
Cyprinus carpio	130	2·93	2·12	1·23	125·2	—	—	274*	FW
				Reptiles					
Malaclemys centrata	163·4	3·8	—	—	136·6	—	115·2	458·8*	SW
Caretta caretta	166	3·5	—	—	109·6	—	30·5	465*	SW
Clemmys leprosa	146	2·6	—	—	146	—	4·7	362*	FW
Sea water[b]	480·2	10·17	10·42	54·71	560·0	28·84	—	—	—
Fresh water[b] soft	0·24	0·005	0·06	0·04	0·22	0·04	—	—	—
hard	2·22	1·46	3·98	1·67	2·54	3·95	—	—	—

Ionic concentrations are given in $mM\ l^{-1}$. Osmotic pressure (in $mOsm\ l^{-1}$) has been calculated from the ionic concentrations given by the authors, except where indicated by *.

[a] Acclimation medium before sampling.

[b] Values for sea water and fresh water are generalized; they may not be identical to the external media of the animals tested.

in all the freshwater species studied up till now, as well as in many stenohaline marine vertebrates which maintain a blood osmotic pressure lower than the osmotic pressure of the surroundings (Table 5-1).

In all these species, inorganic ions—mainly sodium and chloride—can account for most of the blood osmotic pressure. On the other hand, it is interesting to note that in various marine vertebrates, inorganic ions account only for part of the blood osmotic pressure. For instance, the blood of the coelacanthiform *Latimera chalumnae*, and of marine elasmobranchs, is near isosmotic with sea water although the inorganic ions account for about half of the blood osmotic pressure. In these

Table 5-2

Blood ionic composition of a flatfish, a shark, a turtle and a crustacean after acclimation to the media indicated. Concentration of blood constituents in mM l^{-1}; osmotic pressure in mOsm l^{-1}. The animals were maintained in their acclimation medium—sea water (SW), 50% SW, or fresh water (FW)—for periods ranging from 1 week (*N. brevirostris*) to several months (*E. sinensis*) (Compiled from the sources listed below)

Species	Na	K	Cl	Urea	Osmotic pressure	Acclimation medium
Platichthys flesus[a]	141·7	3·4	168·1	—	297	SW
	123·9	2·9	131·7	—	240	FW
Negaprion brevirostris[b]	—	—	310	421	1117	SW
			252	191	726	50% SW
Malaclemys centrata[c]	163·4	3·8	136·6	115·2	458·8	SW
	129·0	3·1	88·0	21·5	271·8	FW
Eriocheir sinensis[d]	536	10·3	520	—	1054	SW
	300	4·9	319	—	615	FW

[a] LAHLOU (1967).
[b] GOLDSTEIN (1970): osmotic pressure calculated, assuming Na^+ concentration to be equal to Cl^- concentration.
[c] GILLES-BAILLIEN (1970).
[d] SCHOFFENIELS and GILLES (1970a).

species, it is mainly urea which makes up the rest of the osmotic pressure. Urea plays also an important part in the extracellular-fluid anisosmotic regulation of stenohaline freshwater elasmobranchs and in euryhaline species. From Table 5-2 it can also be seen that urea is an important factor in the regulation of the blood osmoconcentration in several other vertebrates, such as reptiles, when submitted to osmotic stress.

It thus appears that all species studied are capable of regulating their blood inorganic-ion concentration. This ion regulation (Volume I, Chapter 4) is an integrant part of the extracellular-fluid anisosmotic regulation, and in most species the adjustment of the blood osmotic pressure only concerns the inorganic-ion regulation mechanism. However, some species, among which are those belonging to the class Chondrichthyes, use urea as an osmotic effector. In these species urea

plays a part which is as important as that of inorganic ions in the regulation of the blood osmotic pressure. We shall therefore consider both mechanisms of regulation.

(a) Mechanisms Regulating Blood-Inorganic Solute Concentrations

General Considerations

Since most studies in this field have been conducted on decapod crustaceans and on teleost fishes, this section deals mainly with mechanisms of regulation of blood inorganic-ion content in representatives of these two groups.

In most aquatic animals, extracellular-fluid anisosmotic regulation is primarily effected by the inorganic ions sodium and chloride (Tables 5-1, 5-2). As a matter of fact, the hyperosmotic state observed in freshwater forms or in many euryhaline species acclimated to a diluted medium, as well as the hypo-osmotic state observed in many marine species, can be accounted for mainly by the amount of sodium and chloride present in the blood. These hyper- and hypo-osmotic states are the result of charge and discharge phenomena which are controlled by both passive and active mechanisms. The main passive mechanisms are the permeability to salts and water and the Donnan equilibrium; uptake of salts can be considered as an active factor.

Donnan equilibrium

In some species in which the blood is near isosmotic to the surrounding sea water, the Donnan equilibrium may account for the slight difference in ionic concentrations between blood and external medium (Table 5-1; Volume I: KINNE, 1971). Proteins which cannot permeate the membranes of the animal concerned may induce a Donnan equilibrium affecting the distribution of all ions. Proteins may also affect ion distribution by retaining them in indiffusible complexes. These possibilities have been investigated in marine invertebrates by dialysing body-fluid samples against sea water through a collodion membrane which is permeable to water and ions but not to proteins (ROBERTSON, 1949). In such experiments any difference between the dialysate and the dialysed fluid is due to ion binding or Donnan equilibrium. The results obtained with plasma of *Nephrops norvegicus* are listed in Table 5-3. With the exception of calcium, the residual differences in ionic concentra-

Table 5-3

Nephrops norvegicus. Composition of blood plasma before and after dialysis with sea water. All values expressed as mM kg^{-1} water (After ROBERTSON 1949; modified; reproduced by permission of Company of Biologists Ltd.)

Fluid	Na	K	Ca	Mg	Cl	SO_4
Sea water	453·6	9·6	10·0	51·7	529·9	27·3
Plasma	517·7	7·6	13·8	8·9	518·6	18·5
Dialysate	458·6	9·8	11·1	53·3	524·0	26·8
Dialysate calculated from mean Donnan ratio	458·6	9·7	10·1	52·7	524·0	26·8

tion, remaining after dialysis, can be attributed to a Donnan equilibrium. The fact that the calcium concentration exceeds the Donnan-equilibrium concentration implies some binding of this cation.

Thus a small, but significant, Donnan effect can be found in some species with high blood-protein concentrations. As pointed out by ROBERTSON (1949), this is the case in some crustaceans. But in most other marine invertebrates such an effect could not be demonstrated. Even where the Donnan effect significantly modifies ionic distributions, it accounts only for a small portion of ionic regulation. The differences observed between the ionic composition of the blood and that of sea water must then be attributed to other processes.

Movements of salt and water

Most marine invertebrates and the hagfish are practically isosmotic with sea water and hence have only few problems in ion regulation. On the other hand, sea-water living teleosts, and in some cases also arthropods, maintain a hypo-osmotic state. According to physico-chemical considerations this situation results in outflux of solvent and net movement of solutes towards the solution with the lower thermodynamic energy. Thus a sea-water teleost tends to lose water and to gain inorganic ions (mainly Na^+ and Cl^-). Marine elasmobranchs and coelacanthiforms, which are nearly isosmotic with sea water due to high blood-urea concentrations, still must continuously compensate a gain in inorganic ions and a loss of urea.

Marine species acclimating to a diluted medium exhibit two types of responses: (i) Their blood remains isosmotic with the environmental medium down to the lower limiting salinity (e.g. molluscs, many annelids, some crustaceans and the hagfish); (ii) their blood is maintained hyperosmotic to the surrounding medium. This last case implies a tendency to gain water and to lose inorganic ions.

We thus have to distinguish between animals without extracellular-fluid anisosmotic regulation and those which maintain a blood osmoconcentration different from that of their ambient medium.

In species without extracellular-fluid anisosmotic regulation, the blood remains near isosmotic in all salinities encountered. Examples are euryhaline sea-water molluscs. In some molluscan species, however, blood has been found hyperosmotic in salinities lower than 15‰ (MILNE, 1940; FREEMAN and RIGLER, 1957; TODD, 1964). In fact this osmotic gradient can be maintained only temporarily due to reduction of contact with adverse salinities (Volume I: KINNE, 1971, pp. 939, 941). The influence of such a mechanism on changes in blood osmotic pressure of *Mytilis edulis* during acclimatization to diluted media is shown in Fig. 5-2. It can be seen that the blood is always isosmotic with the perivisceral fluid which is maintained temporarily hyperosmotic to the environmental medium. The same phenomenon has also been observed in gastropods such as *Littorina saxatilis* (AVENS and SLEIGH, 1965) or *Siphonaria pectinata* (MCALISTER and FISHER, 1968) and in many other invertebrates (for further examples, consult KINNE, 1971, p. 941). Hyperosmosis due to reduction of contact with adverse salinities is of considerable importance in the survival of these animals under the temporary adverse conditions which often prevail in intertidal or estuarine environments. However, this mechanism can only help the animal to wait for better conditions during a relatively short period of time.

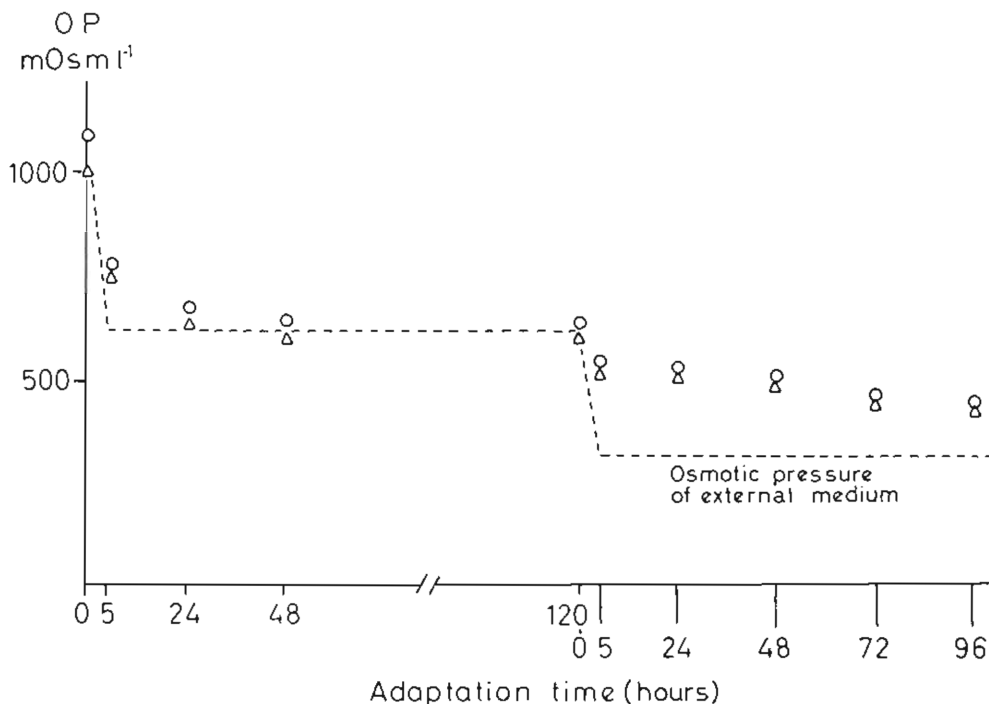

Fig. 5-2: *Mytilus edulis*. Changes in osmotic pressure (OP) of blood (○) and perivisceral fluid (△) during acclimatization to diluted media. (After GILLES, 1972a; modified; reproduced by permission of *Biological Bulletin*.)

Body walls are continuously bathed by the ambient medium and provide avenues for exchange of water and inorganic ions. In marine species which maintain a hypo-osmotic state, the solutes present in the surrounding medium tend to diffuse into the blood. Such diffusion involves mainly sodium and chloride, to a much lesser extent potassium. Movements of sodium chloride have been indicated by KROGH (1939), and demonstrated by radio-isotope techniques. If $^{24}Na^+$ is added to the external medium, the amount of isotope penetrating into the test animal can be measured (Na^+ influx), and after injecting the animal with $^{24}Na^+$, isotope appearance in the surrounding water can be determined (Na^+ efflux). In organisms in salt balance, influx and efflux of ions are identical and measurements of the fluxes can be taken as an indication of the total rate of Na^+ turnover in the animal. This rate constant (K) is a relatively accurate parameter for comparing permeability of different organisms exposed to different environmental conditions. Table 5-4 lists the K values for several fishes. The rate constants for sodium vary greatly from one species to another. For instance, the killifish *Fundulus heteroclitus* when in sea water exchanges about 35% of its total exchangeable sodium every hour, the toadfish *Opsanus tau* only 16%, and the flounder *Platichthys flesus* about 45%. Marine elasmobranchs exchange sodium far less rapidly. K values in these species are below 1%. Also freshwater fishes, or euryhaline species when acclimated to fresh water, exchange sodium very slowly. The same is true of

Table 5-4

Permeability coefficient (K), calculated from total sodium fluxes in various fishes. Total Na fluxes are expressed as μ equivalents 100 g^{-1} fish hr^{-1}. SW: sea water; FW: fresh water (Compiled from the sources indicated)

	Medium	Na flux	K	Author
Platichthys flesus	SW	2600	45	MOTAIS and MAETZ (1965)
	FW	43	< 1	
Fundulus heteroclitus	SW	2020	34	MAETZ and co-authors (1967)
	FW	60	< 0·1	POTTS and EVANS (1966)
Opsanus tau	SW	805	16	LAHLOU and SAWYER (1969)
Carassius auratus	FW	27	< 1	LAHLOU and co-authors (1969a)
Squalus acanthias	SW	90	0·9	BURGER and TOSTESON (1966)
Hemiscyllium plagiosum	SW	75	0·74	CHAN and co-authors (1967)

crustaceans. The permeability of freshwater crustaceans to water and salts is remarkably low when compared to that of sea-water species (GROSS, 1957; SHAW, 1961b; CROGHAN and LOCKWOOD, 1968). Good examples are experiments with gammarid crustaceans carried out by SUTCLIFFE and SHAW (Table 5-5). Since gammarids inhabit marine, brackish and freshwater habitats, this group provides good material for examining mechanisms of ion regulation in animals exhibiting different degrees of adaptation to salinity. Permeability-rate constants obtained for sodium are presented in Table 5-5. They are about four times greater in marine species than in freshwater ones. K values for brackish-water gammarids are of the same order as those obtained for freshwater species. This may be due to the fact that *Gammarus duebeni*, *G. zaddachi* and *G. tigrinus* are not true brackish-water gammarids and often experience very low salinities in their normal habitat.

Table 5-5

Sodium rate constants (hr^{-1} for 200 mg of animal) in gammarids from different environments (Compiled from the sources indicated)

Species	Rate constant	Author
Marine		
Marinogammarus finmarchicus	0·0050	SUTCLIFFE (1968)
Marinogammarus obtusatus	0·0058	SUTCLIFFE (1968)
Brackish		
Gammarus tigrinus	0·0019	SUTCLIFFE (1968)
	0·0021	SUTCLIFFE (1968)
Gammarus zaddachi	0·0014	SUTCLIFFE (1967a)
Gammarus duebeni	0·0009	SUTCLIFFE (1967a)
	0·0005	SUTCLIFFE (1967a)
Limnic		
Gammarus duebeni	0·0011	SUTCLIFFE and SHAW (1968)
Gammarus pulex	0·0014	SUTCLIFFE (1967b)
Gammarus lacustris	0·0014	SUTCLIFFE and SHAW (1967)

From what has been said above, it can be concluded that permeability to salt and water is lower in freshwater than in sea-water species. However, rate constants for salts calculated for euryhaline crustaceans from marine, brackish or freshwater habitats, do not significantly change with acclimation to different salinities (Table 5-6). On the other hand, the H_2O-influx constant for *Carcinus maenas* remains about the same in sea water (0·79), in 70% (0·78) and in 40% sea water (0·72; RUDY, 1967). Presumably reduction of body-wall permeability to salts and water represents an important link in the chain of adaptive evolution to fresh water. However, such a mechanism does not seem to play any role in euryhaline crustaceans passing from a concentrated medium to a more diluted one.

Table 5-6

Sodium permeability in euryhaline crustaceans acclimated to different salinities. —: no data recorded; rate constants are expressed in hr^{-1} for 100 mg of animal. Values for *Astacus pallipes* recalculated from POTTS and PARRY (1964), assuming that blood volume is 35% of the body weight (Compiled from the sources indicated)

Species	External medium (% sea water)	Mean blood Na conc. (mM l^{-1})	Body-wall Na loss for 100 mg of animal (μM hr^{-1})	Rate constant	Author
Carcinus maenas	100	460	2·466	0·0052	SHAW (1961a)
	50	340	1·692	0·0048	
	40	300	1·406	0·0046	
Gammarus zaddachi	—	500	0·665	0·0013	SUTCLIFFE (1968)
	—	300	0·457	0·0015	
	—	252	0·332	0·0013	
Marinogammarus finmarchicus	100	536	2·787	0·0051	SUTCLIFFE (1968)
	50	368	1·864	0·0050	
	20	324	1·520	0·0046	
Astacus pallipes	—	310	0·442	0·0014	BRYAN (1960a, b, c)
	—	186	0·251	0·0013	
	—	310	0·671	0·0021	
	—	180	0·371	0·0020	

The situation appears to be quite different in euryhaline fishes. For instance, MOTAIS (1961a, b) has found that the euryhaline flounder *Platichthys flesus* exchanges about 25% of its total body sodium per hour when in sea water, but only 0·7% when acclimated to fresh water (see also Table 5-4). When transferred from sea water to fresh water the flounders reduce their sodium outflux rapidly; it ceases almost completely after 1 hr. In contrast, in flounders acclimated to fresh water the rate of sodium exchange increases only slowly when transferred to sea water; the whole process requires about 30 hrs. If sea-water acclimated flounders are placed in fresh water for only 30 mins, their rate of sodium exchange drops by 90%; but if immediately returned to sea water, their sodium-exchange rate immediately resumes control values and delayed regulation (observed with freshwater-

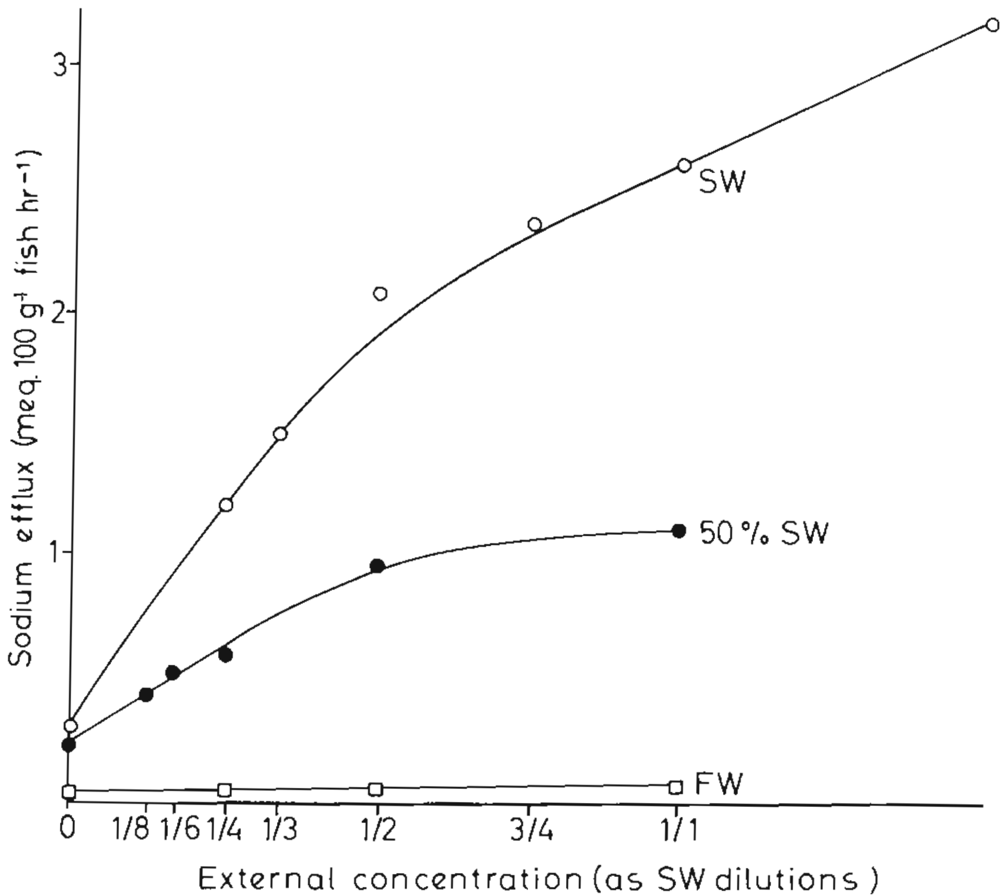

Fig. 5-3: *Platichthys flesus*. Relation between external concentration and efflux of Na^+ in individuals acclimated to sea water (SW), 50% sea water or fresh water (FW). (After MOTAIS and co-authors, 1966; redrawn; reproduced by permission of Rockefeller University Press.)

acclimated flounders) no longer occurs (see p. 278 for details). Fig. 5-3 shows the outfluxes of labelled Na^+ from flounders acclimated to sea water or to fresh water as a function of the Na^+ concentration of the ambient medium. The fluxes were measured during the first 30 mins after transfer to the new media. In sea-water acclimated flounders, the Na outflux changes immediately after transfer, while in freshwater-acclimated individuals, the flux values remain low. This indicates that, in contrast to the situation in euryhaline crustaceans, the permeability of euryhaline fishes can be changed in different salinities. The results suggest, moreover, the existence of a mechanism which can assist osmoregulation during 'long-term' acclimation.

Water exchange has been investigated with the aid of tritiated water. In fresh water, the eel *Anguilla anguilla* exchanges an amount of water equivalent to 42% of its total body water within 1 hr. If transferred to sea water, water exchange

decreases to 29% (MOTAIS and co-authors, 1969). In the freshwater-acclimated *Tilapia mossambica* water exchange amounts to 115% of the body water and in individuals transferred to sea water to 84% (POTTS and co-authors, 1967). This is striking, since osmotic gradients between the external media of these fishes are greater when they are in sea water than when they are in fresh water (see Table 5-1). Transfer of water, however, is less in sea water. Body-wall permeability is thus apparently lower in fishes acclimated to sea water. These observations on overall fluxes of water and salt point to differences between crustaceans and fishes. Although both groups show reduced body-wall ion permeability in their freshwater-living representatives, euryhaline fishes apparently possess a mechanism for adjusting permeability depending on the habitat medium. Such a mechanism seems to be absent in euryhaline crustaceans.

Measurements of total fluxes, influx or outflux through the whole animal, thus yield information on the role of particular structures in extracellular-fluid anisosmotic regulation. Skin (integument), gut, excretory organs, gills, the urinary bladder and salt glands are candidates for such a role and they have been studied for their relative participation in the blood osmotic regulation process.

The Various Structures Implicated

The skin

This section considers skin permeability in fishes and the permeability of corresponding structures in other animals.

The skin of fishes is structurally complex and liberally supplied with mucous glands. These properties suggest a potential barrier to molecular transfer. Unfortunately, there are few direct measurements available to sustain or to contradict such a view. KROGH (1939), considering the facts at hand, concluded that fish skin is not very permeable and that the gills, rather than the skin, represent the principal pathway of solvent and solutes. MOTAIS and co-authors (1969) have measured exchanges of tritiated water across the gills of several freshwater and marine fishes and found that virtually all of the water exchanged could be accounted for by the amount passing through the gill tissue.

The skin of fishes thus seems to be a rather impermeable structure, which, moreover, constitutes an important factor in osmoregulation. When freshwater-acclimated eels *Anguilla anguilla* are handled or dried with a towel they excrete large volumes of urine and eventually die. This is considered to result from skin damage, possibly due to removing part of the coating of mucus on its surface. These findings reveal that in fishes, as in molluscs, 'mechanical' devices help the animal to cope with its osmotic problems.

Practically nothing is known about skin permeability in other animal groups. Ligaturing experiments on aquatic arthropods indicate that the exoskeleton with its chitinous cuticle is quite impermeable (CROGHAN, 1958). Precise measurements of cutaneous permeability are, however, still lacking.

The gut

Water accumulating in euryhaline teleosts exposed to fresh water is excreted by the kidney. Water loss encountered in sea water can be compensated for, at least in

part, by drinking. Prevented from drinking, a marine teleost dies rapidly from dehydration. In euryhaline species, drinking rate changes as a function of salinity. In fresh water, eels *Anguilla anguilla* drink an amount of fluid per hour which is equivalent to 0·14% of their body weight; in sea water, drinking rate increases to 0·33% (MAETZ and SKADHAUGE, 1968). *Tilapia mossambica* drink 0·26% of their body weight in fresh water but 1·11% in sea water (POTTS and co-authors, 1967).

Drinking is also assumed to be important in hypo-osmoregulation in the larvae of *Aedes detritus* (BEADLE, 1939) and of *Limnephilus affinus* (SUTCLIFFE, 1961) as well as in the crabs *Uca pugnax* and *U. pugilator* (GREEN and co-authors, 1959).

Sea-water living mammals and reptiles do not seem to drink. Presumably water loss in these species is so small that they can rely on food intake for water balance. In regard to reptiles this hypothesis will be discussed further on p. 290.

Water absorption occurs in the intestine subsequent to salt absorption. Most of the divalent ions appear to be retained in the gut, though some are absorbed and

Table 5-7

Lophius piscatorius. Composition of gut fluid. All concentrations in mM kg^{-1} of fluid (After SMITH, 1930; modified; reproduced by permission of American Society of Physiologists)

Fluid	Na	K	Ca	Mg	Cl	SO_4
External medium	235	8·0	6·9	29	275	18
Gastric fluid	237	6·6	6·1	18	235	17
Anterior intestinal fluid	127	24	20·6	94	90	125
Posterior intestinal fluid	56	5·5	21·5	98	41	116

later excreted by the kidney. Most of the salt absorbed is sodium chloride. This was already shown by SMITH (1930). His results (Table 5-7) show that considerable absorption of NaCl occurs in the gut of *Lophius piscatorius* (see also BRULL and NIZET, 1953). The residual fluid is hypo-osmotic to the blood but contains a high concentration of magnesium and sulphate. More recently, the process of salt and water movements across the gut has been examined in the gut of teleost fishes perfused *in vivo* as well as in isolated intestine preparations (SHARRATT and co-authors, 1964; OIDE and UTIDA, 1967; SKADHAUGE and MAETZ, 1967). These studies show that water absorption is coupled to the continual transport of sodium occurring in the gut. Moreover, the rate of fluid absorption is higher in euryhaline fishes acclimated to sea water than to fresh water. This regulation of fluid absorption at the gut level, however, is not sufficient to ensure body-volume regulation. Special excretory organs are at play in this process.

The excretory organs

Marine and brackish-water crustaceans produce near-isosmotic urine. In crustaceans in which the blood is isosmotic with the surrounding water, salt loss via urine is replaced by inward diffusion. Acclimation of euryhaline species to diluted media results in an increased urine flow (SHAW, 1961a). However, the urine remains isos-

motic with the medium down to salinities as low as 10% sea water (ROBERTSON, 1953). It must be concluded, therefore, that in these species, urine production does not play any role in blood ionic regulation. In fact, the reverse situation prevails, since sodium loss through urine increases during non-genetic adaptation to diluted media. This has been demonstrated by SHAW (1961a) who acclimated sea-water *Carcinus maenas* to brackish water.

A few marine crustaceans and the teleost fishes have blood which is hypo-osmotic to the ambient water. During extracellular-fluid anisosmotic regulation these species absorb much sodium chloride along with some other ions (p. 272). Sodium-chloride absorption permits water uptake, which, in turn, compensates for water

Table 5-8

Urine flow and water tubular re-absorption in various fishes. GFR: Glomerular filtration rate; FW: fresh water; SW: sea water (Compiled from the sources indicated)

Species	Ambient medium	Volume (ml kg^{-1}hr^{-1})	GFR (ml kg^{-1}hr^{-1})	H_2O tubular re-absorption (%)	Author
Carassius auratus	FW	13·7	20·4	33	MAETZ (1963)
Protopterus aethiopicus	FW	4·9	14	69	SAWYER (1966)
Anguilla anguilla	FW	3·5	4·7	25	SHARRATT and co-authors (1964)
	SW	0·6	1	40	
A. japonica	FW	2·26	2·80	19	OIDE and UTIDA (1968)
	SW	0·38	3·13	88	
Platichthys flesus	FW	1·8	4·2	57	LAHLOU (1967)
	SW	0·6	2·4	75	
Paralichthys lethostigma	FW	2·9	3·88	25	HICKMAN and TRUMP (1969)
	SW	0·22	1·69	87	HICKMAN (1968)

loss. Urine flow may thus be expected to be very low in hypo-osmoregulators. That this is indeed the case is shown by the results presented in Table 5-8. Glomerular filtration rate (GFR) is much lower in sea-water than in freshwater species. Excretory organs of euryhaline species can adapt to prevailing salinity conditions; they tend to eliminate excess water in fresh water and to keep as much water as possible in sea water. However, in sea water, NaCl accumulated during gut-water absorption cannot be eliminated by the kidney. Indeed none of these species (crustaceans nor fishes) which maintain a blood osmotic pressure below the surrounding water have yet been shown to produce hyper-osmotic urine. Their urine is either isosmotic or hypo-osmotic (Table 5-9); even in cases where the urine is isosmotic the results indicate that some sodium re-absorption takes place.

STANLEY and FLEMING (1964) showed that during the first few days of re-acclimation from fresh water to sea water *Fundulus kansae* produces a strongly blood-hyperosmotic urine (serum 242 mOsm l^{-1}; urine 362 mOsm l^{-1}). This finding has aroused considerable interest. However, once the fish is fully acclimated to sea water,

Table 5-9

Urine composition of various aquatic animals. SW: sea water; FW: fresh water (Compiled from the sources indicated)

Species	Ambient medium	mM l^{-1}								Osmotic pressure (mOsm l^{-1})	Author
		Na	K	Ca	Mg	Cl	SO_4	PO_4	Urea		
Anguilla anguilla	SW	64·4	1·58	8·20	28·7	123·0	—	0·62	—		(a)
	FW	13·1	1·14	0·63	0·02	3·3	—	4·47	—		
Paralichthys lethostigma	SW	17·1	1·42	19·3	133	120·6	68·5	9·6	—		(b)
	FW	27·9	2·10	1·1	0·42	6·4	0·008	2·8	—		
Malaclemys centrata	SW	7·9	59·7	—	—	—	—	—	107·4	372·2	(c)
	FW	4·4	16·8	—	—	—	—	—	22·0	107·0	
Balaenoptera borealis	SW	240	3·6	0·3	1·6	180	33	—	380	1320	(d)
Uca pugnax	SW	276	16	17	54	622	47	—	—	1166	(e)
Eriocheir sinensis	SW	—	10·0	10·7	35·0	500	—	—	—		
	FW	—	7·0	4·7	0·7	264	—	—	—		
Maja squinado	SW	462·6	11·4	12·8	44·9	514·0	29·5	—	—		(f)
Sepia officinalis	SW	335·6	10·9	7·0	35·7	540·5	9·8	—	—		(f)

[a] Chester-Jones and co-authors (1969a).
[b] Hickman and Trump (1969).
[c] Gilles-Baillien (1970).
[d] Potts and Parry (1964).
[e] Green and co-authors (1959).
[f] Recalculated from Potts and Parry (1964), assuming a sea water of the following composition (in mM l^{-1}): Na: 453·6; K: 9·6; Ca: 10·0; Mg: 51·7; Cl: 529·9; SO_4: 27·3.

Refer to Tables 5-1 and 5-2 for mean blood ionic composition.

the hyperosmoticity of the urine disappears. Satisfactory interpretation of this phenomenon is not possible at the moment (STANLEY and FLEMING, 1966). HICKMAN and TRUMP (1969) suggested sodium secretion into kidney tubules to assist removal of some of the sodium, which rapidly accumulates during the initial period of adaptation to sea water. It is clear, however, that the excretory organs are not a major exchange site of osmotically important ions (NaCl) in hypo-osmotic regulators.

In sea water, the flounder *Platichthys flesus* and the sea perch *Serranus scriba* have been shown to excrete only 0·1% of the total accumulated sodium through their urine (MOTAIS and MAETZ, 1965). When in fresh water, sodium—badly needed to maintain the blood hyperosmotic state—is lost in the copious amounts of urine formed (even if it is hypo-osmotic). In freshwater species like the gold fish *Carassius auratus*, the daily sodium loss via urine amounts to about 8% of the total body sodium (MAETZ, 1963) and this in spite of the active ion re-absorption in the kidney.

Re-absorption of ions in excretory organs seems, however, to be sufficient for ensuring hyperosmotic blood regulation in some freshwater animals. In insect larvae, such as *Sialis lutaria* in which the permeability of body walls to salts and water is extremely low (SHAW, 1955a), it is impossible to detect active sodium uptake from the surrounding tap water (SHAW, 1955b); re-absorption in the rectum appears to play an important part here in the hyperosmotic regulation. Nevertheless, in most cases, salt re-absorption from urine can only compensate for a small portion of the loss of osmotically important salts.

If excretory organs do not play a significant role in the regulation of osmotically important ions in the blood, it appears that they are of considerable importance in the regulation of other ions such as Mg^{2+}. Kidney tubular secretion of divalent ions was demonstrated long ago. BIETER (1931) showed that the tubular electrolyte-secretory mechanism responds directly and specifically to increases in the concentration of blood magnesium, calcium and sulphate. Injection of a salt of one of these divalent ions into the circulatory system of the toadfish *Opsanus tau* causes significant diuresis (BIETER, 1931). In the southern flounder *Paralichthys lethostigma*, intravascular infusion of $MgCl_2$ results in an increase in both urine Mg^{2+} concentration and urine flow (HICKMAN, 1968; Fig. 5-4). The activity of the Mg^{2+}-secretory mechanism appears to be controlled directly by the amount of Mg^{2+} in the peritubular blood. Efforts to characterize the kidney's divalent-ion secretory mechanism remain inconclusive. From their study on tubular secretion in the goosefish, BERGLUND and FORSTER (1958) concluded that there are at least two separate transport systems, one for cations and one for anions. According to BIETER (1935), injection of $MgSO_4$ into the toadfish *Opsanus tau* causes ipsilateral diuresis. Urine flow in the opposite kidney increased significantly only if the injected dose was so large that some of the salt reached it by recycling through the circulatory system. It seems that hormonal participation can be ruled out since a hormone should have affected the two kidneys in the same way.

Phosphate, sulphate, magnesium, and calcium appear to be secreted in the tubular lumen. However, phosphate excretion appears to be independent of the blood inorganic phosphate level. According to MARSHALL and GRAFFLIN (1933), large phosphate injections did not increase renal excretion of this ion. As for the divalent ions, the mechanism controlling phosphate excretion in the kidney is unknown. It

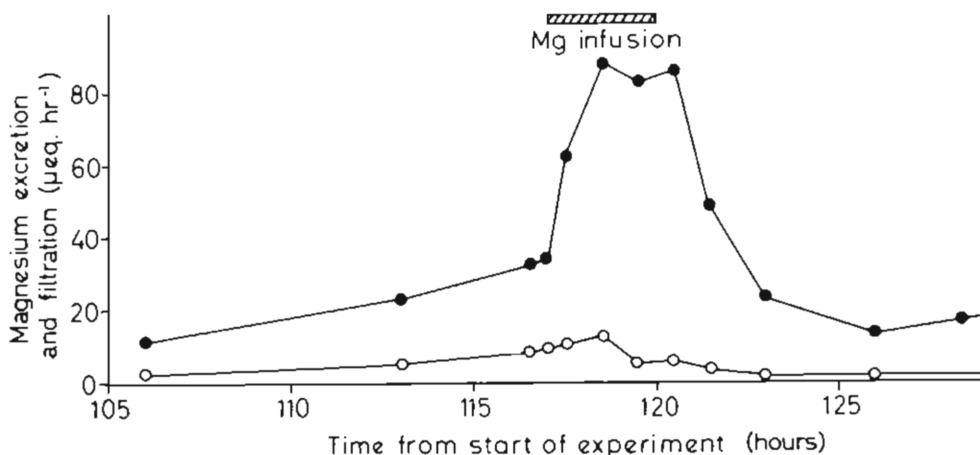

Fig. 5-4: *Paralichthys lethostigma*. Effect of $MgCl_2$ intravascular infusion (hatched bar) on kidney Mg^{2+} excretion (●) and filtration (○). (After HICKMAN, 1969; reproduced by permission of Academic Press.)

is likely, however, that these mechanisms are important in the regulation of blood divalent ion concentration.

The urinary bladder

Besides re-absorption in kidney tubules, the urinary bladder which various species possess may function to modify urine composition. In the flounder *Platichthys flesus*, sodium, potassium and urine osmolality are significantly reduced in the urinary bladder (LAHLOU, 1967). In the toadfish *Opsanus tau*, urine chloride can be reduced to an extremely low level in the bladder (LAHLOU and co-authors, 1969b). In turtles, the urinary bladder also appears to participate in urine concentration (KLAHR and BRICKER, 1965; GONZALES and co-authors 1967). Certainly, the bladder serves not only as a storage organ as previously assumed. Although much work has been done to characterize the nature of movements of water and electrolytes in kidney and urinary bladder of amphibians and mammals (e.g. HAYS and co-authors, 1970; GIEBISCH and co-authors, 1971), not much is known about the mechanisms involved in fishes and turtles (KLAHR and BRICKER, 1965; HICKMAN and TRUMP, 1969).

In summary, excretory organs appear to be of considerable importance in ion and osmoregulation of aquatic species. In freshwater forms, or in euryhaline species acclimated to fresh water, they function primarily as a water excretory device. In marine species, they appear to act chiefly as an excretory device for divalent ions; however, the excretory organs do not appear in most cases to play an important part in the regulation of blood ions of osmotic importance. Presumably these ions are primarily regulated in the so-called extrarenal 'salt moving' structures.

The gill

Ion and water movements. The large amount of sodium chloride transported at the gut level by hypo-osmotic regulators in order to ensure water absorption is not

excreted with the urine. Salt secretion was localized in the head region of fishes by KEYS (1931). Later on it became evident that the gill tissue performed this work.

In the flounder *Platichthys flesus,* ion exchange amounts to about 2600 μeq. Na^{+} 100 g^{-1} body weight hr^{-1} (MOTAIS and MAETZ, 1965). This is equivalent to 40% of the total exchangeable sodium in the fish. However, only 25% of this sodium is absorbed through the gut; 75% of the total sodium movement occurs through the gill. In the sea perch *Serranus scriba,* about 90% of the sodium influx takes place in the gill tissue (MOTAIS and MAETZ, 1964). In these two fishes the gills represent the primary site of sodium exchange; of course, the outflux exceeds the influx by an amount equivalent to that gained in the process of water absorption in the gut. These findings leave only a minor role to the gut in regard to NaCl exchange in teleosts exposed to sea water. Although it had been recognized long before these studies were completed that the gill was the main organ implicated in salt secretion, the importance of passive salt movements through the gills of marine hypo-osmotic species was not generally appreciated.

In *Platichthys flesus* exposed to sea water, MOTAIS and co-authors (1966) reported a sodium influx of 2250 μM and an efflux of 2600 μM. The net flux (−350 μM) exceeds somewhat the amounts calculated from the drinking rate (−100 μM). This discrepancy can probably be explained by technical difficulties. In any case, the results reveal an important diffusional component in the efflux; they also demonstrate a sodium secretion, which may be the result of active transport. In *Anguilla anguilla* acclimated to sea water, MAETZ and CAMPANINI (1966) reported a potential difference of 18 mV, the blood being electropositive to the ambient water. Applying USSING's passive flux ratio formula to the *A. anguilla* in sea water, the expected flux ratio across the gill should be 1·75 for sodium, and 9·1 for chloride (potential difference: +18 mV-medium Na^{+} concentration: 510 mM—medium Cl^{-} concentration: 610 mM—blood Na^{+} concentration: 135 mM—blood Cl^{-} concentration: 145 mM). The observed ratios are 0·9 for both Na^{+} and Cl^{-}. It thus appears that chloride is excreted actively while most of the sodium movement is diffusional. The brine shrimp *Artemia salina* displays very similar mechanisms of salt and water balances. In this crustacean, SMITH (1969a, b) has postulated active chloride movement, but passive sodium exchange across the gill. SMITH discusses sodium exchange in terms of the Nernst equation, assuming equality between influx and outflux. However, in *A. salina,* drinking rate amounts to 2 to 3% of the body weight, and to compensate for the salt absorption in the gut the outflux must be greater than the influx. According to THUET and co-authors (1968), the actual flux ratio in *A. salina* is 0·6; this leads the authors to conclude active sodium secretion, despite the electrical gradient which favours passive sodium efflux.

We may conclude: in sea-water hypo-osmotic regulators, chloride is actively transported outward. In regard to sodium, the situation is more equivocal; although there is a movement against the concentration gradient, the electrical gradient favours a net cation outflux. Moreover, differences between calculated and observed flux ratios do not provide convincing evidence of active transport because of the technical difficulties encountered during such experiments. Sodium extrusion could be purely passive, although present data do not rule out the possibility of active transport.

In regard to potassium movements, MAETZ (1971) reports an influx of 120 μM hr^{-1} 100 g^{-1}, and an efflux of 145 μM hr^{-1} 100 g^{-1} in sea-water acclimated *Platichthys*

flesus. Since renal loss and gut absorption account for only about 1 or 2 μM hr^{-1}, the gill represents the main site for potassium exchange. Considering the potential difference and the concentration ratio, part of the potassium transport in the gill must be achieved by an active mechanism. A similar conclusion has been reached by SMITH (1969a, b) who studied potassium movements in *Artemia salina*. On this basis, a mechanism of active exchange Na^+/K^+ has been postulated by MAETZ (1971) in anisosmotic regulation of hypo-osmotic regulators. Such considerations remain speculative since it is not known if there is active outward movement of sodium at the gill level.

While most conclusions on active sodium transport through the gill are hypothetical, important passive fluxes have been demonstrated unequivocally. In *Anguilla anguilla* acclimated to sea water, passive sodium flux accounts for at least 90% of the total sodium-movement measure. The ecological importance of such passive exchange for *A. anguilla* was first recognized by MAETZ, MOTAIS and co-workers (see following). We have already stressed that, in euryhaline species, sodium turnover is extremely fast in sea water and decreases after transfer to fresh water (p. 270). This phenomenon can be accounted for by changes in activity of sodium-transfer mechanisms at the gill level.

Platichthys flesus exchanges between 25 and 40% of its total exchangeable sodium per hour when in sea water. After transfer to fresh water, sodium-turnover rate drops to 0·7% (MOTAIS, 1961a, b). MOTAIS and co-authors (1965, 1966) compared the Na fluxes in the gills of the euryhaline *P. flesus* to those of the stenohaline *Serranus scriba*. In sea water in- and outflux are high in both fishes. When transferred to fresh water, the rate of loss from *P. flesus* decreases by about 90%; in *S. scriba* by only 40%. *S. scriba* continues to lose salts at this relatively high rate and dies from salt depletion after about 3 hrs. This observation demonstrates an important difference between euryhaline and stenohaline species.

This phenomenon is exemplified, on the basis of efflux values, in 3 *Platichthys flesus*, acclimated to 100% sea water, 50% sea water and fresh water respectively (Fig. 5-3). While the sea-water-acclimated fish can adjust its sodium fluxes rapidly after transfer to a diluted medium, the freshwater-acclimated fish cannot do so. However, if sea-water-acclimated *P. flesus* are transferred to fresh water and, shortly afterwards, re-transferred to sea water, the outflux of sodium decreased abruptly; control values are resumed immediately upon return to sea water (Fig. 5-5). It thus appears that we have to distinguish between phenomena occurring during rapid transfer and during long-term acclimation (immediate response and stabilization; KINNE, 1964a, b). This difference is illustrated by experiments undertaken by MOTAIS and co-authors (1966) and MOTAIS (1967) on the time course of sodium outflux during acclimation of fishes from sea water to fresh water (Fig. 5-6). Immediately after transfer, all fishes studied—euryhaline as well as stenohaline—exhibit a sudden decrease in sodium outflux. The euryhaline *Anguilla anguilla* and *Fundulus heteroclitus* decrease their efflux rates further. The onset of this 'delayed regulation' can be seen about 30 mins after transfer to fresh water. Fig. 5-6 also shows that the extent of the instantaneous efflux reduction may vary from species to species, regardless of the degree of their euryhalinity; for instance, the immediate efflux rate reduction is much larger in the stenohaline *Scorpena porcus* than in the euryhaline *F. heteroclitus*. However *S. porcus* dies in fresh water, presumably because

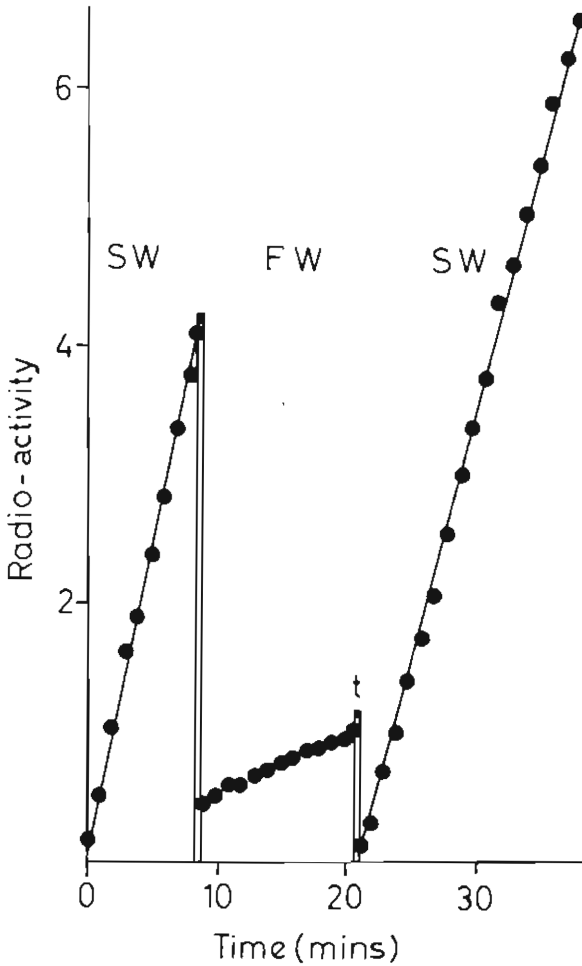

Fig. 5-5: *Platichthys flesus*. Relative appearance rates of $^{24}Na^+$ (radio-activity) in sea-water acclimated fish (SW) transferred (t) to fresh water (FW) and back to sea water (SW). (After MOTAIS and co-authors, 1966; modified; reproduced by permission of Rockefeller University Press.)

of lack of the 'delayed regulation' mechanisms which allow *F. heteroclitus* to recover its mineral balance.

What is the mechanism of such modifications in the fluxes? In *Platichthys flesus*, rapid exchange of sodium across the gill depends directly on salinity (salt content; Fig. 5-3), but not on the osmotic concentration of the ambient medium. If the salt content of the external medium is decreased, but its osmotic concentration maintained by addition of mannitol, the decline in sodium exchange is the same as in ordinary diluted salt. In the stenohaline *Serranus scriba*, however, sodium-exchange rate remains at its sea-water value after transfer to a salt-diluted medium which is

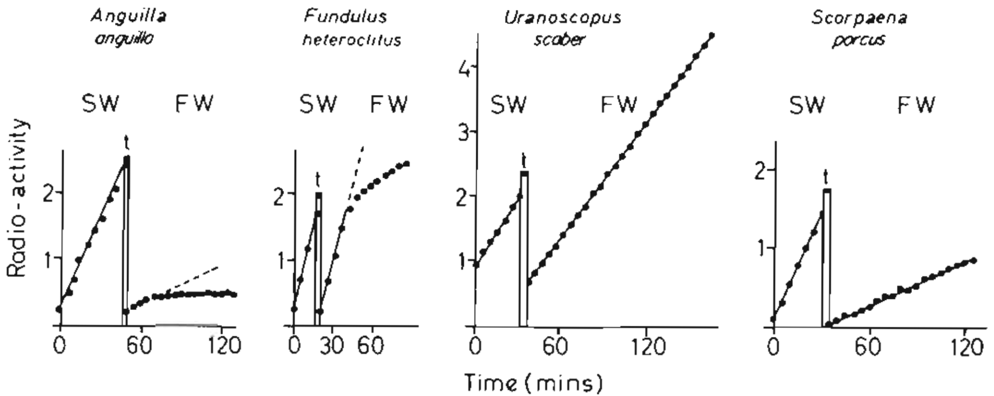

Fig. 5-6: Relative appearance rates of $^{24}Na^+$ (radio-activity) in teleosts (*Anguilla anguilla*, *Fundulus heteroclitus*, *Uranoscopus scaber*, *Scorpaena porcus*) transferred (t) from sea water (SW) to fresh water (FW). (After MOTAIS and co-authors, 1966 and MOTAIS, 1967; modified; reproduced by permission of Rockefeller University Press.)

kept isosmotic with sea water. In this case, the 40% decline observed upon transfer to fresh water can be related to changes in osmotic concentration of the external medium. Thus, in the euryhaline *P. flesus*, sodium turnover across the gill is closely related to the external sodium concentration. This reminds us of the process of 'exchange diffusion' that USSING (1947) suggested to account for rapid exchanges of labelled sodium in frog muscle, and which could not be explained in terms of the energy requirements of an active process. Such exchange–diffusion mechanism could account for about 90% of the sodium movement across the gill of the sea-water-acclimated *P. flesus* but would be practically absent in the stenohaline *S. scriba*. Exchange diffusion, however, is not a specific characteristic of euryhaline species. Significant exchange diffusion has also been observed in the stenohaline *Scorpaena porcus* (MOTAIS and co-authors, 1966).

In conclusion, sodium fluxes in marine fishes can be related to 'exchange diffusion' which plays an important role during transfer to diluted media. However, branchial exchange diffusion is not confined to, or present in, all the euryhaline fishes. On the contrary, the secondary 'delayed regulation' mechanism (whereby gill permeability is gradually restricted after transfer to fresh water) has only been described for euryhaline species. The mechanism of 'delayed' regulation' is unknown. MOTAIS and co-authors (1966) have suggested that it implies either changes in amount or synthesis of a substance inhibiting the action of the carrier which mediates the exchange diffusion.

In experiments with *Artemia salina*, fluxes varied also as though they were largely due to Na^+–Na^+ and Cl^-–Cl^- exchange (THUET and co-authors, 1968). It would thus appear that the mechanisms at work in extracellular-fluid anisosmotic regulation of *A. salina*, when in sea water, are homologous to those of the teleosts. SMITH (1969a, b), however, came to the conclusion that if chloride exchanges were largely due to an exchange component sodium efflux would not occur by exchange but would be the result of a leakage. Much work remains to be done before these import-

ant problems can be solved with sufficient certainty.

It is interesting to consider that if sodium exchange can be decreased upon transfer from sea water to fresh water, the reverse situation is observed when one deals with the water movement occurring in the gill of euryhaline fishes. We have already stated that the gill structure is responsible for a large part of the water movement which occurs in fishes (p. 271). In euryhaline fishes, such as *Anguilla anguilla* or *Platichthys flesus*, water exchange declines from 42 to 31% of the total body water per hour to 29 or 20% when the fish are transferred from fresh water to sea water (MOTAIS and co-authors, 1969). A similar phenomenon has been observed in *Tilapia mossambica* (POTTS and co-authors, 1967) and suggests that gill permeability to water is reduced when the fishes are in sea water.

In euryhaline teleosts exposed to fresh water, accumulating body water is excreted by the kidneys; in sea water the losses encountered are replaced by drinking. Water absorption in the gut is only achieved at the expense of a salt transport. It is the role of the gill to secrete the salts entering the fish due to gut activity and diffusion through outer surfaces. At the same time, water loss via urine is decreased by reducing urine flow rates (LAHLOU, 1967; CHESTER JONES and co-authors, 1969a). In fresh water, intrusion of water is counterbalanced by a decrease in drinking rate and an increase in urine flow; salt loss is reduced to a minimum by decreasing the gill permeability and by excreting a hypotonic urine.

As already stated (p. 269) there are no changes in body-wall permeability in most euryhaline crustaceans during osmotic stress. This is at variance with the situation found in euryhaline teleosts where modifications in permeability of the body wall appear to be of great help in extracellular-fluid anisosmotic regulation process.

JØRGENSEN and DALES (1957), however, suggested that the permeability of the euryhaline polychaete *Nereis diversicolor* to salts and water is less in fresh water than in brackish water. SMITH (1967) found lower water flux in the brackish-water crab, *Rhithropanopeus harrisii*, when acclimated to diluted media; and in preliminary experiments on *Gammarus duebeni*, LOCKWOOD (1970) reported a lower water flux in individuals acclimated to low salinities. Possibly some euryhaline invertebrates are able—as are the teleosts—to reduce salt loss in diluted media via decreased gill permeability. Direct measurements of tissue permeability are still lacking. On the other hand, freshwater forms excrete hypo-osmotic urine while most brackish and marine euryhaline invertebrates produce isosmotic urine; the latter species rely entirely on salt uptake to ensure hyperosmotic regulation.

The nature of ion movements. Sodium inward movement has been studied in many crustacean species such as *Eriocheir sinensis* (SHAW, 1961b), *Artemia salina* (THUET and co-authors, 1968) and *Gammarus zaddachi* (SUTCLIFFE, 1968). A typical result obtained from *G. zaddachi* is shown in Fig. 5-7. The rate of sodium uptake is directly related to the external sodium concentration up to a certain salinity and then tends to level off. Such response has also been observed in regard to Na^+ influx in *A. salina* (SMITH, 1969a, b) and in fishes (MOTAIS and co-authors, 1966).

The relation between sodium influx and external sodium concentration is hyperbolic. This type of curve is reminiscent of the well-known Michaelis–Menten kinetics for enzymes, where the initial velocity of the reaction is equal to the maximum velocity, multiplied by the ratio substrate concentration over substrate

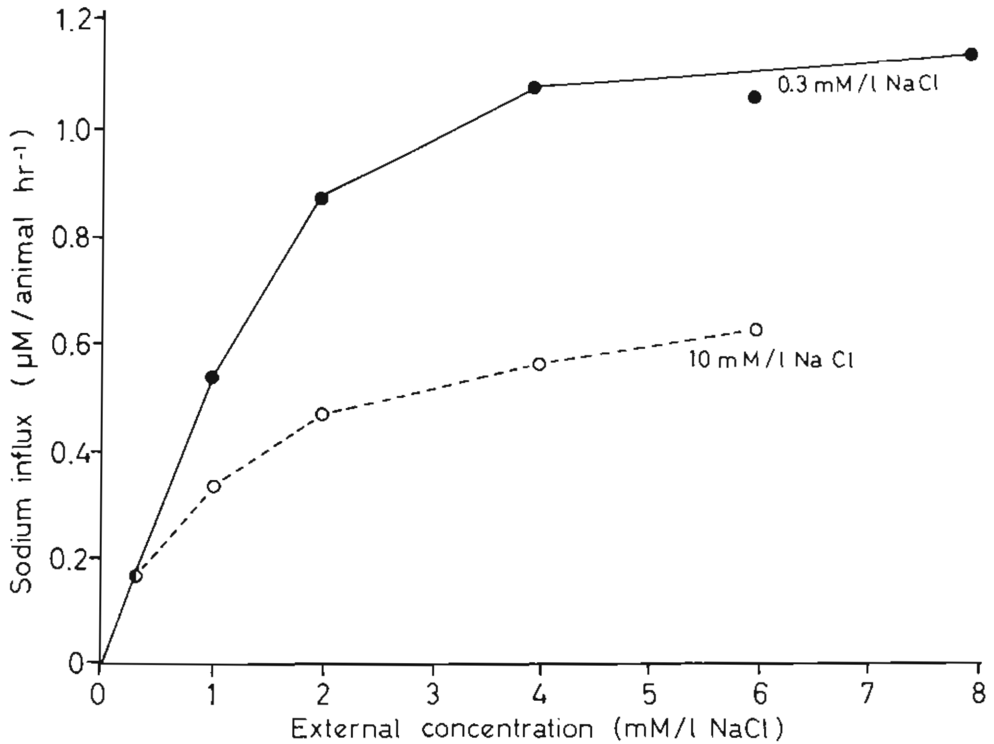

Fig. 5-7: *Gammarus zaddachi*. Relation between external concentration and sodium influx in individuals acclimated to two salinities. (After SUTCLIFFE, 1968; modified; reproduced by permission of Company of Biologists Ltd.)

concentration plus the Michaelis constant. By direct analogy, SHAW (1959b) proposed the following relation

$$f = f_{max} \frac{(\mathrm{Na})}{(\mathrm{Na}) + K_S}$$

where f = influx, f_{max} = maximum influx, (Na) = external sodium concentration, and K_S = constant defined as the external concentration at which the influx is half the maximum value. K_S is directly related to the Michaelis constant and expresses the degree of affinity of the carrier for the ion transported.

The transport system may thus be described in terms of f_{max} and K_S. Table 5-10 gives values of f_{max} and K_S for various crustaceans. From these data it appears that in the course of adaptive evolution to fresh water there has been a progressive increase in affinity of the carrier for the transported ion, since K_S values for fresh-water species are much lower than those recorded in marine or brackish-water species.

Some euryhaline crustaceans have developed an adaptive sodium-transport system; they respond to salt depletion by an increase in f_{max} rather than by a

decrease in K_S (SHAW, 1971a; SUTCLIFFE, 1968; THUET and co-authors, 1968). Such an increase in f_{max} may be related to an increase in carrier concentration. This adaptive process is not present in all euryhaline crustaceans; SUTCLIFFE (1968) found no difference in f_{max} between the influxes of *Marinogammarus finmarchicus* acclimated to 115 mM or to 10 mM NaCl solutions.

Sodium uptake has also been shown to depend on the internal sodium concentration. As an example, a decline from 400 mM l^{-1} to 390 mM l^{-1} suffices to produce, in *Carcinus maenas*, a fourfold increase in the uptake rate of sodium (SHAW, 1961a; see also KINNE, 1971, pp. 916, 917). Thus the rate of sodium uptake appears to be a function of both external and blood sodium concentrations. This invalidates theories based on a simple kinetic model such as that proposed by SHAW (1959b).

Table 5-10

Characteristics of the sodium-uptake mechanism of some crustaceans (Compiled from the sources indicated)

Species	f_{max} (mM kg^{-1} hr^{-1})	K_S (mM l^{-1})	Author
Marine			
Marinogammarus finmarchicus	10	2·5	SUTCLIFFE (1968)
Brackish			
Carcinus maenas	10	20	SHAW (1961a)
Gammarus duebeni	20	1·5	SHAW and SUTCLIFFE (1961)
Limnic			
Gammarus pulex	7·5	0·15	SHAW and SUTCLIFFE (1961)
Potamon niloticus	2	0·1	SHAW (1959a)
Astacus fluviatilis	0·15	0·2	SHAW (1959b)

The overall balance of sodium in euryhaline crustaceans can, therefore, be characterized by the relationship between external and blood-sodium concentrations. This has been done by POTTS and PARRY (1964) on the basis of experimental data presented by SHAW (1959a, b) who studied sodium fluxes in the freshwater decapod *Astacus pallipes*. While we shall not discuss this matter any further, it is interesting to consider that sodium influx, in addition to being dependent on external and internal sodium contents, is apparently also dependent on blood volume; this has been shown by LOCKWOOD (1970) in the amphipod *Gammarus duebeni*. To our knowledge, a dependence of Na^+ influx on blood volume has never been investigated in fishes. A mechanism, initiating variations in sodium uptake subsequent to volume changes, may be of considerable significance with respect to the evolution of ion regulation. Although blood ion regulation is an integrant part of the mechanism of osmoregulation and, therefore, of volume regulation, evidence of a direct link between blood volume and ion fluxes was not presented prior to LOCKWOOD's findings on *G. duebeni*. Is this a general mechanism in animals? How is it controlled? We do not yet know. Further studies are required to solve this important problem.

