THE PREOPTICO-HYPOPHYSIAL NEUROSECRETORY SYSTEM OF THE MEDAKA, *ORYZIAS LATIPES*, AND ITS CHANGES IN RELATION TO THE ANNUAL REPRODUCTIVE CYCLE UNDER NATURAL CONDITIONS

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It has often been mentioned that, in many fishes, hypophysial gonadotropic functions are influenced by environmental factors such as light and temperature which may act on the hypophysis indirectly through the central nervous system (cf. Pickford and Atz, 1957; Jørgensen and Larsen, 1967; Jørgensen, 1968). In lower vertebrates, two pairs of hypothalamic neurosecretory centers, the preoptic and the lateral tuberal nuclei, are presumed to be included in the system of the central nervous control, though the latter nucleus has so far been regarded as a predominant site of control of the hypophysial-gonadal activities in some teleost fishes (Billenstien, 1962; Stahl and Leray, 1962; Öztan, 1963; Honma and Tamura, 1965; Honma and Suzuki, 1968; Peter, 1970). Much more data about the significance of the diencephalic neurosecretion in controlling the hypophysial gonadotropic functions in fishes, however, still remain to be gathered.

The reproductive activities of the medaka, *Oryzias latipes*, are known to be influenced quite sensitively by environmental light conditions (Robinson and Rugh, 1943; Egami, 1954; Yoshioka, 1962, 1963). In the fish, the hypophysial-gonadal relationships have already been established experimentally by Egami and Ishii (1962). Moreover, the previous study of the present writers (Kasuga and Takahashi, 1970) has shown the direct innervation of neurosecretory axons in the meso-adenohypophysis and has suggested a possible neurosecretory control of gonadotropic function in the medaka. In that context, the present writers had an interest in searching the fluctuation of the diencephalic neurosecretion of the medaka in response to seasonal photoperiodic changes. The present paper deals with the seasonal changes of cytological features of the hypothalamic preoptic nucleus and of the meso-adenohypophysial gonadotrophs in relation to the seasonal changes of the gonad of the medaka raised in natural environmental conditions.

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Material and methods

The medaka, *Oryzias latipes*, of the wild type, which had been collected from a pond in Yunokawa in the suburbs of Hakodate, were reared in an outdoor pond in the campus of the Faculty of Fisheries, Hokkaido University, under natural conditions of temperature and light. They were fed on living water fleas and mosquito larvae in summer, and on powdered commercial fish food in other seasons.

Five female fish of 2.6–3.8 cm in body length were sampled from the stock pond for each of the observations done monthly or bimonthly during the period from October 1968 to October 1969 (Table 1). After the measurement of the body length and body weight, the fish were sacrificed by quick decapitation, and their heads were fixed *in toto* with Heidenhain's Susa. Serial paraffin sections of 6 μ in thickness were cut frontally or sagittally, and were stained with Gomori-Halmi's aldehyde fuchsin (AF; Halmi, 1952), Gomori's chrome alum Hematoxylin and phloxine (CH-P), Mallory's Azan, or periodic acid-Schiff reagent (PAS). The ovaries of the fish were weighed and preserved in Heidenhain's Susa. Besides these, a total of 14 females were sacrificed at various times of the year, and the hypothalamo-hypophysial system was examined in view of its ultrastructural characteristics by the method described in the previous report (Kasuga and Takahashi, 1970).

Observations

In the preoptic nucleus (PON) of the hypothalamus of the medaka, like that of other teleost fishes, the neurosecretory cells were distributed and roughly grouped into two parts, viz., pars parvocellularis (PPC) and pars magnocellularis (PMC). The PPC was composed of smaller cells of 4.3–12.7 μ in size and lay on either side of the preoptic recess of the third ventricle, whereas the PMC consisted of larger cells of 6.2–24.0 μ in size and extended dorsally from the top of the PPC to the area near the subcommissural organ. The neurosecretory cell bodies in the PPC (Fig. 8) and PMC (Figs. 3, 4, 5, 6b and 7a) were often seen bordering on the third ventricle or on blood capillaries. They ranged in total number from 150 to 270, averaging about 200, of which approximately 70 cells belonged to the PMC. The neurosecretory cell bodies were generally stainable with AF, but their affinity to the dye was variable in degree in different seasons of the year. In addition, they were generally furnished with large AF-positive globules of about 3 μ in the largest size (Figs. 3 and 4). These globules existed scatteringly among the AF-positive materials in the cytoplasm, so that they were imperceptible in some of the cells or were prominently noticeable in others according to the amount of AF-positive materials present in the respective cells.

The dendrites or axons of the neurosecretory neurons were stained plainly with AF. The neurosecretory nerve tracts extending from PON appeared to follow three different routes in the hypothalamus (Fig. 1). The first, most prominent tract originated principally in the PPC and ran caudally through the latero-ventral region under the commissura horizontalis to terminate in the neurohypophysis (NH). The second tract started from the PMC, and ran caudally curving to the ventral direction to pass through the region between the commissura horizontalis of both sides, and terminated in the NH. The third tract, which was often obscure in histological sections, arose in the PMC and ran toward the caudal direction, but its termination is not resolved by the present study.

The definite neurosecretory cells of the lateral tuberal nucleus of the hypothalamus were not identified in the medaka used in the present study. In the fish sampled in March and April, however, several cells with large round nuclei and thin cytoplasm were dispersedly encountered in the infundibular region (Fig. 2). A preoptico-hypophysial tract was seen to run near these large cells. Since these cells were not stainable with the histological dyes employed, it was impossible to determine whether or not the cells were truly neurosecretory in function.

