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Cytophysiological Studies on Pituitary Gonadotrophs of Some Teleost Fishes, Particularly of the Loach, *Misgurnus anguillicaudatus*

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Introduction

It is well known that, in all vertebrate animals, the pituitary gland controls many aspects of reproduction by secreting various tropic hormones. Among the hormones, those known as gonadotropic hormones (GTH) undoubtedly play leading roles in regulating gonadal activities of vertebrates including teleost fishes. On the basis of these facts, injection of exogenous pituitary preparations (hypophysation) has been widely practiced for current aquaculture in order to better control reproductive conditions, especially such as oocyte maturation and ovulation, in marine as well as freshwater fishes. At present, however, there are contradictory opinions among workers in that field of study about the chemical and biological nature of fish GTH. Elucidation of the problem seems to be advantageous for extending the practical application of hypophysation to fishes of different states of gonadal maturity.

It is evident that the pituitary gland of mammals contains two physiologically distinct gonadotropic hormones, viz. follicle stimulating hormone (FSH) and luteinizing hormone (LH). Also in the pituitaries of non-mammalian tetrapods, the existence of two GTHs, resembling mammalian FSH and LH, has been reported by several workers (Licht et al., 1976). In teleost fishes, however, the problem as to whether the pituitary gland

possesses one or two kinds of GTH(s) is still not settled.

In early biochemical studies, Witschi (1955) bioassayed pituitaries of sharks, garpikes and salmons, and obtained FSH and LH activities from their pituitaries. Otsuka (1956) also detected FSH and LH substances from salmon pituitaries by using mammalian bioassay systems. On the other hand, Fontaine and Gérard (1963) could biochemically prepare only one gonadotropic factor (c-GTH) from the pituitaries of carp, Cyprinus carpio, that was entirely inactive in mammalian assays but was quite active, like mammalian LH, in the frog spermiation assay (Burzawa-Gérard and Fontaine, 1965) and in the goldfish testicular hydration assay (Clemens et al., 1964). Burzawa-Gérard (1971) later succeeded in getting more highly purified c-GTH.

Yamazaki and Donaldson (1968a, b) partially purified, in chinook salmon, Oncorhynchus tshawytscha, a single gonadotropic hormone (s-GTH), which was capable of regulating spermatogenesis, spermiation, vitellogenesis and ovulation in hypophysectomized goldfish, Carassius auratus. The same effect was also reported in Indian catfish, Heteropneustes fossilis (Sundararaj et al., 1971, 1972). The s-GTH as well as mammalian LH was shown to be able to stimulate the day-old chick testis to enhance its radiophosphorus uptake (Donaldson et al., 1972).

Thus, the results obtained in the carp and the salmon have led these authors to emphasize the

possibility of the existence of a single GTH in teleost fishes which is different in its chemical composition from either LH or FSH in higher vertebrates (Yamazaki, 1969; Burzawa-Gérard and Fontaine, 1972; Donaldson, 1973). Later, in fact, Breton et al. (1976) purified only one pituitary GTH (t-GTH) in rainbow trout, Salmo gairdneri, which had physico-chemical characteristics comparable to c-GTH and s-GTH and to mammalian LH. Yoneda and Yamazaki (1976) and Farmer and Papkoff (1977) also reported the presence of a single GTH in chum salmon, Oncorhynchus keta, and in Tilapia mossambica, respectively.

However, there are some studies in which conflicting results were shown as to the kind of gonadotropic factors present in teleost pituitaries. Hattingh and du Toit (1973) identified two active gonadotropic fractions, both of which were able to induce spermiation in the goldfish, from the pituitary gland of mudfish, Labeo umbratus. Haider and Blüm (1977) also obtained two gonadotropic fractions from the pituitary glands of crucian carp, Carassius auratus gibelio. When administered to male swordtails, Xiphophorus helleri, one of the two fractions of the crucian carp induced spermatogenesis and spermiation, and activated testicular interstitium, while the other only stimulated the spermatogenesis without significant effects on spermiation and testicular interstitium.

Recently, two protein fractions with gonadotropic activities were purified from pituitaries of chum salmon, Oncorhynchus keta, by Idler et al. (1975a, b) using the chick testicular radiophosphate uptake assay and the trout gonadal c-AMP production assay. In addition, Campbell and Idler (1976, 1977) reported that a glycoprotein fraction isolated from the pituitary gland of American plaice, Hippoglossoides platessoides, failed to stimulate accumulation of yolk in oocytes while a non-glycoprotein fraction was able to reinitiate the process of vitellogenesis when assayed in hypophysectomized winter flounder, Pseudopleuronectes americanus. The vitellogenic non-glycoprotein hormone and the maturational glycoprotein hormone were isolated further from pituitaries of the American plaice (Ng and Idler, 1978a), the chum salmon (Ng and Idler, 1978b), the winter flounder (Ng and Idler, 1979) and the carp (Idler and Ng, 1979). These studies by Idler and his collaborators seem to imply that the duality of pituitary GTHs may be not confined to those fishes examined by these authors.

A major problem concerning the identification of teleost GTH(s) seems to be the lack of sensitive and specific bioassay methods using teleost fishes as test animals. If such bioassay procedures for piscine GTH(s) be established, biochemical analyses must provide conclusive evidence for determining the kind and nature of the pituitary hormone(s). The biochemical approaches,

however, may encounter another important difficulty in that quite a large number of pituitary glands are needed to get a sufficient amount of purified preparations in order to accomplish reliable bioassays of their hormonal activities. This is reflected in the fact that the purification of piscine GTH(s) has thus far been successful in a few large-sized fish species of a high human availability such as salmons and carp.

Cytological as well as biochemical studies have long been devoted to identify glandular cells in the pituitary gland of various teleost fish, and knowledge of the functional morphology of the gland has been rapidly accumulated for the past quarter of century (Pickford and Atz, 1957; van Oordt, 1968; Ball and Baker, 1969; Sage and Bern, 1971; Nagahama, 1973; Schreibman et al., 1973; Holmes and Ball, 1974; Fontaine and Olivereau, 1975; Leatherland, 1975). Numerous histological and histochemical studies on teleost pituitaires have thus shown that GTH(s) are produced and discharged into circulation from basophilic cells which display prominent cyclic changes of their activity in harmony with the reproductive cycle. In contrast with biochemical studies mentioned above, the cytophysiological studies seem to merit in that different kinds of pituitary cells producing GTHs of different chemical natures, if any, may possibly be identified histochemically regardless of the size and number of specimens available. Moreover,

functional differentiation of the gonadotrophs associated with reproductive cycling of fishes can also be investigated by such studies. Nevertheless, the question of whether the pituitary glands of teleost fishes possess one or two types of gonadotropic cells is still controversial to date.

Since the 1960's, irrespective of widespread introduction of sophisticated histochemical techniques into the study of fish pituitary glands, only one type of gonadotroph has been reported to exist in various teleost species: killifish, Fundulus heteroclitus, and guppy, Lebistes reticulatus (Sokol, 1961); black molly, Poecilia latipinna (Olivereau and Ball, 1964; Sage and Bromage, 1970a, b); black surfperch, Embiotoca jacksoni (Lagios, 1965); catfish, Clarias batrachus (Lehri, 1965; Joy and Sathyanesan, 1979); blind Mexican cave fish, Anoptichthys jordani (Mattheij and van Oordt, 1967; Mattheij, 1970); medaka, Oryzias latipes (Aoki and Umeura, 1970; Kasuga and Takahashi, 1971; Chan, 1977); Japanese eel, Anguilla japonica (Yamamoto et al., 1974); Indian catfish, Heteropneustes fossilis (Baker et al., 1974); Indian carp, Cirrhinus mrigala (Moitra and Sarkar, 1976); zebrafish, Brachydanio rerio (van Ree, 1976); grass carp, Ctenopharyngodon idellus (Anon., 1978a); a cichlid, Tilapia mossambica (Chiba et al., 1978); and a goby, Rhinogobius brunneus (Yoshie and Honma, 1978).

On the other hand, the presence of two types of

pituitary gonadotrophs has been shown histologically and histochemically in European eel, Anguilla anguilla (Olivereau, 1960, 1961, 1967; Olivereau and Herlant, 1960; Olivereau and Fontaine, 1966; Knowles and Vollrath, 1966b), Oncorhynchus spp. (Olivereau and Ridgway, 1962), goldfish, Carassius auratus (Olivereau, 1962), Mugil spp. (Stahl, 1963), sockeye salmon, Oncorhynchus nerka (van Overbeeke and McBride, 1967, 1971; McBride and van Overbeeke, 1969), roach, Leuciscus rutilus (Olivereau, 1969), coho salmon, Oncorhynchus kisutch (Chestnut, 1970), a sparid, Boops salpha (Michele, 1973; Malo-Michele, 1978), chinook salmon, Oncorhynchus tshawytscha (Olivereau, 1972), Symphodus ocellatus and S. mediterraneus (Demal et al., 1974), Lepomis cyanellus and L. macrochrius (Simon and Reinboth, 1974), kokanee salmon, Oncorhynchus nerka (Kawashima et al., 1976), Atlantic salmon, Salmo salar (Olivereau, 1976), Perca fluviatilis macedonica (Dimovska, 1977), rainbow trout, Salmo gairdneri, and brown trout, Salmo fario (Olivereau, 1978).

Current immunohistochemical studies using antibodies raised against the c-GTH or mammalian LH and FSH have been able to locate gonadotrophs in the pituitary gland of the carp (Billard et al., 1971), the sockeye salmon (McKeown and van Overbeeke, 1971), Tilapia marmoratus (Doerr-Schott, 1976), the black molly (Goos et al., 1976), threespined stickleback, Gasterosteus aculaeatus, perch, Perca fluviatilis, and Tilapia mossambica

(Follenius et al., 1978) and platyfish, Xiphophorus maculatus (Schreibman and Margolis-Kazan, 1979). However, the gonadotropic substances used as antigens in the above-cited studies were either purified GTHs of mammalian origin which are undoubtedly different in their chemical nature from piscine GTH, or a single GTH preparation of piscine origin which completely lacks, or even contains, another kind of possible gonadotropic factor. Accordingly, at least at present, the immunocytochemical studies do not seem to be fully efficient in discriminating possible two types of pituitary gonadotrophs in fishes. Similar immunological studies, in separate attempts, using two kinds of GTHs that have been purified from several teleost species as mentioned before must be more fruitful in this regard.

Electron microscopy on the pituitary gland, if combined adequately with the histochemical study, is efficient not only for the identification of different glandular cell types but also for observations of changes in the activity of a given cell type in connection with physiological states of fishes. Nevertheless, the problem as to whether there is only one or two types of gonadotrophs in fish pituitaries has become more ambiguous even after introduction of extensive electron microscopic studies since the 1960's.

In some teleost species, only one type of pituitary gonadotroph was detected by electron microscopy:

the guppy (Follenius and Porte, 1960); Cymatogaster aggregata (Leatherland, 1969); the chum salmon (Nagahama and Yamamoto, 1970); the medaka (Kasuga and Takahashi, 1970); the Japanese eel (Yamamoto and Nagahama, 1973); grey mullet, Mugil cephalus (Abraham, 1974); Tilapia spp. (Leatherland et al., 1974); flounder, Pleuronectes flesus, and minnow, Phoxinus phoxinus (Benjamin, 1975); the black molly (Batten et al., 1975; Benjamin, 1976; Peute et al., 1976); the coho salmon (Nagahama et al., 1977); and the grass carp (Anon., 1978b). On the contrary, two distinct types of gonadotrophs have been demonstrated ultrastructurally in other species of teleosts: the European eel (Knowles and Vollrath, 1966a, c); Zoarces viviparaus (Öztan, 1966); pond loach, Misgurnus fossilis (Jasiński, 1973); the roach (Båge et al., 1974; Ekengren et al., 1978a); and loach, Misgurnus anguillicaudatus (Oota, 1975).

The situation in some other fishes appears to be a matter of much complexity, since the results of ultrastructural observations were quite conflicting as to the kind of gonadotropic cells even in the same species of teleosts. For example, in the sockeye salmon, Oncorhynchus nerka, Nagahama and Yamamoto (1969a) and McKeown and Leatherland (1973) identified only a single type of pituitary gonadotroph, while Cook and van Overbeeke (1972) discriminated two types of the cells. Such are also the cases for the goldfish, Carassius auratus

(Nagahama and Yamamoto, 1969b; Kaul and Vollrath, 1974; Lam et al., 1976: vs. Leatherland, 1972; Leatherland and Sonstegard, 1977), the carp, Cyprinus carpio (Kurosumi et al., 1963: vs. Leatherland and Sonstegard, 1977), and the threespined stickleback, Gasterosteus aculeatus (Leatherland, 1970: vs. Slijkhuis, 1978). Quite recently, Ekengren et al. (1978b) and Peute et al. (1978) reported their ultrastructural observations on pituitary gonadotrophs of the Atlantic salmon and the rainbow trout, respectively, in which they regarded two gonadotropic cell types as different forms or in different stages of activity of a single type of gonadotroph during the sexual cycle of the fishes.

Although, as described above, there have been an extensive body of works concerning the pituitary gonadotrophs of teleost fishes, the problem as to whether teleost fishes possess one or two gonadotropic cell types in the pituitary gland is still controversial at present. The present study is thus conducted to examine if some species of teleosts truly have two distinct types of gonadotrophs in the pituitary gland, and to study the significance of cytological characteristics of the two types of pituitary gonadotrophs in various fish species in order to distinguish their respective function.

First, with respect to gonadotropic cell types of the pituitary gland, gonadotrophs in the loach, Misgurnus anguillicaudatus, are of the writer's special interest

since previous investigations on some species of the genus Misgurnus have indicated the presence of the two types of cells based on light and electron microscopy (Jasiński, 1973; Oota, 1975). However, these studies did not refer to cytological changes of the cells under different physiological conditions of the fishes. In the present study, some experimental attempts were conducted in order to identify and discriminate possible two types of pituitary gonadotrophs in the loach. Ultrastructural changes denoting gonadotropic activities of the cells were studied by stimulating them through gonadectomy and through administrations of GTH-releasing substances, and further by inhibiting them through treatments with sex steroids. The results could indicated the presence of two distinct types of pituitary gonadotrophs in the loach, which will be described in detail in this paper. In addition, careful cytological studies were carried out in the present study on the changes of the two types of pituitary gonadotrophs in both sexes of the loach during the whole reproductive cycle. Physiological significance of the changes analyzed on the basis of some experimental findings will be presented in this paper.

Second, a comparative study on pituitary gonadotrophs of other teleost fishes were made using chum salmon, Oncorhynchus keta, masu salmon, O. masou, rainbow trout, Salmo gairdneri, whitespotted char, Salvelinus leucomaenis,

goldfish, Carassius auratus, and Japanese eel, Anguilla japonica, as materials. In these species of teleosts, some confused results of observations on pituitary gonadotrophs have been obtained by several authors.

Finally, discussion will be made in this paper on the significance of the duality or unicity of pituitary gonadotrophs in teleost fishes, especially on different roles played by two distinct types of gonadotrophs in the course of reproductive development in both sexes of this group of vertebrates.

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salmon, masu salmon, rainbow trout and whitespotted char, the writer is greatly indebted to Associate Professor T. Kubo, Nanae Fish-Culture Experimental Station, Hokkai do University, to Mr. M. Uchiyama, the Mori Branch, Hokkaido Fish Hatchery, and to Mr. Y. Ishikawa, the Yakumo Branch, Hokkaido Salmon Hatchery.

Materials and Methods

The main material used in the present study is the loach, Misgurnus anguillicaudatus. The fish were mainly collected from brooks in the vicinity of Hakodate, Hokkaido. They were subsequently kept in aquaria set in the laboratory under natural light and temperature conditions, and were fed on commercial fish diet once a day.

Besides the loach, 6 species of teleost fishes were also studied in the present study. Goldfish, Carassius auratus, of a Wakin variety were bred and reared in stock ponds of the laboratory. Japanese eel, Anguilla japonica, of the silver catadromous form were captured in rivers in Aomori Prefecture, transported to the laboratory, and kept in aquaria with sea water under natural light condition. About 4-year-old adults of chum salmon, Oncorhynchus keta, were sampled at the Yakumo Branch, Hokkaido Salmon Hatchery. Masu salmon, O. masou, were

obtained from stocks cultured in outdoor fresh-water ponds at the Mori Branch, Hokkaido Fish Hatchery. Rainbow trout, Salmo gairdneri, and whitespotted char, Salvelinus leucomaenis, were both cultured at the Nanae Fish-Culture Experimental Station, Hokkaido University. Specifications of other characteristics of these fishes used in the present study will be given in corresponding sections of this paper.

For the purpose of histological and histochemical investigations of the pituitary gland and the gonad, fishes were killed by quick decapitation and the organs were excised out rapidly. Pieces of gonads were fixed in Bouin's fluid or Bouin-Hollande solution. Serial paraffin sections of the specimens were cut at 8 μm in thickness, and stained with usual Delafield's hematoxylin and eosin. Pituitary glands for light microscopy were fixed in Susa, Zeker-formol, or Bouin-Hollande-sublimate. Serial sagittal sections were made at 6 μm in thickness from paraffin-embedded blocks, and stained with aldehyde fuchsin (AF)-light green-orange G, periodic acid-Schiff (PAS) or Herlant's alcian blue-PAS-orange G (AB-PAS-OG; Herlant, 1960).

For electron microscopical observations, some pituitary glands were immersed in 2.5% glutaraldehyde in 0.05M phosphate buffer (pH 7.4) for 2 hours at 4 °C, and then postfixed in Millonig's osmium tetroxide for 2 hours at 4 °C. Others were prefixed with Karnovsky's

glutaraldehyde-paraformaldehyde mixture (Karnovsky, 1965) in 0.2M 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. After the dehydration through graded ethanols, the glands were embedded in a Epon-epoxy resin mixture. Ultrathin sections, cut with glass knives on a Porter-Blum MT-1 ultramicrotome at the thickness of 50-80 nm, were stained double with uranyl acetate and Reynolds' lead citrate (Reynolds, 1963), and then examined with a Hitachi HS-7 or a HU-12 electron microscope. Parallel semithin sections of the Epon-embedded specimens of about 1 μm thick were stained with methylene blue for light microscopic comparison.

Other methods employed in the present study will be described in detail in respective sections of this paper.

Results

I. Observations in the loach

1. Types of glandular cells in the pituitary gland

(1) General morphology of the pituitary gland

The pituitary gland of the loach, Misgurnus

anguillicaudatus, is composed of two distinct components, the neurohypophysis and the adenohypophysis. The former penetrates into the center of the gland and interdigitates complicatedly with the latter. The adenohypophysis consists of three different lobes, viz. rostral pars distalis (RPD), proximal pars distalis (PPD) and pars intermedia (PI) (Fig. 1). These three lobes comprise eight different types of glandular cells, viz. two types of acidophils, four types of basophils and two types of chromophobic cells (Fig. 2).

The RPD occupies the anterior portion of the gland, and contains one type of acidophilic cells together with chromophobic ones. The acidophils are stained with orange G by AB-PAS-OG staining, and with both light green and orange G by AF staining. The cells are distributed evenly throughout the RPD, and form the largest component of that region. They are elongate or polygonal in shape. Generally they exhibit no particular arrangements, but sometimes they are arrayed in a form of pseudofollicles (Fig. 3). The nucleus, oval or elongate in shape, is situated in a corner of the cell on many occasions. Ultrastructurally, the acidophils have many electron dense, rounded granules, ranging from 250 to 350 nm in size (Fig. 4). The rough endoplasmic reticulum is composed of flat or lamellar cisternae. The Golgi apparatus lying in the juxtanuclear region is rather a few in number. Mitochondria are generally of

round or rod shape and are found throughout the cytoplasm. Basing on these histological and cytological characteristics, the cells of this type may be identified to be prolactin cells.

The remaining type of cells in the RPD shows no specific affinity to both acidic and basic dyes. They are elongate or columnar in shape and exist close to the area bordering the neurohypophysis, forming a 2 or 3 cells thick layer (Fig. 5). The nucleus of the cells is oval in form and is located in the cell periphery. Electron microscopically, the chromophobic cells contain dense, round or oval granules, measuring 150-250 nm in size (Fig. 6). The rough endoplasmic reticulum is composed of long lamellated cisternae. The Golgi apparatus and rod- or round-shaped mitochondria are rarely observed. Judging from these cytological characteristics, they may be regarded as adrenocorticotropic (ACTH) cells.

The PPD is situated in the central portion of the gland. One acidophilic type and three basophilic types of glandular cells are present in the PPD. Acidophilic cells, which are strongly positive to orange G in both the AB-PAS-OG and AF staining, are encountered throughout the PPD. They are oval or round in shape, and their oval nucleus is eccentric in position in most cases (Fig. 7). The cells are characterized ultrastructurally by having electron dense, spherical granules, ranging

from 200 to 400 nm in size, distributed throughout the cytoplasm (Fig. 8). The rough endoplasmic reticulum generally consists of flat cisternae oriented randomly. The Golgi apparatus is scarcely found. Mitochondria are few in number and mostly elongate in shape. They may possibly be somatotropic (STH) cells.

Cytological characteristics of the other three types of basophils existing in the PPD will be described in detail in successive sections of this paper.

The PI is located in the posterior part of the gland, and is composed of two types of cells, viz. chromophobes and basophils. The chromophobic cells are distributed evenly in the PI. They are oval or round in shape with round nuclei (Fig. 9). In electron micrographs, the cytoplasm of the cells are shown to contain spherical or round granules, measuring 150-200 nm in size, of electron densities varying from highly dense to translucent appearance. The rough endoplasmic reticulum consists of flat cisternae. The Golgi apparatus and round-shaped mitochondria are sparse in the cells.

Basophilic cells in the PI are weakly positive to PAS, and is fewer in number than chromophobes. They are angular in shape with round or oval nucleus (Fig. 9). In fine structure, the cells contain many electron dense granules of 200-450 nm in size (Fig. 10). Ill-developed rough endoplasmic reticulum is found scattered throughout

the cytoplasm. The Golgi apparatus and rod-shaped mitochondria are only a few in number. Although precise roles of these two cell types in the PI are uncertain, the chromophobic cells may be related to the production of melanophore stimulating hormone (MSH).

(2) Identification of thyrotrophs

It appears in most teleost fishes that thyrotropic (TSH) and gonadotropic (GTH) cells are commonly present in the PPD and both are basophilic in nature. In the PPD of the pituitary gland of the loach, three basophilic cell types are discernible other than the acidophilic somatotrophs. Then, the first attempt in the present study was to identify TSH cells and GTH cells among the three types of basophils.

In order to distinguish thyrotrophs decisively from other cell types, adult loaches were treated with thiourea which is known to stimulate the release of TSH from the pituitary gland by inhibiting the production of thyroid hormones. A total of 24 adult loaches of both sexes, ranging from 8.5 to 11.3 cm in body length and from 3.1 to 8.6 g in body weight, were used in the thiourea treatment which was carried out during the period from June to August, 1978. The loaches were kept in glass aquaria with aerated water under natural light and temperature conditions. They were fed on commercial fish 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. Half of them were killed after 30 days of the treatment, and half after 60 days. Another group of 3 females and 3 males, which served as controls, were raised ordinarily and were killed 60 days after the start of experiment.

In the fish treated with thiourea for 30 days, some basophilic cells existing in the PPD 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 the thiourea treatment, basophilic cells were further increased in number as well as in size (Fig. 13). Their secretory granules were clearly diminished in amount, and the rough endoplasmic reticulum consisting of parallel lamellae was developed conspicuously throughout the cytoplasm (Fig. 14). Many Golgi apparatus appeared to be very active, and immature granules were often present within the Golgi field. Mitochondria were seen to be much enlarged in size.

The cells activated evidently by the thiourea treatment were found to be localized in the dorsal region of the PPD. In control loaches of both sexes, they showed

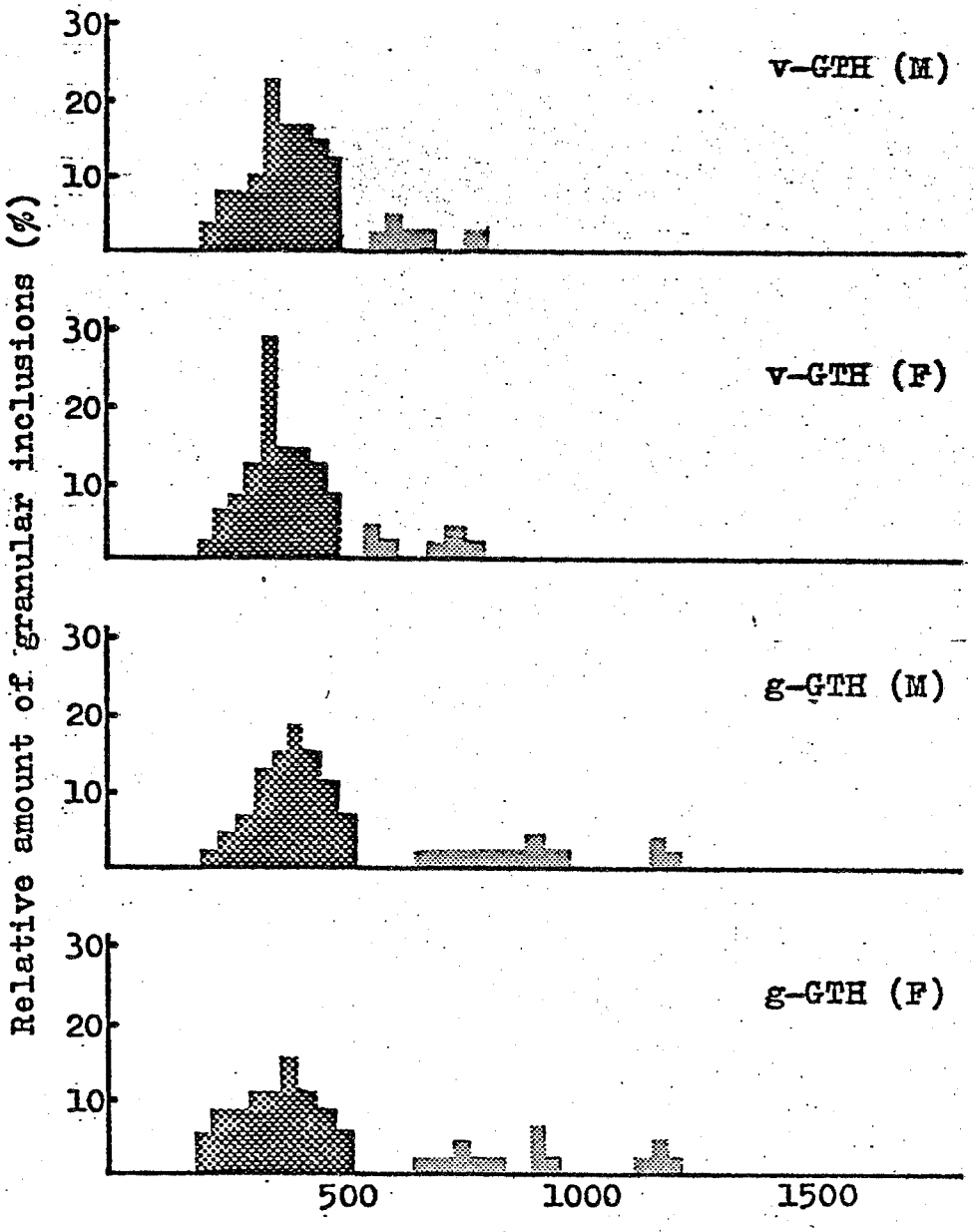
a weak affinity to both alcian blue and PAS in the AB-PAS-OG preparation and to aldehyde fuchsin in the AF preparation. They were few in number, small in size, and mostly angular in shape with round or oval nuclei (Fig. 11). Ultrastructurally, they contained samll, round or oval granules, measuring 80-200 nm in size (Fig. 12). The secretory granules of the cells were observed to be smallest in size compared with those of other cell types of the pars distalis. Lamellated rough endoplasmic reticulum was sparse. The Golgi apparatus and rod- or round-shaped mitochondria were rarely detected. These cytological aspects corresponded well to those of thyrotropic (TSH) cells described so far for other teleost species.

(3) Globular cells and vesicular cells

The remaining two types of basophilic cells in the PPD, other than thyrotrophs, show different localities, staining properties and cytological characteristics from each other. The cells of one type are distributed in the central and ventral parts of the PPD, and are stained intensely with alcian blue and PAS in the AB-PAS-OG preparation. Their nuclei are often irregular in shape and eccentric in position (Fig. 15). The cells are ultrastructurally characterized by having two different kinds of spherical granular inclusions: one of them, designated as large globules, are relatively

low in electron density and noticeably larger in size, measuring 600-1200 nm, while the other, described henceforth as small granules, are highly electron dense and smaller in size, ranging from 200 to 500 nm in diameter (Fig. 16). The rough endoplasmic reticulum of the cells is usually lamellar or irregularly vesicular in form with electron lucent materials. The Golgi apparatus is moderately active, being accompanied sometimes with vesicles charging electron dense contents. Mitochondria are few in number and mostly elongate in shape, and are located near the Golgi field. The cells of this type are designated as the "globular cells" in the present study.

Basophilic cells of the other type encountered in the central and dorsal parts of the PPD are strongly positive to PAS but are almost negative to alcian blue in the AB-PAS-OG preparation. However, the cells show an affinity to aldehyde fuchsin similar to the globular cells in the AF preparation. Their nuclei are generally round in shape and are almost centrally located (Fig. 17). They are marked ultrastructurally by the constant occurrence of numerous cytoplasmic vesicles, which are rounded cisternae of the rough endoplasmic reticulum, with finely granular contents of moderate electron density (Fig. 18). The cytoplasmic vesicles are round in shape and relatively uniform in size, being in sharp contrast to cisternae of the rough endoplasmic



Text-fig. 1. Size distribution of small granules (▨) and large globules (▩) of the two types of gonadotrophs, the vesicular (v-GTH) and globular (g-GTH) cells, in males (M) and females (F) of the loach, Misgurnus anguillicaudatus.

reticulum of the globular cells. The cells also contain large globules of 500-800 nm in size and small granules of 150-450 nm in size, but these granular cytoplasmic inclusions are smaller in size and fewer in number than those of the globular cells. Well-developed Golgi apparatus consisting of small vesicles and a stack of flattened or slightly dilated lamellae sometimes exist in the juxtanuclear region. Mitochondria are mostly roundish in shape, and are scattered throughout the cytoplasm. The cells are termed the "vesicular cells" in the present study. The size distribution of granular inclusions of the globular and vesicular cells in the pituitary gland of the loach is summarized in Text-fig. 1.

The globular and vesicular cells are easily distinguishable from the other six types of glandular cells in the pituitary gland and also from each other by their ultrastructural features as well as their localization and histochemical characteristics. Any intermediate cells between the two types of the cells were not observed by the present light and electron microscopy.

2. Identification of gonadotrophs (GTH cells)

For the purpose of identifying pituitary GTH cells of the loach decisively, two different experiments were carried out in the present study. It is well understood

that release of GTH from the pituitary gland is under the control of releasing hormone(s) of the hypothalamus in teleosts as well as other vertebrates (Schally et al., 1973). Therefore, in the first series of experiments, a synthetic luteinizing hormone-releasing hormone (LH-RH), which has been shown to be potent to selectively activate pituitary GTH cells in some teleosts (Kaul and Vollrath, 1974; Lam et al., 1976; Chan, 1977; Anon., 1978a, b; Ekengren et al., 1978b), was administered to the loach in order to determine the locality of GTH cells by their cytological responses. In addition, clomiphene citrate, a non-steroidal antiestrongen capable of stimulating GTH cells possibly through a promoted release of hypothalamic GTH-releasing hormone, was also used in the experiment. In the second series of experiments, bearing in mind a negative feedback mechanism which controls the activity of pituitary GTH cells, gonadectomy of loaches of both sexes and the treatment of the fish with exogenous sex steroids were done to cause activation and inhibition, respectively, of their GTH cells.

All the four different experiments were done during the periods from May to July when the fish were at later phases of the prespawning period or at the early spawning period. During that period, there were many globular and vesicular cells in the PPD of the pituitary gland of adult control fish regardless of sexes (Fig.

19). The globular cells contained numerous small granules and a few large globules in the cytoplasm. In these cells, the rough endoplasmic reticulum was scattered throughout the cytoplasm, its cisternae being scarcely dilated (Fig. 20). The Golgi apparatus appeared to be highly active, and mitochondria were rather small in number. The vesicular cells also had small granules and large globules, but these granular inclusions were constantly fewer in number than those of globular cells. Their characteristic cytoplasmic vesicles were relatively uniform in size and always contained material of a low electron density (Fig. 20). The Golgi apparatus and mitochondria were rarely observed in the cells.

In the pituitary gland of young female loaches used in some experiments, both the globular and the vesicular cells had already differentiated in the PPD (Fig. 21). In the globular cells, both the two kinds of granular inclusions were found to be smaller in size and fewer in number than those of adult females; small granules being 100-400 nm and large globules 400-1000 nm in size (Fig. 22). The vesicular cells were almost the same in their cytological features as those of control adult females (Fig. 22).

(1) Effects of synthetic LH-RH

Eighteen adult females, 12 young females and 12

adult males, 9.5-12.1, 6.7-7.9 and 8.7-9.9 cm in body length, respectively, were used in the experiment. These fish were divided into 6 groups, each consisting of 3 adult females, 2 young females and 2 adult males. They were kept in separate glass aquaria with constantly aerated water under natural light and temperature varying from 15 to 18 ℃, and were fed on commercial fish pellet once a day. The experiment was commenced on May 30, 1979 and lasted for 3 days.

Synthetic LH-RH (Rutamin, Daiichi Co., Ltd.) was dissolved in 0.6% saline at a concentration of 1 μg/0.01 ml, and injected intraperitoneally at a dose of 1 μg/g body weight. Control fish were injected with 0.6% saline only. The fish of 4 experimental groups were killed 1, 24, 48 and 72 hours after a single injection of LH-RH, and those of 2 control groups were sacrificed 1 and 72 hours.

In the control groups, ovaries of adult females were filled with many oocytes in the tertiary yolk stage, and those of young females had oocytes in the yolk vesicle stage. Control males had testes with germ cell cysts in various spermatogenetic stages along with a large amount of mature spermatozoa. A single injection of LH-RH did not provoke any prominent changes in the gonads irrespective of sex and maturity of treated fish, when examined 72 hours after the injection.

Administration of synthetic LH-RH into adult loaches

led the globular and vesicular cells of the pituitary gland to some structural changes. One hour after the injection, most of the globular cells showed pronounced hypertrophy (Fig. 23). Ultrastructurally, the cells showed a distinct decrease in number of small granules. Although large globules remained unchanged in number, they became low in electron density. The rough endoplasmic reticulum was composed of numerous cisternae dilated conspicuously in various degrees. The nucleus of the globular cells was quite irregular in shape and unusually dense (Fig. 24). Some enlarged Golgi apparatus and mitochondria occurred in the cells. Twenty-four, 48 and 72 hours after the LH-RH injection, some of the globular cells were undergoing pycnotic changes, but others became regranulated with small granules and a few large globules in the cytoplasm (Fig. 25). In the regranulated cells, the rough endoplasmic reticulum developed stacks of flat or lamellar cisternae, the Golgi apparatus appeared to be moderately active, and mitochondria were a few in number (Fig. 25).

Similar but less prominent cytological changes were observed in the globular cells of the LH-RH-treated young female loaches. One hour after the injection, small granules and large globules in the cells were almost unchanged in number, and cisternae of the rough endoplasmic reticulum was as flat as those of unaffected cells. However, the Golgi apparatus in the affected

cells became highly active with immature granules appearing frequently within the Golgi field (Fig. 26). After 24, 48 and 72 hours post-injection, the cells showed a gradual decrease in number of small granules and a progressive dilation of cisternae of the rough endoplasmic reticulum (Fig. 27). The large globules appeared to be unchanged in number and in electron density. The Golgi apparatus was moderately active and mitochondria were seen to be increased in size.

On the other hand, while no notable structural changes were detected in the vesicular cells of adult fish of both sexes after 1,24 and 48 hours of the LH-RH injection (Figs. 23 and 24), they became to exhibit some active features after 72 hours. At that time, the cells increased in number and in size. Small granules in their cytoplasm increased slightly in number (Figs. 28 and 29). Cytoplasmic vesicles of the cells were relatively uniform and small in size in females, but many of them were evidently larger in size in males (compare Fig. 28 with Fig. 29). Well-developed Golgi apparatus and enlarged mitochondria were encountered in the vesicular cells of the LH-RH-injected adult loaches of both sexes.

In the vesicular cells of young females, too, there were no promintnt cytological changes 1 hour after injection (Fig. 26). After 24, 48 and 72 hours, the vesicular cells progressively displayed a slight increase

in size of cytoplasmic vesicles, but the number of small granules and large globules were almost unchanged (Fig. 27). The Golgi apparatus appeared to become somewhat active and mitochondria were increased in size in the cells.

(2) Effects of clomiphene citrate

Thirty six adult female loaches, 9.9-17.3 cm in total length and 4.5-18.1 g in body weight, were collected in middle May, 1976. At the start of experiment, 10 females were sacrificed and served as initial controls. The other 26 fish were divided into control and experimental groups, and were kept in separate glass aquaria with well-aerated water under natural light condition. Water temperature was not regulated and varied from 13 to 15 °C. They were not fed during the experiment.

Clomiphene citrate dissolved in 0.6% saline at the concentration of 1 μg/0.01 ml was injected at a dose of 1 μg/g body weight intraperitoneally into females once a day or every third day. Control fish were injected with 0.6% saline only. All of the experimental and control fish were examined for the occurrence of ovulation by stripping them every day, and those that had been ovulated were autopsied. Fish of the control group were sacrificed at the end of experiments lasting for 10 and 15 days.

In all the fish that had been induced to ovulate by

clomiphene treatment, most of the basophilic cells in the PPD showed a remarkable decrease in their stainability to AF, though thyrotropic cells existing in the dorsal PPD showed no prominent change in the stainability (compare Fig. 30 with Fig. 31). The globular cells were observed to be highly vacuolated, and their nuclei became dark with indented contour (Fig. 33). The vesicular cells were also seen to be prominently vacuolated, but their nuclei exhibited little change (Fig. 33). Ultrastructurally, too, the globular cells showed a distinct decrease in number of small granules. Some large globules in the cells became more electron dense and others appeared to be filled with finely granulated material of rather low electron density (Figs. 34 and 35). The rough endoplasmic reticulum of these cells was composed of numerous cisternae dilated conspicuously in various degrees. The Golgi apparatus was moderately developed and mitochondria were seen to be much elongated.

Changes in the vesicular cells were essentially similar to those found in the globular cells. Small granules in their cytoplasm were clearly diminished in number, while large globules appeared to have increased their electron density and become irregular in shape in some cases (Fig. 36). Cytoplasmic vesicles characteristic of the cells were evidently larger in size than those in the cells of control fish, but the change was less prominent than that in the globular cells in most cases (Fig. 37).

No notable change was detected in mitochondria and the Golgi apparatus, except for a possible increase in size of mitochondria in the vesicular cells.

(3) Effects of castration

Gonadectomy of adult loaches was carried out in October 1977 and in April 1978. The experiment was repeated in June 1978 when the fish were at the late prespawning period, producing essentially similar but more conspicuous results than before. In this case, 10 females and 6 males, ranging from 8.3 to 11.4 cm in body length and from 3.9 to 9.0 g in body weight, were subjected to bilateral surgical gonadectomy through a mid-ventral incision. They were kept in aquaria containing well-aerated 0.6% NaCl solution under natural light and temperature, which ranged from 15 to 20 °C, and were fed commercial fish food once a day. Under these rearing conditions, the fish were well maintained during the experimental period. Autopsy confirmed complete gonadectomy by the absence of gonadal tissue of the fish operated. Another 4 fish of each sex served as controls. Half the number of these fish were killed after 30 and 60 days post-operation for the examination of their pituitaries.

Gonadectomy of the loach at the late prespawning period elicited some structural changes in the globular and vesicular cells of the pituitaries, but the responses

appeared to be different in structural aspects and in degrees between the two cell types and even between the different sex of the fish. In the PPD of females killed 30 days after ovariectomy, the globular cells were observed to be increased in size mainly due to a dilatation to varying extents of cisternae of the rough endoplasmic reticulum. In the cytoplasm of these cells, small granules were slightly decreased in number but large globules appeared to remain unchanged, when compared with those in the cells of control females. The globular cells became to show a pronounced hypertrophy 60 days after ovariectomy (Fig. 38). Cisternae of the rough endoplasmic reticulum were dilated extensively to form an irregular vacuolar system occupying most of the cytoplasm, giving to the cells a spongy feature rather than the so-called signet-ring appearance. The contents of the dilated cisternae were generally transparent. Small granules in these cells became scanty in amount, while large globules showed little change in their number though they appeared to become low in electron density (Fig. 39). An enlargement of Golgi areas and of mitochondria was also noticed in the cytoplasm.

In contrast, any prominent modifications were not detectable in the vesicular cells of ovariectomized fish even after 60 days post-operation (Figs. 38 and 39). It was found only that the cytoplasmic vesicles characteristic of the cell type were somewhat diversified in

size and in electron density as compared with those in the cells of control females.

In castrated males, changes of the globular cells were not so marked as those found in spayed females (Fig. 40). Even after 60 days post-operation, there was only a tenuous progress of a dilatation of cisternae of the rough endoplasmic reticulum in the globular cells. Small granules and large globules in their cytoplasm mostly remained unchanged in number and in electron density, though some of the large globules were seen to show a higher electron density. The Golgi apparatus consisting of numerous parallel lamellae and minute vesicles appeared to be moderately active in these cells (Fig. 41).

The vesicular cells showed evident changes in male loaches subjected to castration. In the cells 30 days after castration, their cytoplasmic vesicles were slightly increased in size, and both small granules and large globules were decreased in number. Cytoplasmic vesicles of the cells 60 days after castration were round in shape and rather uniform in size (Fig. 41), but a few of them were extraordinarily large on some occasions (Fig. 42). These features differed from dilated cisternae of the rough endoplasmic reticulum found in the globular cells affected by castration. Moreover, in the vesicular cells 60 days after castration, small granules were eminently decreased in number and large globules were

scarcely detected (Figs. 41 and 42). The Golgi apparatus was rarely observed in these cells.

(4) Effects of sex steroids

Two series of experiments were carried out using adult male and female loaches as material. In the first series, which was conducted from June to July 1978, 16 loaches of both sexes, 9.0-11.7 cm in body length and 4.2-7.7 g in body weight, were divided into 4 groups of 4 fish each. Estradiol-17β at a dose of 50 μg and testosterone at a dose of 100 μg per fish were administered to 4 females and 4 males, respectively. The second series of experiment began at the end of May 1979, with 12 fish of both sexes of 8.2-11.4 cm in body length and 3.0-8.0 g in body weight. In this series, 3 males and 3 females were injected hetrogeneously with estradiol-17β at a dose of 50 μg and testosterone at a dose of 100 μg per fish, respectively. The hormones, which were previously dissolved in ethanol, were added to 0.6% saline and were injected intraperitoneally into the fish 6 times at 5-day intervals. Autopsies were done on the treated fish along with saline-injected controls 5 days after the last injection. The fish were kept in separate glass aquaria of constantly aerated 0.6% NaCl solution under natural light and temperature, and were fed on commercial pellets once a day.

In both series of experiments, ovaries of control

fish were filled with many oocytes at developmental stages near the end of vitellogenesis, and testes of control fish had many germ cell cysts in various spermatogenetic stages. Following injections of estradiol, the yolk-laden oocytes were seen to be undergoing degeneration. Testes of males treated with estradiol contained a considerable amount of mature spermatozoa, but germ cells in intermediate stages of spermatogenesis were scarcely found in seminal lobules.

In pituitaries of estrogen-treated female loaches, the globular cells were clearly diminished in size (Fig. 43). Their small granules were smaller in size, measuring 100-350 nm, in comparison with those of the cells of control females, though they were increased in amount remarkably (Fig. 44). Large globules remained unchanged in number but became irregular in shape and higher in electron density. Cisternae of the rough endoplasmic reticulum were reduced in amount and were mostly flattened. Ill-developed Golgi apparatus and highly elongated mitochondria were also present in these cells.

On the other hand, only slight changes occurred in the vesicular cells of estradiol-treated females (Fig. 43). Cytoplasmic vesicles of these cells were relatively uniform in size and were similar in aspects to those of the control fish. Granular inclusions of the cells remained almost unaffected by the estrogen treatment, except for large globules that tended to augment their size.

It was noticed, however, that the Golgi apparatus in the vesicular cells of estrogen-treated females appeared to be very active, consisting of extended parallel lamellae and numerous minute vesicles, with immature granules appearing frequently within the Golgi field (Fig. 44).

Similar structural changes occurred also in the two types of cells in male loaches following estrogen treatment (Fig. 45). In the cytoplasm of the globular cells, small granules were clearly increased in number, and large globules appeared to be highly in electron density. The rough endoplasmic reticulum was reduced in amount, and the Golgi apparatus was poorly developed in these cells (Fig. 46). In the vesicular cells, cytoplasmic vesicles were rather uneven in size, but their granular inclusions were almost unchanged in number and in size. The Golgi apparatus was highly active, sometimes being accompanied with immature granules (Fig. 47). These agreed well with the features of the vesicular cells in estrogen-treated female loaches.

Testosterone injected into male loaches at the late prespawning period exerted no notable influences on their testes in which germ cell cysts at various spermatogenetic stages were maintained along with a large amount of spermatozoa accumulated in the lobule lumen. Ovaries of testosterone-treated females also showed little change in histological aspect: many oocytes in the tertiary yolk stage were still as healthy in androgen-

affected ovaries as in control ones.

The testosterone treatment caused some characteristic changes in the globular and vesicular cells of the loach of both sexes (Figs. 48 and 51). In the globular cells of testosterone-treated males and females, there was a notable increment in amount of small granules which were reduced in size to 150-450 nm as was the case for the cells of estrogen-treated females. Large globules were also increased in number and in electron density. Cisternae of the rough endoplasmic reticulum were often observed to be dilated to various extents in the cells of testosterone-treated fish of both sexes, though the dilatation was more conspicuous in males than in females (compare Fig. 49 with Fig. 52). The Golgi apparatus and mitochondria in these cells were not significantly influenced by the androgen treatment.

The testosterone treatment provoked interesting modifications in the vesicular cells in treated males. Cytoplasmic vescicles in these cells were conspicuously diminished in size to become flattened in some places of the cytoplasm. In addition, small granules in their cytoplasm were considerably larger in amount and smaller in size, measuring 100-400 nm, while large globules were increased in number and in electron density. The Golgi apparatus was moderately developed with a few minute vesicles, and mitochondria were somewhat elongated in these cells (Fig. 50). On the other hand, the vesicular

cells of female fish were observed to be almost unresponsive to the testosterone treatment (Fig. 51). Cytoplasmic vesicles of the cells were round in shape and contained somewhat low electron dense contents. The number and the size of their granular inclusions were almost similar to those of the cells of controls. The Golgi apparatus appeared to be moderately active in these cells (Fig. 53).

(5) Conclusion

Following the treatment of prespawning loaches with LH-RH or clomiphene citrate, changes such as cellular hypertrophy, degranulation and dilatation of endoplasmic reticulum were prominent exclusively in the globular cells of the PPD of the pituitary gland. Cells of this type were also conspicuous for their characteristic responses to gonadectomy, though changes of the cells were less prominent in castrated males than in ovariectomized females. In addition, the globular cells became atrophied after the treatment with sex hormones in either sex of the loach. Therefore, it seems reasonable to consider the globular cells to be conventional gonadotrophs.

The vesicular cells of the loach also exhibited some structural changes following the treatment with LH-RH or clomiphene citrate, but the changes were less obvious than those found in the globular cells. The

cells of female loaches responded also to ovariectomy, though the response was rather slight when compared with that of the globular cells. In the vesicular cells of male loaches, by contrast, complete castration brought about an enlargement of cytoplasmic vesicles and a decrease in the amount of both small granules and large globules. Estrogen treatment stimulated the vesicular cells to develop the synthetic activity of the Golgi apparatus in the fish of both sexes. On the other hand, in androgen-treated male loaches, the vesicular cells were observed to regress with notable shrinkage of their cytoplasmic vesicles. These observations indicate that the vesicular cells also relate functionally to gonadal activities in male and female loaches.

The results obtained from the present experiments not only disclose the presence of two distinct types of pituitary gonadotrophs in the loach, but also suggest the difference in functional significance concerning the control of gonadal activities between the two cell types. No transitional types of cells from one to the other of the two cell types were detectable in the pituitary gland by the present experimental studies. It is noted that the two cell types always retain their respective cytological characteristics and remain strikingly distinguishable from each other even in the experimental conditions that would cause extreme functional alterations of gonadotropic cells. It is thus concluded that the loach,

Misgurnus anguillicaudatus, have two distinct types of gonadotropic cells in the pituitary gland, and that the two gonadotropic cell types may play different roles in different stages of sexual development in either sex of the loach.

3. Cytological changes of the two types of gonadotrophs

To solve the problem as to when the two types of gonadotrophs may exert their respective gonadotropic potencies, it seems effective to carry out careful cytological studies on changes of the pituitary cells both during the course of gonadal development in juvenile stages and during the reproductive cycle of adults.

(1) Changes during gonadal development in juvenile loaches

a) Females

Juvenile female loaches killed in November, averaging 5.0 cm in body length and 0.8 g in body weight, had quite immature ovaries in which oogonia were the sole germinal component. In these fish, only a few alcian blue- and PAS-positive cells were distributed exclusively in the central part of the PPD. These cells were mostly oval or elongate in shape with oval-shaped nuclei (Fig. 54). These cells contained many small, electron dense granules of 150-300 nm in size and a few large, less electron dense globules of 400-600 nm in size (Fig. 55).

The rough endoplasmic reticulum was composed of flat cisternae. The Golgi apparatus was poorly developed. Mitochondria were few in number and small in size. These cytological features of the cells appeared to correspond to those of the globular cells. No typical cells of the vesicular type could be detected in the fish. However, some cells with clear cytoplasm were present in the dorsal and central parts of the PPD (Fig. 54). In these cells, cytoplasmic organelles were scarcely present (Fig. 55). These cells were termed the non-granulated cells in the present study. Somatotrophs and thyrotrophs in the fish were observed to be similar in structural features to those of adult fish.

In juvenile females sampled in January, averaging 6.7 cm in body length and 1.5 g in body weight, the ovaries had oocytes in the peri-nucleolus stage. In the pituitary gland of these fish, the globular cells were observed to have slightly increased in number in the ventral and central parts of the PPD (Fig. 56). However, the cells showed little change in their ultrastructural aspects in comparison with those observed in the previous stage (Fig. 57). In their cytoplasm, small granules were slightly enlarged to 200-400 nm in size, and the large globules also became larger in size, attaining 400-800 nm in size. The rough endoplasmic reticulum consisted of flat or small vesicular cisternae. Although the vesicular cells were not be detectable yet in females

at that stage of gonadal development, the non-granulated cells were slightly increased in number (Fig. 56).

Yearling females sacrifieed in April, measuring 8.0 cm in body length and 2.5 g in body weight on an average, had ovaries with many oocytes of the yolk vesicle stage. The globular cells in their pituitaries showed almost the same features as those found in the preceding stage. In these fish, however, some cells with clear vacuoles in the cytoplasm appeared in the central and dorsal parts of the PPD. These cells were strongly positive to PAS, but were almost negative to alcian blue. They were round or oval in shape and had mostly round-shaped nucleus (Fig. 58). The rough endoplasmic reticulum of the cells was composed of rounded vesicular cisternae which contained material of low electron density (Fig. 59). Small granules of 100-300 nm in size were accumulated in groups in their cytoplasm, and a few large globules of 500-800 nm in size were also present. The Golgi apparatus was moderately active and rod-shaped mitochondria occurred in the juxtanuclear region. These cells were thought to be vesicular cells that had differentiated during that stage of the life-cycle, presumably from the non-granulated cells pre-existing in the PPD, since the non-granulated cells could hardly be detected in the pituitary gland of the fish at that stage of development.

In May, ovaries of yearling females, measuring 8.5

cm in body length and 3.0 g in body weight, had many oocytes in the primary yolk globule stage. In the fish, a considerable number of the vesicular cells were found in the dorsal and central regions of the PPD. These cells were rich in cytoplasm which contained many clear vacuoles by light microscopy (Fig. 60). The cytoplasmic vacuoles coincided electron microscopically with rounded vesicles of the rough endoplasmic reticulum with finely granulated contents of a moderate electron density. In the cytoplasm of the vesicular cells, small granules of 100-400 nm in size were slightly increased in number, while large globules appeared to remain unchanged in number and in size (Fig. 62). The Golgi apparatus were rarely observed in the cells. On the other hand, although some of the globular cells at this stage retained a similar morphological aspect to those at the previous stage, others exhibited some activated features: their small granules of 150-450 nm in size were somewhat increased in number, and cisternae of the rough endoplasmic reticulum were dilated to some extents (Fig. 61). The Golgi apparatus was also moderately active in these cells.

In yearling females sampled during the months from June to August, which averaged about 9.0 cm in body length and about 5.0 g in body weight, ovaries were filled with many oocytes advancing to the secondary and tertiary yolk stages. In the pituitaries of these fish,

the globular cells were observed to have increased in number and in size (Fig. 63). In their cytoplasm, a lot of small granules were increased evidently in size attaining 200-500 nm, and large globules were also increased both in number and in size measuring 600-1200 nm (Fig. 64). Cisternae of the rough endoplasmic reticulum were dilated to various extents and contained electron lucent material. The Golgi apparatus was highly active, and sometimes immature granules were present within the Golgi field. In contrast, no notable cytological change was observed in the vesicular cells when compared with those found in yearling females collected in May (Fig. 63). Dilated vesicles of the cells were relatively uniform in size, and both small granules and large globules were few in number (Fig. 65).

