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**Effect of Continuous Light Exposure on Pituitary Gonadotrophs
of the Loach, *Misgurnus anguillicaudatus****

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Abstract

Adult and young loaches, *Misgurnus anguillicaudatus*, of both sexes were exposed to continuous light for periods of 30 and 60 days during the postspawning period. The treatment caused a significant promotion of vitellogenesis and spermatogenesis in affected ovaries and testes of adult fish. In these fish, a remarkable activation of pituitary gonadotrophs of the vesicular cell type was observed electron microscopically. The vesicular cells showed a notable increase both in number and in size and, characteristically, a progressive expansion of their cytoplasmic vesicles. Pituitary gonadotrophs of the globular cell type were also observed to be activated under the influence of constant illumination, but the response was rather slight when compared with that of the vesicular cells even after 60 days of the treatment. In addition, exposure of young females to continuous light did efficiently induce a precocious differentiation of the vesicular cells in their pituitary glands, in parallel with a precocious occurrence of yolk accumulation in ovarian oocytes. The results indicate that the vesicular gonadotropic cells may be implicated primarily in the mechanism of vitellogenesis in females and spermatogenesis in males of the loach.

For the purpose of identifying a gonadotropic cell type(s) of the pituitary gland in teleost fishes, various experimental attempts have hitherto been carried out to cause functional alterations of pituitary cells¹⁾²⁾³⁾. In our previous ultrastructural studies on the pituitary gland of the loach, *Misgurnus anguillicaudatus*, we confirmed, by some experimental means, the presence of two distinct types of gonadotrophs, termed the globular and the vesicular cell, along with thyrotroph and somatotroph in the proximal pars distalis of pituitaries of the fish⁴⁾⁵⁾⁶⁾. Moreover, these studies suggested that each of the two types of gonadotrophs might take different parts in different stages of development and maturation of the gonad of the loach. However, the exact roles played by the two cell types have not been settled yet.

Simon and Reinboth⁷⁾ distinguished two distinct types of gonadotrophs light microscopically in the pituitary gland of *Lepomis macrochirus*. Treatment of the fish with a long photoperiod during their sexually quiescent period caused a stimulated development of the gonad accompanied with a marked hypertrophy of one of the two gonadotropic cell types⁷⁾. This led us to examine whether a continuous exposure of immature or sexually regressed loaches to a long photoperiod could promote

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gonadal development through an increased activity of either of the two types of pituitary gonadotrophs. It was expected that the resulting changes of the cells might give an important clue for discriminating functional differences between the two types of gonadotrophs of the loach. The present study was thus conducted to observe ultrastructural changes of pituitary gonadotrophs of loaches of both sexes subjected to continuous light exposure during their postspawning period.

Material and Methods

The loach, *Misgurnus anguillicaudatus*, used in the present study were collected in the suburbs of Hakodate in late August 1979, when they were at the end of the spawning period. Thirty-two adult loaches, 8.5–12.5 cm in body length and 4.0–13.2 g in body weight, and 30 young fish, 6.5–8.5 cm in body length and 1.8–3.5 g in body weight, of both sexes were selected for the present study. Beginning from 6 days before the start of experiment, they were acclimated to 0.6% NaCl solution in laboratory aquaria with continuous aeration and filtration. The fish could survive laboratory conditions of the present study better in the saline solution than in ordinary freshwater. They were fed daily on commercial fish food throughout the course of the present study.

Continuous light exposure of the fish was begun on September 1 and ended on October 31, 1979, lasting for 60 days. At the start of experiment, 9 adult and 6 young fish of both sexes were killed and they served as initial controls. The remaining 23 adult and 24 young loaches were equally divided into an experimental and a control group, each comprising the same number of males and females except for control adults with 5 males and 6 females. Fish of the experimental group were exposed to constant illumination by two 20-W fluorescent lamps in a room where room temperature was maintained between 18 and 22°C. Fish of the control group were kept under natural light and temperature which ranged from 15 to 20°C during the period of experiment. They were killed 30 and 60 days after the start of experiment, and changes of their gonads and pituitary glands were examined.

Pituitary glands were excised out immediately after decapitation. They were fixed with glutaraldehyde-paraformaldehyde mixture in 0.2 M cacodylate buffer (pH 7.4) for about 3 hours at room temperature, followed by postfixation in 1% osmium tetroxide in the same buffer for about 2 hours at 4°C, and embedded in Epon. Ultrathin sections stained double with uranyl acetate and lead citrate were observed with a Hitachi HU-12 electron microscope. Semithin sections of the Epon-embedded specimens stained with methylene blue were also observed for light microscopic comparison. Gonads of the control and experimental fish were fixed in Bouin's fluid and stained with Delafield's hematoxylin and eosin for histological inspection.

