THE DEVELOPMENT OF THE WEBERIAN SYSTEM AND EARLY EMBRYOLOGY OF PIMEPHALES PROMELAS (OSTEICHTHYES: CYPRINIDAE)

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Dedicated to David Niazi

Scholar, Teacher and

Father

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CHAPTER I

INTRODUCTION

From the standpoint of embryology the literature references concerning the origin and development of the Weberian Apparatus in the Cyprinidae, present controversial ideas as to the derivation of the ossicles. No literature is available concerning the origin of the apparatus in Pimephales promelas. The intention of this study is to test and either confirm or disprove the contradictory statements.

Since the early embryology of this fish has not been studied and since the nature of the work necessitates procurement of pertinent life history stages, an effort is made to describe the embryonic development of Pimephales promelas.

The subfamily Pimephalinae includes the nominal genera Hyborhynchus Rafinesque, Pimephales Rafinesque and Ceratichthys Baird and Girard. The first two are monotypic, including H. notatus (Rafinesque) and P. promelas Rafinesque respectively. Ceratichthys includes only two valid species, because C. callarchus Hubbs and Black is considered as a dubius species, represented only by the holotype. Hay (1888) considered Hyborhynchus synonymous with Pimephales and Bailey (1951) merged the three nominal genera in the genus Pimephales.

Pimephales notatus is intermediate between P. promelas and Ceratichthys. P. promelas has a longer, coiled intestine, while

Ceratichthys has an intestine with a single loop.

Cross (1953) demonstrated the need for the reduction of P. perspicuus to subspecific status under P. vigilax, in accordance with Hubbs' (1951) statement: "Recent work shows that C. perspicuus is conspecific with C. vigilax, as formerly suspected---."

Hubbs and Black (1947) presented characteristics and phylogeny of the Pimephalinae suggesting that this subfamily differs from other cyprinids in two respects: (1) the presence of a blunt, rudimentary spine-like ray attached by a membrane to the first developed dorsal ray, while in other American cyprinids the second ray has the form of a thin splint appressed to the third ray; and (2) nuptial tubercles in the breeding males are limited in number and arranged in one, two or three transverse rows on the snout, whereas in other minnows they occur in different patterns.

For separation of the species of Pimephales the following characters are in agreement with Moore (1957).

P. promelas is characterized by the presence of three rows, a fourth one between the nostrils may be present, of tubercles on the snout of breeding males; caudal spot indistinct or absent; body stout, its greatest depth contained about 3.2 - 4.0 times in standard length; and nuptial chin tubercles may be present.

The other three species have in common the following characteristics: slender body, its greatest depth contained about 3.9 - 5.1 times in standard length; a distinct caudal spot; and breeding males with 1 - 3 rows of nuptial tubercles on the snout. These three species can be separated using the following diagnostic features:

1. P. notatus is characterized by a black peritoneum or

at least black pigment present; upper lip overhung by a fleshy snout, mouth more ventral; and intestine variable in length, but usually with at least one transverse loop across the anterior end of the stomach.

2. P. vigilax and P. tenellus never have a black peritoneum and the gut is always short, without a transverse loop. P. tenellus differs from P. vigilax in having more distinct cross hatching, the absence of black pigment in crotches of the dorsal rays and a more slender body.

On the basis of an osteological study of the Weberian apparatus, Niazi (1960) concluded that the subfamily Pimephalinae can probably be divided into two groups with P. tenellus and P. notatus in one and P. promelas and P. vigilax in the other. These two species groups are supposed to have arisen separately in the evolution of the subfamily, on the basis of character similarities within each. Another conclusion was that the four species did not exhibit differences at the generic level.

CHAPTER II

HISTORICAL REVIEW

A. Fish Embryology

As a group, the teleosts follow a typical pattern in their development, however there are wide variations in the details of organogenesis in individual species.

Wilson (1889) described the embryology of the sea bass Serranus atrarius (Linnaeus). The eggs are pelagic, having a diameter of 1 mm and an incubation period of 75 hours at 15.5 C. The incubation period decreases at higher temperature. The chorion is very thin and horny. A single oil globule is peripherally embedded in the yolk and is always in the uppermost portion of the floating egg. The thin cytoplasmic layer that envelops the yolk in the unfertilized egg becomes concentrated opposite the oil globule after fertilization.

Cleavage is meroblastic and equal as typical in the Teleostei. The first two planes are meridional and at right angles to each other. They do not extend through the yolk and the resulting blastomeres are connected by what is called the central periblast, a thin protoplasmic layer.

A segmentation cavity is recognizable after the third cleavage, having a floor formed of the periblastic layer. Wilson, in describing the formation of the periblast, showed that the marginal cells of the

blastoderm lose their cell outlines and are drawn into the surrounding protoplasm. Periblastic nuclei, according to Hoffmann, work on the yolk in such a way that it becomes assimilable by the cells of the blastodisc. These nuclei do not contribute to any embryonic structure, but disappear concomitantly with the yolk.

The peripherally located cells of the blastoderm start their invagination most rapidly at a point corresponding to the dorsal lip of the amphibian blastopore. This centripetal growth results in the formation of the germ ring. To Wilson, the dorsal lip remains fixed while the rest of the germ ring migrates around and encloses the yolk.

During further growth the embryonic area in the region of the dorsal lip of the blastopore becomes marked from the rest of the blastoderm. This triangular area is called the embryonic shield. The extra-embryonic part of the germ ring, not included in the embryonic shield, becomes thin and flattened. Later the midline of the embryonic shield thickens as the neural keel.

By further development and differentiation the neural keel constricts from the surface ectoderm. The cells line up in two rows leading to the formation of the neural canal. Thus the neural canal is formed by delamination rather than folding as in higher vertebrates.

By the time the neural chord has begun to form in the trunk region, the formation of the notochord and secondary layers have also commenced, further increasing the median thickening. As the endoderm is established, the notochord assumes a cylindrical rod-shaped structure; its cells flatten in antero-posterior direction and become vacuolized. Vacuolization continues until hatching. In the vacuolized notochordal

cells the protoplasm forms a thin peripheral lining in which the nuclei are located with protoplasmic strands scattered across the vacuoles. By this time a well-defined sheath is formed around the notochord, becoming more conspicuous after hatching. The vacuolization, which probably puts an end to cell multiplication, does not extend into the posterior portion of the chorda. In this region the notochordal cells retain their unvacuolized embryonic property. Three days after hatching the chorda terminates in the undifferentiated caudal mass. The posteriorwise growth of the chorda is presumably dependent upon the character of the undifferentiated cellular mass.

The alimentary canal is formed from endodermal lamellae by folding along the midline. Balinsky (1961) observed that the endodermal plate concentrates toward the midline and becomes thicker, but the cavity of the alimentary canal does not appear until a very much later stage. When it does appear, it is formed by a separation of the cells along the middle of the endodermal cord cells.

Kupffer's vesicle is formed at the end of the postanal gut in a manner similar to the formation of the permanent gut, but the postanal gut is formed by thickening rather than folding of endoderm. Later in development, both the postanal gut and Kupffer's vesicle atrophy.

The degree of the embryonic differentiation varies in different species of teleosts. When the germ ring is at the equatorial position in the sea bass, the embryonic shield is just beginning to be visible as a triangular thickened area. At a similar stage in the position of the germ ring, the embryo of the pointed-nosed sole, Parophrys vetula Girard, has lengthened until it extends halfway around the yolk

(Budd, 1940). Once past this equatorial position, the germ ring pivots at the posterior pole, which remains stationary, while the anterior edge of the ring draws away from the head end of the embryo. After completely encircling the yolk, the germ ring pinches together and closes the blastopore. The cells of the germ ring make part of the caudal mass. The pointed-nosed sole has a transparent pelagic egg with a diameter of 0.9 mm. Hatching occurs after 90 hours of incubation at 13.0 C.

Budd (1940) also described the embryology in five other species. Some notes concerning his observations follow.

Pleuronichthys verticalis Jordan and Gilbert, has a pelagic egg of 1.07 mm in diameter, having a hexagonal pattern that extends through the entire thickness of the chorion. Hatching occurs in 86 hours at 13.8 C.

P. decurrens Jordan and Gilbert, has a pelagic egg of 1.44 mm in diameter. The germ ring forms a tight band around the yolk, causing a marked constriction. The incubation period lasts one week at a temperature of 13. 8 C.

P. coenosus Girard, has eggs of 1.88 mm. The embryonic shield never attains great size due to the slight invagination of the germ ring. Differentiation occurs after the closure of the blastopore. Hatching occurs in twelve days of incubation at 13.8 C.

Artedius lateralis (Girard) has a demersal egg, 1.07 mm in diameter, adherent to the substrate in tide-pools. The egg has a characteristic large, single, light cherry red, oil globule. Kupffer's vesicle appears after 73 hours of incubation. Hatching occurs after sixteen days at 15.5 C.

Clinocottus analis (Girard), has a brownish-yellow egg 1.30 mm in diameter. Several large oil globules and scattered groups of small ones are always present. Hatching occurs after 24-30 days of incubation at 15.5 C.

An unusual pink oil globule is characteristic of the egg of Oxyjulis californicus Günther. The eggs are pelagic and spherical with an average diameter of 0.76 mm (Bolin, 1930). Another unusual feature encountered was that about 50 percent of eggs showed two Kupffer's vesicles while the germ ring is equatorial in position. Hatching occurs after 48 hours of incubation.

Fry (1936) reported that the unfertilized egg of the Pacific mackerel, Pneumatophorus diego (Ayers), averaged 1.05 mm in diameter and had a single large oil globule which later in development became pigmented with melanophores. He did not make it clear whether a cellular membrane or the melanophores surround the globule as a network. In the fertilized eggs the very narrow perivitelline space was apt to be overlooked. Melanophores make their appearance after the embryo segmented. Hatching occurred after 50 hours of incubation at 18.5 C.

In the blue gourami, Trichogaster trichopterus (Pallas), a freshwater fish, the pelagic eggs average 0.71 mm in diameter, being characterized by a single oil globule which is always uppermost in the floating egg (Ingersol, unpublished thesis). Hatching occurs after 24 to 26 hours at 23 to 24 C. The germ ring moves around the yolk on all sides instead of pivoting from a stationary point near the dorsal lip of the blastopore as recorded by Wilson (1889).

Oppenheimer (1937), Solberg (1938) and Jones (1939) independently described the embryology of Fundulus heteroclitus. The eggs have an average diameter of 2.0 mm, and are covered by a thick shell-like chorion to which are attached a large number of uniformly distributed adhesive threads. The chorion and the threads are produced by follicular cells in the ovary (Solberg, 1938). He described the germ ring as growing over the yolk to the opposite side from which it started. Jones (1937), using the oil droplet as a landmark, disagreed with Wilson's description of the germ ring movement; while the oil droplet is consistently lateral to the embryonic axis, it is eventually covered by the downward growth of the posterior embryonic shield. The oil droplet may retard the downward progress of the germ ring, but it is completely enclosed before the formation of a distinct blastopore. There is a posterior as well as an anterior epiboly.

The eggs of the gizzard shad Dorosoma cepedianum LeSueur, according to Warner (1940), have a diameter of 0.75 mm, and an adhesive chorion. One hour after fertilization the two-cell stage is reached. Hatching requires 36 hours at 26.6 C, whereas if the temperature dropped to 16.8 C, hatching occurred after 95 hours of incubation. At hatching the larvae lack pigmentation on the body and the eye pigment appears three days after hatching.

The eggs of the goldfish as described by Battle (1940) are spherical, cream-colored, have a diameter of 1.25 mm and are surrounded by an adhesive chorion which permits the eggs to stick to vegetation. The brain is clearly visible and the optic vesicles are partially differentiated by the time the blastopore closes. The heart appears on the anterior surface of the yolk sac after 24 hours. Hatching

occurs in 76 hours at 25 C. Lower temperatures delay hatching as much as 14 days.

Harrington (1947) described the early life history of the bridled shiner Notropis bifrenatus (Cope), a minnow having spherical, demersal eggs of a diameter varying from 1.0 to 1.5 mm. The blastoderm envelops the yolk completely before the rudiment of the embryo is visible, unlike the whitefish which is well differentiated at this stage. Hatching occurs after 56.5 hours at 23.0 C. Eggs of Notropis girardi Hubbs and Ortenburger, developed from the blastodisc stage to hatching in 24 hours (Moore, 1944).

Carr (1942) studied the breeding habits and embryology of the largemouth bass Huro (=Micropterus) salmoides (Lacépède). The demersal eggs have a diameter of 1.60 mm and hatch after 47 hours of incubation at a temperature ranging from 23 to 26 C.

The eggs of the whitefish Coregonus clupeaformis (Mitchell), lack oil globules, by which the relative position of the germ ring can be judged. Price (1934) concluded that the closure of the blastopore occurs as in the sea bass with slight backward growth of the dorsal lip. This indicates that the closure involves epiboly of the ventral lip aided by concrescence and a slight backward growth of the dorsal lip. The unfertilized egg has a diameter of 3.0 mm. They are laid in a cold period, November, and thus the growth is so slow that 24 hours are required to reach the eight-cell stage. The blastopore closes after 22 days at 3.0 C. At this time the embryo is clearly outlined. Eleven pairs of somites and the notochord have differentiated with Kupffer's vesicle reaching its maximum development. By

67 days most of the organs have formed. According to Price (1935) the incubation period is 134 days at winter lake temperature of 1.5 C.

The embryology of the zebrafish Brachydanio rerio (Hamilton) was conveniently divided into 25 morphologically distinct developmental stages by Roosen-Runge (1938). The protoplasm separates from the yolk when the cell first absorbs water. This occurs directly before the first cleavage. The separation consists of streaming of the protoplasm through the yolk toward the blastodisc. At the same time a counter stream of the protoplasm is moving toward the vegetal pole.

The embryology of the live bearer, Platypoecilus maculatus Günther, has been described by Tavolga and Rugh (1947), Turner (1940) and Tavolga (1949). Tavolga and Rugh (1947) state, "the platyfish is truly a viviparous fish." Among Osteichthyes, viviparity is confined to four families of cyprinodonts (Poeciliidae, Goodeidae, Jenysiidae and Anablepidae), to the sea perches (Embiotocidae), a few blennies (Zoarces), and rockfishes (Scleroparei).

The development of the pericardial amnion and pericardial serosa, as described by Tavolga (1949) and by Tavolga and Rugh (1947) for Platypoecilus exhibits a remarkable similarity to the development of the amnion and serosa of avian embryos. Three important differences should be noted. One difference lies in the initial formation of these membranes from a single-layered periblastic ectoderm with a later invasion by mesenchyme to form a typical double-layered sac, an inner amnion and an outer serosa, both somatopleural. Second, there is the presence of a highly developed vascular plexus in the platyfish serosa. Third, the membranes enfold only the head region, and no caudal amniotic fold is present, as in avian embryos.

Fertilization of the ova takes place through a follicular canal leading from the ovarian cavity to each egg. The fertilized ova are retained within the follicles and all the embryonic development takes place there. The follicle ruptures before parturition and the larval fishes find their way from the ovarian cavity through the oviduct and out through the urogenital pore.

Tavolga and Rugh (1947) suspected that the pericardial serosa has a nutritive as well as a respiratory and excretory function.

The oldest papers concerning the micropyle of the fish chorion are those of Mark (1890), who described it in the egg of the gar Lepisosteus, and Eigenmann (1890) who studied the micropyle in nine osseous fishes. Hayes (1949) described the osmoregulatory function of the chorion in the "hardening" of fish eggs. This is a chemical change in the chorion during which it is converted to pseudokeratin to prevent the entry of other sperms.

Bottrell (1962, unpublished thesis) studied the embryology and egg membranes of Hybopsis aestivalis tetranemus Gilbert and, from sectioned mature ovaries, determined that the chorion was one layer in thickness and radially striated much like that reported by Eigenmann (1890) for the golden shiner. Eigenmann supported Mark (1890) who concluded that these striations are the pore canals.

Balinsky (1948) proposed a table of developmental stages for Cyprinidae. He listed 46 stages for the development of the fishes from the unfertilized egg through postlarval stages and included as criteria, characters that are specific for fishes native to Europe. He pointed out that standard length cannot be used to determine the age of the larvae.

Armstrong (1936) indicated that there are two factors that operate to induce hatching in Fundulus heteroclitus eggs: (1) lashing movement of the tail of the embryo and (2) an enzyme liberated from unicellular structures that disappear after hatching. The hatching enzyme is produced within the mouth and pharyngeal cavities. Iwai (1962) in his manuscript dealing with the development of the lateral line cupulae in Tridentiger trigonocephalus (Gill) found that there are scattered small glands over the preorbital, frontal and mandibular regions. The glands were filled with refractive granules before hatching. Rubbing the head against the chorion brings about the release of the contents of these structures, hatching glands, which disappear after hatching.

B. The Weberian Apparatus

The literature concerning the Weberian apparatus has been reviewed by several writers (Krumholz, 1943; Nelson, 1948; Weitzman, 1954; Bridge and Haddon, 1889) and many others.

E. H. Weber (1820) in his paper "De aure et auditu hominis et animalium" was the first to describe the anatomy of the ossicles, having found them in Silurus glanis, (but according to Adams, 1928, they were first seen by Rosenthal). They were named after Weber and termed the Weberian ossicles (Bridge and Haddon, 1889). Isis von Oken (1821) described the anatomy of the apparatus in Cyprinus brama. Reissner (1859) studied the ossicles in some siluroid fishes and Nusbaum (1881) gave a description of the apparatus in some Cyprinidae. One of the first North American investigations was made by Wright (1884) when he described these bones in Amiurus catus (sic).

Krumholz (1943) in citing Wright (1884), introduced some doubt concerning Wright's material by indicating "Amiurus catus (Ameiurus nebulosus)". He may have doubted the identity of Wright's material or falsely considered A. catus a synonym of A. nebulosus. Bridge and Haddon (1889, 1892) gave the initial account of the anatomy of the Weberian ossicles of Nematognathi. They called the bones the "Weberian ossicles" rather than auditory ossicles and described the elastic spring apparatus, "Springfederapparat", of Johannes Müller (1843), an outgrowth of the transverse process of the fourth vertebra in some catfishes. Nusbaum and Sidoriak (1899) described the Weberian apparatus of Cobitis (= Misgurnus) fossilis. Evans (1924-1925) studied the air bladder and Weberian ossicles of Cyprinidae from the standpoint of anatomy and physiology. More recent investigations concerning the Weberian apparatus were made by Hora (1922), Chranilov (1926, 1927, 1929, 1930), Adams (1928), Wunder (1936) and von Frisch (1938). Krumholz (1943) comparatively studied the ossicles in North American ostariophysine fishes. Nelson (1948) studied the comparative morphology and systematic significance of the Weberian apparatus in the Catostomidae.

Ostariophysaea was the name given by Sagemehl (1891) to include those fishes possessing Weberian ossicles. Jordan (1929) defined the series Ostariophysi as "fishes with the anterior vertebrae modified to connect with the air-bladder and enclosing an organ of hearing". This series includes the nominal orders Eventognathi (suckers, minnows and loaches), Nematognathi (catfishes), Heterognathi (characins) and Gymnonoti (electric eels, etc.) the latter occurring in the tropical regions of Central and South America.

In reviewing the literature, it was found that different authors applied different names for the ossicles. The nomenclature proposed by various authors is presented in the table below.

TABLE I

NOMENCLATURE OF THE WEBERIAN OSSICLES BY VARIOUS AUTHORS

Author	Date	Names of Ossicles in Anteroposterior Sequence			
Weber	1820	Claustrum	Stapes	Incus	Malleus
Müeller	1853	Claustrum	Steigbügel	Ambos	Hammer
Bridge and Haddon	1889	Claustrum	Scaphium	Intercalarium	Tripus
Thilo	1908	Hinlage	Deckel	Lenker	Hebel

The nomeclature of Bridge and Haddon is preferred because it avoids any confusion with the non-homologous auditory ossicles of Mammalia.

Many workers supposed that the ossicles originated by simple detachment of the portions of the anterior vertebrae. All theories were based on morphology, an insufficient guide to reveal origins.

CHAPTER III

MATERIALS AND METHODS

Adult fish, juveniles, post larvae, larvae, embryos and fertilized eggs of Pimephales promelas were used in this study. Some adults were used as in my previous investigation (Niazi, 1960, unpublished) for gross morphological examination. Fish used for stripping ranged in length from 45 to 65 and 40 to 55 mm for males and females respectively and were seined from two ponds near Stillwater, Oklahoma. The number of specimens sectioned or otherwise used was 129.

The process of stripping can be summarized as follows: sexually mature fish were selected and placed between the second and fourth fingers, leaving the third finger as support from beneath on the back of the fish. Slight abdominal pressure forces ripe gametes out. Many of the males when so treated, ejected a thin stream of milt with a force similar to a jet from a hypodermic needle.

During the process of stripping, many fishes, especially the males, produced a croaking noise. The origin and function of the sound has not been investigated, but it may have resulted from the pressure of the fingers or from natural causes. It is well known that many fishes normally produce sounds (Delco, 1960; Winn, 1960).

Gravid females stripped much easier than the males. Eggs from many females were mixed with a viscid, jelly-like fluid that spurted

on the finger when little pressure was put on the abdomen. Reference will be made to this material in connection with observations on egg collection.

Gametes from stripped females and males were transferred into a watch glass containing water barely covering its bottom. Sperms were stripped first in one case and the eggs in the other. The transfer of the gametes was done by means of a wet, soft camel hair brush. The contents of the watch glass were then examined with a binocular microscope, to see if fertilization had occurred. The fertilized eggs and other gametes were then placed inside a jar in a bucket of pond water and transported to the laboratory as soon as possible.

Aerated tap water was used instead of pond water since the latter was turbid. The colloidal particles in pond water adhere to the chorion and make observation difficult.

In the summer of 1962 natural spawning, for unknown reasons, was not observed. The usual stripping method was ineffective. Another method, suggested by Dr. R. W. Jones, consisted of mixing teased testicular tissue with stripped eggs, but this also failed to yield fertilized eggs.

