Changes in the Cells of the Adenohypophysis Associated with the Diadromous Migration of the Threespine Stickleback, *Gasterosteus aculeatus* L.*

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Received September 17, 1975

Summary. The adenohypophysis of the threespine stickleback, *Gasterosteus aculeatus*, was studied light-microscopically to determine and estimate the cell types and their function. For these purposes, the adult specimens obtained during the period from migration to spawning were examined. Further, the juveniles caught in the spawning bed were subjected artificially to sea water.

The rostral pars distalis (RPD) consists mainly of two types of cells: dorsally shifted lead hematoxylin (PbH)-positive cells bordering the neurohypophysis correspond to corticotrophs, and antero-ventrally shifted acidophil cells are identified as prolactin cells. The latter undergo marked hypertrophy and active state just at the time of entering the river (February), while no detectable change was seen in the former throughout anadromous migration. The role of prolactin on the osmoregulation in freshwater environment is thus suggested.

The proximal pars distalis (PPD) consists mainly of two cell types: the basophil cells in round shape are regarded as the gonadotrophs and the acidophil cells in ellipsoid shape are considered to be somatotrophs. The size of the gonadotrophs reaches the maximum at the time of spawning. A few AP-positive cells of elongate shape occur in the dorsal region and are identified as thyrotrophs.

In the pars intermedia (PI), two types of cells are discernible: PAS-positive and PAS-negative cells. The latter attained their maximal size in the earliest time of anadromous migration.

Recent progress of piscine endocrinology revealed that the pituitary gland of several teleost fish is concerned with osmoregulation, and the prolactin and ACTH, both elaborated in the cells of the rostral pars distalis (RPD), play an important role under freshwater and/or salt water adaptation (Ball, 1969a, b; Oliverneau and Ball, 1970; Lam, 1972; Ichikawa et al., 1973; etc.). In the green mollies, *Poecilia latipinna*, Ball and Ingleton (1973) demonstrated that the pituitary prolactin content is 6 times greater in freshwater adapted fish than in sea water, and prolactin cells are larger, more numerous and more active in fresh water than in sea water. On the other hand, only the nuclei of the ACTH cells increased in size when the larval guppy, *Lebistes reticulatus*, were immersed in 1/3 sea water.

Using anadromous fish (*Plecopterus altivelis*) and coastal puffer (*Fugu niphobles*), we demonstrated that one of the cells of RPD, probably the prolactin cells, indicated a drastic change during the upstream migration or the freshwater adaptation (Chiba and Honma, 1973; Honma and Yoshie, 1974).

* Contributions from the Sado Marine Biological Station, Niigata University, No. 251. This work was partly supported by a Grant-in-Aid for Fundamental Scientific Research for the Ministry of Education (830405).

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The identification and seasonal and experimentally induced changes in the adeno-hypophysial cells of both freshwater and marine forms of the European and North American threespine stickleback were investigated thoroughly by light and electron microscopy (Leatherland, 1970a, b; Benjamin, 1974a, b; Benjamin and Ireland, 1974). Immunocytological demonstration of the MSH-producing cell was also achieved in the mature specimen of threespine stickleback (Follenius and Dubois, 1974).

In Niigata facing the Japan Sea, marine trachurus form of the stickleback ascends the river to spawn, and the inhabitants near the river prepare a small gill net for stickleback in reedy fields. After breeding all the spent fish die near the spawning beds by early summer.

In order to learn a difference between the Japanese form and European and North American ones, histological observations were undertaken using adult upstream migrants and juvenile downstream migrants.

Material and Methods

Forty-nine adult stickleback, Gasterosteus aculeatus (L.), used in this study were collected by hand net and rod from the rivers of Niigata City, such as the River Agano and Sekiya-Bunsujo canal in the lower reaches of the River Shinano, Lake Kamoko, a brackish lake in Sado Island, and also off the coast of the Sado Marine Biological Station of Niigata University located on the west coast of this island in the Japan Sea. The period of collection lasted from the commencement of upstream migration (February) to the end of spawning (June) in 1973.

Forty juvenile fish before downstream migration were obtained by hand net at the spawning bed of the River Agano during the period from late May to late June, 1973, while an additional 44 juveniles artificially propagated and reared in a glass trough were also used by courtesy of Mr. Norio Kojima, an amateur aquarist in Niigata City.

