Incorporation of Tritiated Thymidine by Teleost Epidermal Cells[1]

Information about the structure and function of epidermis has been obtained mainly from studies of mammalian skin[2-5]. Mammalian epidermis is a stratified squamous epithelium consisting of 4 cell types: relatively undifferentiated basal cells, differentiating spinous and granular cells, and fully differentiated horny cells. The basal cells are mitotically active and constitute the germinative layer of the tissue. A constant number of cells in the epidermis is achieved by the balancing of mitotic reproduction in the basal layer with the desquamation of cells at the surface.

In contrast to the epidermis of mammals, the epidermis of teleosts or bony fish is not distinctly stratified into a progression of differentiating cell types[6-8]. Instead, the epidermis consists of small, basophilic cells which are found throughout the epithelium, from the basal to the most superficial layer, and which surround the much larger mucus-producing cells[9] and club cells[10-12] (Schreckstoffzellen of PFEIFFER). A horny layer or stratum corneum is not regularly found in teleost epidermis and nucleate cells commonly occupy the most superficial layer. The total thickness of the epidermis varies widely depending upon the species and site examined. In species with superficial and prominent scales, the epidermis is draped over the scales and reflects the imbricate arrangement of the scales. Generally, fins are scaleless. At these sites the epidermis is closely apposed to the dermal bony fin rays.

Little is known about the turnover of teleost epidermis, as opposed to the epidermis of other vertebrates. Important for an understanding of the renewal of teleost epidermis is the intraepidermal location of germinative cells; it was the purpose of this study to localize the DNA-synthesizing cells in teleost epidermis.

Tritiated thymidine, a radioactive precursor of deoxyribonucleic acid, has been widely used in studying the dynamics of cell populations[13,14]. This molecule is incorporated by cells which are synthesizing DNA. Such labelled cells can then be localized in tissues by the technique of radioautography.

In one experiment *Carassius auratus* (goldfish) were injected i.p. with tritiated thymidine, each receiving a total of about 300 μc (specific activity 6.7 c/mM). Approximately 90 min elapsed between initial exposure to the label and fixation of caudal fin and flank epidermis. In another experiment, the thin distal part of the caudal fin was exposed to the label in vitro. The transected tip of the tail was placed in a saline solution of tritiated thymidine (100 μc/cm^3) for 30 min, then rinsed in 3 changes of saline to remove unincorporated tritiated thymidine. Tissues from both experiments were fixed in formol-saline and embedded in paraffin. Sections, 6 μ thick, were placed on subbed slides and dipped in Kodak NTB-2 liquid emulsion. After exposure for 14 days at 4°C, the radioautographs were developed in Kodak D-76. Slides were stained either in Delafield's hematoxylin or by the periodic acid-Schiff reaction. In the latter tech-

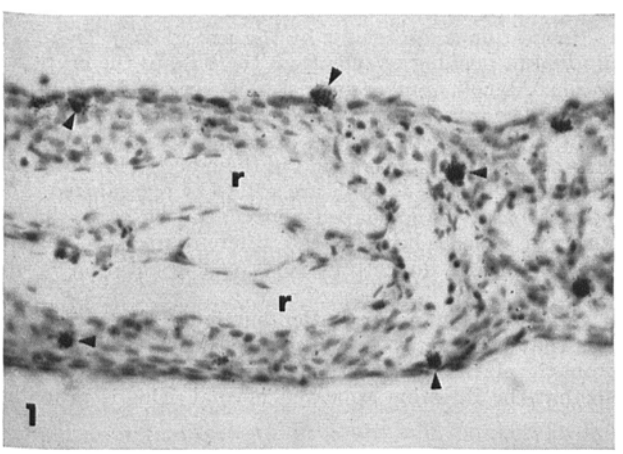

Fig. 1. Transverse section through the caudal fin of *Carassius*. Radioautograph. Some of the labelled cells are indicated by arrows; surface cells as well as cells deeper in the epidermis have incorporated tritiated thymidine. The epidermis covering both sides of the fin is several cells thick. Between the 2 layers of epidermis are dermal components including the bony fin rays (r). Delafield's hematoxylin. × 380. (Focus is at level of silver grains; tissue section is slightly out of focus.)

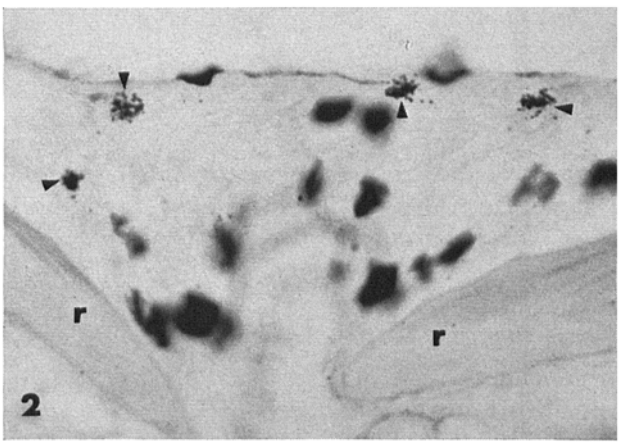

Fig. 2. A more highly magnified field of caudal fin epidermis of *Carassius*. Radioautograph. Labelled cells are marked by arrows; note that 3 are found superficially. Darkly staining mucus-producing cells are scattered throughout the epidermis. Dermal fin rays (r) are seen beneath the epidermis. Periodic acid-Schiff. × 600. (Focus is at level of silver grains; tissue section is slightly out of focus.)