In September, adult females that had spawned measured 9.5 cm in body length and 5.0 g in body weight on an average. In these fish, the globular cells displayed a degenerative feature (Fig. 66). The nuclei of the cells were quite irregular in shape and unusually dense. Large globules in the cells appeared to remain unchanged in number, and they were filled with a finely granulated material of low electron density. Small granules remained unchanged in size and electron density. The rough endoplasmic reticulum became composed of numerous cisternae dilated to various degrees (Fig. 67). The Golgi apparatus were sparse in these cells. On the other

hand, no significant changes were detected in the vesicular cells of postspawning females by light and electron microscopy (Figs. 66 and 68).

b) Males

Juvenile males sampled in November, measuring 5.3 cm in body length and 1.0 g in body weight, had immature testes with spermatogonia only. In these fish, there were only a few globular cells in the central part of the PPD, as was the case in juvenile females of the same period (Fig. 69). The cells contained many small granules of 100-300 nm in diameter together with a few large globules of 400-600 nm in diameter (Fig. 70). Flattened cisternae of the rough endoplasmic reticulum were sparse, the Golgi apparatus were poorly developed, and small-sized mitochondria were located in a corner of the cells. There were also some non-granulated cells in the central and dorsal parts of the PPD in these fish (Figs. 69 and 70).

In juvenile males collected in January, a small number of cysts of the first spermatocyte stage were present in the testis together with many spermatogonia. In the pituitary of the fish, many granulated cells of the vesicular type had been differentiated in the dorsal and central parts of the PPD (Fig. 71). Cytoplasmic vesicles in these cells were relatively uniform in size, but a few of them were extraordinarily large on some occasions (Fig. 72). Small granules of 150-300 nm in size

and large globules of 400-600 nm in size were few in number, the Golgi apparatus appeared to be moderately active, and rod-shaped mitochondria were few in number in these cells. The globular cells in the fish were almost similar in structural features to those observed in the previous stage.

In yearling males sampled during the period from April to August, ranging 7.0-8.0 cm in body length and 2.0-3.5 g in body weight, germ cell cysts in various spermatogenetic stages were present in their testes, and a varying amounts of spermatozoa were always accumulated in expanded lumina of testicular lobules. In the PPD of these fish, the vesicular cells showed little change when compared with those of the preceding period (Fig. 73). In the cytoplasm of these cells, small granules were attained 150-450 nm in size and large globules were measured 500-800 nm in size (Fig. 75). Cytoplasmic vesicles of the cells were uniformly round in shape. The Golgi apparatus was moderately active. In contrast, the globular cells became to exhibit a highly active feature in harmony with gonadal development (Fig. 73). They were increased in size and in number, and their small granules were clearly increased in number and in size, measuring 200-500 nm in diameter, while large globules remained mostly unchanged in number and in electron density. The rough endoplasmic reticulum of these cells was composed of flat or small vesicular cisternae, and was scarcely

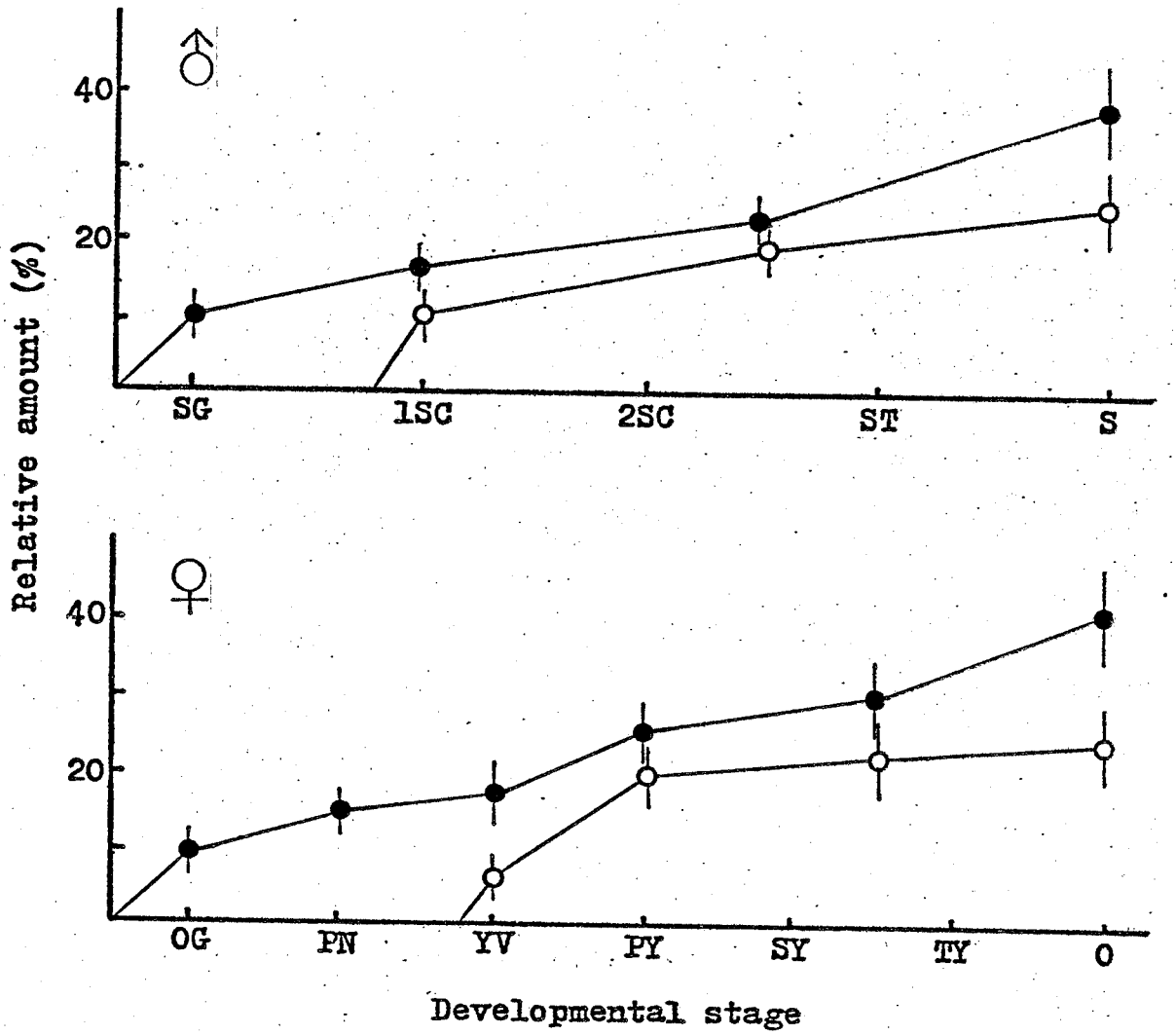
dilated (Fig. 74). The Golgi apparatus was highly active in the cells.

In September, males measuring 8.5 cm in body length and 4.3 g in body weight had already been potentiated to discharge the milt when stripped. In these fish, most of the globular cells of the PPD revealed degenerative changes just like those found in females after spawning (Fig. 76). The nucleus of the cells was of irregular shape and was conspicuously dense. Their cytoplasmic organelles appeared to become condensed and indistinct (Fig. 77). In the vesicular cells, however, such degenerative changes were never detected, and were seen to be similar in feature to those observed in the preceding period. Their characteristic cytoplasmic vesicles were relatively uniform in size, and their granular inclusions remained to be few in number (Fig. 78).

Changes in the relative amount of the globular and vesicular cells in the PPD of the pituitary gland of the loach during the course of gonadal development were summarized in Text-fig. 2. The relative amount of the cells was estimated by counting the whole number of glandular cells occurring in midsagittal sections of the PPD and calculating the percentage of the appearance of each types.

(2) Changes during the reproductive cycle of adult loaches

The gonad of adult loaches changes its weight

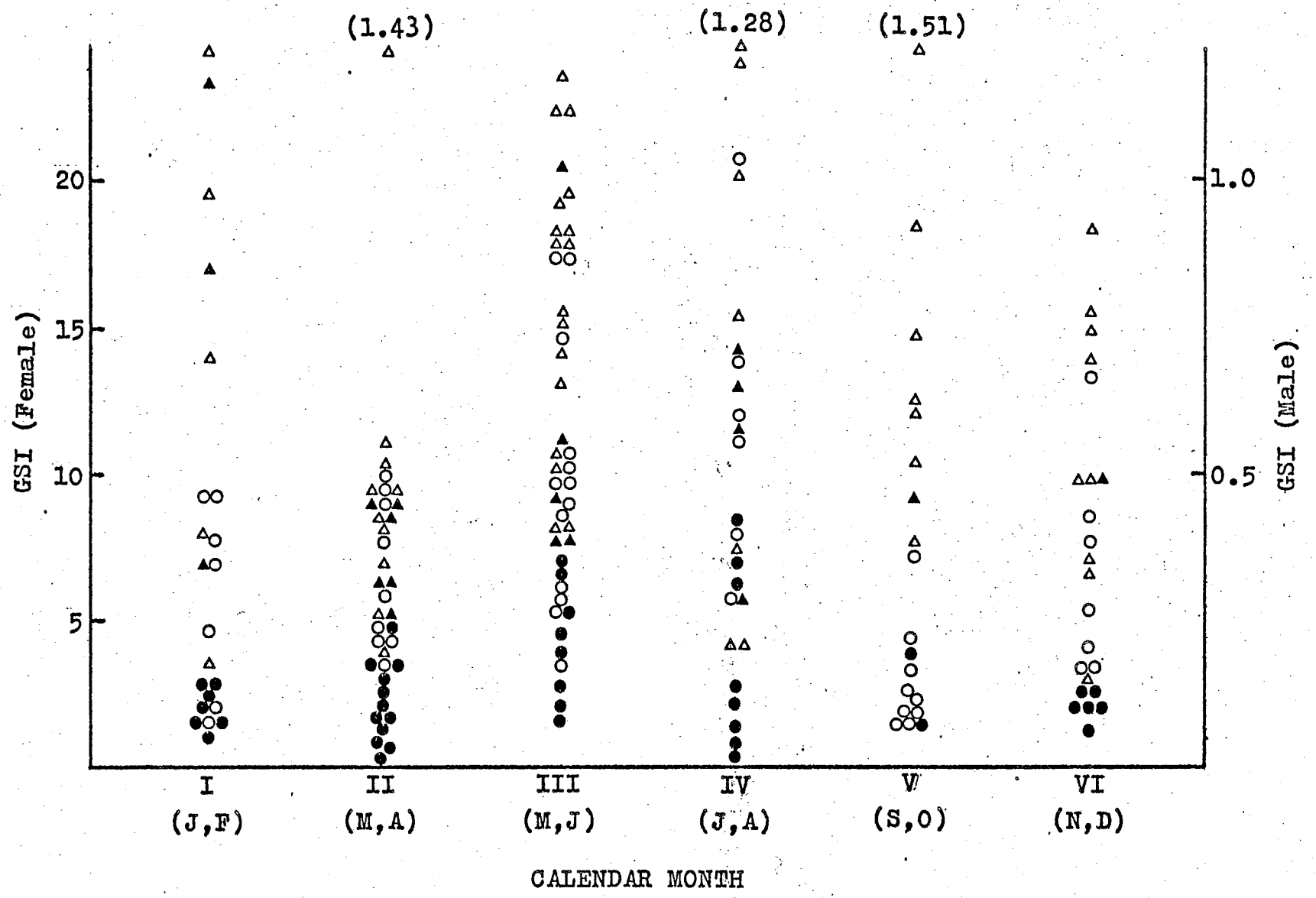


Text-fig. 2. Changes in the relative amount of the globular cells (●) and the vesicular cells (○) in the pituitary gland of female (lower side) and male loaches (upper side) during the course of gonadal development. OG, oogonium stage; PN, peri-nucleolus stage; YV, yolk vesicle stage; PY, primary yolk stage; SY, secondary yolk stage; TY, tertiary yolk stage; O, ovulation stage; SG, spermatogonium stage; 1SC, first spermatocyte stage; 2SC, second spermatocyte stage; ST, spermatid stage; S, spermiation stage. Mean ± SE.

annually in harmony with its maturational state. The change of gonadal weight is shown in Text-fig. 3 in which gonadal weight is expressed in terms of the gonadosomatic index (GSI: gonad weight X 100/body weight). For convenience, in the present study, six sequential stages (I-VI), which were composed of two successive months each, were set through the year. Cytological changes of the gonadotrophs of the loach were examined in each of the six stages of a reproductive cycle using adult females and males larger than 9.5 and 8.3 cm in body length, respectively.

a) Females

In the stage I (January and February), ovaries of adult females had many oocytes in the primary yolk stage or in the yolk vesicle stage. In pituitaries of these fish, a considerable number of the vesicular cells were found in the PPD (Fig. 79). Their characteristic cytoplasmic vesicles were relatively small and uniform in size. In their cytoplasm, both small granules and large globules were few in number, and the Golgi apparatus was rarely observed (Fig. 80). The globular cells in these fish were a few in number (Fig. 79). Their small granules were few in number and small in size, measuring 100-400 nm, and large globules were low in electron density. The rough endoplasmic reticulum was dilated slightly, and the Golgi apparatus was sparse in these cells (Fig. 81).



Text-fig. 3. Annual changes of gonadosomatic index (GSI) of female loaches above (○) or below (●) 9.5 cm in body length, and of male loaches above (△) or below (▲) 8.3 cm in body length.

In females sampled in the stage II (March and April), there were many oocytes advancing to the primary and secondary yolk stages in their ovaries. In these fish, the globular cells were increased slightly in number and in size to exhibit some active features (Fig. 82). The number of small granules in these cell were slightly increased, while large globules remained unchanged in number but became more electron dense. Cisternae of the rough endoplasmic reticulum were not dilated, being composed of irregular vesicles or flattened lamellae. The Golgi apparatus appeared to be moderately active (Fig. 83). On the other hand, there were little structural changes in the vesicular cells in these fish. Cytoplasmic vesicles of the cells were larger in size than before, but were still uniform in shape. Both small granules and large globules were still in a small number. The Golgi apparatus was rarely observed in the cells (Fig. 84).

Adult females killed in the stage III (May and June) had ovaries with many oocytes in the secondary and tertiary yolk stages. In pituitaries of these fish, the globular cells were observed to have clearly increased in number and in size with an evident increment of their granular contents (Fig. 85). In their cytoplasm, small granules were increased evidently in number, while large globules remained unchanged in structural aspects. It was notable in this stage that cisternae of the rough

endoplasmic reticulum became dilated irregularly throughout the cytoplasm. The Golgi apparatus was highly active around which immature granules appeared frequently (Fig. 86). On the other hand, only a slight change occurred in the vesicular cells in these fish. Cytoplasmic vesicles of the cells were relatively uniform but smaller in size than those in the previous stages. Granular inclusions of the cells remained almost unchanged, though large globules were seen to be somewhat high in electron density. The Golgi apparatus was slightly active in the cells (Fig. 86).

In the stage IV (July and August), loaches were at the peak of their spawning period. In pituitaries of the females which had ovulated, the globular cells displayed remarkable changes (Fig. 87). These cells were shrinked considerably among other glandular cells, and their cytoplasm as well as their nucleus was quite irregular in contour and unusually electron dense. In the dark cytoplasm, large globules tended to gather in groups and small granules were clearly decreased in number. The rough endoplasmic reticulum was dilated to various extents and contained materials of moderate electron density. The Golgi apparatus was obscure in the cells (Fig. 88). In contrast, such a remarkable change was never detectable in the vesicular cells (Figs. 87 and 88). Their cytoplasmic vesicles were still small in size. Although small granules were almost unchanged in

number, large globules became rarely observed in the cells. The Golgi apparatus was somewhat active in features in the vesicular cells.

In adult females collected in the stage V (September and October), ovaries had many postovulatory follicles together with yolkless oocytes. In these fish, although some of the globular cells occurring in the PPD were still in the degenerative phase as mentioned above, others became regranulated with many small granules and a few large globules in clear cytoplasm (Figs. 89 and 91), possibly indicating a recovery from functional exhaustion. On the other hand, the vesicular cells were seen to be larger in size and in number than those in the previous stages (Fig. 89). They contained cytoplasmic vesicles which were evidently larger in size than those observed in stage IV. Small granules in their cytoplasm were relatively abundant and existed in groups among the dilated vesicles, while large globules were still few in number. The Golgi apparatus was moderately active (Fig. 90).

In the stage VI (November and December), ovaries of adult females had some postovulatory follicles and many oocytes of the yolk vesicle stage. In the pituitary gland of these fish, although globular cells had mostly been recovered to show distinct regranulation, they were still few in number as those found in stage I (Fig. 92), and their rough endoplasmic reticulum and the Golgi

apparatus were poorly developed (Fig. 93). The vesicular cells exhibited, however, some active features (Fig. 92). Their cytoplasmic vesicles were rather uneven in size, and some of them were atypically dilated (Fig. 93). Small granules were clearly diminished in number and large globules were scarcely detected in these cells.

b) Males

Adult males sampled in the stage I had testes with a large amount of spermatozoa besides a few spermatogonia, primary spermatocytes and spermatids. In pituitaries of these fish the globular cells were observed to be in an inactive state (Fig. 94). The nucleus of the cells was irregular in contour and electron dense. Their granular inclusions mounted in concentrated cytoplasm were relatively small in amount. Cisternae of the rough endoplasmic reticulum were dilated to some extents, and the Golgi apparatus was scarcely detected (Fig. 95). On the other hand, the vesicular cells were seen to present some active features: some of their cytoplasmic vesicles showed a clear hypertrophy, small granules were relatively few in number, and large globules were rarely present (Fig. 95). The Golgi apparatus of these cells was moderately active.

In testes of adult males sampled in the stage II, spermatogonia were clearly increased in number whereas mature spermatozoa were reduced in amount. In the pituitary gland, the globular cells became loaded in expanded

and clear cytoplasm with small granules of 100-300 nm, which were much smaller in size than those in the other stages, and with large globules of 400-600 nm in size (Figs. 96 and 97). The rough endoplasmic reticulum consisted of flat cisternae and was never dilated. The Golgi apparatus was moderately active. The vesicular cells in these fish revealed, however, only a slight change in the size of cytoplasmic vesicles which became to be relatively uniform in size (Figs. 96 and 97).

In the stage III, testes of adult males contained many cysts of spermatocytes, spermatids and spermatozoa together with a decreased number of spermatogonia. In their pituitaries, the globular cells were observed to become numerous and hypertrophied (Fig. 98), and small granules in the cytoplasm were evidently increased in number and in size, measuring 200-500 nm in size. Large globules also became augmented in size to 600-1200 nm. Cisternae of the rough endoplasmic reticulum were not dilated but remained flat. The Golgi apparatus appeared to be highly active in feature (Fig. 99). On the other hand, no notable structural changes were observed in the vesicular cells in these fish (Figs. 98 and 100).

Adult males killed in the stage IV at the peak of their breeding activities, had testes in which germ cell cysts at various spermatogenetic stages were present along with a large amount of spermatozoa accumulated in lobule lumina. In the pituitary gland, the globular

cells seemed to be highly active (Fig. 101). Cisternae of their rough endoplasmic reticulum were dilated to form an irregular vacuolar system (Fig. 102). Small granules in these cells were larger in amount than those in the previous stage, and large globules appeared to be high in electron density. The Golgi apparatus was still active in these cells. In contrast, the vesicular cells showed only slight changes in that a few of their cytoplasmic vesicles, which were mostly uniform in shape, were extraordinarily large on some occasions (Fig. 103).

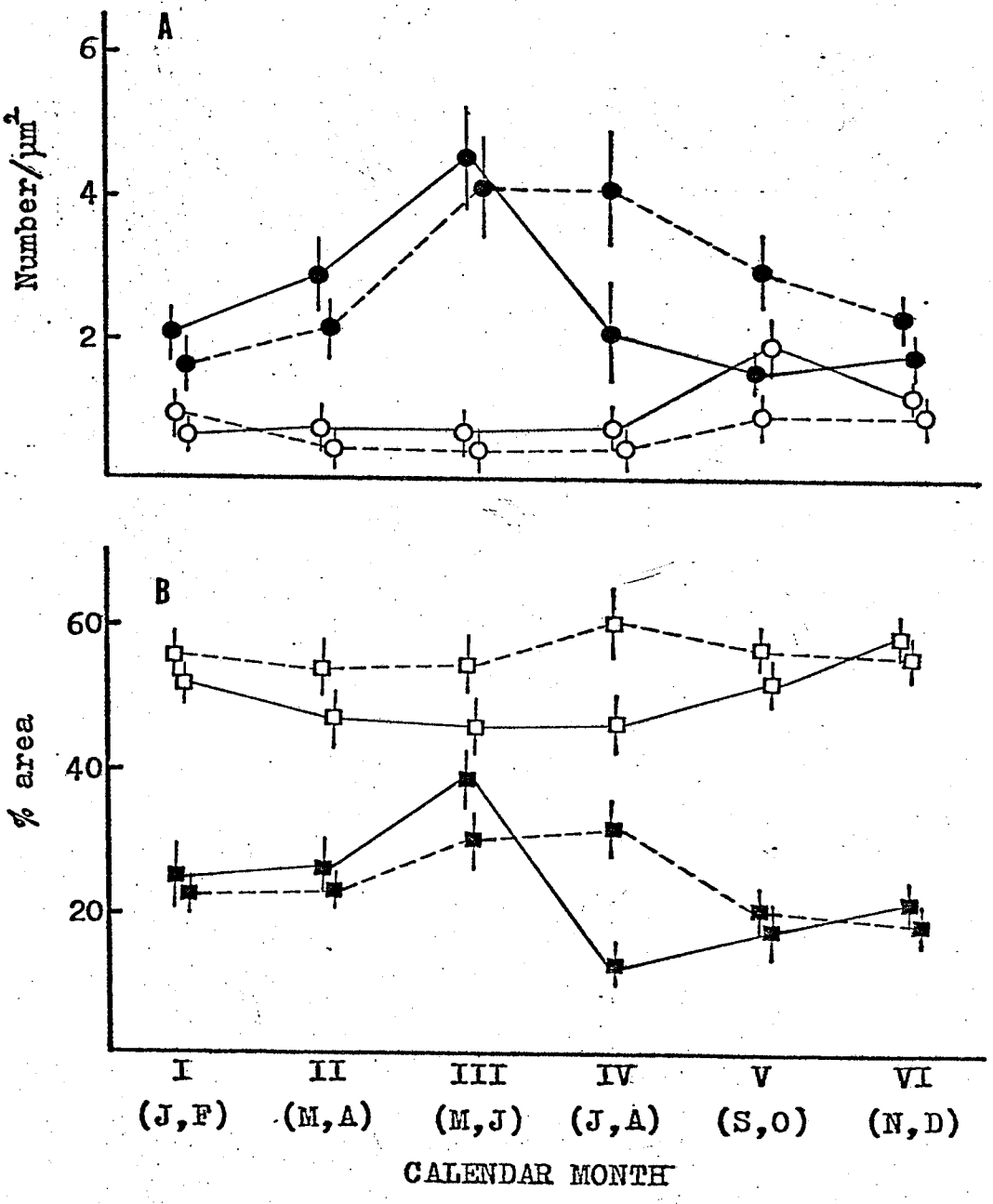
In the stage V when breeding of the loach had almost ceased, testes of adult males had a large amount of spermatozoa in widely expanded lobule lumina, though a small number of cysts of spermatocytes and spermatids still existed in testicular lobules. In the pituitary gland, the globular cells were decreased prominently in size and in number, displaying some degenerative features (Fig. 104). In their cytoplasm which showed shrinkage and was high in electron density, small granules were clearly diminished their number, while large globules were seen less electron dense though they remained unchanged in number. The rough endoplasmic reticulum was reduced in amount and never dilated (Fig. 105). The Golgi apparatus were hardly detected in the cells. On the other hand, the vesicular cells were quite similar in feature to those observed in the preceding stage, except that large cytoplasmic vesicles

appearing in the previous stage did not occur and small granules were somewhat increased in number in these cells at this stage (Fig. 106).

In adult males collected in the stage VI, a small number of first spermatocytes were present in their testes together with a few spermatogonia and many spermatozoa. In pituitaries of these fish, the globular cells were observed still to be in a regressed state (Fig. 107). The cells were quite irregular in contour and had darkly concentrated cytoplasm. Their nucleus was much electron dense and irregular in shape. Small granules, and large globules as well, in the cytoplasm were few in number, and cisternae of the rough endoplasmic reticulum were slightly dilated (Fig. 108). The vesicular cells were observed to increase in size and in number (Fig. 107). Their cytoplasmic vesicles were relatively uniform but slightly reduced in size. Small granules in the cytoplasm tended rather to be accumulated in groups, and large globules made their appearance in the cells of this stage (Fig. 109).

(3) Conclusion

Text-fig. 4 shows results of morphometric studies by a modification of Weibel's (1969) point-counting method on electron micrographs of the two types of pituitary gonadotrophs in the loach. From the figure, quantitative changes of rough endoplasmic reticulum



Text-fig. 4. Fluctuations in the number of small granules (A) and in the relative area of rough endoplasmic reticulum (B) in the globular cells (● and ■) and the vesicular cells (○ and □) of female (——) and male (---) loaches during the reproductive cycle. Mean ± SE.

and small (secretory) granules of these cells observed during the course of annual reproductive cycle are to be seen. It is concluded that the globular and vesicular cells of the loach have their respective gonadotropic potencies in different phases of the reproductive cycle. The globular cells displayed a notable increase in size and in number in harmony with progressive maturation of the gonads in the loach of both sexes. The cells revealed a conspicuous depletion of small granules, extensive dilation of the rough endoplasmic reticulum, and subsequent pycnotic changes of the nucleus in the females and males following ovulation and spermiation. In the loach, the globular cells may secrete a gonadotropin which promotes gonadal changes concerning ovulation and spermiation. The vesicular cells in the loach made their first appearance in the PPD concurrently with the appearance of the sign of exogenous vitellogenesis in ovarian oocytes or with the formation of spermatocytes in the testis. During the advancement of vitellogenesis and spermatogenesis, the vesicular cells did not show any conspicuous changes. The vesicular cells appeared to become active again, a few month after the spawning period, before exogenous vitellogenesis in ovarian oocytes and spermatogenesis in testes had taken place. Therefore, the vesicular cells may secrete a gonadotropin which is implicated in the onset of vitellogenesis and spermatogenesis in the loach.

4. Experimental modifications of gonadotropic cell function

It seems reasonable to consider that the globular cells in the pituitary gland of the loach may correspond to conventional gonadotrophs. The globular cells are conspicuous for their characteristic responses to gonadotropin releasing substances, gonadectomy and sex steroids. Moreover, the cells exhibit noticeable changes in clear association with gonadal maturation in the loach of both sexes. On the other hand, there are still some unclear problems in gonadotropic function of the vesicular cells. The vesicular cells also responded to the above-mentioned experimental treatments, but the responses were not as clear as those of the globular cells. The vesicular cells seemed to become cytologically active concurrently with the appearance of oocytes of the yolk vesicle stage in ovaries and first spermatocytes in testes, and showed little change during the advancement of vitellogenesis and spermatogenesis.

In an attempt to make the function of the vesicular cells clear, some further experiments were carried out using adult and juvenile loaches of both sexes. First, in order to promote vitellogenesis and spermatogenesis in the gonad, the fish were exposed to continuous light during the out-of-breeding season. Second, a starvation treatment was conducted to induce a gonadal regression. Third, to accelerate differentiation of the vesicular

cells in the pituitary gland of juvenile loaches, the fish were treated with sex steroids or human chorionic gonadotropin (HCG).

(1) Effects of continuous light

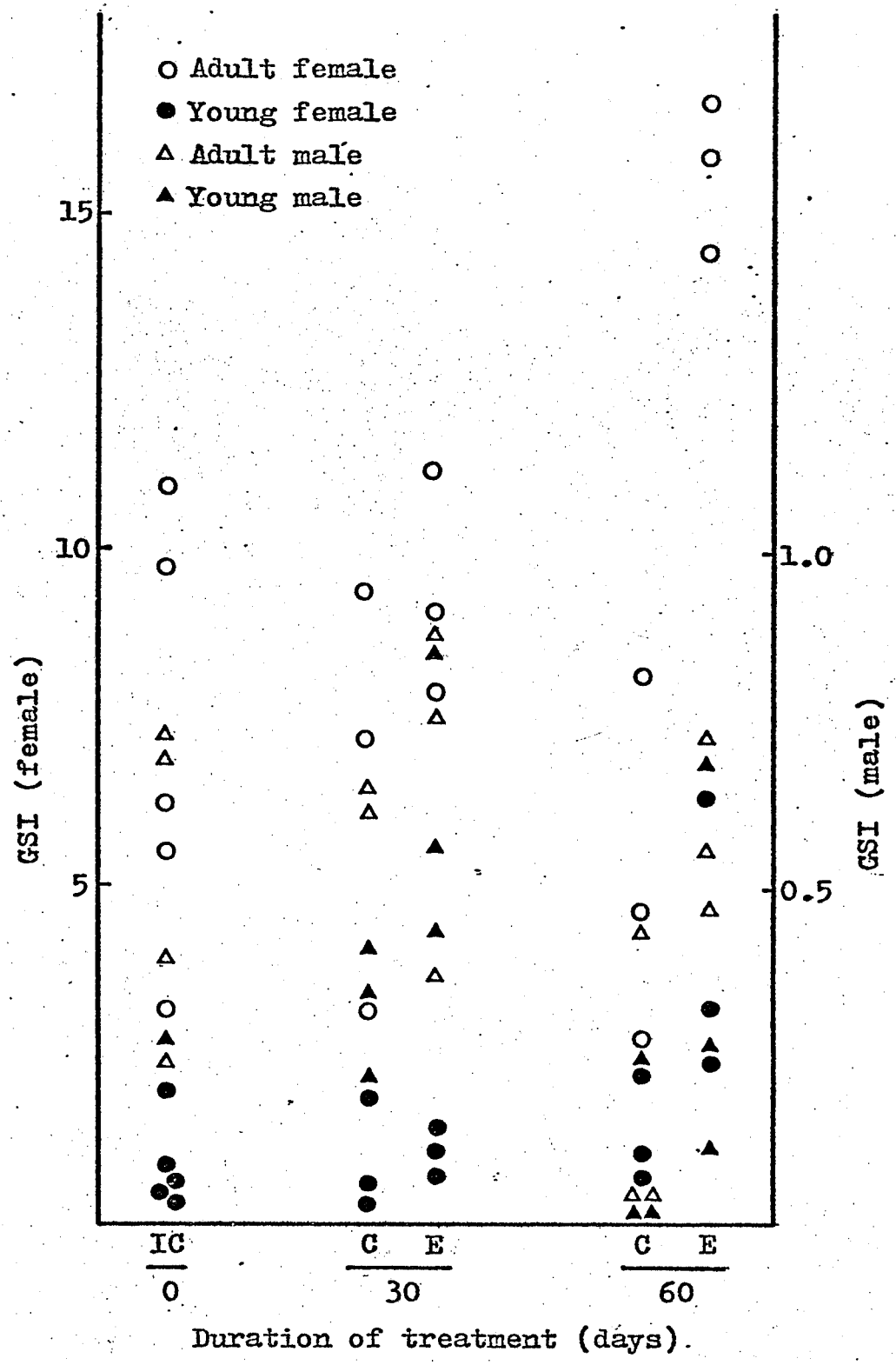
Since the spawning period of the loach is thought to cover about 3 months from June to August, the fish is considered to belong to the so-called long-day type. It is expected, therefore, that a long photoperiod in out-of-breeding season can induce gonadal development in the loach through an activation of gonadotropic functions of their pituitaries.

Thirty-two adult males and females, 8.5-12.5 cm in body length and 4.0-13.2 g in body weight, and 30 young fish of both sexes, 6.5-8.5 cm in body length and 1.8-3.5 g in body weight, were collected in late August when they were at the end of the spawning period. They were subsequently kept in glass aquaria with well-aerated 0.6% NaCl solution and were fed daily on commercial fish diet. The experiment lasted for 60 days from September 1 to October 31, 1979. At the start of experiment, 15 fish of both sexes were sacrificed for initial controls. Another group of 24 fish was exposed to continuous light in a room where room temperature was ranged from 18 to 22 °C. The other group of 23 fish, which served as controls, were kept under natural light and temperature which ranged about 15-20 °C. Fish of the experimental

and control groups were killed 30 and 60 days after the start of treatment.

Changes in GSI of fish of the control and experimental group were indicated in Text-fig. 5. Adult females of the initial control group had ovaries with many postovulatory follicles and yolk-laden, atretic oocytes. In ovaries of young females of the initial control group, the most advanced oocytes were at the peri-nucleolus stage. Both adult and young males of the initial control group had testes with germ cell cysts in various spermatogenetic stages including only a small amount of mature spermatozoa in lobule lumina. No significant difference was found in GSI values between the initial control fish and the control fish examined after 30 days of experiment. Following 60 days of experiment, GSI values of control females and males were decreaced slightly: ovaries of adult females showed only a slight advance in development, the most developed oocytes being in the primary yolk stage, those of young females still had oocytes in the peri-nucleolus stage, and testes of adult and young males were not different in histological aspects from those observed at the start of experiment.

Exposure of the fish to continuous light obviously caused a development of the gonad in both sexes. In the treated fish, GSI values were only slightly higher after 30 days of treatment, but evidently higher after 60 days when compared with those of the control



Text-fig. 5. Changes of gonadosomatic index (GSI) of the loach exposed to continuous light for 30 and 60 days during the post-spawning period. IC, initial control group; C, control group; E, experimental group.

fish (Text-fig. 5). Histologically, ovaries of treated females were filled with many oocytes up to the secondary yolk and tertiary yolk stages after 30 and 60 days of the light exposure, respectively. In ovaries of young females exposed to continuous light, oocytes of the yolk vesicle stage were clearly increased in number, and sometimes oocytes of the primary yolk stage were observed to appear on 60 days of the treatment. Testes of adult and young fish revealed conspicuous acceleration of spermatogenesis and enhancement of sperm content under the condition of constant light, though the changes were more remarkable after 30 days than after 60 days of treatment.

In pituitaries of adult females and males of the control groups, the globular cells were seen to be inactive cytologically (Fig. 110). Some of the cells displayed degenerative aspects with pycnotic nuclei and darkly cencentrated cytoplasm. In others, many small granules and a few large globules were present, and flat cisternae of the rough endoplasmic reticulum were found scattered throughout the cytoplasm (Fig. 111). In contrast, in the vesicular cells of these fish, characteristic cytoplasmic vesicles with contents of moderate electron density showed a dilation to varying degrees, which was suggestive of activated functions of these cells. The cells contained a few large globules and relatively many small granules together with

moderately active Golgi apparatus in the cytoplasm (Fig. 111).

In young females of the control groups, a few globular cells were present, but the vesicular cells were never detected to exist in the PPD (Fig. 112). The two kinds of granular inclusions of the globular cells were observed to be fewer in number than those in the cells of adult females. The rough endoplasmic reticulum of the cells was composed of flat or small vesicular cisternae, and the Golgi apparatus was moderately active (Fig. 113). In the PPD of young males of the control group, however, both the globular and vesicular cells had already existed (Fig. 114). The globular cells appeared to be the same in aspects as those of young females of the same group. The vesicular cells sometimes had large cytoplasmic vesicles (Fig. 115). Small granules were present, but large globules were not observed in these cells. The Golgi apparatus were scarcely detected.

In adult fish of both sexes exposed to continuous light for 30 and 60 days, the globular cells were increased in size and in number (Fig. 116). Their granular inclusions were also increased in number, and large globules were filled with finely granulated material of decreased electron density (Fig. 117). Cisternae of the rough endoplasmic reticulum became dilated conspicuously and the Golgi apparatus appeared to develope 60 days

after the treatment (Fig. 118). The vesicular cells also showed notable increases both in number and in size in males and females subjected to the treatment, and their hypertrophic changes were more prominent than those of the globular cell. Moreover, there were some structural differences in the vesicular cells between females and males affected by continuous light. In females after 30 days of constant illumination, cytoplasmic vesicles of the cells were relatively uniform in size, small granules were increased clearly in number, and large globules were scarcely detected (Fig. 120). In males, however, some cytoplasmic vesicles were extraordinarily large in size and occupied most of the cytoplasm of the cells (Figs. 119 and 121). Sixty days after treatment, cytoplasmic vesicles of the vesicular cells in females were further increased in volume, and small granules were greatly decreased in number (Fig. 122). In males, on the contrary, the size of the cytoplasmic vesicles became to decrease a little, and large globules began to appear in the cytoplasm (Fig. 123).

Similar but less conspicuous structural changes were observed in the globular cells of young males and females treated with continuous light for 30 and 60 days. The globular cells increased in number and in size, and showed some activated features (Fig. 124). Even after 60 days of treatment, the dilatation of the rough endoplasmic reticulum was less in degree, and granular inclusions

in the cells were fewer in amount than those in the globular cells of adult fish (Fig. 125). On the contrary, in the PPD of the pituitary gland of treated females after 30 days of treatment, a small number of the vesicular cells began to appear (Fig. 124). The cells were rich in cytoplasm and contained many small cytoplasmic vesicles. Their granular inclusions were few in number. The Golgi apparatus seemed to be highly active (Fig. 126). In young females killed after 60 days of treatment, the vesicular cells were increased clearly in number and in size, and their cytoplasmic vesicles were evidently larger in size than those in the cells observed after 30 days of treatment (Fig. 127). Small granules and large globules in their cytoplasm still remained unchanged in number. The Golgi apparatus was scarcely found in the cell.

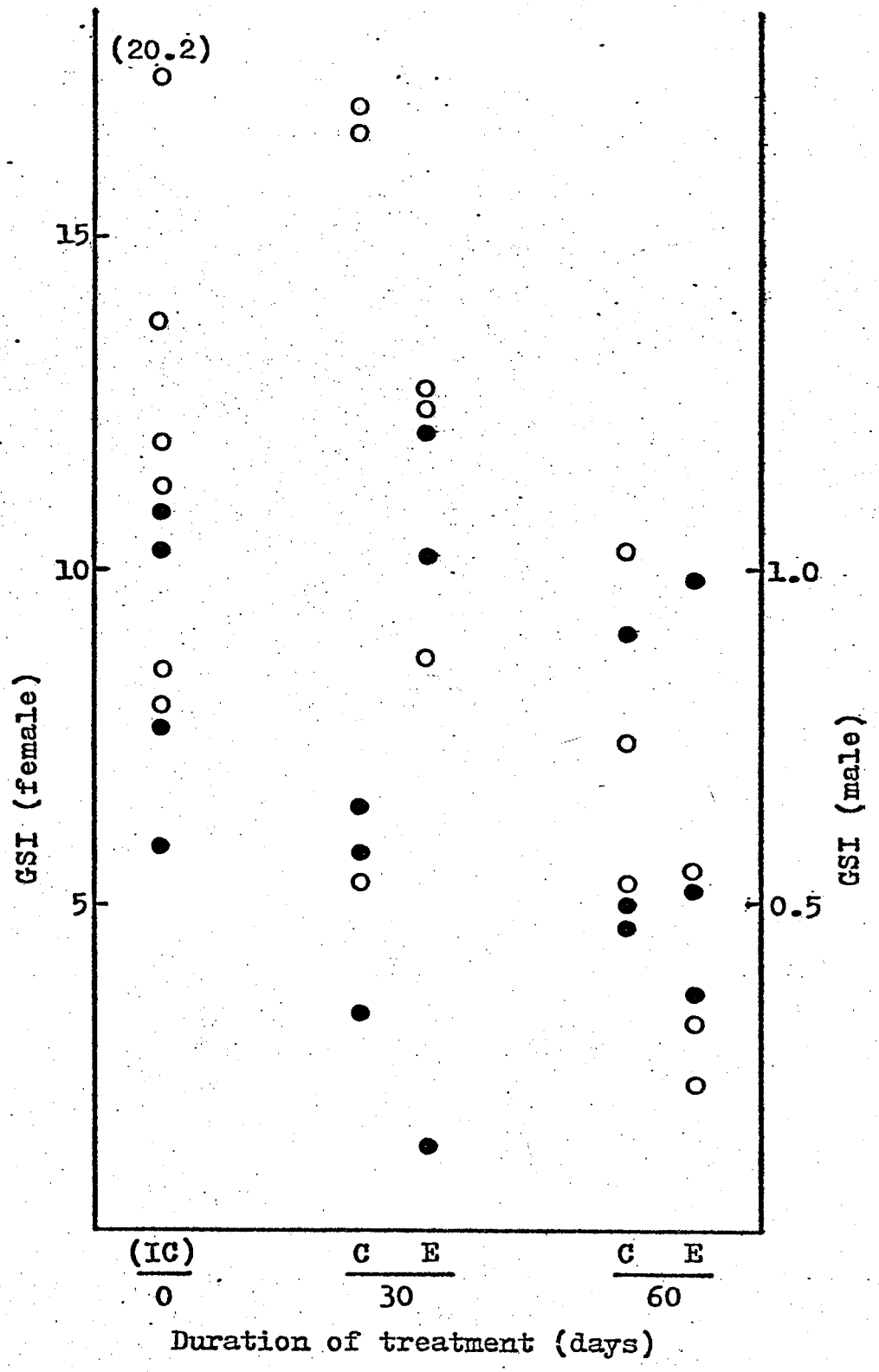
The vesicular cells in young males affected by the treatment for 30 and 60 days showed no progressive change, though their cytoplasmic vesicles were larger in size than those of control males, and large globules began to appear (Fig. 128). The Golgi apparatus of these cells were seen to be highly active.

(2) Effects of starvation

Generally speaking, complete starvation of fishes is known to cause a suppression and regression of ovarian maturation through an inhibition of gonadotropin

release from the pituitary gland. In order to examine the change of pituitary gonadotrophs under such a condition, 24 loaches of 7.7-12.9 cm in body length and 2.3-11.7 g in body weight were divided into experimental and control groups each consisting of 6 females and 6 males. The experiment was carried out from July to September 1979. Both groups of the fish were kept in separate glass aquaria with constantly aerated 0.6% NaCl solution under natural light and temperature. Twelve fish of the experimental group were fasted completely, and were killed after 30 and 60 days of starvation. The other 12 fish of the control group were fed on commercial fish food once a day, and were sacrificed 30 and 60 days after the start of experiment.

Weight of ovaries and testes in terms of GSI varied largely according to individual fish of both experimental and control groups especially on 30 days of experiment, probably because the experiment was carried out during the active breeding months (Text-fig. 6.). However, GSI values of females starved for 60 days were admittedly lower than those of associated control females and of starved females on 30 days of experiment. Histologically, in ovaries of control females on 60 days of experiment, there were still many oocytes in the secondary and tertiary yolk stages together with a few yolk-laden oocytes which were undergoing degeneration. In contrast, ovaries of females starved for 30



Text-fig. 6. Changes of gonadosomatic index (GSI) of female (O) and male (●) loaches starved for 30 and 60 days during the spawning period. IC, initial control group; C, control group; E, experimental group.

and 60 days were filled with many degenerating yolky oocytes. In testes of control males, germ cell cysts in various spermatogenetic stages were still present after 60 days of experiment, while in those of starved males a large amount of spermatozoa and some spermatogonia were the exclusive constituents after 30 and 60 days of starvation.

The pituitary gland of control males and females contained many globular and vesicular cells (Fig. 129). The globular cells had many electron dense small granules and a few, less electron dense, large globules in the cytoplasm. The rough endoplasmic reticulum consisted of flat or slightly dilated cisternae. The Golgi apparatus was moderately active in the cells (Fig. 130). Some of the globular cells in control fish began to exhibit regressive features after 60 days of experiment. The regressed cells showed a shrinkage, becoming irregular in contour with condensed cytoplasm and pycnotic nucleus, and their granular inclusions also revealed degenerative aspects (Fig. 131). The vesicular cells of control males and females had a few small granules and large globules, and their cytoplasmic vesicles were relatively uniform in size containing moderately electron dense contents (Figs. 130 and 131). The vesicular cells in the control fish did not show any notable changes during the experiment.

Contrary to expectation, the starvation treatment

provoked rather slight modifications in both the globular and vesicular cells of the loach of both sexes. In the globular cells, a slight increase in number of the small granules and a slight decrease in amount of the rough endoplasmic reticulum was observed on 30 days of starvation, and these changes became more pronounced after 60 days of the treatment (Fig. 132). Large globules in these cells remained unchanged in number, but became more electron dense. The Golgi apparatus was scarcely detected, and mitochondria were seen to be small in size (Fig. 133).

In the vesicular cells, too, functional alterations were indistinct in ultrastructural figures of granular inclusions and cytoplasmic vesicles, though large globules became rarely observed in these cells after 60 days of complete starvation. The Golgi apparatus and mitochondria were scarcely found in the cells (Fig. 134).

(3) Effects of HCG and of sex steroids on juvenile loaches

In the pituitary gland of juvenile loaches of both sexes, glandular cells of the globular cell type existed in the PPD, while those of the vesicular cell type were indiscernible. Getting a clue to know the nature of the vesicular cells, attempts were conducted to induce cytological differentiation of the vesicular cells in the juvenile fish. The induction of differentiation of pituitary gonadotrophs in immature fish has been observed

under influences of mammalian gonadotropin (Yamamoto and Nagahama, 1973; Yamamoto et al., 1974) and of sex steroids (van Overbeeke and McBride, 1971; Olivereau and Chambolle, 1978; Olivereau and Olivereau, 1979a, b). In the present study, the effect of human chorionic gonadotropin (HCG) and of sex steroids on the pituitary gonadotrophs were examined in juvenile loaches.

a) HCG

Twelve juvenile loaches, ranging 5.6-7.1 cm in body length and 1.1-1.9 g in body weight, were divided into experimental and control groups each consisting of 2 males and 4 females. The experiment was carried out in July and August 1978. They were raised in separate glass aquaria with constantly aerated 0.6% saline solution under natural light and temperature which ranged from 18 to 22 °C. They were fed on commercial fish food once a day. Fish of the experimental group were injected intraperitoneally with 100 IU of HCG per fish every fifth day for 30 days. Fish of the control group were injected with 0.6% NaCl only.

No significant difference was found in values of GSI between the experimental and the control fish of both sexes: mean GSI values were 1.49 for experimental females, 0.33 for experimental males, 1.41 for control females and 0.21 for control males. Histologically, signs of a stimulation of germ cell development could scarcely be detected both in ovaries and in testes. In

affected ovaries, oocytes in the peri-nucleolus stage were the most advanced ones as in ovaries of control fish. In testes, presence of germ cell cysts in various spermatogenetic stages and a small amount of spermatozoa was the common feautre in both experimental and control males.

In pituitaries of control females, there were a few globular cells but not the vesicular cells (Fig. 135). The globular cells had many small granules of 100-400 nm in size and a few large globules of 500-1000 nm in size. Among these granular inclusions, flattened cisternae of the rough endoplasmic reticulum were distributed (Fig. 136). The Golgi apparatus was poorly developed. In pituitaries of control males, on the other hand, both the globular and vesicular cells had already existed (Fig. 137). The vesicular cells contained a few small granules and no large globules, and their cytoplasmic vesicles were relatively uniform in size. The Golgi apparatus was rarely observed, and a few elongate mitochondria were present in the cells (Fig. 138).

In females examined after 6 injections of HCG, a few vesicular cells, which were not present in the pituitary gland of control females, were present in the PPD of their pituitaries (Fig. 139). Small granules in their cytoplasm were seen to be accumulated in groups, and large globules also appeared intermingling with small granules (Fig. 140). Moderately active Golgi

apparatus and rod-shaped mitochondria were also encountered in the cells. In female pituitaries influenced by HCG, the globular cells showed notable increase in number of small granules, and the rough endoplasmic reticulum containing electron translucent contents was dilated to various degrees. The nuclei of the cells became much electron dense (Fig. 140). The Golgi apparatus and elongated mitochondria were sometimes detected in the cells.

In the globular cells of treated males, small granules were increased in amount as in those of treated females. In these cells, however, a dilatation of cisternae of the rough endoplasmic reticulum was less in degree than that found in the globular cells of treated females, and pycnotic changes of the nucleus did not occur (Fig. 141). The vesicular cells in male fish were seen to become more active following the HCG treatment. Many of their cytoplasmic vesicles were evidently larger in size than those in the cells of control males, and the Golgi apparatus and mitochondria were slightly activated (Fig. 142).

b) Sex steroids

Forty-six juvenile loaches of both sexes, measuring 4.2-6.0 cm in body length and 0.6-1.2 g in body weight, were collected in October 1979, and were divided into 2 experimental and 1 control groups. They were kept in separate glass aquaria with constantly aerated 0.6%

saline slolution at 15-18 ℃ under natural light condition. Steroid hormone treatment was carried out by feeding a hormone-containing diet daily to juvenile loaches of the experimental groups. Both testosterone and estrone were dissolved previously in 95% ethyl alcohol and added to the food at the dosage of 50 μg per g diet. A diet for control fish was similarly prepared by adding the same amount of ethyl alcohol to the diet. Aquarium water was changed generally once a weak. Each 3 to 5 fish were killed from both the experimental and control groups at a time, at 10, 20 and 30 days of treatment.

Gonads of control fish were very small in size and quite immature in histology: the maturity of the gonad was the early peri-nucleolus stage for ovaries and the spermatogonial stage for testes. Neither testosterone nor estrone could promote germ cell development in the gonads of both sexes at least by the treatment for 30 days.

In the PPD of the pituitary gland in both sexes of control fish at the end of experiment, a small number of the globular cells were present together with many somatotrophs, thyrotrophs and non-granulated cells (Fig. 143). No typical cells of the vesicular cell type could be detected in the PPD. The globular cells were small in size and had many small, electron dense granules of 100-300 nm in size along with a few large, less electron

dense globules of 400-600 nm in size. The rough endoplasmic reticulum was composed of flat cisternae. The Golgi apparatus and mitochondria were poorly developed (Fig. 144)

Following the testosterone treatment for 10 days the globular cells of the treated fish of both sexes were observed to have slightly increased in number (Fig. 145). Their small granules were also increased in number (Fig. 146). In these fish, some cells of the vesicular cell type made their appearance in the dorsal part of the PPD (Fig. 147). These cells contained only a few minute granules of 50-150 nm in size, and the rough endoplasmic reticulum was composed of round or elongate vesicular cisternae which always contained material of low electron density. Well-developed Golgi apparatus and rod-shaped mitochondria occurred in the cells (Fig. 148). After 30 days of testosterone treatment, the number of globular cells showed a pronounced increase (Fig. 150). Their small granules became large in amount and in size, attaining to 200-400 nm, while large globules remained unchanged. Cisternae of the rough endoplasmic reticulum were often observed to be dilated. The Golgi apparatus was moderately active, and some elongated mitochondria were present in the cells (Fig. 151). The vesicular cells were increased in number in the fish affected by testosterone for 20 and 30 days. Their small granules were increased in number and

in size to 150-350 nm, but large globules were not detectable yet. The cytoplasmic vesicles were relatively uniform in size and round in shape (Fig. 149).

Estrone treatment exterted conspicuous influences on the globular cells of juvenile loaches of both sexes as was the case for testosterone treatment (Fig. 152). Thirty days after the start of treatment, the globular cells were clearly increased in size and in number, and showed progressive dilation of cisternae of the rough endoplasmic reticulum (Fig. 153). Their small granules were larger in size than those of control fish, measuring 200-400 nm in size, and their number was increased remarkably. Large globules tended to augment their size attaining to 500-1000 nm in diameter. The Golgi apparatus appeared to be very active, and mitochondria were seen to be much enlarged in size.

In contrast to testosterone, estrone could not induce a cytological differentiation of the vesicular cells in the pituitary gland of juvenile loaches of both sexes at the end of experiment. However, some cells, which had only a few small granules of 100-300 nm in size and large globules of 400-600 nm in size in their cytoplasm, were observed to occur in the central part of the PPD of estrone-treated fish (Fig. 154). The granular inclusions was quite similar in size to those of the vesicular cells which were caused to be differentiated by testosterone in juvenile males. However, cisternae of the

rough endoplasmic reticulum was not vesicular but flat or long lamellated in appearance, the feature being clearly different from that of the vesicular cells in the testosterone-treated males.

(4) Conclusion

In summary, complete starvation of the loach did not cause evident changes of the globular and vesicular cells in the pituitary gland of either sex of the fish. On the contrary, exposure of male and female loaches to continuous light was effective in making the vesicular cells display highly active features in association with the promotion of gonadal maturation. Although the globular cells were also seen to be activated under the continuous light, the change appeared to be less prominent than that found in the vesicular cells. Moreover, the vesicular cells, which were absent in pituitaries of immature young females, made their appearance following the light treatment. The precocious differentiation of the vesicular cells was also the cases for juvenile loaches treated with HCG or sex steroids. Significance of these findings will be discussed in detail in a later chapter of this paper.

II. Observations in other teleost fishes

1. Salmonid fishes

(1) Presence of two types of possible gonadotrophs in several salmonid fishes

General morphological and cytological characteristics of the pituitary gland of chum salmon, Oncorhynchus keta, masu salmon, O. masou, and whitespotted char, Salvelinus leucomaenis, were almost similar to each other. In the PPD, four different types of glandular cells were distinguishable by light and electron microscopy. One of these four types of cells encountered throughout the PPD was strongly positive to orange G, and were considered to be somatotrophs. They were oval or slightly elongate in shape, and their oval nuclei were eccentric in position (Fig. 155). Ultrastructurally, they had electron dense, spherical granules, ranging from 150 to 350 nm in diameter, in the cytoplasm (Fig. 156). The rough endoplasmic reticulum generally consisted of flat cisternae oriented randomly. The Golgi apparatus was rarely seen and mitochondria were few in number and mostly elongate in shape.