To learn the possible function of the prolactin and ACTH cells and tolerance of the fish in varying salinity, the following experimental procedure was performed. Several adult fish caught from the sea were kept in 1/2 dilute sea water for 3 days, and then transferred to fresh water for 10 days at the aquarium of the Sado Marine Biological Station. On the other hand, the juvenile fish caught near the spawning bed were kept in 1/2 dilute sea water for 3 days, and then transferred to sea water. After this acclimation, the juveniles were killed at the intervals of 10 days, 23 days and 38 days, each of which time includes 3 to 6 individuals.

After decapitation the brain with the hypophysis of the adult fish was removed, and immersed in Bouin-Holland-sublimate solution for 2 days, while the entire head of the juvenile fish was immersed in the same fixing solution for 2 days, and then decalcified with a solution consisting of 5% formic acid in 5% formol for one day. These materials were dehydrated, embedded in paraplast, cut serially 5 to 6 μ thick in sagittal and transverse directions, and stained by various stainings, such as azan trichrome, aldehyde fuchsin (AF)-azan, AF-light green and orange G, periodic acid Schiff (PAS)-light green and orange G, PAS-alcian blue and orange G recommended by El Etreby et al. (1973), PAS-alcian blue-azocarmine and orange G, PAS-lead hematoxylin (PbH) and orange G recommended by Solcia et al. (1969), and PbH-light green and orange G.
To estimate the rate of activity of the glandular cells in the adenohypophysis, the cell index (CI)—(maximum cell length + maximum cell width)/2—of ten cells of each cell type measured and mean grid count (GC)—number of cell nuclei/mm² × 10⁻³—in 40 different regions of the RPD containing the prolactin cells were adopted after Leatherland (1970a).

Fig. 1. Paramedian section of the hypophysis of an adult stickleback, Gasterosteus aculeatus, caught in the mouth of Shin-kawa River, on April 8, 1972. It is easy to distinguish four components of the hypophysis: RPD (rostral pars distalis), PPD (proximal pars distalis), PI (pars intermedia) and N (neurohypophysis). Anterior to the right. Azan stain. ×100

Fig. 2. Part of the RPD to show the palisadal PbH positive cells corresponding to the ACTH (corticotroph, epsilon) cells at the commencement of upstream migration in February. PAS-PbH and orange G stain. ×330
Fig. 3. Ventral part of the RPD occupied chiefly by the layers of acidophil cells corresponding to the prolactin cells (eta cells) \( (P) \). Note the cells of this marine specimen have rich granules stained intensely by azocarmine. The light cells in this picture are the GSH (gonadotroph) \( (G) \) cells of PPD. AF-azan stain. \( \times 330 \)

Fig. 4. Prolactin cells of the specimen caught in the river in April. A decrease in the affinity with dyes and a slight indication of degranulation are seen. AF-azan stain. \( \times 330 \)

Fig. 5. Prolactin cells of the mature specimen caught in the spawning bed in May. Again, a considerable increase in the amount of acidophilic granules is noticed in this picture. AF-azan stain. \( \times 330 \)
Results

The general structural pattern of the hypophysis of the threespine stickleback, form trachurus and form leiurus, have already been described thoroughly by Leatherland (1970a, b) and Benjamin (1974a, b, c), respectively, and the present examination confirmed their description (Fig. 1).

Rostral pars distalis (RPD)

The RPD covering the antero-ventral region of the principal pars distalis (PPD) is a thin layer of glandular cells consisting of two cell types. In general, the posterior end of RPD reaches one third the length of the hypophysis, but it extends one half or more in the fully mature fish (Fig. 1). In the most dorsal part of RPD bordering the anterior projection of neurohypophysis, the palisadal PbH positive cells are found from a single to two-cell layers (Fig. 2). These cells may correspond to the adrenocorticotrophs, i.e., ACTH (=epsilon) cell. However, no change was demonstrated in the affinity of cytoplasm for dyes associated with upstream migration. As shown in Figure 16 for the CI data, it is difficult to detect the significant difference among the fish, such as the earliest migrants in February, active migrants in April and the mature adult individuals in the spawning bed.

