MORPHOLOGICAL STUDIES ON THE PITUITARY OF THE CHUM SALMON, *ONCORHYNCHUS KETA*

(II) CHANGES OF THE PROLACTIN CELLS DURING THE LIFE-CYCLE

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It is well known that the so-called prolactin cells in the pro-adenohypophysis of some teleostean species are active in secretory function to adapt the fish to freshwater (see Ball and Baker, 1969). Ball (1965a) showed that the removal of part of the pro-adenohypophysis in *Poecilia latipinna* impaired the freshwater tolerance of the fish. Moreover, he (1965b) demonstrated that, in *P. latipinna*, ectopic pituitary transplants of the rostral part of the pituitary, composed mainly of the prolactin cells, secreted a hormone in response to the entrance of the grafted fish into freshwater, while the transplants of the posterior part of the gland did not react to the environmental change. These experimental findings strongly suggest that the prolactin cells of teleost fishes secrete a hormone, which is responsible for the adaptation of fishes to the freshwater environment.

As for the function of the prolactin cells in salmonid fishes, some reports have so far been published, mainly dealing with changes of the cells in association with anadromous migration of the fishes. In Atlantic salmon, *Salmo salar*, Olivereau (1954) reported an increased secretory activity of these cells of the fish during the period of their anadromous migration. On the other hand, in sockeye salmon, *Oncorhynchus nerka*, Cook and van Overbeeke (1969) indicated that these cells underwent only slight ultrastructural changes as the fish migrated from the ocean to the spawning grounds. The knowledge hitherto accumulated about the prolactin cells of salmonid fishes is limited to those of the fishes in the later stage of their life-cycle, and seems to be insufficient for obtaining an adequate understanding of physiological significance of these cells in salmonids. Thus, it was decided to investigate the changes of the prolactin cells of the chum salmon, *Oncorhynchus keta*, at different stages of the life-cycle of the fish by light and electron microscopes.

Before proceeding further, we wish to express our hearty thanks to Dr. Seizo Sano, Mr. Kazuhiko Nishino and Dr. Toyohiko Hikita, Hokkaido Salmon Hatchery, and to Messers. Shigeru Hara and Yoshio Ishikawa, the Tokachi Branch of Hokkaido Salmon Hatchery and Mr. Tasturo Kubo, Dr. Juro Yamada and Mr. Tsuneo Nishiyama, Hokkaido Univ. for their friendly collaboration in the collection of the present materials.

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Material and Methods

The chum salmon, *Oncorhynchus keta*, of various ages were used as materials. The fish of 2 months of age cultured in freshwater in the Nanae Fish-Culture Experimental Station, Hokkaido University, were sampled in May 1969, and about 2-year-old fish reared in the same pond were used in June 1968. Those just after entering the sea were obtained in the coastal sea off Mori, southern Hokkaido, in July 1969. Those in the period of feeding migration, which included both sexually immature and maturing fish, were collected in northern Pacific in July and August 1968. Those on the route of the anadromous migration were captured at three points in the Tokachi river, eastern Hokkaido, during the periods from September to November, 1966 and 1967: Atsunai, about 20 km distant from the mouth of the river; Otsu near the mouth of the river; Chiyoda about 45 km from the mouth of the river. In addition, some naturally spawned fish were obtained from the spawning beds in the Yakumo river, southern Hokkaido, in December 1967. Moreover, fish kept for several days after ovulation were collected from the stock raised in an outdoor pond of the Chitose Branch of the Hokkaido Salmon Hatchery in October 1966.

For light microscopic observations, pituitary glands were fixed for about 24 hours with Zenker-formol or Bouin's solution. Then they were cut 5–7 micra in thickness by the usual paraffin method, and stained with various techniques such as Heidenhain's azan, Halmi's aldehyde fuchsin-light green-orange G, and periodic acid-Shiff.

For electron microscopic observations, small pieces of the organs were usually immersed in Millonig's solution or Weber's solution for 2 hours. Some of them were also placed in 6.25% glutaraldehyde in 0.05 M phosphate buffer for 1 hour and then immersed in Millonig's solution for 2 hours. After fixation, the specimens were dehydrated by the quick cold ethanol method with two changes of propylene oxide, and embedded in Epon Epoxy resin mixture. Thin sections were cut with glass knives in a Porter-Blum microtome at a thickness of about 500 to 800Å, and stained with uranyl acetate and Reynolds' lead citrate, and examined with a Hitachi HS–7 electron microscope. Thick sections of about 1 micron for light microscopy were cut by the same method and stained with the method of Richardson *et al.* (1961).

