

Squamous cell carcinoma in rudd *Scardinius erythrophthalmus*: histopathology, ultrastructure, and transmission

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ABSTRACT: The histopathology and ultrastructure of a skin neoplasm in rudd *Scardinius erythrophthalmus* (L.) are described and the neoplasm is diagnosed as squamous cell carcinoma. In the early stage, tumour cells appear singly or in clusters close to the epidermis; then tumour cells are seen in the dermis as epithelial pearls/nests, cords or sheets with central keratin; and with further development of the neoplasm, tumour cells appear as large sheets and masses, enclosing extensive keratinous formations and foci of necrotic cells, infiltrating deeper into the dermis and penetrating the muscle layers. Increasing vascularity and inflammation are associated with all stages of progression. In some cases metastases are observed in the viscera. Electron microscopic examination shows that the neoplastic cells are joined by desmosomes; the cytoplasm contains abundant mitochondria and rough endoplasmic reticulum. The neoplasm was successfully transmitted experimentally to healthy rudd when tumour cells were inoculated subcutaneously. Eight of 19 surviving test fish developed tumour growth at the site of tumour cell injection and/or in a corresponding site on the opposite of the body. One of these 8 fish, which developed a neoplasm, also showed a microscopic internal tumour in the viscera. One of the 19 test fish showed a microscopic tumour in the spleen, even though no skin tumour was visible in this fish. No tumours were found in control fish. In contrast, intraperitoneal injection of tumour cells into healthy rudd did not result in transmission of the neoplasm.

KEY WORDS: Rudd · Squamous cell carcinoma · Transmission · Keratin · Metastasis

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INTRODUCTION

Squamous cell carcinoma is an integumentary neoplasm that has been reported in both freshwater and marine fish (Mawdesley-Thomas 1972, 1975), in gudgeon *Gobio gobio* (Mawdesley-Thomas & Bucke 1967), in salmon *Salmo salar* (Roberts 1972), in brown bullhead *Ameiurus nebulosus* (Baumann et al. 1987), in gulf menhaden *Brevoortia patronus* (Fournie et al. 1987), in rainbow smelt *Osmerus mordax* (Herman 1988, Morrison & MacDonald 1995), in rudd *Scardinius erythrophthalmus* (Hanjavanit et al. 1990), in hybrid sunfish *Lepomis* sp. (Fitzgerald et al. 1991), and in mirror carp *Cyprinus carpio* (Manera & Biavati 1994). In addition the Registry of Tumors in Lower Animals

(Sterling, VA) has registered squamous cell carcinoma case material from twelve further species, including chinook salmon *Onchorhynchus tshawytscha*, yellow bullhead *Ameiurus natalis*, black bullhead *A. melas*, Atlantic rainbow smelt *O. mordax*, goldfish *Carrassius auratus*, white sucker *Catostomus commersoni*, roach *Rutilus rutilus*, bream *Abramis abramis*, American eel *Anguilla rostrata*, Malawi cichlid *Cryptocara moorii*, medaka *Oryzias latipes*, and Congo tetra *Phenacogrammus interruptus*.

The finding of squamous cell carcinoma in rudd in a sample of wild fish from a small lake in Ireland and the histology of the neoplasm have been briefly recorded (Hanjavanit et al. 1990). This paper now reports on (1) the histopathology and ultrastructure of the squa-

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mous cell carcinoma in rudd; and (2) the successful experimental transmission of the disease to healthy rudd by subcutaneous inoculation of tumour cells.

MATERIALS AND METHODS

Histology and light microscopy. Wild rudd were caught, using fyke nets, from 3 small lakes: Ballyhonock Lake (Irish Grid Reference W9973), Lough Aderry (Irish Grid Reference W9473) and College Lake (Irish Grid Reference W9674), East Cork, Ireland. Fish were examined externally and internally for visible signs of neoplasia and other pathology. Diseased and unaffected control fish were fixed whole for 24 h in Bouin's fixative and then preserved in 70% ethanol; the abdominal cavity was first opened to facilitate fixation. Pieces of fixed tissues from skin lesions as well as from normal skin and visceral organs (i.e. intestine, liver, spleen, pancreas, anterior, mid-, posterior portion of kidney, and gonad) were dehydrated with a graded series of ethanol and embedded in paraffin wax. Sections were cut at 5 to 7 μm and serial sections were stained with Ehrlich's haematoxylin and eosin (H&E). Selected sections were stained with Gram stain, Ziehl-Neelsen Method, Gomori Methenamine-Silver Nitrate Method, Periodic Acid-Schiff (PAS) and anti-cytokeratin CAM5.2 (Becton Dickinson) for keratin.

Electron microscopy. For transmission electron microscopy, tissues of skin lesions from freshly killed fish were fixed with cold 2% paraformaldehyde/2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 1.5 h, washed 3 times with cold 0.1 M phosphate buffer for 2 h, and then post-fixed with cold 1% osmium tetroxide in the same buffer for 1.5 h. The tissues were dehydrated rapidly in a graded series of acetone and then embedded in epon/araldite. Ultrathin sections were cut on glass knives with an LKB ultratome III. They were then mounted on 400 mesh copper grids and were stained with 2% uranyl acetate and Reynold's lead citrate. Sections were examined and photographed on a Corinth 500 electron microscope operating at 60 kV.

Transmission trial. Samples of wild, healthy rudd for the experiment were obtained from Carrigtwohill quarry pond (Irish Grid Reference W840720) from which tumour-bearing fish were never observed in the wild. The neoplastic fish, used to produce the tumour whole cell suspension, were collected from Lough Aderry, in which tumours had previously been observed in the wild population (Hanjavanit et al. 1990). The diagnosis of each squamous cell carcinoma was confirmed from histopathological examination.

Excised cutaneous tumours from 5 wild fish, aged 2 to 3 yr, were pooled in sterile phosphate buffered

saline (PBS) on ice. The tumour samples were chopped into small pieces. Homogenisation was carried out by hand in a sterile Griffiths tube homogeniser. The homogenate was then inoculated into the test fish. The viability of cells in suspension was tested using trypan blue. Numbers of viable cells were determined using a Neubauer haemocytometer.

Expt 1. Transmission by intraperitoneal inoculation of tumour cells: Twenty-six healthy rudd: 21 female and 5 male, 11.2 to 13.0 cm in length, aged 1 to 4 yr were each given 0.2 ml containing 1.35×10^6 viable cells ml^{-1} intraperitoneally. Twenty-six control fish (19 female and 7 male, 10.6 to 13.0 cm, aged 3 yr) were injected i.p. with 0.2 ml sterile PBS. The 2 groups of fish were held separately in aquaria with well-aerated, dechlorinated water. The fish were fed with a diet of commercial trout pellets once a day; water in the aquaria was changed once a week. Water temperatures ranged with ambient temperature from 4.5 to 20.0°C over the duration of the experiment. Both groups of fish were sampled every 3 mo.