Hoping to obtain fertilized eggs by natural spawning in the laboratory, sexually mature individuals were placed in an aquarium of about 30 gallons capacity under incandescent light. The fish retreated under a board provided as a nesting site, but refused to spawn. Substitution of pond water for tap water and indirect sunlight for incandescent light failed to yield satisfactory results. Several attempts were made to strip the females in the laboratory. No fertilizable egg was obtained if stripping process was effected a few hours after the

fishes were collected. This phenomenon has been reported also in stripping the females of Notropis bifrenatus (Harrington, 1947). It was found that the gravid females contained undersized eggs. During the summer of 1962 the pond contained huge numbers of the predaceous backswimmer Notonecta. Failure to obtain eggs directly from the pond during 1962 may be associated with the presence of the large population of the backswimmer. This needs to be investigated as well as the reason for the size regression of the eggs.

Pimephales promelas is a nest builder and, according to Markus (1934), the male chooses the location of the nest. The eggs are laid on the underside of objects that are horizontal or nearly so. Markus found only one instance where a nest was built on a stake that was at right angles to the surface of the water where no normally suitable site was available. Eggs may be found under any sizable object that permits the activity of the male. Wynne-Edwards (1933) found nests on the undersides of many of the pads of the yellow water lily, Nuphor advena. According to Markus (1934), several batches of eggs first laid in the spring and the last group of eggs laid at the end of spawning season, dropped off, the day after they had been deposited. These eggs, upon examination, were found undeveloped.

In connection with my studies, boards, with smooth surfaces, placed in the pond in shallow water with aquatic vegetation were accepted as spawning sites. No eggs were found on the undersides of rocks in the bottom of the pond or on the parts of submerged vegetation.

There seems to be no fixed time preferable for spawning, but it was found that early morning was the best time to collect the earliest

stages of development; where temperature is near 70 F. This is in agreement with Markus (1934) who made the following general statement: "Eggs were always deposited during the night." The females seemed to prefer a flat-surfaced floating board to deposit their eggs, unless a board was not available. The number of spawning areas were reduced to collect more eggs from fewer boards. This indicates that one nest can be used by more than one female. The eggs were collected from the boards by means of a sharp scalpel. They were then transferred to a jar containing aerated tap water, filtered pond water or regular pond water. The jars were placed in a bucket of pond water and transported to the laboratory. Eggs thus collected, always had adherent sediment. Such eggs are difficult to clean but, by holding them with fine forceps and rubbing with a rough-edged needle, the sediment was removed. Picking the wooden particles with fine forceps often ruptured the chorion. The containers, in which the eggs were placed, were cleaned thoroughly to avoid foreign particles. After cleaning, the eggs were separated into groups of developmental stages in shallow containers and placed in an incubator.

After hatching, the prolarvae were collected by a wide-mouthed eye dropper and transferred to one-gallon aquaria labeled as to time and date of hatching. Eating started when the yolk sac was consumed. The food used consisted of commercially prepared micrograin, powdered egg yolk, and infusoria tablets.

Measurements of larvae were taken with a calibrated ocular micrometer. Juveniles and adults were measured with dividers.

Four fixatives were utilized in preparing specimens for this study: ten percent formalin; Tellysnicky's fluid was used for specimens having

a length less than six millimeters, since the solution does not tend to harden the yolky substance; Bouin's fluid was used as a general fixative; and Smith's fluid was used for early yolky stages.

After fixation the specimens were washed in running water if they were treated with formalin or Smith's fluid. In the latter case the washed specimens were preserved in three percent formalin. Other specimens were preserved in 50 percent isopropanol. The shortest method of dehydration with equally satisfactory results was found by using three changes of tetrahydrofuran with 30-minute intervals. Infiltration methods utilized were those generally used in microtechnical works; but the use of tetrahydrofuran, modified from Salthouse (1958) as an enhancing agent, shortened the time at oven temperatures. In this modification the specimen was transferred from the third change of tetrahydrofuran into a mixture of equal parts of tetrahydrofuran and Tissuemat in a tightly-capped jar and left for 30 minutes in a 60 C oven. The specimen was then transferred to pure Tissuemat and left in the oven for another 30 minutes.

Embedding was done under a binocular microscope to facilitate orientation of specimens in Tissuemat.

Tetrahydrofuran is highly volatile at room temperature. Material placed in a tightly-capped jar containing a solution of the material and Tissuemat are rapidly infiltrated by positive vapor pressure.

Embedded specimens were serially sectioned with a rotary microtome at 10 microns in the transverse, sagittal and frontal planes. The stiffness, sharpness and angle of the blade were of high importance in obtaining satisfactory sections.

Microscope slides were cleaned in dichromate cleaning solution and thoroughly rinsed in distilled water. The ribbon was cut into strips arranged in successive rows on the slides. The stips were affixed to the slides with a gelatin fixative (Sass, 1940). The strips were flooded with this mixture and placed on a 55 C warming table to flatten. The solution was then either drained or blotted with a damp paper towel and allowed to dry at least 48 hours. Sass' solution proved to be excellent in that sections seldom fell off the slide during staining.

Hematoxylin and eosin was not as satisfactory as Mallory's triple connective tissue stain. The method as presented in Guyer (1953) was modified to get the best possible differentiation. The modifications are summarized below. The slides were left for 15 seconds in the acid fuchsin followed by a distilled water wash, and transferred for three minutes in the second solution after which they were passed through two changes of 90 percent isopropanol, followed by three changes of 95 percent isopropanol, upgraded and cleared in xylene, and mounted in Piccolyte. It was found that the two changes of the 90 percent alcohol are necessary to give good differentiation. The longer the slides were left in the second change the better the results.

Specimens longer than 10 mm were decalcified in two percent hydrochloric acid in 80 percent alcohol for not more than five days, depending on size, before dehydration, clearing and embedding. Adult specimens for morphological study were stained with alizarin red-S (Hollister, 1934).

Photographs were taken with a Kodak 35 mm Pony IV camera mounted on a Spencer trinocular microscope.

No attempts were made to maintain a constant incubation temperature, however, the temperature fluctuated between 76 and 80 F.

Some of the eggs were kept in the incubator at 79 $\pm$ 1 F, except for short intervals while checking.

CHAPTER IV

OBSERVATIONS

A. The Morphology of the Adult Weberian System of Pimephales promelas

The Weberian system of Pimephales promelas consists of four functional units, (Plate 1-Fig. 1). These are:

(1) the transforming unit (Weberian chamber)

(2) the Weberian ossicles (pars auditum)

(3) the supporting unit (pars sustentaculum) and

(4) the registering unit (membranous labyrinth).

1. The Transforming Unit (Weberian chamber). The retroperitoneal, two-chambered, gas bladder is connected by a long, narrow-lumened pneumatic duct originating slightly to the left dorsal surface of the esophagus and connected to the anteroventral surface of the posterior chamber. The chambers are joined through a narrow ductus communicans surrounded by a sphincter innervated by a branch from the vagus nerve. The anterior or Weberian chamber consists of two coats. The outer, tunica externa, exhibits a pear-shaped slit anteroventrally occupied by the ventral portion of the ossa suspensoria (Plate I, Fig. 2). This part of the ossa is called the central plate (Evans, 1924-1925). On either side of this slit the external coat is thickened forming a ligament that passes downward to attach to the blunt extremities of

the ossa ventrally. The outer coat extends backward over the internal coat to the ductus communicans behind which the tunica interna only constitutes the posterior chamber (Plate II, Fig. 4).

2. The Weberian Ossicles (pars auditum). The pars auditum consists of four ossicles on each side of the first three vertebrae, named in anteroposterior order, the claustrum, scaphium, intercalarium and tripus.

The claustrum (Plate I, Fig. 1 and 4) is an inverted cup-shaped bone with a slightly convex triangular crest-like projection on its convex surface. The crest is connected posterodorsally by connective tissue to the anterolateral surface of the saddle-like second neural arch. The claustrum covers more than half of the posterior scaphial cavity leaving an aperture for communication with the atrium sinus imparis.

The scaphium (Plate I, Fig. 1 and 3), according to Krumholz (1943) consists of four parts:

(1) concha stapedis, a cup-shaped cavity;

(2) a rounded prominance from the outer lateral surface, of the concha, to which the interossicular ligament is attached;

(3) the ascending process;

(4) the articulating process.

The articulating process is a short knob fitted into a pit on the dorsolateral surface of the first centrum. The ascending process is on a straight line with the articulating process, and extends dorsoposteriorly at about 45° from the vertical. Anteriorly it lies under the second neural arch and posteriorly in front of the anterior

edge of the third neural pedicle. It is connected to both by connective tissue. The concave surface of the scaphium is directed anterodorsally toward the spinal cord. As seen from the convex surface, the scaphium bears a shallow groove extending at right angles from the axis of the ascending and articulating processes to the anterior rim of the cup. A foramen, of undetermined function, near the base of the articular process, is present. The articular processes of the scaphium and intercalarium were considered by Niazi (1960) and Niazi and Moore (1962) to be homologous with the pyramidal roots of the third neural pedicle and fourth basidorsal. The ascending processes of the scaphium and intercalarium were considered as postzygapophyses of the first and second vertebrae respectively.

The intercalarium (Plate I, Fig. 1 and 5) is cresentic in shape in that the shaft is curved upward. Through its alar condyle, of Krumholz (1943), it is connected by the interossicular ligament to the ventroposterior osseous nipple-like projection of the scaphium anteriorly and to the anterior ramus of the tripus posteriorly. The intercalarium articulates with the second centrum by a rounded, short, articulating process fitted into a socket on the dorsolateral surface of the centrum. On the other side of this articular process there is a slender extension which is directed upward and backward to the anteroventral edge of the third neural pedicle.

The tripus (Plate I, Fig. 1 and 6) is the largest bone in the series and consists of five parts.

1. The body is convex on its ventral and concave on its posterodorsal surface, with a concave shelf in front.

2. The anterior ramus is firmly continuous with the interossicular ligament.
3. The articular process is located on the medial side of the body and articulates with the ventrolateral surface of the third centrum anteroventrally to posterodorsally. The process has a groove along its surface into which a ridge from the lateral surface of the third centrum is fitted.
4. The posterior ramus, thinner than the anterior ramus, extends posteriorly beneath the medial extension of the fourth pleural rib.
5. The transformator process, a delicate hooklike recurved extension, is embedded in the tunica externa of the anterior air chamber ("Weberian air-chamber" of Chranilov). Anteriorly the transformator process, near its termination, is connected to the os suspensorium by a small triangular, unstriated muscle, the tensor tripodis of Bridge and Haddon (1889), Evans (1924-1925). The apex of the tensor tripodis is connected with the os suspensorium. Two good characters can be used as specific peculiarities to distinguish the tripus of this species.
   (1) A sharply declivous shelf extending below the portion of the anterior ramus and dorsally with a deep concavity.
   (2) The body is usually not fenestrated.

3. The Supporting Unit (pars sustentaculum). The pars sustentaculum consists of the first four modified vertebrae, none of which

is fused with others in the genus Pimephales. The first vertebra has a disc-like centrum with two, big, pit-like depressions on its dorso-lateral side to hold the articular processes of the scaphia; one very small pit lies on the mid-ventral surface. The vertebra has two short ribs near the anterior margin of the centrum, extending forward and then outward. A long ligament connects their extremities to the posterior edges of the supracleithra. The ribs are firmly fused to the centrum while distally they penetrate the horizontal myosepta. Watson (1939) called them dorsal ribs in the goldfish. The first vertebra lacking a neural arch, is joined anteriorly to the conical centrum-like proatlas of the basioccipital (Harrington, 1955) and posteriorly with the second centrum. The anterior face of the centrum is slightly concave. Its posterior face is extended dorsally backward, producing a concavity.

The second vertebra consists of a centrum, neural arch and long ribs. The anterior face of the centrum extends anteriorly in its ventral portion and the ribs are fused to the centrum anteroventrally. Dorsolaterally the second centrum has a pit on each side into which the articulating processes of the intercalaria are fitted. The second centrum has one midventral pit.

The third, a ribless, vertebra consists of the amphicoelous centrum, the neural pedicles and the neural complex. Laterally viewed, the centrum has the aspect of three cones with their apexes meeting at a common locus in the lateroventral surface. This configuration presents two grooves in the lateral surface, one extending diagonally about 50° downward and backward; the other characterized by a ridge extending dorsoposteriad about 50° with it. The groove in the articulating process

of the tripus fits over the ridge within this groove, producing a hinge. The upper cone, duplicated on the opposite side, is inverted and hollow to receive the pyramidal root of the neural pedicle. The posterior wall of the upper cone extends posteriad over the posterior face of the centrum. The two upper cones are separated by an antero-posterior medianly placed bridge. The bridge is widest posteriad and pointed anteriad. A ventromedian bridge joins the anterior and posterior faces of the centrum and is constricted in its middle. The neural pedicles consist of three parts, the pyramidal roots each fitting into the upper cone dorsolaterally on the centrum. The stem extends a short distance dorsal with a pit near its base. The third part is the body which extends anteriorly over the second centrum. The dorsal and anterior edges of the body are thick. The body articulates anterodorsally with the second neural arch, dorsally with the basal arch of the neural complex and posterodorsally with the prezygapophysis of the fourth vertebra.

The neural complex is treated as having three parts, the basal arch, the stem and the boat (Niazi, 1960). The basal arch articulates ventrolaterally with the neural pedicle, anteriorly with the second neural arch and posteriorly with the fourth neural arch. The short stem extends dorsally as a compressed crest. The boat is the uppermost portion and opens posteriorly. The general shape of the neural complex shows great variability. The more constant features are briefly stated below.

1. The boat is deep, its anterior, big, blade extends in front of the boat and the stem, reaching over the posterior portion of second neural arch.

2. The stem is wide and short, being sculptured with many pits.

The fourth vertebra is the least modified, consisting of the following parts: (1) the centrum, (2) the pleural ribs, ossa suspensoria and (3) the neural arch with its spine. The amphicoelous centrum viewed laterally shows three pyramids, dorsal, anterior and posterior. Dorsally the centrum shows two pyramidal cavities with a posteriorly and anteriorly widened, narrow bridge. The pyramidal roots of the fourth neural arch are fitted into the dorsolateral pyramidal cavities while the heads of the pleural ribs are accommodated by the latero-ventral cavities. Posteriorly the neural arch gives off a post-zygapophysis. The neural spine is usually short, being connected by a ligament to the posterior edge of the neural complex. The pleural ribs, widest near their articulation with the fourth centrum, extend downward, slightly backward and outward from the fourth centrum. Usually there is a depression, just under the articulation of the rib, containing three or four pits separated by struts. From the point where the ribs extend anterolaterally, a knob articulates with the proximal part of the fifth rib by a strong ligament. The pleural rib divides into two portions, the rib proper and the ossa suspensoria, an impression acquired when only the adult is considered. The ossa suspensoria extend posteromesad then turn abruptly downward, without making connection with each other. The space between the ossa is wider dorsally than ventrally. The ossa are slightly inclined forward in about 18°. The space between the ossa and the fourth centrum accommodates the dorsal aorta. The posterior ramus of the tripus passes beneath the median extension of the fourth pleural ribs. The tensor tripodis is inserted on the transformator process of the tripus

and has its origin on the ossa (Evans, 1924-1925).

4. The Registering Unit (membranous labyrinth). The utriculo-saccular canal issues from the floor of the utriculus below the crus commune. The canal opens into the saccular roof, in front of which the saccular cavity extends for a short distance. Posterior to the opening, is the anterior edge of the medianly situated saccular macula. In a transverse section passing through the region, the anteriorly narrow lagena is widely separated from the sacculus and is dorsolateral to the saccular cavity.

The ductus endolymphaticus issues from the median region, dorsal to the saccular macula. Laterally, the lagena opens into the saccular cavity. Two common fibrous partitions project internally between the two sacs, which join posteriorly to separate the two cavities again as they do anteriorly.

Anteriorly, the dorsomedian lagenal macula becomes medianly situated posteriad. The two endolymphatic ducts project in a medioposterior direction rather than median as usually illustrated in the literature. The two join forming the sinus endolymphaticus which is bathed in the perilymph of the sinus cavum imparis which is continuous with the perilymph around the membranous labyrinth through a tiny canal on each side, overlooked by previous investigators, at the junction of the two endolymphatic ducts. The lagena becomes wider posteriad and extends further back than the posteriorly narrow sacculus.

The sinus imparis extends posteriorly to the endorhachis of the perineural tube where it projects on both sides as the atria sinus imparis which are enclosed by the claustra and scaphia.

The lapillus lies on the floor of the utriculus, with its ventral, convex surface on its macula. The asteriscus, the largest otolith in the species, stands on its edge with its convex surface laterad and the plane surface medial toward the lagenal macula. The sagitta occupies the median edge of the sacculus. Its plate-like anterior expansion lies higher than its needle-like posterior end.

B. The Eggs of Pimephales promelas

The earliest eggs were collected 22 May 1961; spawning continued until the first half of September. In some of the egg collections made from nests, small, thin, transparent, yellowish plates by which the eggs were attached to the board were found. Many females, when stripped ejected a yellowish fluid with the eggs. Observations indicate that the material hardens when ejected in the water and serves as an adhesive agent secreted when the eggs are ready to be oviposited. Eggs of females, not producing the fluid, were unfertilizable and undersized. The gametes are ejected by both sexes on the underside of the boards. It was reported by Markus (1939) that early and late batches of eggs dropped off and did not develop.

The eggs are laid in chains (Plate III, Fig. 1) arranged in a single layer. Markus (1934) and Wynne-Edwards (1933) found some eggs in two layers. Variable numbers of eggs were found per board but four hundred eggs per board was not uncommon. Such nests contain different stages in embryonic development. It is very probable that more than one female uses the same nest. Markus (1934) and Wynne-Edwards (1933) reported egg diameters of 1.15 and 1.30 mm respectively. The micropyle, apparently, was overlooked by both.

The eggs are adhesive, demersal and of variable diameter depending on whether they are mature or not. The eggs collected by stripping had an average polar diameter of 0.963 mm and equatorial diameter of 0.946, being almost spherical. In one region the chorion is depressed like a funnel from the center of which seven striations radiate outwardly (Plate III, Fig. 2). The depressed region of the chorion is called the micropyle and is, according to Rugh (1960), the only possible point of insemination. The yolk is opaque, milky-yellowish in color. When such unfertilized eggs were placed in water the chorion, in the region of the micropyle, began to bulge. The chorionic expansion remains restricted to the micropylar region.

Another phenomenon was noticed when some eggs were mixed with milt. The chorion became completely separated by a clear perivitelline fluid and the cytoplasmic material collected under the region of the micropyle. Cytoplasmic caps showed buds projecting into the perivitelline fluid. Some buds were pinched into the perivitelline fluid making it turbid, others were reabsorbed into the cap (Plate III, Fig. 3).

A similar situation was noticed by R. W. Jones (personal communication) in the eggs of Brachydanio rerio. Such zygotes may or may not develop into a normal embryo. In P. promelas such abnormal eggs did not develop beyond the "fertilized" egg stage. The unusual behavior is apparently an uneven flow of the cytoplasm, caused, in case of the zebrafish egg, by mechanical injury during siphoning the eggs from an aquarium. Equivalent factors such as the effect of the brush in cleaning operation may be involved in the eggs of P. promelas. The

unusual behavior may result from a rise in temperature during transportation of the eggs.

C. The Fertilized Egg and Developmental Stages

The first change occurring after the mixing of the gametes is the separation of the chorion from the yolk, in the region of the micropyle, by a fluid-filled perivitelline space. The change starts fifteen minutes after the mixing. When the eggs were checked in the laboratory, thirty minutes later, the whole cytoplasm was collected in the form of a cone establishing bipolarity. The egg in the one-cell stage (Plate III, Fig. 4) remains so for the next ten minutes. The telolecithal, fertilized eggs have the following average measurements in millimeters: chorionic diameter, 1.286; the equatorial diameter of yolk, 0.972; polar diameter, 0.648; and the height of the cytoplasmic cap 0.412. A variable number of differently shaped, superficially embedded oil globules in the yolk were seen nearer the vegetal pole. Eggs from the same female show variability in number and shape of globules. In unfertilized eggs, globules were not seen and it is believed that their appearance is brought about by pressure inside the chorion resulting from the filling of the perivitelline space.

With the advance of embryonic development the chorion becomes less adhesive because of a chemical hardening involving conversion of the chorion to pseudokeratin. Cleavage is meroblastic and extremely discoidol. The following information, as far as timing is concerned, is based on observations of developing embryos. The times given represent average intervals and are not in reference to a single embryo. The temperature during embryonic development varied

between 76 and 80 F.

Two-celled ovum (Plate III, Fig. 5)

Forty minutes after fertilization, the blastodisc starts its first meridional cleavage and is completed in seven minutes.

Four-celled ovum (Plate III, Fig. 6)

Approximately one hour and five minutes after fertilization the embryo was in the four-cell stage. The second meridional cleavage cuts across the plane of the first at right angles. It starts ten minutes after the completion of the first division and is completed within ten minutes. The four resulting globular blastomeres are equal in size and persist for about ten minutes.

Eight-celled ovum (Plate IV, Fig. 1)

One hour and twenty-five minutes after fertilization, the third cleavage started. This is also meridional and parallel to the plane of the first. The resulting eight blastomeres, smaller than in earlier stages, are arranged in two rows of four cells each. Oppenheimer (1937) suggested that the long axis of the eight cells may become the future embryonic axis.

Sixteen-celled ovum (Plate IV, Fig. 2)

The fourth cleavage is complete approximately one hour and fifty minutes after fertilization. The plane of the cleavage is parallel to the second and at right angles to the first and third. According to Wilson (1889), during this stage the segmentation cavity is established as the central cells pull away from the central periblast, a thin protoplasmic layer on the yolk connecting the peripheral cells

at their bases. It is formed as a result of failure of cleavage furrows to cut through the blastoderm. After this stage cleavage becomes irregular.

Blastula (Plate IV, Fig. 3)

After an incubation period of about two hours and forty minutes the elevated cap of the blastoderm is deeply constricted from the edge of the yolk. The situation is equivalent to the early high blastula stage (Hisaoka and Battle, 1958). Viewed dorsally from the animal pole, the albuminous blastoderm is oval in outline, being constricted in two opposite regions. The cell boundaries stand out as dark lines in lateral view. Approximately three hours after fertilization the cellular mass becomes more compact as the cells get smaller and the constriction between the cellular mass and the yolk lessens conspicuously. One hour after this change the blastoderm flattens, with a smooth edge, on the surface of the yolk. The margin of the blastoderm blends with the yolk curvature and thirty minutes later the egg assumes a spherical shape.