Results

General morphology of the prolactin cells of the chum salmon

The pituitary gland of the adult chum salmon consists of four zones as already reported by the present writers (1970), i.e. pro-adenohypophysis, meso-adenohypophysis, meta-adenohypophysis and neurohypophysis. In midsagittal

sections of the gland, the pro-adenohypophysis occupies a large part of the anterior aspect of the gland. The component glandular cells of this zone can be divided into two cell types, i.e. prolactin cells and corticotrophs. The prolactin cells are easily distinguishable from the corticotrophs in their tinctorial properties and in the mode of cellular arrangements.

The prolactin cells are columnar in shape and are arranged in the form of follicles which have narrow lumina in the center. Some cilia and microvilli are seen projecting from the cells into the follicular lumen. They are stained with azocarmine G or light green but have no affinity to lead hematoxylin. The nucleus is situated on the opposite side of the follicular lumen and generally contains one or two acidophilic nucleoli. Under the electron microscope, the cells contain secretory granules measuring from 200 to 350 mμ in diameter, which are generally round in shape. A well developed Golgi apparatus appe ars distributed in the perinuclear region distal to the nucleus. A well developed rough-surfaced endoplasmic reticulum lies in the cytoplasm. Mitochondria of short or long rod in shape are found to be distributed sparsely throughout the cytoplasm. Moreover, the prolactin cells are in strict contact with each other by tight junctions and desmosomes.

The prolactin cells of juvenile chum salmon

The pituitary glands of 2-month-old fry are largely different in histological features from those of the adult fish. Although the gland of the fry is small and flat in shape and is closely contiguous to the ventral side of the brain floor, it is possible to discriminate the pro-, meso-, meta-adenohypophysis and neurohypophysis histologically (Figs. 1 and Fig. 2). The glands of the fry measure from 400 to 500 μ in the major axis and from 150 to 200 μ in the minor axis. Although the cells of the pro-adenohypophysis display a similar staining property to those of the other lobes, a follicular arrangement of the former cells make it possible to distinguish them from the latter (Fig. 1). The peripheral cytoplasm of the prolactin cells was stained weakly with azocarmine G. Under the electron microscope, many membrane-bound and, in general, highly electron dense secretory granules are recognized around the nucleus (Fig. 9). The granules, being 300 mμ in maximum diameter, are various in size and generally round but sometimes oval or elongated in shape. Slightly dilated, multilayered rough-surfaced endoplasmic reticulum is recognized around the nucleus. A well developed Golgi apparatus consisting of many vacuoles and several vesicles is distributed in the distal cytoplasm near the nucleus. The secretory granules at the beginning of formation occur sometimes in the sacs of the Golgi apparatus.

In specimens of approximately 1 month after entering the sea, the pituitary gland nearly doubles its dorso-ventral measurement, measuring from 500 to 600

μ in the major axis and from 300 to 400 μ in the minor axis. Owing to the multiplication and enlargement of component cells, follicles of the cells increase in number and size. Thus, the pro-adenohypophysis becomes much more prominent in histological features than that in the foregoing stage (Fig. 2). Although the basal cytoplasm around the nucleus increases the staining affinity to azocarmine G, the distal cytoplasm still remains unstainable with the dye. Materials stained positively with aldehyde fuchsin are recognized in the lumen of the follicles. Under the electron microscope, the secretory granules are slightly increased in number and are various in size (Fig. 10). The Golgi apparatus and the rough-surfaced endoplasmic reticulum do not remarkably change in features as compared with the specimens of the same age kept in fresh water.

The prolactin cells of adult fish kept for two years in fresh water successively after hatching

Unfortunately, no pituitary material of this group of fish was available for light microscopic studies. The prolactin cells contain numerous, highly electron dense granules of round or elongated shape. The multilayered rough-surfaced endoplasmic reticulum is well developed in the distal cytoplasm near the nucleus, being often arranged in concentrically organized whorls (Fig. 11). A well developed Golgi apparatus is also recognized near the nucleus, with repeated occurrence of immature granules in the Golgi lamellae.