In *Anguilla anguilla* acclimated to fresh water, Na^+ turnover is much lower than in sea-water acclimated eels. This is mainly due to adaptive modifications in gill permeability. The branchial Na^+ influx is about 20 to 50% higher than the efflux (MAETZ, 1971). This can account, at least partly, for the ionic balance maintained in fresh water. The influx of Na^+ had been studied in several euryhaline teleosts acclimated to fresh water; it appears to depend—as in crustaceans—on both external and internal Na^+ concentrations (BOURGUET and co-authors, 1964; MAETZ, 1971). For the flounder *Platichthys flesus* the dependence of Na^+ influx on external Na^+ concentration is shown in Fig. 5-8. The uptake presumably occurs at the gill level.

So far we have concentrated on water and sodium exchanges. But, as already shown in Table 5-1, the chloride ion is deeply implicated in extracellular-fluid

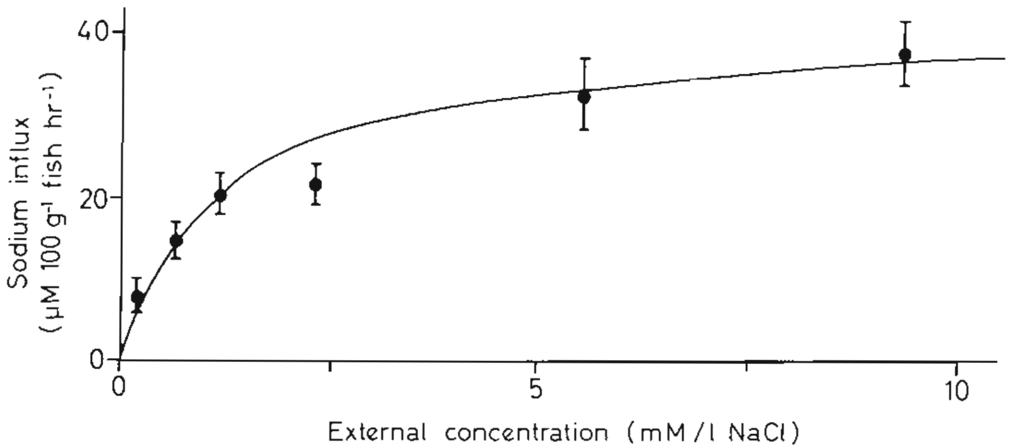

Fig. 5-8: *Platichthys flesus*. Relation between external concentration and sodium influx in freshwater-acclimated individuals. (After MAETZ, 1971; redrawn; reproduced by permission of The Royal Society.)

anisosmotic regulation. Originally it was assumed that chloride movements accompanied sodium movements. However, by placing salt-depleted animals in different solutions, KROGH (1939) demonstrated that sodium and chloride transport can be independent. This view has been confirmed by many authors working on a variety of species, invertebrates (see SCHOFFENIELS and GILLES, 1970a, for a review) as well as vertebrates (GARCIA-ROMEU and MAETZ, 1964; KERSTETTER and co-authors 1970). The hypothesis that HCO_3^- is the endogenous ion exchanged for Cl^- was first proposed by KROGH (1939). Few pertinent studies deal with invertebrates and almost nothing is known about the mechanism of chloride transport in this group. For details, the following papers should be consulted: SHAW (1960), STOBBART (1967), THUET and co-authors (1968).

In fishes, addition of bicarbonate to the external medium inhibits specifically Cl^- uptake. This has been shown, for example, in *Carassius auratus* and interpreted by MAETZ and GARCIA ROMEU (1964) as an indication of competition of HCO_3^-

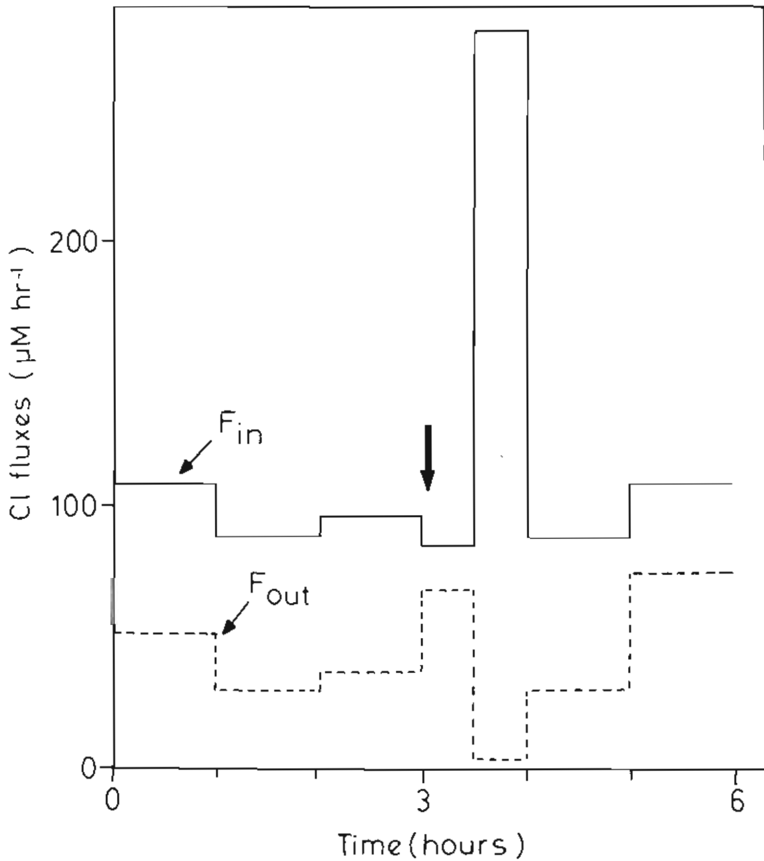

Fig. 5-9: *Carassius auratus*. Effect of intraperitoneal injection of HCO_3^- (heavy arrow) on Cl^- influx (F_{in}) and outflux (F_{out}). (After MAETZ and GARCIA-ROMEU, 1964; modified; reproduced by permission of The Royal Society.)

added to the external medium with Cl^- for the transport mechanism. Variations in chloride influx can also be induced by modifying the HCO_3^- blood load via intraperitoneal injections of potassium bicarbonate (Fig. 5-9). It appears, therefore, that the chloride active-uptake mechanism implicates an exchange of HCO_3^- versus Cl^-. DEJOURS (1969) presents compelling evidence that in *C. auratus* such exchange may be obligatory. Although the work on chloride uptake has been performed mainly on goldfish, there is every reason to assume that the same mechanism is operative also in other fish species.

Several hypotheses have been put forward to account for the net Na^+ uptake in fishes acclimated to fresh water.

The hyperbolic relationship between influx values and external sodium concentration are generally interpreted as indicating a carrier-mediated transport system, although SMITH (1969a, b) argued that this may not always be the case when discussing the hyperbolic relationship in *Artemia salina*. Since the mechanism promotes a net transport of sodium from the outside to the inside against a concen-

Table 5-11

Sodium fluxes in gills of *Eriocheir sinensis* and *Maja squinado*

Na_{out}/Na_{in}	1·0					0·76				
Medium (mM NaCl)	450					340				
Perfusate (mM NaCl)	450					450				
Species and gill part	In[c]	Out[c]	PD[d]	Ratio[e] Experimental	Ratio[e] Calculated	In[c]	Out[c]	PD[d]	Ratio[e] Experimental	Ratio[e] Calculated
Maja squinado	4·2	4·2	0	1·0	1·0	3·2	3·5	0	0·91	0·76
Eriocheir sinensis										
Anterior										
(a)	1·15	1·1	3·0	1·0	0·87	—	—	—	—	—
(b)	0·38	0·26	3·0	1·4	0·87	—	—	—	—	—
Posterior										
(a)	0·14	0·06	– 15·0	2·3	1·8	—	—	—	—	—
(b)	0·68	0·02	– 29·0	28·0	2·9	—	—	—	—	—

[a] After KING and SCHOFFENIELS (*in*: SCHOFFENIELS and GILLES, 1970a).
[b] Perfusate: balanced salt solution (LOCKWOOD, 1961); medium: composed of balanced salt solution diluted with distilled water; flux values given as mM min^{-1} per gill; means of 2 to 3 readings.

tration gradient, it cannot be accounted for by passive transport. The active nature of sodium influx in the gill of *Eriocheir sinensis* is documented in Table 5-11. The flux ratio in the posterior gills of *E. sinensis* always exceeds the flux ratio calculated according to USSING (1947). Only the posterior gills can actively transport sodium. In the gill of the stenohaline *Maja squinado* the calculated flux ratio is in agreement with the experimentally observed flux ratio, both in isosmotic medium and in twice-diluted medium. This indicates that sodium movement through the gill of this crab is a passive phenomenon. *M. squinado* is incapable of extracellular-fluid anisosmotic regulation. As far as fishes are concerned, the evidence at hand points to an active transport mechanism for both Na^+ and Cl^-. The ratio of influx to outflux may be as high as 2·5 for Cl^- and 5·5 for Na^+ in salt-depleted freshwater *Carassius auratus* for a ratio of internal to external concentrations averaging 300 for Na^+ and 650 for Cl^-. Applying USSING's criterion to calculate the potential difference that would explain such ratios in terms of passive transport, a difference of about 180 mV positive inside for chloride and negative inside for sodium should occur. Such high potential difference has never been observed, not even in euryhaline species such as *Blennius pholis* or *Pholis gunnellus* (HOUSE, 1963; EVANS, 1969). In *Anguilla anguilla*, MAETZ and CAMPANINI (1966, see also MAETZ, 1971) reported potentials of about 200 mV negative with respect to the blood for individuals acclimated to fresh water.

Thus in species capable of extracellular-fluid anisosmotic regulation in diluted media, active transport occurs in the gill. What are the physiological and biochemical characteristics of this Na^+ transport mechanism?

Ammonia is the principal 'waste' product of aquatic animals; in fishes it is excreted primarily by the gills, the site of cation absorption (see Volume III:

0·4					0·2					0·02				
180					90					9				
450					450					450				
			Ratio[e]					Ratio[e]					Ratio[e]	
In[c]	Out[c]	PD[d]	Experimental	Calculated	In[c]	Out[c]	PD[d]	Experimental	Calculated	In[c]	Out[c]	PD[d]	Experimental	Calculated
1·3	3·1	− 0·5	0·43	0·41	0·8	2·9	− 1·0	0·27	0·21	—	—	—	—	—
0·82	1·1	− 5·0	0·78	0·60	0·53	0·78	− 27·0	0·65	0·58	—	—	—	—	—
—	—	—	—	—	0·26	0·29	− 27·0	0·88	0·58	0·15	0·37	− 68·0	0·39	0·2
0·12	0·09	− 15·0	1·3	0·9	0·08	0·1	− 16·0	0·80	0·37	—	—	—	—	—
—	—	—	—	—	0·34	0·04	− 25·0	8·8	0·50	0·13	0·06	− 25·0	2·1	0·05

[c] In: appearance of ^{22}NaCl in perfusate; Out: appearance of ^{24}NaCl in medium.
[d] PD: potential difference between perfusate and medium; sign given as blood relative to medium.
[e] Ratio: calculated $(Na^+_{out}/Na^+_{in})e^{-(2F/RT)\Delta E}$; measurements taken at 10 to 20 min intervals.

KINNE, in press). This suggests that in the fish gill, as in the mammalian kidney, both mechanisms (Na^+ absorption and NH_3^+ excretion) are linked.

The rates of ammonia excretion across the gills of various fishes (15 to 100 μM hr^{-1} 100 g^{-1}) appear to be sufficient to account for the rate of Na^+ influx generally observed. However, except for the trout, both rates have never been measured simultaneously. KERSTETTER and co-authors (1970) measured both rates in *Salmo gairdnerii* and found that Na^+ influx equals the ammonia excretion. This may be an argument for an obligatory NH_4^+/Na^+ exchange. Attempts have been made to demonstrate such obligatory exchange in experiments where the ammonia-excretion rate was altered either by adding ammonium salts to the outside medium or by injecting ammonium salts into the intraperitoneal cavity. The first procedure depresses ammonia excretion, the second hastens ammonia clearance via the gill. Both experimental interventions are specific in so far as the Cl^- uptake remains more or less unchanged. Such studies have been performed on freshwater fishes (MAETZ and GARCIA-ROMEU, 1964; KERSTETTER and co-authors, 1970), and on euryhaline species acclimated to fresh water (GARCIA-ROMEU and MOTAIS, 1966; MOTAIS, 1967).

In all these cases, a depression of sodium uptake is observed and the sodium balance becomes negative when ammonia is added to the external medium. After rinsing and elimination of the ammonia added, sodium uptake is resumed. Intraperitoneal injection of $(NH_4)_2SO_4$ is followed by an increase in Na^+ uptake and net absorption. These results suggest that NH_4^+/Na^+ exchange takes place in the gills of freshwater-acclimated teleosts; however, the question of whether this exchange is obligatory remains unanswered. Obligatory exchange seems unlikely in the freshwater fishes (DE VOOYS, 1968; KERSTETTER and co-authors, 1970).

NH_4^+/Na^+ exchange has been suggested in crustaceans (SHAW, 1960; SCHOFFENIELS and GILLES, 1970a). Upon examination of the facts at hand (and there have been no new results since), SHAW (1960) suggested that Na^+/NH_4^+ exchange in crustaceans constitutes the 'usual' mechanism; but he adds that there is *a priori* no reason for supposing that the exchange process involves only a single ion species. An alternative possibility would be an H^+/Na^+ exchange. For instance, in the crayfish *Astacus pallipes* SHAW (1960) observed that H^+ is 10 times more effective than NH_4^+ in depressing the Na^+ uptake. A similar effect of external acidity has also been observed in fishes (PACKER and DUNSON, 1970). KERSTETTER and co-authors (1970) investigated the possibility of a Na^+/H^+ exchange in *Salmo gairdnerii*. They followed (as an indication of H^+ excretion) the changes in pH of the chloride-free external medium of *Salmo gairdnerii* in which the Na^+ uptake rate was experimentally submitted to changes by varying the Na^+ concentration of the ambient medium. The results obtained are listed in Table 5-12. There is no correlation

Table 5-12

Salmo gairdnerii. Effect of external Na^+ concentration on Na^+ fluxes, NH_4^+ output and pH changes. 12° to 13° C. Average values of determinations and standard deviations (After KERSTETTER and co-authors, 1970; modified; reproduced by permission of Rockefeller University Press)

External NA^+ (mM l^{-1})	Na^+ influx (μM hr^{-1}100 g^{-1} fish)	Na^+ efflux (μM hr^{-1}100 g^{-1} fish)	NH_4 excretion (μM hr^{-1}100 g^{-1} fish)	ΔpH
7·0	33·4 ± 6·3	29·8 ± 5·7	15·4 ± 2·4	−0·31 ± 0·08
1·0	17·9 ± 1·1	16·0 ± 2·9	17·4 ± 0·6	−0·07 ± 0·04
0·1	6·2 ± 1·5	11·6 ± 2·8	12·4 ± 1·8	+0·07 ± 0·08

between Na^+ influx and NH_4^+ output, indicating that if Na^+/NH_4^+ exchange exists, it is not obligatory. At the highest external Na^+ concentration, the pH decreases significantly while the Na^+ uptake increases. The H^+ output, calculated from the pH shift, is about 10 μM. This accounts for about one third of the Na^+ influx. A large part of the remaining Na^+ influx may be accounted for by the NH_4^+ excreted. At 1 mM external sodium concentration, NH_4^+ excretion accounts for the whole Na^+ influx and no significant pH change can be recorded.

We may conclude that, in addition to NH_4^+/Na^+ exchange, H^+/Na^+ exchange may take place in the gills of crustaceans and fishes. The source of NH_4^+ is probably amino-acid deamination (p. 313). A possible source of the H^+, exchanged in combination with NH_4^+ against Na^+, may be CO_2 hydration producing carbonic acid. This reaction is catalysed by carbonic anhydrase, and it is known that inhibition of the activity of this enzyme is followed by a depression of Na uptake (MAETZ, 1956; KERSTETTER and co-authors, 1970).

The gills thus play an important role in the salt balance achieved by crustaceans and teleosts during extracellular-fluid anisosmotic regulation. However, different species have developed different structures to ensure proper salt balances.

The 'salt' glands of aquatic reptiles

Salt-secreting supra-orbital glands have been discovered in reptiles by SCHMIDT-NIELSEN and FÄNGE (1958). The sea-water turtle *Caretta caretta* produces 'tears' of highly concentrated salts. The salt-secreting glands assist marine reptiles to cope with a major problem: ion regulation. As shown in Table 5-1, the blood composition of reptiles is similar to that of teleosts. Species living in sea water are hypo-osmotic. Similar to fishes, reptiles are unable to produce hyperosmotic urine (Table 5-9); they rely entirely on extrarenal excretion to ensure salt balance.

In marine reptiles, the quantities of salt excreted by salt glands exceed by far those excreted in urine; salt-gland excretion makes up more than 90% of the total salt loss in the green turtle *Chelonia mydas* (HOLMES and MCBEAN, 1964), and in the yellow-bellied sea snake *Pelamis platurus* (DUNSON, 1968). In the marine iguana *Amblyrhyncus cristatus*, salt-gland secretion accounts for about 75% of the total loss. The fluid produced is hyperosmotic, and the electrolyte levels can be higher than in sea water. Sodium concentration in the secreted fluid amounts to 784 meq. l^{-1} in *Malaclemys centrata*, to 878 meq. l^{-1} in *Caretta caretta* (SCHMIDT-NIELSEN and FÄNGE, 1958) and to 685 meq. l^{-1} in *C. mydas* (HOLMES and MCBEAN, 1964); it can go up to 969 meq. l^{-1} in the marine iguana *A. cristatus* (DUNSON, 1969).

Injection of hyperosmotic solutions of NaCl, KCl and the cholinergic drug methacholine can all bring about secretion. HOLMES and MCBEAN (1964) found that injections of amphenone, which reduces the rate of formation of corticosteroids in reptiles and mammals, reduced extrarenal salt secretion in *Chelonia mydas*. Osmotic and cholinergic drugs exert similar actions in birds (PEAKER, 1971); secretion is possibly controlled by changes in plasma concentration, stimulating a parasympathetic nerve supply to the gland. As shown in birds, secretion requires continuous acetylcholine stimulation; interruption of the nervous transmission while the gland is secreting immediately inhibits secretion. We shall not explore this question any further since nothing is known about the intimate mechanisms of salt secretion by the salt gland in birds or reptiles. Readers interested in a more detailed discussion should consult the review by PEAKER (1971; see also HANWELL and co-authors, 1971).

Although most marine reptiles possess salt glands for controlling their blood ionic concentration they can support great changes in their body fluid concentration. This capacity of reptiles has been studied mainly in terrestrial species. When they lack water *Trachysaurus rugosus* and *Amphibolorus ornatus* retain electrolytes and wait for more adequate supplies of water which they then utilize for renal excretion of excess of solutes. BRADSHAW and SHOEMAKER (1967) found that plasma sodium level of *A. ornatus* can rise to as much as 300 meq. during summer drought. A summer rainstorm caused these lizards to run about and to drink rapidly the available water. Similar behaviour has been observed in the brackish-water turtle diamondback terrapin, *Malaclemys centrata*. This turtle, when kept in sea water, accumulates sodium (Table 5-1) although it has functional salt glands. When returned to fresh water *M. centrata* drinks and rapidly excretes excess solutes through its salt glands (BENTLEY and co-authors, 1967). In crocodilians, which sometimes encounter sea water and which do not secrete 'salty tears' (DUNSON, 1970), such a capacity to withstand temporarily great changes in blood ionic

concentration may be of ecological importance; these animals can indeed have easy access to fresh water when returning to their normal habitat.

Reptiles in sea water can also compensate their water loss by raising their blood osmolarity by urea accumulation (Table 5-1). Nevertheless, blood always stays hypo-osmotic to sea water and they remain faced with the problem of water loss. It has been assumed that turtles in sea water do not drink and that they rely on food to maintain their water balance. This supposes very low water loss. However, besides the loss through renal and salt-gland excretion cutaneous water loss must be added. In *Pseudemys scripta*, kept in 33‰ NaCl solution, BENTLEY and SCHMIDT-NIELSEN (1966) recorded a water loss through the skin of 10 mg cm^{-2}/day.

About 10 years ago the University of Liège (Belgium) acquired a loggerhead turtle, *Caretta caretta*. Since then the turtle has been kept in a sea-water tank with no fresh water available. It is fed mostly on marine crabs and fillets of marine fishes; the latter may provide small amounts of diluted fluids although the turtle swallows large quantities of sea water when eating. In view of the water loss, both through the skin and during renal and salt-gland excretion processes, it seems unlikely that food can be the only water supply of this turtle.

Freshwater reptiles do not possess salt glands. Apparently they rely on food to replenish their salt loss. In some freshwater turtles, however, the pharynx has been shown to be implicated in ion uptake from the diluted ambient medium (DUNSON and WEYMOUTH, 1965).

It thus appears that the problem of how reptiles in sea water maintain their salt and water balances has not yet been completely solved. Further studies are needed to bring more light on this problem.

Up to now we have considered the regulation of extracellular-fluid inorganic-ion concentration. Let us now consider another mechanism which assists various species to maintain a proper water balance.

(b) Mechanisms Regulating Blood-Organic Solute Concentrations

Osmoregulatory Function of Amino Acids

Use of amino acids as blood osmotic effectors is, to our knowledge, restricted to the insects. The participation of inorganic ions in osmoregulation of haemolymph concentration tends to decrease with the evolutionary level of the insect (SUTCLIFFE, 1962, 1963); the most primitive forms such as the apterygote *Petrobius maritimus* exhibit a nearly exclusive participation of inorganic ions as osmotic effectors (LOCKWOOD and CROGHAN, 1959); in exopterygotes (Ephemeroptera, Odonata, Dictyoptera, Heteroptera and, to a lesser extent, Orthoptera, Isoptera and Dermaptera) organic molecules, such as amino acids, assist in establishing the osmotic pressure of the haemolymph. In endopterygotes (Megaloptera, Neuroptera, Mecoptera, Trichoptera and Diptera), organic ions and molecules account for about half of the osmotic pressure. In *Dytiscus marginalis* blood concentrations of inorganic ions and amino acids appear to be regulated during osmotic stress (SCHOFFENIELS, 1950, 1960a). The mechanisms by which amino acidemia is controlled are not known.

Osmoregulatory Function of Urea

Urea is used for osmotic purposes by several animal groups. In addition to chondrichthian fishes, in which urea may constitute as much as 50% of the osmotic effectors present in the body fluids, urea retention occurs in the coelacanth *Latimeria chalumnae* (Table 5-1). Urea participation in osmoregulation has also been demonstrated in the turtle *Malaclemys* (Table 5-1) as well as in amphibians, such as *Rana cancrivora*, *Bufo viridis*, and *Xenopus laevis* when submitted to osmotic stress (BALINSKY and co-authors, 1961; MCBEAN and GOLDSTEIN, 1967; GORDON and TUCKER, 1968; JANSSENS and COHEN, 1968).

Chondrichthians and *Latimeria chalumnae* maintain the low salt–blood concentrations characteristic of most vertebrates. Consequently as in the other marine vertebrates, salts tend to enter by diffusion and must be actively excreted; calcium, magnesium and sulphate are removed by the kidney, sodium and chloride by the rectal gland. The secretion of the rectal gland, although isosmotic with the blood, consists almost entirely of sodium chloride. The mechanisms of ion regulation in the forms mentioned appear thus to be the same as in teleosts, except for the structure responsible for extra renal movements of sodium chloride.

The ability of various species to accumulate urea in order to avoid or to minimize water balance problems has been correlated to the presence of urea-synthetizing mechanisms effecting the so-called urea cycle (Fig. 5-10). HUNTER (1929) reported the presence of one of the urea cycle enzymes, arginase, in a number of fishes including several chondrichthians. BALDWIN (1960) provided evidence supporting the existence in elasmobranchs of all the enzymes of the cycle except carbamoyl phosphate synthetase, which he could not detect. BROWN (1965) presented strong evidence for the presence of carbamoyl phosphate synthetase in the liver of various elasmobranchs; his finding was later confirmed by WATTS and WATTS (1966). The urea cycle, however, is not the only possible source of urea. Using ^{14}C precursors, SCHOOLER and co-authors (1966) compared the importance for urea formation of the cycle and of the purine metabolism pathway in *Squalus acanthias*. They concluded that the urea cycle is the major pathway for urea formation in this species. READ (1967) presented evidence for the existence of urea-cycle enzymes in the chimaera *Hydrolagus colliei*. The urea cycle is also known in amphibians and reptiles. It appears that all species able to accumulate urea possess a functional urea cycle. The cycle operates in the liver; however, some of its enzymes have also been reported in other tissues (CAMPBELL, 1961; READ, 1970). Of course this constitutes no proof that the cycle is functioning in these tissues. The activities reported are much lower than those recorded for the liver enzymes; it thus appears that the liver is the main organ of urea formation.

The mechanisms responsible for regulating the urea level in the body fluids have not yet been fully investigated. Obviously, urea concentration can be regulated by controlling the rates of urea synthesis and/or excretion.

Fully aquatic amphibians such as *Xenopus laevis* are normally ammonotelic (see also Chapter 3) and excrete only little urea. However, in conditions of restricted water availability (estivation, hyperosmotic media), the amphibian accumulates mainly urea and not ammonia. BALINSKY and co-authors (1967a, b) have shown that during estivation, the activity of carbamoyl phosphate synthetase increases

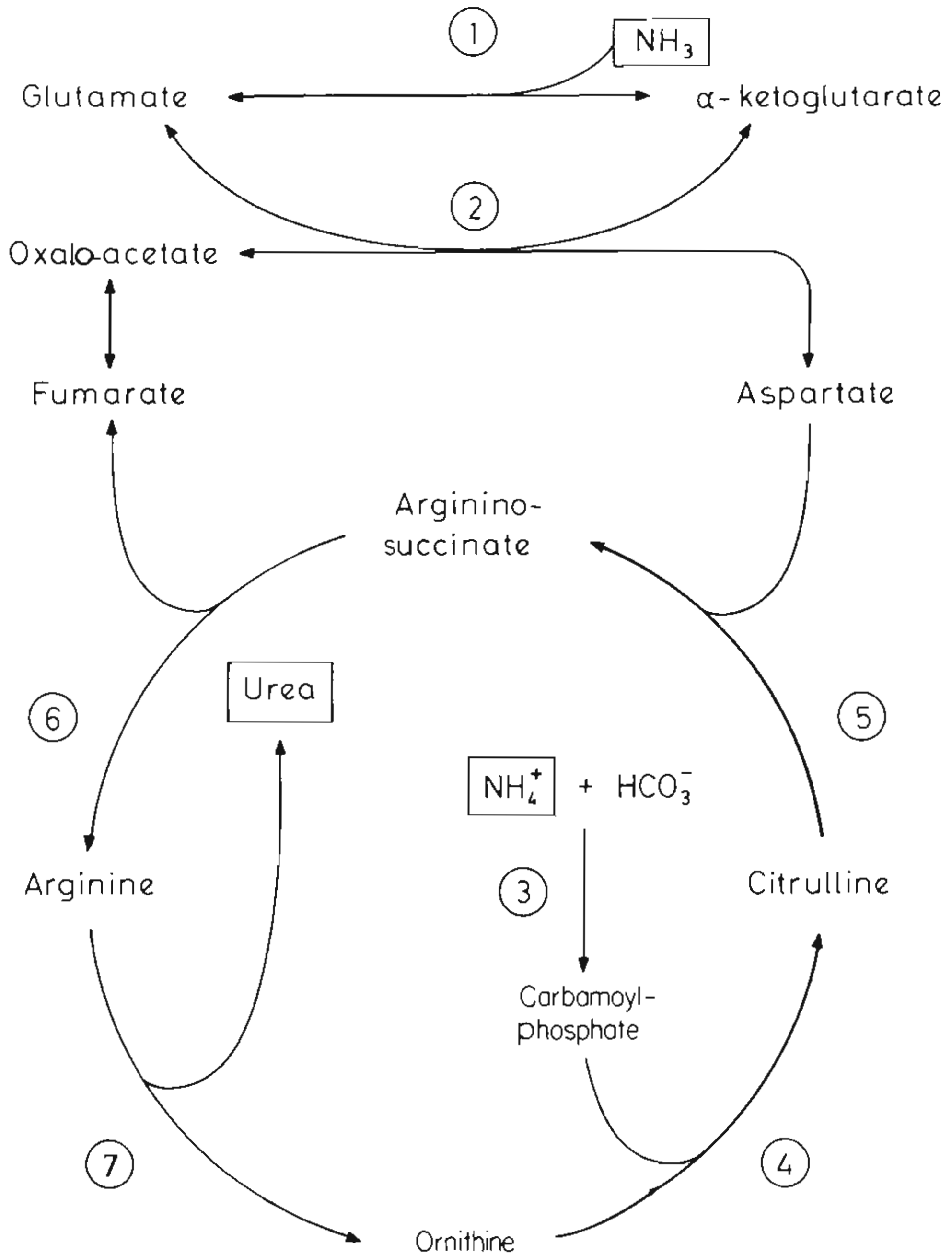

Fig. 5-10: Urea cycle. 1: Glutamate dehydrogenase; 2: aspartate aminotransferase; 3: carbamoylphosphate synthetase; 4: ornithine carbamoyl-transferase; 5: argininosuccinate synthetase; 6: argininosuccinate lyase; 7: arginase. (Based on the sources cited in text.)

about six-fold over its level of activity in *X. laevis* kept in water. Upon return of estivating individuals to water, enzyme activity resumes its control value. This indicates that at least part of the urea accumulation, observed during water restriction, is due to increased activity of the urea cycle. However, as shown in Fig. 5-11, initial increase in carbamoyl phosphate synthetase activity in *X. laevis* exposed to a hyperosmotic medium is slower than that of urea (accumulation + excretion). It may be concluded, therefore, that, while changes in urea cycle activity can assist in urea accumulation, they are not the prime cause of this process. In fact, urine flow is depressed to 1/10 of the level found in freshwater *X. laevis* and urea excretion

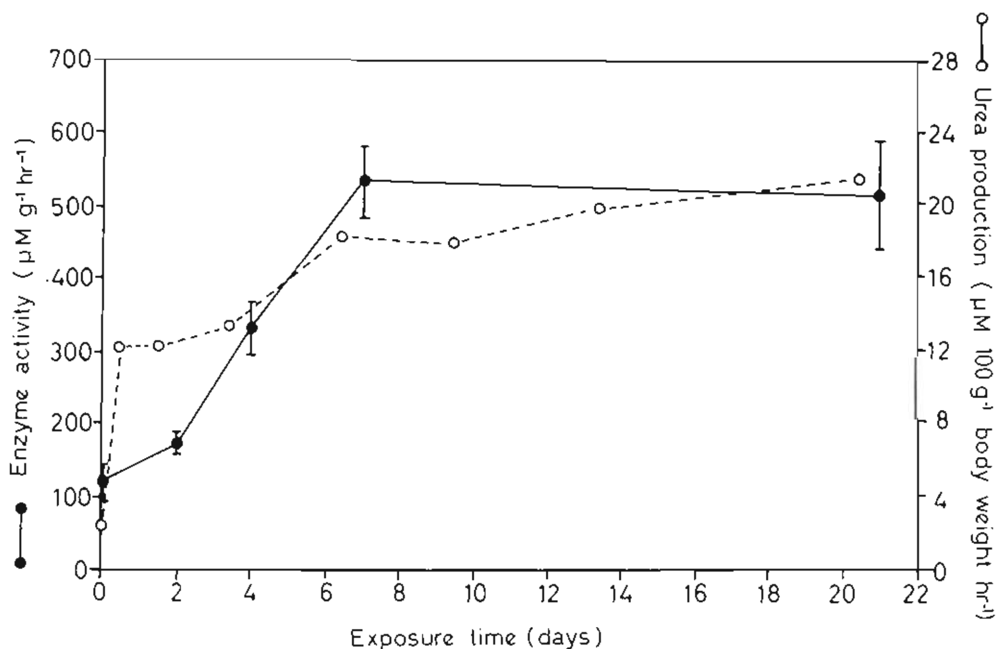

Fig. 5-11: *Xenopus laevis*. Changes in carbamoylphosphate synthetase activity (closed circles) and in urea production (accumulation + excretion, open circles) during acclimation to a hyperosmotic medium (300 mOsm l^{-1}). (After GOLDSTEIN, 1970; reproduced by permission of Excerpta Medica Foundation.)

is drastically reduced immediately after transfer to the hyperosmotic external medium (GOLDSTEIN, 1970).

The situation is similar in elasmobranchs. Studying salinity effects on regulation of blood urea levels in the lemon shark *Negaprion brevirostris* and the little skate *Raja erinacea*, GOLDSTEIN (1970) found no decrease in the rate of urea production during acclimation to diluted sea water. There occurs, however, a 55% decrease in blood-urea concentration. This decrease could be accounted for by the increase in ^{14}C urea from body fluids which occurs after transfer of the fishes to diluted medium. In sharks, the rate of urea loss in sea water is 1·7% per day, it increases to 4·8% per day in diluted media. It is likely that most of this loss occurs through extrarenal pathways. Hence the gills seem as likely a site of urea concentration regulation as

the kidneys. Even in elasmobranchs in steady state with the surrounding sea water, urea loss occurs mainly through the gills (GOLDSTEIN and FORSTER, 1965). In the chimera, *Hydrolagus colliei*, nearly all the urea appears to be lost extrarenally. Although concentration of urea-N in the urine of this species amounts to 144 $\pm$ 55 mg%, urine flow is reduced (about 0·15 ml kg^{-1} hr^{-1}); this gives an average urea renal-excretion rate of about 0·22 mg urea–N kg^{-1} hr^{-1}, while the total loss is about 1·78 mg urea–N kg^{-1} hr^{-1}. Extrarenal loss appears to play an important part in the urea balance of elasmobranchs. Unfortunately, most of the investigations have been focused on the kidneys by studying renal loss and urea re-absorption (KEMPTON, 1953); to our knowledge, urea transport across the gill tissue has never been studied.

(c) Hormones and Extracellular-Fluid Anisosmotic Regulation

It is well established, and has long been known, that the mammalian kidney function is regulated by hormonal actions. Interest in the effects of hormones on ion and osmoregulation therefore developed early. Research has largely been restricted to euryhaline fishes. Only KATO and KAMEMOTO (1969) have briefly commented on the possible involvement of eyestalk hormone in the regulation of the urine flow in *Procambarus clarkii*.

In various fishes changes in activity of the pars intermedia of the pituitary complex have been reported during migration from or to sea water (OLIVEREAU, 1954, 1968; VOLLRATH, 1966). These results suggest some implication of this structure in the mechanisms at work during migration. However, it is well known that fishes undergo colour changes during migration and that the pars intermedia secretes hormones implicated in melanin dispersion. With the facts in hand up to now, it is difficult to decide whether the changes in activity of the PAS-positive cells of the pituitary complex are directly related to an osmoregulatory function.

Transitory changes in neurohypophysis histochemistry or anatomy have been observed in fishes upon transfer to media of various salinities (LEDERIS, 1963, 1964; OLIVEREAU, 1967). These changes have been interpreted as indicating release or retention of neurohypophysial factors in response to osmotic stress. In support of this there is evidence that vasotocin affects water and sodium movements in fishes (Table 5-13).

This effect can be assigned to the increase in the urine flow which probably occurs as a result of the increase in the glomerular filtration (MAETZ, 1963; SAWYER, 1965).

Vasotocin and related compounds have also been shown to affect sodium movements across the gill tissue of various fishes (Table 5-13). It is possible, however, that this effect is not direct but is mediated through changes in branchial circulation (MAETZ and RANKIN, 1969). There has been little work on possible circulatory effects of hormones in teleosts. Recently LAHLOU and co-authors (1969b) reported a pressor effect of arginine vasotocin in *Opsanus tau*. These considerations make the physiological significance of the neurohypophysial peptides in osmoregulation quite vague and judgement on such a possible role should be reserved.

Adrenalectomy has been realized successfully only in *Carassius auratus* (ETOH and EGAMI, 1963), and *Anguilla anguilla* (CHESTER-JONES and co-authors, 1964). This operation seems to affect sodium movements across the gill (BENTLEY and

Table 5-13

Effects of hormonal factors on salt and water movements in fishes. FW: fresh water; SW: sea water (Compiled from the sources indicated)

Species		Kidney		Gill sodium	Treatment	Author
		Water	Sodium			
Protopterus aethiopicus	(FW)	Loss	Loss		Vasotocin	(a)
		0	0		Mesotocin	
Carassius auratus	(FW)	Loss	Loss	Gain	Vasotocin	(b)
	(FW)			Loss	Prolactin	(c)
Anguilla anguilla	(FW)	Loss	Loss		Mesotocin, vasotocin, isotocin, vasopressin	(d)
	(FW)		0	Gain or loss	Aldosterone-cortisol	(e)
	(SW)			Loss	Cortisol	
	(FW)			Loss	Prolactin-ACTH	(f)
Samputa fluviatilis	(FW)	0	Loss	0	Vasotocin	(g)
	(FW)		0	Gain	Aldosterone	
	(FW)		0	0	Cortisol	
Fundulus heteroclitus	(FW)			Loss	Prolactin	(h)

[a] SAWYER (1966).
[b] MAETZ and co-authors (1964).
[c] LAHLOU and SAWYER (1967).
[d] CHESTER JONES and co-authors (1969a).
[e] MAYER and co-authors (1967).
[f] BUTLER (1967).
[g] BENTLEY and FOLLETT (1962, 1963).
[h] BURDEN (1956).

FOLLETT, 1962, 1963; MAYER and co-authors, 1967; MOTAIS, 1967; CHAN, 1969). The effects of adrenalectomy have been recently reviewed by CHESTER-JONES and co-authors (1969b).

Since the early findings of BURDEN (1956) and PICKFORD and PHILLIPS (1959) that *Fundulus heteroclitus* living in fresh water dies after removal of its pituitary but survives if prolactin is injected, much work has been devoted to the effect of prolactin on osmoregulation. This substance (together with prolactin-like compounds found in fishes) appears to be unique in promoting survival of *F. heteroclitus* in fresh water (BALL and PICKFORD, 1964; PICKFORD and co-authors, 1965; EMMART and co-authors, 1966). On the other hand, the data obtained suggest that the main deficiency after hypophysectomy concerns conservation of electrolytes and that the ion loss can be retarded by injection of prolactin. However, as pointed out by BALL (1969), many fishes, euryhaline or stenohaline, can easily survive in fresh water after hypophysectomy. This suggests that in these species, the involvement of prolactin in the hyperosmotic regulation may not be as important as in *F. heteroclitus*. In the goldfish *Carassius auratus*, for instance, hypophysectomy causes a marked fall in plasma electrolytes due to enhanced extrarenal loss (LAHLOU and SAWYER, 1967). The fish is able to survive, however, perhaps because its tissues are able to tolerate considerable changes in electrolyte composition of the body fluids. This possibility, however, has never been investigated. In *Anguilla anguilla*, another fish which can survive in fresh water after hypophysectomy, OLIVEREAU and CHARTIER-BARADUC (1966) observed a slow reduction in plasma electrolytes which can be retarded by maintenance therapy with ovine prolactin. However, if prolactin is used at low physiological doses, such an effect cannot be demonstrated (BUTLER, 1967; CHAN and co-authors, 1968).

Prolactin seems to act in the control of extrarenal loss of ions (see for instance ENSOR and BALL, 1968). It may be that in *Anguilla anguilla* the prolactin-activated restriction of sodium efflux is only of minor importance compared with other mechanisms largely non-endocrine. Whether the prolactin effect could be related in some way to the 'delayed regulation' mechanism specifically found in various euryhaline fishes after transfer from sea water to fresh water is actually unknown. This problem should merit some attention although it is difficult at present to generalize not only on the physiological importance of the prolactin effect but also on the site of action of prolactin. Indeed, if in various species prolactin appears to affect the extrarenal loss of sodium, it has been shown that in *Fundulus kansae* it is mainly the renal loss of sodium which is affected (STANLEY and FLEMING, 1966, 1967).

On the other hand, nothing is known about the mode of action of these hormones. Do they act directly on the cells of the gill epithelium or do they act indirectly, for instance through changes in the microcirculation of the target organ as suggested by SCHAYER (1964)? These aspects have not yet been explored. There is, however, a preliminary report of a study conducted on isolated gill filaments which suggests that salt secretion in the cell can be achieved in the absence of neurohypophysial hormones (HIRANO and co-authors, 1967). Much more information is needed before definitive conclusions can be drawn about the physiological importance and about the eventual mechanisms of the hormonal control of extracellular-fluid anisosmotic regulation.

(d) Morphological and Biochemical Studies of Salt Active Movements

Most hypo-osmotic regulators possess specific structures for active salt secretion: crustaceans and fishes, gill; elasmobranchs, rectal gland; reptiles, nasal or suborbital 'salt' gland. Hyperosmotic regulators reveal particular structures for active salt uptake: the gill of teleosts, the anal papillae of insect larvae and the pharynx of freshwater turtles.

Histological studies on glands responsible for active salt movements have shown that the cellular organization of these epithelia is quite similar. Indeed, the ultra-

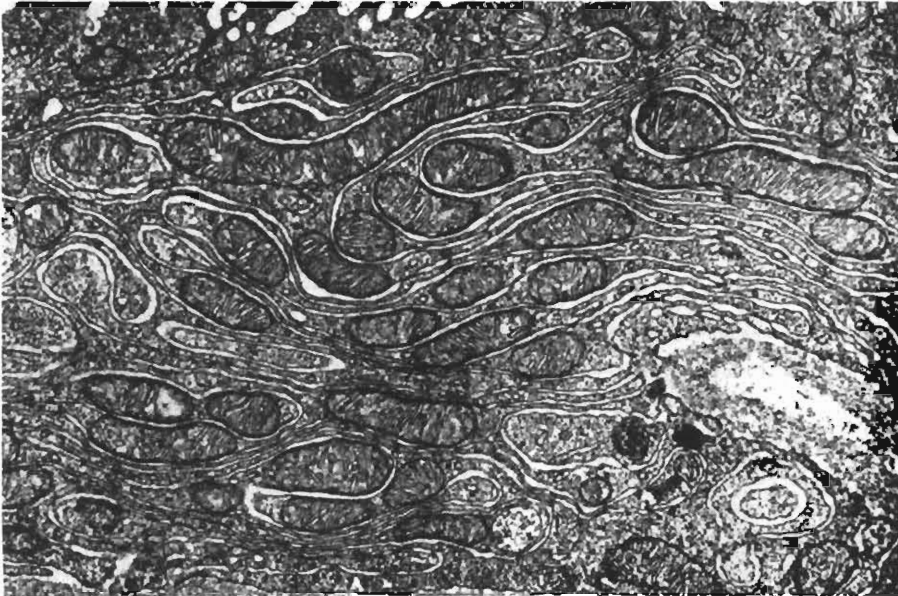

Fig. 5-12: *Gecarcinus lateralis.* Fine structure of 'osmoregulatory' gill tissue. Note the interdigitating folds highly populated with mitochondria. ×17,000. (After COPELAND, 1968; reproduced by permission of American Society of Zoologists.)

structure of the cells features an abundance of mitochondria and a highly developed labyrinth of granular membranes. The mitochondria are closely associated with double membranes of interdigitating folds of the cells. The differences in fine structure between the various types of 'salt moving' epithelia appear to be restricted mainly to form and shape of the membranous labyrinth which serves as extension of the exchange area.

The ultrastructure of the 'osmoregulatory' gill tissue of *Gecarcinus lateralis* is illustrated in Fig. 5-12. Similar structures have been discovered in the anal papillae of insect larvae (COPELAND, 1964a, b; SOHAL and COPELAND, 1966; COPELAND and FITZJARRELL, 1968), and in the 'salt' gland of marine turtles (ELLIS and ABEL, 1964) and birds (KOMNICK, 1963). Apparently, cells of the gill filaments of fishes and the rectal gland of elasmobranchs also reveal similar organization (CONTE, 1969). This particular structure of the epithelia responsible for the active movements of salts

had led COPELAND to suggest the existence of a mitochondrial pump which is implicated in the salt active movements.

On the other hand, volume changes of the salt gland of marine birds during long periods of unusually intensive osmotic work is a well-known phenomenon (PEAKER, 1971). A close correlation between the size of anal papillae and salinity has been demonstrated in insect larvae. Structure modifications related to salinity concern not only the volume of the organ but also its ultrastructure. As demonstrated by SOHAL and COPELAND (1966), the absorption of electrolytes from the medium may be regulated in larvae of *Aedes aegypti* by varying the surface area of the plasma membrane of the epithelial cells. In larvae raised in an isotonic medium fewer mitochondria lay between the folds of the plasma membrane than in larvae raised in hypotonic solutions. Investigations on the ultrastructure of the gills of *Fundulus heteroclitus* acclimated to sea water or to fresh water also indicate morphological changes in mitochondria-rich cells during salinity adaptation (DOYLE and GORECKI, 1961).

The extent of morphological changes in 'salt moving' glands seems to depend on the intensity of the work performed. This led CONTE (1969) to suggest changes in the renewal rates of osmotically active cells during salinity acclimation and he attempted to measure turnover rates of gill cell populations in the salmon *Oncorhynchus kisutch* by following the kinetics of ^{3}H-thymidine incorporation in DNA. He interpreted his results as indicating much faster DNA renewal in sea water than in freshwater-acclimated fishes. In the same way, the higher incorporation of ^{3}H-leucine in the protein of the gills of the platyfish *Xiphophorus maculatus* when acclimatized to 1/3 sea water has been interpreted as a indication of a larger protein synthesis related to the mechanism of acclimatization to salinity changes (HOLTZMAN and SCHREIBMAN, 1970). It must be noted, however, that the platyfish *X. maculatus* should have a more important osmotic problem in fresh water than in 1/3 sea water. Therefore, protein metabolism, if it were related only to the synthesis of a proteic material required for active transport, should be higher in the freshwater animals than in those acclimatized to 1/3 sea water. Recently, CONTE and co-authors (1973) showed a relationship between rate of incorporation of ^{14}C leucine into proteins of *Artemia salina* nauplii and salinity of the external medium. In this case, elevating the level of NaCl from 0·05 M to 0·50 M carries a decrease in ^{14}C incorporation. However, further increase in NaCl concentration (up to 2·5 M) has no effect. In this respect it is interesting to consider that protein turnover, as indicated by incorporation of ^{14}C from labelled glucose, is higher in the gills of *Callinectes sapidus* in sea water than in the gills of animals acclimatized to 50% sea water (Table 5-14). This crustacean, however, has no problems of extracellular-fluid anisosmotic regulation in sea water. It maintains a blood hyperosmotic state only when in diluted media and an active movement of salts at the gill level is to be expected only in the diluted medium. Moreover, it is clear from the results of Table 5-14 that a higher incorporation of ^{14}C into protein is observed not only at the gill level but also in the nerve. Although the incorporation rate appears to be much faster in the gill than in the other tissues, no significant changes in the activity of protein when expressed as % of the total tissue activity can be demonstrated in the muscle or hepatopancreas. This may be due to the fact that in these tissues protein turnover is much lower than in the gill; hence the experimental values

obtained would also be much lower. Nevertheless, it appears that changes in ^{14}C incorporation into proteins as related to salinity occurs not only in the gill but also in at least one other tissue of *Callinectes sapidus*.

It may be misleading, therefore, to consider the increased incorporation of labelled compound into protein or nucleoproteins observed in sea-water-acclimated animals as directly related to the phenomenon of ion regulation. A number of factors are known to influence protein and nucleoprotein turnover in mammalian tissues (e.g. MUNRO, 1970), notably in amino-acid pattern or electrolyte composition of the tissue. This may provide an explanation for the changes in protein labelling observed in *Callinectes sapidus* tissues since it is known that the amino-acid pool

Table 5-14

Callinectes sapidus. Incorporation of ^{14}C glucose in different fractions of various tissues of individuals acclimated to sea water (SW) or to double-diluted sea water (SW/2). Crabs were given 15 μC of the labelled compound in a single injection. Activity expressed in DPM mg^{-1} wet weight tissue (Original)

Tissue	Proteins (TCA precipitate)	Soluble* fraction	Total activity	Activity in proteins as % of total activity
Gill				
SW	476·78	524·20	1126	42·35
SW/2	177·47	520·40	794	22·34
Muscle				
SW	30·32	264·00	378	8·01
SW/2	23·66	239·00	316	7·48
Nerve				
SW	97·42	1409·80	1865	5·22
SW/2	64·78	1475·00	1760	3·70
Hepatopancreas				
SW	81·02	599·00	849	9·53
SW/2	71·13	531·80	741	9·59

* Soluble fraction consisted of the dialysate of the soluble material after 24 hrs dialysis.

in the tissues of crustaceans is subject to changes during salinity stresses (p. 308). In the same way, amino-acid content of fish tissues increases with increasing salinity (ANDERS, 1967; HUGGINS and COLLEY, 1971; LASSERRE and GILLES, 1971); it has also been demonstrated that amino acids stimulate DNA replication in fishes (SIEGER and co-authors, 1969). Such relationships may provide an explanation for the fact that DNA turnover and protein turnover are higher in gills of fishes acclimated to high salinities. Thus, much work remains to be done to determine whether the observed changes can be related directly to the mechanism responsible for active salts movements. Nevertheless changes in the structure of salt-moving organs can be demonstrated upon acclimation of euryhaline species to various media. Protein metabolism must thus be involved in such gross changes. MAETZ and co-authors (1969) showed that injection of actinomycin D into sea-water-acclimated *Anguilla anguilla* caused a decrease in the sodium turnover rate from 30%

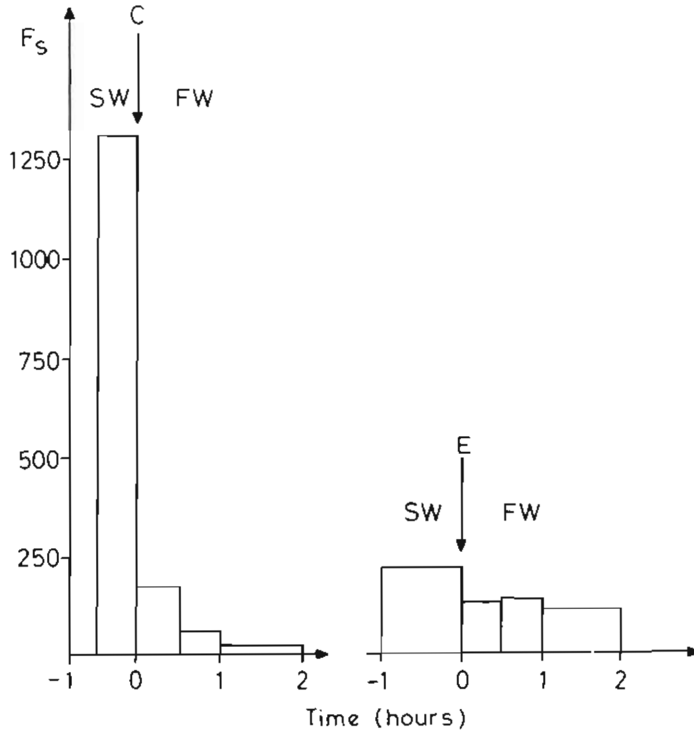

Fig. 5-13: *Anguilla anguilla*. Effect of actinomycin D on Na^+ efflux during transfer from sea water (SW) to fresh water (FW). C: controls, E: experimentals. Sodium efflux (F_s) is given in μeq. hr^{-1} 100 g^{-1}. (After MAETZ and co-authors, 1969; modified; reproduced by permission of Microforms International Marketing Corp.)

hr^{-1} to 1 to 2% hr^{-1} within 4 to 5 days (Fig. 5–13); the treated fishes (experimentals) died on the 6th day with abnormally high plasma sodium and chloride levels. Injection into freshwater eels has no measurable effect upon the sodium influx, but the sodium efflux increases tremendously. This brings about a slow decrease in the blood electrolyte content which amounts to 15 to 30% after 7 to 9 days. Generally, freshwater-treated eels survive longer than sea-water ones. Such results favour the idea of a mechanism for ion movement which is dependent on the protein-metabolism machinery. However, actinomycin D had no effect on influx in freshwater eels, even though part of the influx is active. In contrast, in the sea-water eels sodium turnover is drastically modified. It appears, therefore, that the exchange diffusion parameter which is responsible for more than 90% of the sodium movement in the sea-water eels is highly sensitive to actinomycin D. Moreover, MAETZ and co-authors (1969) showed that the so-called 'delayed regulation' mechanism (p. 278), which can be demonstrated during transfer of *A. anguilla* from sea water to fresh water, is almost non-existent in treated fishes (Fig. 5-13).

It is not known if the effects of actinomycin D can be related directly to its action on the mechanism of intracellular-fluid anisosmotic regulation. MAETZ and co-

authors (1969) found an argument in favour of this view in the fact that mitomycin does not affect sodium exchanges while puromycin and cycloheximide induce modifications in the osmoregulation mechanism completely different from the ones observed with actinomycin D; that is, puromycin induces an increase in the efflux re-adjustment occurring during transfer from fresh water to sea water while actinomycin D treatment produces a decrease in the re-adjustment velocity. MAETZ and co-authors (1969) explained these differences by considering that the synthesis or the activity of the sodium carrier is regulated at the translation level in a way similar to that proposed by TOMKINS and co-authors (1966), see also GILLES and GILLES-BAILLIEN (1974), to explain enzyme induction in mammalian liver. Of course, as stressed by MAETZ and his colleagues, this consideration is speculative. Nevertheless, it is interesting to notice that, in their studies, MAETZ and co-authors (1969) present arguments that actinomycin D does not act primarily on a mechanism implicated in active salt transport, but on the carrier implicated in passive sodium movements at the gill level. Actinomycin D appears, therefore, to act first on the mechanism of rapid adaptation to salinity changes. Whether or not it exerts an action on the other secondary mechanisms which assist the animal to adjust to salinity changes during long-term acclimation is not known.

Since the discovery by SKOU (1957) of a Na^+/K^+ sensitive ATPase, studies have been carried out indicating that, in many cases, the activity of this enzyme is related to sodium transport. As shown in Table 5-15, the activity of the enzyme in the gills

Table 5-15

ATPase activity in gills of limnic, marine and euryhaline teleosts (After KAMIYA and UTIDA, 1969; modified; reproduced by permission of Microforms International Marketing Corporation)

Species	ATPase activity (μM Pi hr^{-1} mg^{-1} protein)	
	Na^+/K^+	Mg^{2+}
Limnic		
Carassius auratus	11·0 ± 2·3*	65·4 ± 10·8
Cyprinus carpio	9·4 ± 2·7	138·9 ± 7·3
Tribolodon hakonensis	13·8 ± 5·2	60·1 ± 5·4
Marine		
Atherina tsurugae	41·6	25·0
Trachurus japonicus	53·1 ± 9·4	24·6 ± 5·0
Girella punctata	33·6 ± 4·5	28·3 ± 1·3
Euryhaline		
Anguilla japonica		
fresh water	8·8 ± 1·0	4·9 ± 0·4
1 week in sea water	38·5 ± 1·6	4·6 ± 0·3
Salmo gairdnerii irideus		
fresh water	8·5 ± 2·3	11·9 ± 1·8
1 week in sea water	30·9 ± 3·8	13·1 ± 2·5

* Standard error of mean.

of stenohaline teleosts is higher in marine species than in freshwater ones. In contrast, the activity of the Mg^{2+} ATPase is higher in freshwater than in sea-water species. This has led various workers to examine possible changes in the activity of these enzymes in euryhaline teleosts during acclimation to various media. The activity of the Na^+/K^+ ATPase is higher in fishes acclimated to sea water than in those acclimated to fresh water. No such correlation could be observed between MgATPase activity and salinity (Table 5-15). In fact, when Japanese eels *Anguilla japonica* are transferred from fresh water to sea water, the Na^+/K^+ ATPase activity of their gills increases markedly during the first week of acclimation and the activity remains high for a long period (UTIDA and co-authors, 1966; KAMIYA and UTIDA, 1968). Similar results have been reported in *Fundulus heteroclitus* by EPSTEIN and co-authors (1967) not only in the gill tissue but also in the gut which plays an important part in the extracellular-fluid anisosmotic regulation of teleosts in sea water (JAMPOL and EPSTEIN, 1970). Moreover, EPSTEIN and co-authors (1971) recently showed that injection of cortisol in freshwater-acclimated eels *Anguilla rostrata* induces a rise in the activity of Na^+/K^+ ATPase in both gill and gut at levels comparable to those described for the sea-water acclimated animals. We have already discussed the limited evidence suggesting the involvement of hormones in the osmotic regulation process. The work of EPSTEIN and co-authors (1971) is a first approach to this problem at the biochemical level. It would be interesting to know if cortisol produces its effect only on specific target organs, such as gill and gut, or if the effect is general. This remark also pertains to studies on Na^+/K^+ ATPase. To the reviewer's knowledge, changes in Na^+/K^+ ATPase activity have only been studied in gill, gut and kidney, but never in organs which are not directly implicated in the regulation process.

From the results presented above it is tempting to speculate on possible correlations between hormonal control of Na^+/K^+ ATPase activity and high-level sodium efflux through the gills of teleosts in sea water. However, such a relationship remains to be demonstrated. We have already seen that most of the sodium efflux at the gill level can be accounted for by passive movements and that active movement accounts only for a very small part of the total sodium efflux. In various cases, we are not even sure if there is an active movement at all (p. 277). On the other hand, during transfer from fresh water to sea water, the increase in sodium efflux stabilizes in a few days (p. 269; MAETZ and co-authors, 1969), while it takes more than a week (possibly more than a month) for the gill Na^+/K^+ ATPase to reach its normal sea-water level (KAMIYA and UTIDA, 1968).

It appears that the changes in Na^+/K^+ ATPase activity are not implicated in the mechanisms which allow the euryhaline teleost to withstand rapid changes in salinity. Rather, they may be part of a mechanism which assists osmoregulation during long-term acclimation. This is reminiscent of the changes observed in the ultra-structure of the salt-moving glands. It is interesting to recall here that changes in activity can be recorded at the level of membrane-bound enzymes other than Na^+/K^+ ATPase. The concentration of succinic dehydrogenase, as determined by histochemical techniques, appears to be higher in the gill of *Fracturus mediterraneus* acclimated to hyperosmotic media (GINETSINSKII and co-authors, 1965). NATOCHIN and BOCHAROV (1962) reported an increase in succinic dehydrogenase level in the gill of different Salmonidae after acclimation to sea water. Using biochemical

techniques, CONTE and TRIPP (1970) recently studied the succinic dehydrogenase of the chinook salmon *Oncorhynchus tshawytscha*, the starry flounder *Pleuronectes stellatus* and the staghorn sculpin *Leptocottus armatus*. They found that the specific activity of the enzyme is much higher in marine sculpin than in flounder and salmon acclimated to fresh water or sea water. The time spent by the two euryhaline fishes in both media, however, was not recorded. No significant changes in specific activity could be observed during short-term acclimation (12 hrs) from one medium to the other. This may be partly due to the large individual variations in activity. Nevertheless, it is still possible that modifications of the enzyme level could be detected after long-term acclimation.

