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The embryology of the paradise fish, macropodus opercularis Linnaeus

Lewis M. Mulkay
Brigham Young University - Provo

THE EMBRYOLOGY OF THE PARADISE FISH, *MACROPODUS OPERCULARIS* LINNAEUS

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INTRODUCTION

The purpose of this study was to describe the normal embryological development of the Paradise Fish, Macropodus opercularis, Linnaeus. It was undertaken to learn the ages at which various structures and systems appeared and to determine whether or not the typical pattern of teleostean development was followed. Allen (1951) used M. opercularis for his investigation of anomalies caused by x-irradiation. In order to understand more fully the anomalies produced by x-rays in this species, it was felt that a more detailed knowledge of the normal development should be known.

The natural habitats for the Paradise Fish are the rice paddies, small rivers, and streams of China (Innes, 1955). Its economic importance is probably as a mosquito control in those areas, since its size is too small for it to be of value as a food source. The ease of laboratory breeding and the great number of offspring produced (up to 1200 eggs per breeding) make the Paradise Fish a convenient organism for studies in both experimental and descriptive embryology.

According to Berg (1947) the Paradise Fish is a member of the order Perciformes, family Anabantidae (ex parte Labyrinthici). Anabantidae are characterised by having a complex mass of labyrinths in the auditory area. These

labyrinths function to trap and store air which the fish gulps into its mouth. The air then moves out over the gills and out through the operculum. This extra respiratory process helps the Paradise Fish to live in the warm, muddy waters of its native rice paddies in spite of the low oxygen content found there.

The literature pertaining to Macropodus opercularis deals primarily with taxonomy. There are a few studies of particular structures such as: chromatophores (Dalton and Goodrich, 1937), air-breathing organs (Das, 1927), color patterns (Goodrich and Smith, 1937), respiratory labyrinths (Ito, 1950), chloride secreting cells (Liu, 1942), and hydrostatic apparatus (Peters, 1946). No detailed study on the normal embryology has been found in literature at this time. Only superficial studies relating to the natural history features, gross anatomical observations and notes on development can be found (Boulart, 1872 and Pouchet, 1872). Dr. Hans M. Peters (1956) of the University of Tuebingen, Tuebingen, Germany, referred to work to be published about Macropodus opercularis, but thus far it has not been found in the literature by this author.

METHODS AND MATERIALS

The fish used for the present study were kept in three gallon and twenty gallon aquaria. The temperature was maintained between 24° and 28°C. Some of the newly hatched embryos were retained to observe their gross development. These were first fed infusoria and powdered lettuce. The powdered lettuce served primarily as a food for bacteria, which in turn served as food for infusorial organisms, and it was the infusoria that served as the first food for the fry as they developed. When the fry were old enough, they were fed brine shrimp and ground liver.

After the eggs and embryos were collected, they were placed in Bouin's solution. The specimens were then progressed stepwise, through a series of alcohols to dehydrate them (Brauer, 1955). Cedal-oil was used instead of the usual clearing agent, Xylol, in order to prevent hardening of the yolk material. Following clearing, the embryos were embedded in paraffin wax, mounted on wooden blocks, and sectioned at four to six microns. They were then stained with haematoxylin and counterstained with eosin (Brauer, 1955).

Approximately one hundred and fifty embryos in varying stages of development were examined with an Olympus binocular compound microscope at magnifications of 40, 100, 200, and 400

diameters. Illumination was provided by a Bausch and Lomb model PR27 illuminator that was modified by various blue and neutral filters.

In order to illustrate appropriate developmental stages, certain sections were photographed with a Zeiss-Ikon camera using Kodak, Panatomix X, FX135 film. Various exposure times were used depending upon the magnification used and the intensity desired.

ORGANOLOGY

This section includes a description of the normal stages of development of the major organs, presented in unit form for each organ discussed. The sequence of development of each organ is described from initial appearance or organisation to the stage of development as found at four or five days of age. For the most part this includes the major changes in each of the organs discussed. Not every organ of M. opercularis is discussed nor is any one discussed completely. The organs are taken up in the following order: brain, eye, ear, notochord, heart, and gut.

The Brain

The brain is one of the first definite structures to be recognised in the developing embryo. It progresses from a primitive neural keel, through the formation of a large ellipsoidal rod, and on to a sequence of first opening to form the neurocoels, then a subsequent closing of the neurocoels. As viewed in cross section the shape of the brain changes from a flat, wedge-shaped keel to an ellipsoidal rod, to a triangular adult brain.