The prolactin cells of adult fish caught in the northern Pacific

The observations of this group of fish were done only by light microscope. The fish of this group could be divided, based on the degree of development of the gonad, into sexually immature and maturing adults.

In sexually immature fish, the pro-adenohypophysis is increased considerably in volume along with the increase in number of the follicles of the prolactin cells as compared with that of the fry. The cytoplasm of the cells is generally stained weakly with azocarmine G (Fig. 3). The lumen of the follicles includes an amorphous material stained with aniline blue. Moreover, many mitotic figures of the cells can be observed in the follicles, this constituting the most prominent characteristic of the pro-adenohypophysis of the immature adults (Fig. 3).

In sexually maturing fish, the pro-adenohypophysis is observed to undergo an advanced change. The follicles of the prolactin cells are much more in number than those of the immature fish. The cells with the basal cytoplasm stained strongly with azocarmine G are increased in number and the distal cytoplasm of some cells become to be stained weakly with the same dye (Fig. 4). The nuclei do not change in shape but become to contain a few prominent nucleoli stained

strongly with azocarmine G. Mitotic figures are scarcely observable in the prolactin cells in this stage.

The prolactin cells of adult fish captured in the coastal sea

Mainly owing to a further development of the follicles of the prolactin cells, the pro-adenohypophysis reaches its largest size. The cells of the fish of this group are greatly different especially in their staining property from those of the sexually maturing fish caught in the northern Pacific. The cells are increased in size and their cytoplasm becomes mostly to be stained strongly with azocarmine G (Fig. 5). Under the electron microscope, the basal cytoplasm of the cells are found occupied by numerous, highly electron dense granules of round, oval or elongated shapes. Moreover, the most remarkable characteristic of the cells is a well developed rough-surfaced endoplasmic reticulum arranged in concentrically organized whorls (Fig. 12). The Golgi apparatus develops moderately, though it hardly contains secretory granules in the process of maturation. The distal cytoplasm is provided with a small number of granules, a slightly dilated rough-surfaced endoplasmic reticulum, and a moderate number of mitochondria of round, oval or elongated shapes.

The prolactin cells of adult fish captured at the mouth of river

The pro-adenohypophysis of the fish of this group seems to be similar in size to that of the fish obtained in the coastal sea, though the prolactin cells stained strongly with azocarmine G are larger in number in the former than those in the latter (Fig. 6). Under the electron microscope, the cells contain numerous, highly electron dense granules which are distributed throughout the cytoplasm (Fig. 13). No remarkable difference could be found in the size of the secretory granules between the cells of the fish of the river and those of the sea. The release of secretory granules by extrusion through the cell membrane could be hardly observed. The well developed rough-surfaced endoplasmic reticulum arranged in concentrically organized whorls could still be observed in the distal cytoplasm near the nucleus. No changes could be recognized in the ultrastructural aspect of the Golgi apparatus. Few newly formed secretory granules could be found in the Golgi apparatus.

The prolactin cells of fully matured or spent fish

Although the prolactin cells do not remarkably change in comparison with those of the specimens of the previous stage of the life-cycle (Fig. 7), some of the cells invade into the meso-adenohypophysis. The cytoplasm can be stained strongly with azocarmine G in the majority of the cells, while it notably lessens the staining affinity for the dye in some of them. In the spent fish, many cells show only

a weak affinity for azocarmine G. Occasionally, the cells begin to shrink (Fig. 8), and the nucleus is indefinite in shape and shows various stages of pyknotic changes. Under the electron microscope, little change could be seen in these cells. Although the cells contain many highly electron dense granules of relative uniformity in size throughout the cytoplasm, those which are devoid of secretory granules are sometimes observed in the follicles. A multilayered rough-surfaced endoplasmic reticulum is still observed to be situated around the nucleus (Fig. 14).

