AN AUTORADIOGRAPHIC STUDY OF THE HYPOPHYSIS OF *CARASSIUS AURATUS* BY ADMINISTRATION OF $Na_2S^{35}O_4$ AFTER TREATMENT WITH THIOUREA OR THYROXINE

In previous reports we showed that, after administration of $Na_2S^{35}O_4$ to *Carassius auratus*, the radioactive sulfate was concentrated in the anterior hypophyseal cells which are stained by Alcian blue (AB+) and by the periodic acid Schiff technique (PAS) (*Path. Biol.* **10:** 425, 1962; *C. R. Acad. Sci.* **254:** 1513, 1962; *C. R. Soc. Biol.* **156:** 1924, 1962).

We know that these PAS+ and AB+ cells become hypertrophied under the influence of thiourea, and that, in contrast, they shrink and appear more condensed during treatment with thyroxine. We thought it would be of interest to study, by autoradiography the effects of previous treatment of fish with thiourea or thyroxine (Herlant, *Biol. Med.* **51:** 205, 1962) on the incorporation of radioactive sulfate into these cells.

We used 15 fish (*Carassius auratus*) in this study: 11 were treated with thiourea (0.3 gm/liter) for 15 days and 4 for 9 months. The other 4 fish were kept in a bath of thyroxine (1.4 mg/liter) for 9 months.

Fish were killed 24 hours after a single intraperitoneal injection of $Na_2S^{35}O_4$ (2 to 5 μc per gram of body weight).

The hypophyses were fixed in 10% formol. Sec-

FIG. 1. Autoradiograph of an hypophysis of *Carassius auratus* after treatment with thyroxine for 9 months and administration of $Na_2S^{35}O_4$.

FIG. 2. The section from Figure 1, PAS stain

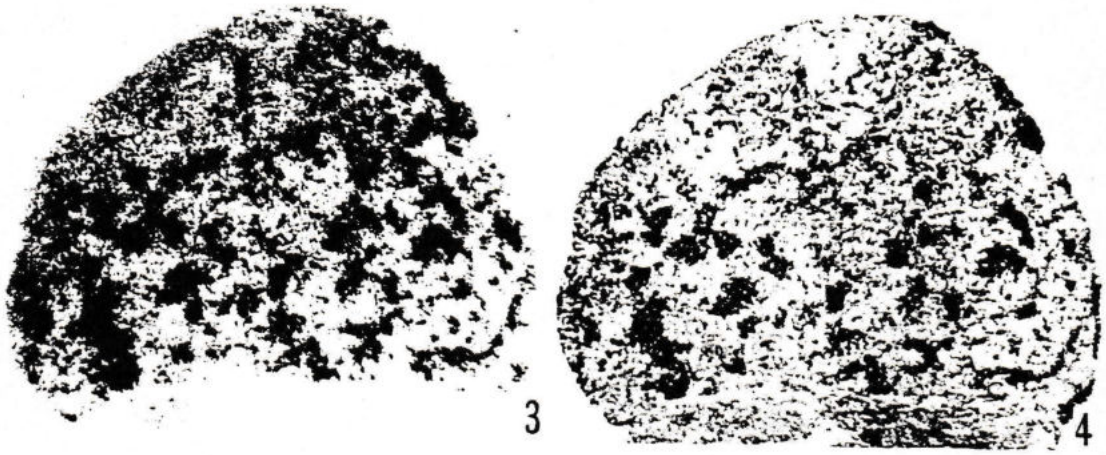

FIG. 3. Autoradiograph of an hypophysis of *Carassius auratus* after treatment with thiourea for 9 months and administration of $Na_2S^{35}O_4$.

FIG. 4. The section from Figure 3, PAS stain

tions were covered with stripping film (Kodak AR-10). They were exposed for periods of 15 days to 2 months and then developed. Pictures were taken of the autoradiographs. The photographic emulsion was removed and the sections stained by the periodic acid Schiff technique or with Alcian blue at pH 0.2 after oxidation by permanganate-sulfuric acid. We could then compare the intensity of incorporation of S^{35} in a cell with its histochemical properties.

