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Presence in the Intestinal Lumen of Marine Fish of Corpuscles with a High Cadmium-, Zinc- and Copper-Binding Capacity: A Possible Mechanism of Heavy Metal Tolerance

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ABSTRACT: In various species of unfed fish (*Anguilla anguilla, Myoxocephalus scorpius, Serranus cabrilla, Moena chryselis, Scorpaena* sp.), white mucous corpuscles were observed in the intestinal lumen. This material is regularly evacuated. The corpuscles contain high concentrations of Ca and Mg; these elements are probably present in the form of carbonates precipitated from sea water contained in the intestine. In fish intoxicated with $CdCl_2$, $ZnCl_2$ or $CuCl_2$ added to sea water, the corpuscles contain enormous concentrations of these metals; although the weight of the corpuscles is small (about 0.1 % of the total wet weight of *Anguilla anguilla*), they may contain most of the Cd body burden. Corpuscles from non-intoxicated fish bind heavy metals *in vitro*: exposed to Cd, Zn or Cu enriched solutions, they retain these metals to the same extent as *in vivo*. It thus appears that metals are accumulated in the corpuscles directly from ingested sea water with a reduction in respective metal concentrations of the intestinal liquid. Intestinal corpuscles seem therefore to limit the entry of metals through the intestinal wall and to protect the fish against potentially hazardous concentrations of heavy metals.

INTRODUCTION

In context with experimental studies of Cd accumulation in the eel *Anguilla anguilla* (Noël-Lambot and Bouquegneau, 1977; Noël-Lambot et al., 1978) we observed in the intestinal lumen of unfed fish, white mucous corpuscles with a very high Cd content. This material, regularly evacuated through the anus, was termed 'intestinal corpuscles'. The nature of this material and the mechanisms of Cd-, Zn- and Cu-binding in eels and other teleost species have been investigated.

MATERIAL AND METHODS

Individuals of the fish species *Anguilla anguilla, Myoxocephalus scorpius, Serranus cabrilla, Moena chryselis* and *Scorpaena* sp. were placed in polyethylene bags containing aerated natural sea water (5 l ind.$^{-1}$) to which various doses of $CdCl_2$, $ZnCl_2$ and/or $CuCl_2$ were added. Before intoxication, freshwater eels (45 to 60 cm in length) were adapted for 1 week to sea water. The water was changed daily; its temperature was about 18 °C. Its initial content in heavy metals was: 0.003 ppm Cd, 0.017 ppm Zn, 0.014 ppm Cu. The fish were not fed.

Stomach and intestine were clamped by means of artery clips, cut off and their contents were collected. Intestinal corpuscles were separated manually from the intestinal liquid and then stored in a deep freezer or analysed immediately.

Cd and Ca analyses were made by atomic absorption spectrophotometry (A.A.S., Perkin Elmer, Model 103) after mineralization for 12 h at 80 °C in 65 % HNO_3 (2.5 ml HNO_3 g^{-1} wet weight) and twentyfold dilution. Owing to the high Ca and Na content of the intestinal corpuscles and the interference of both elements with Cd determination by A.A.S. (Pulido et al., 1966; Friberg et al., 1974), this method was only used for samples of corpuscles with Cd concentration higher than 4 ppm w.wt. Corpuscles with lower Cd concentrations were analysed by anodic stripping voltammetry (MASA-2014 ESA) after ashing with microwave activated oxygen (calcinator Tracerlab 600) and dissolution in concentrated HCl (Gillain and Duyckaerts, 1977). One sample of intestinal corpuscles was also analysed by spark source mass spectrography (AEI, type MS702R) following the method described by Gauneau (1975).

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RESULTS

Nature of Intestinal Corpuscles

The material named 'intestinal corpuscles' was observed in unfed fish living in natural sea water enriched, or not, with heavy metals. Absent in eels living in fresh water, it appears in the first hours after transfer into sea water. The intestinal corpuscles consist of tangled white threads enveloped in a material of mucous (Figs 1 and 2). They are exclusively found in the intestinal lumen or in the surrounding water where they are eliminated. Often, their appearance changes according to the part of the intestine occupied: mucoid corpuscles predominate in the anterior intestine, chalky corpuscles in the posterior region (in Fig. 2 both materials occur in the same corpuscle).