Discussion

It has recently become widely accepted that the prolactin cells of teleost fishes secrete a hormone which is essential for the adaptation of fishes to the freshwater condition (see Ball and Baker, 1969). As for the salmonid fishes, the presence of the prolactin cells have been shown by light microscopy (Woodman, 1939; Olivereau, 1954; Honma, 1960; Robertson and Wexler, 1962a, b; van Overbeeke and McBride, 1967) and also by electron microscopy (Follenius, 1963; Cook and van Overbeeke, 1969; Nagahama and Yamamoto, 1969, 1970). It is interesting to note that, in the case of immunohistochemical analysis of the sockeye pituitary gland, the eta cells are the only cells capable of binding antiovine prolactin (McKeown and van Overbeeke, 1969), as are the cases in the pituitary glands of *Poecilia latipinna* (Emmart *et al.*, 1966; Emmart and Mossakowski, 1967) and *Carassius auratus* (Emmart, 1969). Recently, Donaldson *et al.* (1968) showed that the injection of the salmon pituitary powder in hypophysectomized goldfish brought about the recovery of mean plasma osmolarity to the normal level. Thus, there seems little doubt that the prolactin cells of salmonid fishes also secrete a hormone which is essential for the adaptation of fishes to the freshwater environment.

It is certain that in some teleost fishes the prolactin cells become inactive in secretory function after the transfer of the fish from fresh water to sea water (Ball and Olivereau, 1964; Olivereau and Ball, 1964; Dharmamba and Nishioka, 1968). Moreover, Knowles and Vollrath (1966) indicated that, in the adult migratory eel, the activity of the prolactin cells decreased when the fish moved from the river into the sea. In the present study, the prolactin cells of seawater fry seem to become slightly intensified in staining affinity as compared with those of freshwater fry. However, it may be reasonable to assume that these changes are responsible for the development of the pituitary glands accompanied by the growth of the fish. Moreover, the prolactin cells of the freshwater fry were considerably undeveloped when compared with those of the adult fish. So far as we know, there is no investigation concerning the functional significance of the prolactin cells during the seaward migration of the chum salmon fry. Therefore,

we can not emphasize at the present time that the prolactin cells are responsible for the adaptation of the chum salmon fry to the freshwater condition.

On the other hand, it is well known that the prolactin cells of teleost fishes become active after the transfer of the fish from sea water to fresh water (Olivereau and Ball, 1964; Ball and Olivereau, 1964; Ball and Pickford, 1964; Dharmamba and Nishioka, 1968). Moreover, in Atlantic salmon (Olivereau, 1954) and in sockeye salmon (van Overbeeke and McBride, 1967), the increased secretory activity of the prolactin cells have been exhibited as soon as they enter the river. In the present study, the active features of secretory function of the cells became recognizable in sexually maturing fish caught in the coastal sea, became more prominent in the specimen caught at the mouth of the river and were maintained during anadromous migration. These findings strongly support the view that the prolactin cells play an important role in the adaptation of the anadromous chum salmon to the freshwater environment.

However, under experimental conditions, cytological changes were noticeable 24 hours after the transfer of the fish from sea water to fresh water (Ball and Olivereau, 1964). Moreover, in the medaka, *Oryzias latipes*, the abundance of granule formation in the Golgi apparatus and the frequent extrusion of the granules into the intercellular space were recognized 1–3 hours after the transfer of the fish from sea water to fresh water (Nagahama, 1970; unpublished). In the present study, however, the ultrastructure of the prolactin cells had already indicated an increased activity even in the fish from the coastal sea and did not undergo considerable change during the entrance of the fish into the river. Cook and van Overbeeke (1969) also reported that there was little, if any, structural change in the sockeye prolactin cells during migration. Thus, it is possible that there exists the preadaptation mechanism under the natural conditions of anadromous migration. In this respect, an interesting hypothesis has recently been demonstrated in the stickleback by Leatherland (1970). According to him, the activity of the prolactin cells "seems to be regulated by two factors: the first acting primarily by controlling hormone synthesis and causing the cells to increase in size and number to the condition of spring fish collected in the sea prior to migration (possibly controlled by photoperiod acting via the hypothalamus), and the second regulating hormone release (possibly governed by the salinity of the ambient environment)". We could not observe, however, the frequent discharge of the secretory granules from the prolactin cells of fish caught at the mouth of the river, though the accumulation of the secretory granules had obviously progressed in the fish caught in the coastal sea. Further additional investigations may be necessary to substantiate more firmly the dual control of the secretory function of the prolactin cells of the chum salmon pituitary.