The other 3 types of glandular cells in the PPD were basophilic in nature. Basophilic cells of the first type were localized in the dorsal region and showed a weak affinity to both alcian blue and PAS. They were few in number, small in size, and mostly angular in shape. They had central nuclei of an indented contour (Fig. 155). In fine structure, they contained small, round or oval granules, measuring 100-200 nm in size, which were

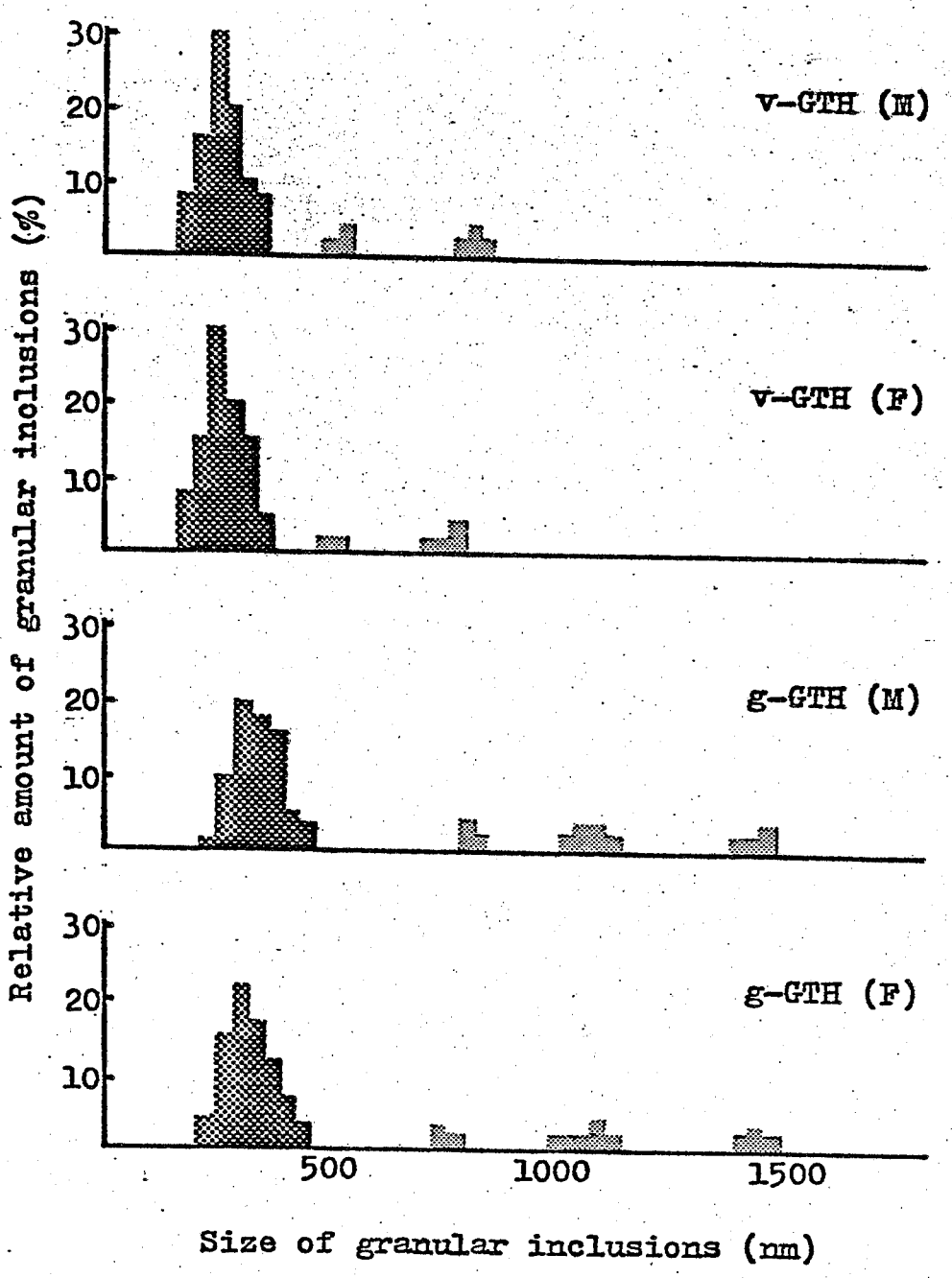
the smallest among those found in all granulated cells existing in the PPD. Lamellated rough endoplasmic reticulum was sparse. In general, the Golgi apparatus and rod- or round-shaped mitochondria were rarely observed in the cells (Fig. 156). They were identified as thyrotrophs.

Of the other two types of basophils, those localized in the central and ventral regions of the PPD were stained intensely with both alcian blue and PAS. Some of the cells were found also in the prolactin follicles of the rostral pars distalis. Their nuclei were irregular in shape, being concaved complicatedly on some occasions (Figs. 157, 159 and 161). The cells were characterized ultrastructurally by the occurrence of a few large globules in the cytoplasm, 800-1500 nm in diameter, along with numerous small granules, 200-400 nm in diameter (Figs. 158, 160 and 162). The rough endoplasmic reticulum was dilated to various extents and contained electron lucent material. In these ultrastructural aspects, the basophilic cells are fairly identical with those of the "globular" gonadotrophs of the loach described in the preceding sections, and will be also called the globular cells in the following descriptions.

Basophilic cells of the remaining type, which were distributed exclusively in the central and dorsal parts of the PPD, were strongly positive to PAS but almost negative to alcian blue. The nucleus of the cells was

in most cases oval or elongate in shape (Figs. 157, 159 and 161). They were rich in cytoplasm which was characteristically loaded by many clear vacuoles, or rounded vesicular cisternae of the rough endoplasmic reticulum. The cytoplasmic vesicles always contained material of moderate electron density (Figs. 158, 160 and 162). The cells were also possessed of two kinds of granular inclusions: small granules, measuring 100-300 nm in diameter, were always few in number, and existed occasionally in groups in narrow interstices among the cytoplasm vesicles; large globules, ranging 500-800 nm in diameter, were quite a few in number. The cells of this type may correspond to the "vesicular" gonadodtophs of the loach, and will be described also as the vesicular cells in this paper. The size distribution of granular inclusions of the two types of gonadotrophs in the pituitary gland of the salmonid fish is shown in Text-fig. 7.

No typical intermediate cell types between the globular and vesicular cells were detectable light and electron microscopically in the chum salmon, the masu salmon and the whitespotted char as was the case in the loach. These two cell types were evidently different from possible somatotrophs and thyrotrophs in their ultrastructure as well as in their staining properties and localization in the PPD. From their light and electron microscopic characteristics, they are determined to be similar to those identified as distinct types of gonadotrophs in other



Text-fig. 7. Size distribution of small granules (▩) and large globules (▩) of the two types of gonadotrophs, the vesicular (v-GTH) and globular (g-GTH) cells, in males (M) and females (F) of salmonid fishes.

salmonid fishes such as coho salmon, Oncorhynchus kisutchi (Chestnut, 1970), sockeye salmon, O. nerka (Cook and van Overbeeke, 1972), Atlantic salmon, Salmo salar (Olivereau, 1976), and rainbow trout, S. gairdneri, and brown trout, S. fario (Olivereau, 1978).

(2) Changes of two types of possible gonadotrophs in the whitespotted char during gonadal development

It has been suggested in the afore-mentioned studies on the loach that the two types of pituitary gonadotrophs may take their respective gonadotropic part in different phases of sexual cycle of the fish of each sex. Thus a study was further extended to compare the morphological results in the loach with those in salmonids and to substantiate gonadotropic roles played by the two types of possible gonadotrophs. The whitespotted char, Salvelinus leucomaenis, was used as material for their ease in culturing and breeding in the pond.

Two-year-old females, 20.5-36.5 cm in body length and 130-620 g in body weight, were sampled 3 fish at a time, monthly from October 1977 to April 1978. In addition, a total of 39 1-year-old fish of both sexes, 16.4-29.4 cm in body length and 65-300 g in body weight, were also collected during the months from April 1978 to September 1978.

a) Changes in female chars

One-year-old females killed in April and May had

ovaries with many oocytes in the early yolk vesicle stage. In these fish, a small number of the globular cells were distributed exclusively in the central part of the PPD (Fig. 163). They contained many small, electron dense granules of 150-350 nm in size and a few large, less electron dense globules of 400-1000 nm in size (Fig. 164). The rough endoplasmic reticulum was composed of flat or small vesicular cisternae with electron lucent contents. The Golgi apparatus was moderately active, and mitochondria were a few in number and small in size. No typical cells of the vesicular type could be detected in 1-year-old females in this period. However, there existed some cells with clear cytoplasm in the dorsal and central parts of the PPD (Fig. 163). In these cells, cytoplasmic organelles were scarcely present. These cells were termed non-granulated cells as in the case of the loach.

In June, ovaries of female yearlings had oocytes in the late yolk vesicle stage. The globular cells were observed to be almost the same in features as those observed in the preceding months. In these fish, some cells with the rough endoplasmic reticulum composed of rounded vesicular cisternae appeared in the dorsal and central parts of the PPD (Fig. 165). In these cells, granular inclusions were scarcely present in the cytoplasm. They were thought to be the vesicular cells that had differentiated, during that stage of the life-cycle,

presumably from non-granulated cells pre-existing in the PPD.

In female yearlings collected in July, ovaries had many oocytes in the primary yolk stage. In these fish, a considerable number of the vesicular cells were found in the dorsal and central regions of the PPD (Fig. 166). They were rich in cytoplasm containing, by light microscopy, many clear vacuoles which coincided with rounded vesicles of the rough endoplasmic reticulum. The rounded vesicles always contained material of low electron density. In the vesicular cells, small granules of 100-300 nm in size were gathered in groups, large globules of 500-800 nm in size were few in number, and well-developed Golgi apparatus and rod-shaped mitochondria appeared in the juxtanuclear region (Fig. 167). The globular cells did not show any prominent changes in their structural aspects in comparison with those observed during the preceding months.

In female yearlings sampled in August and September, ovaries were filled with many oocytes advancing to the secondary and tertiary yolk stages. In these fish, the globular cells were observed to have increased in number and in size (Fig. 168). Moreover, a few cells of the globular cell type also existed among prolactin cells localized in the rostral pars distalis. In the cytoplasm of the globular cells in the PPD, small granules evidently increased in size, attaining to 200-400

nm (Fig. 169), and large globules also increased in size, measuring 800-1500 nm. Cisternae of the rough endoplasmic reticulum were dilated to various extents and contained electron lucent material. The Golgi apparatus was highly active, and sometimes immature granules were present within the Golgi field. On the other hand, no notable ultrastructural changes were observed in the vesicular cells when compared with those found in female yearlings collected in July. Dilated vesicles characteristic of the cells were relatively uniform in size.

In October, the fish were at the peak of the spawning period. In pituitaries of the females with ovulated eggs in their coelomic cavity, the globular cells displayed remarkable changes (Fig. 170). Small granules in their cytoplasm were clearly diminished in number, and large globules were seen to become more electron dense. Cisternae of the rough endoplasmic reticulum were dilated extensively so that they occupied most of the cytoplasm (Fig. 171). On the other hand, changes were not significant in the vesicular cells of females at the spawning stage.

Females killed in November and December had ovaries with yolkless oocytes and many postovulatory follicles. In these fish, the globular cells displayed pycnotic changes of the nucleus which was quite irregular in shape and unusually electron dense (Fig. 172). Large globules in their cytoplasm were increased in number and

were observed to coalesce with one another, becoming irregular in shape (Fig. 173). On the contrary, the vesicular cells were almost similar in structural features to those observed in October, never exhibiting such regressive changes in that postspawning period.

In January, 3 months after the termination of spawning period, ovaries of 2-year-old females came to have many oocytes of the yolk vesicle stage. In these fish, although some of the globular cells still retained a regressed state, others became regranulated with small granules of 150-350 nm in size in the cytoplasm. A few large globules of 400-1000 nm were also present in these cells. The rough endoplasmic reticulum consisted of flat or small vesicular cisternae, and the Golgi apparatus appeared to be moderately active in these cells (Fig. 176). On the other hand, the vesicular cells were seen to be larger in size than before (Fig. 174). In these cells, rounded cisternae of the rough endoplasmic reticulum were rather uneven in size, and some of them were atypically dilated (Fig. 175). Small granules in their cytoplasm were relatively abundant and existed in groups among the dilated vesicles, while large globules were scarcely detected in these cells. Well-developed Golgi apparatus consisting of minute vesicles and a stack of flattened lamellae were notable.

During the successive months up to April, ovarian oocytes of 2-year-old fish were maintained in the yolk

vesicle stage. During that period, no significant alterations were observed in the globular cells. The vesicular cells regained their typical cytological aspects in February: rounded vesicles of the rough endoplasmic reticulum became uniform in size and small granules became fewer in number than those found in January. They stayed almost unchanged during the successive months.

b) Changes in male chars

One-year-old males sampled in April and May had immature testes with spermatogonia as the sole germinal component. In their pituitaries, there were only a few globular cells besides thyrotrophs and somatotrophs in the central part of the PPD, as was the case for the pituitary of yearling female chars of the same period.

In male yearlings collected in June, a small number of cysts of the first spermatocytes were present together with many spermatogonia in their testes. In pituitaries of these fish, a considerable number of the vesicular cells were distributed in the dorsal and central parts of the PPD (Fig. 177). Rounded cisternae of the rough endoplasmic reticulum in these cells were evidently larger in size than those in the cells of females sampled in July (Fig. 178, compare with Fig. 167). Small granules of 100-300 nm in size and large globules of 500-800 nm in size were few in number in the cytoplasm. The globular cells in these fish were very

similar in fine structure to those of male chars examined in the preceding months.

During the subsequent course of testicular development, the vesicular cells displayed a pronounced decrease in the size of rounded cisternae of the rough endoplasmic reticulum, but small granules and large globules were almost unchanged in number (Fig. 180). In contrast, the globular cells began to exhibit highly active features in harmony with gonadal development. In August and September, a large amount of spermatozoa had accumulated in widely expanded lumina of testicular lobules in the testis. During these months, small granules and large globules in the vesicular cells of the pituitary gland were clearly increased in number (Fig. 179), though some of the large globules were seen to become less electron dense. In the globular cells, the rough endoplasmic reticulum was extended to various degrees, and the Golgi apparatus was quite active to form immature granules (Fig. 180).

In summary, the two distinct types of pituitary gonadotrophs, the globular and vesicular cells, displayed some characteristic changes in association with gonadal development in the whitespotted char of both sexes. In the pituitaries of immature yearlings of each sex, cells of the globular type with immature granular inclusions existed in the PPD, while those of the vesicular type were indiscernible in that stage of gonadal immaturity.

The vesicular cells made their first appearance concurrently with the beginning of exogenous vitellogenesis in ovarian oocytes or with the development of the first spermatocytes in testes of the fish. During the course of subsequent development of the gonads, the globular cells showed a notable increase both in number and in size, and displayed a degranulation and a dilation of endoplasmic reticulum followed by some atrophic changes at the time of spawning. On the contrary, the vesicular cells showed no significant alterations in their structure during the latter phase of gonadal maturation. Thus, as in the case of the loach, it seems certain that the two types of gonadotrophs play different roles at different stages of gonadal development in the salmonid fish.

(3) Observation on gonadotroph in the rainbow trout

Some confusing results of observations have been obtained in the pituitary gonadotrophs of the rainbow trout, Salmo gairdneri. Whereas Olivereau (1978) presented cytological and cytochemical evidence for the presence of two types of the cells, Peute et al. (1978) regarded the two types as different forms or stages of activity of a single type of gonadotroph on the basis of ultrastructural findings obtained during the sexual cycle of the fish. In order to explain the discrepancy, a careful cytological observation was conducted on

pituitary gonadotrophs in the rainbow trout which had been cultured in outdoor ponds continuously following hatching. Three females and two males, 30.2-35.0 cm in body length and 515-845 g in body weight, were collected in March 1979 when they were at the peak of their spawning period.

In the rainbow trout of both sexes, contrary to expectation, cells of the vesicular type were not detectable electron and light microscopically in the pituitary gland. Many basophilic cells, which were strongly positive to both alcian blue and PAS, were distributed throughout PPD, and some cells of this type were also found in the rostral pars distalis of the pituitary gland. They were round or oval in shape and their nuclei were concaved or undulated in contour frequently (Fig. 181). The cells contained many small, electron dense granules of 200-400 nm in size and a few large, less electron dense globules of 600-1200 nm in size. Cisternae of well-developed rough endoplasmic reticulum were dilated to various extents, and contained electron lucent material. The Golgi apparatus was moderately active and rod shaped mitochondria were located near the Golgi field (Fig. 182). The cytological characteristics of these cells corresponded fairly to those of the globular cells mentioned already for other salmonid fishes.

On the other hand, some cells which showed a weak affinity to alcian blue and PAS were found intermingled

among the globular cells in the PPD. They were oval or elongate in shape, and their nuclei were irregular in form. Ultrastructurally, the cells were devoid of any granular inclusions. Their rough endoplasmic reticulum consisted of numerous irregularly vesicular cisternae containing electron lucent material, and the Golgi apparatus and mitochondria were hardly detected in the cells (Fig. 182). The cells differed clearly in these features from the vesicular cells of other salmonids, and were considered to be cells of the globular type in which granular inclusions had depleted almost completely.

The results may suggest that, in the rainbow trout, only one type of gonadotroph, which corresponds with the globular cells in other salmonids, is present in the pituitary gland. However, Olivereau (1978) described the presence of two types of pituitary gonadotrophs, and Peute et al. (1978) also found two forms of pituitary gonadotrophs in some stages of gonadal development in the rainbow trout. Further work should be done in order to explain the discrepancy by examining pituitary gonadotrophs of the fish in various stages of reproductive cycle.

2. Goldfish

In the goldfish, Carassius auratus, too, some authors suggested the presence of two types of gonadotrophs

(Olivereau, 1962; Leatherland, 1972; Leatherland and Sonstegard, 1977) while others could find only one gonadotropic cell type in the pituitary gland (Nagahama and Yamamoto, 1969b; Nagahama, 1973; Kaul and Vollrath, 1974). In the present study, 2 adult males and 4 adult females, measuring 6.2-10.2 cm in body length and 9.1-39.0 g in body weight, were sacrificed in June 1979 when they were at the peak of the spawning period, and their pituitaries were examined light and electron microscopically for the identification of gonadotropic cells.

Pituitary gonadotrophs of the goldfish were stained intensely with aldehyde fuchsin in AF preparation and with alcian blue and PAS in AB-PAS-OG preparation, and were found to be distributed throughout the PPD. The cells were mostly round or elliptical in shape, and their nucleus was round or oval in shape (Fig. 183). Ultrastructurally, the cells were characterized by the presence of two kinds of granular inclusions, i.e. many small, electron dense granules of 200-350 nm in size and a few large, less electron dense globules of 1000-2500 nm in size. The rough endoplasmic reticulum was generally composed of vesicular cisternae with contents of moderate electron density. In the matured fish examined, the Golgi apparatus was highly active and sometimes immature granules at the beginning of formation occurred within the Golgi field. Mitochondria were mostly oval or elongate in shape and distributed throughout the

cytoplasm (Fig. 184).

Thus the pituitary gonadotroph in the goldfish is provided with cytological characteristics of both the globular and vesicular cells occurring in the loach: the gonadotroph of the goldfish resembles the globular cells in respect to its granular inclusions and is further similar to the vesicular cells in the feature of the rough endoplasmic reticulum. These observations may imply that a single type of gonadotroph in the goldfish could have two different gonadotropic potencies that are to be ascribable to the two types of gonadotrophs as in the case of the loach.

3. Eel

It has been shown that, in male Japanese eel, Anguilla japonica, there is only one type of gonadotroph in the pituitary gland (Yamamoto and Nagahama, 1973; Yamamoto et al., 1974). On the other hand, in European eel, Anguilla anguilla, two types of gonadotrophs have been detected to occur in the pituitary gland (Olivereau and Herlant, 1960; Knowles and Vollrath, 1966a, b). As known generally, eels in the fresh water phase of their life history remain to be quite immature in terms of gonadal development and also of pituitary morphology which is far prior to full differentiation of gonadotropic cells. This seems to make exact identification of

pituitary gonadotropic cell type(s) difficult and confused. In this respect, it is interesting to note that some authors could observed a precocious differentiation of pituitary gonadotrophs in male Japanese eels which were induced to mature by the treatment with mammalian gonadotopins (Yamamoto and Nagahama, 1973; Yamamoto et al., 1974). As described before, in juvenile females of the loach, too, the administration of HCG was effective in causing an accelerated differentiation of the vesicular gonadotrophs as well as an activation of the globular gonadotrophs. In the present study, pituitary gonadotrophs of Japanese eel were examined in silver females subjected to the treatment with a fish GTH preparation for the induction of ovarian maturation.

Among 14 females examined in the present study, 2 fish served as initial controls, and the remaining 12 were induced to mature by weekly intramuscular injections of 2 mg powder of aceton-dried chum salmon pituitaries per 100 g body weight. They were sacrificed at various times during the course of the treatment lasting for 14 weeks.

GSI value was only about 2% for initial control females. In these fish, a small number of possible gonadotrophs were distributed exclusively in the PPD of the pituitary gland. The cells were oval or elongate in shape, and were characterized light microscopically by having a few, darkly stained globules in lightly stained

cytoplasm. The nucleus of the cells was large in size, oval or round in shape, with an indistinct nucleolus (Fig. 185). 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. 186). 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 during the course of progressive ovarian maturation, after 5 to 8 injections with salmon pituitaries. They ranged in GSI from 10 to 40%. In their pituitaries, gonadotrophs were observed to have drastically increased in number and in size with an evident increment in the content of small granules in the cytoplasm (Fig. 187). Their nucleus was also enlarged with a prominent nucleolus. Furthermore, a few of the cells were encountered in the follicles of prolactin cells in the rostral pars distalis of the pituitary. In the gonadotropic cells of the fish with GSI of 10-20%, small granules were enlarged to 100-300 nm in size and became electron dense. Large globules in these cells also became large in size, attaining 1000-1500 nm in diameter, and became similarly electron dense. Cisternae

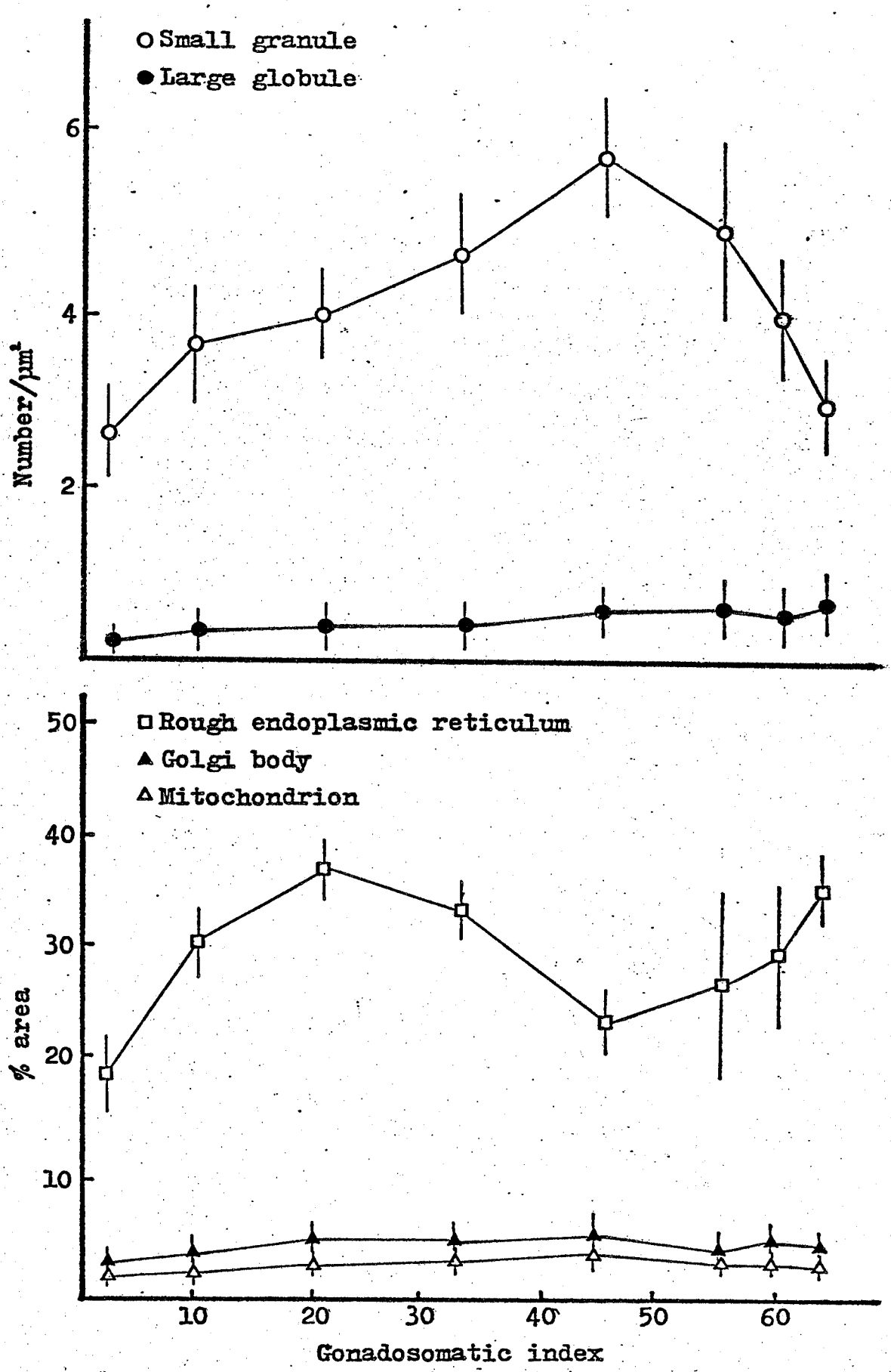
of the rough endoplasmic reticulum were dilated widely throughout the cytoplasm (Fig. 188).

In 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. The dilatation of cisternae of the rough endoplasmic reticulum was, however, less in degree (Fig. 189) when compared with that found in the cells of the fish with GSI of 10–20% (Fig. 188). 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 pituitary gland of an eel which had ovulated following 10 injections of salmon pituitaries, almost all gonadotrophs were provided with many small vacuoles in the cytoplasm, and their small granules were clearly decreased in amount (Fig. 190), only a small amount of 200–400 nm in size being left in the cells (Fig. 191). Large globules in these cells appeared to remain unchanged in number, and were seen to become 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. Many small granules in the process of formation

were still observed within the Golgi field.

The remaining 5 fish could not ovulate even after 10 to 14 injections of the pituitary extract and, at autopsy, had ovarian eggs in various states of progressive overripeness. GSI of these overripe eels ranged from about 50 to 70%. Pituitary gonadotrophs of these fish showed extensive variations in their cytological features, being in contrast to the uniform changes of the cells found in the fish ovulated spontaneously (Fig. 192). While some 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. 193). In these cells, large globules appeared to be highly electron dense and were sometimes irregular in shape. 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, and 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 those observed in the gonadotrophs of the fish after



Text-fig. 8. Fluctuations in the number of cytoplasmic granular inclusions and in the relative area of cytoplasmic organelles in gonadotrophs of female Japanese eels during the course of artificial maturation. Mean ± SE.

ovulation.

A morphometric study on electron micrographs of pituitary gonadotrophs of the female Japanese eel clearly indicated the changes of cell organelles and granular inclusions of the cells as shown in Text-fig. 8.

The present observation confirmed that, in silver female Japanese eel, only one type of adenohypophysial cells displayed some characteristic changes in association with ovarian maturation induced artificially.

Discussion

To date, a considerable interest has been focused on a question of whether the pituitary gland of teleost fishes contains one or two kinds of gonadotropic hormones. In early biochemical studies on teleost gonadotropins, both follicle stimulating hormone (FSH) and luteinizing hormone (LH) with similar activities to those of mammals had been demonstrated in pituitary extracts (Witschi, 1955; Otsuka, 1956; Fontaine and Chauvel, 1961). Fontaine and Gérard (1963) purified, however, only one gonadotropic factor in the pituitary of carp, Cyprinus carpio. Later, they convinced of the possibility that only one kind of gonadotropin was produced in the carp pituitary (Burzawa-Gérard and Fontaine, 1965; Burzawa-Gérard, 1971). Yamazaki and Donaldson (1968a, b)

partially purified, from the pituitary gland of chinook salmon, Oncorhynchus tshawytscha, a single gonadotropin which was proved to have a full range of gonadotropic effects in goldfish, Carassius auratus (Donaldson et al., 1972) and in Indian catfish, Heteropneustes fossilis (Sundararaj et al., 1971, 1972). These findings have collaborated in advancing the view of the existence of a single gonadotropin in teleost pituitary gland in contrast to the presence of LH and FSH in the gland of higher vertebrates (Yamazaki, 1969; Burzawa-Gérard and Fontaine, 1972; Donaldson, 1973; Fontaine, 1976). On the other hand, two different kinds of gonadotropic hormones have recently been isolated from the pituitary of American plaice, Hippoglossoides platessoides (Ng and Idler, 1978a), chum salmon, Oncorhynchus keta (Ng and Idler, 1978b), winter flounder, Pseudopleuronectes americanus (Ng and Idler, 1979) and the carp (Idler and Ng, 1979); one is vitellogenic hormone which is able to stimulate the ovary to incorporate vitellogenin, and the other is maturational hormone which can evoke oocyte maturation and ovulation.

Similar conflicting results have been obtained also from cytological studies on gonadotropic cells of teleost pituitaries (Pickford and Atz, 1957; van Oordt, 1968; Ball and Baker, 1969; Sage and Bern, 1971; Nagahama, 1973; Schreibman et al., 1973; Holmes and Ball, 1974; Fontaine and Olivereau, 1975; Leatherland, 1975).

Several light microscopic studies on the pituitary gland have mentioned the presence of both LH- and FSH-secreting cells in grey mullet, Mugil cephalus (Stahl, 1963), some species of Lepomis (Simon and Reinboth, 1974) and Perca fluviatilis macedonica (Dimovska, 1977). In early electron microscopic studies, too, similar findings have been reported in European eel, Anguilla anguilla (Knowles and Vollrath, 1966a, c) and Zoarces viviparas (Öztan, 1966).

Olivereau and her cooperators appear to be most eager to present light microscopic evidence for the presence of two types of pituitary gonadotrophs in teleost fishes such as the European eel (Olivereau, 1960, 1961, 1967; Olivereau and Herlant, 1960; Olivereau and Fontaine, 1966), the goldfish (Olivereau, 1962), some species of Oncorhynchus (Olivereau and Ridgway, 1962), the grey mullet (Olivereau, 1968), roach, Leuciscus rutilus (Olivereau, 1969), the chinook salmon (Olivereau, 1972), Atlantic salmon, Salmo salar (Olivereau, 1976), rainbow trout, S. gairdneri, and brown trout, S. fario (Olivereau, 1978). Basing on these extensive studies, she suggests that the two types of gonadotrophs in teleosts differ in their cytochemical nature from LH- and FSH-secreting cells in higher vertebrates, and that each of the two teleostean gonadotrophs may play roles at different stages of gonadal development. Similar suggestion has been given by Cook and van Overbeeke (1972)

in their ultrastructural studies on sockeye salmon, Oncorhynchus nerka, which confirmed their early light microscopic observations on the same species (van Overbeeke and McBride, 1967, 1971; McBride and van Overbeeke, 1969). Some other studies have also shown the existence of two types of pituitary gonadotrophs light microscopically in coho salmon, Oncorhynchus kisutch (Chestnut, 1970) and Boops salpa (Michele, 1973; Malo-Michele, 1978), and electron microscopically in the goldfish (Leatherland, 1972; Leatherland and Sonstegard, 1977), the roach (Bağe et al., 1974; Ekengren et al., 1978a), the carp (Leatherland and Sonstegard, 1977) and threespined stickleback, Gasterosteus aculeatus (Slijkhuis, 1978).

However, some authors have found only one type of pituitary gonadotroph even in teleost species in which two types of cells were identified as gonadotropic as cited above: the sockeye salmon (Nagahama and Yamamoto, 1969a; McKeown and Leatherland, 1973), the glodfish (Nagahama and Yamamoto, 1969b; Kaul and Vollrath, 1974; Lam et al., 1976), the chum salmon (Nagahama and Yamamoto, 1970) and the threespined stickleback (Leatherland, 1970). Among them, Nagahama (1973) described that pituitary gonadotrophs of several species of teleosts were characterized by having two different kinds of secretory granules in their cytoplasm, and suggested that each of them might be related separately to the control of fish

reproduction, one to ovulation and the other to vitellogenesis. A similar conception was expressed about pituitary gonadotrophs of grass carp, Ctenopharyngodon idellus (Anon., 1978a, b).

On the other hand, several cases have been reported that a single type of pituitary gonadotroph may show a wide variety in cytological aspects even in fishes at a given stage of sexual development. For example, in the grey mullet, Abraham (1974) described several gonadotropic cell variants which had a series of extensive cytological differences. Some other authors have also come to admit the possibility of the presence of two types of gonadotrophs in some species of Tilapia (Leatherland et al., 1974) and in black molly, Poecilia latipinna (Batten et al., 1975). Quite recently, Ekengren et al. (1978b) and Peute et al. (1978) observed two ultrastructurally different forms of pituitary gonadotrophs in the Atlantic salmon and the rainbow trout, respectively, during the reproductive cycle, and they regarded the two forms as in different stages of activity of a single type of gonadotroph.

It seems likely that at least a cause of the above-described discrepancies of the observations can be sought in the fact that many of the studies concerned have dealt only with the pituitary gland of fishes at a certain fixed stage of gonadal development. It seems obvious that a time-course study of the changes of

pituitary cells throughout the gonadal development and maturation is efficient to confirm the contention about the entity of pituitary gonadotrophs of fishes. In addition, information available so far about fish gonadotrophs seems to be lacking in experimental supports which may fail to give exact discrimination of functional gonadotropic cells from other glandular cells of the pituitary gland. Such experimental work may further favor the determination of respective roles of different types of gonadotrophs, if present, in controlling gonadal activities.

In the proximal pars distalis (PPD) of pituitary gland of the loach, Misgurnus anguillicaudatus, examined in the present study, one acidophilic cell type and three basophilic cell types were discernible light microscopically. As the acidophils comprised a large area of the PPD and were the only granulated, PAS-negative cells, they were definitely identified as somatotrophs. Basophilic cells of one type, which were located mostly in the dorsal region of the PPD and showed a weak affinity to basic dyes, were clearly distinguished from the other two types of basophilic cells by their scantiness, by their smaller size and by their granular contents of much smaller size. They were the only cells that displayed conspicuous cytological changes under the influence of a goitrogen: following the treatment with thiourea, they showed a cellular hypertrophy, a marked

development of lamellar rough endoplasmic reticulum, a striking decrease in the number of secretory granules, and an activation of the Golgi apparatus. These responses to thiourea treatment have been assigned to the thyrotrophs of Channa punctatus (Belsare, 1965), guppy, Poecilia reticulata (Sage, 1967; Pandey and Leatherland, 1970), the sockeye salmon (McBride and van Overbeeke, 1975), catfish, Clarias batrachus (Joy and Sathyanesan, 1979), the goldfish (Nagahama and Yamamoto, 1969b; Leatherland, 1972; Kaul and Vollrath, 1974) and medaka, Oryzias latipes (Nagahama, 1973).

Of the basophils of the other two types, those which were situated in the central and ventral regions of the PPD were stained intensely with both AB and PAS, while those localized in the central and dorsal regions were strongly positive to PAS but were almost negative to AB in AB-PAS-OG preparation. By the staining with AF, however, these two types of basophils showed a similar affinity to AF, and it was impossible to distinguish them decisively. In light microscopic studies of the Atlantic salmon, the rainbow trout and the brown trout, Olivereau (1976, 1978) has shown that one kind of gonadotroph which predominated in the anterior region of the PPD was positive to both AB and PAS, while the other kind of gonadotroph distributed in the central and dorsal PPD was weakly positive to PAS. The light microscopic images of the two kinds of gonadotrophs in these salmonids

fairly resemble those of the two types of basophilic cells found in the loach.

Moreover, among the other six species of fishes examined in the present study, the chum salmon, Oncorhynchus keta, the masu salmon, O. masou, and the whitespotted char, Salvelinus leucomaenis, possessed two types of basophils which exhibited almost the same histological and histochemical natures as those of the loach. The occurrence of such two types of putative gonadotrophs have been noticed also in the coho salmon (Chestnut, 1970), the sockeye salmon (van Overbeeke and McBride, 1967, 1971; McBride and van Overbeeke, 1969), Perca sp. (Dimovska, 1977) and Boops sp. (Michele, 1973; Malo-Michele, 1978).

The two types of basophils in the loach and in the three salmonid fishes differed from each other in their fine structure, too. The alcian blue- and PAS-positive cells were characterized by having a few large globules together with many small, secretory granules in their cytoplasm, so they were designated as the globular cells. They may correspond to the globule-containing cells or the globular gonadotrophs described in the goldfish (Nagahama and Yamamoto, 1969b; Kaul and Vollrath, 1974), the pond loach (Jasiński, 1973), and the several other salmonids (Nagahama and Yamamoto, 1969a, 1970; Cook and van Overbeeke, 1972; McKeown and Leatherland, 1973). The PAS-positive and AB-negative cells were conspicuous

for the constant occurrence of numerous vesicular cisternae (cytoplasmic vesicles) of the rough endoplasmic reticulum, with moderately electron dense contents, throughout the cytoplasm together with rather a scanty number of granular inclusions. Cells of this type appear to correspond to the vesicular cells which have been regarded as the second type of gonadotroph in the sockeye salmon (Cook and van Overbeeke, 1972). In the present study, too, they were termed the vesicular cells. The presence of the vesicular gonadotrophs with similar ultrastructural characteristics has been noticed also in the European eel (Knowles and Vollrath, 1966a, c) the goldfish (Leatherland, 1972; Leatherland and Sonstegard, 1977), and the pond loach (Jasiński, 1973).

Up to date, various experimental attempts have been carried out to cause functional alterations of pituitary gonadotrophs for the purpose of clear identification of the cells in teleost fishes (cf. Nagahama, 1973; Schreibman et al., 1973; Holmes and Ball, 1974; Fontaine and Olivereau, 1975). In the present study, several experimental treatments were conducted to identify pituitary gonadotrophs of the loach decisively using adult and young fish of both sexes.

In synthetic LH-RH-treated adult loaches of either sex, the globular cells were characterized by a conspicuous decrease in number of the small granules accompanied with a prominent dilatation of cisternae of the rough

endoplasmic reticulum. Similar responses of pituitary gonadotrophs to LH-RH treatment have been demonstrated in the goldfish (Kaul and Vollrath, 1974; Lam et al., 1976), ayu, Plecoglossus altivelis (Hirose and Ishida, 1974), the medaka (Chan, 1977), the grass carp (Anon., 1978a, b), and the Atlantic salmon (Ekengren et al., 1978b). Furthermore, following ovulation induced by clomiphene citrate, the cells of this type of female loaches also displayed a marked degranulation and a considerable dilation of the endoplasmic reticulum. These changes of the globular cells in the loach are fairly consistent with those found in pituitary gonadotrophs of the goldfish immediately after their spontaneous ovulation (Nagahama and Yamamoto, 1969b). It has been shown that both synthetic LH-RH and clomiphene citrate are able to induce a significant release of pituitary gonadotropin in several teleost fishes (Breton et al., 1975; Lam et al., 1975; Crim et al., 1976; Billard and Peter, 1977; Singh and Singh, 1979), and that a surge of circulating gonadotropin occurs just prior to ovulation in the goldfish (Breton et al., 1972; Stancey et al., 1979) and salmonid fishes (Crim et al., 1973, 1975; Hirose and Wakabayashi, 1978). Therefore, it seems reasonable to consider that the globular cells of the loach are conventional gonadotrophs and that quantitative alterations of the small granules of their cytoplasm may be directly involved in releasing of gonadotropin.

The vesicular cells of the loach were also induced to change their fine structure by the treatment with LH-RH, though their response was not so conspicuous and prompt as that of the globular cells. Structural modifications of the vesicular cells were evident also in the fish following clomiphene-induced ovulation, though the changes were much less in degree when compared with those of the globular cells in that case, too. Anyway, these observations may indicate that functions of the vesicular cells as well as the globular cells in loach pituitaries are admittedly under the control of hypothalamic gonadotropin-releasing factors and may thus be considered as gonadotropic in nature.

The existence of a feedback control mechanism in the pituitary-gonadal axis in teleosts has been demonstrated in studies on cytological changes of pituitary gonadotrophs after the removal of gonads or after the treatment with exogenous sex steroids (cf. Reinboth, 1972; Nagahama, 1973; Dodd, 1975). Gonadectomy has been shown to elicit drastic changes of pituitary gonadotrophs light microscopically in the Indian catfish (Lehri, 1965; Sundararaj and Nayyar, 1969; Rao et al., 1972), the sockeye salmon (McBride and van Overbeeke, 1969), and electron microscopically in the goldfish (Nagahama and Yamamoto, 1969b) and Gillichthys mirabilis (Zambrano, 1971). In the loach, it was the globular cells that responded intensively to gonadectomy, though the changes

of the cells were less obvious in castrated males than in ovariectomized females. The vesicular cells of female loaches also responded to ovariectomy, but they exhibited only a little changes even 60 days after the operation. In castrated male loaches, in contrast, the vesicular cells became to display an enlargement of cytoplasmic vesicles and a decrease in the amount of both small granules and large globules in their cytoplasm. In the castrated male loaches, ultrastructural modifications were seen to be more prominent in the vesicular cells than in the globular cells.

Only the report by Simon and Reinboth (1974) has hitherto dealt with the effect of gonadectomy on different types of possible gonadotrophs in fish pituitaries. They detected, in Lepomis cyanellus and L. macrochirus, light microscopic differences between two types of pituitary gonadotrophs in their response to the treatment: one cell type displayed evident changes leading to the occurrence of the so-called signet-ring cells after gonadectomy of both sexes, while the other cell type did not react to gonadectomy. It is interesting to note that there is apparent similarity between the results of Lepomis and those of the loach in which gonadectomy could exerted a significant influence on the globular cells but not on the vesicular cells especially in females, though no difference in response of the cells was noticed between males and females in Lepomis.

In Lepomis macrochirus, treatment of males and females with estrogen failed to produce any light microscopic changes in both types of pituitary gonadotrophs (Simon and Reinboth, 1974). In the present study, however, following the treatment of female and male loaches with estrogen and androgen, the globular cells became atrophied as was the case for the conventional type of pituitary gonadotroph of sex hormone-treated goldfish (Nagahama, 1973; Kaul and Vollrath, 1974), and other several species of teleosts (Sundararaj and Goswami, 1968; Sage and Bromage, 1970b; van Overbeeke and McBride, 1971; Viswanathan and Sundararaj, 1974; Peute et al., 1976). The vesicular cells of the loach were also observed to regress with notable shrinkage of their cytoplasmic vesicles, in androgen-treated males but not in androgen-treated females. On the contrary, the vesicular cells were stimulated to develop synthetic activities of the Golgi apparatus by estrogen treatment of loaches of both sexes.

The experimental findings mentioned above may confirm that both the two types of pituitary cells of the loach have functional relations with gonadal activities of the fish. In addition, clear differences existing between the two cell types in their responses to LH-RH, clomiphene, gonadectomy, and exogenous sex steroids may favor the assumption that each of the two distinct types of gonadotrophs may have separate actions on sexual

development in loaches of either sex. Opposed to this view is the oppinion advanced by Peute et al. (1978), who emphasized that, in their study on the rainbow trout, a single type of pituitary gonadotroph may ultrastructurally assume either the "globular" or the "cisternal (vesicular)" stage accompanied with some intermediate stages in association with changes of their activity. In the loach, however, no transitional cells from one to the other type of the cells were evident in the present ultrastructural observations. It was rather noted that the two cell types always retained their respective cytological characteristics, remaining striking contrast from each other, even in the experimental conditions that would force the gonadotropic cells to evoke extreme functional alterations.

To substantiate the assumption that the two types of gonadotrophs in the loach may play their respective roles at different stages of gonadal maturation, changes of the pituitary cells in relation to gonadal development and reproductive cycle of the fish should be studied carefully. In the pituitary gland of juvenile loaches of both sexes, the globular cells with immature cytoplasmic granules existed in the central parts of the PPD, but the vesicular cells could not be detected in the gland. Although the globular cells did not show any conspicuous changes in early stages of gonadal development, they displayed a notable increase in size and in

number in harmony with progressive maturation of ovaries and testes of the loach. Moreover, they were conspicuous for a depletion of small, secretory granules, extensive dilation of the endoplasmic reticulum, and subsequent pycnotic changes of the nucleus following ovulation and spermiation. This indicates that the globular cells of the loach may secrete a gonadotropin which promote later stages of gonadal maturation, especially at the time of ovulation and spermiation. Similar changes of pituitary gonadotrophs of the globular type during gonadal development were noted in various other fishes by many investigators (Olivereau and Herlant, 1960; Sokol, 1961; Olivereau and Ridgway, 1962; Robertson and Wexler, 1962a, b; Lagios, 1965; Olivereau, 1967, 1976, 1978; van Overbeeke and McBride, 1967; Kasuga and Takahashi, 1971; Demal et al., 1974; Sasayama and Takahashi, 1975; Kawashima et al., 1976; Moitra and Sarkar, 1976; Dimovska, 1977; Malo-Michele, 1978).

The vesicular cells were indiscernible in the pituitary gland of juvenile loaches with ovaries at the perinucleolus stage or with testes at the spermatogonial stage. The cells made their first appearance in the PPD concurrently with the appearance of oocytes in the yolk vesicle stage in the ovary or of the first spermatocytes in the testes. They revealed little cytological alterations during the period of advance of vitellogenesis and spermatogenesis, or even at the time of

ovulation and spermiation which were accompanied with remarkable modifications of the globular cells. The vesicular cells appeared to become active again, exhibiting the development of Golgi apparatus and the accumulation of cytoplasmic granules, a few months after the spawning period, sometime before exogenous vitellogenesis in ovarian oocytes or spermatogenesis in testes had taken place. Cook and van Overbeeke (1972) suggested that the vesicular cells of sockeye salmon pituitaries may have their essential gonadotropic potency in early stages of gonadal development and become largely degranulated near the time of full maturation when the globular gonadotrophs abound in the gland. In the Atlantic salmon, no vesicular gonadotrophs are detected in the pituitary gland of immature parrs, and then they become apparent and highly active when the gonadosomatic index is still very low (Olivereau, 1976). Olivereau (1978) has mentioned similar cytological alteration of the two types of pituitary gonadotrophs in the rainbow trout and the brown trout, too.

Peute et al. (1978) reported that, in the rainbow trout, a predominant occurrence of pituitary gonadotrophs of the vesicular stage was also correlated with vitellogenesis in females and spermatocyte formation in males, and that these cells were observed to be shifted into the globular stage by gradual regranulation, leading to absolute predominance of the globular cells toward the

spawning season. In contrast, in the loach examined in the present study, no intermediate cell types between the two types of gonadotrophs were detectable in the pituitary gland. This was also the case for the two types of gonadotrophs of the whitespotted char observed in the present study.

In the whitespotted char, the globular cells showed a notable increase both in number and in size parallel with progressive maturation of the gonad. At the time of spawning, they displayed a degranulation and a dilation of the endoplasmic reticulum, followed by some atrophic changes. On the other hand, the vesicular cells made their first appearance concurrently with the appearance of oocytes of the late yolk vesicle stage in ovaries or of first spermatocytes in testes. During the successive course of gonadal development, they showed little change until they were activated again when ovarian oocytes were at the late yolk vesicle stage about 3 months after spawning.

Thus, possible explanation of the function of the vesicular cells in female fish may be that the secretion of the vesicular cells is implicated in vitellogenesis in ovarian oocytes, and that, once the vitellogenesis has been initiated, its subsequent course extending over a long period of time depends only on a rather stable maintenance of secretion of the vesicular cell hormone(s). The globular cells may also have an action on vitello-

genesis, presumably through stimulation of the secretion of estrogen which has been shown to increase in the blood with the advancement of vitellogenesis (Lambert et al., 1978). Precise roles played by the vesicular cells in testicular development of male fish remain to be clarified. However, Hyder and his collaborators (Hyder, 1970; Hyder et al., 1974, 1979) suggested that two pituitary hormones (a FSH-like and a LH-like hormone) might control testicular development in Tilapia: the FSH-like principle is probably active largely in early phases of testicular growth, while the LH-like principle probably exerts its maximal effect on the testis at the spermiation period. It is interesting to compare this suggestion given in Tilapia with that obtained in the present study in terms of that there may be some correlation between the FSH-like activity and a function of the vesicular cells and between the LH-like activity and a function of the globular cells in male fishes.

On the other hand, Simon and Reinboth (1974) reported that the treatment of Lepomis with a long photoperiod caused different histological changes in two distinct types of pituitary gonadotrophs. This led the present writer to consider the possibility that the exposure of loaches to continuous light may accelerate vitellogenesis and spermatogenesis in their post-spawning period, possibly through an activation of the vesicular cells in the

pituitary gland. In fact, the treatment was apparently effective in accelerating the maturation of gonads in adult and young loaches of both sexes. In the treated loaches, the vesicular cells showed an increase both in number and in size and, characteristically, a progressive increase in size of their cytoplasmic vesicles. Although the globular cells of adult loaches of both sexes also displayed activated features following the treatment, their changes appeared to be less prominent in comparison with those of the vesicular cells. In addition, it is interesting to note that, in young females exposed to constant light, the vesicular cells, which had been indiscernible at the start of treatment, were induced to be differentiated simultaneously with a precocious occurrence of exogenous vitellogenesis in ovarian oocytes.

Furthermore, it is expected that experimental treatments to inhibit gonadotropic activities of the pituitary gland result in an inactivation of the vesicular cells which may reflect on certain ultrastructural changes of the cells. Complete starvation of some fishes has been shown to cause gonadal regression through "psudohypophysectomy" of the fish (Clements and Reed, 1967; Bogenschutz and Clemens, 1967; Nagahama, 1973). By complete starvation of loaches lasting for 60 days, severe retrogressive changes of ovaries and testes were caused. In pituitaries of the starved loaches, the

globular cells showed an increase in number of small granules and a reduction in amount of the endoplasmic reticulum, indicating a subsidence of their secretory and synthetic activities. In contrast, no notable structural change was detectable in the vesicular cells of the starved fish even after 60 days of the treatment. These facts probably suggest that a secretion of the globular cells may have a basic significance in the progress of gonadal development whereas that of the vesicular cells may take parts in certain particular processes of gonadal maturation. This further implies different mechanisms controlling gonadotropic activities of the two distinct types of pituitary gonadotrophs of the loach.

It was mentioned previously that a precocious differentiation of the vesicular cells was induced by the treatment of young female loaches with continuous light. Comparable results were obtained also in juvenile female loaches injected with HCG. At the start of experiment, pituitaries of the loaches were provided with only a few, small gonadotrophs of inactive globular cell types. After 6 injections of HCG, while the globular cells became to be highly active, a few cells of the vesicular cell type began to appear in the pituitaries. Furthermore, oral administration of testosterone to juvenile loaches of either sex could also accelerate the differentiation of the vesicular cells. Although estrone

administration failed to bring about such an apparent effect, some cells having granular contents similar to those of the vesicular cells appeared in affected pituitary glands. The globular cells of the juvenile loaches treated with either testosterone or estrone exhibited a highly activated aspect to synthesize their granular inclusions.

Thus it is concluded that both exogenous gonadotropin and sex steroids can induce a precocious differentiation of the vesicular cells and an activation of the globular cells in the pituitary gland of juvenile loaches. At least in regard to the activation of pituitary gonadotrophs by exogenous hormones, this agrees with the results obtained in previous studies on immature fishes treated with fish pituitary extracts (Anguilla anguilla, Olivereau and Fontaine, 1966; Olivereau, 1967), with mammalian gonadotropin (Anguilla japonica, Yamamoto and Nagahama, 1973; Yamamoto et al., 1974), or with sex steroids (Oncorhynchus nerka, van Overbeeke and McBride, 1971; Anguilla anguilla, Olivereau and Chambolle, 1978; Sokolowska et al., 1978; Olivereau and Olivereau, 1979a, b). In the present study, too, pituitary gonadotrophs of silver females of the Japanese eel showed a progressive increase in size and in number with a prominent accumulation of secretory granules during the course of artificial maturation caused by injections of salmon pituitaries.

In a series of recent studies on immature European eels, Olivereau and Olivereau (1979a, b) suggested that estradiol acted on gonadotrophs to stimulate hormone synthesis by a positive feedback. In addition, it has been demonstrated that pituitary gonadotropin levels are raised following administration of testosterone in Atlantic salmon parr (Crim and Peter, 1978) and in juvenile rainbow trout (Crim and Evans, 1979). Consequently it seems likely that sex steroids, either administered from outside or triggered to be secreted by the action of exogenous gonadotropins, may be involed in the regulation of the globular cells. Moreover, a possible development of non-granulated pituitary cells into the vesicular cells, or more precisely, the initiation of synthesis of secretory products in the former cells, is also likely to be promoted by sex steroids in juvenile loaches. These assumptions are to be substantiated by further studies. It is tempting, however, to speculate that, during immature, subadult stages of fishes, the vesicular cells become to function as gonadotropic progressively by an action of sex steroids which have been discharged into circulation by the stimulation of hormone(s) of the globular cells.

Some conflicting results were obtained concerning the type of pituitary gonadotrophs of several teleost fishes examined in the present study. Whereas the presence of two distinct types of pituitary gonadotrophs was

confirmed in the loach, the chum salmon, the masu salmon and the whitespotted char, only one type of gonadotroph was found in the rainbow trout, the Japanese eel and the goldfish. It is of special interest that a single type of pituitary gonadotroph of the goldfish are provided with cytological characteristics which are seen to combine those of the two types of gonadotrophs of the loach. Only one type of gonadotroph has been repeatedly demonstrated in the goldfish under various experimental conditions (Nagahama and Yamamoto, 1969b; Kaul and Vollrath, 1974; Lam et al., 1976). In contrast, Olivereau (1962), Leatherland (1972) and Leatherland and Sonstegard (1977) have presented evidence for the existence of two types of gonadotrophs in the same species.