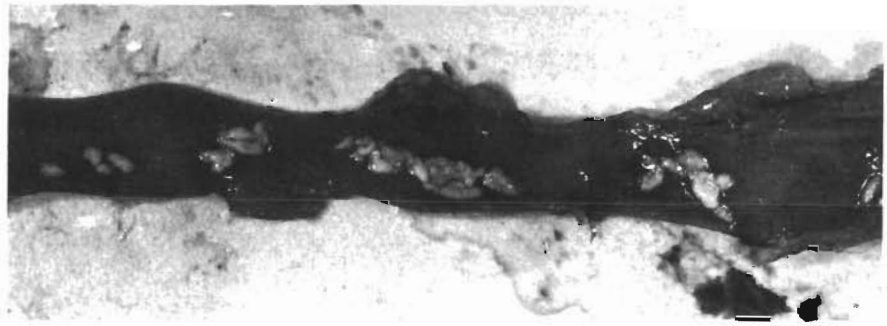

Fig. 1. *Anguilla anguilla.* Longitudinal section of intestine showing intestinal corpuscles (1.5 ×)

Organic matter is not an abundant constitutent of the corpuscles as indicated by the small weight loss during calcination of the dry matter. Microscopic examination shows that intestinal corpuscles consist of granules (about 1 μm in diameter). These granules are very abundant in the chalky portions of the corpuscles, whereas in the mucous portions they are grouped in scattered islets. These granules, which are the main constituents of the corpuscles, remain unaltered during prolonged conservation (several months) at room temperature. The granules have a high carbonate content as indicated by their disappearance accompanied by a gas emission upon treatment by acids. In the intestinal corpuscles formed in the eels during the first hours following transfer to sea water, next to the granules one can observe some crystals, probably of calcite (average size: 0.1 mm).

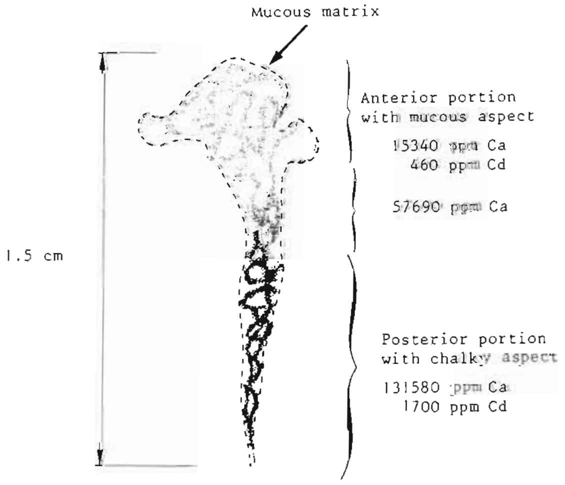

Fig. 2. *Anguilla anguilla.* Intestinal corpuscle of an individual exposed for 4 d to sea water containing 13 ppm Cd. Ca and Cd concentrations (ppm w.wt.) are given for various parts

Table 1. *Anguilla anguilla.* Analysis of intestinal corpuscles from individuals living in natural sea water

Element	ppm dr.wt.*	ppm w.wt.**
Mg	126 000	
Ca	75 000	
Na	20 500	
S	5 400	
K	1 300	
Sr	700	
P	600	
Si	150	
As	74	
Zn	46	39–98 (m = 63)
Ti	31	
Fe	27	
F	20	
Cu	—	4–27 (m = 20)
Al	15	
Cr	11	
Br	10	
Pb	—	3–12 (m = 7)
Ba	5	
Sb	4	
Cd	—	0.3–0.5 (m = 0.4)

* Mass spectrography analysis (n = 1)
** Anodic stripping voltammetry analysis (n = 4)

