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Effect of Thiourea Treatment on Pituitary Basophilic Cells
of the Loach, *Misgurnus anguillicaudatus*

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Abstract

Adult males and females of the loach, *Misgurnus anguillicaudatus*, were immersed in 0.01% thiourea solution for periods of 30 and 60 days. The treatment produced a pronounced hypertrophy, hyperplasia and hyperemia of the thyroid glands and prominent cytological changes of one type of basophilic cells which were distributed in the proximal pars distalis (PPD) of the pituitary gland. The cells were situated mainly in the dorsal parts of the PPD and usually showed a weak affinity to both alcian blue and PAS. Ultrastructurally, they were characterized by having secretory granules, measuring 80-200 nm in size, which were the smallest in size of all the glandular cells of the PPD. After the thiourea treatment, they showed a marked development of lamellar rough endoplasmic reticulum, a striking decrease in the number of secretory granules, and an activation of the Golgi apparatus, being thus defined to be thyrotropic in function. No other cell type of the PPD exhibited such remarkable changes after the treatment, except for a few gonadotropic cells of the globular type which appeared to be suppressed in function by thiourea treatment.

The question of whether the pituitary gland of teleost fishes contains one or two types of gonadotrophs is still controversial. Whereas some of the studies made so far on teleost pituitaries have shown only one type of gonadotropic cells, others have stressed the occurrence of two distinct types of the cells in several species^{1),2),3),4)}. The present writer also reported ultrastructural evidence indicating that among three kinds of basophilic cells located in the proximal pars distalis of the pituitary gland of the loach, *Misgurnus anguillicaudatus*, two cell types more or less displayed some structural changes after artificially induced ovulation⁵⁾. Of these two types of pituitary basophils, the one referred to as globular cells showed pronounced changes following ovulation, corresponding to the globular gonadotrophs described in other teleosts⁶⁾. The other, however, designated as vesicular cells, exhibited less prominent changes after ovulation, preventing their being clearly defined as a distinct type of gonadotroph. As a first step for clarification, the experiment described in this paper was intended to distinguish them decisively from thyrotropic cells which were to be distributed also in the proximal pars distalis of the pituitary gland of the loach.

It is well known that thiourea stimulates thyrotropin release from the pituitary gland through an inhibition of the production of thyroid hormones. Therefore, the use of this drug appears to make it possible to identify the thyrotrophs from other cell types of the pituitary gland. In fact, the goitrogen has been

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administrated to various teleosts, with consistent results including hyperactivity of pituitary thyrotrophs and ensuing histological stimulation of the thyroid gland⁷⁾. Ultrastructural studies carried out so far in goldfish, *Carassius auratus*^{8),9)} and medaka, *Oryzias latipes*¹⁰⁾ have characterized the changes of pituitary thyrotrophs influenced by goitrogenic treatment. The present study deals with observations on ultrastructural changes of pituitary glandular cells of the loach subjected to treatment with thiourea.

Before going further the writer wishes to express his appreciation to Professor H. Takahashi, Faculty of Fisheries, Hokkaido University, who supervised the present study and criticized the manuscript.

Material and Methods

A total of 24 adult loaches, *Misgurnus anguillicaudatus*, ranging from 8.5 to 11.3 cm in body length and from 3.1 to 8.6 g in body weight, were collected in the suburbs of Hakodate in May 1978, when they were at a later phase of the pre-spawning period. The loaches were then acclimated to laboratory conditions for about two weeks prior to experimental treatment. They were kept in a glass aquarium with well-aerated water under natural light and temperature conditions, and were fed on commercial diets once a day throughout the experiment.

At the start of experiment, 4 females and 4 males were sacrificed and served as initial controls. Another group of 6 females and 4 males was immersed in 0.01% thiourea solution, which was changed every two days, and half were killed after 30 days of treatment, and half after 60 days. One other group, 3 females and 3 males, which served as controls, were raised in ordinary water and were killed 60 days after the start of the experiment.

The pituitary glands were removed immediately after decapitation. About one-half of the glands were preserved in Bouin-Hollande-sublimate for about 3 days, cut at 6 μ m in thickness and stained with alcian blue-PAS-orange G. Others were prefixed with Karnovsky's glutaraldehyde-formaldehyde mixture in 0.2 M cacodylate buffer (pH 7.4) for about 3 hours at room temperature, postfixed in 1% osmium tetroxide in the same buffer (pH 7.4) for about 2 hours at 4°C, and embedded in Epon. Ultrathin sections stained doubly with uranyl acetate and lead citrate were observed with a Hitachi HU-12 electron microscope. Parallel semithin sections of the Epon-embedded glands were stained with methylene blue for light microscopy. The gonad and thyroid gland of the control and treated fish were fixed in Bouin's fluid and prepared routinely for histological inspection of their activity.