Apparently, not only Na^+/K^+ ATPase but also other membrane-bound enzymes may change in activity upon long-term acclimation to different salinities. Moreover, concomitant changes in ultrastructure of the salt-moving glands can be observed. These changes mainly concern unfolding of the plasma membrane and shape and number of mitochondria. Possibly, both phenomena are related. Whether such changes are directly or indirectly related to active salt movements is unknown. It seems clear that they are related to extracellular-fluid anisosmotic regulation during long-term acclimation.

Modifications in shape and number of mitochondria indicate the presence of a mitochondrial pump as suggested by COPELAND (1968); but such modifications may also occur in response to the increased energy requirements by the active transport mechanism. From this view, the increase in the level of membrane-bound enzymes observed during transfer of euryhaline teleosts to sea water could be visualized as being related to active chloride transport, for instance, and not necessarily related to an active outward movement of sodium (which, in various cases, remains to be demonstrated).

In conclusion, the biochemical studies conducted thus far have failed to provide a clear picture of the molecular basis of extracellular-fluid anisosmotic regulation. However, they indicate lines of investigation which may prove rewarding. We must distinguish between short- and long-term mechanisms of acclimation to salinity stress. During long-term acclimation, biochemical mechanisms implicated in the regulation of the enzyme level appear to be at play. Such a mechanism has been studied only in micro-organisms. Recent studies on mammalian liver show that regulation of the protein level (enzymes) can be achieved by mechanisms completely different from those described in micro-organisms (for reviews see SCHIMKE, 1969; GILLES and GILLES-BAILLIEN, 1974). Euryhaline species appear to be good material for studying such a problem and for demonstrating the importance of non-genetic and genetic adaptation (Volume I, Chapter 4). Much work remains to be done, particularly on possible relations between hormonal status, enzyme (and possibly carrier) level and regulation of ion fluxes; the results obtained so far are encouraging.

Blood ionic regulation is an important evolutionary acquisition for coping with changes in the thermodynamical activity of the ambient water. It protects the cells of multicellular animals against critical changes in volume and inorganic-ion concentration. However, a number of aquatic organisms lack such a mechanism. Their survival under salinity stress depends on the capability of the cells to tolerate osmotic stress.

(3) Intracellular-Fluid Isosmotic Regulation

The preceding section has documented that most aquatic multicellular animals have only a limited capability of extracellular-fluid anisosmotic regulation. Even in so-called homeosmotic species, modifications in blood osmotic pressure of 20 to 30% can be observed upon transfer from sea water to fresh water (Fig. 5-1). Penetration of marine animals into brackish or fresh waters, requires the cells to tolerate considerable changes in solute composition of their surrounding fluid.

(a) Cell-Volume Regulation During Osmotic Stress

For many years, the notion of isosmoticity between cell and surrounding fluid has been opposed by many physiologists on the basis of experiments on tissue slices incubated in presence or absence of metabolic inhibitors. Since the cells take up water from an isotonic medium when their metabolism is impaired, it was concluded that their osmotic pressure is higher than that of their environment. Direct measurements of tissue osmotic pressure led ROBINSON (1950) to the same conclusion. However, CONWAY and MCCORMACK (1953) questioned the technique used by ROBINSON and showed that, when using adequate techniques, the intracellular osmotic pressure of various tissues is equal to that of the surrounding medium.

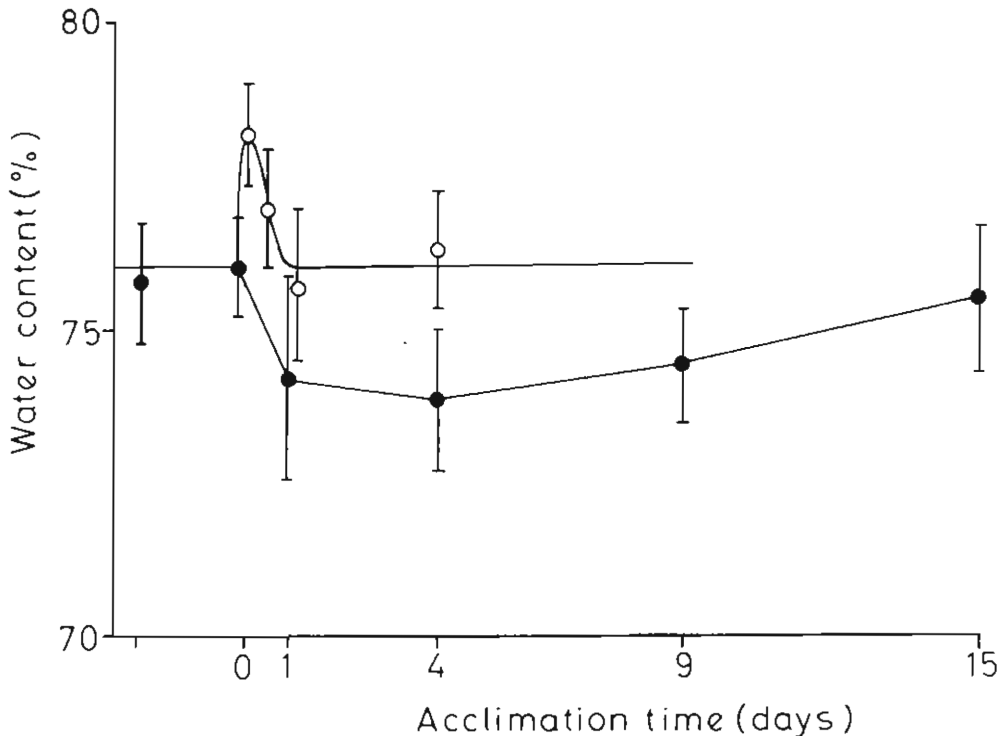

Fig. 5-14: *Eriocheir sinensis*. Volume regulation of muscle tissue during acclimation from sea water to fresh water (○) or from fresh water to sea water (●). All values expressed as percent of tissue water. (Original.)

Moreover, it has been shown that the volume changes observed under the influence of metabolic inhibitors are a direct consequence of the abolition of the active transport of cations (TOSTESON and HOFFMAN, 1960). In fact, most recent evidence indicates that the cell is in isosmotic equilibrium with its surrounding medium. Hence it may be expected that a change in blood osmoconcentration induces a concomitant adjustment of cell osmotic pressure. Such adjustment may occur either by passive osmosis or by intervention of an active phenomenon regulating the amount of the intracellular osmotic effectors. In this way, changes in cellular volume due to water exchange would be, at least partly, avoided. That such a control mechanism exists is shown by the regulation of volume recorded in various tissues of euryhaline species submitted to osmotic stress. An example is shown in Fig. 5-14 for muscle of the Chinese crab *Eriocheir sinensis* during transfer to different salinities. Upon acclimation from sea water to fresh water the osmotic pressure of the blood falls from about 1100 mOsm l^{-1} to 550 mOsm l^{-1} (Table 5-2). This induces tissue swelling. The swelling phase is only transitory, however, and the muscle resumes its control volume very rapidly (Fig. 5-14). In contrast, during transfer from fresh water to sea water, the tissue shrinks slowly and volume re-adjustment is completed only after about 2 weeks. Rapid volume re-adjustment following hypo-osmotic stress has also been observed in the osmoconformer *Modiolus* sp. (PIERCE, 1971). In these experiments, PIERCE was unable to show

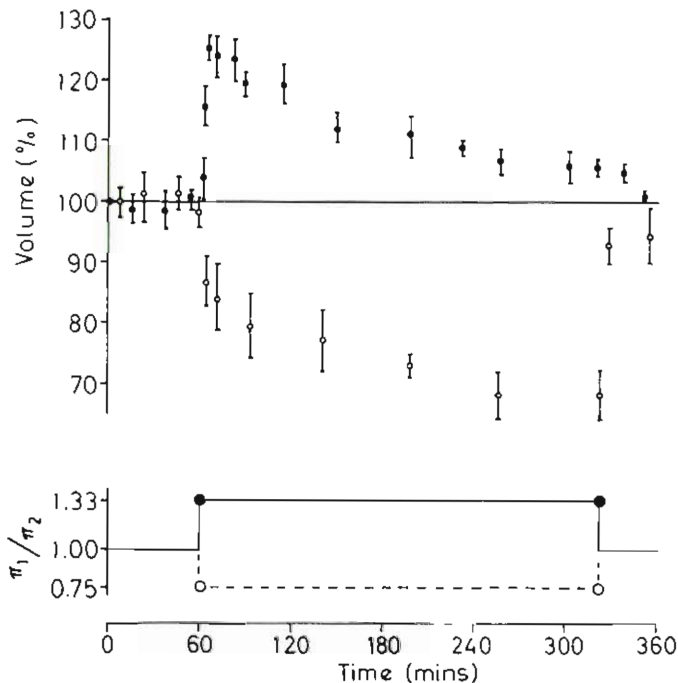

Fig. 5-15: *Callinectes sapidus*. Volume regulation in isolated axons during hyperosmotic (○) or hypo-osmotic (●) shock. (After GERARD and GILLES, 1972b; reproduced by permission of Experientia.)

any volume regulation by the molluscs during hyperosmotic stress. However, he recorded volume changes within a 48-hr period, and we have seen that re-adjustment takes much longer in the case of *Eriocheir sinensis* muscle. The results of PIERCE (1971), therefore, do not exclude the possibility of volume regulation following hyperosmotic stress in *Modiolus* sp.

Such results point out two important facts: (i) In euryhaline species of *Modiolus* or in *Eriocheir sinensis*, the cells do not simply swell or shrink passively with the osmotic changes in the extracellular fluids. There exists, in fact, a mechanism of tissue-volume regulation. (ii) Regulation is much faster under hypo-osmotic than under hyperosmotic stress. This may indicate the presence of different mechanisms in the two processes.

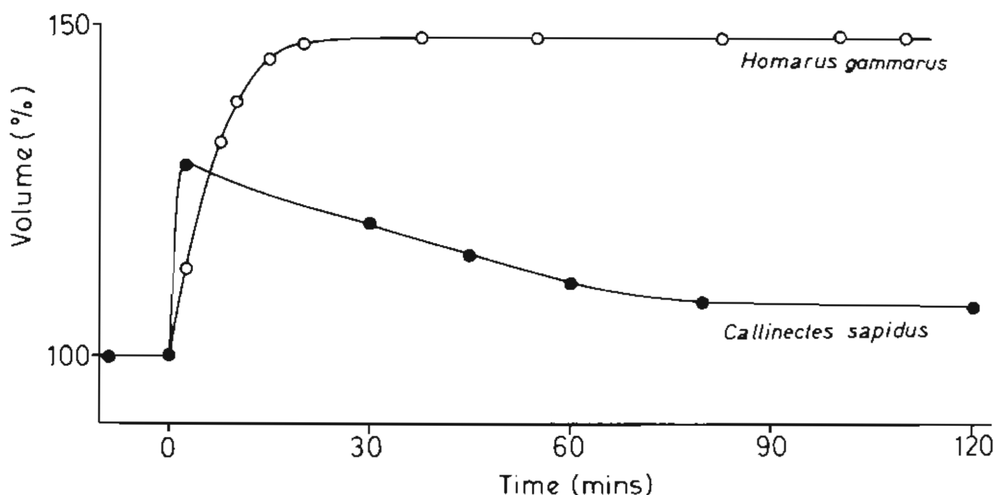

Fig. 5-16: Effect of a hypo-osmotic shock (π_1/π_2 approximately equal to 1·5) on the volume of isolated muscle fibres of the euryhaline *Callinectes sapidus* and the stenohaline *Homarus gammarus* (Syn.: *H. vulgaris*). (Original; drawn after data from GAINER and GRUNDFEST, 1968, and LANG and GAINER, 1969.)

Cell-volume regulation has also been observed in tissues isolated from euryhaline species and submitted to osmotic stress in artificial sea waters (FUGELLI, 1967; LANG and GAINER, 1969; GERARD and GILLES, 1971, 1972b; GILLES, 1973). Fig. 5-15 illustrates this phenomenon in isolated axons of the blue crab *Callinectes sapidus*. During hypo-osmotic shock, an initial swelling occurs which is rapidly regulated, the axon resuming its control volume within a few hours. During hyperosmotic shock, on the other hand, the tissue shrinks and no volume regulation can be observed even after 7 hrs. This is reminiscent of the situation encountered with whole animals in which rapid regulation of swelling is observed while regulation following shrinkage of the tissue is much slower. The experiments performed on isolated tissues point to the fact that hormonal control is not primarily implicated in cell-volume regulation.

Interestingly, the osmotic responses of isolated muscle fibre of the stenohaline lobster *Homarus gammarus* is completely different. Fig. 5-16 shows osmotic respon-

ses of muscle fibres isolated from the euryhaline blue crab *Callinectes sapidus* and from the stenohaline lobster *H. gammarus* when submitted to hypo-osmotic stress. After 2 hrs, the muscle fibre of the euryhaline crab has largely regulated its volume, but no volume regulation can be observed yet in the muscle fibre of the stenohaline lobster. In fact, GAINER and GRUNDFEST (1968) have shown that the muscle fibre of *H. gammarus* behaves as a perfect osmometer following the linear Van't Hoff relation over a fairly wide range of medium osmotic pressures. Such a difference in osmotic responses between tissues of a euryhaline and a stenohaline species may be of ecological significance and would provide a physiological basis for explaining, at least partly, the capability of various species with limited ability for extracellular-fluid anisosmotic regulation to withstand considerable changes in salinity.

Euryhalinity and stenohalinity (Volume I, Chapter 4), however, may not be related to an 'all or nothing' capacity for volume regulation. Presumably, rather the relative efficiency of such a mechanism determines the degree of euryhalinity of the animal. More species must be studied before a definite statement can be made.

Cell-volume regulation appears to play an important part in the ecology of euryhaline species. Since the cell is always isosmotic with its surrounding fluid, it must be assumed that in these species the intracellular water content is controlled by a mechanism regulating the level of intracellular osmotic effectors. FLORKIN (1962) has termed this phenomenon 'isosmotic intracellular regulation'. Before attempting to analyse the mechanisms at work in intracellular isosmotic regulation, it is necessary to know the important osmotic effectors encountered in various tissues.

(b) Cellular Osmotic Effectors

In body fluids of most species, the osmotic pressure is largely made up of inorganic ions (salts). However, as early as 1901, FREDERICQ noticed that the total tissue concentration in inorganic ions is in general lower than that of the surrounding blood. The cell's osmotic salt deficit is compensated for by organic substances (Fig. 5-17).

Osmotic balance sheets, obtained for various tissues from several species, show that a variety of organic substances act as osmotic effectors. The osmotic effectors in muscle tissue of two molluscs are listed in Table 5-16. In the marine *Sepia officinalis*, organic compounds account for more than half the muscle osmotic pressure. Organic phosphates and amino acids, together with some other amino compounds, appear as important organic effectors. Some of the organic compounds undoubtedly play also an important role in cellular ion balance. Isethionic acid and dicarboxylic amino acids are, for instance, of prime importance for the ion balance in *Loligo pealii* nerves (DEFFNER and HAFTER, 1960). On the other hand, ionic balance is achieved mainly by phosphate in the muscle of another squid, *Sepia officinalis* (ROBERTSON, 1965). Thus, the organic molecules involved in cation–anion balance vary from species to species, and in the same group from tissue to tissue. The same is true for the organic compounds used by the cells as osmotic effectors. It is now well established, however, that amino acids and some amino compounds such as taurine or glycine-betaine are generally present (for reviews, see SCHOFFENIELS and GILLES, 1970a, 1972).

Table 5-16 further reveals that the amino-acid content in muscles is much higher in the marine *Sepia officinalis* than in the limnic *Anodonta cygnea*. This is also the case for betaine and taurine; though found in large amounts in marine species, the latter compound is not generally present in limnic forms (SIMPSON and co-authors, 1959). Apparently, molecules, together with the inorganic ions play an important part in the intracellular-fluid isosmotic regulation. Not much is known about the means by which inorganic-ion regulation is achieved at the cellular level. The potassium concentration within the muscle cell can be regarded as being maintained by the association of this cation with a fixed anion. Thus the mechanisms by which sodium and chloride are excluded from the cell are most important in ionic regulation in marine species. Unfortunately, nothing is known about these mechanisms (LOCKWOOD, 1962; POTTS and PARRY, 1964).

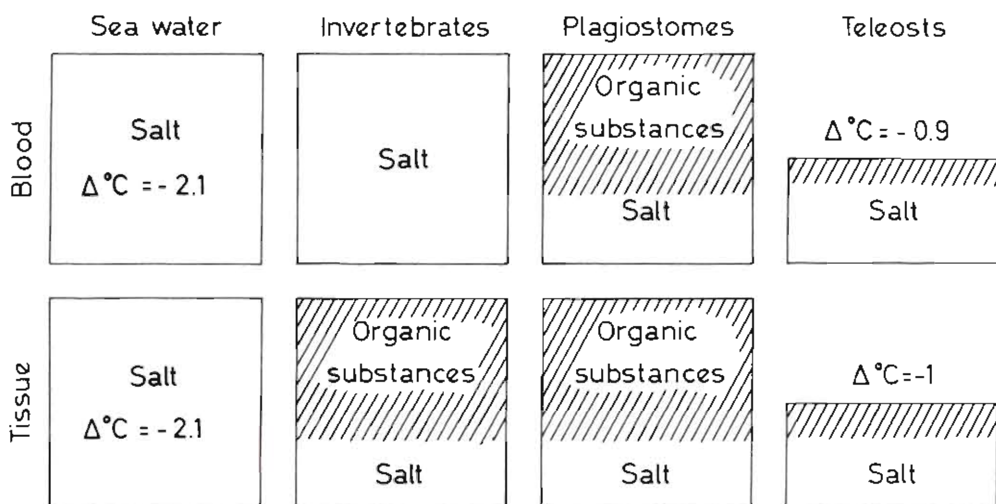

Fig. 5-17: Osmotic concentration in sea water and in blood and tissue of marine animals. (After FREDERICQ, 1901; modified.)

Present knowledge makes it difficult to elaborate on the mechanisms of regulation of organic osmotic effectors such as quaternary ammonium derivatives or organic phosphates. Hitherto, most investigations have been concerned with the role of amino acids in intracellular-fluid isosmotic regulation. Amino acids are indeed the most common organic osmotic effectors; in some cases their concentration can account for more than 50% of the total cellular osmotic pressure.

(c) Amino Acids as Osmotic Effectors

The amount of amino acids in the muscle of the limnic *Anodonta cygnea* is much lower than that in the muscle of the marine *Sepia officinalis* (Table 5-16). This observation suggests that free amino acids play an important role in osmoregulation of intracellular fluids. It appears of interest, therefore, to compare the performance of euryhaline crabs with poor capacities for extracellular-fluid anisosmotic regulation exposed to different salinity (Table 5-17). In the muscle of sea-water acclimated

Callinectes sapidus, the amino-acid concentration when expressed in mOsm kg^{-1} of intracellular water, accounts for about 70% of the total osmotic pressure of the tissue. The remaining osmotic pressure can be accounted for almost completely by inorganic ions. After acclimation of the crab to double-diluted sea water, the inorganic-ion concentration decreases only slightly with the exception of chloride which undergoes a decrease much larger than sodium and potassium. In contrast, there is a large decrease at the amino-acid level which accounts for most of the decrease observed in the total osmotic pressure of the intracellular fluid. That active processes are involved and not simply dilution processes due to tissue swelling is shown by the fact that: (i) tissue water content does not change significantly and (ii) the percentage of variation varies from one amino acid to the other. Furthermore, with the exception of arginine, the so-called non-essential amino acids play

Table 5-16

Osmotic effectors in muscle of two molluscs. All concentrations expressed as mM kg^{-1} water (Compiled from the sources indicated)

Effector	*Sepia officinalis* mantle muscle[a]	*Anodonta cygnea* fast adductor muscle[b]
Inorganic compounds		
Sodium	30·8	6·6
Potassium	189	18·4
Calcium	1·88	11·5
Magnesium	19·0	3·9
Ammonium	2·2	—
Chloride	45·0	3·9
Sulphate	2·0	—
Inorganic phosphate	92·0	6·9
Organic compounds		
α-amino nitrogen	483	10·5[c]
Taurine	—	0
Trimethylamine oxide	86·1	—
Betaine	108·2	—
Arginine phosphate	4·3	8·4
Adenosine triphosphate	8·2	1·7
Remaining acid-soluble phosphate	12·2	2.8
Reducing sugar	2·9	—
Glycerol	4·2	—
Osmotic pressure due to inorganic compounds (mOsm kg^{-1} water)	588·76	80·40
Osmotic pressure due to organic compounds (mOsm kg^{-1} water)[d]	787·88	26·00
Total cellular osmotic pressure (mOsm kg^{-1} water)	1376·64	106·40

[a] ROBERTSON (1965).
[b] POTTS (1958).
[c] Only carboxylic acids determined by method of VAN SLYKE and co-authors (1941).
[d] Osmotic pressure of organic compounds has been calculated assuming that Δ of a molar solution of some organic compounds such as amino acids is $-1·86$ °C (PROSSER and co-authors, 1950).

the largest part as osmotic effectors. However, so-called essential amino acids, such as methionine, phenylalanine, lysine or histidine, also participate in intracellular-fluid isosmotic regulation.

In recent years many studies have dealt with the free amino-acid content in euryhaline species exposed to variations in salinity. The results obtained demonstrate that the tissue level of free amino acids varies with the ambient osmotic pressure. This phenomenon has been recorded in all phyla studied (for a review, see SCHOFFENIELS and GILLES, 1970a). It has also been documented for euryhaline fishes (CHOLETTE and co-authors, 1970; HUGGINS and COLLEY, 1971; LASSERRE

Table 5-17

Callinectes sapidus. Osmotic components in the muscle of individuals acclimated 4 weeks to sea water (SW) or to double-diluted sea water (SW/2) (After GERARD and GILLES, 1972a; reproduced by permission of North Holland Publishing Company)

	SW		SW/2	
Compounds	Wet weight of tissue (μM g^{-1})	Intracellular water (mOsm kg^{-1} H_2O)	Wet weight of tissue (μM g^{-1})	Intracellular water (mOsm kg^{-1} H_2O)
Amino acids				
Taurine	37·86	69·41	20·49	37·30
Aspartate	4·26	7·80	1·67	3·03
Threonine	not determined			
Serine	28·57	52·37	2·82	5·12
Glutamate	5·55	10·16	1·86	3·38
Proline	40·37	74·01	26·79	48·76
Glycine	197·45	361·98	154·91	282·01
Alanine	20·51	37·60	12·40	22·57
Cysteine	1·16	2·12	0·89	1·61
Valine	2·90	5·30	3·08	5·60
Methionine	3·22	5·89	0·64	1·16
Isoleucine	0·97	1·77	0·89	1·61
Leucine	1·87	3·42	1·92	3·50
Tyrosine	1·09	2·00	0·64	1·15
Phenylalanine	2·32	4·25	0·38	0·69
Lysine	1·87	3·43	0·05	0·08
Histidine	1·35	2·47	0·45	0·82
Arginine	74·43	136·44	52·36	95·32
Total	422·53	780·42	282·24	513·71
Inorganic ions				
Na	105·16	39·5	85·71	28·4
K	143·40	186·0	96·16	162·2
Cl	98·68	45·6	77·33	25·9
Total		271·1		216·5
Blood osmotic pressure (mOsm l^{-1})		1100		850
Water content (% H_2O)				77·80

and GILLES, 1971) under osmotic stress as well as for several protozoans, e.g. the marine ciliate *Miamiensis avidus* (KANESHIRO and co-authors, 1969) and *Tetrahymena pyriformis* (STONER and DUNHAM, 1970). These findings suggest the possibility that amino-acid participation in intracellular-fluid isosmotic regulation is a general mechanism present in all organisms.

It is also important to note that euryhalinity of several species depends only on their capacity for intracellular-fluid isosmotic regulation. The sea-star *Asterias rubens* does not show measurable extracellular-fluid anisosmotic regulation; neither do *Arenicola marina, Mytilis edulis, Libinia emarginata* nor the hagfish *Myxine glutinosa*. In all these euryhaline species, intracellular-fluid isosmotic regulation is found. Apparently, intracellular-fluid isosmotic regulation can be considered as a basic mechanism to which, in several species, anisosmotic extracellular-fluid regulation has added a new range of possibilities (see also KINNE 1971, p. 897).

Which amino acids participate in intracellular-fluid isosmotic regulation? Table 5-18 compiles amino-acid concentrations in muscle tissue of several euryhaline animals exposed to their normal environment, as well as after acclimation to different salinities. The results show that acclimation to the new salinity affects the concentration of all amino acids determined. However, the largest variations are recorded in the so-called non-essential amino acids: alanine, aspartic acid, glutamic acid, glycine and proline. An exception is arginine which cannot be synthesized by many of the species listed in Table 5-18 and which exhibits large variability.

Table 5-18 also indicates that the participation of the amino-acid pool in the establishment of the cell's osmotic pressure varies from species to species. In most of the marine invertebrates studied, amino-acid concentration ranges from about 150 to about 350 mM kg^{-1}, although it is much lower in the molluscs *Purpura lapillus* and *Acanthochitona discrepans*. Obviously, other organic molecules participate in the regulation process in these species. In the vertebrates, the total amino-acid content of the muscle tissue is low. This is understandable when considering that, in these species, the blood is normally kept hypo-osmotic to the environmental medium. The role of organic molecules as osmotic effectors is less important than in invertebrates nearly isosmotic with the surrounding sea water.

Most studies on participation of amino acids in intracellular-fluid isosmotic regulation have been conducted on muscle tissues. GERARD and GILLES (1972a) measured the concentration of amino acids in blood, hepatopancreas, gill, nerve and muscle of the euryhaline crab *Callinectes sapidus*, acclimated to sea water or to 50% sea water. They found that the amino-acid level is much lower in blood than in tissues. Among the tissues examined, muscle and nerve entertain a larger pool of amino acids than gill or hepatopancreas. The high total amount of amino acids in these tissues is accounted for mainly by the high concentration of proline, glycine, alanine and glutamic acid. Upon acclimation of sea-water *C. sapidus* to 50% sea water, the total amount of amino acids decreases in all tissues except the blood. This decrease affects the concentration of all amino acids determined and is much larger in muscle or nerve than in gill or hepatopancreas. These results indicate that the participation of the amino-acid pool in the regulation of the osmotic equilibrium which the cell maintains with its surrounding fluid is a general phenomenon in tissues of *C. sapidus*.

Table

Amino-acid concentration in tissues of euryhaline animals acclimated to different salinities. determined; SW: sea water; FW: fresh water; *: values represent lysine + histidine muscle tissue (Compiled

Amino acids	Worms						Molluscs							
	Nereis diversicolor[a]		*Perinereis cultrifera*[a]		*Arenicola marina*[b]		*Mytilus edulis*[c]		*Mya arenaria*[d]		*Purpura lapillus*[e]		*Acanthochitona discrepans*[f]	
	SW	20% SW	SW	50% SW	SW	50% SW	SW	50% SW	SW	50% SW	SW	50% SW	SW	50% SW
Alanine	36·3	3·1	34·3	11·7	74·5	22·0	18·4	13·0	159·1	60·8	8·0	1·3	1·8	1·7
Arginine	0·1	0·1	0·2	0·1	0·2	0·7	1·9	8·4	22·8	15·2	5·3	18·3	3·4	3·1
Aspartic acid	7·1	3·1	7·2	6·6	13·8	14·6	9·7	1·7	1·8	2·2	2·2	0·9	1·5	1·0
Glutamic acid	14·3	6·2	14·8	7·9	12·4	6·4	9·7	10·6	15·2	15·6	4·4	2·7	4·0	4·7
Glycine	57·8	6·7	177·1	96·9	224·6	99·6	61·8	18·4	94·5	69·0	5·3	1·0	0·6	0·7
Histidine	2·0	0·7	1·6	0·6	1·2	8·7	1·8	1·2	—	0·5	0	0	traces	traces
Isoleucine	1·9	0·4	0·9	0·4	0·5	0·2	0·3	0·4	2·2	0·8	—	—	traces	0·1
Leucine	1·7	0·5	1·2	0·5	0·7	0·3	0·4	0·6	2·6	1·2	0·8	0·1	traces	0·2
Lysine	2·8	2·0	0·8	0·4	1·8	1·5	2·8	1·7	3·5	3·2	0·4	traces	0·3	0·4
Phenylalanine	0·5	0·2	0·4	0·3	0·4	0·3	0·5	0·3	2·7	0·4	traces	traces	traces	0·1
Proline	45·8	5·0	25·3	17·6	—	—	6·3	2·8	2·6	1·2	3·5	2·0	0·4	0·4
Serine	—	—	—	—	—	—	6·0	3·0	5·7	5·4	3·1	0·2	0·7	0·9
Taurine	—	—	—	—	—	—	58·1	44·5	23·4	17·2	38·3	21·8	13·4	14·0
Threonine	4·3	1·4	7·9	3·6	1·9	0·9	4·3	1·9	4·2	2·9	0·3	0·2	0·4	0·4
Tyrosine	1·4	0·3	0·6	0·4	0·3	0·1	0·7	0·5	3·0	0·8	0·3	0·1	0·1	0·1
Valine	3·8	0·6	2·0	1·1	0·6	0·2	0·9	0·5	3·4	1·3	1·0	0·3	0·2	0·2
Total	182·5	30·9	267·3	148·8	331·2	150·6	193·6	109·5	346·7	197·7	72·9	48·9	26·8	28·0

[a] JEAUNIAUX and co-authors (1961b).
[b] DUCHÂTEAU-BOSSON and co-authors (1961).
[c] BRICTEUX-GRÉGOIRE and co-authors (1964).
[d] VIRKAR and WEBB (1970): results in mM kg^{-1} tissue water.
[e] HOYAUX and co-authors (1974).
[f] GILLES (1972a).
[g] DUCHÂTEAU and co-authors (1959).
[h] GILLES (1970a).

It can thus be concluded that, together with inorganic ions, a variety of organic molecules, including amino acids, are involved in the regulation of the cell's volume by playing an active part in controlling the osmotic pressure of the intracellular fluid.

(d) Mechanisms of Regulation of the Intracellular Amino-Acid Pool

When *Callinectes sapidus* is transferred from sea water to double-diluted sea water, the blood-ammonia concentration increases considerably, while the amino-acid concentration in the tissues decreases (Fig. 5-18). Ammonia-blood concentration increases abruptly by a factor of 10 and remains high at least during the first 4 days. Similarly, variations in nitrogen excretion related to salinity stress have been recorded by NEEDHAM (1957) in *Carcinus maenas*, by JEUNIAUX and FLORKIN (1961) in *Eriocheir sinensis* and by EMERSON (1969) in euryhaline molluscs. JEUNIAUX and FLORKIN (1961) showed that all variations in nitrogen excretion can be accounted for by modification of ammonia excretion. These results favour the idea of deamination of the amino acids during hypo-osmotic shock. It is not clear, however, whether this increased deamination occurs in all tissues separately or if the amino acids are first released from the tissues and then degraded in a specific organ. Studies on *C. sapidus* blood (GERARD and GILLES, 1972a) reveal a small but significant variation in various blood-amino acids. For instance, there is a transitory

5-18

Values expressed in mM kg^{-1} tissue wet weight unless otherwise indicated; —: not
+ an undetermined compound. Except for worms and *Asterias rubens*, results refer to
from the sources indicated)

Crustaceans								Echinoids		Fishes						Reptiles	
Carcinus maenas[a]		*Libinia emarginata*[h]		*Eriocheir sinensis*[i]		*Leander squilla*[j]		*Asterias rubens*[k]		*Crenimugil labrosus*[l]		*Paralichthys lethostigma*[l]		*Anguilla anguilla*[m]		Diamond back terrapin[n]	
SW	50% SW	SW	50% SW	SW	FW	SW	30% SW	SW	60% SW	SW	FW	SW	FW	SW	FW	SW	FW
19·3	16·0	26·2	17·9	71·9	18·1	7·9	6·3	1·7	—	3·6	0·8	4·1	0·9	1·4	0·7	2·1	0·4
34·6	32·9	14·4	21·0	54·7	36·5	25·8	24·7	1·3	1·0	0·2	0·1	0·4	0·2	0·2	0·4	0·4	0·1
2·5	2·0	6·0	3·7	11·7	3·6	0	0·8	1·1	0·5	1·2	0·1	1·6	0·1	0·2	0·2	0·1	0·1
31·9	19·9	4·9	2·7	28·2	10·3	0	0	1·4	1·7	1·1	0·3	1·8	0·4	0·9	0·4	3·1	0·2
139·6	75·3	56·1	18·6	108·5	57·0	80·0	57·0	73·0	95·0	11·5	2·5	8·0	1·3	10·9	4·5	3·8	1·2
0	0·2	traces	traces	—	—	0	0	0	0	0·8	0·6	0·5	0·2	0·4	0·3	1·0	1·1
0·7	0·7	0·4	0·3	3·2	1·0	traces	traces	traces	traces	0·2	0·2	0·2	0·2	0·1	0·1	0·2	0·05
0·8	0·9	0·5	0·2	5·4	1·7	traces	traces	traces	traces	0·4	0·4	0·2	0·2	0·4	0·4	0·3	0·07
1·0	2·5	0·8	2·8	18·5	14·3*	0·9	0·7	traces	traces	1·0	1·1	1·1	1·0	0·9	1·0	0·9	0·3
0·3	0·3	0·3	0·2	traces	0	traces	0	traces	traces	0·1	0·1	0·2	0·1	traces	traces	0·1	0·03
59·3	28·3	25·4	2·3	23·7	4·7	29·5	22·6	0	0	0·8	0·3	1·4	0·6	0·6	0·5	0·7	traces
—	—	1·9	1·1	6·3	2·6	5·0	3·5	traces	traces	1·3	0·4	1·9	0·5	0·7	0·6	1·4	0·1
—	—	0·9	0·8	27·7	20·5	24·0	22·4	39·0	22·0	23·3	6·4	28·4	16·5	1·4	1·6	35·2	15·4
1·0	1·0	1·8	—	15·3	4·4	0	traces	traces	traces	0·4	0·3	0·6	0·4	0·5	0·5	traces	0·08
0·2	0·2	0·6	0·2	traces	0	traces	0	traces	traces	0·2	0·1	0·4	0·1	0·8	0·2	0·3	0·07
1·0	1·0	1·4	0·4	6·9	0	traces	traces	traces	traces	0·3	0·2	0·4	0·3	0·2	0·2	1·2	0·8
294·2	182·6	141·5	72·2	382·0	174·7	173·1	138·0	221·0	122·4	46·4	13·9	51·2	23·0	19·6	11·6	51·7	20·00

[i] Bricteux-Grégoire and co-authors (1962); results in mM kg^{-1} tissue water.
[j] Jeuniaux and co-authors (1961a).
[k] Jeuniaux and co-authors (1962).
[l] Lasserre and Gilles (1971); results in mM kg^{-1} tissue water.
[m] Huggins and Colley (1971): results in mM kg^{-1} tissue water.
[n] Gilles-Baillien (1973).

increase in proline concentration the first day during acclimation to a reduced salinity. Such a feature has also been observed in *E. sinensis* (Vincent-Marique and Gilles, 1970a, b). Increase in blood amino-acid content has also been observed in various polychaetes during hypo-osmotic shocks (Clark, 1968). Thus, in various euryhaline invertebrates under hypo-osmotic stress, there occurs, together with a decrease in the tissue amino-acid content, an increase in the blood level of various amino acids as well as an increase in blood ammonia or ammonia excretion. These results indicate the possibility that at least part of the amino acids are released from the tissues and deaminated in a specific organ. That amino acids are not released as such to the environment is indicated by the fact that, in *E. sinensis*, the variation in nitrogen excretion can be totally accounted for by a change in ammonia output (Jeauniaux and Florkin, 1961). Nothing is known about the organ which deaminates the amino acids. In crustaceans, hepatopancreas and the antennal glands appear to be likely candidates for this function. However, in a study of the fate of the proline released by *E. sinensis* into the blood during hypo-osmotic stress, Vincent-Marique and Gilles (*in*: Schoffeniels and Gilles, 1970a) show only important proline oxidase activity in the gill tissue of this crustacean; the activity is particularly high in the posterior gill pairs. It is interesting to recall here that, in *E. sinensis*, the three posterior gill pairs are those responsible for active sodium movement in reduced salinities. Perhaps proline degradation in these branchiae may be related to this phenomenon. Proline oxidation can be a

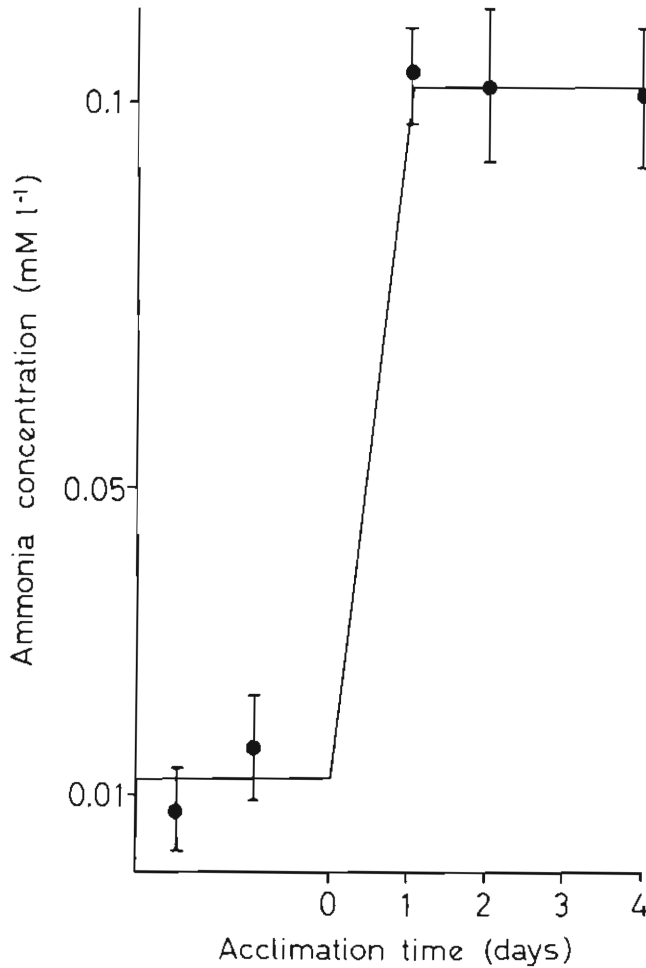

Fig. 5-18: *Callinectes sapidus*. Changes in blood ammonia concentration during re-acclimation from sea water to double-diluted sea water. (Original: drawn after data from GERARD and GILLES, 1972a.)

readily available source of energy (BURSELL, 1966). On the other hand, we have seen that active uptake of sodium probably implicates an exchange with NH_4^+ ions. Amino-acid deamination would provide the gills with the NH_4^+ ions required in this exchange process. Further experiments are needed however to critically assess this interesting problem.

The results discussed show that intracellular-fluid isosmotic regulation is likely to be achieved in a number of ways. Control of amino-acid concentration by regulating their deamination in different tissues and/or in a specific organ following release from other tissues appears, however, to be an important mechanism facilitating cellular osmotic adjustments in tissues of euryhaline invertebrates under osmotic stress.

These conclusions, reached from results obtained on whole animals, have been confirmed and extended by studies at the subindividual level (isolated tissues). GILLES and SCHOFFENIELS (1969a) have studied ammonia and amino-acid patterns in axons of *Eriocheir sinensis* and in their ambient media, following hypo- or hyperosmotic shocks. The results (Tables 5-19 and 5-20) have been recalculated per units dry weight to avoid introduction of changes due to volume modifications which are known to occur during short-term experiments on isolated tissues (Figs 5-14 to 5-16).

Table 5-19

Eriocheir sinensis. Effect of osmotic shocks on the amino-acid concentration in isolated axons. All values expressed in μM 100 mg^{-1} dry weight (Recalculated from GILLES and SCHOFFENIELS, 1969a; reproduced by permission of Microforms International Marketing Corporation)

Amino acid	Hypo-osmotic stress		Hyperosmotic stress	
	Experimentals	Controls	Experimentals	Controls
Taurine	5·38	7·03	6·79	6·25
Aspartic acid	10·25	23·65	18·23	16·22
Threonine	0·87	1·04	0·64	0·49
Serine	3·10	3·34	1·33	1·56
Glutamic acid	2·62	3·03	4·52	3·72
Proline	6·94	17·78	4·73	2·50
Glycine	3·24	3·83	2·22	2·27
Alanine	8·07	12·16	8·81	5·61
Cystine	traces	traces	0·50	traces
Valine	0·52	0·58	0·43	0·40
Methionine	traces	0·10	0·44	0·23
Isoleucine	0·30	0·50	0·33	0·27
Leucine	0·32	0·45	0·42	0·42
Tyrosine	0·21	0·30	0·18	0·15
Phenylalanine	0·24	0·30	0·17	0·15
Lysine	0·42	0·39	0·42	0·46
Histidine	0·49	0·51	0·15	0·15
Arginine	1·45	3·03	3·64	3·78
Ammonia	2·19	10·00	0·71	16·17

After 3 hrs of hypo-osmotic stress, the concentration of the amino acids in the axons has decreased considerably (Table 5-19). In the same way, ammonia concentration sharply decreases while the ammonia level in the incubating medium increases by an amount corresponding to that lost by the tissue (Table 5-20). As far as amino acids are concerned, an increase in concentration can be recorded for some of them in the ambient medium (Table 5-20). However, this is not a general phenomenon; no significant changes have been recorded for alanine, glutamic acid or serine. During hyperosmotic shock, there is a small increase in the total concentration of amino acids in the tissue. This increase pertains mainly to aspartic acid, serine, glutamic acid, proline and alanine. That the decrease in amino-acid concentration during hypo-osmotic stress is much larger than the increase observed during

Table 5-20

Eriocheir sinensis. Effect of osmotic shocks on the amount of amino acids released into the ambient medium by isolated axons. Amounts of amino acids and ammonia released during 3 hrs are expressed in μM 100 mg^{-1} dry weight of axon (Recalculated from GILLES and SCHOFFENIELS, 1969a; reproduced by permission of Microforms International Marketing Corporation)

Amino acid	Hypo-osmotic shock		Hyperosmotic shock	
	Experimentals	Controls	Experimentals	Controls
Taurine	0·83	0·70	0·48	0·48
Aspartic acid	11·17	11·11	8·40	9·04
Threonine	0·18	0·22	traces	traces
Serine	0·83	0·88	0·46	0·47
Glutamic acid	1·43	2·43	2·14	2·50
Proline	19·34	7·93	1·80	0·79
Glycine	1·25	1·09	1·30	1·51
Alanine	5·99	6·06	2·14	2·63
Cystine	traces	traces	traces	traces
Valine	0·38	0·29	0·09	0·26
Methionine	traces	traces	traces	traces
Isoleucine	0·30	0·15	0·29	0·43
Leucine	0·36	0·20	0·38	0·54
Tyrosine	0·22	0·15	0·10	0·22
Phenylalanine	0·27	0·19	0·15	0·25
Ammonia	17·39	8·75	6·92	12·10

hyperosmotic stress is reminiscent of the fact that volume regulation is much faster after hypo-osmotic shock than after a hyperosmotic shock. The different types of cell volume regulation, depending on the osmotic stress the tissue has to cope with, may thus be related, at least partly, to the capacity of the tissue to regulate its amino-acid level more or less rapidly. During hyperosmotic shock, there is a small decrease in the amount of amino acids in the ambient medium. This decrease cannot, however, account for the increase in concentration recorded in the tissue at the level of the so-called non-essential amino acids. Hence, the change in the intracellular concentration of these amino acids cannot be explained solely on the basis of modifications in amino-acid fluxes in or out of the cell. An intracellular mechanism must be at work which regulates the concentration of some amino acids, among them the ones playing a prominent part in cell-volume adjustments. The fact that, during hyperosmotic stress, there is a significant decrease in the ammonia level in both tissue and medium, suggests that this mechanism involves modification of the amination or deamination rate of the amino acids.

The mechanisms controlling the fluxes of amino acids are far from being elucidated. Several studies conducted on isolated tissues have revealed that ninhydrin-positive substances are released into the ambient medium during volume re-adjustment following hypo-osmotic stress. This is the case in *Pleuronectes flesus* erythrocytes (FUGELLI, 1967), *Callinectes sapidus* muscle fibres (LANG and GAINER, 1969) and in isolated heart muscle of *Modiolus modiolus* (PIERCE and GREENBERG,

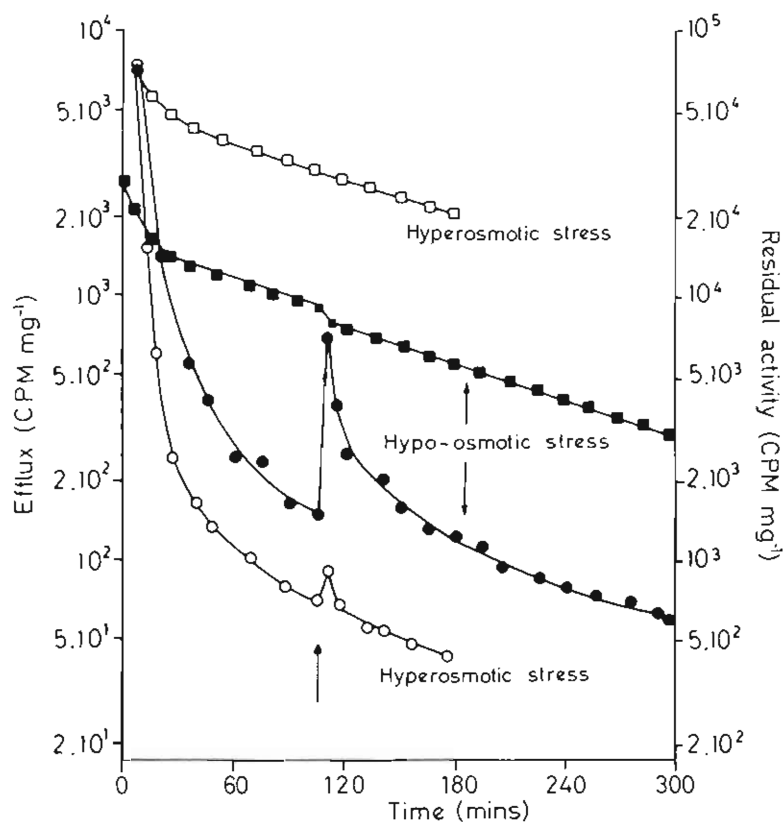

Fig. 5-19: *Callinectes sapidus*. Effect of hypo-osmotic and hyperosmotic stresses on the residual activity (squares) and efflux (circles) of radio-active material from isolated axons preloaded with alanine-U-^{14}C. (After GERARD and GILLES, 1972b; modified; reproduced by permission of Experientia.)

1970). In preliminary experiments, we have followed the outflux of radio-active material from isolated axons of *C. sapidus*, loaded with labelled alanine and submitted to hypo- or hyperosmotic stresses. The results obtained are presented in Fig. 5-19. During hypo-osmotic stress, there is an important increase in the efflux of radio-active material immediately after stress application. Subsequently, the outflux progressively slows down and returns to values only slightly above the controls. This is best shown when considering the half-renewal times calculated from the slope of the residual-activity curve corresponding to the intracellular compartment of the axon: the period is 135 mins during hypo-osmotic shock and 610 mins for the controls. As discussed elsewhere (GERARD and co-authors, 1973), the large transitory increase in outflux observed immediately after application of osmotic stress can probably be related, at least partly, to a modification of the extracellular space due to changes in axonal volume.

During hyperosmotic stress, on the other hand, the results illustrated in Fig. 5-19 reveal no significant change in the efflux. The increase in amino-acid concentration

observed under these conditions, therefore, must be accounted for by some other mechanism. On the basis of changes in blood proteins and amino acids during acclimation of *Carcinus maenas* to concentrated media, SIEBERS and co-authors (1972) have suggested a transport of amino acids from haemolymph into the cells. When studying rates of incorporation of ^{14}C leucine into proteins of *Artemia salina* nauplii, CONTE and co-authors (1973) showed that uptake of leucine depends on salinity when leucine $5 \cdot 10^{-7}$ M is present in the external medium. Such dependency disappears for leucine concentrations higher than 10^{-4} M. The effect of salinity on amino-acid uptake may thus be dependent upon the blood concentration of the amino acid transported. Whether or not modifications of influx may be part of the mechanism of regulation of intracellular amino-acid concentration remains to be demonstrated. Studies on inward movements and active transport of amino acids following osmotic shock are needed to illuminate this problem.

Table 5-21

Orconectes limosus. Concentration (mg 20 g^{-1} of animal) of various substances in fresh water and after 10 days acclimation to a salinity of 650 mOsm l^{-1} (After SIEBERS, 1972; modified; reproduced by permission of Springer-Verlag, Berlin)

Substance	Fresh water	650 mOsm l^{-1}
Inorganic ions	99	170
Amino acids	62 ± 6	107 ± 10
Neutral sugars	23 ± 15	50 ± 16
Organic acids	85	117
Proteins	960 ± 68	909 ± 54
Lipids	149 ± 10	150 ± 12
Polysaccharides	4 ± 3	6 ± 3

Nevertheless, increase in amino-acid content of isolated tissues, incubated in hyperosmotic media, which did not initially contain amino acids, favours the idea of an intracellular mechanism of isosmotic regulation (SCHOFFENIELS, 1960b; GILLES and SCHOFFENIELS, 1969a; BEDFORD, 1971; see also p. 315).

We have already pointed out that a mechanism involving changes in intracellular metabolism appears to be at work in the control of the concentration of various amino acids. Such an intracellular mechanism may implicate control of the steady state between amino acids and proteins and/or control of synthesis-degradation rates of amino acids. We have seen that there are important modifications in ammonia excretion and blood concentration in euryhaline animals exposed to osmotic stress. This can be considered as an argument in favour of a mechanism controlling rates of synthesis or degradation of amino acids. However, this does not rule out the possibility of a regulation of the steady state between proteins and amino acids. Another possibility which could account for the changes in ammonia concentration is that the amino acids are deaminated to their keto-precursors which are then stored in the form of high-molecular-weight compounds.

Such a mechanism could be involved during hypo-osmotic stress, the reverse process occurring during hyperosmotic stress.

SIEBERS (1972) studied the fate of various compounds during intracellular-fluid isosmotic regulation in *Orconectes limosus* (Table 5-21). Differences in concentration between individuals acclimated for 10 days to fresh water or to a medium of 650 mOsm l^{-1} were mainly found at the level of low-molecular-weight organic compounds. No significant variations could be observed in total lipids, polysaccharides or proteins. This would provide an argument in favour of the view that the changes occurring in the amino-acid pool are not related to modifications in the equilibrium between them or their ketoprecursors and proteins, or other high-molecular-weight compounds. However, SIEBERS studied whole animals; hence it is possible that changes in high-molecular-weight compounds could have occurred at the level of specific organs.

Lipid composition is known to be quite different in marine and freshwater crustaceans. Oil extracted from marine species contains a relatively high proportion of long-chain, polyunsaturated fatty acids, whereas the lipid of freshwater forms characteristically yields a relatively larger quantity of saturated ^{16}C and ^{18}C fatty acids (O'CONNOR and GILBERT, 1968). Whether such a difference could be of significance in intracellular-fluid isosmotic regulation in a euryhaline animal under osmotic stress remains to be seen. Such a switch from saturated to unsaturated fatty acid, if it exists in euryhaline animals, may be of interest for meeting energy requirements under osmotic stress. Anyway, it is likely, from the results of SIEBERS (1972) that lipids do not directly participate in the regulation of the amino-acid level occurring during cell-volume adjustment in euryhaline animals.

The same appears to be true as far as the equilibrium amino acids versus proteins is concerned, since no significant changes in protein concentration can be observed on whole *Orconectes limosus* (Table 5-21). Similar results have been reported by GILLES and SCHOFFENIELS (1969a) who measured protein concentration in isolated surviving axons of *Eriocheir sinensis* submitted to osmotic stress. This view is also supported by results obtained when measuring the variation in free amino acids before and after hydrolysis of proteins. Table 5-22 shows that when *E. sinensis* is re-acclimated from fresh water to sea water, increase in free alanine is accompanied

Table 5-22

Eriocheir sinensis. Changes in free and total alanine and proline in muscle after 6 days re-acclimation from fresh water to sea water. All values expressed as mg amino acid 100 g^{-1} dry muscle (After FLORKIN and SCHOFFENIELS, 1969; modified; reproduced by permission of Academic Press)

Crabs	Amino acid	Free amino acid			Total amino acid		
		Fresh water	Sea water	Variation	Fresh water	Sea water	Variation
1	Alanine	1764	2899	+ 1135	5582	6777	+ 1195
3		1794	3183	+ 1389	5633	6594	+ 961
1	Proline	1966	2575	+ 609	4451	4614	+ 163
2		953	1755	+ 802	3783	4531	+ 748
3		718	1446	+ 728	3707	4569	+ 862

by a parallel increase in total alanine. While free proline increases markedly in the muscle, the amount of total proline increases proportionally. This indicates that the amount of proline or alanine obtained from protein hydrolysis does not vary significantly. Similarly, BEDFORD (1971) showed that the increase in amino-nitrogen occurring in isolated foot muscle of the mollusc *Melanopsis trifasciata* submitted to hyperosmotic stress, is paralleled by an increase in total nitrogen. This also provides an argument that the increase in amino acids may not be related to a decrease in other intracellular nitrogenous compounds.

The possibility of modifications in synthesis and/or degradation rates of amino acids has been investigated in some detail at the individual and subindividual levels.

MUNDAY and his colleagues have studied salinity effects on the incorporation of various labelled amino acids and of labelled acetate into the soluble and insoluble fractions of several tissues of *Carcinus maenas* either *in vivo* after injection of the crab with the labelled compound, or *in vitro* after incubation of the tissue in a medium containing the labelled substrate. The results obtained have been reviewed recently by CHAPLIN and co-authors (1970); in regard to whole animals, they are difficult to interpret because the variations in the percentage of total radiocarbon recovered depend on the time elapsed between injection and the extraction of the tissue. However, the results suggest a more rapid turnover rate of the labelled marker into the metabolic pools of various compounds in the soluble fraction at lower salinity.

The experiments *in vitro* are more easily interpreted: the proportion of the radioactivity recovered from labelled glutamate has been estimated at the level of several Krebs cycle intermediates (malic acid, α-ketoglutaric acid, succinic acid) and at the level of one amino acid (alanine). Values are also given for a compound (U_8) unknown at the time, which has since been identified as 2-hydroxyglutarate

Table 5-23

Carcinus maenas. Distribution of ^{14}C and U-^{14}C glutamic acid in leg muscle tissue of individuals acclimated to 100% sea water or to 40% sea water. After a 48-hr acclimation the muscle tissue was removed and incubated for 3 hrs with 1 μ C. (U-^{14}C)-glutamate. Values are counts per 100 sec per 50 mg tissue; ± standard error of the mean of 7 observations (After CHAPLIN and co-authors, 1970; modified; reproduced by permission of Scientechnica)

Substance	Sea water 100%	Sea water 40%
Glutamate	26,010 ± 926	15,388 ± 1,412
Glutamine	875 ± 291	312 ± 78
Alanine	2,120 ± 302	3,503 ± 437
2-Hydroxyglutarate (U_8)	11,424 ± 1,044	11,998 ± 1,316
Malate	1,361 ± 241	1,834 ± 468
α-Ketoglutarate	4,741 ± 507	1,282 ± 253
Succinate	2,471 ± 202	3,845 ± 435
Total less glutamate	19,992	22,775
Utilization (in %)	57·4 ± 2·9	74·8 ± 3·1

(HUGGINS and BOULTON, 1971). Values obtained for leg muscle after 3 hrs of incubation are shown in Table 5-23. They demonstrate clearly that muscle, extracted from a crab acclimated to 40% sea water, utilizes glutamic acid faster than the same tissue isolated from a 100% sea-water crab. Except for 2-hydroxyglutarate, the proportion of radio-activity recovered on the isolated compounds is lower in the case of sea-water *Carcinus maenas*. However, since these experiments deal only with recovered radio-activity, it is difficult to assess the mechanism responsible for the changes in radio-activity.

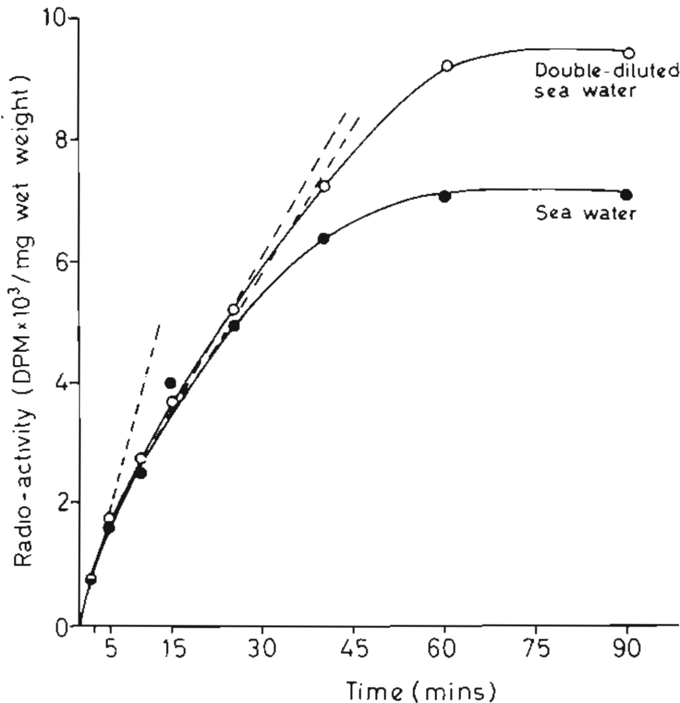

Fig. 5-20: *Callinectes sapidus*. Uptake of (U-^{14}C) glucose by isolated axons in sea water and in double-diluted sea water. (Original.)

Recently we have studied salinity effects on incorporation of ^{14}C from labelled glucose into amino acids of isolated surviving axons of *Callinectes sapidus*. Before starting the study, the possibility of changes in the influx rate of the marker has been considered. As shown in Fig. 5-20, the salinity is without significant effect on the rate of entrance of labelled glucose into isolated axons of *C. sapidus*. The steady state achieved after about 60 mins is at a higher level in axons incubated in reduced salinities as compared to the sea-water controls. This may indicate higher utilization of glucose by the tissue in the lower salinity (see also p. 325).

Hypo-osmotic stress leads to a decrease in the concentration of all the measured amino acids. At the same time, there occurs an increase in specific activity which can be calculated for the amino acids labelled from glucose. This increase may be interpreted as the result of an increased synthesis of amino acids or as indicating a

Table 5-24

Callinectes sapidus. Effect of osmotic stress on concentration, radio-activity and specific activity of amino acids in isolated axons of crabs incubated with (U-^{14}C)-glucose. Concentrations are given in μM 100 mg^{-1} dry weight, radio-activities in CPM 100 mg^{-1} dry weight, and specific activities in CPM μM^{-1} amino acid (Original)

Amino acid	Hypo-osmotic stress						Hyperosmotic stress					
	Experimentals			Controls			Experimentals			Controls		
	Concen-tration	Activity	Specific activity	Concen-tration	Activity	Specific activity	Concen-tration	Activity	Specific activity	Concen-tration	Activity	Specific activity
Taurine	6·37	—	—	10·16	—	—	11·64	—	—	9·35	—	—
Aspartic acid*	61·25	40,289·86	—	148·05	47,606·54	—	93·06	80,729·28	—	86·23	45,706·47	—
Serine	·94	23,419·80	24,783	1·82	33,115·73	18,185	2·27	29,230·62	12,871	1·35	14,358·26	10,604
Glutamic acid	9·55	56,808·62	5,945	17·83	43,641·19	2,448	11·64	57,101·36	4,906	10·39	45,783·87	4,408
Proline	4·04	—	—	16·09	—	—	2·04	—	—	traces	—	—
Glycine	3·36	—	—	6·45	—	—	5·14	—	—	3·85	—	—
Alanine	6·37	147,732·10	23,192	14·79	173,478·61	11,722	9·77	275,729·27	28,202	4·47	139,944·62	31,280
Valine	traces			·15	—	—	·54			·41	—	—
Methionine	traces			traces			traces	—	—	traces	—	—
Isoleucine	traces			traces			·22	—	—	·16	—	—
Leucine	traces			traces			·45	—	—	·37	—	—

* Aspartic acid plus an undetermined compound.

fast removal of amino acids from the metabolic pool at the beginning of the experiment. Under these conditions, synthesis from glucose would occur in a pool of smaller size, thus leading to increased specific activity. Such a mechanism would, moreover, account for the decrease in the concentration of all the amino acids determined, not only those which can be synthesized from glucose.

