Bull. Fac. Fish. Hokkaido Univ.
29(4), 299–307. 1978.

Changes of Pituitary Gonadotrophs Associated with Artificial Maturation in Silver Females of the Japanese Eel, *Anguilla japonica*

Hiroshi UEDA* and Hiroya TAKAHASHI*

Abstract

In the pituitary gland of silver females of the Japanese eel, *Anguilla japonica*, only one type of glandular cells was observed to be gonadotropic in nature. The gonadotrophs of the eels which were induced to mature by injections of salmon pituitaries showed a remarkable increase in size and in number as compared with those of untreated, immature females. The hypertrophied gonadotrophs revealed a prominent accumulation of secretory granules, which were increased also in size and in electron-density, an activated formation of granules, and a dilatation of cisternae of the rough endoplasmic reticulum. No evident sign of secretion was detectable in the hypertrophied gonadotrophs of maturing eels. However, in the cells of the eel which had ovulated spontaneously, a distinct degranulation and a marked dilatation of cisternae of the rough endoplasmic reticulum occurred extensively, suggesting a release of hormonal contents from the cells. In the eels which had failed to ovulate following complete maturation, changes of pituitary gonadotrophs were not so prominent and uniform as those in the cells of the ovulating eel, implying a possible disturbance of the mechanism implicated in pituitary gonadotropin release in these fish.

Previous researches in our laboratory have shown that, in silver females of the Japanese eel, *Anguilla japonica*, gonadal maturation leading to ovulation is induced effectively by repeated injections of salmon pituitaries. However, the treatment was not always successful in eliciting spontaneous ovulation in the fully matured eels, resulting in rapid overripening of ovarian eggs followed by a decline of their fertilizing and developing potencies in many cases.[1)]

In male Japanese eels, gonadotrophs of the pituitary gland become activated in parallel with the maturation caused by administrations of mammalian gonadotropins[2)]. Although similar findings have been presented in female European eels, *A. anguilla*, which are induced to mature by treatment with carp pituitary preparations[3,4)], no report has been concerned so far with changes of pituitary gonadotrophs in association with the induced maturation of female Japanese eel. Furthermore, it is interesting to examine whether or not any disturbance of endogenous gonadotropic function may be responsible for a failure of spontaneous ovulation in artificially matured female Japanese eels.

The present paper deals with ultrastructural observations on changes of pitutiary gonadotrophs of female Japanese eels subjected to the gonadotropic treatment, with special regard to those observed in the females which could not ovulate their eggs after complete maturation.

* *Laboratory of Fresh-Water Fish-Culture, Faculty of Fisheries, Hokkaido University*
(北海道大学水産学部淡水増殖学講座)

We are very grateful to Mr. Y. Sugimoto and other members of the laboratory for their collaboration in carrying out the experimental maturation of the eels used in the present study.

Material and Methods

Silver females of the Japanese eel, *Anguilla japonica*, used in the present study were captured in rivers of Aomori Prefecture in October 1976. After gradual acclimatization to sea water in the laboratory within a week after collection, they were kept in aquaria containing about 2.5 tons of filtered and aerated sea water at 18±1°C under natural light condition. They were not fed during the experiment.

Fourteen fish in total were examined in this study. Except for 2 fish which served as initial controls, they were induced to mature through weekly intramuscular injections of 2 mg powder of acetone-dried chum salmon pituitaries per 100 g body weight.

