# LIGHT AND ELECTRON MICROSCOPIC STUDIES ON THE PINEAL ORGAN OF THE GOLDFISH, *CARASSIUS AURATUS* L.

Hiroya TAKAHASHI*

Photosensory nature of the pineal organ in lower vertebrates has been repeatedly demonstrated by many authors in various species (for references, see Dodt, 1966). In some teleost fishes, the pineal organ has been evidenced as to be photosensitive in nature as based on the experiments on light-mediated behaviour (Scharrer, 1928; Hoar, 1955), on pigmentary responses of fishes (Frisch, 1911; Hoar, 1955; Rasquin, 1958), or on electrophysiological studies (Dodt, 1963; Motte, 1964; Morita, 1966). Ultrastructural characteristics of teleost pineal organs, which have been studied quite recently in several species (*Salmo irideus*, Breucker and Horstmann, 1965; *Anguilla vulgaris*, Oksche and Vaupel-von Harnack, 1965; *Mugil auratus* and *Uranoscopus scaber*, Rüdeberg, 1966; *Phoxinus laevis*, Oksche and Kirschstein, 1967; *Sardina pilchardus sardina*, Rüdeberg, 1968), seem also to indicate photosensory function of the organ. On the other hand, a certain secretory activity in the pineal organ has been declared in a few teleosts by some authors (Grunewald-Lowenstein, 1956; Hafeez and Ford, 1967; Rüdeberg, 1968). In fact, light and electron microscopic structure of teleost pineal organ varies to a considerable degree in different species, which might allow one to suspect that the organ is endowed with a certain function, often of secretory nature, other than photoreceptive nature. To elucidate the problem, morphological as well as physiological informations on the pineal function in teleost fishes seem to be still insufficient.

The goldfish, *Carassius auratus*, seem to be fit for experimental studies of such purposes, for they are easy to rear and breed in the laboratory and are enough tolerant to the various experimental treatments. Furthermore, permanent exposure of the pineal organ to light in the goldfish, which is due to complete lack of black pigmentation in the tissues overlying the organ, seems to offer an interesting character for the research of pineal function. So far as the writer knows, however, no report except a brief description made by Pflügfelder (1964) has hitherto been published about the morphology of the pineal organ of the goldfish. The present report deals with light and electron microscopic structure of the pineal organ of the goldfish. Some additional observations are also reported concerning the ultrastructural modifications of the pineal organ found in aged

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

goldfish.

Before proceeding further, the writer wishes to express his hearty thanks to Professor Kiichiro Yamamoto, Hokkaido University, for his invaluable criticism given throughout the course of the present study. Writer's thanks are also due to Messrs. H. Onozato, O. Hiroi, and Y. Nagahama for their kind help.

## Material and Methods

A total of 47 goldfish, *Carassius auratus*, including 0- and one-year-old fish of both sexes, were used in the present study on the pineal organ, i.e. 33 for light microscopy and 14 for electron microscopy. They were exclusively of an orange-red variety known as "Wakin", and were ranged from 35 to 70 mm in body length. Besides these, from two-year-old stock three fish of 110–121 mm in body length were also employed for electron microscopic observations. All of them were reared in outdoor ponds or in aquaria set in the greenhouse under the conditions of natural illumination, and fed with commercial pelleted diet for fish.

For light microscopic observations, the fish were decapitated, the skull was opened, and the pineal organ, which remained attached to the roof of the cranium, was removed by cutting the pineal stalk and the underlying adipose tissue by fine scissors, and fixed in Bouin's fluid, Zenker-formol solution and Heidenhain's Susa. In some cases where the small animals were employed, the whole head except the lower jaw was preserved in the fixatives. After the decalcification, if necessary in 2.5% solution of trichloroacetic acid, the specimens were cut transversally or longitudinally into serial sections of 6–8 $\mu$ in thickness by ordinary paraffin method. The sections were stained with Delafield's hematoxylin-eosin, Heidenhain's iron hematoxylin-light green, and Mallory's triple stain for general histological purposes. Gomori's chrome alum hematoxylin-phloxine (CH), Gomori-Halmi's or Holmgren's (Holmgren, 1958) paraldehyde fuchsin (AF), periodic acid-Schiff (PAS) and alucian blue (AB)-PAS techniques were also used to check the chemical nature of presumed secretory material in the organ. In addition, gallocyanin stain following fixation with formol-alcohol was adopted for the demonstration of ganglion cells in the organ.

For electron microscopic observations, the roof of the cranium with the pineal organ attached to it was removed from the head immediately after the decapitation, and was set upside down under the dissecting microscope. After quick removal of dispensable adipose tissue surrounding the pineal organ by the aid of a fine sucking tube, the cranial plate was flooded inside with a fixative, then the pineal organ was carefully separated from the cranial plate by fine forceps and immersed in the same fixative, an ice-cooled 1% $OsO_4$ solution of Millonig (1961), for 1–2 hours. The fixed specimens were dehydrated by graded

alcohols and embedded in Epon. Ultrathin sections were obtained using a Porter-Blum microtome and stained double with uranyl acetate and lead hydroxide (Karnovsky, 1961) or lead citrate (Reynolds, 1963). Observations were made by a Hitachi HS–7 electron microscope. Besides, sections of 0.75–1 $\mu$ thickness of Epon-embedded tissues were stained according to the method of Richardson *et al.* (1960) and examined light microscopically.

## Observations

*Light microscopy*: Pineal organ of the adult goldfish is enclosed in a capsule of connective tissue rich in blood capillaries, and leans over the telencephalon with thick layer of adipose tissue in between. The organ is composed of a distal vesicle and a stalk, which show a T-shaped arrangement. Pineal vesicle, the cross bar of the T, is easily recognizable in living fish through the tissues covering the pineal area. The vesicle is a flattened, spindle-shaped body with a wide lumen in the center, lying transversally just anterior to the cartilageous rib and closely attached to the overlying bony skull (Fig. 1). It is connected at its median ventro-posterior wall of the lumen with the roof of diencephalon by a slender stalk, the stem of the T, which has a central canal contiguous to the lumen of the third ventricle. The proximal part of the stalk which is inclined rostrally is compactly surrounded by the dorsal sac and ends on the brain at the region directly caudad to the habenular commissure, but the opening of the canal into the ventricle is mostly obscure in the adult fish.