Summary

Cytological changes of the prolactin cells of the pro-adenohypophysis at different stages of the life-cycle of the chum salmon, *Oncorhynchus keta*, were investigated by light and electron microscopes. During the seaward migration of the salmon fry, the prolactin cells were considerably undeveloped in cytological features when compared with those of the adult fish, though in the specimens caught in the sea the cells seemed to undergo slight changes accompanied by the growth of the fish. The functionally active features of the prolactin cells became recognizable in sexually maturing fish in the coastal sea: the cells were enlarged in size, and were found to contain numerous highly electron dense granules and a well developed rough-surfaced endoplasmic reticulum which was arranged in concentrically organized whorls. Moreover, their activity became more prominent in the specimens caught at the mouth of the river, and was maintained during the period of the anadromous migration. Accordingly, it is likely that the prolactin cells may play an important role in accomplishing the adaptation of the adult chum salmon to the freshwater condition in the course of anadromous migration. Moreover, the fact that the cytological active features of the cells have already been recognized before the fish enters into the river suggests the possible occurrence of a certain physiological change preparatory for the coming environmental alterations in the migratory fish.

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Explanation of Plates

PLATE I

Fig. 1 Sagittal section of pituitary gland of 2-month-old fry. Follicular arrangement of the prolactin cells (arrow) is observed. ×100

Fig. 2 Sagittal section of pituitary gland of fry just after entering the sea. Prolactin follicles (arrows) become conspicuous. ×100

Fig. 3 Prolactin cells of sexually immature fish caught in the northern Pacific. Mitotic figure (arrow) is found in a follicle. ×500

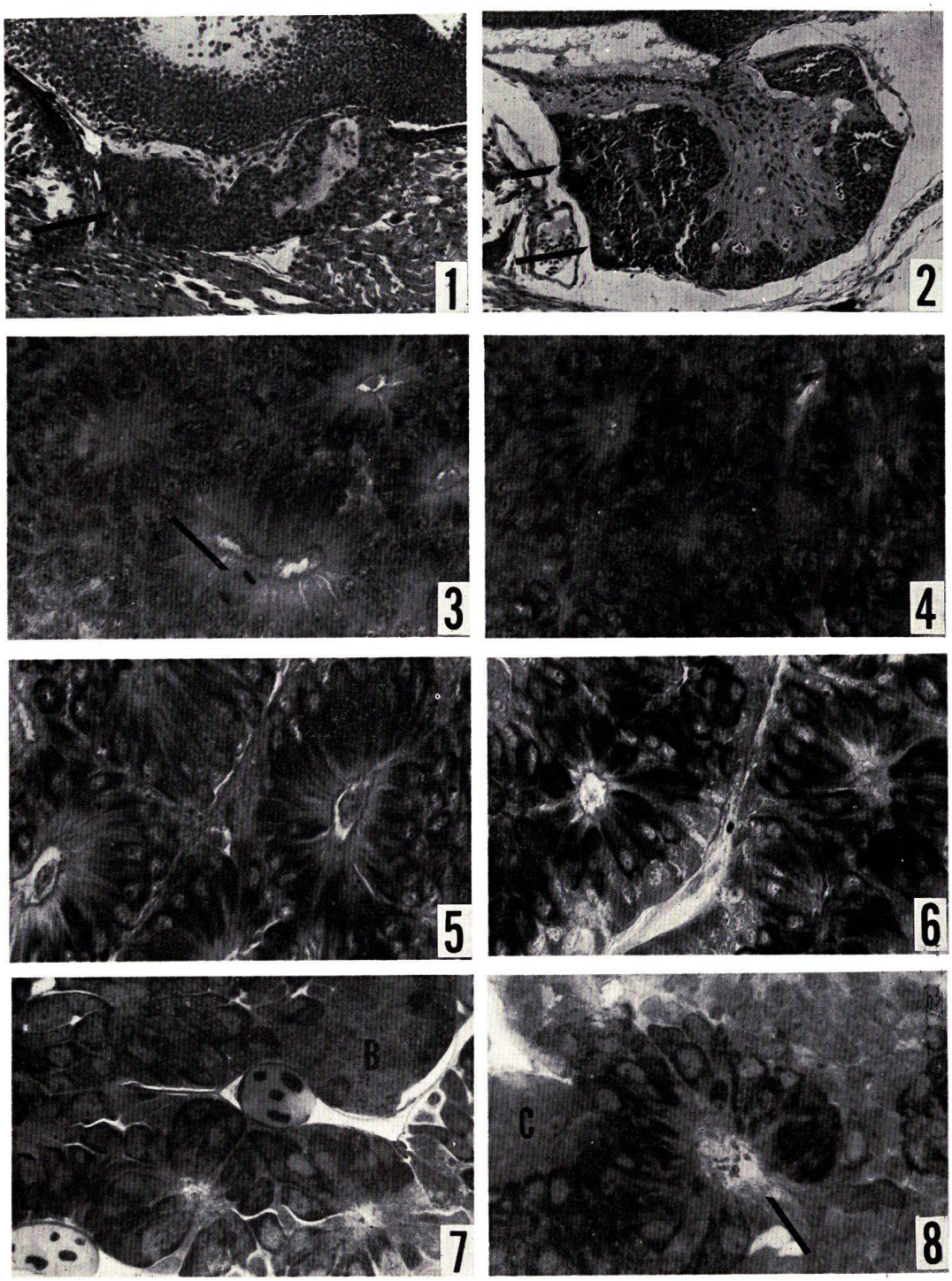
Fig. 4 Prolactin cells of sexually maturing fish in the northern Pacific. The cells are increased significantly in staining affinity when compared with sexually immature fish. ×500