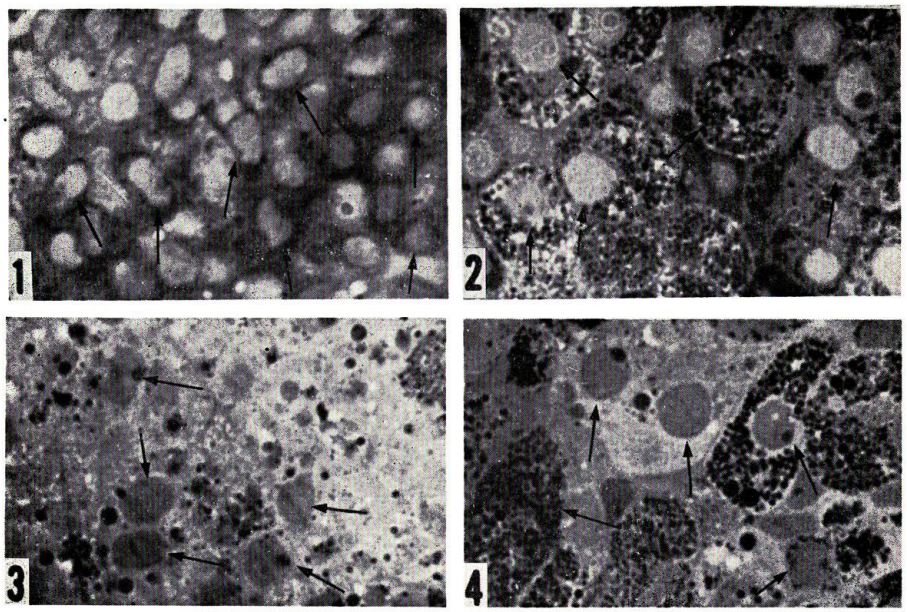
The fish were sacrificed at various times during the course of the treatment. Their pituitary glands were removed immediately after decapitation, and were immersed in 2.5% glutaraldehyde in 0.05M phosphate buffer (pH 7.4) followed by postfixation in Millonig's OsO_4 solution, each for 2 hours at 4°C. After dehydration through a graded ethanol series, the glands were embedded in Epon. Ultrathin sections were stained with uranyl acetate and Reynolds' lead citrate, and examined with a Hitachi HU-12 electron microscope. Parallel sections of the gland of about 1 μm thick were stained with methylene blue for light microscopy.

Results

The gonadosomatic index (GSI; ovarian weight×100/body weight) of the initial control fish was about 2%. In these fish, a small number of gonadotrophs were distributed exclusively in the proximal pars distalis of the pituitary gland. The cells were mostly oval or elongate in shape, and were characterized light-microscopically by having a few, darkly stained globules in a lightly stained cytoplasm (Fig. 1). The nucleus of the cells was large in size, oval or round in shape, with an indistinct nucleolus. Ultrastructurally, the cells contained many small granules, 100–200 nm in size, of varying electron-densities, and a few large globules, 500–800 nm in size, of low electron-density (Fig. 5). The rough endoplasmic reticulum of these cells was composed of flat or lamellar cisternae. The Golgi apparatus lying in the juxtanuclear region were rather a few in number and appeared to be moderately active. Mitochondria were generally of round or rod shape and were found throughout the cytoplasm.

Six fish were killed at some stages of artificially induced ovarian maturation. These fish had been injected 5 to 8 times with salmon pituitary powder, and ranged in GSI from about 10 to 40%. In these fish, gonadotrophs were observed to have drastically increased in number and in size with an evident increment in amount of their small granular contents (Fig. 2). The nucleus of the cells was also enlarged with a prominent nucleolus. Furthermore, a few of them were encountered intermingling with prolactin cells of a follicular arrangement in the rostral pars distalis of the pitutiary. In the gonadotropic cells of the fish with GSI