Table 1 lists the results of mass spectrography and

Table 2. *Anguilla anguilla*. Cd concentration of intestinal corpuscles from individuals intoxicated for 8 d or more (see text) in sea water plus $CdCl_2$

Cd in water (ppm)	n	Cd in corpuscles (ppm w.wt.) Extreme values	Mean	Maximum concentration factor*
0.003	4	0.3– 0.5	0.4	167
0.020	2	2.9– 3.1	3.0	155
0.060	2	1.8– 3.5	2.6	58
0.13	7	5.0– 27.0	22.0	207
1.00	4	7.0– 71.0	55.0	71
13.00	7	141.0–1700.0	396.0	131
100.0	7	33.0–3397.0	942.0	34

* Conc. factor = Cd in corpuscles (ppm w.wt.)/Cd in water (ppm)

Table 3. *Scorpaena* sp. Cd, Zn and Cu concentrations of intestinal corpuscles from scorpionfish in absence of intoxication or after 1 d intoxication in sea water containing 1 ppm of Cd, 1 ppm of Zn and 1 ppm of Cu

Condition	Cd	Zn	Cu	Cd	Zn	Cu
	(ppm w.wt.)			(ppm dr.wt.)		
Control	<1	25.3	4.5	<4	94.9	16.9
1 d intoxication	99.0	158.6	78.6	220.3	353.1	175.0

Table 4. *Anguilla anguilla*. Cd distribution in individuals intoxicated for 8 d in sea water containing 13 ppm Cd (n = 4)

Organs	Weight of organs (g)	Cd conc. (ppm or $\mu g\ g^{-1}$ w.wt.)	Cd load (μg)*
Muscles	75.5	0.2	18.9
Skin	10.6	0.6	6.9
Bones	6.6	0.2	1.6
Digestive tract	2.1	4.8	10.1
Liver	1.2	10.3	12.4
Kidneys	0.7	5.7	4.0
Fat	0.7	0.3	0.2
Plasma	0.6	0.8	0.5
Gills	0.5	4.0	2.0
Intestinal fluid	0.4	3.0	1.2
Blood cells	0.3	–	–
Spleen	0.2	–	–
Air bladder	0.2	–	–
Bile	0.1	0.4	<0.1
Heart	0.1	–	–
Gastric fluid	0.1	10.0	1.0
Intestinal corpuscles	0.1	396.0	39.6
Total	100.0	0.98	98.5

* From metal concentrations in different organs and from weight fractions of these organs, the metal load (expressed in μg) of each organ can be calculated for a fish whose weight is reduced to 100 g. The total body load is determined by summation of the load of each organ

anodic stripping voltammetry (ASV) analyses of intestinal corpuscles of eels from unpolluted sea water. Concentrations of Mg and Ca are high and some metals, present as traces in sea water, are found at comparatively high concentrations: As, Al, Cd, Cr, Cu, Fe, P, Pb, Sb, Ti, Zn.

A.A.S. analyses confirm the existence of high concentrations of Ca in the intestinal corpuscles, especially in their more opaque areas (Fig. 2), which is where the microgranules are most numerous. The variations of Cd concentrations in the corpuscles of intoxicated eels follow approximately those of Ca (Fig. 2). This probably explains the pronounced differences between Cd concentrations in corpuscles collected in identical conditions (Table 2).

Heavy Metals Accumulation in Intestinal Corpuscles

Metal concentrations in the intestinal corpuscles from fish submitted for 1 d or longer to different Cd, Zn and Cu concentrations are shown in Tables 2 and 3. Metal concentrations in the corpuscles similar to those presented in Tables 2 and 3 are already attained during the first hours of intoxication (see below). They then remain nearly constant during intoxication and decline rapidly after the fish are returned to clean water.

Cd concentrations in intestinal corpuscles from intoxicated fish are generally higher than in any other organ. This is why, in spite of their low weight (ca 0.1 g), the intestinal corpuscles account for a large fraction of the total Cd content in the fish. The study of Cd distribution in the different organs of eels during experimental intoxication shows that after 8 d in sea water containing 13 ppm Cd, the intestinal corpuscles contain almost as much Cd as do the remaining body parts (Table 4; see also Noël-Lambot and Bouquegneau, 1977).

