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DEVELOPMENT OF A GOBY, *CHAENOGOBIUS UROTAENIA*, WITH SPECIAL REFERENCE TO THE GILL AND THE CHLORIDE CELL

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I. Introduction

All fish, except the myxinoids, must perform the function of osmotic regulation, because the environment is, to their blood, hypotonic in fresh water and is hypertonic in sea water. Endocrinological studies have been carried out on some fishes, for instance the eel, the alewife, the anadromous smelt, the three spine stickelback and *Fundulus*, in relation to osmotic regulation (Black 1957, Hoar 1952, '57).

The hormones of the neurohypophysis and thyroids are associated with the regulation of water metabolism or salt blance, but the direct agent for chloride excretion or absorption may be chloride cells. It is accepted by many authors that the site of chloride excretion or absorption is the gill, and the chloride cell plays the function for extra-renal electrolyte excretion in teleosts (Copeland 1948, Getman 1950, Hamada 1967, Pettengill and Copeland 1948, Philpott and Copeland 1963).

The functions of osmoregulation are of interest not only as physiological subjects but also from the point of view of animal evolution. The present paper is a part of the studies in order to find a clue to make clear the relation between the function of osmotic regulation and that of the chloride cells. It is the main purpose to give a detailed account of organogenesis of the gill and the chloride cell of *Ch. urotaenia*, and the early development is at the same time traced.

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II. Materials and Methods

Eggs which were attached with a bundle of adhesive filaments in one layer to the under surface of the piece of an earthen pipe were obtained from a stream in Minato-Machi, Hakodate, and reared in the laboratory at the University. The water temperature in the stream was 13° to 15°C at the time of collection. The

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eggs, both unfertilized and newly fertilized were brought from the stream, and the latter were cultured in watch-glasses (10 to 20 eggs per a watch-glass). The water temperature was not controlled, accordingly it ranged from 10° to 20°C. Some of the fry just after hatching were reared in fresh water continuously, and others were transferred into sea water, Cl 18.425 to 20.210 o/oo. After the living eggs and embryos were grossly observed, the materials were fixed in Bouin's fluid. The fry and the juveniles were also fixed in the same fluid. The chorion was removed after fixation. These materials were dehydrated, cleared, embedded in parafin (56° to 58°C melting point), and sectioned, 5 or 10 microns thick. These sections were stained with Delafield haematoxylin and eosin or Heidenhain's iron haematoxylin and eosin (or orange G, light green). Some of them were stained with Mallory's triple stain.

III. Observations of Living Embryos

The development of *Ch. urotaenia* ovum, from before fertilization until the time of hatching, was grossly observed. The eggs spawn in a stream were used in this observation. Since the time of fertilization is not sure, the eggs formed before the accumulation of the protoplasm at the animal pole were deemed as the eggs just after fertilization.

1. *The unfertilized ovum.* About 1000 unfertilized eggs were found once. Those were attached in one layer to the under surface of a borken piece of earthen pipe. Then, the female was observed under it but no male was found. The ovum is about 1.1 mm in diameter. It is homogenous, opaque and plae yellow in colour (Fig. 1).

At fertilization, the chorion is separated from the vitelline membrane of the ovum. The yolk becomes clear with the development of the perivitelline space. The protoplasm which covers probably the entire surface of the yolk is concentrated at one pole, there being a bundle of adhesive filaments, of the ovum. This concentration of protoplasm is the blastodisc. Globules of oil appear and become large in size (Fig. 2).

2. *One-celled ovum.* The blastodisc becomes thicker, more prominent and presents an appearance such as a vault turned over within a few hours. The yolk becomes clearer (Fig. 3).

3. *Two-celled ovum.* The blastodisc is divided into two cells of equal size by a furrow, which is meridional. The first cleavage furrow is apparent in most of the eggs preserved 2 hours and 30 minutes after fertilization (Fig. 4).

4. *Four-celled ovum.* The second cleavage furrow, also meridional, is perpendicular to the first, and divides the blastoderm into four cells of equal size. The furrow appears about 3 hours after fertilization (Fig. 5).

5. *Eight-celled ovum.* The third cleavage furrow, which is double, is parallel

to the first, consequently the blastoderm is divided into eight cells which form two rows of four cells each. The blastoderm at this stage has become elongated in the axis of the second furrow of cleavage. Each of the eight cells is, nearly, of euqal sized (Fig. 6).

6. *Sixteen-celled ovum.* The fourth division consists of two parallel furrows to the second cleavage one. The blastoderm is divided into four rows of four cells each. The sixteen cells are of unequal size. The arrangement of the cells becomes irregular in some degree (Fig. 7).

7. *Thirty-two-celled ovum.* At this stage, the fifth cleavage furrow divides the cells meridionally. The arrangement of the cells is irregular, unequal in size and peripheral cells of the blastoderm protrude beyond the circumference of the yolk sphere. No cells appear to be divided horizontally (Fig. 8).

8. *Blastula.* The blastoderm is similar, in the early stage, in shape externally to the blastodisc of one-celled stage but the blastomeres become microscopic in size. With the advance of the cleavage, the blastoderm becomes flat and begins to spread over the yolk. The blastoderm which is opaque and pale yellow in colour, becomes gradually clear and pale in colour (Fig. 9).

9. *Gastrula.* When the blastoderm continues to spread over the yolk, it becomes thinner. After a while the peripheral part of the blastoderm is somewhat thickened. This thickened rim is the germ ring (Figs. 10 and 11).

10. *Appearance of the embryonic body.* The rim becomes thick at one portion. This is the initial formation of the embryonic shield which becomes the future posterior pole of the embryo. The shield increases in size and elongates (Fig. 12).

11. *Head fold and closure of blastopore.* The shield swells in the anterior end to form the forebrain. The rudiment of optic vesicles clearly appears on both lateral side of the head. Later in this stage the yolk is covered by the blastoderm (Fig. 13).

12. *Formation of the optic vesicle, notochord, Kupffer's vesicle and somite.* The optic vesicle is well developed and the mid-brain expands exceedingly, possessing no true cavity. The notochord is developed in the anterior region of the embryo. The differentiation of the somites begins after the Kupffer's vesicle has appeared. Kupffer's vesicle lies before the tail bud and sinks into the yolk. This vesicle disappears after about 17 hours. The auditory placode becomes visible (Fig. 14).

13. *Formation of the optic cup, lens placode and auditory vesicle.* The optic cup is well developed and the lens placode is clearly visible. At this stage, the auditory placode is not yet detached from the surface ectoderm, and it takes already the form of the vesicle. The placode of the lateral line organ is also formed behind the auditory vesicle at this time. The head and the tail loosen from the yolk. At the latter part of this stage, the embryo begins to exhibit muscular

contrictions (Fig. 15).

14. *Heart beating.* The heart begins to beat when the auditory vesicle is clearly visible. About this time, the lens is detached from the surface ectoderm. The somites are about 37 in number. The metencephalon is clearly visible. The pectoral fin buds appear just behind the level of the auditory vesicle and above the surface of lateral somatopleure as masses of undifferentiated tissue covered by a thin one-celled layer of epithelium (Fig. 16).

15. *Blood circulation.* The pigmentation in the retina, that has begun at the same time as the gathering of cells in the region of the pectoral fin, becomes established and the eyes are visble to the naked eye. Meanwhile, definite blood vessles containing blood cells are traceable throughout the body. An aortic arch extends through each gill arch, though the primary gill filaments is still not formed. The blood circulation through these vessels can be clearly observed. The tail moves actively. The pectoral fin changes in shape from a bud to a flattened round structure. The gut reaches the proctodeum (Fig. 17).