The AF-positive materials were seen as granules or strands permeating among meta- and meso-adenohypophysial cells including the gonadotrophs distributed in the periphery of the meso-adenohypophysis. The materials were, however, most abundantly accumulated in the posterior half of the NH and they formed large droplets designated as Herring bodies especially in the region near the infundibulum. The Herring bodies were variable in size but tended to grow larger as the accumulation of neurosecretory material in the NH became more advanced. Sometimes the AF-positive material in the Herring body was assembled along the periphery, leaving a clear circular spot in the center of the body (Fig. 21, inset). Such a type of the Herring body made its frequent appearance especially at the time when the neurosecretory material was fairly abundant in the NH.

In order to check the annual changes in the PON quantitatively, the size of 50 and 30 neurosecretory cells of the PPC and PMC, respectively, were measured in 3 specimens with an ocular micrometer at each observation. Furthermore, the size of the largest Herring bodies existing in the NH was also measured to represent the degree of accumulation of the AF-positive material in that region of the pituitary gland. The degree of gonadal maturation of each fish was determined in terms of the gonadosomatic index (GSI; gonad weight$\times$100/body weight).

The histological features of the hypothalamo-hypophysial system of the medaka changed dynamically in harmony with the changes of reproductive activities. In the fish examined during the spawning period extending from May to late August or early September, the meso-adenohypophysial gonadotropic (GTH) cells were stainable with AF (Fig.15). The preoptic neurosecretory cells were

Table 1. Gonadosomatic index (GSI), the size of the neurosecretory cells of the preoptic nucleus and the size of the largest Herring bodies in the neurohypophysis of the medaka, *Oryzias latipes*, examined at various times during a year. The number of animals in each sampling was 5. In the time marked with an asterisk, stock pond was covered with ice.

Date of samplings	Water temp. (°C)	GSI Mean (Range)	Neurosecretory cells Mean (Range) (μ)		Largest Herring body Mean(Range) (μ)
			Pars magnocellularis	Pars parvocellularis	
'68 Sep. 20	20.0	2.49(1.83- 3.13)	12.7(9.6-16.8)	7.7(6.5- 9.7)	15(12-18)
Oct. 11	8.5	1.82(1.31- 3.05)	10.6(7.2-19.2)	6.1(4.3- 9.6)	17(14-19)
Dec. 6	*	1.80(1.57- 2.25)	11.8(10.0-14.9)	7.6(6.7- 9.6)	6(5- 7)
'69 Feb. 10	*	2.40(1.47- 5.42)	11.2(8.4-14.4)	7.4(6.0- 9.6)	7(5-10)
Mar. 12	*	3.05(2.04- 4.05)	14.6(10.8-21.6)	8.6(6.0-12.0)	4(3- 4)
Apr. 14	12.4	2.58(1.94- 3.14)	15.7(9.6-24.0)	9.1(6.0-12.7)	10(6-16)
May 22	13.0	8.80(6.05-11.00)	13.5(9.1-18.0)	8.2(6.0-10.8)	7(7- 8)
Jul. 16	21.5	11.25(8.17-15.50)	12.8(8.4-14.4)	8.1(6.0-12.0)	6(5- 7)
Sep. 9	22.5	2.62(2.32- 3.02)	11.2(7.7-14.4)	7.5(5.3- 9.0)	17(14-24)
Oct. 7	11.6	2.79(2.26- 3.16)	12.3(9.1-17.5)	8.0(5.3-10.8)	19(15-22)

moderate in size, as indicated in Table 1, and were provided with numerous AF-positive globules in the cytoplasm (Fig. 3). The Herring bodies in the NH were at most 6 to 7 μ in diameter in the largest ones (Fig. 9).

In the post-spawning refractory period of September and October (cf. Yoshioka, 1966), the meso-adenohypophysis still retained the GTH-cells which were clearly stained with AF (Figs. 16 and 17). In this period, remarkable changes were noticed in the hypothalamus and in the NH. The nuerosecretory cells in both the PPC and PMC were of the smallest size as compared with those in other periods of the reproductive cycle. In 1968 that fact occurred in October while in 1969 it was in September, and there was a tendency to show gradual increase in size in October. In addition, some of the cells showed localized accumulation of neurosecretory material around the nucleus, the peripheral zone of the cells remaining unstainable with AF (Fig. 4). The AF-positive globules in the neurosecretory cells showed no appreciable change in their features as compared with those seen in the spawning period. In the NH, the Herring bodies became the largest in size, reaching to about 20 μ in diameter, and the AF-positive granules packed the NH more abundantly than before (Figs. 10 and 11).

The gonadosomatic indices (GSI) of the female medaka remained small throughout the resting period from November to February. In the resting period, the GTH-cells in the meso-adenohypophysis were decreased in size and lost their affinity to AF (Fig. 18). On the contrary, the PON cells recovered in size by Decemb reand subsequently remained unchanged until February. The AF-positive globules in these cells were rarely detected in the cytoplasm, otherwise the cells were similar in their stainability to AF to those observed in the refractory period. The Herring bodies in the NH diminished in size and amount (Fig. 12). During the pre-maturation period covering March and April, the fish still had ovaries of small GSI value. In these fish, the GTH-cells were also still unstainable with AF (Figs. 19 and 20). The PON cells, however, increased markedly in size in March (Fig. 6a, b) and attained their largest size in April both in the PPC (6.0–12.7 μ, mean 9.1 μ; Fig. 8) and in the PMC (9.6–24.0 μ, mean 15.7 μ; Fig. 7a, b). These cells had each a large round nucleus in which two nucleoli were visible on some occasions. The staining response of cytoplasmic neurosecretory material to AF was rather weak in March, giving the cytoplasm a granular appearance, whereas it was most intense in April in comparison with that in the other periods. Large AF-positive globules were scarcely noticed in their cytoplasm. Furthermore, especially in the PMC, the cells with the above-mentioned changes were often encountered in contact with blood capillaries (Fig. 6b). The neurosecretory cells decreased in size during the successive months of the early spawning period, and cytoplasmic globules made their evident appearance as described before. In the NH, the large Herring bodies were not detectable in March (Fig. 13), but they reappeared temporarily in April (Fig. 14), with a distinct decrease in amount and size in the early spawning period.