Expt 2. Transmission by subcutaneous inoculation of tumour cells: Thirty-five healthy rudd aged 2 to 4 yr of age were each inoculated subcutaneously with 0.1 ml containing 1.03×10^6 viable cells ml^{-1} . Two sites were injected in each fish, one at the base of the dorsal fin on the left hand side, and the other on the peduncle of the caudal fin on the right hand side because the bases of the fins, and the caudal peduncle were the sites most frequently involved in tumours in the wild fish (Fig. 1). Sixteen of these fish died immediately after inoculation; the remaining 19 fish ranged from 10.3 to 12.0 cm and included 4 females and 15 males. Twenty-eight control fish (12 female and 16 male, 8.3 to 12.5 cm in length, aged 2 to 3 yr) were each injected subcutaneously with 0.1 ml sterile PBS in the same sites as the experimental fish. The 2 groups of fish were held separately, and maintained under the same conditions as in Expt 1, and were sampled every 3 mo.

Pathology. Samples of 5 to 7 fish from each of the control and test groups were examined histologically after 3, 6, 9, and 12 mo. Fish were fixed whole in Bouin's fixative for 24 h and then preserved in 70%

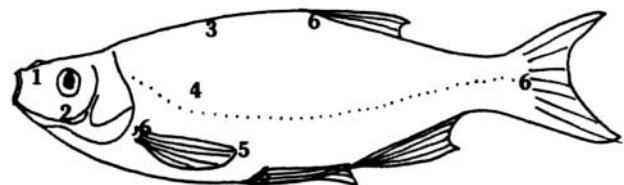


Fig. 1. *Scardinius erythrophthalmus*. Diagram showing most frequent sites of tumours in the skin of rudd. 1: snout; 2: anterior to operculum; 3: dorsal side; 4: mid-body above lateral line; 5: mid-body behind pectoral fin; 6: bases of fins

ethanol. Pieces of fixed tissue from all skin lesions, including skin and underlying muscle beneath the base of the dorsal fin and the peduncle of caudal fin of both sides, and viscera (i.e. intestine, liver, spleen, pancreas, anterior, mid-, posterior portion of kidney, and gonad) were processed routinely, sectioned at 5 μm , and stained with H&E.

RESULTS

Pathology of the spontaneous neoplasms

External tumours, single or multiple, were widely scattered on the surface of the fish body. Tumours were located as follows (Fig. 1): (1) on the snout; (2) anterior to the operculum; (3) on the dorsal side of the body; (4) mid-body above the lateral line; (5) mid-body behind the pectoral fin; and (6) at the bases of fins, most frequently at the base of the caudal fins. Tumours were usually oval or hemispherical in shape, globular and raised from the body surface, and firm to the touch. Tumours as small as 0.2 \times 0.2 cm and up to 1.1 \times 1.4 cm were measured. They were deep reddish brown in colour.

Light microscopy

The epidermis of the neoplastic skin lesions was mainly composed of many layers from basal, columnar cells to squamous cells; in comparison with normal skin the mucous cells were reduced in number and the club cells were completely absent. Tumour cells appeared singly or in clusters of epithelial cells, which were found below the basal cell layer of epidermis. The clusters were not found in normal dermis nor in the healthy tissues. They occurred in groups or strands of 2 to 32 cells in section. Sometimes they showed an irregular arrangement in the stroma. The cells were large and ovoid or spindle-shaped. Their cytoplasm was slightly basophilic, showing large, round or elongated nuclei with central prominent nucleoli. At high magnification, it could be seen that the cytological features of spindle cells, composing the deeper portion of the tumour, were similar to those of malignant cells found in the dermis. There was moderate vascularity and inflammation throughout the lesion.

In the early stages the tumour was seen in the vicinity of the basement membrane, even though the integrity of the basement membrane was lost and some neoplastic cells had extended into the underlying tissue. This pattern is illustrated in Fig. 2, which shows a neoplasm originating from the lower portion of the epidermis with evidence of focal active cell division.

As the neoplasm progressed, masses of tumour cells were visible invading the dermis. In some cases, the adjacent covering epidermis appeared to have sloughed off. In further instances, no evidence of epidermal cell division was apparent, perhaps because of the plane of sections; infiltrating neoplastic columns appeared deep in underlying tissues and had apparently separated from the epidermis. The tumour cells coalesced into a mass in the dermis. The tumour cells were arranged in clusters, cords, or sheets of pleomorphic squamous cells showing individual cell keratinisation and an infiltrating growth pattern (Fig. 3). Individual neoplastic cells were large, round or elongated and slightly fusiform in shape with slightly basophilic cytoplasm, pleomorphic nuclei and prominent nucleoli. Nuclei and cytoplasm ranged in size from 4.90 to 10.78 μm and from 7.84 to 19.60 μm respectively in greatest diameter ($n = 50$), and the nucleus:cytoplasm ratio was 1:1.6. Mitotic structures were not abundant, but were occasionally seen. Numerous isolated mucous cells were scattered throughout the mass of

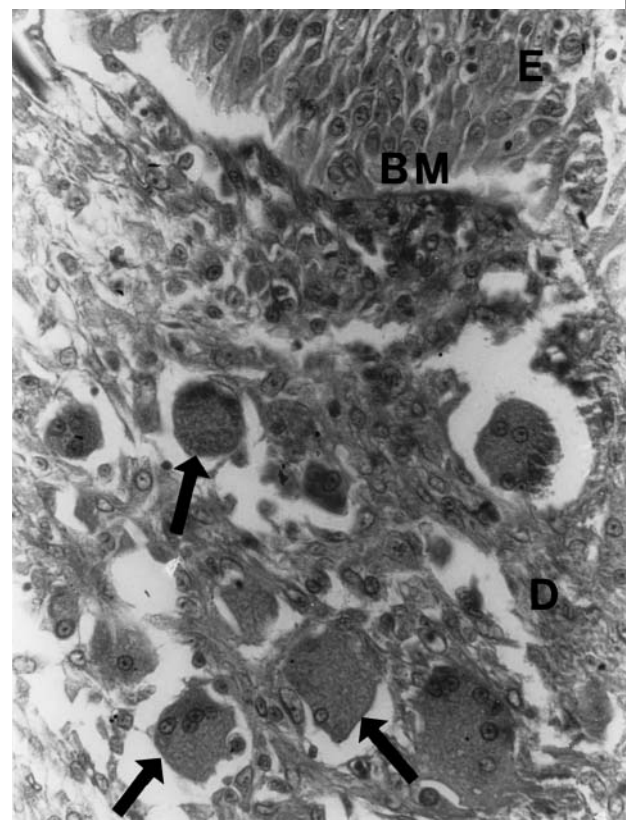


Fig. 2. *Scardinius erythrophthalmus*. Histological section of skin tumour of rudd (H&E). BM = basement membrane, D = dermis, E = epidermis. Skin tumour showing clusters of squamous cells (arrows) arising from basal cells and invading the dermis ($\times 200$)