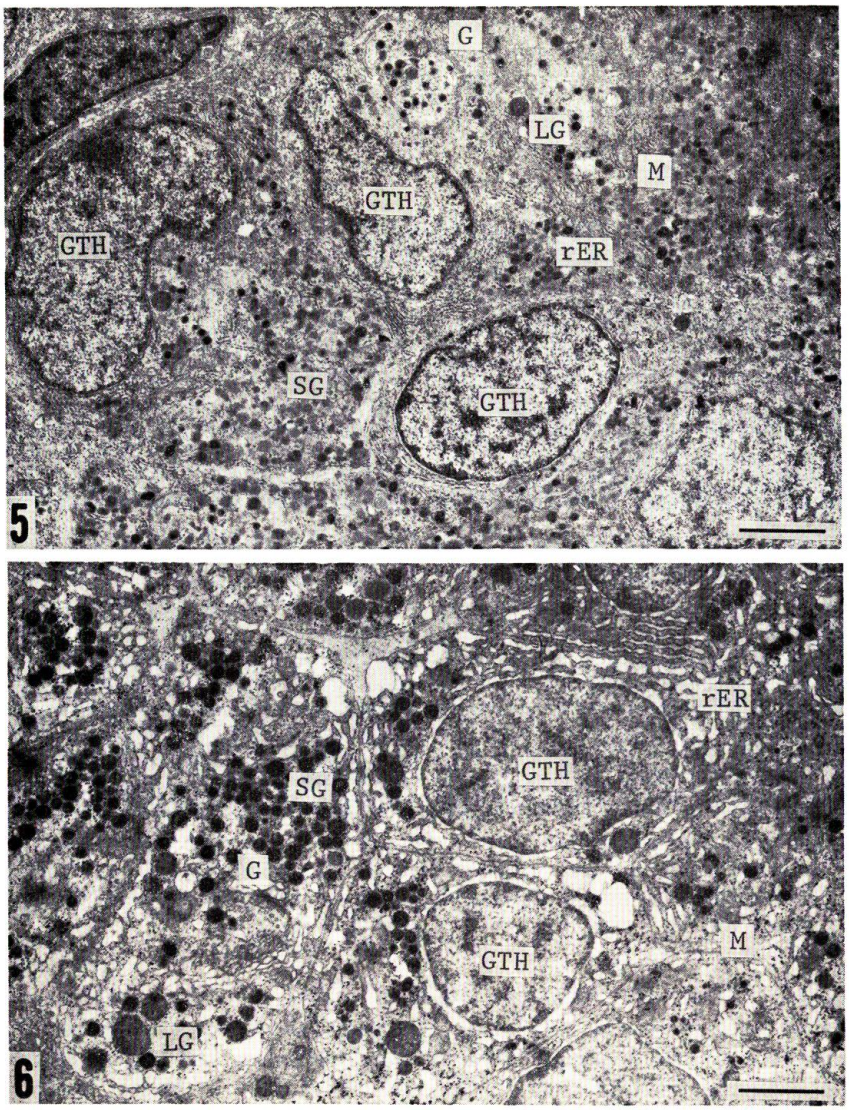
of 10–20%, the small granules were enlarged to 100–300 nm in size and showed an increase also in their electron-density (Fig. 6). Large globules in these cells also became larger in size, attaining 1000–1500 nm in diameter, and became more electron-dense. The cisternae of the rough endoplasmic reticulum were dilated widely throughout the cytoplasm.



Figs. 1–4. Light-microscopic pictures of Epon-embedded, methylene blue-stained sections of the pitutiary gland of an initial control (Fig. 1), an eel with GSI of 30% (Fig. 2), an eel ovulated spontaneously (Fig. 3), and an overripe eel with GSI of 60% (Fig. 4). Arrows indicate gonadotropic cells. × 1050.