During hyperosmotic shock, the radio-activity and the concentration of the amino acids increase (Table 5-24). On the other hand, no significant change can be recorded in specific activity. These results can be interpreted in terms of a decreased release rate in the presence of continuous entering of amino acids into the metabolic pool. It is unlikely that increased synthesis can account for the increased amino-acid level, since this would lead to increase in specific activity. These results thus demonstrate that possible changes in synthesis rate do not play an important role in the regulation of the amino-acid pool. Control of the intracellular amino-acid level appears to be achieved mainly by regulation of the removal of amino acids from the metabolic pool.

Release of amino acids from the metabolic pool can be regulated either by controlling outflux or degradation. Control of outward movement of amino acids occurs during hypo-osmotic stress (p. 317); however, this process can only account for increased removal of amino acids from the metabolic pool and not for decreased removal during hyperosmotic stress. Moreover, we have provided evidence indicating that even in the case of hypo-osmotic shock, an active metabolic process effects the large quantitative variations in different amino acids. It appears that this metabolic process mainly implicates a control of the degradation rate of the amino acids. In order to test this hypothesis, we have studied the effect of osmotic stress on $^{14}CO_2$ production by isolated axons preloaded with various U-^{14}C amino acids (Table 5-25).

The results obtained show that isolated axons can oxidize arginine and leucine to CO_2, although these amino acids are not known to be synthesized by crustaceans (SCHOFFENIELS and GILLES, 1970b). However, it is well known that various essential amino acids can be degradated by peripheral tissues in a variety of organisms (MUNRO, 1970).

During hypo-osmotic stress, there is increased $^{14}CO_2$ production from the ^{14}C-amino acids. At the same time, oxygen consumption and CO_2 production also increase.

These findings indicate an increased amino-acid catabolism in isolated axons under hypo-osmotic stress. When the tissues are submitted to hyperosmotic stress the catabolic activity decreases as indicated by the decrease in $^{14}CO_2$ production. During these experiments no significant changes in the specific activity calculated for CO_2 could be recorded. This suggests that the changes in $^{14}CO_2$ production are not due to a mechanism controlling the activity of degradation of some specific amino acids, but rather to a general modification of their oxidative metabolism. Such a conclusion is supported by the changes in oxygen consumption and in total CO_2 production. Another argument in favour of this conclusion is the increase in $^{14}CO_2$ production from labelled glucose or pyruvate observed in isolated axons of *Callinectes sapidus* when submitted to hypo-osmotic shock (GILLES, unpublished). Also interesting in this context is that CO_2 production from labelled glucose or pyruvate is much higher in the nerve cord of the limnic *Astacus fluviatilis* than in

Table 5-25

Callinectes sapidus. Effect of osmotic stress on CO_2 production, oxygen consumption and $^{14}CO_2$ production of isolated axons of crabs incubated with various labelled amino acids. SW: sea water; SW/2: double-diluted sea water; dw: dry weight (After GILLES, 1972b; modified; reproduced by permission of Pergamon Press Ltd.)

(U-^{14}C) Amino acid	Hypo-osmotic stress								Hyperosmotic stress							
	$^{14}CO_2$ CPM hr^{-1} mg^{-1} dw		QO_2 μl hr^{-1} mg^{-1} dw		QCO_2 μl hr^{-1} mg^{-1} dw		CO_2 specific activity CPM μM^{-1} CO_2		$^{14}CO_2$ CPM hr^{-1} mg^{-1} dw		QO_2 μl hr^{-1} mg^{-1} dw		QCO_2 μl hr^{-1} mg^{-1} dw		CO_2 specific activity CPM μM^{-1} CO_2	
	SW	SW/2	SW	SW/2	SW	SW/2	SW	SW/2	SW/2	SW	SW/2	SW	SW/2	SW	SW/2	SW
Glutamate	85·91	113·08	5·56	6·38	4·16	4·86	462·56	521·25	114·03	75·25	2·65	1·92	2·05	1·43	1245·88	1179·80
Arginine	36·31	44·81	2·16	2·42	1·62	1·91	501·98	525·60	32·67	23·87	2·35	1·60	1·84	1·20	397·60	445·53
Leucine	17·76	21·13	2·77	3·46	2·09	2·62	190·17	180·54	17·50	14·34	2·53	1·71	2·04	1·30	192·19	247·07
Alanine	371·11	595·96	4·88	10·38	3·86	7·96	2152·60	1677·70	192·94	140·02	2·89	2·09	2·17	1·53	1991·58	2050·04
Aspartate	165·40	219·35	4·08	5·62	3·00	4·30	1234·91	1142·62	223·74	179·81	4·06	3·34	2·92	2·58	1716·28	1561·05
Serine	31·76	51·18	3·60	5·35	2·68	4·10	265·44	279·55	127·61	84·31	3·34	1·98	2·59	1·49	1103·65	1267·39

the marine *Homarus gammarus* (GILLES and SCHOFFENIELS, 1969b). This may be indicative of faster oxidative metabolism in species living in waters of reduced salinities.

The responses of isolated axons of *Callinectes sapidus* thus suggest that changes in oxidative metabolic activity are implicated in the control of the amino-acid level during osmotic stress. That such a mechanism is also effective in whole organisms is indicated by the results of SIEBERS (1972), who showed a decrease in CO_2 production in *Orconectes limosus* during acclimation to sea water. According to SIEBERS, not only the concentration of amino acids, which is reduced in fresh water, but also the level of other low-molecular-weight compounds such as carbohydrates or organic acids, are related to the intermediary metabolism.

Similarly, the conversion of radiocarbon from glutamic acid to $^{14}CO_2$ increases by 50% in tissues of *Carcinus maenas* acclimated to 40% sea water, compared with those in 100% sea water (HUGGINS and BOULTON *in*: CHAPLIN and co-authors, 1970). These findings suggest that oxidative metabolism is higher in low salinities.

Modification in catabolic activity during osmotic stress is also shown by redox changes in respiratory-chain components. GILLES and JÖBSIS (1972) found changes in the redox state of pyridine nucleotides (NADH) of isolated muscle of *Callinectes sapidus* submitted to osmotic stress (Figs 5-21 and 5-22). They show that during osmotic stress, there is no specific change in the spectrum of respiratory-chain components in the range 400 to 640 mμ, except for a general drift in the baseline. This drift can be associated with light scattering due to changes in fibre volume. However, there is a specific modification in the pyridine nucleotides oxido-reduction level as indicated by extinction changes in the range 330 to 390 mμ. These changes are reversible; if a muscle preparation is submitted to hypo-osmotic stress, the pyridine nucleotides are reduced whereas they are oxidized when the tissue is placed back in the control medium (Fig. 5-21). On the other hand, during hyperosmotic

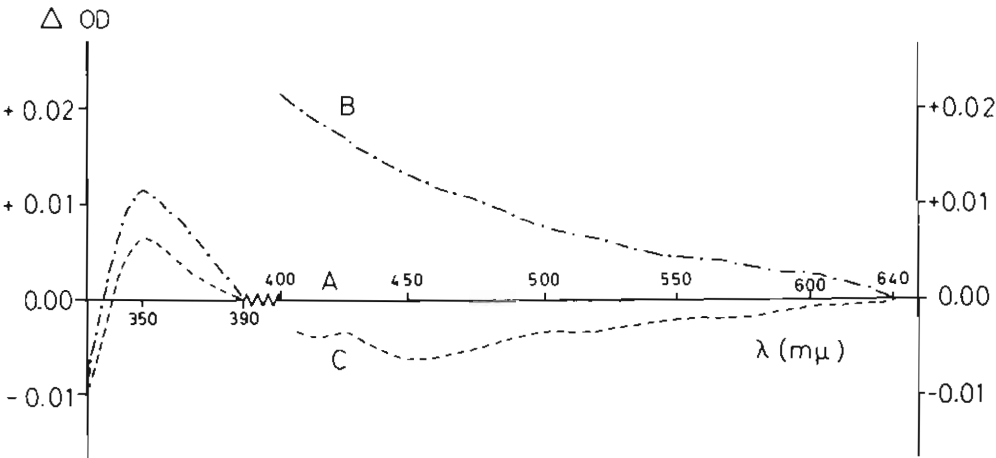

Fig. 5-21: *Callinectes sapidus*. Effect of hypo-osmotic stress on oxidoreduction of respiratory-chain components in isolated muscle fibres. A: baseline (control conditions); B: hypo-osmotic stress; C: return to control conditions. (After GILLES and JÖBSIS, 1972, modified; reproduced by permission of Pergamon Press Ltd.)

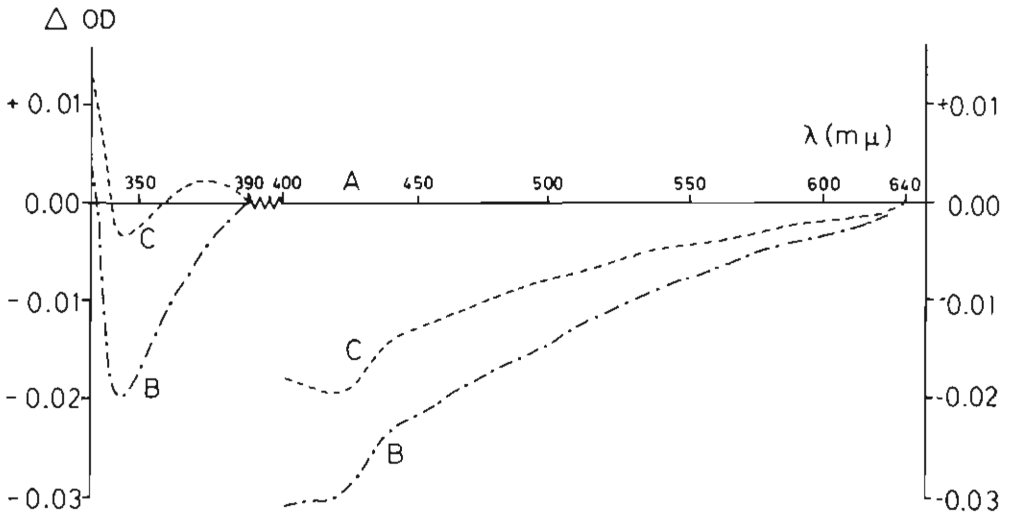

Fig. 5-22: *Callinectes sapidus*. Effect of hyperosmotic stress on oxidoreduction level of respiratory-chain components of isolated muscle fibres. A: baseline (control conditions); B: hyperosmotic stress; C: return to control conditions. (After GILLES and JÖBSIS, 1972; modified; reproduced by permission of Pergamon Press Ltd.)

stress, there is an oxidation of NADH. When the tissue is returned to control conditions, the pyridine nucleotides become reduced again and resume the original redox state (Fig. 5-22). These changes in the NADH oxidoreduction level during osmotic stress are consistent with the changes in metabolic activity during cellular osmotic adjustment. During hypo-osmotic shock, the amino-acid concentration decreases; this can be associated with an increase in the activity of the amino-acid degradation pathways. Such increase leads to the formation of reducing equivalents which can account for the reduction of pyridine-nucleotides. During hyperosmotic stress, the increase in amino-acid concentration can be related to a decrease in their degradation. This causes decreased CO_2 production and a decrease in O_2 consumption. Such a phenomenon may well account for decreased production of reducing equivalents which would be responsible for oxidation of pyridine nucleotides.

The results discussed point to a basic relationship between changes in the level of organic osmotic effectors and changes in oxygen consumption during osmotic stress. According to the proposed scheme, oxygen consumption should decrease in animals withstanding hyperosmotic stress and increase in low salinities. To our knowledge, this is true in most cases where the animal has been studied during the acclimation period (POTTS and PARRY, 1964; SCHOFFENIELS and GILLES, 1970a; GILLES, 1972c; SIEBERS and co-authors, 1972), although various studies show that many species display different relationships once acclimated to the new medium (KUENEN, 1939; ELIASSEN, 1953; GILCHRIST, 1956; Volume I, Chapter 4).

What are the primary causes of the changes in metabolic activity during acclimation to different salinities?

It is evident from experiments on isolated tissues that hormonal control is not primarily implicated in the regulation of the cellular osmotic pressure. Support for

this view has also been provided by DUCHÂTEAU and FLORKIN (1962), who showed that removal of the eyestalk gland does not alter isosmotic regulation in *Eriocheir sinensis* exposed to osmotic stress.

On the other hand, increase in saline osmolarity by addition of sucrose causes a decrease in amino-nitrogen level of isolated axons of *Eriocheir sinensis* instead of the slight increase recorded when increase in osmolarity is achieved by increasing the ionic concentration (SCHOFFENIELS, 1960b). Similarly, incubation of isolated foot muscle of *Melanopsis trifasciata* in media of increasing salt concentration, but of constant osmotic pressure, induces an increase in the intracellular amino-nitrogen content (BEDFORD, 1971). These findings indicate that it is not the osmotic pressure *per se* which is responsible for regulating the amino-acid level during adjustment to high salinities. This conclusion is substantiated by experiments which show that veratrine—a modifier of the intracellular ionic composition—affects amino-acid metabolism as indicated by the increase in incorporation of ^{14}C from glucose into various amino acids (GILLES and SCHOFFENIELS, 1964). It can be suggested, therefore, that it is the change in ionic concentration, to which the cell is submitted during acclimation, which controls the mechanism responsible for amino-acid concentrations.

Ionic composition may control either the level or the activity of key enzymes implicated in the metabolic pathways. As to the first possibility, CHAPLIN and co-authors (1965) have not been able to demonstrate a significant difference in the total glutamate dehydrogenase activity between tissues of *Carcinus maenas* acclimated to 50% sea water and to 100% sea water. While this seems to indicate that the change in amino-acid concentration is not caused by changes in key-enzyme concentrations, REDDY and co-authors (personal communication) found changes in the level of alanine and aspartate amino-transferase in aquatic gastropods adjusting to different salinities. Hence more information is needed before definite conclusions can be drawn as to the possibility of a regulation at the enzyme level during osmotic adjustment in euryhaline animals.

As to the possibility of enzyme-activity control, the ionic composition of the incubation medium can affect the amino-acid metabolism either directly or indirectly through an intermediary substance. The latter relates to the kind of mechanism generally used to explain hormonal action, postulating the production of an intermediary effector such as 3′, 5′-AMP. So far no attempt has been made to test such a possibility. Up to now, most studies have been concerned with the direct effect of the ionic composition on the activity of various enzymatic systems implicated in the amino-acid metabolism.

The degradation of most amino acids can be summarized in two important sequences (Fig. 5-23). The first is the deamination of the amino acids to their ketoprecursors. The second is the oxidation to CO_2 of the ketoprecursors.

The amino group resulting from the deamination process can be stored in the form of a 'carrier' compound (glutamine–alanine) or can be released in the form of free ammonia. The changes observed in the level of ammonia released from isolated tissues or whole organisms when submitted to osmotic shocks point to the importance of this last system in the osmoregulation process. Besides oxidation, transamination constitutes an important pathway of ammonia liberation from many amino acids (Fig. 5-23). The effect of ion concentration on activity of enzymes implicated

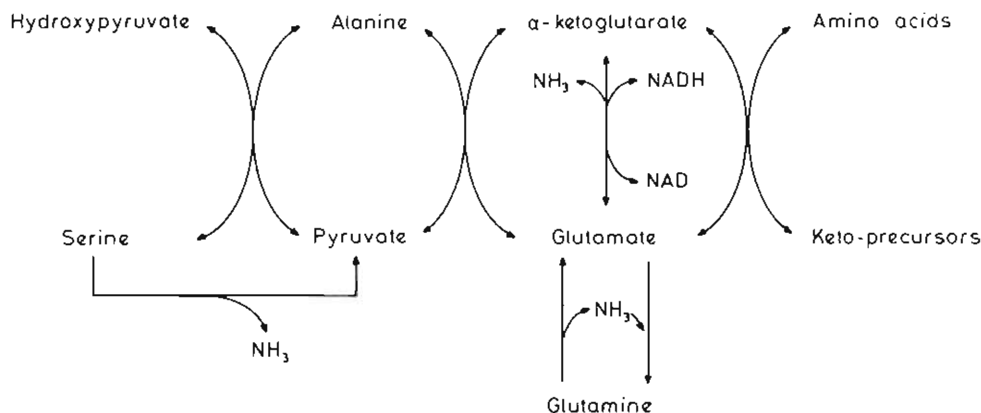

Fig. 5-23: Main pathways of ammonia disposal in the amino-acid metabolism. (After GILLES, 1969; redrawn; reproduced by permission of Archives internationales de Physiologie et de Biochemie.)

in the deamination of amino acids as well as in the Krebs cycle has therefore been studied. Since intracellular concentration of chloride is primarily affected by osmotic stress (see Table 5-17), the influence of NaCl on enzyme activity has been first examined in muscle preparations from stenohaline and euryhaline animals (SCHOFFENIELS and GILLES, 1963, 1970a; FLORKIN and SCHOFFENIELS, 1969; GILLES and GILLES-BAILLIEN, 1974). The results obtained are briefly summarized in Table 5-26. Increasing amounts of NaCl in the incubating medium causes a decrease in the activity of isocitrate dehydrogenase, malate dehydrogenase and oxaloacetate decarboxylase (Table 5-26). This indicates a decreased activity of the Krebs cycle and its anaplerotic pathways with increasing NaCl concentration. Such interpretation is in agreement with the inhibition of the cycle observed when incubating different tissues in the presence of high NaCl concentrations or under conditions inducing a modification of the intracellular ionic content (TUSTANOFF and STEWART, 1965; GILLES and SCHOFFENIELS, 1968a, b; HUGGINS and MUNDAY, 1968). Such interpretation is also in line with the decreased oxygen consumption and CO_2 production of isolated axons of *Callinectes sapidus* when submitted to hyperosmotic stress (p. 323).

As far as the enzymes of the deamination process are concerned, glutamate dehydrogenase activity increases with increasing NaCl concentration up to 400 mM. On the other hand, the activity of serine hydrolyase decreases while the activity of aspartate aminotransferase is not affected. This last observation confirms results of HUGGINS and MUNDAY (1968) who, moreover, showed that the activity of alanine aminotransferase of *Carcinus maenas* tissues remains also unaffected by an increase in NaCl concentration. It can be tentatively concluded, therefore, that increase in ionic level induces augmented glutamate formation. Under the same conditions of increased ionic concentration, serine hydrolase activity is inhibited, thus leading to reduced deaminating activity. In turn, the increased amount of glutamate induces increase in the amount of the other amino acids, the activity of the aminotransferases being unaffected (Fig. 5-22). This concept is in agreement with observations showing an increase in the free amino-acid pool and

Table 5-26

Effect of NaCl on the activity of various enzymes extracted from muscles of the lobster *Homarus gammarus* and the crayfish *Astacus fluviatilis*. Values are percentages of control activity (100%) (Compiled from the sources indicated)

Enzyme	Animal	NaCl concentration (mM)							Author
		0	50	100	200	300	400	600	
Glutamate dehydrogenase	Lobster	100	130	160	180	—	250	160	SCHOFFENIELS and GILLES (1970a)
(EC 1.4.1.2)	Crayfish	100	150	230	270	—	310	220	
Aspartate aminotransferase	Lobster	100	95	108	100	—	95	—	GILLES (1969)
(EC 2.6.1.1)	Crayfish	100	100	100	95	—	102		
Serine hydrolyase (EC 4.2.1.13)	Lobster	100	74	36	12	—	—	—	GILLES (1969)
	Crayfish	100	63	38	3	—	—	—	
Aspartate decarboxylase	Lobster	100	—	—	99	—	104	—	GILLES and SCHOFFENIELS (1966)
(EC 4.1.1.11)	Crayfish	100	—	—	95	—	86	—	
3-Glycerophosphate dehydrogenase	Lobster	100	—	145	117	93	50	33	SCHOFFENIELS (1968)
(EC 1.1.1.8)	Crayfish	100	—	366	315	217	195	116	
Lactic dehydrogenase	Lobster	100	—	58	12	5·1	4·1	5·1	SCHOFFENIELS (1968)
(EC 1.1.1.27)	Crayfish	100	94	66	47	34	28	—	
Pyruvate (5×10^{-4}M)	Lobster	100	125	125	125	125	125	—	SCHOFFENIELS (1968)
Pyruvate (10^{-2}M)	Crayfish	100	100	100	78	78	68	—	
Malate dehydrogenase	Lobster	100	115	113	105	—	78	—	GILLES (1969)
(EC 1.1.1.37)	Crayfish	100	103	112	83	—	—	—	
Malate hydrolyase (EC 4.2.1.2)	Lobster	100	142	117	70	—	—	—	GILLES (1969)
	Crayfish	100	74	57	30	—	—	—	
Isocitrate dehydrogenase	Lobster	100	83	75	52	—	25	—	GILLES (1969)
(EC 1.1.1.42)	Crayfish	100	87	73	63	—	50	29	
Malate dehydrogenase (EC 1.1.1.40)	Lobster	100	80	69	59	—	48	16	GILLES (1969)
	Crayfish	100	68	58	51	—	43	31	
Oxalo-acetate decarboxylase	Lobster	100	98	95	89	—	85	80	GILLES and SCHOFFENIELS (1966)
(EC 4.1.1.3)	Crayfish	100	96	86	72	—	65	58	
Glyoxylate reductase (EC 1.1.1.26)	Lobster	100	—	42	24	15	10	6	SCHOFFENIELS (1968)
	Crayfish	100	—	30	15	7	6	3	

a decrease in ammonia production during acclimation of euryhaline animals to high salinities.

There is no difference in the effect of NaCl on the activity of these enzymes whether they are extracted from the muscle of the crayfish *Astacus fluviatilis* or of the lobster *Homarus gammarus*. It seems, however, that the stenohalinity of *H. gammarus* is in some way related to its inability to regulate the concentration of its intracellular osmotic effectors. In contrast to the situation encountered with euryhaline species there is no volume regulation of muscle tissue under hypo-osmotic stress (p. 306). When studying substances inducing modification in the intracellular ionic content we have shown that compounds, such as veratrine, induce increased labelling of amino acids from radio-active glucose in isolated nerve cord of *H. gammarus* (GILLES and SCHOFFENIELS, 1968b). This parallels the increased labelling found in the amino acids of *Callinectes sapidus* axons when submitted to hyperosmotic shock. It must be noted, however, that in *C. sapidus* axons, this increase in labelling is accompanied by a concomitant increase in amino-acid concentration. On the other hand, veratrine, although it induces increased amino-acid labelling, is unable to promote a concentration increase in isolated nerve cord of *H. gammarus*. This is at variance with its effect on the same tissue isolated from the euryhaline crayfish *A. fluviatilis*, where an increase in the amount of most amino acids can be recorded (Table 5-27).

These results indicate that, in euryhaline species, modification of the intracellular ionic content causes impairment between anabolism and catabolism of amino acids; in stenohaline species, there would be a stimulation of both processes thus leading

Table 5-27

Effect of veratrine sulphate (4 μM) on amino-acid concentration (μM 100 mg^{-1} wet weight) in the nerve cord of the lobster *Homarus gammarus* and the crayfish *Astacus fluviatilis* (After GILLES and SCHOFFENIELS, 1968b; modified; reproduced by permission of Archives internationales de Physiologie et de Biochemie)

Amino acid	*Homarus gammarus*		*Astacus fluviatilis*	
	Control	Veratrine	Control	Veratrine
Taurine	1·845	1·843	0·047	0.068
Aspartic acid	3·281	3·438	0·510	0·379
Threonine	—	—	0·045	traces
Serine	0·563	0·556	0·241	0·242
Glutamic acid	0·796	0·723	0·209	0·292
Proline	1·372	1·297	0·064	0·098
Glycine	2·148	2·038	0·145	0·192
Alanine	1·125	1·146	0·239	0·393
Cystine	0·102	0·089	—	—
Valine	—	—	0·032	0·040
Methionine	0·020	0·021	—	—
Isoleucine	0·046	0·044	0·021	0·027
Leucine	0·052	0·057	0·020	0·034
Tyrosine	0·056	0·059	0·026	0·035
Phenylalanine	0·039	0·039	0·023	0·027

to increased radio-activity but not to a net increase in amino-acid concentration. In this respect, it is interesting to consider that at low NaCl concentrations (0–50 mM) differences exist between the effect of increasing NaCl concentration on the activity of serine hydrolyase, depending on whether it is extracted from tissues of the euryhaline crayfish *Astacus fluviatilis* or of the stenohaline lobster *Homarus gammarus*. In lobster tissues, these different effects may account for increased amino-acid turnover without changes in concentration since the activity of both the aminating enzyme (glutamate dehydrogenase) and the deaminating enzyme (serine hydrolyase) are activated by increase in NaCl concentration (Fig. 5-26). In a euryhaline species the decrease in serine hydrolyase activity together with the increase in glutamate dehydrogenase activity would account for the impairment between anabolism and catabolism which leads to the increase in amino-acid concentration. Differences are also observed when considering the effect of NaCl on the activity of lactic dehydrogenase and 3-glycerophosphate dehydrogenase (Table 5-26). These enzymes are important among those controlling extramitochondrial ratio NADH/NAD and transfer of reducing equivalent, originating in the cytoplasm, to the mitochondrial respiratory chain. A difference between euryhaline and stenohaline species seems, therefore, to be related to the control of reducing equivalents. This problem has not been studied further.

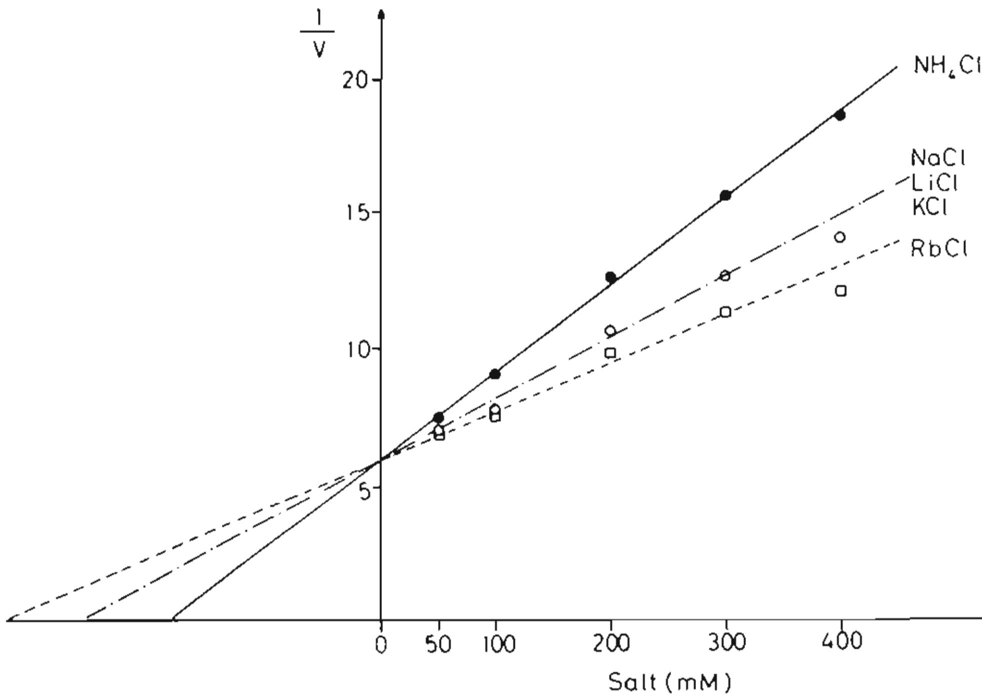

Fig. 5-24: *Mytilus californianus*. Effect of various cationic species on the activity of mantle tissue succinate dehydrogenase. Reaction velocity (v) is expressed in μM succinate min^{-1} mg^{-1} protein. Cl^- is used as the anionic species. (After GILLES and co-authors, 1971; modified; reproduced by permission of Pergamon Press Ltd.)

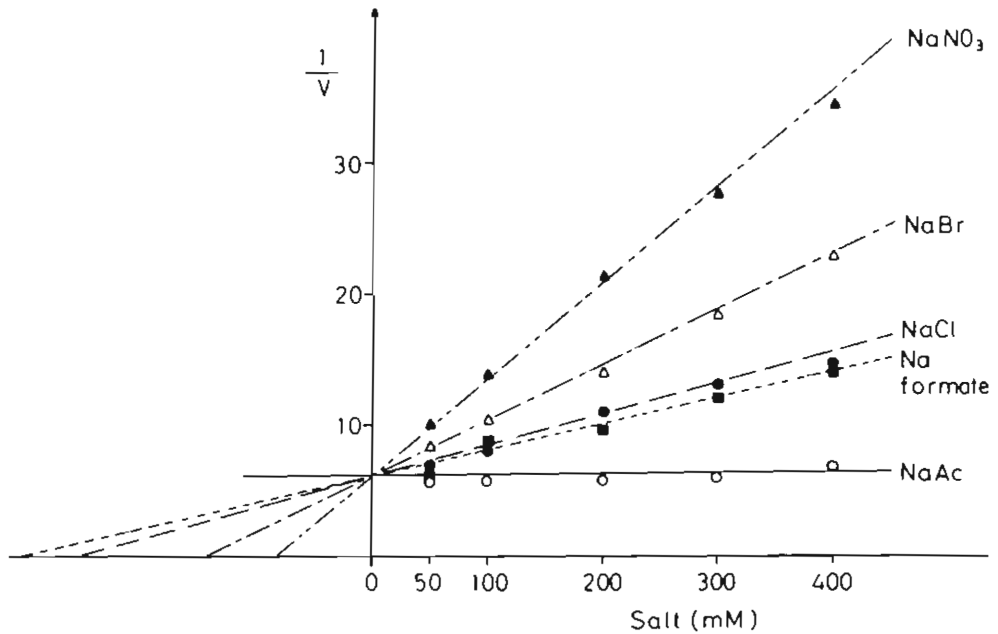

Fig. 5-25: *Mytilus californianus*. Effect of various anionic species on the activity of mantle tissue succinate dehydrogenase. Reaction velocity (v) is expressed in μM succinate min^{-1} mg^{-1} protein. Na^+ is used as the cationic species. (After GILLES and co-authors, 1971; modified; reproduced by permission of Pergamon Press Ltd.)

The above results show that some of the biochemical events which occur when a euryhaline animal is transferred from low to high salinities or when isolated tissues are submitted to hyperosmotic stress may, possibly, be explainable by ionic concentration effects on the activity of enzymatic systems related to the amino-acid metabolism. The new equilibrium attained between anabolism and catabolism could account for the subsequent modification of nitrogen excretion in *Carcinus maenas* (p. 312); but not for the transitory change in ammonia excretion in *Eriocheir sinensis* (p. 313). This last observation might be explained, however, by the effect of compounds other than inorganic ions which can modulate the enzymatic activity (possibly some allosteric effector implicated in a feedback control of the activity of enzymes implicated in the deamination process). The possibility that such regulatory processes could be at work in the control of enzyme activity in euryhaline invertebrates has never been investigated. The modulation that NADH exerts on the effect of inorganic ions on the activity of mammalian glutamate dehydrogenase, if it occurs with the enzyme from invertebrate sources, may provide us with an example of such a control mechanism (CORMAN and KAPLAN, 1967).

The question now arises: how can NaCl affect the activity of different enzymes? Since the first demonstrations of an NaCl effect in physiological concentration on the activity of glutamate dehydrogenase (SNOKE, 1956; SCHOFFENIELS and GILLES, 1963), different studies have been devoted to this problem (CHAPLIN and co-authors, 1965; SCHOFFENIELS, 1966; CORMAN and KAPLAN, 1967; GILLES, 1969; GILLES and co-authors, 1971). These studies first showed that various salts of monovalent

cations used at the same concentration as NaCl induce different effects. This is an argument showing that the effect of NaCl is independent of a variation of the ionic strength of the incubating medium. The effect must thus be related to a specific effect of the cationic species, or of the anionic species, or of both. In fact, various studies (CHAPLIN and co-authors, 1965; GILLES, 1969; GILLES and co-authors, 1971) have shown that it is mainly the anionic species which is important in modulating enzymatic activity. This is exemplified in Figs 5-24 and 5-25 in the case of the succinic dehydrogenate of *Mytilus californianus*.

It is worth noting, moreover, that when studying various anionic species, the series obtained when placing the anions in order of increasing effectiveness is the same for every enzymatic system studied up to now (MASSEY, 1953; WARREN and CHEATUM, 1966; CHIMOSKEY and GERGELY, 1968; GILLES, 1969; GILLES and co-authors, 1971); that is $Ac^- < Cl < Br^- < NO_3^-$. This order is essentially similar to the order in which these anions disrupt the structure of diverse macromolecules as determined by physical methods (BRAHMS and BREZNER, 1961; TONOMURA and co-authors, 1962; VON HIPPEL and WONG, 1964; WARREN and co-authors, 1966).

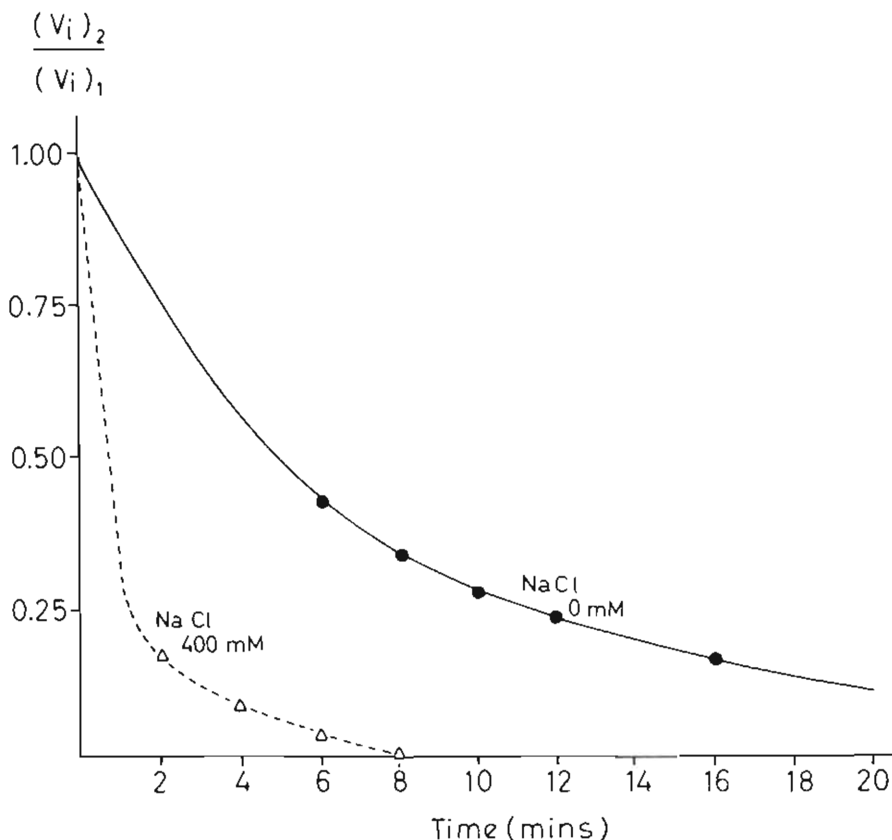

Fig. 5-26: *Homarus gammarus*. Effect of NaCl on the thermosensitivity of muscle glutamate dehydrogenase. Values are expressed as ratio of reaction velocity after heating the enzyme at 52° C for the time indicated $(v_i)_2$ to reaction velocity in absence of heating $(v_i)_1$. (Original.)

This is an argument to consider the effect of salts as resulting from changes in the structure of the enzyme protein rather than from some indirect effect such as association ion–substrate, change in substrate pK or electrostatic shielding effect of the salt at the active centre. The changes in thermosensitivity of glutamate dehydrogenase brought about by addition of NaCl to the incubation medium (Fig. 5-26) also favour this view.

To sum up, the control of the amino-acid level which occurs during cell-volume regulation can be explained partly on the basis of modulation by the ionic concentration of the activity of enzymes concerned with the amino-acid metabolism. Although the results obtained are very encouraging, the proposed scheme has to be considered mainly as a working hypothesis. More information on the intracellular localization of the enzymes, on the ionic composition prevailing locally, on the nature of the effect of salts on the enzyme activity and also on the modulation of this effect by various compounds are obviously needed. The relationships suggested above, if they explain satisfactorily some of the observations, by no means exclude other regulatory mechanisms. We have, for instance, pointed out the role of the transport mechanisms in regulating the amino-acid level. Studies in this field are almost totally lacking and information about the control of solutes transport is needed before a more complete picture can be produced.

(4) Conclusions

In the course of evolution, multicellular aquatic animals have developed effective mechanisms to control their cellular volumes and ionic composition. These processes imply the possibility to regulate osmolarity and ionic content of blood and/or tissues. Regulation at the cellular level (intracellular-fluid isosmotic regulation) is found in every species studied thus far while effective control at the blood level (extracellular-fluid anisosmotic regulation) is restricted to a few animals. Apparently, intracellular-fluid isosmotic regulation represents an evolutionary primitive mechanism to which, in some species, the mechanism of extracellular fluid anisosmotic regulation has added a new range of possibilities (see also Volume I, Chapter 4).

Intracellular-fluid isosmotic regulation maintains the isosmotic equilibrium between cells and their ambient fluids, thus avoiding excessive changes in cellular volume. This mechanism implicates control of the level of both inorganic and organic osmotic effectors among which amino acids are of general occurrence. Up to now, most pertinent studies have been devoted to the control of the intracellular amino-acid level. It appears that this control is not primarily governed by hormones and that it involves regulation of amino-acid transport and metabolism.

Nothing is known about the mechanisms controlling amino-acid transport. As far as metabolism is concerned, it appears that the ionic concentration of the intracellular medium affects the activity of enzymatic systems implicated in the cell oxidative metabolism thus bringing about a modification in the level of these organic osmotic effectors.

In many species, this primitive mechanism is assisted by a regulation of the ionic concentration and osmolarity of the body fluids.

Blood osmolarity is generally accounted for by inorganic ions (most Na^+ and Cl^-). In some animals, however, organic compounds such as urea or amino acids act

as blood osmotic effectors. This is mainly the case in some insects and fishes—chondrichthians and coelacanthiforms—although urea also occurs in other vertebrates in different conditions of dehydration.

In chondrichthians, regulation of urea blood concentration appears to be primarily achieved by modifications in transport of this metabolite at the gill and kidney levels. Also changes in the level of enzymes participating in urea synthesis in the liver may participate in this process as shown by long-term acclimation experiments.

Regulation of blood Na^+ and Cl^- concentrations is the result of passive and active charge and discharge phenomena.

Control of the movements of these ions is effected mostly at the level of 'salt moving' structures; the gut and the gills in fishes and crustaceans, anal papillae in some insect larvae, pharynx in some freshwater turtles, nasal salt gland in marine turtles or birds. Kidneys and related structures or skin do not play an important part in these NaCl balance processes. Skin permeability is indeed generally low and kidney is mainly implicated in control of plurivalent ions and in water regulation in hyperosmotic regulators.

Our present understanding of the mechanisms of extracellular-fluid anisosmotic regulation comes mostly from studies on euryhaline fishes. While the physiological events occurring during transfer of these species to reduced or increased salinities have been quite well described, our knowledge of their molecular basis remains rather speculative. Apparently, one must distinguish between mechanisms controlling immediate responses to osmotic stress and those implicated in long-term acclimation. Immediate response involves modifications in permeability and active transport of salt. Changes in ultrastructure of the 'salt moving' organs as well as in the activity of different membrane-bound enzymes should be related to long-term responses. Attempts have been made to relate the increase in Na^+/K^+ sensitive ATPase of gill tissues during transfer of euryhaline fishes to increased salinities with sodium transport. Mechanisms relating Na^+/K^+ ATPase activity, hormonal control and sodium efflux, through the gills of euryhaline teleosts when in sea water have been discussed. However, such relationships remain to be demonstrated. We are not even sure, in many cases, whether there is active outward Na^+ movement. On the other hand, it seems possible that increase in the level of membrane-bound enzymes—together with modifications in shape and number of mitochondria—are related to long-term responses assisting, during long-term acclimation, the energy requirements of the extracellular anisosmotic fluid regulation process.

Euryhalinity does not result solely from control of enzymic systems. Obviously, it results from a number of adaptations, morphological and biochemical, implicated in the regulation of blood and cellular fluid osmolarity. The mechanisms by which these adaptations occur constitute a wide field of research, rewarding not only with regard to our knowledge on the ecological potential of euryhaline species, but also on the mechanisms of acclimation to environmental changes which is one of the central problems of biology today.

Literature Cited (Chapter 5)

ANDERS, F. (1967). Tumour formation in platyfish-swordtail hybrids as a problem of gene regulation. *Experientia*, **23**, 1–10.

ANDREWS, P. (1967). Über den Blutmechanismus des Flußkrebses *Orconectes limosus* und seine Veränderung im Laufe des Jahres. *Z. vergl. Physiol.*, **57**, 7–43.

AVENS, A. C. and SLEIGH, M. A. (1965). Osmotic balance in gastropod molluscs. I. Some marine and littoral gastropods. *Comp. Biochem. Physiol.*, **16**, 121–141.

BALDWIN, E. (1960). Ureogenesis in elasmobranch fishes. *Comp. Biochem. Physiol.*, **1**, 24–37.

BALINSKY, J. B., CHORITZ, E. L., COE, C. G. L. and SCHANS, G. S. VAN DER (1967a). Urea cycle enzymes and urea excretion during the development and metamorphosis of *Xenopus laevis*. *Comp. Biochem. Physiol.*, **22**, 53–58.

BALINSKY, J. B., CHORITZ, E. L., COE, C. G. L. and SCHANS, G. S. VAN DER (1967b). Amino acid metabolism and urea synthesis in naturally aestivating *Xenopus laevis*. *Comp. Biochem. Physiol.*, **22**, 59–68.

BALINSKY, J. B., CRAGG, M. M. and BALDWIN, E. (1961). The adaptation of amphibian waste nitrogen excretion to desiccation. *Comp. Biochem. Physiol.*, **3**, 236–244.

BALL, J. N. (1969). Prolactin (fish prolactin or paralactin) and growth hormone. *In* W. S. Hoar and D. J. Randall (Eds), *Fish Physiology*, Vol. II. Academic Press, New York. pp. 207–240.

BALL, J. N. and PICKFORD, G. E. (1964). Pituitary cytology and freshwater adaptation in *Fundulus heteroclitus*. *Anat. Rec.*, **148**, 358.

BEADLE, L. C. (1939). Regulation of the haemolymph in the saline water mosquito larva, *Aedes detritus*. *J. exp. Biol.*, **16**, 346–362.

BEDFORD, J. J. (1971). Osmoregulation in *Melanopsis trifasciata*. IV. The possible control of intracellular isosmotic regulation. *Comp. Biochem. Physiol.*, **40A**, 1015–1027.

BENTLEY, P. J., BRETZ, W. L. and SCHMIDT-NIELSEN, K. (1967). Osmoregulation in the diamondback terrapin *Malaclemys terrapin centrata*. *J. exp. Biol.*, **46**, 161–167.

BENTLEY, P. J. and FOLLETT, B. K. (1962). The action of neuro-hypophysial and adrenocortical hormones on sodium balance in the cyclostome *Lampetra fluviatilis*. *Gen. comp. Endocrinol.*, **2**, 329–335.

BENTLEY, P. J. and FOLLETT, B. K. (1963). Kidney function in a primitive vertebrate, the cyclostome *Lampetra fluviatilis*. *J. Physiol., Lond.*, **169**, 902–918.

BENTLEY, P. J. and SCHMIDT-NIELSEN, K. (1966). Cutaneous water loss in reptiles. *Science, N.Y.*, **151**, 1547–1549.

BERGLUND, F. and FORSTER, R. P. (1958). Renal tubular transport of inorganic divalent ions by the aglomerular marine teleost, *Lophius americanus*. *J. gen. Physiol.*, **41**, 429–440.

BIETER, R. N. (1931). The action of some diuretics upon the aglomerular kidney. *J. Pharmac. exp. Ther.*, **43**, 399–406.

BIETER, R. N. (1935). The action of diuretics injected into one kidney of the aglomerular toadfish. *J. Pharmac. exp. Ther.*, **53**, 347–349.

BOURGUET, J., LAHLOU, B. and MAETZ, J. (1964). Modifications expérimentales de l'équilibre hydrominéral et osmorégulation chez *Carassius auratus*. *Gen. comp. Endocrinol.*, **4**, 563–576.

BRADSHAW, S. D. and SHOEMAKER, U. H. (1967). Aspects of water and electrolyte changes in a field population of *Amphibolurus* lizards. *Comp. Biochem. Physiol.*, **20**, 855–865.

BRAHMS, J. and BREZNER, J. (1961). Interaction of myosin A with ions. *Archs Biochem. Biophys.*, **95**, 219–228.

BRICTEUX-GRÉGOIRE, S., DUCHÂTEAU-BOSSON, G., JEUNIAUX, C. and FLORKIN M. (1962). Constituants osmotiquement actifs des muscles du crabe chinois *Eriocheir sinensis*, adapté à l'eau douce ou à l'eau de mer. *Archs int. Physiol. Biochim.*, **70**, 273–286.

BRICTEUX-GRÉGOIRE, S., DUCHÂTEAU-BOSSON, G., JEUNIAUX, C. and FLORKIN, M. (1964). Constituants osmotiquement actifs des muscles adducteurs de *Mytilus edulis* adaptée à l'eau de mer ou à l'eau saumâtre. *Archs int. Physiol. Biochim.*, **72**, 116–123.

BROWN, G. W., JR. (1965). Ornithine carbamoyltransferase in liver of the dipnoan *Protopterus aethiopicus*. *Science, N.Y.*, **149**, 1515.

BRULL, L. and NIZET, E. (1953). Blood and urine constituents of *Lophius piscatorius* L. *J. mar. biol. Ass. U.K.*, **32**, 321–328.

BRYAN, G. W. (1960a). Sodium regulation in the crayfish *Astacus fluviatilis*. I. The normal animal. *J. exp. Biol.*, **37**, 83–99.

BRYAN, G. W. (1960b). Sodium regulation in the crayfish *Astacus fluviatilis*. II. Experiments with sodium-depleted animals. *J. exp. Biol.*, **37**, 100–112.

BRYAN, G. W. (1960c). Sodium regulation in the crayfish *Astacus fluviatilis*. III. Experiments with NaCl-loaded animals. *J. exp. Biol.* **37**, 113–128.

BURDEN, C. E. (1956). The failure of hypophysectomized *Fundulus heteroclitus* to survive in fresh water. *Biol. Bull. mar. biol. Lab., Woods Hole*, **110**, 8–28.

BURGER, J. W. and TOSTESON, D. C. (1966). Sodium influx and efflux in the spring dogfish *Squalus acanthias*. *Comp. Biochem. Physiol.*, **19**, 649–653.

BURSELL, E. (1966). Aspects of the flight metabolism of tsetse flies (*Glossina*). *Comp. Biochem. Physiol.*, **19**, 809–818.

BUTLER, D. G. (1967). Effect of ovine prolactin on tissue electrolyte composition of hypophysectomized freshwater eels (*Anguilla anguilla*). *J. Fish. Res. Bd Can.*, **24**, 1823–1826.

CAMPBELL, J. W. (1961). Studies on tissue arginase and ureogenesis in the elasmobranch, *Mustelus canis*. *Archs Biochem. Biophys.*, **93**, 448–455.

CHAN, D. K. O., CHESTER JONES, I. and MOSLEY, W. (1968). Pituitary and adrenocortical factors in the control of the water and electrolyte composition of the freshwater European eel (*Anguilla anguilla* L.). *J. Endocr.*, **42**, 91–98.

CHAN, D. K. O., PHILLIPS, J. G. and CHESTER JONES, I. (1967). Studies on electrolyte, changes in the lip shark, *Hemiscyllium plagiosum* (BENNETT) with special reference to the hormonal influence on the rectal gland. *Comp. Biochem. Physiol.*, **23**, 185–198.

CHAPLIN, A. E., HUGGINS, A. K. and MUNDAY, K. A. (1965). Ionic effects on glutamate dehydrogenase activity from beef liver, lobster muscle and crab muscle. *Comp. Biochem. Physiol.*, **16**, 49–62.

CHAPLIN, A. E., HUGGINS, A. K. and MUNDAY, K. A. (1970). The effect of salinity on the metabolism of nitrogen containing compounds by *Carcinus maenas*. *Int. J. Biochem.*, **1**, 385–400.

CHESTER JONES, I., CHAN, D. K. O. and RANKIN, J. C. (1969a). Renal function in the European eel (*Anguilla anguilla* L.): changes in blood pressure and renal function of the freshwater eel transferred to sea water. *J. Endocr.*, **43**, 9–19.

CHESTER JONES, I., CHAN, D. K. O., HENDERSON, I. W. and BALL, J. N. (1969b). The adrenocortical steroids, adrenocorticotropin and the corpuscules of Stannius. In W. S. Hoar and D. J. Randall (Eds), *Fish Physiology*, Vol. II. Academic Press, New York. pp. 321–376.

CHESTER JONES, I., CHAN, D. K. O. and RANKIN, J. C. (1969c). Renal function in the European eel (*Anguilla anguilla* L.): effects of the caudal neurosecretory system, corpuscules of Stannius, neurohypophysial peptides and vasoactive substances. *J. Endocr.*, **43**, 21–31.

CHESTER JONES, I., HENDERSON, I. W. and MOSLEY, W. (1964). Methods for the adrenalectomy of the European eel (*Anguilla anguilla* L.). *J. Endocr.*, **29**, 155–156.

CHIMOSKEY, J. E. and GERGELY, J. (1968). Effect of ions on sarcoplasmic reticulum fragments. *Archs Biochem. Biophys.* **128**, 601–605.

CHOLETTE, C., GAGNON, A. and GERMAIN, P. (1970). Isosmotic adaptation in *Myxine glutinosa* L. I. Variations of some parameters and role of the amino acid pool of the muscle cells. *Comp. Biochem. Physiol.*, **33**, 333–346.

CLARK, M. E. (1968). A survey of the effect of osmotic dilution on free amino acids of various polychaetes. *Biol. Bull. mar. biol. Lab., Woods Hole*, **134**, 252–260.

CONTE, F. P. (1969). Salt secretion. In W. S. Hoar and D. J. Randall (Eds), *Fish Physiology*, Vol. I. Academic Press, New York. pp. 241–292.

CONTE, F. P., PETERSON, G. L. and EWING, R. D. (1973). Larval salt gland of *Artemia salina* nauplii. *J. comp. Physiol.*, **82**, 277–289.

CONTE, F. P. and TRIPP, M. J. (1970). Succinic acid dehydrogenase activity in the gill epithelium of euryhaline fishes. *Int. J. Biochem.*, **1**, 129–138.

CONWAY, E. J. and MCCORMACK, J. I. (1953). The total intracellular concentration of mammalian tissues compared with that of the extracellular fluid. *J. Physiol., Lond.*, **120**, 1–14.

COPELAND, D. E. (1964a). A mitochondrial pump in the cells of the anal papilliae of mosquito larvae. *J. Cell Biol.*, **23**, 253–263.

COPELAND, D. E. (1964b). Salt absorbing cells in gills of crabs *Callinectes* and *Carcinus*. *Biol. Bull. mar. biol. Lab., Woods Hole*, **127**, 367–368.

COPELAND, D. E. (1968). Fine structure of salt and water uptake in the land-crab *Gecarcinus lateralis*. *Am. Zool.*, **8**, 417–432.

COPELAND, D. E. and FITZJARRELL, A. T., (1968). The salt absorbing cells in the gills of the blue crab (*Callinectes sapidus* RATHBUN) with notes on modified mitochondria. *Z. Zellforsch. mikrosk. Anat.*, **92**, 1–22.

CORMAN, L. and KAPLAN, N. O. (1967). Kinetic studies of dogfish liver glutamate dehydrogenase with diphosphopyridine nucleotide and the effect of added salts. *J. biol. Chem.*, **242**, 2840–2846.

CROGHAN, P. C. (1958). The osmotic and ionic regulation of *Artemia salina* (L.). *J. exp. Biol.*, **35**, 219–249.

CROGHAN, P. C. and LOCKWOOD, A. P. M. (1968). Ionic regulation of the Baltic and fresh water races of the isopod *Mesidotea* (*Laduria*) *entomon* (L.). *J. exp. Biol.*, **48**, 141–158.

DEFFNER, G. G. J. and HAFTER, R. E. (1960). Chemical investigations of the giant nerve fibers of the squid. III. Identification and quantitative estimation of free organic ninhydrin-negative constituents. IV. Acid-base balance in axoplasm. *Biochim. biophys. Acta*, **42**, 189–205.

DEJOURS, P. (1969). Variations of CO_2 output of a freshwater teleost upon change of the ionic composition of water. *J. Physiol., Lond.*, **202**, 113–114 P.

DENTON, E. J. (1971). Examples of the use of active transport of salts and water to give buoyancy in the sea. *Phil. Trans. R. Soc. (B)*, **262**, 277–287.

DOYLE, W. and GORECKI, D. (1961). The so-called chloride cell of the fish gill. *Physiol. Zool.*, **34**, 81–85.

DUCHÂTEAU, G. and FLORKIN, M. (1962). Régulation isosmotique intracellulaire chez *Eriocheir sinensis* après ablation des pédoncules oculaires. *Archs int. Physiol. Biochim.*, **70**, 393–396.

DUCHÂTEAU, G., FLORKIN, M. and JEUNIAUX, C. (1959). Composante aminoacide des tissus chez les crustacés. I. Composante aminoacide des muscles de *Carcinus maenas* L. lors du passage de l'eau de mer à l'eau saumâtre et au cours de la mue. *Archs int. Physiol. Biochim.*, **67**, 489–500.

DUCHÂTEAU-BOSSON, G., JEUNIAUX, C. and FLORKIN, M. (1961). Rôle de la variation de la composante amino-acide intracellulaire dans l'euryhalinité d'*Arenicola marina* L. *Archs int. Physiol. Biochim.*, **69**, 30–35.

DUNSON, W. A. (1968). Salt gland secretion in the pelagic sea snake *Pelamis*. *Am. J. Physiol.*, **215**, 1512–1517.

DUNSON, W. A. (1969). Electrolyte excretion by the salt gland of the Galapagos marine iguana. *Am. J. Physiol.*, **216**, 995–1002.

DUNSON, W. A. (1970). Some aspects of electrolyte and water balance in three estuarine reptiles, the diamondback terrapin, American and 'salt water' crocodiles. *Comp. Biochem. Physiol.*, **32**, 161–174.

DUNSON, W. A. and WEYMOUTH, R. D. (1965). Active uptake of sodium by soft shell turtles (*Trionyx spinifer*). *Science, N.Y.*, **149**, 67–69.

ELIASSEN, F. (1953). The energy metabolism of *Artemia salina* in relation to body size, seasonal rhythms and different salinities. *Natur. vitensk bekke*, **11**, 3–17.

ELLIS, K. and ABEL, J. (1964). Intracellular channels in the salt-secreting glands of marine turtles. *Science, N.Y.*, **144**, 1340–1342.

EMERSON, D. N. (1969). Influence of salinity on ammonia excretion rates and tissue constituents of euryhaline invertebrates. *Comp. Biochem. Physiol.*, **29**, 1115–1133.

EMMART, E. W., PICKFORD, G. E. and WILHELMI, A. E. (1966). Localization of prolactin within the pituitary of a cyprinodont fish, *Fundulus heteroclitus* (LINNAEUS) by a specific fluorescent antiovine prolactin globulin. *Gen. comp. Endocrinol.*, **7**, 571–583.

ENSOR, D. M. and BALL, J. N. (1968). Prolactin and freshwater sodium fluxes in *Poecilia latipuma* (Teleostei). *J. Endocr.*, **41**, 16.

EPSTEIN, F. H., CYNAMON, M. and MCKAY, W. (1971). Endocrine control of Na-K-ATPase and sea-water adaptation in *Anguilla rostrata*. *Gen. comp. Endocrinol.*, **16**, 323–328.

EPSTEIN, F., KATZ, A. and PICKFORD, G. (1967). Sodium and potassium activated adenosine triphosphatase of gills: role in adaptation of teleosts to salt water. *Science, N.Y.*, **156**, 1245–1247.

ETOH, H. and EGAMI, N. (1963). Effect of hypophysectomy and adrenalectomy on the length of survival time after X-irradiation in the goldfish, *Carassius auratus*. *Proc. Japan Acad.*, **39**, 503–506.

EVANS, D. H. (1969). Sodium, chloride and water balance of the intertidal teleost, *Pholis gunnellus*. *J. exp. Biol.*, **50**, 179–190.

FLORKIN, M. (1960). Blood chemistry. In T. H. Waterman (Ed.), *The Physiology of Crustacea*, Vol. I. Academic Press, New York. pp. 141–159.

FLORKIN, M. (1962). La régulation isosmotique intracellulaire chez les invertébrés marins euryhalins. *Bull. Acad. r. Belg. Cl. Sci.*, **48**, 687–694.

FLORKIN, M. and SCHOFFENIELS, E. (1969). *Molecular Approaches to Ecology*, Academic Press, New York.

FREDERICQ, L. (1901). Sur la concentration moléculaire du sang et des tissus chez les animaux aquatiques. *Bull. Acad. r. Belg. Cl. Sci.*, **1901**, 428.

FREEMAN, R. F. H. and RIGLER, F. H. (1957). The response of *Scrodicularia plana* (DA COSTA) to osmotic pressure changes. *J. mar. biol. Ass. U.K.*, **36**, 553–567.

FUGELLI, K. (1967). Regulation of cell volume in flounder (*Pleuronectes flesus*) erythrocytes accompanying a decrease in plasma osmolarity. *Comp. Biochem. Physiol.*, **22**, 253–260.

GAINER, H. and GRUNDFEST, H. (1968). Permeability of alkali metal cations in lobster muscle. A comparison of electrophysiological and osmometric analyses. *J. gen. Physiol.*, **51**, 399–425.

GARCIA-ROMEU, F. and MAETZ, J. (1964). The mechanism of sodium and chloride uptake by the gills of a freshwater fish, *Carassius auratus*. I. Evidence for an independent uptake of sodium and chloride ions. *J. gen. Physiol.*, **47**, 1195–1207.

GARCIA-ROMEU, F. and MOTAIS, R. (1966). Mise en évidence d'échanges Na^+/NH_4^+ chez l'anguille d'eau douce. *Comp. Biochem. Physiol.*, **17**, 1201–1204.

GERARD, J. F., DANDRIFOSSE, G. and GILLES, R. (1973). Changes in the extracellular space of *Callinectes sapidus* axons during osmotic shocks. (In preparation.)

GERARD, J. F. and GILLES, R. (1971). Osmoregulation and amino acid efflux in the estuarine crab *Callinectes sapidus*. In D. S. Gorsline (Ed.), *Second National Coastal and Shallow Water Research Conference*, Abstract volume. University Press, University of Southern California, Los Angeles. p. 82.