Results

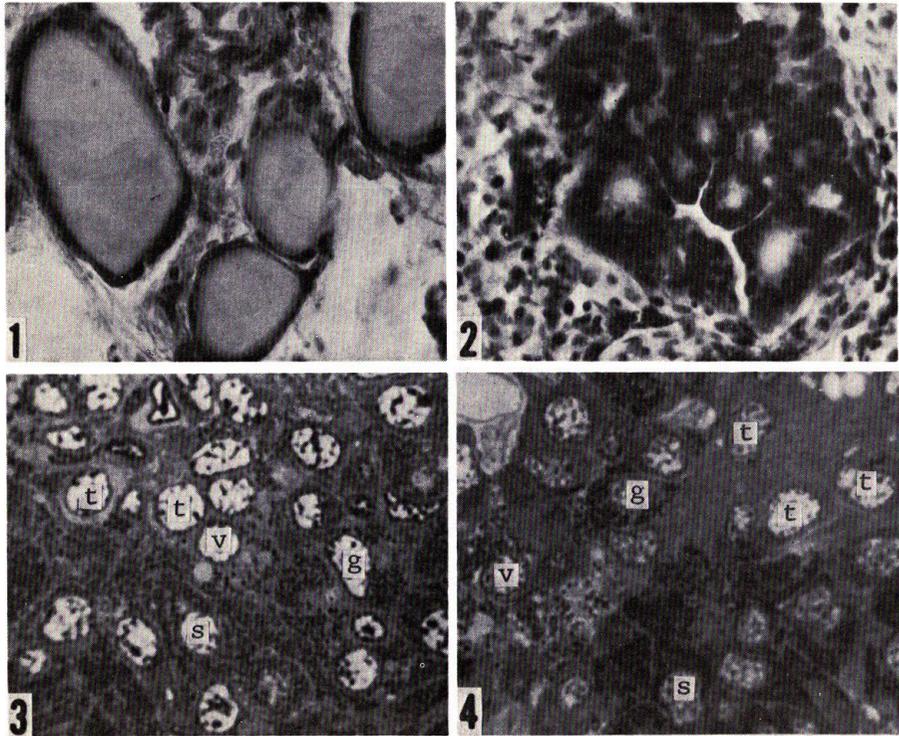
Females and males of the initial control fish had ovaries with many oocytes in the tertiary yolk stage and testes with germ cell cysts in various spermatogenic stages, respectively. No notable changes were detected in the gonads of both sexes after 60 days of thiourea treatment.

The thyroid gland of the loach was composed of a number of separate

follicles which were distributed in the pharyngeal region. In the follicles of control fish of both sexes, epithelial cells were low cuboidal in shape, averaging $5\ \mu\text{m}$ in height, and the follicle lumina were packed homogeneously with colloid which was intensely eosinophilic, indicating as a whole a low histological activity (Fig. 1).

Thiourea induced a remarkable hypertrophy, hyperplasia and hyperemia of the thyroid gland in both sexes. In the fish killed after 30 days of thiourea treatment, the epithelial cells were increased from 8 to $12\ \mu\text{m}$ in height, and the colloid was decreased both in amount and in stainability. The hyperactive features were much pronounced in the thyroid gland of the fish treated for 60 days: follicular epithelium measured $15\ \mu\text{m}$ in height, and the follicles were almost devoid of eosinophilic colloid (Fig. 2).

In the proximal pars distalis (PPD) of the pituitary gland of the loach, only one type of basophilic cells displayed prominent changes following thiourea treatment, regardless of sex of the fish treated. The cells were situated mainly in the



Figs. 1 and 2. Sections of thyroid follicles of a control (Fig. 1) and a thiourea-treated loach (Fig. 2), 60 days after the start of treatment. Hematoxylin-eosin stain. $\times 400$.
Figs. 3 and 4. Sections of Epon-embedded pituitary glands of a control (Fig. 3) and a thiourea-treated loach (Fig. 4), 60 days after the start of treatment, demonstrating globular gonadotrophs (g), somatotrophs (s), thyrotrophs (t) and putative gonadotrophs of vesicular type (v). Methylene blue stain $\times 1000$.

dorsal region of the PPD and showed a weak affinity to both alcian blue and PAS. In control animals, these cells were few in number, small in size, and mostly angular in shape (Fig. 3). Ultrastructurally, the cells were characterized by having small, round or oval secretory granules measuring 80–200 nm in size. The granules were observed to be the smallest in size compared with those found in the other granulated cell types of the PPD. Lamellated rough endoplasmic reticulum was sparse. The Golgi apparatus and rod- or round-shaped mitochondria were observed to be scarce (Fig. 5).