The peripheral region of RPD is occupied exclusively with the layers of acidophil cells, i.e., prolactin (=eta) cells, almost round in shape. According to the developmental stages, this layer, better called sheath, extends more posteriorly toward the pars intermedia (PI) through the ventral surface of the PPD. The cell of the marine adult specimen with immature and/or maturing gonads has the rich granular cytoplasm stained strongly with azocarmine (Fig. 3). In the cell of the specimen caught in April just at the time of the upstream migration a slight indication of degranulation is seen, and a decrease in the affinity with dyes is noticed (Fig. 4). The cell of this stage, oval to long ovoid in shape, has a marginally shifted nucleus, and the size of the cell increases considerably as compared with the marine specimen. In the specimen caught in the spawning bed in May, the rate of staining affinity and the amount of cytoplasmic granules increases again to some extent. Most of the cells are rounded in shape (Fig. 5).

The prolactin cell GC of freshwater fish is significantly smaller when compared with marine fish (p<0.01) (Fig. 16). Among the specimens in fresh water throughout the period from upstream migration to postspawning, the GC is the smallest in the specimen of February just in the commencement of upstream migration, while the greatest is in the marine specimen. However, there is no significant difference between the cytologic figures and GC of the fish transferred from sea water to fresh water for 10 days and the fish caught in sea water (Fig. 16).

The prolactin cell of the juvenile fish is smaller in size and the staining affinity of the cytoplasm for dyes is weaker than in adult fish (Fig. 6-9). In the juvenile fish transferred to sea water for 10 days, no appreciable difference is detected in the zonal area of the prolactin cells when compared with freshwater fish (Fig. 6, 7). However, it is remarkably reduced in the juvenile fish kept in sea water for 23 days until late June: the zone consists only of one to two cell layers (Fig. 8, 9).
Fig. 6. Prolactin cells (P) of the juvenile fish caught in the spawning bed in early June. The affinity for acidic dye is still weak in this stage. AF-azan stain. ×330

Fig. 7. Prolactin cells of juvenile fish transferred to sea water for 10 days. Not so appreciable change is detected by this short treatment. AF-azan stain. ×330

Fig. 8. Prolactin cells of juvenile fish transferred to sea water for 23 days. Now, the cell size and the cell layers are remarkably reduced, and increase in the affinity for acid dye resulted. AF-azan stain. ×330

Fig. 9. Prolactin cells of juvenile fish caught in the spawning bed in late June. A marked difference is noticed when compared with the specimen of the same developmental stage shown in Figure. 8 AF-azan stain. ×330
Fig. 10. Dorsal part of the landlocked specimen caught in Aizu District to show the small basophil cells facing the neural elements. These cells may correspond to the TSH (thyrotroph) cells (arrows). PAS-alcian blue and orange G stain. ×330

Fig. 11. Part of the PPD of the marine specimen showing roughly the dorsally shifted orangenophil cells corresponding to STH (somatotroph) cells (S) and the ventrally shifted basophil cells, i.e., GSH (gonadotroph) cells (G). PAS-light green and orange G stain. ×120

Fig. 12. Part of the PPD of the mature specimen caught in the spawning bed. Degranular and degenerative change are noticed in the GSH cells (G). PAS-alcian blue and orang G stain. ×120
Fig. 13. Enlarged view of the degenerative change of GSH cells. Dilation of the cell size, degranulated chromophobes and distortion of nuclear shape are noticed in this picture. PAS-alcian blue and orange G stain. ×330

Fig. 14. Part of the PPD of juvenile fish consisted mainly of acidophil STH cells (S). PAS-alcian blue and orange G stain. ×330

Fig. 15. Part of the PI of mature fish to show two types of cells, such as PAS-positive (type 1) (arrows) and PAS-negative (type 2) ones. PAS-alcian blue and orange G stain. ×330
Proximal pars distalis (PPD)

Three types of cells are discernible in the PPD by their shapes and tinctorial responses. The dorsal region facing the neurohypophysis contains a small number of smaller basophils, somewhat elongate in shape. The cell is demonstrated as AF, alcian blue and PAS positive, but it is considerably difficult to discriminate this cell from the gonadotroph without experimental procedure, such as thiourea treatment. On the contrary, a mass of well developed thyrotrophs is seen in the landlocked trachurus form collected in the Aizu District adjacent to the Niigata District (Fig. 10). More detailed information on this aspect in relation to thyroid activities will be reported elsewhere.