Gastrulation (Plate IV, Fig. 4 and 5)

Five hours after fertilization epiboly begins. The peripheral cells start their involution to form a thickened edge of the germ ring which becomes clear in the next hour. The germ layer is the thickened edge of the blastoderm, which continues in epiboly to cover one-half of the yolk seven hours and twenty minutes after incubation began. One area in the germ ring is slightly thicker than the rest as a result of cellular involution. This is the beginning of the embryonic shield and is equivalent to the dorsal lip of the blastopore in amphibian embryos.

Viewed from the animal pole, the embryonic shield is a clear area and from it the embryo proper will develop. No embryonic axis is visible. The edge of the germ ring is closer to the animal pole in the region of the embryonic shield.

Ten hours and fifteen minutes after fertilization epiboly has continued so that more than three-fourths of the yolk is covered. The embryonic axis is barely visible but a large yolk plug is present.

Closure of the blastopore (Plate IV, Fig. 6) and (Plate V, Fig. 1)

As the germ ring advances toward the vegetal pole the ventral edge of the embryonic shield slows in its migration. As a result of retardation the uncovered yolk is pear-shaped rather than circular in appearance. An average incubation period of eleven hours is required before the embryonic axis almost encircles the yolk. Forty-five minutes later one mesodermal somite makes its appearance. The brain is recognizable.

Optic vesicle stage (Plate V, Fig. 2)

After fourteen hours of incubation the optic anlagen are recognizable as lateral expansions from the prosencephalic region (viewed dorsally they look like an arrowhead). Three to four pairs of somites usually can be detected. The notochord extends from the rhombencephalic region to the undifferentiated caudal mass.

Otic placode (Plate V, Fig. 3)

The otic placodes make their appearance in the region of the metencephalon, after about 21-22 hours of incubation. The posterior yolk mass shows partial constriction. Kupffer's vesicle, not clearly

recognizable in the species, makes an appearance in the 17-somite (average) stage. The tail region begins to round, being elevated terminally only. A small pericardial cavity, located below the head, rests on the yolk. The first muscular contraction usually starts within the next hour when the tail bud becomes partially freed from the yolk sphere. In the C-shaped embryo the muscular contractions start in the region of first myotomes. Fifteen minutes later the contraction involves the whole region directly behind the spherical yolk mass. The contraction is expressed in such a way that the tail and head regions come closer together.

Optic-cup stage (Plate V, Fig. 4)

The optic vesicle starts invaginating to form the optic cup and the lens occupies the mouth of the cup. The 20-somite stage was reached after about 24 hours of incubation exactly as in the zebrafish (Hisaoka and Battle, 1958). The embryo has increased in length posteriorly, freeing 0.324 mm of the caudal region. The otic vesicles are dorsoventrally compressed and have a thickened rim. The perivitelline fluid becomes turbid.

Heart beat

Within 27.5 hours of incubation, the heart starts beating irregularly. One hour later its beats average eleven per minute. The S-shaped tubular heart can be seen through the left eye below the head. There is no circulation, but a fluid is seen inside as the heart contracts and relaxes. The embryo forms almost a complete circle and about the posterior one-fourth of its length is freed from the yolk. Aggregations of yellowish white cells, the blood islands, can be seen

on the yolk. The pectoral fins, the first fins to appear, are represented by tiny pads in this 26-somite stage.

Circulation

After thirty hours of incubation the blood cells are seen in a slowly moving stream, common cardinals, on the sides of the yolk sac. The blood cells are similar in shape and color to the constituents of the blood islands, the components of which are washed into the blood stream. In the dorsal aorta, a single row of blood cells is also seen streaming. The median fin fold can be seen only in the tail region. One hour later the blood stream moved faster inside the body, but was still slow in the common cardinals. The precursors of the otoliths were seen, in some embryos in this stage, as very tiny dots. The tail has elongated considerably so that it touches the head.

Otoliths (Plate V, Fig. 5)

The otoliths become clear as two concretions in each otic vesicle representing the lapillus and the sagitta and not the asteriscus and sagitta as Harrington (1955) reported. This fact is supported when larvae were studied in serial sections, as will be discussed later. The retinae may become pigmented, although the degree of pigmentation is subject to considerable variation in different embryos. The pericardial cavity enlarges, becoming more anterior as incubation progresses. The pectoral fins become more elongated. The choroid fissure becomes clearer as retinal pigmentation increases.

After these stages the embryo is in the prehatching stage, not assignable to a definite time (Plate V, Fig. 6).

After about forty-five hours of incubation the blood islands have disappeared and the blood with a yellowish-red color moves faster in definitely outlined vessels. The pectoral fins are elongated and narrower at their bases, having a leaf-like appearance. The median fin fold becomes more prominent and some mesenchymal cells aggregate in it. The retina possesses more pigment. Few exceptional embryos hatched after 48 hours of incubation. Usually the embryonic activity, lashing movements of the tail, increases. The hatching glands are seen as bright nodules anteriorly in the head region.

Within fifty-six hours of incubation the heart is beating at an average rate of 120 per minute and increases to 138 per minute after two hours.

After seventy hours of continuous incubation, many embryos hatched. The temperature was maintained at 79 $\pm$ 1 F. Other embryos of the same age did not hatch although kept in the incubator.

Hatching is usually tail first (Plate VI, Fig. 1), but a few embryos hatched head first (Plate VI, Fig. 2). The newly hatched prolarvae are transparent except for the retinal and blood color; a few embryos hatched with unpigmented retinae. The total length of the prolarvae varied between 4.0 and 4.5 mm, with 32 to 34 pairs of somites. The heart is located anterior to the reduced yolk sac. A few stellate chromatophores, only on the yolk sac, are clearly visible one hour after hatching. The fin fold is completely formed on the dorsal surface and is continuous with the rounded caudal fin into which the notochord protrudes as a straight rod. This fold extends anteriorly on the median ventral surface, forming a post- and a short preanal portion. The incubation period varies between 84 and 120 hours at a temperature

range between 76 and 80 F. One hour after hatching the prolarvae move upwardly almost to the surface, in an inclined line, then they stop wiggling and fall to the bottom of the container and start another trip. Heart beat averages 158 per minute. The eyeballs are not co-ordinated, each moving independently of the other.

CHAPTER V

THE DEVELOPMENT OF THE WEBERIAN SYSTEM OF PIMEPHALES PROMELAS

Length is the only possible criterion correlated with ontogenetic differentiation. Age of prolarvae, larvae and postlarval stages, cannot be used as a criteria. The number of somites cannot be used because organogenetic development is not uniformly correlated with the number of somites as they are added. Specimens raised in aquaria and comparable in size to those seined from the pond show exactly the same degree of differentiation.

The Weberian system consists of four functional units. Development of the units was studied, using serial sections of life history stages of graded lengths, and is outlined below.

A. The Developmental Relationship of the Transforming Unit in P. promelas

The main objective of the developmental investigation was to reveal the relationships between the classical units of the apparatus and the air bladder (anterior chamber). Thus the posterior chamber will not be considered in detail except where it shows specific peculiarities that have not been described in other species.

The darkly colored primordium of the air bladder is microscopically detectable toward the end of the first day after hatching.

It is inflated early in the third day and lies above the remnant of the incompletely consumed yolk. The primitive air bladder represents the posterior chamber of the adult fish. It increases rapidly in length and toward the end of the third day averages 0.49 mm in length. The anterior (Weberian) chamber develops, 14 to 16 days after hatching, from an anterior diverticulum from the posterior primitive chamber. Diagramatically the developmental stages of the gas bladder are represented in Plate II, Figs. 2, 3, and 4. In the 3.75 mm stage its primordium is represented by a tiny dorsal esophageal diverticulum (Plate II, Fig. 2). It is surrounded by mesodermal material similar and continuous with that around the gut. In the newly hatched (Plate VI, Fig. 3), 4.10 mm embryo, the externally undetectable chamber has a narrow lumen projecting from the dorsal surface of the gut. The stage is equivalent to the 11.2 mm larvae of Catostomus commersoni described by Nelson (1959).

The increase in the length of the larvae is accompanied by structural changes in the developing air bladder. The first of the changes takes place when the larva attains the length of 4.40 mm. A peculiar intraepithelial substance makes an appearance in this stage (Plate VI, Fig. 4) and stains yellow with Mallory triple connective tissue stain. The material is located on the sides of the cross-shaped lumen. Low columnar epithelium protrudes into the lumen from four directions. Another change includes the appearance of concentrically arranged connective tissue fibers around the bladder. In the posterior region of the chamber, the epithelial lining is partially stained, suggesting the presence of gas secreting cells. The pneumatic duct enters the primitive chamber anteriorly and slightly to the left.

As more yolk is consumed the gut occupies a more median position.

The yellow staining, structureless material increases when the larva reaches the length of 4.65 mm, and the lumen enlarges its cross-shaped cavity, with a squamous epithelial lining (Plate VI, Fig. 5). This yellow material is located on the sides which project into the lumen outside the lining and is lacking anteriorly and posteriorly. The upper and lower lining protrudes to a lesser degree than the lateral. Outside the yellow material, low cuboidal epithelium becomes thinner dorsally and thicker laterally. Another characteristic of the stage involves thickened, lateral, crescentic fibers on both sides of the primitive chamber.

The yellow material disappears when the larva attains a length of 5 mm. The fibers fuse to form the wall of the chamber, the lining flattens markedly as in the membranous labyrinth, and the only low columnar epithelium and distinct concentric fibers left are around the junction of the pneumatic duct with the chamber.

The anterior chamber begins to show as a hollow anterior diverticulum above the opening of the pneumatic duct in larvae ranging in length between 5 and 6 mm. In the 5.75 mm stage (Plate VI, Fig. 6), the anterior chamber is represented by a cuboidal lining surrounded by colorless, structureless intraepithelial material surrounded by thin concentric fibers, forming the tunica interna, a continuation of the posterior chamber. The tunica externa, composed of mesenchyme, occurs only outside the interna of the anterior chamber. When the anterior sac is formed it has an oval, dorsoventrally compressed shape, its two, low, columnar, epithelial layers come closer together anteriorly

and posteriorly while they are separated in the rest of the sac.

In the 6.25 mm stage (Plate VII, Fig. 1), the concentric fibers become more compact and few are applied to the outer epithelial layer. The intraepithelial substance increases and consequently the lumen narrows. The mesenchyme layer is thin dorsally and ventrally.

The third cartilaginous basiventral, connected through a mesenchymatous band to the tunica externa, is revealed in sagittal sections (Plate VII, Fig. 2).

The intraepithelial material in the anterior chamber disappears completely and in the 7 mm stage the concentric fibers become more compact on the outer epithelal layer (Plate VII, Fig. 3). The enlarged lumen, of the anterior chamber, is now lined by squamous epithelium, and its tunica externa consists of compact mesenchyme. In the posterior chamber the epithelium and the surrounding tissue have already consolidated to form a single-layered wall.

In the 7.5 mm stage the anterior chamber is composed of tunica externa and tunica interna. The externa exhibits an anterior fibrous thickening in which the ossa suspensoria are embedded and becomes clearer as fish length increases (Plate VII, Fig. 4). The ductus communicans clearly has a sphincter of smooth muscles innervated by a branch from the vagus nerve (Plate VII, Fig. 5).

The Initial Inflation of the Air Bladder. The manner in which the teleost swim bladder is initially inflated is open to controversy. Vogt (1842) states that it is accomplished by gulping air from the surface. Jacobs (1937) according to Johnston (1953) believes that gulping stimulates the activity of the gas-secreting tissue of the swim bladder.

The initial supply of gas is believed to be derived from cellular breakdown (Power, et al., 1932; McEwen, 1940).

With the controversial ideas in mind, two types of experiments were conducted using larvae of P. promelas. In one experiment, some newly-hatched larvae were placed in culture dishes filled with water and covered by cloth to prevent the larvae from reaching the surface. The whole process was performed under the water. In the second experiment a few prehatching embryos were placed in cloth-capped dishes submerged in the aquaria. Hatching occurred inside the dishes and the larvae remained trapped in them. Other larvae were left free in another aquarium. The latter swam to the surface, but whether they gulped air or not is a matter of conjecture. Under these experimental conditions, all the larvae developed normal, gas-filled bladders in about the same time period as those larvae with access to the surface.

B. The Development of the Weberian Apparatus

In the region of the first four vertebrae, of the newly-hatched embryos (4.0 - 4.5 mm), the notochord and its sheaths are surrounded by a thin mesenchymatous layer. The cartilaginous arcualia of the first three vertebrae appear when the larvae attain an average length of 5.0 mm and the rest of the arcualia are completed at the 6 mm stage (Plate VII, Fig. 2). The centra start to ossify when the larvae attain an average length of 6.25 mm.

1. Scaphium. In larvae of 6.25 mm, the scaphium rudiment is represented only by the first basidorsal which has elongated dorsally on the side of the spinal cord (Plate VIII, Fig. 3). It is continuous with the mesenchymal sheath dorsally and ventrally. The saccus

paravertebralis starts here as a very small space separating the centrum from the musculature. In 6.5 mm larvae the scaphium shows the first sign of the ossified concha stapedis. The concha stapedis is represented by a tiny bony strip extending anterodorsally (Plate VIII, Fig. 4), embedded in mesenchyme and not in direct osseous connection with the cartilaginous first basidorsal. The sacculi evaginate medially to form the ductus endolymphaticus (Plate VIII, Fig. 5). The horizontal plates of the exoccipitals begin to ossify and the saccus endolymphaticus projects as a small posterior protrusion from the united endolymphatic ducts. Another important correlation is that the perineural material starts to show its fibrous nature only on the dorsum of the first centrum between the two basidorsals. The concha stapedis becomes enlarged and connected by osseous substance with the first basidorsal at the 6.75 mm stage (Plate VIII, Fig. 6). The ascending process of the scaphium is formed in cartilage and the atria sinus imparis are distinguishable in the scaphial cavities. The conchae have thin mesenchymal sheaths through which the interossicular ligament, appearing in this stage, merges with them on their lateral edges. The ligament, earlier was represented by a mesenchymal band. In the 7.38 mm stage the perineural material thickens above the first centrum and the scaphial cavities enlarge in an anterodorsal direction. The atrium widens as a result of this enlargement and the constriction of the perineural material between the first two basidorsals. The perineural material assumes the fibrocartilage aspect in the 8 mm stage. Following the horizontal plate of the exoccipital in a posterior direction, the cavum sinus imparis is roofed by the floor of the perineural tissue. A fibrocartilage,

the endorhachis of Kindred (1919), forms a compact, thick pad resting on the dorsum of the first centrum. The roof and side walls of perineural tube remain thin. Its thick floor contains two capillaries diverging posteriad, while converging into one anteriad, and leave the pad at the posterior limit of the first centrum. The first spinal nerve issues directly behind the first basidorsals, through the perineural tube which lacks a roof behind this region. The saccus paravertebralis enlarges as the fish attain greater length. The perineural tube is not a posterior extension from the exoccipital, since its floor overlaps the latter (Plate IX, Fig. 1).

The first basidorsals form the ascending and articulating processes of the scaphia. Each articulating process, with a thin bony coat, fits into a shallow pit of the first centrum. On attaining the length of 8.5 mm, the scaphium assumes its adult shape. A nipple-like prominence on the lateral surface is added by ossification of the interossicular ligament at its attachment with the concha (Plate IX, Fig. 2). The ascending process ossifies also. The articulating processes gradually move into the deepened pits lined by thin fibrous layers, acting as a synovial lubricant, at the 9.0 mm stage (Plate IX, Fig. 3).

2. Claustrum. This ossicle has the shortest and fastest developmental history. Its mesenchymal primordium is continuous with that of the scaphium in their earliest stage. At 7.38 mm the primordium starts to show a faint staining reaction of osseous material. It starts to ossify in the 8.0 mm stage as a narrow bony strip embedded in mesenchyme (Plate IX, Fig. 4). Anteriorly its crest is higher

while posteriorly it descends almost to the level of the first basidorsal. Dorsally it is attached to the second neural arch by connective tissue and assumes the adult, inverted-Y shape, on reaching the 9.0 mm stage (Plate IX, Fig. 5).

3. Intercalarium. The second basidorsal makes an appearance as a cartilaginous nodule when the larva attains an average length of 5.0 mm. It elongates upward as an ascending cartilaginous process at the 6.25 mm stage. Between the 6.5 and 7.0 mm stages, an ossified nodule appears in the interossicular ligament very close to the second basidorsal (Plate VIII, Fig. 6). The two are joined by mesenchyme which ossifies to form the shaft of the intercalarium at 7.0 mm (Plate IX, Fig. 6). The original tiny nodule in the interossicular ligament is called the alar condyle (Krumholz, 1943) or the manubrium incudis (Watson, 1939). The cartilaginous, articulating process of the intercalarium elongates and its shaft increases its length in 8.0 mm specimens. The cartilaginous ascending process ossifies in the 8.5 mm stage, and its articular process lies in a pit similar to that described for the scaphium.

4. Tripus. In the 6.25 mm stage the small cartilaginous third basiventral is connected by a mesenchymatous band to the tunica externa of the Weberian chamber (Plate VII, Fig. 2). Anteriorly it is connected by a similar mesenchymatous band extending anteriorly to the scaphoid rudiment. The interossicular ligament appears at the 7.0 mm stage clearly attached to the anterior part of the third basiventral. The arcualia are covered by a small bony cap. From its posteroventral surface the third basiventral is also attached to the fourth basiventral

by a fibrous band. At the 7.65 mm stage, the tunica externa shows a thickened fibrous anterodorsal part in which a tiny bone, the transformator process of the tripus, is embedded. From the transformator process a fibrous band, in which a bony strip is embedded, extends anteriorly and contributes to the posterior ramus of the tripus (Plate X, Fig. 1). Anteriorly the fibrous band contributes to the peripheral portion of the body of the tripus and its anterior ramus. Mediodorsal to the ligament, mesenchyme connects it to the third basiventral. This mesenchymatous tissue may represent a phylogenetic remnant of the third rib. In front of the anterior ramus the ligament widens. The third basiventral extends diagonally anteroventrad along the lateral surface of the third centrum. The ossified part of the body of the tripus, at 8.0 mm, extends lateroventrad from this mass and, in cross section, gives the impression of a pleural rib attached to the basiventral. During this developmental period the anterior ramus elongates as a result of more ossification in the interossicular ligament. On reaching the 9.5 mm stage the tripus attains adult shape (Plate X, Fig. 2). The basiventral remains cartilaginous but is reduced and coated by bone. The ossified body of the tripus is located in the saccus paravertebralis, traversed by areolar connective tissue. The fibrous tissue from the third basiventral is attached to the fourth basiventral at the region where the median extensions of the ossa issue.

The tensor tripodis (Plate X, Fig. 3) is a triangular muscle that connects each transformator process to its respective os suspensorium. This muscle was detected first in a specimen 14.5 mm long, but it is probably formed earlier. Failure to find it is probably due to its close proximity to the transformator process and its tiny size

in earlier stages. Examination with oil immersion objective reveals that it is smooth muscle rather than ligamentous as considered by Nelson (1948).

Ossification of the first four centra starts when the larva attains an average length of 6.25 mm. The first two vertebrae possess dorsal ribs but no basiventrals were detected. Since they can be mesenchymatous and the ribs fuse where the arcualia should be, their presence may be obscured. The first rib is very short and attached by a ligament to the supracleithrum; it penetrates the horizontal myoseptum and retains its position as development proceeds. It ossifies between the 7.0 and 8.0 mm stages and is represented by a tiny osseous projection. The second rib in larvae of 9.0 mm is greatly elongated, reaching near the subcutaneous region. In the 7.0 mm stage a cartilaginous mass, dorsally in contact with the third basidorsal, appears above the first three centra. The third basidorsal remains cartilaginous and forms the third neural pedicle, with the neural complex above it. The third neural pedicles as well as the base of the neural complex are covered by a bony coat, but in continuous cartilaginous connection with the rest of the dorsal mass. In the 7.0 mm stage a median cartilaginous extension from the dorsal occipital region extends posteriad above the first centrum between the spinal cord and the dorsal cartilaginous mass. In the 7.65 mm stage, the portion of the cartilaginous mass above the third centrum projects slightly upward as a hump (Plate X, Fig. 1). The dorsal median ligament, between two longitudinal muscles, extends from the posterior surface of the skull to the hump. Another muscle extends from the hump in a posterior direction. A frontal section shows a Y-shaped skeletogenous septum in which the development of the neural

complex can be followed. In the 8.5 mm stage, fibrous strands are seen in the skeletogenous septum. The hump develops a bony spine (third neural) at the 9.0 mm stage. Interspinous, cartilaginous structures appear in the 10.5 mm stage. The most anterior one is above the fourth neural spine and connected by a fibrous sheath of the dorsal ligament. The neural complex is represented by a tiny anterior bony projection from the third neural spine. The neural complex assumes the adult shape, a boat open posteriorly and a blade projecting anteriorly, in the 14.5 mm stage (Plate X, Figs. 4 and 5). In the alizarin stained specimens, the second neural arch stains as bone and is separated by a transparent space from the base of the neural complex. The latter is separated similarly from the third neural pedicles. Sectioned specimens show that all the structures are cartilaginous, but coated with thin bone which breaks in the transparent portions referred to above.

The development of the ossa suspensoria has already been mentioned in connection with the development of the Weberian chamber. At the stage of 10.0 mm a central plate is joined by bony connection to the fourth pleural rib which ossifies at the 8.5 mm stage. The extension issues where the fibrous band from the third basiventral attaches to the fourth basiventral. Other than bearing the ossa, the fourth vertebra does not show principal modification. Other modifications in the first four vertebrae include the cartilaginous fourth and third pre- and postzygapophyses. Those of the third vertebra are part of the third neural pedicles.