The neural keel is formed of ectoderm on the surface of the blastodisc, laying along the long body axis. At 11 hours (Fig. 1) it is shallow but wide in cross section (5-8 cells

deep). At the same time that the neural keel appears, Kupffer's Vesicle appears in the posterior region (Figs. 8, 31). At 12 hours the neural keel is narrower and deeper (12-15 cells deep). By 16 hours the brain has changed from a keel to a solid ellipsoidal structure anteriorly, the anlage of the forebrain (Fig. 3). The posterior region remains in the form of a keel-shaped structure.

The forebrain is a solid structure at 16 hours, as described above. The process of cavitation of the forebrain is initiated at 19 hours, when a vertical slit appears along the midline (Figs. 9, 10, 24). At 20 hours the infundibulum appears at the base of the diencephalon (Fig. 4) and the brain is flexed (Fig. 3), expressing the pontine, apical, and nuchal flexures. The optic stalk (diencephalon) is hollow at 23 hours (Fig. 11). By 25 hours the epiphysis (Fig. 4) is formed at a point dorsal to the pontine flexure (diencephalon). at 35 hours white matter is found in the lateral areas of the telencephalon and diencephalon.

The midbrain follows somewhat the same time pattern as the forebrain. It is a solid ellipsoid at 16 hours (Fig. 25) and is opened by cavitation by 19 hours when a vertical slit appears along the midline. The mesencephalon develops a wide cavity by 20 hours, forming the third ventricle (Fig. 3). The optic lobes (Fig. 2) form by cavitation of the mesencephalon by 21 hours. By 25 hours the cavity of the optic lobes widen considerably but are narrow again by 28 hours. Also at 28 hours the floor of the mesencephalon is very thick, and

white matter is visible along the ventral part of the central nervous system from the level of the ears to the posterior region of the spinal cord. By 60 hours the cavity of the optic lobes have been dorso-ventrally reduced to mere horizontal slits. By 90 hours (Fig. 19) only a small band of grey matter remains surrounding the neurocoel of the midbrain.

While the forebrain and midbrain are developing together, the hindbrain lags its anterior counterparts. At 16 hours the hindbrain is still in a stage between the neural keel and solid ellipsoid mentioned above. The spinal cord is still in the neural keel stage at this time. At 19 hours when the more anterior part of the brain is cavitating, the hindbrain remains solid. However, by 21 hours the brain is open throughout, all parts having opened by the process of cavitation. The roof of the mylencephalon is very thin, only one cell layer thick, by 25 hours. At 31 hours it may be noticed that the fourth ventricle, which has been as wide as the prosocoel and mesocoel, begins to diminish in diameter in the posterior region of the myelencephalon. At 35 hours white matter may be found ventral to the fourth ventricle (Fig. 42) and the lumen of the fourth ventricle is very small. The fourth ventricle is nearly squeesed out of existence by 48 hours (Fig. 25).

The white matter is visible from the level of the ears (mesencephalon) to the posterior region of the spinal cord at 28 hours (Fig. 34). At 35 hours the white matter is lateral to the telencephalon and diencephalon and is ventral to the mesencephalon and rhombencephalon. By 48 hours the white

matter has increased to such a degree that it represents one-fourth the area of the brain and one-third the area of the spinal cord (in cross section). The white matter fills one-half of the brain area as viewed in cross section by 84 hours (Fig. 37). Finally by 90 hours the white matter is more abundant dorsally as well as ventrally so that only a small amount of grey matter may be found in the medial, mid-dorsal area and surrounding the ventricles of the optic lobes (Fig. 7).

The brain is underlayed by a cartilage band (with central vertebrae and lateral wings), the anlage of the brain case and spinal column (Fig. 54). A cavity exists between this cartilage and the now triangular brain (Fig. 54). The brain has now undergone a change in general shape from ellipsoidal to triangular (in cross section). By 84 hours all existing neurocoels of the brain are mere slits and only a mid-ventral cavity remains in the diencephalon (Fig. 19).

The spinal cord follows the same pattern as that set forth for the hindbrain. The times of its cavitation (neural canal) and closure are the same as for the hindbrain. Two canals develop early as a result of the single neural canal closing medially (Fig. 32). Later (Figs. 43, 45, 46, 47) the more dorsal of the cavities closes at the same time as does the fourth ventricle (31-35 hours).