Results

Changes in the gonadosomatic index (GSI: gonad weight \times 100/body weight) of fish of the initial control, control and experimental groups were indicated in Fig. 1. In the initial control group, adult females had ovaries containing many

postovulatory follicles and atretic, yolk-laden oocytes, and young females had ovaries in which the most advanced oocytes were at the peri-nucleolus stage; adult and young males had testes with germ cell cysts in various spermatogenic stages including a small amount of mature spermatozoa in lobule lumina. No notable difference was found in GSI values between fish of the initial control group and those of the control group after 30 and 60 days of experiment. Even in the control group after 60 days of experiment, ovaries of adult females showed

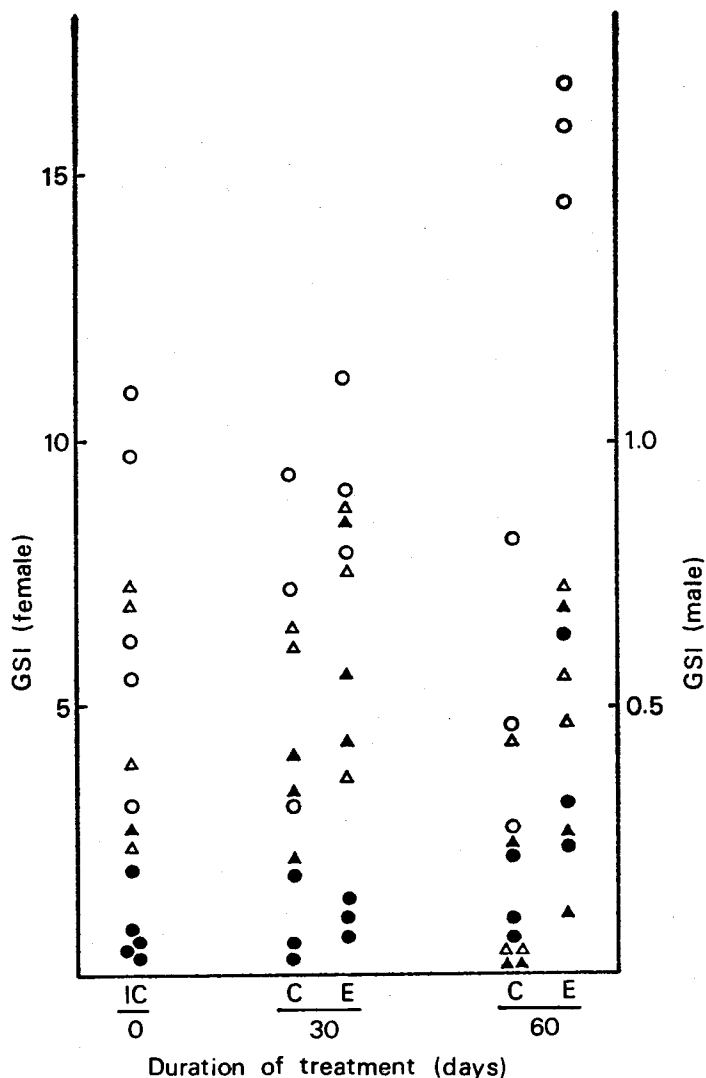


Fig. 1. Changes of gonadosomatic index (GSI) of adult female (O), adult male (Δ), young female (●) and young male (▲) loaches exposed to continuous light for 30 and 60 days during postspawning period. IC, initial control group; C, control group; E, experimental group.