C. The Development of Vertebrae

The development of the unmodified vertebrae follow the same pattern described by Mookerjee et al. (1940) for Lebistes reticulatus. Directly after hatching the vertebral column of P. promelas is represented by the notochord and its sheaths surrounded by mesenchyme. The centrum is formed by the ossification of the sheaths together with the noncellular perichordal material. The notochordal nuclei are scattered in the protoplasmic strands that traverse the vacuolated center. The nuclei, the notochordal epithelium of Mookerjee et al. (1940), are concentrated more toward the periphery of the notochord and are responsible for the secretion of the intervertebral ligament. The secretion becomes fibrous in later stages of development. Intervertebrally the notochord bulges as a knob, being surrounded by unossified perichordal material, best seen in a frontal section (Plate VII, Fig. 6). In later stages some of the notochordal strands ossify, but never to the degree of occupying the whole space (Plate VIII, Fig. 1).

The neural arches start as mesenchyme, and the arcualia appear as small, cartilaginous nodules, not sitting directly on the notochord but separated by a thin mesenchyme layer. This takes place when the larva is 5 to 6 mm in length. In front and behind the arcualia the neural arch remains mesenchymatous to ossify later as the pre- and post-zygapophyses. The rest of the arch is preformed in cartilage.

The notochordal epithelium inside may be regarded as endosteal whereas the mesenchymal covering of the centra and arcualia is periosteal. The bone of the fish is accellular and the explanation of bone formation is probably based on direct secretion of osseous material with movement

of the cells away from the secreted material, as in dentine formation. Another possibility is that the "osteoblasts" lose their power to secrete the osseous substance and are trapped by secretion of other "osteoblasts". It seems very probable that they do not reach the osteocyte stage, but rather are reabsorbed or destroyed directly after being trapped. Acellular bone is not uncommon in fishes and needs special study.

D. The Development of the Membranous Labyrinth of *P. promelas*

The development of the membranous labyrinth in the bony fishes differs basically from that of amniotes because the otic placode originates only from the interior sensory layer of the ectoderm without invagination as in the amniotes. It rather forms a thickened mass of cells and later delaminates into a pear-shaped vesicle lacking an external opening. The vesicle represents the major contributor to the adult labyrinth. The vesicles later enlarge and expand in different directions forming a complicated structure that deserves the term "labyrinth". It becomes surrounded by mesenchymal cells (Balinsky, 1961).

Successive stages in the differentiation of the labyrinth were studied, utilizing at least three specimens for each stage.

3.75 mm embryo (Plate XI, Fig. 1)

The original otic vesicle of the 3.75 mm embryo is slightly constricted into the dorso-medial pars superior and a ventral pars inferior; they are widely joined. The utricular part of the pars superior shows a small ovoid macula and a tiny lapillus which exhibits

a different staining reaction than the adult otolith. The utricular macula is continuous through columnar epithelium to the region where the ampulla of the anterior vertical semicircular canal will be formed later. The labyrinth shows a double-layered fold from its dorsolateral edge posteriorly. The whole posterior wall of the utriculus is lined with high columnar epithelium. In the posterior ventral part of the labyrinth, the columnar epithelium is connected to a median macula, judged by its location to represent the saccular macula since its sagitta is also present; the sagitta stained similar to the lapillus. Both otoliths are spherical, and are only recognized through association with their maculae. The anterior vertical semicircular canal is separated only in its anteroventral portion. The ampullary crista is recognizable as an internal projection. The horizontal canal is represented only by a lateral utricular evagination. The posterior vertical canal has not developed. The whole labyrinth is sheathed by a thin mesenchymal layer which secretes the perilymph later and contributes to the formation of the trabeculae to support the membranous labyrinth (Rugh, 1951).

4.0 - 4.5 mm stage

The major changes that occur on reaching the length of 4.5 mm are: (1) the vertical semicircular canals become partially separated ventrally from the utriculus, and laterally from the horizontal canal, (2) the utriculo-saccular duct begins to show its fibrous nature, (3) the utricular and saccular maculae are defined, and the columnar epithelium disappears from the ventral wall of both, leaving an extremely flattened squamous epithelial lining, and (4) the trabeculae first appear above

the vertical semicircular canals only while the rest of the labyrinth is still sheathed by mesenchyme.

5.0 - 6.0 mm stage (Plate XI, Fig. 2)

In this stage the three semicircular canals are completely separated from the utriculus, having cristae in their ampullae. The lapillus has enlarged and the sagitta and lapillus begin to show the staining reaction of the adult otolith. The sacculus is still widely joined to the utriculus. The lagena is not separated; it starts to form in the 6.5 mm stage and is clearly separated in 7 mm stage.

7 mm stage

The pars inferior shows a dorsoventral membranous partition in its posterior portion. The median sacculus is separated from the newly-formed, laterally located, lagena which has the asteriscus close to the macula on the external side of the separating membrane. Another important change is the union of the two endolymphatic ducts. The ducts evaginate during the 6.5 mm stage from a region anterodorsal to the succular macula (Plate VIII, Fig. 5). These ducts are directed posteromedially and are surrounded by perilymph. The horizontal plate of the exoccipital start to ossify. The membranous labyrinth is held in place by trabeculae. The utriculo-saccular duct is constricted, however, its fibrous wall has not thickened. The anterior vertical canal is surrounded, except on its median side, by cartilage. The horizontal canal is laterally completely surrounded by a cartilaginous ring, through which the canal opens into the utriculus. No trace of a process projecting from the pars superior (crus commune) to the skull wall, as found in the drum (Schneider, 1962), was seen.

10 mm stage (Plate XI, Fig. 3)

In the 10 mm stage the adult labyrinth is attained. The labyrinth is described here in detail since no literature is available, and because the morphology of the labyrinth in fishes is poorly understood and superficially referred to by previous investigators, dealing with the Weberian "apparatus", who have followed Chranilov (1927).

The Semicircular canals (Plate II, Fig. 1)

1. The anterior vertical semicircular canal is the first part of the ear encountered in cross sections. The canal ascends in front of its ampulla where its crista is ventrally located and innervated. The anterior ascending portion is laterally located. The canal is surrounded by cartilage except ventromedianly where trabeculae hold it in the perilymph. The canal passes posteriorly and joins the crus commune. Such a section shows the sacculus below the utriculus.

2. On the same transverse section that passes through the anterior edge of the utricular macula the anterior edge of the horizontal ampulla is also seen. This canal projects from the surface of the utriculus. Its crista is ventrolaterally located. Posteriorly, but still in the ampulla, the ventrolateral crista shows a mound-like cupula projecting dorsally from the thickest portion of the crista. The canal curves backward lateral to its ampulla and is enclosed in cartilage, except on its median surface. This cartilage mass extends dorsally above the anterior vertical canal. Posterior to this region the lateral canal is completely enclosed in a cartilaginous tube while the anterior vertical canal remains uncovered ventromedianly. As the lateral canal passes posteriorly, it occupies a higher level opposite the crus commune.

The posterior end of the canal joins the utriculus below the anterior edge of the ampulla of the posterior vertical canal. Posteriorly the canal passes a short distance behind its utricular opening.

3. The posterior ampullary crista is ventroposteriorly located. The posterior vertical canal passes dorsolaterally behind the crus commune. The ampulla projects from the utriculus posterolaterally, passing posteriorly and soon turning dorsoanteriorly. It extends behind the posterior opening of the horizontal canal. Posteriorly the canal occupies a more lateral position. The posterior limit of the canal reaches above the region where the sacculus and lagena separated, thus reaching above the ductus endolymphaticus (Plate IX, Fig. 4).

The utriculus

Behind the region where the ampulla of the anterior vertical canal projects, the utricular roof is flat, while the floor is arc-shaped. It is ovoid in cross section anteriorly where its macula occupies a lateral position in the floor. The anterior border of the macula consists of low neuroepithelial cells. The sensory patch becomes thicker and occupies the center of the utricular floor where the anterior edge of the lapillus reaches. In this region the lapillus is medially pointed where the macula is thick while its blunt lateral end is toward the thin portion of the macula. In the more posterior region, both the macula and the lapillus assume a uniform thickness. The lapillus is separated from its macula by a transparent space. Posterior to this uniformly thickened sensory area the macula becomes thicker laterally while the lapillus is thickest in the middle portion and tapering at its extremities. Near its posterior edge the lapillus thickens laterally

while the median edge curves upward. At the posterior extremity the lapillus occupies a more median position, being wider laterally. In serial cross sections the lapillus occupies an area in the utricular floor from the region lateral to the horizontal crista and ends where the crista is highest. The utricular macula behind the lapillus is thinner and laterally displaced. Posteriorly the utriculus has a narrow lumen above the saccular cavity. The median wall is straight while the rest of the wall is cresentic. The roof is thicker in the region of the crus commune.

The Sacculus (Plate XI, Fig. 4)

The anteriormost end of the sacculus is cup-shaped in cross section, surrounded by trabeculae and a bony roof, becoming ovoid in a more posterior region, with the narrow end on the dorsolateral side. Directly behind, the dorsomedian and lateroventral wall of the sacculus thickens. In a section passing through the crus commune, the sacculus below is surrounded by thick fibrous tissue, posteriorly extending dorsally to the utriculus forming the anterior edge of the utriculo-saccular duct. Behind this an invaginated fibrous mound projects into the saccular cavity from its lateroventral wall. The anteriorly thin saccular macula occupies a median position in a section passing through the crus commune. The opening of the utriculo-saccular duct in the utricular floor assumes an oval shape, thickened laterally, because the duct extends in a ventro-posterior direction. On its way to the sacculus, this duct penetrates the bony roof over the sacculus and the lagena. Below its thickened upper wall and narrow canal, the lumen of the duct widens. The opening of this duct is also thickened on the saccular

roof. This saccular opening consists of a thickened fibrous crescent that thins gradually posteriad. Below this opening the saccular macula thickens and occupies the dorsal portion of the median wall, with the anterior end of the sagitta close by. Posteriorly the saccular macula descends on the median wall and the sagitta is wider in section, being pointed dorsally and ventrally and occupying the upper portion of the median wall.

The ductus endolymphaticus issues from the dorsomedian surface of the sacculus and extends posteromediad. The endolymphatic ducts of both sides join halfway between the sacculi under the brain. The saccular macula is thick in this region and the sagitta occupies a more ventral position. From this it is apparent that the sagitta extends anteroposteriad in a dorso-ventral aspect respectively along the median saccular macula.

As the sacculus narrows posteriorly, the lagena enlarges, filling the space in the recessus sacculi. The sacculus in its posterior region is surrounded by perilymph except laterally where it shares the wall with the lagena.

The lagena (Plate XI, Figs. 3 and 5)

Its anteriormost portion lies below the junction of the descending and ascending parts of the posterior vertical canal, dorsolaterad to the sacculus and separated from it by fibrous tissue. As the lagena enlarges posteriorly, this fibrous mass becomes thinner and thinner permitting the confluence of the lagena and the sacculus. The fibrous tissue is restricted dorsally at the region of confluence. The fibrous mass projects internally from the dorsal wall and assumes an inverted T shape. The median arm of the (T) connects to the ventral wall of

the ductus endolymphaticus. The lateral arm meets another fibrous fold from the ventrolateral surface, resulting in separation again of the sacculus and lagena. At the separation, the lagenar cavity enlarges, while the saccular cavity narrows. The vagus nerve issues and passes ventrolaterad between the posterior part of the posterior vertical canal dorsally, and the pars inferior ventrally. The lagenar macula is located on the dorsomedian wall. A small canal penetrates through the fibrous tissue dorsomedian to the lagena and opens anteriorly into the perilymph, while posteriorly it opens lateral to the saccus endolymphaticus into the perilymph of the cavum sinus imparis. The bony roof over the ductus endolymphaticus is also joined to the crest of the basioccipital to house the ductus endolymphaticus. A cross section here passes through the posterior end of the sagitta and the asteriscus begins to appear as the sagitta ends. The heavy asteriscus is located on the median wall of the lagena next to its thin macula. Behind the asteriscus the macula is thin while the saccular macula is thick. The saccus endolymphaticus extends 10 microns behind the posterior limit of the posterior, blunt end of the lagena.

The otoliths of adult P. promelas (Plate II, Fig. 1) agree in general shape with other cyprinid otoliths described by Harrington (1955) in Notropis bifrenatus and by Adams (1940) in four genera of ostariophysine fishes and need not be repeated here.

The histology of the adult labyrinth is attained on reaching the 10 mm stage. The wall of the labyrinth consists of an outer fibrous layer and an inner extremely flat squamous epithelium which is replaced in the maculae and cristae by neuroepithelium of supporting and sensory cells.

As in most other teleosts the brain of P. promelas is surrounded by a large quantity of areolar connective tissue. The tissue fills the cavities which contain the semicircular canals and the whole auditory labyrinth and serves as perilymph, with which it is continuous.

CHAPTER VI

DISCUSSION AND CONCLUSIONS

A. Eggs and Embryology

One might assume that, since P. promelas is an egg-laying and nest-building species, spawning from May to late September in Oklahoma, collection of its eggs would be easy. This was true during the summer of 1961. The eggs were available and were easily collected from the pond. During the summer of 1962 not even a single egg was collected from any ponds that had been checked for all the possible sites of oviposition reported. Two methods of stripping were used to obtain fertilized eggs, none of which was successful. The few postlarval stages collected indicate low spawning activity for summer of 1962. By telephone, I learned that a commercial hatchery in Missouri experienced similar spawning failure in 1962.

The temperature during the summer (1962) was high and may be considered as one of the factors involved in reducing the population size in its early stages of development. Possibly the high temperature may have caused the eggs to drop from the surface on which they were deposited. Another factor, that may have contributed to spawning failure, was the large population of predatory aquatic insects, especially the backswimmers.

Failure to find eggs on boards, and the failure to get fertilizable eggs by stripping, during the summer of 1962, may suggest also an annual fluctuation or periodic oscillation in the reproductive cycle. However, this needs to be investigated carefully.

The first time eggs were collected, in 1961, was in the middle of May when the water temperature fluctuated between 65 and 68 F. The number of eggs per nest is extremely variable, as reported by Markus (1934). It also seems certain that more than one female uses the same nest, judging by the number of eggs and different stages of embryonic development. A single male cares for the nest, both under natural conditions and in the laboratory.

The eggs are deposited in a single layer usually in the form of chains. Wynne-Edwards (1933) and Markus (1934) found two layers in some nests, but I made no such observations. Parental care by the male did not seem to be necessary under laboratory conditions where sedimentation was at a minimum. However, the brushing activity of the male seems to be essential in the natural habitat since the fathead is very much at home in turbid water. The male in its activities may protect against predation, agitate the water and keep the nest free from sediment.

Wynne-Edwards (1932) believed, incorrectly, that the male turns the eggs over to permit access to oxygen. Markus (1934) hesitated to accept this idea because in case of two layers of eggs the male could not have access to the upper layer. I reject the idea because the eggs are firmly attached to lower surface of the floating object, in one layer and cannot be rolled. Parker (1962, unpublished thesis) reported that eggs of P. vigilax are also firmly attached to stones and

undersides of floating boards.

The diameter of the fertilized eggs obtained by stripping averaged 1.286 mm. Markus reported an average diameter of 1.15 mm, Wynne-Edwards, 1.30 mm while Greeley (1927) gave the size as 1/16 inch (= 1.59 mm). Possibly the differences may be attributed to population or racial differences or to differences in method. The three authors did not report the presence of the micropyle. Parker (1962) did not report it in P. vigilax, although present (personal observation) and Fish (1932) did not report it for P. notatus.

This study has shown that P. promelas develops in much the same manner as other described teleostean embryos, but differs from other species in some respects.

Two different views have been expressed concerning the nature of the migration of the edge of the germ ring. Wilson (1889) maintained that the posterior pole (dorsal lip) remains in a stationary position while the rest of the ring moves around the yolk from one end to the other. Budd (1940) is in agreement with Wilson. Jones (1937), Solberg (1938), Tavolga and Rugh (1947) and Price (1934) observed that in some species the rim of the germ ring moves downward although the dorsal lip may be retarded in its movement. My observations support the second view and that the dorsal lip of the blastopore is slightly retarded in its migration. This is evidenced by the pear-shaped late yolk plug and also with regard to the stationary oil globules.

Directly after hatching pigmentation is mainly restricted to the retina. Few dot-like melanophores, easily overlooked, are present on the yolk sac. Bottrell (1960, unpublished thesis) reported complete

absence of melanophores in the newly hatched embryos of Hybopsis aestivalis.

Since the incubation period varies, the newly hatched embryos show variation in length (4.0 - 4.5 mm) and number of somites (32-34), as reported by Battle (1940) in the goldfish.

B. The Weberian System

In all of the previous investigations, the Weberian "apparatus" has been described as consisting of two functional units, the pars auditum and the pars sustentaculum, based principally on the morphological study of dissected adult specimens. The developmental study of the "apparatus" reveals more than the two classical units. The "apparatus" from the standpoints of physiology and developmental differentiation is a rather complicated system consisting of four units which are closely related and functionally interdependent. The evidences in support of the Weberian system idea are: (1) since the anterior air chamber (Weberian chamber) contributes to the ossa suspensoria (part of the pars sustentaculum) and since this chamber plays the initial role of transmission of pressure waves, then it is unavoidable that it be considered as an important functional unit in the system as a whole; (2) since the ostariophysines have peculiar modifications in their membranous labyrinths, and since these peculiarities are correlated with the development of the anterior ossicles, then the labyrinth must be considered as an important unit in the system; (3) added to these two important units are the two classical ones, the pars sustentaculum and pars auditum.

The serial sections of the earliest stage studied revealed no trace of the endolymphatic duct. This leads to a conclusion in agreement with Balinsky (1961) that the otic vesicle originates from a sunken solid cellular mass of the otic placode. The placode by delamination forms the otic vesicle, unlike the shark situation in which the endolymphatic duct persists in the adult. During the late embryonic stages each vesicle possesses two dark otoliths. On sectioning such specimens the otoliths proved to be the lapillus of the utriculus and the sagitta in the sacculus, with different staining reaction and shapes than adult otoliths. This finding is in disagreement with Harrington (1947) who stated that in Notropis bifrenatus the otoliths represent the lapillus and asteriscus. One of the otoliths could not be the asteriscus, since the lagena develops later than the sacculus.

The embryonic otoliths are believed to be secreted by the auditory epithelium which exerts one of its phylogenetic functions, that of calcareous secretion, for dermal purposes. This embryonic activity according to Ayers (1892) gradually gives way as the adult function of the cells becomes more pronounced.

There seems to be conflicting opinion as to the manner by which the semicircular canals are formed. Valentine (1835) believed the canals start as a blind pocket of the utriculus, growing in a semicircular manner until they come again in contact to open in the utriculus. He believed also that the posterior canal was the first to form, then the anterior canal. Rathke (1839) found that in the adder Tropidonotus natrix each canal is formed by closing the deges of a fold in the utricular wall. The edges of the grooves unite first near the middle and grow toward the ends. Their separation from the utriculus is done by resorption of the tissue composing the fused folds. These

observations were confirmed by later investigators of the nineteenth century. In P. promelas the utriculus is drawn into folds which eventually stick together and become perforated while the original cavities along the folds remain open at both ends. The first of the canals to separate are the verticals which originate from a common fold. The anterior canal seems to be slightly advanced in its separation. The first portion to assume the canal shape is the ampulla and starts at the 3.75 mm stage. Von Noorden (1883) found in the herring that the cristae acquire their adult form before the ampullae close over them, while in the salmon the canals are fully formed before the cristae complete development. P. promelas is similar to the salmon in this respect.

On reaching the stage of 6 mm the lapillus and the sagitta show the staining reaction of the adult otolith. The origin of the otolithic material in the adult is believed to be from the endolymph (Ayers, 1892). It seems that the embryonic otoliths have different composition than those of the adult. When the lagena starts to separate and its asteriscus is formed, the latter exhibits the adult staining reaction directly, in the 7 mm stage. The late separation of the lagena reveals the recent evolutionary history as is also true for late separation of the lateral semicircular canal.

Watson (1939) illustrated the two endolymphatic ducts projecting from the sacculi as straight and perpendicular to the longitudinal axis of the body. He did not refer in his text to the level of projection of these ducts from the medial walls of the sacculi. He labeled his diagram as a modification from Chranilov (1927), but on comparing the two, no modifications were detected except that he shortened the saccus

endolymphaticus. In P. promelas the endolymphatic ducts project from the dorsomedian wall of the sacculi in front of its macula. The ducts of the two sides converge in a posteromedian direction and unite in a large saccus endolymphaticus which projects backwards into the cavum sinus imparis to a region slightly behind the section that passes in the posterior termination of the lagena. The lagena extends behind the sacculus. Watson's figure also failed to reveal the relation of the lagena to the sacculus. Lagler et al. (1962) show the membranous labyrinth of cyprinid fishes based on von Frisch (1938). His diagram more closely represents the labyrinth of P. promelas, except that von Frisch, as well as Watson, did not show the continuation of the perilymph in the cavum sinus imparis as being confluent with the labyrinthine perilymph as in P. promelas.

Manning (1923) studied the structure of the ear of the goldfish and stated that the sacculus and lagena form two distinct sacs joined by a minute canal. This is also true in P. promelas, except the canal is not minute. Watson (1939), although using Manning's paper as one of his references, drew a single sac and left it without a label.

In taking into consideration the differentiation of the scaphium, there seems to be a definite correlation between it and the backward extension of the saccus endolymphaticus since both of these events occur in the 7 mm stage.

From the foregoing discussion it is clear that the endolymph is continuous throughout the membranous labyrinth since the pars superior and inferior are joined by the utriculo-saccular duct. Schneider (1962) reported that the endolymph in the pars superior is not continuous with that in the pars inferior in two species of drum.

The asteriscus tends to be the largest otolith in P. promelas. This is in agreement with Adams (1940) and Frost (1925), and in disagreement with Harrington (1955).