GERARD, J. F. and GILLES, R. (1972a). The free amino-acid pool in *Callinectes sapidus* tissues and its role in the osmotic intracellular regulation. *J. exp. mar. Biol. Ecol.*, **10**, 125–136.

GERARD, J. F. and GILLES, R. (1972b). Modification of the amino acid efflux during the osmotic adjustment of isolated axons of *Callinectes sapidus*. *Experientia*, **28**, 863–864.

GIEBISCH, G., BOULPAEP, E. L. and WHITTEMBURY, G. (1971). Electrolyte transport in kidney tubule cells. *Phil. Trans. R. Soc.* (*B*), **262**, 175–196.

GILCHRIST, B. M. (1956). The oxygen consumption of *Artemia salina* (L.) in different salinities. *Hydrobiologia*, **8**, 54–63.

GILLES, R. (1969). Effect of various salts on the activity of enzymes implicated in amino acid metabolism. *Archs int. Physiol. Biochim.*, **77**, 441–464.

GILLES, R. (1970a). Osmoregulation in the stenohaline crab '*Libinia emarginata*' LEACH. *Archs int. Physiol. Biochim.*, **78**, 91–99.

GILLES, R. (1970b). Intermediary metabolism and energy production in some invertebrates. *Archs int. Physiol. Biochim.*, **78**, 313–326.

GILLES, R. (1972a). Osmoregulation in three molluscs: *Acanthochitona discrepans* (BROWN), *Glycymeris glycymeris* (L.) and *Mytilus edulis* (L.). *Biol. Bull. mar. biol. Lab., Woods Hole*, **142**, 25–35.

GILLES, R. (1972b). Amino-acid metabolism and isosmotic intracellular regulation in isolated surviving axons of *Callinectes sapidus*. *Life Sci.*, **11** (II), 565–572.

GILLES, R. (1972c). Oxygen consumption as related to the amino-acid metabolism during osmoregulation in the blue crab *Callinectes sapidus*. *Neth. J. Sea Res.*, **7**, 280–289.

GILLES, R. (1973). Osmotic behavior of isolated axons of a euryhaline and a stenohaline crustacean. *Experientia*, **29**, 1354–1355.

GILLES, R. and GILLES-BAILLIEN, M. (1974). *The Regulation of the Amino-Acid Pool*. Scientechnica, Bristol, England. (In preparation.)

GILLES, R., HOGUE, P. and KEARNEY, E. B. (1971). Effect of various ions on the succinic dehydrogenase activity of *Mytilus californianus*. *Life Sci.*, **10** (II), 1421–1427.

GILLES, R. and JÖBSIS, F. F. (1972). Isosmotic intracellular regulation and redox changes in the respiratory chain components of *Callinectes sapidus* isolated muscle fibers. *Life Sci.*, **11** (II), 877–886.

GILLES, R. and SCHOFFENIELS, E. (1964). Action de la vératrine, de la cocaïne et de la stimulation électrique sur la synthèse et sur le pool des acides aminés de la chaîne nerveuse ventrale du homard. *Biochim. biophys. Acta*, **82**, 525–537.

GILLES, R. and SCHOFFENIELS, E. (1966). Décarboxylation des acides aspartique et oxaloacétique chez le homard et l'écrevisse. *Bull. Soc. Chim. biol.*, **68**, 397–417.

GILLES, R. and SCHOFFENIELS, E. (1968a). Fixation de $^{14}CO_2$ par les acides aminés de la chaine nerveuse ventrale du crustacé *Homarus vulgaris* M. EDW. *Archs int. Physiol. Biochim.*, **76**, 441–451.

GILLES, R. and SCHOFFENIELS, E. (1968b). Influence du NH_4Cl et du sulfate de vératrine sur la synthèse et le pool des acides aminés au niveau de la chaîne nerveuse ventrale de deux crustacés (*Homarus vulgaris* M. EDW. et *Astacus fluviatilis* F.). *Archs int. Physiol. Biochim.*, **76**, 452–464.

GILLES, R. and SCHOFFENIELS, E. (1969a). Isosmotic regulation in isolated surviving nerves of *Eriocheir sinensis* MILNE EDWARDS. *Comp. Biochem. Physiol.*, **31**, 927–939.

GILLES, R. and SCHOFFENIELS, E. (1969b). Metabolic fate of glucose and pyruvate in the nerve cord of *Homarus vulgaris* (M. EDW.) and *Astacus fluviatilis* (F.). *Comp. Biochem. Physiol.* **28**, 417–423.

GILLES-BAILLIEN, M. (1970). Urea and osmoregulation in the diamondback terrapin *Malaclemys centrata centrata* (LATREILLE). *J. exp. Biol.*, **52**, 691–697.

GILLES-BAILLIEN, M. (1973). Isosmotic regulation in various tissues of the diamondback terrapin *Malaclemys centrata centrata* (LATREILLE). *J. exp. Biol.*, **59**, 39–43.

GINETSINSKII, A. G., VASIL'YEVA, V. F. and NATOCHIN, YU. V. (1965). The reaction of fish to change in the salinity of the medium. In J. W. S. Pringle (Ed.), *Essays on Physiological Evolution*. Pergamon Press, Oxford. pp. 92–106.

GOLDSTEIN, L. (1970). Urea metabolism and osmoregulation in euryhaline elasmobranchs and Amphibia. In B. Schmidt-Nielsen and D. W. S. Kerr (Eds), *Urea and the Kidney*. Excerpta Medica Foundation, Amsterdam. pp. 243–251.

GOLDSTEIN, L. and FORSTER, R. P. (1965). The role of uricolysis in the production of urea by fishes and other aquatic vertebrates. *Comp. Biochem. Physiol.*, **14**, 567–576.

GONZALES, C. F., SHAMOO, Y. E. and WYSSBROD, H. R. (1967). Electrical nature of Na transport across the isolated turtle bladder. *Am. J. Physiol.*, **213**, 333–340.

GORDON, M. S. and TUCKER, V. A. (1968). Further observations on the physiology of salinity adaptation in the crab-eating frog (*Rana cancrivora*). *J. exp. Biol.*, **49**, 185–193.

GREEN, J. W., HARSCH, M., BARR, L. and PROSSER, C. L. (1959). The regulation of water and salt by the fiddler crabs, *Uca pugnax* and *Uca pugilator*. *Biol. Bull. mar. biol. Lab., Woods Hole*, **116**, 76–87.

GROSS, W. J. (1957). An analysis of response to osmotic stress in selected decapod Crustacea. *Biol. Bull. mar. biol. Lab., Woods Hole*, **112**, 43–62.

GUTKNECHT, J. and DAINTY, J. (1968). Ionic relations of marine algae. *Oceanogr. mar. Biol. A. Rev.*, **6**, 163–200.

HANWELL, A., LINZELL, J. L. and PEAKER, M. (1971). Salt-gland secretion and blood flow in the goose. *J. Physiol., Lond.*, **213**, 373–387.

HAYS, R. M., HARKNESS, S. H. and FRANKI, N. (1970). The movement of urea and other small molecules across the toad bladder. In B. Schmidt-Nielsen and D. W. S. Kerr (Eds), *Urea and the Kidney*. Excerpta Medica Foundation, Amsterdam. pp. 149–158.

HICKMAN, C. P. (1968). Urine composition and kidney function in Southern flounder, *Paralichthys lethostigma*, in sea water. *Can. J. Zool.*, **46**, 439–455.

HICKMAN, C. P. and TRUMP, B. F. (1969). The kidney. In W. S. Hoar and D. J. Randall (Eds), *Fish Physiology*, Vol. I. Academic Press, New York. pp. 91–239.

HIPPEL, P. H. VON and WONG, K. Y. (1964). Neutral salts: the generality of their effects on the stability of macro-molecular conformations. *Science, N.Y.*, **145**, 577–580.

HIRANO, T., KAMIYA, M., SAISHU, S. and UTIDA, S. (1967). Effects of hypophysectomy and urophysectomy on water and sodium transport in isolated intestine and gills of the Japanese eel, *Anguilla japonica*. *Endocr. jap.*, **14**, 182–186.

HOLMES, W. N. and DONALDSON, E. M. (1969). The body compartments and the distribution of electrolytes. In W. S. Hoar and D. J. Randall (Eds), *Fish Physiology*, Vol. I. Academic Press, New York. pp. 1–89.

HOLMES, W. N. and MCBEAN, R. L. (1964). Some aspects of the electrolyte excretion in the green turtle, *Chelonia mydas mydas*. *J. exp. Biol.*, **41**, 81–90.

HOLTZMAN, S. and SCHREIBMAN, M. P. (1970). Effect of environmental salinity on the incorporation of H^3-leucine in gills of a freshwater teleost, *Xiphophorus maculatus*. *Am. Zool.*, **10**, 518.

HOUSE, C. R. (1963). Osmotic regulation in the brackish water teleost *Blennius pholis*. *J. exp. Biol.*, **40**, 87–104.

HOYAUX, J. M., GILLES, R. and JEUNIAUX, C. (1974). Osmoregulation in three gastropod snails. (In preparation.)

HUGGINS, A. K. and BOULTON, A. P. (1971). 2-hydroxyglutarate in crustaceans. *Biochem. J.*, **125**, 1141–1143.

HUGGINS, A. K. and COLLEY, L. (1971). The changes in the non-protein nitrogenous constituents of muscle during the adaptation of the eel *Anguilla anguilla* L. from fresh water to sea water. *Comp. Biochem. Physiol.*, **38B**, 537–541.

HUGGINS, A. K. and MUNDAY, K. A. (1968). Crustacean metabolism. *Adv. comp. Physiol. Biochem.*, **3**, 271–378.

HUNTER, A. (1929). Further observations on the distribution of arginase in fishes. *J. biol. Chem.*, **81**, 505–511.

JAMPOL, L. M. and EPSTEIN, F. H. (1970). Sodium–potassium activated adenosine triphosphatase and osmotic regulation by fishes. *Am. J. Physiol.*, **218**, 607–611.

JANSSENS, P. A. and COHEN, P. P. (1968). Biosynthesis of urea in the estivating African lungfish and in *Xenopus laevis* under conditions of water-shortage. *Comp. Biochem. Physiol.*, **24**, 887–898.

JEUNIAUX, C., BRICTEUX, S. and FLORKIN, M. (1961a). Contribution des acides aminés libres à la régulation osmotique intracellulaire chez deux crustacés euryhalins, *Leander serratus* et *Leander squilla*. *Cah. Biol. mar.*, **2**, 373–379.

JEUNIAUX, C., BRICTEUX-GRÉGOIRE, S. and FLORKIN, M. (1962). Régulation osmotique intracellulaire chez *Asterias rubens*. Rôle du glycocolle et de la taurine. *Cah. Biol. mar.*, **3**, 107–113.

JEUNIAUX, C., DUCHÂTEAU, G. and FLORKIN, M. (1961b). Variation de la composante aminoacide des tissus et euryhalinité chez *Perinereis cultrifera* et *Nereis diversicolor*. *J. Biochem., Tokyo*, **49**, 527–531.

JEUNIAUX, C. and FLORKIN, M. (1961). Modification de l'excrétion azotée du crabe chinois au cours de l'adaptation osmotique. *Archs int. Physiol. Biochim.*, **69**, 385–386.

JØRGENSEN, C. B. and DALES, R. P. (1957). The regulation of volume and osmotic regulation in some nereid polychaetes. *Physiologica comp. Oecol.*, **4**, 357–374.

KAMIYA, M. and UTIDA, S. (1968). Changes in activity of sodium potassium activated adenosine-triphosphatase in gills during adaptation of the Japanese eel to sea water. *Comp. Biochem. Physiol.*, **26**, 675–685.

KAMIYA, M. and UTIDA, S. (1969). Sodium–potassium activated adenosine-triphosphatase activity in gills of fresh water, marine and euryhaline teleosts. *Comp. Biochem. Physiol.*, **31**, 671–674.

KANESHIRO, E. S., HOLZ, G. G. and DUNHAM, P. B. (1969). Osmoregulation in a marine ciliate, *Miamiensis avidus*. II. Regulation of the intracellular free amino acids. *Biol. Bull. mar. biol. Lab., Woods Hole*, **137**, 161–169.

KATO, K. N. and KAMEMOTO, F. I. (1969). Neuroendocrine involvement in osmoregulation in the grapsoid crab *Metopograpsus messor*. *Comp. Biochem. Physiol.*, **28**, 665–674.

KEMPTON, R. T. (1953). Studies on the elasmobranch kidney. II. Reabsorption of urea by the smooth dogfish, *Mustelus canis*. *Biol. Bull. mar. biol. Lab., Woods Hole*, **104**, 45–56.

KERSTETTER, T. H., KIRSCHNER, L. B. and RAFUSE, D. D. (1970). On the mechanisms of sodium ion transport by the irrigated gills of rainbow trout (*Salmo gairdneri*). *J. gen. Physiol.*, **56**, 342–359.

KEYS, A. B. (1931). Chloride and water secretion and absorption by the gills of the eel. *Z. vergl. Physiol.*, **15**, 364–388.

KINNE, O. (1964a). Non-genetic adaptation to temperature and salinity. *Helgoländer wiss. Meeresunters.*, **9**, 433–458.

KINNE, O. (1964b). Animals in aquatic environments: crustaceans. In D. B. Dill, E. F. Adolph and C. G. Wilber (Eds), *Handbook of Physiology*, Sect. 4, Adaptation to the environment. American Physiological Society, Washington, D.C. pp. 669–682.

KINNE, O. (1971). Salinity: Animals—Invertebrates. In O. Kinne (Ed.), *Marine Ecology*, Vol. I, Environmental Factors, Part 1. Wiley, London. pp. 821–995.

KINNE, O. (in press). Concepts, management and technology. In O. Kinne (Ed.), *Marine Ecology*, Vol. III, Cultivation. Wiley, London.

KLAHR, S. and BRICKER, N. S. (1965). Energetics of anaeobie sodium transport by the fresh water turtle bladder. *J. gen. Physiol.*, **48**, 571–580.

KOMNICK, H. (1963). Elektronenmikroskopische Untersuchungen zur funktionellen Morphologie des Ionentransportes in der Salzdrüse von *Larus argentatus*. I. Teil: Bau und Feinstruktur der Salzdrüse. *Protoplasma*, **56**, 274–314.

KROGH, A. (1939). *Osmotic Regulation in Aquatic Animals*, University Press, Cambridge.

KUENEN, D. J. (1939). Systematical and physiological notes on the brine shrimp, *Artemia*. *Archs néerl. Zool.*, **3**, 365–449.

LAHLOU, B. (1967). Excrétion rénale chez un poisson euryhalin, le flet (*Platichthys flesus* L.): caractéristiques de l'urine normale en eau douce et en eau de mer et effets de changements de milieu. *Comp. Biochem. Physiol.*, **20**, 925–938.

LAHLOU, B., HENDERSON, I. W. and SAWYER, W. H. (1969a). Sodium exchanges in goldfish (*Carassius auratus* L.) adapted to a hypertonic saline solution. *Comp. Biochem. Physiol.*, **28**, 1427–1433.

LAHLOU, B., HENDERSON, I. W. and SAWYER, W. H. (1969b). Renal adaptations by *Opsanus tau*, a euryhaline aglomerular teleost, to dilute media. *Am. J. Physiol.*, **216**, 1266–1272.

LAHLOU, B. and SAWYER, W. H. (1967). Sodium and chloride balance in hypophysectomized goldfish, *Carassius auratus* L. *Am. Zool.*, **7**, 720.

LAHLOU, B. and SAWYER, W. H. (1969). Sodium exchanges in the toadfish, *Opsanus tau*, a euryhaline aglomerular teleost. *Am. J. Physiol.*, **216**, 1273–1278.

LANG, M. A. and GAINER, H. (1969). Isosmotic intracellular regulation as a mechanism of volume control in crab muscle fibers. *Comp. Biochem. Physiol.*, **30**, 445–456.

LASSERRE, P. and GILLES, R. (1971). Modification of the amino acid pool in the parietal muscle of two euryhaline teleosts during osmotic adjustment. *Experientia*, **27**, 1434–1435.

LEDERIS, K. (1963). Hormonal and ultrastructural changes in the hypothalamo-neurohypophysial system following osmotic stimulation. *Gen. comp. Endocrinol.*, **3**, 714–715.

LEDERIS, K. (1964). Fine structure and hormone content of the hypothalamo-neurohypophysial system of the rainbow trout (*Salmo viridens*) exposed to sea water. *Gen. comp. Endocrinol.*, **4**, 638–661.

LOCKWOOD, A. P. M. (1961). Ringer solutions and some notes on the physiological basis of their ionic composition. *Comp. Biochem. Physiol.*, **2**, 241–289.

LOCKWOOD, A. P. M. (1962). The osmoregulation of Crustacea. *Biol. Rev.*, **37**, 257–305.

LOCKWOOD, A. P. M. (1970). The involvement of sodium transport in the volume regulation of the amphipod crustacean *Gammarus duebeni*. *J. exp. Biol.*, **53**, 737–751.

LOCKWOOD, A. P. M. and CROGHAN, P. C. (1959). Composition of the haemolymph of *Petrobius maritimus* LEACH. *Nature, Lond.*, **184**, 370–371.

MCALISTER, R. O. and FISHER, F. M. (1968). Response of the false limpet, *Siphonaria pectinata* LINNAEUS (Gastropoda, pulmonata) to osmotic stress. *Biol. Bull. mar. biol. Lab., Woods Hole*, **134**, 96–117.

MCBEAN, R. L. and GOLDSTEIN, L. (1967). Ornithine-urea cycle activity in *Xenopus laevis*: adaptation in saline. *Science, N.Y.*, **157**, 931–932.

MACROBBIE, E. A. C. (1970). The active transport of ions in plant cells. *Q. Rev. Biophys.*, **3**, 251–294.

MAETZ, J. (1956). Les échanges de sodium chez le poisson *Carassius auratus* L. Action d'un inhibiteur de l'anhydrase carbonique. *J. Physiol., Paris*, **48**, 1085–1099.

MAETZ, J. (1963). Physiological aspects of neurohypophysial function in fishes with some reference to the Amphibia. *Symp. zool. Soc. Lond.*, **9**, 107–140.

MAETZ, J. (1971). Fish gills: mechanisms of salt transfer in fresh water and sea water. *Phil. Trans. R. Soc. (B)*, **262**, 209–249.

MAETZ, J., BOURGUET, J., LAHLOU, B. and HOURDRY, J. (1964). Peptides neurohypophysaires et osmorégulation chez *Carassius auratus*. *Gen. comp. Endocrinol.*, **4**, 508–522.

MAETZ, J. and CAMPANINI, G. (1966). Potentiels trans-épithéliaux de la branchie d'anguille *in vivo* en eau douce et en eau de mer. *J. Physiol., Paris*, **58**, 248.

MAETZ, J. and GARCIA-ROMEU, F. (1964). The mechanism of sodium and water uptake by the gills of a fresh water fish *Carassius auratus*. II. Evidence for NH_4^+/Na^+ and HCO_3^-/Cl^- exchanges. *J. gen. Physiol.*, **47**, 1209–1227.

MAETZ, J., MAYER, N. and CHARTIER-BARADUC, M. M. (1967). La balance minérale du sodium chez *Anguilla anguilla* en eau de mer, en eau douce et au cours de transfert d'un milieu à l'autre: effets de l'hypophysectomie et de la prolactine. *Gen. comp. Endocrinol.*, **8**, 177–188.

MAETZ, J., NIBELLE, J., BORNANCIN, M. and MOTAIS, R. (1969). Action sur l'osmorégulation de l'anguille de divers antibiotiques inhibiteurs de la synthèse des protéines ou du renouvellement cellulaire. *Comp. Biochem. Physiol.*, **30**, 1125–1151.

MAETZ, J. and RANKIN, J. C. (1969). Quelques aspects du rôle biologique des hormones neurohypophysaires chez les poissons. *Colloques int. Cent. natn. Rech. scient.*, **177**, 45–55.

MAETZ, J. and SKADHAUGE, E. (1968). Drinking rates and gill ionic turnover in relation to external salinities in the eel. *Nature, Lond.*, **217**, 371–373.

MARSHALL, E. K., JR. and GRAFFLIN, A. L. (1933). Excretion of inorganic phosphate by the algomerular kidney. *Proc. Soc. exp. Biol. Med.*, **31**, 44–46.

MASSEY, V. (1953). Studies on fumarase. 2. The effects of inorganic anions on fumarase activity. *Biochem. J.*, **53**, 67–71.

MAYER, N., MAETZ, J., CHAN, D. K. O., FORSTER, M. E. and CHESTER JONES, I. (1967). Cortisol, a sodium excreting factor in the eel (*Anguilla anguilla* L.) adapted to sea water. *Nature, Lond.*, **214**, 1118–1120.

MILNE, A. (1940). Some ecological aspects of the intertidal area of the estuary of the Aberdeenshire Dee. *Trans. R. Soc. Edinb.*, **50**, 107–140.

MOTAIS, R. (1961a). Les échanges de sodium chez un téléostéen euryhalin, *Platichthys flesus flesus* LINNÉ: cinétique de ces échanges lors des passages d'eau de mer en eau douce et d'eau douce en eau de mer. *C.r. hebd. Séanc. Acad. Sci., Paris*, **253**, 724–726.

MOTAIS, R. (1961b). Cinétique des échanges de sodium chez un téléostéen euryhalin (*Platichthys flesus flesus* LINNÉ) au cours de passages successifs eau de mer-eau douce-eau de mer, en fonction du temps de séjour en eau douce. *C.r. hebd. Séanc. Acad. Sci., Paris*, **253**, 2609–2611.

MOTAIS, R. (1967). Les mécanismes d'échanges ionique branchiaux chez les téléostéens. *Annls Inst. océanogr., Monaco*, **45**, 1–84.

MOTAIS, R., GARCIA ROMEU, F. and MAETZ, J. (1965). Mécanisme de l'euryhalinité. Etude comparée du flet (euryhalin) et du serran (sténohalin) au cours du transfert en eau douce. *C.r. hebd. Séanc. Acad. Sci., Paris*, **261**, 801–804.

MOTAIS, R., GARCIA-ROMEU, F. and MAETZ, J. (1966). Exchange diffusion effect and euryhalinity in teleosts. *J. gen. Physiol.*, **50**, 391–422.

MOTAIS, R., ISAIA, J., RANKIN, J. C. and MAETZ, J. (1969). Adaptive changes of the water permeability of the teleostean gill epithelium in relation to external salinity. *J. exp. Biol.*, **51**, 529–546.

MOTAIS, R. and MAETZ, J. (1964). Action des hormones neuro-hypophysaires sur les échanges de sodium (mesurés à l'aide du radio-sodium Na^{24}) chez un téléostéen euryhalin: *Platichthys flesus* L. *Gen. comp. Endocrinol.*, **4**, 210–224.

MOTAIS, R. and MAETZ, J. (1965). Comparaison des échanges de sodium chez un téléostéen euryhalin (le flet) et un téléostéen sténohalin (le serran) en eau de mer. Importance relative du tube digestif et de la branchie dans ces échanges. *C.r. hebd. Séanc. Acad. Sci., Paris*, **261**, 532–535.

MUNRO, H. N. (Ed.) (1970). *Mammalian Protein Metabolism*, Vol. IV, Academic Press, New York.

NATOCHIN, U. and BOCHAROV, G. (1962). Activation of sodium excreting cells in the gills of pink and chum salmon during adaptation to life in salt water. *Vop. Ikhtiol.*, **2**, 687–692.

NEEDHAM, A. E. (1957). Factors affecting nitrogen excretion in *Carcinides maenas* (PENNANT). *Physiologia comp. Oecol.*, **4**, 209–239.

O'CONNOR, J. D. and GILBERT, L. I. (1968). Aspects of lipid metabolism in crustaceans. *Am. Zool.*, **8**, 529–539.

OGLESBY, L. (1969). Inorganic components and metabolism; ionic and osmotic regulation: Annelida, Sipuncula and Echiura. In M. Florkin and B. T. Scheer (Eds), *Chemical Zoology*, Vol. IV. Academic Press, New York. pp. 211–310.

OIDE, M. and UTIDA, S. (1967). Changes in water and ion transport in isolated intestines of eel during salt adaptation and migration. *Mar. Biol.*, **1**, 102–106.

OIDE, M. and UTIDA, S. (1968). Changes in intestinal absorption and renal excretion of water during adaptation to sea water in the Japanese eel. *Mar. Biol.*, **1**, 172–177.

OLIVEREAU, M. (1954). Hypophyse et glande thyroide chez les poissons. Etude histophysiologique de quelques corrélations endocriniennes, en particulier chez *Salmo salar* L. *Annls Inst. océanogr., Monaco*, **29**, 95–296.

OLIVEREAU, M. (1967). Reactions observées chez l'anguille maintenue dans un milieu privé d'électrolytes, en particulier au niveau du système hypothalamo-hypophysaire. *Z. Zellforsch. mikrosk. Anat.*, **80**, 264–285.

OLIVEREAU, M. (1968). Etude cytologique de l'hypophyse du Muge, en particulier en relation avec la salinité extérieure. *Z. Zellforsch. mikrosk. Anat.*, **87**, 545–561.

OLIVEREAU, M. and CHARTIER-BARADUC, M. M. (1966). Action de la prolactine chez l'anguille intacte et hypophysectomisée II. Effets sur les électrolytes plasmatiques (sodium, potassium et calcium). *Gen. comp. Endocrinol.*, **7**, 27–36.

PACKER, R. K. and DUNSON, W. A. (1970). Effects of low environmental pH on blood pH and sodium balance of brook trout. *J. exp. Zool.*, **174**, 65–72.

PEAKER, M. (1971). Avian salt glands. *Phil. Trans. R. Soc. (B)*, **262**, 289–300.

PICKFORD, G. E. and PHILLIPS, J. G. (1959). Prolactin, a factor in promoting survival of hypophysectomized killifish in fresh water. *Science, N.Y.*, **130**, 454–455.

PICKFORD, G. E., ROBERTSON, E. E. and SAWYER, W. H. (1965). Hypophysectomy, replacement therapy, and the tolerance of the euryhaline killifish, *Fundulus heteroclitus*, to hypotonic media. *Gen. comp. Endocrinol.*, **5**, 160–180.

PIERCE, S. K. (1971). Volume regulation and valve movements by marine mussels. *Comp. Biochem. Physiol.*, **39A**, 103–177.

PIERCE, S. K. and GREENBERG, M. J. (1970). Free amino acid efflux from mussel hearts: a demonstration of volume regulation. *Am. Zool.*, **10**, 518.

POTTS, W. T. W. (1958). The inorganic and amino acid composition of some lamellibranch muscles. *J. exp. Biol.*, **35**, 749–764.

POTTS, W. T. W. and EVANS, D. H. (1966). The effects of hypophysectomy and bovine prolactin on salt fluxes in fresh water adapted *Fundulus heteroclitus*. *Biol. Bull. mar. biol. Lab., Woods Hole*, **131**, 362–368.

POTTS, W. T. W., FOSTER, M. A., RUDY, P. P. and PARRY HOWELLS, G. (1967). Sodium and water balance in the cichlid teleost, *Tilapia mossambica*. *J. exp. Biol.*, **47**, 461–470.

POTTS, W. T. W. and PARRY, G. (1964). *Osmotic and Ionic Regulation in Animals*, Pergamon Press, Oxford.

PROSSER, C. L., BISHOP, D. W., BROWN, F. A., JAHN, T. L. and WULFF, V. J. (1950). *Comparative Animal Physiology*, Saunders, Philadelphia.

READ, L. J. (1967). Enzymes of the ornithine-urea cycle in the chimaera *Hydrolagus colliei*. *Nature, Lond.*, **215**, 1412–1413.

READ, L. J. (1970). Nitrogen metabolism in chondrichthyean and agnathan fishes. In B. Schmidt-Nielsen and D. W. S. Kerr (Eds), *Urea and the Kidney*. Excerpta Medica Foundation, Amsterdam. pp. 23–34.

ROBERTSON, J. D. (1949). Ionic regulation in some marine invertebrates. *J. exp. Biol.*, **26**, 182–200.

Robertson, J. D. (1953). Further studies on ionic regulation in marine invertebrates. *J. exp. Biol.*, **30**, 277–296.

Robertson, J. D. (1954). The chemical composition of the blood of some aquatic chordates, including members of the Tunicata, Cyclostomata and Osteichthyes. *J. exp. Biol.*, **31**, 424–442.

Robertson, J. D. (1965). Studies on the chemical composition of muscle tissue. III. The mantle muscle of cephalopod molluscs. *J. exp. Biol.*, **42**, 153–175.

Robinson, R. R. (1950). Osmoregulation in surviving slices from the kidneys of adult rats. *Proc. R. Soc.* (*B*), **137**, 378–402.

Rudy, P. P. (1967). Water permeability in selected decapod Crustacea. *Comp. Biochem. Physiol.*, **22**, 581–589.

Sawyer, W. H. (1965). Evolution of neurohypophysial principles. *Archs Anat. microsc. Morph. exp.*, **54**, 295–312.

Sawyer, W. H. (1966). Diuretic and natriuretic responses of lungfish (*Protopterus aethiopicus*) to arginine vasotocin. *Am. J. Physiol.*, **210**, 191–197.

Schayer, R. W. (1964). A unified theory of glucocorticoid action. *Perspect. Biol. Med.*, **10**, 409–418.

Schimke, R. T. (1969). On the role of synthesis and degradation in regulation of enzyme levels in mammalian tissues. *Curr. Top. Cell Regul.*, **1**, 77–124.

Schmidt-Nielsen, K. and Fänge, R. (1958). Salt glands in marine reptiles. *Nature, Lond.*, **182**, 783–785.

Schoffeniels, E. (1950). La régulation de la pression osmotique et de la chlorémie chez les larves d'odonates. *Archs int. Physiol.*, **58**, 1–4.

Schoffeniels, E. (1960a). Rôle des acides aminés dans la régulation de la pression osmotique du milieu intérieur des insectes aquatiques. *Archs int. Physiol. Biochim.*, **68**, 507–508.

Schoffeniels, E. (1960b). Origine des acides aminés intervenant dans la régulation de la pression osmotique intracellulaire d'*Eriocheir sinensis* Milne Edwards. *Archs int. Physiol. Biochim.*, **68**, 696–698.

Schoffeniels, E. (1966). The activity of the L-glutamic acid dehydrogenase. *Archs int. Physiol. Biochim.*, **74**, 665–676.

Schoffeniels, E. (1968). The control of intracellular hydrogen transport by inorganic ions. *Archs int. Physiol. Biochim.*, **76**, 319–343.

Schoffeniels, E. and Gilles, R. (1963). Effect of cations on the activity of L-glutamic acid dehydrogenase. *Life Sci.*, **2**, 834–839.

Schoffeniels, E. and Gilles, R. (1970a). Osmoregulation in aquatic arthropods. In M. Florkin and B. T. Scheer (Eds), *Chemical Zoology*, Vol. V, Part A. Academic Press, New York. pp. 255–286.

Schoffeniels, E. and Gilles, R. (1970b). Nitrogenous constituents and nitrogen metabolism in arthropods. In M. Florkin and B. T. Scheer (Eds), *Chemical Zoology*, Vol. V, Part A. Academic Press, New York. pp. 199–227.

Schoffeniels, E. and Gilles, R. (1972). Ionoregulation and osmoregulation in Mollusca. In M. Florkin and B.T. Scheer (Eds), *Chemical Zoology*, Vol. VII. Academic Press, New York. pp. 393–420.

Schooler, J. M., Goldstein, L., Hartman, S. C. and Forster, R. P. (1966). Pathways of urea synthesis in the elasmobranch *Squalus acanthias*. *Comp. Biochem. Physiol.*, **18**, 271–281.

Sharratt, B. M., Bellamy, D. and Chester Jones, I. (1964). Adaptation of the silver eel (*Anguilla anguilla* L.) to sea water and to artificial media together with observations on the role of the gut. *Comp. Biochem. Physiol.*, **11**, 19–30.

Shaw, J. (1955a). The permeability and structure of the cuticle of the aquatic larva of *Sialis lutaria*. *J. exp. Biol.*, **32**, 330–352.

Shaw, J. (1955b). Ionic regulation and water balance in the aquatic larva of *Sialis lutaria*. *J. exp. Biol.*, **32**, 353–382.

Shaw, J. (1959a). Salt and water balance in the East African freshwater crab, *Potamon niloticus* (M. Edw.). *J. exp. Biol.*, **36**, 157–176.

Shaw, J. (1959b). The absorption of sodium ions by the crayfish *Astacus pallipes* Lereboullet. I. The effect of external and internal sodium concentrations. *J. exp. Biol.*, **36**, 126–144.

SHAW, J. (1960). The absorption of chloride ions by the crayfish *Astacus pallipes* LEREBOULLET. *J. exp. Biol.*, **37**, 557–572.

SHAW, J. (1961a). Studies on the ionic regulation in *Carcinus maenas* L. I. Sodium balance. *J. exp. Biol.*, **38**, 135–153.

SHAW, J. (1961b). Sodium balance in *Eriocheir sinensis* M. EDW. The adaptation of the Crustacea to fresh water. *J. exp. Biol.*, **38**, 154–162.

SHAW, J. and SUTCLIFFE, D. W. (1961). Studies on sodium balance in *Gammarus duebeni* LILLJEBORG and *G. pulex pulex* (L.). *J. exp. Biol.*, **38**, 1–15.

SIEBERS, D. (1972). Mechanismen der intrazellulären isosmotischen Regulation der Aminosäurekonzentration bei dem Flußkrebs *Orconectes limosus*. *Z. vergl. Physiol.*, **76**, 97–114.

SIEBERS, D., LUCU, C., SPERLING, K. R. and EBERLEIN, K. (1972). Kinetics of osmoregulation in the crab *Carcinus maenas*. *Mar. Biol.*, **17**, 291–303.

SIEGER, F., SIEGER, M., PRÜSSING, R. and ANDERS, F. (1969). Amino acids as stimulating agents of DNA replication in melanomas. II. Stimulation in *in situ* melanomas. *Experientia*, **25**, 778–779.

SIMPSON, J. W., ALLEN, K. and AWAPARA, J. (1959). Free amino acids in some aquatic invertebrates. *Biol. Bull. mar. biol. Lab., Woods Hole*, **117**, 371–381.

SKADHAUGE, E. and MAETZ, J. (1967). Etude *in vivo* de l'absorption intestinale d'eau et d'électrolytes chez *Anguilla anguilla* adapté à des milieux de salinité diverses. *C.r. hebd. Séanc. Acad. Sci., Paris*, **265**, 347–350.

SKOU, J. C. (1957). The influence of some cations on an adenosine triphosphatase from peripheral nerves. *Biochim. Biophys. Acta*, **23**, 394–401.

SLYKE, D. D. VAN, MCFADYEN, D. A. and HAMILTON, P. (1941). Determination of free amino acids by titration of the carbon dioxide formed in the reaction with ninhydrin. *J. biol. Chem.*, **141**, 671–680.

SMITH, H. W. (1930). The absorption and secretion of water and salts by marine teleosts. *Am. J. Physiol.*, **93**, 480–505.

SMITH, P. G. (1969a). The ionic relations of *Artemia salina*. I. *J. exp. Biol.*, **51**, 727–738.

SMITH, P. G. (1969b). The ionic relations of *Artemia salina*. II. *J. exp. Biol.*, **51**, 739–758.

SMITH, R. I. (1967). Osmotic regulation and adaptative reduction of water permeability in a brackish water crab *Rhithropanopeus harrisi* (Brachyura, Xanthidae). *Biol. Bull. mar. biol. Lab., Woods Hole*, **133**, 643–658.

SNOKE, J. E. (1956). Chicken liver glutamic dehydrogenase. *J. biol. Chem.*, **223**, 271–276.

SOHAL, R. S. and COPELAND, E. (1966). Ultrastructural variations in the anal papillae of *Aedes aegypti* (L.) at different environmental salinities. *J. Insect Physiol.*, **12**, 429–439.

STANLEY, J. G. and FLEMING, W. R. (1964). Excretion of hypertonic urine by a teleost. *Science, N.Y.*, **144**, 63–64.

STANLEY, J. G. and FLEMING, W. R. (1966). Effect of hypophysectomy on the function of the kidney of the euryhaline teleost, *Fundulus kansae*. *Biol. Bull. mar. biol. Lab., Woods Hole*, **130**, 430–441.

STANLEY, J. G. and FLEMING, W. R. (1967). Effect of prolactin and ACTH on the serum and urine sodium levels of *Fundulus kansae*. *Comp. Biochem. Physiol.*, **20**, 199–208.

STOBBART, R. H. (1967). The effect of some anions and cations upon the fluxes and net uptake of chloride in the larva of *Aedes aegypti* (L.) and the nature of the uptake mechanisms for sodium and chloride. *J. exp. Biol.*, **47**, 35–37.

STONER, L. C. and DUNHAM, P. B. (1970). Regulation of cellular osmolarity and volume in *Tetrahymena*. *J. exp. Biol.*, **53**, 391–399.

SUTCLIFFE, D. W. (1961). Studies on salt and water balance in caddis larvae (Trichoptera). I. Osmotic and ionic regulation of body fluids in *Limnephilus affinis* CURTIS. *J. exp. Biol.*, **38**, 501–520.

SUTCLIFFE, D. W. (1962). The composition of haemolymph in aquatic insects. *J. exp. Biol.*, **39**, 325–343.

SUTCLIFFE, D. W. (1963). The chemical composition of haemolymph in insects and some other arthropods in relation to their phylogeny. *Comp. Biochem. Physiol.*, **9**, 121–135.

SUTCLIFFE, D. W. (1967a). Sodium regulation in the amphipod *Gammarus duebeni* from brackish water and fresh water localities in Britain. *J. exp. Biol.*, **46**, 529–550.

SUTCLIFFE, D. W. (1967b). Sodium regulation in the freshwater amphipod, *Gammarus pulex* (L.). *J. exp. Biol.*, **46**, 499–518.

SUTCLIFFE, D. W. (1968). Sodium regulation and adaptation to fresh water in gammarid crustaceans. *J. exp. Biol.*, **48**, 359–380.

SUTCLIFFE, D. W. and SHAW, J. (1967). The sodium balance mechanism in the freshwater amphipod, *Gammarus lacustris* SARS. *J. exp. Biol.*, **46**, 519–528.

SUTCLIFFE, D. W. and SHAW, J. (1968). Sodium regulation in the amphipod *Gammarus duebeni* LILLJEBORG from freshwater localities in Ireland. *J. exp. Biol.*, **48**, 339–358.

THUET, P., MOTAIS, R. and MAETZ, J. (1968). Les mécanismes de l'euryhalinité chez le crustacé des salines *Artemia salina*. *Comp. Biochem. Physiol.*, **26**, 793–818.

TODD, M. E. (1964). Osmotic balance in *Hydrobia ulvae* and *Potamopyrgus jenkinsi* (Gastropoda hydrobiidae). *J. exp. Biol.*, **41**, 665–677.

TOMKINS, G. M., THOMPSON, S., HAYASHI, S., GELEHRTER, T., GRANNER, D. and PETERKOFSKY, B. (1966). Tyrosine transaminase induction in mammalian cells in tissue culture. *Symp. quant. Biol.*, **31**, 349–360.

TONOMURA, Y., SEKIYA, K. and IMAMURA, K. (1962). The optical rotatory dispersion of myosin A. I. Effect of inorganic salts. *J. biol. Chem.*, **237**, 3110–3115.

TOSTESON, D. C. and HOFFMAN, J. F. (1960). Regulation of cell volume by active cation transport in high and low potassium sheep red cells. *J. gen. Physiol.*, **44**, 169–194.

TUSTANOFF, E. R. and STEWART, H. B. (1965). The effect of neutral salts on particle preparations from rat liver. II. Effects of neutral salts on tricarboxylic acid cycle reactions. *Can. J. Biochem.*, **43**, 359–372.

USSING, H. H. (1947). Interpretation of the exchange of radiosodium in isolated muscle. *Nature, Lond.*, **160**, 262–263.

UTIDA, S., HIRANO, T., OIDE, M., KAMIYA, M., SAISYU, S. and OIDE, H. (1966). Na^+–K^+ activated adenosinetriphosphatase in gills and Cl^--activated alkaline phosphatase in intestinal mucosa with special reference to salt adaptation of eels. *Abstr. Pap., Proc. 11th Pacif. Sci. Congr., Tokyo*, **7**, 55.

VINCENT-MARIQUE, C. and GILLES, R. (1970a). Changes in the amino-acid concentration in blood and muscle of *Eriocheir sinensis* during hypoosmotic stress. *Life Sci.*, **9**(I), 509–512.

VINCENT-MARIQUE, C. and GILLES, R. (1970b). Modification of the amino acid pool in blood and muscle of *Eriocheir sinensis* during osmotic stress. *Comp. Biochem. Physiol.*, **35**, 479–485.

VIRKAR, R. A. and WEBB, K. L. (1970). Free amino acid composition of the soft-shell clam *Mya arenaria* in relation to salinity of the medium. *Comp. Biochem. Physiol.*, **32**, 775–784.

VOLLRATH, L. (1966). The ultrastructure of the eel pituitary at the elver stage with special reference to its neurosecretory innervation. *Z. Zellforsch. mikrosk. Anat.*, **73**, 107–131.

VOOYS, G. G. N. DE (1968). Formation and excretion of ammonia in Teleostei. I. Excretion of ammonia through the gills. *Archs int. Physiol. Biochim.*, **76**, 268–273.

WARREN, J. C. and CHEATUM, S. G. (1966). Effect of neutral salts on enzyme activity and structure. *Biochemistry*, **5**, 1702–1707.

WARREN, J. C., STOWRING, L. and MORALES, M. (1966). The effect of structure-disrupting ions on the activity of myosin and other enzymes. *J. biol. Chem.*, **241**, 309–316.

WATTS, D. C. and WATTS, R. L. (1966). Carbamoyl phosphate synthetase in the Elasmobranchii: osmoregulatory function and evolutionary implications. *Comp. Biochem. Physiol.*, **17**, 785–798.

6. MECHANISMS OF EVOLUTION AND POPULATION GENETICS

J. L. Gooch

(1) Introduction

Most of the key insights into the genetics of the evolutionary process have been derived from investigations of a relatively few terrestrial species representing a fraction of protist, plant, and animal taxa. The dual purpose of modern evolutionary genetics has been to perfect and advance these insights and to determine the universality of genetic phenomena discovered in this narrow taxonomic focus. As a corollary to the recognition of the evolutionary kinship of all organisms, evolutionists have generally assumed that the genetic systems of all organisms above the organizational level of the Monera are broadly similar. Research supports this assumption. Mendelian segregation based on chromosomal reshuffling has been discovered throughout the biological spectrum. Moreover, seemingly exceptional genetic mechanisms, such as polyploidy, chromosome diminution, and novel means of sex determination are now viewed as variations on a common theme.

There has never been any reason to postulate a difference in the genetic systems of marine and terrestrial organisms. During the heyday of *Drosophila* transmission genetics, eye mutants of the estuarine amphipod *Gammarus chevreuxi* figured in investigations of gene transmission and interaction (summarized in Sexton and Clark, 1936). However, the apparent uniformity of the open ocean, with its less prominent barriers to movement and dampened environmental fluctuations, provided the basis for speculation that evolution and speciation might be subtly different there. Zeuner (1958) argued from fossil evidence that the tempo of evolution is much slower in the sea than on land. Sympatric speciation or speciation by distance over a continuously occupied range has been proposed for marine organisms (Kohn, 1960; Wieser, 1960; Day, 1963). Buzzati-Traverso (1958) suggested that unidirectional gene flow along defined current systems promotes speciation in planktonic organisms (the contrary argument, that such gene flow tends to maintain genetic continuity, is developed by Scheltema, 1971).

It is increasingly evident that barriers to free movement are widespread in the sea. These include temperature and salinity differences of water masses, low nutrient regions, inimical substrate types, countervening currents, and the dwarfing of dispersal efforts by oceanic distances. Nevertheless, the sea is probably less heterogeneous than the terrestrial environment generally, although it is debatable whether it is more uniform than many terrestrial environments, such as the far-ranging deciduous forest of eastern North America prior to European settlement.

The potential of genetic studies for illuminating processes of evolution and ecological dynamics in the sea has been stressed by Buzzati-Traverso (1958, 1960), Ray (1960), and Battaglia (1965). The difficulties besetting the marine

geneticist have been formidable. In the editorial introduction of *Speciation in the Sea* (HARDING and TEBBLE, 1963, p. 5) it is stated:

> 'The nature of the sea as an environment imposes experimental limitations which never arise in terrestrial habitats and which are difficult to overcome. The almost complete lack of genetic information and the very great difficulties in the way of obtaining it, makes the distinction between phenotypic and genotypic differences very difficult to assess.'

Of course, much is known of physiological variation in geographically separated populations, but the genetic components of this variation are difficult to disentangle from physiological adaptive mechanisms. Conventional genetic analysis entails (i) the discovery of phenotypic variants of potential genetic origin, and (ii) testing for Mendelian segregation of the traits through controlled breeding. The

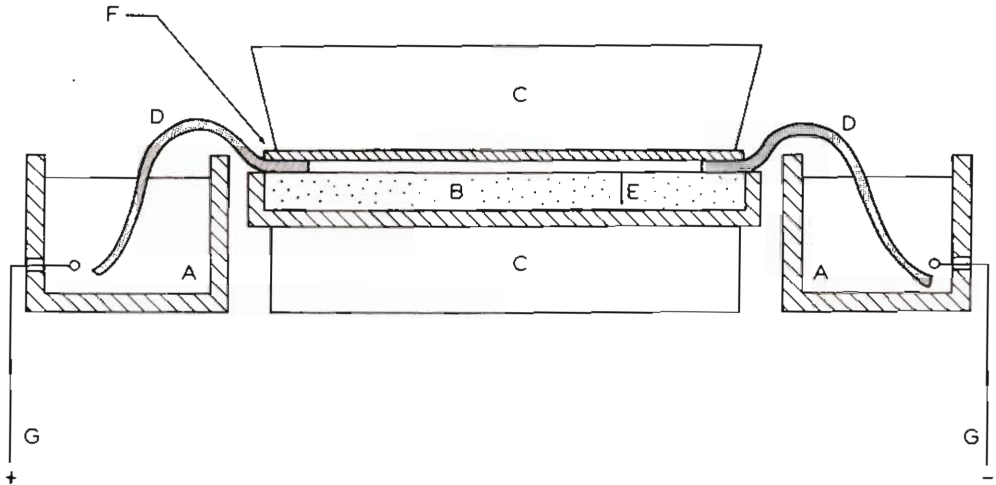

Fig. 6-1: Apparatus for horizontal starch or polyacrylamide electrophoresis. A: Electrode trays filled with buffer; B: gel 5 to 10 mm thick; C: ice tray for cooling; D: paper or sponge wicks; E: slit for insertion of samples; F: thin glass plate; G: connections to DC power supply. (Original.)

literature employing these methods is sparse, and primarily involves a few extensively studied species. Nevertheless, it can be predicted that marine genetics will make large strides in the immediate future. This is partly due to increased scientific efforts along all fronts of marine biology and through increasing sophistication of methods of cultivating marine organisms through their life cycle (Volume III).

Perhaps most important are the techniques of electrophoresis genetics that have become available during the last decade. The staining of specific proteins on starch, agar, polyacrylamide, and cellulose acetate strips following electrophoresis reveals characteristic migration patterns (Fig. 6-1). Protein mobility is largely determined by molecular configuration and electrical charge, which in turn are dependent on the amino-acid sequence. Since the latter is the direct product of the genetic template, the electrophoresis phenotype lies very close to the core of genetic information of

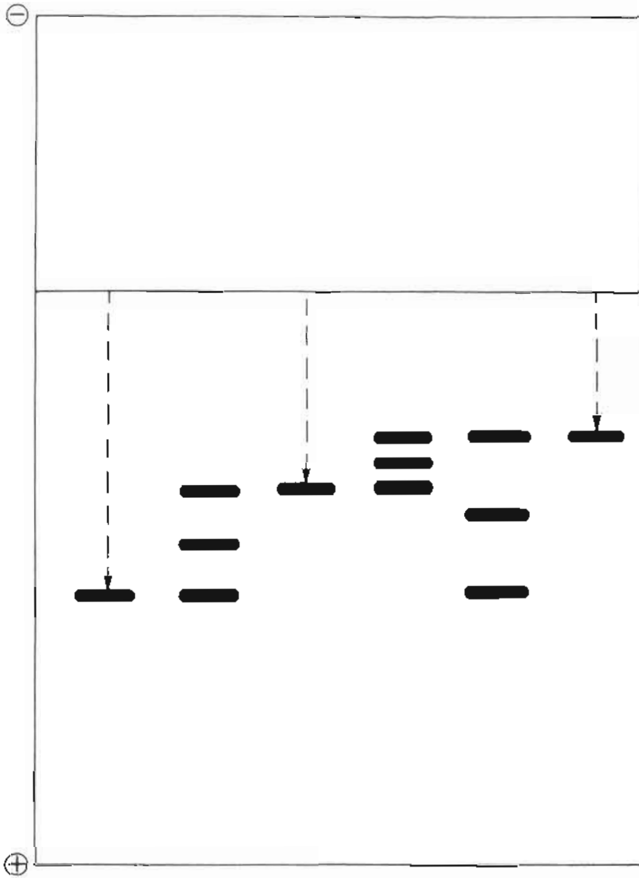

Fig. 6-2: Starch gel with stained band patterns (zymogram). Depicted are the segregating protein products of a single triallelic locus. Broken arrows indicate mobilities for homozygous individuals. The three heterozygous combinations are shown with intermediate hybrid bands, indicating that the primary gene products are probably polypeptides associated as dimers. (Original.)

individuals. It was recognized that certain patterns of migration of a specific protein could be ascribed to the allelic variation of a controlling gene locus (SCHWARTZ, 1960; Fig. 6-2). Electrophoresis genetics can and does reveal cryptic genetic variability with relatively little ambiguity of interpretation, and it bypasses the necessity of successful controlled mating (although this is extremely desirable when possible) in organisms that cannot be bred in the laboratory and those with unconscionably long life cycles. The method detects one-locus polymorphisms of possible adaptive significance and of value as marker genes for populations, stocks, and races. Unlike conventional marine genetics, it is also possible to sample significant portions of the genome—20 to 30 loci—for estimates of genetic variability

and studies of interactions between loci. When intraspecific genetic variation is known, the amount of genetic differentiation between related species can be estimated (Table 6-1), and this has been done in a preliminary fashion for one marine species-pair of asteroids (SCHOPF and MURPHY, 1973).

Through the introduction of electrophoresis genetics it can be asserted that the number of significant papers on population genetics of marine organisms has doubled during the last five years. Moreover, it will probably double again when investigations underway in numerous laboratories have reached completion. The present picture is fragmentary, and no principles unknown in terrestrial studies have emerged.

This chapter first presents a discussion and evaluation of a portion of the literature that deals with genetic aspects of evolution in oceans and coastal waters. The fact that this literature has never been brought together before necessitates an organizational framework. The remainder of the chapter is a treatment of principles of evolutionary genetics in relation to marine biota.

Table 6-1

Electrophoresis genetics as a tool in assigning individuals to closely related species in *Drosophila*. Each number represents the percentage of enzyme loci that are diagnostic between two species. Above the diagonal: 99% or higher probability of correct diagnosis at each locus; below the diagonal: 99·9% probability (After AYALA and POWELL, 1972; reproduced by permission of National Academy of Sciences, USA)

	D. pseudoobscura	*D. persimilis*	*D. miranda*	*D. azteca*
D. pseudoobscura		15·4	27·8	47·6
D. persimilis	3·8		22·2	52·4
D. miranda	16·7	16·7		38·9
D. azteca	33·3	28·6	33·3	

(2) Population Genetics Studies of Marine Taxa

(a) Gastropoda

The dogwhelk *Nucella* (= *Purpura*; *Thais*) *lapillus* has a widespread Atlantic boreal and temperate distribution (MOORE, 1936) as an intertidal carnivore of rocky shores (FEARE, 1970). Shell colour, sculpture, size, and thickness are often variable geographically. External morphology as affected by diet and selective environmental factors has received considerable attention (COLTON, 1916, 1922; AGERSBORG, 1929; MOORE, 1936; KITCHING and co-authors, 1966; LARGEN, 1966; BERRY and CROTHERS, 1968; FEARE, 1970).

Populations of *Nucella lapillus* along the Breton peninsula of France are chromosomally polymorphic (STAIGER, 1954, 1957). Metaphase and anaphase I figures of individuals from wave-exposed shores usually have a haploid number of 13, and

those from sheltered shores typically are of an $n = 18$ configuration. Eight acrocentric chromosomes of the two forms are identical; however, 5 metacentric chromosomes of form 13 are represented by 10 acrocentrics in form 18. Some or all of the acrocentrics may actually be telocentrics (BROWN, 1972). Chromosomal polymorphism arising from Robertsonian alterations, involving centric fusion or fission, is not uncommon within species. Form 13 can be derived from form 18 by fusion through or near the centromeres of the 10 acrocentric (or telocentric) chromosomes. The genomes of the two forms would be, of course, practically identical. Heritable changes could occur through (i) alteration of gene action due to positional rearrangement; (ii) loss of genic material during breakage; or (iii) loss of acentric fragments. Recombination might also occur more freely in form 18.

STAIGER (1954, 1957) found that many populations of *Nucella lapillus* are polymorphic for chromosome number, with individuals possessing a range of intermediate karyotypic counts. Usually such populations occupy habitats intermediate between maximally exposed and sheltered. Gradients of exposure on the Breton coast were found to be paralleled by clines in chromosome number. Samples of snails collected only 5 m apart sometimes differed in karyotype. In the vicinity of Roscoff (France), microgeographic chromosomal variability was marked, but between Primel and Locquirec form 13 predominated in all habitats. The adaptive value of chromosomal polymorphism is thus not invariably linked to wave exposure, or else form 18 has been unable to colonize the Primel-Locquirec region. HOXMARK (1970) examined chromosomes of small samples of *N. lapillus* at Roscoff and also at three widely separated localities on the western and southern coasts of Norway. At Roscoff, form 18 is most numerous, as STAIGER had also found nearly two decades earlier, although some aberrant karyotypes with fewer than 13 and more than 18 chromosomes were recorded. Most metaphase figures from Norwegian localities show 13 bivalents, but again chromosome counts are quite variable. At Espegrend, near Bergen (Norway), four stations exhibiting an exposure gradient comparable to those of Brittany were collected. Form 13 strongly predominates on all shores irrespective of exposure. Obviously the correlation of karyotype with wave exposure is far from universal along European coasts.

The karyotyped Breton populations of *Nucella lapillus* were also measured for shell size and, by means of relative shell/body weight, for shell thickness (STAIGER, 1957). Shell size was greatest near Roscoff in populations homogeneous for either 13 n or 18 n karyotypes. However, the chromosomally polymorphic populations characteristic of intermediate exposure had the thickest shells, possibly as a heterotic effect. Nevertheless, it is not clear that chromosomal polymorphism influences shell size or thickness, since these attributes could arise from differences in diet, predation, or other factors that might occur along the exposure gradient. Roscoff ($n = 18$) and the nearby Ile Verte ($n = 13$) were investigated for shell weight by HOXMARK (1971). These populations, contrasting in exposure and karyotype, differ little in mean shell weight, but Roscoff individuals of a given shell weight are much more heavy-bodied. On the other hand, two form 13 Norwegian populations, one sheltered and the other exposed, are uniform for shell weight/body weight ratio. This evidence does suggest that karyotype differences accentuate difference in shell morphology and points out the need for additional study of this species.

Variation in shell morphology and colour is also characteristic of other gastropods. Phenotypic polymorphism is often particularly striking in limpets and species of the large genus *Littorina*. Intraspecific pattern and shape differences have been discussed in the limpet genera *Patina* (GRAHAM and FRETTER, 1949), *Patella* (FISCHER-PIETTE, 1935; EVANS, 1947, 1953), and *Acmaea* (TEST, 1945). It has been difficult to separate genetic from environmental components of variability in field studies. The role of intrapopulation genetic differentiation is most evident in experiments on *Acmaea digitalis* made near Cape Arago on the coast of Oregon,

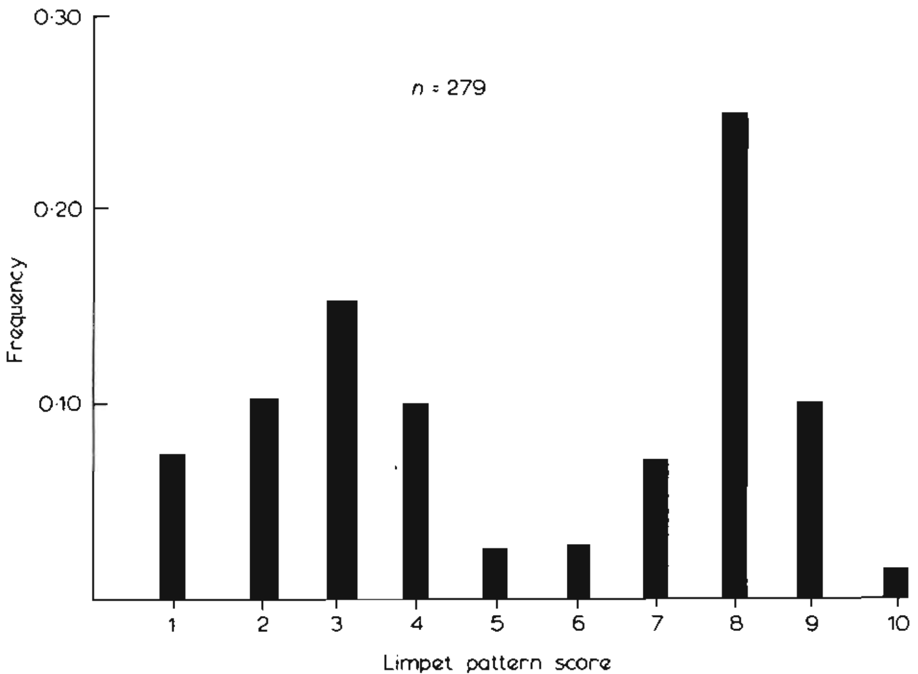

Fig. 6-3: *Acmaea digitalis*. Bimodal frequency distribution of patterns in individuals larger than 10·0 mm at Cape Arago, Oregon (USA). Low scores denote lightest limpets. (After GIESEL, 1970; modified; reproduced by permission of Society for the Study of Evolution.)

USA (GIESEL, 1970). Limpets exist as two form complexes at this rocky intertidal locality: one form is light in shell colour and is associated with beds of gooseneck barnacles *Pollicipes polymerus*; the other is darkly patterned and occurs on rock faces in the high intertidal zone. Each form is cryptic only on its own substrate. Shell colour and pattern are probably controlled genetically to some degree, since new growth of experimentally broken shells has the same colour. Limpets less than 4·0 mm in length of *Pollicipes*-, rock- and intermediate forms were found to be indiscriminately mixed, but successively larger size classes exhibited increasing bimodality of distribution, until individuals over 10 mm were sharply confined to colour-matching substrates (Fig. 6-3). The development of the bimodal distribution

of colour forms is ascribed, in part, to disruptive selection. Oystercatchers and other avian predators were most numerous when the distributional bias was strongest, indicating that the chief agent of disruptive selection has probably been the differential destruction of non-substrate matching individuals. Under such a selection regime it might be expected that individuals would become behaviourally polymorphic, each preferring substrates on which their shells display crypsis. This has, in fact, happened according to GIESEL's experiments with small and transplanted limpets. Individuals showed behavioural affinities to matching substrates and actively sought them out. Prolonged disruptive selection should also promote genetic differentiation and isolation of the substrate forms. This, however, has not occurred in the *A. digitalis* under study.