Fig. 5 Prolactin cells of sexually maturing fish caught in the coastal sea. The cells are large and their cytoplasm are stained strongly with azocarmine G. ×500

Fig. 6 Prolactin cells of sexually maturing fish caught at the mouth of the river. The cytoplasm is stained strongly with azocarmine G. ×500

Fig. 7 Photomicrograph of 1 μ section of the pro-adenohypophysis of mature fish caught near the spawning ground. The prolactin cells are still stained with acid dyes. B, basophil. Azur 11-methylene blue. ×500

Fig. 8 Photomicrograph of 1 μ section of the pro-adenohypophysis of spent fish. Some prolactin cells (arrow) lost their staining affinity. C, corticotroph. ×500



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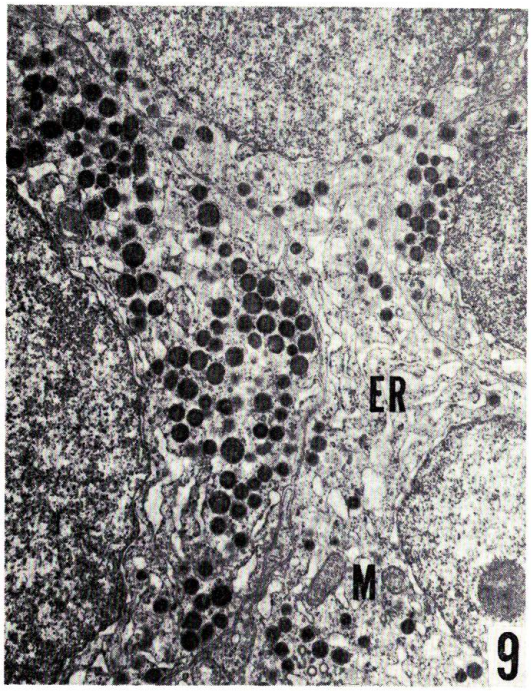