The swim bladder of P. promelas belongs to the physostomous group of Tracy (1911). The general manner of development in P. promelas is similar to that described by Nelson (1959) for Catostomus commersoni. The anterior chamber originates as an anterior diverticulum from the posterior chamber dorsal to the entrance of the pneumatic duct. There are some differences in detail, between the two species. One of the differences is the appearance, in P. promelas, of an intraepithelial substance in the posterior chamber of 4.40 mm larvae. This material, lacking anteriorly and posteriorly, increases when the larvae attain the length of 4.65 mm and disappears at the 5 mm stage. The function, composition and origin of the material is unknown, but I believe it is a cellular product since the epithelial lining changes from columnar to low cubiodal and finally squamous epithelium as the material increases in quantity. Lagler et al. (1962) state that the initial filling of the gas bladder in most physostomes cannot take place if the larvae have no access to the atmosphere. The experiments performed in this study indicate that there are exceptions to the rule since the initial inflation of the posterior chamber did not seem to be dependent on air gulping. Furthermore it is believed that the surfacing of Pimephales larvae is probably nothing more than the usual behavior of young animals.

Whether the larvae gulp air is not easy to prove. Johnston (1953) states that the initial filling of the swim bladder in Micropterus salmoides, a physoclist, is effected by secretion from the vacuolated

columnar epithelial lining of the chamber. The inflation took place before the atrophy of the pneumatic duct. McEwen (1940) is in agreement with Powers (1932) that the initial inflation is effected by disintegration of organic compounds producing carbon dioxide within the embryonic, epithelial organ. Disintegration is initiated or augumented in some fishes by bacteria.

Another characteristic described by Nelson (1959), is the presence of the "intertunic connective tissue transient matrix" in the early stage of the anterior chamber. In P. promelas this matrix is intraepithelial in the tunica interna and makes its appearance at 5.75 mm, increases in the 6.0 mm stage and disappears at 7.0 mm. Since Thilo (1908), according to Nelson (1959), found the same material in the cyprinid fishes he studied, it may be characteristic of cypriniform fishes. Nelson considered the matrix along with the cardiac jelly of the developing vertebrate heart, but its function remains unknown.

The tunica externa thickens anteriorly and, at 8.0 mm, ossifies to form the ossa suspensoria. Niazi (1960) and Evans (1924-1925) agree that the central plate of the ossa occupies a pear-shaped slit anteriorly in the tunica externa. Neither reached a conclusion since their works were not developmental in nature.

The development of the vertebral column of P. promelas was studied beginning immediately after hatching. It was represented only by the notochord and its sheaths, being surrounded externally by the closely-applied perichordal mesenchyme. Watson (1939) reported a similar condition in the goldfish, except that no mention was made of the perichordal mesenchyme. Butler (1960) found the notochord as the only representative of the vertebral column in Pantosteus plebius

of 8 mm and younger stages. The first three arcualia in P. promelas appear when the larvae attain an average length of 5.0 mm. Ossification starts at the 6.25 mm stage. In Pantosteus the arcualia appear at 9.0 mm and in the goldfish the 8.0 mm stage.

There are two opinions regarding the fate of the notochordal sheaths. Faruqi (1935) believed they degenerate without sharing in the formation of the centrum. Ramanujam (1929) believed that in the herring the centrum originates in two parts, the first by ossification of the outer part of the fibrous sheath, and the other outside the first by membranous ossification of the surrounding connective tissue. My observations are in disagreement with both workers because: (1) the sheaths take part in the formation of the centrum, and (2) there is no space between the inner bony part of the fibrous sheath and the outer perichordal bony tube. To Ramanujam the centrum must look like two ossified tubes one inside the other, whereas, in P. promelas and Lebistes reticulatus, as reported by Mookerjee et al. (1940), there is no such space and the entire notochordal sheaths, with the outer perichordal ring, ossify as a single bony ring.

Ramanujam (1929) stated that the fibrous notochordal sheath between each two centra becomes the intervertebral ligament. It is a well known fact that the notochordal sheaths originally are a secretory product of the notochordal epithelium and as such could not have secretory ability. They cannot be modified into ligament since they can be seen in the intervertebral region. The only possible origin of this ligament is from the notochordal epithelium. This secretion becomes fibrillar in later stages and forms the intervertebral ligament. In

passing outward from the notochord the following layers are encountered: notochordal vacuoles, notochordal epithelium, intervertebral ligament, elastica interna (fibrous), elastica externa (cuticular), non cellular perichordal layer and the outermost connective tissue binding material. This is in complete agreement with the findings of Mookerjee et al. (1940). The notochordal sheaths and the noncellular perichordal layer bulge in the intervertebral region. The notochord of Pimephales promelas is persistent through life, but in certain regions it is traversed by osseous trabeculae.

Most of the previous workers considered the arches as major contributors to the centrum. Ramanujam (1929) and Faruqi (1935) stated that the arches in teleosts do not rest directly on the notochordal sheaths and thus cannot contribute to the formation of the centrum. Mookerjee confirmed this in his study of the development of the vertebral column of teleosts (1940) and Anura (1931). His finding is that the early sclerotomes are restricted to the posterior half of each myotome, the anterior half is left for the spinal nerve. The sclerotomes form the cartilaginous arcualia in front and behind which the neural arch is continuous as membrane bone contributing to the pre- and postzygapophyses. Thus the neural arches are not restricted to the region which forms a complete ring around the spinal cord. They are continuous through the pre- and postzygapophyses and not widely apart as Faruqi (1935) reported. I agree with Ramanujam (1929) that as growth proceeds the cartilaginous cells of the arcualia decrease in number dorsoventrally. Faruqi (1935) found no cartilage cells in the neural arches of the haddock. This is explainable since in teleosts the supression of cartilage is of frequent occurrence (Goodrich, 1930).

One other point to be added is that the pre- and postzygapophyses are to be considered as part of the arch rather than of the centrum as Ramanujam (1929) and Faruqi (1935) had reported.

There are two kinds of ribs in vertebrates. Dorsal ribs which extend between the epaxial and hypaxial muscles; and ventral or pleural ribs that lie internal to the hypaxial muscles and external to the peritoneal lining. According to Hyman (1942), Emelianov (1935) described two modes of ossification of ribs. The first is as membrane bone, the other endochordal in origin. He made another distinction between the two kinds, saying that dorsal ribs form independently some distance from the vertebra and grow toward it, while pleural ribs start very close to the vertebra and grow away from it. Goodrich (1930) stated that dorsal ribs (epipleural) are usually attached by ligaments.

None of the ribs in P. promelas are preformed in cartilage and all ribs except the first two are considered as pleural ribs. Hyman (1942) stated that teleostean ribs may be formed at any level in the myosepta; the extra ribs according to Emelianov are dorsal ribs.

In P. promelas the first two ribs extend in the horizontal myosepta. This is a diagnostic characteristic of dorsal ribs and has been described by Budgett (1902) in Polypterus.

The first rib is connected by a ligament with the supraceithrum; this supports the idea of considering it a dorsal rib (Goodrich (1930). The first rib is believed to have composite origin, since in some adult specimens osseous nodules were embedded in the ligament. The first rib must be an ossified, partly sesamoid, dorsal rib. That the two first ribs are dorsal is in disagreement with Berg (1947) who stated that cyprinoid fishes have no dorsal ribs, he called them parapophyses.

Hyman (1942) defines parapophyses as "lateral projections for the attachment of the lower head of two headed ribs". Thus Berg's (1947) terminology is not applicable to the condition in P. promelas. Considering them as dorsal ribs is also in disagreement with Niazi (1960), Sisk (1962) and Niazi and Moore (1962), who rejected this idea on the basis of Berg (1947). Another reason for the rejection was based on Emelianov who stated that ribs change their position with regard to the muscles during development. No observations were made during this investigation in support of Emelianov's statement, however this may be correct in other species.

The first two ribs have been treated differently by different authors. Wright (1884), Bridge and Haddon (1889), Hora (1922), Chranilov (1926), Adams (1928) and Nelson (1948) called them transverse processes. Koh (1931) considered them as ribs, Mookerjee (1952) considered them as lateral processes and Ramaswami (1955) agreed with Watson that they are dorsal ribs.

The origin of the neural complex also has been a source of disagreement. Butler (1960) reported that in the adult Pantosteus the neural spines of the fused second, third and fourth vertebrae form a large continuous neural spine. Watson (1939) found that early in development there is a cartilaginous mass above the second and third centra which he thought represents fused neural spines and basidorsals of the second, third and fourth vertebrae together with the first three interspinous bones and possibly the neural spine of the first vertebra. This mass, Watson thought, gives rise later to two parts, the compound neural arch with a spine and the neural spine of the fourth. He decided that the compound neural spine represents the fused neural

spine of the second and third with the first three interspinous bones and possibly the neural spine of the first, but later, in contradiction, he mentioned that the first vertebra does not take part in the formation of the cartilaginous mass.

Ramaswami (1955) stated that in Notropis cornutus the neural complex represents the fused second and third neural spines, although he showed a similar situation in Pimphales where the second and third centra are not fused. Taranetz (1946) observed the structure in several cyprinid species but avoided the issue by calling it an apophysis, any of the vertebral projections according to Hyman (1942). Adams (1928) did not mention the compound neural spine in Ictiobus urus (= I. niger). Nelson (1948) believes that it represents the neural arches and spines of the third plus possible interspinous elements. Niazi (1960) and Niazi and Moore (1962) stated that the neural complex represents a fusion of the third neural spine and possibly interspinous bones, if they exist.

The origin of the neural complex in P. promelas may be stated after considering the facts below.

1. In the adult and postembryonic stages no centra are fused with others.
2. The interspinous elements start as cartilaginous structures and the anteriormost one is located behind the region of the neural complex. Neither cartilaginous nor osseous structures were detected in front of the region of the complex.
3. The dorsal longitudinal ligament (fibrous) extends between the posterior surface of the skull to the hump of the dorsal

> cartilaginous mass. On either side of the ligament there is a large muscle. Another muscle extends from the hump posteriad, best seen in the cross section. Thus there is left a Y-shaped skeletogenous septum, its stem directed anteriad to the skull, and its arms posteriad in the future position of the neural complex. The area between the arms shows thin fibrous connective tissue below the posteriorly directed muscle.

The conclusions of Harrington (1955) and Kindred (1919) that the median dorsal fibrous septum, between the dorsolateral muscles of the two sides of the body, ossifies to form the spina occipitis of the supraoccipital, may also be considered.

With regard to the origin of the neural complex, in Pimephales promelas my conclusions are: (1) no interspinous elements are involved; (2) the neural spine of the third vertebra contributes only to the stem; (3) the boat and the blade are independent of vertebral structures derived by ossification in fibrous connective tissue of the dorsal ligament. I disagree with Watson (1939) who stated that the dorsal cartilaginous mass contributes to the adult neural spine. In P. promelas the neural spines have sclerotomic origin independent of the neural arch. This is in agreement with Mookerjee et al. (1940).

My observations concerning the origin of the ossa suspensoria are in disagreement with all previous workers. Ramaswami (1955) stated that the ossa project from the vertebral surface of the fourth centrum. Watson (1939) considered them hemapophyses. Goodrich (1930) and Berg (1940) regarded them as fourth ribs. Adams (1928) believed that they represent the haemal process. Niazi and Moore (1962), on the basis of adult structure, considered the ossa as parts of the fourth pleural

ribs. The ossa, as indicated earlier, consist of two parts. The dorsoventrally extending medially located central plate and the mediolaterally extending parts which join each os to its respective fourth pleural rib. The central plate has its origin as an ossification in the anteriorly thickened fibrous tunica externa. The lateral part is membrane bone, originally mesenchyme, and joins the central plate to the proximal portion of the fourth pleural rib. The two components ossify and fuse. Thus the ossa have double origin not revealed by study of the adult.

Three theories concerning the origin of the Weberian ossicles have been proposed. Weber (1820) and many other earlier investigators regarded them as homologous to the mammalian auditory ossicles. St. Hilaire (1824), Müller (1843), Beaudelot (1868), Sagemehl (1884a,b, 1891), Nusbaum (1908a,b) Grassi (1883) and Sidoriak (1898) considered them to be entirely derived from the anterior vertebrae. Wright (1884), held that they are derived in part from ossification of ligaments and in part from the anterior vertebrae. All these theories were based on morphology which alone is an insufficient guide. Hora (1922) homologized the ossicles as follows: claustrum and scaphium, as parts of the first neural arch; intercalarium, neural arch II; and the tripus, transverse process plus rib of III and IV.

The findings of Watson (1939) and Butler (1960) will be taken into consideration in discussing the origin of the ossicles in P. promelas.

The claustrum, a membrane bone in P. promelas, starts to ossify in the 8.0 mm stage as a narrow strip of bone located dorsal to the first basidorsal and joined to it by mesenchyme. It assumes adult shape at 9.0 mm and has a single origin as reported by Butler (1960)

and Watson (1939). In homologizing the structure, Watson (1939) considered it as an intercalated structure, in agreement with Kindred (1919), although the presence of intercalaries was not revealed in his preparations. The ossicle is formed late, as compared with the other ossicles, being converted directly into bone at the posterior edge of the centrum. According to Ramanujam (1929) intercalaries occur in teleosts. According to MacBride (1932) the intercalaries alternate with the neural arches but do not extend so far dorsad. Gadow and Abbot (1895) defined the intercalaries as extensions of the basidorsals upwards and basiventrals downwards to separate from the main mass of arcualia.

Since originally the mesenchymal primordia of the claustra are indistinguishable from the rest of the perichordal sheath, it is logical to conclude that the intercalarium when fully formed is a separated part of the first basidorsal. In Amiurus the claustrum is a cartilaginous structure (Kindred, 1919) while in P. promelas it never passes through a cartilage stage, another example of cartilage suppression in teleosts (Goodrich, 1930). Matveiev (1929) explains the presence of intercalaries as a persistent, primitive character in Cyprinidae, a secondary recapitulation as a result of having modification in the anterior vertebrae.

The origin of the scaphium does not seem to cause any trouble to most of the previous investigators since the majority agreed that it represents a modified part of the first neural arch, however one thing has been overlooked by all previous workers. In P. promelas as in the goldfish, the concha stapedis starts as a membranous bony rod embedded in mesenchyme and not in direct osseous connection with the cartilaginous first basidorsal. Later in development it attaches to its respective basidorsal. The first basidorsal extends dorsally as the

ascending process which ossifies later in ontogeny. The original arcualia represent the articulating processes. The adult concha shows a peg-like process from its outer surface to which the interossicular ligament attaches. This nipple is an ossification, not previously reported, in the interossicular ligament at its point of attachment to the outer surface of the cup and takes place at the 8.5 mm stage.

The first representative of the intercalarium, is the second basidorsal which appears at 5.0 mm. An ossification in the interossicular ligament opposite this region appears between the 6.5 and 7.0 mm stages, being connected by mesenchyme with its cartilaginous arcualia.

The tripus starts with the appearance of the third basiventral. The interossicular ligament in earliest stages can be traced from the scaphoid rudiment to the third basiventral and back to the tunica externa. Near the third basiventral is a small mass of mesenchyme, believed to represent a rudiment of a rib, of uncertain type. Although such a rib is unknown in Pimephales, ribs are of mesenchymatous origin and this may represent one. Butler (1960) concluded that it represents a dorsal rib and went further to state that this process arises at the junction of the third myoseptum with the horizontal skeletogenous septum, while his figure shows it in the region of the sixth myotome, not in the myoseptum. His conclusion was based on the absence of a rib in the region of the third vertebra.

In P. promelas the interosseous ligament contributes to the peripheral portion of the body of the tripus, its anterior and posterior rami, while the mesenchymatous mass contributes to its body. The third basiventral becomes the articular process and probably part of

the body. The transformator process originates as direct ossification in the tunica externa. Watson (1939) applied the term "transformator process" to that region of the tripus posterior to its body, which, as here considered, includes the posterior ramus, and the transformator process. It is apparent that Watson believed that the third rib gives rise to the posterior ramus. The rib according to him is membrane bone as is true in P. promelas, but Butler (1960) reported that it is preformed in cartilage.

Each transformator process is joined to its respective os suspensorium by a triangular smooth muscle, the tensor tripodis, not a ligament as described by Nelson (1948).

The use of the tripus as a taxonomic character (Robins and Raney, 1956; Miller, 1963) in separating closely related species seems to be of doubtful usefulness since the bone shows variations within a species and even from side to side in the same individual.

It is interesting that the development of the ossicles is very rapid and that they assume their adult forms in an individual having a length of only 9.5 mm in comparison with Carassius auratus and Pantosteus plebius in which the ossicles assume their adult form at the 15.0 mm stage. The earlier attainment of the adult forms of the ossicles in P. promelas is explainable since the maximum length which the fish attains during its life is much smaller than either Carassius or Pantosteus.

Another interesting point is that the differentiation of these ossicles follows the natural phenomenon of axial gradients, basic in all developmental processes. The late differentiation of the claustrum has been noted to occur late in Carassius, Pantosteus and Pimephales however in the latter the differentiation of this ossicle soon catches

the others. The comparison of the lengths of the fishes at which each ossicle assumes its adult form is presented below for the three species. The length in millimeters is indicated in parenthesis.

Watson (1939) - Carassius auratus

scaphium (11), intercalarium and tripus (15), claustrum (?).

Butler (1960) - Pantosteus plebius

intercalarium (11), claustrum (12), tripus and scaphium (15).

In the present study - Pimephales promelas

scaphium and intercalarium (8.5), claustrum (9.0), tripus (9.5).

C. Phylogeny

Since Martin (1963) agrees with Watson (1939) that the Siluridae are more primitive than the Cyprinidae, it seems appropriate at this time to consider the probable phylogenetic relationships among ostariophysine fishes.

Since the function of the Weberian ossicles is to convey vibrations from the air bladder to the ear in hydrostatic or auditory functions, the ostariophysi seem to be a successful evolutionary unit. All the Ostariophysi are fresh-water forms except a few Siluroids. Of the known 2180 (approximate) fresh-water Teleostei, there are only about 600 in which the Weberian ossicles are absent. Thus the number of ostariophysine species is about five times as great as all other species of fresh-water physotomes, nearly five times greater than the species of fresh-water physoclists, and about three times the combined total of physoclists plus the non-ostariophysine physotomes. The families Cyprinidae and Siluridae are by far the richest, both in species and genera, the former including one-third (724), and the latter about

one-fourth (572), of all the known fresh-water species. In the Indian and Neotropical regions, where fresh-water fishes attain their maximum speciation, the Ostariophysi outnumber all the remaining species. The great rivals of the Ostariophysi, among fresh-water fishes, are the Salmonidae and Cyprinodontidae, but owing to differences of habit or of geographical distribution, a considerable number of them do not come into direct competition with the former. The conclusion which can be drawn here is that the ostariophysines are the dominant families of the fresh-water Teleostei.

The order Cypriniformes (series Ostariophyseae of Jordan, 1923) first appear in the Upper Cretaceous, Berg (1947). The series Ostariophysi was divided into four orders by Jordan (1923), whereas Berg (1947) considered four sub-orders. The geological history of the constituents is included in Table II.

Darlington (1948) suggested the simplified dispersal lines for the Ostariophysines which are shown in Table III.

The order Clupeiformes has its geologic horizon in the Middle Triassic to recent. They probably were of marine origin and are still very numerous in the sea. This order, according to Berg (1947), represents an artificial assemblage with wide diversity. One of the extinct sub-orders of the group is the Lycopteroidei (from the lower Cretaceous of Mongolia and Northern China) with many cyprinid characteristics. The largest otolith is the asteriscus instead of the sagitta as in clupeids; the small, rounded scales with a central focus and numerous radii as in Phoxinus (Cyprinidae).

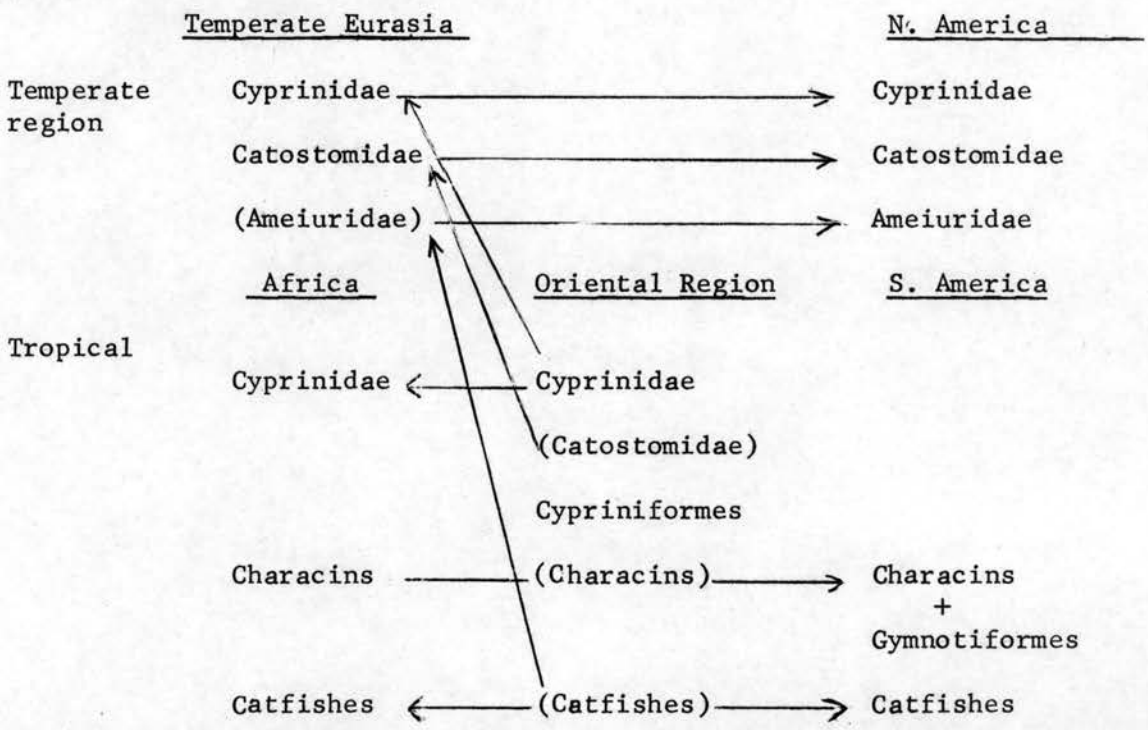
TABLE II

GEOLOGICAL HISTORY OF THE CYPRINIFORMES,
BASED MAINLY ON BERG, 1947

Era	Periods	Epochs	
	Quarternary	Recent Pleistocene	
Cenozoic		Pliocene	
	Tertiary	Miocene	Siluridae Europe and Asia
			Characidae of Peru
		Oliogocene	Amiuridae N. America to Guatemala
		Eocene	Catostomidae *catostomus* of Mongolia Central and N. America N. E. Siberia
			Ariidae, *Amiurus*
		Paleocene	Cyprinidae Siluroidei
Mesozoic		Upper Cretaceous	distinct characinoid family in California and Wyoming Some Ariidae
		Lower Cretaceous of Mongolia and N. China	The sub-order Lycopteroidei *Lycoptera* (extinct)

TABLE III

DISPERSAL LINES OF OSTARIOPHYSINES,
ACCORDING TO DARLINGTON, 1948



Names in paranthesis indicate former occurrence of a group not existing in it now.