Origin of Cd Found in Intestinal Corpuscles of *Anguilla anguilla*

It is assumed that the Cd present in the intestinal corpuscles originates either directly from the sea water ingested or is excreted through the digestive barrier. The second hypothesis postulates a mechanism other than Cd elimination in the bile because the latter is not sufficient to account for the high Cd contents of the intestinal corpuscles (Table 4).

Table 5. *Anguilla anguilla*. Mean Cd concentration of intestinal corpuscles, taken from untoxicated individuals and then placed for 6 h in sea water or distilled water plus $CdCl_2$. Corpuscles were quickly rinsed with distilled water before being analysed

Type of water	Cd in water (ppm)	n	Cd in corpuscles (ppm w.wt.)
Sea water	0.06	2	3
Distilled water	0.90	1	88
Sea water	0.90	3	89
Distilled water	90.00	7	2188

Table 5 indicates that, *in vitro*, the intestinal corpuscles rapidly bind Cd^{2+} ions present in sea water or in distilled water. Compared to Table 2, Table 5 indicates that Cd accumulation in the intestinal corpuscles is as important *in vitro* as *in vivo* for similar external Cd concentrations.

Table 6 reveals that, in the case of short intoxication, high Cd concentrations in the corpuscles are attained, although the metal penetrates the body only in minute amounts. It therefore seems improbable that a mechanism to eliminate the pollutant is already operative. Experiments involving intramuscular $CdCl_2$ injections prove that the Cd in intestinal corpuscles does not result from an excretory process. Indeed, in that case only very small amounts of Cd are found in the corpuscles (Table 7).

Table 6. *Anguilla anguilla*. Cd concentrations in organs after brief intoxication in sea water plus $CdCl_2$

Material	6 h in 1 ppm Cd n = 3	2 h in 100 ppm Cd n = 2	6 h in 100 ppm Cd n = 2•
Muscles	–	–	0.6
Skin	–	–	1.5
Oesophagus	–	–	2.7
Stomach	–	–	1.7
Duodenum	–	–	1.4
Liver	–	–	3.2
Kidneys	–	–	2.2
Blood	–	–	2.6
Gills	–	–	3.4
Brain	–	–	0.5
Total body	–	–	0.8
Intestinal corpuscles	55.4	231–3400	33–3000
Gastric fluid	0.8	54.4	44.5
Intestinal fluid	0.15	2.7	1.3

• For the last 3 lines n = 5

Table 7. *Anguilla anguilla*. Distribution of Cd 60 h after single intramuscular injection of 800 µg Cd. Means of 2 individuals. Weight of each individual: 170 g

Material	Cd (ppm w.wt.)
Muscles	1.0
Skin	3.7
Oesophagus	4.9
Stomach	6.4
Duodenum	23.9
Intestine	8.1
Liver	132.5
Bile	10.6
Kidneys	38.8
Fat	3.4
Plasma	11.8
Blood cells	6.2
Gills	15.0
Spleen	24.0
Brain	1.2
Intestinal fluid	1.2
Intestinal corpuscles	< 5

All this information indicates that the Cd found in intestinal corpuscles mainly comes from the water ingested by the fish. This important fixation on the corpuscles reduces the Cd content in the water inside the intestine. Tables 4 and 6 show that the Cd concentration in the gastric fluid is only slightly less than in sea water, but that intestinal-fluid concentration is much smaller. This significant lowering of the Cd concentration in the fluid in contact with the intestinal wall probably limits the amount of metal absorption at this level.