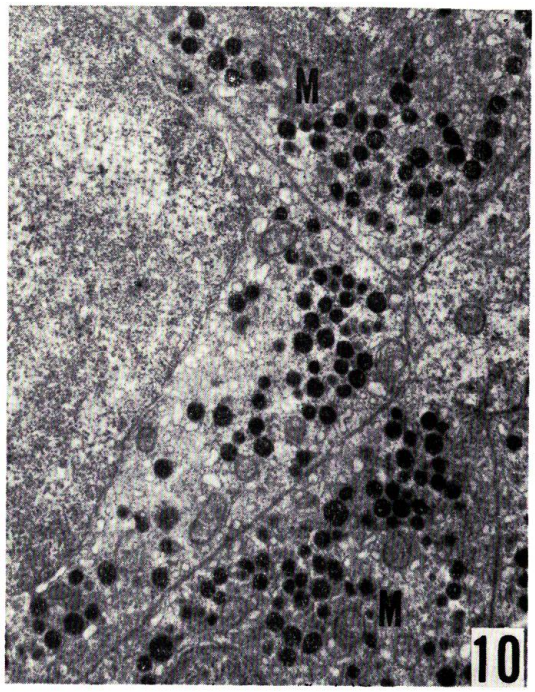

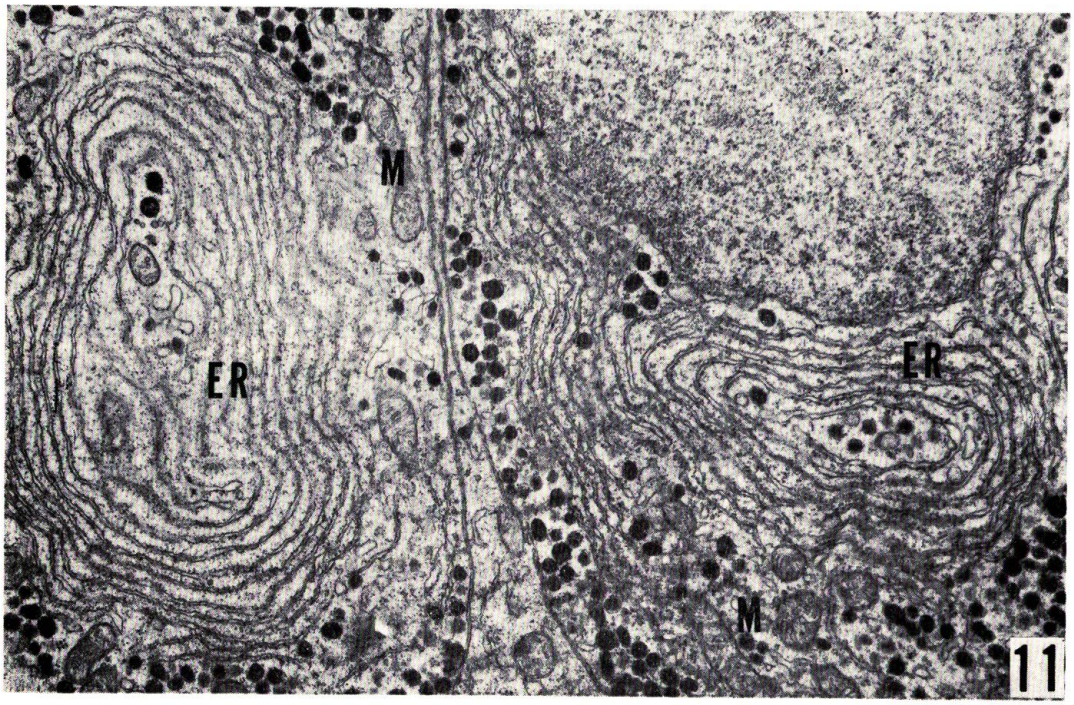

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PLATE II

Fig. 9 Prolactin cells of 2-month-old fry. The secretory granules are various in size and density. A little dilated endoplasmic reticulum (ER) is observed. M, mitochondrion. ×9,000

Fig. 10 Prolactin cells of fry just after entering the sea. The secretory granules are various in size and density. A little dilated endoplasmic reticulum (ER) is observed around the nucleus. M, mitochondrion. ×9,000

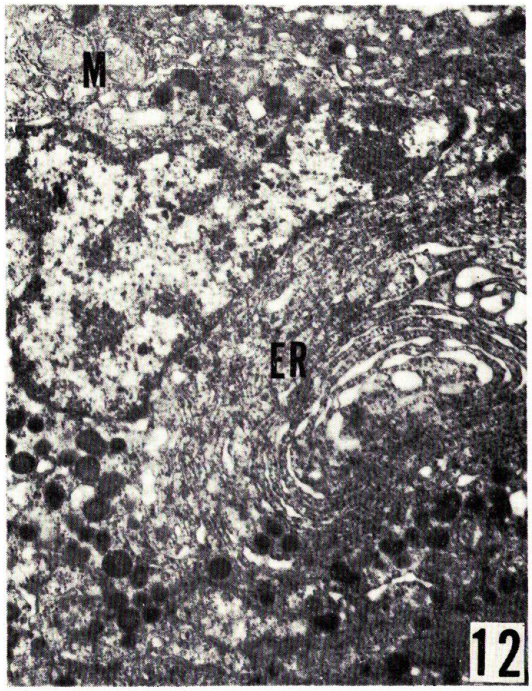
Fig. 11 Prolactin cells of sexually immature 2-year-old fish cultured in fresh water. Notice the well developed rough-surfaced endoplasmic reticulum (ER). M, mitochondrion. ×13,000

