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Excessively Enlarged Thyroid Follicles of the Threespine Stickleback, *Gasterosteus aculeatus aculeatus*, Reared in Freshwater

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Abstract A histological examination of the thyroid glands of the threespine stickleback was carried out through various stages from the fry just hatched to the young kept for 110 days. The glands were highly active as judged from the histological criterion in the juveniles kept for about 20 days in freshwater. The juveniles survived in sea water began to have deposited guanine in the skin. In freshwater, the epithelial wall of the thyroid in the stickleback at the age of one month was irregular in shape and folded up irregularly. Then the fish which were transferred into sea water developed a heavy coat of guanine crystals. The thyroid follicles of the specimens kept continuously in freshwater beyond one month or more were excessively enlarged, and the interstitial connective tissue had markedly decreased in amount. Nucleus-like bodies and colloid-like droplets were often found in the granulated and eosinophilic colloid of the follicular lumen. The colloid-like droplets were stained pink with eosin and orange or brownish orange with Mallory's triple stain. Although small follicles were scattered in the branchial arch, the larger follicles were united with one another.

When the fish were transferred into sea water, a spontaneous involution of the enlarged thyroid occured.

Introduction

Hoar and Bell (1950) reported that the thyroid gland of the chum salmon fry retained in freshwater for two or three months is extremely hyperplastic. They also observed that the thyroid of pink salmon behaves similarly to that of the chum, and that the thyroids of yearling coho and sockeye display heightened activity in the seaward migration. Hoar (1952) found that the thyroids of landlocked alewives and smelt are always more active than those of the same fish taken in coastal areas.

In this study, the histological changes of the thyroid of the threespine stickleback were observed, and discussed in relation to the thyroid tumor.

Materials and methods

A pair of stickleback were obtained from the seashore of Nanaehama, Hakodate, Hokkaido at the beginning of May, 1970. They spawned in the glass tank and their eggs were hatched on June 6. The hatched fry were reared in a glass tank filled with city water aerated for 3 days. The water temperature was not controlled and the iodine content of the water was not tested.

Fifty-three individuals (♂ 8, ♀ 16, undistinguishable specimens 29) were used in the present experiment. The histological pictures of the thyroid of nine specimens which were kept in sea water from 2 to 63 days before fixation was compared with those of fish kept in freshwater. They were fed on brine shrimp in the stage of fry and on water flea and aquatic oligochaetes during juvenile and young stages.

The materials used for histological observation were fixed in Bouin's fluid. Formalin (10%) was also used to fix some juveniles and youngs, and fixatives containing osmium tetroxide to fix some fry just after hatching. The living fish were put in fixative and preserved for 24 hrs. The specimens were sectioned serially at 10 μ and stained with hematoxylin and eosin or with Mallory's triple stain. The PAS technique and Feulgen's staining were also used to reveal the nucleus-

like bodies and the colloid in the follicular lumen.

Results

The fish, about 5 to 8 mm in length, reared for 18 days in freshwater after hatching, have a well-developed thyroid under the basibranchials. The follicles are enlarged and irregular in form. The epithelial cells are low columnar, about 8 μ in height, and contain a spherical nucleus adjacent to the basement membrane. The cytoplasm is stained faintly with hematoxylin. The follicular lumen of the thyroid contains a small quantity of granulated eosinophilic colloid (Fig. 1A). There is no such granulated colloid in the lumen of the fish kept for 9 to 10 days after hatching.

In the fish kept in freshwater for a month (about 14 mm in total length), their thyroid follicles surrounding the ventral aorta are considerably enlarged and their lumina are filled moderately with a granulated eosinophilic colloid. Almost all of the epithelial cells are columnar, 11.2 to 13.0 μ in height, though some of them are cuboidal. The nucleus of the columnar cell is located near the free surface of the cell, and is always spherical in form (about 4.0 μ in diameter). The nucleus contains a conspicuous nucleolus. The epithelial wall is irregular in shape, accompanied with infolding in varying degrees (Fig. 1B). The stickleback become silvery by the deposition of guanine in the skin, and they can survive in sea water with no sign of distress. Namely, they may tolerate the change of osmotic pressure in ambient water. When the fish were kept continuously in freshwater, the follicles become large in size.