The family Megalopidae (suborder Elopoidei) has an air bladder connected to the ear. According to Goodrich (1930), in Megalops the diverticulae of the air bladder are lodged in the prootic. In the suborder Notopteroidei the air bladder is also connected to the ear, but in Notopterus the diverticulae are separated by a membrane alone whereas in the Hyodontidae the diverticulae penetrate into the perilymph.

It is a well accepted fact that the Clupeiformes are much more primitive than the Cypriniformes, but both possess the following characters.

1. Air bladder vibrations are conveyed to the membranous labyrinth.

2. The asteriscus is the largest otolith.

3. Lycopterid scales are similar to those of Phoxinus.

4. Dorsal and pleural ribs are present.

5. Physotomous.

It seems probable to postulate that a primitive form related to Lycopterus was ancestral to the cyprinids. Another possibility would be to consider a primitive form related to the Megalopidae. If it is true that such clupeid represent the ancestral form, the question is then how such direct connection, between the air bladder and the ear, was replaced by the indirect transmission system through the Weberian ossicles. One may postulate the mode of phylogenetic development of the Weberian system as follows.

1. Depending on the size of the asteriscus, the direct connection of the air bladder to the ear, and the type of the fossil scales of certain clupeids it seems possible that a primitive lycopterid, megalopid or a closely related form represents the ancestor of the Cypriniformes.

2. In evolution the air bladder retreated backwards. However, since its connection to the ear seems to have a high selective value, the Ostariophysi became the largest group of fresh-water fishes. The mechanism of shock wave transmission took different modes, and necessitated changes in the ossicles.

3. The mode of air bladder retreat probably took two directions: (a) complete retreat resulting in the formation of the Weberian chamber having a rounded anterior surface instead of the original two ancestral diverticulae as in the Division Cyprini of Berg (cyprinids, catostomids, characids and Gymnotids); (b) incomplete retreat, either

as an intermediate stage or rather in a different evolutionary line diverging early in phylogeny to become the Division Siluri of Berg.

4. The two types of retreat may have been accompanied by two other modifications: (a) the remnants of the retreated diverticulae gave rise to the interossicular ligament, which, as the present investigation indicates, gives rise to the nipple of the scaphium, the alar condyle of the intercalarium and the tripus, except the more median portion of its body and its articulating process; (b) the establishment of the ductus endolymphaticus and the saccus and sinus imparis and their atria are all correlated and associated with the differentiation of the scaphial cups.

It seems logical to divide the Cypriniformes into two distinct orders equivalent to the Divisions of Berg (1947). Jordan (1923) went farther and divided the Series Ostariophyseae into four orders, Heterognathi (Characinoidei), Gymnonoti (Gymnotoidei), Evantognathi (Cyprinoidei) and Nematognathi (Siluroidei). Jordan's Heterognathi, Gymnonoti and Evantognathi have many common characters which differ from the Nematognathi.

1. In the first three, the parietals, symplectic and subopercular are present, but these are absent in the Siluroidei.
2. The third vertebra is never coalesced with the fourth (in the first three groups) whereas in the Siluroidei the second, third and fourth (sometimes the fifth) vertebrae are ankylosed.
3. The body is covered by scales or naked, never with bony plates, whereas in the Siluroidei the body is covered with bony plates or naked.

Some Siluroidei show primitive characters such as having superficial dermal bones on the head. Plotosus has ampulae of Lorenzini, a selachian feature, here unique among Teleostomi.

The following characteristics of the Weberian system will also set the Siluroidei apart from the Cyprini.

1. The lateral growth of the air bladder and its frequent possession of caecae.
2. The spring apparatus
3. The intercalarium which never articulates with the second vertebra, being extremely variable.
4. The rudimentary first centrum.

Since the intercalarium in the Cobitidae and Siluridae does not articulate with the second centrum, Watson (1939) concluded that the families are more primitive than the Cyprinidae (goldfish) in which the ossicle does articulate, although not early in ontogeny. Such a condition was found in Pantosteus plebius (Butler, 1960) and Pimephales promelas of the present work which is in disagreement with Watson's conclusion. Watson (1939) and Martin (1963) considered the Weberian apparatus of Cyprinoidei (catostomids and cyprinids) as an indirect system. The intercalarium articulates with the vertebral column and the tripus possesses a transformator process, reversing the direction of motion as result of its attachment by a tensor tripodis to the os suspensorium. In the siluroid direct system the intercalarium does not articulate with the second centrum and the tripus does not possess a transformator process. Watson and Martin both concluded that the indirect system evolved from the direct one. Wright (1884) thought the direct siluroid system is a specialization of the indirect one.

However, Wright stated that the posterior part of the tripus is an ossification in the tunica externa and this is a transformator process by definition. Bridge and Haddon (1889) indirectly mentioned the transformator process as follows:

> "The relation of each of the lateral cavities of the air bladder to the crescentic process of the tripus imbedded in its dorsal wall are almost precisely the same in each half of the anterior chamber in a normal siluroid".

I reject the conclusions of Watson (1939) and Martin (1963) and also of Wright (but in a different sense) for the following reasons.

1. In the Siluroidei "normales" the claustrum is occasionally absent and if present it is a very small spicule. If we accept the idea that the claustrum represents a secondary recapitulation, due to the modification of the anterior vertebrae to form the Weberian ossicles, then the conclusion is that such an intercalary must be present at the start and any deviation from the standard shape of the bone is to be looked upon as degeneration during specialization. This phenomenon is not uncommon in fishes, and there are many examples demonstrating it, such as the degeneration of the air bladder in the percids, the evolution of physoclistous from physostomous fishes...etc. The intercalarium also may be absent in the siluroids.

2. The lateral sacs of the air bladder may become completely separated. The air bladder is very rudimentary in the silurid genus Cetopsis so that J. Müller denied its presence (Bridge and Haddon, 1889).

3. The Siluroidei exhibit many advanced characters over the cyprinoids: the fusion of higher number of centra; the peculiar type of the air bladder, which also shows diversified characters; the pneumatic duct is very generally, but not invariably, present; and the spring structure.

The air bladder in siluroids exhibits a far higher degree of specialization in its relation to the Weberian apparatus than in any other Ostariophysi (Bridge and Haddon 1889). This fact renders it specially liable to degeneration, when the necessity for the exercise of its special function has, from any change of habit on the part of the fish, ceased to exist. Bridge and Haddon believed that the degeneration is due to the assumption of a ground habit where a hydrostatic structure is not necessary.

All the above statements support the view that the Siluroidei are more advanced than the Cyprinoidei. I believe that the siluroids probably were derived from a form closely similar, but not necessarily closely related to the cyprinoid prototype and that two distinct orders are recognizable.

CHAPTER VII

SUMMARY

1. The stripped eggs were not fertilizable after the fish were transported to the laboratory. The stripping process proved to be impractical in securing fertilized eggs.

2. The early embryology of P. promelas was studied from the fertilized egg stage through hatching. The embryonic development of this fish is not different from other teleost species described.

3. The eggs possess a star-shaped, slightly depressed micropyle.

4. The migration of the dorsal lip of the blastopore is slower than the rest of the germ ring, resulting in a pear-shaped yolk plug before the closure of the blastopore.

5. The initial inflation of the air bladder does not seem dependent on gulping air by the larvae, since developing embryos not having access to the atmospheric air developed normally filled air bladders.

6. The membranous labyrinth does not possess an external endolymphatic duct opening; this duct does not seem to exist in bony fishes. The adult otoliths are similar in shape to those of other cyprinids.

7. The sacculus and the lagena are incompletely separated by a partition. The sacculus joins the utriculus through a thick-walled utriculo-saccular duct.

8. The saccus endolymphaticus extends posteriorly, in sections,

beyond the lagena. The latter extends behind the sacculus. Each endolymphatic duct originates as an evagination from the dorsomedian wall of each sacculus. The two ducts are directed in a posteromedian direction.

9. The development of the vertebral column was studied from a stage directly after hatching. The intervertebral ligament originates in a secretion from the notochordal epithelium.

10. The neural complex represents an ossification in the dorsomedian skeletogenous septum plus the third neural arch and spine. No interspinous element contributes to its formation.

11. The first two centra bear dorsal ribs. The first rib is partially sesamoid.

12. The claustrum is considered as an intercalary.

13. The scaphium originates from the usual part of a neural arch. A nipple-like ossification in the anterior end of the interossicular ligament is added to the concha. The first basidorsal gives rise to the ascending and articulating processes of the scaphium.

14. The second basidorsal forms the ascending and articulating processes of the intercalarium, whereas the manubrium incudis is an ossification in the interossicular ligament.

15. The parts of the tripus originate as follows:

a. The interossicular ligament contributes to the anterior and posterior rami, and to the peripheral part of the body whereas the rest of the latter arises from a mass of mesenchyme which may represent a remnant of the third rib.

b. The third basiventral contributes mainly to the articulating process of the tripus and partly to its body.

c. The transformator process originates as a direct ossification in the anteriorly thickened, fibrous, tunica externa.

16. The os suspensorium has two origins: the median central plate as a direct ossification in the anteriorly-thickened fibrous tunica externa, ventromedian to the transformator processes of the tripus, and the dorsolateral portion joining to the fourth pleural rib is a median extension from the latter.

17. The Weberian apparatus constitutes two units in the more complex Weberian system consisting of four functional units: the ear, the air bladder, plus the two classical units.

18. The four units are physiologically as well as developmentally correlated and each is necessary for normal functioning of the system as an effective device with high selective value.

19. The development of the four units is very rapid compared with that of Carassius auratus and Pantosteus plebius.

CHAPTER VIII

SUGGESTIONS FOR FURTHER STUDY

1. A micro-chemical analysis of the gaseous contents and the composition of the yellow-staining material in the newly formed posterior chamber.

2. A micro-chemical analysis of the transient material of the anterior chamber.

3. A study of the early development of the auditory vesicles before hatching.

4. A study of bone formation in Pimephales.

5. An investigation of a possible annual fluctuation in the reproductive potential of Pimephales and its relation to temperature and predation.

6. A study of sound production in Pimephales, its source and biological significance.

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A P P E N D I X

PLATE I

Figure 1 - Posterolateral view of three units of the Weberian System of P. promelas, 65 mm.

Figure 2 - Posterior view through the tunica externa of 45 mm specimen.

Figure 3 - Scaphium, 58 mm specimen.

Figure 4 - Claustrum, 55 mm specimen.

Figure 5 - Intercalarium, 62 mm specimen.

Figure 6 - Tripus, 43 mm specimen.

Figure 7 - A diagramatic representation of the Weberian System.

Legend:

AC - Anterior vertical semicircular canal
ACH - Anterior chamber
AM - Ampulla
AR - Anterior ramus
ARP - Articulating process
ASI - Atrium sinus imparis
ASP - Ascending process
B - Body
BA - Base
BL - Blade
BO - Boat
C - Crest
C1,2,3,4 - Centra
CC - Crus commune
CER - Cerebellum
CP - Cup
CPO - Central plate of os suspensorium
CS - Concha stapedis
DE - Ductus endolymphaticus
DR - Dorsal rib
E - Eye
F - Foramen
HC - Horizontal semicircular canal
IL - Interossicular ligament
INS - Interspinous structure
INT - Intercalarium
LG and AST - Lagena and Asteriscus
LP - Lapillus
MI(AC) - Manubrium incudis (Alar condyle)
N - Nipple
NA - Neural arch
NC - Neural complex
NO - Nostril
NP - Neural pedicle
NS - Neural spine
NT - Nuptial tubercle
OFL - Olfactory lobe
OP - Optic nerve
OPL - Optic lobe
OS - Os suspensorium
PC - Posterior vertical semicircular canal
PIN - Pineal body
PLD - Perilymphatic duct
PR - Posterior ramus
4th PR - 4th pleural rib
SA - Shaft
SA and SG - Sacculus and sagitta
SC - Scaphium
SI - Sinus imparis
T - Tripus
TE - Tunica externa
TP - Transformator process
TT - Tensor tripodis
U - Utriculus
ZY(PR,PO) - Pre- and Post-zygapophyses

PLATE-I

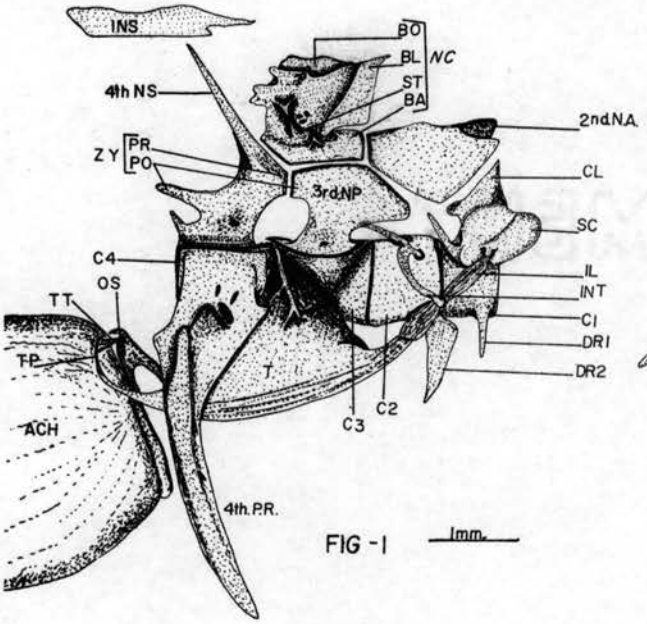


FIG-1

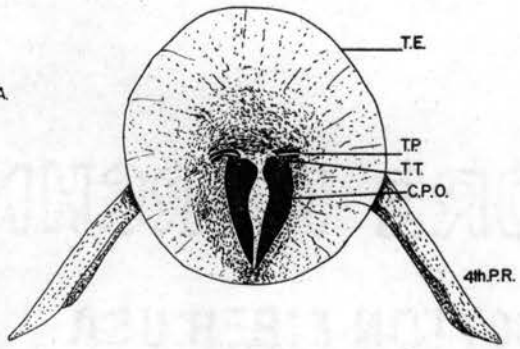


FIG-2

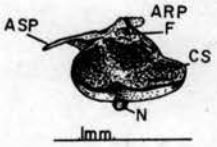


FIG-3

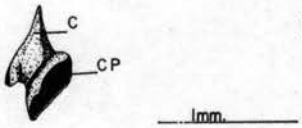


FIG-4

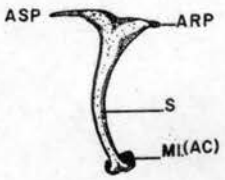


FIG-5

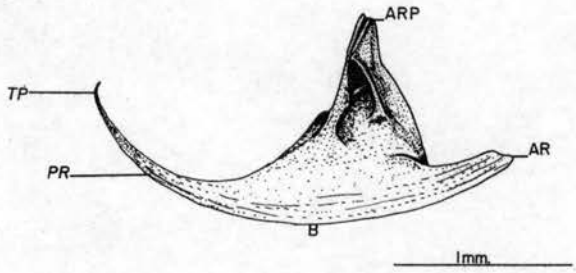


FIG-6

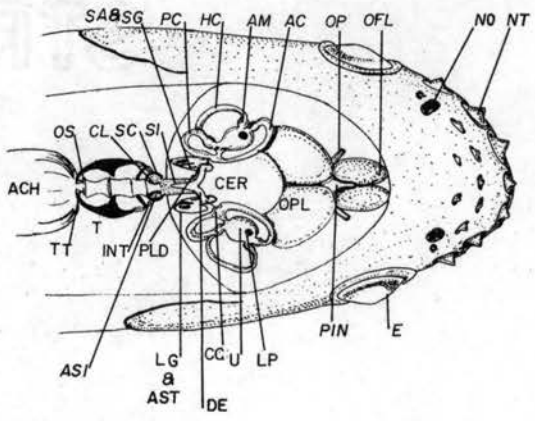


FIG-7

PLATE II

Figure 1 - Diagrammatic reconstruction of the membranous labyrinth of P. promelas based on serial sections and dissected specimens. The otoliths in dorsal view.

Figure 2, 3, and 4 - Successive developmental stages of the air bladder. (Diagrammatic)

Legend:

AC - Anterior vertical semicircular canal
ACH - Anterior chamber
AM - Ampulla
AST - Asteriscus
CC - Crus commune
CPO - Central plate of os suspensorium
DC - Ductus communicans
DE - Ductus endolymphaticus
G - Gut
HC - Horizontal semicircular canal
LG - Lagena
LP - Lapillus
PACH - Primordium of anterior chamber
PCH - Posterior chamber
PD - Pneumatic duct
PLD - Perilymphatic duct
SA - Sacculus
SE - Saccus endolymphaticus
SG - Sagitta
SI - Sinus imparis
TE - Tunica externa
TI - Tunica interna
TP - Transformator process
U - Utriculus
US - Utriculosaccular duct

PLATE-II

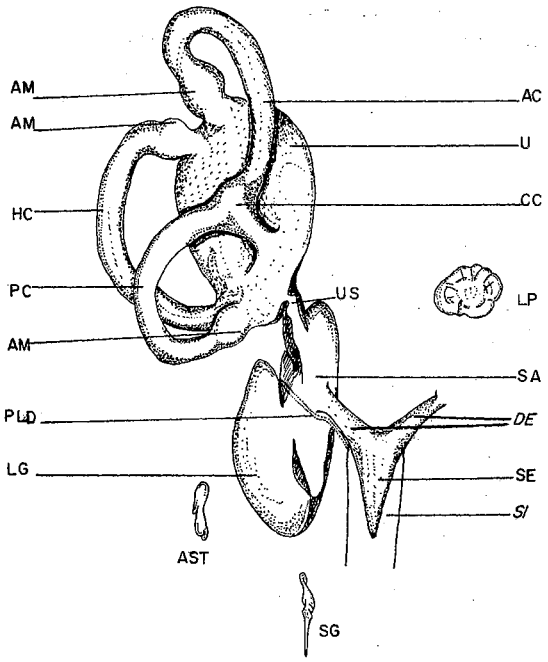


FIG-1

FIG-2

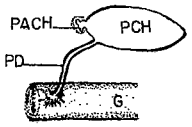


FIG-3

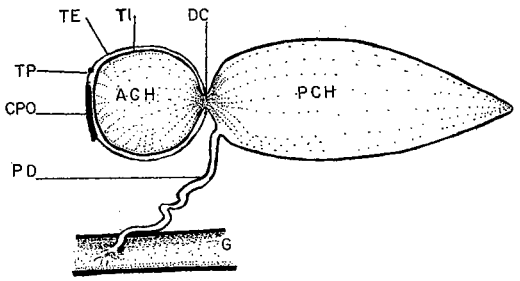


FIG-4

PLATE III

Average time intervals are given from fertilization

Figure 1 - Chain of eggs of P. promelas.

Figure 2 - The micropyle of the unfertilized egg.

Figure 3 - Abnormality of a cleaving egg.

Figure 4 - The single cell stage; 30 minutes. Note the oil globules on the yolk.

Figure 5 - The two-celled ovum; 47 minutes.

Figure 6 - The four-celled ovum; 65 minutes.

PLATE - III

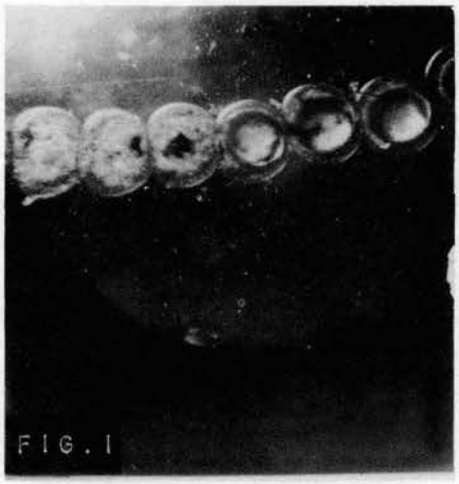


FIG. 1

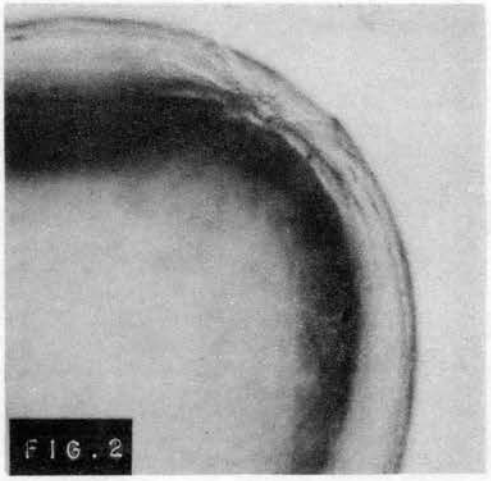


FIG. 2

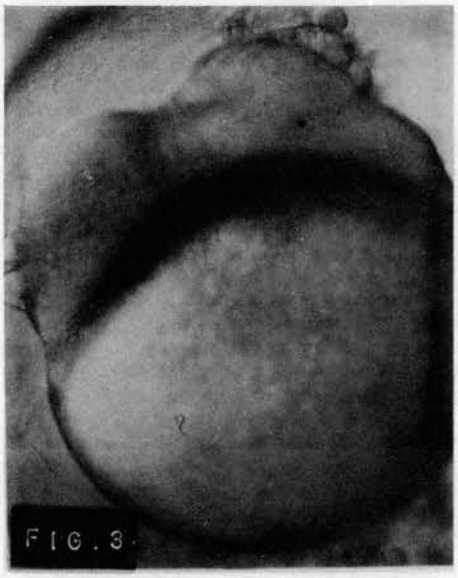


FIG. 3

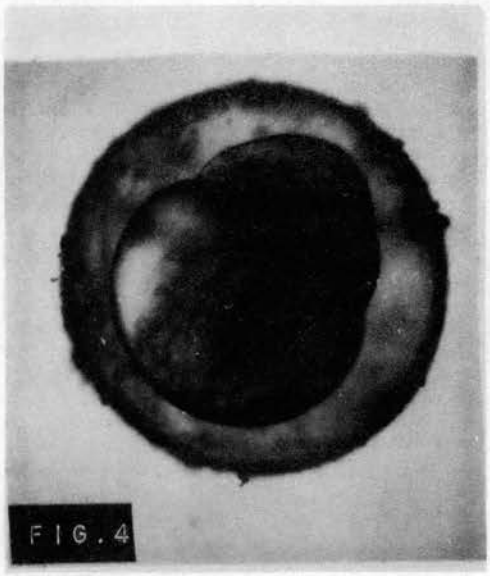


FIG. 4

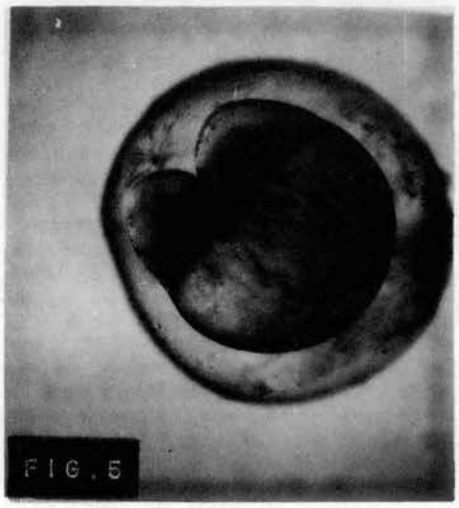


FIG. 5

FIG. 6

PLATE IV

Average time intervals are given from fertilization

Figure 1 - Eight-celled ovum; lateral view; 85 minutes.