Figure 3 illustrates and quantifies this fixation of Cd on the corpuscles of the eel and its consequences for Cd distribution in the digestive lumen. After a 6-h exposure to 100 ppm Cd, the intestinal corpuscles retain more than 99 % of the Cd present in the intestinal lumen. This amount of trapped Cd is even greater than the total Cd accumulated by all the tissues of the eel during 6 h of intoxication. Indeed the total body load calculated from the Cd concentrations in the different organs (Table 6) is 78.5 µg Cd for eels weighing 100 g. If the Cd body load prior to intoxication (Noël-Lambot, 1980) is subtracted from this value, one obtains a Cd accumulation of 78.5 µg − 15 µg

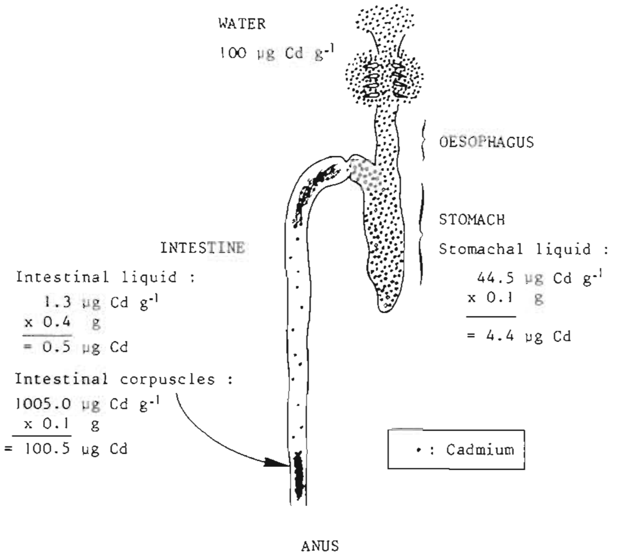

Fig. 3. *Anguilla anguilla*. Cd concentration inside the digestive tract of individuals intoxicated during a 6-h exposure to sea water containing 100 ppm Cd. For each constituent the Cd concentration (ppm = $\mu g\ g^{-1}$) is given, as well as the load (μg) equal to the product of the concentration and the weight of the constituent considered. The loads are calculated for eels whose weight is adjusted to 100 g

= 63.5 µg; now 100.5 µg Cd are trapped in the corpuscles (Fig. 3).

Since $CaCO_3$ is an important constituent of the intestinal corpuscles, it was interesting to find out its binding capacity toward Cd. We could observe this was of the same order of magnitude as that of the intestinal corpuscles. Indeed, when amorphous $CaCO_3$ precipitate (calcium carbonate precipitate, for analysis of silicates, MERCK) is immersed in a $CdCl_2$ solution, an important part of the Cd can be found in the precipitate (Table 8).

DISCUSSION AND CONCLUSIONS

The results described in this paper principally concern *Anguilla anguilla*, but we could observe similar corpuscles in all the teleosts we investigated: *Myoxocephalus scorpius, Serranus cabrilla, Scorpaena* sp., *Moena chryselis*.

Gardner and Yevich (1970) observed during lethal intoxication of the euryhaline fish *Fundulus heteroclitus* – in sea water containing 50 ppm Cd – elimination through the anus of a 'white mucoid material, tubular in shape. Microscopic examination of this anal exudate indicated a mucous mass containing cellular debris'. The Cd content of this material was not measured. To our knowledge, this is the only information on our subject in literature.

The formation of intestinal corpuscles can probably be explained by precipitation of salts – mainly Mg and Ca carbonates – present in water swallowed by the fish, on an organic support made of mucus and cellular debris. Indeed, as Ca concentrations in the gastric and intestinal walls are very low (89 and 44 ppm respectively) it seems improbable that the Ca found in the corpuscles has been secreted through the digestive mucosa. High intestinal pH probably plays a role in carbonate precipitation: the pH of eel gastric and intestinal fluids is 6.5 and 8.5 respectively. Such high pH of the sea water contained in the intestine modifies the dissociation equilibrium of carbonic acid (Riley and Chester, 1971) and enhances the precipitation of low-solubility carbonates. It must be pointed out that most surface sea waters are oversaturated in $CaCO_3$ (Pytkowicz, 1965) and also that the water volume ingested by sea fish is large, water absorption in the intestine compensating the loss of water resulting from hypotonicity (e. g. Maetz, 1971).