16. *Hatching.* The fry just after hatching is about 5 mm in total length, and swims actively. The yolk has decreased in amount. The diameter of the yolk sphere, round in shape, is less than one half of it for the one-celled ovum. The pectoral and caudal fin rays are not formed yet. The alimentary canal is straight (Fig. 18).

IV. Development of the Gill

When the embryos reached 1.6 mm in length, the anterior end of the neural cord forms an enlarged solid mass of cells. This is the anlage of the forebrain. A pair lateral out growths of this anlage are the rudiments of the optic vesicles (Fig. 19). At this time, the mesoderm becomes differentiated into paired lateral lamellae in the posterior region of the optic anlage (Fig. 20). The pharyngeal anlage is still not visible, though the entoderm underlaying the posterior region of the optic anlage has thickened to form the pharyngeal pouch. The neural keel is wedgy in shape in the trunk region. Anterior to Kupffer's vesicle, the notochord is not round in the cross section, but it is nearly square (Fig. 21). The Kupffer's vesicle having reached its maximum development, lies just before the tail bud. It is semielliptical in cross section. This vesicle sinks into the yolk, and consists of a syncytium with large nuclei in the region attached to the yolk mass. And the upper wall is formed with a layer of columner cells (Fig. 22).

1. *Formation of first and second pharyngeal pouches.* By the time that the optic vesicles are folded into optic cups with a thickened sensory layer, the entodermal layer posterior to the optic cup begins to fold up itself on either side to initiate the formation of the first and second pairs of pharyngeal pouches. The brain and spinal cord are laterally compressed and there is a slight crevice in the median

region.

a) When the embryo has reached 1.8 mm in length, the inner layer of the optic vesicle is invaginated and the ectoderm covering the optic vesicle forms a lens placode. A pair of the first pharyngeal pouches present solid sac-like pouches which consist of a layer of columner entodermal cells oriented to form the sac (Fig. 23). Just under the first pouch, the heart rudiment is formed. The location of the rudiment is well in agreement with it in the chum salmon which was shown by Mahon and Hoar (1956). They traced the development of the heart from a mesodermal lamella of the intermediate cell mass in the head region. But the present study could not clearly substantiate the origin of the cardiac rudiment. The auditory placode is now developed into veiscle structures whose wall is composed of a single row of columner cells concentrically arranged, but the cavity is not visible and is as yet in contact with the surface ectoderm. Just under it the second pharyngeal pouch is found. The first pharyngeal pouch possesses no cavity but the second pouch begins to form a narrow cavity and reaches already to the surface ectoderm (Fig. 24). There are the formation of the visceral grooves corresponding to the development of the pouches. The grooves appear to enter the mesoderm in a wedgy shape. When the second pharyngeal pouch reaches the surface ectoderm, the entoderm massed toward the mid-line begins to form the tubular gut throughout the length of the embryo. The wall of the gut consists of a layer columner cells. Posterior to the auditory vesicle, a thickening of the nervous layer of ectoderm can be noticed. This is the anlage of the lateral line organ. At this time, lateral plate mesoderm develops the splanchnic and the somatic layers (Fig. 25). As the lens separated from the surface ectoderm migrates into the optic cup (Fig. 26), the second pharyngeal pouch breaks through the surface ectoderm.

b) When the embyro has reached 2.5 mm in length, the brain has undergone significant differentiation. The mid-brain is divided into two distinct optic lobes, and the hind brain is differentiated into the metencephalon and myelencephalon. The differentiation into the telencephalon and diencephalon of the forebrain occurs after the differentiation of the hind brain. At the level anterior of the auditory vesicle, there is a first pharyngeal pouch which has broadened as compared with a 1.8 mm embryo although the development of the pouch is retarded (Fig. 27). The second pharyngeal pouch situated just under the auditory vesicle possesses a cavity and breaks through the surface ectoderm (Figs. 28 and 29). The auditory vesicle is well developed and is already separated from the surface ectoderm. The third pharyngeal pouch does not appear yet. The anlage of the lateral line organ extended backward, reaches 80 microns in length (Fig. 30).

c) In some of the embryos which had passed 3 days after fertilization, the lens

begins to differentiate into the lens epithelium and the fiber cells (Fig. 31). Anterior to the auditory vesicle, a pair of the first pharyngeal pouches which were elevated on either side in form of an "V" in the previous stage, is now situated on the same horizontal plane with the gut. And the sac-like shape of the pouch transforms into a flattened shape as indicated in Figure 32. The height of columnar cells composing the wall are low in comparison with that of the previous stage. A pair of dorsal aortae appear above these pouches and extend backward to fuse in the midline beneath the notochord beyond the level of the myelencephalon. The first pouch reaches the surface ectoderm and appears to break the surface ectoderm. The auditory vesicle is well developed. The wall of it becomes thin, especially in the dorso-lateral wall. The second pharyngeal pouch is also placed on a level with the gut, slightly anterior to the center of the auditory vesicle (Fig. 33). There is a cell mass under the auditory vesicle. This is an anlage of auditory ganglion. The surface ectoderm of the visceral groove corresponding to the pouch appear to be invaginated into the gill cleft (Figs. 33 and 34). The mesenchymal cells in the mandibular and the hyoid arches begin already to differentiate into chondroblast.

2. *Formation of third and fourth pharyngeal pouches.* The anlagen of pectoral fins appear, five days after fertilization, above the surface of the lateral somatopleure behind the head as an aggregation of blastema cells covered with a thin layer of epithelium (Figs. 38 and 39). They are oval in transverse section and parallelogramic in frontal section. So far as the author has observed, the pectoral fins do not arise as out growths of the epidermis from the body wall. In the notochord, the cells are undergoing vacuolation from tip to backward beyond the level of the vent. As a consequence, the protoplasm of the notochordal cells is reduced to a thin peripheral layer as indicated in Figures 38 and 39. Somatic mesoderm begins to differentiate into the myotomes with myofibrils in the trunk region of the embryo (Fig. 38). The pigmentation in the retina begins at this time. Meanwhile the third and the fourth pharyngeal pouches are well developed. The third pouch lies posterior to the auditory vesicle (Fig. 36). The fourth pharyngeal pouch is situated at the level of the anlage of the pectoral fin (Figs. 37 and 39). Both of the thrid and the fourth pouches break through the surface ectoderm to form the open gill clefts.

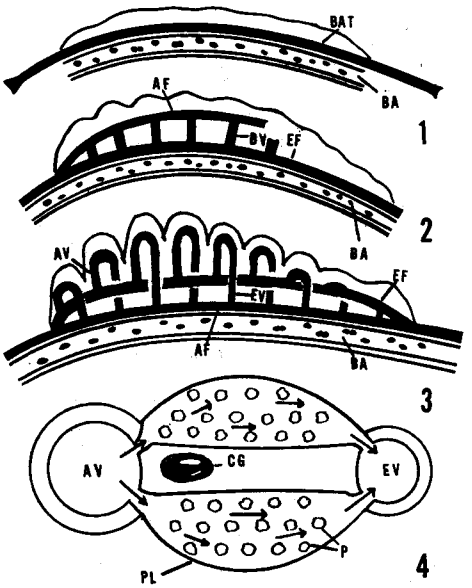
3. *Formation of branchial arches and operculum.* When the embryo has reached 4.0 mm in length after six days since fertilization, four pairs of branchial arches are separated by five well developed pairs of branchial clefts (Fig. 40). In each arch, a ridge of primary gill filaments is not visible. The hyoid arch does not grow backward to form the operculum but sidewards. At this time, the retina acquire the histologic differentiation into layers. The operculum extends already backward to cover the first pair of branchial arches in some of the embyros,

reaching 4.2 mm in length. The cartilage is visible in the arches, and the gill buds are observed on their outside.