Extremely enlarged follicles, 0.58 mm in the major axis of the lumen and 0.27 mm in the minor one, are found in two male fish kept in freshwater for 46 days (17.4 mm in total length). Their follicles are not scattered but congregated, and the connective tissue is thin and contains capillaries. The epithelial cell varies from columnar (8.4 μ in height) to flat (2.1 μ in height). Colloid-like granules, stained deeply pink with eosin and orange with Mallory's triple stain, are sometimes found in the apex of the cell. Large cells are occasionally found in their epithelia. These cells are spherical or oval in shape (10.8 to 13.4 μ in diameter). The cytoplasm is stained pale pink with eosin and pale violet with Mallory's triple stain (Fig. 1D). The nucleus is spherical, 3.6 to 3.8 μ in diameter. These large cells are quite alike with a chloride cell in shape. The granules of the thyroid colloid are stained brownish violet and partially orange with Mallory's triple stain. Eosinophilic homogeneous colloid-like droplets are often found in the granulated colloid (Fig. 1E). Nucleus-like bodies are also detected in the lumen (Fig. 1F). They are basophilic and are usually oval or spherical.

A spontaneous involution of the enlarged thyroid occured after the stickleback was transferred into sea water. The thyroid shows a mild or quiescent activity as judged from the picture of the fish, 17.4 to 19.0 mm in total length, which were kept from 7 to 12 days after transferring from freshwater to sea water. The follicles are scattered in the connective tissue around the ventral aorta, and the largest one is 0.19 mm in the major axis of the lumen. Although the epithelium is still irregular, the follicles begin to turn oval (Fig. 2A and B). The epithelial cell varies from flat to cuboidal, 3.4 to 7.5 μ in height, and contains a nucleus (3.2 to 3.5 μ in diameter) located near the basement membrane. The follicular lumen is filled with a non-granulated acidophilic colloid. In the thyroids fixed with formalin, the vacuoles occur in the margin of the colloid.

Thyroid of the fish, 19.8 to 21.7 mm in total length, kept in freshwater from 39 to 61 days is similar to that of the fish kept in freshwater for 46 days (Fig. 2C and D). On the other hand, a mildly active thyroid was observed in one fish kept for 24 days in sea water after being kept for 37 days in freshwater. The follicular lumen is filled with a homogeneous colloid, and is 0.28 mm in the major axis. The epithelial cell varies from cuboidal to flat, 5.1 to 2.3 μ in height (Fig. 2E and F). The nucleus is spherical, 3.7 μ in diameter, or oval, 4.5 μ in the long diameter and 18 μ in short one.

In the fish kept in freshwater more than 61 days, the epithelial cell varies from columnar to flat, 12.5 to 2.5 μ in height. The