Through the electron microscope, a huge number of neurosecretory granules were seen deposited in the neurosecretory axons of the NH, which were identified as light microscopical Herring bodies. The Herring body measured 20 μ in size in extreme cases, and sometimes the constituent secretory granules were concentrated to the periphery of the body, leaving a light zone with sparse cytoplasmic inclusions in the center (Fig. 21). The Herring body of this type may correspond to the light microscopical Herring body of a vesicular structure described before. The neurosecretory granules which packed the Herring bodies were mostly of 1,400–1,800 Å in size. In the specimens sampled in the resting period, however, some of the Herring bodies were observed to contain electron lucent vesicles of the neurosecretory granules of possible origin (Fig. 25). In addition to the ordinary inclusions such as neurotubules and mitochondria in the Herring body, peculiar cytoplasmic inclusions were noted in the body particularly in the fish sampled in October and in the resting period. These inclusions were various in features, being those like multivesicular bodies in some cases and those resembling multilamellate ones in others (Figs. 22, 23 and 25). These peculiar inclusions were frequently observed to include some neurosecretory granules and minute electron dense particles in the core. They were most abundantly seen in the specimens examined in October and then decreased in amount during the winter months.

In the pituicyte of the first type (Kasuga and Takahashi, 1970), numerous oil droplets were noticeable during the winter months from October to February (Fig. 24), but they were rather scarce in the pituicytes of the fish sampled in the other months.

Discussion

In some teleost fishes, the photoperiod is known to be a primary environmental factor which is concerned with the cyclic alterations of reproductive activities. Such is also the case of the medaka, *Oryzias latipes*. The breeding period of the medaka employed in this study usually ranges over 4 summer months from May to August. During that period the fish spawns almost daily at the time soon after the onset of day-time. Actually, the occurrence of ovulation and spawning of the fish has an intimate correlation with daily photoperiodicity, and some authors have confirmed the correlation by applying experimentally conducted photoperiods to the fish in the breeding period (Robinson and Rugh, 1943; Egami, 1954; Yoshioka, 1963). In view of the known pituitary control over the gonadal function in fishes (Pickford and Atz, 1957), it is natural to consider that the photic stimuli may probably be mediated by the hypothalamic neurosecretory system to effectuate their influence on the pituitary function.

The results of the present observation revealed an interesting fluctuation in the neurosecretion of the hypothalamic preoptic nucleus harmonized with the annual changes of the meso-adenohypophysial GTH-cell and the gonad in the medaka under natural conditions. During the spawning period, the accumulation of AF-positive material was marked neither in PON cells nor in the NH, implying an active discharge of the material needed for maintaining the daily reproductive activities. Similar observations were made in *Fundulus heteroclitus* by Sokol (1961). In a subsequent sexually quiescent period of September and October with shortened day-length, the PON cells decreased in size with a meager amount of AF-positive material, whereas the NH accumulated a considerable amount of the material which appears as large Herring bodies. During the successive 4 winter months in which the gonad remained small in size, the PON cells showed few changes but a slight tendency to increase their size, while the AF-positive material in the NH was clearly lessened in amount and the gonadotropic cells in the meso-adenohypophysis lost their affinity to AF. These findings may suggest that, at the initiation of this sexually quiescent period, the lowering down in degree of the synthesis of neurosecretory material in the PON and the suppression of discharge of the material from the NH occur in association with the shortening of the day-length, which may presumably have a concern with the inactivation of the pituitary gonadotropic function through this period.

According to Yoshioka (1963), the critical length of the time of illumination to attain actual spawning of the medaka lies between 12.0 to 13.0 hours of light a day. Even in January, if the fish are raised in the constant water temperature of 25°C, a treatment with 14 hours of artificial light per day is effective in inducing the spawning less than 2 weeks after the start of that treatment (Kasuga, unpublished data). Under natural conditions, however, the sexually inactive state conceivable from low GSI value and lost AF-affinity of GTH-cells was still sustained in the fish during the period of March and April when natural day-length had exceeded 13 hours. In these fish, on the other hand, PON cells showed fairly activated synthesis of neurosecretory material, whereas the NH became loaded with many large Herring bodies in April. Considering the markedly low water temperature of the outdoor stock pond during these months, one of the possible explanations of the findings is that, while the neurosecretory cells have already been activated to function by increasing day-length, the release of the substance for stimulating gonadotropic activities of the adenohypophysis is prevented as a result of the low environmental temperature.

The significance of hypothalamic neurosecretory function which mediates between the photoperiodic signal and the gonad is widely conceived in vertebrates (Wurtman, 1967). The two known neurosecretory centers of lower vertebrates, the preoptic and the lateral tuberal nuclei, seem to be included in that mechanism, though relative importance of the two centers in the regulation of reproductive activities may be different in different species of teleosts. So far as the writers know, rather many studies have been inclined to stress the role played by LTN in the control of gonadotropic activities of the pituitary gland (Billenstien, 1962; Stahl and Leray, 1962; Öztan, 1963; Honma and Tamura, 1965; Honma and Suzuki, 1968). Recently Peter (1970) described an effectiveness of electric lesion of a part of the LTN, but not of the PON, in inducing gonadal atrophy in *Carassius auratus*. In the medaka, too, it is likely that the LTN also plays a role in the control of secretion of gonadotropins from the pituitary gland. The present study failed to identify definite neurons of the LTN. Ultrastructurally, however, gonadotrophs of the meso-adenohypophysis of the medaka keep an evident connection including a synaptoid contact with Type B neurosecretory fibers (Kasuga and Takahashi, 1970) which are presumed to be originated from LTN (Knowles and Vollrath, 1965).