4. *Formation of primary gill filaments.* Primary gill filaments are developed on the outside of the arch and take position in a line at a time near hatching fry reaching about 5.2 mm in length. They never grow on the foreside and the hinder side. Accordingly a gill septum is not developed and the filaments do not face each other to form the shape of a "V". The filaments are however leaning alternately in the opposite direction in the gill of an adult. And so the filaments of the gill of an adult fish take, at a glance, the appearance of a "V". An aortic arch extends through each gill arch at the base of the filament and blood cells can be clearly observed in these vessels (Fig. 42). The arch is surrounded with a layer of low cuboidal cells with a large oval shaped nucleus. The cells of a layer covering the filament are flattened, in a high degree, as compared with other cells.

5. *Formation of afferent and efferent arteries.* When the fry which has been reared for 24 hours in fresh water after hatching, has reached about 5.4 mm in length, the gill filament becomes long by the multiplication of the cells. Meanwhile, an artery is formed in the region newly grown in addition to the vessel formed previously (Figs. 43 and 44). This is an afferent branchial artery and the vessel extending through the arch at the base of the filament is an efferent branchial artery. So far as the author investigated, it has never been observed that the aortic arch is divided into the afferent and the efferent branchial arteries. Then a blood vessel becomes organized, which ascends from the efferent artery through the filament to the afferent artery and connects together both arteries (Fig. 45 and Text-fig. I). This vessel forms the afferent and the efferent filament arteries in the future. The filament is covered, at that time, with a layer of squamous cells. The cells in the filament are arranged concentrically in some degree. The fourth branchial arch is retarded in the organization of the blood vessel as compared with the other arches. In the fourth arch, the organization of the afferent branchial artery begins 2 days after hatching (Figs. 46, 47 and 48). By the time that the afferent and the efferent arteries are formed, the anlage of the opercular gill appears on the inner surface of the operculum as a solid cell mass covered with a layer of squamous cells (fig. 49).

6. *Formation of afferent and efferent filament arteries.* The vessel which connects together the afferent and the efferent branchial arteries extends its length, and the afferent branchial artery shifts to the efferent branchial artery. As a result, the vessel ascends from the afferent branchial artery to the tip of the filament and descends from there to the efferent branchial artery. The ascending vessel is an afferent filament artery and the descending vessel is an efferent filament artery (Text-fig. I, Figs. 50 and 54). The situation of the afferent and



Text-fig. I. Diagram of successive development of blood vessel in the gill of *Chaenogobius urotaenia*. Fig. 4 shows the transverse section of a gill filament of an adult. Arrows indicate blood frow

AF, afferent branchial artery; AV, afferent filament artery; BA, branchial arch; BAT, branchial artery (aortic arch); BV, filament vessel; CG, supporting cartilage of the gill filament (gill ray); EF, efferent branchial artery; EV, efferent filament artery; P, pilaster cell; PL, platelet (respiratory plexus)

the efferent arteries in the gill filaments arranged in a line is reverse alternately in the position as shown in Text-figure I. The organization stated above, begins 4 days after hatching. By this time the differentiation of the vessels occurs in the primary opercular gill filament (Fig. 51).

7. *Appearance of chloride cells in the gill filament.* There appear buds of lamellae in the gill filaments of the fry which has been reared in sea water for 18 days after hatching, as the mass of a few round or oval cells (Fig. 52). The supporting cartilage (gill ray) now can be seen in the filament. At that same time, larger cells, about 16 microns in diameter, appear mainly in the proximal portion of the filament. These cells are round or oval and are filled with granular cytoplasm. A vacuole can often be detected. On the other hand, the juvenile fish acquires the same opercular gills as that of the adult in structure in about 20 days after hatching (Figs. 53 and 55). In the gill, the lamellae (leaflet, gill platelet) are made up of the following type of cells and membrane: the pilaster cells supporting two walls of the lamellae, the basement membrane underlaying the epithelium and the epithelial cells being squamous. There is a layer of cells between the

epithelium and the basement membrane in the lamellae of the opercular gill filament. In the juvenile fish kept in sea water, these cells increase in size, reaching about 13 microns in diameter (Fig. 55). Sometimes the cells located in the proximal portion of the opercular gill filament possess the same pit as that of the cell localized at the base of the gill filament of the adult kept in sea water (Fig. 56). In the young fish kept in fresh water, these cells shrink in size (Fig. 53).

8. *Appearance of the cell with a pit in the gill filament.* The buds of the lamellae of the gill are now organized into the epithelium, the basement membrane and the pilaster cell, in the juvenile fish which has been reared for about 25 days in sea water after hatching. Namely, the lamellae are nearly the same as those of the adult in structure. The distinct difference is however found between the gill filament of the juvenile and that of the adult. The cell with a pit is found in the crescent-shaped cavity at the base of the filament in the adult (Hamada 1967). In the fry, the cell with a pit is however extended on the side of the filament supplied with afferent blood (Figs. 57 and 58). This cell is large, about 13 microns in diameter, and the cytoplasm stain into a slightly dirty violet in Mallory's triple stain. The nucleus is oval and situated towards the periphery of the cell.

The respiratory system is entirely developed in the gill of the juvenile kept for 34 days in sea water after hatching (Figs. 59, 60, 61 and 62). The cell with a pit as stated above is however still observed at the side of the filament.

9. *Disappearance of the cell with a pit at the side of the filament.* A crescent-shaped cavity is formed at the base of the gill filament of the juvenile kept in sea water for about 47 days after hatching. At this time, the cell with a pit is not observed on the side of the filament (Fig. 63), but is located restrictedly within the crescent-shaped cavity. When the juvenile is kept in fresh water, the pit of the cell disappears (Figs. 64 and 65).

V. Discussion

It has been assumed by M.G. Zaks and M.M. Sokolova (1961) that the development of the so-called chloride-secretory cells causes the sockeye salmon to descend to the sea. The present paper is, as stated earlier, a part of the studies in order to find a clue to make clear the relation between the descending or ascending migration of fishes (function of osmotic regulation) and the chloride cells. This paper is also the first detailed embryological account of the development of the genus of *Chaenogobius*.

According to Oppenheimer (1937), the fifth cleavage plane divides the peripheral ring of cells meridionally and the central cells horizontally in the egg of *Fundulus heteroclitus*. In the pond smelt, the fifth cleavage plane divides the monolayered blastomeres horizontally (Yamada 1968). In the egg of *Ch. urotaenia*,

the fifth cleavage furrow divides the cells meridionally however. As a result, the blastoderm is flattened and spread out on the yolk, and peripheral cells of the blastoderm protrude beyond the circumference of the yolk sphere (Fig. 8). This may be characteristic in the cleavage of the egg of this species. In a succeeding stage, the blastoderm becomes round, compact and becomes similar in shape externally to the blastodisc of an one-celled ovum.

By the time the solid optic anlagen are greatly enlarged, the Kupffer's vesicle reaches its maximum size. In the platyfish, this vesicle appears before the formation of a pair of optic anlagen, and it reaches it maximum development about the time when a pair of lateral growth of forebrain, optic buds, are present (W.N. Tavolga 1949). In other teleosts, for instance *Coregonus clupeaformis*, *Oncorhynchus keta* and *Fundulus heteroclitus*, the Kupffer's vesicle reaches also similarly its maximum size at the time that the optic anlagen have enlarged (Mahon and Hoar 1956, Oppenheimer 1937 and Price 1934). According to Saito (1950), the first sign of the proctodaeum in the chum salmon appears as a depression of the ectoderm at the base of the tail bud in a very early stage, in which the enteron is still a solid band of entodermal cells. But it could not be observed in the embryo of *Ch. urotaenia* at this stage, and there is the Kupffer's vesicle at the base of the tail bud. This is in agreement with the observation on chum salmon by Mahon and Hoar.