Several cases have been reported to show that a single type of pituitary gonadotroph may vary in its cytological aspects rather widely even in fish at a given stage of sexual development. For example, Knowles and Vollrath (1966c) described five types of gonadotropic cells in the migrating European eel, Anguilla anguilla, and Abraham (1974) mentioned several gonadotropic cell variants with a series of extensive cytological differences in the mullet, Mugil sp. In their ultrastructural studies on Tilapia sp. and the black molly, Poecilia latipinna, Leatherland et al. (1974) and Batten et al. (1975) also reported great variabilities observed in the activity of gonadotrophs of the same pituitary gland.

A general survey of the results obtained in the present study and those appeared in many literatures on pituitary gonadotrophs of various teleost fishes may lead to the following conclusions. Teleost fishes can be classified into 5 groups in terms of the type of gonadotropic cells occurring in the pituitary gland: 1) those with only a single type of gonadotroph which does not reveal any different cytological "forms" during gonadal development (Anguilla japonica, Oryzias latipes); 2) those with one type of gonadotroph which may assume some different cytological aspects depending on maturational stages of the gonad (Mugil, Poecilia latipinna, Tilapia); 3) those with a single type of gonadotroph which possesses cytological characteristics that combine, in the same cells, those of two distinct types of gonadotrophs (Carassius auratus); 4) those with a single type of gonadotroph which exhibits two different forms accompanied with some intermediate forms in association with changes of their activity (Salmo gairdneri, Salmo salar); and 5) those with two distinct types of gonadotrophs which are not accompanied with any intermediate cell types between the two (Misgurnus anguillicaudatus, Oncorhynchus keta, Oncorhynchus masou, Rutilus rutilus, Salvelinus leucomaenis).

So far as the present writer knows, only Haider and Blüm (1977) have thus far presented a hypothesis about the evolutionary aspect of the pituitary-gonadal axis

of lower vertebrates. They postulate that evolutionary changes of the pituitary gonadotropin system in vertebrates may have occurred at three levels, namely gonadotropic cells in the pituitary, gonadotropic hormone molecule, and gonadotropin receptor in the gonad, which have undergone a change independently of each other. They further suggest that different phases of combined changes at the three levels are in play in different species, genera, or families of the same animal class in accordance with the selective value gained during the process of evolution. They are of the opinion that neither the theory of unicity nor the concept of duality can be generalized for the whole lower vertebrates. It is interesting to consider the above-mentioned five types of pituitary gonadotrophs as representing a sequential change in the differentiation of gonadotropic function of the cells.

In conclusion, the present study presented evidence of the existence of two distinct types of pituitary gonadotrophs in the loach, each of which may play roles at different stages of the development and maturation of ovaries and testes of the fish. It is interesting to make a comparison between hormones of the two types of pituitary gonadotrophs determined in the present study and the two different kinds of gonadotropic hormones isolated recently from pituitary extracts of several teleost fishes by Idler and his coworkers (Ng and Idler,

1978a, b, 1979; Idler and Ng, 1979). Immunocytochemical methods employing antibodies generated against each of the two kinds of gonadotropins are considered to be the most available method to prove this possibility. Furthermore, the present study revealed some differences in ultrastructural aspects of the two types of gonadotrophs between males and females of the loach. It remains to be elucidated by further studies whether these differences are due to the different physiological conditions of the ovary and the testis of the fish or to presumable sex-linked differences in biochemical nature of the hormones produced. The latter possibility has been indicated by some studies in which gonadotropins of separate natures were isolated from male and female pituitaries of salmonid fishes (Oncorhynchus keta, Idler et al., 1975a, b; Oncorhynchus tshawytscha, Breton et al., 1978). Finally, the present study indicates that the type of pituitary gonadotrophs may be different among fish species of the same family such as Cypridae and Salmonidae. Further work aiming at understanding these phenomena is to be done using various fishes of both sexes in various stages of their reproductive cycle.

Summary

One of the current controversial problems in

teleost reproductive physiology has centered around the question as to whether teleost fishes possess one or two types of gonadotropic cells in the pituitary gland. The main aim of the present study is to identify pituitary gonadotrophs cytophysiologically and to clarify their functional relationships to gonadal development mainly in loach, Misgrunus anguillicaudatus, and in chum salmon, Oncorhynchus keta, masu salmon, Oncorhynchus masou, whitespotted char, Salvelinus leucomaenis, rainbow trout, Salmo gairdneri, goldfish, Carassius auratus, and Japanese eel, Anguilla japonica.

1. In the proximal pars distalis (PPD) of pituitary gland of the loach, two distinct types of basophilic cells with different localities, staining properties and ultrastructural characteristics are present along with the cells identified as thyrotrophs and somatotrophs. Ultrastructural changes of the two types of basophils, termed the globular cell and the vesicular cell according to their ultrastructural features, were examined in male and female loaches which had been subjected to the injection of synthetic LH-RH or clomiphene citrate, to the gonadectomy or to the treatment with sex steroids during their prespawning period. Results of these treatments indicate that both the two types of pituitary cells have functional relation to gonadal activities of the fish.

2. During the course of gonadal development and

reproductive cycle of the loach the globular cells, which had already existed in the PPD of juvenile loaches, became activated in parallel with gonadal maturation, and showed remarkable changes at the time of ovulation or spermiation, thus denoting themselves to be the conventional gonadotrophs. In contrast, the vesicular cells made their first appearance in the PPD of young loaches concurrently with initiation of vitellogenesis and spermatogenesis in the gonad, and revealed little change during subsequent periods of advancing gonadal maturation. It is suggested that the two types of gonadotrophs may take different parts in different stages of development and maturation of the ovary and the testis of the loach.

3. Exposure of loaches to continuous light during the postspawning period could recover and promote vitellogenesis and spermatogenesis, and caused a concomitant activation of functions of the vesicular cells. Complete starvation of loaches during the spawning period could cause regression of the gonad, but failed to exert any notable influences on the vesicular cells. It is inferred that the vesicular cells may be implicated essentially in the initiation and successive maintenance of gametogenesis in the loach.

4. Administrations of human chorionic gonadotropin or testosterone to juvenile loaches did efficiently induce a precocious differentiation of the vesicular

cells in the PPD, which implied a certain relation of sex steroids with functioning of the vesicular cells.

5. Among the other six species of teleost fishes examined in the present study, three salmonids, namely the chum salmon, the masu salmon and the whitespotted char, had two distinct types of pituitary gonadotrophs with similar cytological characteristics to those of the loach. The two gonadotropic cell types of the whitespotted char showed characteristic changes during gonadal development, which fairly coincided with those observed in the loach. On the other hand, only one type of gonadotroph was found in the pituitary gland of the rainbow trout examined at the peak of their spawning period. A single type of pituitary gonadotroph was discernible in the goldfish, too. However, the cell of the goldfish possessed cytological characteristics which seemed to combine those of the vesicular cells and the globular cells of the loach. In the pituitary gland of the Japanese eel induced to maturation artificially, only one type of glandular cells was observed to be gonadotropic in nature.

6. The results were discussed in terms of the possibility that different species of teleost fishes might have one of the following 5 different kinds of pituitary gonadotrophs, which might bear some evolutionary significance in the development of the pituitary-gonadal axis controlling the reproduction of fishes: 1) a single type

of gonadotroph which does not reveal any different cytological "forms" during gonadal development; 2) a single type of gonadotroph which may assume some different cytological aspects depending on maturational stages of the gonad; 3) a single type of gonadotroph which possesses cytological characteristics which combine those of two distinct types of gonadotroph; 4) a single type of gonadotroph which exhibits two different forms accompanied with some intermediate forms in association with changes of their activity; 5) two distinct types of gonadotrophs which are not accompanied with any intermediate cell types between the two.

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Abbreviations

a, ACTH: adrenocorticotropic cell

g, g-GTH: globular cell (gonadotropic cell)

g, GTH: gonadotropic cell

G: Golgi apparatus

LG: large globule

M: mitochondrion

n, NGC: non-granulated cell

NH: neurohypophysis

p, Pro: prolactin cell

PI: pars intermedia

p1, PI-1: chromophobic cell of pars intermedia

p2, PI-2: basophilic cell of pars intermedia

PPD: proximal pars distalis

rER: rough endoplasmic reticulum

RPD: rostral pars distalis

SG: small granule

ST: stellate cell

s, STH: somatotropic cell

t, TSH: thyrotropic cell

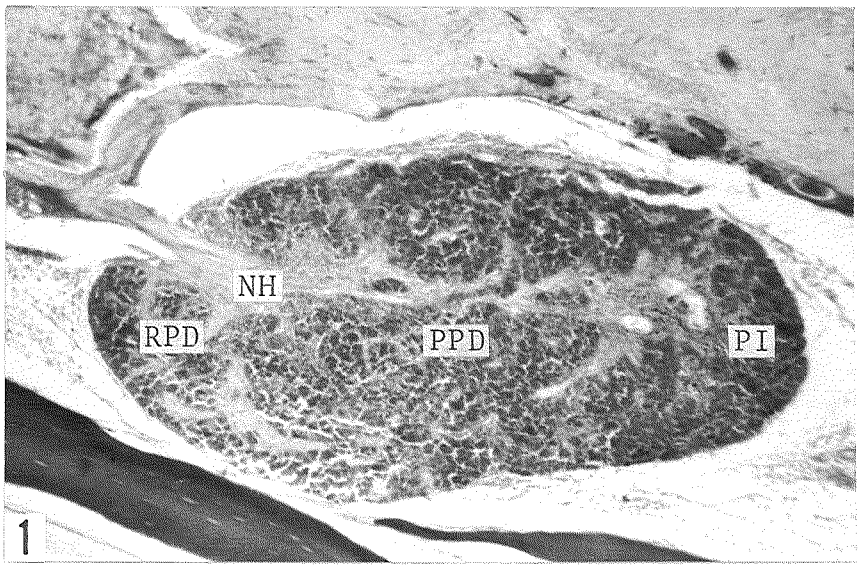
v, v-GTH: vesicular cell (gonadotropic cell)

V: vesicular cisterna (cytoplasmic vesicle) of rough endoplasmic reticulum

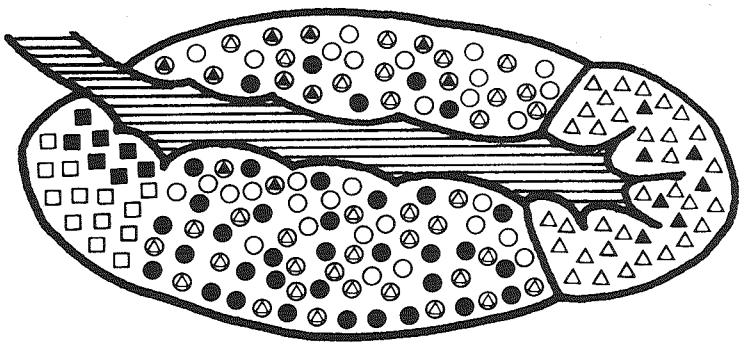
Scale on electron micrograms: 1 μm.

Plate 1

Figs. 1 and 2. Median sagittal section through the pituitary gland of the loach, Misgurnus anguillicaudatus. Fig. 1: AF stain X150. Fig. 2: ■, ACTH cell; ●, globular cell; △, PI-1 cell; ▲, PI-2 cell; □, prolactin cell; ⊘, somatotropic cell; ⊙, thyrotropic cell; ○, vesicular cell.



1



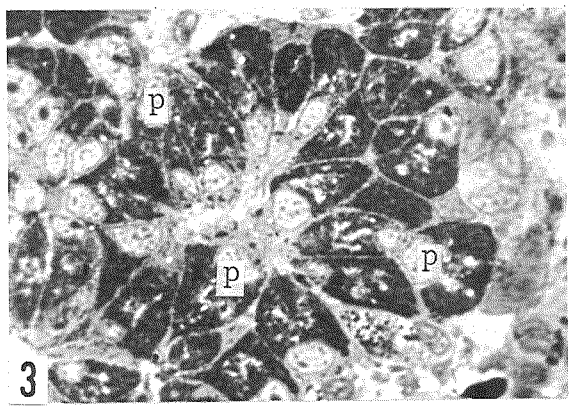
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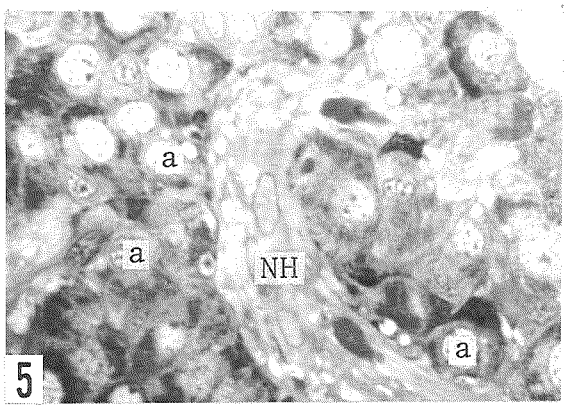
Plate 2

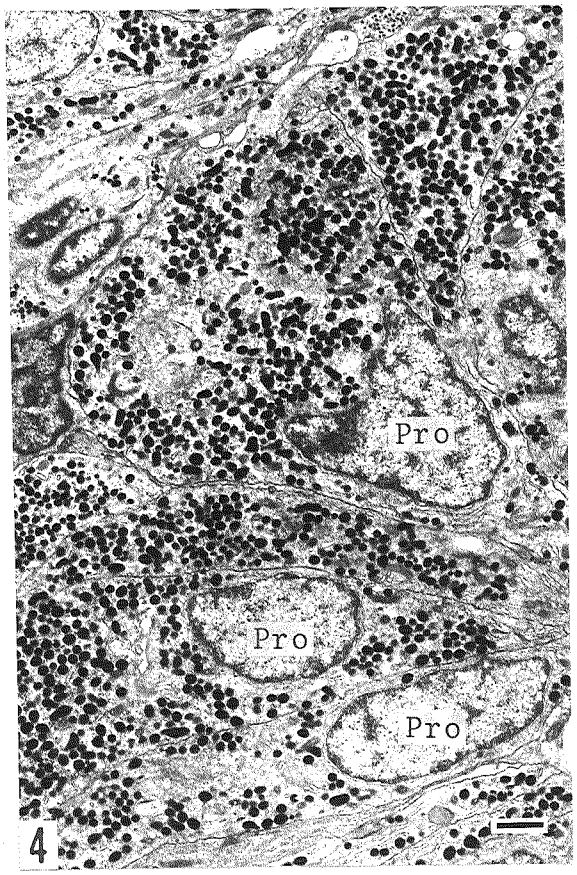
Figs. 3-6. Glandular cells in the RPD of the pituitary gland of adult loaches. Fig. 3 and 5: methylene blue stain of Epon-embedded, semithin sections, X1000.

Fig. 3 and 4. Prolactin cell. A psudo-follicles of the cells is shown in Fig. 3.

Fig. 5 and 6. Adrenocorticotropic cells.


p
p
p
3


a
a
NH
a
5


Pro
Pro
Pro
4

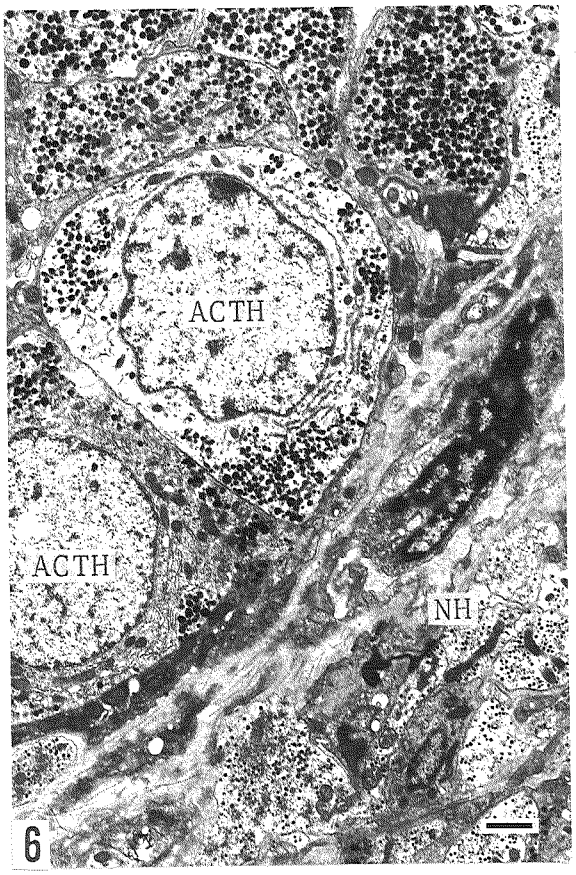
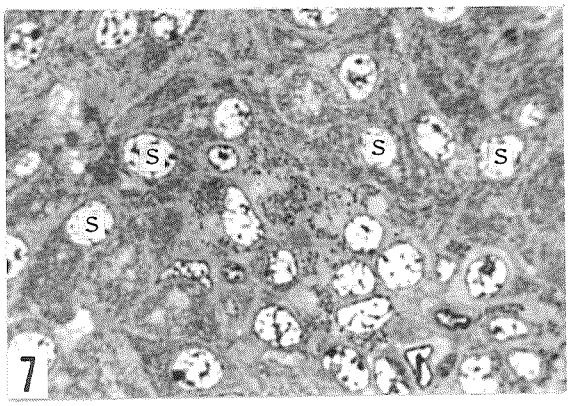

ACTH
ACTH
NH
6

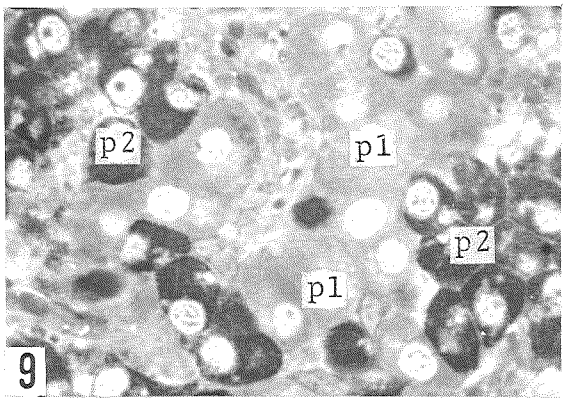
Plate 3

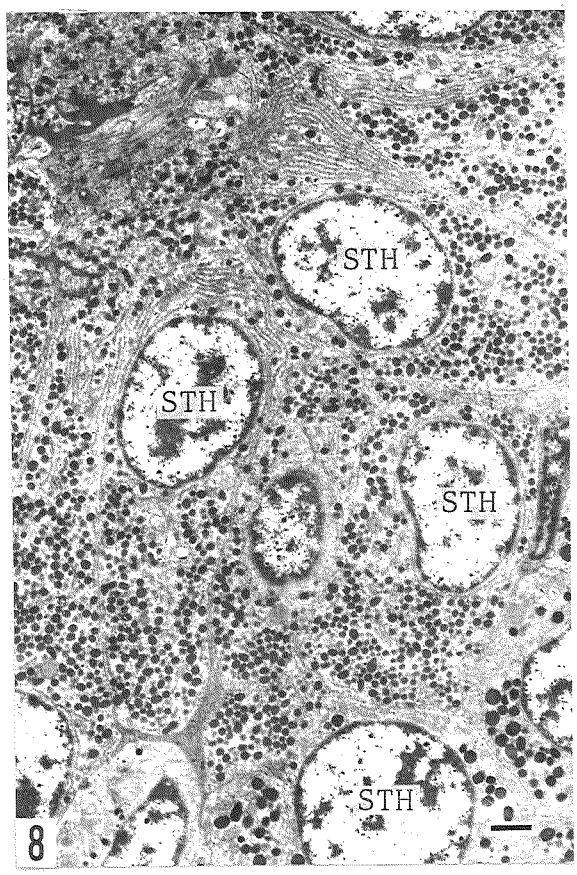
Figs. 7-10. Glandular cells in the PPD (Figs. 7 and 8) and in the PI (Figs. 9 and 10) of the pituitary gland of adult loaches. Figs. 7aand 9: methylene blue stain of Epon-embedded, semithin sections, X1000.

Figs. 7 and 8. Somatotropic cells (s and STH).

Figs. 9 and 10. Chromophobic cells (p1 and PI-1) and basophilic cells (p2 and PI-2).


s
s
s
s
s
7

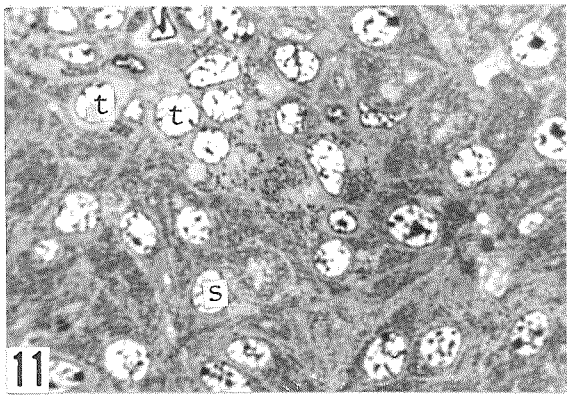

p2
p1
p2
p1
9

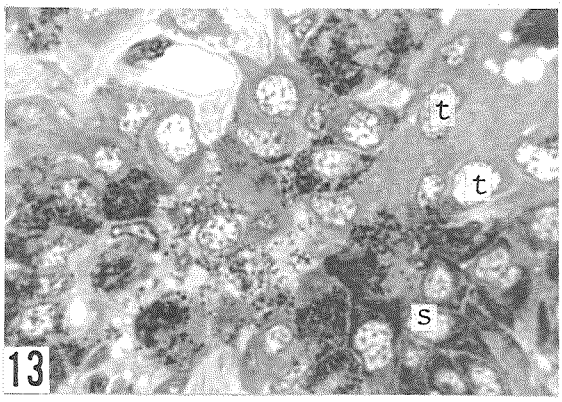

STH
STH
STH
STH
8

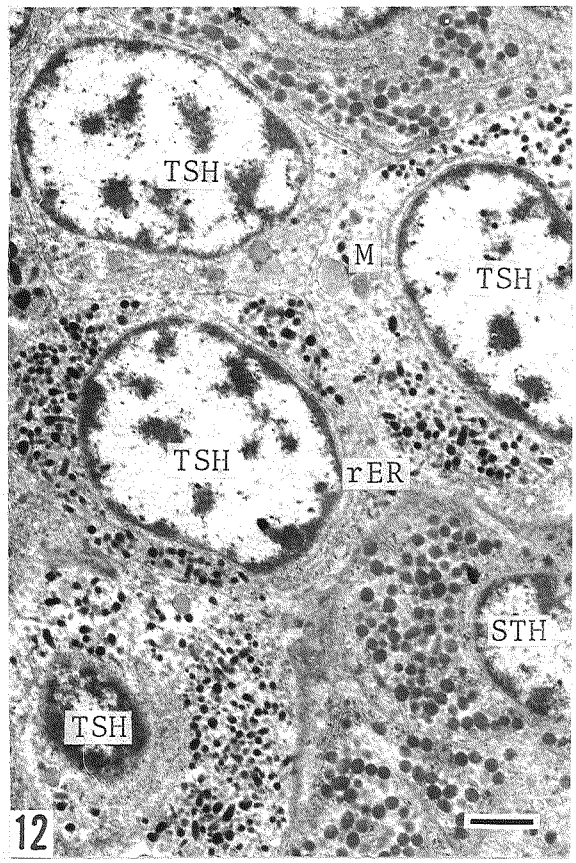

PI-1
PI-2
10

Plate 4

Figs. 11-14. Thyrotrophs (t and TSH) in the PPD of the pituitary gland of control (Figs. 11 and 12) and thiourea-treated (Figs. 13 and 14) loaches after 60 days of treatment. Figs. 11 and 13: methylene blue stain of Epon-embedded, semithin sections, X1000.


t
t
s
11


t
t
s
13


TSH
M
TSH
TSH
rER
STH
TSH
12

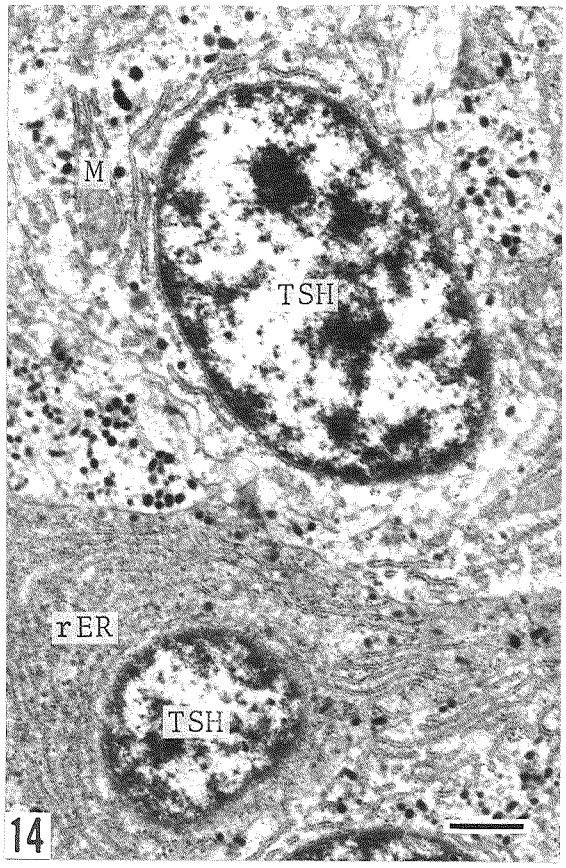
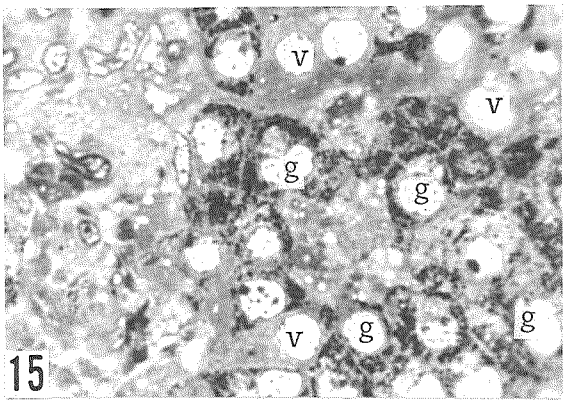
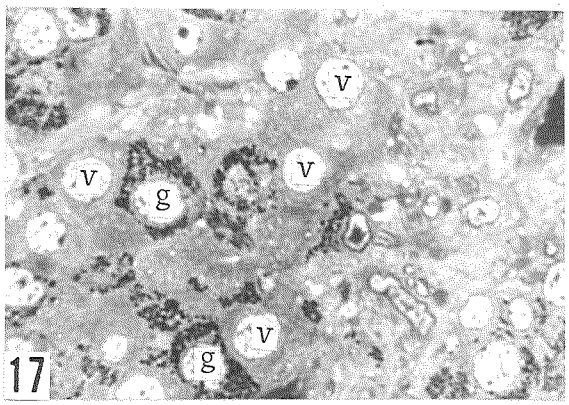

M
TSH
rER
TSH
14

Plate 5

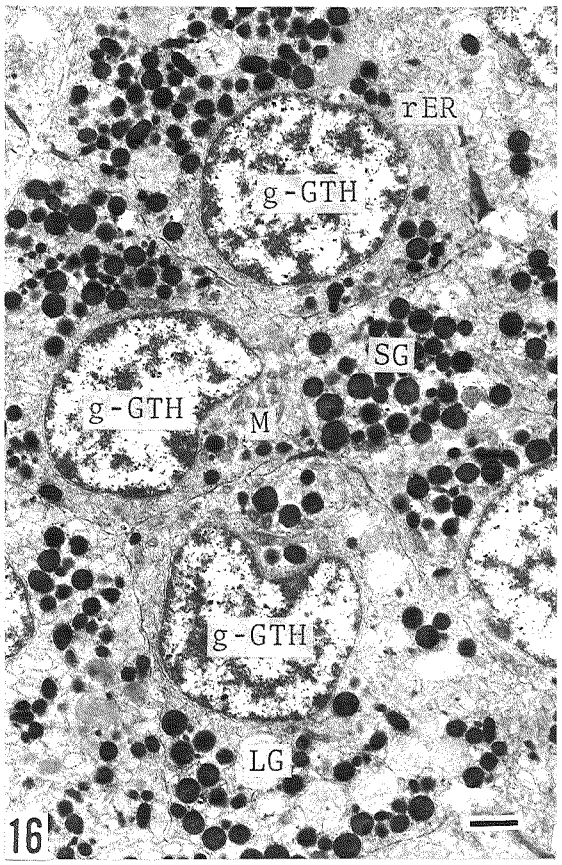
Figs. 15-18. Globular cells (g and g-GTH) and vesicular cells (v and v-GTH) occurring in the PPD of the pituitary gland of adult loaches. Figs. 15 and 17: methylene blue stain of Epon-embedded, semithin sections, X1000.



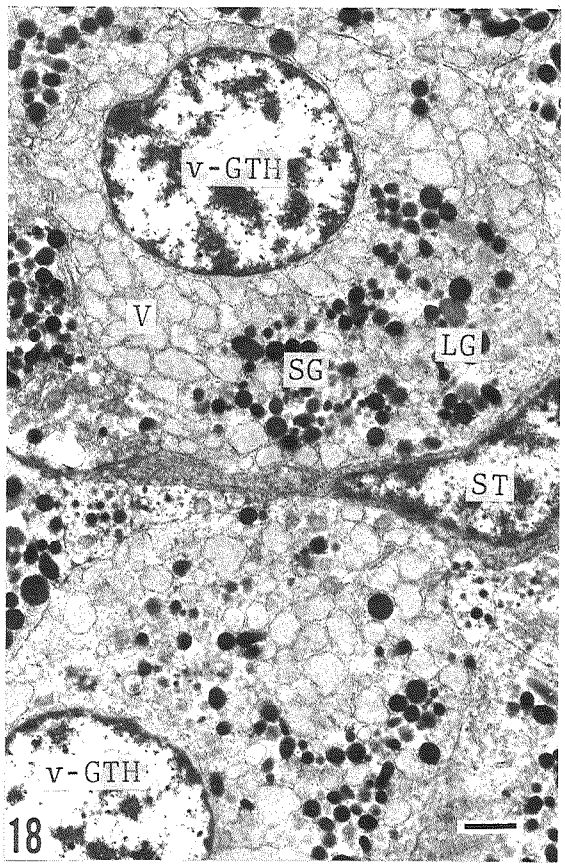
15



17



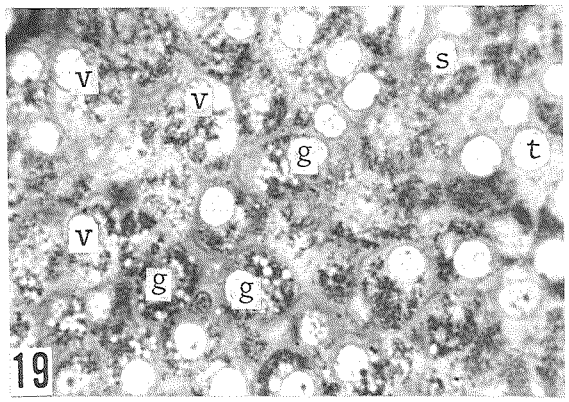
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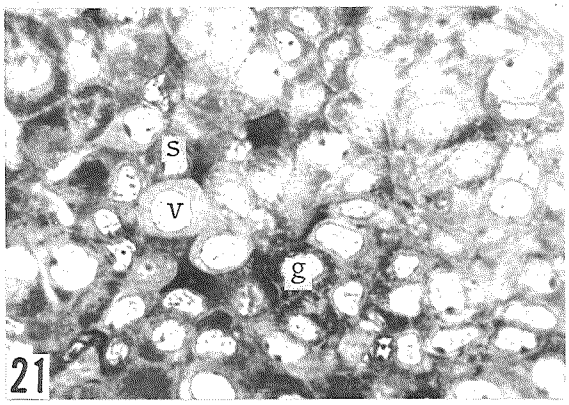


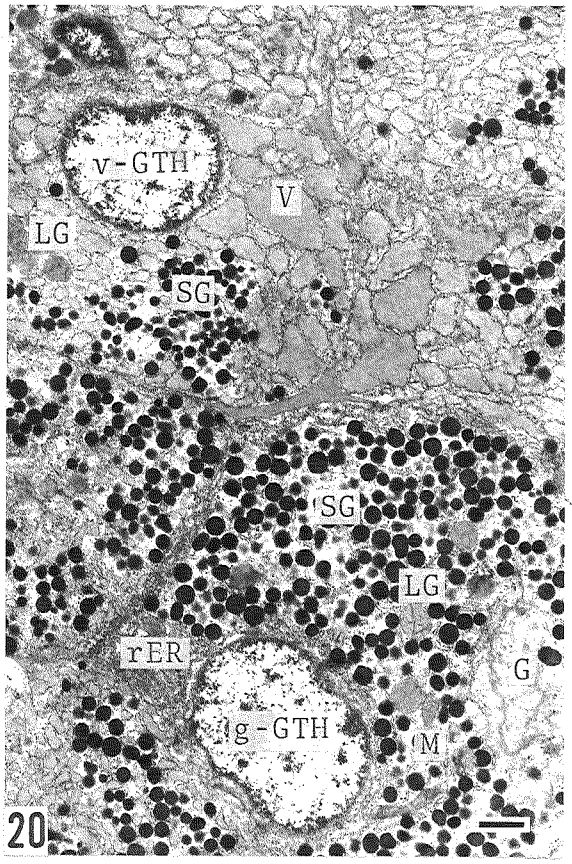
18

Plate 6

Figs. 19–22. Globular cells (g and g-GTH) and vesicular cells (v and v-GTH) of adult (Figs. 19 and 20) and young (Figs. 21 and 22) loaches of the control groups serving in the experiments done at the late prespawning or the early spawning period (May–July). Figs. 19 and 21: methylene blue stain of Epon-embedded, semithin sections, X1000.


v
v
s
g
t
v
g
g
19


s
v
g
21


v-GTH
V
LG
SG
SG
LG
rER
G
g-GTH
M
20

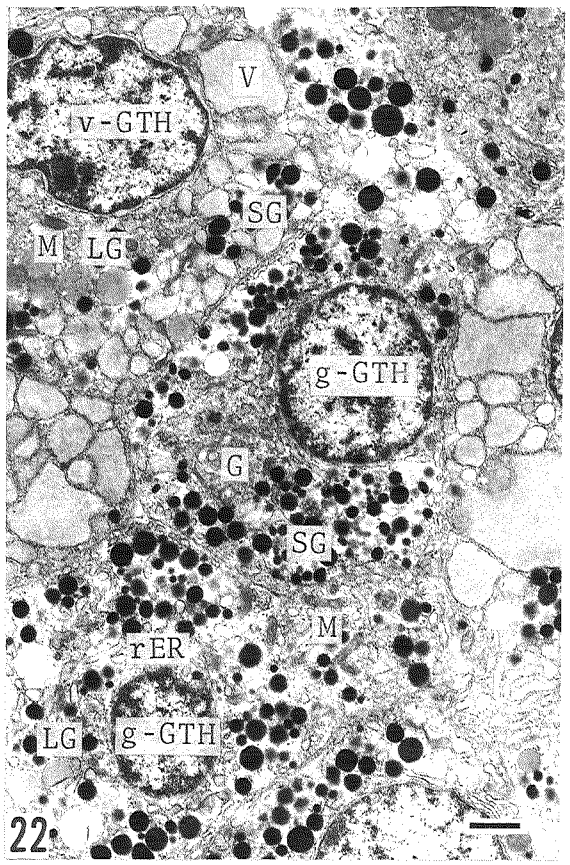

V
v-GTH
SG
M
LG
g-GTH
G
SG
M
rER
LG
g-GTH
22

Plate 7

Figs. 23-25. Globular cells (g and g-GTH) and vesicular cells (v and v-GTH) of LH-RH-treated adult loaches sampled 1 hour (Figs. 23 and 24) and 48 hours (Fig. 25) after injection.
Fig. 23: methylene blue stain of an Epon-embedded, semithin section, X1000.

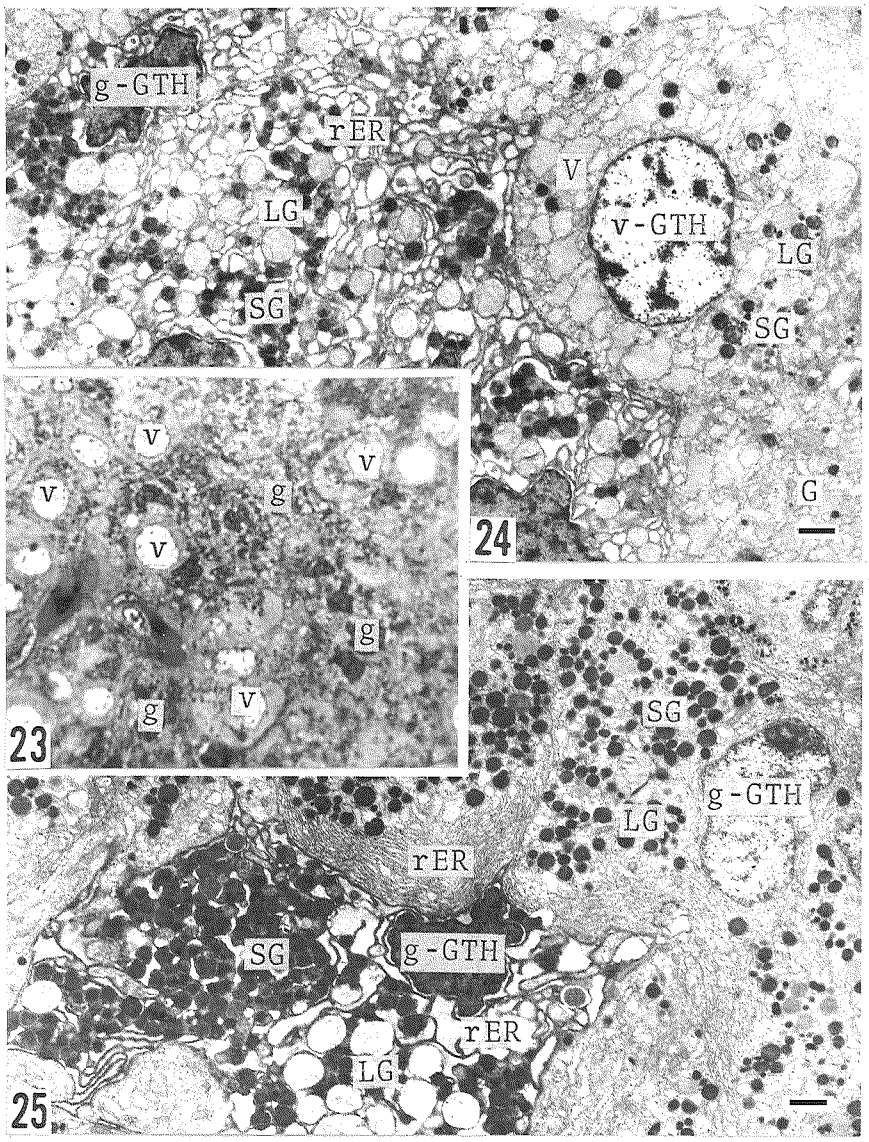
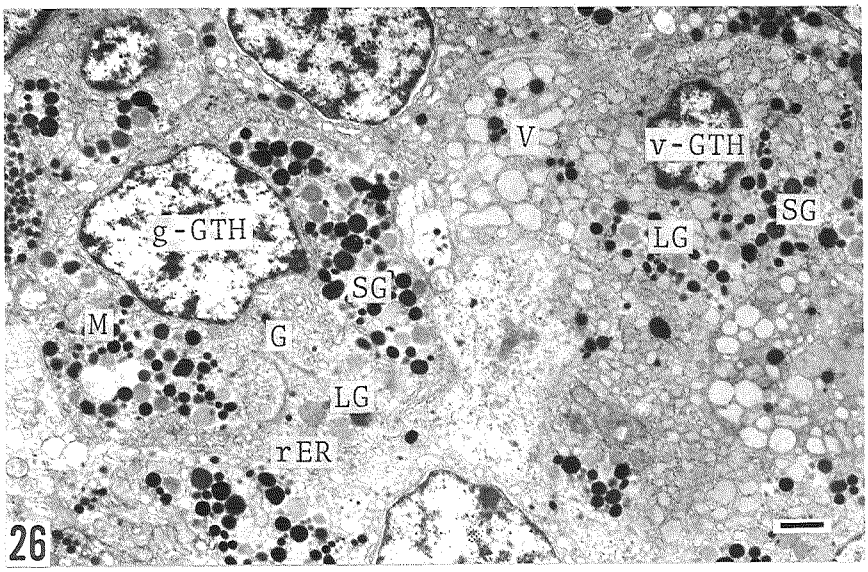

g-GTH
rER
LG
SG
V
v-GTH
LG
SG
G
24
v
v
v
g
v
g
g
v
23
SG
g-GTH
LG
rER
SG
g-GTH
rER
LG
25

Plate 8

Figs. 26 and 27. Globular cells (g-GTH) and vesicular cells (v-GTH) of LH-RH-treated, young female loaches sampled 1 hour (Fig. 26) and 24 hours (Fig. 27) after injection.


V
v-GTH
SG
g-GTH
LG
SG
M
G
LG
rER
26

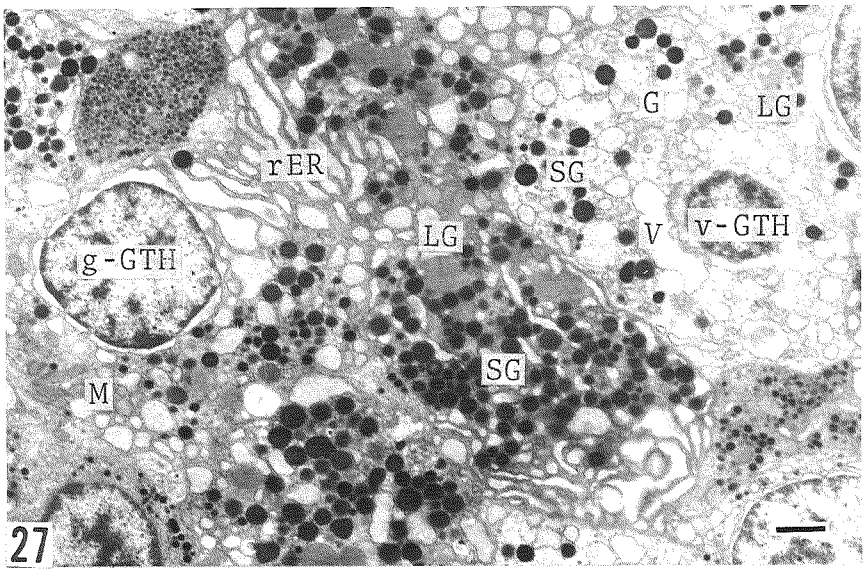
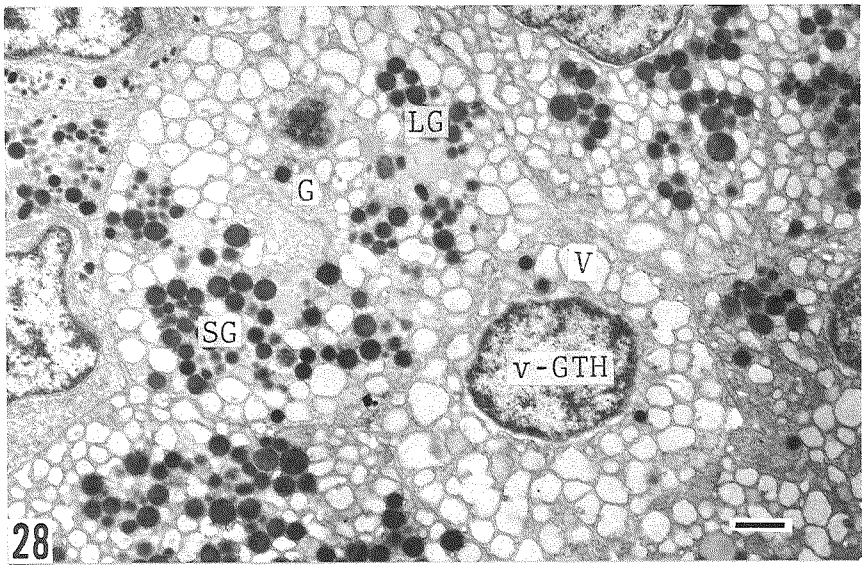

G
LG
rER
SG
LG
V
v-GTH
g-GTH
SG
M
27

Plate 9

Figs. 28 and 29. Vesicular cells (v-GTH) of LH-RH-treated adult female (Fig. 28) and male (Fig. 29) loaches sampled 72 hours after injection.


LG
G
V
SG
v-GTH
28

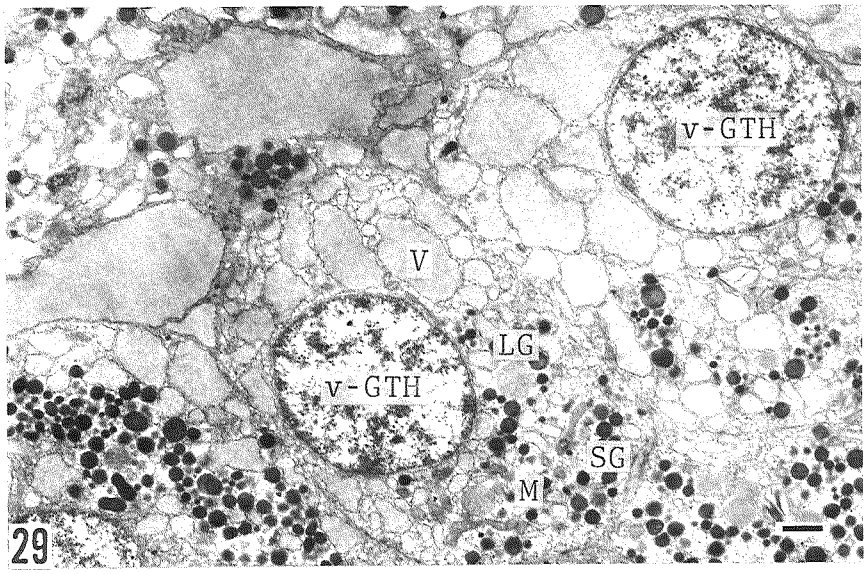
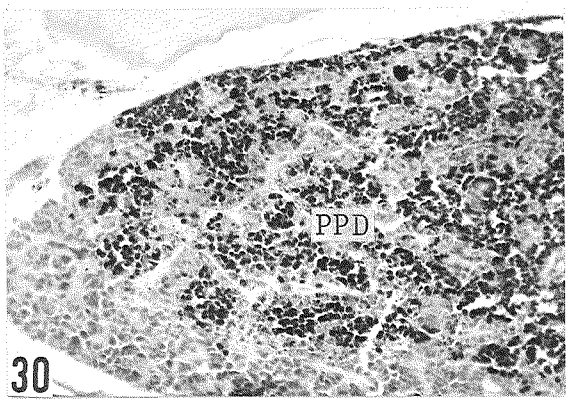
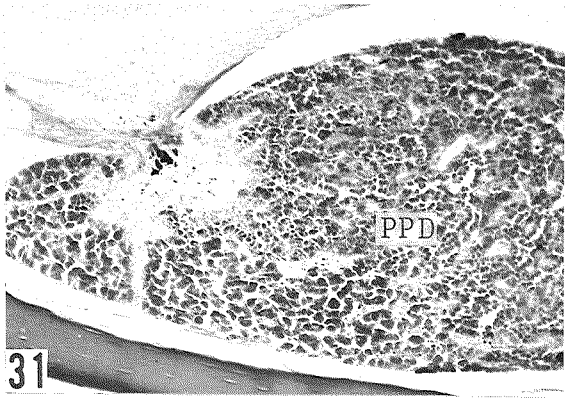
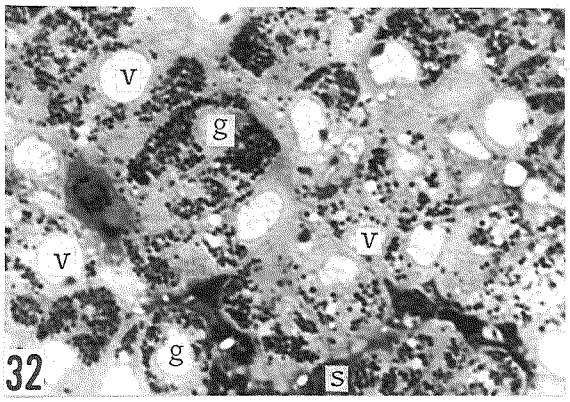

v-GTH
V
LG
v-GTH
SG
M
29

Plate 10

Figs. 30-33. Sagittal sections through the pituitary gland of a saline-injected (Figs. 30 and 32) and a clomiphene-injected (Figs. 31 and 33) female loach, showing light microscopic changes of globular (g) and vesicular (v) cells in the PPD. Figs. 30 and 31: AF stain of paraffin embedded sections, X150. Figs. 32 and 33: methylene blue stain of Epon-embedded, semithin sections, X1000.


PPD
30


PPD
31


v
g
v
v
g
s
32

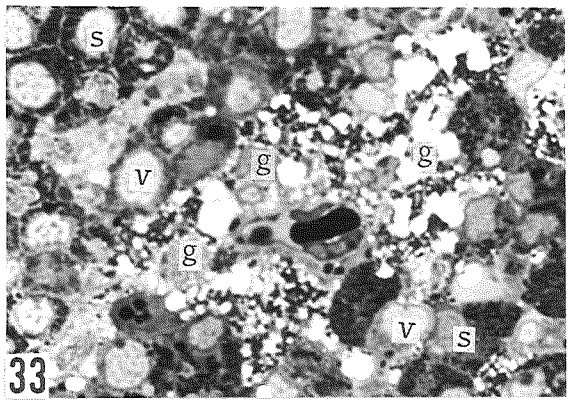
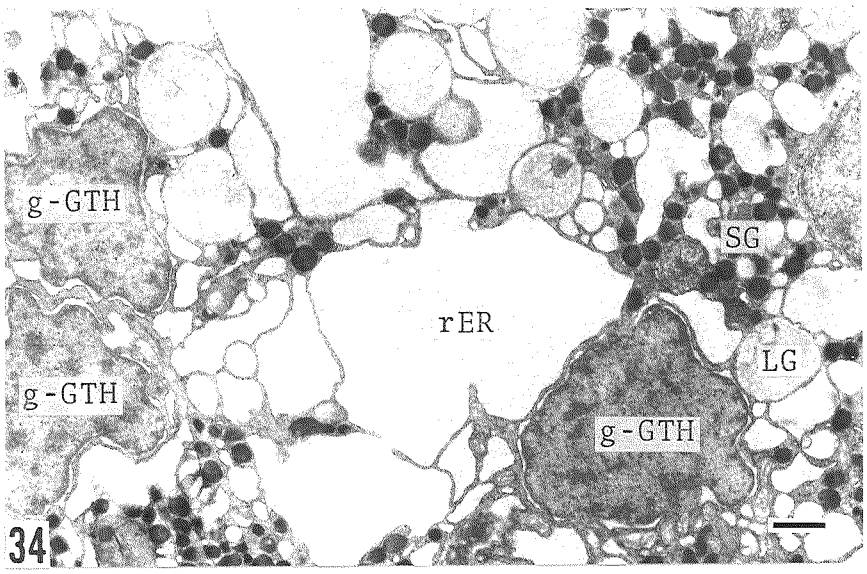

s
v
g
g
g
v
s
33

Plate 11

Figs. 34 and 35. Globular cells (g-GTH) of clomiphene-treated female loaches . In Fig. 34, an extreme expansion of cisternae of the rough endoplasmic reticulum is shown.


g-GTH
SG
rER
LG
g-GTH
g-GTH
34

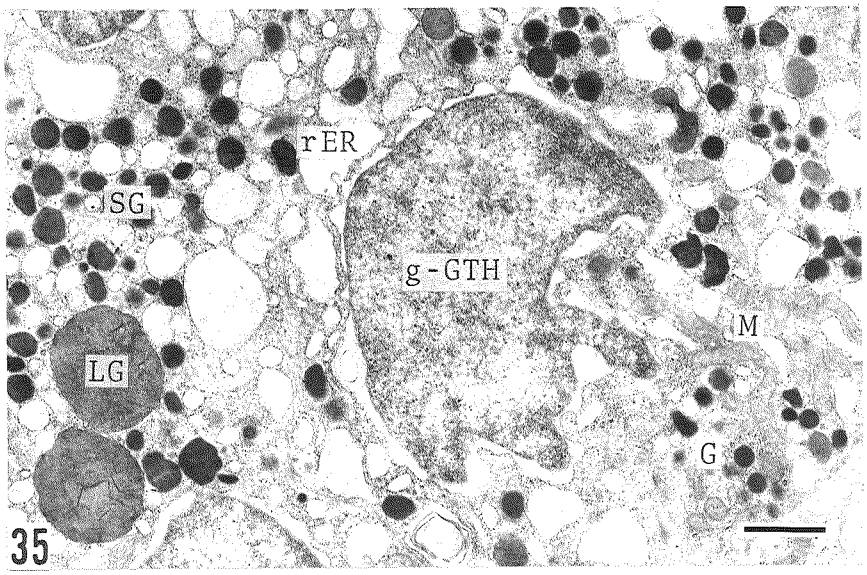
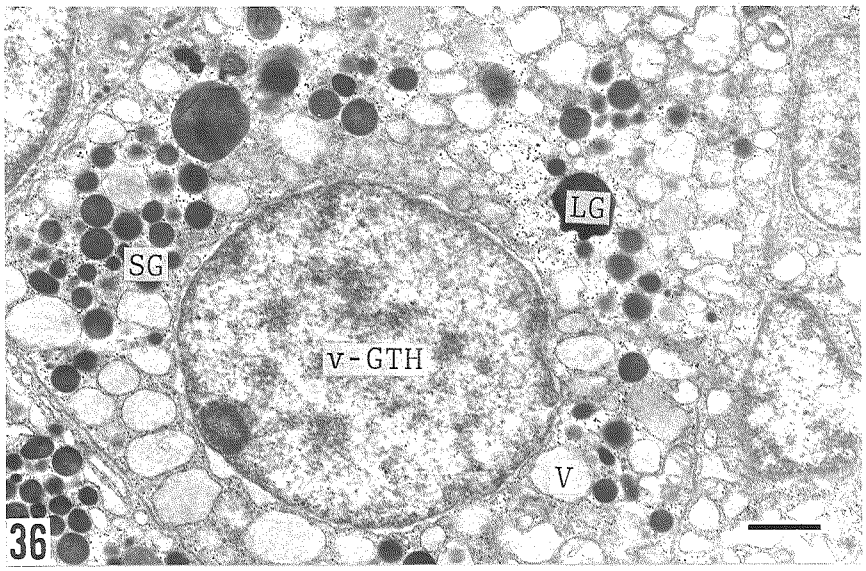

rER
SG
g-GTH
M
LG
G
35

Plate 12

Figs. 36 and 37. Vesicular cells (v-GTH) of clomiphene-treated female loaches.