Some interesting development in conjunction with Kupffer's vesicle may be noted. Kupffer's vesicle (Fig. 8) appears as a cavity at 11 hours under the presumptive spinal cord (a neural keel at this time). At 17 hours the vesicle is

lined dorsally by columnar cells and ventrally by periblast. At 19 hours the spinal cord touches the dorsal surface of Kupffer's vesicle (Fig. 31). Kupffer's vesicle for some reason unknown to the author, simply disappears at 18 hours.

The Eye

The eye first makes its appearance as paired optic buds (Fig. 8), which protrude laterally from the brain (diencephalon) at 16 hours. At 17 hours an optocoel appears in each bud to form the optic vesicle (Fig. 9), which enlarges during the next two hours. By 19 hours the vesicles indent to form the optic cups and the lens placode develops as an inward thickening of the ectodermal layer at a point opposite the center of the optic vesicles (Fig. 10). The optocoels persist for a short time even though the optic cups are now formed (19 hours). The lens remains attached as a placode (Fig. 12) until 23 hours when it is freed. Also at 23 hours the optic stalk, which is an extension of the brain, is complete from the brain to the optic cup and is hollow (Fig. 11).

The retina grows until, at 33 hours, it is bent around the lens so that it encircles the lens except at the region of the cornea. Also at this time the texture of the lens takes on a loose appearance, denoting the morphogenesis from a homogenous mass of undifferentiated cells to individual fiber-like cells (Fig. 6). The lens has the appearance of three laminated concentric spheres at 35 hours (Fig. 22). Pigment first appears in the sclera at 37 hours (Fig. 13). The retina

at this time remains unpigmented. At 39 hours the piliform layer of the retina forms (Fig. 14) and four distinct layers are visible in the retina (Fig. 15): (1) inner nuclear layer, (2) piliform layer, (3) outer nuclear layer, (4) rod and cone layer. The optic chiasma is first seen and is complete at 42 hours (Figs. 19, 20, 21, 23). The retractor lentis (Figs. 14, 21) is evidenced at 45 hours as it appears as a continuation of the optic nerve. (It later is differentiated as a muscle although it appears to be nerve-like at this early stage due to its position and origin). It is readily visible from 45-54 hours and is visible in the early adult several days old, but becomes indistinct between 54 and 132 hours (the limit of this study).

By 57 hours the rods and cones begin to lengthen, thereby decreasing the cavity between themselves and the pigmented layer of the sclera (Fig. 15). The piliform layer also widens at 57 hours. The rods and cones complete their lengthening by 60 hours and the now more prevalent pigment granules invade the area between each rod and cone so that the pigment seems to overlay the rods and cones (Fig. 17). Also at this time the iris starts to form and be pigmented, and the cornea begins to bulge outward (Fig. 17), probably due to the formation of the aqueous humor.

The iris is definitely well shaped and pigmented by 90 hours (Fig. 22) and by 108 hours the bulging of the cornea has markedly increased. The only further change by 132 hours is a size increase.

The Ear

The ear develops as a solid triangle at 18 hours (Fig. 24), as a result of the organization of mesenchyme material (neural crest material) which lies alongside the brain. At 19 hours a lumen forms in the organized group of cells resulting in the formation of an otic vesicle with walls of uniform thickness. The triangle appears as an ellipsoid in sagittal or frontal sections, with the long axis being parallel to the long axis of the embryo. The ear thins along the dorsal-external wall, so that by 27 hours the walls are no longer of uniform thickness (Fig. 42). This results in a triangular structure with a base thicker than the sides and the top. Enlargement of the ear takes place so that by 28 hours it is a comparativoly large single chamber. The otic ganglia (Figs. 26, 44, 48) may be seen in juxtaposition to the mid-ventral surface of the otic vesicle at 30 hours. The ear at this time is composed of two to three layers of cuboidal cells at its base. The side walls thin dorsally so that the vesicle has only one flattened cell layer at its apex. The endolymphatic duct is first indicated as a pointed anterio-dorsal constriction of the otic vesicle at 33 hours (Fig. 46). In the ear there are three maculae that function with the lateral line. These maculae are cone shaped clusters of cuboidal cells, with the apex inward toward the center of the lumen of the ear. These maculae appear at 39 hours and are

located mid-ventrally, latero-ventrally, and mid-dorsally on the inner surface of the otic vesicle (Figs. 35, 44). The first indication of chambering in the ear is at 51 hours when a shelf (Fig. 26) begins to form in the antero-dorsal area, separating off the endolymphatic area. The shelf is nearly complete at 54 hours and is complete by 57 hours (Fig. 27). A second shelf, at a right angle to the first shelf, quickly follows; for, at 60 hours, the ear is a three-chambered structure (Fig. 28). These three chambers may be distinguished as the endolymphatic duct (antero-dorsal), utricle (dorsal chamber of the large part of the vesicle), and the sacculus (ventral chamber of the large part of the vesicle). By 90 hours (Fig. 29) the ear has walls of uniform thickness one cell thick, and is a large structure rivaling the brain and eyes for space in the head. By 108 hours the shelves have become thickened to two to three cell layers. By 132 hours only size changes can be seen to differ from the 108 hour stage.