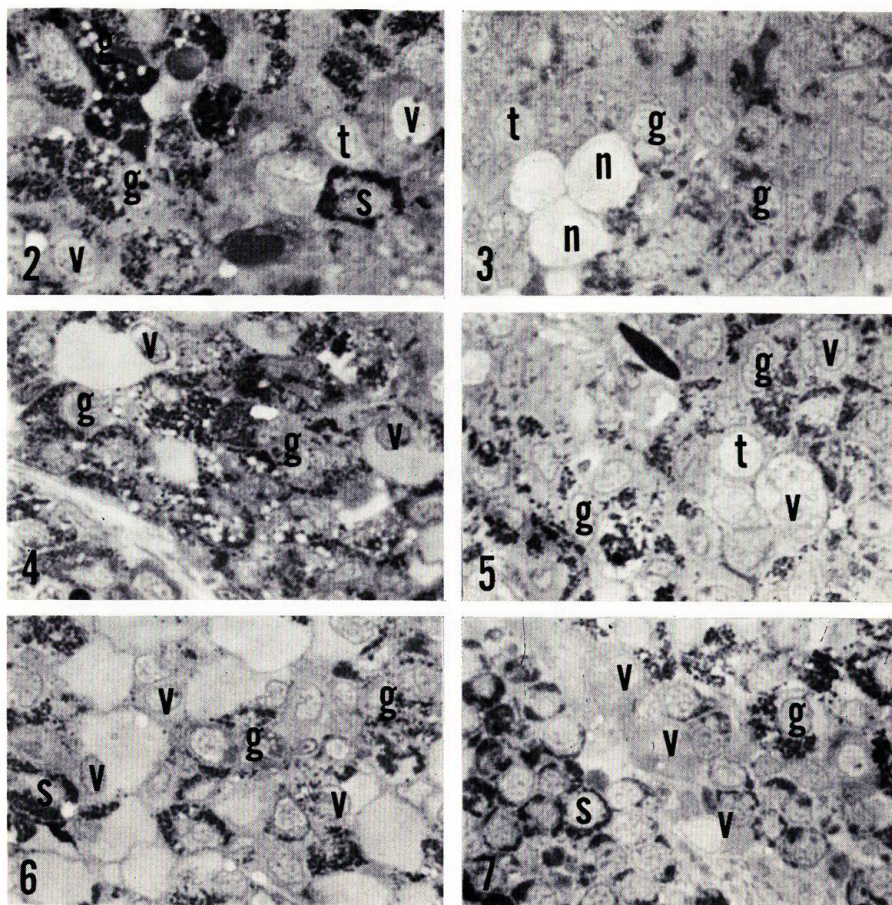
only a slight development with a small number of oocytes advancing to the primary yolk stage, those of young females still had oocytes at the peri-nucleolus stage, and testes of adult and young males were histologically the same as those of fish of the initial control group.

Exposure of loaches 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 fish (Fig. 1). Histologically, ovaries of treated adult females were filled with many oocytes developing to the secondary and tertiary yolk stages after 30 and 60 days of light exposure, respectively. In ovaries of treated young females, oocytes of the yolk vesicle stage clearly increased in number, and sometimes oocytes of the primary yolk stage were observed to appear after 60 days of the treatment. Testes of adult and young fish of the experimental group revealed a conspicuous acceleration of spermatogenesis and an increase in amount of mature spermatozoa, though the changes were more remarkable after 30 days than after 60 days of treatment.

In the loach of both sexes, there are two types of gonadotropic cells in the proximal pars distalis (PPD) of the pituitary gland⁽⁴⁾⁵⁾⁶⁾. Of the two types of gonadotrophs, cells of one type are characterized ultrastructurally by having many large globular inclusions together with small secretory granules in their cytoplasm. They are designated as the globular cells. Cells of the other type are marked ultrastructurally by a constant occurrence of numerous rounded vesicles in the cytoplasm. The cells are termed the vesicular cells.

In the PPD of adult females and males of the control group after 60 days of experiment, the globular cells were seen to be inactive cytologically (Fig. 2). Some of the cells displayed degenerative aspects with pyknotic nuclei and darkly concentrated 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. 8). By contrast, in the vesicular cells of these fish, characteristic cytoplasmic vesicles with contents of moderate electron density showed a dilation to varying degrees. The cells contained a few large globules and relatively many small granules together with moderately active Golgi apparatus in the cytoplasm (Fig. 8).