The surface ectoderm borken by branchial pouches appears to be invaginated into the pouch. This may be related to the location where the rudiments of gill filaments grow. According to Nakamura (1966), the filaments grow on the foreside and the hinder side of the branchial arch in teleosts. The filaments grow however on the outside of the arch and take position in a line in *Ch. urotaenia*. They never grow on the foreside or the hinder side. On the other hand, the surface ectoderm covering the hyoid groove does not appear to be invaginated into the pouch. This may be important in connection with the fact that the pseudobranchia is not found in *Ch. urotaenia*. As stated before, when four pairs of branchial arches and five apris of branchial clefts are well developed, the hyoid arch grows lateral. After the hyoid has projected laterally, it grows backward to cover the branchial arches (Figs. 40 and 41). This fact indicates that the growth of the outer surface of the operculum is quicker than that of the inner surface. As a result, the surface ectoderm covering the hyoid arch may be left on the inner surface of the operculum. The development of the opercular gill in *Ch. urotaenia* may be related to this fact. The facts stated above maybe indicate that the gill filament is developed from the ectoderm covering the visceral arches.

As stated before, after the branchial arteries and the filament arteries have been formed, there appear buds of lamellae in the filament and the large cells make their appearance (Fig. 52). These cells resemble the morphological characters of the chloride cell which was found in the gill filament of the common eel by

Keys and Willmer (1932). Similar cells are also found in the gills of the chum salmon and the sockeye salmon (Zaks and Sokolova 1961, Natochin and Bocharov 1962). In the more developed gill filament, large cells with a tubular pit appear on the side of the filament supplied with afferent blood (Figs. 57 to 60). They are alike, as far as the morphological characters and the location situated are concerned, to the chloride cell in the gill of *Fundulus heteroclitus* which can survive in a wide range of salinities. When the gill of *Ch. urotaenia* has developed to maturity, these cells disappear from the side of the filament. They are located within the crescent-shaped cavity formed at the base of the filament. Hamada (1967) reported that *Ch. urotaenia* tolerates a sudden change of the media from fresh water to 100% sea water and survives with no signs of distress. He described the cells with a pit located within the crescent-shaped cavity at the base of the gill filament as chloride cells. According to him, they are columnar and possess a tube-like pit. These cells are strongly responsible to change of salinity of the media. The facts stated above suggest that *Ch. urotaenia* lived in past age a parallel life to the fishes belonging to the genus of *Oncorhynchus* and *Fundulus*. After that, maybe they acquired the strong ability to tolerate a sudden change of salinity.

Though the function of the opercular gill is not evident, there is no dobut that they play the function of extra-renal excretion of salts. Because they are the same cells covered with the squamous epithelium, along with the chloride cells of the gill all over their surface. Moreover, the chloride cell in the opercular gill becomes obese when they are adapted to sea water, and shrink in size when transfered into fresh water (Figs. 53 and 55).

It is difficult to determine when the chloride cells begin to play their osmotic function, for *Ch. urotaenia* is euryhaline through their life from hatching time to death. They are swept down the stream to the sea just after hatching. Accordingly, they must excrete the sea water salt to maintain their body fluid concentration normally soon after they hatched out. It is about 2 weeks after hatching that the chloride cells appears in the gill. The opercular gill also is not yet completely differentiated. No specific sites for osmoregulation can be identified, at present, in the fry. As was described by Schelbourne (1957), osmotic work may be a function of the whole epidermis at that early stage.

VI. Summary

1. *Ch. urotaenia* in its major embyrological processes is in general typical of the teleosts.

2. In the cleavage of the blastomeres, the fifth cleavage furrow divides the cells meridionally and the arrangement of the cells is irregular, unequal in size (Fig. 8).

3. When the optic vesicles are folded into optic cups, the entodermal layer

posterior to the optic cup begins to form the first and second pairs of pharyngeal pouches.

4. The hyoid arch extends backward to cover the branchial arches after it grows lateral (Fig. 40 and 41).

5. The gill filaments grow on the outside of the branchial arch (Figs. 41 and 42).

6. After hatching, an artery is formed in the apical region of the gill filament in addition to the aortic arch formed previously at the base of the filament. These are afferent and efferent branchial arteries (Figs. 50 and 54).

7. When buds of lamellae appear in the gill filaments, the large cells appear in the filament. These cells are chloride cells (Fig. 52).

8. When the buds of the lamellae of the gill have been organized into the epithelium, the basement membrane and the pilaster cell, the cells with a pit appear on the side of the filament (Figs. 57 and 58).

9. The opercular gill is developed on the inner surface of the operculum. They are responsible for the change of salinity of the media (Figs. 53, 55 and 64).

