STUDIES ON THE ADRENAL GLANDS OF TELEOSTS—V.
EXPERIMENTALLY INDUCED HISTOLOGICAL ALTERATIONS
IN THE INTERRENAL CELLS OF FISHES*

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The cortical cells of mammalian adrenals and the interrenal cells of avian adrenals contain ascorbic acid, and lipid droplets composed mainly of neutral fat and cholesterol, and these substances are supposed to have close relationship to the production or secretion of cortical hormones. However, these substances are not detected histochemically in the interrenal cells of teleosts, as reported in the previous paper¹⁾.

On the histochemically unique interrenal cells of teleosts, cellular alterations induced were investigated under some experimental conditions such as the injection of mammalian adrenocorticotrophic hormone (ACTH), immersion in dilute sea water or rearing in thiourea solution.

Experimental methods

In the following experiments goldfish, *Carassius auratus*, were used.

Effects of mammalian ACTH: 24 animals weighing 18–24 gm were injected intraperitoneally with 1 I.U. of ACTH (N. V. Organon's "Cortrophine") once a day for 1, 2, 5, 8 or 10 successive days. As control, 10 animals were injected with the hormone's solvent only. These animals were sacrificed at 6 hours after the last injection.

Effects of thiourea: 15 animals were raised in 0.1% thiourea solution for 15, 42 or 50 days. For control, other 5 animals were raised in normal water for 42 days.

Effects of rearing in dilute sea water: 25 animals were used. These animals were divided into 4 groups and raised by the following procedures.

Group 1. 5 animals were raised in 1/5 sea water*** for 5 days.

Group 2. 5 animals were raised in 1/5 and then 1/3 sea water for 5 days respectively.

Group 3. 5 animals were raised in 1/5, 1/3 and then 2/5 sea water for 5 days respectively.

Group 4. 10 animals were raised in 1/5, 1/3, 2/5 and then 1/2 sea water for 5 days respectively. Two goldfish of this group were raised further in 1/1 sea water for 3 hours after having reared for five days in 1/2 sea water.

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*** 1/5 sea water; one part of sea water is diluted by four parts of fresh water.

Here, the microscopic examinations were made on the histological alterations of the interrenal cells induced by rearing in sea water of various salinities.

The head kidneys containing the interrenal cells were fixed in BOUIN's or ZENKER-formol fluids and imbedded in paraffin. Sections were stained with MAYER's acid haemalum-eosin, MALLORY's triple stain, and GOMORI's chrome-haematoxylin phloxine. To discriminate the pigment granules appearing in the interrenal cells treated with thiourea solution, bleaching with 10% hydrogen peroxide was also employed.

Results

1) Effects of mammalian ACTH: No remarkable alterations were observed in the histological structure of the interrenal cells injected with 1 or 2 I.U. of mammalian ACTH. However, the hypertrophy of the interrenal cells is conspicuous in the fishes injected with 5, 8 or 10 I.U. of this hormone and the nuclei of these cells increase in size. Furthermore, the nucleoli are hypertrophied and the chromatin decreases in number in most cases (Fig. 2-1). Such changes are similar to those of RASQUIN²⁾ on the characin, *Astyanax mexicanus* and of CHAVIN³⁾ on the goldfish, *Carassius auratus*. In this connection, OLIVEREAU and FROMENTIN⁴⁾ reported that the atrophy of interrenal cells in the eel, *Anguilla anguilla*, is observed after hypophysectomy.

To show more clearly the hypertrophy of the interrenal cells induced with ACTH, the size of nuclei is measured in five goldfish injected with 10 I.U. of this hormone and compared with that of interrenal cells in control. This measurement was done on 200 interrenal cells in each goldfish, and the results are shown in Fig. 1. From

Fig. 1. Distribution curve of the nuclear diameter of interrenal cells in control and mammalian ACTH injected goldfish.

this figure, it is evident that the hypertrophy of interrenal cells of goldfish is induced with the administration of mammalian ACTH.

2) Effects of thiourea: The hypofunctional state of the interrenal cells occurs in the goldfish raised in 0.1% thiourea solution for 42 or 50 days, and also shrinkage of cytoplasm and pycnosis of nuclei are observed. The most conspicuous change, however, taking place in these cells, is the appearance of yellowish brown pigment granules in the cytoplasm, especially around the nuclei (Fig. 2-2).