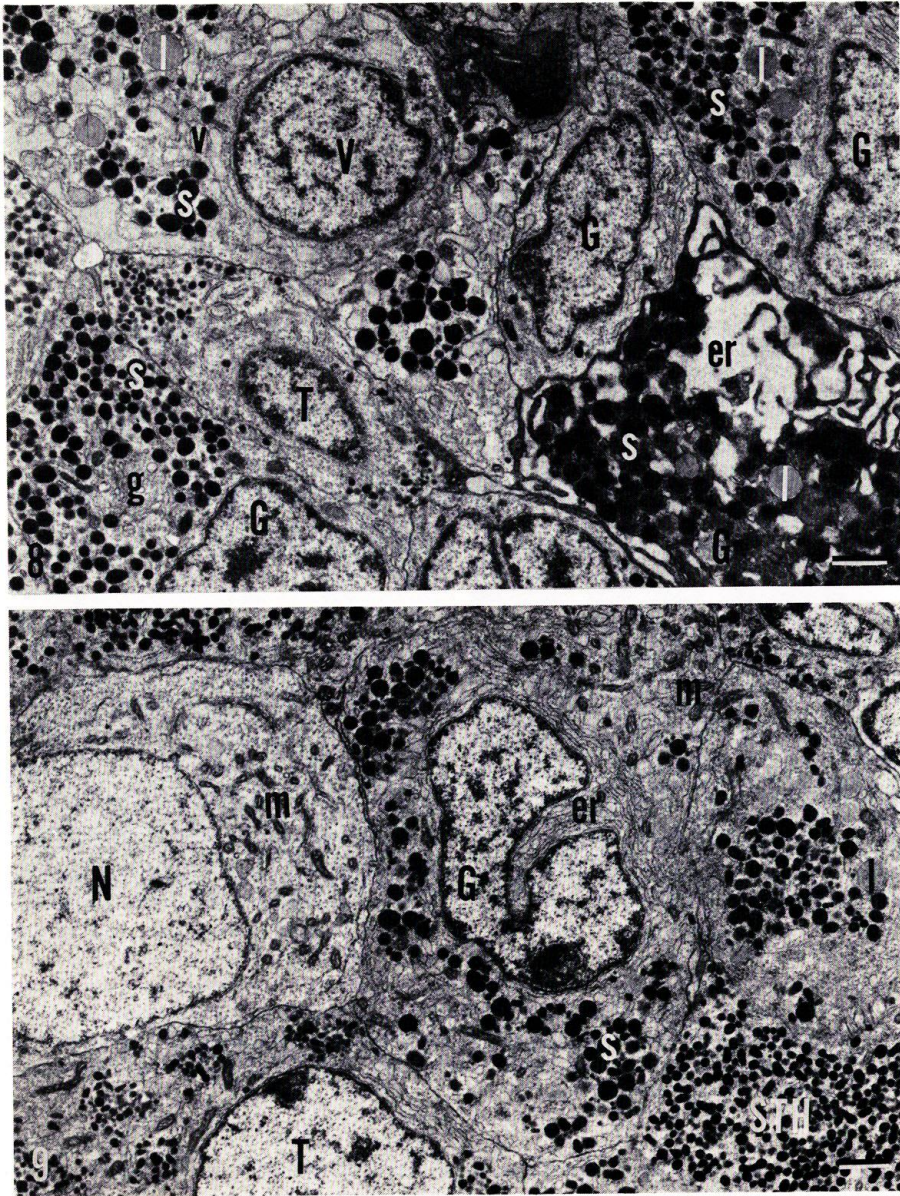
In the PPD of young females of the control group after 60 days of experiment, a few globular cells were present together with many somatotrophs, thyrotrophs and non-granulated cells, but the vesicular cells were never detected to exist (Fig. 3). Large globules and small granules of the globular cells were observed to be fewer in number than those in the cells of adult females of the control group. The rough endoplasmic reticulum of the cells was composed of flat or small vesicular cisternae, and the Golgi apparatus was moderately active (Fig. 9). In the non-granulated cells, cytoplasmic organelles were scarcely developed except for many electron dense mitochondria (Fig. 9). In the PPD of young males of the control group after 30 and 60 days of experiment, both the globular and the vesicular cells had already existed. The globular cells appeared to be the same in aspects as those of young females described above. The vesicular cells sometimes had large cytoplasmic vesicles. Small granules were present, but large globules were