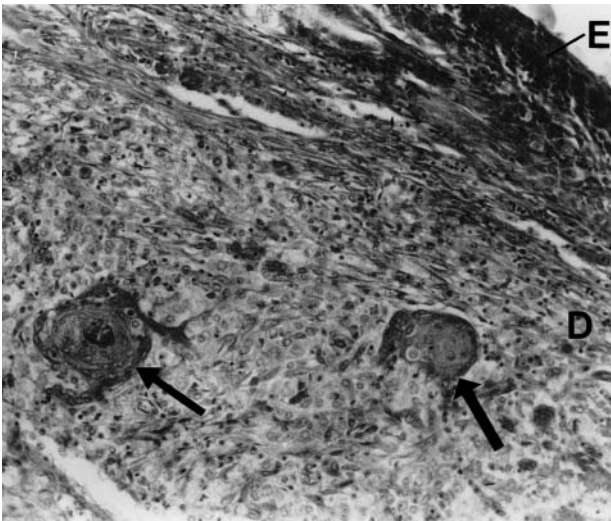


Fig. 3. *Scardinius erythrophthalmus*. Histological section of skin tumour of rudd (H&E). D = dermis, E = epidermis. Skin tumour showing nests of epithelial cells (arrows) in the dermis ($\times 73$)

tumour tissue and these cells were positive for the PAS reaction. There were also foci of necrosis and irregular deposits of keratin within the tumour tissue. In some cases, the tumour cells were arranged in cell-nests of stratified squamous epithelium, with central keratin forming characteristic 'epithelial pearls' (Fig. 3). Infiltration by inflammatory cells could be observed within the tumour mass. Numerous small blood vessels were noted in the neoplasm, and blood cells were localised in some tissue spaces. Some of the tumours showed small patches of melanin pigment scattered irregularly near the surface. At this stage, the tumours found in the dermis appeared as a uniform sheet of pleomorphic squamous cells.

The most advanced tumours appeared as large sheets and masses, which showed a very high proliferative activity of squamous cells with keratinisation (Fig. 4). Extensive necrotic changes usually developed in the centre of tumour mass. Scattered lymphocytes were also noted with varying frequency in the necrotic area. Marginal areas of keratinising tumour sometimes were papilloma-like, and in some areas of the tumour, especially towards the central keratin area, cells showed pronounced nuclear polymorphism with vacuolation. In some cases, masses of neoplastic cells were seen to infiltrate and often replace much of skeletal muscle.

Numerous isolated normal mucous cells were scattered throughout the tumour tissue. All stages of tumour progression exhibited associated inflammatory cells and vascularity. In some cases granulomata were observed adjacent to the tumours (Fig. 5).

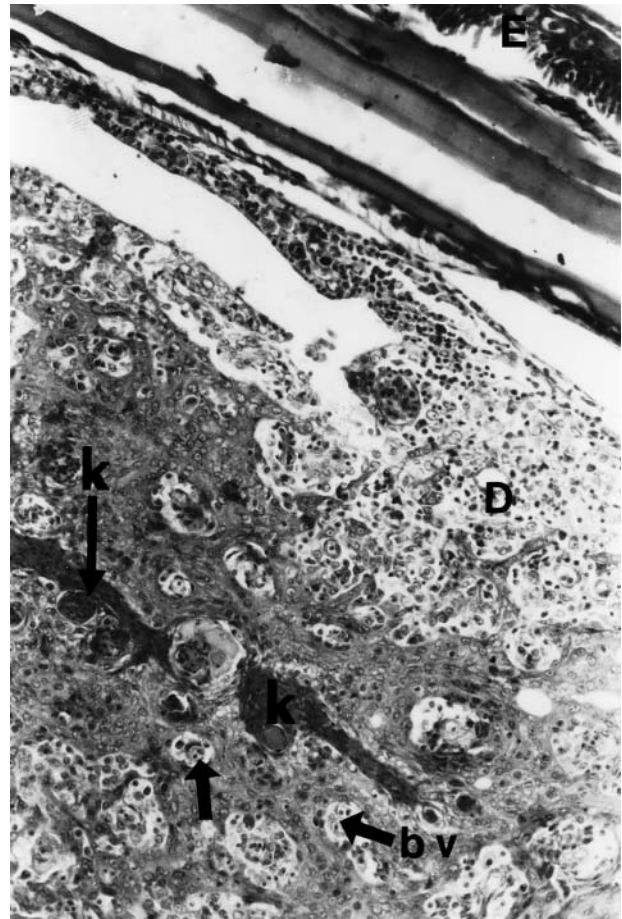


Fig. 4. *Scardinius erythrophthalmus*. Histological section of skin tumour of rudd (H&E). D = dermis, E = epidermis. Skin tumour showing mass of squamous cell carcinoma in the dermis. Note keratinous material (k), blood vessel (bv, arrow) ($\times 130$)

The monoclonal anti-cytokeratin, CAM5.2, which binds specifically to cytokeratin peptide 8 in tissue sections, showed a positive staining reaction (Fig. 6). It revealed prominent, dark-brown cytoplasmic staining of keratin, characterising squamous cell carcinoma, at its origin from the lower region of the epidermis and extending downwards into the dermis.

While internal tumours were not observed macroscopically, they were found on histological screening of visceral organs such as intestine, pancreas, spleen, kidney and ovary, though not in liver or testis. The visceral tumour growths were focal or multifocal, and they were massive and destructive to surrounding tissue (Fig. 7). Generally, neoplastic cells were large and round to oval in shape. Nuclei were pleomorphic, vacuolated and with somewhat prominent nucleoli. Mitotic figures were not observed. Keratinised cells and some necrotic cells were usually observed in the squamous cell tumour mass.