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Explanation of Plate

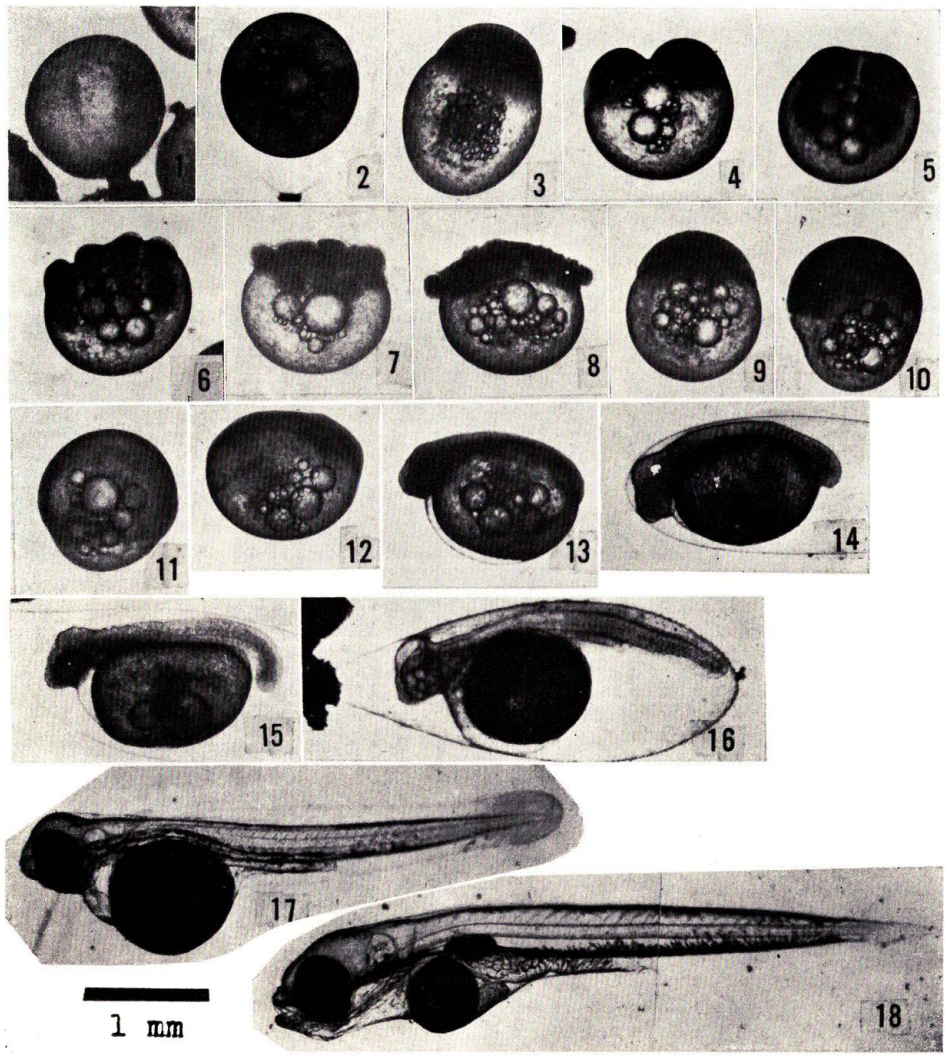
Key for All Figures

af, afferent branchial artery
ag, auditory ganglion
at, otic anlage
av, afferent filament artery
ba, branchial arch
ba_1, first branchial arch
bat, branchial artery
bc, blood cell
bv, filament vessel
cc, chloride cell
cd, cell in division
cg, supporting cartilage of gill filament (gill ray)
co, cartilage of operculum
cp, chloride cell with a pit
cr, heart rudiment
ef, efferent branchial artery
ev, efferent filament artery
f, forebrain
g, gut
som, somatic lateral plate mesoderm
spl, splanchnic lateral plate mesoderm
ha, hyoid arch
kv, Kupffer's vesicle
llo, anlage of lateral line organ
m, mesoderm
ma, mandibular arch
mt, metencephalon
my, myelencephalon
nc, neural cord
nch, notochord
nk, neural keel
oa, optic anlage
ob, opercular gill
oba, anlage of opercular gill
pp_1, first pharyngeal pouch
pp_2, second pharyngeal pouch
pp_3, thrid pharyngeal pouch
pp_4, fourth pharyngeal pouch
pc, pericardial cavity
pfa, anlage of pectoral fin

PLATE I

Photomicrographs of living *Chaenogobius urotaenia* embryos. The magnification on all of these figures is given in the measure indicated below the left of the plate. The blastoderms and embryos are photographed through the chorion except Figure 17 which is the photograph of the embyro after the removal of the chorion

1. Unfertilized ovum
2. Fertilized ovum
3. One-celled ovum, 1 hr
4. Two-celled ovum, 1 hr., 50 min
5. Four-celled ovum, 2 hrs., 25 min
6. Eight-celled ovum, 3 hrs., 30 min
7. Sixteen-celled ovum, 4 hrs., 30 min
8. Thirty-two-celled ovum, 5 hrs., 55 min
9. Blastula, 23 hrs., 55 min
10. Early gastrula, 28 hrs., 15 min
11. Late gastrula, 32 hrs
12. Formation of embryonic shield 48 hrs., 50 min
13. Formation of embryo, 72 hrs
14. Formation of optic vesicle, Kupffer's vesicle appearing, 80 hrs
15. Head and tail free from yolk, Kupffer's vesicle disappearing, Formation of optic cup. 97 hrs
16. Formation of lens. Formation of auditory vesicle, Heart beating, 125 hrs
17. Formation of ventral aorta and blood cells, Anus opening, 220 hrs
18. Just after hatching, 282 hrs



K. HAMADA: Development of *Chaenogobius urotaeaia*

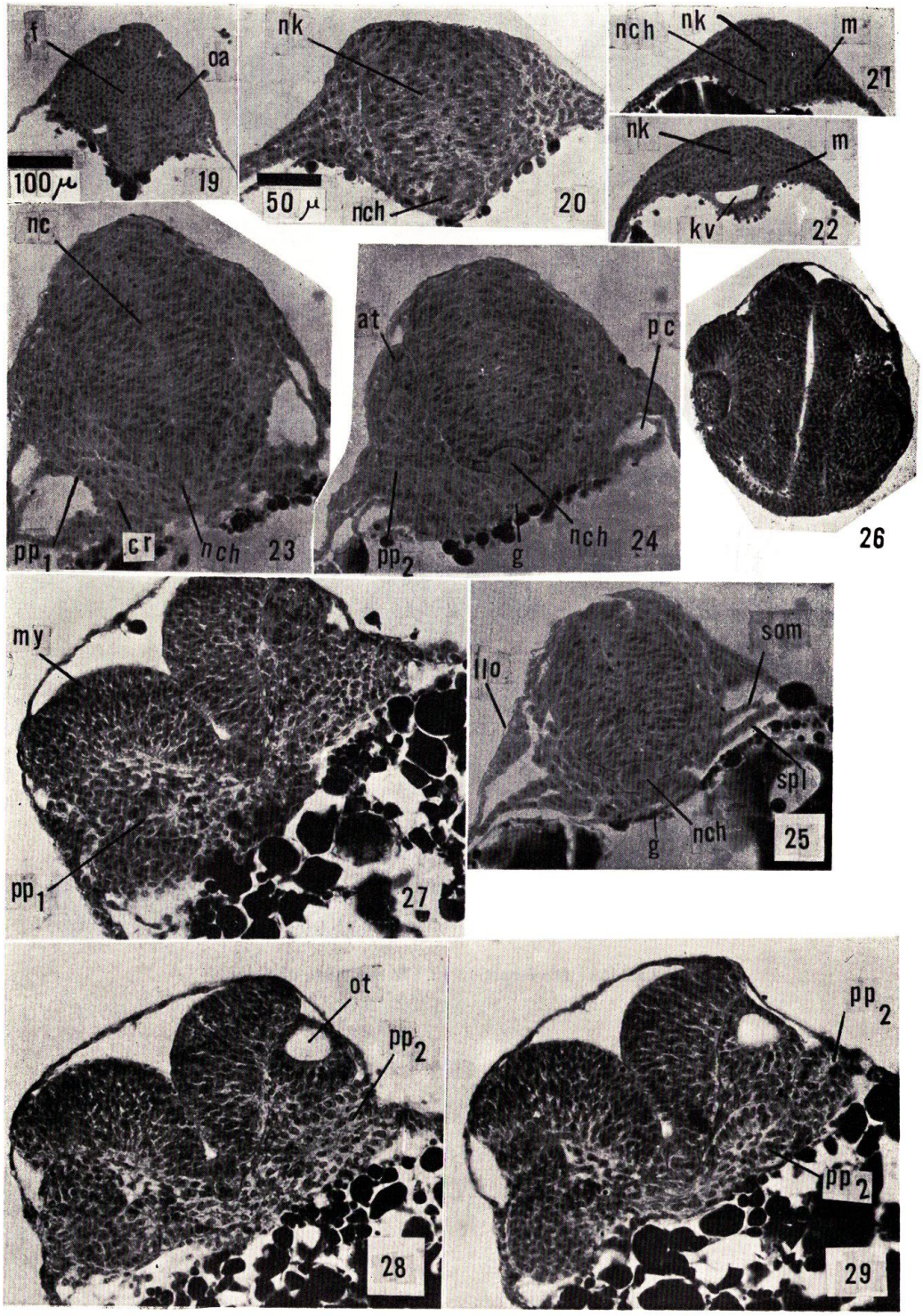


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PLATE II

The magnification on Figs. 19, 21, 22 and 26 is given in the measure shown in Fig. 19. On all of other figures, the measure is indicated in Fig. 20. Figs. 19, 20, 21 and 22 are transverse sections through a 1.6 mm embryo. Two days after fertilization

19. Section through optic anlagen (oa) and forebrain (f)
20. Section through tip of notochord (nch) and neural keel (nk)
21. Section anterior to Kupffer's vesicle
22. Section through Kupffer's vesicle (kv)

Figs. 23, 24 and 25 are transverse sections through a 1.8 mm embryo. Three days after fertilization

23. Section through first pair of pharyngeal pouches (pp_1)
24. Section through second pair of pharyngeal pouches (pp_2). Aduitory anlagen (at), pericardial cavity (pc) and gut (g) are seen
25. Section through mid body region. Somatic (som) and splanchnic lateral plate (spl) formation. Lateral line organ anlagen (llo) are seen

Figs. 26 to 30 are transverse sections through a 2.5 mm embyro. Six days after fertilization