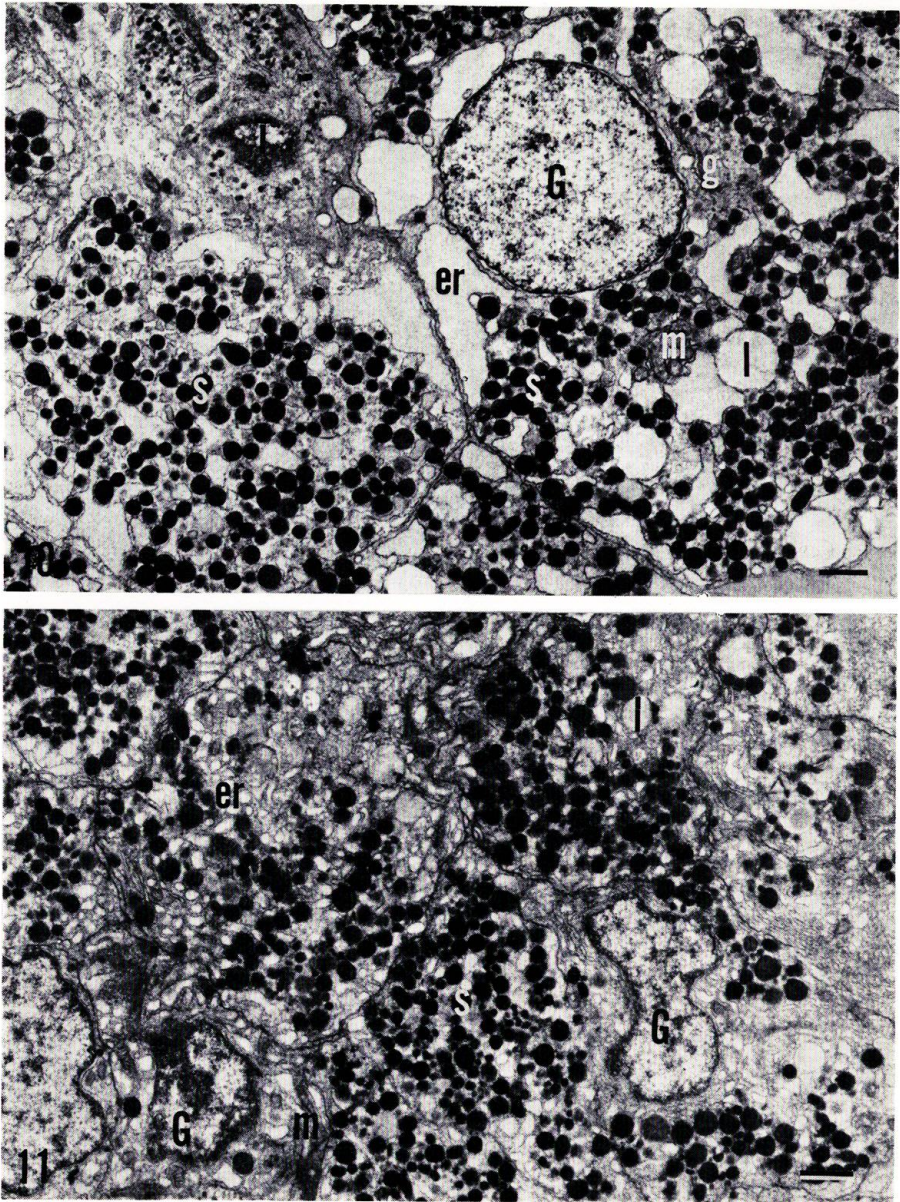
Figs. 2-7. Light microscopic pictures of Epon-embedded, methylene blue-stained sections of pituitary glands of control (Figs. 2 and 3) and light-exposed (Figs. 4-7) loaches. $\times 1000$. 2, control adult, 60 days of experiment; 3, control young, 60 days of experiment; 4, experimental adult, 30 days of experiment; 5, experimental young, 30 days of experiment; 6, experimental adult, 60 days of experiment; 7, experimental young, 60 days of experiment. *g*, globular cell; *n*, non-granulated cell; *s*, somatotroph; *t*, thyrotroph; *v*, vesicular cell.

not observed, and the Golgi apparatus was scarcely detected in the cytoplasm of these cells.