The wall of the pineal vesicle is an irregularly doubled layer of pineal parenchymal cells and is thicker in ventral and lateral regions than in dorsal one. In most cases the wall lacks distinct convolutions or folds, though indefinable cellular connections are occasionally encountered between the dorsal and the ventral walls. The bilayered wall is mainly composed of three types of cells. Those of the first type are gourd-shaped sensory cells of 20–24 $\mu$ in length, with a neck near the middle of the length of each cell (Fig. 2). The basal part of the cell lies in the inner layer of the wall and is provided, at its basal extremity, with a nucleus containing a prominent nucleolus. The nucleus is roundish but somewhat bilobed in shape, with its concave edge facing to the proximal part of the cell. The inner segment proximal to the basal nucleated part is seen clearly protruded from the wall into the lumen. In these parts of the sensory cell, while fine fibrillar structure was sometimes observed to run longitudinally in sections fixed with Bouin and stained with Mallory's stain, no special stainability was demonstrated by the stainings with AF, AB, CHP, or PAS.

Closely adjacent to the distal end of the inner segment, the outer segment exists as a vesicular body of various roundish shapes projecting in the lumen. In

1 $\mu$ sections of $OsO_4$-fixed, Epon-embedded materials, the outer segment is well preserved as a markedly basophilic body which has a definite connection with the inner segment, lapping over the latter like a dome on some occasions (Fig. 3). By ordinary histological procedures, the outer segment is stained positively with iron hematoxylin, aniline blue, AF, and PAS, but not with CH and AB, revealing stronger response to the former dyes in the periphery than in the center of the body, and thus showing a vesicular appearance (Fig. 4).

Cells of the second type constitute the outer layer of the wall, and are regarded as the supporting cells. The main bodies of the cells each with a rather elongated bilobular nucleus are arrayed deep in the wall, being in contact with the capillary bed enveloping the pineal (Fig. 2). Each of the cells sends its cytoplasm inwards between the basal nucleated parts of the sensory cells and bounds the lumen on the apical border of the elongated cytoplasm. The cytoplasm of the supporting cell is usually noticed to be stained faintly in comparison with that of the sensory cell, but no notable cytological characteristics in the cell were detected by the light microscopic methods used in this study. Besides the supporting cells of this type, certain cells are rarely distinguished in the outer layer of the wall, on the basis of their small roundish nuclei which are stained deep with hematoxylin. These cells might be another type of cells of supporting nature, although their exact character is uncertain because of the indistinct contour of these cells in ordinary histological preparations.

The third type of cells are the ganglion cells. These cells are rather small in number and are dispersed in the wall of the pineal vesicle under the inner sensory cell layer, each often making a small bulge towards the lumen. They are large and round in shape, being 13–17 $\mu$ in diameter, and are characterized by the presence of spherical nucleus of 8–10 $\mu$ in diameter with a nucleolus near the nuclear membrane (Fig. 5). These cells contain certain spots of deep blue in colour in the cytoplasm after the staining with gallocyanin, indicating the accumulation of Nissl substance (Fig. 6). In addition to the ganglion cell, there are some peculiar masses of complicated fibrous structure in the wall of the vesicle. The fibrous mass appears in many cases as a nodular swelling of the wall as shown in Figs. 7 and 8. These are neuropile zones where basal processes of sensory cells and unmyelinated nerve fibers are gathered together to keep synaptic contact with each other, as is to be demonstrated later by electron microscopy. Ganglion cells are not always present in the neuropile zones, but are frequently found near those regions.

In the lumen of the pineal vesicle there occur a few inclusions such as free cells, outer segments detached from sensory cells, and a certain coagulated material. The intraluminal free cells are more or less of common occurrence in the pineal vesicle, appearing near the protruded outer segments frequently (Fig. 9). They vary in shape and size, and more than two types are discernible among them.

Some of them are seen to include various amounts of particles stained positively with AF and PAS in the cytoplasm (Fig. 10).

Intraluminal coagula are rather rarely encountered in the goldfish pineal. These are usually irregular thread-like or granulated masses lying along the margin of the lumen with fine reticular connections to the wall (Fig. 11). This mass shows the same stainability to the afore-mentioned histological stains as the outer segment of the sensory cell displays, and is present mostly in the region where the vesicular figures of the outer segment are indetectable. These findings imply that the coagulum is of disintegrated outer segments of pineal sensory cells in origin.

*Electron microscopy*: Electron microscopically, as well as light microscopically, pineal sensory cell is composed of an outer segment, an inner segment, and a basal nucleated part which extends a cytoplasmic process probably into the neuropile zone. The cell has a neck-like constriction between the basal nucleated part and the inner segment, and at the region of the neck the cell is provided with desmosomal junctions which connect the cell with supporting cells (Fig. 12). The inner segment of the sensory cell protrudes into the pineal lumen, with the outer segment overlapping on its apical portion.

The lobulated nucleus of the sensory cell occupies the distal half of the basal nucleated part. In the nucleus, aggregates of chromatin granules show irregular concentrations along the inner nuclear membrane and are distributed dispersedly in the center. The perinuclear cytoplasm contains mitochondria, Golgi-complexes, rough endoplasmic reticulum, small vesicles, free ribosomes, and lysosomal bodies of various sizes and appearances (Fig. 12). Mitochondria are of a tubular type and are generally of a short rod-shape, though a variety of the shapes are noticeable on many occasions. The Golgi-complexes are in some cases composed of parallel flattened lamellae and associated vesicles, but are often seen to be constituted from distended lamellae and vesicles arranged irregularly. The lysosomal bodies are about 500 m$\mu$ in size, but often exceed 800 m$\mu$. Some of them contain finely granulated substance of moderate electron-density, while others have homogeneous, highly electron-dense substance, in the limiting membrane. Fibrous structure and electron-dense aggregates are sometimes included in these lysosomal bodies. The basal nucleated part is furnished with a basal prolongation of cytoplasm where a few mitochondria, small vesicles, and bundles of long fibrils are noticed (Fig. 13). The fibril bundles run longitudinally in the central part of the cytoplasmic prolongation, rarely penetrating into the perinuclear cytoplasm.