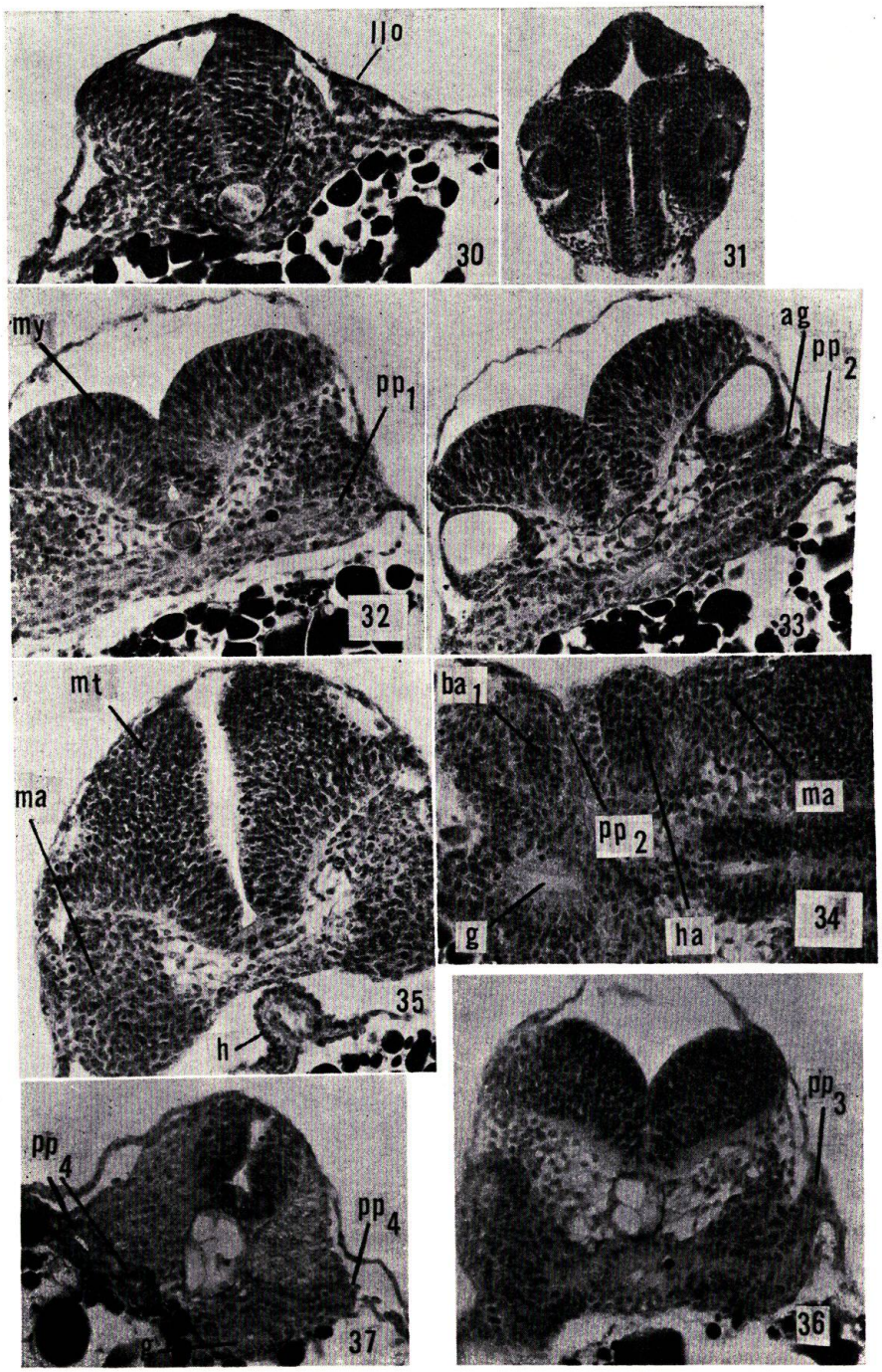
26. Section through optic cup. Lens formation
27. Section through first pair of pharyngeal pouches
28. Section through second pair of pharyngeal pouches. Owing to plane sectioning this is not visible on one side
29. Section through second pair of pharyngeal pouches (pp_2)

PLATE III

30. Section through region behind the head. Lateral line organ anlage more developed can be seen

Figs. 31 to 35 are sections through a 2.5 mm embryo and Figs. 36 and 37 are sections through 3 mm embryo. Fig. 34 is frontal section through embryo and all others are transverse section. Three days after fertilization. The magnification on figures except Fig. 31 is given as the same measure indicated in Fig. 20. Fig. 31 is equal to Fig. 19 in mangification

31. Section through optic cups. Lens developing
32. Section through first pair of pharyngeal pouches which have reached surface ectoderm
33. Section through second pair of pharyngeal pouches. Pouch breaks through surface ectoderm. There is a lumen in gut
34. Section through visceral arches (transverse section through arches), Second pharyngeal pouch breaks through surface ectoderm
35. Section through mandibular arch (ma). Heart beating
36. Section through 3rd pharyngeal pouch. Five days after fertilization
37. Section through 4th pharyngeal pouch. The pouch shown on left exhibits the appearance of the segmental duct, but it is due to the flexion of the pouch. It results from the fixation of the embryo. The detailed tracing assures its fact. Five days after fertilization



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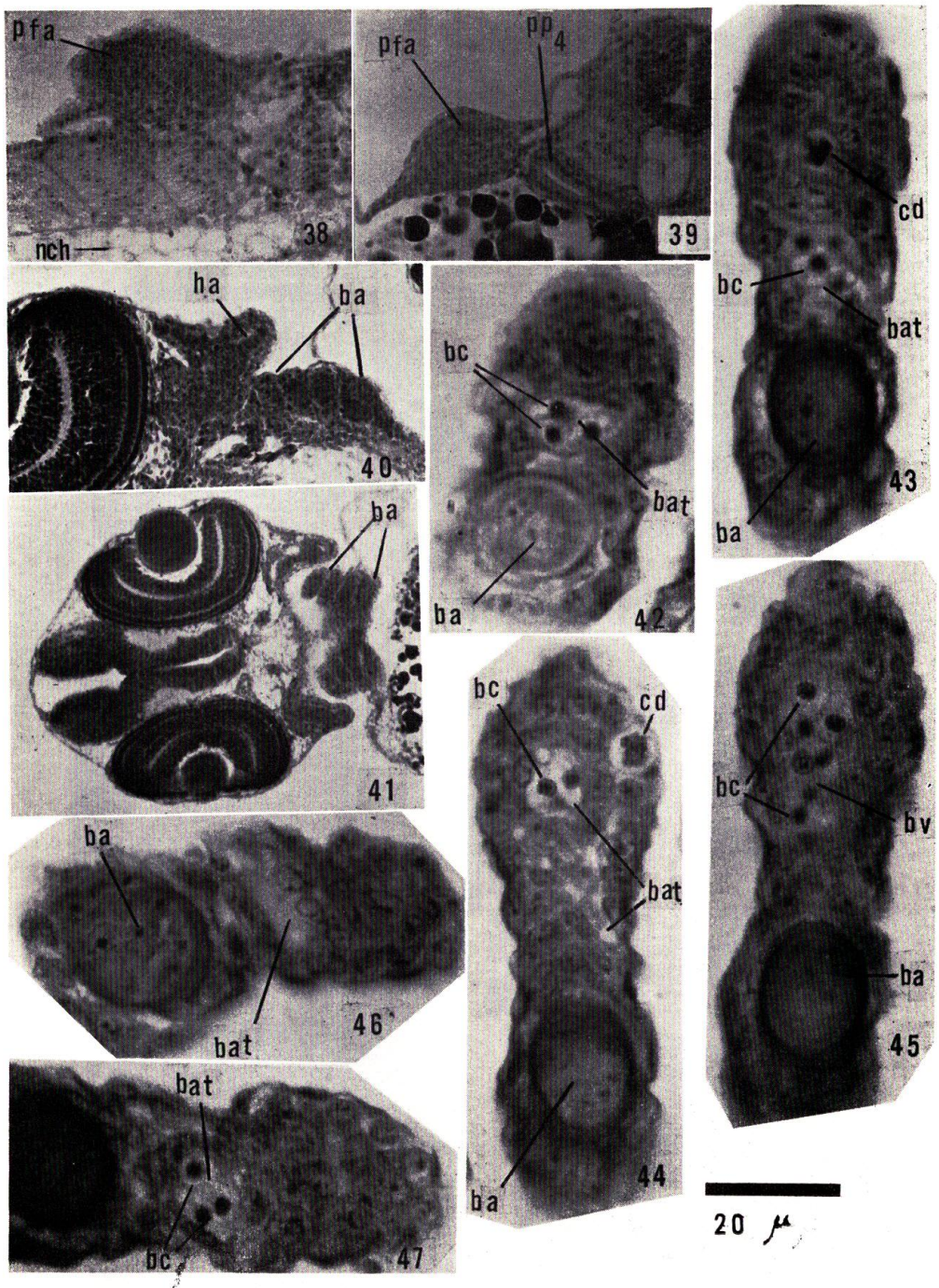