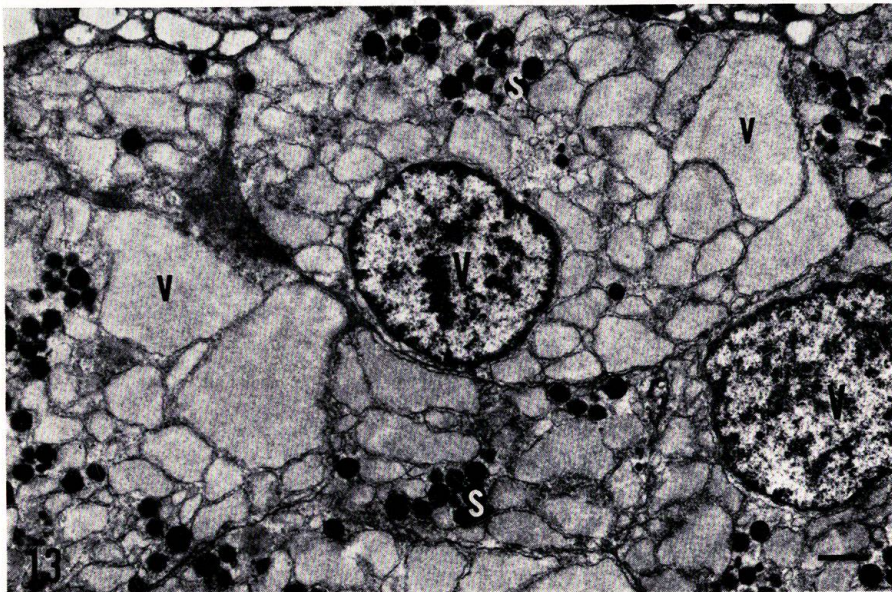
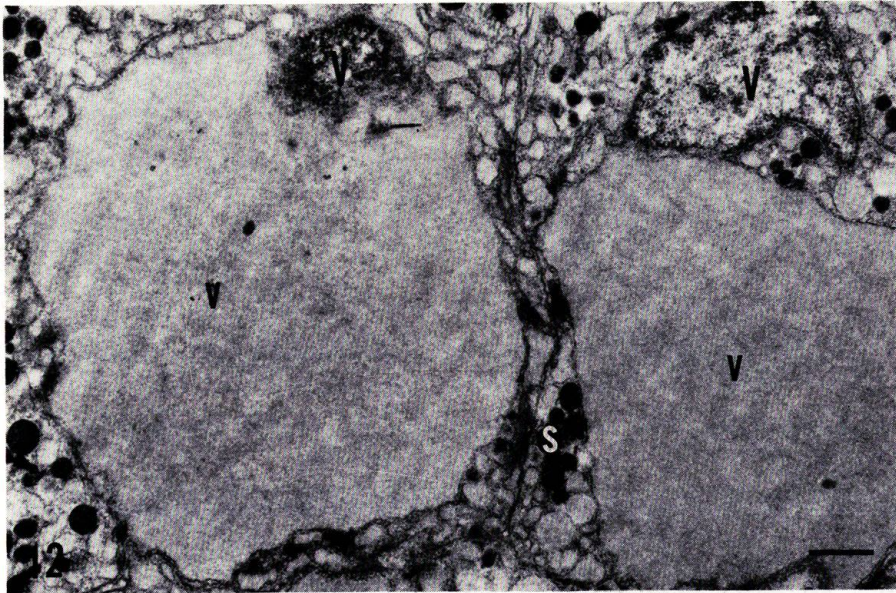
In the PPD of adult loaches of both sexes exposed to continuous light, the globular cells progressively increased in size and in number (Figs. 4 and 6). Their granular inclusions also increased in number, and large globules became filled with finely granulated material of decreased electron density (Fig. 10). After 60 days of treatment, cisternae of the rough endoplasmic reticulum of the globular cells were dilated to various extents and the Golgi apparatus appeared to develop their activity (Fig. 10). The vesicular cells also showed a notable increase both in



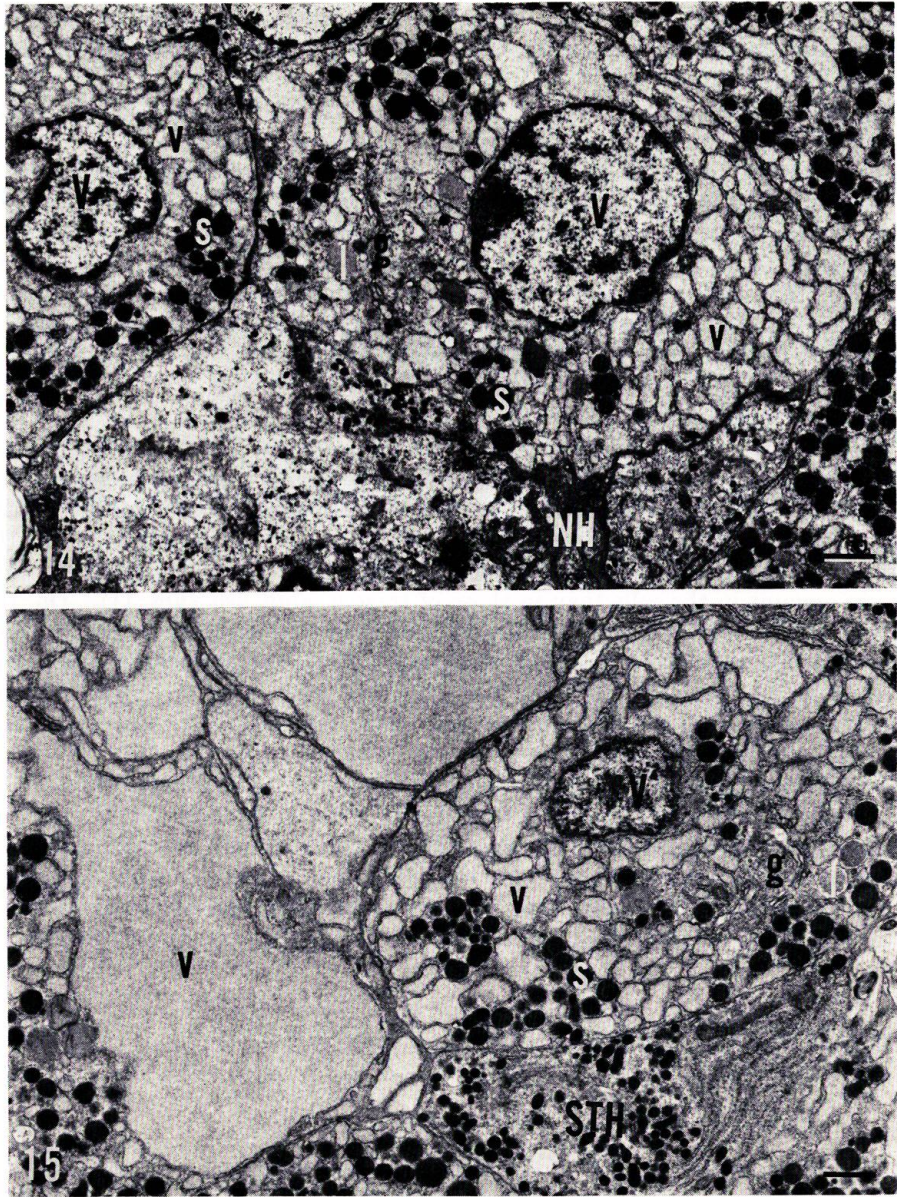
Figs. 8 and 9. Electron micrographs of globular cells (*G*) and a vesicular cell (*V*) in pituitaries of an adult female (Fig. 8) and a young female (Fig. 9) locah of the control group after 60 days of experiment. *er*, rough endoplasmic reticulum; *g*, Golgi apparatus; *l*, large globule; *m*, mitochondrion; *N*, non-granulated cell; *s*, small granule; *STH*, somatotroph; *T*, thyrotroph; *v*, cytoplasmic vesicle. Scale, 1 μ m.