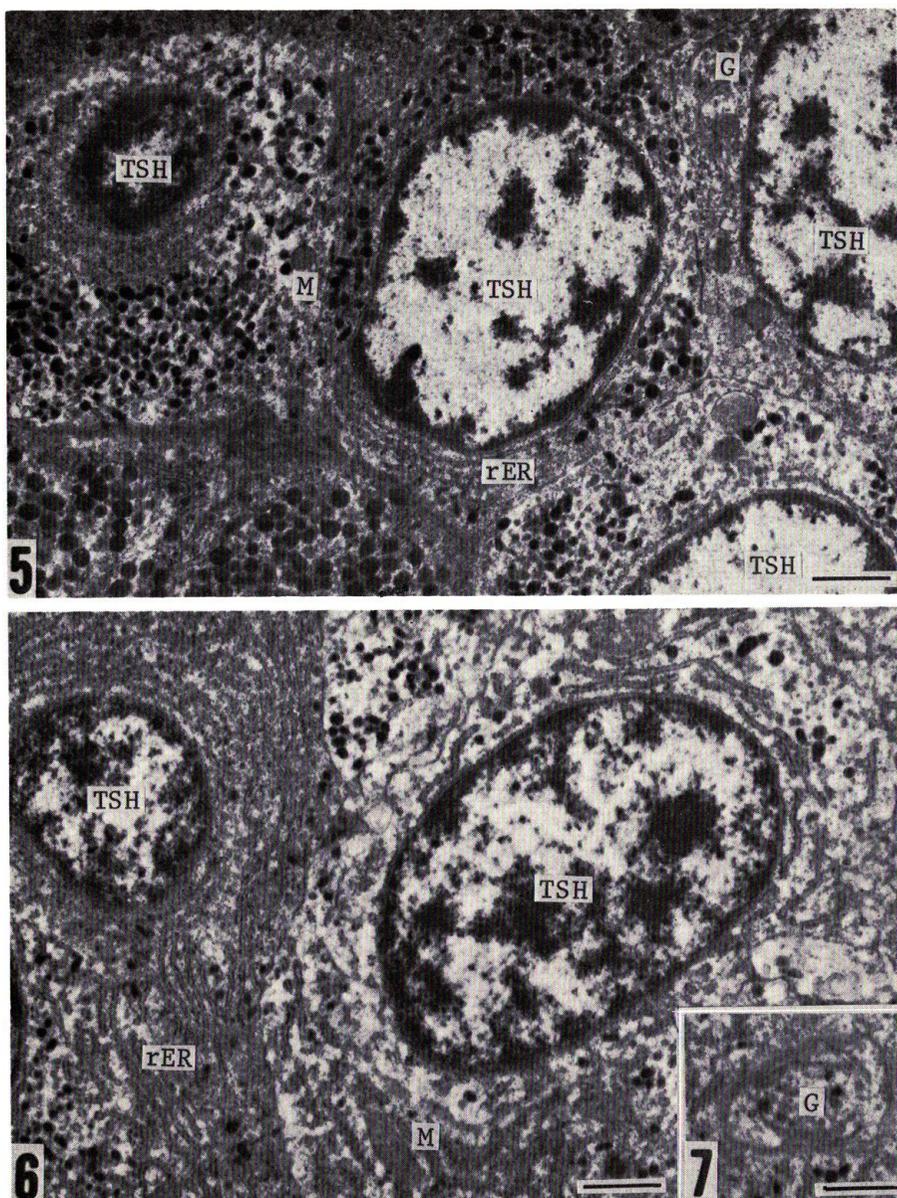
In the fish treated with thiourea for 30 days, some of the basophilic cells were seen to be increased in size. In these cells secretory granules were decreased slightly in number, and the rough endoplasmic reticulum came to consist of long lamellated cisternae. Sometimes well-developed Golgi apparatus could be found near the nucleus. In the fish killed after 60 days of thiourea treatment, basophilic cells were further increased in number as well as in size (Fig. 4). Their secretory granules were clearly diminished in amount, the rough endoplasmic reticulum consisting of parallel lamellae was developed conspicuously throughout the cytoplasm, and many Golgi apparatus appeared to be very active. Immature granules were often present within the Golgi field. Mitochondria were seen to be much enlarged in size (Figs. 6 and 7). The observed changes denote that the cells under question are decisively thyrotropic in function.