The inner segment is characterized by a loose cluster of mitochondria of a tubular type in its apical region (Fig. 15). In some cases the cluster is made up of mitochondria gathered compactly and entangled complicatedly with each other.

Other cytoplasmic organelles found in the inner segment are similar in features to those present in the basal nucleated part, but lysosomal bodies are fewer in number and Golgi-complexes are rather rare in the former than in the latter. Besides these, centrioles are present along the outer margin of the inner segment, sometimes representing an evident arrangement of a diplosome (Fig. 17d and e). In addition, it is worthy to note that on some occasions a few complicated membrane materials are seen in the inner segment (Fig. 14a and b), for this may denote a possible phagocytotic action latent in this part of the sensory cell.

The outer segment is constructed, in typical cases, from a stack of 40–50 flattened sacs which overlap on top of each other and, as a whole, on the inner segment like a dome of 2–2.5 $\mu$ in height and 3–4 $\mu$ in width (Fig. 16). The sacs are each measured to be 150–200 Å in thickness. It is connected at one margin of the dome with the margin of the inner segment on the same side by a short, narrow connecting piece. Further the outer segment is fastened to the inner segment by slender, vertically elongated cytoplasmic processes probably arising from the inner segment and running closely along the outer margin of the dome (Fig. 17 a-c). The connecting piece is a ciliary process in nature, through which nine outer filaments of the cilia run starting from the centriole in the inner segment and extending into a narrow space in the outer segment. The lamellated sacs of the outer segment open into that ciliary space, the membrane of one sac being each contiguous to those of the adjacent sacs at the edges bordering on the ciliary space. Only the membranes of the innermost and the outermost sacs are directly contiguous to the plasma membrane of the connecting piece. The feature of the outer segment seems to be identical with that of the cone photoreceptor of the retina rather than that of the rod.

Most outer segments display, however, a wide variety of transfigurations from the typical ones, i.e. the lamellated sacs are partially replaced by vesicles and tubules especially in the region neighbouring the ciliary space (Figs. 16 and 17 a-c), while in some cases the vesicles and tubules are seen sandwiched between the lamellated sacs (Fig. 17f), further on many occasions the stack of the sacs as a whole is contorted to show atypical, complicated shapes (Fig. 17b, d and e). These transfiguration of the outer segment were widely observed in the pineal organ of all the goldfish examined without having any concern with sex, sexual maturation and the season of samplings of the fish.

The supporting cell bodies are extended through the whole depth of the wall of pineal vesicle, isolating the basal nucleated parts of sensory cells from each other. The nucleus of the supporting cell is of elongated, bilobed shape and is present generally in the basal region of the cell which faces on the pericapillary space in the connective tissue capsule of the organ. The perinuclear cytoplasm

contains rod-shaped mitochondria of a tubular type, a few Golgi-complexes, sparse rough endoplasmic reticulum, many vesicles of smooth endoplasmic reticulum, small vesicles, free ribosomes, ill-developed fibrillar elements, and lysosomal bodies (Fig. 18). Rarely a few lipid droplets and phagosomes with membrane scars are also seen. These organelles except the lipid droplet are commonly encountered in the distal cytoplasm where the supporting cells are connected with each other or with the sensory cells by desmosomal junctions (Fig. 17a). The supporting cell extends thick, short cytoplasmic processes into the pineal lumen (Fig. 19b). These processes are frequently loaded with many small vesicles of less than 500 Å in size. No definite evidence of secretory function is, however, exhibited in the supporting cells.

The ganglion cells are difficult to be detected in electron microscopic pictures owing to their comparative scantiness in number. Only on two occasions the cells were examined in this study concerning their fine structures. The cell is characterized by a large, round nucleus with aggregates of chromatin granules uniformly distributed over the nuleoplasm (Fig. 20). Nuclear pores are observable in many places on the nuclear membrane (Fig. 20, inset), and frequent evagination of the outer nuclear membrane into the cytoplasm is also seen. The perinuclear cytoplasm is rich in organelles of various types. Many small mitochondria, lamellar rough endoplasmic reticulum, and free ribosomes are found distributing in the cytoplasm, while Golgi-complex is poorly developed. Free polysomal ribosomes are noticed to be accumulated in some parts of the cytoplasm. From the cells, at least two axonal or dendritic processes arise, and neurotubules are evident only in one of them.

The neuropile zone shows a complicated feature in which many unmyelinated nerve fibers and many processes originated from pineal parenchymal cells are entangled with each other. Here can be noticed synaptic structure (Fig. 21): several electron-dense synaptic ribbons and numerous synaptic vesicles are noted in the presynaptic part but not in the postsynaptic part. Probably due to differences in the planes of section, some of the synaptic ribbons show a parallel arrangement while others display a perpendicular arrangement to the membrane of deeply indented synaptic cavity. The cell membrane bearing the synaptic apparatus appears to be a little higher in electron-density than the other. The longest synaptic ribbon is measured to be 300–350 Å in thickness and more than 1 $\mu$ in length, and is generally encircled by synaptic vesicles of about 500 Å in diameter (Fig. 21, inset). The cytoplasmic processes loaded with the synaptic apparatus often possess masses of mitochondria of peculiar constitution (Fig. 22). In many cases several mitochondria, each with longitudinally packed cristae, are arrayed compactly side by side, and sometimes they are bent to form a concentric ring.

It is quite difficult to determine which type of the cells is equipped with the

synaptic apparatus, owing to the discontinuity between the processes and the cell bodies in the thin sections. It is probable, however, that the apparatus may be in the terminals of the sensory cell processes, because some processes contain numerous minute vesicles of possible synaptic nature together with bundles of fibrils which are similar in feature to those found in the proximal region of the sensory cell process.