On the other hand, it has been ascertained that the PON displays distinct changes in its activity in concurrence with experimentally altered photoperiods in some fishes such as *Porichthys notatus* (Sathyanesan, 1965) and *Plecoglossus altivelis* (Honma and Suzuki, 1968). In particular in the medaka, *Oryzias latipes*, light seems to be one of major environmental factors of regulation of gonadal activities, since gonadal maturation and spawning are brought about in response to a long photoperiod experimentally applied in a sexually quiescent period, and *vice versa*, under the water temperature of 18–21°C (Yoshioka, 1963). Histological observations of the pituitary gland of the medaka show deep invasion of preoptic neurosecretory fibers charged with AF-stainable material among gonadotropic cells, which is supported also by electron microscopy (Kasuga and Takahashi, 1970). If the results of the present study are taken together into consideration, these facts seem to point out a hypophysiotropic action of the PON in terms of regulation of gonadal activities. At present, however, it is left uncertain whether the PON actually relates to the execution of gonadal maturation. Some authors ascribe mainly the stimulation of the spawning to the secretory products of the PON (Egami and Ishii, 1962; Honma and Suzuki, 1968). All these remain to be studied by further experiments.

Finally it is interesting to note that, especially in the medaka examined in October when artificial photic stimuli failed to stimulate gonadal maturation (Yoshioka, 1966), many multilamellate bodies were observed intermingled with secretory granules in some of the neurohypophysial Herring bodies by electron microscopy. These peculiar inclusions were detectable in the Herring bodies throughout the months of the refractory and the resting periods with a tendency to decrease in amount. The appearance of such characteristic inclusions in the neurosecretory axons might reflect the occurrence of progressive degeneration of neurosecretory granules hindered in discharging their component substance. In addition, during these months, electron lucent vesicles of secretory granules in probable origin were also repeatedly encountered in other Herring bodies which were clearly demarcated from axon endings in fine structure. Pituicytes surrounding these Herring bodies were seen to become loaded with many oil droplets in the cytoplasm. Kurosumi *et al.* (1964) suggested that, in the neural lobe of the rat pituitary, the pituicytes were capable of taking the lipid component of disintegrated membrane of depleted neurosecretory granules and of accumulating it as lipid droplets in the cytoplasm. These phenomena seem to imply a possible fate followed by some secretory granules stored in the Herring body without getting a chance to release their component substance during the sexually active period.

Summary

Seasonal cytological fluctuations of the preoptic (PON) neurosecretory cells of the hypothalamus, the neurohypophysis (NH), and the meso-adenohypophysial gonadotropic (GTH) cells were examined light and electron microscopically in the female medaka, *Oryzias latipes*, of the wild type which were raised under natural conditions of light and temperature.

In March and April (pre-maturation period), when GTH cells showed little affinity to aldehyde fuchsin (AF) and the ovaries were small in size, AF-positive

neurosecretory material was heavily accumulated in the PON cells and, especially in April, in the NH, which implied activated synthesis and inhibited discharge of the material probably resulting from increasing day-length and from low water temperature, respectively. During the subsequent months from May to August (spawning period), the material was prominently small in amount in both the PON and the NH, possibly as a result of its active release. In September and October (refractory period) the neurosecretory material was again accumulated in the NH, but not in the PON, which was suggestive of lowered synthesis and blocked release of the material occurring in association with decreasing day-length. During the months from November to February (resting period), GTH-cells became to lose their affinity to AF and the ovaries remained small in size, and concomitantly only scanty amounts of the AF-positive material was found in both the PON and the NH, which probably reflected the quiescence of neurosecretory activity.

From these findings, probable contribution of the secretion of the PON to the regulaton of hypophysial-gonadal activities in the medaka was suggested. Some comments were also given on the appearance of multilamellate bodies and electron lucent vesicles in the so-called Herring bodies in the NH during the refractory and the resting period.

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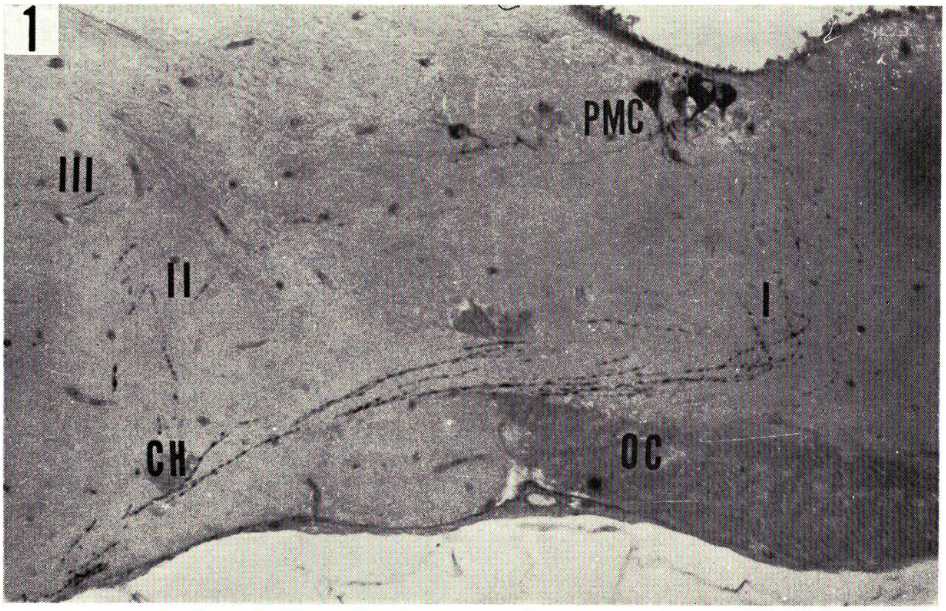
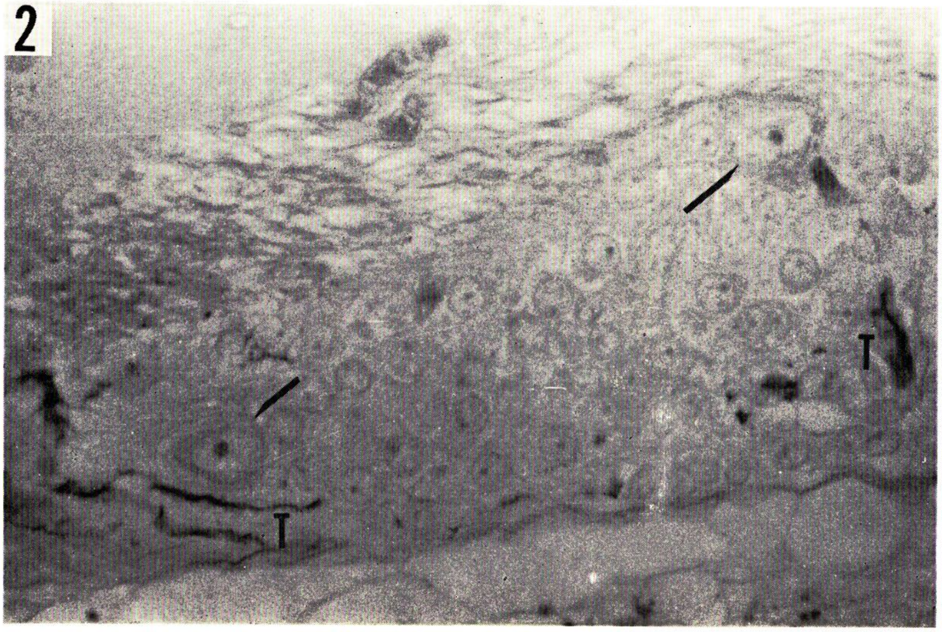
Explanation of Plates