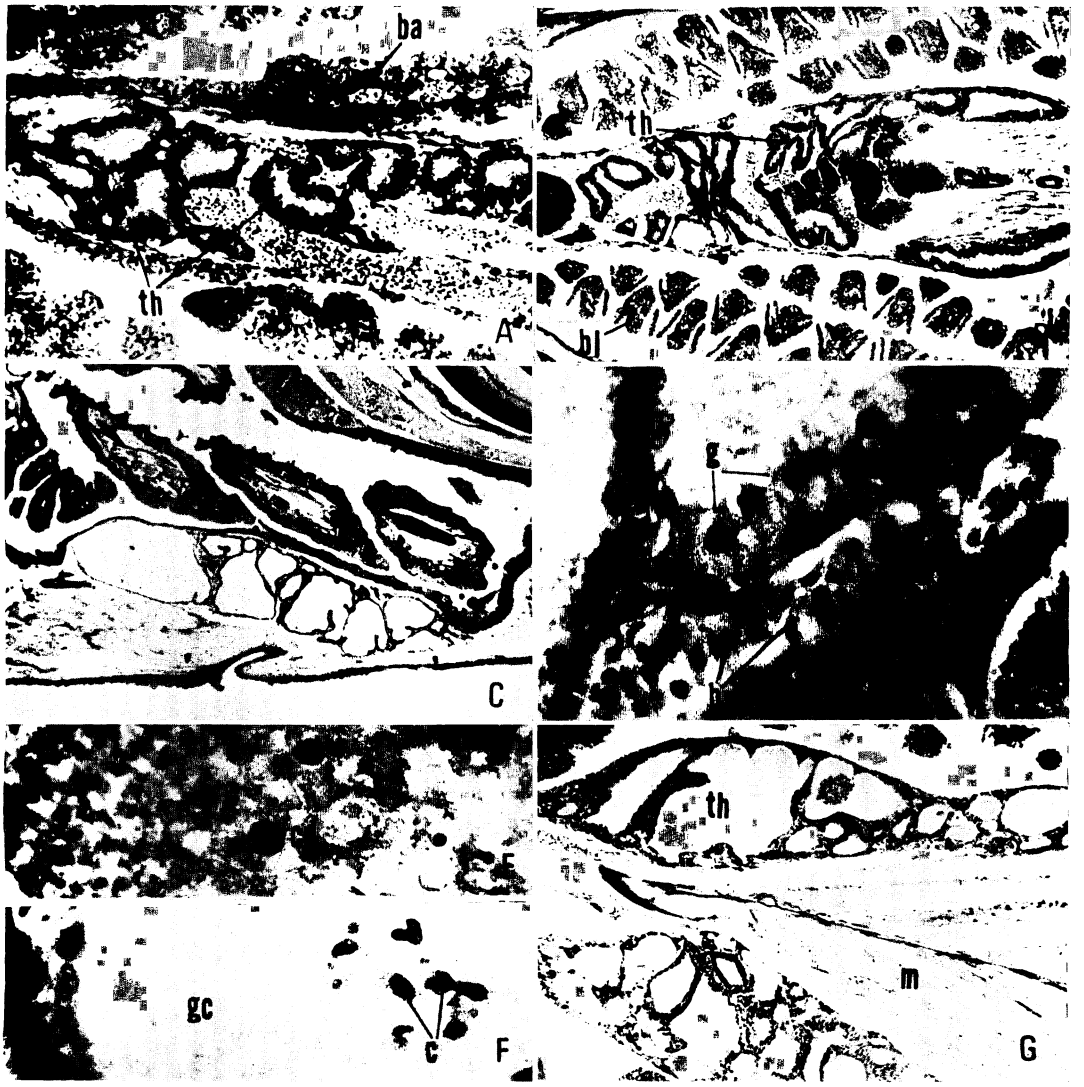

Fig. 1, A. Frontal section of the thyroid region of a stickleback kept for 26 days in freshwater. Total length 10.2 mm, female. ×177. B. Frontal section of the thyroid region of a stickleback kept for 30 days in freshwater. Total length 14.3 mm, female. ×35. C. Sagittal section of the thyroid region of a stickleback kept for 46 days in freshwater. Total length 17.4 mm, male. ×35. D, E, and F. Higher magnification of C. ×435. G. Frontal section of the thyroid region of a stickleback kept for 49 days in freshwater. Total length 21.7 mm, female. ×86. All stained with Hematoxylin and eosin. b, blood cell; ba, branchial arch; bl, gill lamella; c, nucleus-like bodies; cd, colloid-like droplets; g, large round cells; gc, granulated colloid; m, muscle; th, thyroid follicle.

cytoplasm is stained reddish violet with hematoxylin and eosin. The nucleus is spherical (3.2 to 4.3 μ in diameter) or oval (4.3 μ in the long diameter and 3.2 μ in the short one), and is stained weakly with hematoxylin. The follicular lumen, 0.79 mm in the major axis, is filled with a granulated eosinophilic colloid. However, there is an interesting histological picture in the gland of the female fish kept in freshwater for 110