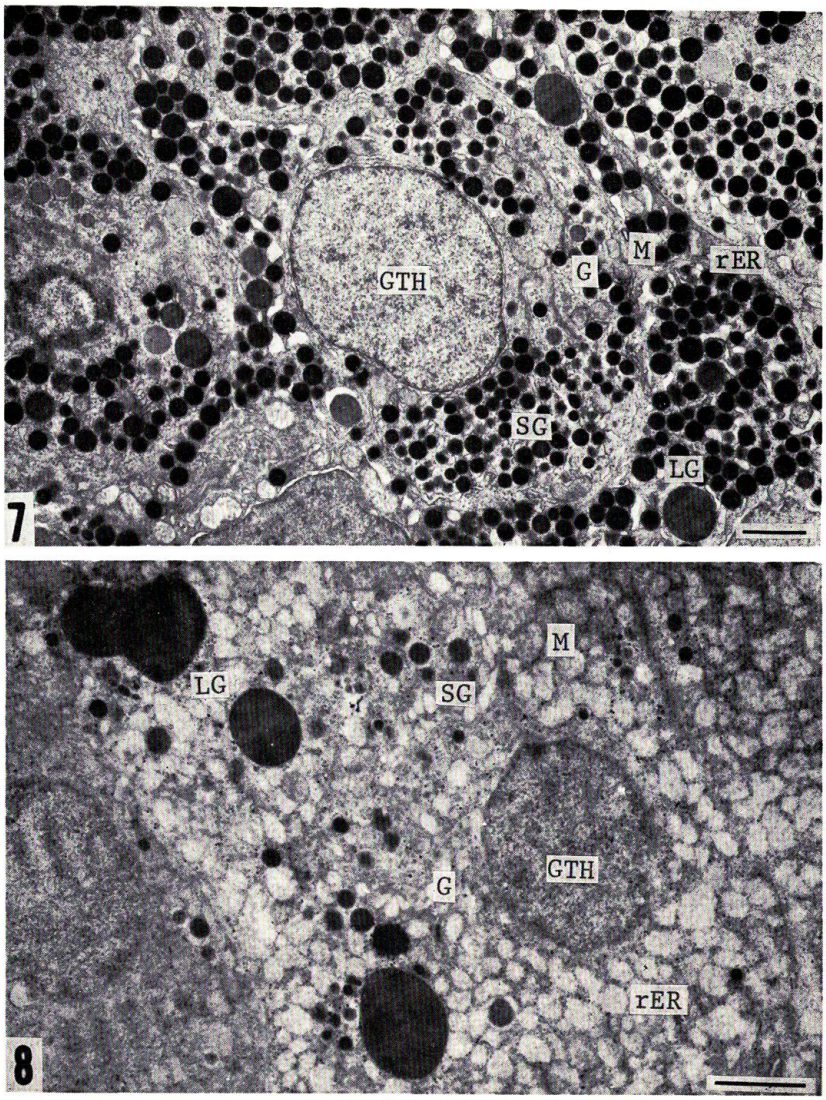
In the gonadotrophs of the fish attaining 30–40% in GSI, small granules were further increased in amount and in size, reaching 200–400 nm in diameter, but the dilatation of cisternae of the rough endoplasmic reticulum was less in degree (Fig. 7) when compared with that found in the cells of the fish with GSI of 10–20% (Fig. 6). The Golgi apparatus appeared to be very active, consisting of several long lamellae, a few vacuoles and many small vesicles with electron-dense contents. Immature granules at the beginning of their formation were often present within the Golgi field. Mitochondria were seen to be much elongated.

In the present study only one fish spontaneously ovulated fully matured eggs following 10 injections of salmon pituitaries. In the pituitary gland of the eel after ovulation, almost all gonadotrophs were provided with many small vacuoles in the cytoplasm, and their small granules were clearly decreased in amount (Fig. 3). Electron-microscopically, small granules of 200–400 nm in size were left in only a small amount in the cells of the ovulating eel (Fig. 8). Large globules in these cells appeared to remain unchanged in number, but were seen to become



Figs. 5 and 6. Electron micrographs of gonadotrophs in the pituitary gland of an initial control (Fig. 5) and an eel with GSI of 10% (Fig. 6). *G*, Golgi apparatus; *LG*, large globule; *M*, mitochondrion; *rER*, rough endoplasmic reticulum; *SG*, small granule. Scales, 1 μm

much electron-dense and irregular in shape. The rough endoplasmic reticulum showed a marked dilatation of constituent cisternae containing somewhat electron-opaque material, which gave a vacuolated appearance to most of the cytoplasm. No notable change was detected in the ultrastructural aspect of the mitochondria and the Golgi apparatus. Many small granules in the process of formation were still observed within the Golgi field.



Figs. 7 and 8. Electron micrographs of gonadotrophs in the pituitary gland of an eel with GSI of 30% (Fig. 7) and an eel ovulated spontaneously (Fig. 8). *G*, Golgi apparatus; *LG*, large globule; *M*, mitochondrion; *rER*, rough endoplasmic reticulum; *SG*, small granule. Scales, 1 μm

The remaining 5 fish, which had been subjected to 10–14 injections of salmon pituitaries, could not ovulate their eggs. At autopsy, they had ovarian eggs which were in various states of progressive overripeness. The GSI of these overripe eels ranged from about 50 to 70%. Pituitary gonadotrophs of these eels showed extensive variations in their histological features, in contrast to the uniform changes of the cells found in the fish after ovulation (Fig. 4). While some