LG
SG
v-GTH
V
36

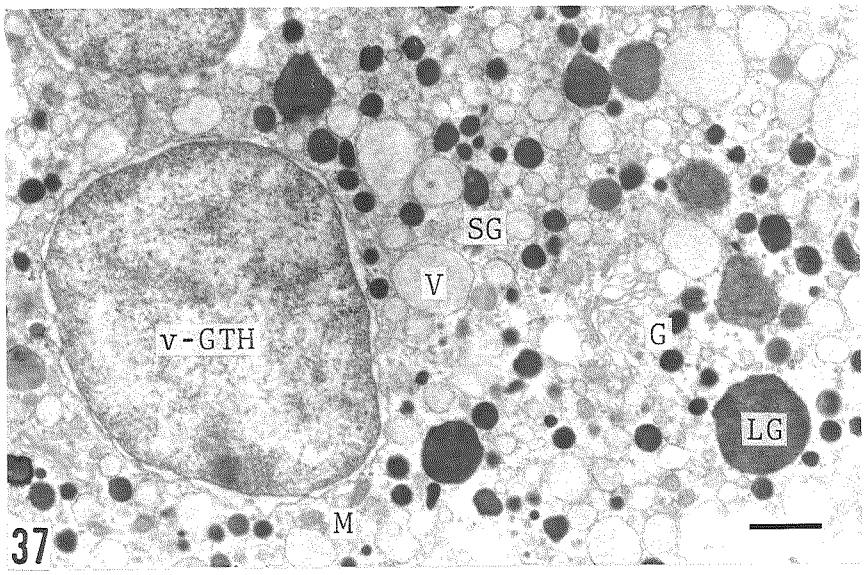

SG
V
v-GTH
G
LG
M
37

Plate 13

Figs. 38 and 39. Globular cells (g and g-GTH) and vesicular cells (v and v-GTH) of ovariectomized loaches examined 60 days after operation. Fig. 38: methylene blue stain of an Epon-embedded, semithin section, X1000.

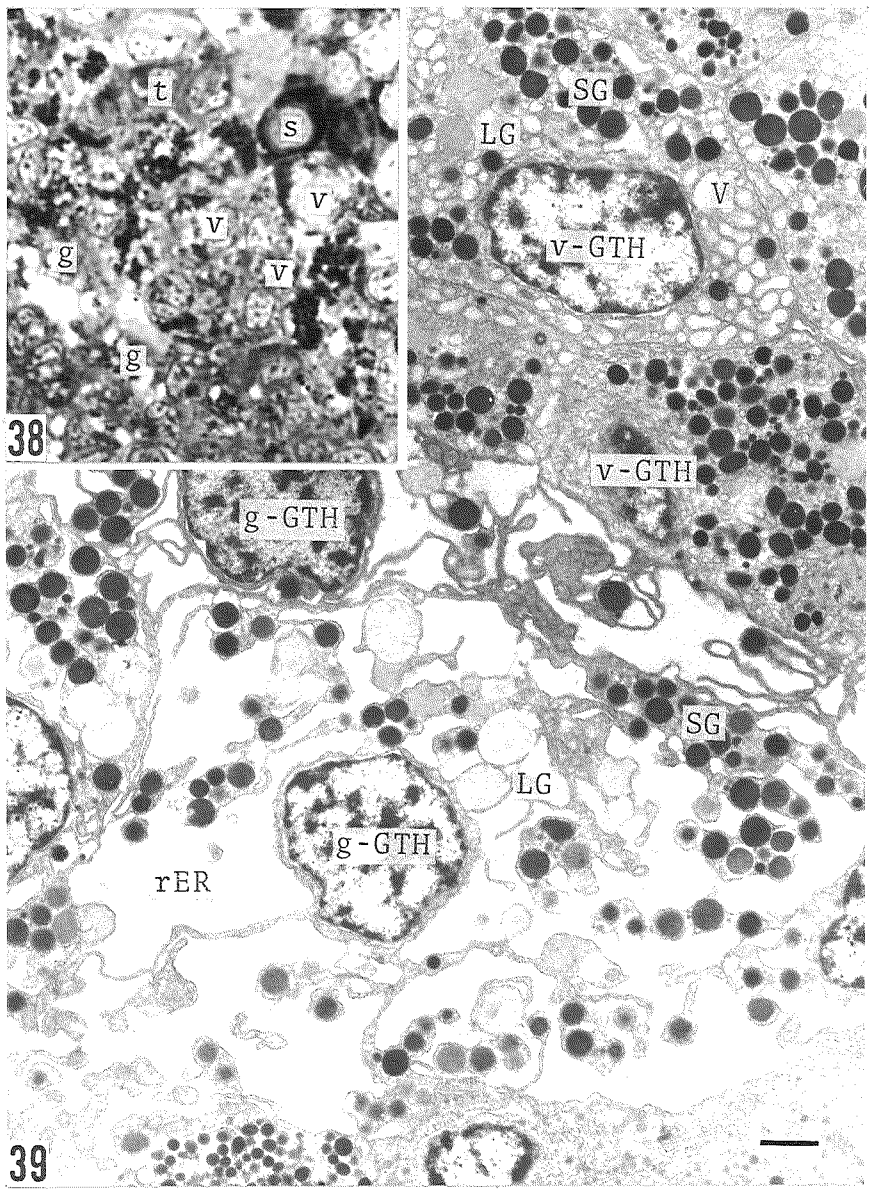

t
s
v
v
v
g
g
38
SG
LG
V
v-GTH
v-GTH
g-GTH
SG
LG
g-GTH
rER
39

Plate 14

Figs. 40–42. Globular cells (g and g-GTH) and vesicular cells (v and v-GTH) of castrated male loaches examined 60 days after operation. In Fig. 42, an atypically expanded cytoplasmic vesicle is shown. Fig. 40: methylene blue stain of an Epon-embedded, semithin section, X1000.

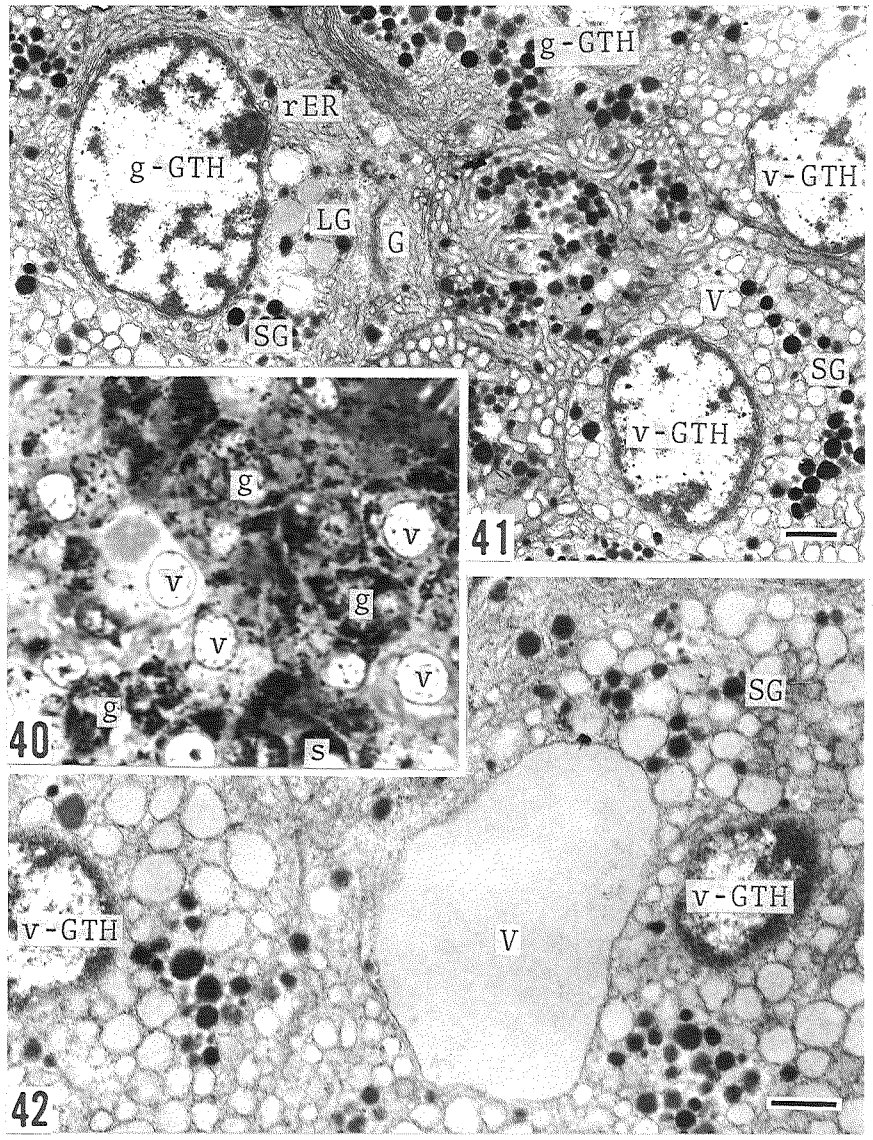

g-GTH
rER
g-GTH
v-GTH
LG
G
V
SG
SG
v-GTH
41
g
v
v
g
v
v
g
40
s
SG
v-GTH
V
v-GTH
42

Plate 15

Figs. 43 and 44. Globular cells (g and g-GTH) and vesicular cells (v and v-GTH) of female loaches injected with estradiol. Fig. 43: methylene blue stain of an Epon-embedded, semithin section, X1000.

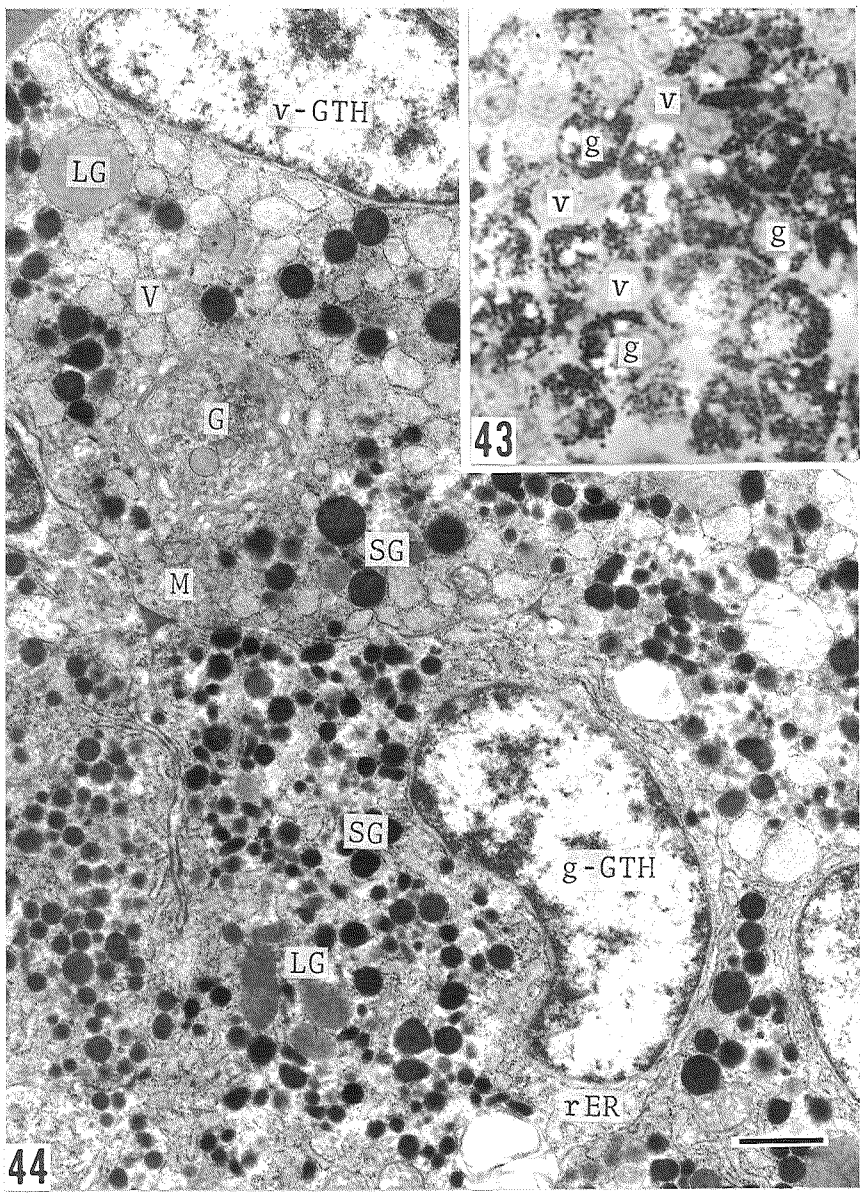

v-GTH
LG
V
G
SG
M
SG
LG
g-GTH
rER
44
v
g
v
g
v
g
43

Plate 16

Figs. 45–47. Globular cells (g and g-GTH) and vesicular cells (v and v-GTH) of male loaches injected with estradiol. Fig. 45: methylene blue stain of an Epon-embedded, semithin section, X1000.

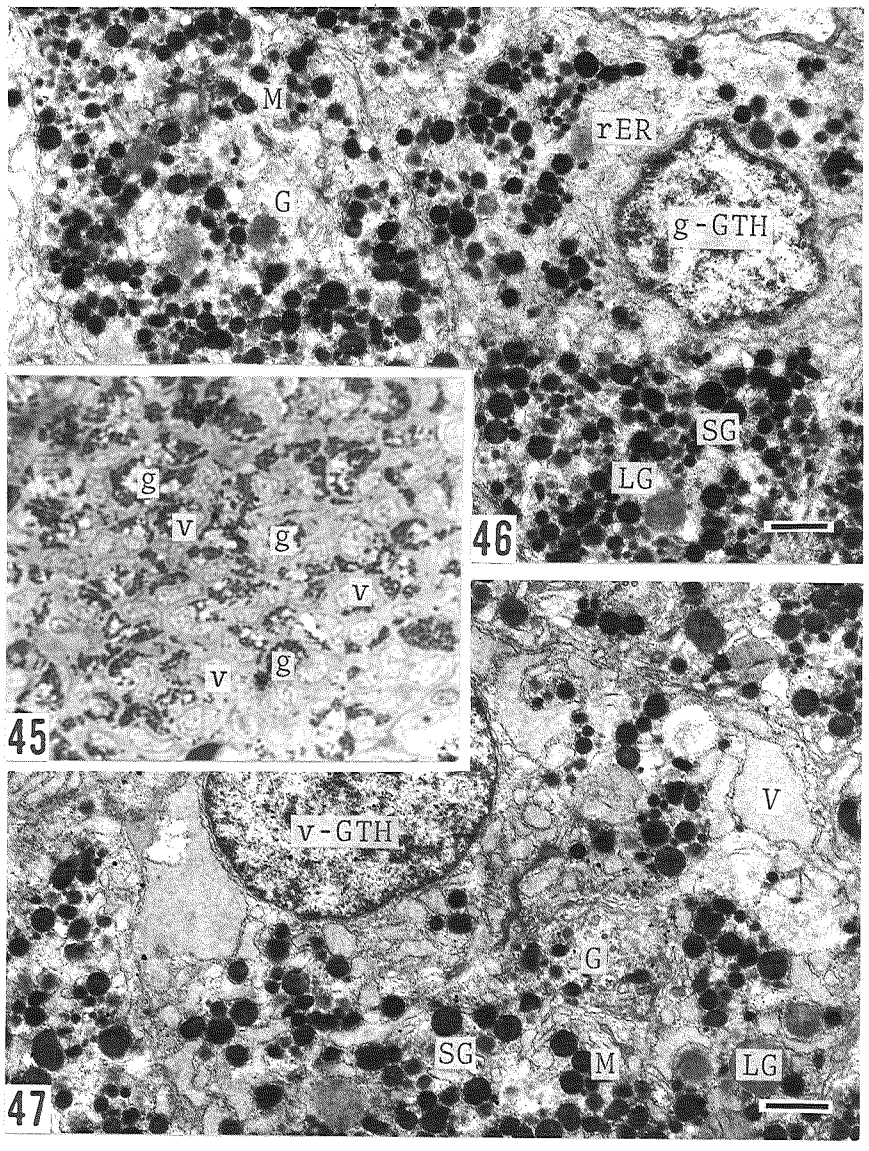

M
G
rER
g-GTH
SG
LG
46
g
v
g
v
v
g
45
v-GTH
V
G
SG
M
LG
47

Plate 17

Figs. 48-50. Globular cells (g and g-GTH) and vesicular cells (v and v-GTH) of male loaches injected with testosterone. Fig. 48: methylene blue stain of an Epon-embedded, semithin section, X1000.

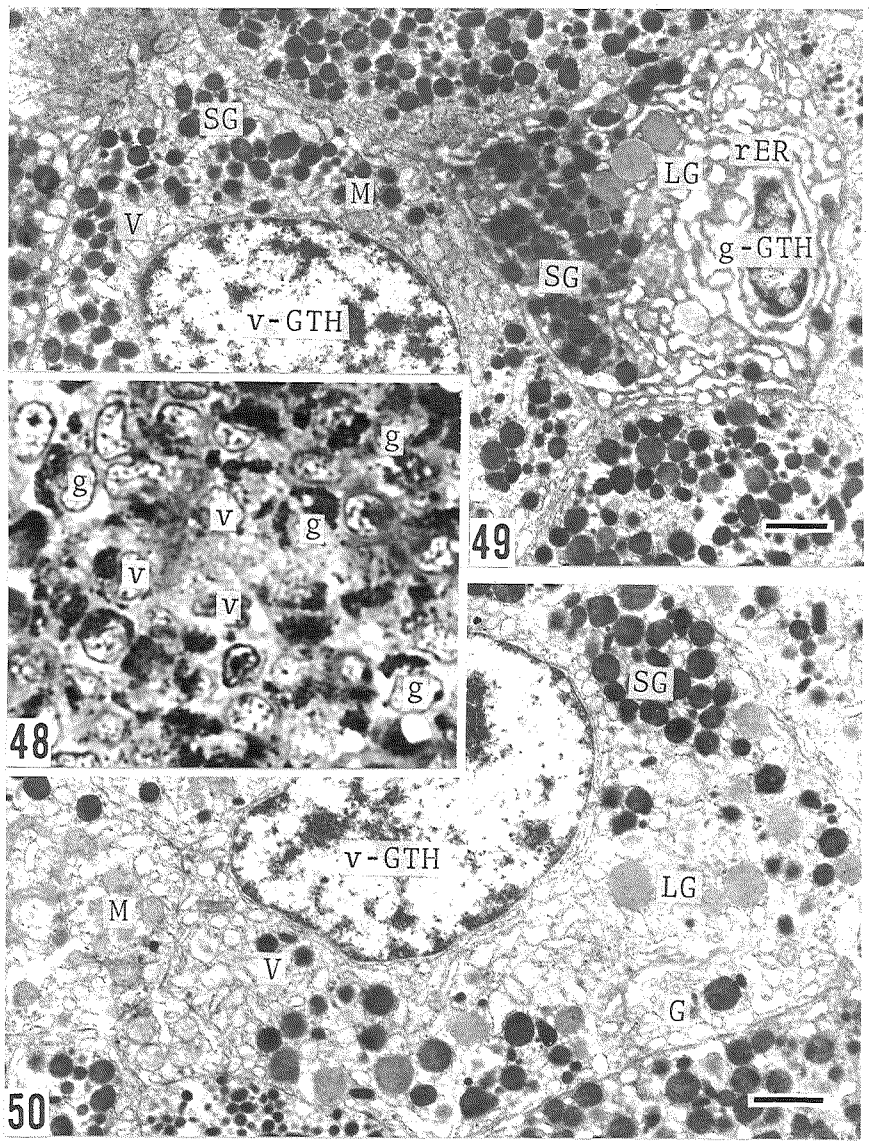

SG
M
V
v-GTH
LG
rER
g-GTH
SG
49
g
g
v
g
v
v
g
48
SG
v-GTH
M
V
LG
G
50

Plate 18

Figs. 51-53. Globular cells (g and g-GTH) and vesicular cells (v and v-GTH) of female loaches injected with testosterone. Fig. 51: methylene blue stain of an Epon-embedded, semithin section, X1000.

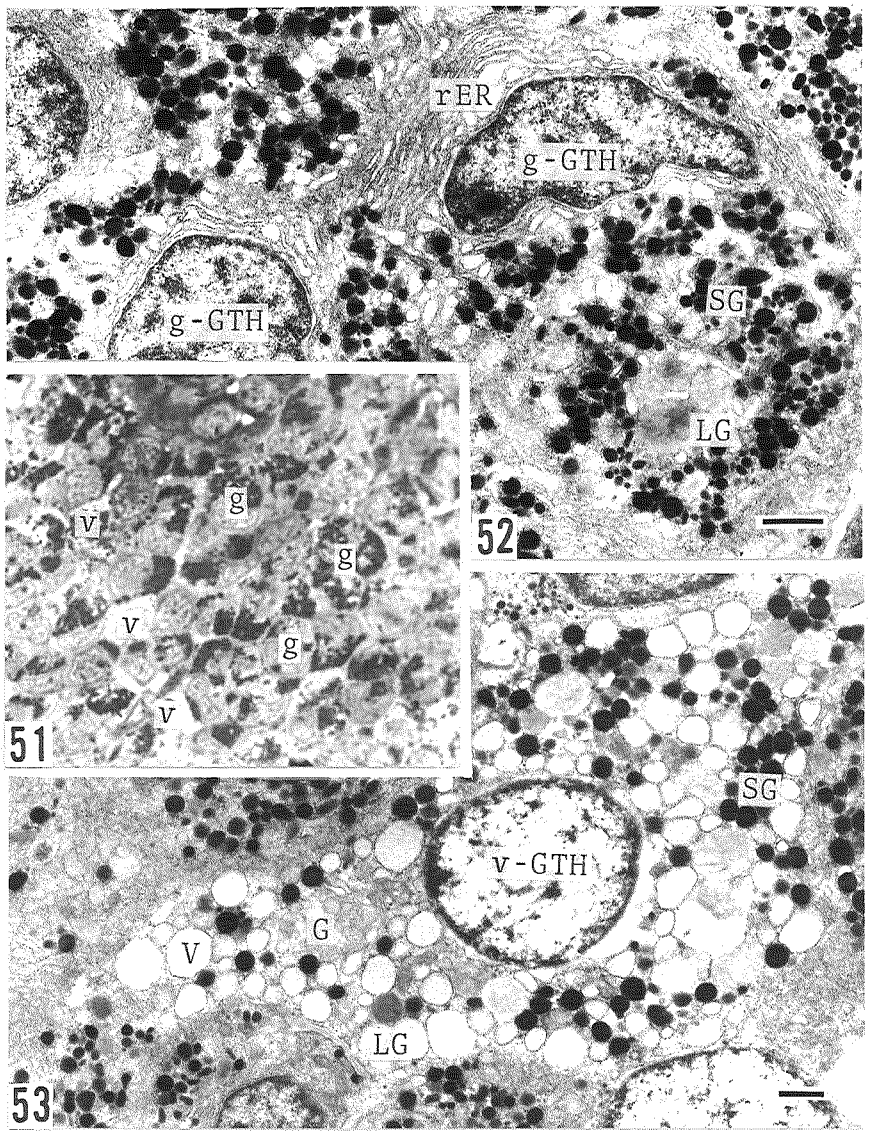
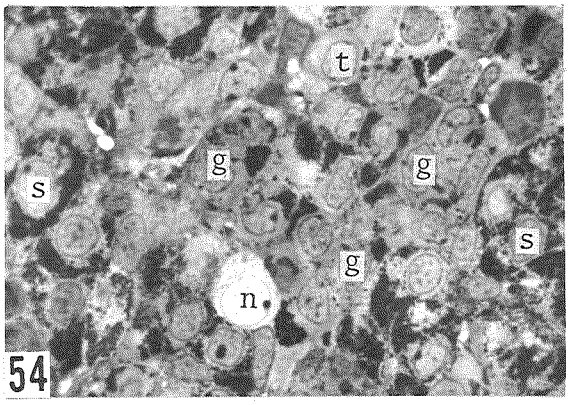
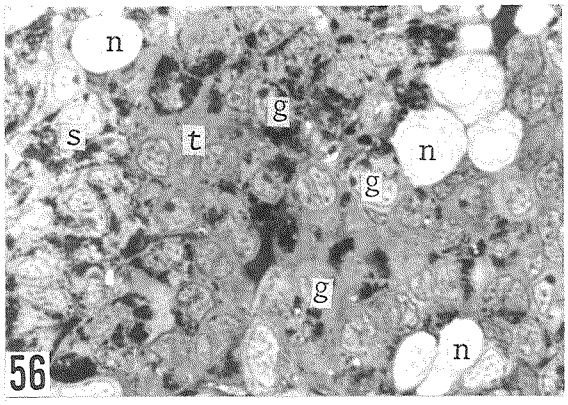

rER
g-GTH
g-GTH
SG
LG
52
g
v
g
v
g
v
51
SG
v-GTH
G
V
LG
53

Plate 19

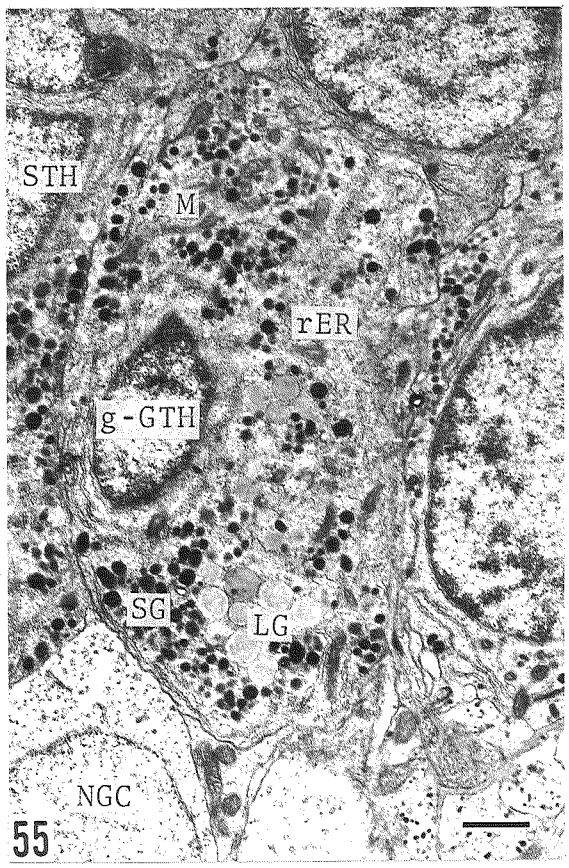
Figs. 54-57. Globular cells (g and g-GTH) and non-granulated cells (n and NGC) of juvenile loaches having oocytes in the oogonial stage, sampled in November (Figs. 54 and 55) and in the peri-nucleolus stage, sampled in January (Figs. 56 and 57). Note the absence of typical vesicular cells in pituitaries of these quite immature fish. Figs. 54 and 56: methylene blue stain of Epon-embedded, semithin sections, X1000.



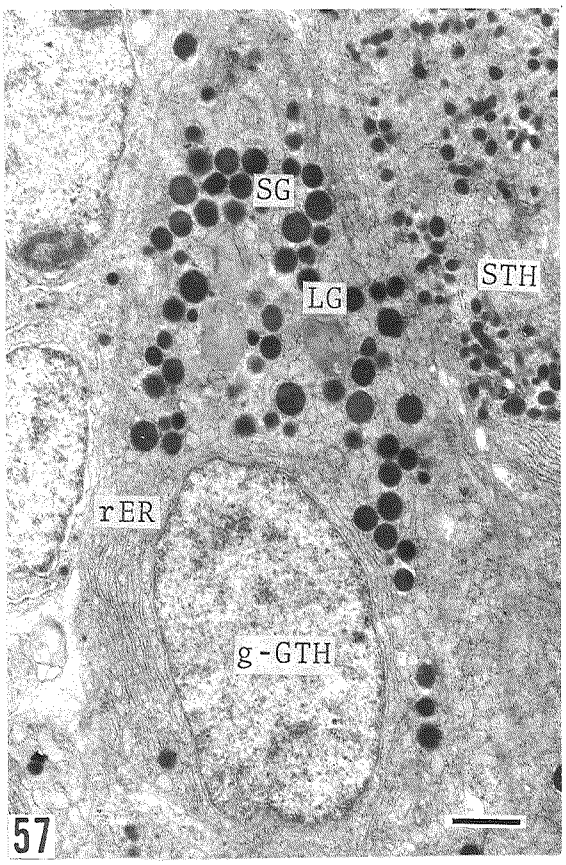
54



56



55



57

Plate 20

Figs. 58 and 59. Globular cells (g) and vesicular cells (v and v-GTH) of yearling female loaches having oocytes in the yolk vesicle stage, sampled in April. Fig. 58: methylene blue stain of an Epon embedded, semithin section, X1000.

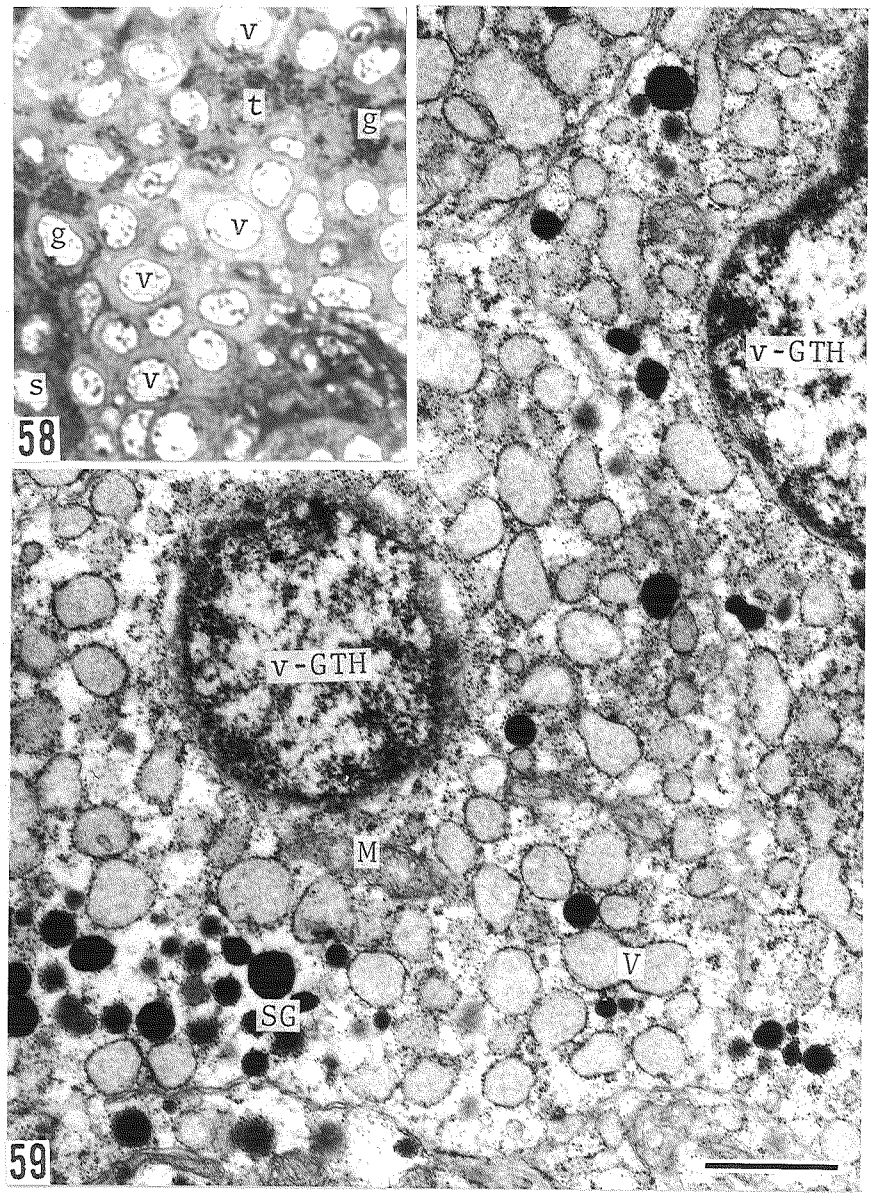

v
t
g
g
v
v
s
v
58
v-GTH
v-GTH
M
V
SG
59

Plate 21

Figs. 60–62. Globular cells (g and g-GTH) and vesicular cells (v and v-GTH) of yearling female loaches having oocytes in the primary yolk stage, sampled in May. Fig. 60: methylene blue stain of an Epon-embedded, semithin section, X1000.

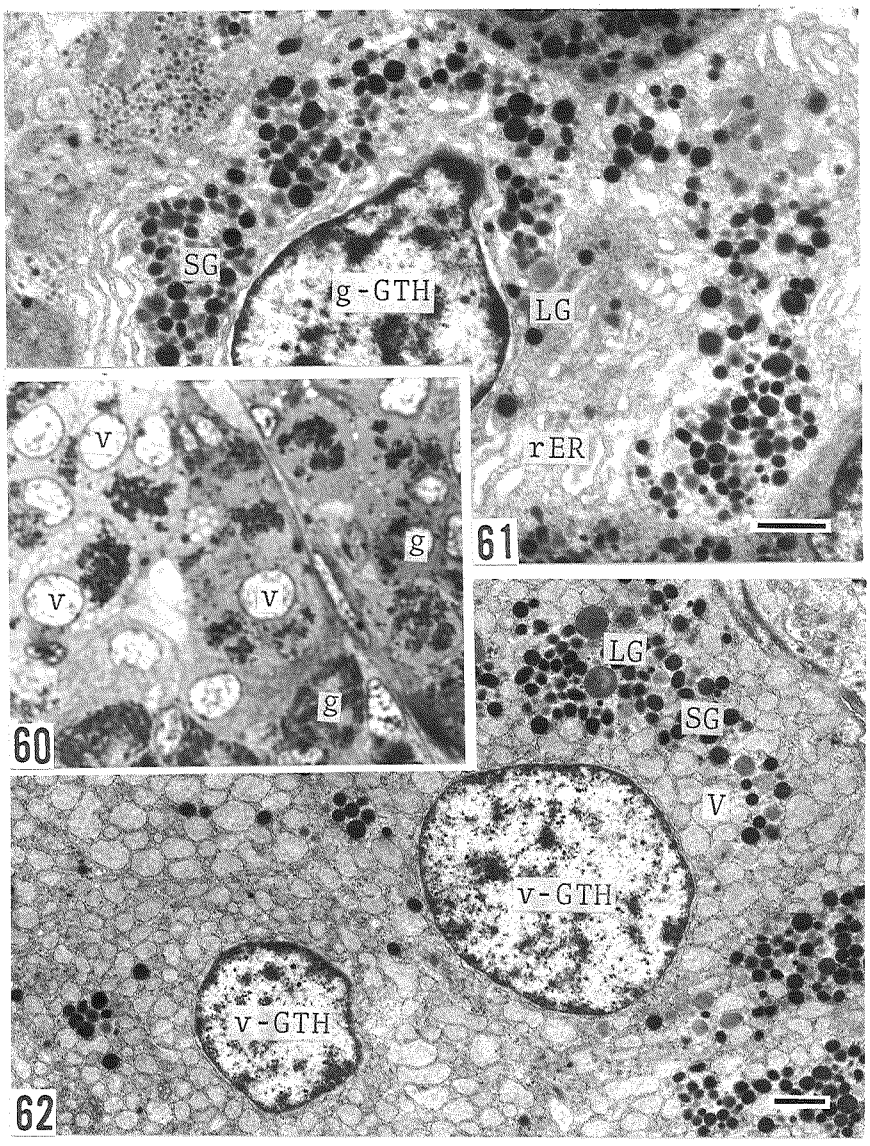

SG
g-GTH
LG
rER
61
v
v
v
g
g
60
LG
SG
V
v-GTH
v-GTH
62

Plate 22

Figs. 63-65. Globular cells (g and g-GTH) and vesicular cells (v and v-GTH) of yearling female loaches having oocytes in the tertiary yolk stage, sampled in July. Fig. 63: methylene blue stain of an Epon-embedded, semithin section, ×1000.

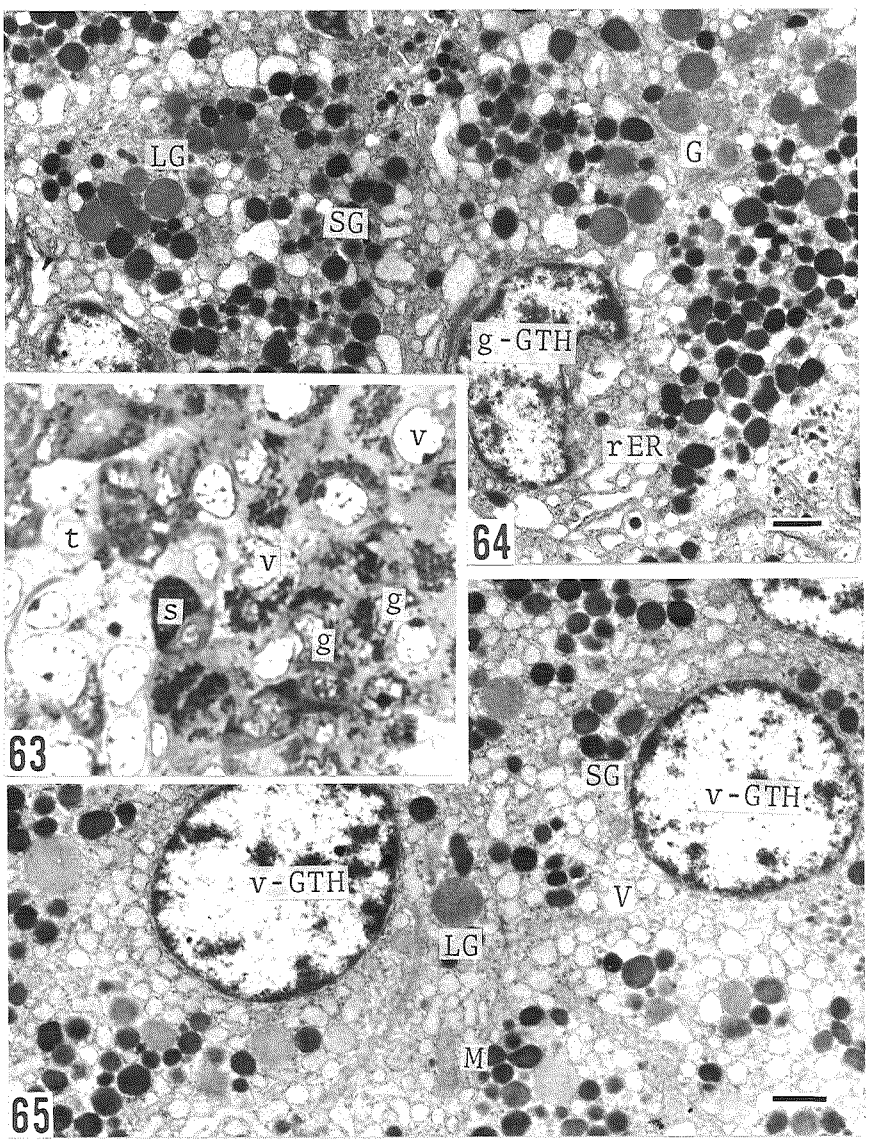

LG
SG
G
g-GTH
rER
64
v
t
v
s
g
g
63
SG
v-GTH
v-GTH
V
LG
M
65

Plate 23

Figs. 66-68. Globular cells (g and g-GTH) and vesicular cells (v and v-GTH) of postspawning loaches, sampled in September. Fig. 66: methylene blue stain of an Epon-embedded, semithin section, X1000.

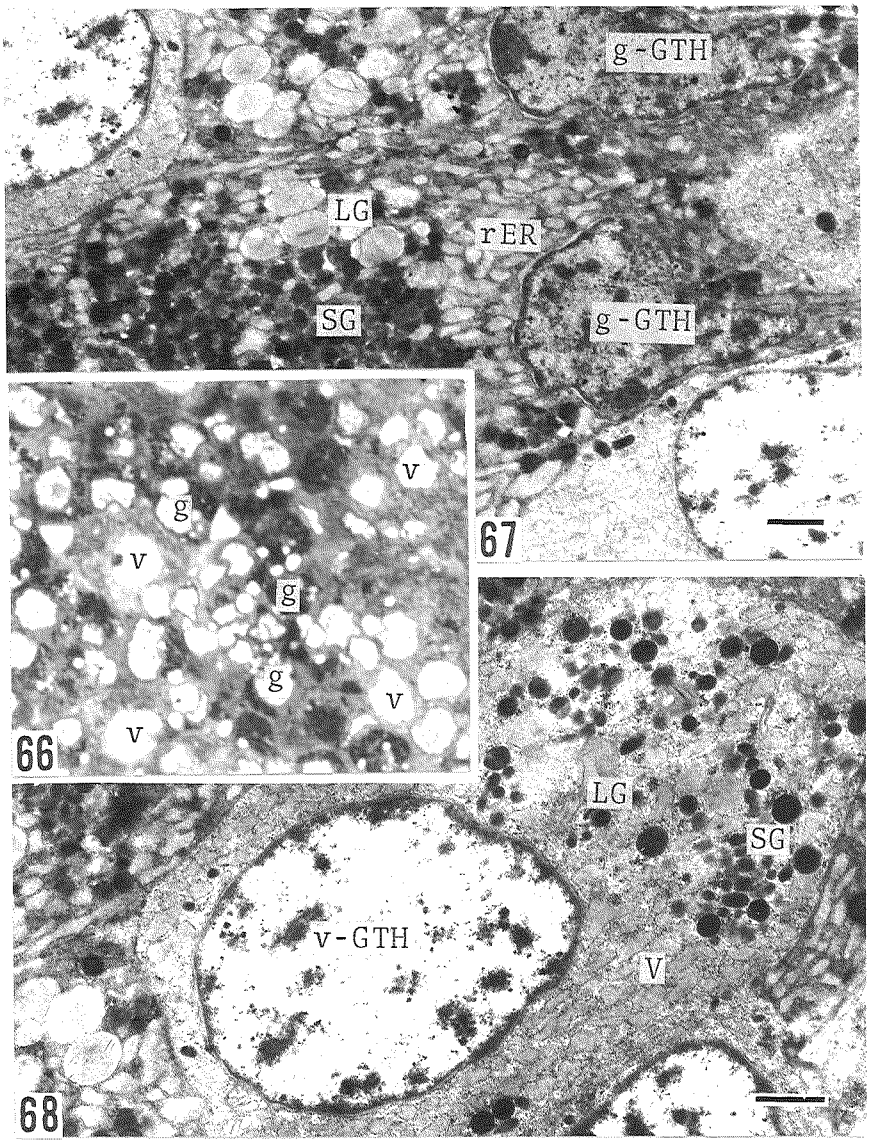
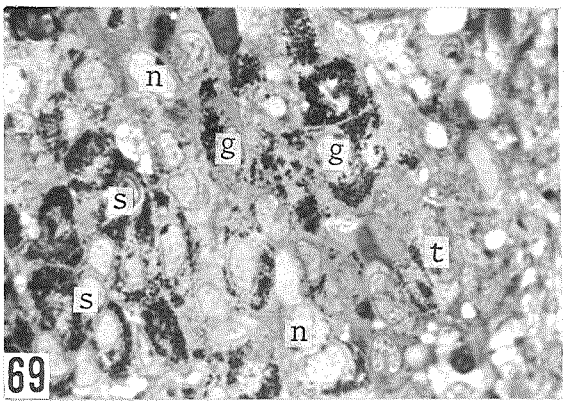
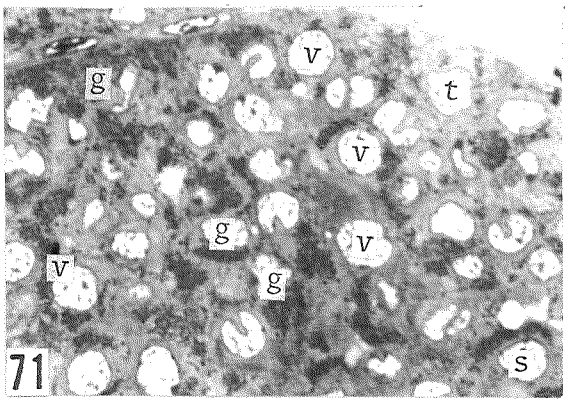
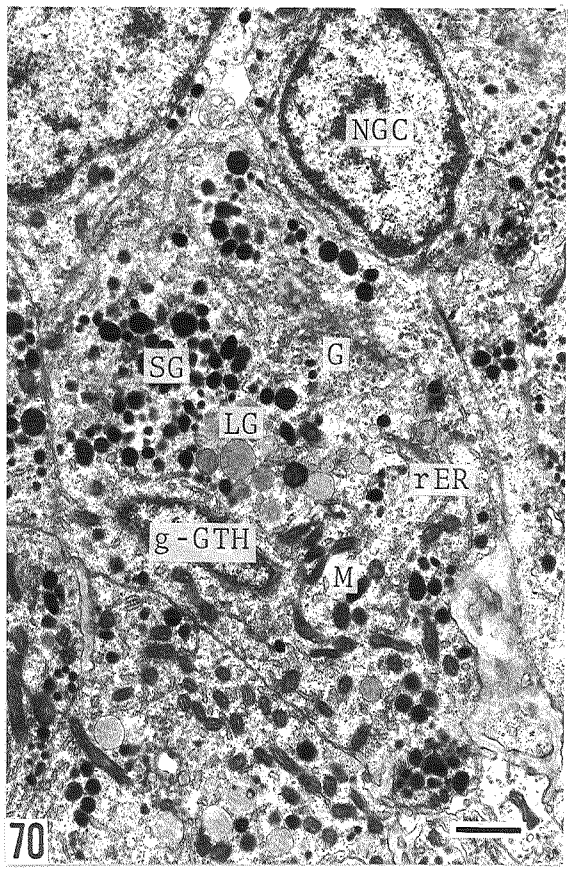

g-GTH
LG
rER
SG
g-GTH
67
v
g
v
g
g
v
v
66
LG
SG
v-GTH
V
68

Plate 24

Figs. 69-72. Globular cells (g and g-GTH), non-granulated cells (n and NGC) and vesicular cells (v and v-GTH) of juvenile male loaches having testes with spermatogonia only, sampled in November (Figs. 69 and 70), and with a small number of first spermatocytes, sampled in January (Figs. 71 and 72). Figs. 69 and 71: methylene blue stain of Epon-embedded, semithin sections, X1000.


n
g
g
s
t
s
n
69


g
v
t
v
g
v
v
g
s
71


NGC
SG
G
LG
rER
g-GTH
M
70

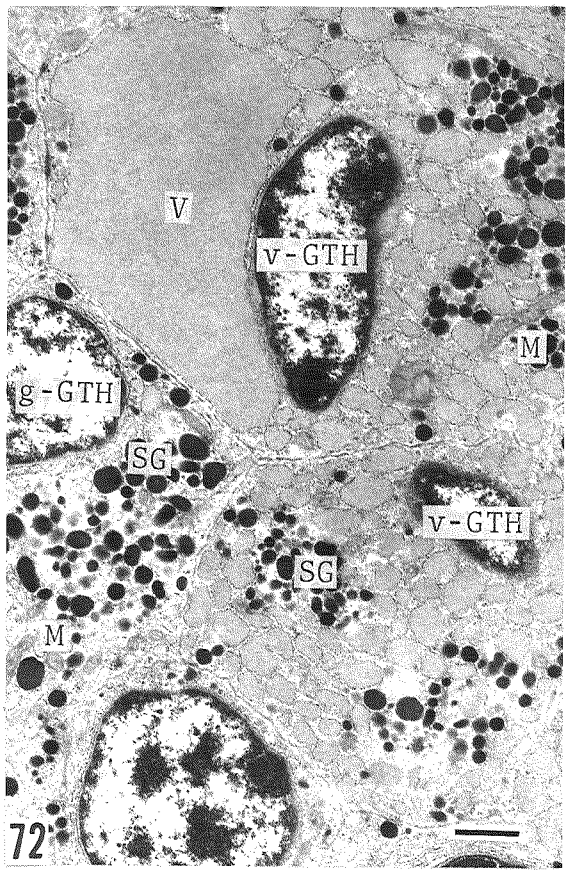

V
v-GTH
M
g-GTH
SG
v-GTH
SG
M
72

Plate 25

Figs. 73-75. Globular cells (g and g-GTH) and vesicular cells (v and v-GTH) of yearling male loaches having germ cell cysts in various spermatogenetic stages, sampled in July. Fig. 73: methylene blue stain of an Epon-embedded, semithin section, X1000.

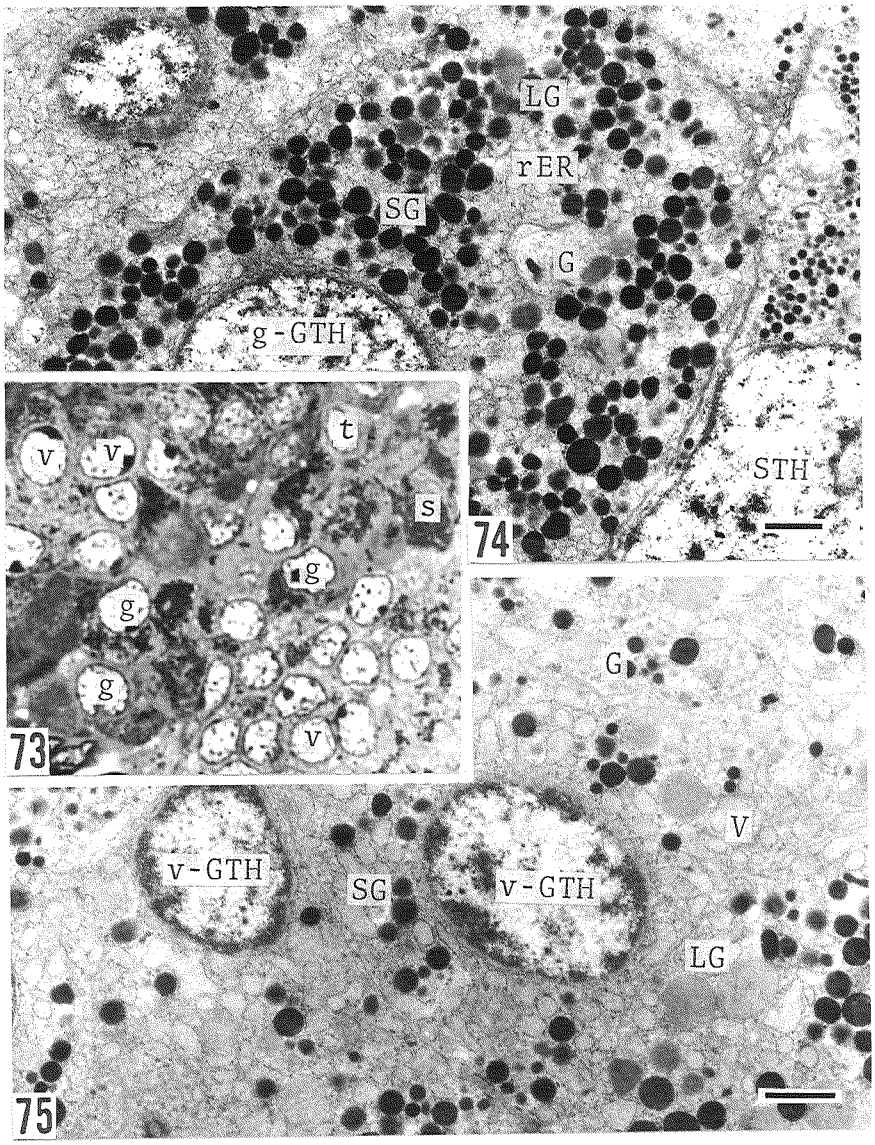

LG
rER
SG
G
g-GTH
STH
74
v
v
t
s
g
g
g
v
73
G
V
v-GTH
SG
v-GTH
LG
75

Plate 26

Figs. 76-78. Globular cells (g and g-GTH) and vesicular cells (v and v-GTH) of mature spermiating loaches, sampled in September. Fig. 76: methylene blue stain of an Epon-embedded, semithin section, X1000.