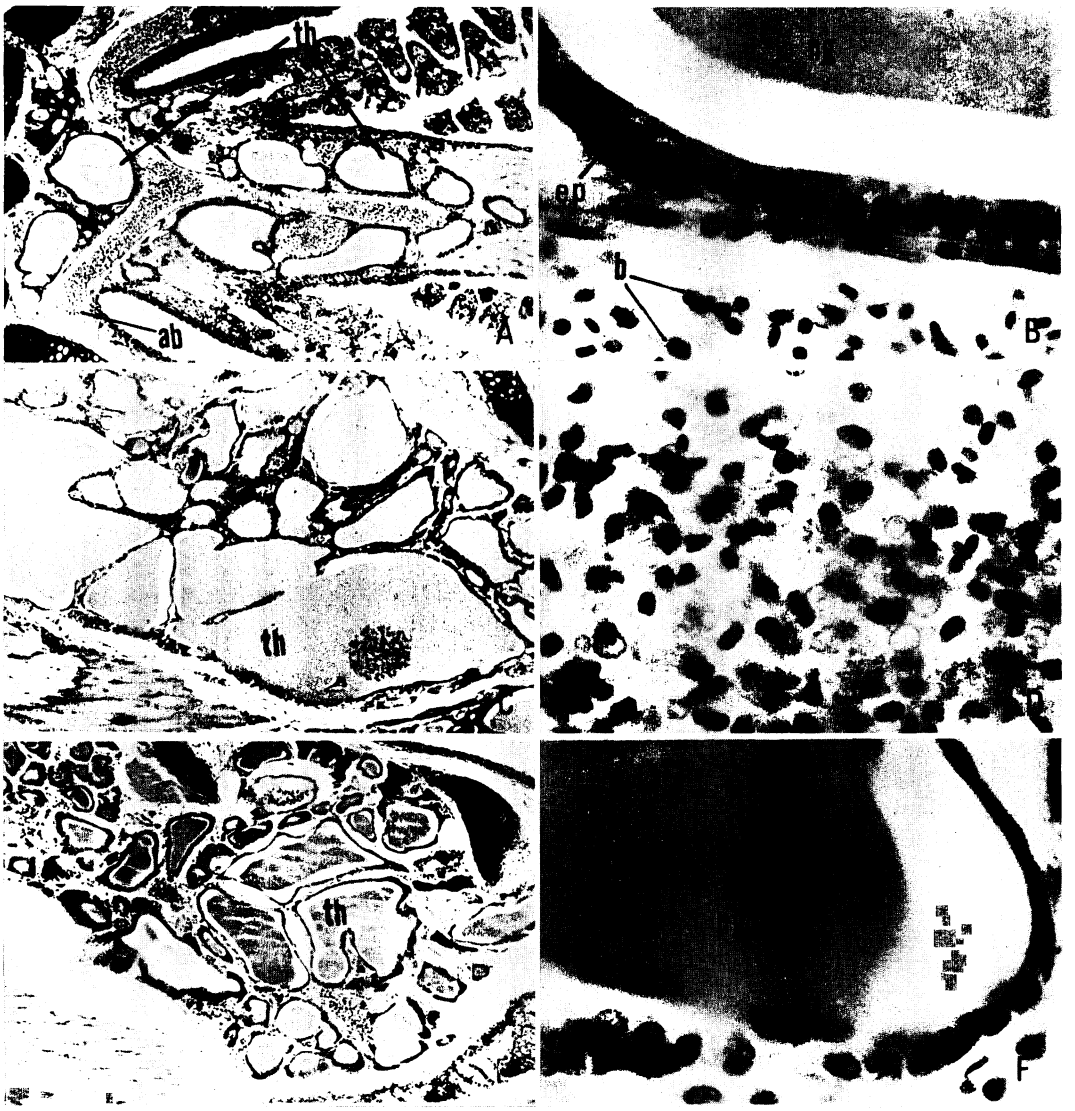

Fig. 2. A. Frontal section of the thyroid region of a stickleback kept for 12 days in sea water after rearing for 37 days in freshwater. Total length 19.0 mm, male. ×86. B. Higher magnification of A. ×435. C. Sagittal section of the thyroid region of a stickleback kept for 61 days in freshwater. Total length 19.8 mm, male. ×86. D. Higher magnification of the colloid of C. ×435. E. Sagittal section of the thyroid region of a stickleback kept for 24 days in sea water after rearing for 37 days in freshwater. Total length 22.4 mm, female. ×86. F. Higher magnification of E. ×435. All stained with hematoxylin and eosin. ab, afferent branchial artery; b, blood cell; ep, cuboidal epithelial cells; ng, non-granulated colloid; th, thyroid follicles.

days: the nucleus is often located at the free surface of the epithelial cells and nucleus-like bodies are found in the colloid (Fig. 3A, B and C). The nucleus-like bodies are basophilic and nearly identical with the nucleus of the epithelial cell in shape and in size (Fig. 3D). In the columnar epithelial cells, the nucleus is located in the free surface of the cell (Fig.

3C). Both of nucleus and nucleus-like bodies clearly show a positive Feulgen's reaction (Fig. 4A, B, C and D). The colloid accumulated in the lumen has weak affinity for the periodic acid-Schiff's reagent. Excessively enlarged follicles are observed in the female fish kept for 110 