Among gastropods the genus *Littorina* has received disproportionate attention from evolutionary geneticists. *L. picta* inhabits exposed locations of the Hawaiian supratidal zone. It displays colour variability and is strongly polymorphic for sculpture, varying continuously from virtually smooth to strongly ribbed. STRUHSAKER (1968) studied the sculpture morphs in relation to environmental and physiological factors and interpreted the variation as an example of adaptive, balanced polymorphism. Smooth shells predominate on low-angle rocky beaches subjected to strong horizontal wave action, and sculptured forms are concentrated on high-angle beaches more protected from the surf. Gradational forms occupy intermediate locations. Observations of one transect suggest that post-veligers, just settled from the planktonic larval phase, and juveniles are less segregated by habitat than adults. Selection rather than larval choice is thus indicated (Fig. 6-4).

Many authors have noted morphological variation in prosobranch gastropods that correlate with environment, but have been unable to assess the relative genetic and environmental contributions to the variation. STRUHSAKER (1968), however, was able to cultivate larval *Littorina picta*, and established that progeny inherit the sculpture pattern of adults. Morphs reared under identical experimental conditions also differ physiologically in appropriate ways, e.g., extreme sculptured forms tolerate high temperature and salinity better than smooth forms, but withstand immersion in sea water less well.

The life cycles of *Acmaea digitalis* and *Littorina picta* have fundamental similarities. Pelagic larvae of both species appear to randomly colonize spatially and temporally heterogeneous rocky coasts. Subsequent selection brings older age classes of snails in harmony with their micro-environment through mortality of ill-matching morphs, operating by visually hunting predators in *A. digitalis* and through a combination of physical factors in *L. picta*. Maintenance of the polymorphisms is assured by heterogeneity of the environment and by the species' continued capacity to withstand the wasteful mortality that attends the accommodation of each generation to this heterogeneity. The proposed di-locus epistasis in *Mytilus edulis* (MITTON and co-authors, 1973) and the shifting heterozygote frequency in *Modiolus demissus* (KOEHN and co-authors, 1973) discussed below probably also reflect the accommodation of surviving larval or young adult genotypes to environmental heterogeneity.

The boreally distributed littorinids of the European and North American coasts, *Littorina obtusata* and *L. saxatilis*, also inhabit heterogeneous intertidal areas; but they have abolished larval dispersal and hence avoid the huge mortality of random

larval settlement. In this they resemble *Nucella lapillus*; also like that species they exhibit considerable intra- and interpopulation phenotypic variability. *L. obtusata* populations are remarkably polymorphic for colour. Green, yellow, orange, brown and black morphs, or 'varieties', commonly occur side-by-side. The relationships of the species and some of its morphs to environmental factors have been discussed by DAUTZENBERG and FISHER (1915), BARKMAN (1956), and SACCHI in a series of papers (1961 a, b, 1963, 1964, 1966). SACCHI demonstrated that morphs differ in desiccation resistance (1963), activity in full daylight (1961a,

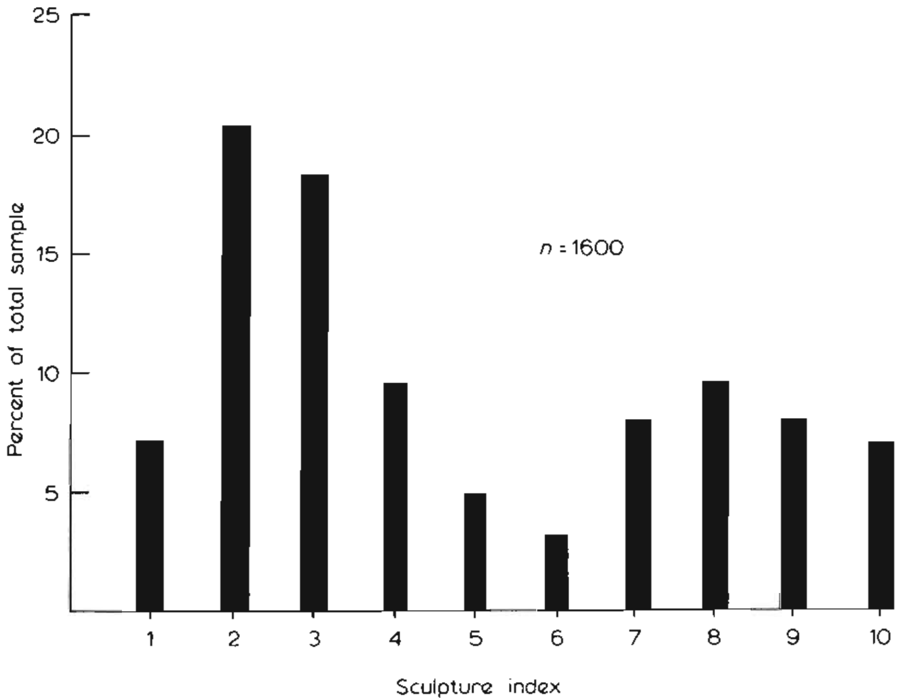

Fig. 6-4: *Littorina picta*. Bimodal frequency distribution of populations of adult individuals from Oahu, Hawaii. Low scores indicate smoothest shells. (After STRUHSAKER, 1968; redrawn; reproduced by permission of Society for the Study of Evolution.)

1963) and other behavioural attributes; also in distribution on wave-exposed and sheltered shores (1961b), and in susceptibility to predation by gulls (1961a). Morph distribution and behaviour were generally similar on opposite sides of the English Channel, at Roscoff, France, and at Plymouth, England (1966).

Littorina saxatilis is ovoviviparous and therefore seems to lack effective means of dispersal. Nevertheless, it ranges from the Black Sea westward to the Atlantic coasts of Europe, Iceland, Greenland, and North America south to Virginia. Shell colour and sculpture are highly polymorphic within populations, and have led to the description of several 'varieties' in European populations (FISCHER-PIETTE and GAILLARD, 1961; FISCHER-PIETTE and co-authors, 1964; JAMES, 1968) with charac-

teristic microgeographic distributions. Several electrophoresis-genetics studies have involved *L. saxatilis*. Thirteen Danish populations were surveyed for a two-allele system interpreted as a haemoglobin locus (WIUM-ANDERSEN, 1970) in *L. rudis* (= *L. saxatilis*). Most samples ($n = 17$ to 63) conform to the Hardy–Weinberg equilibrium. The allele HbI is always highest in frequency, but the frequency varies irregularly among the Danish islands. An apparent cline of increasing frequency was found from south to north along the western coast of Jutland. Bands derived from gels stained for general protein are highly variable in pattern, in contrast to relatively constant patterns found in *L. littorea* and *L. striata*. Band variability is taken by the author to reflect the underlying genetic variability of *L. rudis* in the inshore estuarine areas where samples were taken.

The interpretation of band variability is problematical when it does not conform to the characteristic patterns of protein products of homozygotes and heterozygotes. Increasing band complexity over a geographic transect may indicate the presence of new alleles, but it may also represent *in vivo* or *in vitro* isozymic instability springing from physical or physiological causes. Conceivably, such patterns may also be caused by changing patterns of gene activation and repression, which might correlate with environmental changes.

SNYDER and GOOCH (1973) have assayed seven populations of *Littorina saxatilis* on or near Cape Cod, Massachusetts (USA), for allele frequencies at a diallelic peptidase locus (Pep) and a triallelic phosphoglucose isomerase locus (Pgi), using standard starch gel techniques. Genotype distributions of most populations fit the Hardy–Weinberg equilibrium. A population inhabiting a shingle beach near the Woods Hole municipal dock was very significantly deficit for heterozygotes at the Pgi locus in 1971 samplings, but not so in 1972. These findings may indicate intermittent selection against heterozygotes. The Wahlund effect is an unlikely explanation in this sedentary species (see p. 363).

Contingency table chi-square tests indicate very significant interpopulation heterogeneity in allele frequency at the Pgi locus and not quite significant heterogeneity at the Pep locus. Allele frequencies do not vary clinally, nor are systematic differences apparent between stations in Buzzards Bay or the colder water of Cape Cod Bay. Either natural selection or random drift could account for the Pgi heterogeneity. Drift is the less likely alternative, since (i) populations are large (although bottlenecking in winter or during severe storms may occur), (ii) the populations are highly polymorphic (five segregating loci detected; GOOCH, unpublished), (iii) allele frequencies at the Pep locus have not differentiated, and (iv) the frequencies of colour and sculpture morphs are similar at many sites.

Littorina saxatilis occurs sympatrically with *L. littorea* and *L. obtusata* from Labrador to well south of Cape Cod. *L. littorea* contrasts with its congeners in its possession of a planktonically dispersed larva. BERGER (1972) tested the hypothesis that demes should be more genetically isolated in species in which dispersal is limited. Two to three polymorphic esterase loci were detected in the three littorinid species, and allele frequencies were obtained from 14 stations from Prince Edward Island (Canada) to Buzzards Bay, south of Cape Cod (USA). An index of genetic heterogeneity amounting to summed genotype differences of pairs of population samples averaged over number of loci was used to compare interpopulation and interspecific differences. About three to four times greater interpopulation heterogeneity was found in *L. saxatilis* and *L. obtusata* than in *L. littorea*.

Table 6-2

Nassarius obsoletus. Individuals from Long Island Sound (USA) partitioned into size classes. Genetic data for Lc-1 locus. All size classes conform to the Hardy–Weinberg equilibrium. Allele frequency is homogeneous over size classes $\chi^2_{(5)} =$ 1·8, p >0·8) (After GOOCH and co-authors, 1972; reproduced by permission of Biological Bulletin)

Size class (mm)	Sample size	Allele frequency		Genotype distribution			Chi-square accord with Hardy–Weinberg equilibrium
		$Lc\text{-}1^{1\cdot 01}$	$Lc\text{-}1^{1\cdot 05}$	1·01/1·01	1·01/1·05	1·05/1·05	
8–9	32	0·578	0·422	9	19	4	1·5, p >0·20
10–11	65	0·523	0·477	16	36	13	0·8, p >0·30
12–13	78	0·545	0·455	24	37	17	0·1, p >0·70
14–15	95	0·584	0·416	34	43	18	0·4, p >0·50
16–17	68	0·566	0·434	20	37	11	0·9, p >0·30
18–21	30	0·600	0·400	11	14	5	0·02, p >0·80
Pooled	368	0·561	0·439	114	186	68	0·3, p >0·50

Table 6-3

Littorina saxatilis. Averaged probabilities* of genotypic identity between population samples (lower half matrix) and occurrence of a unique genotype (upper half matrix) for the Pgi and Pep loci. Indices after HEDRICK (1971). Cape Cod localities: NAU, Naushon Island; WEE, Weepecket Island; MUN, Municipal dock beach at Woods Hole; NOB, Nobska Point; BAR, Barlow's Landing; BSB, Barnstable; NAH, Nahant, north of Boston (After SNYDER and GOOCH, 1973; reproduced by permission of Springer-Verlag, Berlin)

	NAU	WEE	MUN	NOB	BAR	BSB	NAH
NAU		*·01*	*·04*	*·05*	*·05*	·04	*·00*
		·05	*·00*	*·05*	*·15*	·11	*·00*
WEE	*·96*		*·08*	*·05*	*·05*	·04	*·02*
			·01	*·00*	*·05*	·04	*·01*
MUN	*·76*	*·87*		*·03*	*·00*	·00	*·00*
				·05	*·08*	·19	*·04*
NOB	*·95*	*·89*	*·62*		*·00*	·00	*·02*
					·16	·02	*·05*
BAR	*·91*	*·97*	*·90*	*·81*		·09	*·04*
						·17	*·05*
BSB	·88	·81	·45	·94	·66		·04
							·02
NAH	*·87*	*·94*	*·93*	*·77*	*·95*	·62	

*Non-italicized values represent Pgi locus only

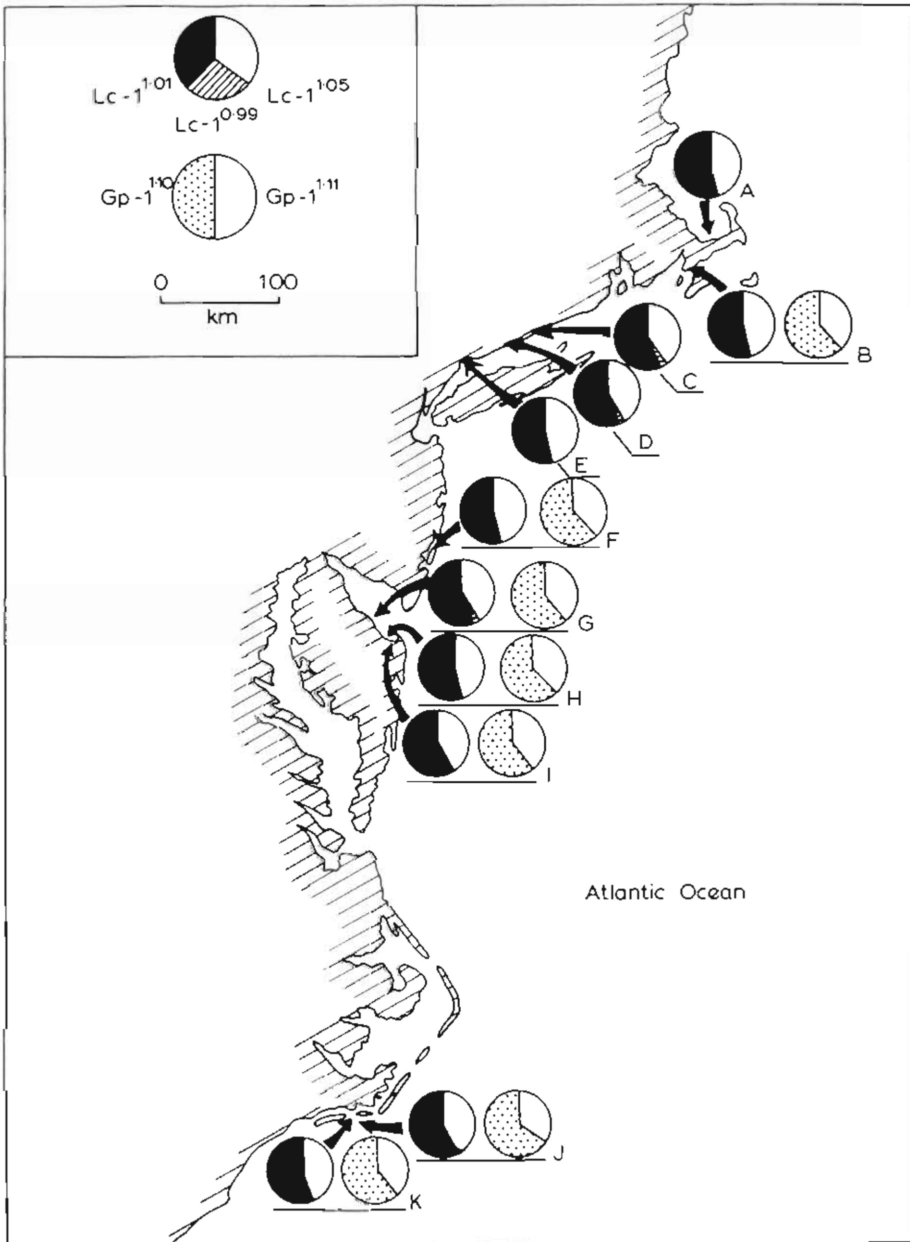

Fig. 6-5: *Nassarius obsoletus*. Transect from Cape Cod, Massachusetts, to Beaufort, North Carolina (USA). Allele frequencies at the Lc-1 (lactate dehydrogenase) locus are indicated as proportions of shoreward circles and those of the Gp-1 (general protein) locus by the seaward circles. (After GOOCH and co-authors, 1972; reproduced by permission of *Biological Bulletin*.)

Additional weight is given to the hypothesis by comparing interdeme heterogeneity in *Littorina saxatilis* (SNYDER and GOOCH, 1973) and in *Nassarius obsoletus* (GOOCH and co-authors, 1972). *N. obsoletus* is an abundant intertidal and subtidal prosobranch with a range from the Gulf of St. Lawrence, Canada, to northern Florida, USA (SCHELTEMA, 1964). There is a pelagic larva capable of remaining waterborne for several weeks at least (SCHELTEMA, 1965, 1967); in this regard *N. obsoletus* is the analog of *L. littorea*. Eleven localities along a transect of about 1000 km from Cape Cod to Beaufort, North Carolina were surveyed for allele frequencies at a lactate dehydrogenase locus (Lc-1) and 7 of the stations were also sampled at general protein locus (Gp-1) (Fig. 6-5). All samples conform to Hardy–Weinberg expectations and allele frequencies at both loci are statistically homogeneous over shell size classes (and hence age classes) in selected populations, and also homogeneous over interpopulation comparisons (Table 6-2). These data suggest that there is minimum genetic differentiation of widely separated adult populations, and gene flow owing to pelagic transport of the veliger larva may reasonably be taken as the cause.

Table 6-4

Nassarius obsoletus. Averaged probabilities of genotypic identity between population samples (lower half matrix) and the occurrence of a unique genotype (upper half matrix) for the Lc-1 and Gp-1 loci. Localities A–K as in Fig. 6–5; L is Assateague, Maryland, USA (After SNYDER and GOOCH, 1973; reproduced by permission of Springer-Verlag, Berlin)

	A	B	C	D	E	F	G	H	I	J	K	L
A		·00	·01	·01	·00	·00	·03	·00	·00	·00	·02	·00
		·00	·00	·00	·00	·00	·00	·00	·00	·00	·00	·00
B	·99		·01	·01	·00	*·00*	*·01*	*·00*	*·00*	*·00*	*·02*	·00
			·00	·00	·00	*·06*	*·00*	*·00*	*·00*	*·00*	*·00*	*·00*
C	·96	·98		·01	·00	·00	·01	·00	·00	·00	·02	·00
				·01	·01	·01	·00	·01	·01	·01	·00	·01
D	·96	·99	·99		·00	·00	·01	·00	·00	·00	·02	·00
					·01	·01	·00	·01	·01	·01	·01	·01
E	·99	·99	·98	·99		·00	·03	·00	·00	·00	·02	·00
						·00	·00	·00	·00	·00	·00	·00
F	·87	*·93*	·94	·97	·93		*·08*	*·03*	*·03*	*·01*	*·06*	*·04*
							·00	*·00*	*·00*	*·00*	*·00*	*·00*
G	·90	*·97*	·97	·99	·96	*·97*		*·00*	*·00*	*·00*	*·01*	*·00*
								·01	*·01*	*·01*	*·01*	*·01*
H	·89	*·95*	·95	·98	·95	*·99*	*·99*		*·00*	*·00*	*·02*	*·00*
									·00	*·00*	*·00*	*·00*
I	·96	*·99*	·99	·99	·99	*·97*	*·99*	*·98*		*·00*	*·02*	*·00*
										·00	*·00*	*·00*
J	·98	*·99*	·99	·99	·99	*·95*	*·96*	*·96*	*·99*		*·02*	*·00*
											·00	*·00*
K	·99	*·99*	·98	·98	·99	*·92*	*·96*	*·94*	*·98*	*·98*		*·02*
												·00
L	·91	*·98*	·99	·99	·96	*·98*	*·99*	*·99*	*·99*	*·98*	*·96*	

*Italicized values represent values averaged over loci, other values for only Lc-1 locus

The regional scale of genetic differentiation in *Littorina saxatilis* and *Nassarius obsoletus* is most directly compared through an index of genetic similarity. Table 6-3 records the probability of genotypic identity of HEDRICK (1971) for all pairwise population comparisons in *L. saxatilis*, and Table 6-4 does the same for *N. obsoletus* (SNYDER and GOOCH, 1973). Index probability values are variable and appear not to decrease over short distances in *L. saxatilis*. By contrast, they are uniformly high over long distances in *N. obsoletus*.

(b) Bivalvia

The blue mussel *Mytilus edulis* has long been a favoured subject of experimental ecology and physiology. This abundant intertidal bivalve, with a nearly worldwide distribution (STUBBINGS, 1954), was early regarded as a good candidate for genetic study (RAY, 1960). A number of recent papers have made it one of the most extensively studied marine invertebrates.

The diploid chromosome number of *Mytilus edulis* of the Pacific coast of the USA is 28 (AHMED and SPARKS, 1970); a 2 *n* number of 24 is reported from the Atlantic coast (MENZEL, 1968). Autosomal structural polymorphism exists in Pacific coast populations, appearing in mitotic and meotic figures as asymmetrical bivalents (AHMED and SPARKS, 1970). These probably represent structural re-arrangements, such as pericentric inversions, that cause intrachromosomal shifts in centromere position. Similar figures were observed in *M. californianus*, and aneuploid sets also occur. Nothing is known of the adaptive value of the polymorphisms. The high frequency of non-standard arrangements (5–10% aneuploid sets; under 5% meiotic plates with asymmetrical bivalents) suggests maintenance by natural selection analogously to structural polymorphism in *Drosophila*, *Moraba*, and other insect genera.

MILKMAN and BEATY (1970), MILKMAN (1971), KOEHN and MITTON (1972), LEVINTON (1973), and MITTON and co-authors (1973) have described four segregating loci in *Mytilus edulis* of the Atlantic coast of North America, using a variety of electrophoresis techniques. A survey of a triallelic leucine aminopeptidase polymorphism (Lap) southwest of Cape Cod, Massachusetts (USA), revealed only small differences in allele frequency. The frequency of the 'slow' allele was within the range 0·53 and 0·59 among 6 samples (MILKMAN and BEATY, 1970; reconfirmed by MILKMAN, 1971). This uniformity disappeared when sampling was extended to waters off Long Island and in Cape Cod Bay. 'Slow' allele frequency was low in cold waters of Cape Cod Bay in both 1970 and 1971; locality to locality differences proved to be large and unsystematic rather than clinal, indicating local differences in selection intensity or reflecting settlement patterns from different spawning sources. Throughout the area studied, population samples usually exhibited heterozygote deficiencies ascribed to the undetected segregation of a 'silent' allele (MILKMAN and BEATY, 1970).

Near the Cape Cod Canal juvenile populations displayed much lower frequencies of the 'slow' allele than the intermingled large mussels in both 1970 and 1971 studies. It was concluded that the populations were of mixed provenance, different size samples representing settling of larvae of differing geographic and genetic origin.

Allele frequencies and current patterns suggest an origin southwest of Cape Cod for earlier-settling larvae, and a predominantly northern input after late August. MILKMAN (1971) proposes the term population circuitry for the current cycling of larval populations among recruiting adult populations.

Genetic variation at the Lap locus along the Atlantic coast of North America was also noted by MITTON and co-authors (1973), but an aminopeptidase locus (Ap = peptidase and leucine-alanyl peptidase of other authors) does not show significant variation from Maine to Virginia. This study focused more closely on the frequencies of combined genotypes at the Lap and Ap loci as a possible indicator of epistasis. Over 1000 *Mytilus edulis* were collected at the mouth of the Nissequogue River in Long Island Sound, New York. Three common Lap alleles, Lap^s, Lap^m and Lap^f, and three common Ap alleles, Ap^s, Ap^m and Ap^f, were characterized. Combined genotypes at the two loci may show three relationships: (i) alleles may segregate independently into random di-locus genotype combinations without subsequent distortion of combination frequencies by selection; (ii) certain allele combinations of linked loci may occur in higher than random frequency (linkage disequilibrium) and result in biased genotype frequencies; and (iii) random di-locus genotypes may be generated, after which epistatic interactions permit selection to act on genotypic combinations and bias surviving di-locus genotypes.

A contingency table comparison of genotypic combinations in pooled samples indicates significant departure ($p < \cdot 005$) from randomness. This information is not sufficient to distinguish between linkage disequilibrium and epistasis, and the linkage relationship of the loci is not known. However, di-locus genotype frequencies should be stable over age classes if generated through linkage disequilibrium, whereas they would change progressively in populations continuously selected for favourably interacting epistatic combinations. In one of three microhabitat samples the excess and deficiency of certain combinations increased in large *Mytilus edulis* high in the intertidal zone compared to smaller low-intertidal mussels. This evidence points to epistasis as the mechanism of the di-locus genotype distortion. It is suggestive that the loci involved both have peptidases as products; but the *in vivo* functioning of the enzymes is obscure and no biochemical basis of the epistasis is proposed. This study underlines the long-standing concept of the 'unity of the genotype' and that overall fitness is not a simple additive function of alleles of fixed selective value at multiple loci. The selective value of each allele has a component contributed by multiple inter-locus interactions throughout the genome.

In the same series of studies, KOEHN and MITTON (1972) utilized Lap and malate dehydrogenase (Mdh) polymorphisms to investigate parallel genetic variation in *Mytilus edulis* and the ribbed mussel *Modiolus demissus*. *M. demissus* has a three-allele polymorphism at the Lap locus similar to that of *M. edulis*, as was first noted by MILKMAN and BEATY (1970). Ribbed mussel populations are also diallelic at the presumably homologus Mdh locus. Samples of 72 to 95 individuals of each species were taken at four sites in the estuaries at the mouth of the Nissequogue River. Individuals of the two species are closely associated there and face very similar physical conditions. Localities differ in temperature, salinity and probably in plankton composition.

Alleles of the two loci have unlike mobilities in the two species, but, as defined by relative mobilities, they occur in nearly identical rank order of abundance. The

authors propose that the genetic response of these presumably homologous loci to common heterogeneity of the physical environment could also be very similar: the species might employ parallel genetic-adaptive strategies. Genotype distributions at the Lap locus are significantly different in four of six inter-locality pairwise comparisons in *Mytilus edulis* and in two of six in *Modiolus demissus*. In contrast, the four between-species within-locality comparisons reveal no significant differences (Table 6-5). If the assumption that gene homology is determinable by rank order of enzyme mobility is valid, the data are consistent with the hypothesis of parallel strategies, even though the selective factors of the environment are not known. More far-reaching conclusions must await more complete investigation.

Table 6-5

Pairwise comparisons of genotypic distributions at the Lap locus in samples of *Mytilus edulis* and *Modiolus demissus* (After KOEHN and MITTON, 1972; modified; reproduced by permission of University of Chicago Press)

Comparisons between the species at different localities:				
Modiolus demissus	*Mytilus edulis*			
	I	II	III	IV
I	—	6·11*	12·48**	3·77
II	0·99	—	9·38**	4·29
III	8·98*	6·80*	—	19·88**
IV	3·14	3·96	3·08	—
Comparisons at the same locality:	I	II	III	IV
M. edulis vs. *M. demissus*	0·747	3·14	5·54	5·37

$^{*}P < \cdot05$
$^{**}P < \cdot01$

Modiolus demissus from one of these localities evidence genotypic selection over age classes (KOEHN and co-authors, 1973). Smaller mussels from a 0·66 m vertical span in the intertidal zone exhibit a heterozygote deficiency at a tetrazolium oxidase locus. Genotype distribution shifts significantly to a heterozygote excess among larger individuals. This holds in both the high- and low- intertidal. These data suggest the maintenance of a stable polymorphism through heterozygote superiority in mature mussels, but are uninformative as to the mode of selection and the causes of heterozygote deficiency in the young. If larvae are recruited from several populations differing in allele frequency their pooled genotype distribution will show a lower frequency of heterozygotes than the Hardy–Weinberg equilibrium (Wahlund effect). The Cape Cod Canal populations of *Mytilus edulis* exhibit the Wahlund effect (MILKMAN, 1971). However, regional allele frequencies at the tetrazolium oxidase locus appear too uniform for a Wahlund effect equal to the observed heterozygote deficiency. The age-dependent trends in heterozygote frequency could occur through differing heterozygote proportions over successive waves of larval settlement or by a reversal from differential heterozygote mortality to

heterozygote survival in young mussels. As the authors point out, both explanations entail selective discrimination of heterozygotes beginning either with the larva or the recently settled individual.

The hypothesis that less adaptive genetic polymorphism exists in relatively stable, predictable environments has recently been tested by LEVINTON (1973). Two multi-allelic loci, Lap and Phi (phosphohexose isomerase), were scored from starch gel patterns of 50 to 85 individuals each of six bivalve species. The species fall along a gradient of environmental predictability, particularly in regard to temperature and salinity, from the epifaunal and intertidal *Mytilus edulis* to the infaunal and subtidal *Nucula proxima*. Absolute number of alleles (A) and effective number of alleles (n_e) are used as indices of genetic variability. At both loci the values of A and n_e decline steadily with depth below the substrate (Table 6-6). The hypothesis is borne out for these loci of these populations; by extrapolation, it predicts increasing genetic monomorphism in more stable environments.

Table 6-6

Genetic variability at two enzyme loci in epifaunal and infaunal bivalves. A: number of alleles; n_e: effective number of alleles; N: number of bivalves in sample (After LEVINTON, 1973; modified; reproduced by permission of American Association for the Advancement of Science. Copyright 1973)

Species	Occurrence	Phi locus			Lap locus		
		A	n_e	N	A	n_e	N
Mytilis edulis	epifaunal, intertidal	7	3·9	70	5	3·0	52
Modiolus demissus	semi-infaunal, intertidal	6	2·6	62	4	2·5	66
Mercenaria mercenaria	shallow-infaunal, intertidal	6	2·5	55	4	2·6	60
Macoma balthica	medium infaunal (10 cm), intertidal	3	2·1	85	4	2·2	50
Mya arenaria	deep infaunal (15–30 cm) intertidal	3	1·7	58	3	1·1	64
Nucula proxima	infaunal, subtidal (depth = 20 m)	2	1·2	71	—	—	—

Contradictory results were obtained by GOOCH and SCHOPF (1972). As a part of a genetic study of deep-sea species, genetic variability was estimated in the protobranch bivalves *Nuculana pontonia* and *Malletia* sp. dredged from 1000 to 1200 m off southern California, USA. As Table 6-12 shows, genetic variability is high in these inhabitants of an extremely stable and uniform environment, whether measured by proportion of polymorphic loci or by mean heterozygosity. Mean heterozygosity is the proportion of loci at which an average individual is heterozygous (LEWONTIN and HUBBY, 1966); a population monomorphic at half of its loci and segregating for two alleles in equal frequency at the other half would have a mean heterozygosity of 0·25, assuming a Hardy–Weinberg equilibrium. As will be discussed later, it is obvious that the level of genetic variability is more than a simple function of environment variability.

Two electrophoretic studies of allele frequency variation in bivalves sampled along latitudinal gradients have reached completion. O'GOWER and NICOL (1968) elucidated a two-allele haemoglobin locus from stained zymogram patterns in

Anadara trapezia; they obtained allele and genotype frequencies in seven populations from the southeastern coast of Australia. Samples of approximately 100 to 200 bivalves were collected in quiet shallow-water habitats in bays and inlets from Point Vernon, Queensland in the north to Mallacoota Inlet, Victoria, in the south, a transect of about 1400 km. Genotype frequencies in all samples conform to the Hardy–Weinberg distribution. Geographically variable allele frequencies exist, and there is significant clinal diminution in the frequency of the 'fast' allele southward. The five intermediate stations follow a nearly linear plot of allele frequency versus latitudinal distance. The two terminal stations, however, deviate from this relationship. The clinal segment of the transect is swept by the East Australian Current, which originates in the warm Coral Sea and cools in its southward movement. The correlation of allele frequency with temperature suggests that the haemoglobin morphs are unequally advantageous in a temperature gradient. The terminal populations are influenced by other current systems; hence the relationship between allele frequency and temperature may be complex.

PESCH (1972) reports the presence of a presumed lactate dehydrogenase locus (Ldh) polymorphism in the quahog *Mercenaria mercenaria* and the closely related *Mercenaria campechiensis*. *M. mercenaria* was taken in samples of about 50 individuals from four localities in a transect from the Bideford River, Prince Edward Island, Canada to Wadmalaw Island, South Carolina, USA. *M. campechiensis* was collected near Beaufort, North Carolina and from Tampa Bay, Florida. Band patterns are consistent with an interpretation of 5 alleles, designated A to E in *M. mercenaria*, and 7 alleles, the same A to E and also F and G, in *M. campechiensis*. The number of alleles increases steadily southward from the Bideford River with 2 to Tampa Bay with 7. The A and B alleles are always highest in frequency, A increasing from 0·50 to 0·62 southward, and B decreasing from 0·50 to 0·23 in a clinal manner.

The lactate dehydrogenase patterns in these bivalves are unusual in two respects. First, all 54 individuals in the Bideford River sample yield two bands. The author's interpretation is that selection may have retained only heterozygotes (making it a balanced lethal system), or that the locus is duplicated locally. Second, all but one population have significant ($p < 0{\cdot}05$) or very significant ($p < 0{\cdot}01$) excesses of heterozygotes. Possibly band patterns are non-genetic artefacts and these genotype frequencies are spurious; if patterning is interpreted correctly, this is a remarkable example of heterozygote superiority, and the system deserves further investigation.

(c) Arthropoda

Members of the phylum Arthropoda have figured less prominently in studies of marine genetics than have the molluscs. To date, arthropod studies have centred principally on two groups that display colour variants (polychromatism), namely shallow-water harpacticoid copepods of the genus *Tisbe*, and intertidal isopods, particularly of the genus *Sphaeroma*.

Present knowledge of *Tisbe* genetics comes primarily from the work of BATTAGLIA and his school. *Tisbe reticulata* is a benthic inshore form often found on algae and *Zostera* species. Colour morphs are distinguishable from patches of pigment in the

hypodermis of the cephalothorax and thoracic segments. The patches vary in size, pattern, colour and intensity (Fig. 6-6). Seven important colour forms and a number of minor variants exist near Roscoff (France), and are under the control of several independent genes (BOCQUET, 1951). *T. reticulata* also lives in the Lagoon of Venice (Italy), an environment that experiences much more short-term variability in temperature and salinity than does Roscoff. Only four of the major colour forms are found there, *violacea, maculata, trifasciata* and *punctata* (BATTAGLIA, 1957). One multi-allelic locus accounts for all the Venetian morphs (BATTAGLIA, 1958). Roscoff and Lagoon of Venice populations also differ physiologically; Venetian *T. reticulata* are much more tolerant to variation in salinity, and some incompatibility exists in crosses between the geographical forms (BATTAGLIA, 1957).

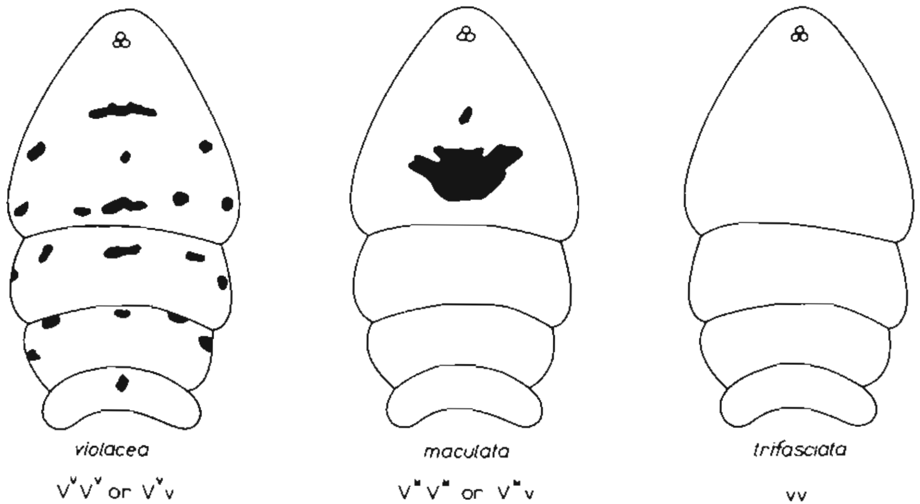

Fig. 6-6: *Tisbe reticulata*. Major colour morphs of males from the Lagoon of Venice (Italy). Only cephalothorax and free thoracic segments are depicted. (After BATTAGLIA, 1958; modified; reproduced by permission of Society for the Study of Evolution.)

Density of *Tisbe reticulata* in culture affects genotype frequency. In the F_2 generation of crosses of homozygous *violacea* (V^vV^v) with *maculata* (V^mV^m) in uncrowded cultures there is a slight but significant excess of heterozygote survivors (V^vV^m), and the heterozygote excess becomes large in severely crowded cultures (Table 6-7). This appears to be an unusually clear example of heterosis, and one in which heterozygote superiority is increasingly manifest in a deteriorating environment. The physiological basis is not known.

The adaptive values of these alleles change when other aspects of the environment are varied, and new equilibrium frequencies are struck correspondingly. Populations initiated at 80% V^vV^v and 20% V^mV^m, or the converse frequencies, both equilibrate at about 60% V^m at 18° C, but they equilibrate instead at about 40% V^m at 23° to 24° C (BATTAGLIA and LAZZARETTO, 1967). When parental cope-

pods from the Lagoon of Venice during the summer reproduce in culture at 6° C lower than environmental temperature, the F_1 generation shows an increase in the frequency of V^m, as in the equilibrium experiments (BATTAGLIA and LAZZARETTO, 1967). These demonstrations of natural selection acting on allele frequencies at individual loci parallel in many respects those of DOBZHANSKY and co-workers on wild and population cage experiments with *Drosophila* karyotypes.

Tisbe clodiensis occurs sympatrically with *T. reticulata* in the Lagoon of Venice, and has similar ecological and culture requirements. A diallelic colour polymorphism, with the alleles P and p, is ascertainable in females (BATTAGLIA and FAVA, 1968). The P alleles do not respond like the V series; in an equilibrium experiment similar to the one above, P decreased from all initial frequencies. Heterozygote advantage is nevertheless suggested by the persistence of P in mass cultures. Actually, P is quite common in Lagoon populations and it remains at relatively high

Table 6-7

Tisbe reticulata. Relative viability of the genotypes V^vV^v, V^vV^m, and V^mV^m in laboratory cultures based on deviations of genotype frequencies from Hardy–Weinberg equilibrium. Genotype viability is density-dependent under these conditions, but heterozygote superiority is exhibited even in low crowding (After BATTAGLIA, 1958; modified; reproduced by permission of Society for the Study of Evolution)

Culture conditions	Genotype		
	V^vV^v	V^vV^m	V^mV^m
High crowding	0·660	1	0·615
Medium crowding	0·675	1	0·758
Low crowding	0·893	1	0·901

frequency in uncrowded cultures. Moreover, P increases in frequency when *T. clodiensis* is grown in mixed cultures with *T. reticulata* (BATTAGLIA and FINCO, 1969). It appears that selective factors at this locus are primarily biotic rather than physical (BATTAGLIA and FINCO, 1969; BATTAGLIA, 1970), in contrast to the demonstrated selective role of physical factors at the V locus in *T. reticulata*.

Tisbe also resembles *Drosophila* in that isolated populations can be mated in culture to determine the degree of reproductive isolation. Geographically more remote *T. clodiensis* tend to show decreasing reproductive success in Mediterranean populations (BATTAGLIA and VOLKMANN-ROCCO, 1969; Fig. 6-7), but over an even greater range *T. furcata* are highly interfertile (BATTAGLIA, 1967). The correlation between morphological differentiation and reproductive incompatibility is sometimes poor; the Mediterranean species *T. reluctans* and *T. persimilis* are strikingly similar morphologically, but are intersterile (VOLKMANN-ROCCO and

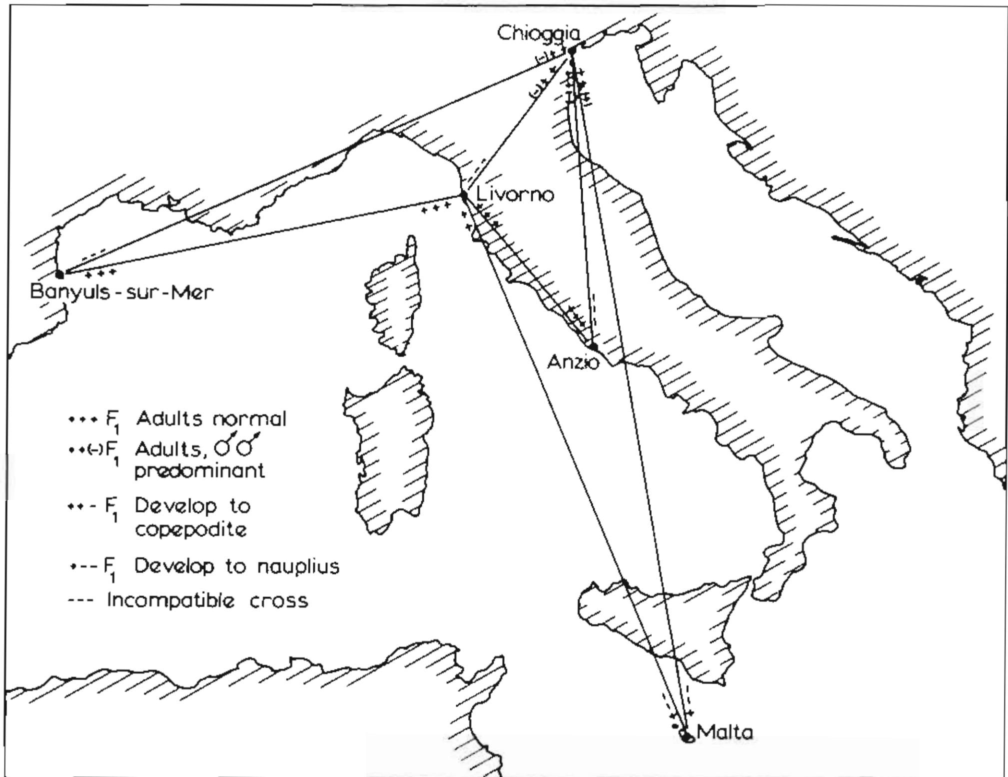

Fig. 6-7: *Tisbe clodiensis*. Reciprocal crosses of central Mediterranean populations and relative degree of reproductive isolation. Direct distance between localities is an imperfect indicator of mating and breeding success. (After BATTAGLIA and VOLKMANN-ROCCO, 1969; modified; reproduced by permission of the authors.)

FAVA, 1969), and the incompatibility gradient in *T. clodiensis* is also not marked by morphological change.

Preliminary studies on chromosomes of copepods have shown interesting variability. Of two *Pseudocalanus* forms in landlocked fiords of Baffin Island, the larger proves to be polytene and is probably evolutionarily derived from the smaller (WOODS, 1969). Polyteny has lengthened and otherwise modified the life cycle, which may be adaptive under prevailing conditions. Triploidy is known in nauplii of *Calanus finmarchicus* (HARDING and MARSHALL, 1955).

Calanus finmarchicus has also figured in an electrophoretic study. This species and its North Atlantic congener, *C. helgolandicus*, are barely distinguishable morphologically. MANWELL and co-authors (1967) electrophoresed and stained individuals of both species obtained from British coastal waters, examining, in all, eleven enzyme systems. Band phenotypes were not resolved into gene systems. However, a comparison of band patterns revealed only 15 bands in common and 24 to 28 that were different between the species (Fig. 6-8). This constitutes presumptive evidence of genetic isolation and reinforces the division of the two forms into separate species that had been proposed on morphological grounds. It also shows the potential usefulness of electrophoresis in the resolution of difficult taxonomic problems.

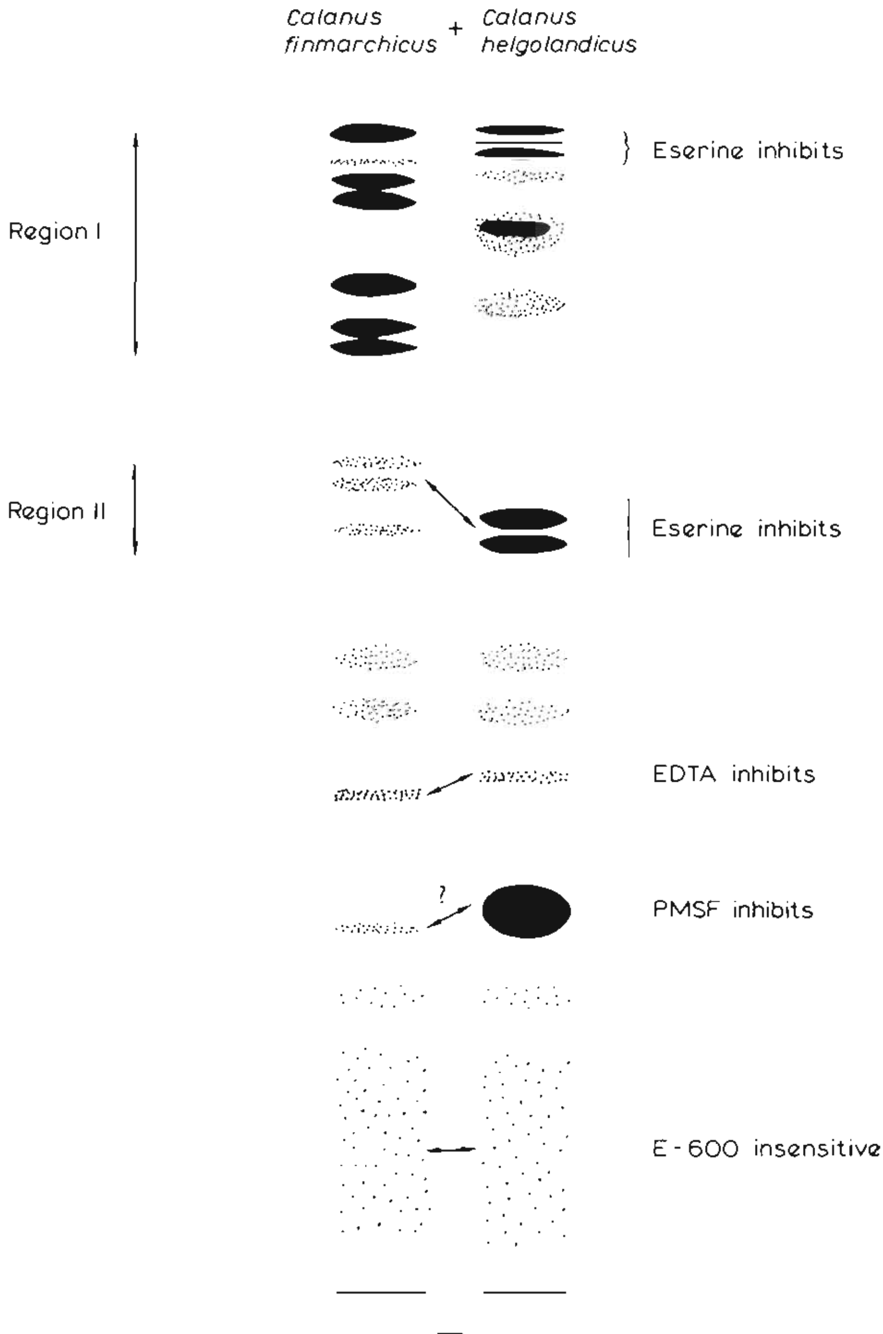

Fig. 6-8: Comparison of esterase patterns of *Calanus finmarchicus* and *C. helgolandicus* stained with α-naphthyl acetate on starch gels. Inferred enzyme homology is indicated by arrows, and is based on similarity of band patterns, mobility, and on response to specific inhibitors. (MANWELL and co-authors, 1967; modified; reproduced by permission of Marine Biological Association of the United Kingdom.)

Intertidal and estuarine genera of Isopoda such as *Jaera* and *Sphaeroma* are conspicuously polymorphic for colour with individuals displaying stripes or patches of black, grey, orange, white, pink or pale yellow hues. Since isopods brood their progeny in a marsupium and the adults are relatively sedentary, they are in many respects the crustacean analogs of *Littorina saxatilis* and *L. obtusata*.

At Roscoff and other Atlantic coastal localities *Jaera marina* exists in 4 to 6 morphological forms, each occupying a narrowly stratified intertidal band (BOCQUET, 1953, 1954). Although the variants possess somewhat similar colour-morph series—presumably controlled by homologous genes—and interbreed with little difficulty in the laboratory, they do not readily hybridize in nature. In the opinion of BOCQUET (1954), the forms are not merely microgeographic ecotypes, but are incipient or full species. Some of the forms, according to BOCQUET, may have speciated microgeographically or ecologically, with increasing subdivision of the intertidal biotope into narrow niche spaces. Interestingly, colour polymorphism is most prominent in the mid-tidal forms or species, which are subjected to the greatest habitat heterogeneity, and declines toward both higher and lower intertidal levels.

Colour inheritance of *Sphaeroma serratum* has come under intensive investigation, especially along the coast of Brittany, where a complex of colour morphs is controlled by five pairs of genes, some of which segregate independently (BOCQUET and co-authors, 1951; HOESTLANDT, 1955). Morph and genotype frequencies vary geographically, but have generally remained stable over a period of years. Genetic diversity for polychromatism increases southward (TEISSIER, 1969). In other species of *Sphaeroma*, LEJUEZ (1966) has demonstrated that the genes controlling colour are tightly linked, with only rare crossovers. The linkage blocs are thus inherited as units and constitute supergenes.

The distribution of colour phenotypes of *Sphaeroma rugicauda*, a common, high-intertidal salt marsh isopod of the English coast, has been investigated by WEST (1964) and BISHOP (1969). Colour forms much like those of *S. serratum* are typically taken in intrapopulation collections (Fig. 6-9). Several non-epistatically interacting genes control the morphs, and only one instance of close linkage has been noted (WEST, 1964). In the same study WEST collected 46 stations over most of Great Britain, determined morph ratios and calculated allele frequencies. Frequencies were found to vary irregularly, with occasional indications of local clines or correlation with temperature. Ecologically disparate sites might or might not differ genetically. Localities sampled over a two-year period generally remained genetically homogeneous, but 7 Pembrokeshire populations underwent a parallel change in the frequency of *yellow* and 2 other genetic morphs, suggesting the action of a common selective mechanism.

BISHOP (1969) collected *Sphaeroma rugicauda* principally from localities near the estuary of the Dee, paying particular attention to the *yellow* morph. Matings performed on *yellow* × *yellow* conform closely to a 2:1 ratio, showing that these isopods are probably heterozygotes and that the homozygous *yellow* is lethal. The *yellow* morph nevertheless persists in natural populations, although at such low frequencies that most matings would probably be with the dominant morph, grey. A clue to the adaptive value of yellow heterozygotes is given by seasonal observations of adults and their annual progeny class; the frequency of *yellow* in the latter is twice as high as in the parents during the winter. Laboratory experi-

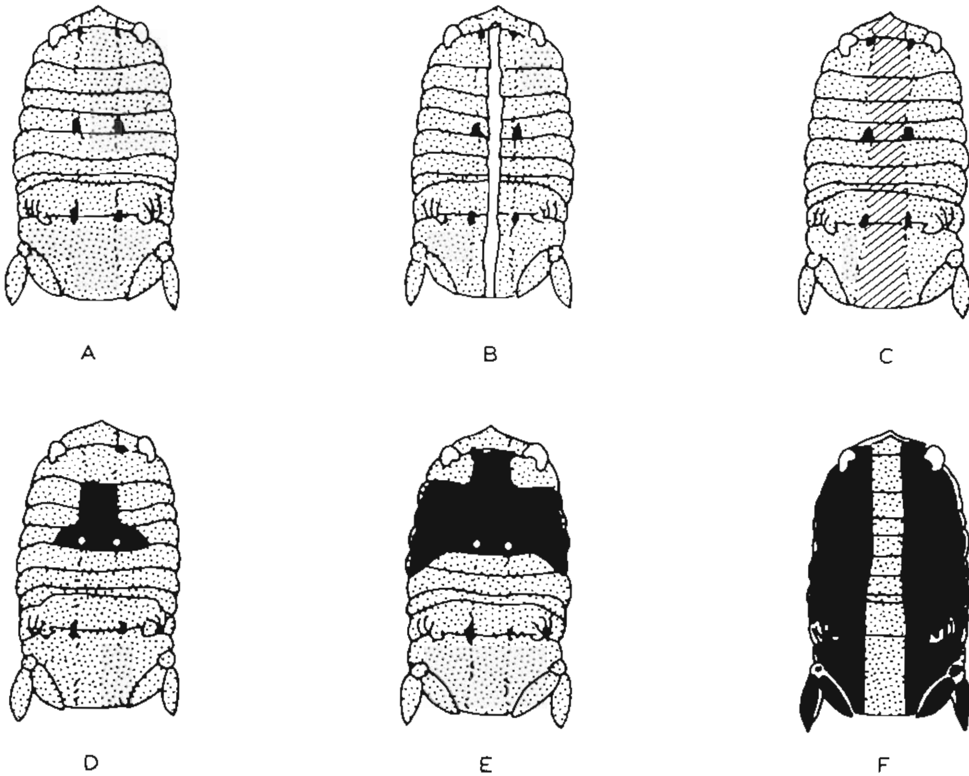

Fig. 6-9: *Sphaeroma rugicauda.* Major morphs or phenotypic forms. A: *grey*; B: *yellow*; C: *red*; D and E: *pattern* (two extremes); F: *stripe* (unusually black sides). Stippling indicates scattered pale and dark chromatophores; white: pale yellow or pink; hatched: orange-red; black: black. (After WEST, 1964; reproduced by permission of Society for the Study of Evolution.)

ments at 1 to 2° C also establish the greater viability of *yellow* than *grey*. With the onset of warmer weather *yellow* decreases in frequency. These observations support the hypothesis that the yellow allele is involved in a temporally balanced polymorphism.

Although much less is known of genetic systems in other arthropods, recent electrophoretic studies have made some headway. The landlocked mysid shrimp *Mysis relicta* in two Swedish lakes possesses a complex esterase locus that is interpreted as segregating for 5 alleles (FÜRST and NYMAN, 1969). Allele frequencies differ between Lake Mälaren and Lake Skiren populations, although the rank order in frequency is the same. Interest in this locus derives chiefly from a marked deficiency of heterozygotes in both lakes compared to equilibrium distributions (Fig. 6-10). An undetected 'silent' allele could account for the deficit. If it is real, the Wahlund effect is an unlikely explanation because genetically differentiated mysids are not thought to exist in the lakes. The remaining possibilities—inbreeding and selection against heterozygotes—are not entirely satisfactory; the former because mysid populations are large and the latter because this mode of selection

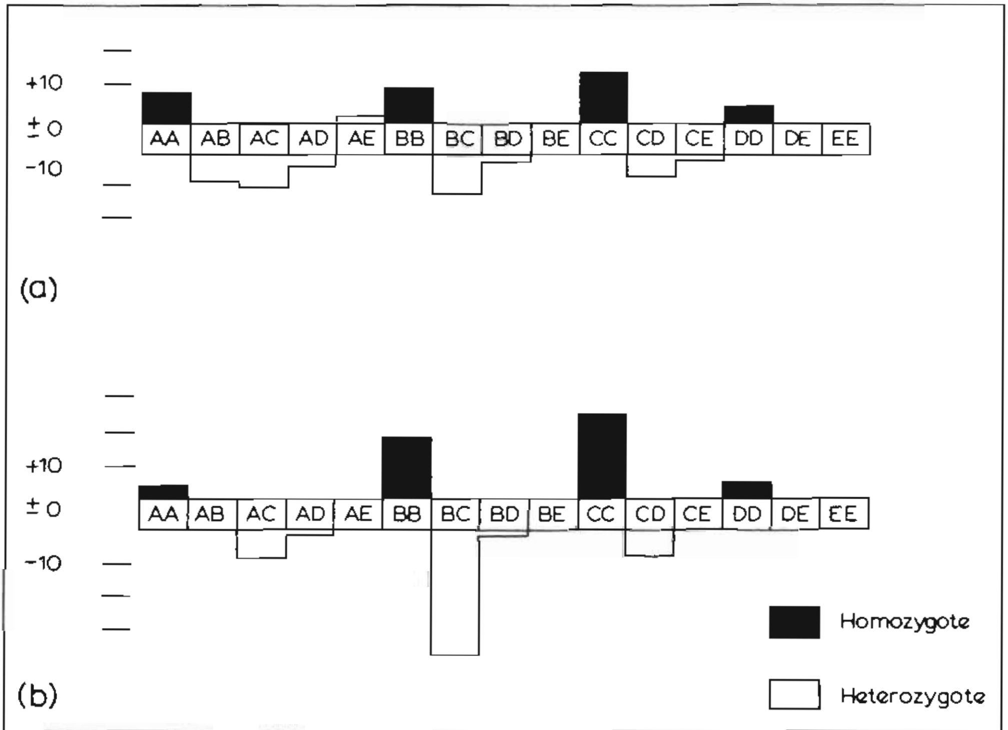

Fig. 6-10: *Mysis relicta*. Proportional excess of homozygotes in Lake Mälaren (a) and Lake Skiren (b). (After FÜRST and NYMAN, 1969; redrawn; reproduced by permission of Institute of Freshwater Research, Drottningholm, Sweden.)

would destroy the stability of the polymorphism unless, as perhaps in *Modiolus demissus* (KOEHN and co-authors, 1973), there is a recurrent shift to selection against homozygotes.

In another esterase study, BARLOW and RIDGWAY (1971) obtained allele frequencies at a triallelic locus in inshore (Boothbay Harbor, Maine) and offshore (near Hudson Canyon) samples of the American lobster *Homarus americanus*. Unlike *Mysis relicta*, the samples fit the Hardy–Weinberg equilibrium and inspection of the data gives no indication of allele frequency differences. No correlation of band phenotype or inferred genotype was evident with sex, size, or moult-cycle stage. An amylase locus polymorphism is reported in 4 populations of another decapod, the brackish-water shrimp *Palaemonetes varians*, along a 140 km transect on the coast of Denmark (CHRISTENSEN and LOMHOLT, 1972). Interpopulation genetic differences are surprisingly great; 2 alleles exist in some populations and 4 in others. Allele frequencies match Hardy–Weinberg expectations in all cases. Preliminary results of electrophoresis of 8 loci in the fiddler crabs *Uca pugilator* and *U. pugnax* from the Atlantic and Gulf of Mexico coasts of the USA show that both species are actually sibling pairs (SELANDER and co-authors, 1971). Cape Cod and South Carolina *U. pugnax* populations are homogenous at phosphoglucose isomerase and phosphoglucomutase loci, but allele frequencies at a glutamate-

oxaloacetate locus are heterogeneous. Interlocus discordance in frequency distributions probably indicates stabilizing selection at the former loci and differentiating selection at the latter.

The arthropod with the most completely characterized genome presently is *Limulus polyphemus*, the 'phylogenetically relic' survivor of the ancient subclass Xiphosura. Of 25 electrophoretically demonstrated loci, 4 to 9 (16 to 36%) are polymorphic in one or another of 4 population samples in a transect stretching from Cape Cod to Panama City, Florida, on the Gulf of Mexico (SELANDER and co-authors, 1970). At most loci allele frequencies are homogeneous over Atlantic or Gulf populations (Table 6-8). Comparison of pooled populations of Atlantic with Gulf *L. polyphemus* show large differences, particularly in the larger number of polymorphic loci in the Gulf.