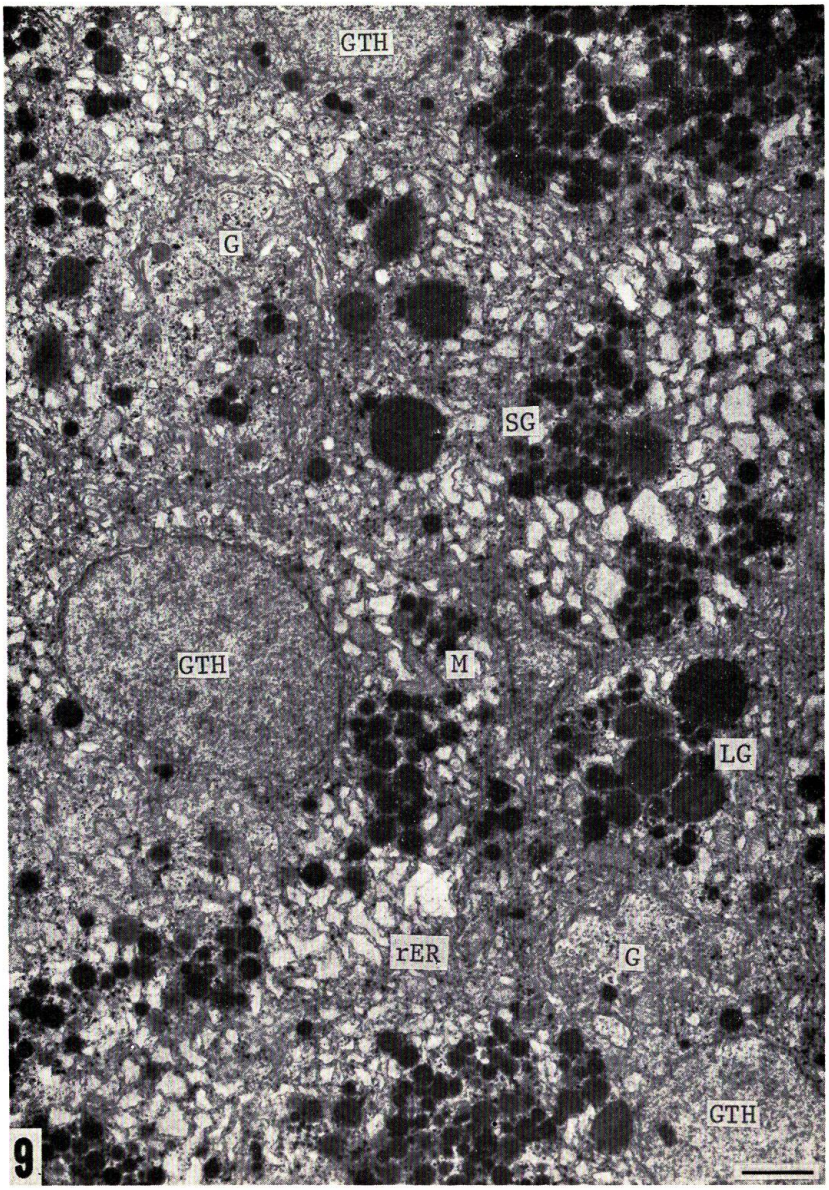


Fig. 9. Electron micrograph of gonadotrophs in the pituitary gland of an overripe eel. *G*, Golgi apparatus; *LG*, large glouble; *M*, mitochondrion; *rER*, rough endoplasmic reticulum; *SG*, small granule. Scale, 1 μm

of the cells were packed still with small granules in their hypertrophied cytoplasm, others displayed more or less a cytoplasmic vacuolization accompanied with a decrease in amount of small granules, though the change was generally less in degree than that occurring after spontaneous ovulation (Fig. 9). In general, small

granules remaining in these cells were much more in amount and higher in electron-density as compared with those observed in the gonadotrophs after ovulation. Large globules appeared to be highly electron-dense and were sometimes irregular in shape. The cisternae of the rough endoplasmic reticulum were dilated to various extents, but the change was less prominent than that found in the cells of the ovulating eel. Some elongated mitochondria were present in the cytoplasm, but they were often irregular in shape. In the Golgi field, there were a few long lamellae and many vacuoles with electron-lucent contents. Evident signs of active formation of secretory granules were no more found in these Golgi fields, which contrasted with the feature of Golgi apparatus observed in the gonadotrophs of the fish after ovulation.