days in freshwater (Fig. 5A). The lumen is 1.56 mm in the long diameter, and filled with a granulated colloid. Almost all the epithelial cells are flat (5.4 to 2.8 μ in height) except for a few columnar cells (13.0 to 16.0 μ in height). Such large follicles are situated in pairs on each side of the ventral aorta, and small follicles are located around the large follicles. The follicles are united together one to another. Some small follicles are scattered in the branchial arch and the areolor tissue beneath the epithelium of the floor of the pharynx, and are rarely found in the muscle.

The involution of the thyroid occurs in the fish kept in sea water for 63 days after surviving in freshwater for 47 days (Fig. 5B, C and D). The follicle decreases in size, 0.33 mm in the major axis of the lumen, and is scattered in the connective tissue around the ventral aorta. The epithelial cell of the large follicle varies from cuboidal to flat, 6.0 to 2.4 μ in height, and the eosinophilic colloid is slightly granulated. While in the small follicles, the epithelial cell is usually cuboidal (6.9 to 5.6 μ in height) and sometimes flat (2.8 μ in height). The follicles do not unite together. The colloid is homogeneous and eosinophilic, and shows a positive response to the periodic acid-Schiff's reaction.

Discussion

Baggerman (1960) concluded that in stickleback the blood level of the thyroid hormone is able to induce the changes in salinity