Intraluminal cells are various in shape and size, but mostly enclose many lysosomal dense bodies and other peculiar inclusions, probably phagosomal in nature, in the cytoplasm (Fig. 23a and b). The presence of well-developed Golgi-complexes is also the characteristic of these cells. In addition to the intraluminal cells, the fragments of outer segment of the sensory cells are also occasionally observed in the lumen of the pineal. Otherwise, no structures indicative of secretory products are detected in the pineal lumen.

*Ultrastructural modifications of the pineal parenchymal cells in aged goldfish*: In one male and two females of two-year-old goldfish, the pineal organ showd interesting modifications in ultrastructure of some component cells. Histologically the wall of the pineal vesicle became thicker as a result of disarrangement of the parenchymal cells. Ultrastructurally, too, it was very difficult to distinguish sensory cells from supporting cells, although some basal nucleated parts of the sensory cell were definitely recognizable. Transformation in the shape of the cell nuclei seems to have occurred.

The most prominent phenomenon is that the wall is provided with many basophilic particles in the region bordering on the connective tissue capsule of the organ (Fig. 24, inset). This structural alteration is conspicuous especially in the proximal area to which the pineal stalk applies, and is also noticeable in the wall of the stalk. The basophilic particles found in the wall are ultrastructurally peculiar, tightly whirled membrane systems (Fig. 24). They appear frequently near the cell nuclei, and measure 3–5 $\mu$ in diameter. The membrane whorls include complicated, highly electron-dense structure of mostly membraneous nature in the cores. In some cases, small mitochondria, vesicles and concentric lamellae of, probably, rough endoplasmic reticulum are also contained in the core (Fig. 25a). Peripheral lamellae or sacs of the whorls have an evident connection with vesicles which are numerously present in the cytoplasm around the whorl (Fig. 25b). The cells which keep these peculiar structure are judged to be the supporting cells basing on the topographic situation of the structure.

The cell membrane of the parenchymal cells have many characteristic infoldings or invaginations bordering on the pericapillary space (Fig. 24). This phenomenon is sometimes encountered in the pineal organ of younger goldfish, but seems to occur more frequently and more prominently in that of the aged ones. No definite connection is observed, however, between the vesicles around the membrane whorl

and the infolded plasma membrane. Further minute vesicles of about 500 Å and dense bodies of 300–600 mμ in size are often found in the cytoplasm near the infolded membrane, but no evidence of secretion into the pericapillary space was detected in the present cases.

An appearance of disorganized outer segments of the sensory cells also constitutes the structural modification of the organ (Fig. 26 a and b). Outer segments, which have become diminished in number, are in most cases constructed from tubules and vesicles arranged irregularly, being almost completely devoid of the typical lamellated sacs. Although some outer segments still retain the stack of lamellated sacs, the disorganization is remarkably spread over these outer segments. In the associated inner segments, mitochondria are present only sparsely, scarcely taking the clustered feature. In the basal nucleated part of the sensory cell, well-developed Golgi-complexes with lamellae, sometimes beaded, and numerous vesicles of 400–600 Å in size, are conspicuous (Fig. 27). In some ones, mitochondria of a tubular type contained electron-dense materials in their matrices.

## Discussion

Pineal organ of the goldfish has not been decidedly proved to be photoreceptive in function. From the morphological basis obtained in the present study, however, the organ must be photosensory, for it bears fundamental resemblances in ultrastructure of constituent cells to that of *Salmo irideus* (Breucker and Horstmann, 1965) and *Phoxinus laevis* (Oksche and Kirschstein, 1967), in both of which the pineal organ is evidently sensitive to light (cf. Dodt, 1966). Although the pineal organ of the goldfish appears to be rather simple in morphological construction, three different types of cells, namely sensory cells, supporting cells and ganglion cells, are clearly noted even by light microscopy.

Photosensory cells of the retinal cone type seem to be a common occurrence in the pineal organ of teleost fishes so far examined. A typical dome-like stack of lamellated sacs found in the sensory cell of the goldfish pineal is quite parallel in feature to that of *Sardina pilchardus sardina* (Rüdeberg, 1968) and *Phoxinus laevis* (Oksche and Kirschstein, 1967). Atypical outer segments with irregularly piled lamellae are also common to the pineal organs of these fishes. In the goldfish, however, partial replacement of the flattened sacs of outer segments by tubules and vesicles seems to occur more generally than in the other two fishes. Similar but more severe modification of the outer segment has been reported in *Salmo irideus* (Breucker and Horstmann, 1965), which was regarded by these authors as a possible indication of secretion into the pineal lumen.

On the other hand, Kamer (1965), Oksche and Kirschstein (1967), Rüdeberg

(1968) and others stressed that such a tubular or vesicular disorganization of the outer segment might be an artifacts resulting from improper fixation of the pineal organ. The present writer has also experienced the labile nature of sensory cell structures, especially of the outer and inner segment, which is liable to give variable cytological features according to various conditions of the fixation. Nevertheless, it is interesting to note that, in the present cases, slight disorganization of the afore-mentioned type was repeatedly seen restricted within the region near the ciliary space in the outer segment, and that the change was much variable in degree in the same organ and even in the neighbouring outer segments.

Considering a pronounced structural modification of the outer segment found in the aged goldfish, which will be discussed later, it seems likely that a slight structural deviation from the typically lamellated, dome-like outer segment may naturally occur in the pineal sensory cell of the goldfish of younger age. Further it cannot be denied that degeneration and renewal of the outer segment may take place in the pineal organ as suggested by Kamer (1965), since in the present cases the inner segments were sometimes seen to include disintegrated membranes as was also the case in *Sardina* (Rüdeberg, 1968). Frequent appearances of phagocyte-like intraluminal cells in the goldfish pineal may be explained along this line of consideration. In the frontal organ of a frog, *Rana pipiens*, Kelly and Smith (1964) has described the presence of "macrophages" which are involved in phagocytosis of degenerated outer segments.