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PLATE IV

Figs. 38 and 39 are frontal and transverse section through pectoral fin of 3 mm embyros respectively. The magnification of these figures being equal to those of Fig. 20.

38. Section through pectoral fin anlage (pfa)

39. Section through pectoral fin anlage and 4th pharyngeal pouch. Owing to plane sectioning the pouch seems to be a sac, but it has already broken the surface ectoderm. Five days after fertilization

Figs. 40 and 41 are transverse sections through branchial arches of a 4.0 and a 4.2 mm embryos respectively. The magnification is equal respectively to Figs. 20 and 19. Figs. 42 to 48 are transverse sections through branchial arches of fry. The magnification is given in the measure shown below the right of the plate

40. Section through branchial arches. Six days afer fertilization

41. Section through branchial arches. Six days after fertilization

42. Section through 3rd branchial arch of a fry just after hatching. About 5.2 mm in total length

43. 44. 45. Section through 3rd branchial arches of a fry reared for 24 hours in fresh water after hatching. About 5.4 mm in total length. Gill filaments is more developed in comparison with Fig. 42

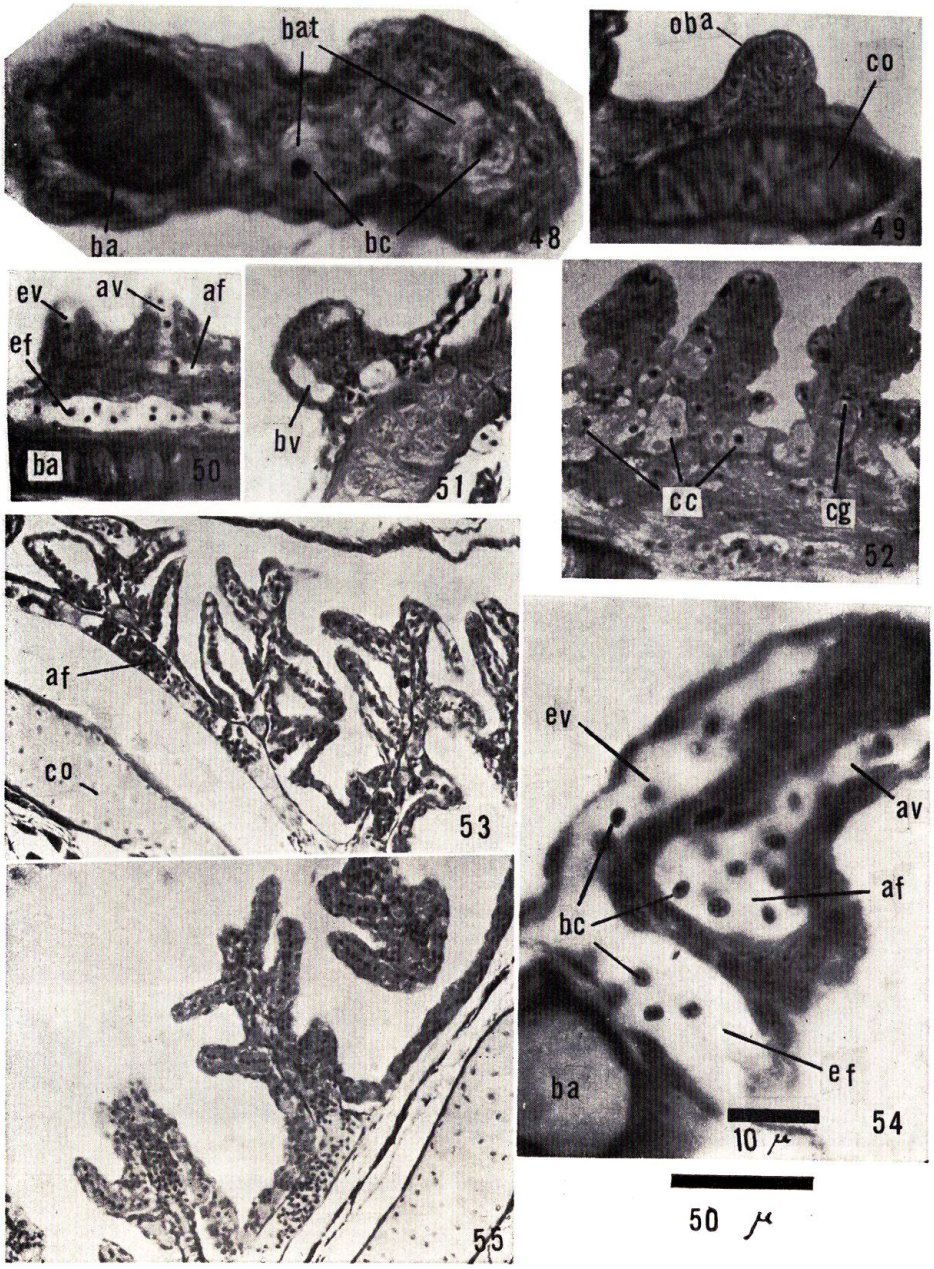
46. 47. Section through 4th arches of a about 5.5 mm fry reared 2 days in fresh water after hatching

PLATE V

48. Section through 4th branchial arch. Two arteries are seen. The same individual with Fig. 47

Figs. 49 and 51 are transverse section through operculum and anlage of opercular gill. The magnification of figures is given in the measure shown below the right of plate. Figs. 53 and 55 are frontal section through operculum, and opercular gills are sectioned in parallel to filaments. The magnification is the same as in Fig. 20. Figs. 50 and 52 are sagittal sections through branchial arch and gill filament. The magnification is the same as in Figs. 49 and 51

49. Section through anlage of opercular gill of a 5.5 mm fry reared for 2 days in fresh water after hatching
50. Section through gill filament of a 5.5 mm fry reared for 4 days in fresh water after hatching
51. Section through anlage of opercular gill of a 5.7 mm fry reared for 5 days in fresh water after hatching
52. Section through gill filament of a 7.0 mm fry reared for 18 days in sea water after hatching
53. Section through opercular gill of a 16.7 mm juvenile reared for 19 days in sea water and 16 days in fresh water after hatching
54. Section through branchial arch of a 6.7 mm fry reared for 13 days in sea water after hatching
55. Section through opercular gill of a 20.0 mm juvenile reared for 25 days in sea water after hatching