Figure 2 - Sixteen-celled ovum; 110 minutes.

Figure 3 - Blastula; 2 hours, 40 minutes.

Figure 4 - Gastrulation; epiboly and involution continue; 5 hours, 40 minutes.

Figure 5 - Blastoderm enveloping about one-half the yolk sphere; 7 hours, 20 minutes.

Figure 6 - Early stage in the closure of the blastopore; 9 hours, 45 minutes. Note pear-shaped yolk plug and embryonic shield (ES).

PLATE-IV

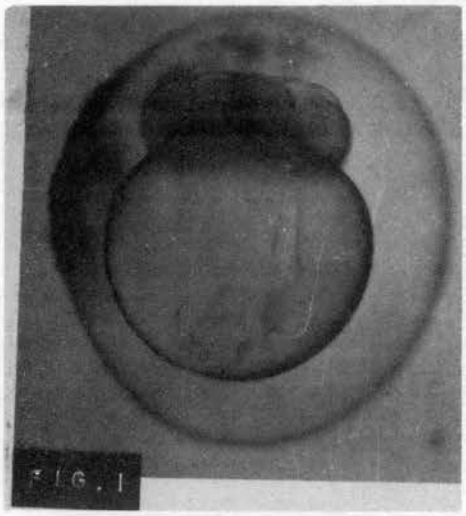
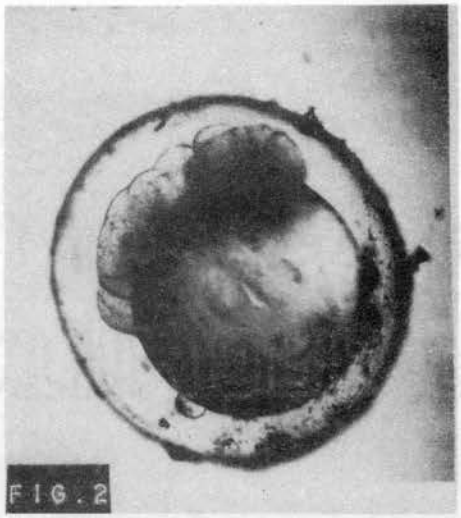


FIG. 1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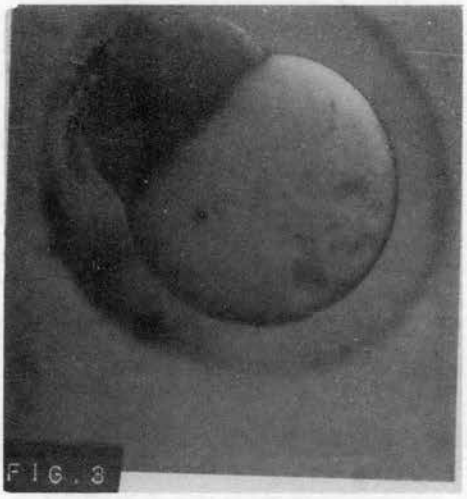


FIG. 3

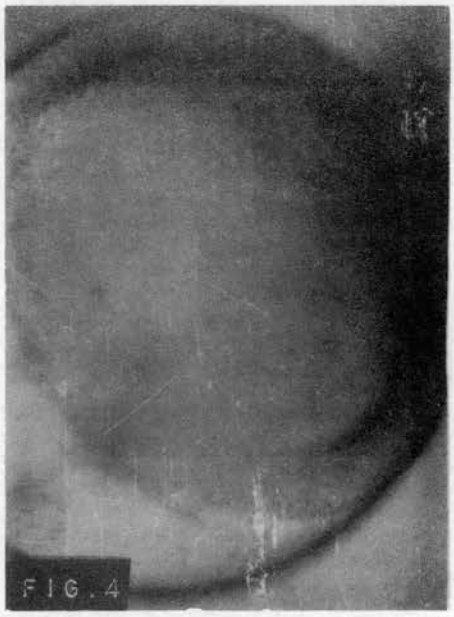


FIG. 4

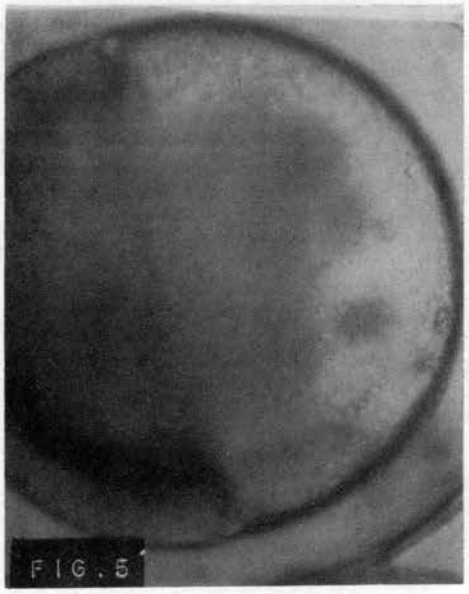


FIG. 5

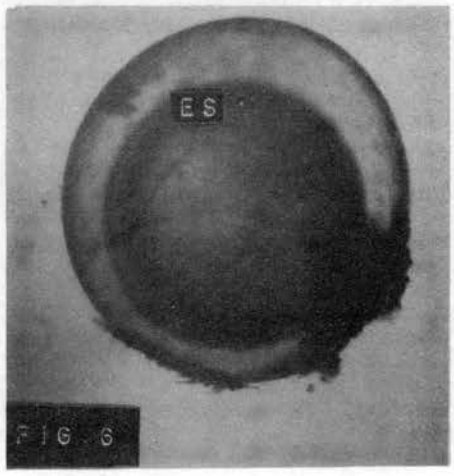


FIG. 6

PLATE V

Figure 1 - Closure of blastopore; one to two somites present; gastrulation ends; 11 hours, 45 minutes.

Figure 2 - Optic vesicle stage; three pairs of somites; 14 hours.

Figure 3 - Otic placode; Kupffer's vesicles; 17 somites, tail beginning to elevate from the yolk. First muscular contraction starts within one hour in this stage; the yolk sphere shows a slight constriction posteriorly; 21 - 22 hours.

Figure 4 - Optic cup stage, with lens; dorsal view; 20 somites; 24 hours.

Figure 5 - Otoliths (asteriscus and lapillus). Pectoral fin bud formed; common cardinals; choroid fissure shown as dark area ventrally located in the eye. 31 somites; 31 hours.

Figure 6 - Prehatching stage.

PLATE-V

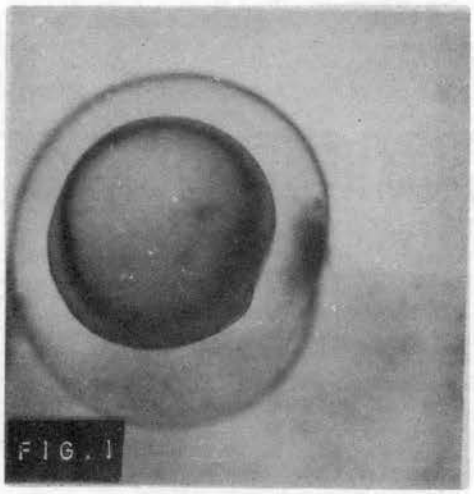


FIG. 1

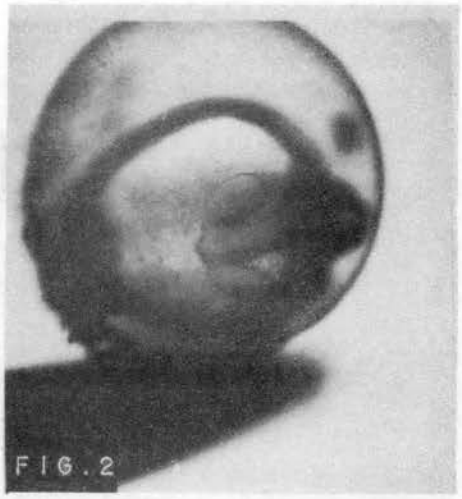
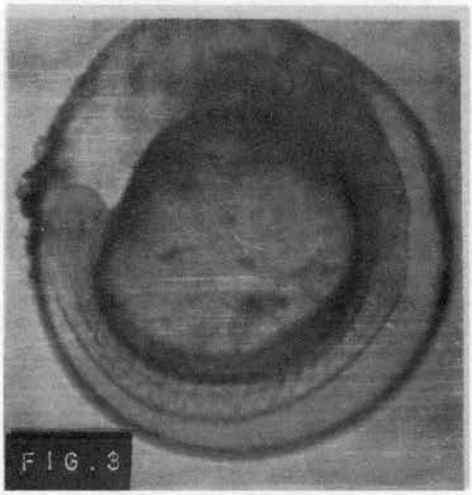


FIG. 2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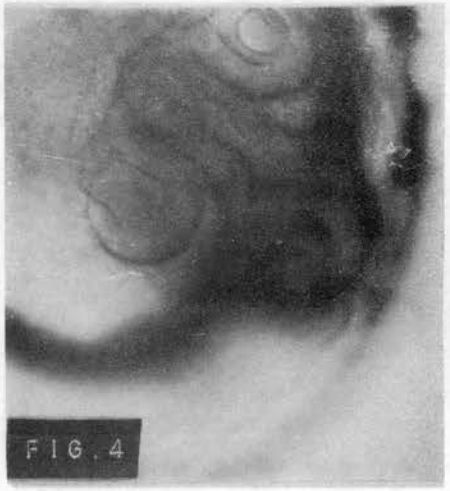
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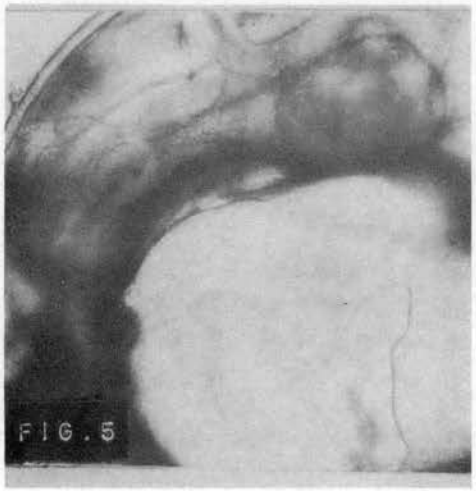
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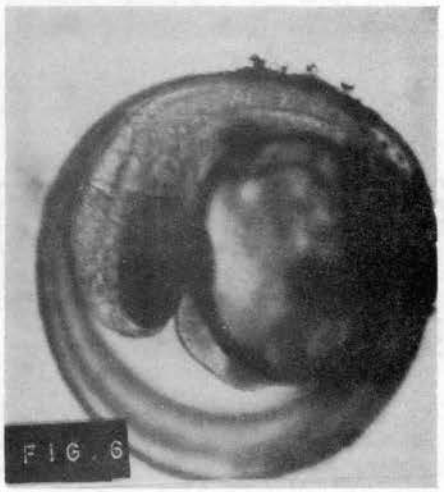


FIG. 6

PLATE VI

Scale line represents 50 microns

Figure 1 - Hatching, tail first.

Figure 2 - Hatching, head first.

Figure 3 - Cross section; 4.10 mm; the primitive chamber of newly hatched embryo.

Figure 4 - Cross section; 4.40 mm stage; the intraepithelial material and the concentric connective tissue fibers in the primitive posterior chamber.

Figure 5 - Cross section; 4.65 mm stage; the primitive posterior chamber; increased intraepithelial material; enlarged lumen; increased concentric fibers.

Figure 6 - Frontal section; 5.75 mm stage; the newly formed anterior air chamber.

Legend:

AC - Anterior chamber
CF - Concentric connective tissue fibers
G - Gut
IE - Intraepithelial material
LC - Low columnar epithelium
MS - Mesenchyme of tunica externa
N - Notochord
PC - Posterior chamber
SE - Squamous epithelial lining
Y - Yolk platelets

PLATE-VI

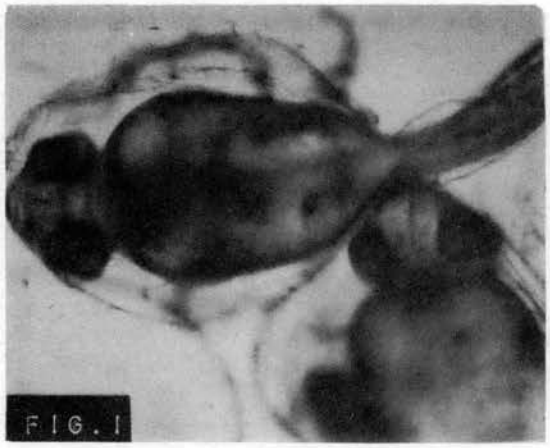


FIG. 1

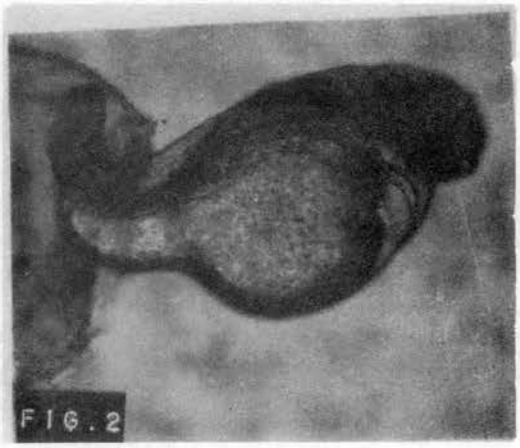
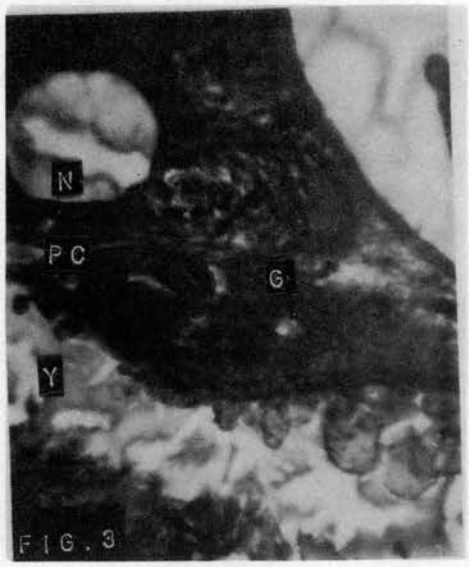


FIG. 2



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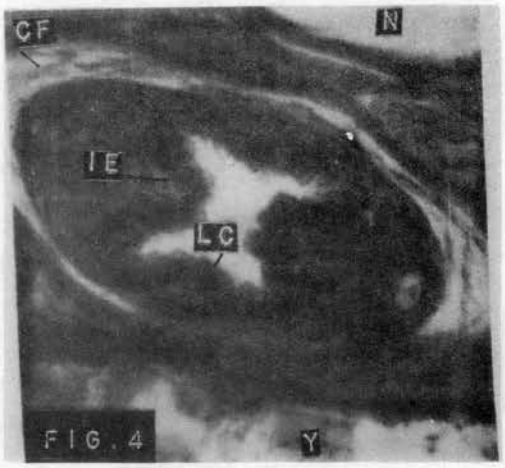


FIG. 4

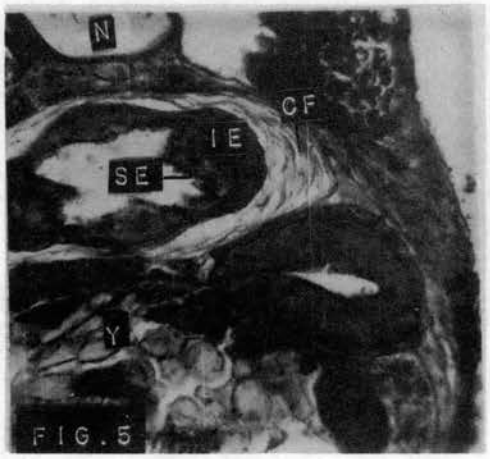


FIG. 5

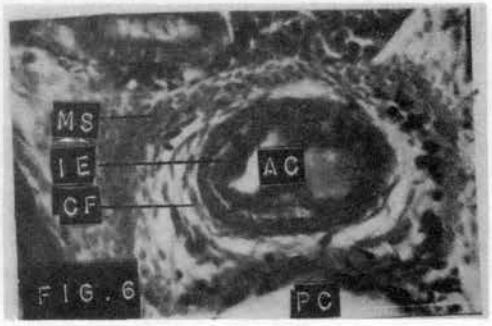


FIG. 6

PLATE VII

Scale line represents 50 microns

Figure 1 - Cross section; 6.25 mm stage; occlusion of the lumen of the anterior chamber by increased amount of transient material.

Figure 2 - Sagittal section; 6.25 mm stage; the third basiventral is connected by mesenchyme to the tunica externa.

Figure 3 - Cross section; 7.0 mm stage; anterior chamber.

Figure 4 - Frontal section; 10.0 mm stage; the ossa suspensoria.

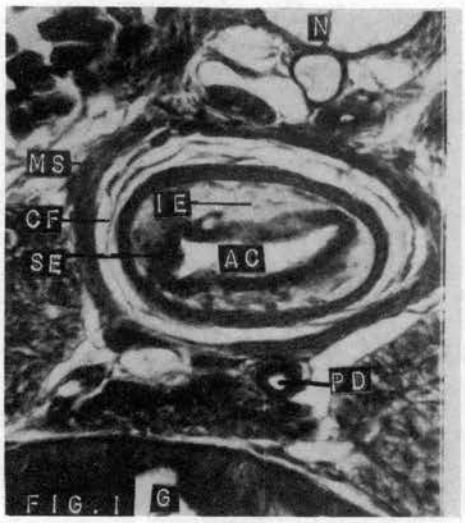
Figure 5 - Frontal section; 8.5 mm stage; the ductus communicans.

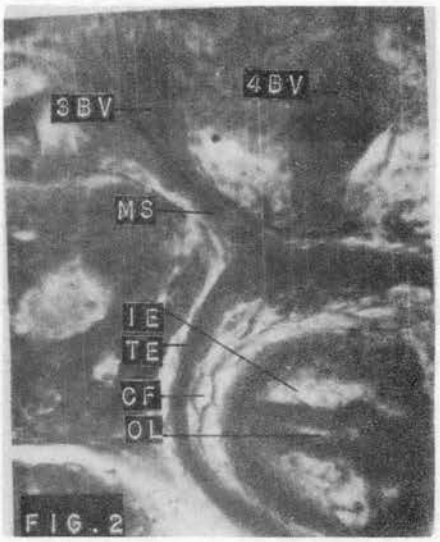
Figure 6 - Frontal section; 10 mm stage; the intervertebral region.

