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

[1] N. B. FRIEDMAN, Archs Path. *34*, 749 (1942).
[2] C. P. LEBLOND, C. E. STEVENS and R. BOGOROCH, Science *108*, 531 (1948).
[3] F. D. BERTALANFFY, Gastroenterology *43*, 472 (1962).
[4] Y. HYODO, Annotnes zool. japon. *37*, 104 (1964).
[5] Y. HYODO, Radiat. Res. *24*, 133 (1965).

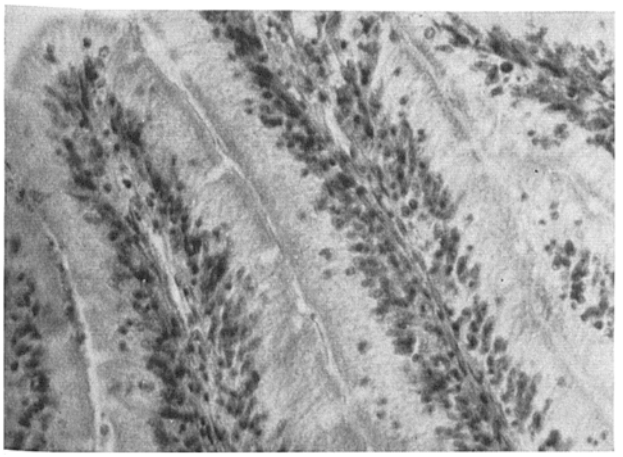

Fig. 1. Cross section of the intestine showing the normal arrangement of cells in the villi. × 320.

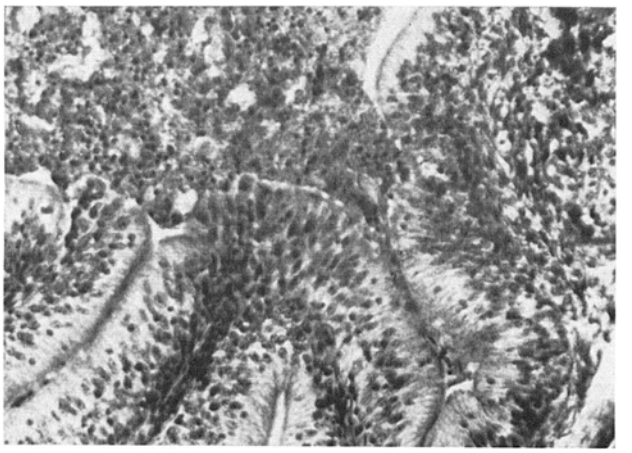

Fig. 3. Cross section of the intestine showing the extrusion of the villous cells. Cells further out in the lumen are extremely autolysed. × 320.

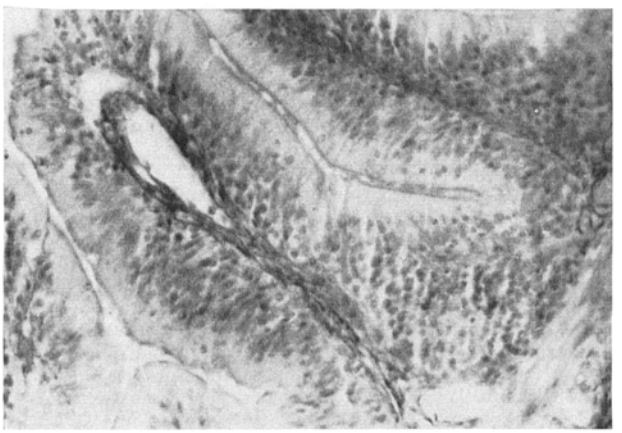

Fig. 2. Cross section of the intestine showing the nuclei in the crypts which are loosely packed and indistinct. × 320.

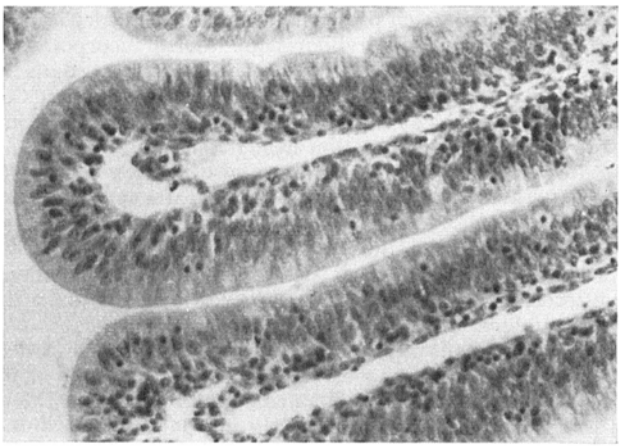

Fig. 4. Cross section of the intestine showing empty spaces along the pathway of cell migration. × 320.

due to the fact that the intestine had become adapted or conditioned to the radiocalcium uptake.

The population of epithelial cells on the villi is dynamic[6,7]. Under normal conditions it is in a steady state. It is known that radiation can prevent the production of viable cells in a number of ways[8]. The greater vulnerability to irradiation of the epithelium of the intestinal crypts in comparison to that of the surface layer has attracted considerable attention. It has been explained on the ground that the young and dividing cells are specially susceptible to the effects of radiation[1]. Although the results of the present experiment are in accord with this view, they do not support any of the disagreeing mammalian views on the goblet cells. MONTAGNA and WILSON[9] found that in mice irradiated with 1000 r X-rays, the number of goblet cells decreased after 4 h. LESHER[10] found an actual increase in goblet cell numbers in both ileum and colon of mice after continuous irradiation. In *H. fossilis*, there is a total absence of goblet cells 4 h after irradiation. They reappear only on the fourth day[11].

Zusammenfassung. Der Vorgang der Strahlenschädigung wurde an der Epithelzellschicht des Darmes des Süsswasserfisches *Heteropneustes fossilis* untersucht. Schon 2 h nach der Strahleneinwirkung ist eine Veränderung der Zellform und des Zellkerns zu erkennen. Die Zellen an den Spitzen der Fasern werden als erste in das Lumen ausgepresst. Becherzellen sind besonders strahlenanfällig und verschwinden schon nach kurzer Zeit. Die Neubildung beginnt vom 2. Tag an, und Becherzellen sind vom 4. Tag an wieder zu beobachten.

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[6] N. B. FRIEDMAN, J. exp. Med. *81*, 553 (1945).
[7] C. P. LEBLOND and C. E. STEVENS, Anat. Rec. *100*, 357 (1948).
[8] H. QUASTLER, F. G. SHERMAN, G. BRECHER and E. P. CRONKITE, Int. Con. Peaceful Uses atom. Energy 202 (1958).
[9] W. MONTAGNA and J. W. WILSON, J. natn. Cancer Inst. *15*, 1703 (1955).
[10] S. LESHER, J. natn. Cancer Inst. *19*, 413 (1957).
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