Notochord

During the early life of M. opercularis, the primary source of axial support is from the notochord. Later, of course, the bony vertebral column develops to give major support.

The notochord develops from the mesodermal plate region ventral to the central nervous system. At 16 hours the notochord (Fig. 30) first appears as a small solid rod laying below

the posterior one-half of the central nervous system. The notochord is small (two to three cells in diameter), round (in cross section), and solid by 17 hours (Figs. 24, 41). By 20 hours the notochord changes from a rod composed of small cells to a dorso-ventrally flattened ellipsoid of taller cells. The notochord nearly reaches its characteristic vacuolated (Battle, 1944) appearance by 25 hours (Fig. 32). By 30 hours the notochord is definitely vacuolated in appearance and is round in cross section, rather than ellipoidal as it was previously (Fig. 33). At 35 hours the notochord, in cross section, is composed of three to four cells (Fig. 44) at the level of the ears, but posteriorly it is larger, being composed of five to six cells (Fig. 42). By 39 hours the entire notochord is composed of only three large cells in cross section (Figs. 54, 57). For comparative size of the notochord, it may be stated that at 48 hours the spinal cord and notochord are the same diameter in cross section (Fig. 43).

The Heart

No indication of the heart as a structure occurs before 23 hours. At this time a bulb or short tube appears under the left eye along the anterior margin of the yolk sac. This unusual positioning of the early heart is also reported in Trichogaster trichopterus, another member of the family Anabantidae, by Ingersoll (1953). Two hours later (25 hours) the heart has moved its dorsal attachment posteriorly, closer to the level of the ears. At 29 hours the heart is only a

tube (Fig. 34) bent anteriorly, near the juncture of the presumptive truncus arteriosus and presumptive ventricle, so that the ventricle hangs ventrad and the presumptive atrium extends latero-posteriorly toward the extra-embryonic area. A very slight thickening of the anterior walls of the tube is noticed by 30 hours indicating the truncus arteriosus and ventricle. The first indication of differentiation is at 35 hours, distinguished by the walls of the heart proper being of uniform thickness and the walls of the truncus arteriosus being slightly thicker than the heart. Also at 35 hours, the extra-embryonic blood sinuses are still open to the atrium and no actual valve exists between the atrium and ventricle. The ventricle is a structure of thicker walls than the atrium by 40 hours (Fig. 35). Also at 40 hours the atrio-ventricular valve is formed (Fig. 35) and the atrium is larger (distended) than the ventricle (compact). At 48 hours the heart is still linear (Fig. 36), definitely two-chambered, but not folded into an "S" shape. By 54 hours the folding has started (Fig. 37), resulting in the ventricle shifting from its anterior location to a position ventral and posterior to the atrium. Also at 54 hours the valve between the ventricle and truncus arteriosus is present as well as the atrio-ventricular valve. At 60 hours the atrium overlays the caudal one-fourth of the ventricle. By 70 hours the atrium covers one-half of the ventricle and at 84 hours three-fourths of the ventricle is overlayed (Fig. 38). By 108 hours five-sixths of the ventricle is covered. No change is observed by 132 hours (four days

post hatching). By 252 hours (nine days post hatching) the atrium completely covers the ventricle from the entrance of the truncus arteriosus to the posterior curvature of the ventricle (Fig. 39). The final change in the heart development may be seen completed by 324 hours (twelve days post hatching) wherein the atrium not only covers all of the dorsal surface of the ventricle but also overhangs the posterior surface of the now bulbous ventricle.

The Gut

The first indication of gut differentiation is noted as a longitudinal thickening or swelling of the endodermal layer below the notochord. This swelling is noticed in the posterior region of the embryo at 21 hours (Fig. 40). By 22 hours the hindgut and midgut are solid ellipsoidal rods. The hindgut and midgut are more rounded by 23 hours (Fig. 41).