Discussion

The present observation confirmed that, in silver female Japanese eels, *Anguilla japonica*, only one type of adenohypophysial cells displayed some characteristic changes in association with artificially induced ovarian maturation. This agrees well with the previous observation made by Yamamoto and Nagahama[2] on the pituitary gland of pond-cultured males of the same species subjected to artificial maturation. On the other hand, two types of possible gonadotrophs have been shown to exist in the pituitary gland of the European eel, *Anguilla anguilla*[5]: one with electron-dense granules of about 190 nm in diameter and the other with electron-lucent granules of about 130 nm in diameter[6]. The sizes of the two kinds of granules existing in these two types of gonadotrophs are almost equal to that of the small granules in pituitary gonadotrophs of immature females of the Japanese eel serving as initial controls in the present study.

In the silver female Japanese eels at the beginning of treatment, ovaries were quite immature and pituitary glands were provided with only a few, small and inactive gonadotrophs with immature granular contents. In the fish receiving injections of salmon pituitary powder, gonadotrophs displayed a notable increase in size and in number as ovarian maturation proceeded. In addition, the gonadotrophs in maturing eels became to occur also in the rostral pars distalis of the pituitary where no gonadotrophs were present in untreated, immature females. These results agree on all the essential points with those of light microscopic observations on pituitary gonadotrophs of female European eels induced to mature with carp pituitary preparations[3,4]. The same phenomenon has been presented also for artificially matured males of the European eel[7] and the Japanese eel[2].

In maturing European eels, light-microscopic signs of the activation of pituitary gonadotrophs are represented by a marked hypertrophy of the cells with nuclear and nucleolar enlargement and production followed by a discharge of their specific glycoprotein granules[4]. The hypertrophy of pituitary gonadotrophs of maturing female Japanese eels was ultrastructurally accompanied with a marked accumulation of small, secretory granules, an activation of the Golgi apparatus, and a dilatation of cisternae of the rough endoplasmic reticulum. In addition, the small granules themselves became evidently larger in size than those found in gonadotrophs of immature fish. These characteristics are interpreted as signifying that the activity of synthesis of a secretory product in the gonadotrophs is highly

stimulated by the treatment with the pituitary preparation. Large cytoplasmic globules of the gonadotrophs also showed some changes in maturing eels, but the significance of the changes was uncertain. It has been suggested that such large cytoplasmic globules may be lytic in nature[8].

The results as a whole may imply that, in the eel treated with pituitary preparations, endogenous hormone is released from the activated gonadotrophs and plays a stimulating role at least partially in the ovarian maturation of the treated eels. In the present study, however, no signs denoting secretory activities were detectable in the hypertrophied gonadotrophs of maturing females. It has been demonstrated that a purified carp gonadotropin is capable of stimulating considerable ovarian growth even when given to hypophysectomized silver females of the European eel[9], though this does not negate the possibility of a partial contribution of endogenous gonadotropin to ovarian maturation in the above-mentioned cases.

Pituitary gonadotrophs of the eel which had ovulated spontaneously following the pituitary treatment were characterized by a marked degranulation along with a remarkable dilatation of cisternae of the rough endoplasmic reticulum. Similar changes of the cells have been noticed in the European eel[3], the goldfish, *Carassius auratus*[10], and the loach, *Misgurnus anguillicaudatus*[11], after ovulation and spawning, and have been explained to be decisive indications of an augmented release of their hormonal contents. In this context, it is interesting to note that, in the female eels which had failed to ovulate following complete maturation of ovarian eggs, cytological changes of pituitary gonadotrophs were not so prominent and uniform as in the ovulating eel. Moreover, in contrast to a persistent formation of granules in the gonadotrophs after ovulation, no synthetic activity was detected in the Golgi field of the cells in the overripe eels. The observations suggest that the occurrence of spontaneous ovulation in the treated eel may depend on an efficient discharge of endogenous gonadotropin, and that a certain impediment to the mechanism of gonadotropin synthesis and release, due to the repeated treatment with piutitary preparations, may be ascribable to the failure of ovulation from which ovarian overripeness ensues.