1. After treatment with thyroxine examination of the autoradiographs shows that the blackness, indicating zones of high radioactivity, corresponds to the PAS and AB+ cell groups. In contrast, the anterior hypophyseal areas which lack PAS+ and AB+ material show only a low level of radioactivity (Figs. 1 and 2).

2. After treatment with thiourea the autoradiographs show diffuse and irregular areas of radioactivity. The most radioactive areas correspond to PAS+ and AB+ cells. The more diffuse appearance of the radioactivity parallels the greater dispersion of the PAS+ and AB+ cells (Figs. 3 and 4).

We can see in *Carassius auratus* treated with thiourea or with thyroxine, as was seen in the control fish studied previously (2), an intense incorporation of $S^{35}O_4^=$ in the PAS+ and AB+ anterior hypophyseal cells after administration of $Na_2S^{35}O_4$.

This technique, however, allowed us to determine neither the biochemical substrates on which the $S^{35}O_4^=$ is fixed nor the relationship between the amount of radioactive sulfate incorporated and the total number of $SO_4^=$ groups present in these cells.

Thus, these pictures do not authorize us to exclude eventual variations of the rapidity of turnover of the $SO_4^=$ groups present in the PAS+ and AB+ prehypophyseal cells.

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Département des Applications Biologiques du Centre de Recherches Nucléaires de Strasbourg-Cronenbourg et Institut d'Histologie de la Faculté de Médecine de Lille (France)

[1] With the technical collaboration of Miss G. Gerlinger.

FLUORESCENT MICROSCOPY OF THIN SECTIONS AS AN ADJUNCT TO ELECTRON MICROSCOPY*

Routine examination of alternate sections by electron and light microscopy is a valuable technique in cytochemical analysis of cellular details. Most workers cut "thick" sections for light microscopy from plastic blocks before cutting ultrathin sections for examination with the electron microscope. The use of such "thick" sections reveals the topography of the tissue within the

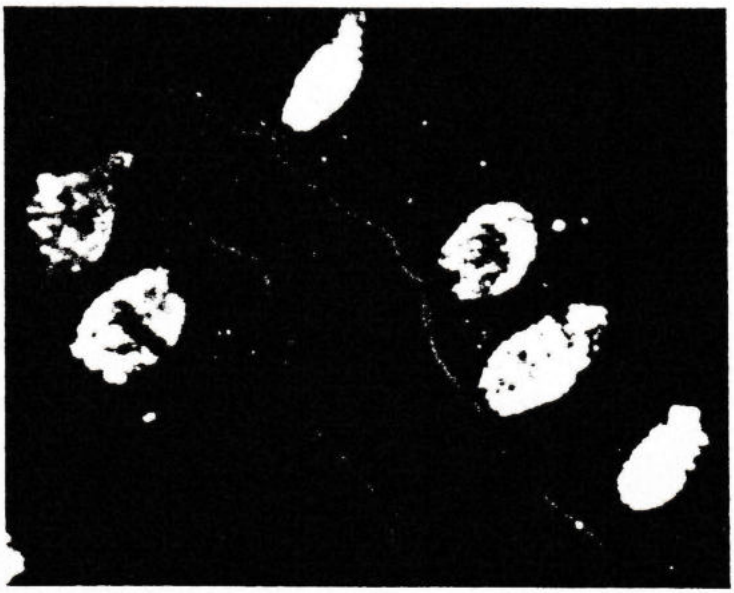

FIG. 1. Fluorescent photomicrograph of a 0.2 μ section of duodenum. Stained with a periodic acid-Schiff type reagent prepared from acriflavine. Note the detail shown in the goblet cells, the striate border and the small positive granules in the epithelial cells. ×900.