PLATE I

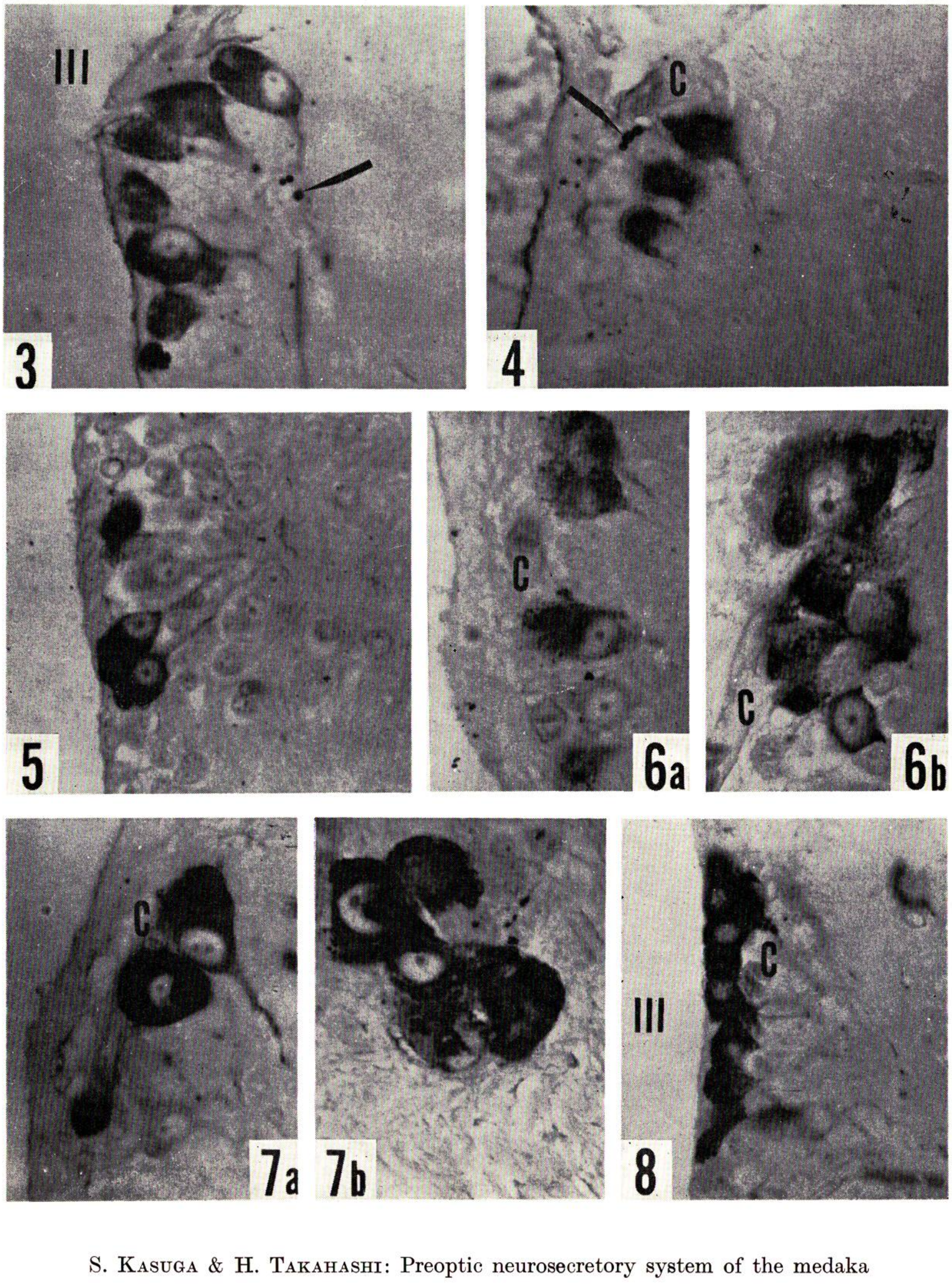
Fig. 1. Sagittal section through the hypothalamus, showing three types of neurosecretory tracts. CH, commissura horizontalis; OC, optic chiasm; PMC, cells of pars magnocellularis; I, II and III, neurosecretory tracts. AF. $\times 190$

Fig. 2. Sagittal section through the ventral hypothalamic region near the infundibulum. Large nuclei (arrows) are seen closely adjacent to preoptic neurosecretory fibers (T). AF. $\times 770$





S. KASUGA & H. TAKAHASHI: Preoptic neurosecretory system of the medaka



S. KASUGA & H. TAKAHASHI: Preoptic neurosecretory system of the medaka

PLATE II

All figures are sections through the preoptic nucleus to show annual changes of neurosecretory cell bodies in size and stainability to the dye AF. Arrows indicate AF-positive globules in the neurosecretory cell bodies. ×560. III, third ventricle; C, blood capillary

Fig. 3. Pars magnocellularis (PMC) of a specimen sacrificed in July. Large AF-positive globules (arrow) are noticed in the cell body.