In the interrenal cells of goldfish, raised in 0.1% thiourea solution for 15 days, these pigment granules have not appeared

yet. In the control fish no pigment granules were observed. In GOMORI's chrome-haematoxylin phloxine stained preparations, the yellowish brown colour of these granules is bleached by the oxidizing agent ($\text{KMnO}_4 + \text{H}_2\text{SO}_4$) used in the pretreatment, therefore the presence of these pigment granules becomes obscure. Moreover, these pigment granules are bleached by the treatment of 10% hydrogen peroxide for 24 hours. Therefore, they may be melanin pigment.

3) Effects of rearing in the dilute sea water: No conspicuous alterations of the interrenal cells were observed in the goldfish of groups 1-3 but remarkable degenerated interrenal cells are detected in goldfish in group 4 (Fig. 2-3). In these degenerated tissues the interrenal cells are atrophied and disorderedly arranged, chromatin is increased in number and also the nuclei become unclear. Moreover, the distinct pycnosis of nuclei is detected in the interrenal cells of two goldfish, which are raised in 1/1 sea water for 3 hours after rearing in 1/2 sea water for 5 days (Fig. 2-4).

Fig. 2. Interrenal cells of goldfish, *Carassius auratus* L. 1. Administered with ACTH ("Cortrophine" 1 I.U. per day for 10 days). 2. Reared in 0.1% thiourea solution for 42 days. 3. and 4. Reared in dilute sea water. Detail in paper. $\times 380$.

Discussion

It is well known that the size of nuclei is changeable according to the functional

activity of cells (KRANTZ⁵¹), and that the volume of nuclei in the cortical cells of mammalian adrenals is increased in the accelerating and decreased in the lowering of activity (KNIGGE⁶¹). Therefore, the hypertrophy of interrenal cells induced by ACTH-administration may indicate the hyperfunctional activity of these cells. Using bonito, *Katsuwonus pelamis* and tunny, *Thunnus thynnus*, Iro et al⁷¹ reported that the ACTH-like hormone is contained in the hypophysis of teleosts. On the other hand, FONTAINE and HATEY^{81,91} ascertained that the cortisone-like steroid hormones are present in the blood of Atlantic salmon, *Salmo salar*. From the author's observations as well as the above mentioned reports, therefore, it is assumed that the activity of interrenal cells is controlled by the interrenal stimulating hormone secreted from the hypophysis and the hypophysis-interrenal system exists in teleosts such as the hypophysis-adrenal cortex system in mammals.

In the administration of ACTH on reptiles and amphibia, it is said that the increasing of dosage induces the degeneration of interrenal cells in consequence of over-secretion of ACTH (MILLER, M. R.)^{101,111}. Therefore, the degeneration of the interrenal cells of goldfish reared in dilute sea water may be explained as follows. The secretion of interrenal-stimulating hormone is hastened by the sudden changes of osmotic pressure of environmental water, consequently the over-secretion of hormones from the interrenal cells occurs, and finally these cells are exhausted and thus degeneration takes place in the interrenal cells.

Thiourea is one of the antithyroid drugs and it suppresses the function of the thyroid glands of mammals. Moreover, GOLDSMITH et al¹²¹ and SCOTT¹³¹ reported that this substance is also effective as the antithyroid drug to teleosts. On the other hand, it is said that these drugs suppress indirectly the function of the adrenal cortex of mammals in consequence of the hypofunction of the thyroid glands. In the author's experiment, the hypofunction of the interrenal cells of goldfish occurs when rearing with thiourea solution. Also in the shrunk cytoplasm appear a good deal of melanin pigment granules relating probably to the degeneration of interrenal cells. For such reasons, it is assumed that a close relationship exists between the thyroid gland and the interrenal cell group of teleosts. It is well known that the thyroid glands of teleosts take part in the osmo-regulation and have close relationship to migration (HOAR and BELL¹⁴¹, HOAR¹⁵¹). In these cases, subsequently, it is supposed that the histological alterations occur in the interrenal cells having close relationship to the thyroid glands. On this respect, further studies are being carried out.

Summary

- 1) The mammalian ACTH is effective for teleosts and accelerate the function of interrenal cells. Consequently the conspicuous hypertrophy of nuclei occurs.
- 2) Elevating the salinity of dilute sea water and continuing the rearing of gold-

fish, the conspicuous degeneration of interrenal cells occurs at last.

3) In goldfish reared in 0.1% thiourea solution for long periods (42-50 days), the interrenal cells show the hypofunctional state and many melanin pigment granules appear in the cytoplasm.

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