Apart from the above problem, pineal sensory cells of the goldfish appear to illustrate some structural patterns basic to the cells of teleost pineal organs. First, as Breucker and Horstmann (1965) has described in *Salmo*, the outer segment is observed to be closely surrounded by finger-like processes of the inner segment in origin. Similar figure has been exhibited in the pineal of *Sardina*, but Rüdeberg (1968) has explained this as "the basal sac covers the greater part of the free surface of the outer segment". In the present writer's case, however, there were no indications of the basal lamella covering the outer surface of the outer segment. Secondly, the connecting piece of the sensory cell always shows a marginal location in the goldfish as in *Salmo*, *Sardina* and *Phoxinus*. Rüdeberg (1966) demonstrated the outer segment which had the centrally inserted connecting piece in *Uranoscopus scaber*, but the outer segment of this type was in no case observed in the goldfish pineal. Thirdly, second centriole is not rarely present in the inner segment of the sensory cell. Rüdeberg (1968) has also described the presence of the true diplosome in the pineal sensory cell of juvenile *Mugil*.

Since ganglion cells are generally scarce in number in teleost pineal organs, only one report has dealt with ultrastructure of the cell (*Mugil auratus*, Rüdeberg, 1966). In the goldfish pineal, light microscopic detection of the ganglion cell is rather easy. The ganglion cell is seen dispersedly without arranging in groups

as in the pineal of *Uranoscopus*, and its ultrastructural characteristics appear to be quite similar to those of the cell found in *Mugil*. The present observation failed to trace the axons from the ganglion cell, but it is believed that the axons are one of the constituents of the neuropile zone where they keep a synaptic connection with the basal processes of the sensory cell. The pineal organ of *Phoxinus* lacks neuropiles and synapses (Oksche and Kirschstein, 1967), whereas that of *Uranoscopus* and *Mugil* seem to develop the neuropile zone (Rüdeberg, 1966). In the goldfish pineal, the neuropile zones are very prominent probably owing to the simpler construction of the organ.

Accumulation of peculiar clusters of mitochondria in the neuropile zone is the first record in the pineal organs of teleost fishes. Kelly and Smith (1964) has described that, in the frontal organ of a frog *Rana pipiens*, mitochondria within basal processes of the photoreceptor cell are sometimes associated in pairs. It remains uncertain, however, whether or not the clustered mitochondria have a relation to photosensory function of the pineal organ in the goldfish.

Synaptic ribbons and vesicles present in the neuropile zone of the goldfish pineal are similar to, and more evident than, those seen in the pineal of *Sardina*. On the other hand, Rüdeberg (1966) reported the absence of synaptic structures in *Uranoscopus* and probable transformation of synapse into desmosome-like structure in *Mugil*, which he suggested to be a sign of involution of pineal sensory cells associated with possible reduction of photosensitivity of the organ in adult fishes.

It is considered that the pineal organ of teleost fishes in which the whole pineal area is exposed to light is highly photosensitive (Breder and Rasquin, 1950; Rasquin, 1958), and in this regard the pineal of adult fishes with thicker cranial covering may be less potent in photosensitivity than that of juveniles. If so, are there any morphological changes, which may in turn imply a functional deviation from the original photoreceptivity, in the pineal organ of adult fishes in comparison with that of juveniles? So far as the writer knows, only a few reports have been concerned with such a problem. Rüdeberg (1966, 1968) could not find ultrastructurally significant differences between the pineal of adult and juvenile *Mugil auratus*, while Hafeez and Ford (1967) observed a decrease in number of pineal sensory cells in adult *Oncorhynchus nerka* as compared with those in juveniles. A phenomenon seemingly comparable to the case of the salmon was exhibited by Kelly (1965) in the pineal of a newt *Taricha torosa*, in which the pineal organ of the animals in full adult stage displayed many degenerated outer segments of the sensory cells and became predominated by supporting cells in its histological construction.

The goldfish used in the present study had the pineal area exposed permanently to light owing to the lack of melanin pigment in the overlying tissues. Although

definite physiological evidence has not been presented so far, histological and cytological characteristics of the goldfish pineal admittedly point to the sensory nature as mentioned earlier. The organ of the aged goldfish is, however, quite peculiar at least in a morphological sense. The structural modifications found in their pineal parenchymal cells cannot be regarded simply as degenerative signs, because well-developed Golgi-complexes in the sensory cells denote an activated state of cellular metabolism. The pineal organs of younger fish, which had been reared in the same condition and had been sacrificed at the same time as the aged goldfish did, did not display any of the modifications. These findings may be interpreted as signifying that some functional alteration may occur in the pineal organ of the goldfish at a certain period of their life time. At present, however, it is only a matter of speculation because of the present observations made on a limited number of specimens of a restricted age.

As for the origin and fate of the peculiar membrane whorls observed in the pineal supporting cells of the aged goldfish, no information was gained in the present study. Of special interest is, however, unusual accumulation of vesicles which are connected with peripheral lamellated sacs of the whorls. Only in this respect the membrane whorl has a resemblance to the "myeloid body", which is similar in feature to that found in the retinal pigment epithelium, in the supporting cell of amphibian pineal organs (Kelly and Smith, 1964; Kelly, 1965; Charlton, 1968; Ueck, 1968).

An occurrence of apocrine secretion in the pineal organ of teleost fishes has been stated by several authors (Grunewald-Lowenstein, 1956; Rasquin, 1958; Hafeez and Ford, 1967). According to their histochemical observations, the exocrine secretion appears to occur in association with disintegration of sensory cells, and secretory material is stainable with PAS and AF just like the outer segment is. In the pineal of the goldfish, too, there occurred occasionally fibrous or granulated mass stained positively by PAS and AF in the lumen. Electron microscopically this mass was not at all observed in the pineal lumen but is distinguished as the outer segment or its fragments detached from the inner segment of the sensory cell. These outer segments are in some cases considered to be in the course of disintegration actually, but in other cases are evidently the artifacts caused by the treatments for fixation of the organ as pointed out by Pflügfelder (1964). Intraluminal processes of the supporting cell may play a role in presumed apocrine secretion as suggested by Rüdeberg (1968), for some of the processes were filled with minute vesicles. Further studies will make it possible to judge exact functional significance of the vesicle-loaded processes of the supporting cells.