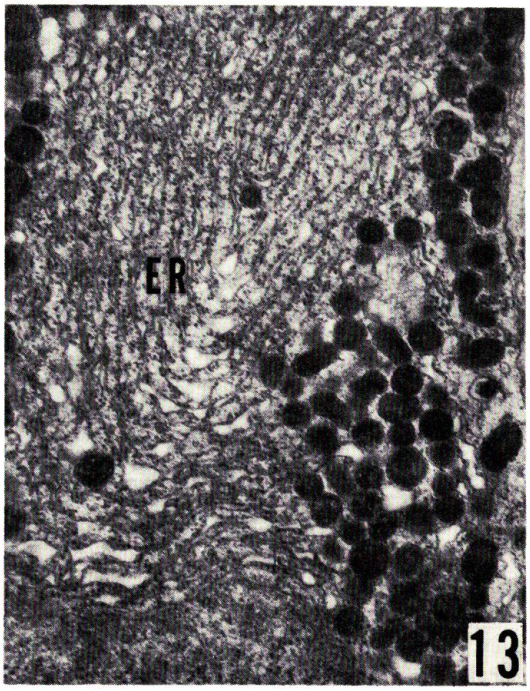
PLATE III

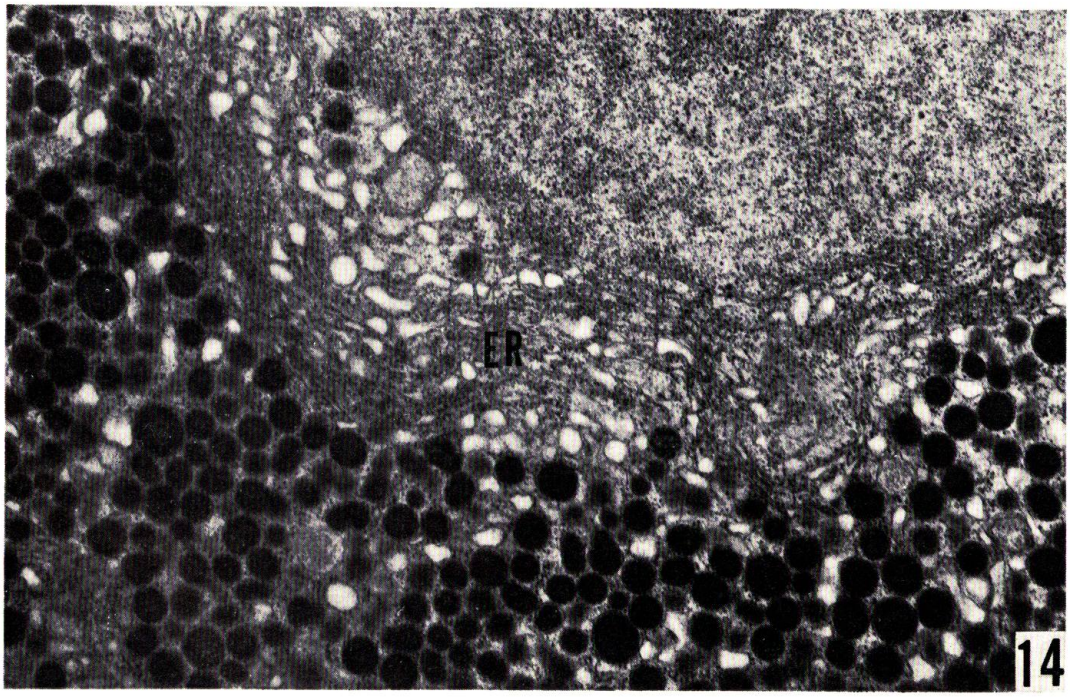
Fig. 12 Portion of a prolactin cell of sexually maturing fish caught in the coastal sea. Notice the well developed rough-surfaced endoplasmic reticulum (ER) arranged in concentrical whorls. M, mitochondrion. ×13,500

Fig. 13 Portion of a prolactin cell of sexually maturing fish caught at the mouth of the river. Notice the well developed rough-surfaced endoplasmic reticulum (ER). ×18,000

Fig. 14 Portion of a prolactin cell of sexually mature fish caught near the spawning ground. Notice the well developed endoplasmic reticulum showing lamellar arrangement (ER). ×18,000







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