At 26 hours the pharynx consists of solid wings of thickened endoderm lateral to the central axis of the foregut (Fig. 42). The esophagus is a solid rod between thepharynx and the anterior end of the midgut. The next change occurs at 32 hours when the hindgut and midgut open by cavitation (Fig. 43). The esophagus makes no change at this time, and at 33 hours is visible as a small solid rod between the posterior part of the pharyngeal wings (Fig. 44). At 36 hours a lateral split appears in the wings resulting in the opening of the pharynx (Fig. 48), but the esophagus remains solid. Also at this time the anus opens externally but an "anal plug" remains

at a position just anterior to the anal flexure of the hindgut (Figs. 45, 46, 47). The Wolffian ducts (Fig. 49) are open and ready to function so that possibly urination may take place at this time, 36 hours. The liver diverticulum (Fig. 50), occurs at 41 hours and seems to be preferential in outpocketing to the left, but occasionally it outpockets to the right. The "anal plug" is still present at 41 hours (Figs. 45, 46, 47). At 45 hours the esophagus may be seen as a solid rod extending from the open pharynx to the liver diverticulum. At 46 hours the "anal plug" is removed. The esophagus opens at 48 hours which results in the gut being open throughout. The gut is a straight tube at 57 hours (Fig. 51) but by 60 hours becomes a coiled tube (Figs. 52, 55). At this time the esophagus takes on its characteristic villous appearance (Figs. 53, 54). The stomach distends at 69 hours to form its usual enlargement in the digestive tract (Figs. 55, 56, 57). The walls of the stomach also have a villous appearance (Fig. 56). By 90 hours the gut has increased its coil so that in cross section three separate lumens may be seen.

HOURLY SUMMARY OF DEVELOPMENT

This section is a brief summary of major growth patterns discussed as they concurrently develop in periods of five hours. Included in this section is a brief summary of some events not discussed in the section on organology.

Hours 1-5

Stages of cleavage.

Hours 6-10

The blastodisc is now large and flattened. The process of epiboly advances the germ ring to cover one-half to two-thirds of the yolk surface and gastulation continues to form the primitive germ layers. Mesoderm begins to free itself from the ectoderm to form the primitive blood cells. At the close of this period the neural keel takes shape and is flanked by undifferentiated somites.

Hours 11-15

The notochord (Fig. 1) begins to take shape as a solid rod, beginning in the caudal region and differentiating cephalad to underlie the presumptive cranial region. The neural keel forms along the midline of the gastrula first as a shallow, but wide wedge, then deepens to form a neat wedge of neural material with the apex of the wedge downward, (Fig. 30).

Hours 16-20

The brain (Fig. 24) forms first as a large, solid, ellipsoidal rod, then hollows by cavitation (Fig. 9) forming the metencoel and myelencoel. The optic buds (Fig. 8) push out from the brain as solid balls at first connected to the brain, later becoming separate and hollow. The optic vesicles (Fig. 9) form from the optic buds by the formation of the optocoels. The ear (Fig. 24) appears as a solid cone organized from head mesenchyme. Kupffer's vesicle (Figs. 8, 31) forms in the posterior region as an ellipsoidal cavity, lined dorsally with columnar cells and ventrally with periblast. Later in this period, the notochord underlies the entire embryo and progressively changes shape from a small, solid, round rod (Fig. 24) to a larger rod with cells more columnar in shape. Also later, the infundibulum (Fig. 4) forms; the mesocoel (Fig. 3) widens greatly; the brain becomes flexed (Fig. 3); the otic vesicles become hollow; the optic vesicles begin to indent (Fig. 10) forming the optic cups; the lens takes shape as a lens placode (Fig. 10) and remains attached to the epidermis. The tail frees itself from the yolk, but the head region remains attached.

Hours 21-25

The brain opens throughout its entire length. The optic lobes (Fig. 2) cavitate in the metencephalon and open wide by the end of this period. The myelencephalon develops a very thin roof, and the epiphysis (Figs. 3, 4) forms in the diencephalon. The lens (Figs. 4, 12) becomes large and free

from the epidermis. The retinal region of the eye is very thick and homogenous (Figs. 4, 12). The notochord becomes more dorso-ventrally flattened and the cells of the notochord become more vacuolated (Figs. 32, 33, 40, 41). The heart forms as a bulb extending downward under the left eye and later in this period moves its dorsal point of attachment posteriorly and medially closer to the ear. The hindgut (Fig. 40) appears as a thickening under the notochord during the early part of this period. Later the midgut and hindgut develop as a solid rod in their respective areas (Fig. 41). The notochord is flanked by now differentiated somites, (Figs. 32, 33). The head frees itself from the yolk but remains in a very close juxtaposition. The extra embryonic blood cells are very numerous and evidenced in both the peripheral and subcephalic regions. The dorsal finbud is just visible late in this period. The paired Wolffian ducts form ventral to the notochord and hollow out, forming a very small lumen within each.