Among the other two types of basophilic cells occurring in the PPD of the loach, those that were designated as "globular" gonadotropic cells in the previous study⁵⁾ occasionally showed some changes following thiourea treatment. They were distributed in the central and ventral parts of the PPD, and were stained intensely with alcian blue and PAS. Although the changes were not detected by light microscopy on the PPD of the thiourea-treated fish of both sexes, it was observed by electron microscopy that some of the globular cells came to be provided with an increased amount of small secretory granules of 200–400 nm in size, slightly dilated rough endoplasmic reticulum, and the nucleus which was seen to become electron-dense (Fig. 9). The remaining type of basophils in the PPD were cells termed as "vesicular" cells and regarded as putative gonadotrophs in the previous study. They were found mainly in the central and dorsal parts of the PPD, and were strongly positive to PAS but negative to alcian blue. These cells were observed to be completely unresponsive to the present thiourea treatment electron microscopically as well as light microscopically (Fig. 9).

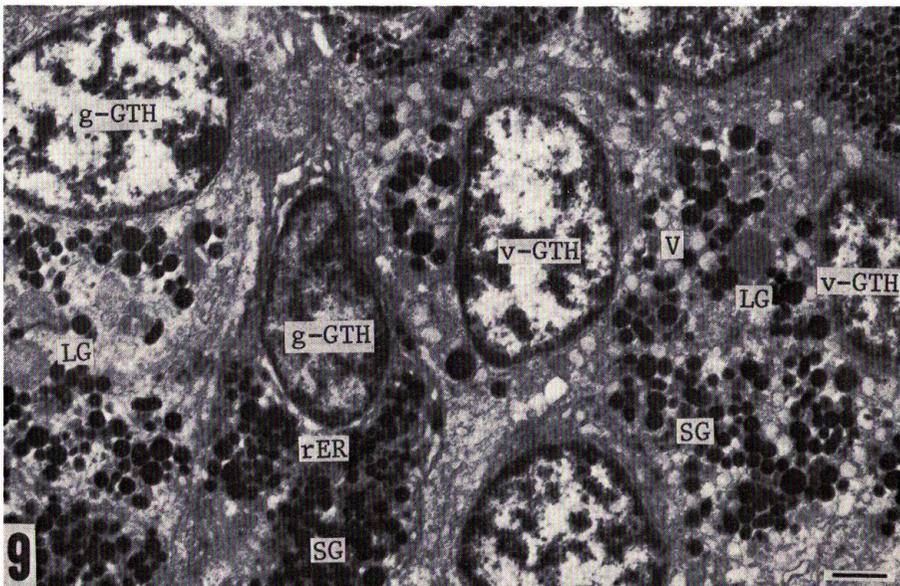
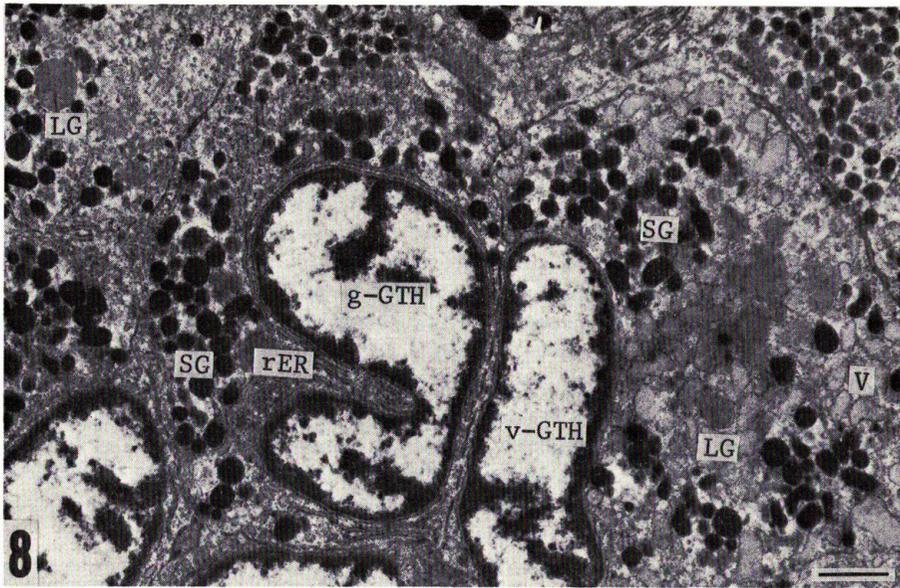
Discussion