The present study also failed to detect any evidence of endocrine secretion in the pineal organ. Pflügfelder (1954, 1964) observed a hyperplastic development of the thyroid and a change in cellular component of the pituitary gland in pineal-

ectomized guppy and goldfish, and considered that the pineal might play an important role in controlling the endocrine system indirectly through its photosensitive ability. Quite recently, however, Peter (1968) failed to demonstrate the effect of pinealectomy on the pituitary, gonad, thyroid, and interrenal tissue in the goldfish. In fact, no report on the ultrastructural basis has supported endocrine secretion of the pineal organ of teleost fishes.

There still remains, however, the possibility that the pineal organ has some secretory function in some fishes such as *Anguilla vulgaris* (Oksche and Vaupel-von Harnack, 1965), and that the organ may gain secondary function such as secretion in a certain period of life time. Further work is expected to be done on the pineal organ of various fishes in different biological conditions.

## Summary

The pineal organ of the goldfish, *Carassius auratus*, was observed by light and electron microscope. The pineal of 0- and one-year-old fish is provided with histological and cytological construction indicative of evident photosensitive nature. Four types of cells are discriminated in the organ: sensory cells, supporting cells, ganglion cells and intraluminal free cells. The sensory cell displays the lamellated outer segment which shows a variety of lamellar configuration but fundamentally resembles that of the retinal cone. The ganglion cell is comparatively easily observable in the goldfish pineal. Synaptic ribbons and vesicles together with clusters of mitochondria with longitudinally packed cristae are found in the prominent neuropile zones. Intraluminal cells seem to play a role in removing disintegrated outer segments from the pineal lumen. No definite evidence of exocrine as well as endocrine function was detected in the organ.

The pineal organ of two-year-old goldfish exhibited some modified structure in their pineal organs: disorganization in arrangement of the parenchymal cells; an appearance of peculiar membrane whorls and associated numerous vesicles in the perinuclear region of the cells; a decrease in number of the outer segment and its profound vesicular or tubular disintegration; and a notable development of Golgi-complexes in the sensory cell perikaryon. The possibility of some functional alteration of the pineal organ at a certain period of life time of the goldfish was suggested and discussed.

## Literature

Breder, C.M. & Rasquin, P. (1950). A preliminary report on the role of the pineal organ in the control of pigment cells and light reactions in recent teleost fishes. *Science* **111**, 10–12.

Breucker, H. & Horstmann, E. (1965). Elektronenmikroskopische Untersuchungen am Pinealorgan der Regenbogenforelle (*Salmo irideus*). *Progr. Brain Res.* **10**, 259–269.

Charlton, H.M. (1968). The pineal gland of *Xenopus laevis* Daudin: a histological, histochemical, and electron microscopic study. *Gen. Comp. Endocrinol.* **11**, 465–480.

Dodt, E. (1963). Photosensitivity of the pineal organ in the teleost, *Salmo irideus* (Gibbons). *Experientia* **19**, 642–643.

——— (1966). Vergleichende Physiologie der lichtempfindlichen Wirbeltier-Epiphyse. *Nova Acta Leopoldina N.F.* **31**, 219–235.

Frisch, K. von (1911). Beiträge zur Physiologie der Pigmentzellen in der Fischhaut. *Pflügers Arch. Ges. Physiol.* **138**, 319–387.

Grunewald-Lowenstein, M. (1956). Influence of light and darkness on the pineal body in *Astyanax mexicanus* (Filippi). *Zoologica, N.Y.* **41**, 119–128.

Hafeez, M.A. & Ford, P. (1967). Histology and histochemistry of the pineal organ in the sockeye salmon, *Oncorhynchus nerka* Walbaum. *Can. J. Zool.* **45**, 117–126.

Hoar, W.S. (1955). Phototactic and pigmentary responses of sockeye salmon smolts following injury to the pineal organ. *J. Fish. Res. Bd. Canada* **12**, 178–185.

Holmgren, U. (1958). Secretory material in the pineal body as shown by aldehyde-fuchsin following performic acid oxidation. *Stain Technol.* **33**, 148–149.

Kamer, J.C. Van de (1965). Histological structure and cytology of the pineal complex in fishes, amphibians and reptiles. *Progr. Brain Res.* **10**, 30–48.

Karnovsky, M.J. (1961). Simple methods for "staining with lead" at high pH in electron microscopy. *J. Biophys. Biochem. Cytol.* **11**, 729–732.

Kelly, D.E. (1965). Ultrastructure and development of amphibian pineal organs. *Progr. Brain Res.* **10**, 270–287.

——— & Smith, S.W. (1964). Fine structure of the pineal organs of the adult frog, *Rana pipiens*. *J. Cell Biol.* **22**, 653–674.

Millonig, G. (1961). Advantages of a phosphate buffer for $OsO_4$ solutions in fixation. *J. Appl. Phys.* **32**, 1637.

Morita, Y. (1966). Entladungsmuster pinealer Neurone der Regenbogenforelle (*Salmo irideus*) bei Belichtung des Zwischenhirns. *Pflügers Arch. Ges. Physiol.* **289**, 155–167.

Motte, I. de la (1964). Untersuchungen zur vergleichenden Physiologie der Lichtempfindlichkeit geblendeter Fische. *Z. Vergl. Physiol.* **49**, 58–90.

Oksche, A. & Kirschstein, H. (1967). Die Ultrastruktur der Sinneszellen im Pinealorgan von *Phoxinus laevis* L. *Z. Zellforsch.* **78**, 151–166.

——— & Vaupel-von Harnack, M. (1965). Vergleichende elektronenmikroskopische Studien am Pinealorgan. *Progr. Brain Res.* **10**, 237–258.

Peter, R.E. (1968). Failure to detect an effect of pinealectomy in goldfish. *Gen. Comp. Endocrinol.* **10**, 443–449.

Pflügfelder, O. (1954). Wirkungen partieller Zerstörungen der Parietalregion von *Lebistes reticulatus*. *Roux' Arch. Entwickl.-Mech.* **147**, 42–60.