Hours 26-30

The lumens of the optic lobes (Fig. 5) become reduced (they narrow dorso-ventrally). The ear enlarges and thins dorsally but remains a single chamber (Fig. 41). The floor of the mesencephalon develops to its characteristic thick appearance. White matter is visible at the level of the ears and extends posteriorly along the brain and spinal cord. Otic ganglia (Fig. 26) form ventro-medial to the ear. The notochord (Fig. 42) completes its vacuolation and is round in cross section. The heart (Fig. 34) becomes a bent tube and

thickens anteriorly, differentiating the truncus arteriosus. The midgut and hindgut remain solid and the pharangeal wings enlarge ventrad, giving the first indication of the foregut, (Fig. 42). The Wolffian ducts and glomerulae are distinct and the ducts extend caudad in a straight line from the glomerulae. The ducts join posteriorly to enter the cloacal cavity which is open but not complete since the excretory and digestive systems remain separate; the hindgut is still a solid structure. The lateral line forms spottily along the midlateral epidermis. The somites are segmented into sclerotomes (Figs. 43, 52). The dorsal and ventral finbuds are distinct. Anteriorly the nasal pits indent.

Hours 31-35

The fourth ventricle and the dorsal region of the spinal neurolcoel begin to diminish. White matter increases, occupying the lateral region of the telencephalon and diencephalon and the ventral region of the mesencephalon, metencephalon, and myelencephalon. The optic cup (retina) becomes bent anteriolaterally locking the now heterogenous lens within its boundary (Fig. 13). The ear delimits the endolymphatic region (Fig. 48) by becoming pointed dorso-anteriorly. Cranial ganglia appear in the region posterior to the eye. The notochord extends cephlad to a point anterior to the ears. The notochord is now 3-4 cells thick (Fig. 44) in the region of the ears and is 5-6 cells thick in the posterior region. Due to the development of the valve between the ventricle and the truncus arteriosus, the heart is now two-chambered (See Figs. 32, 39 for late view of

the valve). The walls of the atrium and ventricle are of a uniform thickness, but the walls of the truncus arteriosus are thicker. The midgut and hindgut open during this period (Fig. 43). The pharangeal wings enlarge ventrad and the esophagus develops as a solid rod posterior to the pharangeal wings (Fig. 44). The midgut and hindgut open, but a series of partitions (Fig. 51) make them appear as if they were composed of linearly arranged compartments. The anus (Figs. 46, 47) is now open to the outside. An anal plug (Figs. 45, 46) about 1 mm in length is present just anterior to the flexure where the gut bends ventrad to exit at the anal papilla. The glomerulae are more well formed (Fig. 49) and the Wolffian ducts curve laterally in a question mark shape. Posteriorly the Wolffian ducts enter the cloacal cavity. The lateral finbuds are just beginning to appear during this period; the dorsal and ventral finbuds are much more distinct. Pigment occurs in the body lining in scattered locations. (Das, 1927, reports that no pattern of pigmentation occurs until the embryo is 9-12 days old.) Vitelline veins are evident surrounding the yolk sac. The lateral line is more prevalent, but it is still very spotty.

Hours 35-37

Hatching takes place at this time (36 hours at 80°F).

Hours 36-40

White matter may be seen to form the floor of the mesencephalon, metencephalon, and myelencephalon. The retina becomes laminated, forming the piliform layer, inner and outer nuclear layers, and the layer of rods and cones (See Fig. 14).

The sclera of the eye (Figs. 13, 14, 15) is now pigmented with very dark purplish-black melanophores. The optic nerve is complete from the brain to the eye, forming the optic chiasma (Figs. 19, 20, 21). The notochord is composed of three cells, in cross section, and is very small in diameter. The atrium and ventricle of the heart are distinct, having definite differences in their wall thickness (See Fig. 36). The atrio-ventricular valve (Figs. 36, 38, 39) is very evident at this time. Later in this period, the atrium distends while the ventricle remains compact. The pharynx opens in the mid-region but the mouth, pharangeal wings (posterior), and esophagus remain solid. The midgut and hindgut are open at this time. The anus is open to the outside, by way of the anal papilla (Fig. 47) but an anal plug remains (Figs. 45, 46). The nasal pits are very evident and quite deep. The lateral, (Fig. 49) ventral, and dorsal fin-buds are advancing in size. The lateral line is still formed only in clusters along the lateral epidermis. Yolk is present in the yolk sac.