The PPD is mainly occupied by two types of cells intermingled with each other. The orangenophil (acidophil) cells in ovoid or ellipsoid shape show no noticeable

![Figure 16](image-url)

**Fig. 16.** Histogram to show seasonal changes in the glandular cells of pars distalis. Left two are the rostral components of ACTH (epsilon) and prolactin (eta) cells. Right two are the proximal components of STH (acidophil) and GSH (basophil) cells. These are indicated as cell index (CI) and grid count (GC). See the text page 3. F February, A April, M May. Blank bar: sea water specimen, bar with oblique line: fresh water specimen.
change in the staining affinity and cell size during the course of upstream migration. However, in the specimen from the breeding place, a comparatively large number of these acidophils are inclined to occupy a rather dorsal region of the PPD near the neurohypophysis, and are gathered to form cell islands (Fig. 11, 12).

The round basophils as the third component are stained deeply with AF, alcian blue and PAS, and are located chiefly in the ventral region of the PPD of adult fish, although a considerable number of the cells immigrated into the dorsal region interspersing among the acidophils (Fig. 13). The size of the cell and the accumulation of the granules gradually increased month by month in accordance with the maturation of the gonads, and reached the maximum in the specimen from the spawning bed ($P<0.01$) (Fig. 16). During and after the spawning season, chromophobes with enlarged nuclei are increased in number as a process of degranulation, and several mitotic figures are still encountered in the specimen of mature fish. Following this change, degenerative pictures, such as the dilation of the cell, distortion and further disappearance of the nucleus, are detected (Fig. 13).

In addition, in the PPD of juvenile fish, no basophils are demonstrated, while the chromophobes are seen (Fig. 14).

**Pars intermedia (PI)**

Using a combination of PAS-PbH with a counter stain, such as light green and orange G, two types of cells are identified without difficulty in the PI: the PAS positive (but PbH negative) cell round in shape scattered singly (type 1) and the remaining PAS negative (PbH positive) cell (type 2) (Fig. 15). By AF-azan triple stain, these are differentiated as AF-weak positive and faint orangenophil cells. During the migratory period, no detectable changes were recognized in the cell size and affinity for dyes in the PAS positive cell, whereas a remarkable increase in the size of the PAS negative cell was seen in the migratory freshwater fish, in particular, in the earliest migrants, than in the sea water fish (Fig. 17). There are also found two types of cells in the PI of juvenile fish. However, a significant difference was not noted in the number of cell and nucleus size between the juvenile fish transferred to sea water for 10 days and the fish kept in fresh water.
Discussion

The pituitary cytology of ichthyoform animals has extensively been documented and reviewed by Ball (1969b), Ball and Baker (1969) and Holmes and Ball (1974). By both light and electron microscopy seven to eight cell types have been discernible in the adenohypophysis of the teleosts. The pituitary gland of both marine and freshwater threespine stickleback has also been investigated thoroughly by Leatherland (1970a, b), Benjamin (1974a, b), Benjamin and Ireland (1974) and Follenius and Dubois (1974), and the assumed prolactin secretory cell showed pronounced changes at different stages of the migratory cycle of the marine type (Leatherland, 1970a). Nearly identical changes associated with seasons were reported in the freshwater type (Benjamin, 1974b). In the present examination of the Japanese marine type, it was evident that the prolactin cell may play some part in osmoregulation in freshwater environment. The size of the cell became greatest in the earliest migrant in February and a further decrease occurred gradually toward the spawning period. A gradual increase in the amount of acidophil granules of the prolactin cell also occurred toward the spawning period after decrease at the time of entering the river. Moreover, it seemed probable that it takes more than ten days to reveal any histological evidence in response to the environmental change of salinity or osmosis. We have already reported that for histological response of prolactin cells to environmental salinity a considerably long time may be required in the cases of puffer and Ayu (Chiba and Honma, 1973; Honma and Yoshie, 1974). Therefore, it may be assumed that the changes in synthesis and storage of granules to be expected from the histological data might be too slow in rate for such an acute release of secretory granules as required on the occasion of upstream migration.