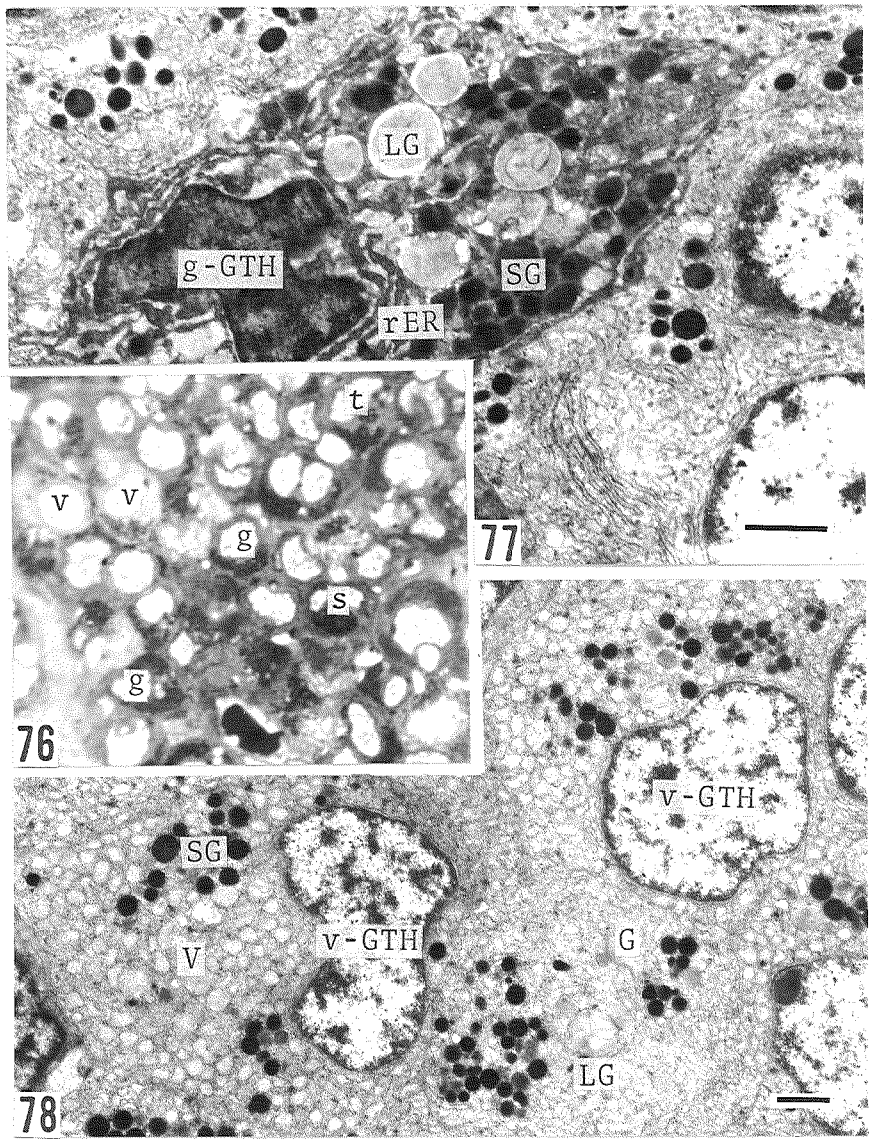

LG
g-GTH
SG
rER
77
t
v
v
g
s
g
76
v-GTH
SG
V
v-GTH
G
LG
78

Plate 27

Figs. 79-81. Globular cells (g and g-GTH) and vesicular cells (v and v-GTH) of adult female loaches of the stage I. Fig. 79: methylene blue stain of an Epon-embedded, semithin section, X1000.

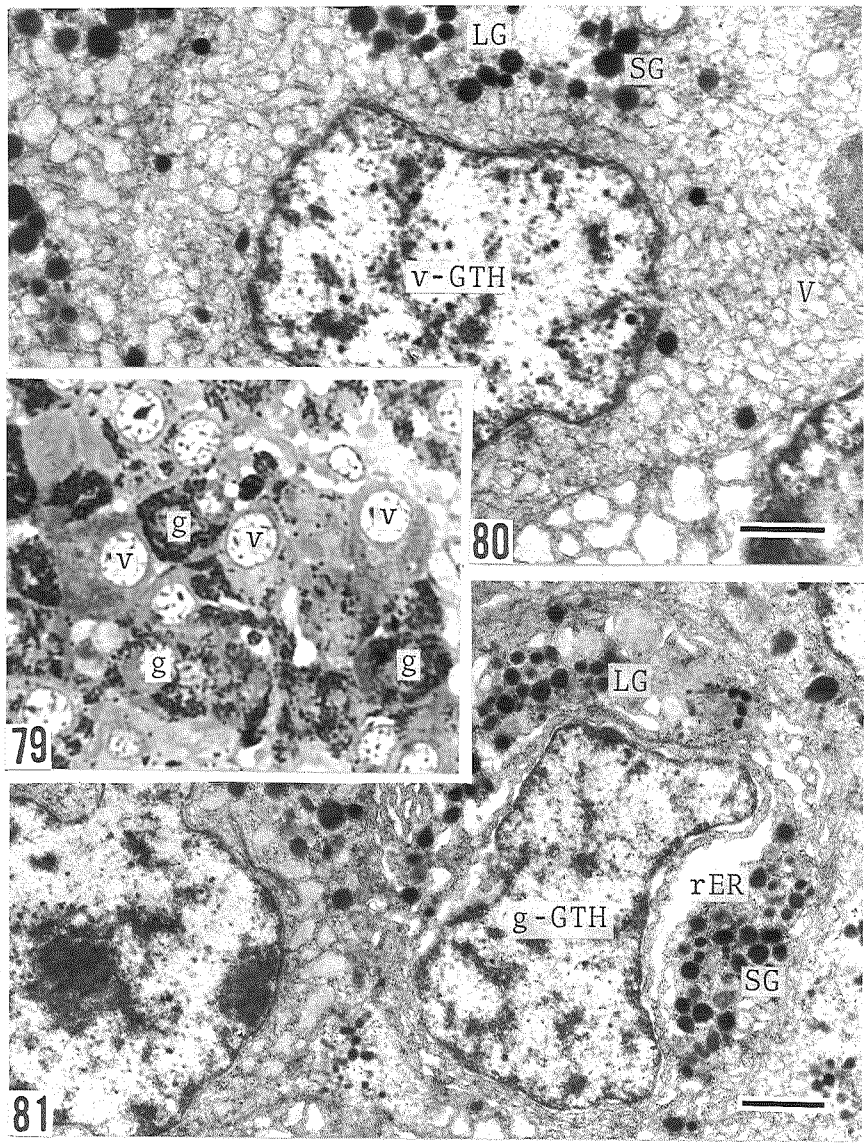

LG
SG
v-GTH
V
80
v
g
v
v
v
g
g
79
LG
rER
g-GTH
SG
81

Plate 28

Figs. 82–84. Globular cells (g and g-GTH) and vesicular cells (v and v-GTH) of adult female loaches of the stage II. Fig. 82: methylene blue stain of an Epon embedded, semithin section, X1000.

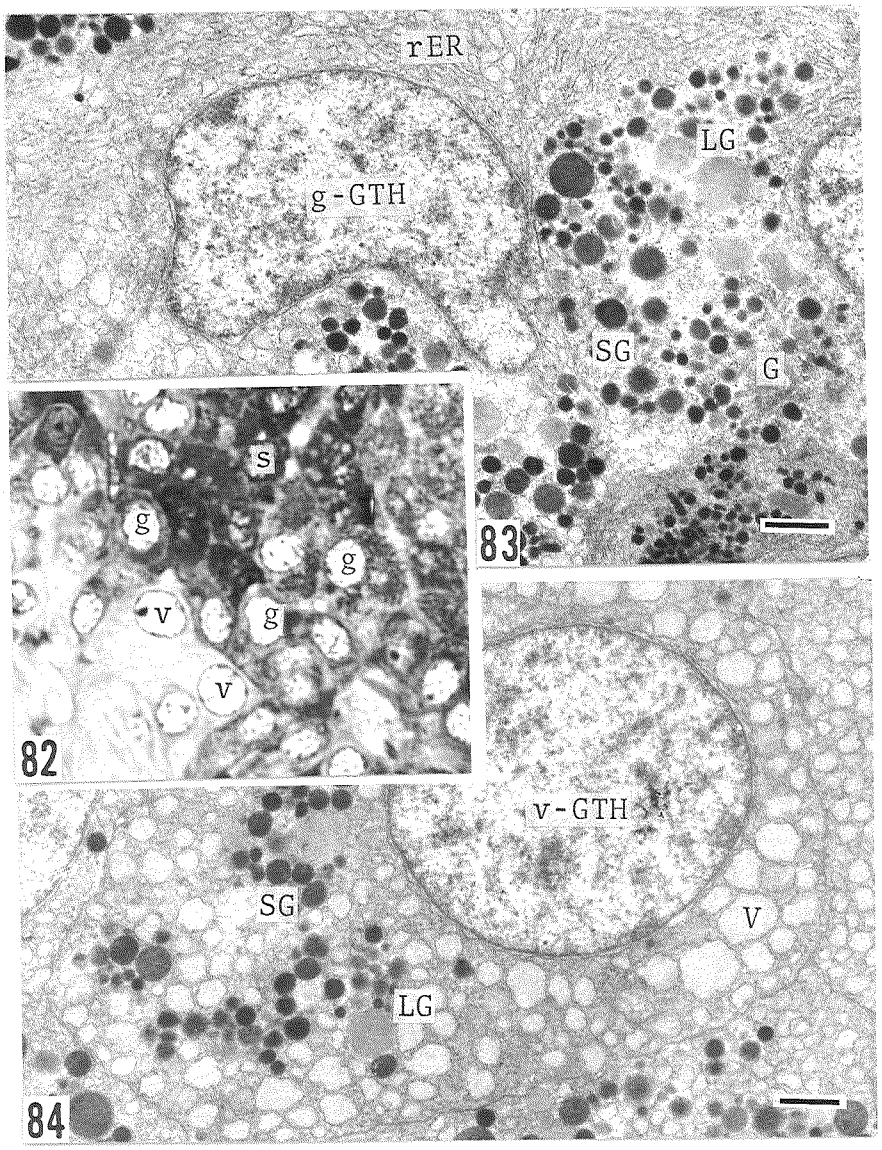

rER
g-GTH
LG
SG
G
83
s
g
g
v
g
v
82
v-GTH
SG
V
LG
84

Plate 29

Figs. 85 and 86. Globular cells (g and g-GTH) vesicular cells (v and v-GTH) of adult female loaches of the stage III. Fig. 85: methylene blue stain of an Epon-embedded, semithin section, X1000.

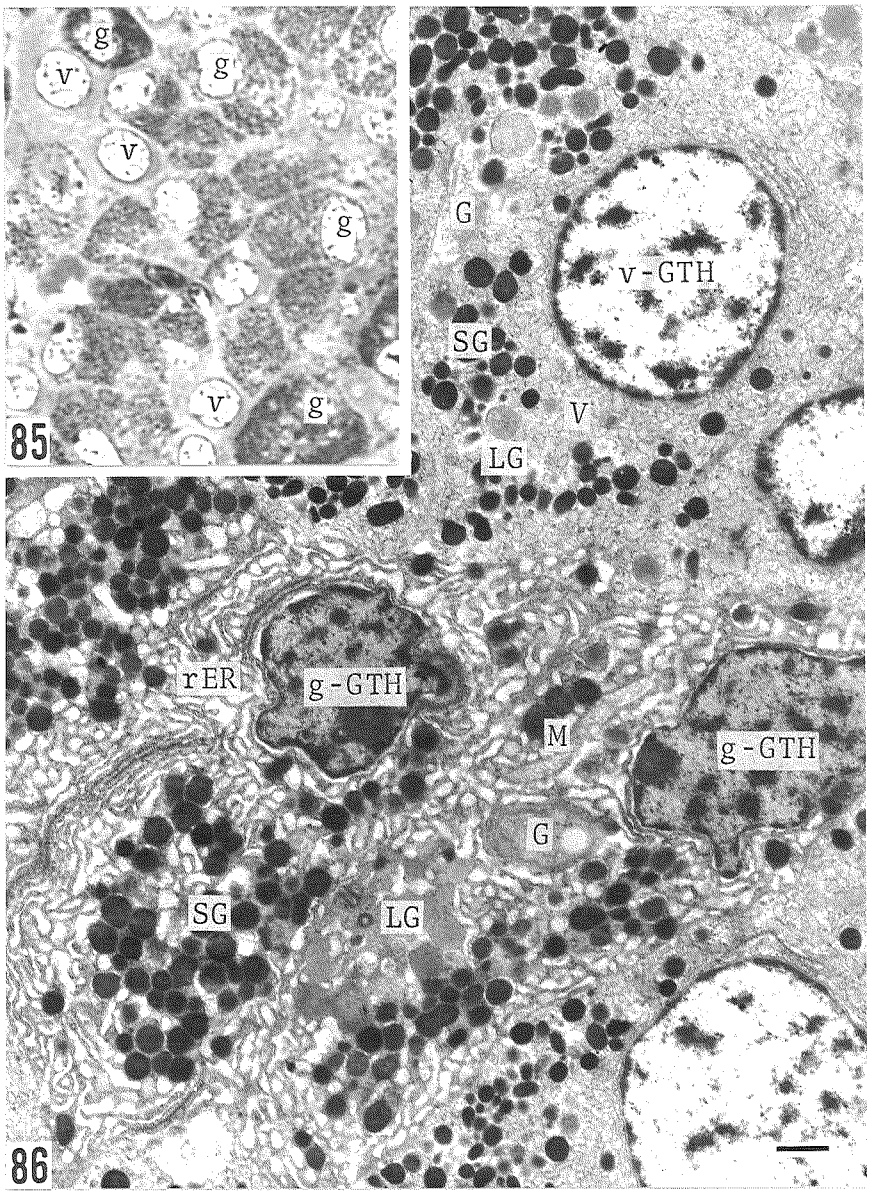

g
v
g
v
g
v
g
85
G
v-GTH
SG
V
LG
rER
g-GTH
M
g-GTH
G
SG
LG
86

Plate 30

Figs. 87 and 88. Globular cells (g and g-GTH) and vesicular cells (v and v-GTH) of adult female loaches of the stage IV. Fig. 88: methylene blue stain of an Epon-embedded, semithin section, X1000.

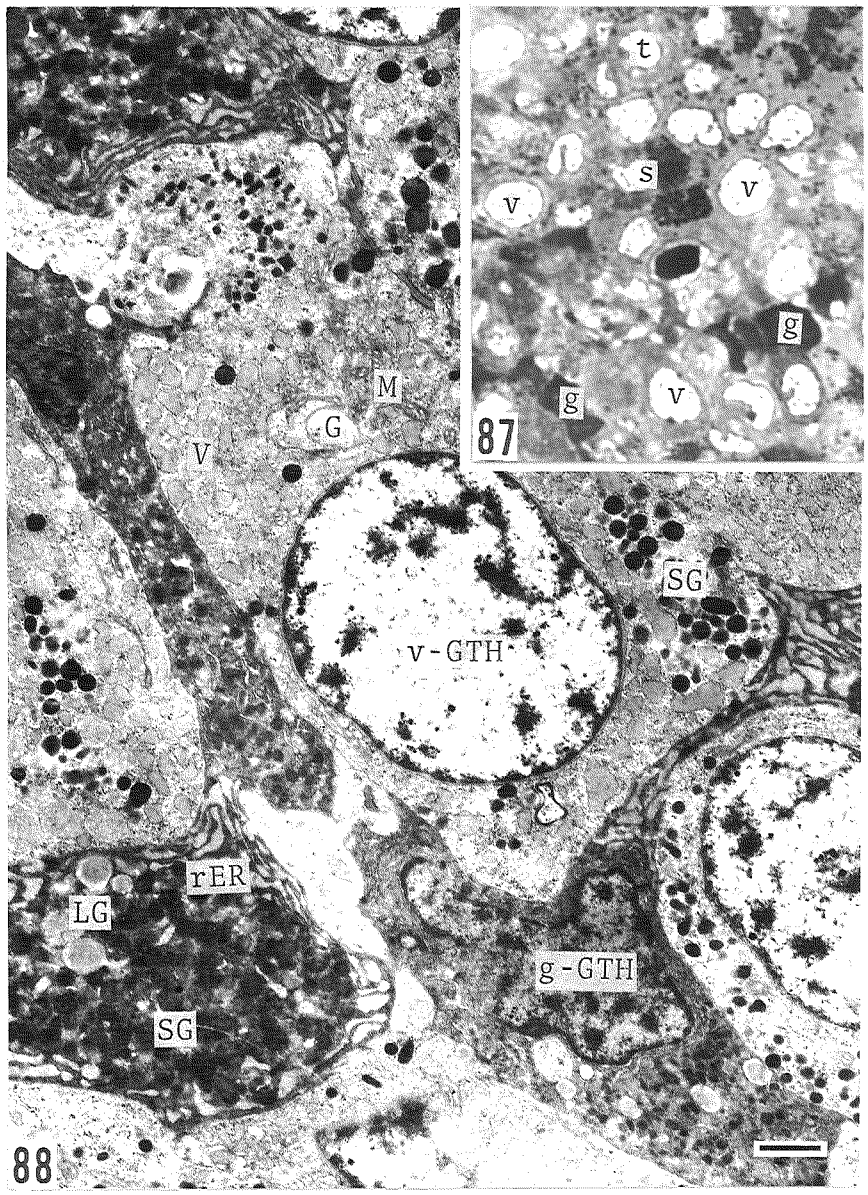

t
s
v
v
g
g
v
87
M
G
V
SG
v-GTH
rER
LG
g-GTH
SG
88

Plate 31

Figs. 89-91. Globular cells (g and g-GTH) and vesicular cells (v and v-GTH) of adult female loaches of the stage V. Fig. 89: methylene blue stain of an Epon-embedded, semithin section, X1000.

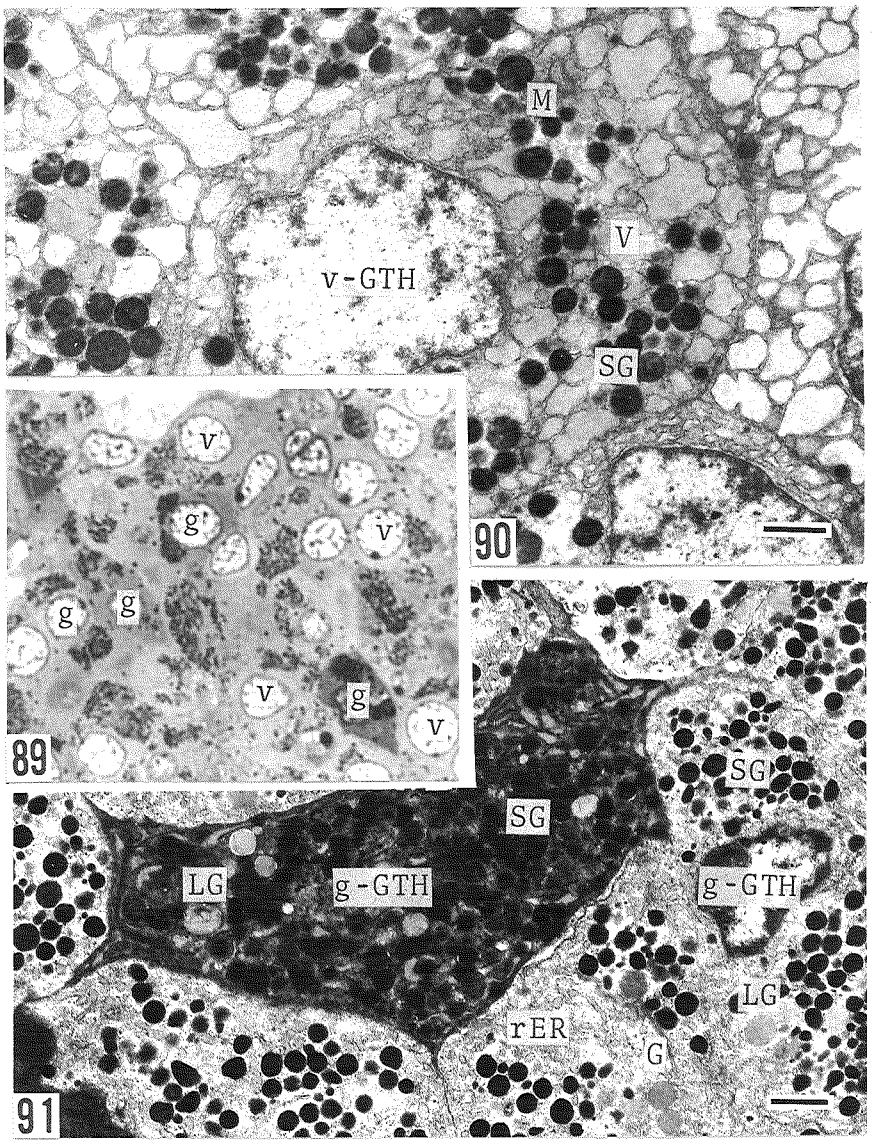

M
V
v-GTH
SG
90
v
g
v
g
g
v
g
v
89
SG
SG
LG
g-GTH
g-GTH
LG
rER
G
91

Plate 32

Figs. 92 and 93. Globular cells (g and g-GTH) vesicular cells (v and v-GTH) of adult female loaches of the stage VI. Fig. 92: methylene blue stain of an Epon-embedded, semithin section, X1000.

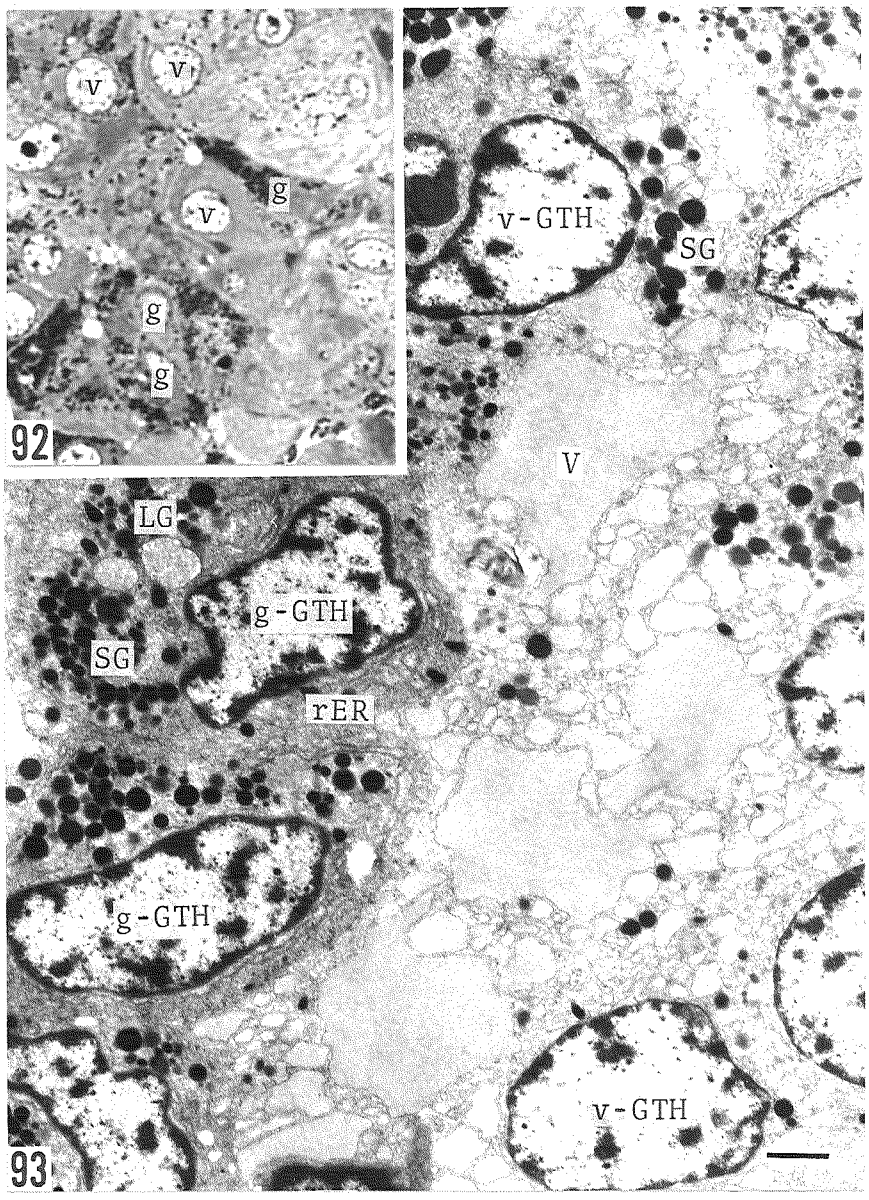
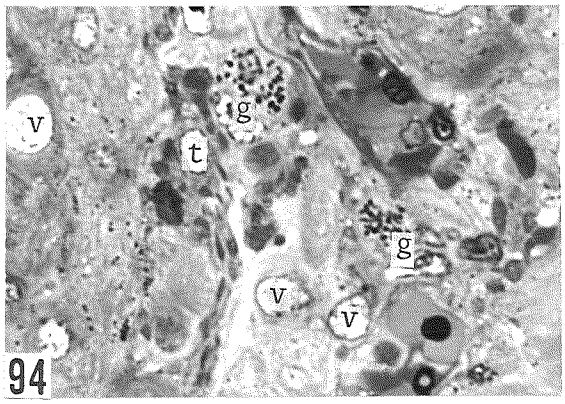
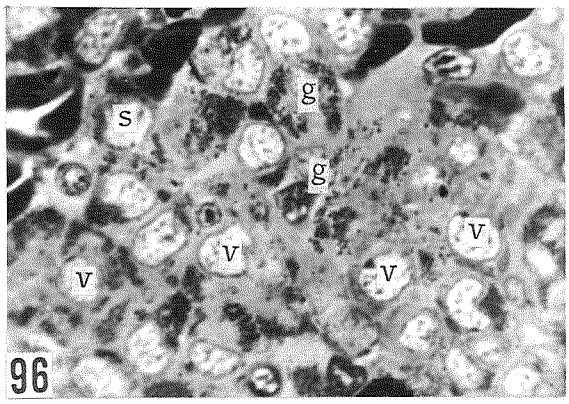
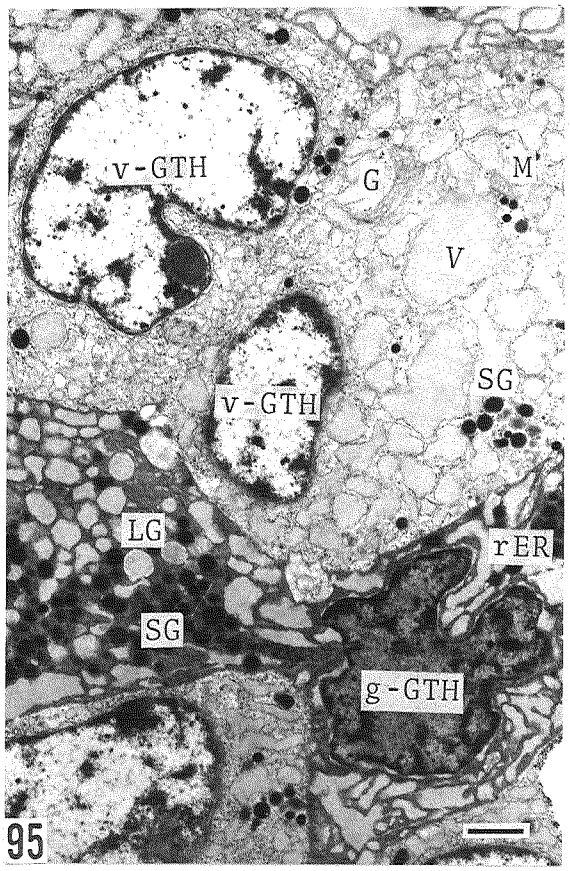

v
v
v
g
v
g
g
92
v-GTH
SG
V
LG
g-GTH
SG
rER
g-GTH
v-GTH
93

Plate 33

Figs. 94-97. Globular cells (g and g-GTH) and vesicular cells (v and v-GTH) of adult male loaches of the stage I (Figs. 94 and 95) and of the stage II (Figs. 96 and 97). Figs. 94 and 96: methylene blue stain of Epon-embedded, semithin sections, ×1000.


v
g
t
g
v
v
94


s
g
g
v
v
v
v
v
96


v-GTH
G
M
V
SG
v-GTH
LG
rER
SG
g-GTH
95

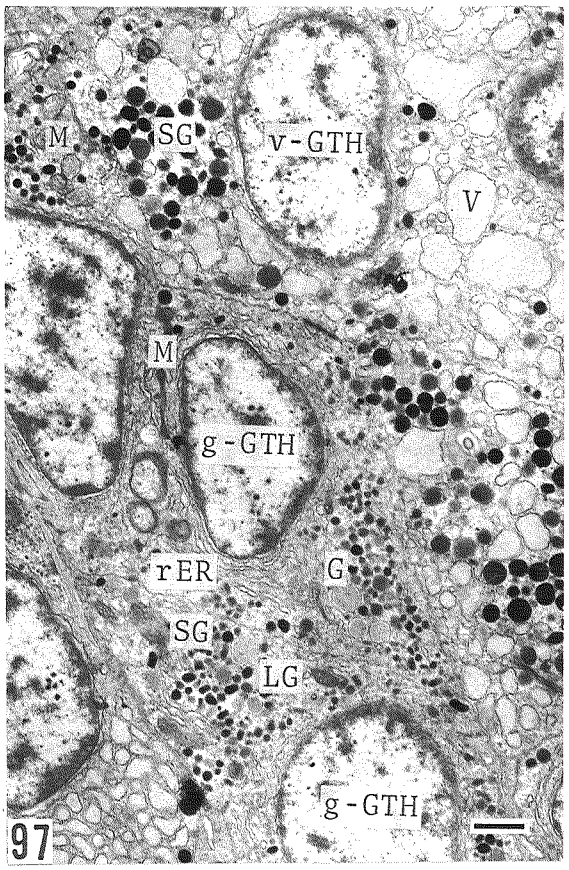

M
SG
v-GTH
V
M
g-GTH
rER
G
SG
LG
g-GTH
97

Plate 34

Figs. 98-100. Globular cells (g and g-GTH) and vesicular cells (v and v-GTH) of adult male loaches of the stage III. Fig. 98: methylene blue stain of an Epon-embedded, semithin section, X1000.

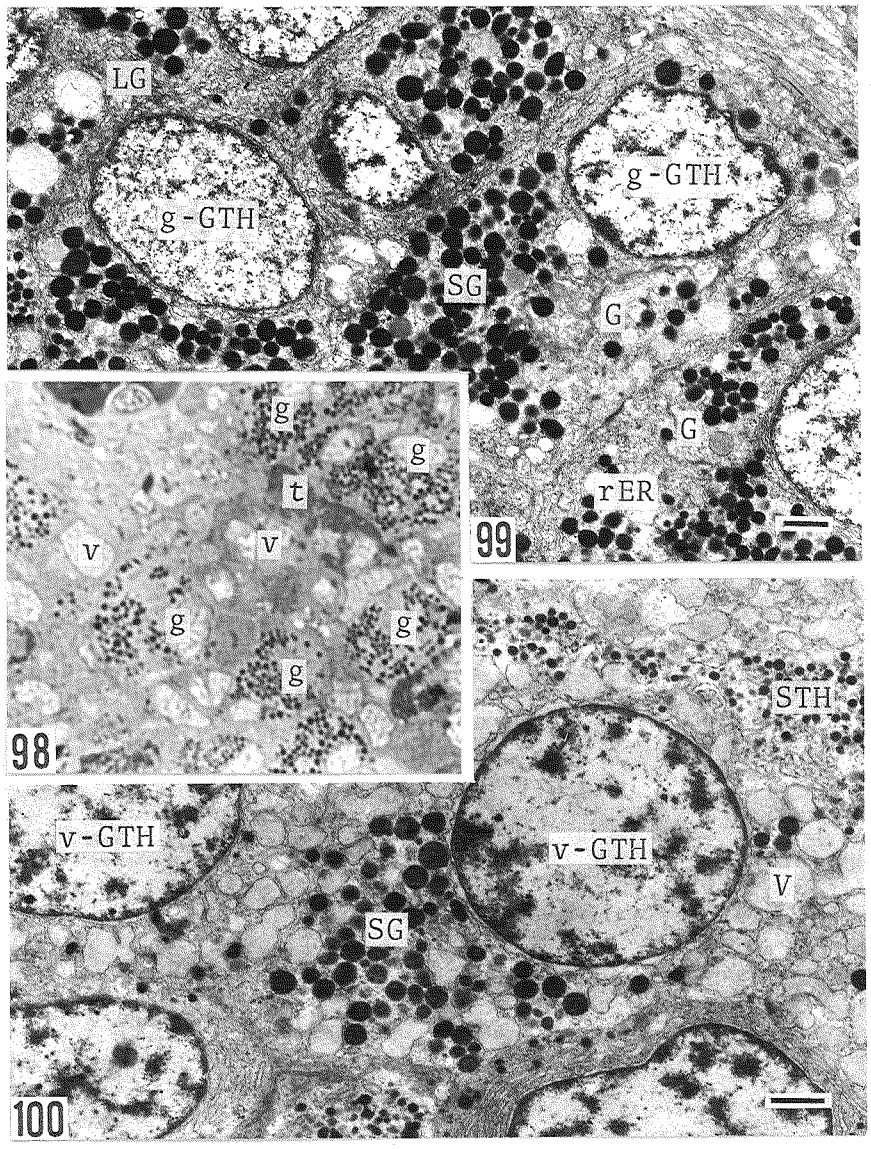

LG
g-GTH
g-GTH
SG
G
G
rER
99
g
g
t
v
v
g
g
g
98
STH
v-GTH
v-GTH
V
SG
100

Plate 35

Figs. 101-103. Globular cells (g and g-GTH) and vesicular cells (v and v-GTH) of adult male loaches of the stage IV. Fig. 101: methylene blue stain of an Epon-embedded, semithin section, X1000.

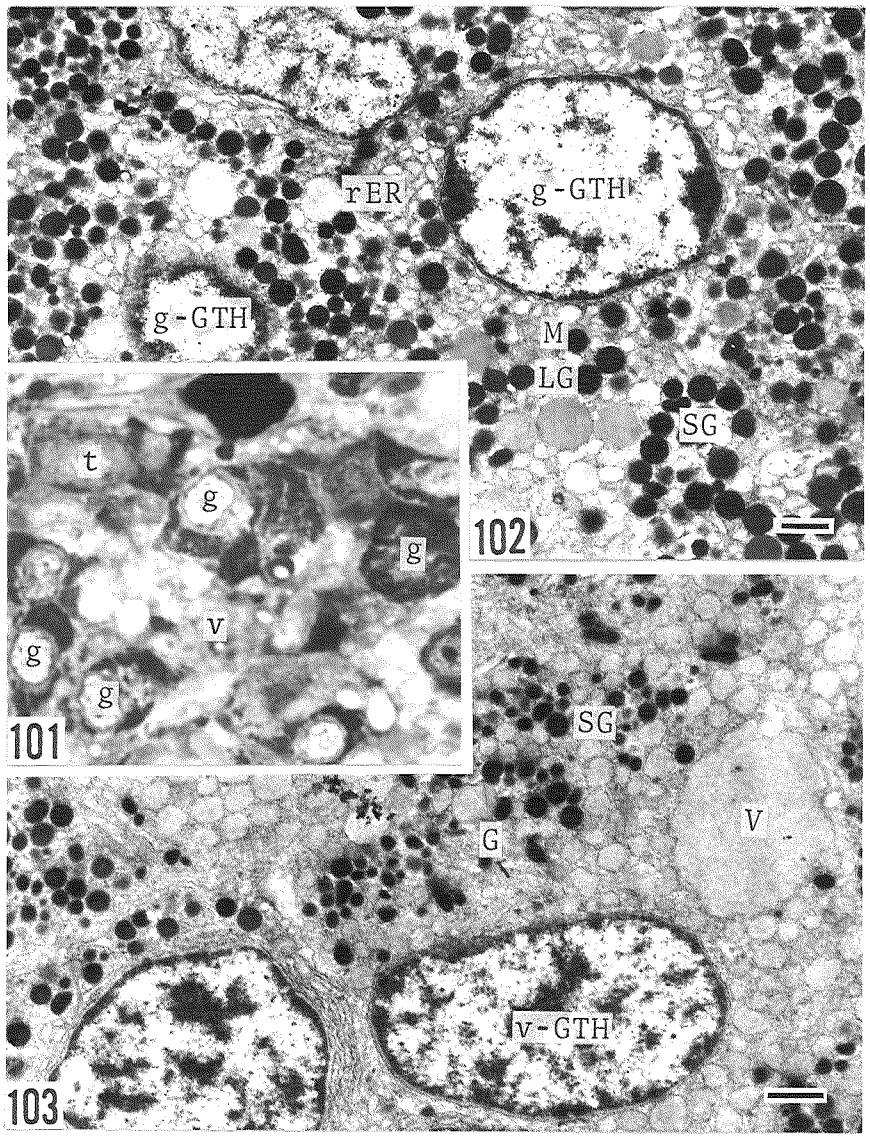

rER
g-GTH
g-GTH
M
LG
SG
102
t
g
g
v
g
g
101
SG
V
G
v-GTH
103

Plate 36

Figs. 104-106. Globular cells (g and g-GTH) and vesicular cells (v and v-GTH) of adult male loaches of the stage V. Fig. 104: methylene blue stain of an Epon-embedded, semithin section, X1000.

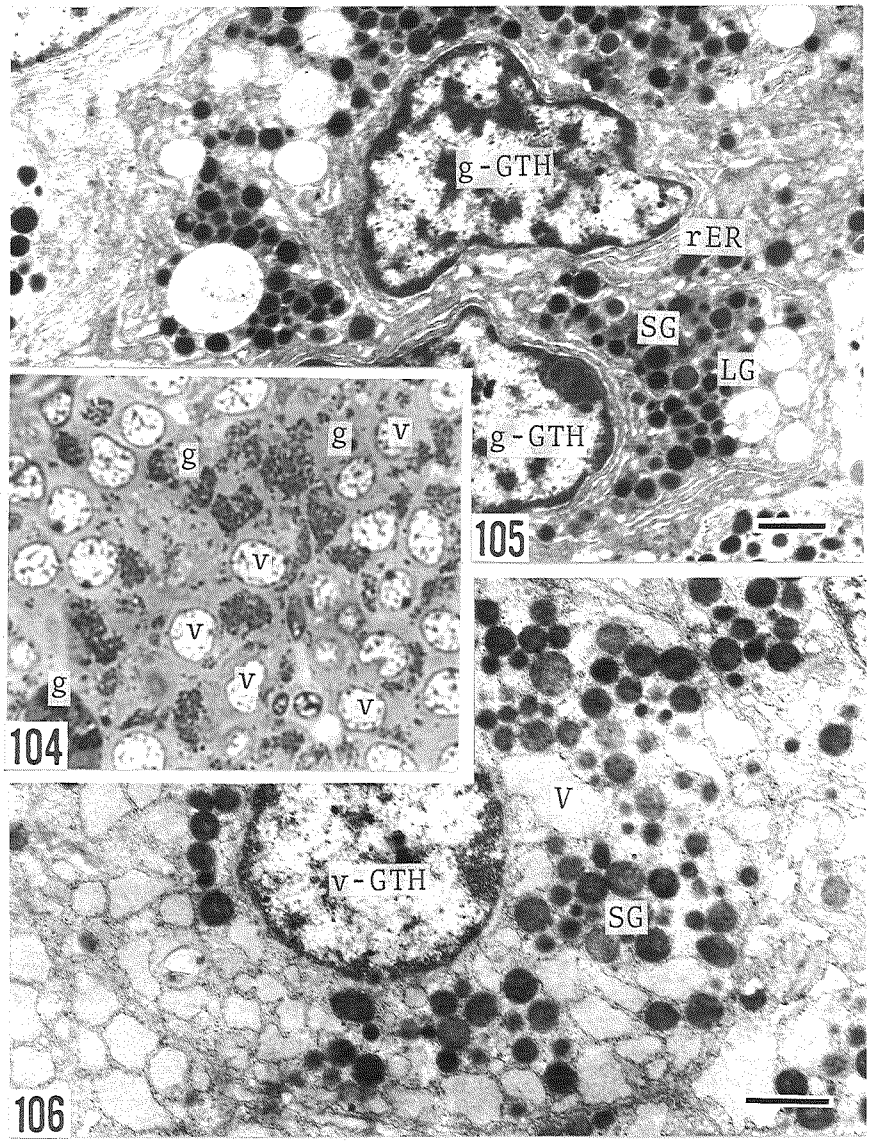

g-GTH
rER
SG
LG
g-GTH
105
g
g
v
v
v
g
v
v
104
V
v-GTH
SG
106

Plate 37

Figs. 107-109. Globular cells (g and g-GTH) and vesicular cells (v and v-GTH) of adult male loaches of the stage VI. Fig. 107: methylene blue stain of an Epon embedded, semithin section, X1000.

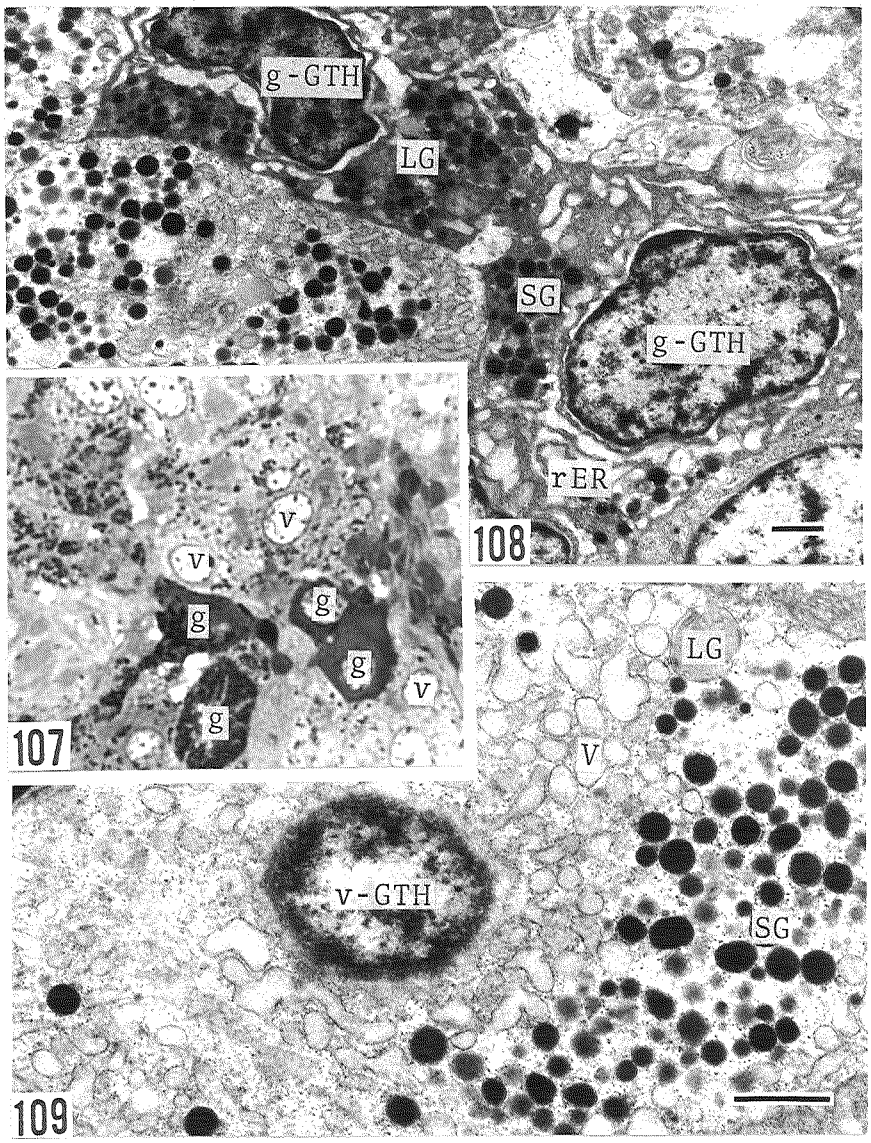

g-GTH
LG
SG
g-GTH
rER
108
v
v
g
g
g
g
v
107
LG
V
v-GTH
SG
109

Plate 38

Figs. 110 and 111. Globular cells (g and g-GTH) vesicular cells (v and v-GTH) of adult female loaches of the control group serving in the experiment with continuous light exposure. Fig. 110: methylene blue stain of an Epon-embedded, semithin section, X1000.

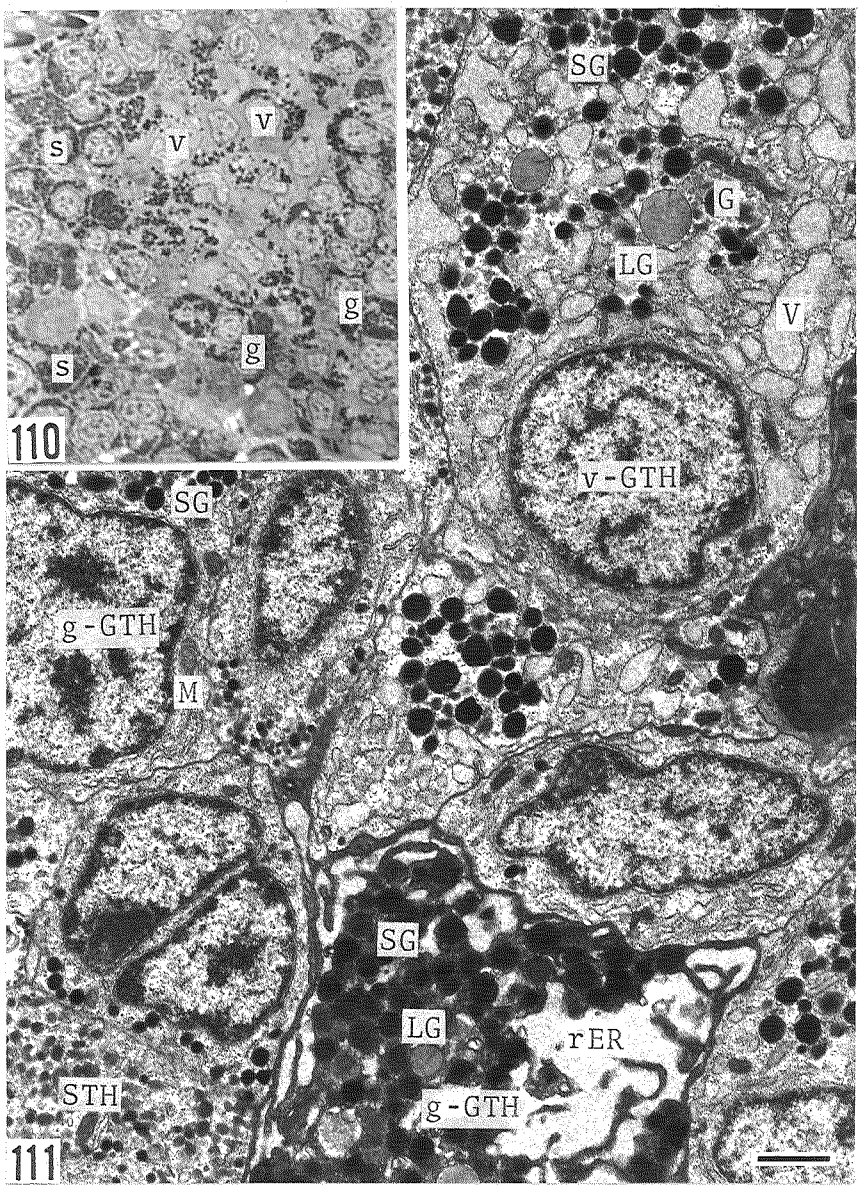
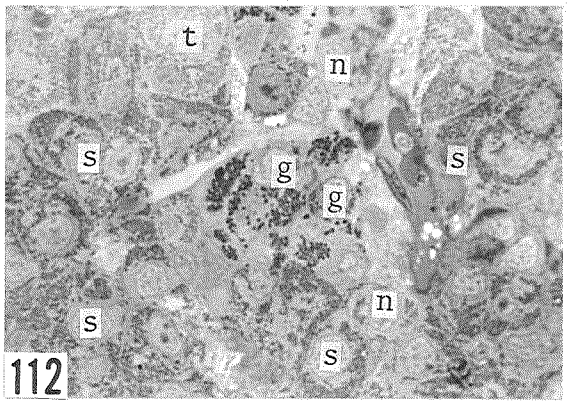
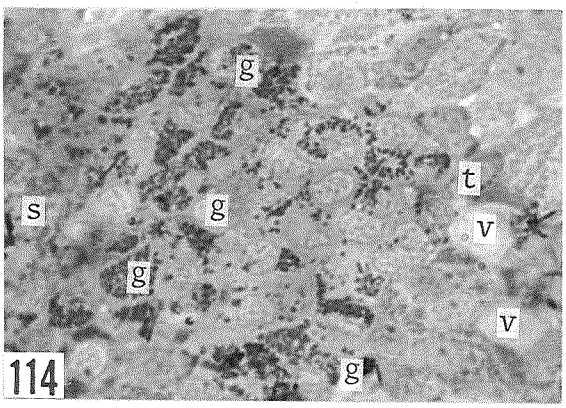

s
v
v
g
g
s
110
SG
G
LG
V
v-GTH
SG
g-GTH
M
SG
LG
rER
STH
g-GTH
111

Plate 39

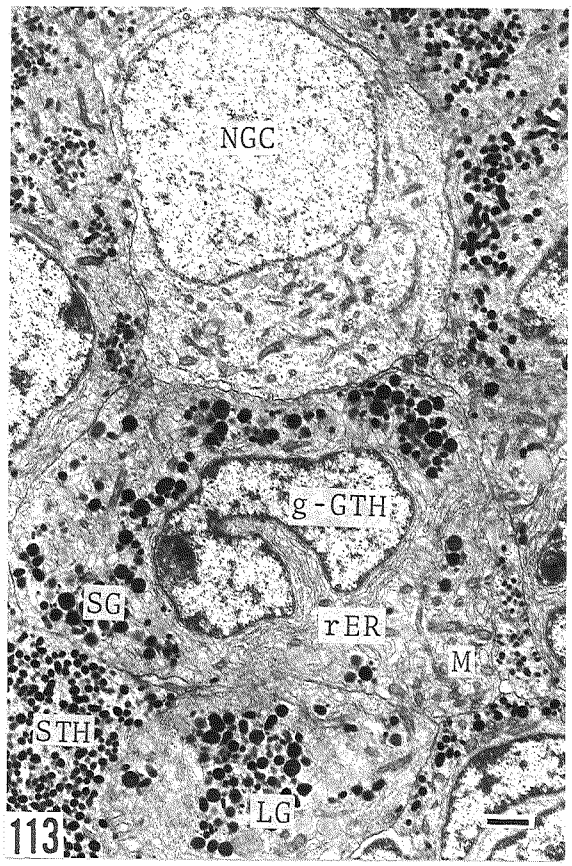
Figs. 112-115. Globular cells (g and g-GTH), non-granulated cells (n and NGC) and vesicular cells (v and v-GTH) of young female (Figs. 112 and 113) and young male (Figs. 114 and 115) loaches of the control group serving in the experiment with continuous light exposure. Note the absence of the vesicular cells in the female and the presence of the cells in the male. Figs. 112 and 114: methylene blue stain of Epon-embedded, semithin sections, X1000.



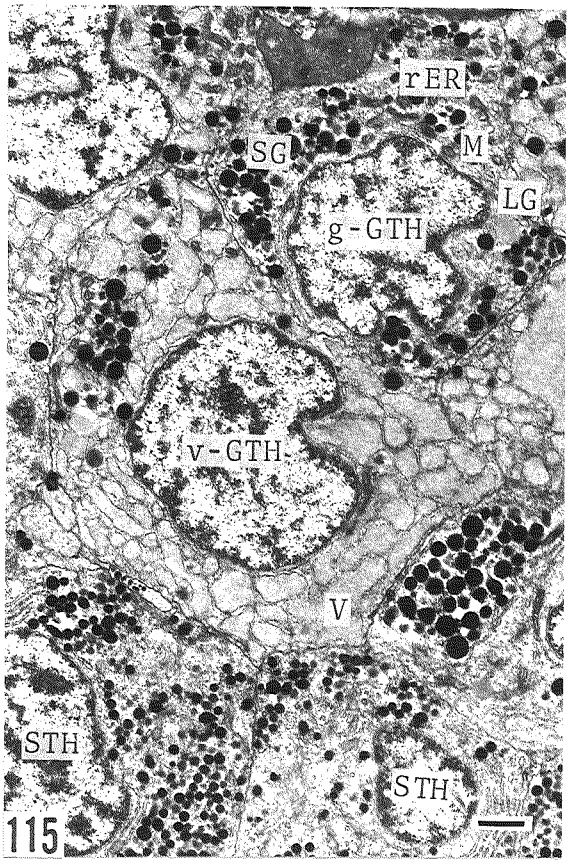
112



114



113



115

Plate 40

Figs. 116-118. Globular cells (g and g-GTH) of of adult female loaches exposed to continuous light for 30 (Figs. 116 and 117) and 60 days (Fig. 118). Fig. 116: methylene blue stain of an Epon-embedded, semithin section, X1000.