It is concluded that the administration of chum salmon pituitaries can bring about a differentiation and activation of synthesizing and, probably, secretory functions of gonadotropic cells in the pituitary gland of silver females of the Japanese eel. It remains to be clarified whether the pituitary preparation containing gonadotropin can affect the gonadotrophs directly or indirectly through steroid hormones produced in the gonad which is induced to mature by the treatment. The possibility of the latter is indicated by the facts that sex steroids can activate pituitary gonadotrophs of gonadectomized, immature sockeye salmon, *Oncorhynchus nerka*[12], and that steroid hormones can induce an evident differentiation of basophilic cells in the pituitary gland of juvenile Japanese eels[13].

References

1) Sugimoto, Y., Takeuchi, Y., Yamauchi, K. and Takahashi, H. (1976). Induced maturation of female Japanese eels (*Anguilla japonica*) by administration of salmon pituitaries, with notes on changes of oil droplets of matured eels. *Bull.*

Fac. Fish. Hokkaido Univ. **27**, 107–120. (In Japanese with English summary).

2) Yamamoto, K. and Nagahama, Y. (1973). Cytological changes in the adenohypophysis of fresh-water cultivated male Japanese eels, *Anguilla japonica*, induced to maturation by transfer to sea water and Synahorin injection. *Bull. Jap. Soc. Sci. Fish.* **39**, 585–594. (In Japanese with English summary).
3) Olivereau, M. and Fontaine, M. (1966). Etude cytologique de l'hypophyse de l'Anguille femelle mûre. *C.R. Soc. Biol.* **160**, 1374–1378.
4) Olivereau, M. (1967). Observations sur l'hypophyse de l'Anguille femelle, en particulier lors de la maturation sexuelle. *Z. Zellforsch.* **80**, 286–306.
5) Olivereau, M. and Herlant, M. (1960). Etude de l'hypophyse de l'Anguille mâle au cours de la reproduction. *C.R. Soc. Biol.* **154**, 706–709.
6) Knowles, F. and Vollrath, L. (1966). Changes in the pituitary of the migrating European eel during its journey from rivers to the sea. *Z. Zellforsch.* **75**, 317–327.
7) Olivereau, M. (1960). Hypophyse et activité sexuelle et thyroïdienne chez l'Anguille mâle. *J. Physiol., Paris* **52**, 181–182.
8) Holmes, R.L. and Ball, J.N. (1974). *The Pituitary Gland: a comparative account.* 397p. Cambridge Univ. Press, Cambridge.
9) Fontaine, Y.A., Lopez, E., Delerue-Le Belle, N., Fontaine-Bertrand, E., Lallier, F. and Salmon, C. (1976). Stimulation gonadotrope de l'ovaire chez l'Anguille (*Anguilla anguilla* L.) hypophysectomisée. Morphologie, activités adényl cyclase et phosphodiestérase de l'adénosine monophosphate cyclique. *J. Physiol., Paris* **72**, 871–892.
10) Nagahama, Y. and Yamamoto, K. (1969). Basophils in the adenohypophysis of the goldfish (*Carassius auratus*). *Gunma Symp. Endocrinol.* **6**, 39–55.
11) Ueda, H. and Takahashi, H. (1977). Promotion of ovarian maturation accompanied with ovulation and changes of pitutiary gonadotrophs after ovulation in the loach, *Misgurnus anguillicaudatus*, treated with clomiphene citrate. *Bull. Fac. Fish. Hokkaido Univ.* **28**, 106–117.
12) van Overbeeke, A.P. and McBride, J.R. (1971). Histological effects of 11-ketotestosterone, 17α-methyltestosterone, estradiol, estradiol cypionate, and cortisol on the interrenal tissue, thyroid gland, and pituitary gland of gonadectomized sockeye salmon (*Oncorhynchus* nerka). *J. Fish. Res. Bd. Canada* **28**, 477–484.
13) Oota, H. (1978). Sex differentiation and development of the gonad in the Japanese eel, *Anguilla japonica*, and the European eel, *A. anguilla*, with special regard to the effect of exogenous sex steroids. Thesis M. Fish. Sci., Hokkaido University, Japan. (In Japanese).