[1] This investigation was supported by U.S.P.H.S. Grant No. AM 05779 of the National Institute of Arthritis and Metabolic Diseases.

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nique it was important that the sections be stained before application of the emulsion.

Histologic examination of the radioautographs showed incorporation of tritiated thymidine by epithelial cells throughout the epidermis, from the basal to superficial layers (Figures 1 and 2). Particularly dense labelling was found after the in vitro exposure to tritiated thymidine. (Both micrographs included in this report are from this experiment.) Occasionally silver grains were found over the nuclei of mucous cells. Labelled club cells were not observed.

Discussion. Studies of mammalian epidermis have shown that only premitotic *basal* cells incorporate tritiated thymidine after exposure to this radioactive precursor of DNA. On the other hand, as found in this study, tritiated thymidine is incorporated by cells in all levels of teleost epidermis after only a single brief exposure to the DNA precursor. The short duration of the experiments, 30 and 90 min, excludes the possibility that labelled cells in the more superficial levels of the epidermis could correspond to postmitotic migrating cells. The observed indiscriminate distribution of labelled cells indicates that cells capable of synthesizing DNA, and presumably mitotically active, reside at all levels in teleost epidermis, suggesting that mitoses are not restricted to basal cells as in the epidermis of other vertebrates.

Zusammenfassung. Zur Lokalisierung der potentiell synthetisierenden mitotischen Epidermiszellen wurden Knochenfische mit H^3-Thymidin injiziert, bzw. ihre Epidermis mit H^3-Thymidin inkubiert. Radioaktiv markierte Zellen wurden in allen Epidermisschichten gefunden. Diese wahllose Verteilung der DNS-synthetisierenden Zellen in der Knochenfischepidermis steht im Gegensatz zu Befunden bei anderen Wirbeltieren, wo die radioaktive Markierung nur auf die Basalschicht beschränkt bleibt.

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Pattern of Cell Damage Due to Ionizing Radiation in the Epithelial Lining of the Intestine in *Heteropneustes fossilis* Bloch

A continuous massive movement of the cells from the intestinal crypts to the tips of the villi has been demonstrated after irradiation[1,2]. Extrusion zones in the human intestine were also shown to exist by BERTALANFFY[3]. So far as we are aware, there are only a few papers on the effects of irradiation on the piscine intestinal epithelium[4,5]. The present work was undertaken in order to examine the phenomenon of external post-irradiation intestinal changes with particular attention to the atrophy and regeneration of the intestinal epithelium in *Heteropneustes fossilis* Bloch, a silurid fish.

2 aquaria containing equal volumes of water were set up. 32 fish, weighing 10 ± 2 g, were placed in one of the aquaria and Ca^{45} was added to give an activity of 3820 counts/min/ml. The other aquarium containing an equal number of fish served as control. 3 fish were sacrificed at various intervals up to 12 days. No deaths occurred during the experimental period. Portions of intestine from both control and experimental fish were fixed in Bouin's fluid, sectioned at 6 microns and stained with hematoxylin and eosin, Heidenhans' iron hematoxylin and Mallory's phosphotungstic acid hematoxylin.

Figure 1 shows the arrangement of cells in control intestinal epithelium. 2 h after Ca^{45} treatment, transformation in cell and nuclear shape is evident. The epithelium at the side of the villi is intact and uniform and the goblet cells are seen in normal position. However, at the extreme tips of certain villi, the epithelial regularity is interrupted. On the other hand, the cells which occur at the base of the crypt tend to be loosely organized (Figure 2). The connective tissue of the mucosa and the submucosa, as well as the intermuscular and subserosal stromal elements show slight hydropic degenerations. The blood vessels that are situated at the base of the submucosa are hyperaemic. Hyaline degeneration of blood vessels occurs in the lamina propria and the periphery. The muscle coats are also affected.

Within 4–8 h after radiocalcium treatment, the epithelia lining investing the villi becomes disorganized. Goblet cells disappear. The procession of epithelial cells from the crypts of the villi to their tips, where the cells are desquamated into the lumen, continues. The lumen is now filled with exudate. The cells of the lumen nearest to the villus tips closely resemble cells in the 'extrusion zones' of the villus tips (Figure 3). The number of cells per villus was markedly decreased. The villus cell population was reduced because of the absence of a compensatory supply of new cells from the crypts. The areas of active cell proliferation are occupied by vacuoles. With the continuous streaming exit of cells into the lumen, empty spaces become greatly marked (Figure 4). These spaces, which are first noticeable in the regeneration zones, extend to the central part of the intestinal villus, i.e. spaces prevail along the pathway of cell migration.

Reparation is discernible by the second day. There is a gradual in-pushing of cells from the base of the crypt inwards. As cell number increases, spaces in the villi decrease. The presence of exuded debris in the lumen is now greatly diminished; it may even be scanty or totally lacking. No breakage is found in the mucosal lining. None the less, the epithelial cells are still to be reorganized. The normal basal round-shaped and central elongate-shaped nuclei of the cells have not yet differentiated into various tiers. However, in certain parts cell orientation is observable.

Goblet cells reappear on the fourth day. In spite of the fact that greater uptake of Ca^{45} into the intestine occurred from the sixth day onwards, complete recovery prevailed so that the intestine appeared normal. This is probably

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