——— (1964). Wirkungen lokaler Hirnläsionen auf Hypophyse und Thyreoidea von *Carassius gibelio auratus* Bloch. *Roux' Arch. Entwickl.-Mech.* **155**, 535–548.

Rasquin, P. (1958). Studies in the control of pigment cells and light reactions in recent teleost fishes. *Bull. Amer. Mus. Nat. Hist.* **115**, 1–68.

Reynolds, E.S. (1963). The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J. Cell Biol.* **17**, 208–212.

Richardson, K.C., Jarett, L. & Finke, E.H. (1960). Embedding in epoxy resins for ultrathin sectioning in electron microscopy. *Stain Technol.* **35**, 313–323.

Rüdeberg, C. (1966). Electron microscopical observations on the pineal organ of the teleosts *Mugil auratus* (Risso) and *Uranoscopus scaber* (Linné). *Pubbl. Staz. Zool. Napoli* **35**, 47–60.

——— (1968). Structure of the pineal organ of the sardine, *Sardina pilchardus sardina* (Risso), and some further remarks on the pineal organ of *Mugil* spp. *Z. Zellforsch.* **84**, 219–237.

Scharrer, E. (1928). Die Lichtempfindlichkeit blinder Elritzen. I. Untersuchungen über das Zwischenhirn der Fische. *Z. Vergl. Physiol.* **7**, 1–38.

Ueck, M. (1968). Ultrastruktur des pinealen Sinnesapparatus bei einigen Pipidae und Discoglossidae. *Z. Zellforsch.* **92**, 452–476.

## Explanation of Plates

## PLATE I

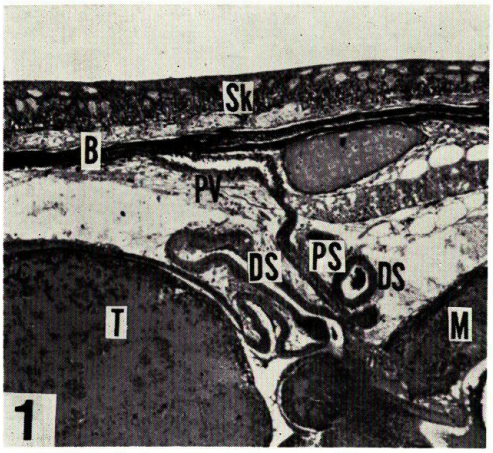
Abbreviations. *B*, bone of the skull. *C*, Blood capillary. *DS*, dorsal sac. *IS*, inner segment of sensory cell. *L*, lumen of pineal vesicle. *LC*, intraluminal cell. *M*, mesencephalon. *OS*, outer segment of sensory cell. *PN*, basal nucleated part of sensory cell. *PS*, pineal stalk. *PV*, pineal vesicle. *S*, supporting cell. *Sk*, head skin. *T*, telencephalon.

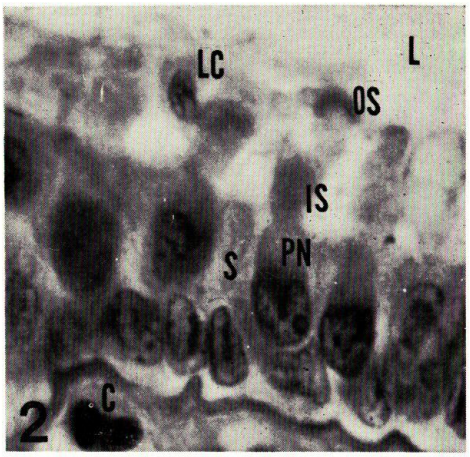
Fig. 1. Median sagittal section through the pineal area of 98-day-old goldfish, showing gross structure of the pineal area. Bouin, Delafield's hematoxylin-eosin, × 65.

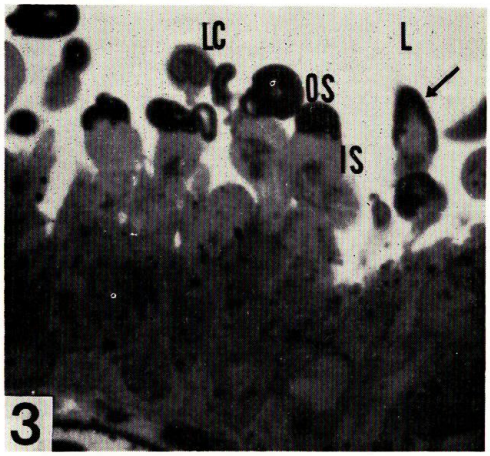
Figs. 2 and 3. Structure of the bilayered wall lining the lumen of pineal vesicle, indicating evident protrusion of inner and outer segments of the sensory cells into the lumen. Typical dome-like attachment of the outer segment to the inner segment is shown by an arrow in Fig. 3. Fig. 2, Bouin, AF with nuclear stain, × 1,600. Fig. 3, $OsO_4$, Richardson's stain, × 1,600.

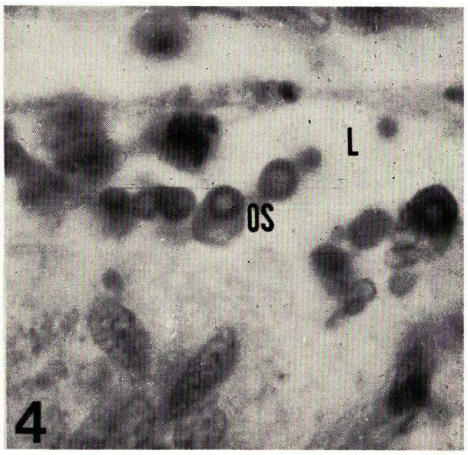
Fig. 4. Outer segments of a vesicular appearance in the pineal lumen. Bouin, AF, × 1,600.