Hours 41-45

The neurocoels continue to decrease in volume. The optic chiasma is very evident and slightly larger than in the previous period. It is late in this period and early in the next that the retractor lentis attaches to the medial surface of the lens (Figs. 14, 19, 21). The notochord lengthens slightly so that it now extends anteriorly to the point of the pontine flexure. The ear is still a single chamber, though now it is very large and thin-walled. The liver diverticulum (Fig. 50) appears as

an outpocketing of the gut, at the junction of the foregut and midgut. Later the liver enlarges and lies under the gut. The esophagus is still not hollow, and the anal plug still persists. The Wolffian ducts are now complete, entering the cloaca behind the anal flexure. The mouth is closed and yolk persists in the yolk sac.

Hours 46-50

White matter, though only ventral, occupies one-fourth of the area of the brain and one-third of the spinal cord as viewed in cross section. The neurocoels are now nearly compressed out of existence, being only slits in most cases (Figs. 6, 7, 35, 37). The spinal cord and notochord are of the same diameter and circumference. The heart remains a linear two-chambered structure (Fig. 36). The gut opens throughout during this period with the removal of the anal plug and opening of the esophagus. Pigment becomes evident at the top of the brain. The lateral fins now have central support of cartilage. The gill arches form during this time and the operculum forms to cover the gill arches (Fig. 6). The nasal pits become nares, opening externally. The liver, now large, fills most of the cavity below the intestinal tract. The first indication of the tongue rudiment forms late in this period.

Hours 50-72 (Days 2-3)

The brain alters its shape throughout its existence so that during this time, it takes a triangular shape (Figs. 6, 7). The cavities of the optic lobes are mere slits (Figs. 6, 7).

Cartilage, the forerunner of the braincase and spinal column (Fig. 28), underlays the brain and spinal cord. The layer of rods and cones extends peripherally (Figs. 15, 21, 22) and gives an appearance of being loosely arranged. Also during this time, pigment from the sclera invades the layer of rods and cones (Figs. 17, 18, 22, 23). The iris forms, hollows, and becomes pigmented (Figs. 17, 18, 19, 22). The cornea begins to bulge outward (Fig. 17) as it appears in the adult. The ear changes from two to three chambers (Figs. 26, 27, 28, 29). The heart makes a major change during this time by beginning to move into its characteristic "S" shape (Fig. 37). The atrium moves dorsally to a position dorsal to the ventricle. By the end of this time the atrium covers or overlays one-half of the ventricle (Fig. 38). The atrio-ventricular valve and the valve between the ventricle and truncus arteriosus are both formed and completed during this time (Fig. 38). The truncus arteriosus, dorsal aorta, and cardinal veins are visible also. Vitelline veins are still present in the embryo at this age. The gut is open throughout as mentioned before, but alters its shape; it starts the period as a straight tube (Fig. 51) but lengthens (Fig. 55) so that it is folded into coils (Figs. 52, 56, 57) due to the restricted space. The stomach distends forming a pouch in the tube larger than the intestine (Figs. 56, 57). The lateral line is visible in the region of the eye. Posteriorly a pair of tubes appears in the same position as the Wolffian ducts and connects to them. These tubes, however, are smaller in diameter and may be the

beginning of the mesonephros. Various cartilage groups form in the mouth. The gill arches and gill clefts which started to form in the last period complete their development.

Hours 73-96 (Days 3-4)

Only minor changes are noticed during this time. All cavities of the brain are now mere slits. The brain fills the head area and rivals the eyes for space. White matter encompasses one-half of the area of the brain (in cross section), and extends the length of the spinal cord. During the latter part of this period, white matter may be found both dorsal and ventral in the brain. Only a little amount of grey matter surrounds the neurocoels (Figs. 6, 7). The pigment in the iris increases and darkens. The curvature of the cornea increases. The atrium continues to move over the ventricle so that it now covers three-fourths of the ventricle. The operculum progresses over about three-fourths of the area of the gill arches. The liver is now a large structure occupying most of the body cavity around the gut. Periblast and yolk may be observed in various places even at this late age, as late as 84 hours. The tongue takes definite shape and can be recognised as such.