It has been claimed that stable, slowly changing forms in the fossil record evolved gradually because of a lack of genetic variability (discussed in SIMPSON, 1944). SELANDER and co-authors (1970) point out that high levels of polymorphism in

Table 6-8

Limulus polyphemus. Allele frequencies at 7 of 9 polymorphic loci in 4 populations (After SELANDER and co-authors, 1970; modified; reproduced by permission of Society for the Study of Evolution)

Allele	Atlantic coast		Gulf of Mexico coast	
	Woods Hole	Chincoteague	Panacea	Panama City
	N = 17	N = 14	N=19	N = 14
*Mdh-1*ᵃ	—	0·04	—	—
*Mdh-1*ᵇ	0·88	0·86	0·97	1·00
*Mdh-1*ᶜ	0·12	0·11	0·03	—
*Mdh-2*ᵃ	0·76	0·79	0·66	0·39
*Mdh-2*ᵇ	0·24	0·21	0·34	0·61
*Gpd-1*ᵃ	1·00	1·00	0·92	1·00
*Gpd-1*ᵇ	—	—	0·08	—
*Idh-1*ᵃ	0·21	0·21	0·13	0·14
*Idh-1*ᵇ	0·79	0·79	0·84	0·82
*Idh-1*ᶜ	—	—	0·03	0·04
*Idh-2*ᵃ	1·00	1·00	0·87	0·75
*Idh-2*ᵇ	—	—	0·13	0·25
*Pgm-1*ᵃ	0·68	0·57	0·92	0·82
*Pgm-1*ᵇ	0·32	0·39	0·08	0·18
*Pgm-1*ᶜ	—	0·04	—	—
*Pgm-2*ᵃ	0·03	—	0·08	0·07
*Pgm-2*ᵇ	0·97	1·00	0·92	0·93

Mdh-1 = NAD-malate dehydrogenase (mitochondrial).
Mdh-2 = NAD-malate dehydrogenase (supernatant).
Gpd-1 = α-glycerophosphate dehydrogenase.
Idh-1 = NADP-isocitrate dehydrogenase (supernatant).
Idh-2 = NADP-isocitrate dehydrogenase (mitochondrial).
Pgm-1 and *Pgm-2* = phosphoglucomutase enzyme loci.

Limulus polyphemus are antithetic to this hypothesis. The true explanation for slowly evolving lines may lie with extended temporal uniformity of organism-environment relationships.

(d) Other Invertebrates

Annelida. Little is known of the genetics of marine annelids. In the archiannelid *Dinophilis gyrociliatus* two size mutants of male-producing eggs have been reported from laboratory cultures (TRAUT, 1968). A comprehensive study of the physiological and developmental genetics of an eye pigment locus and its mutant allele, *or*, in the polychaete *Platynereis dumerilii* is under way (FISHER, 1969, 1971a, b). Blue, brown, and orange pigments in the branchial crown of the serpulid polychaete *Pomatoceros triqueter* are monofactorially inherited (FØYN and GJØEN, 1954). This would appear to be a good marker gene for field studies, as most of the genotypes are recognizable by inspection (blue, however, is dominant to brown).

Ectoprocta. The population genetics of ectoprocts of the Cape Cod region have recently received attention. Six of 11 esterase, malate dehydrogenase, and leucine aminopeptase loci (55%) are polymorphic in *Bugula stolonifera*, a species sporadically common in the fouling community of quiet water habitats (GOOCH and SCHOPF,

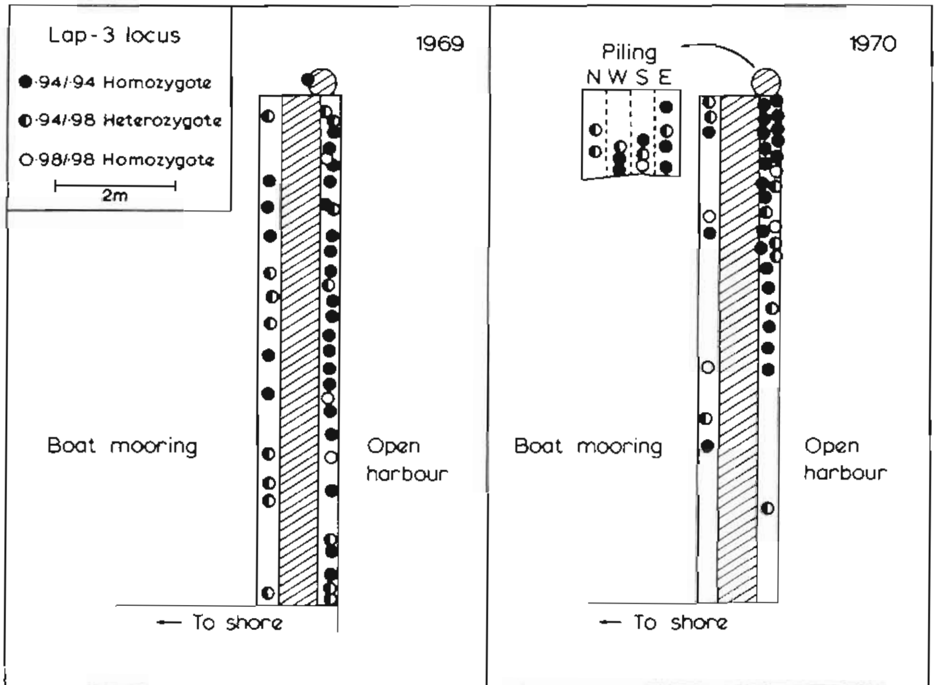

Fig. 6-11: *Schizoporella errata*. Distribution of Lap-3 genotypes on a floating dock at Green Pond on Cape Cod, Massachusetts (USA). White area available for colonization. (After GOOCH and SCHOPF, 1971; modified; reproduced by permission of *Biological Bulletin*.)

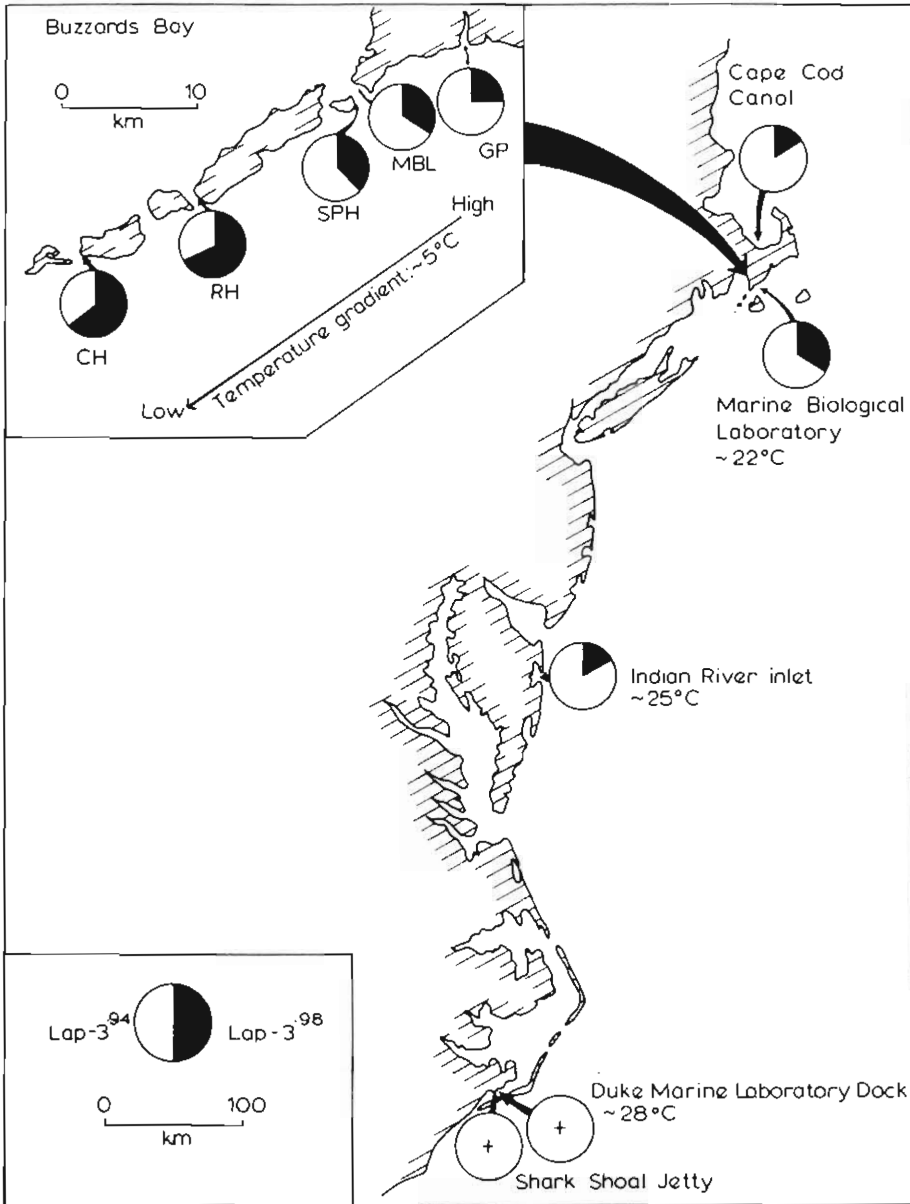

Fig. 6-12: *Schizoporella errata*. Transect from Cape Cod to Beaufort (USA). There is a general increase in frequency of the Lap-$3^{.98}$ allele parallel to decreasing summer water temperature. CH: Cuttyhunk; RH: Robinsons Hole; SPH: Sheep Pen Harbor; MBL: Marine Biological Laboratory dock; GP: Green Pond. (After GOOCH and SCHOPF, 1971; modified; reproduced by permission of *Biological Bulletin*.)

1970). The same enzyme systems yield 2 of 8 (25%) polymorphic loci in the more abundant fouling-community species *Schizoporella errata* (Syn: *S. unicornis*), with a diallelic leucine aminopeptidase locus (Lap-3) proving most useful in analyses (GOOCH and SCHOPF, 1970). Some workers have concluded that the majority of ectoprocts are hermaphroditic (HYMAN, 1959); but the usual agreement of genotype frequencies with the Hardy–Weinberg equilibrium in *B. stolonifera* and *S. errata* indicates that these species are outbreeders.

The sessile nature of ectoproct colonies makes it possible to map their microgeographic distribution accurately. During 1969 and 1970, colonies on pilings, rocks,

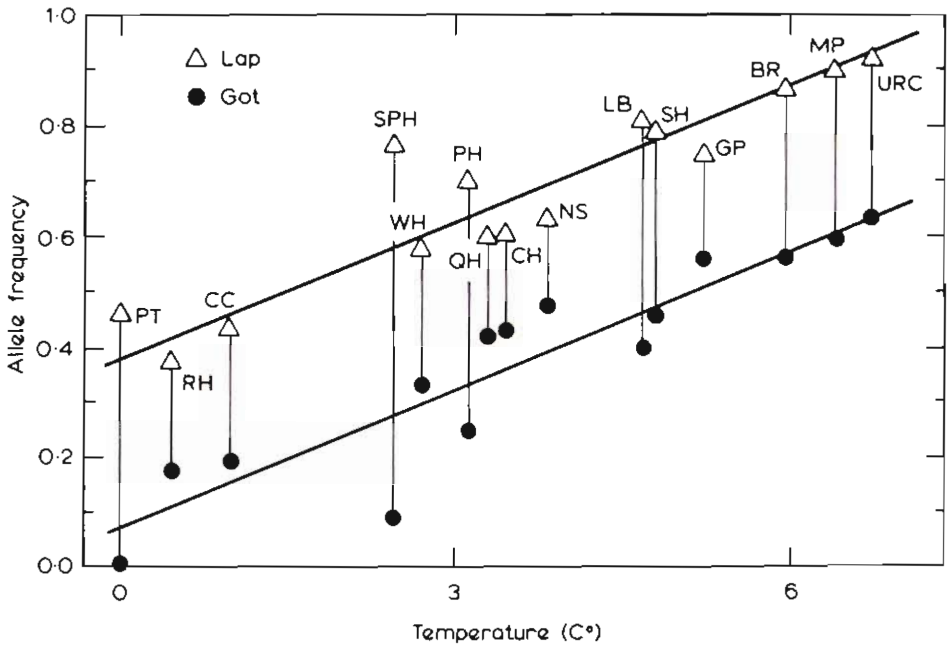

Fig. 6-13: *Schizoporella errata*. Allele frequencies at the Lap and Got loci along the southern coast of Cape Cod (USA). Abbreviations represent 15 localities along a summer temperature transect. Connected points are from the same locality. Allele frequencies co-ordinately increase in a temperature related cline. (After SCHOPF, unpublished.)

and a floating dock near Woods Hole, Massachusetts (USA) were mapped, and genotypes were related to depth and wave exposure (GOOCH and SCHOPF, 1970, 1971). A significant excess of heterozygotes at Lap-3 occurred on the landward, more polluted side of a floating dock at Green Pond in 1969, but distributional bias was no longer evident in 1970 sampling (Fig. 6-11). Small 1969 collections from the Marine Biological Laboratory dock, Woods Hole, showed a possible excess of the Lap-3$^{\cdot 98}$ allele with increasing depth; otherwise no consistent patterns of genotype distribution were found.

On a regional scale, the Lap-3$^{\cdot 98}$ allele increases clinally southeastward for 20 km along the Elizabeth Islands and parallel to a local lowering of summer water

temperature of about 5 C° (SCHOPF and GOOCH, 1971b). Lower temperature may also account for the increase of this allele in colder, deeper water at the MBL dock. Also, according to the temperature-selection hypothesis this local cline should be superimposed on a regional decrease in the frequency of Lap-$3^{\cdot 98}$ from north to south. Such is the case, with Lap-$3^{\cdot 98}$ disappearing entirely from populations between Indian River inlet, Delaware and Beaufort, North Carolina (GOOCH and SCHOPF, 1971; Fig. 6-12). One locality only, in the cold Cape Cod Canal, gives an anomalously low frequency of the ·98 allele. However, it experiences daily temperature changes of up to 11 C° during tidal ebb and flow, and the relationship of allele frequencies to such temperature variation is problematical (SCHOPF and GOOCH, 1971b).

In a study by SCHOPF (1973), the number of surveyed stations was increased to 15, stretching along the entire Cape Cod coastline of Buzzards Bay and Vineyard Sound. An additional diallelic locus, Got-3 (glutamate oxalo-acetate transaminase) was also surveyed. Frequencies of both loci vary clinally and co-ordinately over the transect, which differs by over 6 C° in summer water temperature (Fig. 6-13). Temperature is often a good predictor of allele frequencies, although a few localities yield slightly discordant correlations. Data on environmental factors are too scanty to shed light on these discrepancies.

Echinodermata. *Asterias vulgaris* is an abundant sea-star over the northern Atlantic coast of North America, and it is replaced southward by the closely related *A. forbesi*. The two species overlap in range from Maine to Cape Hatteras, where occasional hybrid individuals distinguishable by intermediate morphological characters are found. Twenty-six gene loci were inferred from electrophoretic band patterns of 10 protein systems (SCHOPF and MURPHY, 1973). Following NEI (1971), the value of I (probability of identity of proteins compared between two species) was found to be 0·59. Identity is presumed from band phenotypes of equal mobility, following HUBBY and THROCKMORTON (1968) and the study of sibling species of copepods (MANWELL and co-authors, 1967). SCHOPF and MURPHY (1973) ascribe genetic differences to fission of a wide-ranging Tertiary ancestral species into Pleistocene isolates north and south of a barrier coincident with Cape Cod and Georges Bank. Post-Pleistocene warming and sea-level rise has re-established range overlap, but genetic differentiation of the magnitude above has led to reproductive isolation and species integrity, notwithstanding some hybridization.

The deep-sea ophiuroid *Ophiomusium lymani* and other echinoderms have been the subjects of genetic-evolutionary studies by DOYLE (1972) and GOOCH and SCHOPF (1972). These investigations will be treated in the section on genetic variability.

Chordata-Urochordata. The subtidally dwelling compound ascidian genus *Botryllus* is a well-known subject of studies on development (BERRILL, 1941a, b; OKA and WATANABE, 1957a, b), developmental genetics (MILKMAN, 1967), and immunogenetics (BURNET, 1971). SABADDIN (1959, 1969) and SABADDIN and GRAZIANI (1967a, b) have elucidated the Mendelian basis of colour morphs of Mediterranean *B. schlosseri*; segregation at 4 loci accounts for orange, reddish, and blue pigment cells and singly or double-banded intersiphonal bands. These

polychromatic characters would be good genetic markers of possible selective significance. SABADDIN and GRAZIANI (1967b) note gene-controlled phenotypes in 2 localities in the Lagoon of Venice, Venice and Chioggia (Italy); phenotypes remain relatively uniform in frequency over a 5-month interval, but at Chioggia the frequency of colonies lacking orange pigment and those with double intersiphonal bands was distinctly different on pilings and on *Zostera* beds 1300 m distant.

(e) Fishes

Marine fishes are adapted to their diverse environments through a multitude of morphological and physiological modifications. Genetic variation within species, however, is usually somewhat cryptically expressed in the anatomy. Much effort has gone into the description of variation in otolith banding and number of scales, fin rays, and vertebrae in commercial fishes such as species of *Gadus*, *Salmo* and *Clupea*, in a search for reliable markers of local populations and races. Meristic characters, which are fixed in number from early in life and are not dependent upon nutrition, have proved more useful than age-variable traits. Unfortunately, meristic traits are profoundly influenced by the environment; salinity (HEUTS, 1949), oxygen concentration (TÅNING, 1952), and other factors may be important; but above all temperature is a modifier of vertebra and fin-ray counts (TÅNING, 1952; MARCKMANN, 1954; LINDSEY, 1961; FOWLER, 1970; for details consult Volume I). The problem of determining the genetic residue after removing the environmental component of meristic variation has remained persistently refractory. Nevertheless, population meristics are a record of the environment experienced in larval life, and two populations that differ meristically bear evidence of different early life histories (LINDSEY, 1961; Volume I: GARSIDE, 1970).

Another phase of the search for stable and reliable population markers was inaugurated with serological techniques. The blood cells of higher vertebrates possess a number of antigens, each of which is the product of a specific gene; for each independent multi-allelic gene there is a parallel series of similar yet distinguishable antigens. The antigens of the ABO blood group locus of man are an example. Antigens are identified by their agglutination reaction with serum antibodies, which may be manufactured to a higher titer in the same species (iso-immune antiserum) or in a different species (hetero-immune antiserum) by prior injection of the antigen. The techniques of immunogenetics permit the antigenic typing of individuals and, if there are no complicating factors, the classification of individuals for blood-group genotypes. Immunogenetic markers have the advantage over meristic traits of being directly and simply inherited and little subject to environmental influence (STORMONT, 1961). On the other hand, distinct gene-controlled antigenic blood groups are apparently uncommon in fishes, and many researchers have failed to distinguish clear genetic markers. Immunogenetics of blood groups has been reviewed comprehensively by CUSHING (1964) and DE LIGNY (1969). CUSHING's review is a particularly thorough account of immunogenetic methodology.

The usefulness of meristic and immunogenetic methods in genetic evolutionary studies is far from fully exploited; even so, the advent of electrophoresis genetics over a decade ago in the haemoglobin work of SICK (1961) and others have provided the tool *par excellence* for discovering and following marker genes. The literature of

electrophoresis genetics of fishes is now very large, although some of it is preliminary and fragmentary. It has been reviewed by LOVE (1970) and in great detail by DE LIGNY (1969), as well as in a few works devoted to specific groups such as tuna (FUJINO, 1970). The discussion that follows is a selective sampling of investigations to date on the evolutionary genetics of marine fishes and it makes no attempt at comprehensiveness. It is hoped that emphasis falls on studies that are relatively complete and that illuminate evolutionary processes.

The cod *Gadus morhua* has an extensive temperate and boreal North Atlantic range. As the egg and larval stages are pelagic and the adult is a powerful swimmer, regional genetic homogeneity might be expected in this species. On the other hand, mean vertebrae and fin ray numbers differ transoceanically (SCHMIDT, 1930) and tagged North Sea individuals seem not to migrate out of prescribed areas (BEDFORD, 1966).

A haemoglobin locus (SICK, 1961; common alleles HbI^1 and HbI^2) and a serum transferrin locus (MØLLER, 1966; MØLLER and NAEVDAL, 1967; JAMIESON, 1968; common alleles Tf^A, Tf^B, Tf^{C1}, Tf^C, Tf^D, and Tf^E) have been utilized in gene marker studies of *Gadus morhua* populations over much of the species range. The frequency of HbI^1 is about 0·10 in the Barents Sea and increases in a rough cline southward along the Norwegian coast until frequencies of 0·59 to 0·69 are reached in the Skagerak (FRYDENBERG and co-authors, 1965). HbI^1 frequencies remain relatively homogeneous into the Kattegat and the Danish Belt Sea (SICK, 1965a) and, to some extent, across the North Sea. Northwestward, the semi-isolated cod populations near the Faroe Islands have dropped to about 0·06 to 0·10 in HbI^1 frequency and the allele is even rarer off Iceland and West Greenland, averaging about 0·01 to 0·02 (SICK, 1965b). Proceeding southward along the North American coast as far as the state of Maryland, HbI^1 again rises in frequency, but only to 0·07 to 0·08 (SICK, 1965b). Both Norwegian and North American clinal trends in increase of HbI^1 roughly parallel temperature, but are not equivalent, i.e., temperatures off Maryland are much higher than those off the southern end of the Norwegian cline, but have allele frequencies like those of the Barents Sea.

The same North Atlantic region presents a less clear-cut picture at the multi-allelic transferrin locus in *Gadus morhua*. The Tf^B, Tf^{C1} and Tf^D alleles exist in high frequency off Canada and diminish irregularly but steadily to the East (JAMIESON, 1968). Tf^{C1} and Tf^D become rare east of Iceland. Tf^C describes the opposite course, declining from frequencies of over 0·80 in the North Sea to 0·03 to 0·18 near Canada (JAMIESON, 1970). Other alleles show no easily discernible trends. Intensive regional sampling indicates considerable homogeneity of allele frequencies; heavy sampling of North Sea populations from 1962 to 1970 reveals little temporal or spatial genetic heterogeneity, although pooling data results in significant heterozygote deficiency at both loci (JAMIESON and THOMPSON, 1972).

While the environmental determinants of this genetic change are not known, the gradual geographic shift of allele frequencies is in keeping with established dispersal abilities of cod. The situation is quite different near the Lofoten Islands off northwestern Norway, where external anatomy and otolith rings indicate that spawning cod are mixtures of migrants from the Barents Sea and members of stationary coastal populations (ROLLEFSEN, 1954). Even though the two populations spawn in the same area approximately simultaneously, studies of allele

frequencies at the haemoglobin, transferrin, and a blood type locus indicate that fish distinguished by otoliths are also genetically different (MØLLER, 1968). Further studies may reveal behavioural or other isolating mechanisms that maintain the genetic integrity of the Barents Sea and coastal populations (MØLLER, 1968).

An even more striking example of genetic differentiation of contiguous *Gadus morhua* populations is provided by a study of SICK (1965b). Cod range throughout the Baltic Sea, even into the cold and semi-fresh eastern embayments. The frequency of HbI^1 is close to 0·61 in the western Baltic Sea, which communicates with the Kattegat and North Sea with similar HbI^1 frequencies. This broad, genetically homogeneous belt gives way suddenly in the central Baltic Sea, where allele frequencies become highly variable, and population samples show homozygote excesses owing, probably, to the presence of a mixture of individuals from genetically differentiated populations. The zone of genetic heterogeneity does not coincide with unusual hydrographic variability. In the eastern Baltic Sea, allele frequencies again become homogeneous; but here HbI^1 is only 0·03 and HbI^2 is the predominant allele.

The sharpness of this genetic discontinuity is hardly matched elsewhere in the literature of marine genetics. Such a sharp transitional zone may result from sympatric evolution, or represent a secondary zone of hybridization. FORD (1971) has documented apparent examples of sympatric evolution, in which adjoining populations of the butterfly *Maniola jurtina* are thought to differ in wing spot-pattern because the spot-controlling genes have different adaptive values in the genetic complexes of the two populations. Selection acts so differently in the populations that gene flow is unable to break down the integrity of the genetic complexes. SICK (1965b) presents a speculative case for secondary hybridization. The Baltic Sea contained freshwater about 7000 years ago and was presumably uninhabited by cod. Prior to that time there was probably oceanic communication between the Baltic Sea and the White Sea. White Sea cod with low frequency of HbI^1, as in the present Barents Sea, may have colonized the Baltic Sea. When the Baltic Sea became fresh this now-isolated population existed in the salt-water 'Kattegat Fjord' refugium until renewed connection with the sea enabled it to move back into the Baltic Sea. The belt of hybridization was established when oceanic cod invaded the Baltic Sea, and it remained narrow because genetic exchange has been limited between the formerly isolated and now genetically differentiated races of cod.

These studies on cod appear to indicate broad genetic intergradation in the open seas, although they leave open the possibility of small discontinuities between separate stocks. Negligible or small regional allelic differences in oceanic fishes have been noted by others (RIDGWAY and co-authors, 1970: esterases of Atlantic herring *Clupea harengus harengus*; JAMIESON and co-authors, 1971: esterases of North Sea mackerel *Scomber scombrus*; EDMUNDS and SAMMONS, 1971: tetrazolium oxidase of North Atlantic bluefin tuna *Thunnus thynnus*; TSUYUKI and co-authors, 1969: transferrins of Pacific halibut *Hippoglossus stenolepis* from Alaskan and British Columbia waters; JOHNSON and co-authors 1971: phosphoglucomutase in Pacific Ocean perch *Sebastodes alutus*, from off the northwest coast of the USA).

However, the cod studies also point out the potentialities for sharper differentiation along coasts and in inland seas. Opportunities for geographic, ecological, temporal and ethological barriers to arise between populations are inherently

greater in and adjacent to discontinuous freshwater drainage, as is evidenced by explosive speciation in Amazonian and African drainages (LOWE-MCCONNELL, 1969). Homing fishes that spawn in fresh water, such as the anadromous Salmonidae, may be expected to show considerable genetic differentiation.

Along these lines, serum transferrin patterns on starch-agar gels and breeding experiments give evidence of two common (Tf^A, Tf^B) and several rare alleles in Atlantic salmon *Salmo salar* of streams from Labrador south to Maine (MØLLER, 1970, 1971). These investigations involved over 5500 specimens from 56 samples of

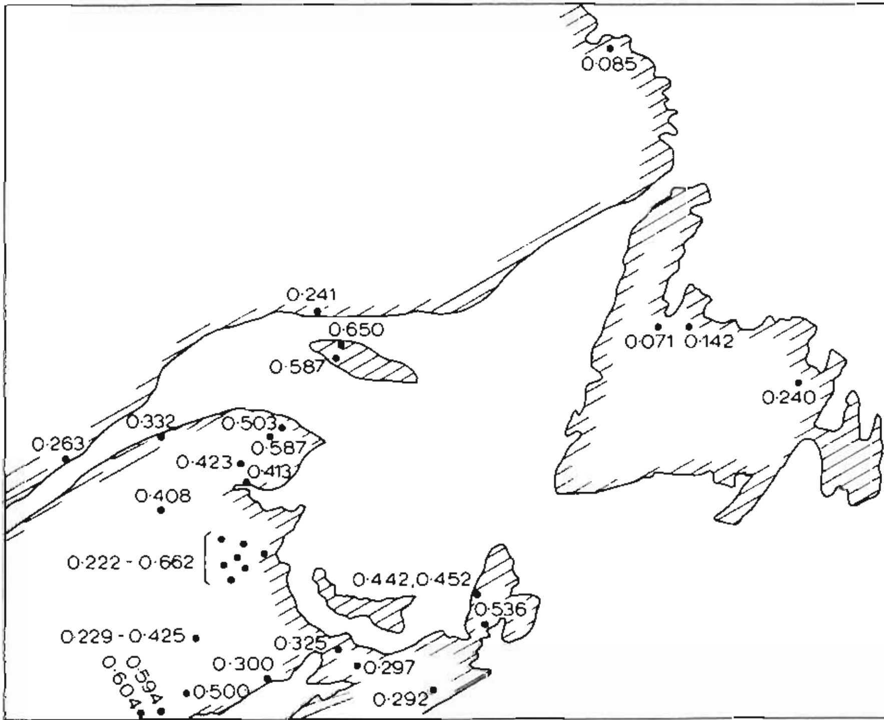

Fig. 6-14: *Salmo salar*. Frequency of the Tf^A allele in Atlantic salmon at localities over eastern Canada and Newfoundland. (After MØLLER, 1971; modified; reproduced by permission of Fisheries Research Board of Canada.)

38 localities. Regional allele frequencies are highly variable. Tf^A is generally low in frequency in Newfoundland and Labrador (0·085 to 0·240) and high in frequency on Anticosti Island (2 localities: 0·587; 0·650). There is little indication of regional patterns elsewhere (Fig. 6-14). Areas extensively stocked are scarcely distinguishable from those inhabited mostly by indigenous fish. As only six population samples differ significantly from a Hardy–Weinberg genotypic distribution, it is surmised that the majority of populations maintain their distinctness during migrations (MØLLER, 1971). This inference is corroborated by frequency discontinuities between nearby populations, as at Mingan, Quebec ($Tf^A = 0{\cdot}241$) and McDonald on Anticosti Island ($Tf^A = 0{\cdot}650$) across the Mingan Passage. However, population mixing would

yield heterozygote deficiencies, and 5 of the deviant samples show heterozygote deficits, indicating some interbreeding or possibly inadvertent collection of genetically distinct but simultaneously migrating stocks. Differences in allele frequency occur within river systems as well as between separate drainages. The complex Miramichi and St. John river systems in New Brunswick are inhabited by several genetically differentiated stocks.

These results are paralleled by the findings from a three-allele transferrin locus (UTTER and co-authors, 1970) in coho salmon *Oncorhynchus kisutch*. Salmon were taken from four streams emptying into Puget Sound, Washington, USA, and from three tributaries of the Columbia River, in all cases adjacent to hatcheries. The Tf^B allele exceeds 0·10 in frequency at most of the Puget Sound localities, but is absent in Columbia River samples. In the same comparison, Tf^A increases in frequency by about 0·60, and Tf^C declines concordantly. Moreover, Puget Sound localities show regional genetic heterogeneity, much as in *Salmo salar*.

The efficacy of freshwater barriers in genetic differentiation is evident in the landlocked rainbow trout *Salmo gairdnerii* of Kokanee Creek, British Columbia.

Table 6-9

Salmo gairdnerii. Distribution of LDH genotypes and allele frequencies in rainbow trout from above and below the falls on Kokanee Creek, Canada (After NORTHCOTE and co-authors, 1970; modified; reproduced by permission of Fisheries Research Board of Canada)

Location	Number	Genotype distribution			Allele frequency	
		CC	CC′	C′C′	C	C′
Below Falls	57	11	29	17	·447	·553
Above Falls	64	27	27	10	·633	·367

NORTHCOTE and co-authors (1970) obtained meristic determinations and allele frequencies at a diallelic lactate dehydrogenase locus from populations immediately above and below a waterfall dating from the late Pleistocene. The populations differ in mean number of parr marks, scale rows, and vertebrae count, although the environments are very similar. There is also a significant discontinuity in allele frequency (difference of 0·19; Table 6-9).

Genetic variability that does not parallel environmental heterogeneity in obvious ways is the rule in marine genetics studies to date. A noteworthy exception involves a diallelic (alleles A and A′) lactate dehydrogenase locus in the crested blenny *Anoplarchus purpurescens* of Puget Sound, USA, and its entrance (JOHNSON, 1971). *A. purpurescens* is abundant in rocky intertidal habitats of Puget Sound, and the closely related *A. insignis* has, on the whole, a more northern and deeper-water distribution. A transect of 23 population samples of *A. purpurescens* from the Strait of Georgia southward to near Tacoma, Washington, about three degrees of latitude, reveals a clinal increase in the frequency of A′ from less than 0·05 to over 0·26 (Figs 6-15, 6-16). Although there is some heterogeneity of allele frequency among nearby samples, the correlation of A′ frequency and temperature is quite

Fig. 6-15: *Anoplarchus purpurescens*. Sampling localities from Strait of Georgia, Canada to head of Puget Sound, USA. Locality 21 is Penrose Point. (After JOHNSON, 1971; modified; reproduced by permission of *Heredity, London*.)

striking. Perhaps in keeping with its more northerly range, *A. insignis* seems to lack the A′ allele altogether. At sites near Penrose Point and other localities evidence of a decline in A′ frequency in deeper, colder water, at least during the spring season, was obtained. Repeated sampling showed genetic heterogeneity to be meaningfully associated with year classes, e.g., the high frequency of A′ in the 1967 year class coincides with an unusually hot summer of that year. *A. purpurescens* larvae were raised at 4° C and at 16° C in the laboratory and assayed for allele frequency; the results—significant excess of the expected homozygote class in the majority

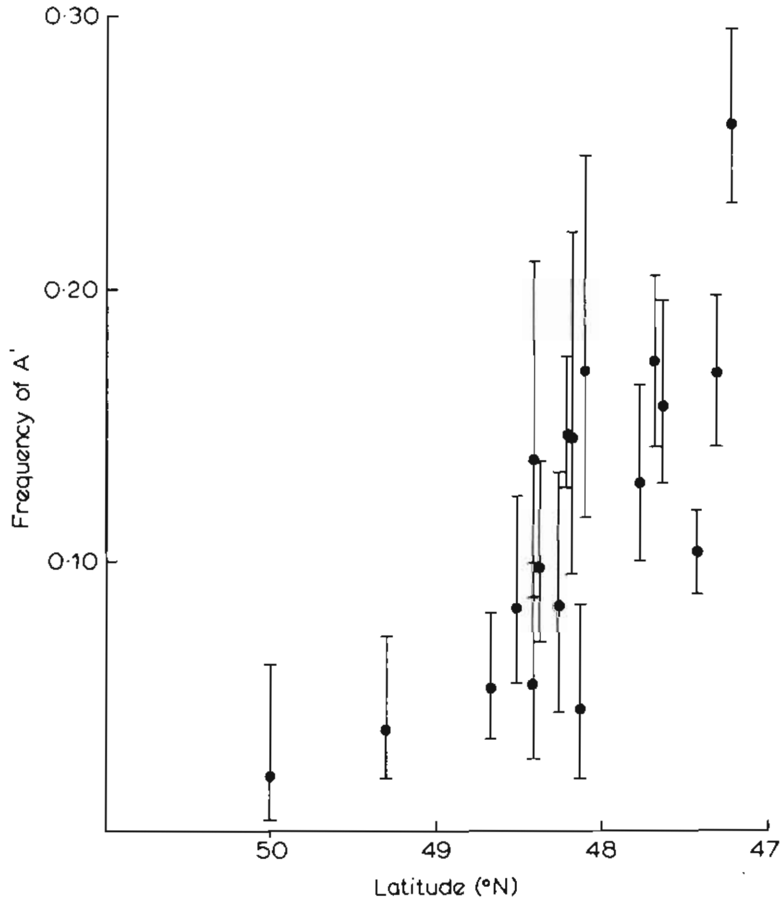

Fig. 6-16: *Anoplarchus purpurescens*. Latitudinal cline in frequency of the A′ allele. Vertical lines: 90% confidence intervals. (After JOHNSON, 1971; modified; reproduced by permission of *Heredity, London*.)

of clutches—lend additional corroboration to the conclusion that temperature is an important selective agent. Experiments also implicate oxygen availability and possibly other factors in selection and underline the complexly interacting nature of real environmental situations.

Considerable interest has focused on the life history of Atlantic eels since the researches of SCHMIDT (1922, 1925) provided evidence that populations spawning in overlapping sections of the Sargasso Sea belong to two oppositely migrating species: *Anguilla rostrata* of North America and *Anguilla anguilla* of Europe. This interpretation was challenged by TUCKER (1959), who held that the American and European eels belong to the same species sharing common breeding grounds. Further literature, reviewed and summarized by JONES (1968), has not settled the controversy, but most investigators have accepted the classical arguments of SCHMIDT.

A number of authors have attempted to discriminate American and European eels by electrophoresis methodology. If these populations are conspecific they should be genetically identical except for selective differences that have arisen during migration of elvers and in adult life. The earliest investigation (SICK and co-authors 1962) discovered no differences in chromosome numbers or haemoglobin pattern, but more recent work (SICK and co-authors, 1967) established the likelihood that North American populations are diallelic at a haemoglobin locus, with one of the alleles occurring in low frequency. This rare allele is absent from European samples. FINE and co-authors (1964) and DRILHON and co-authors (1966, 1967) investigated serum transferrin patterns and found them to be similar in type in trans-Atlantic populations, but widely differing in frequencies of types. The patterns were interpreted as a three-allele polymorphism, but the occurrence of occasional anomalous patterns has called this into question (KOEHN, 1972). Erythrocyte, plasma, and liver esterase and plasma protein band patterns have also been employed (PANTELOURIS and PAYNE, 1968; PANTELOURIS and co-authors, 1970, 1971). Although there is again some difficulty in genetic interpretation of band patterns (KOEHN, 1972), the frequencies of pattern types differ strikingly between American and European eels, and smaller differences are evident among widely separated European samples. In some cases qualitative discontinuities, rather than mere frequency differences, were noted in transoceanic comparisons (PANTELOURIS and co-authors, 1971).

The combined weight of so much isozyme work lends support to the two-species hypothesis. Nevertheless, band patterns that are not clearly allozymic, i.e., resulting from segregation of alleles, may be liable to environmental influences (as is well known for many esterase systems) and thus suffer the same disadvantages as other aspects of the phenotype in ecological, evolutionary and taxonomic studies.

A recent thorough investigation of polymorphic gene loci in the American eel (WILLIAMS and co-authors, 1973) bears on the Atlantic eel problems generally. The leptocephalus larva of *Anguilla rostrata* is dispersed from its restricted spawning grounds over a coastal range of 50 degrees of latitude, from the northern shoulder of South America to the Arctic. Genetic differentiation of eels must, therefore, be due to natural selection operating since the last spawning episode, unless breeding adults actually belong to self-perpetuating stocks. The latter is unlikely, for it necessitates navigational equipment to locate an unfamiliar region and the ability to swim counter to strong and complex current systems. Of 5 polymorphic loci examined (mostly in young elvers) over a transect from St. John, Newfoundland to Vero Beach, Florida, 1 (an esterase locus) varies unsystematically in allele frequency, 1 (malate dehydrogenase) exhibits little geographic variation, and 3 (alcohol dehydrogenase, phosphohexose isomerase, and sorbitol dehydrogenase) loci vary consistently with latitude. Geographic differences in allele frequencies commonly reach or exceed 0·10, and the majority of these differences are statistically significant. This investigation stands as an unusually clear example of the magnitude of genetic differentiation that can arise by natural selection in a single generation. It undermines the generally agreed idea, as the authors point out, that genetic discontinuities are automatically indicators of stock, race or species boundaries. It therefore re-opens the Atlantic eel problem in that it suggests that isozyme band or genetic differences observable in trans-Atlantic comparisons could possibly

have arisen by selective mechanisms over very short periods. The implications of this finding are further discussed below.

(3) Genetic Differentiation

(a) Population Structure

As has been pointed out by ROSS (1962), MAYR (1963) and others, organisms are not continuously distributed throughout their species range like a carpet. They occur instead in local aggregations. Such local populations or demes may be held together by bonds of breeding, defence, or sociability; or individuals may be fortuitously associated by common requirements for food, shelter, or substrate. Proximity usually leads to high frequency of interbreeding and makes the local population the primary genetic unit below the level of the species. The idealized population distribution of wild *Drosophila* approximates a 'bed of nails' (WALLACE, 1968) with 'nails' representing peaks of population density and intervening troughs as inter-deme areas sparsely inhabited by migrants. This model of patchy peaks of abundance is equally applicable to marine animals: the benthic fauna clustered on preferred substrates or in favourable currents; the plankton associated with discrete water masses; the groups or schools of nektonic cephalopods, fishes or mammals.

Natural selection within genomes and within and among local populations is complex and acts at many levels (consult LEWONTIN, 1970, for a discussion of this problem). In a very general sense, some components of selection operate uniformly over populations and others are more strictly local. The more uniform the abiotic and biotic environment, the greater is the predominance of regional components of selection. Wide-ranging species, or those occupying heterogeneous environments, experience more varied or localized modes of selection. The amount of gene exchange among local populations via migrating larvae or adults controls the genetic cohesiveness of the species. Diminished gene flow due to intrinsically limited dispersal capabilities or to environmental barriers leads to genetic differentiation of populations and ultimately to speciation.

The rate of genetic differentiation of isolated and gene-exchanging populations is poorly understood. Computer-simulated species divided into intermating neighbourhoods differentiate internally in the absence of selection (ROHLF and SCHNELL, 1971). It has been claimed, however, that regional selection pressures are often sufficiently uniform to prevent significant genetic differentiation of even isolated populations (EHRLICH and RAVEN, 1969). A major trend of recent years has been the repeated discovery of unexpectedly high selection coefficients in natural populations (Table 6-10). Selection of this intensity in a heterogeneous environment could produce sympatric genetic divergence, although not necessarily of the magnitude leading to speciation. Genetic divergence of adjacent cliff-top and cliff-face populations of the grass *Agrostis stilonifera* (ASTON and BRADSHAW, 1966) and populations of *Anthoxanthum odoratum* adapted and unadapted to heavy-metal-contaminated soil (McNEILLY and ANTONOVICS, 1968; ANTONOVICS and BRADSHAW, 1970) exists in the face of heavy gene flow. Selection maintains abrupt or steeply clinal boundaries only metres or fractions of metres wide. These findings

Table 6-10

Estimates of coefficients of selection in natural conditions (After ANTONOVICS, 1971; reproduced by permission of American Scientist)

Example	Coefficient of selection	Basis of estimation	Author
Selection for tolerance on mine soil: (a) *Agrostis tenuis* along transection on copper mine	0·54–0·65	Seed/adult comparison	Calculated from MCNEILLY (1968)
(b) *Holcus lanatus* on a lead/zinc mine	0·46		ANTONOVICS (unpublished)
Selection against copper tolerance on pasture: *Agrostis tenuis* along transect downwind from a mine	0·27–0·62	Seed/adult comparison	Calculated from MCNEILLY (1968)
Selection against unspotted type in overwintering leopard frog *Rana pipiens*	0·23–0·38	Change in morph frequency with time	MERRELL and RODELL (1968)
Selection against heavily banded water snakes *Natrix sipedon* compared with relatively unbanded individuals	0·77	Young/adult comparison	EHRLICH and CAMIN (1960)
Selection for toothsize in the fossil horse *Mercychippus primus*	0·27–0·61	Neonatal/yearling comparison	VAN VALEN (1965b)
Advantage of melanic form of *Biston betularia* over other forms in Great Britain (various regions)	0·05–0·35	Change in morph frequency with time	KETTLEWELL (1958)
Selection against larvae and pupae of *Maniola jurtina* destined to give females with 2 or more spots	0·69–0·74	Artificially reared/naturally flying comparison	DOWDESWELL (1961)
Selection against banded *Cepaea nemoralis* in woodlands	0·19	Living/destroyed population comparison	CAIN and SHEPPARD (1954)
Selection against brown *Cepaea nemoralis* on dunes	0·06	Change in morph frequency with time	CLARKE and MURRAY (1962)
Selection for single-banded *Cepaea nemoralis* on dunes	0·05	Change in morph frequency with time	CLARKE and MURRAY (1962)

suggest that local components of selection, if intense enough, do bring about evolution of populations.

(b) Genetic Differentiation in Marine Species

The pelagic larva is the primary agent of distribution and gene flow in many benthic species (THORSON, 1950, 1961). The recruitment or impoverishment of adult populations depends on the number, condition, and timing of larvae reaching the site (COE, 1956). Similarly, the focus of origin of larval dispersal is a function of the distribution of spawning adults. Larval dispersal may be augmented by movements of adults in nektonic species. Larvae do not figure at all in the dispersal of *Littorina saxatilis*, *L. obtusata*, *Nucella lapillus*, and other species that begin independent life as young adults.

Models of marine species may be formulated in which the local components of natural selection are opposed by gene flow. Species that freely broadcast larvae into the plankton stream should possess regionally homogeneous gene pools. Heavy gene flow would retard or prevent genetic adaptation to purely local conditions, and the larvae of each local population would enter a recruitment pool drawn on by numerous populations. Such species would be expected to (i) possess the same alleles at similar frequencies over large areas, (ii) evolve genetic mechanisms that confer physiological flexibility and broad ecological tolerances on individuals, and (iii) show relatively little geographic differentiation in fundamental morphology and physiological mechanisms, particularly under controlled laboratory conditions.

Species in which gene flow is limited should undergo genetic differentiation as each local population becomes increasingly adapted to the peculiarities of the local habitat. In wide-ranging species that lack efficient dispersal, the continued reduction in gene flow, which would transmit a load of inadaptive genes, might be the best adaptive strategy (VERMEIJ, 1972). Random drift may also affect allele frequencies in small, isolated populations. The constellation of characters of the locally adapted species should be (i) the presence of different allele frequencies or even different alleles regionally, (ii) narrow physiological and ecological adaptation to the immediate environment, and (iii) the development of distinct morphological and/or physiological differences among populations.

The legitimacy and usefulness of these models can only be partially tested. Most marine species, of course, occupy an intermediate position between these polar extremes. SCHELTEMA (1971) collected from the North Atlantic Ocean the veligers of 10 species of prosobranch gastropods with amphi-Atlantic adult distributions and compared dispersal abilities with morphological differentiation of the separated populations. He found a generally inverse correlation between larval abundance in oceanic plankton collections and morphological differentiation. These results are consistent with his hypothesis that in species with long-lived and abundant larvae a degree of genetic continuity may be maintained over long distances.

Table 6-11 tabulates estimates of genetic and chromosomal differentiation with distance in species that sharply contrast in dispersal abilities. The estimates are semi-quantitative approximations, based on 1 to 4 loci only. The species *Gadus morhua* to *Mytilus edulis* in that table have the potential for wide dispersal.

Table 6-11

Estimates of genetic and chromosomal differentiation correlated with distance (Compiled from the sources indicated)

Species	Dispersal ability	Regional genetic differentiation	Author
Gadus morhua	High in egg, larval and adult stages	Large differences in allele frequencies at 2 loci over trans-oceanic distances; uniform or gently clinal regionally	SICK (1965a, b), JAMIESON (1968)
Uca pugnax	Moderately high; long-term pelagic larva	Frequencies relatively uniform at 2 loci over 1300 km transect, but variable at a third	SELANDER and co-authors (1971)
Littorina littorea	Moderately high; pelagic larva of several weeks	Allele frequencies at 2 loci similar over 250 km transect	BERGER (1972)
Nassarius obsoletus	Moderately high; pelagic larva of several weeks	Allele frequencies at 2 loci nearly identical over 1000 km transect	GOOCH and co-authors (1972)
Mytilus edulis	Moderately high; pelagic larva of weeks	Relative uniformity at 1 locus over 1000 km transect; moderate variability at another over small area	MITTON and co-authors (1973), MILKMAN (1971)
Schizoporella errata	Limited; short-term pelagic larva	Large difference in allele frequency at 1 locus over 1000 km transect	GOOCH and SCHOPF (1971); SCHOPF (1973)
Littorina saxatilis	Low; no larval dispersal	Moderate difference in allele frequencies at 4 loci over regional distances	BERGER (1972), SNYDER and GOOCH (1973)
Littorina obtusata	Low; no larval dispersal	Allele frequencies moderately variable at 3 loci over 250 km transect	BERGER (1972)
Nucella lapillus	Low; no larval dispersal	Chromosomal morphs differentiated over hundreds of metres	STAIGER (1954, 1957)
Sphaeroma rugicauda	Low, limited to adult	Allele frequencies differ moderately over British coastline	WEST (1964)

Differences in allele frequencies are negligible or low over hundreds of kilometres in *G. morhua*, *Nassarius obsoletus*, and *Littorina littorea*. Results are discordant in *Uca pugnax* and *M. edulis*, in which some loci show little geographic differentiation and others vary on either a microgeographic or regional scale. Examples of this nature presumably indicate that some loci are undergoing selection by components of the environment that differ among localities and others are not. Gene flow maintains uniformity at the latter loci, and differentiating selection dominates at the former.

Schizoporella errata has weak powers of dispersal and gives evidence of some local and considerable regional genetic variation. The species from *Littorina saxatilis* to *Sphaeroma rugicauda* in Table 6-11 do not disperse as larvae; adults are relatively sedentary, and thus fall near the model of the locally adapted species. In each species the evidence for regional and usually local differentiation is good.

These data are consistent with the hypothesis that the fissuring of marine species into local populations is strongly dependent on dispersal and gene flow. However, the contrast in genetic differentiation across the table is not extremely marked. The freshwater amphipod *Gammarus minus* inhabits limestone areas of the eastern USA. Genetic differentiation of spring and cave populations, only 10 km apart, is often of major proportions; some adjacent populations have completely different complements of alleles at two multi-allelic loci (HETRICK and GOOCH, 1973; GOOCH, unpublished). Differences of this magnitude are presently unknown in the sea.

Data that would establish the relationship between morphological and physiological variation over distance with dispersal potential and gene flow are not available to the reviewer.

(c) Natural Selection

Unsystematic genetic changes are effected among populations by random drift, and all genetic differentiation is dampened by gene flow. Natural selection may create patterns when selectively important factors of the environment are systematically arranged. Adaptation to gradationally changing environmental factors may arise through a progressive geographic shift in genotype or phenotype, i.e., a cline. A cline may also come about by gene diffusion into an area between genotypically or phenotypically contrasting populations. Clines in allele frequency have been reported in the bivalve *Anadara trapezia* (O'GOWER and NICOL, 1968), the ectoproct *Schizoporella errata* (GOOCH and SCHOPF, 1971; SCHOPF and GOOCH, 1971b; SCHOPF, 1973), and the fishes *Gadus morhua* (FRYDENBERG and co-authors, 1965), *Anoplarchus purpurescens* (JOHNSON, 1971) and *Anguilla rostrata* (WILLIAMS and co-authors, 1973). These examples are discussed more fully above. Considering also the well-documented cline at an esterase locus in a freshwater fish of the Colorado Plateau, *Catostomus clarki* (KOEHN and RASMUSSEN, 1967; KOEHN, 1969), the majority of allele frequency clines presently known appear to exist in aquatic organisms. It may be that clinal patterns are a special characteristic of adaptation in the sea, where light, temperature, salinity, and substrate variation (Volume I) tend to be more gradational than on land.

The marine clines above all follow a generally north-south course, and so all correlate, to some degree, with temperature variation. It would be a mistake, however, to cast temperature in the role of direct selective factor without additional

evidence. Temperature influences many physical and physiological processes and also the biotic composition of communities (Volume I, Chapter 3); any of these factors or even environmental aspects fortuitously associated with temperature could be involved. The most convincing examples for direct action of temperature are *Anoplarchus purpurescens*, where experimental temperature variation alters allele frequency (JOHNSON, 1971), and *Schizoporella errata*, in which a local reverse cline in temperature superimposed on a regional cline is reproduced by trends in allele frequency at 2 loci (GOOCH and SCHOPF, 1971; SCHOPF, 1973).

Contiguous marine populations may exist with genetic boundaries that are steeply clinal, irregularly gradational, or sharply discontinuous. In each case an explanation is required why gene exchange does not blur the contact into a broad cline and eventually erase genetic differences altogether. The contact may be transitory and of recent origin, perhaps as the result of man-made disturbance; this can only be determined through on-site investigation. It may also be persistent and stable, representing either a directional discontinuity in selection pressures or a zone of secondary hybridization between formerly isolated populations. The first explanation is indicated where the genetic discontinuity coincides with an environmental discontinuity. The recent well-documented evidence for sympatric evolution through strongly opposed selection pressures even in the face of high gene flow reinforces this conclusion. The demonstration of rapid genetic differentiation in *Anguilla rostrata* from a presumably homogeneous gene pool by WILLIAMS and co-authors (1973) is of particular interest. These authors point out that the recognition of selection pressures sufficiently intense to produce discontinuities in allele frequencies without isolation destroys the argument that genetic differentiation must presuppose prior separateness and isolation of populations. They are unquestionably correct where differences in frequencies of isozyme patterns or alleles of widely separated populations have led to conclusions of raciation or speciation (as in much of the Atlantic eel literature). A problem remains where discontinuities of allele frequencies and morphology appear unrelated to the environment — as in the cod populations of the middle Baltic Sea (SICK, 1965b) and the Barents Sea and coastal Norway (MØLLER, 1968). The alternatives of strong but cryptic selection or the existence of imperfectly compatible genetic stocks remain; they can only be discriminated in very thorough studies.

The operation of natural selection on a microgeographic scale is evident in a number of investigations. A very fine selective discrimination of phenotypes occurs in the intertidally stratified forms of *Sphaeroma serratum* (BOCQUET, 1953). Karyotypic differences sometimes exist between adjacent subcolonies of *Nucella lapillus*, even in the absence of ecological barriers (STAIGER, 1957). Genotypic differences in *Schizoporella errata* inhabiting a floating dock occur over sections only a few metres apart (GOOCH and SCHOPF, 1970).

More striking are examples of microgeographic differentiation in species with strong powers of dispersal. These are the marine counterparts to the microgeographic genetic divergence discovered in species of *Agrostis* and *Anthoxanthum*. Particularly instructive are the light and dark form complexes of *Acmaea digitalis* (GIESEL, 1970) and the sculpture morphs of *Littorina picta* (STRUHSAKER, 1968). The pelagic larvae of these species appear to settle indiscriminately over a mosaic of suitable and unsuitable habitats. Selection to maintain microgeographic dis-

crimination must operate intensely against mismatching morphs and must be renewed every generation. It is probable that only species with enormous reproductive potential can withstand such high mortality. Many authors have noted that a correlation often exists between increasing reproductive potential of species and decreasing provision for the survival of each offspring. Here may be found a major advantage of abolishing the dispersal stage. The availability of an environment to support the preceding generation through its reproductive cycle constitutes the best assurance of environmental suitability for the following generation.

(4) Genetic Variability in Populations

(a) Mechanisms of Maintenance

A long-standing controversy among population geneticists concerning the level of genetic variability in natural populations has been brought to a close during the last decade. Formerly, the majority of geneticists reasoned that the fittest genotypes must be homozygotes or else segregational genetic loads would be intolerable, and they assigned most variable loci to the transient polymorphic class. Others were impressed with the observation, as old as Darwin, that morphological variability is universal among organisms, and that this variability has a heritable component—demonstrated by the phenotypic plasticity of domestically bred plants and animals. Discoveries of numerous recessive mutant alleles, including lethals (SPENCER, 1947), and of segregating inversion karyotypes in wild *Drosophila* populations reinforced the viewpoint that gene pools were heterogeneous at a considerable fraction of their loci.

The evidence of electrophoresis genetics has proved incontrovertibly that genetic variability of wild populations is high, indeed higher than most speculation had allowed. Estimates for most species range between 20 to 60% polymorphic loci (compilations in KOJIMA and co-authors, 1970; SELANDER and co-authors, 1970; GOTTLIEB, 1971), with a scattering of higher and lower values. Low levels of variability are likely to be associated with small, inbred populations. Table 6-12 presents estimates of proportion of polymorphic loci for shallow-water and deep-sea marine animals. The range of 0·22 to 0·55 is not detectably different from values for the genera *Drosophila*, *Mus*, *Homo*, and other terrestrial organisms. The proportion of polymorphic loci can be a misleading index of genetic variability since it does not distinguish among polymorphic loci that differ in number of alleles or proportion of heterozygotes. A more accurate index, the proportion of loci heterozygotes per individual, also appears in Table 6-12. Again the estimates are in line with those of terrestrial organisms.

Several mechanisms have been proposed for the maintenance of high levels of genetic variability in populations. These are considered in the following paragraphs.

(i) Neutral allele hypothesis. A new allele originates when a mutational event —such as the substitution of one nucleotide base for another—occurs in a gene, and the primary gene product, usually a protein, is altered as a consequence. The stepwise series is: altered DNA → altered gene product → altered phenotype. It has been proposed that many nucleotide substitutions are without significant effect

Table 6-12

Estimates of genetic variability in marine animals (Compiled from the sources indicated)

Species	Number of loci surveyed	Proportion of loci polymorphic per population	Proportion of loci heterozygous per individual	Author
Mollusca				
Nuculana pontonia	11	0·45	0·23	Gooch and Schopf (1972)
Malletia sp.	9	0·22	0·12	Gooch and Schopf (1972)
Littorina saxatilis	10	0·50	—	Berger (1972), Snyder and Gooch (1973), Gooch (unpublished)
Nassarius obsoletus	9	0·44	—	Gooch and co-authors (1972), Gooch (unpublished)
Arthropoda				
Pandalopsis ampla	12	0·33	0·07	Gooch and Schopf (1972)
Munidopsis diomedeae	10	0·30	0·12	Gooch and Schopf (1972)
Limulus polyphemus	25	0·25	0·06	Selander and co-authors (1970)
Ectoprocta				
Bugula stolonifera	11	0·55	—	Gooch and Schopf (1970)
Schizoporella errata	11	0·18	—	Gooch and Schopf (1970, 1971)
Echinodermata				
Ophiomusium lymani	6	0·33	—	Gooch and Schopf (1972)

on the protein product (due to code degeneracy and interchangeability of some amino acids); hence some phenotypes are not distinguishable by natural selection (Kimura, 1968; King and Jukes, 1969). These virtually neutral alleles are seen as accumulating randomly in populations and as contributing a large share of genetic variability assayed by electrophoresis. The theoretical and molecular arguments of this hypothesis have come under criticism (Clarke, 1970; Maynard-Smith, 1970; Richmond, 1970). There is also a growing body of evidence that selective action occurs at many polymorphic loci in field and experimental populations (Koehn, 1969; Prakash and co-authors, 1969; Ayala and co-authors, 1972; Clegg and Allard, 1972). Since the frequencies of neutral alleles are stochastically determined, any pattern in allele frequencies, such as a cline, makes this interpretation suspect. The possibility remains that an unknown proportion of genetic variability in natural populations consists of nearly neutral alleles, but this would not explain the maintenance of genetic variability generally, nor is the inability to demonstrate selection at a locus proof of its selective neutrality.

(ii) Transient polymorphism. Natural selection may substitute one allele for a pre-existing one until it is nearly fixed in the population. A polymorphism exists

during the transition. Long-time populations of stable habitats are unlikely to contain transient polymorphisms, which should have characterized initial adaptation to the habitat. Populations of habitats disturbed by man or other agencies, on the other hand, may be undergoing adaptive allele substitution at numerous loci.

(iii) Heterozygote superiority. Whenever the fitness of a heterozygous genotype at a locus exceeds that of the homozygotes, allele frequencies eventually come to a stable equilibrium. This is true even if one homozygote is lethal (adaptive value or fitness of zero), for as the allele becomes less common it is found increasingly in high-fitness heterozygous combination and is rarely exposed to selection in the homozygous form. This appears to be the mechanism of maintenance of the lethal allele *yellow* in *Sphaeroma rugicauda* (BISHOP, 1969). It has been argued that heterozygous advantage or heterosis is usually an evolved condition (ANTONOVICS, 1971; FORD, 1971), or that it is innate, with a largely physiological basis. Aspects of the latter view have been presented by GOOCH and SCHOPF (1972) for deep-sea invertebrates.

Heterosis has been repeatedly demonstrated in domesticated animals and laboratory populations, but authenticated examples of single-locus heterosis in wild populations are not numerous. Many examples of genetic polymorphisms with probable heterotic bases have been cited by MAYR (1963), DOBZHANSKY (1970) and FORD (1971). The tetrazolium oxidase locus in *Modiolus demissus* (KOEHN and co-authors, 1973) and the *V* locus in *Tisbe reticulata* (BATTAGLIA, 1958) are examples from marine habitats. Undoubtedly the best understood example of single-locus heterozygote superiority was revealed by the work of ALLISON (1964) in the inheritance of sickle-cell haemoglobins in malarial regions of Africa. One class of homozygous individuals is highly susceptible to endemic falciparum malaria and the other class of homozygotes succumbs to sickle-cell anaemia. Both alleles remain in the population because heterozygotes suffer only a mild anaemia and are partially protected from malaria, and thus contribute disproportionally to the gene pool of succeeding generations.

Heterozygote superiority is detected in populations by an excess of heterozygotes over equilibrium frequencies. Failure to find this excess does not rule out heterozygote superiority, since heterozygote deficiency generated by inbreeding or migration may mask its effects.