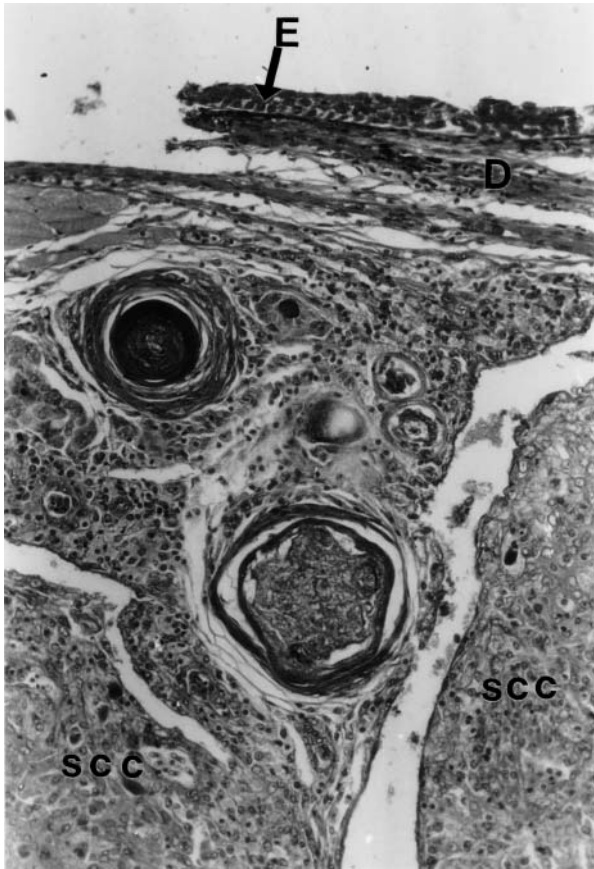


Fig. 5. *Scardinius erythrophthalmus*. Histological section of skin tumour of rudd (H&E). D = dermis, E = epidermis. Granulomata adjacent to squamous cell carcinoma (scc) ($\times 114$)

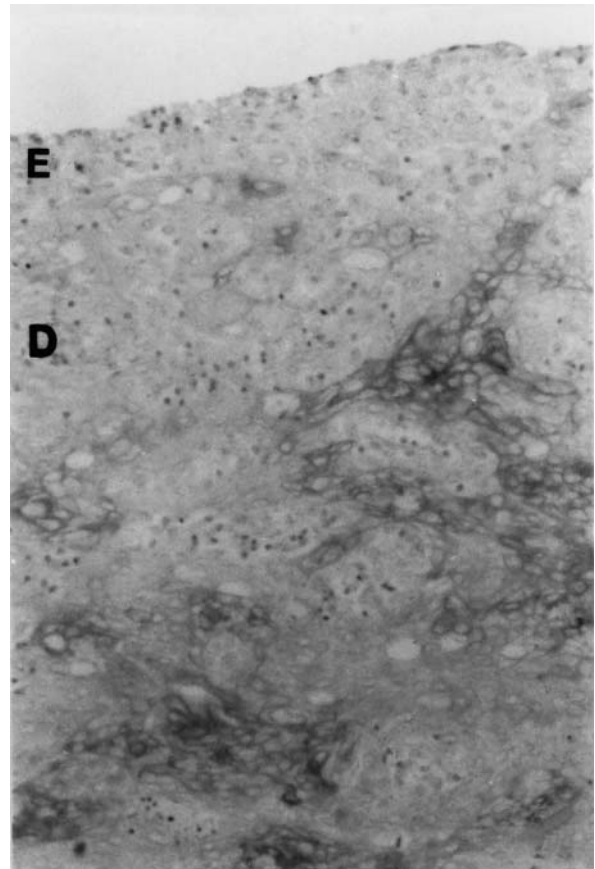


Fig. 6. *Scardinius erythrophthalmus*. Histological section of skin tumour of rudd (H&E). D = dermis, E = epidermis. Anti-cytokeratin staining of skin tumour showing the presence of keratin (dark areas) ($\times 101$)

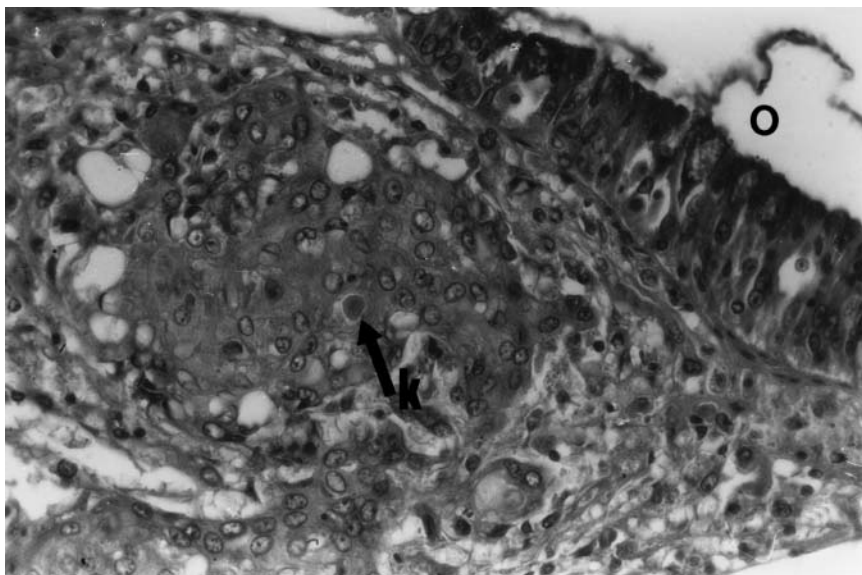


Fig. 7. *Scardinius erythrophthalmus*. Cross-section of posterior kidney showing tumour mass adjacent to the opisthonephric duct (O). Note keratinous material (k) ($\times 219$)

No bacterial or fungal infections were observed from any Gram-stained, Ziehl-Neelsen-stained or Gomori Methenamine–Silver Nitrate-stained tissues of the skin or visceral lesions.

Electron microscopy

The tumour consisted mainly of enlarged, rounded, oval, elongated or polygonal cells, which had large nuclei which occupied almost two-thirds of each cell, reflecting the high nuclear:cytoplasmic ratio seen in the light microscopy. The nuclei were polymorphic, and varied from rounded to elongated or irregularly shaped, with evenly dispersed euchromatin and prominent nucleoli (Fig. 8). In some cells a few heterochromatic patches were scattered throughout the nuclei. Nuclear indentations were present in some

neoplastic cells (Fig. 9). The cytoplasm of neoplastic cells generally had various organelles and a fine granular appearance. Mitochondria were abundant and tended to concentrate in aggregates; they showed variability in size and shape, from round-oval to elongated-tubular, with a double-membrane envelope and swollen intracrystal space. In some areas, swollen mitochondria were also observed. Abundant rough endoplasmic reticulum was present and sometimes grossly dilated. Numerous free ribosomes, polyribosomes and glycogen granules were scattered throughout the cytoplasm. A Golgi apparatus was sometime visible in the neoplastic cell. Lysosomes, myelin figures, lipid droplets and intercellular spaces were also found.