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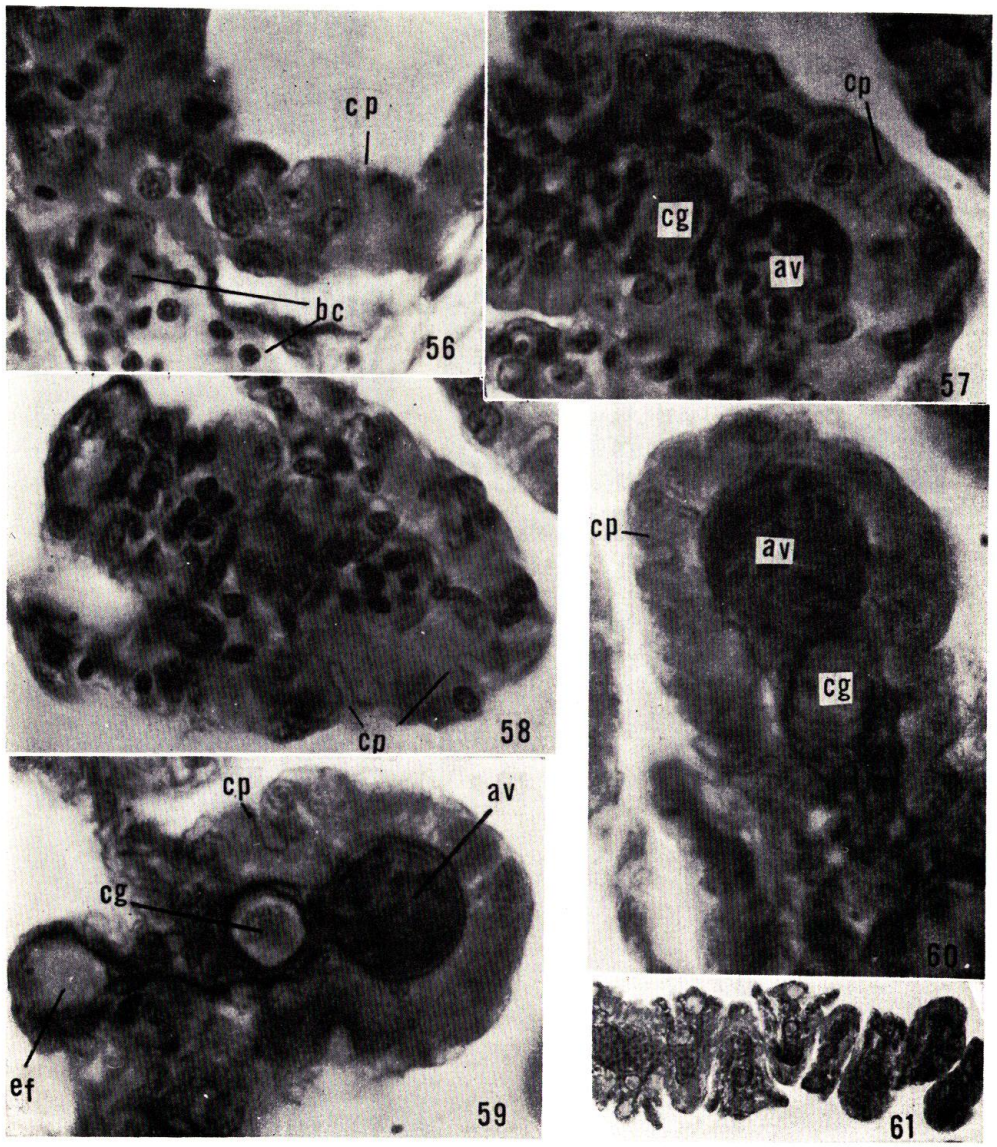

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PLATE VI

Fig. 56 shows proximal portion of opercular gill filament as shown in Fig. 55. Figs. 57 to 60 are transverse sections through gill filaments. The magnification of Figs. 56 to 60 is the same as in Fig. 42, and Fig, 61 is like Fig. 20

56. Section through chloride cell with a tubular pit

57. 58. Section through gill filament of a 20 mm juvenile reared for 25 days in sea water after hatching. Chloride cells are seen

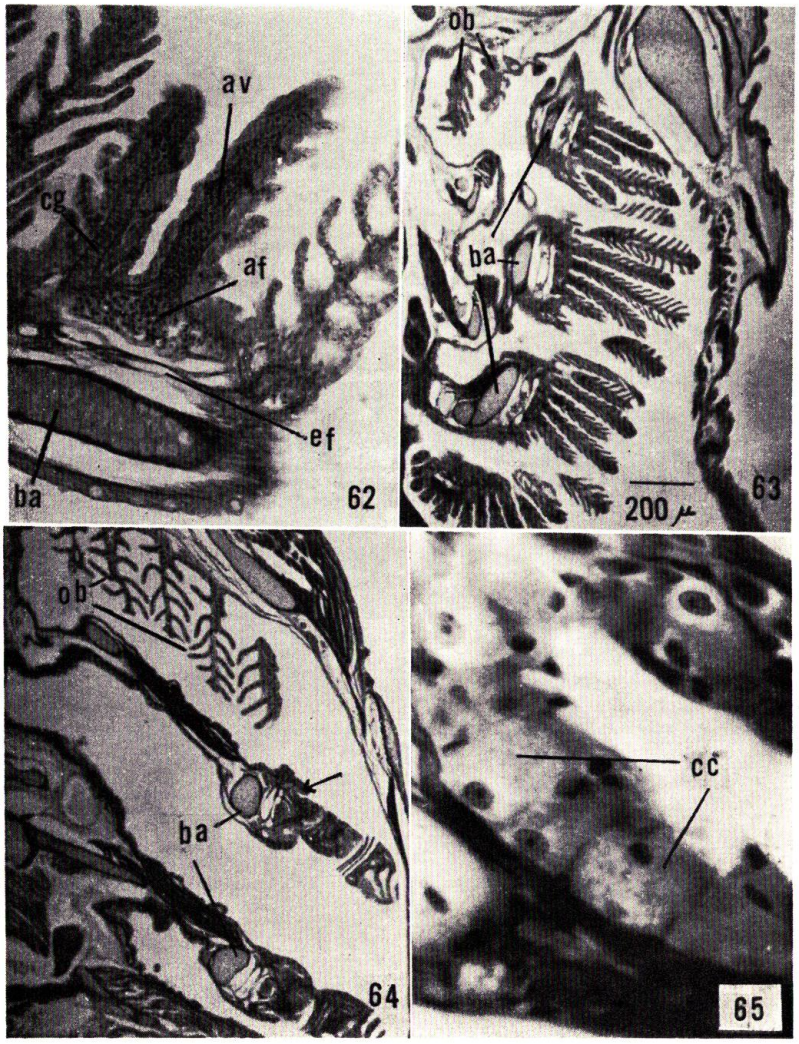
59. 60. Section through gill filament of a 18 mm juvenile reared for 34 days in sea water after hatching. Chloride cells are seen.

61. Section through gill filaments. The same individual as above

PLATE VII

Figs. 62 and 63 are longitudinal sections of gill filaments. Fig. 64 is transverse section through branchial arches. Fig. 65 is the proximal porition of gill filament shown in Fig. 64. Fig. 62 is like Fig. 20 in magnification. The magnification of Figs. 63 and 64 is given in the measure shown in Fig. 63. Fig. 65 is like Fig. 42 in magnification

62. Section through gill filaments of a juvenile about 18 mm reared for 34 days in sea water after hatching
63. Section through gill filaments of a juvenile about 26 mm reared for 47 days in sea water after hatching
64. Section through branchial arch of a juvenile about 28.5 mm reared for 45 days in sea water and 28 days in fresh water after hatching. Arrow indicates the position of next figure
65. Section through the cavity at the base of gill filament shown in Figure 64. Chloride cells without tubular pit are seen



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