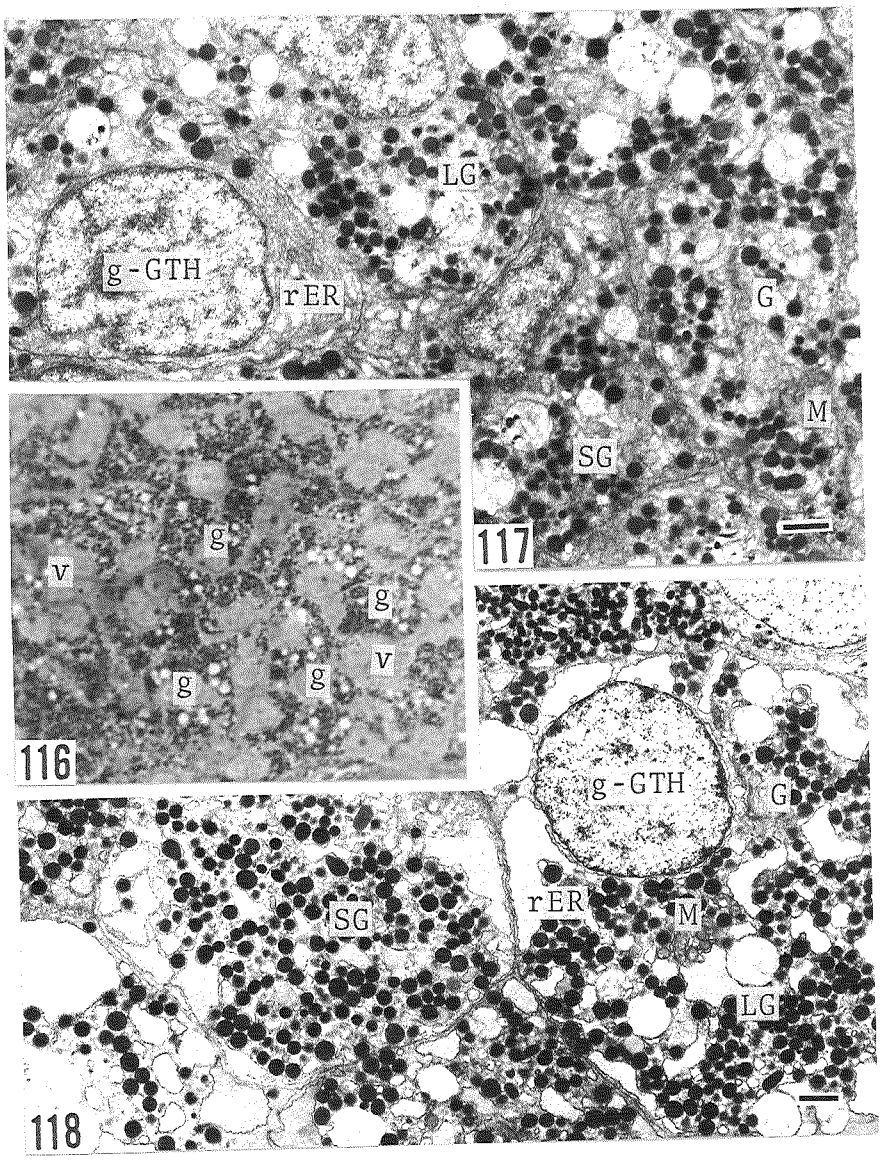

LG
g-GTH
rER
G
M
SG
117
g
v
g
g
g
v
116
g-GTH
G
rER
M
SG
LG
118

Plate 41

Figs. 119-121. Vesicular cells (v and v-GTH) of adult male (Figs. 119 and 121) and adult female (Fig. 120) loaches exposed to continuous light for 30 days. Fig. 119: methylene blue stain of an Epon-embedded, semithin section, X1000.

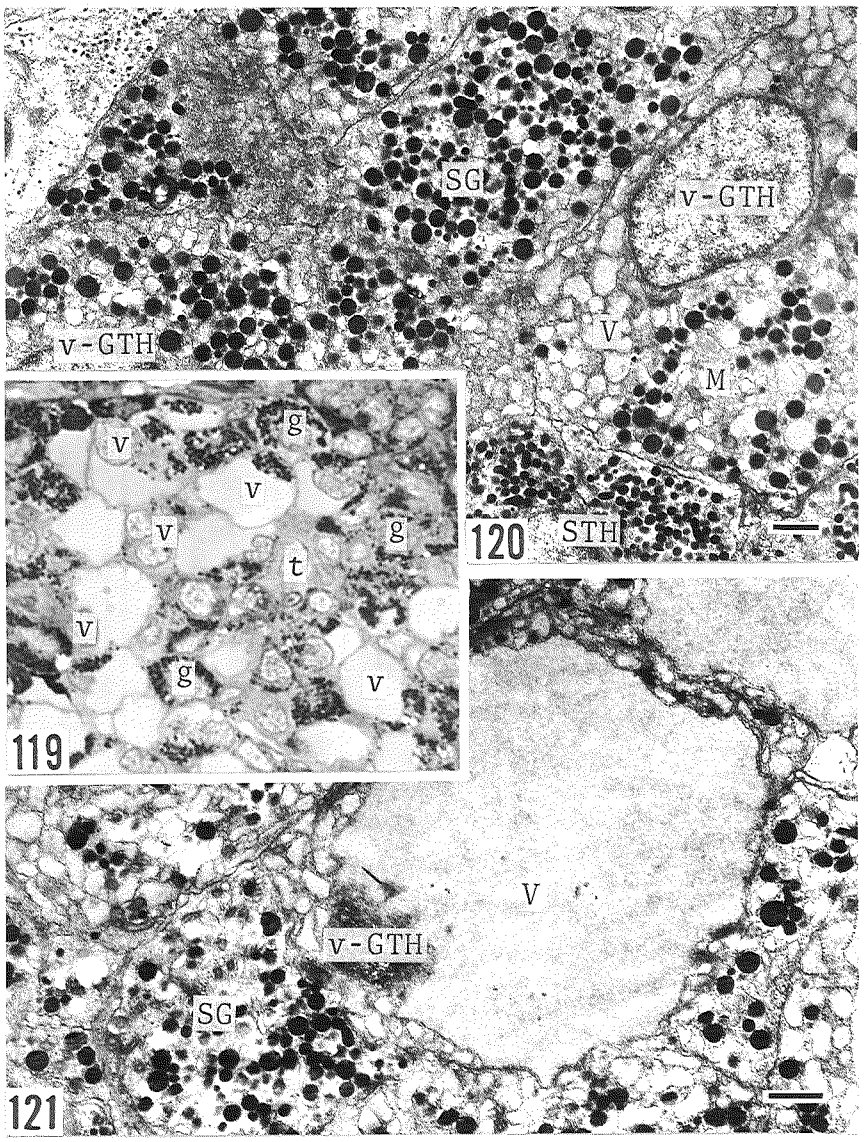
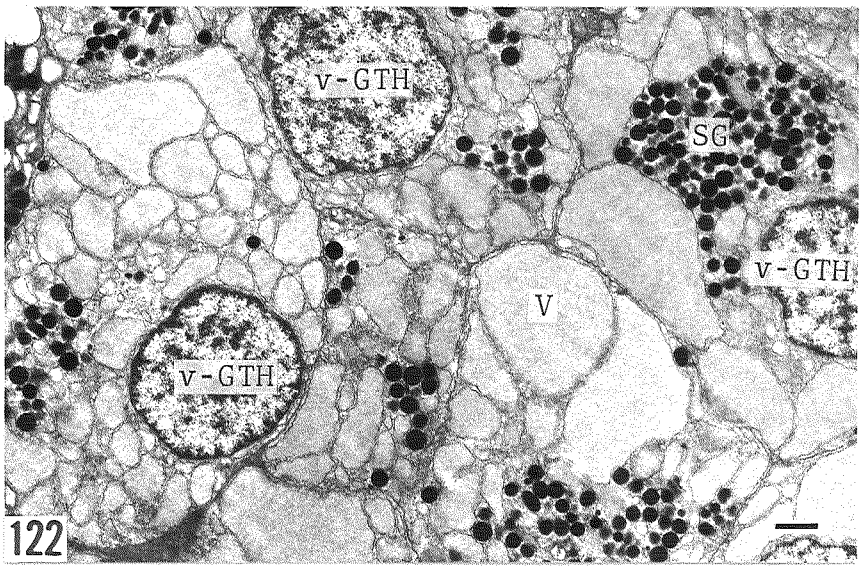

SG
v-GTH
v-GTH
V
M
120
STH
g
v
v
v
g
t
v
g
v
119
V
v-GTH
SG
121

Plate 42

Figs. 122 and 123. Globular cell (g-GTH) and vesicular cells (v-GTH) of adult female (Fig. 122) and adult male (Fig. 123) loaches exposed to continuous light for 60 days.


v-GTH
SG
v-GTH
V
v-GTH
122

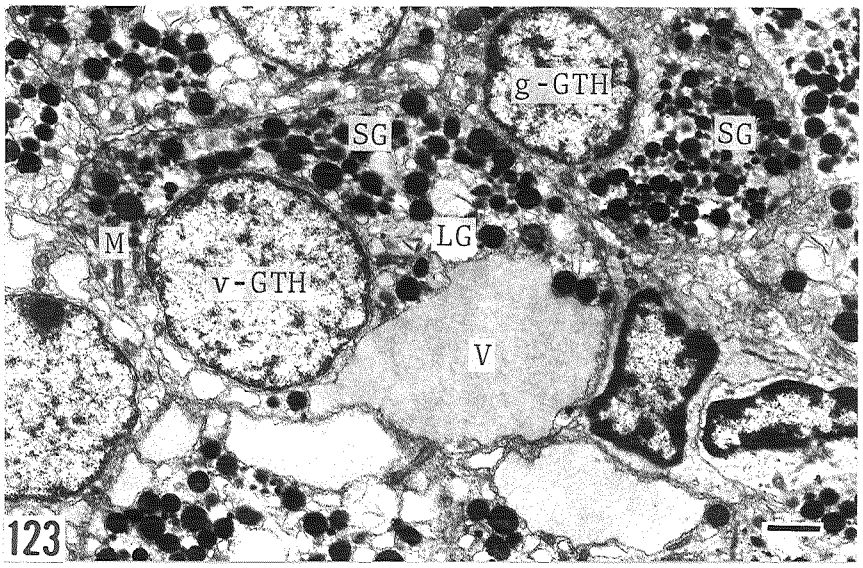

g-GTH
SG
SG
M
LG
v-GTH
V
123

Plate 43

Figs. 124-126. Globular cells (g and g-GTH) and vesicular cells (v and v-GTH) of young female loaches exposed to continuous light for 30 (Figs. 124 and 126) and 60 days (Fig. 125). Fig. 124: methylene blue stain of an Epon-embedded, semithin section, X1000.

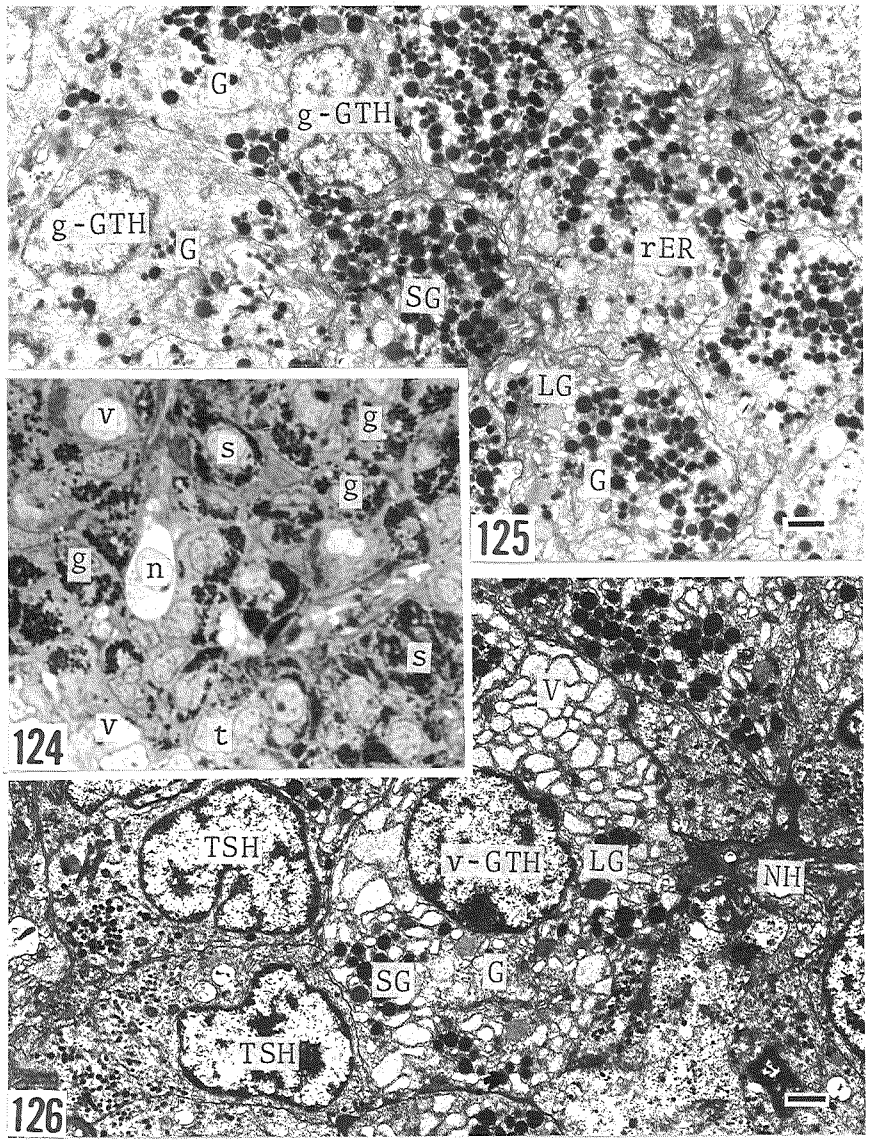

G
g-GTH
g-GTH
G
v
SG
rER
LG
G
125
v
s
g
g
g
n
s
v
t
124
V
TSH
v-GTH
LG
NH
SG
G
TSH
126

Plate 44

Figs. 127 and 128. Globular cell (g-GTH) and vesicular cells (v-GTH) of young female (Fig. 127) and young male (Fig. 128) loaches exposed to continuous light for 60 days.

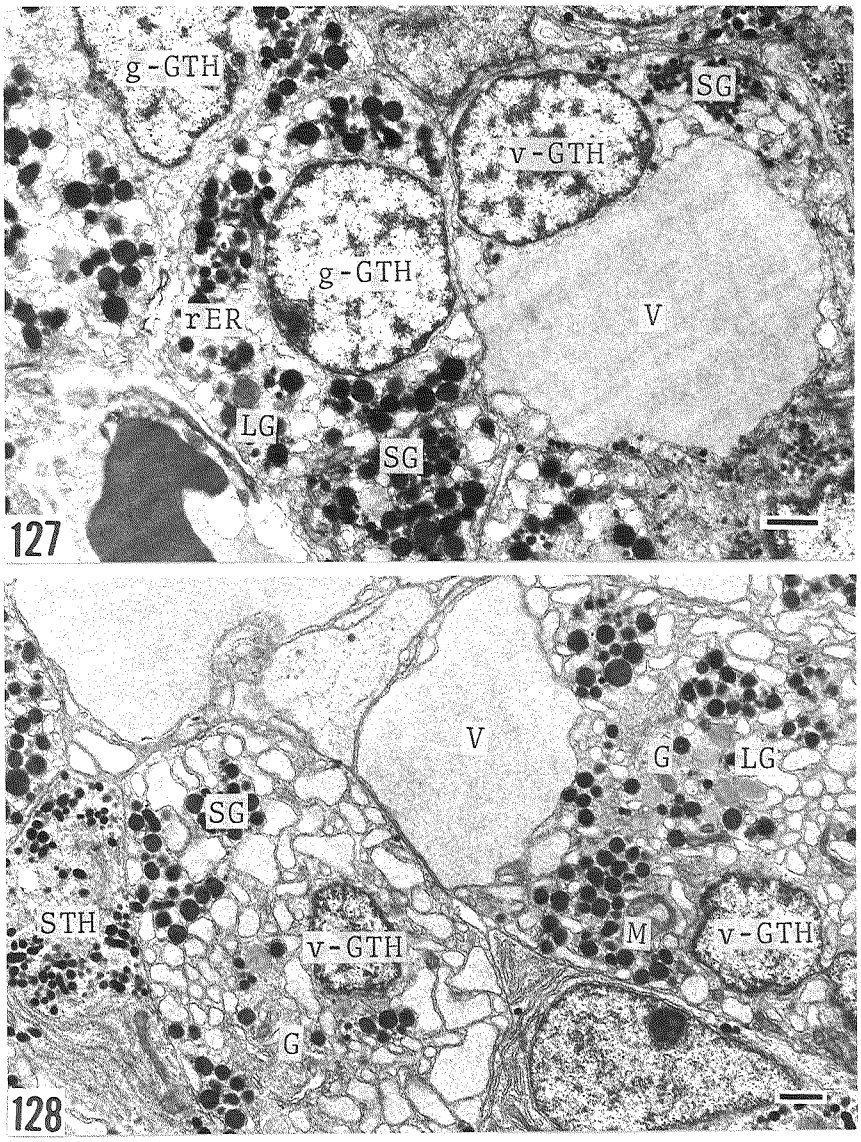

g-GTH
SG
v-GTH
g-GTH
rER
V
LG
SG
127
V
G
LG
SG
STH
v-GTH
M
v-GTH
G
128

Plate 45

Figs. 129-131. Globular cells (g and g-GTH) and vesicular cells (v and v-GTH) of female loaches of the control group sampled at 30 (Figs. 129 and 130) and 60 days (Fig. 131) of the starvation experiment. Fig. 129: methylene blue stain of an Epon-embedded, semithin section, X1000.

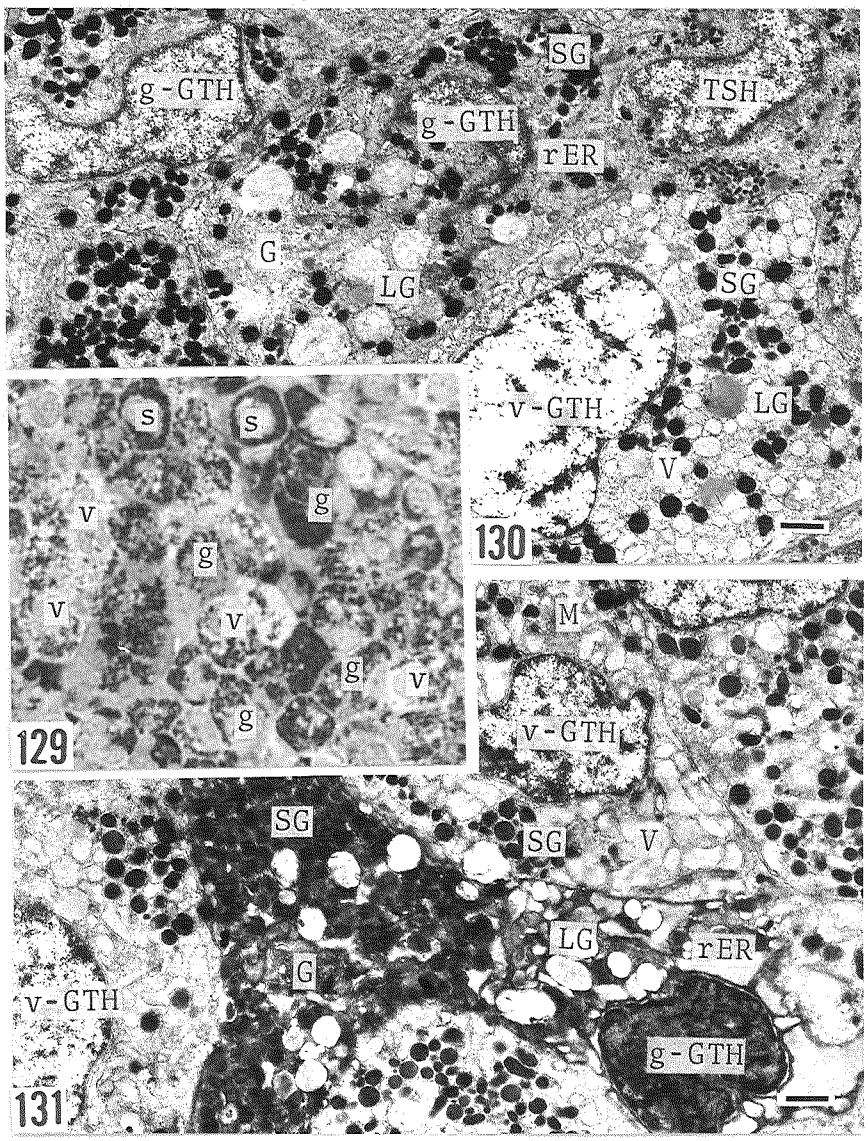

g-GTH
SG
TSH
g-GTH
rER
G
LG
SG
v-GTH
LG
V
130
s
s
v
g
g
v
v
g
v
g
129
M
v-GTH
SG
SG
V
LG
rER
G
v-GTH
g-GTH
131

Plate 46

Figs. 132-134. Globular cells (g and g-GTH) and vesicular cells (v and v-GTH) of female loaches starved for 60 days. Fig. 132: methylene blue stain of an Epon-embedded, semithin section, X1000.

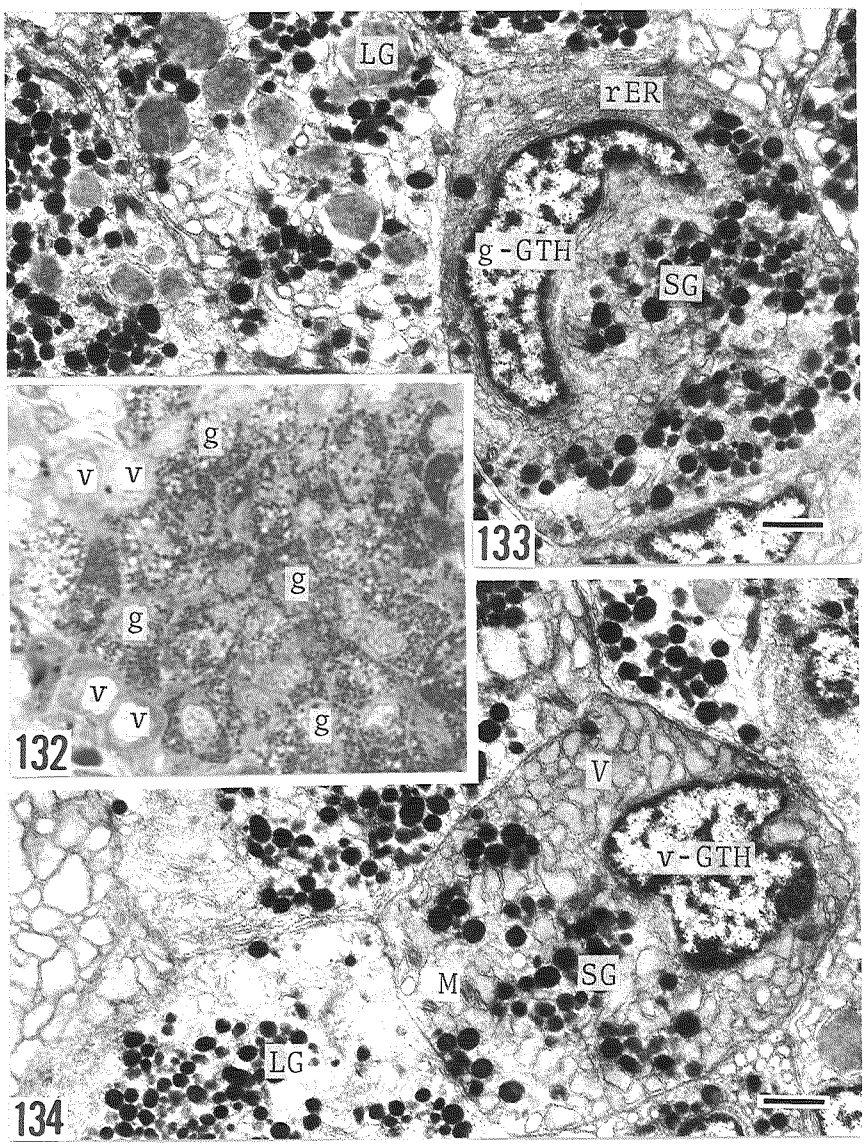
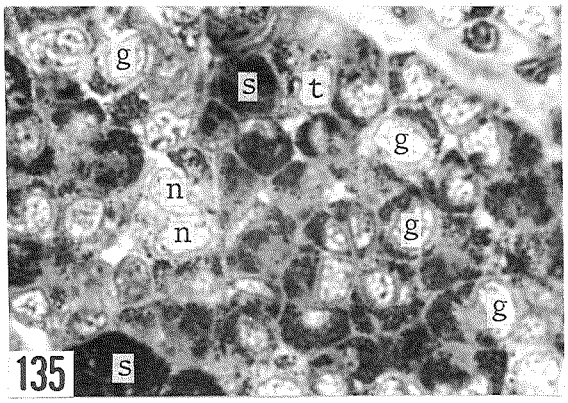
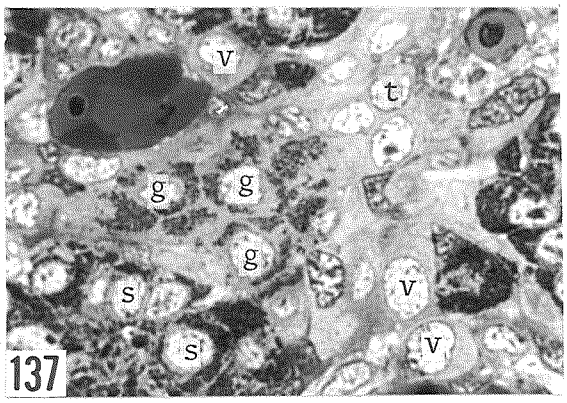

LG
rER
g-GTH
SG
133
g
v v
g
g
v
v
g
132
V
v-GTH
M
SG
LG
134

Plate 47

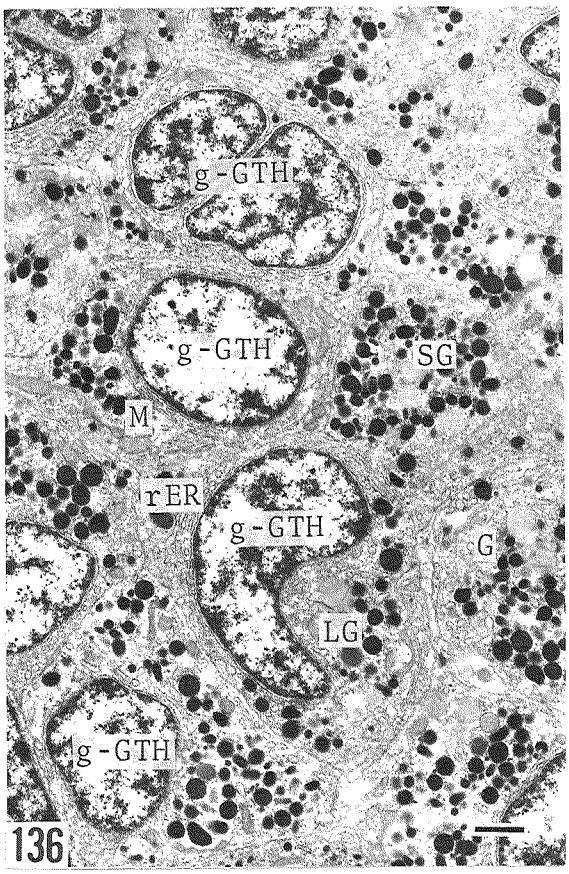
Figs. 135-138. Globular cells (g and g-GTH) and vesicular cells (v and v-GTH) of young female (Figs. 135 and 136) and young male (Figs. 137 and 138) loaches of the control group serving in the treatment with HCG. Figs. 135 and 137: methylene blue stain of Epon-embedded, semithin sections, X1000.



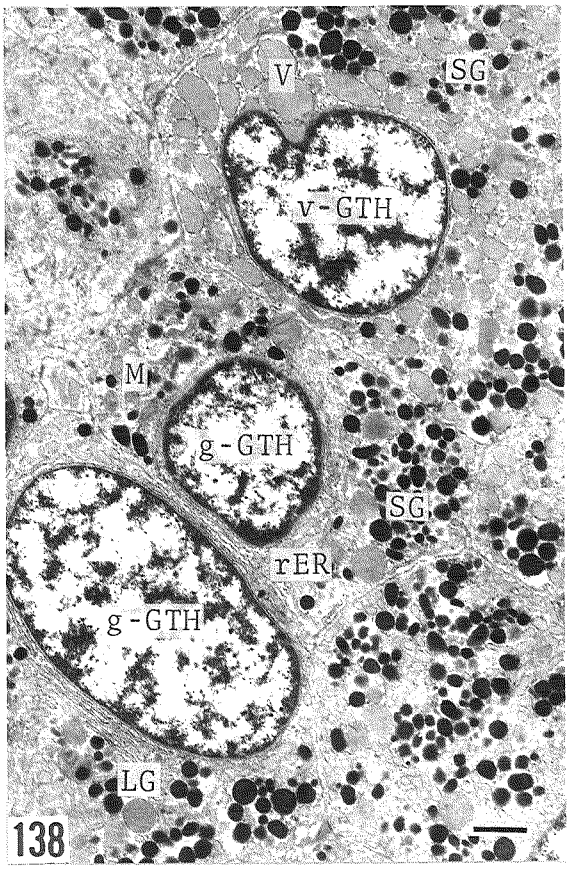
135



137



136



138

Plate 48

Figs. 139 and 140. Globular cells (g and g-GTH) and vesicular cells (v and v-GTH) of HCG-injected, young female loaches. Fig. 139: methylene blue stain of an Epon-embedded, semithin section, X1000.

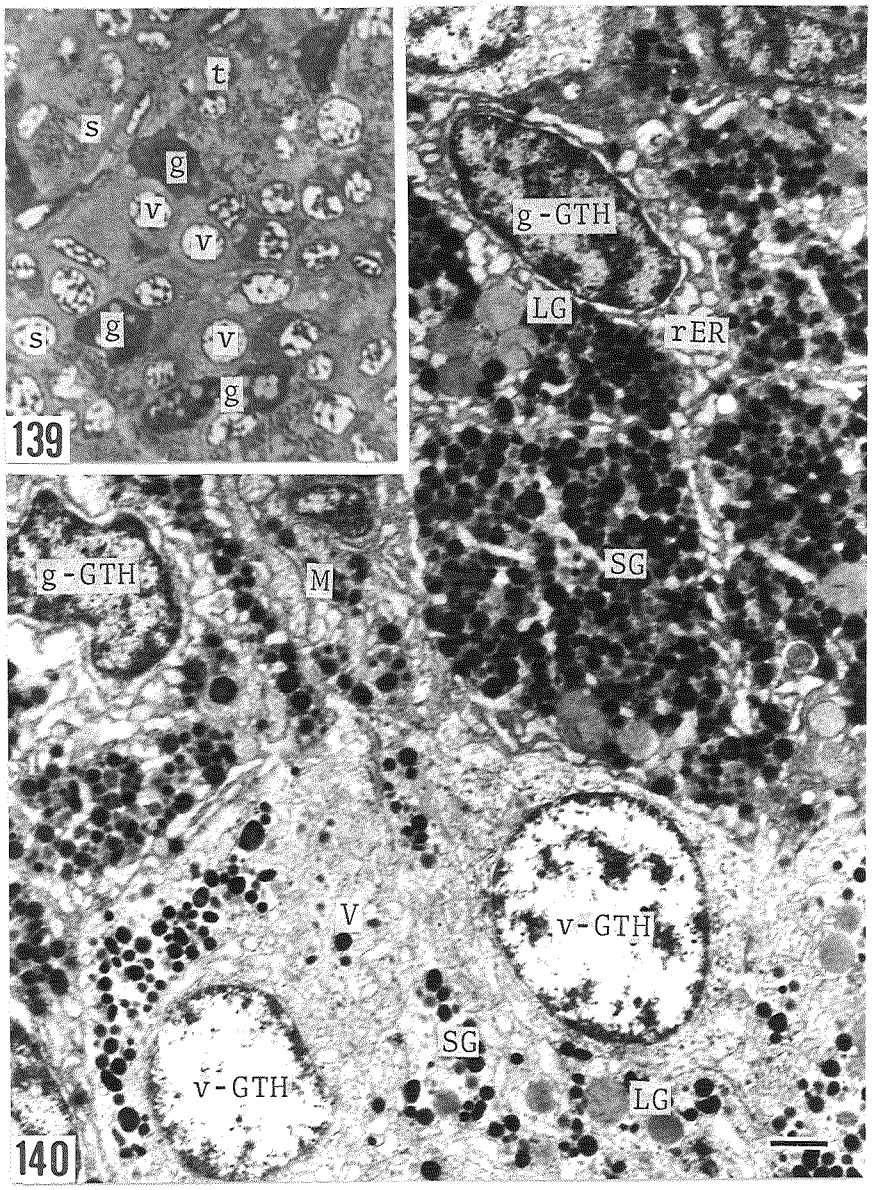

t
s
g
v
v
s
g
v
g
139
g-GTH
LG
rER
SG
g-GTH
M
V
v-GTH
SG
v-GTH
LG
140

Plate 49

Figs. 141 and 142. Globular cells (g-GTH) and vesicular cells (v-GTH) of HCG-injected, young male loaches.


M
SG
g-GTH
g-GTH
LG
rER
141

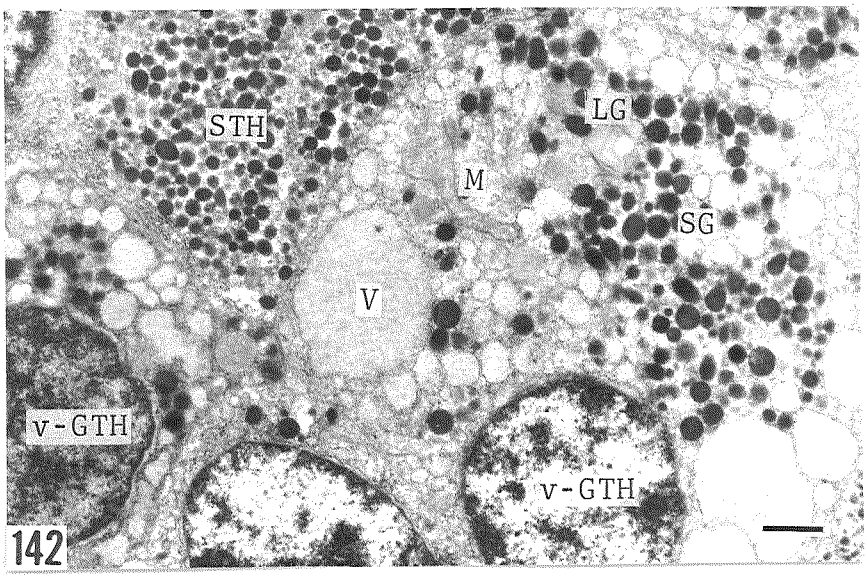
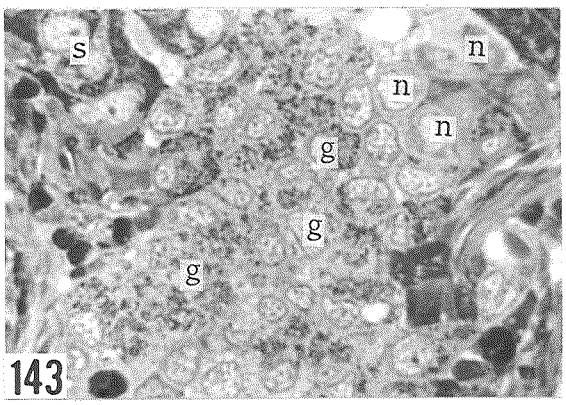
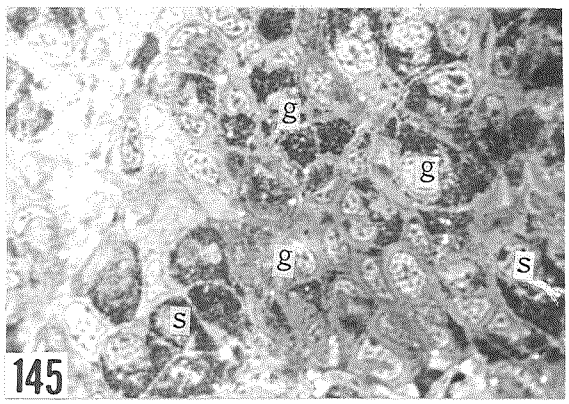
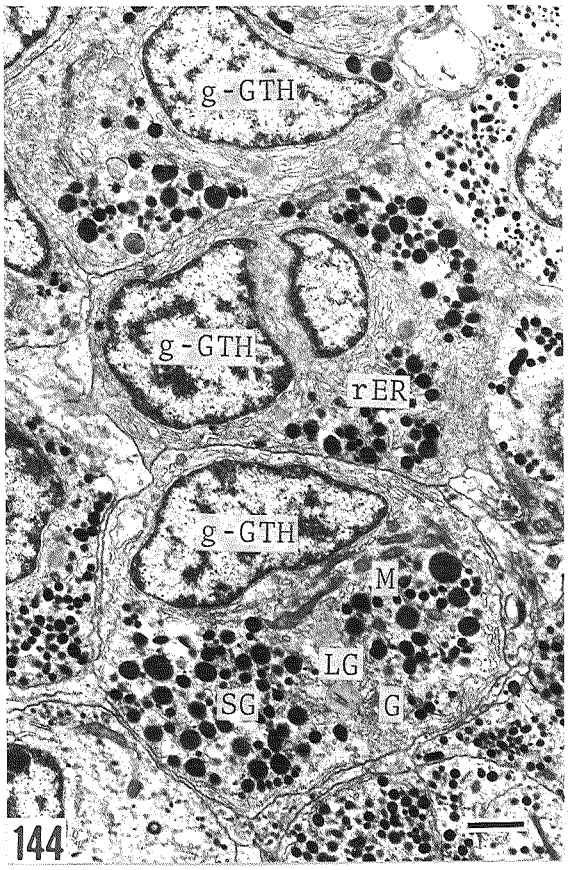

STH
LG
M
SG
V
v-GTH
v-GTH
142

Plate 50

Figs. 143-146. Globular cells (g and g-GTH) of control (Figs. 143 and 144) and testosterone-treated (Figs. 145 and 146) juvenile loaches examined 10 days after the start of treatment. Figs. 143 and 145: methylene blue stain of Epon-embedded, semithin sections, X1000.


s
n
n
n
g
g
g
143


g
g
g
s
s
145


g-GTH
g-GTH
rER
g-GTH
M
LG
SG
G
144

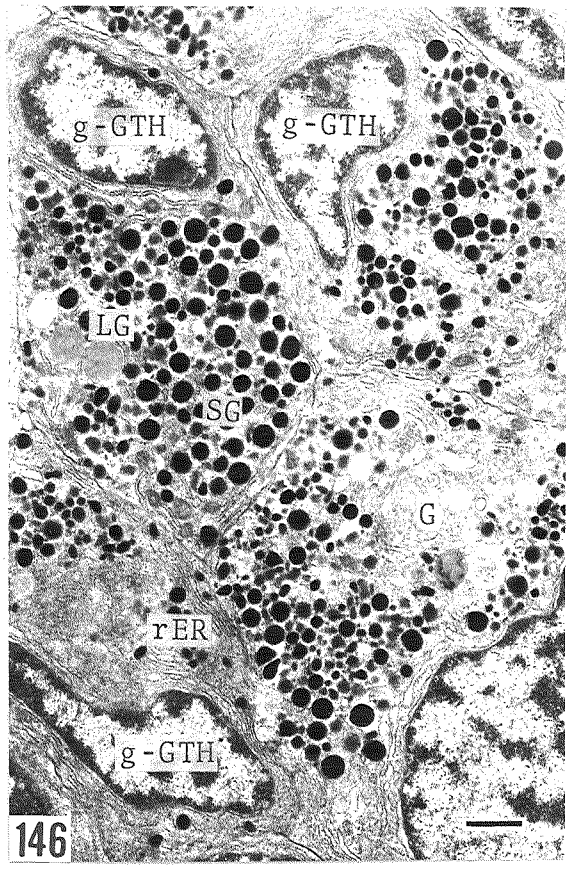

g-GTH
g-GTH
LG
SG
G
rER
g-GTH
146

Plate 51

Figs. 147-149. Vesicular cells (v and v-GTH) of juvenile loaches treated with testosterone for 10 (Figs. 147 and 148) and 30 days (Fig. 149). Fig. 147: methylene blue stain of an Epon-embedded, semithin section, X1000.

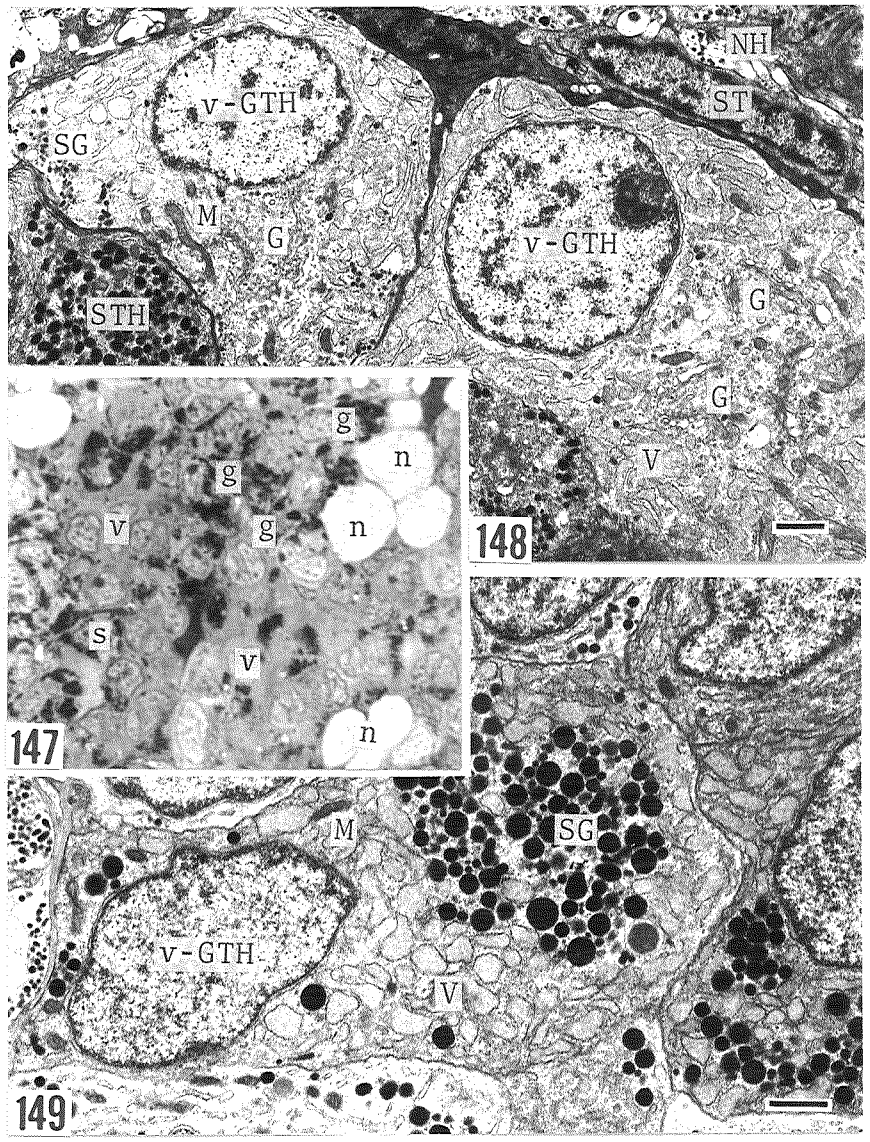

v-GTH
SG
M
G
STH
NH
ST
v-GTH
G
G
V
148
g
n
g
v
g
n
s
v
n
147
M
SG
v-GTH
V
149

Plate 52

Figs. 150 and 151. Globular cells (g and g-GTH) of juvenile loaches treated with testosterone for 30 days. Fig. 150: methylene blue stain of an Epon-embedded, semithin section, X1000.

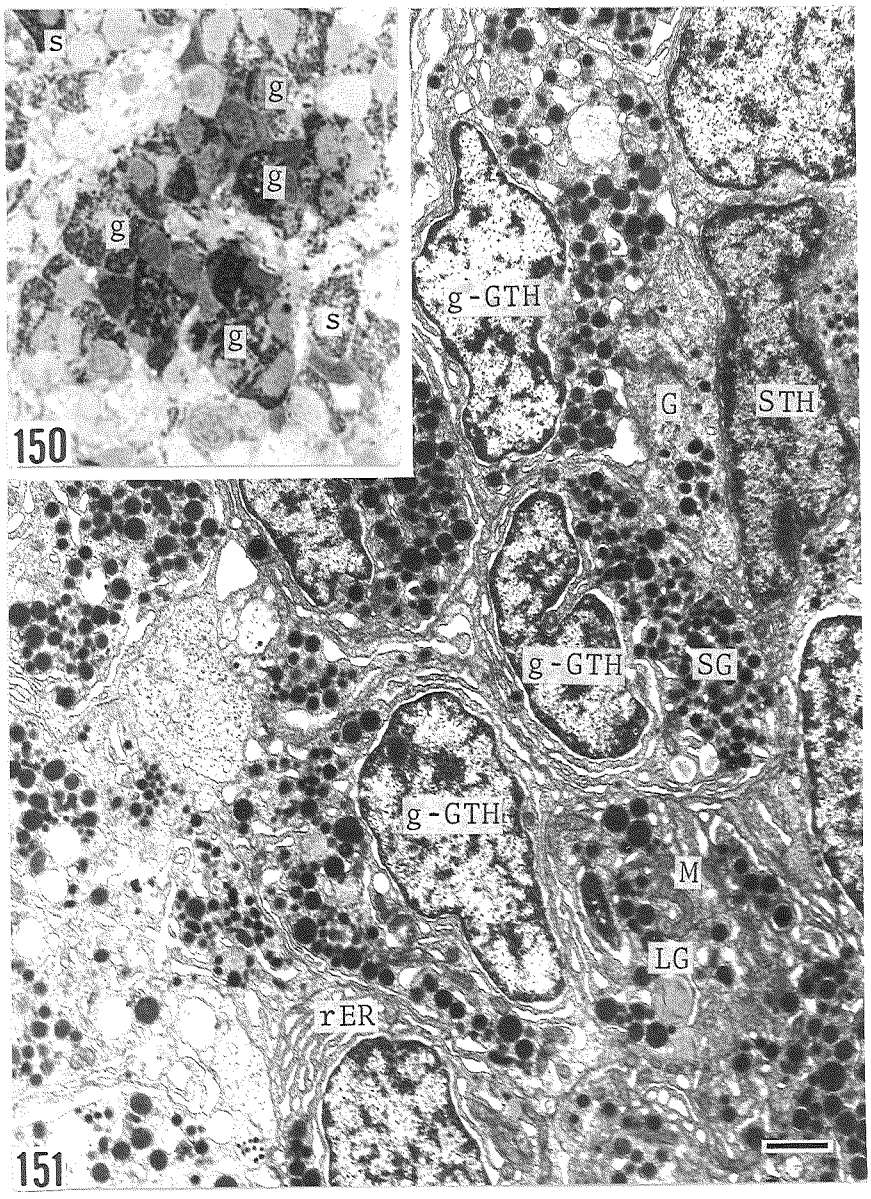

s
g
g
g
g
s
150
g-GTH
G
STH
g-GTH
SG
g-GTH
M
LG
rER
151

Plate 53

Figs. 152-154. Globular cells (g and g-GTH) and possible vesicular cells (v-GTH?) of juvenile loaches treated with estrone for 30 days. Fig. 152: methylene blue stain of an Epon-embedded, semithin section, ×1000.

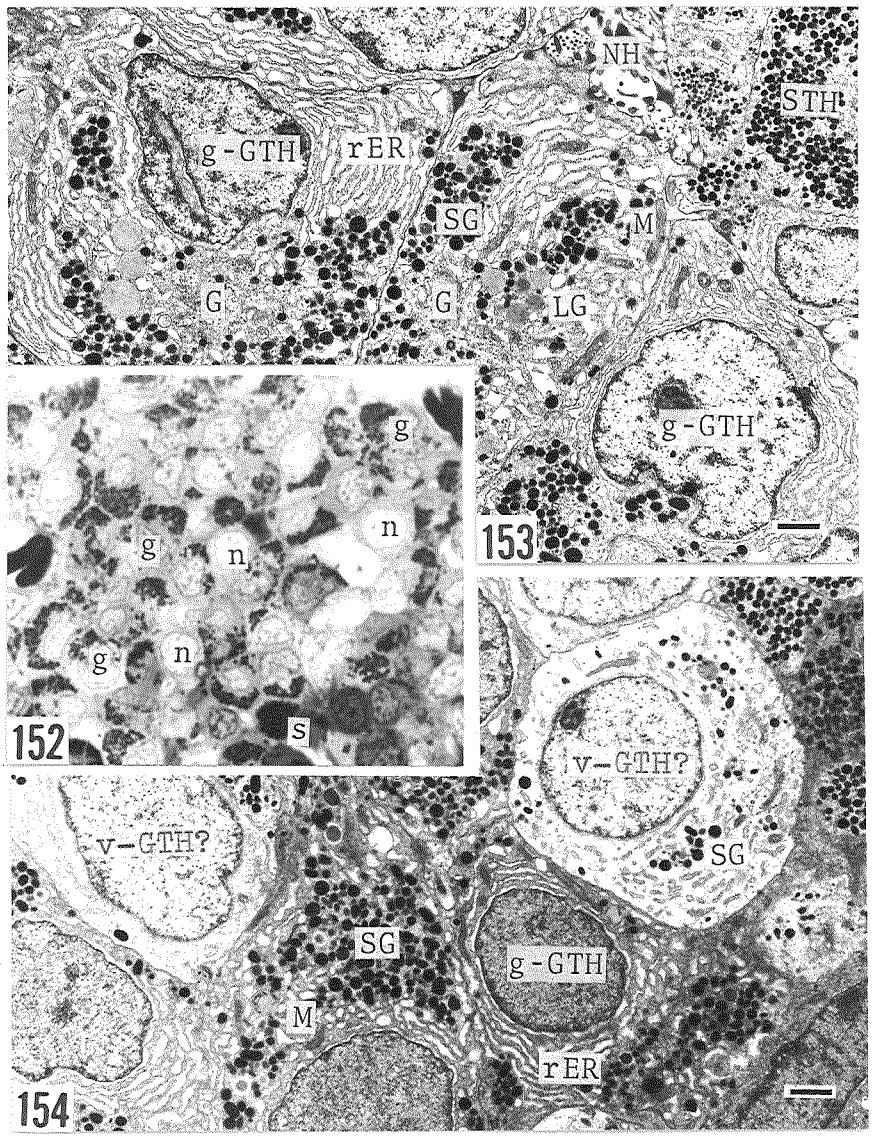

NH
STH
g-GTH
rER
SG
M
G
G
LG
g-GTH
153
g
n
g
n
g
n
s
152
v-GTH?
SG
v-GTH?
SG
g-GTH
M
rER
154

Plate 54

Figs. 155 and 156. Globular cells (g and g-GTH), somatotrophs (s and STH) and thyrotrophs (t and TSH) of female masu salmon, Oncorhynchus masou. Fig. 155: methylene blue stain of an Epon-embedded, semithin section, X 1000.

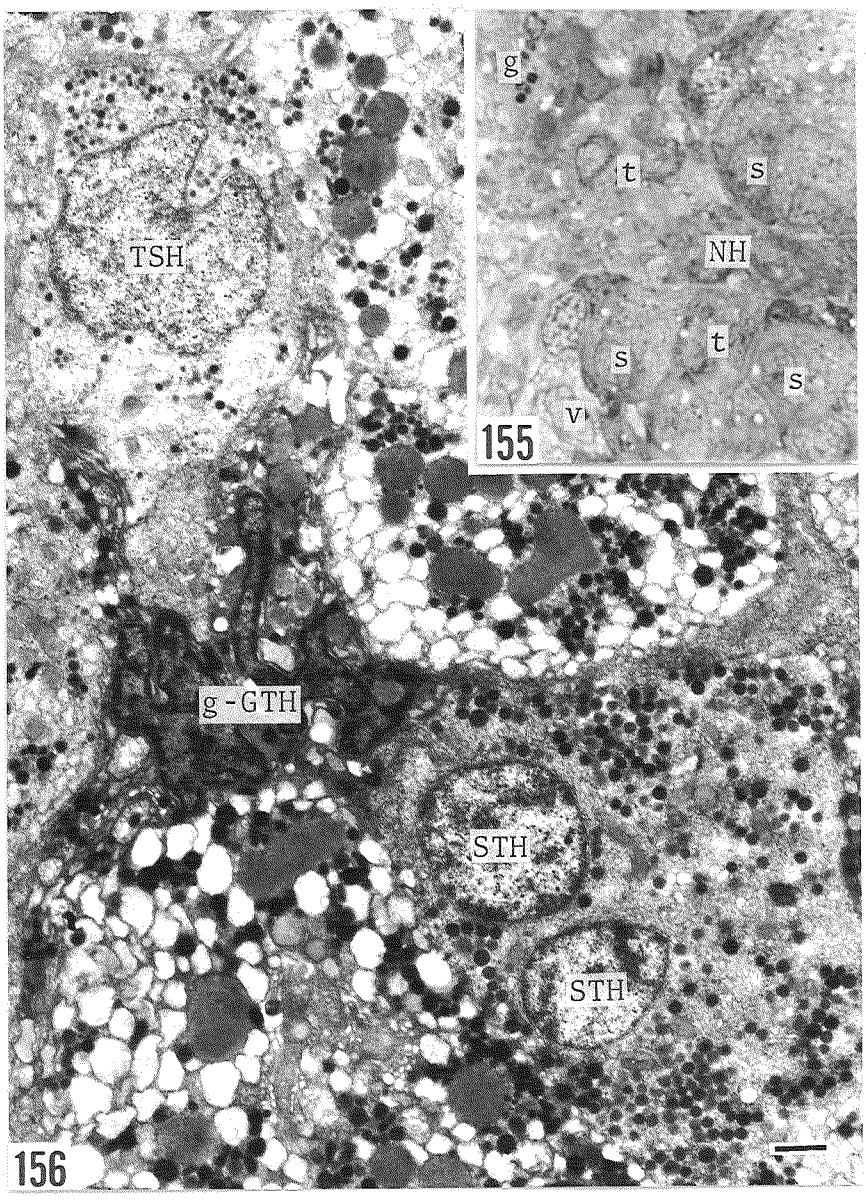

g
t
s
NH
t
s
s
v
155
TSH
g-GTH
STH
STH
156

Plate 55

Figs. 157 and 158. Globular cells (g and g-GTH) and vesicular cells (v and v-GTH) of female chum salmon, Oncorhynchus keta. Fig. 157: methylene blue stain of an Epon-embedded, semithin section, X1000.