Fig. 4. PMC in October. The peripheral zone of the cells are not stainable with AF.

Fig. 5. PMC in February. AF-positive globules are rarely seen in the neurosecretory cells in this season.

Fig. 6a, b. PMC in March in a frontal (a) and a sagittal (b) plane. The neurosecretory cell bodies are increased in size with neurosecretory materials extensively dispersed in the cell bodies.

Fig. 7a, b. PMC in April in a frontal (a) and a sagittal (b) plane. The cell bodies are largest in size and show strongest affinity to AF as compared with those in other seasons. In Fig. 7a, two nucleoli are visible in a nucleus.

Fig. 8. Pars parvocellularis in April, indicating neurosecretory cell bodies bordering on the third ventricle and the blood capillary

PLATE III

All figures are sections through the neurohypophysis (NH) to show annual changes in the amount of AF-positive neurosecretory material. ×560. C, blood capillary

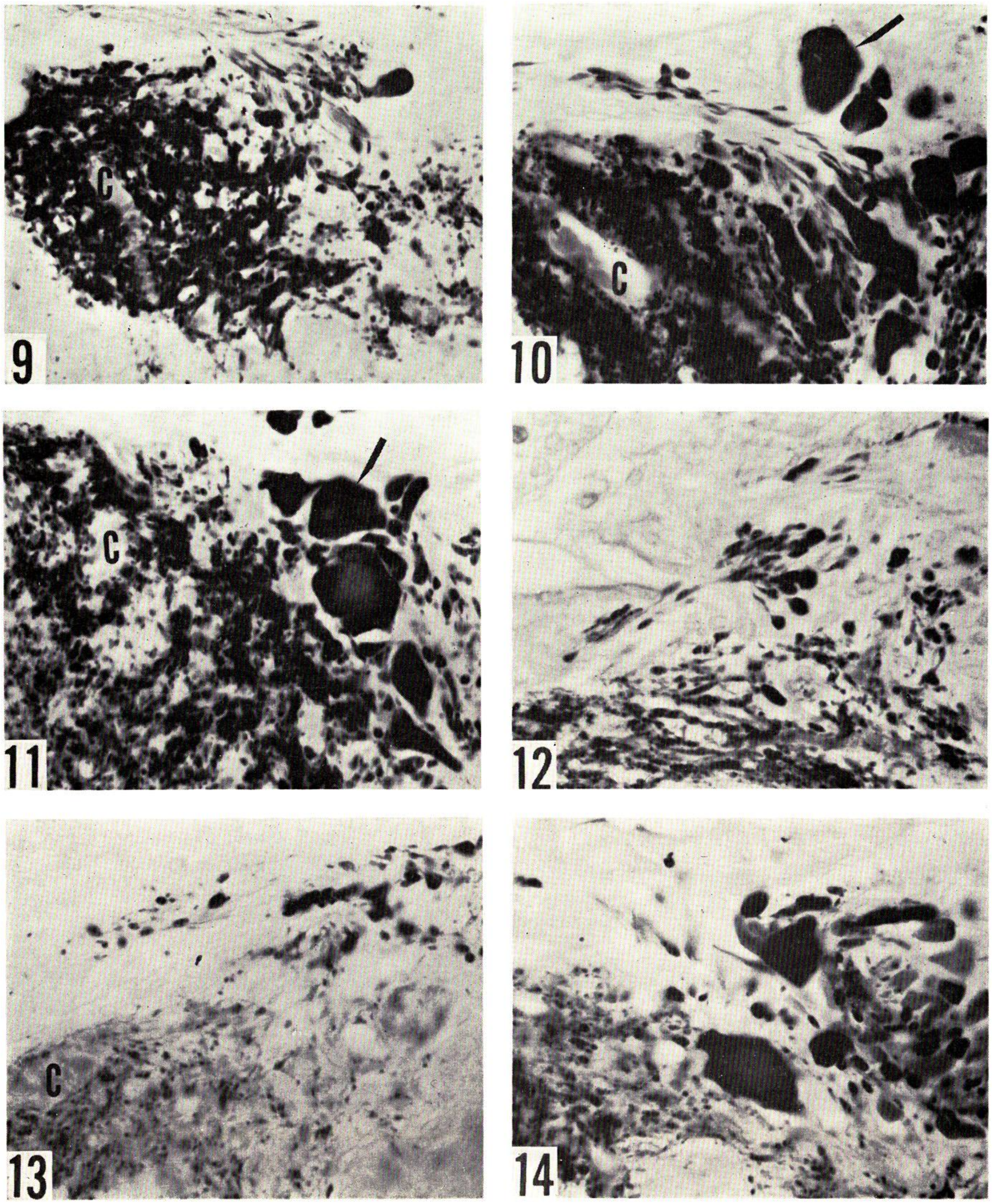
Fig. 9. NH of a specimen sacrificed in July. The large Herring body is not detectable in the NH.

Figs. 10 and 11. NH in September (10) and October (11). Neurosecretory materials are heavily accumulated in the NH causing the appearance of the large Herring bodies (arrows).

Fig. 12. NH in December. Neurosecretory materials are markedly decreased in amount in the NH.

Fig. 13. NH in March. Neurosecretory materials are still meager in amount in the NH.

Fig. 14. NH in April. The large Herring bodies are temporarily present in the NH in this period.