Leatherland (1970a) stated that the ACTH cells may be involved in anadromous migration, even though changes in histology and CI were less marked than in the prolactin cells. The present examination revealed no pronounced change throughout the materials obtained. Accordingly, it is difficult to know whether or not the ACTH cells may play some part in osmoregulation accompanying anadromous migration, since no information was secured about the mode of life of still immature fish that lived in the offing of the Japan Sea.

The basophils in the ventral region of the PPD of adult threespine stickleback seemed to be differentiated from the chromophobes in the juvenile fish. The CI increases gradually in accordance with maturation of the gonads, and further degradation of the cell is seen in the spawning fish. Therefore, the basophils are presumed to correspond to the gonadotrophic cells.

Although Leatherland (1970a) described the possible thyrotrophs in elongate form stained intensely with alcian blue, PAS and aniline blue, the present examination does support this situation. By thiourea treatment, a considerable number of basophilic cells in the dorsal region of the PPD bordering the neurohypophysis are highly stimulated indicating the dilation of cell size and degranulation of cytoplasm. A rather large number of basophils in ellipsoid or wedge shape were demonstrated in the case of the Japanese landlocked form caught in the breeding season. Therefore, we consider that the cells in question may correspond to the thyrotrophs. By electron microscopy, Cook and Van Overbeeke (1972) discussed that the chromophobes...
in the pars distalis of the sockeye salmon are probably thyrotrophs, though previous light microscopy has failed to demonstrate this type of cell in the same species (Van Overbeeke and Mc Bride, 1967; Mc Bride and Van Overbeeke, 1969). Recently, Mc Bride and Van Overbeeke (1975) revealed a marked activation of the thyroid associated with activation of PAS-positive cells in the dorso-caudal area of the PPD of gonadectomized sockeye salmon, and they considered that these cells might be thyrotrophs. To clarify this problem, further investigations should be carried out in combination with experimental procedures.

The PPD of the juvenile stickleback was occupied chiefly by acidophils, most of which were diagnosed as somatotrophs. Using larval guppy, Ichikawa et al. (1973) observed that the nuclei of both somatotrophs and ACTH cells were enlarged when the fish removed from the mother were transferred into fresh water. Therefore, they suggest that the hormones elaborated in these cells might be required in the process of adaptation to an osmotically new environment. In the present investigation, on the other hand, no marked changes were noticed in the histological picture and CI during the course of upstream migration. The acidophil regarded as somatotroph in the PPD of sockeye salmon seemed to have undergone little or no change during the course of migration and spawning (Van Overbeeke and Mc Bride, 1967). Accordingly, whether or not the somatotropin is involved in the osmoregulation is the problem remaining to be solved.

The fact that the CI of the PAS-negative (type 2) cells in the PI of freshwater stickleback, especially the fish caught in the commencement of migration, are larger than in the marine one seemed to be concerned also in the process of osmoregulation. However, these cells were few in number in the juvenile stage of stickleback, especially in freshwater juveniles, while the PAS-positive (type 1) cells were more numerous (Leatherland, 1970b). Therefore, he considered that the type 2 cells are involved in some aspect of spawning, although the detailed mechanism is still obscure. In the present transfer experiment, there is no significant difference between the proportion of the number of PAS-negative cells to PAS-positive ones and also the nucleus size of the juvenile fish kept in sea water and in the fish in fresh water. Further studies are needed to clarify this problem.

両側回遊に伴なうイトヨの腺性下垂体の細胞にみられる変化

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新潟産イトヨの腺性下垂体の細胞型と、それらの機能を決定するために、遊河回遊の前後から放卵放精期までにわたる成魚の下垂体に現われる変化を観察した。また産卵床附近で捕えた降海前の幼魚についても、海水移行実験を試み、比較した。

端葉前部は、神経部に接する鈍ヘマトキシリン好性の間胚組織刺激細胞と、周縁部に並ぶ酸好性のプロラクトシン産生細胞からなる。後者は成魚、幼魚ともに、海水（塩）中で活動的であったので、淡水（塩）中の浸透圧調整に関与することが推定された。

端葉後部には3種の細胞が区別された。すなわち主として腹方に存在する大型円形の塩基好性の生殖腺刺激細胞、中央部に存在する桶円形で酸好性の生長ホルモン分泌細胞,
References


———: Seasonal variation in the structure and ultrastructure of the pituitary gland in the marine form (Trachurus) of the threespine stickleback, Gasterosteus aculeatus L. II. Proximal pars


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