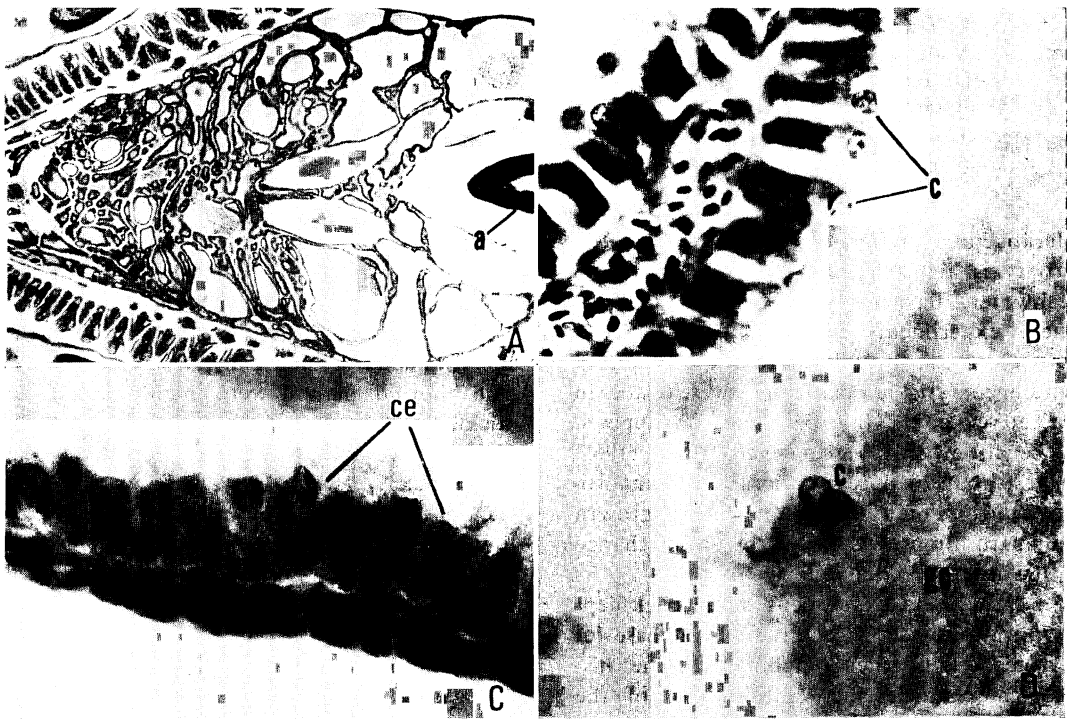

Fig. 3. A. Frontal section of the thyroid region of a stickleback kept for 110 days in freshwater. Total length 26.3 mm, female. Hematoxylin and eosin. ×35. B. Higher magnification of the epithelium of A. ×435. C. Higher magnification of the thyroid epithelium of A. ×435. D. Higher magnification of the thyroid colloid of A. ×435. a, bulbus aorta; c and ce, nucleus; gc, granulated colloid.