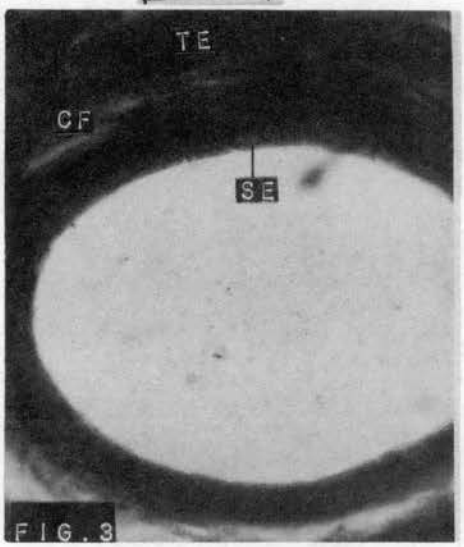
Legend:

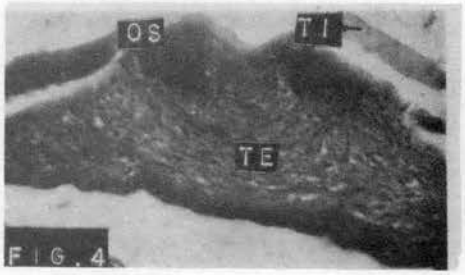
AC - Anterior chamber
BV 3 and 4 - Third and fourth basiventral
C - Centrum
CF - Concentric connective tissue fibers
CT - Noncellular perichordal layer
EI - Elastica interna
EX - Elastica externa
G - Gut
IE - Intraepithelial material
MS - Mesenchyme
N - Notochord
NE - Notochord epithelium
OL - Occluded lumen of the anterior chamber
OS - Os suspensorium (central plate)
P - Noncellular perichordal material
PC - Posterior chamber
PD - Pneumatic duct
SE - Squamous epithelium
SM - Smooth muscle
TE - Tunica externa
TI - Tunica interna
TL - Intervertebral ligament

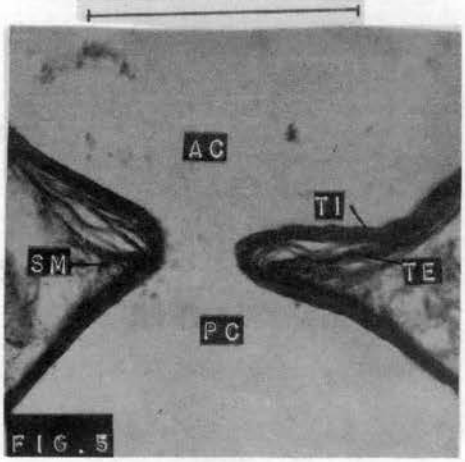
PLATE-VII


N
MS
CF
IE
SE
AC
PD
FIG. 1 G


3BV
4BV
MS
IE
TE
CF
OL
FIG. 2


TE
CF
SE
FIG. 3


OS
TI
TE
FIG. 4


AC
TI
SM
TE
PC
FIG. 5

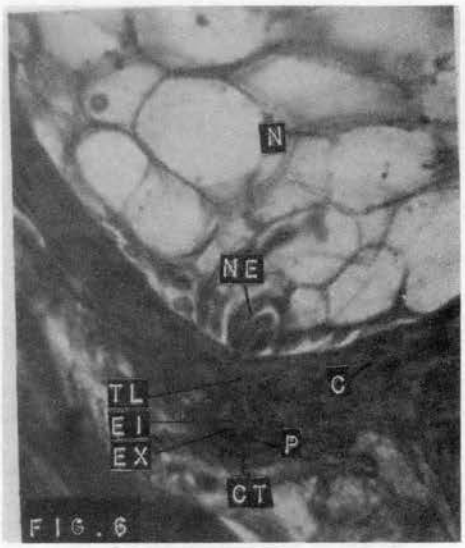

N
NE
TL
EI
EX
C
P
CT
FIG. 6

PLATE VIII

Scale line represents 50 microns

Figure 1 - Cross section; 13 mm stage; ossified notochordal trabeculae.

Figure 2 - Frontal section; 6.0 mm stage; the first three basidorsals.

Figure 3 - Cross section; 6.25 mm stage; the first basidorsal.

Figure 4 - Cross section; 6.50 mm stage; the concha stapedis.

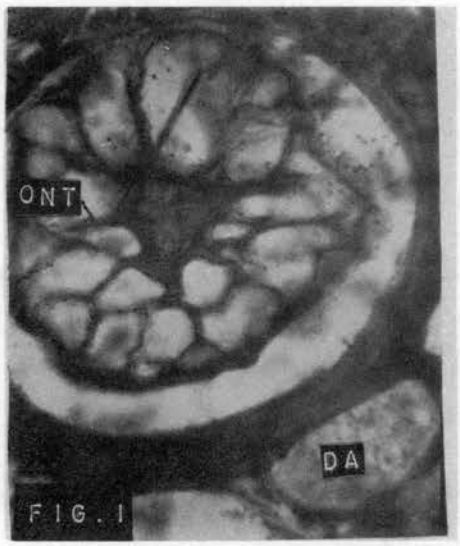
Figure 5 - Cross section; 6.50 mm stage; the evaginations of the sacculi.

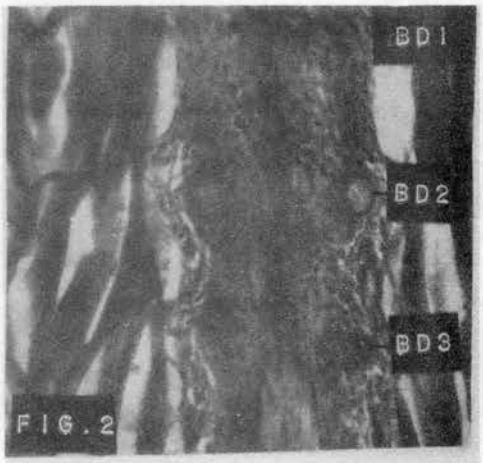
Figure 6 - Sagittal section; 6.75 mm stage; the concha stapedis, manubrium incudis.

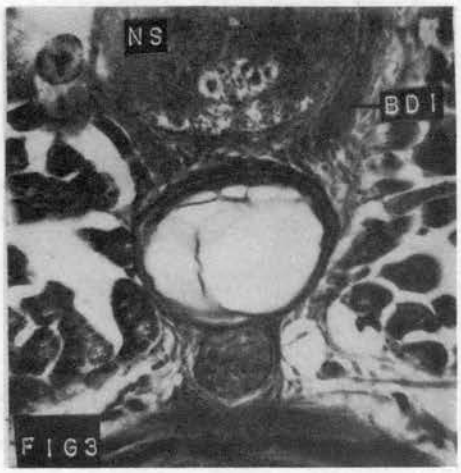
Legend:

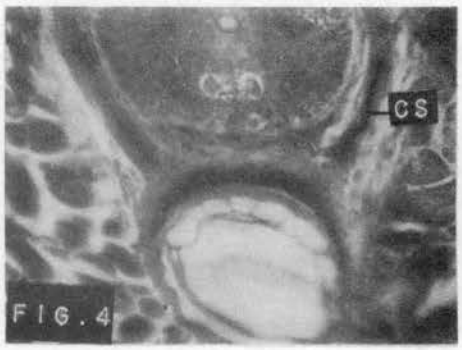
ASI - Atrium sinus imparis
BD - Basidorsal
CS - Concha stapedis
DA - Dorsal aorta
DE - Ductus endolymphaticus
MI - Manubrium incudis
N - Notochord
NS - Nerve cord
ONT - Ossified notordal trabeculae
PL - Perilymph
PN - Perineural tube
SAM - Saccular macula
SN - Spinal cord

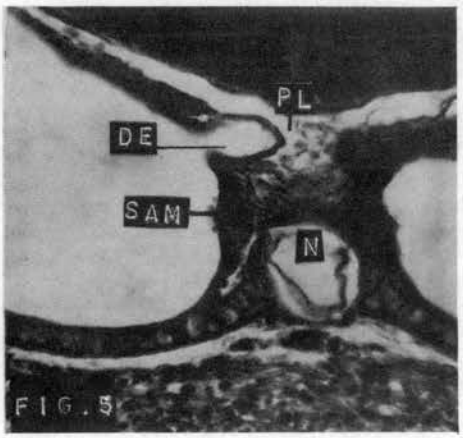
PLATE-VIII


ONT
DA
FIG. 1


BD1
BD2
BD3
FIG. 2


NS
BD1
FIG3


CS
FIG. 4


PL
DE
SAM
N
FIG. 5

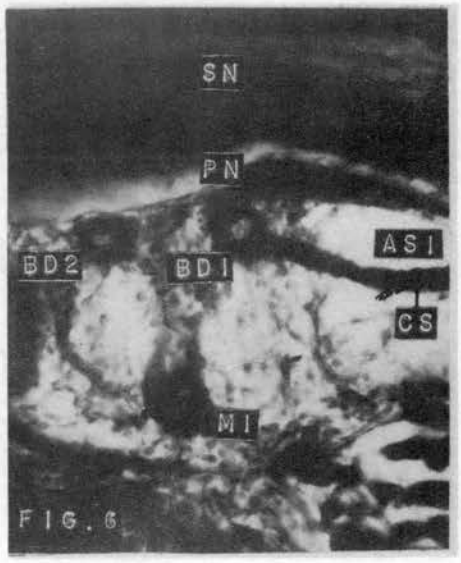

SN
PN
BD2
BD1
ASI
CS
MI
FIG. 6

PLATE IX

Scale line represents 50 microns

Figure 1 - Sagittal section; 8.25 mm stage; the perineural tube is not an extension from the horizontal plate of the exoccipitals.

Figure 2 - Frontal section; 8.5 mm stage; the ossified nipple of concha stapedis.

Figure 3 - Cross section; 9.0 mm stage; the synovial cavity in the articulation of the scaphium.

Figure 4 - Cross section; 8.0 mm stage; the claustrum as a faint bony strip embedded in mesenchyme.

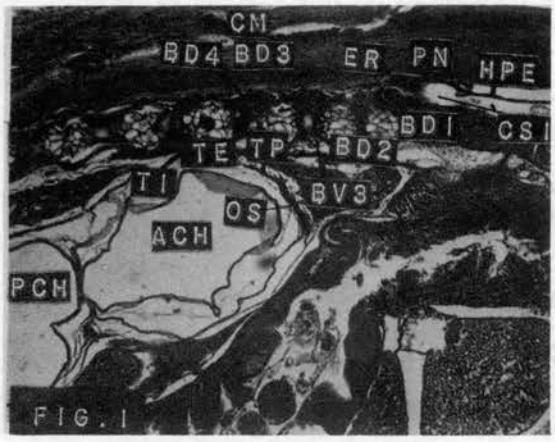
Figure 5 - Cross section; 12.0 mm stage; the proatlas. Adult claustrum. The sac-like perilymphatic atrium sinus imparis is also shown.

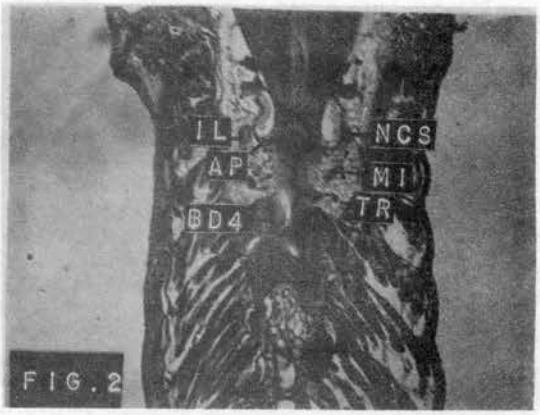
Figure 6 - Frontal section; 7.0 mm stage; the osseous connection between the manubrium incudis and the articulating process of the intercalarium. Compare with (Plate VII, Figure 6).

Legend:

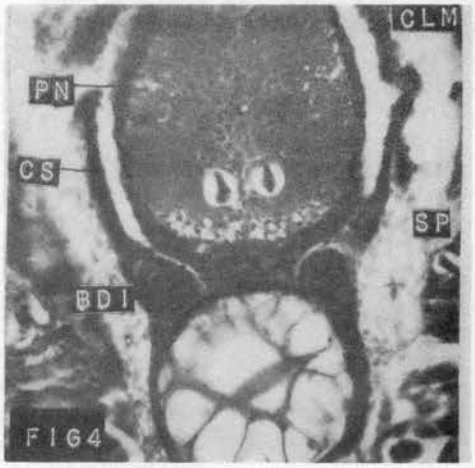
ACH - Anterior chamber
AP - Articular process
ASI - Atrium sinus imparis
BD 1,2,3,4 - Basidorsals
BV 3,4 - Basiventral
CL - Claustrum
CLM - Ossified primordium of the claustrum embedded in mesenchyme
CM - Cartilage mass
CS - Concha stapedis
CSI - Cavum sinus imparis
ER - Endorhachis
HPE - Horizontal plate of exoccipitals
IL - Interossicular ligament
KP - The keratinized roof of esophagus
MI - Manubrium incudis
NA - Neural arch
NCS - Nipple of concha stapedis
OA - Occipital arch
OS - Os suspensorium
P - Esophagus
PA - Proatlas
PB - Pharyngeal process of basioccipital
PCH - Posterior chamber
PN - Perineural
SP - Saccus paravertebralis
SYC - Synovial cavity
TE - Tunica externa
TI - Tunica interna
TP - Transformator process
TR - Tripus

PLATE-IX


CM
BD4 BD3
ER
PN
HPE
BDI
CSI
TE TP
BD2
TI
BV3
OS
ACH
PCH
FIG. 1


IL
NCS
AP
MI
BD4
TR
FIG. 2


OA
2NA
CL
ER
IL
SYC
SP
PB
FIG. 3


CLM
PN
CS
SP
BDI
FIG4


OA
2NA
CL
CS
ASI
IL
PA
SP
FIG. 5
KP

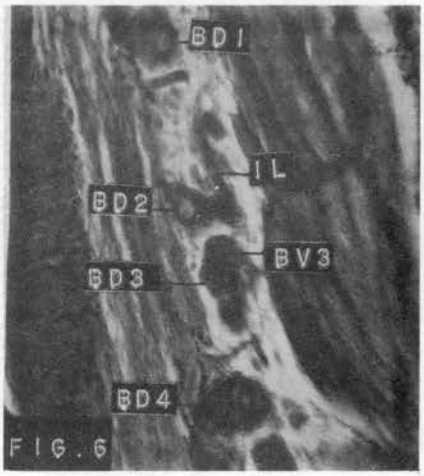

BDI
IL
BD2
BV3
BD3
BD4
FIG. 6

PLATE X

Scale line represents 50 microns

Figure 1 - Sagittal section; 7.65 mm stage; the mesenchyme connecting the peripherally fibrous partly ossified portion of the interossicular ligament.

Figure 2 - Cross section; 9.5 mm stage; adult stage of tripus. The fibrous band extends from the posterior part of the 3rd basiventral to the fourth basiventral.

Figure 3 - Frontal section; 14.5 mm stage; the tensor tripodis.

Figure 4 - Frontal section; 14.5 mm stage; the neural complex.

Figure 5 - Cross section; 14.5 mm stage; the neural complex.

Legend:

AC - Anterior chamber
AR - Anterior ramus of tripus
B - Body
BA - Base of neural complex
BD - Basidorsal
BO - Boat of neural complex
BV - Basiventral
C - Centrum
CS - Concha stapedis
DLL - Dorsomedian longitudinal ligament
FB - Fibrous band
G - Gut
HCM - Hump of cartilage mass
IL - Interossicular ligament
MI - Manubrium incudis
MT - Mesenchyme between interossicular ligament and third basiventral
OC - Occipital region of skull
OS - Os suspensorium
N - Notochord
NCOM - Neural complex
3NP - 3rd neural pedicle
PR - Posterior ramus of tripus
SP - Saccus paravertebralis
ST - Stem of neural complex
TP - Transformator process of tripus
TR - Tripus
TT - Tensor tripodis

PLATE-X

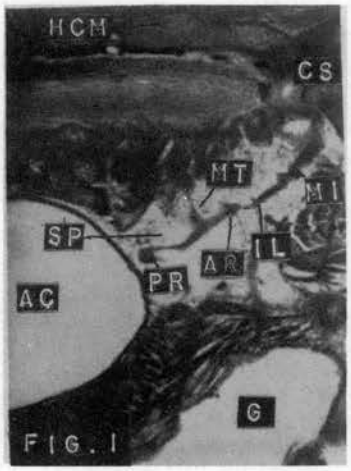


FIG. 1

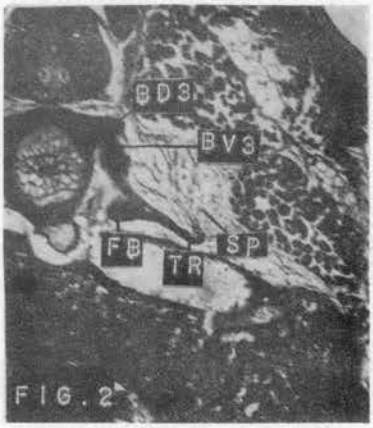


FIG. 2

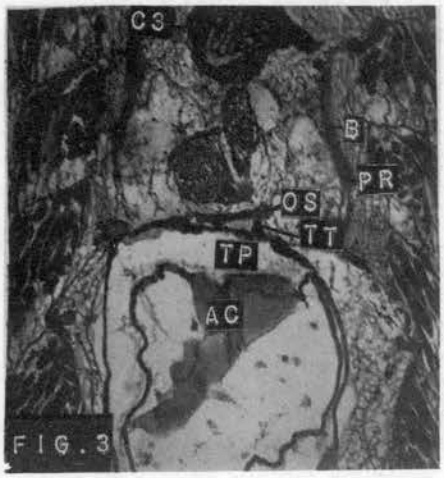


FIG. 3

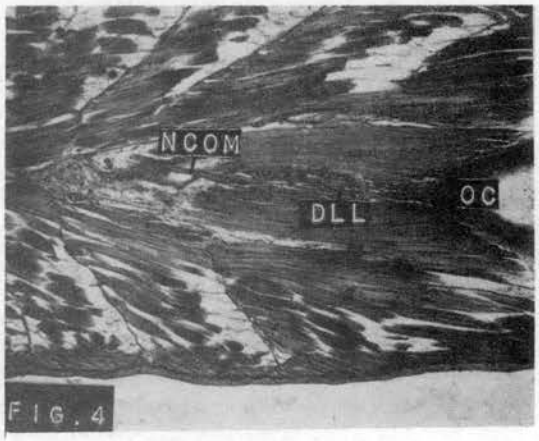


FIG. 4

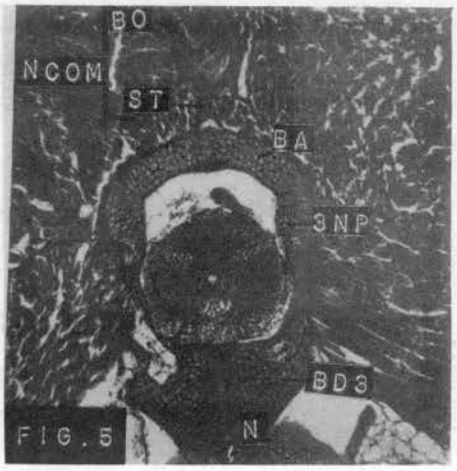


FIG. 5

PLATE XI

Scale line represents 50 microns

Figure 1 - Cross section; 3.75 mm embryo; the right pars superior. H. E. stain.

Figure 2 - Cross section; 5.75 mm stage; the crus commune.

Figure 3 - Frontal section, 10 mm stage; the membranous labyrinth.

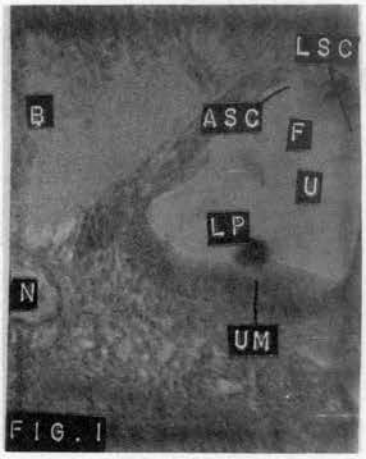
Figure 4 - Cross section; 13.0 mm stage; the membranous labyrinth.

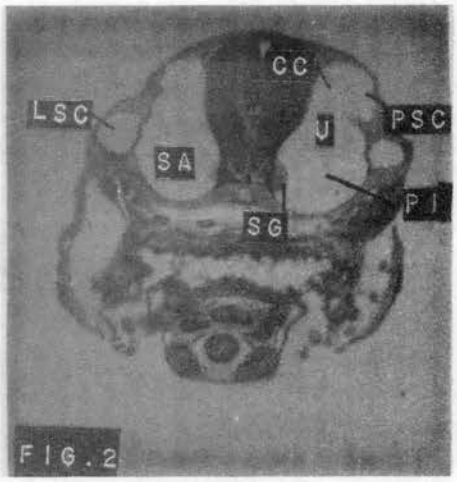
Figure 5 - Cross section; 13.0 mm stage; the relationship between the cavum sinus imparis and saccus endolymphaticus.

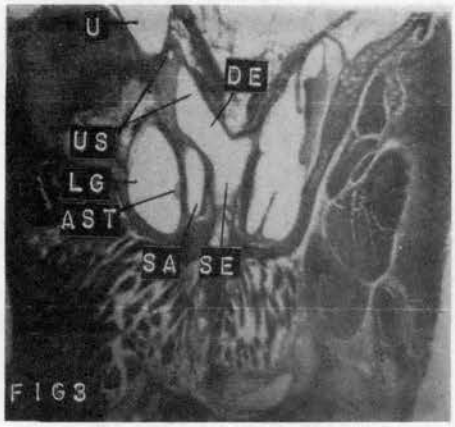
Legend:

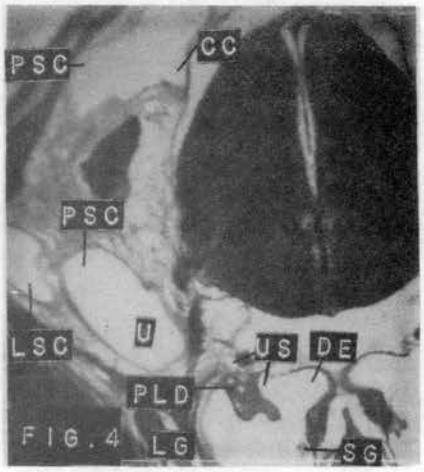
ASC - Anterior semicircular canal
AST - Asteriscus
B - Brain
CC - Crus commune
CSI - Cavum sinus imparis
DE - Ductus endolymphaticus
F - Fold to form the canals
HO - Horizontal plate of the exoccipitals
LG - Lagena
LP - Lapillus
LSC - Lateral semicircular canal
N - Notochord
PI - Pars inferior
PLD - Perilymphatic duct
PSC - Posterior semicircular canal
SA - Sacculus
SE - Saccus endolymphaticus
SG - Sagitta
U - Utriculus
UM - Utricular macula
US - Utriculosaccular duct

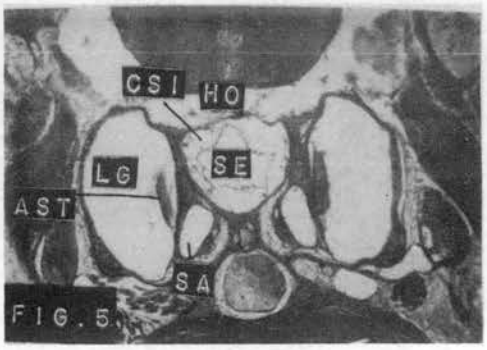
PLATE-XI


LSC
B
ASC
F
U
LP
N
UM
FIG. I


CC
LSC
U
PSC
SA
PI
SG
FIG. 2


U
DE
US
LG
AST
SA
SE
FIG3


PSC
CC
PSC
LSC
U
US
DE
PLD
FIG. 4
LG
SG


CSI
HO
LG
SE
AST
SA
FIG. 5.

VITA

Anwar David Niazi

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Doctor of Philosophy

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