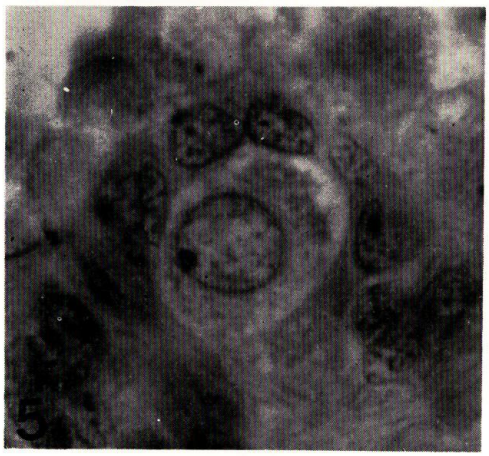
Figs. 5 and 6. Ganglion cells embedded in the wall of pineal vesicle. Two neuronal processes are extended from the cell into the surrounding tissue, as indicated by arrows in Fig. 6. Fig. 5, Bouin, AF with nuclear stain, × 1,600. Fig. 6, formol-alcohol, gallocyanin, × 1,600.

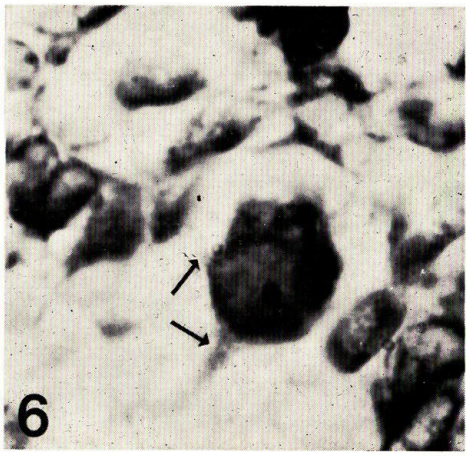


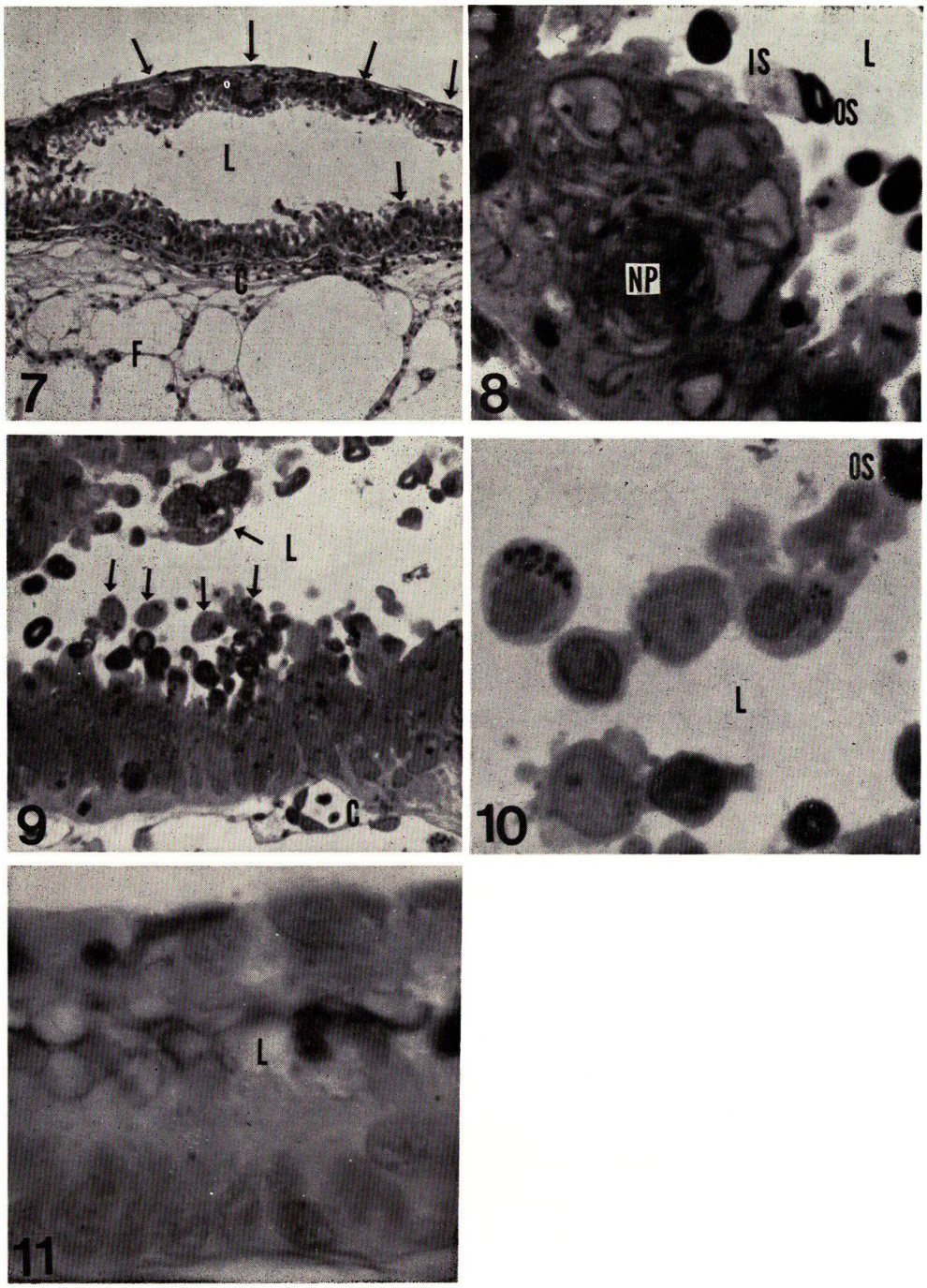




H. TAKAHASHI: The pineal organ of the goldfish




H. TAKAHASHI: The pineal organ of the goldfish

# PLATE X

Fig. 26a, b. Distintegrated outer segments (*OS*) of sensory cells in the pineal organ of two-year-old goldfish. *CP*, connecting piece; *IS*, inner segment of sensory cell; *L*, pineal lumen. a, × 16,000. b, × 13,000.

Fig. 27. Basal nucleated part of sensory cell in the pineal organ of two-year-old goldfish. *D*, desmosomal junctional zone; *G*, Golgi-complex; *Ly*, lysosomal body; *M*, mitochondrion; *Np*, nucleus of sensory cell; *S*, supporting cell. × 12,000.