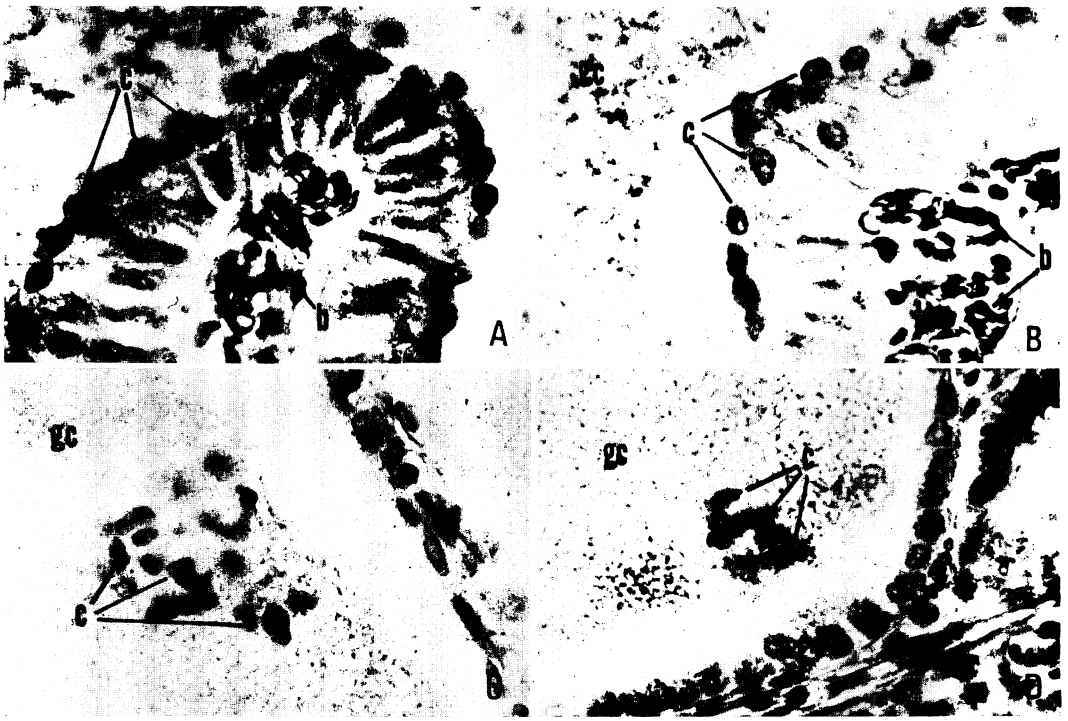

Fig. 4. A and B. The thyroid epithelial cells of a stickleback kept for 110 days in freshwater. Total length 26.3 mm, female. Nucleus (c) on the apex of cell. Granulated colloid (gc). Feulgen's staining and light green. ×435. C and D. Nuclei in the thyroid colloid of a stickleback kept for 110 days in freshwater. Total length 23.7 mm, female. Feulgen's staining and light green. ×435. c, nucleus; gc, granulated colloid.

tolerance and preference. When the thyroid activity is high, the sticklebacks migrate to freshwater. Baggerman (1962) also stated that, in the experiments concerned with the influence of the thyroid hormone on salinity preference, sticklebacks migrate seaward in the fall when the thyroid gland is supposed to be little active. However, Hoar (1965) suggests that the onset of migration in anadromous teleosts may depend on general growth and maturation of the fish, but not change of the thyroid activity.

In the present experiment, stickleback kept in freshwater show a gradual increase of thyroid activity after hatching. In the histological characteristics, the thyroid of the stickleback reared in freshwater about for a month are similar to the gland of *Poecilia formosa* which are given a pituitary transplant (Ball et al., 1965). In stickleback inhabiting natural environment, the thyroid activity may be heightened when the stickleback take their departure to the sea. Migrants retained in freshwater or landlocked animals demonstrate an increased activity or hyperplasia of the thyroid (Hoar and Bell, 1950; Hoar, 1952). The thyroid glands were highly active in sticklebacks kept in freshwater for a month. However it is obscure that the change in thyroid activity in the juvenile and yound sticklebacks may be related to their physiological adjustment to ambient water.

There is a question whether the excessively enlarged thyroid may be goitrous or not. Thyroid goiter and very marked hyperplasia are common in artificially reared fish. These changes may be associated with a lack of iodine (Gaylord and Marsh, 1912; Mawdesley-Thomas, 1972; Marine and Lenhart, 1910a, b, 1911b; Reichenbach-Klinke and Elkan, 1965; Robertson and Chaney, 1953; Wellings, 1969). When the sticklebacks are transferred into sea