S. Kasuga & H. Takahashi: Preoptic neurosecretory system of the medaka

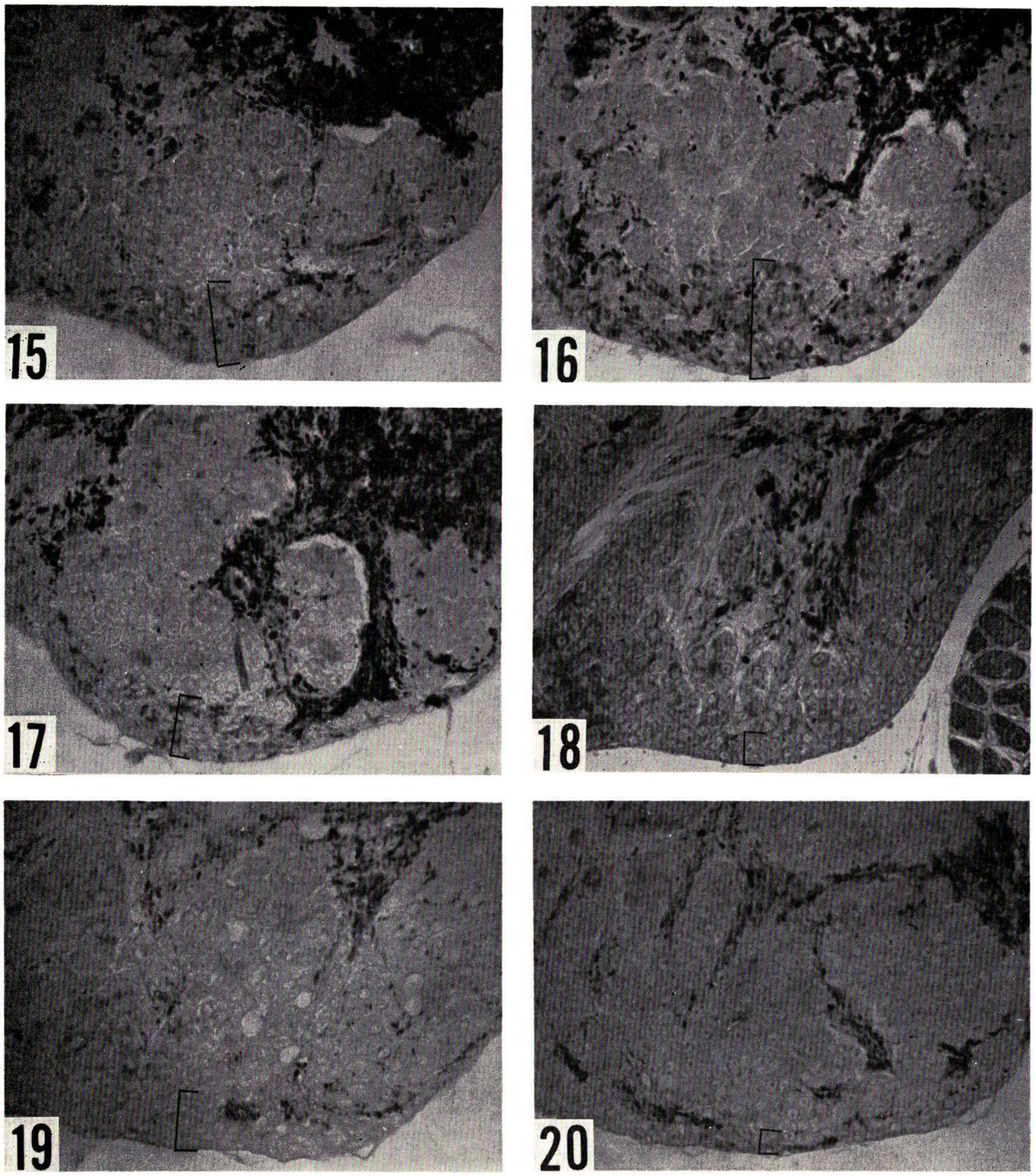


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PLATE IV

All figures are sections through the meso-adenohypophysis to show annual changes of the staining affinity of gonadotropic (GTH) cells to AF. Zones of the GTH-cells is indicated by hook-shaped lines in each figure. ×375

Fig. 15. GTH-cells of a fish sacrificed in July. The cells are stained with AF.

Figs. 16 and 17. GTH-cells in September (16) and October (17). The cells remain to be stainable with AF.

Fig. 18. GTH-cells in December. The cells lose their affinity to AF.

Figs. 19 and 20. GTH-cells in March (19) and April (20). The cells are unstainable with AF.

PLATE V

Fig. 21. Electronmicrograph showing a large Herring body (HB) in the neurohypophysis. C, blood capillary; P, pituicyte. OsO_4. $\times 5,000$
Inset: Light microscopical picture of Herring bodies in the neurohypophsysis. AF. $\times 880$