Interestingly, some neoplastic cells were attached to each other by desmosomes and were separated by intercellular spaces (Fig. 9). Prominent intermediate cytoplasmic filaments with mean \pm SD = 8.9 ± 1.8 nm in

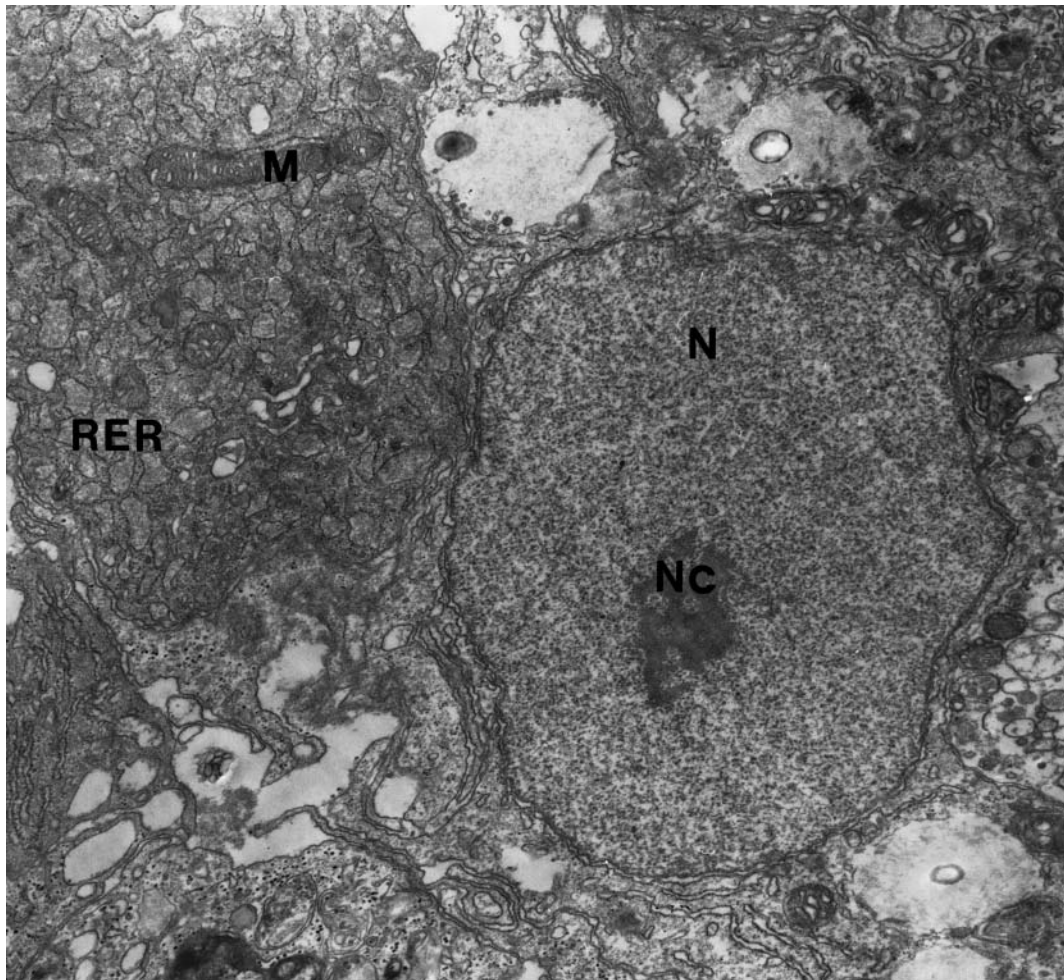


Fig. 8. *Scardinius erythrophthalmus*. Electron micrograph of rudd tumour showing neoplastic cell with large, euchromatic nucleus (N) and prominent nucleolus (Nc), cytoplasm with mitochondria (M), abundant rough endoplasmic reticulum (RER) and numerous ribosomes. Note the RER is grossly dilated ($\times 7500$)

diameter (range 8.0 to 12.0 nm, $n = 20$) were present in the cytoplasm, with amounts varying from cell to cell. Occasionally complex membrane foldings were observed which may represent interdigitation of adjacent cells.

Another cell type found in the neoplastic tissue was the mucous cell, which gave a positive result with PAS staining. The mucous cells appeared normal, and easily distinguished from the neoplastic cells by their oval shapes and by the composition of the vesicles, whether round or ovoid in structure and of various sizes and degrees of electron lucidity (Carr & Toner 1982). These vesicles represented mucus droplets, which occupied a large amount of the cytoplasm and pushed the nucleus aside to the one end of the cell; the vesicles appeared to

be closely related to both Golgi apparatus and rough endoplasmic reticulum. A few mitochondria were seen, while abundant glycogen granules were scattered throughout the cytoplasm. In addition, erythrocytes and macrophages were occasionally encountered.

Transmission trial

Expt 1. Transmission by intraperitoneal inoculation of tumour cells

No evidence of neoplasia was observed either macroscopically or microscopically throughout 12 mo in either control or test fish. Inflammatory lesions were ob-