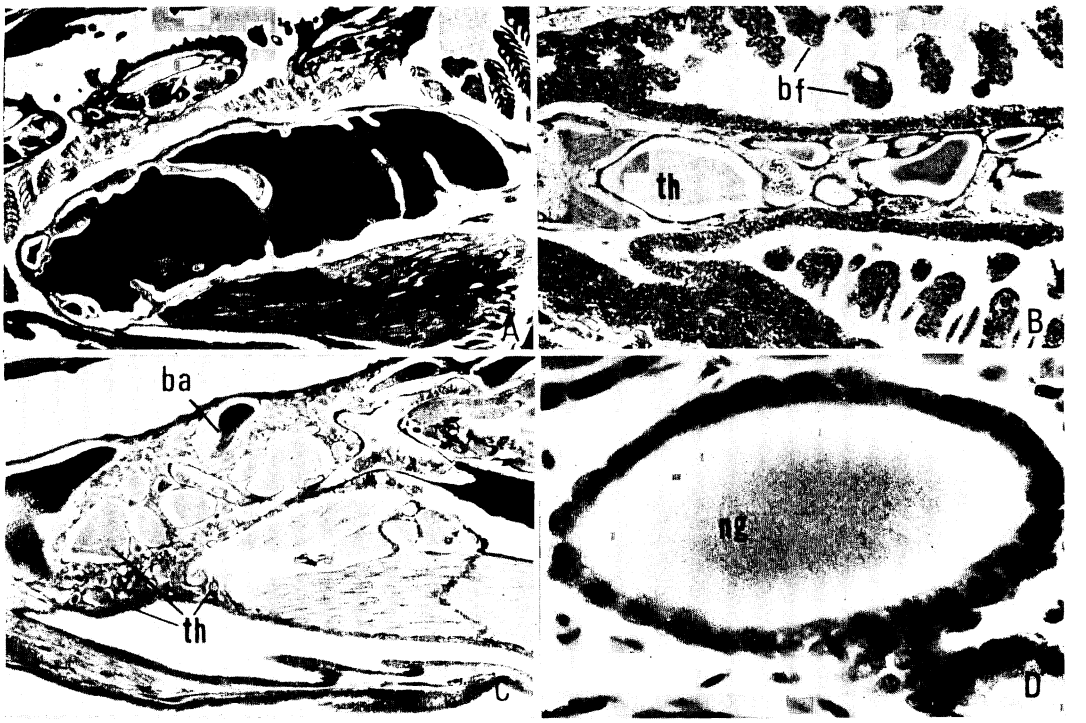

Fig. 5. A. Sagittal section of the thyroid region of a stickleback kept for 110 days. Total length 23.7 mm, female. Mallory's triple stain. ×35. B. Frontal section of the thyroid region of a stickleback kept for 63 days in sea water after rearing for 47 days in freshwater. Total length 24.6 mm, male. Hematoxylin and eosin. ×86. C. Sagittal section of the thyroid region of a stickleback kept for 63 days in sea water after rearing for 47 days in freshwater. Total length 24.0 mm, male. Hematoxylin and eosin. ×35. D. Higher magnification of C. ×435. ba, branchial arch; bf, gill filament; ng, slightly granulated colloid; th, thyroid follicle.

water, a involution of the enlarged follicle occurs. This fact suggests that the thyroid of the stickleback reared in freshwater is an endemic goitrous change. The involution of the thyroid may be associated with sea water containing about 45 to 60 microgams of iodine per liter. In the colloid goiter found in the domesticated adult Scotch sea trout, the alveoli are greatly increased in amount and in size. The epithelial cells are very flattened and the lumina are compactly filled out with large masses of colloid (Gaylord and Marsh, 1912). These changes are similar to the present results obtained from the stickleback.

In the thyroid of the stickleback kept for 110 days in freshwater, the nucleus is located on the apex of the columnar epithelial cell and the nucleus-like bodies are found in the thyroid colloid. It was described above that the follicles of the stickleback kept in freshwater for a long period, are united together one to another, and the nucleus of the epithelial cell seems to be moving toward the free surface. According to Gorbman and Bern (1962), it is possible that a holocrine secretion occurs in the thyroid. Marine and Lenhart (1911a) reported that the lumen of the colloid goitrous thyroid of the brook trout contains large number of leucocytes, shed epithelial cells and granular albuminous debris. Nucleus-like bodies and colloid-like droplets found in the colloid of the thyroid of the stickleback may be indicative of holocrine secretion and the disintegration of the epithelial cells, since the nucleus-like bodies show clearly positive reaction to Feulgen's staining and are nearly

identical with the nucleus of epithelial cells in shape and in size. From the facts indicating of the protrusion of the nucleus from the cell, it is likely that the nucleus-like body is derived from the protrusion of the thyroid epithelial cell containing the nucleus.

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淡水中で飼育したイトヨ幼魚における甲状腺沪胞の拡大

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海で採捕した雌雄一対のイトヨを実験室で飼育産卵させた．ふ化した仔魚を用い甲状腺組織像の変化を観察した．ふ化後約一カ月で甲状腺組織像は高い分泌活動を示す．魚体は皮ふにグアニンを沈着し銀白色となり，海水に適応するようになる．さらに淡水中で飼育を続けると沪胞は拡大し，沪胞にはさまれた結合織は毛細血管をともなった薄い層となる．沪胞腔は顆粒状コロイドでみたされる．コロイド中には沪胞上皮細胞の全分泌を示すと思われる核およびコロイド様小滴がみられる．イトヨを海水へ移すと淡水中の飼育によって異常に拡大した甲状腺沪胞は正常な組織像を示すようになる．

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