Gardner and Yevich (1970) observed an increase in mucous cell activity in the intestinal tract of fish after Cd exposure. It is therefore possible that formation of intestinal corpuscles could be more abundant in the presence of Cd. This would then constitute an interesting protective mechanism against intoxication by heavy metals (see also below).

We have shown that the intestinal corpuscles bind very rapidly cadmium and some other heavy metals present in the ingested water. It seems that this bind-

Table 8. Distribution of Cd between liquid and solid phases of a suspension of $CaCO_3$ in distilled water (D.W.) or sea water (S.W.) containing 1 ppm Cd. $CaCO_3$ precipitate was immersed for 2 d in 50 ml of a $CdCl_2$ solution; the mixture was intermittently stirred and then filtered; Cd was measured in the filtrate and in the precipitate; pH of the mixture was measured at the beginning and at the end of the experiment. This experiment was repeated

Nature of the mixture	Cd in water (µg)	Cd in precipitate (µg)	pH t_0	pH t_2
1 ppm Cd in D.W.	50.0		5.58	–
1 ppm Cd in D.W. + 2g $CaCO_3$	<0.5	50.4	9.60	8.50
1 ppm Cd in S.W.	50.0		7.96	–
1 ppm Cd in S.W. + 0.5g $CaCO_3$	33.7	16.6	7.99	8.29
1 ppm Cd in S.W. + 2g $CaCO_3$	15.0	33.6	7.99	8.22

ing takes place in the inorganic constituents of the corpuscles. Indeed we have established that the mineral portions of the intestinal corpuscles contained higher Cd concentrations than the mucous portions and that $CaCO_3$ precipitates had a high affinity for cadmium ions. The important accumulation of heavy metals in intestinal corpuscles can probably be explained by interactions – for instance adsorption, ionic exchange or complexation – between cadmium ions and some mineral constituents of the corpuscles. Complexation of Cd^{2+} by carbonates has been described by Lake and Goodings (1958) and by Gardiner (1974). The possible precipitation of cadmium as carbonate on the organic support of the corpuscles or its coprecipitation with carbonate minerals must also be considered, because of the low solubility of this salt and the alkaline pH of the intestinal fluid.

A similar mechanism has been proposed by Salomon and Mook (1978, cited by Förstner, 1979) who observed in the transition region from the Rhine to the Ijssel Sea in Holland, that a decrease of the dissolved loads of Zn, Cd and Ni is directly dependent on the precipitation of carbonate minerals, which is chiefly a result of an increase in pH. Coprecipitation experiments on calcium carbonates performed by Popova (1961, cited by Förstner, 1979) show that heavy metal carbonates of low solubility – such as $CdCO_3$, $PbCO_3$ and $ZnCO_3$ – are completely eliminated from solution as a result of $CaCO_3$ precipitation. Without a carrier substance, in this case $CaCO_3$, the metal cations would not have been precipitated.

Cadmium fixation in the intestinal corpuscles lowers considerably the Cd content of the fluid next to the intestinal wall and thus probably limits its absorption by the mucosa. From this point of view, intestinal corpuscles constitute a protective mechanism against Cd. Digestive absorption of Cd is extremely limited in all organisms studied so far (Kumada et al., 1972; Benayoun et al., 1974; Bouquegneau et al., 1976; Valberg et al., 1976). This element is thus found in high concentration in the feces. In fish, Cd fixation in the intestinal corpuscles probably contributes to this mechanism.

Moreover, in the same way as the exuvia of crustaceans (Martin, 1970; Benayoun et al., 1974), the fecal pellets of molluscs and crustaceans (Boothe and Knauer, 1972; Benayoun et al., 1974) or the feces of tunicates that can contain as much as 1500 ppm Cd w.wt. for 0.5 ppm Cd in sea water (Noël-Lambot, unpubl.), the intestinal corpuscles from fish may play an important role in the vertical transfer of Cd and other heavy metals in the marine environment.

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