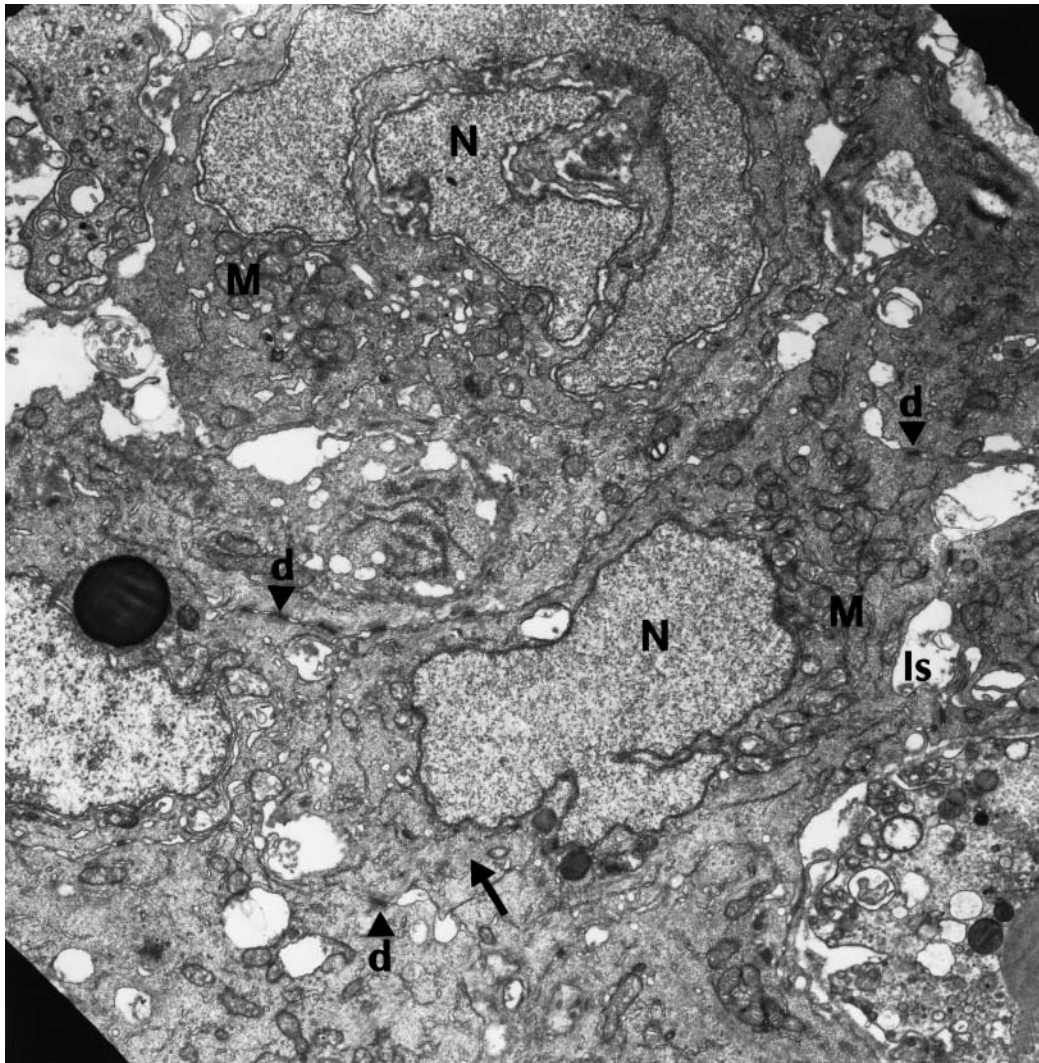


Fig. 9. *Scardinius erythrophthalmus*. Electron micrograph of rudd tumour showing 2 neoplastic cells with indented nuclei (N), cytoplasm with abundant mitochondria (M), intermediate cytoplasmic filaments (arrow). Note desmosomes (d) (arrowhead) and intercellular spaces (Is) ($\times 4528$)

served histologically in both groups. Table 1 shows the number of fish showing inflammation in the viscera. The most prevalent inflammatory lesions noted in the study were in the pancreas. In summary, 4 out of 26 control fish had inflammation only in the pancreas, 4 only in the spleen, and 4 in both the pancreas and spleen. In contrast, 21 out of 26 test fish had inflammation only in the pancreas, 1 out of 26 test fish showed inflammation only in the spleen, and 4 out of 26 test fish had inflammatory lesions in both the pancreas and spleen. The histologic lesion consisted largely of macrophages, lymphocytes and fibrocytes. There were numerous capillaries and red blood cells scattered throughout the inflammatory area. Necrotic cells and melanin pigment were observed. In addition, the inflammation was found in the spleen. The histologic structure was similar to that observed in the pancreas, but in some cases the affected area consisted of epithelioid cells forming concentric multilayered whorls and interspersed with eosinophilic stroma. Areas of necrosis were present within the matrix of the lesion. Pyknotic cells, macrophages and lymphocytes were visible diffusely throughout areas of necrosis. Melanin pigment was occasionally present within the lesion. In some cases, the inflammatory lesions were not only found within and surrounding the pancreas, but they were

also spread along the liver and intestine and affected the intestinal serosa, muscularis and submucosa.

Expt 2. Transmission by subcutaneous inoculation of tumour cells

Of the 35 test fish, 16 died immediately after inoculation and none of the controls died. The histopathological findings in the 19 fish surviving the entire treatment are presented in Table 2. Skin tumours were first visible after 3 mo injection in the test fish at the site and/or at the corresponding site on the side of the body opposite to the injection (Fig. 10). Tumours were fleshy, raised, and deep red to dark brown. The sizes of tumours varied from 0.1 to 1.0 cm in width \times 0.3 to 0.8 cm in length. After 3 mo, 2 out of 7 test fish showed macroscopic skin tumours; histological sections of viscera of these fish showed that 1 of the 7 had also developed an internal tumour. By 6 mo 2 out of 7 test fish showed skin tumours. Between 7 and 9 mo 4 out of 5 test fish developed the external tumours; 1 also showed a microscopic tumour in the spleen. No signs of either external or internal tumours were found by histological examination in any of the control fish throughout the study period.

Table 1. *Scardinius erythrophthalmus*. Development of lesions in rudd (no. fish with lesion/no. fish examined) following intraperitoneal (i.p.) inoculation of tumour cells. Control fish i.p. injected with sterile PBS; experimental fish i.p. injected with tumour cells

Time after treatment (mo)	Control fish				Experimental fish			
	Skin tumour	pancreas only	spleen only	pancreas + spleen	Skin tumour	pancreas only	spleen only	pancreas + spleen
0–3	0/7	0/7	0/7	0/7	0/7	7/7	0/7	0/7
4–6	0/6	0/6	0/6	1/6	0/6	6/6	0/6	0/6
7–9	0/6	2/6	1/6	1/6	0/7	6/7	0/7	1/7
10–12	0/7	2/7	3/7	2/7	0/6	2/6	1/6	3/6

Table 2. *Scardinius erythrophthalmus*. Development of squamous cell carcinoma in rudd (no. fish with lesion/no. fish examined) following subcutaneous (s.c.) inoculation of tumour cells. Control fish s.c. injected with sterile PBS; experimental fish s.c. injected with tumour cells. scc = squamous cell carcinoma, int. = internal, inflam. = inflammation. Study started with 28 control fish, all of which survived throughout the experiment, and 35 test fish, of which 19 survived the full treatment

Time after treatment (mo)	Control fish				Experimental fish			
	skin scc only	int.scc only	skin + int. scc	int. inflam.	skin scc only	int.scc only	skin + int. scc	int. inflam.
0–3	0/7	0/7	0/7	0/7	1/7	0/7	1/7 ^a	0/7
4–6	0/7	0/7	0/7	1/7	2/7	0/7	0/7	1/7
7–9	0/7	0/7	0/7	1/7	4/5	1/5 ^b	0/5	0/5
10–12	0/7	0/7	0/7	1/7	–	–	–	–

^aInternal tumour located between the intestine and pancreas
^bInternal tumour developed in the spleen

Histological examination of skin tumours showed that they were composed of masses of squamous epithelium with keratinisation in the dermis, and in some cases the tumour cells infiltrated and sometimes