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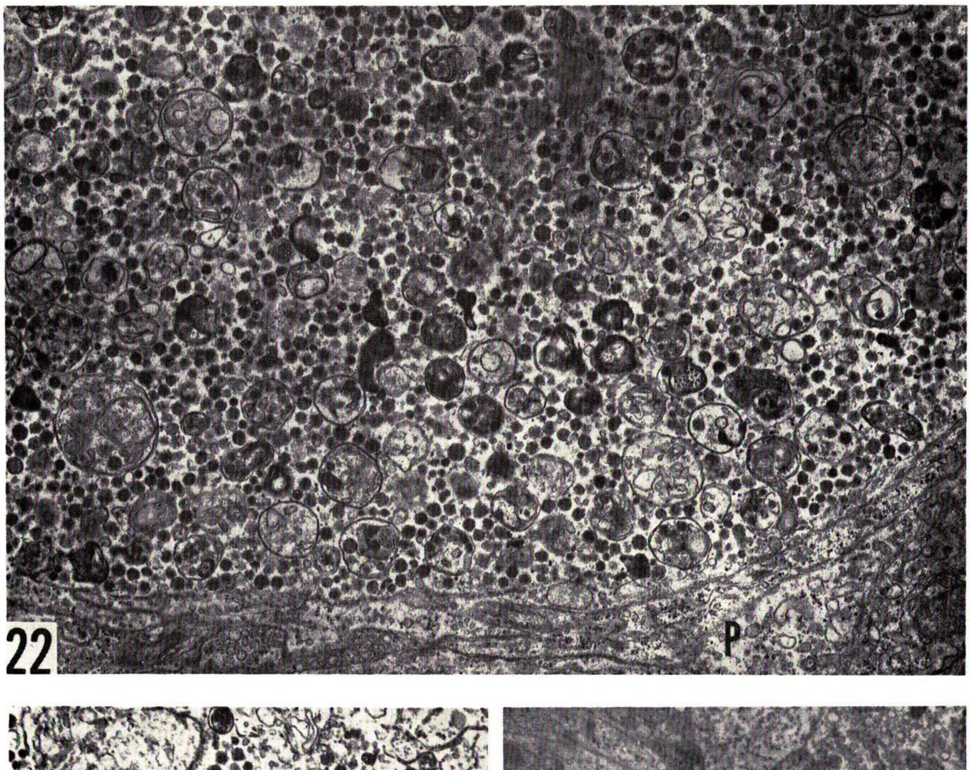

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PLATE VI

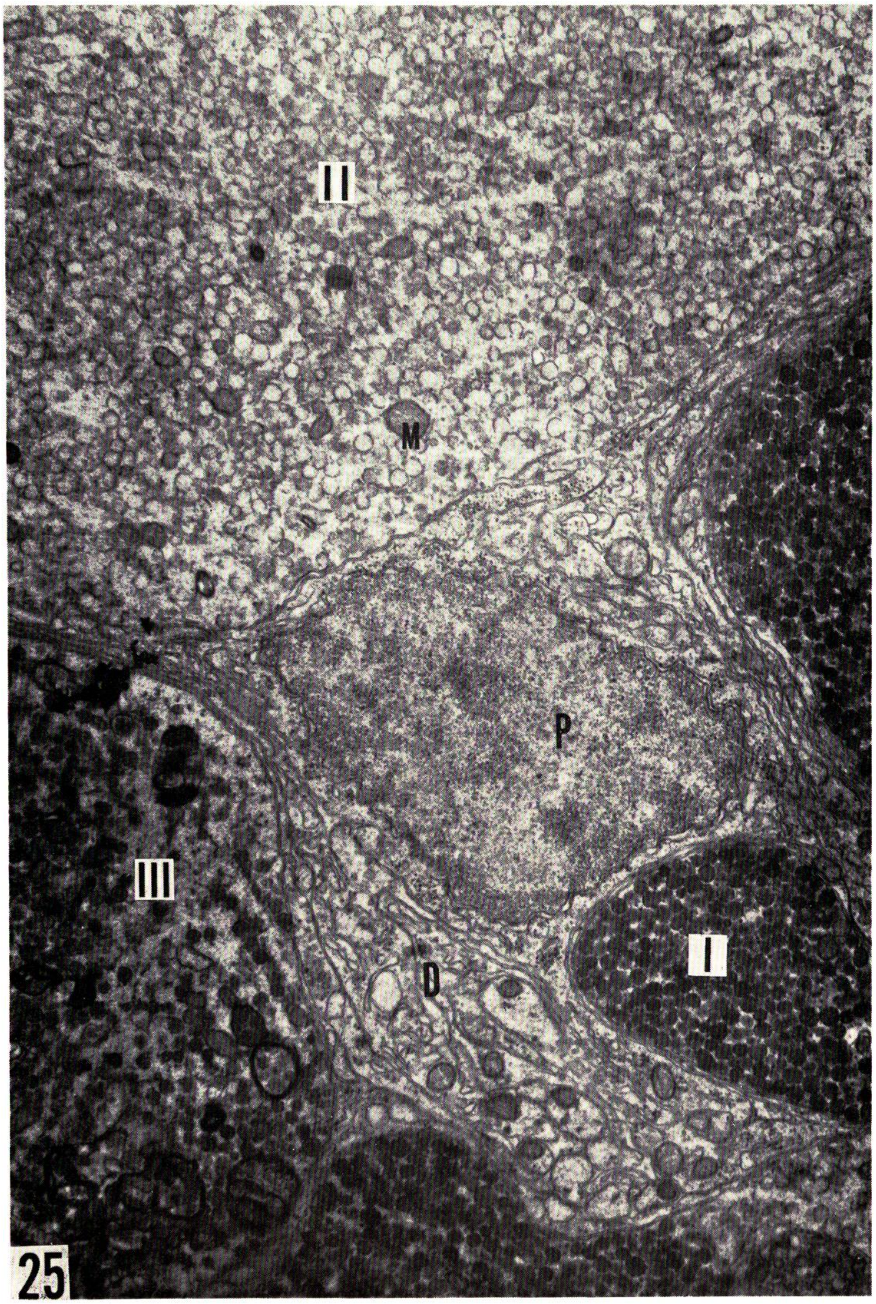
Fig. 22. A Herring body containing peculiar inclusions of multilamellate bodies together with numerous neurosecretory granules, found in the fish sacrificed in October. P, pituicyte. OsO_4. ×12,000

Fig. 23. A well-developed multilamellate body including secretory granules in the core in the fish examined in October. OsO_4. ×12,000

Fig. 24. Pituicytes with abundant oil droplets (OD) in the fish sampled in February. P, pituicyte. OsO_4. ×4,500

PLATE VII

Fig. 25. Three types of Herring bodies with electron dense secretory granules (I), electron lucent vesicles (II), and multilamellate bodies (III), respectively, in the fish examined in February. D, desmosomal junction; M, mitochondrion; P, pituicyte. OsO_4. $\times 15{,}000$



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