Figs. 10 and 11. Electron micrographs of globular cells (*G*) in pituitaries of an adult female (Fig. 10) and a young female (Fig. 11) loach exposed to continuous light for 60 days. *er*, rough endoplasmic reticulum; *g*, Golgi apparatus; *l*, large globule; *m*, mitochondrion; *s*, small granule. Scale, 1 μ m.



Figs. 12 and 13. Electron micrographs of vesicular cells (V) in pituitaries of an adult male (Fig. 12) and an adult female (Fig. 13) loach exposed to continuous light for 30 and 60 days, respectively. s, small granule; v, cytoplasmic vesicle. Scale, 1 μ m.



Figs. 14 and 15. Electron micrographs of vesicular cells (*V*) in pituitaries of a young female (Fig. 14) and a young male (Fig. 15) loach exposed to continuous light for 30 and 60 days, respectively. *g*, Golgi apparatus; *l*, large globule; *NH*, neurohypophysis; *s*, small granule; *STH*, somatotroph; *v*, cytoplasmic vesicle. Scale, 1 μ m.

number and in size in treated males and females, and their hypertrophic changes were more prominent than those seen in the globular cells of the same specimens (Figs. 4 and 6).

Moreover, there were some differences in ultrastructural changes of the vesicular cells between females and males of the experimental group. In the cells of females examined after 30 days of light exposure, cytoplasmic vesicles were relatively uniform in size while small granules clearly increased in number, and large globules were scarcely detected. In the cells of males, however, some cytoplasmic vesicles were extraordinarily large in size and occupied most of the cytoplasm (Fig. 12). After 60 days of treatment, cytoplasmic vesicles of the vesicular cells in females evidently expanded, and small granules greatly decreased in number (Fig. 13). In the vesicular cells of males, on the contrary, the size of the cytoplasmic vesicles decreased slightly, and large globules began to appear in the cytoplasm.

Similar but less conspicuous ultrastructural changes were observed in the globular cells of young males and females treated with continuous light. The globular cells increased in number and in size, and showed some active features (Figs. 5 and 7). Even after 60 days of treatment, however, a dilatation of the rough endoplasmic reticulum was meager in degree and granular inclusions were fewer in amount in the cells than in those of treated adult fish (Fig. 11). On the contrary, in the PPD of treated females after 30 days of treatment, a small number of the vesicular cells were observed to appear and the non-granulated cells had decreased in number (Fig. 5). The vesicular cells were rich in cytoplasm and contained many small cytoplasmic vesicles. Their granular inclusions were few in number, and the Golgi apparatus seemed to be highly active (Fig. 14). In young females killed after 60 days of treatment, the vesicular cells clearly increased in number and in size (Fig. 7). Their cytoplasmic vesicles were evidently larger in size than those examined after 30 days of treatment. Small granules and large globules in their cytoplasm remained unchanged in number, and the Golgi apparatus was scarcely found in the cells. The vesicular cells in the PPD of young males of the experimental group showed no progressive change, though their cytoplasmic vesicles were larger in size than those of the cells of treated young females, and large globules began to appear (Fig. 15). The Golgi apparatus of these cells appeared to be highly active.