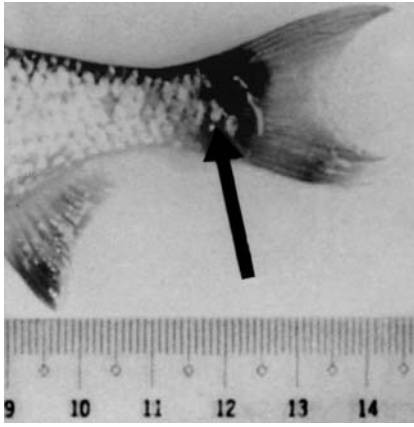


Fig. 10. *Scardinius erythrophthalmus*. Tail region of rudd showing tumour growth on left side 3 mo after subcutaneous inoculation with tumour cells on right side

destroyed the surrounding muscle tissue (Fig. 11). Keratinised material occurred within the centre of tumour mass (Fig. 12). Normal mucous cells were also observed. The tumour was well vascularized. Inflammatory cells consisting of macrophages and lymphocytes were present at the periphery of the neoplastic mass and interspersed in the muscle. The microscopic examination of the visceral organs of 1 fish which had a skin tumour showed a tumour mass lying between the intestine and pancreas. The cellular structure consisted of a mass of epithelial cells with large pleomorphic nuclei and 1 or 2 nucleoli, as well as keratinised cells. Mucous cells and necrotic cells were noted in the tumour mass. In another case of a fish without a detectable skin tumour, neoplastic nodules in the spleen were observed (Fig. 13). The histological structure of external and internal tumours was clearly similar to those observed in the fish with spontaneous tumours, and the experimental tumours were diagnosed as squamous cell carcinomas. In addition, inflammatory lesions were also observed from histological examination, in the pancreas and spleen of both some control and test fish.

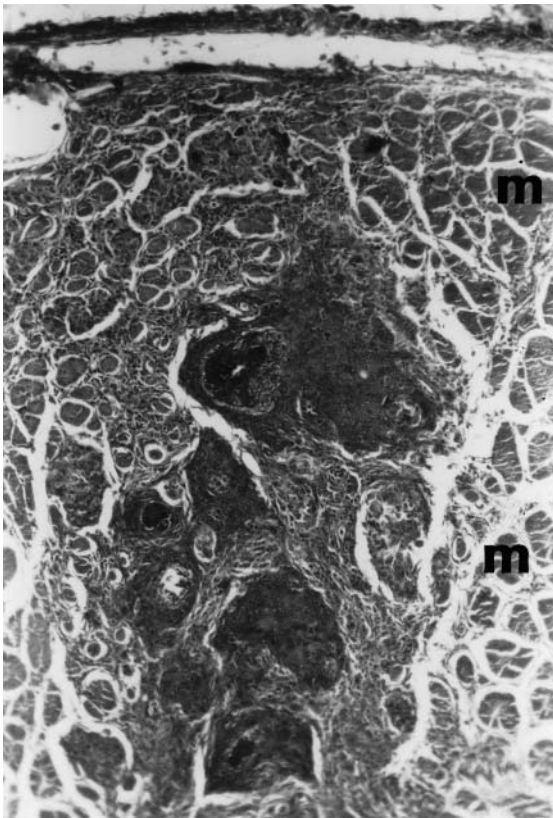


Fig. 11. *Scardinius erythrophthalmus*. Histological section of skin tumour from rudd 3 mo after subcutaneous inoculation with tumour cells, showing tumour mass infiltrating the adjacent skeletal muscle (m) (H&E; $\times 54$)

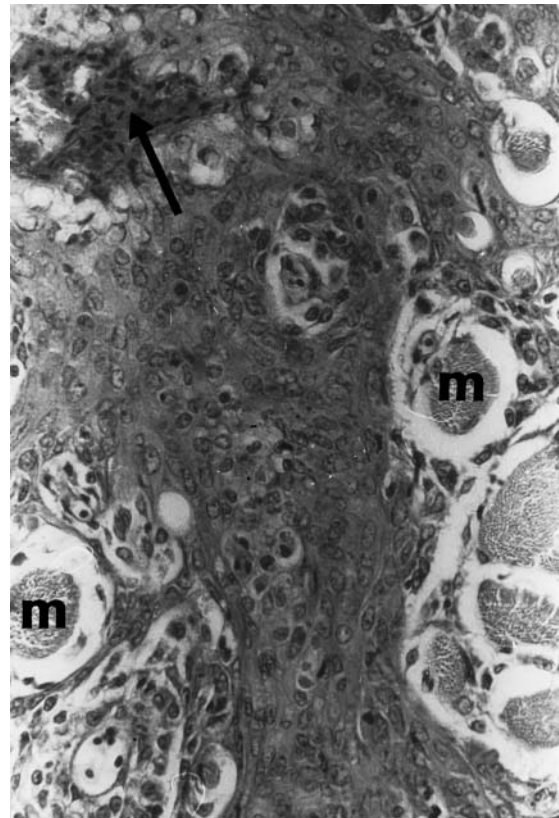


Fig. 12. *Scardinius erythrophthalmus*. Higher magnification of Fig. 11 showing mass of squamous cell carcinoma with keratinous material and necrotic cells (arrow) (m = muscle) (H&E; $\times 212$)

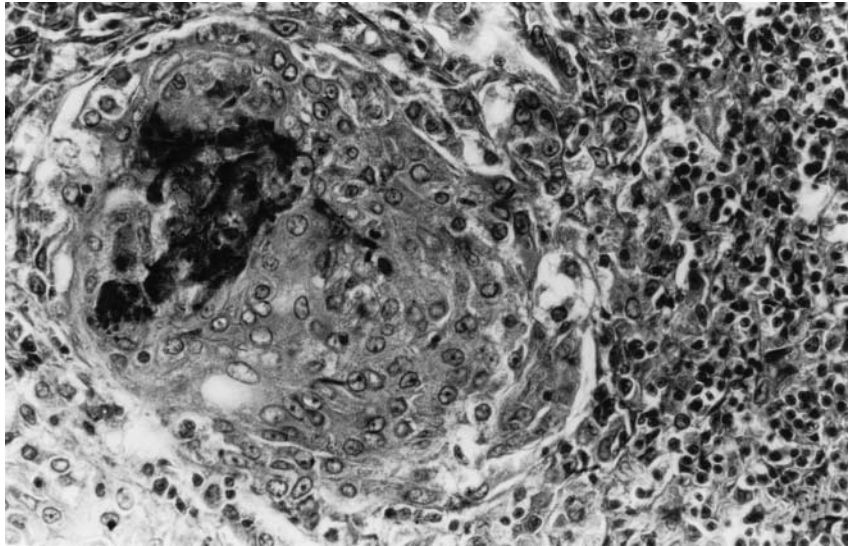


Fig. 13. *Scardinius erythrophthalmus*. Histological section of spleen from rudd 9 mo after subcutaneous inoculation with tumour cells, showing tumour nodule with central keratin and necrotic cells (H&E; $\times 230$)