The thiourea treatment caused severe changes in the thyroid gland of the loach, *Misgurnus anguillicaudatus*, leading to pronounced hypertrophy, hyperplasia and hyperemia of the gland. Such changes of the thyroid gland after thiourea treatment have been noticed also in several other teleosts⁷⁾, and have been reasonably taken as reflecting a stimulated secretion of thyrotropic hormone from pituitary thyrotropic cells.

In the previous study, the author could not decisively distinguish thyrotrophs from gonadotrophs in the pituitary gland of the female loach⁵⁾. In the present study, however, pituitary thyrotrophs of the loach could be clearly identified with



Figs. 5-7. Electron micrographs of pituitary thyrotrophs of a control (Fig. 5) and a thiourea-treated loach (Figs. 6 and 7), examined at 60 days of thiourea treatment. *G*, Golgi apparatus; *M*, mitochondrion; *rER*, rough endoplasmic reticulum. Scale, 1 μ m.



Figs. 8 and 9. Electron micrographs of pituitary gonadotrophs of a control (Fig. 8) and a thiourea-treated loach (Fig. 9), observed at 60 days of thiourea treatment, demonstrating globular gonadotrophs (g-GTH) and putative gonadotrophs of the vesicular type (v-GTH). *LG*, large globule; *rER*, rough endoplasmic reticulum; *SG*, small granule; *V*, vesicular cisternae of the rough endoplasmic reticulum. Scale, 1 μ m.

the aid of thiourea treatment. The cells were located mostly in the dorsal region of the PPD and showed a weak affinity to both alcian blue and PAS, being similar in these aspects to those identified as thyrotrophs in several other species of teleosts^{1),4)}.

The thyrotrophs of the loach are characterized ultrastructurally by having cytoplasmic granules of 80–200 nm in size which are the smallest among those found in all granulated cells existing in the PPD of the pituitary gland, as already described by Oota¹¹⁾. Similar ultrastructural characteristics of pituitary thyrotrophs have been reported also in pond loach, *Misgurnus fossilis*¹²⁾.

Ultrastructural changes of pituitary thyrotrophs after thiourea treatment have also been observed in goldfish, *Carassius auratus*^{8),9)} and medaka, *Oryzias latipes*¹⁰⁾. In both cases there occurred the disappearance of secretory granules, marked dilatation of the rough endoplasmic reticulum, and the development of Golgi apparatus in the thyrotrophs affected by thiourea. In the present study, too, thyrotrophs of the thiourea-treated loach of both sexes became to be occupied by well-developed rough endoplasmic reticulum which was not dilated but consisted of parallel lamellae. The secretory granules were prominently decreased in number, and the formation of new secretory granules was observed to be frequent around the well-developed Golgi apparatus.

No other cell types in the PPD of the pituitary gland of thiourea-treated loaches exhibited such remarkable changes as those found in the thyrotrophs, but some of the cells of a possible gonadotropic type that are termed as the globular cells showed an increase in number of small granules and pycnotic changes of the nucleus. Similar ultrastructural changes were seen to occur in the same type of pituitary cells of the loach treated with sex steroid (Ueda, unpublished). Although no notable change was caused in the gonads of both sexes subjected to thiourea treatment in the present study, thiourea has been shown to have variable effects on the gonads of other teleosts⁷⁾. In *Channa punctatus*¹³⁾ and guppy, *Poecilia reticulata*¹⁴⁾, thiourea was shown to be effective in inhibiting gonadal maturation by blocking the activity of pituitary gonadotrophs which showed regressive changes.

On the other hand, putative gonadotrophs of the other type, or the vesicular cells, of the pituitary gland of the loach displayed no notable ultrastructural changes following thiourea treatment, demonstrating a clear functional distinction between them and the thyrotrophs. The possible gonadotropic nature of the vesicular cells of the loach is to be substantiated by experimental studies which are in progress.

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