(iv) Selection in heterogeneous environments. Selection in an environment that is spatially or temporally variable may co-ordinately vary in rate or direction. Temporal variability, as of temperature, salinity, or resource availability, may cause an otherwise transient polymorphism to persist over long periods, although fixation will eventually be attained in most environments (consult HALDANE and JAYAKAR, 1963, for conditions necessary for stable polymorphism in a temporally varying environment). Spatial heterogeneity coupled with gene flow can indefinitely maintain genetic variability. A population that physically encompasses a mosaic of environments simultaneously experiences different modes of selection in each habitat. Migration insures that alleles favoured in each habitat are interchanged throughout the population. As a first approximation the overall frequency of an allele should be proportional to the frequency of microhabitats in which it is favoured. The ability of environmental heterogeneity in time and space to maintain genetic variability was demonstrated by POWELL (1971) in experimental populations of *Drosophila willistoni*.

(v) Frequency-dependent selection. If the fitness of a genotype increases as a function of its declining frequency in a population, a stable polymorphism will result. This phenomenon is evident at the *esterase-6* locus in *Drosophila melanogaster* (YARBROUGH and KOJIMA, 1967) and in several visible mutants of *Drosophila* in which genotypes are increasingly successful in mating as they become rarer. In mimetic polymorphism of butterflies, the fitness of a mimetic morph depends on its frequency relative to that of the cryptic form (FORD, 1971), and the rarer forms of prey of visually hunting predators may suffer less than proportional predation because they do not fit the 'search-images' of the predators (CLARKE, 1962).

The amount of genetic variability maintained by heterosis, frequency-dependent selection, and selection in heterogeneous environments is regarded by most evolu-

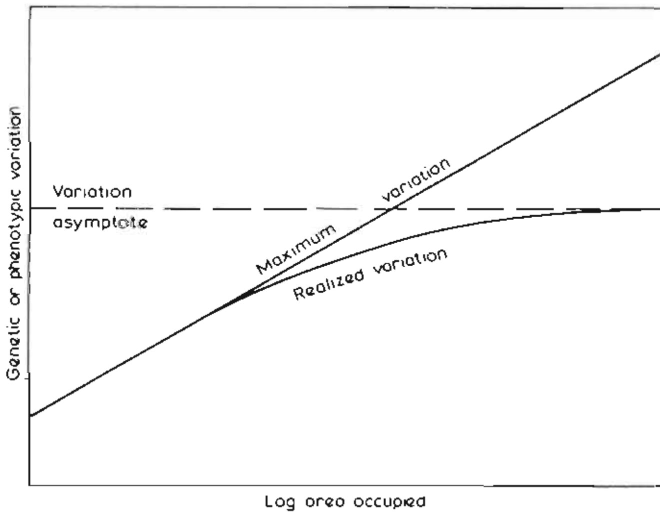

Fig. 6-17: Hypothetical relationship between genetic or phenotypic variation and area occupied. The rate of increase of realized variation declines and approaches an asymptote as area becomes so large that few new alleles reach the population. (*After* SOULÉ, 1971; modified; reproduced by permission of *Taxon*.)

tionists as adaptive, but the significance of this variability in relation to the environment is yet to be established. It has been proposed that high genetic variability permits the exploitation of a wider range of habitat resources and a consequent expansion of the ecological niche (VAN VALEN, 1965a). If the population is nearly panmictic the niche will be broadened and a continuous array of genotypes will occupy continuously intergrading microhabitats. If gene flow is low, the niche will be subdivided and individual populations will express only a fraction of the total genetic variability. In the latter case gene flow might impose a genetic load on populations. Genetic variability may be generated through differential selection over the heterogeneous environment, as stated earlier; or it may be the response to competitive pressure among contiguous individuals of a species (ANTONOVICS, 1971), the intraspecific counterpart of the competitive exclusion principle.

An alternative hypothesis of genetic variability states that much of it is an artefact of gene flow (SOULÉ, 1971). Perhaps the centre of the range of a species is more variable genetically than the periphery, not only as a reflection of occupancy of more habitats, but also because random gene flow centripetally tends to pile up more allelic diversity there (Fig. 6-17). In general, augmented genetic diversity is expected in a population as the area of species-occupied territory adjacent to it increases. According to this gene-flow hypothesis, some of the genetic variability would not be specifically adaptive unless high genetic variability *per se* is adaptive and migration rates are selectively adjusted to keep it high.

The concept of the intrinsic adaptiveness of high genetic variability is often cited by evolutionists, usually without recourse to causal explanations of the origin of variability. The core of the argument is that high genetic variability is a form of insurance against environmental perturbations. Among the multitude of genotypes arising each generation from recombination and syngamy exist combinations potentially adaptive over a wider range of environmental conditions than the population actually experiences. Under a new environmental stress the appropriate alleles are mobilized, enabling a fraction, at least, of the population to survive and propagate. The 'price' for this insurance is exacted each generation as a segregational load of non-adapted genotypes. This genetic load may, in fact, be spurious because low-fitness genotypes may comprise most of the inevitable ecological deaths, the 'ecological load' of each generation (TURNER and WILLIAMSON, 1968; WALLACE, 1970).

(b) Genetic-adaptive Strategies in Marine Organisms

The phenomena of genetic variability, species diversity, and environmental predictability are linked together in hypotheses of genetic-adaptive strategies. SANDERS (1968) distinguishes physically controlled communities with low species diversity and biologically accommodated communities typified by high diversity. In the former, environmental variation is often erratic and severe. A premium is placed on adaptation to fluctuating physical stress and adaptation to the biotic community is secondary. Species that successfully adapt are relatively few and each is able to appropriate a large share of resources and to maintain large numbers. Shallow continental shelf and estuarine communities are of this type, even in regions of high productivity. Biologically accommodated communities occupy uniform or highly predictable habitats, in which time and stability permit the evolution of complex and subtle species interactions. Species become specialists and their population sizes are small. An example is the deep sea, with its low biomass but high species diversity. The extreme physically controlled and biologically accommodated communities are at opposite poles of a continuum, and most communities have intermediate characteristics.

Contrast in species diversity in environments along the continuum is demonstrated by GRASSLE (1967, 1973). Grab samples of mud and sand transecting part of the continental shelf and slope off Cape Lookout, North Carolina, yielded species diversities (measured as H, the Shannon diversity index; SHANNON and WEAVER, 1963) of 4·68 (shelf sample B), 5·14 (slope sample A) and 5·41 (slope sample B). Productivity and resource availability increases in the opposite direction, toward the inshore, more physically controlled communities.

GRASSLE (1973) bases adaptive strategies of marine species on the amount of genetic variability. Species of more physically controlled communities possess high genetic variability. This is presumably generated by environmental heterogeneity and maintenance is made easier by large population size. Genetic variability is viewed in its insurance aspect; some genotypes—temporary and fortuitous elites—survive each unpredictable stress and perpetuate the species. Species of more biologically accommodated communities contain less genetic variability by virtue

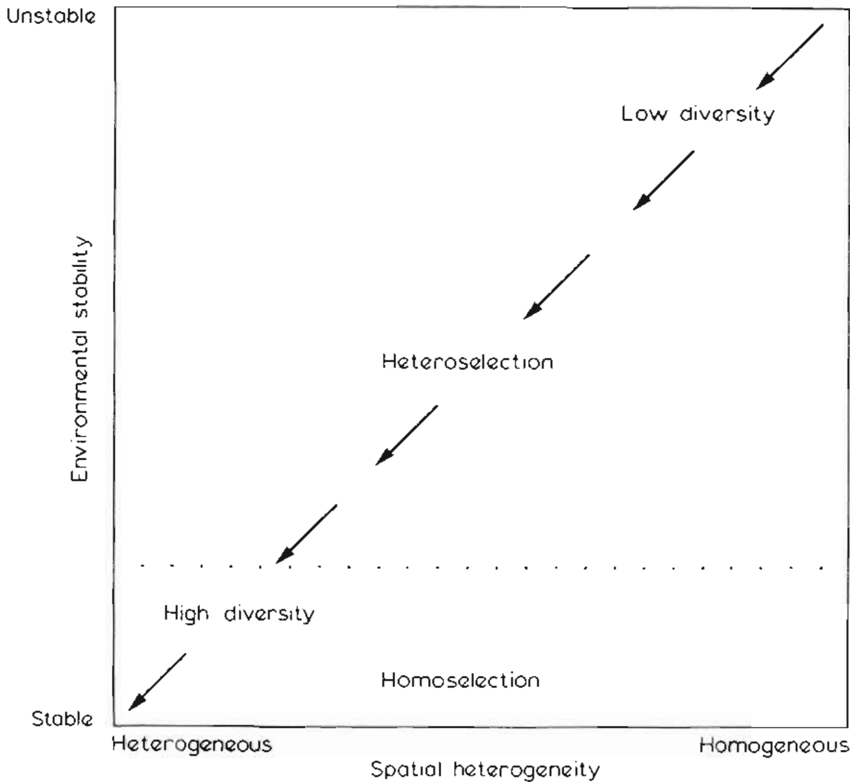

Fig. 6-18: Homoselection and heteroselection as genetic-adaptive strategies of species. According to this hypothesis genetic variability declines with increasing temporal stability of the environment. Spatial heterogeneity promotes high species diversity. (After BRETSKY and LORENZ, 1970; modified; reproduced by permission of Geological Society of America.)

of smaller populations and attendant inbreeding. It is thus predicted that stable environments such as the deep sea will have genetically impoverished faunas relative to those of the shelf. At one point this hypothesis is contradictory. It associates high genetic variability with populations that suffer drastic alternating size reductions and expansions, conditions that should purge alleles from the gene pool during each phase of small size and inbreeding. It remains to be explained how genetic variability is so rapidly replenished after each bottleneck.

A similar but more far-reaching hypothesis of genetic-adaptive strategies is posited by BRETSKY and LORENZ (1970). They assume that environmental stability in time promotes selection for homozygosity, or **homoselection** (used in a different sense than CARSON, 1959), and that environmental instability generates genetic variability through heteroselection (Fig. 6-18). Environments such as the continental shelf and estuaries are primarily heterogeneous and unpredictable, and species of these communities contain high genetic variability, again as insurance for species survival.

Prolonged homoselection in stable high-diversity communities leads to fine adjustment in species relationships, but also increases vulnerability to extinction in periods of environmental vicissitude. There is evidence that diverse faunas of stable habitats in the fossil record have had less geological persistence than low-diversity heteroselected communities, which, to some extent, were pre-adapted to environmental change (BRETSKY, 1969).

(c) Genetic Variability in Marine Environments

The intertidal zone is a highly variable and unstable marine habitat (KNOX, 1963; GREEN, 1969) with widely fluctuating populations (COE, 1956; KNOX, 1963) and might be expected to possess the lowest species diversities. However, much of this variation is due to the predictable tidal cycle, in contrast to lesser but more erratic variability in the subtidal zone such as in temperature, salinity, and visitation by nektonic predators (GREEN, 1969). This author noted decreased diversity in selected subtidal habitats as compared with those of the intertidal. Variability in time and space becomes muted with increasing depth and the deep sea is perhaps the most stable major habitat on earth, although it is by no means monotonous and changeless (SANDERS and co-authors, 1965; SANDERS, 1968; SANDERS and HESSLER, 1969; Volume V).

Much evidence reviewed in this chapter points to the adaptive nature of polymorphism in the intertidal and subtidal environments. Particularly the chromosomal polymorphism of *Nucella lapillus* (STAIGER, 1954, 1967), the colour and sculpture variants of *Acmaea digitalis* (GIESEL, 1970) and *Littorina picta* (STRUHSAKER, 1968), the *V*-locus polymorphism in *Tisbe reticulata* (BATTAGLIA, 1958, 1965), and the protein polymorphisms in *Schizoporella errata* (GOOCH and SCHOPF, 1970, 1971; SCHOPF and GOOCH, 1971b), *Modiolus demissus* (KOEHN and co-authors, 1973), and *Anoplarchus purpurescens* (JOHNSON, 1971) suggest this conclusion. The decline in genetic heterogeneity at 2 loci in intertidal to infaunal bivalves demonstrated by LEVINTON (1973) is consistent with adaptive strategy of reduced genetic variability in uniform environments.

Data are presently insufficient to distinguish levels of genetic variability in intertidal versus shallow-water species. Estimates of levels of polymorphism are available for a few deep-sea species, and they are at variance with the hypotheses of BRETSKY and LORENZ (1970), GRASSLE (1973) and the findings of LEVINTON (1973).

The cosmopolitan brittle star *Ophiomusium lymani* is polymorphic at an esterase locus, with 2 common alleles (DOYLE, 1972). Collections were made at 4 stations at about 2000 m on the continental slope off North Carolina. Genotype frequencies indicate significant heterozygote deficiencies, which could represent inbreeding pop-

ulations or be an artefact of including several populations in each sample (Table 6-13). Allele frequencies are not homogeneous geographically, and most of the heterogeneity appears to relate to depth. These data show that polymorphism exists at great depths and suggest an adaptive pattern of allele frequencies. SCHOPF and GOOCH (1971a) obtained preliminary evidence for high levels of polymorphism in Atlantic continental shelf species. Next a comprehensive investigation of genetic variability in deep-sea species was undertaken (GOOCH and SCHOPF, 1972), including 4 Atlantic continental slope species from off New England and 4 species from the San Diego Trough of the Pacific Ocean. The 8 species—belonging to 6 classes of the phyla Sipunculida, Mollusca, Arthropoda, and Echinodermata—were surveyed for an aggregate of 74 loci, of which 17 were clearly polymorphic, and 18 were inferred as provisionally polymorphic from band variability on gels (Table 6-14). Genetic variability stands at a minimum of 17 of 56 loci (30%), excluding the provisionally polymorphic loci, and at 35 of 74 loci (47%) including the latter. The

Table 6-13

Ophiomusium lymani. Esterase genotype and allele frequencies in a transect off North Carolina (USA) at approximately 2000 m. Collection localities arranged in order from south to north (After DOYLE, 1972; reproduced by permission of Pergamon Press Ltd.)

Collection	AA : AB : BB	$P_B \pm \sigma$*	Chi-Square fit to Hardy–Weinberg
2027	65: 8:2	0·080 ± 0·022	5·69**
2028	44:10:6	0·183 ± 0·035	11·80†
2020	73: 4:0	0·026 ± 0·028	0·06
2031	18: 3:0	0·071 ± 0·040	0·124
Pooled	200:25:8	0·088 ± 0·013	25·59†

*Maximum likelihood estimate of relative frequency of B allele.
**$0·01 < p < 0·025$.
†$p < 0·005$.

primitive infaunal protobranch *Nuculana pontonia* from the San Diego Trough exists in a markedly predictable and stable environment. Yet the mean number of alleles at 5 loci is 3·2 and mean heterozygosity is 0·230, one of the highest values recorded for any organism. These data are difficult to reconcile with the extant hypotheses that environmental predictability and uniformity lead to homoselection and loss of genetic variability in oceans and coastal waters (GOOCH and SCHOPF, 1972; SCHOPF and GOOCH, 1972).

Unless there is more heterogeneity of the continental slopes and deep-sea floor than has been supposed, another explanation must be found for these high levels of genetic variability in the deep-sea fauna. Allele neutrality cannot be ruled out, although evidence from other sources, as stated earlier, has undermined support for this hypothesis. Another possibility is that the protein products of different alleles function optimally at different hydrostatic pressures along the continental slope (GOOCH and SCHOPF, 1972). Experiments suggest that enzymatic stability

Table 6-14

Tabulation of loci in 8 deep-sea species. Loci are scored as monomorphic (i), polymorphic (ii), and provisionally polymorphic (iii). Percent polymorphism is estimated as (ii) + (iii) or, more conservatively, as (ii) only, excluding (iii) from the calculations. Estimates of percent polymorphic loci averaged over species are unweighted means (After GOOCH and SCHOPF, 1972; reproduced by permission of Society for the Study of Evolution)

Species	Monomorphic (i)	Polymorphic (ii)	Provisionally polymorphic (iii)	Percent polymorphic (ii) + (iii)	(ii)
Pacific Ocean					
Pandalopsis ampla	8	4	3	47	33
Munidopsis diomedeae	7	3	2	42	30
Nuculana pontonia	6	5	1	50	45
Malletia sp.	7	2	1	30	22
	28	14	7	42	32
Atlantic Ocean					
Ophiomusium lymani	4	2	2	50	33
Echinus affinus	2	0	2	50	0
Psolus sp.	3	1	5	67	25
Sipunculid	2	0	2	50	0
	11	3	11	54	15

and rate processes are affected by pressure (HOCHACHKA, 1971; PENNISTON, 1971; Volume I, Chapter 8). Gene flow among differently depth-adapted populations would establish high levels of polymorphism. Only on the abyssal plain might monomorphism prevail. The changes of allele frequencies with depth noted by DOYLE (1972) are consistent with this pressure-allele hypothesis.

A further possibility is that the genetic variability is heterotic and that heterozygote superiority is innate and physiological rather than resulting from a balance of environmental factors (GOOCH and SCHOPF, 1972). A number of mechanisms of physiological heterosis have been conceived (SCHWARTZ and LAUGHNER, 1969; BERGER, 1971; SCHWARTZ, 1971; SING and BREWER, 1971; FINCHAM, 1972).

Literature Cited (Chapter 6)

AGERSBORG, H. P. K. (1929). Factors in the evolution of the Prosobranchiata mollusc *Thais lapillus*. *Nautilus*, **43**, 45–49.

AHMED, M. and SPARKS, A. K. (1970). Chromosome number, structure and autosomal polymorphism in the marine mussels *Mytilus edulis* and *Mytilus californianus*. *Biol. Bull. mar. biol. Lab., Woods Hole*, **138**, 1–13.

ALLISON, A. C. (1964). Polymorphism and natural selection in human populations. *Cold Spring Harb. Symp. quant. Biol.*, **29**, 137–150.

ANTONOVICS, J. (1971). The effects of a heterogeneous environment on the genetics of natural populations. *Am. Scient.*, **59**, 593–599.

ANTONOVICS, J. and BRADSHAW, A. D. (1970). Evolution in closely adjacent plant populations. VIII. Clinal patterns at a mine boundary. *Heredity, Lond.*, **25**, 349–362.

ASTON, J. L. and BRADSHAW, A. D. (1966). Evolution in closely adjacent plant populations. II. *Agrostis stolonifera* in maritime habitats. *Heredity, Lond.*, **21**, 649–664.

AYALA, F. J. and POWELL, J. R. (1972). Allozymes as diagnostic characters of sibling species of *Drosophila*. *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1094–1096.

AYALA, F. J., POWELL, J. R., TRACEY, M. L., MOURÃO, C. A. and PEREZ-SALAS, S. (1972). Enzyme variability in the *Drosophila willistoni* group. IV. Genetic variations in natural populations of *Drosophila willistoni*. *Genetics, Princeton*, **70**, 113–139.

BARKMAN, J. J. (1956). On the distribution and ecology of *Littorina obtusata* (L.) and its subspecific units. *Archs néerl. Zool.*, **11**, 22–86.

BARLOW, J. and RIDGWAY, G. J. (1971). Polymorphisms of esterase isozymes in the American lobster (*Homarus americanus*). *J. Fish. Res. Bd Can.*, **28**, 15–21.

BATTAGLIA, B. (1957). Ecological differentiation and incipient intraspecific isolation in marine copepods. *Année Biol.*, **33**, 259–268.

BATTAGLIA, B. (1958). Balanced polymorphism in *Tisbe reticulata*, a marine copepod. *Evolution, Lancaster, Pa.*, **12**, 358–364.

BATTAGLIA, B. (1965). Advances and problems of ecological genetics in marine animals. *Int. Conf. Genet.*, **11** (2), 451–463.

BATTAGLIA, B. (1967). Genetic aspects of benthic ecology in brackish water. In G. H. Lauff (Ed.), *Estuaries*. A.A.A.S., Washington, D.C. pp. 574–577. (*Publs Am. Ass. Advmt Sci.*, **83**.)

BATTAGLIA, B. (1970). Cultivation of marine copepods for genetic and evolutionary research. *Helgoländer wiss. Meeresunters.*, **20**, 385–392.

BATTAGLIA, B. and FAVA, G. (1968). Prime osservazioni sulla genetica di popolazioni naturali e sperimentali di *Tisbe clodiensis* n. sp. (Copepoda, Harpacticoida). *Riv. Biol.*, **61**, 3–19.

BATTAGLIA, B. and FINCO, G. (1969). Fattori biotici e selezione naturale in Copepodi del genere *Tisbe*. *Atti Ist. veneto Sci.* (*Sci. mat. nat.*), **127**, 363–370.

BATTAGLIA, B. and LAZZARETTO, I. (1967). Effect of temperature on the selective value of genotypes of the copepod *Tisbe reticulata*. *Nature, Lond.*, **215**, 999–1001.

BATTAGLIA, B. and VOLKMANN-ROCCO, B. (1969). Gradienti di isolamento riproduttivo in popolazioni geografiche del copepode *Tisbe clodiensis*. *Atti. Ist. veneto Sci.* (*Sci. mat. nat.*), **127**, 371–381.

BEDFORD, B. C. (1966). English cod tagging experiments in the North Sea. ICES, C. M. 1966/G9, Gadoid Fish Committee. 9 pp. (mimeo.)

BERGER, E. M. (1971). A temporal survey of allelic variation in natural and laboratory populations of *Drosophila melanogaster*. *Genetics, Princeton*, **67**, 121–136.

BERGER, E. M. (1972). The distribution of genetic variation in three species of *Littorina*. *Biol. Bull. mar. biol. Lab., Woods Hole*, **143**, 455.

BERRILL, N. J. (1941a). The development of the bud in *Botryllus*. *Biol. Bull. mar. biol. Lab., Woods Hole*, **80**, 169–184.

BERRILL, N. J. (1941b). Size and morphogenesis in the bud of *Botryllus*. *Biol. Bull. mar. biol. Lab., Woods Hole*, **80**, 185–193.

BERRY, A. J. and CROTHERS, J. H. (1968). Stabilizing selection in the dog-whelk (*Nucella lapillus*). *J. Zool.*, **155**, 5–17.

BISHOP, J. A. (1969). Changes in genetic constitution of a population of *Sphaeroma rugicauda* (Crustacea: Isopoda). *Evolution, Lancaster, Pa.*, **23**, 589–601.

BOCQUET, C. (1951). Recherches sur *Tisbe* (=*Idyaea*) *reticulata* n. sp. Essai d'analyse génétique du polychromatisme d'un Copépode Harpacticoide. *Archs Zool. exp. gén.*, **87**, 335–416.

BOCQUET, C. (1953). Recherches sur le polymorphisme naturel des *Jaera marina* (FABR.) (Isopodes asellotes). *Archs Zool. exp. gén.*, **90**, 187–450.

BOCQUET, C. (1954). Evolution of a superspecies of marine isopods. *Syst. Zool.*, **4**, 149–162.

BOCQUET, C., LEVI, C. and TEISSIER, G. (1951). Recherches sur le polychromatisme de *Sphaeroma serratum* (F.). *Archs Zool. exp. gén.*, **87**, 245–297.

BRETSKY, P. W. (1969). Central Appalachian Late Ordovician communities. *Bull. geol. Soc. Am.*, **80**, 193–212.

BRETSKY, P. W. and LORENZ, D. M. (1970). An essay on genetic-adaptive strategies and mass extinctions. *Bull. geol. Soc. Am.*, **81**, 2449–2456.

BROWN, W. V. (1972). *Textbook of Cytogenetics*, C. V. Mosby Co., St. Louis.

BURNET, F. M. (1971). 'Self-recognition' in colonial marine forms and flowering plants in relation to the evolution of immunity. *Nature, Lond.*, **232**, 230–235.

BUZZATI-TRAVERSO, A. A. (1958). Genetische Probleme der marinen Biologie. *Revue suisse Zool.*, **65**, 461–483.

BUZZATI-TRAVERSO, A. A. (1960). Perspectives in marine biology. In A. A. Buzzati-Traverso (Ed.), *Perspectives in Marine Biology*. University of California Press, Berkeley. pp. 613–621.

CAIN, A. J. and SHEPPARD, P. M. (1954). Natural selection in *Cepaea*. *Genetics, Princeton*, **39**, 89–116.

CARSON, H. L. (1959). Genetic conditions which promote or retard the formation of species. *Cold Spring Harb. Symp. quant. Biol.*, **24**, 87–105.

CHRISTENSEN, B. and LOMHOLT, B. (1972). Amylase heterogeneity in *Palaemonetes varians* (LEACH) (Crustacea, Decapoda). *Ophelia*, **10**, 63–65.

CLARKE, B. (1962). Balanced polymorphism and the diversity of sympatric species. *Publs Syst. Ass.*, **4**, 47–70.

CLARKE, B. (1970). Darwinian evolution of proteins. *Science, N.Y.*, **168**, 1009–1011.

CLARKE, B. and MURRAY, J. (1962). Changes of gene frequency in *Cepaea nemoralis*. 2. The estimation of selective values. *Heredity, Lond.*, **17**, 467–476.

CLEGG, M. T. and ALLARD, R. W. (1972). Patterns of genetic differentiation in the slender wild oat species *Avena barbata*. *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1820–1824.

COE, W. R. (1956). Fluctuations in populations of littoral marine invertebrates. *J. mar. Res.*, **16**, 212–232.

COLTON, H. S. (1916). On some varieties of *Thais lapillus* in the Mount Desert region. A study of individual ecology. *Proc. Acad. nat. Sci. Philad.*, **68**, 440–454.

COLTON, H. S. (1922). Variation in the dog whelk, *Thais* (*Purpura auct.*) *lapillus*. *Ecology*, **3**, 146–157.

CUSHING, J. E. (1964). The blood groups of marine animals. *Adv. mar. Biol.*, **2**, 85–131.

DAUTZENBERG, P. and FISCHER, H. (1915). Etude sur *Littorina obtusata* et ses variations. *J. Conch., Paris*, **62**, 87–128.

DAY, J. H. (1963). The complexity of the biotic environment. In J. P. Harding and N. Tebble (Eds), *Speciation in the Sea*. The Systematics Association, London. pp. 31–49.

DOBZHANSKY, T. (1970). *Genetics of the Evolutionary Process*, Columbia University Press, New York.

DOWDESWELL, W. H. (1961). Experimental studies on natural selection in the butterfly *Maniola jurtina*. *Heredity, Lond.*, **16**, 39–52.

DOYLE, R. W. (1972). Genetic variation in *Ophiomusium lymani* (Echinodermata) populations in the deep sea. *Deep Sea Res.*, **29**, 661–664.

DRILHON, A. J., FINE, J. M., BOFFA, G. A., AMOUCH, P. and DROUHET, J. (1966). Les groupes de transferrine chez l'Anguille. Différences phénotypiques entre les anguilles de l'Atlantique et les anguilles Mediterranéennes. *C.r. hebd. Séanc. Acad. Sci., Paris*, **262**, 1315–1318.

DRILHON, A. J., FINE, J. M., BOFFA, G. A., AMOUCH, P. and DROUHET, J. (1967). Les groupes de transferrines chez *Anguilla anguilla*. Etude de deux populations d'origine géographique différente. *C.r. hebd. Séanc. Acad. Sci., Paris*, **265**, 1096–1098.

EDMUNDS, P. H. and SAMMONS, J. I. (1971). Genetic polymorphism of tetrazolium oxidase in bluefin tuna, *Thunnus thynnus*, from the western North Atlantic. *J. Fish. Res. Bd Can.*, **28**, 1053–1055.

EHRLICH, P. R. and CAMIN, J. H. (1960). Natural selection in middle island water snakes (*Natrix sipedon* L.). *Evolution, Lancaster, Pa.*, **14**, 136.

EHRLICH, P. R. and RAVEN, P. H. (1969). Differentiation of populations. *Science, N.Y.*, **165**, 1228–1232.

EVANS, R. G. (1947). Studies on the biology of British limpets. Part I., The genus *Patella* in Cardigan Bay. *Proc. zool. Soc. Lond.*, **117**, 411–423.

EVANS, R. G. (1953). Studies on the biology of British limpets. The genus *Patella* on the south coast of England. *Proc. zool. Soc. Lond.*, **123**, 357–376.

Feare, C. J. (1970). Aspects of the ecology of an exposed shore population of dogwhelks *Nucella lapillus* (L.). *Oecologia* (*Berl.*), **5**, 1–18.

Fincham, J. R. S. (1972). Heterozygous advantage as a likely general basis for enzyme polymorphisms. *Heredity, Lond.*, **28**, 387–391.

Fine, J. M., Drilhon, A., Amouch, P. and Boffa, G. A. (1964). Existence de groupes sériques chez *Anguilla anguilla* L. mise en évidence par electrophorese et autoradiographie de plusiers types de transferrines. *C.r. hebd. Séanc. Acad. Sci., Paris*, **258**, 753–756.

Fischer, A. (1969). Eine Pigmentmangelmutante bei dem Polychaeten *Platynereis dumerilii*. *Molec. gen. Genet.*, **104**, 360–370.

Fischer, A. (1971a). Der Einfluß des *or*-Gens auf die Pigmentierung bei *Platynereis dumerilii* (Polychaeta). I. Pigmentierungsverlauf und Lokalisation der Farbstoffe. *Wilhelm Roux Arch. EntwMech. Org.*, **168**, 226–241.

Fischer, A. (1971b). Der Einfluß des *or*-Gens auf die Pigmentierung bei *Platynereis dumerilii* (Polychaeta). II. Pigmentierung als Wirkung mütterlicher oder generationseigener *or*-Gene. *Wilhelm Roux Arch. EntwMech. Org.*, **168**, 242–268.

Fischer-Piette, E. (1935). Systématique et biogéographie—les Patelles d'Europe et d' Afrique du Nord. *J. Conch., Paris*, **79**, 5–66.

Fischer-Piette, E. and Gaillard, J. M. (1961). Études sur les variations de *Littorina saxatilis*. II. Modifications des caractères au long des estuaires et rias. *Conchol., News*, C, 10–37.

Fischer-Piette, E., Gaillard, J. M. and James, B. L. (1964). Études sur les variations de *Littorina saxatilis*. VI. Quelques cas qui posent de difficiles problemes. *Cah. Biol. mar.*, **5**, 125–171.

Ford, E. B. (1971). *Ecological Genetics*, 3rd ed., Chapman and Hall, London.

Fowler, J. A. (1970). Control of vertebral number in teleosts—an embryological problem. *Q. Rev. Biol.*, **45**, 148–167.

Føyn, B. and Gjøen, I. (1954). Studies on the serpulid *Pomatoceros triqueter* L. *Nytt Mag. Zool.*, **2**, 85–90.

Frydenberg, O., Møller, D., Naevdal, G. and Sick, K. (1965). Haemoglobin polymorphism in Norwegian cod populations. *Hereditas*, **53**, 257–271.

Fürst, M. and Nyman, L. (1969). Isoenzyme polymorphism in *Mysis relicta* Lovén. *Rep. Inst. Freshwat. Res., Drottningholm*, **49**, 44–48.

Fujino, K. (1970). Immunological and biochemical genetics of tunas. *Trans. Am. Fish. Soc.*, **99**, 152–178.

Garside, E. T. (1970). Temperature: Animals—Fishes (Structural responses). In O. Kinne (Ed.), *Marine Ecology*, Vol. I, Environmental Factors, Part 1. Wiley, London. pp. 561–573.

Giesel, J. T. (1970). On the maintenance of a shell pattern and behavior polymorphism in *Acmaea digitalis*, a limpet. *Evolution, Lancaster, Pa.*, **24**, 98–119.

Gooch, J. L. and Schopf, T. J. M. (1970). Population genetics of marine species of the phylum Ectoprocta. *Biol. Bull. mar. biol. Lab., Woods Hole*, **138**, 138–156.

Gooch, J. L. and Schopf, T. J. M. (1971). Genetic variation in the marine ectoproct *Schizoporella errata*. *Biol. Bull. mar. biol. Lab., Woods Hole*, **141**, 235–246.

Gooch, J. L. and Schopf, T. J. M. (1972). Genetic variability in the deep sea: relation to environmental variability. *Evolution, Lancaster, Pa.*, **26**, 545–552.

Gooch, J. L., Smith, B. S. and Knupp, D. (1972). Regional survey of gene frequencies in the mud snail *Nassarius obsoletus*. *Biol. Bull. mar. biol. Lab., Woods Hole*, **142**, 138–156.

Gottlieb, L. D. (1971). Gel electrophoresis: new approach to the study of evolution. *Bioscience*, **21**, 939–944.

Graham, A. and Fretter, V. (1949). The life history of *Patina pellucida* (L.). *J. mar. biol. Ass. U.K.*, **25**, 590–601.

Grassle, J. F. (1967). *Influence of Environmental Variation on Species Diversity in Benthic Communities*, University Microfilms, Ann Arbor, Michigan.

Grassle, J. F. (1973). Species diversity, genetic variability and environmental uncertainty. *Archo Oceanogr. Limnol.* (in press).

Green, R. H. (1969). Population dynamics and environmental variability. *Am. Zool.*, **9**, 393–398.

HALDANE, J. B. S. and JAYAKAR, S. D. (1963). Polymorphism due to selection of varying direction. *J. Genet.*, **58**, 237–242.

HARDING, J. P. and MARSHALL, S. M. (1955). Triploid nauplii of *Calanus finmarchicus*. *Nature, Lond.*, **175**, 175.

HARDING, J. P. and TEBBLE, N. (Eds) (1963). *Speciation in the Sea*, The Systematics Association, London.

HEDRICK, P. W. (1971). A new approach to measuring genetic similarity. *Evolution, Lancaster, Pa.*, **25**, 276–280.

HETRICK, S. and GOOCH, J. L. (1973). Genetic variation in populations of the freshwater amphipod *Gammarus minus* SAY in the central Appalachians. *Bull. natn. speleol. Soc.* **35**, 17–18.

HEUTS, N. J. (1949). Racial divergence in fin ray variation patterns in *Gasterosteus aculeatus*. *J. Genet.*, **49**, 183–191.

HOCHACHKA, P. W. (1971). Enzyme mechanisms in temperature and pressure adaptation of offshore benthic organisms: the basic problem. *Am. Zool.*, **11**, 425–435.

HOESTLANDT, H. (1955). Etude de populations de *Sphaeroma serratum* le long de littoral de la Grande-Bretagne. *C.r. hebd. Séanc. Acad., Sci., Paris*, **240**, 916–919.

HOXMARK, R. C. (1970). The chromosome dimorphism of *Nucella lapillus* (Prosobranchia) in relation to wave exposure. *Nytt Mag. Zool.*, **18**, 229–238.

HOXMARK, R. C. (1971). Shell variation of *Nucella lapillus* in relation to environmental and genetical factors. *Norw. J. Zool.*, **19**, 145–148.

HUBBY, J. L. and THROCKMORTON, L. H. (1968). Protein differences in *Drosophila*. IV. A study of sibling species. *Am. Nat.*, **102**, 193–205.

HYMAN, L. (1959). *The Invertebrates*, Vol. V, McGraw-Hill, New York.

JAMES, B. L. (1968). The characters and distribution of the subspecies and varieties of *Littorina saxatilis* (OLIVI) in Britain. *Cah. Biol. mar.*, **9**, 143–165.

JAMIESON, A. (1970). Cod transferrins and genetic isolates. *Eur. Conf. Anim. Blood Grps Biochem. Polymorph., Warsaw*, **11**, 533–538.

JAMIESON, A., LIGNY, W. DE and NAEVDAL, G. (1971). Serum esterases in mackerel, *Scomber scombrus* L. *Rapp. P.-v. Réun. Cons. int. Explor. Mer*, **161**, 109–117.

JAMIESON, A. and THOMPSON, D. (1972). Blood proteins in North Sea cod. *Eur. Conf. Anim. Blood Grps Biochem. Polymorph, Budapest*, **12**, 571–585.

JOHNSON, A. G., UTTER, F. M. and HODGINS, H. O. (1971). Phosphoglucomutase polymorphism in Pacific ocean perch, *Sebastodes alutus*. *Comp. Biochem. Physiol.*, **39B**, 285–290.

JOHNSON, M. S. (1971). Adaptive lactate dehydrogenase variation in the crested blenny, *Anoplarchus*. *Heredity, Lond.*, **27**, 205–226.

JONES, F. R. H. (1968). *Fish Migration*, Edward Arnold, London.

KETTLEWELL, H. B. D. (1958). A survey of the frequencies of *Biston betularia* (L.) (Lep.) and its melanic forms in Great Britain. *Heredity, Lond.*, **12**, 51–72.

KIMURA, M. (1968). Genetic variability maintained in a finite population due to mutational production of neutral and nearly neutral isoalleles. *Genet. Res.*, **11**, 247–269.

KING, J. L. and JUKES, T. H. (1969). Non-Darwinian evolution. *Science, N.Y.*, **164**, 788–798.

KITCHING, J. A., MUNTZ, L. and EBLING, F. J. (1966). The ecology of Lough Ine. XV. The ecological significance of shell and body forms in *Nucella*. *J. Anim. Ecol.*, **35**, 113–126.

KNOX, G. A. (1963). Problems of speciation in intertidal animals with special reference to New Zealand shores. In J. P. Harding and N. Tebble (Eds), *Speciation in the Sea*. The Systematics Association, London. pp. 7–29.

KOEHN, R. K. (1969). Esterase heterogeneity: Dynamics of a polymorphism. *Science, N.Y.*, **163**, 943–944.

KOEHN, R. K. (1972). Genetic variation in the eel: a critique. *Mar. Biol.*, **14**, 179–181.

KOEHN, R. K. and MITTON, J. B. (1972). Population genetics of marine pelecypods. I. Ecological heterogeneity and adaptive strategy at an enzyme locus. *Am. Nat.*, **106**, 47–56.

KOEHN, R. K. and RASMUSSEN, D. I. (1967). Polymorphic and monomorphic serum esterase heterogeneity in catostomid fish populations. *Biochem. Genet.*, **1**, 131–144.

KOEHN, R. K., TURANO, F. J. and MITTON, J. B. (1973). Population genetics of marine pelecypods. II. Genetic differences in microhabitats of *Modiolus demissus*. *Evolution*, **27**, 100–105.

KOHN, A. J. (1960). Problems of speciation in marine invertebrates. In A. A. Buzzati-Traverso (Ed.), *Perspectives in Marine Biology*. University of California Press, Berkeley. pp. 571–588.

KOJIMA, K., GILLESPIE, J. and TOBARI, Y. N. (1970). A profile of *Drosophila* species' enzymes assayed by electrophoresis. I. Number of alleles, heterozygosities, and linkage disequilibrium in glucose-metabolizing systems and some other enzymes. *Biochem. Genet.*, **4**, 627–637.

LARGEN, M. J. (1966). Some aspects of the biology of *Nucella lapillus*. Unpubl. Ph.D. Thesis, University of London.

LEJUEZ, R. (1966). Comparaison morphologique, biologique et génétique de quelques espècies du genre *Sphaeroma* latrielle (Isopodes flabelliferes). *Archs Zool. exp. gén.*, **107**, 469–667.

LEVINTON, J. (1973). Genetic variation in a gradient of environmental variability: marine Bivalvia (Mollusca). *Science, N.Y.*, **180**, 75–76.

LEWONTIN, R. C. (1970). The units of selection. *A. Rev. Ecology Systematics*, **1**, 1–18.

LEWONTIN, R. C. and HUBBY, J. L. (1966). A molecular approach to the study of genic heterozygosity in natural populations. II. Amount of variation and degree of heterozygosity in natural populations of *Drosophila pseudoobscura*. *Genetics, Princeton*, **54**, 595–609.

LIGNY, W. DE (1969). Serological and biochemical studies on fish populations. *Oceanogr. mar. Biol. A. Rev.*, **7**, 411–513.

LINDSEY, C. C. (1961). The bearing of experimental meristic studies on racial analyses of fish populations. *Proc. Pacif. Sci. Congr.*, **10**, 54–58.

LOVE, R. M. (1970). *The Chemical Biology of Fishes*, Academic Press, London.

LOWE-MCCONNELL, R. H. (1969). Speciation in tropical freshwater fishes. *Biol. J. Linn. Soc.*, **1**, 51–75.

MCNEILLY, T. (1968). Evolution in closely adjacent plant populations. III. *Agrostis tenuis* on a small copper mine. *Heredity, Lond.*, **23**, 99–108.

MCNEILLY, T. and ANTONOVICS, J. (1968). Evolution in closely adjacent plant populations. IV. Barriers to gene-flow. *Heredity, Lond.*, **23**, 205–218.

MANWELL, C., BAKER, C. M. A., ASHTON, P. A. and CORNER, E. D. S. (1967). Biochemical differences between *Calanus finmarchicus* and *C. helgolandicus*. *J. mar. biol. Ass. U.K.*, **47**, 145–169.

MARCKMANN, K. (1954). Is there any correlation between metabolism and number of vertebrae (and other meristic characters) in the sea trout (*Salmo trutta trutta*)? *Meddr Kommn Danm. Fisk.-og Havunders.*, **1**, 1–9.

MAYNARD-SMITH, J. (1970). Population size, polymorphism and the rate of non-Darwinian evolution. *Am. Nat.*, **104**, 231–237.

MAYR, E. (1963). *Animal Species and Evolution*, Belknap Press, Cambridge, USA.

MENZEL, R. W. (1968). Chromosome number in nine families of pelecypod mollusks. *Nautilus*, **82**, 45–50, 53–58.

MERRELL, D. J. and RODELL, C. F. (1968). Seasonal selection in the leopard frog, *Rana pipiens*. *Evolution, Lancaster, Pa.*, **22**, 284–288.

MILKMAN, R. (1967). Genetic and developmental studies on *Botryllus schlosseri*. *Biol. Bull. mar. biol. Lab., Woods Hole*, **132**, 229–243.

MILKMAN, R. (1971). Genetic polymorphism and population dynamics in *Mytilus edulis*. *Biol. Bull. mar. biol. Lab., Woods Hole*, **141**, 397.

MILKMAN, R. and BEATY, L. D. (1970). Large-scale electrophoretic studies of allelic variation in *Mytilus edulis*. *Biol. Bull. mar. biol. Lab., Woods Hole*, **139**, 430.

MITTON, J. B., KOEHN, R. K. and PROUT, T. (1973). Population genetics of marine pelecypods. III. Epistasis between functionally related isoenzymes of *Mytilus edulis*. *Genetics, Princeton*, **73**, 487–496.

MØLLER, D. (1966). Polymorphism of serum transferrin in cod. *FiskDir. Skr.* (*Ser. Havunders.*), **14**, 51–60.

MØLLER, D. (1968). Genetic diversity in spawning cod along the Norwegian coast. *Hereditas*, **60**, 1–32.

MØLLER, D. (1970). Transferrin polymorphism in Atlantic salmon (*Salmo salar*). *J. Fish. Res. Bd Can.*, **27**, 1617–1625.

MØLLER, D. (1971). Genetic diversity in salmon. ICES, C. M. 1971/M 14, Anadromous and Catadromous Fish Committee. 16 pp. (mimeo).

MØLLER, D. and NAEVDAL, G. (1967). Transferrin polymorphism in fishes. *Eur. Conf. Anim. Blood Grps Biochem. Polymorph., Paris*, **10**, 367–372.

MØLLER, D. and NAEVDAL, G. (1969). Studies on hemoglobins of some gadoid fishes. *FiskDir. Skr. (Ser. Havunders.)*, **15**, 91–97.

MOORE, H. B. (1936). The biology of *Purpura lapillus* and shell variation in relation to environment. *J. mar. biol. Ass. U.K.*, **21**, 69–81.

NEI, M. (1971). Interspecific gene differences and evolutionary time estimated from electrophetic data on protein identity. *Am. Nat.*, **105**, 385–398.

NORTHCOTE, T. G., WILLISCROFT, S. N. and TSUYUKI, H. (1970). Meristic and lactate dehydrogenase genotype differences in stream populations of a rainbow trout below and above a waterfall. *J. Fish. Res. Bd Can.*, **27**, 1987–1995.

O'GOWER, A. K. and NICOL, P. I. (1968). A latitudinal cline of haemoglobins in a bivalve mollusc. *Heredity, Lond.*, **23**, 485–492.

OKA, H. and WATANABE, H. (1957a). Colony-specificity in compound ascidians as tested by fusion experiments. *Proc. imp. Acad. Japan.*, **33**, 657–659.

OKA, H. and WATANABE, H. (1957b). Vascular budding, a new type of budding in *Botryllus*. *Biol. Bull. mar. biol. Lab., Woods Hole*, **112**, 225–240.

PANTELOURIS, E. M. and PAYNE, R. H. (1968). Genetic variation in the eel. I. The detection of haemoglobin and esterase polymorphisms. *Genet. Res.*, **11**, 319–325.

PANTELOURIS, E. M., ARNASON, A. and TESCH, F. W. (1970). Genetic variation in the eel. II. Transferrins, haemoglobins and esterases in the eastern North Atlantic. Possible interpretations of phenotypic frequency differences. *Genet. Res.*, **16**, 277–284.

PANTELOURIS, E. M., ARNASON, A. and TESCH, F. W. (1971). Genetic variation in the eel. III. Comparisons of Rhode Island and Icelandic populations. Implications for the Atlantic eel problem. *Mar. Biol.*, **9**, 242–249.

PENNISTON, J. T. (1971). High hydrostatic pressure and enzymic activity: inhibition of multimeric enzymes by dissociation. *Archs Biochem. Biophys.*, **142**, 322–332.

PESCH, G. (1972). Isozymes of lactate dehydrogenase in the hard clam, *Mercenaria mercenaria*. *Comp. Biochem. Physiol.*, **43B**, 33–38.

POWELL, J. R. (1971). Genetic polymorphisms in varied environments. *Science, N.Y.*, **174**, 1035–1036.

PRAKASH, S., LEWONTIN, R. C. and HUBBY, J. L. (1969). A molecular approach to the study of genic heterozygosity in natural populations. IV. Patterns of genic variation in central, marginal, and isolated populations of *Drosophila pseudoobscura*. *Genetics, Princeton*, **61**, 841–858.

RAY, D. L. (1960). Some marine invertebrates useful for genetic research. In A. A. Buzzati-Traverso (Ed.), *Perspectives in Marine Biology*. University of California Press, Berkeley. pp. 497–512.

RICHMOND, R. C. (1970). Non-Darwinian evolution: a critique. *Nature, Lond.*, **225**, 1025–1028.

RIDGWAY, G. J., SHERBURNE, S. W. and LEWIS, R. D. (1970). Polymorphism in the esterases of Atlantic herring. *Trans. Am. Fish. Soc.*, **99**, 147–151.

ROHLF, F. J. and SCHNELL, G. D. (1971). An investigation of the isolation-by-distance model. *Am. Nat.* **105**, 295–324.

ROLLEFSEN, G. (1954). Observations on cod and cod fisheries of Lofoten. *Rapp. P.-v. Réun. Cons. perm. int. Explor. Mer*, **136**, 40–47.

ROSS, H. H. (1962). *A Synthesis of Evolutionary Theory*, Prentice-Hall, Englewood Cliffs, N.J.

SABADDIN, A. (1959). Analisi genetica del policromatismo di *Botryllus schlosseri*. *Boll. Zool.*, **26**, 221–243.

SABADDIN, A. (1969). The compound ascidian *Botryllus schlosseri* in the field and in the laboratory. *Pubbl. Staz. zool. Napoli*, **37** (Suppl.), 62–72.

SABADDIN, A. and GRAZIANI, G. (1967a). New data on the inheritance of pigments and pigmentation patterns in the colonial ascidian *Botryllus schlosseri* (PALLAS). *Riv. Biol.*, **60**, 559–598.

SABADDIN, A. and GRAZIANI, G. (1967b). Microgeographical and ecological distribution of colour morphs of *Botryllus schlosseri* (Ascidiacea). *Nature, Lond.*, **213**, 815–816.

SACCHI, C. F. (1961a). Contribution a l'étude des rapports écologie-polychromatisme chez un Prosobranche intercotidal, *Littorina obtusata*. *Cah. Biol. mar.*, **2**, 271–290.

SACCHI, C. F. (1961b). Relazioni ecologia-policromatismo nel Prosobranco intertidale *L. obtusata* (L.). II. Richerche biometriche. *Boll. Zool.*, **28**, 517–528.

SACCHI, C. F. (1963). Contribution a l'étude des rapports écologie-polychromatisme chez un Prosobranche intercotidal, *Littorina obtusata*. III. Données experimentales et diverses. *Cah. Biol. mar.*, **4**, 299–313.

SACCHI, C. F. (1964). Relazioni tra ecologia e policromatismo nel Prosobranco intertidale *L. obtusata* (L.). IV. Studio sulla Ria di Vigo. *Archo zool. ital.*, **49**, 93–156.

SACCHI, C. F. (1966). Contribution a l'étude des rapports entre variabilité et écologie chez un prosobranche intertidal *Littorina obtusata*. V. Recherches dans le Plymouth Sound (Angleterre S.-W.). *Cah. Biol. mar.*, **7**, 281–294.

SANDERS, H. L. (1968). Marine benthic diversity: a comparative study. *Am. Nat.*, **102**, 243–282.

SANDERS, H. L. and HESSLER, R. R. (1969). Ecology of the deep-sea benthos. *Science, N.Y.*, **163**, 1419–1424.

SANDERS, H. L., HESSLER, R. R. and HAMPSON, G. R. (1965). An introduction to the study of the deep-sea benthic faunal assemblages along the Gay Head–Bermuda transect. *Deep Sea Res.*, **12**, 845–867.

SCHELTEMA, R. S. (1964). Feeding habits and growth in the mud snail *Nassarius obsoletus*. *Chesapeake Sci.*, **5**, 161–166.

SCHELTEMA, R. S. (1965). The relationship of salinity to larval survival and development in *Nassarius obsoletus*. *Biol. Bull. mar. biol. Lab., Woods Hole*, **129**, 340–354.

SCHELTEMA, R. S. (1967). The relationship of temperature to the larval development of *Nassarius obsoletus* (Gastropoda). *Biol. Bull. mar. biol. Lab., Woods Hole*, **132**, 253–265.

SCHELTEMA, R. S. (1971). Larval dispersal as a means of genetic exchange between geographically separated populations of shallow water benthic marine gastropods. *Biol. Bull. mar. biol. Lab., Woods Hole*, **140**, 284–322.

SCHMIDT, J. (1922). The breeding places of the eel. *Phil. Trans. R. Soc.* (*B*), **211**, 179–208.

SCHMIDT, J. (1925). The breeding places of the eel. *A. Rep. Smithson. Inst*, **1924**, 279–316.

SCHMIDT, J. (1930). Racial investigations. X. The Atlantic cod (*Gadus callarias*) and local races of the same. *C.r. Trav. Lab. Carlsberg*, **18**, 1–72.

SCHOPF, T. J. M. (1973). Survey of genetic differentiation in a coastal zone invertebrate: the ectoproct *Schizoporella errata*. *Biol. Bull. mar. biol. Lab., Woods Hole*, **145**, 78–87.

SCHOPF, T. J. M. and GOOCH, J. L. (1971a). A natural experiment using deep-sea invertebrates to test the hypothesis that genetic homozygosity is proportional to environmental stability. *Biol. Bull. mar. biol. Lab., Woods Hole*, **141**, 401.

SCHOPF, T. J. M. and GOOCH, J. L. (1971b). Gene frequencies in a marine ectoproct: a cline in natural populations related to sea temperature. *Evolution, Lancaster, Pa.*, **25**, 286–289.

SCHOPF, T. J. M. and GOOCH, J. L. (1972). A natural experiment to test the hypothesis that loss of genetic variability was responsible for mass extinctions of the fossil record. *J. Geol.*, **80**, 481–483.

SCHOPF, T. J. M. and MURPHY, L. S. (1973). Protein polymorphism of the hybridizing seastars *Asterias forbesi* and *Asterias vulgaris* and implications for their evolution. *Biol. Bull. mar. biol. Lab., Woods Hole*, **145**, 589–597.

SCHWARTZ, D. (1960). Genetic studies on mutant enzymes in maize: synthesis of hybrid enzymes by heterozygotes. *Proc. natn. Acad. Sci. U.S.A.*, **46**, 1210–1215.

SCHWARTZ, D. (1971). Genetic control of alcohol dehydrogenase—a competition model for regulation of gene action. *Genetics, Princeton*, **67**, 411–425.

SCHWARTZ, D. and LAUGHNER, W. J. (1969). A molecular basis for heterosis. *Science, N.Y.*, **166**, 626–627.

SELANDER, R. K., JOHNSON, W. E. and AVISE, J. C. (1971). Biochemical population genetics of fiddler crabs (*Uca*). *Biol. Bull. mar. biol. Lab., Woods Hole*, **141**, 102.

SELANDER, R. K., YANG, S. Y., LEWONTIN, R. C. and JOHNSON, W. E. (1970). Genetic variation in the horseshoe crab (*Limulus polyphemus*), a phylogenetic 'relic'. *Evolution, Lancaster, Pa.*, **24**, 402–414.

SEXTON, E. W. and CLARK, A. R. (1936). Summary of the work on the amphipod *Gammarus chevreuxi* that SEXTON carried out at the Plymouth Laboratory (1912–1936). *J. mar. biol. Ass. U.K.*, **21**, 357–414.

SHANNON, C. E. and WEAVER, W. (1963). *The Mathematical Theory of Communication*, University of Illinois Press, Urbana.

SICK, K. (1961). Haemoglobin polymorphism in fishes. *Nature, Lond.*, **192**, 874–896.

SICK, K. (1965a). Haemoglobin polymorphism of cod in the Baltic and the Danish Belt Sea. *Hereditas*, **54**, 19–48.

SICK, K. (1965b). Haemoglobin polymorphism of cod in the North Sea and the North Atlantic Ocean. *Hereditas*, **54**, 49–69.

SICK, K. M., BAHN, E., FRYDENBERG, O., NIELSEN, J. T. and WETTSTEIN, D. VON (1967). Haemoglobin polymorphism of the American fresh water eel *Anguilla*. *Nature, Lond.*, **214**, 1141–1142.

SICK, K., WESTERGAARD, M. and FRYDENBERG, O. (1962). Haemoglobin pattern and chromosome number of American, European, and Japanese eels (*Anguilla*). *Nature, Lond.*, **193**, 1001–1002.

SIMPSON, G. G. (1944). *Tempo and Mode in Evolution*. Columbia University Press, New York.

SING, C. F. and BREWER, G. J. (1971). Evidence for non-random multiplicity of gene products in 22 plant genera. *Biochem. Genet.*, **5**, 243–251.

SNYDER, T. and GOOCH, J. L. (1973). Genetic differentiation in *Littorina saxatilis* (Gastropoda). *Mar. Biol.*, **22**, 177–182.

SOULÉ, M. (1971). The variation problem: the gene glow hypothesis. *Taxon*, **20**, 37–50.

SPENCER, W. P. (1947). Mutations in wild populations of *Drosophila*. *Adv. Genet.*, **1**, 359–402.

STAIGER, H. (1954). Die Chromosomendimorphismus beim Prosobranchier *Purpura lapillus* in Beziehung zur Ökologie der Art. *Chromosoma*, **6**, 419–478.

STAIGER, H. (1957). Genetical and morphological variation in *Purpura lapillus* with respect to local and regional differentiation of population groups. *Année Biol.*, **33**, 251–258.

STORMONT, C. (1961). Serological criteria for allelism in blood-typing studies. *Proc. Pacif. Sci. Congr.*, **10**, 186–187.

STRUHSAKER, J. W. (1968). Selection mechanisms associated with intraspecific shell variation in *Littorina picta*. (Prosobranchia: Mesogastropoda). *Evolution, Lancaster, Pa.*, **22**, 459–480.

STUBBINGS, H. G. (1954). Biology of the common mussel in relation to fouling problems. *Research, Lond.*, **7**, 222–229.

TÅNING, A. V. (1952). Experimental study of meristic characters in fishes. *Biol. Rev.*, **27**, 169–193.

TEISSIER, G. (1969). Génétique des populations de *Sphaeroma serratum*, Isopode littoral. *Pubbl. Staz. zool. Napoli*, **37** (Suppl.), 135–145.

TEST, A. R. (1945). Ecology of California *Acmaea*. *Ecology*, **26**, 379–405.

THORSON, G. (1950). Reproductive and larval ecology of marine bottom invertebrates. *Biol. Rev.*, **25**, 1–45.

THORSON, G. (1961). Length of pelagic larval life in marine bottom invertebrates as related to larval transport by ocean currents. In M. Sears (Ed), *Oceanography*, A.A.A.S., Washington, D.C. pp. 455–474. (*Publs Am. Ass. Advmt Sci.*, **67**.)

TRAUT, W. (1968). Genetische Analyse zweier Mutanten von *Dinophilis gyrociliatus* (Archiannelida) mit veränderter Eigrösse. *Helgoländer wiss. Meeresunters.*, **18**, 296–316.

TSUYUKI, H., ROBERTS, E. and BEST, E. A. (1969). Serum transferrin systems and the hemoglobins of the Pacific halibut (*Hippoglossus stenolepis*). *J. Fish. Res. Bd Can.*, **26**, 2351–2362.

TUCKER, D. W. (1959). A new solution to the Atlantic eel problem. *Nature, Lond.*, **183**, 495–501.

TURNER, J. R. G. and WILLIAMSON, M. H. (1968). Population size, natural selection and genetic load. *Nature, Lond.*, **218**, 700.

UTTER, F. M., AMES, W. E. and HODGINS, H. (1970). Transferrin polymorphism in coho salmon (*Oncorhynchus kisutch*). *J. Fish. Res. Bd Can.*, **27**, 2371–2373.

VAN VALEN, L. (1965a). Morphological variation and width of ecological niche. *Am. Nat.*, **94**, 377–390.

VAN VALEN, L. (1965b). Selection in natural populations. III. Measurement and estimation. *Evolution, Lancaster, Pa.*, **19**, 514–528.

VERMEIJ, G. J. (1972). Endemism and environment: some shore molluscs of the tropical Atlantic. *Am. Nat.*, **106**, 89–102.

VOLKMANN-ROCCO, B. and FAVA, G. (1969). Two sibling species of *Tisbe* (Copepoda, Harpacticoida): *Tisbe reluctans* and *T. persimilis* n. sp. Research on their morphology and population dynamics. *Mar. Biol.*, **3**, 159–164.

WALLACE, B. (1968). *Topics in Population Genetics*, Norton and Co., New York.

WALLACE, B. (1970). *Genetic Load*, Prentice-Hall, Englewood Cliffs, N.J.

WEST, D. A. (1964). Polymorphism in the isopod *Sphaeroma rugicauda*. *Evolution, Lancaster, Pa.*, **18**, 671–684.

WIESER, W. (1960). Problems of species formation in benthic microfauna of the deep sea. In A. A. Buzzati-Traverso (Ed), *Perspectives in Marine Biology*. University of California Press, Berkeley. pp. 513–518.

WILLIAMS, G. C., KOEHN, R. R. and MITTON, J. B. (1973). Genetic differentiation without isolation in the American eel, *Anguilla rostrata*. *Evolution, Lancaster, Pa.*, **27**, 192–204.

WIUM-ANDERSEN, G. (1970). Haemoglobin and protein variation in three species of *Littorina*. *Ophelia*, **8**, 267–273.

WOODS, S. (1969). Polyteny and size variation in the copepod *Pseudocalanus* from two semi-landlocked fiords on Baffin Island. *J. Fish. Res. Bd Can.*, **26**, 543–556.

YARBROUGH, K. and KOJIMA, K. (1967). The model of selection at the polymorphic *esterase-6* locus in cage populations of *Drosophila melanogaster*. *Genetics, Princeton*, **57**, 677–686.

ZEUNER, F. E. (1958). *Dating the Past. An Introduction to Geochronology*, 4th ed., Methuen and Co., London.

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