DISCUSSION

The histological examination of the skin tumour in rudd *Scardinius erythrophthalmus* (L.) revealed neoplasia at various stages of progression; sometimes early and more advanced stages could be seen in different lesions in the same fish. The histological morphology of the tumour is similar to those observed in gudgeon *Gobio gobio* (Mawdesley-Thomas & Bucke 1967), in gulf menhaden *Brevoortia patronus* (Fournie et al. 1987), in rainbow smelt *Osmerus mordax* (Herman 1988) as well as in hybrid sunfish *Lepomis* sp. (Fitzgerald et al. 1991), in having sheets, cords and whorls of epithelial cells, and epithelial pearls with keratin, and numerous central cores consisting of keratinised material. The presence of epithelial nests within the neoplasm commonly occurs in mammalian squamous cell carcinoma and is a characteristic of well-differentiated squamous cell carcinomas in domestic animals (Goldschmidt et al. 1998) and humans (Wheater et al. 1985). The characteristic presence of keratin pearls within the neoplasm (Erlandson 1994) has previously been reported in the hybrid sunfish neoplasm by Fitzgerald et al. (1991). Immunocytochemical staining of tissue sections showed cytokeratin peptide 8-positive antibody-based staining, consistent with this characterisation. Therefore, the histological evidence confirmed that the skin tumours of rudd reported in this study were squamous cell carcinomas. The mitotic figures indicated a proliferating cell population. In this study the tumours occurred internally as well as externally in some cases. The cellular characteristics of the internal tumours were simi-

lar to the neoplasm of skin, and were also diagnosed as squamous cell carcinomas. The internal tumours appear to be metastases. The metastatic capability was compatible with the fact that the vascularisation of the tumours was greater in the more advanced tumours (Cheville 1994, Leek & Albertsson 2000). Metastasis was reported in the liver of a hybrid sunfish (Fitzgerald et al. 1991) but metastasis of cyprinid neoplasms is rare (Hoole et al. 2001) and of fish neoplasms in general is relatively uncommon (Hayes & Ferguson 1989). An inflammatory response to the neoplasms was evident. Inflammation is the basic protective response to tissue damage of whatever cause (Roberts 1989); the most common causes of tissue damage are microbes and their toxins, physical and chemical trauma, immune reactions, and death of cells from circulatory insufficiency such as at the core of rapidly growing tumours.

On electron microscopy the neoplastic features of the tumour observed included cellular pleomorphism, the enlargement and irregularity of the nucleoli, the swollen mitochondrial intracrystal spaces, the numerous polyribosomes, the abundance of intermediate filaments, which were probably tonofilaments, the fact that the Golgi apparatus was rarely seen, and the presence of necrotic cells (Ghadially 1985, Henderson et al. 1986, Cheville 1994, Erlandson 1994, Ghadially 1997, Cheng & Hudson 2002, Sato et al. 2002). The characteristics consistent with squamous cell carcinoma included the presence of desmosomes, keratin and tonofilaments. By the criteria used for human squamous cell carcinoma, the neoplasm appeared relatively well differentiated, the abundant mitochondria and rough endoplasmic reticulum, the persistence,

though reduction in the number, of desmosomes, and the intermediate filaments.

In the first transmission trial, intraperitoneal inoculation of viable carcinoma cells did not result in tumour development in healthy rudd over the study period of 12 mo.

In the second transmission experiment, none of the control fish developed neoplasia over the study period of 12 mo, but subcutaneous inoculation of neoplastic cells in the test fish resulted in tumours. Among the 19 test fish which survived inoculation, 9 fish developed tumours (47%): 3 fish of the 3 mo sample, 2 fish of the 6 mo sample, and 4 of the 5 fish in the 9 mo sample. The histology of the transmitted tumours was clearly similar to the spontaneous tumours. Sonstegard (1977) failed to transmit papillomas in white suckers by percutaneous inoculation with viable tumour explants.

The transmitted tumours were located either at the site of inoculation or at the corresponding site on the opposite side of the body. One of those sampled at 3 mo had an internal squamous cell tumour but no macroscopically visible external tumour; and one of those sampled at 9 mo had a tumour, presumably metastatic, in the spleen in addition to an external skin tumour. The fact that the transmission of the tumour was successful via the skin route but not by the peritoneal cavity suggests that skin-related factors are important in the progression of the squamous cell carcinoma.

Of the 35 test fish inoculated subcutaneously with tumour cells in Expt 2, 16 died immediately. None of the control fish died. The deaths may have been due to the stress of handling and inoculation, but since no control fish died, the deaths may also have been the result of a reaction to the cell homogenate. In this regard, the skin location again appears significant, since the test fish in Expt 1, inoculated intraperitoneally, all survived.

The development of inflammatory lesions in the viscera over time in both control and test fish was interesting. In Expt 1 higher numbers of test than control fish had inflammatory lesions (Table 1), which suggested that the inoculated cells, while they did not transplant, generated an inflammatory response. However some of the fish in Expt 2 also had visceral inflammatory lesions (Table 2), even though the inoculation route was via the skin. These observations overall do not clearly indicate a primary cause of the visceral inflammatory lesions.

No evidence of bacterial or fungal involvement was found in either spontaneous or transmitted tumours. The lakes in which rudd with tumours were found are in an agricultural area and are eutrophic, but unaffected by industrial effluents. The tumours observed in the experimental fish could have resulted either from

the multiplication of the transplanted cells or from an oncogenic agent, perhaps a virus, carried by the transplanted cells into healthy host fish. However preliminary experiments designed to transmit the tumours cell-free to healthy rudd, using homogenised tumours cells filtered through a 0.22 µm filter, were unsuccessful (Hanjavanit 1991). Epidermal neoplasms in fish include both plaque-type, and smooth, globular lesions. Among the plaque types there is evidence for viral aetiology (Harshbarger et al. 1993), e.g. herpesvirus in common carp *Cyprinus carpio* (Sano et al. 1985) and in rainbow smelt *Osmerus mordax* (Morrison et al. 1996), retrovirus in walleye *Stizostedion vitreum* (Walker 1969), and a picornavirus in European smelt *O. eperlanus* (Ahne et al. 1990). The globular types, including squamous cell carcinomas, tend to be prevalent in polluted environments, and evidence of a viral aetiology is weak (Harshbarger et al. 1993). The aetiology of the squamous cell carcinoma in the rudd remains to be elucidated.

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