Discussion

The results of the present study show that a continuous light exposure of loaches was apparently effective in accelerating the development of gonads in adult and young fish of both sexes. The treatment caused recrudescence of vitellogenesis and promotion of spermatogenesis in adult fish of the postspawning period. In addition, a precocious occurrence of vitellogenesis in ovarian oocytes was induced in young females exposed to constant light. Since the spawning period of loaches used in the present study lies in the months from June to August, maturation of their gonads probably depends on long day-length in nature. Suzuki and Yamaguchi⁸⁾ suggested, however, that the loach was more sensitive to temperature than

to light in terms of a stimulation of gonadal development. In the present study, fish of the experimental group were kept in water temperature somewhat higher than that of fish of the control group. Accordingly, the stimulated maturation of the gonad of experimental animals may be at least in part ascribed to the effect of water temperature.

Simon and Reinboth⁷⁾ could identify two different types of gonadotropic cells in the pituitary gland of *Lepomis macrochirus*, as a result of histological and histochemical studies combined with several experimental ones. They reported that, among the two gonadotropic cell types, one was activated simultaneously with a stimulated development of gonads by exposure of the fish to a long photoperiod, while the other did not react to the light treatment but displayed evident changes after gonadectomy of the fish. In the present study, too, two distinct types of pituitary gonadotrophs of the loach showed different cytological responses to the continuous light treatment. In treated adult loaches of both sexes, 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 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 in the PPD at the start of treatment, were induced to be differentiated simultaneously with a precocious occurrence of exogenous vitellogenesis in ovarian oocytes.

The globular cells of the loach are thought to be the conventional gonadotropic cells. The cells displayed remarkable changes in response to artificially induced ovulation, gonadectomy, and administration of sex steroids⁴⁾⁶⁾. The ultrastructural changes of the globular cells are quite similar to those described for pituitary gonadotrophs of various other teleosts subjected to similar experiments⁹⁾¹⁰⁾¹¹⁾¹²⁾¹³⁾¹⁴⁾¹⁵⁾¹⁶⁾. The globular cells exhibit noticeable changes at the time of natural spawning (Ueda, in press). These findings indicate that the globular cells may secrete a gonadotropin which has its dominant roles in later stages of gonadal maturation, especially at the time of ovulation and spermiation.

Although the vesicular cells of the loach also showed some changes under the conditions of the above-mentioned experiments, the responses were always rather slight as compared with those occurring in the globular cells⁴⁾⁶⁾. In the present study, by contrast, the vesicular cells of the fish exposed to continuous light were induced to make a prominent change in their fine structure coinciding with the acceleration of vitellogenesis and spermatogenesis, and the change was more conspicuous and appeared more promptly than that seen in the globular cells. Although the vesicular cells were characterized by the lack of remarkable cytological changes throughout the reproductive cycle, the peak of their activity was observed to be accompanied with the initiation of vitellogenesis and spermatogenesis (Ueda, in press). Thus a possible explanation of the gonadotropic function of the vesicular cells in the loach may be that the secretion of the vesicular cells is implicated primarily in the initiation and successive maintenance of gametogenesis. Similar suggestions have been given by histological and cytological observations on pituitary gonadotropic cells of the vesicular cell type in salmonid fishes¹⁷⁾¹⁸⁾¹⁹⁾.

On the other hand, Peute *et al.*²⁰⁾ reported that, in rainbow trout, *Salmo gairdneri*, a predominant occurrence of pituitary gonadotrophs of the cisternal (vesicular) stage was 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 cells of the globular stage toward the spawning season. By contrast, in the loach examined in the previous studies and in the present study, no intermediate cell types between the two types of gonadotrophs were detectable in the pituitary gland. Such are also the cases for two types of gonadotrophs in other teleost fishes such as roach, *Rutilus rutilus*²¹⁾²²⁾, masu salmon, *Oncorhynchus masou*²³⁾, and threespined stickleback, *Gasterosteus aculeatus*²⁴⁾.

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