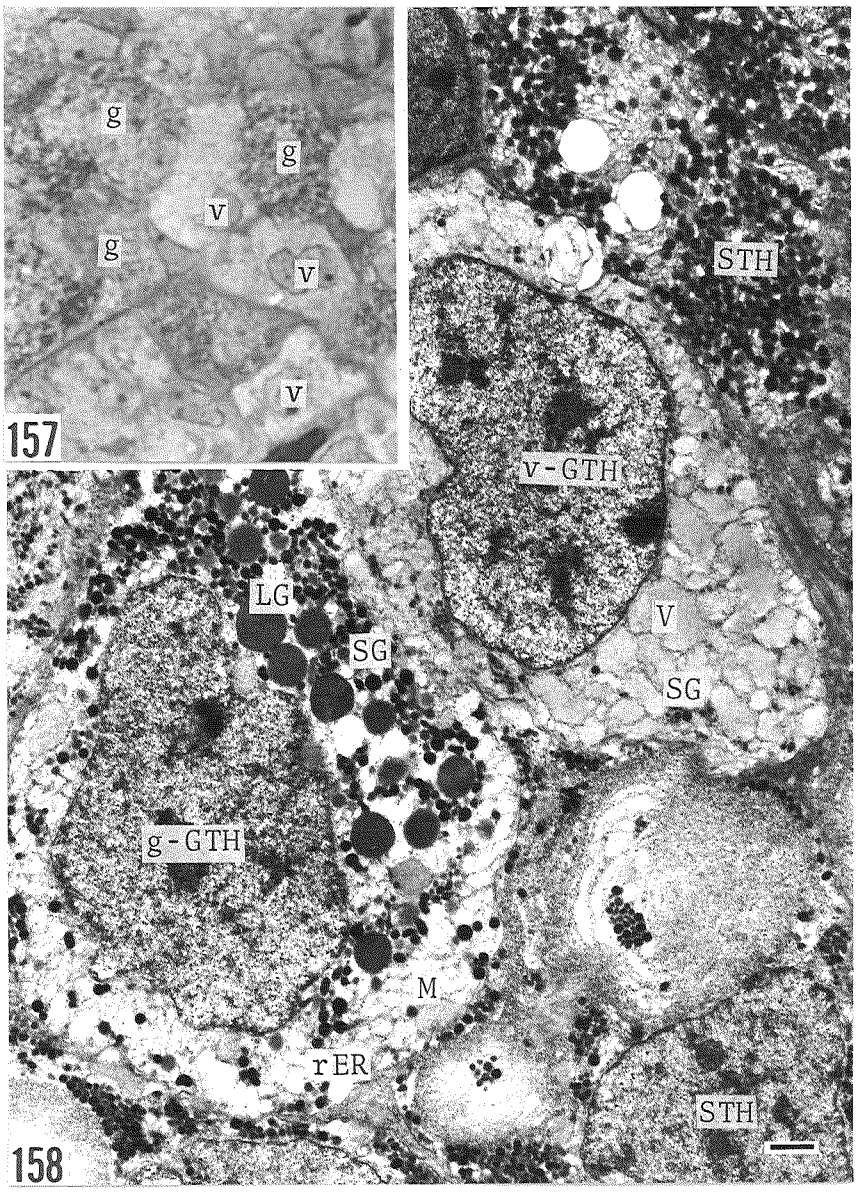

g
g
v
g
v
v
157
STH
v-GTH
LG
SG
V
SG
g-GTH
M
rER
STH
158

Plate 56

Figs. 159 and 160. Globular cells (g and g-GTH) and vesicular cells (v and v-GTH) of female masu salmon, Oncorhynchus masou. Fig. 159: methylene blue stain of an Epon-embedded, semithin section, X1000.

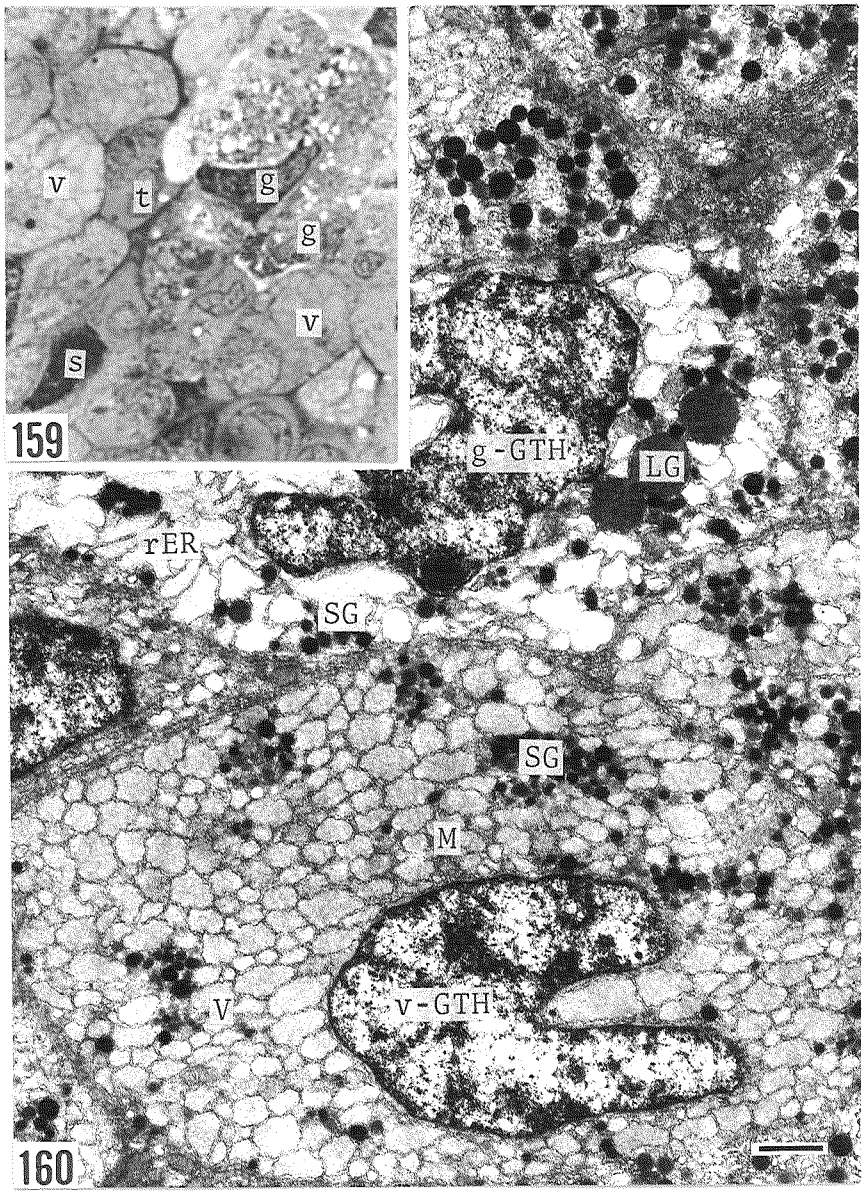

v
t
g
g
v
s
159
g-GTH
LG
rER
SG
SG
M
V
v-GTH
160

Plate 57

Figs. 161 and 162. Globular cells (g and GTH) and vesicular cells (v and v-GTH) of female whitespotted char, Salvelinus leucomaenis. Fig. 161: methylene blue stain of an Epon-embedded, semithin section, X1000.

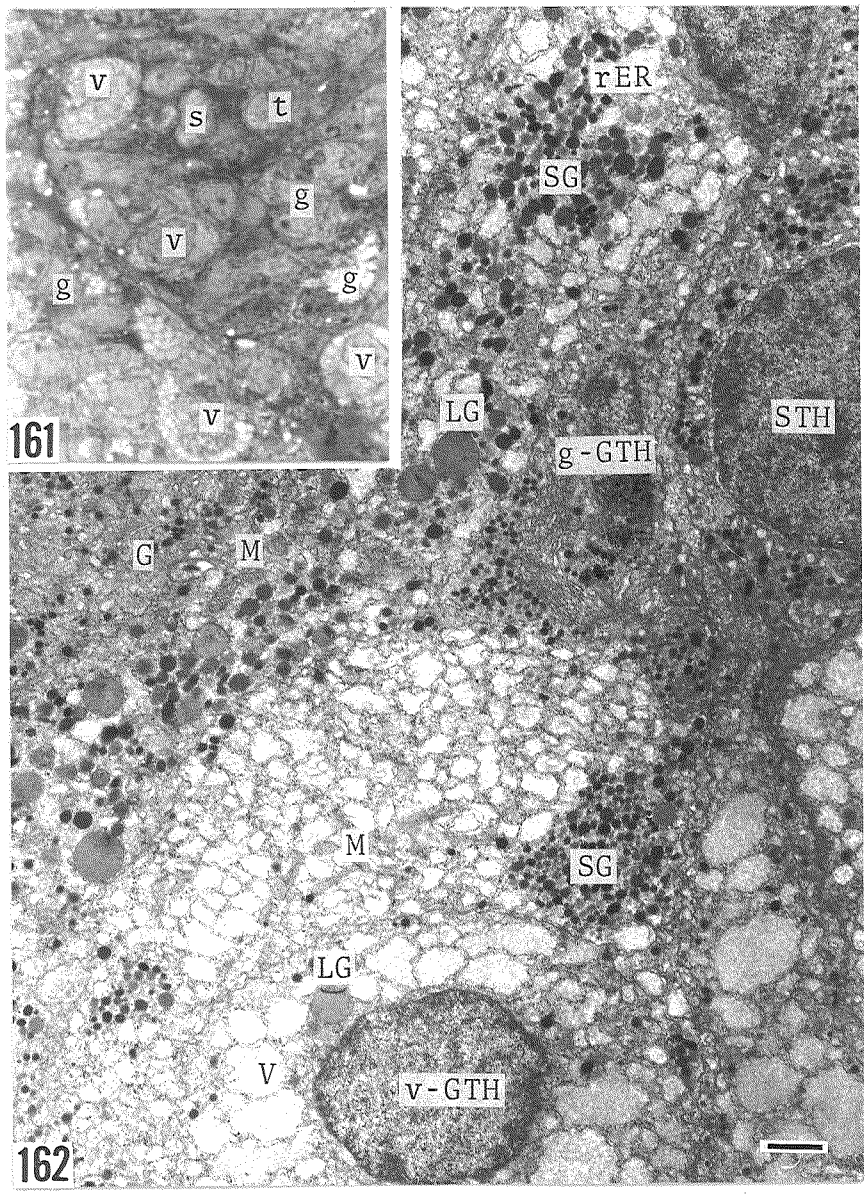

v
s
t
g
v
g
g
v
v
161
rER
SG
LG
STH
g-GTH
G
M
M
SG
LG
V
v-GTH
162

Plate 58

Figs. 163-165. Globular cells (g and g-GTH) and vesicular cells (v-GTH) of female whitespotted chars sampled in May (Figs. 163 and 164) and June (Fig. 165). Fig. 163: methylene blue stain of an Epon-embedded, semithin section, X1000.

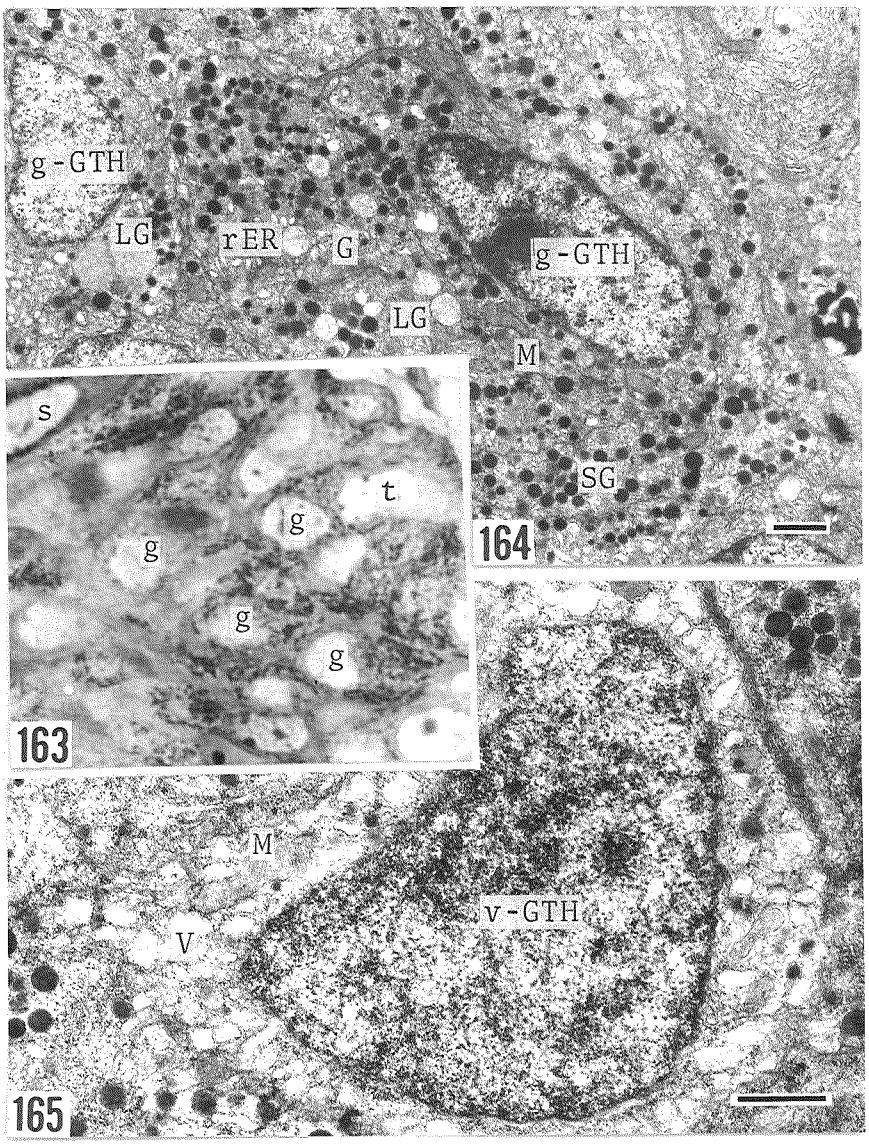
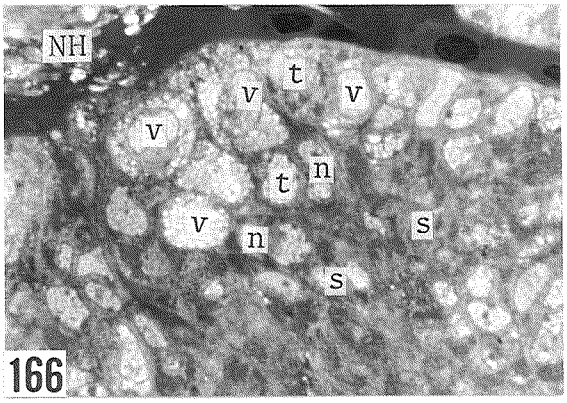
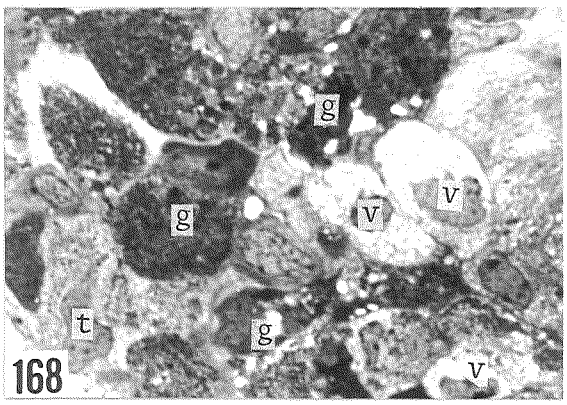

g-GTH
LG
rER
G
g-GTH
LG
M
SG
164
s
t
g
g
g
g
163
M
V
v-GTH
165

Plate 59

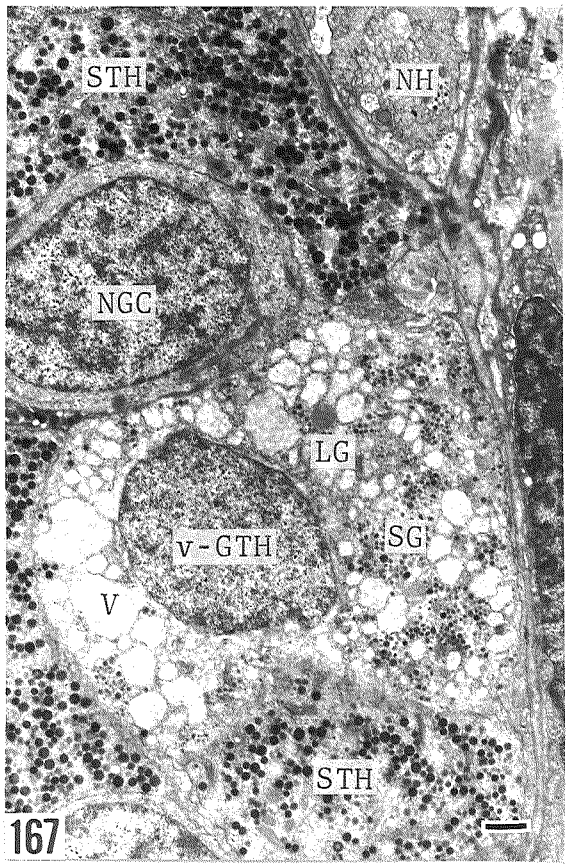
Figs. 166-169. Globular cells (g and g-GTH), non-granulated cells (n and NGC) and vesicular cells (v and v-GTH) of female whitespotted chars sampled in July (Figs. 166 and 167) and September (Figs. 168 and 169). Figs. 166 and 168: methylene blue stain of Epon-embedded, semithin sections, ×1000.



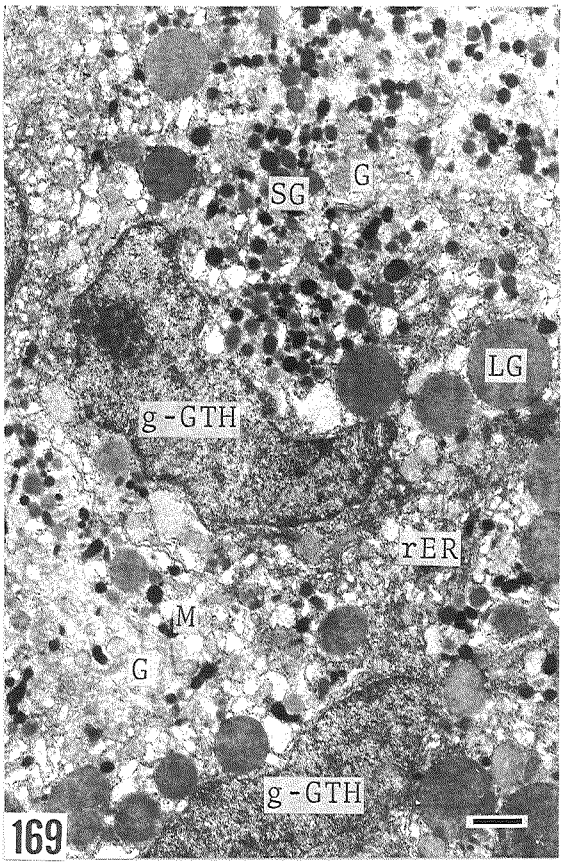
166



168



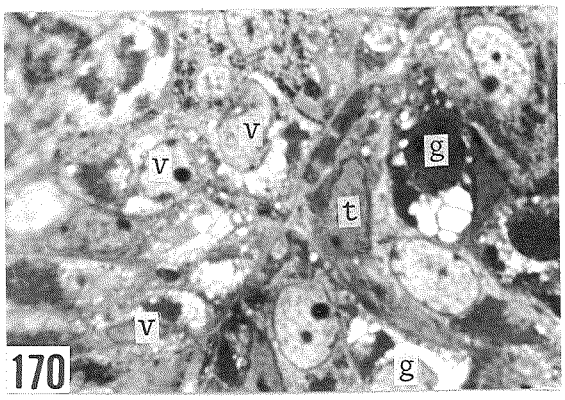
167



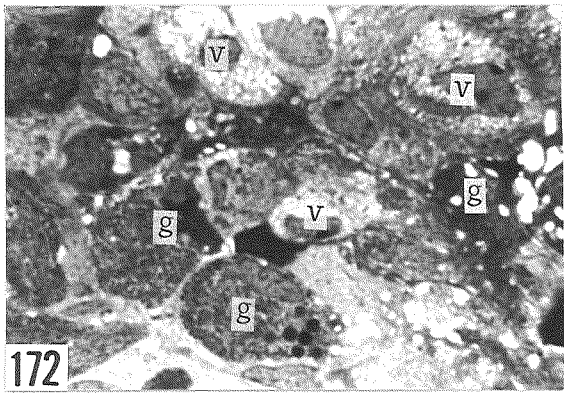
169

Plate 60

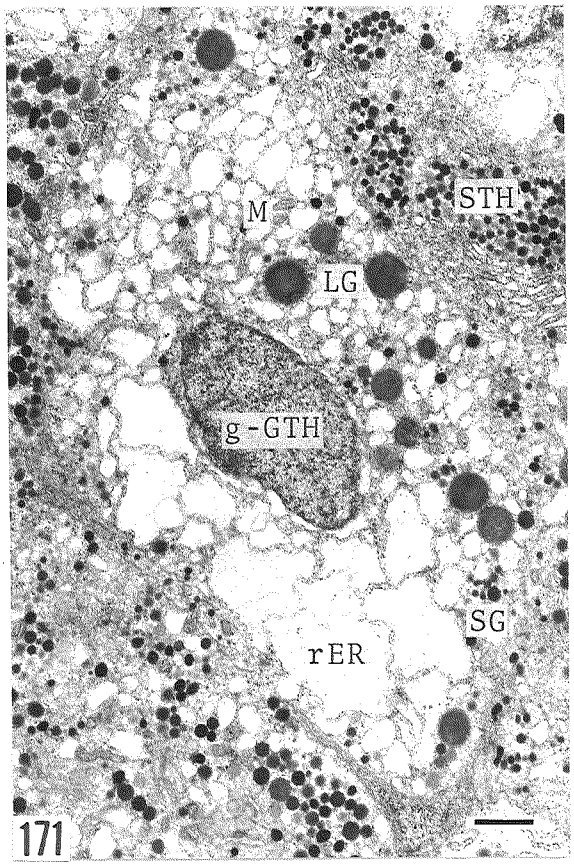
Figs. 170-173. Globular cells (g and g-GTH) of female whitespotted char sampled in October (Figs. 170 and 171) and November (Figs. 172 and 173). Figs. 170 and 172: methylene blue stain of Epon-embedded, semithin sections, X1000.



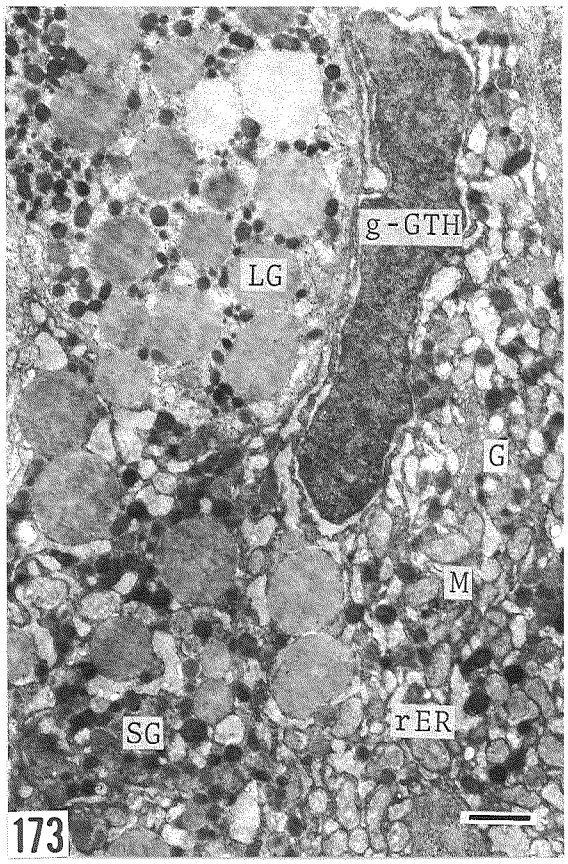
170



172



171



173

Plate 61

Figs. 174-176. Globular cells (g-GTH) and vesicular cells (v and v-GTH) of female whitespotted chars sampled in January. Fig. 174: methylene blue stain of an Epon-embedded, semithin section, X1000.

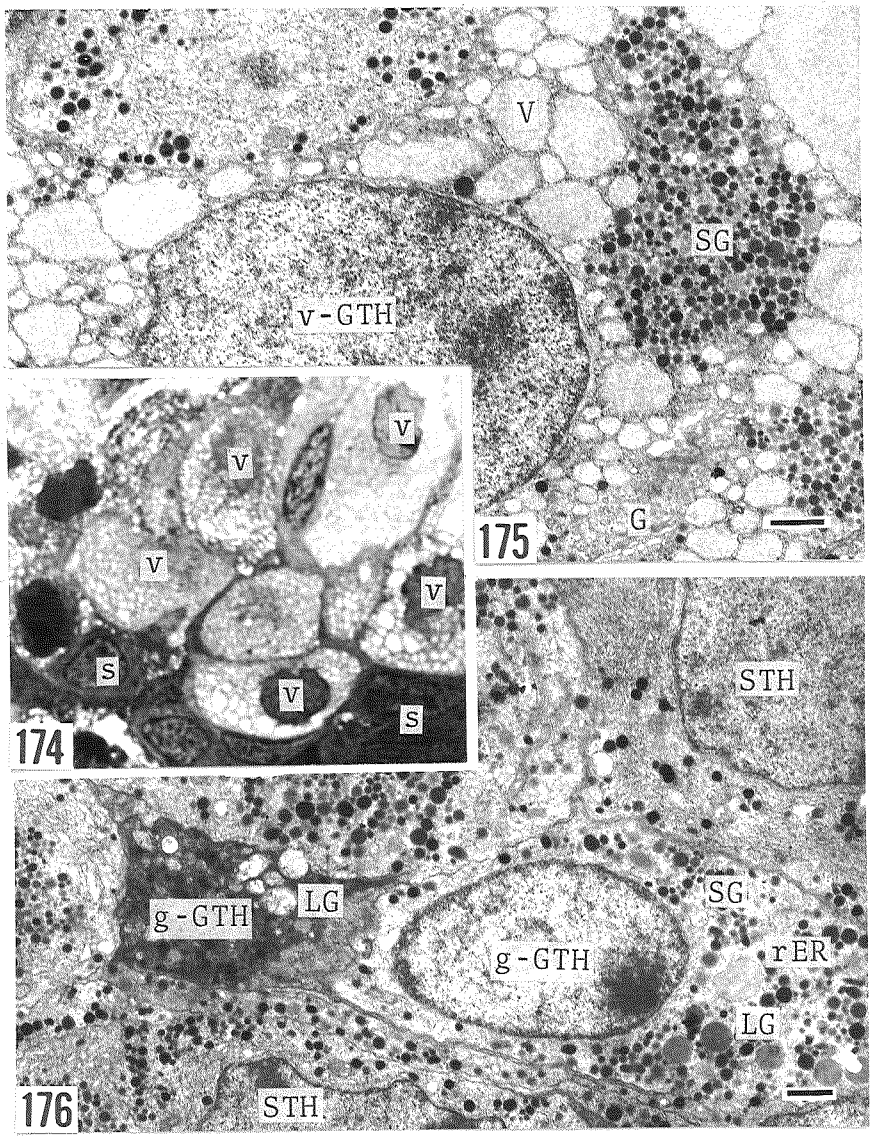

V
SG
v-GTH
G
175
v
v
v
v
v
s
s
174
STH
SG
g-GTH
LG
g-GTH
rER
LG
176
STH

Plate 62

Figs. 177-180. Globular cells (g and g-GTH) and vesicular cells (v and v-GTH) of male whie-spotted chars sampled in June (Figs. 177 and 178) and September (Figs. 179 and 180). Figs. 177 and 179: methylene blue stain of Epon-embedded, semithin sections, X1000.

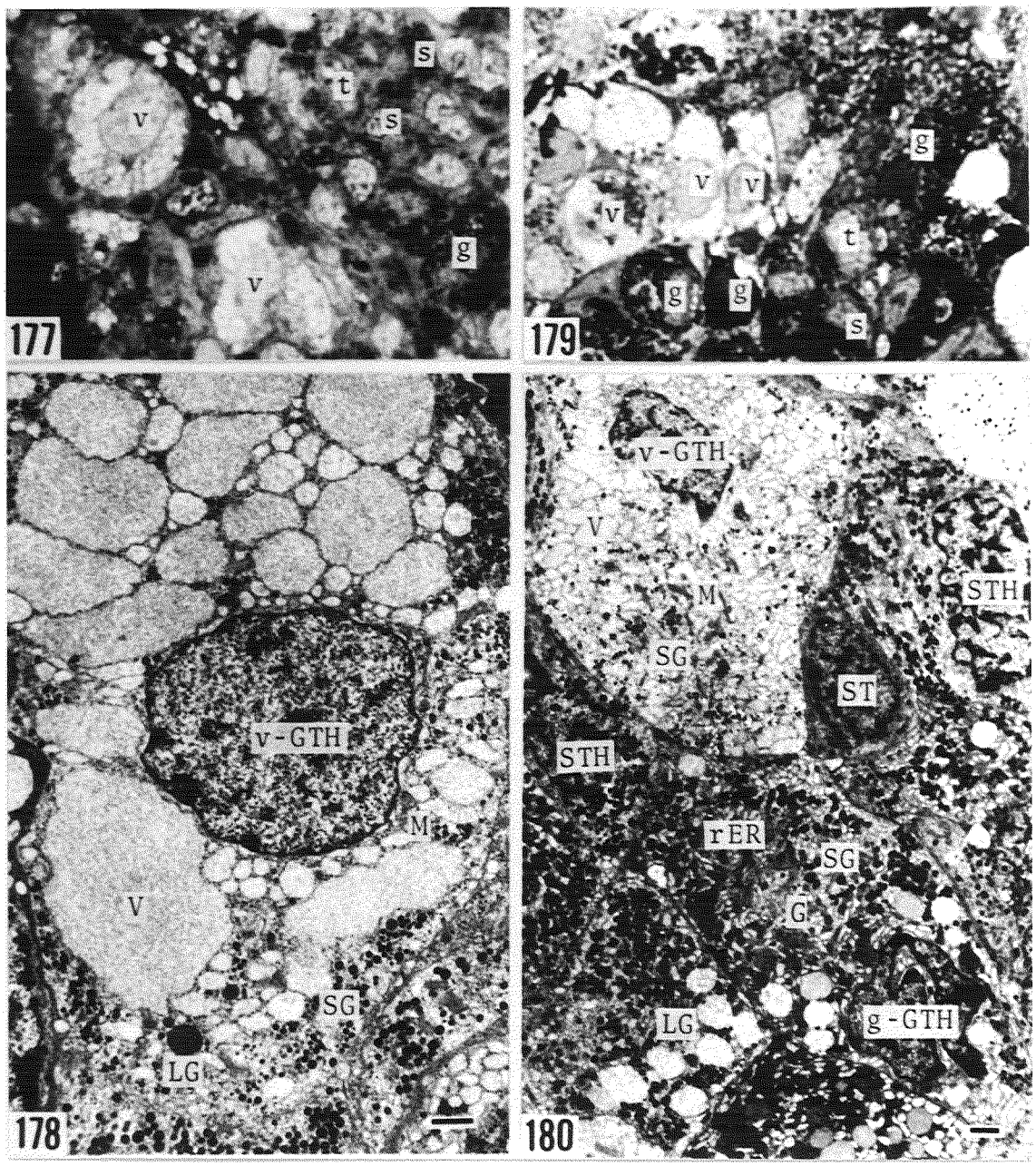

177
s
t
s
v
g
v
179
g
v
v
v
t
g
g
s
178
v-GTH
M
V
SG
LG
180
v-GTH
V
M
STH
SG
ST
STH
rER
SG
G
LG
g-GTH

Plate 63

Figs. 181 and 182. Gonadotrophs (g and GTH) of female rainbow trout, Salmo gairdneri. Fig. 181: methylene blue stain of an Epon-embedded, semithin section, X1000.

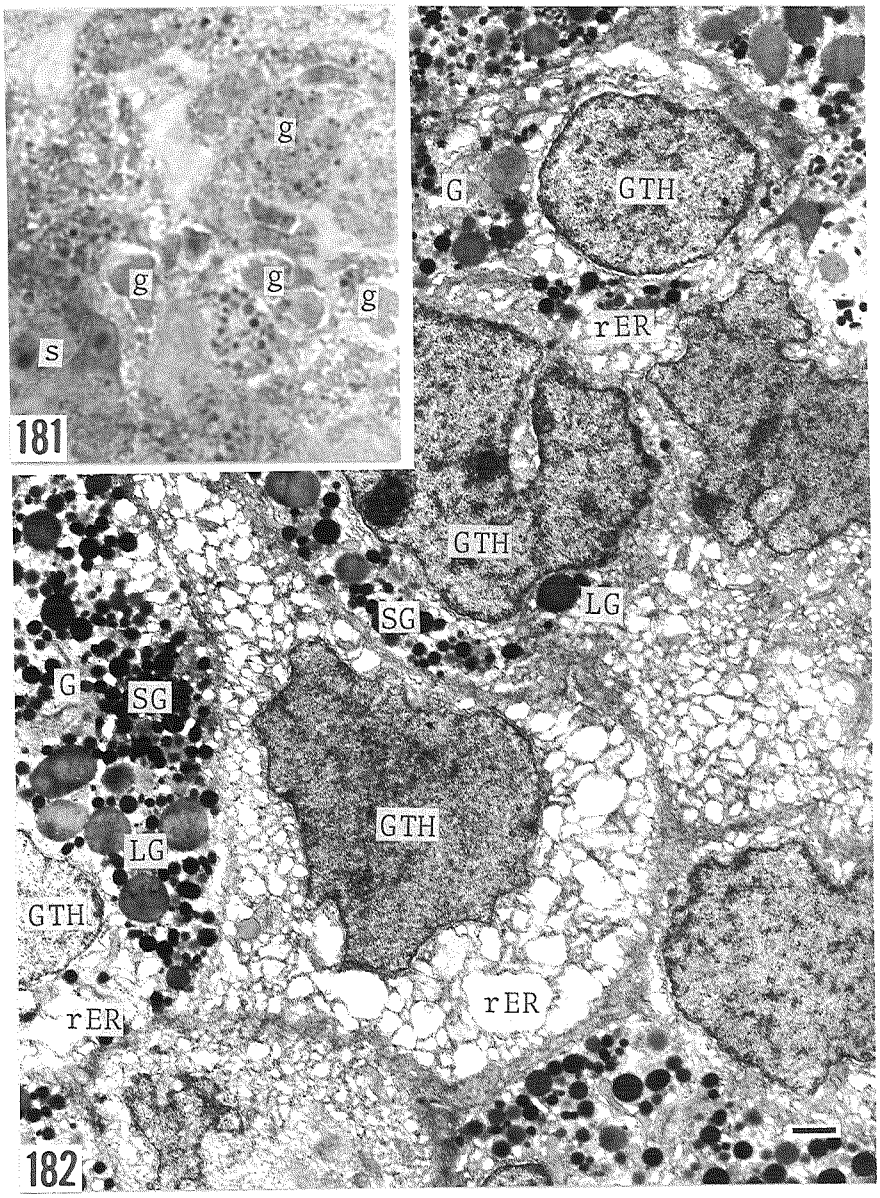

g
g
g
g
s
181
G
GTH
rER
GTH
LG
SG
G
SG
LG
GTH
GTH
rER
rER
182

Plate 64

Figs. 183 and 184. Gonadotrophs (g and GTH) of male goldfish, Carassius auratus. Fig. 183: methylene blue stain of an Epon-embedded, semithin section, X 1000.

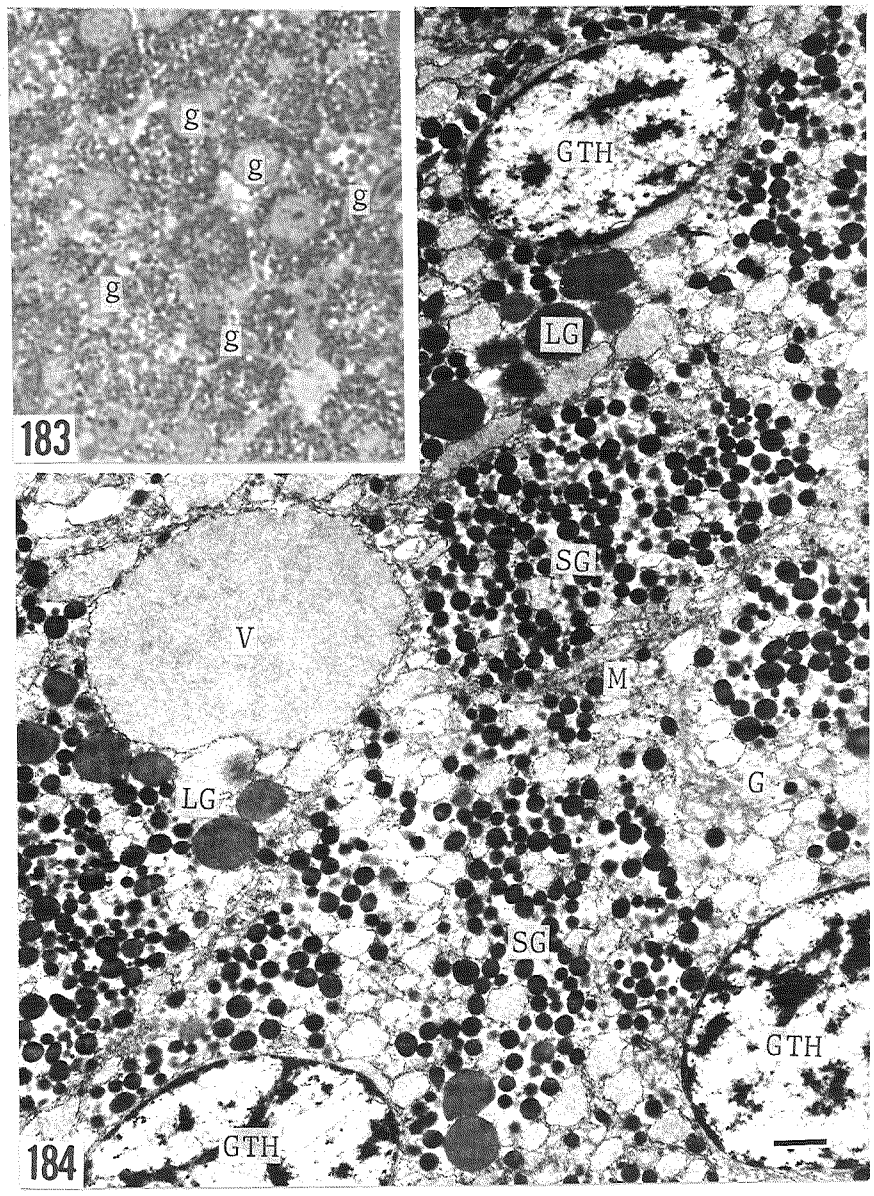
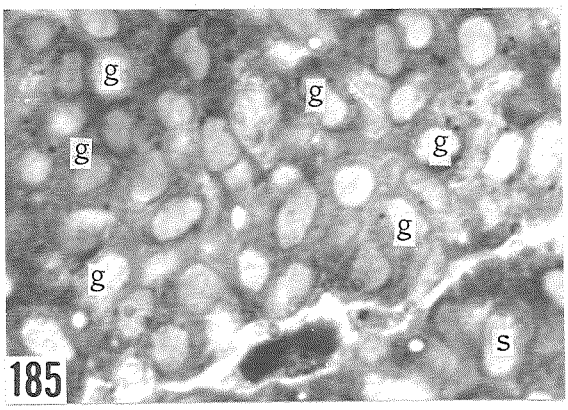
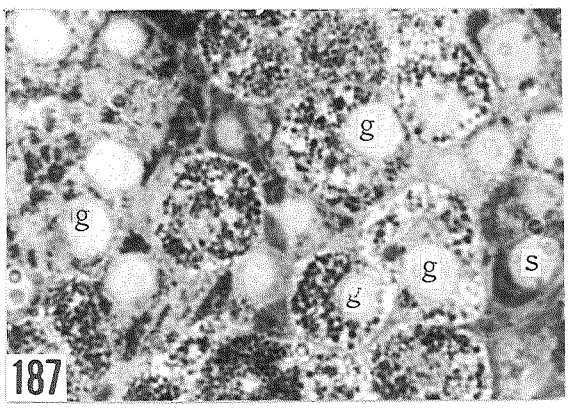
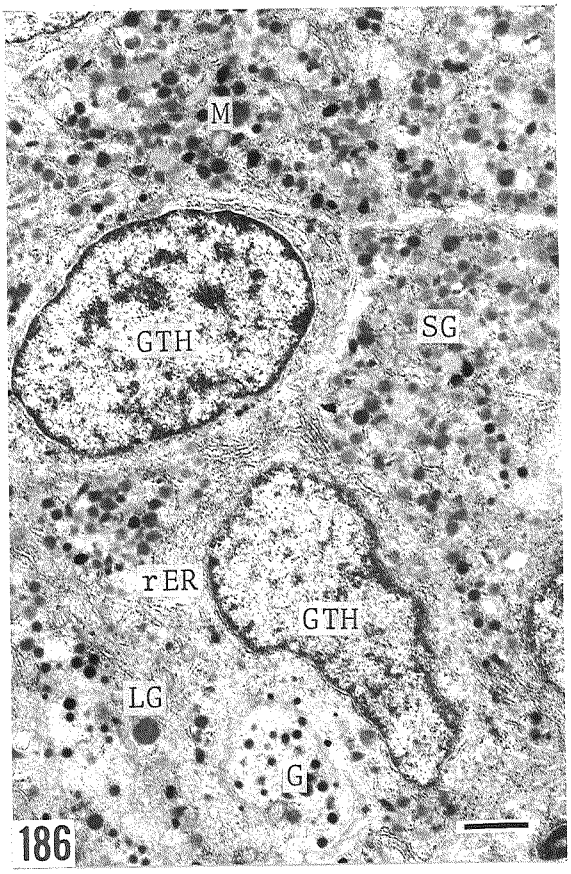

g
g
g
g
g
183
GTH
LG
SG
V
M
G
LG
SG
GTH
GTH
184

Plate 65

Figs. 185-188. Gonadotrophs (g and GTH) of female Japanese eel, Anguilla japonica, of initial control group (Figs. 185 and 186), and those matured artificially to GSI of 10% (Fig. 186) and 20% (Fig. 187). Figs. 185 and 187: methylene blue stain of Epon-embedded, semithin sections, X1000.


g
g
g
g
g
g
s
185


g
g
g
g
g
s
187


M
GTH
SG
rER
GTH
LG
G
186

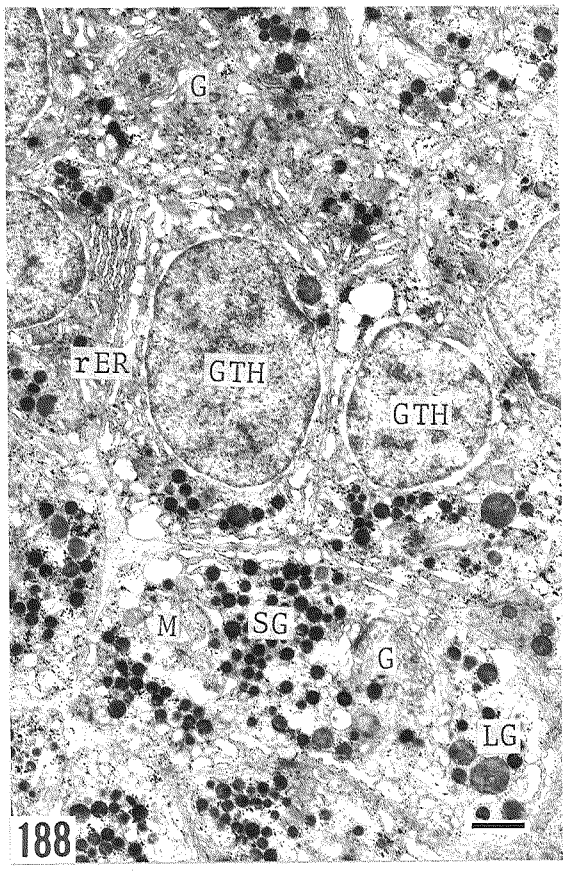

G
rER
GTH
GTH
M
SG
G
LG
188

Plate 66

Fig. 189. Gonadotrophs (GTH) of a female Japanese eel matured artificially to GSI of 30%.

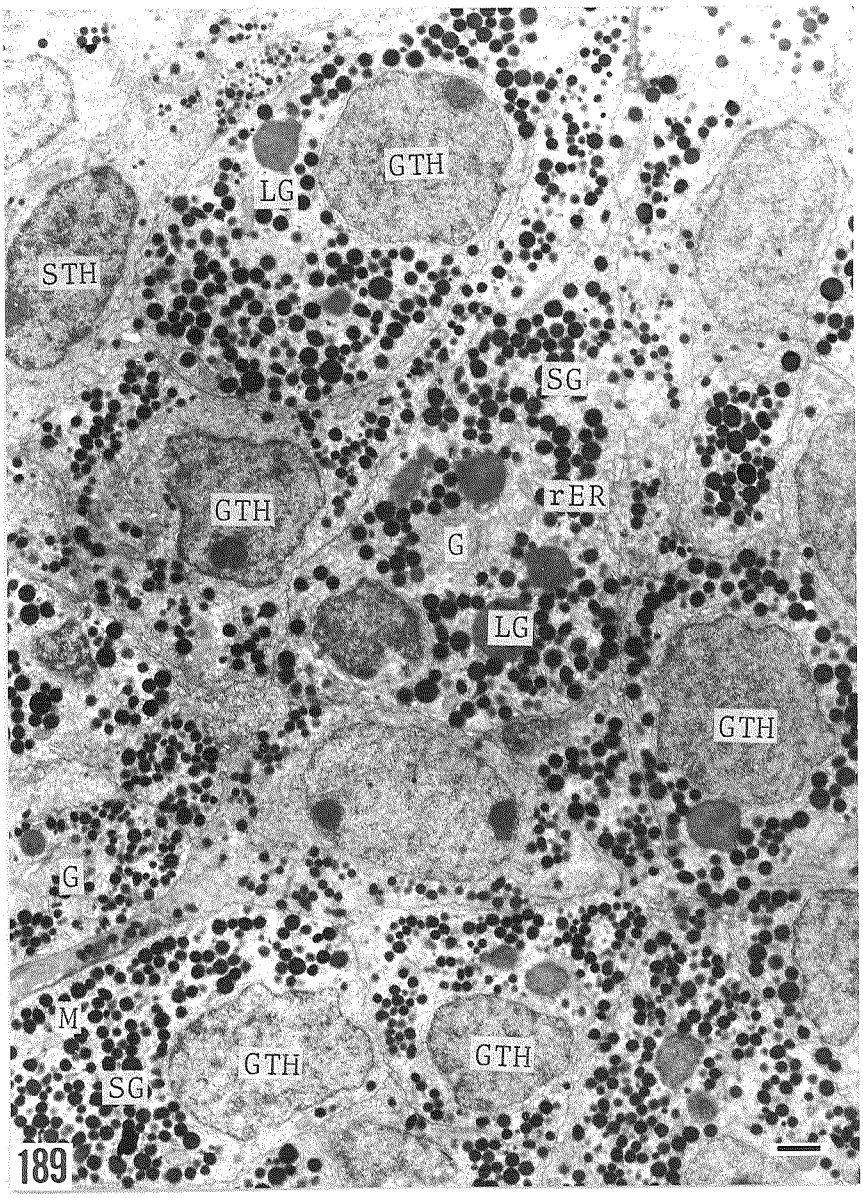
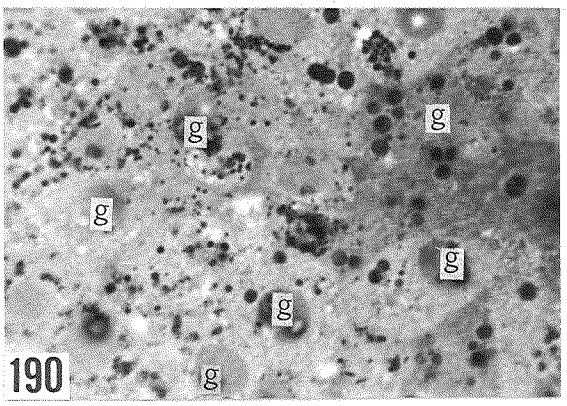
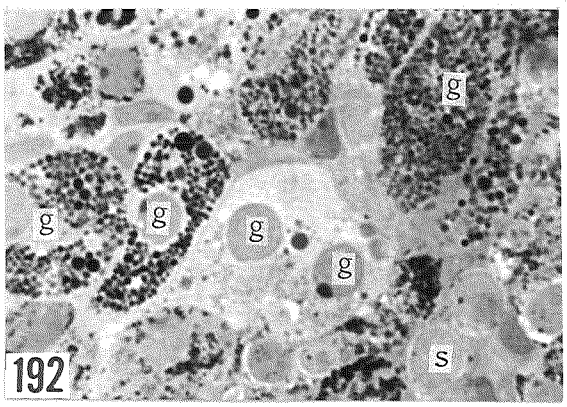
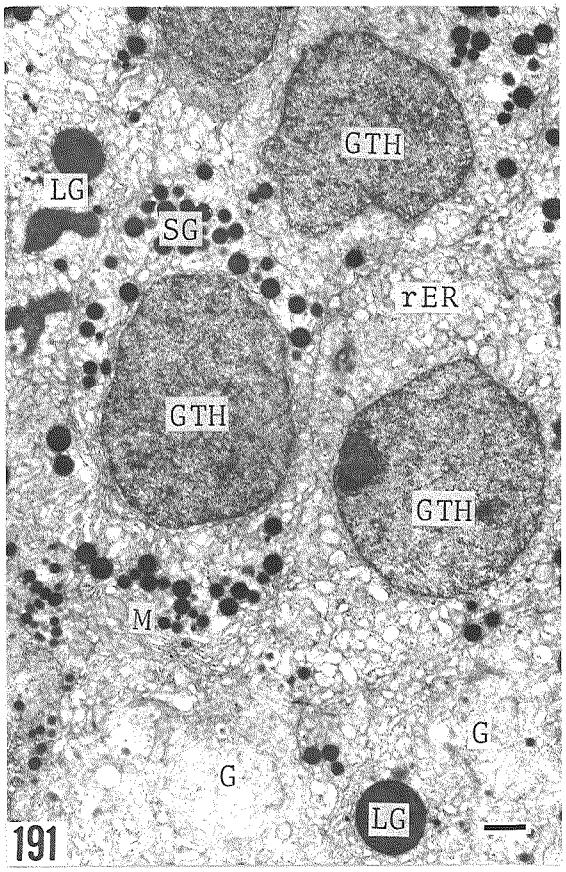

GTH
LG
STH
SG
GTH
rER
G
LG
GTH
G
M
GTH
GTH
SG
189

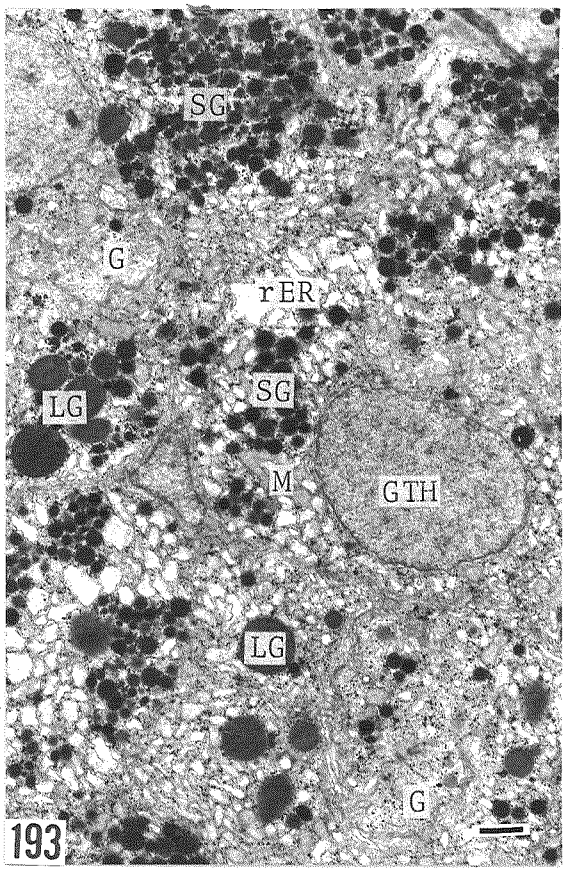
Plate 67

Figs. 190-193. Gonadotrophs (g and GTH) of female Japanese eels ovulated spontaneously (Figs. 190 and 191) and those matured artificially to GSI of 60% (Figs. 192 and 193). Figs. 190 and 192: methylene blue stain of Epon-embedded, semithin sections, X1000.


g
g
g
g
g
g
g
190


g
g
g
g
g
s
192


GTH
LG
SG
rER
GTH
GTH
M
G
G
LG
191


SG
G
rER
LG
SG
M
GTH
LG
G
193