Hours 97-120 (Days 4-5)

Pigment which earlier invaded the layer of rods and cones now covers about one-half of each rod or cone (Figs. 22, 23). The cornea becomes greatly bulged in comparison to the head of the embryo. The lens touches the inner surface of the c ornea. The shelves of the ear become heavy or thick in appearance. The atrium covers five-sixths of the ventricle. The mouth is de-

finitely open, and it may be surmised that the gill arch-respiratory system is functioning. The iris is completed and pigmented. The ear is a three-chambered organ (Fig. 29). The gills complete their development. The operculum completes its development and assumes its protective position over the gills. The lateral fins (Figs. 56, 57) have now grown to a size equal to one-half the length of the body. The tail fin is not yet completely developed at this time. The Wolffian ducts are now small in comparison to the rest of the body. The liver continues to enlarge. The yolk is completely absorbed during this time.

Later, by 9 days the atrium completely covers the dorsal surface of the ventricle (Fig. 39) and by 12 days the atrium overlaps the posterior curvature of the ventricle.

TABLE 1

A tabular summary, showing comparative development of certain stages of Dundulus heteroclitus, Trichogaster trichopterus, and Macropodus opercularis. The figures below each species represent the age in hours for the corresponding developmental stages. Data for Fundulus and Trichogaster as well as the terminology of the stages are taken from Hodges and Behre (1953).

F.	T.	M.	Developmental Stages
19½	---	6	Blastoderm about one-half over surface of yolk. Middle gastrula.
21	---	7	Blastoderm about two-thirds over surface of yolk.
22	---	8	Blastoderm about three-fourths over surface of yolk.
---	7	9	Germ ring forms.
23	---	10	Embryonic shield condenses to form keel.
---	8½	11	Embryonic shield well formed.
24	12½	16	Optic vesicle first visible as expansion of forebrain. Large yolk plug.

F.	T.	M.	Developmental Stages
31	---	17	Optocoel develops.
33	---	18	Auditory placode forms. Optocoel connects across brain.
34	---	19	Optic cup forms, and lens develops. Neurocoel develops. About 10 somites.
---	14½-15	31-35	Body melanophores first appear.
84	---	37	Retinal pigmentation begins. Urinary vesicle formed. Caudal fin begins to develop.
90	---	41	Liver develops. Cartilage begins to differentiate.
144	---	((84)	Air bladder develops.
240	---	(90)	Mouth opens.
264	22-24	35-37	Hatching. Pigmentation of bladder.
288	---	100	Yolk is completely absorbed.

Note: The figures included in the parenthesis are out of sequence with the hatching time as compared to the others.

DISCUSSION

For the most part Macropodus opercularis follows the pattern of development referred to by most authors (Wilson, 1889; Mahon and Hoar, 1956; Battle, 1944) as the typical teleostean development. The brain, the eye, the musculature, the fins, the notochord, the gill system, and the operculum all follow the typical pattern in M. opercularis.

The lining of Kupffer's vesicle by columnar cells dorsally and periblast ventrally seems to differ from the patterns established by other fishes. Mahon and Hoar (1956) report that in Oncorhyncus keta (Walbaum), the Chum Salmon, Kupffer's vesicle is lined both dorsally and ventrally by columnar cells, thereby setting Kupffer's vesicle off entirely from the yolk and periblast. Another feature of Kupffer's vesicle, as found in M. opercularis, but not reported by authors working with other teleosts, is the contact dorsally of Kupffer's vesicle by the notochord and spinal cord. This was found in only a few specimens but may be more prevalent than indicated, since not all specimens were sectioned identically.

The typical formation of the teleost gut as reported by Wilson (1889) and Mahon and Hoar (1953) is by a process of folding of the entodermal lamella along the median line to form

the bubular gut. In M. opercularis the gut forms by cavitation of a solid rod. The first region to differentiate as a solid rod is the hindgut. This is followed in turn by the midgut, pharynx, and esophagus. The process of cavitation follows the same sequence but with a varible time lag, e.g., the midgut is just beginning to differentiate as a solid rod at the same time that the hindgut is hollowing out to form a tube. The hindgut and midgut do not open throughout initially. Instead partitions exist for ten to twelve hours resulting in compartments along the gut. An anal plug blocks the entrence of the hindgut into the cloacal cavity thereby allowing wastes to exit from the Wolffian ducts through the cloaca and anal papilla to the outside.

The liver diverticulum in M. opercularis buds off the dorsal or dorso-lateral surface of the midgut. This is similar to that reported by Wilson (1889) for the Sea Bass but differs from that reported by Mahon and Hoar (1953) for the Chum Salmon.

The most significant variance in development of M. opercularis is the position of the developing heart. Ingersoll (1953) has reported the same pattern of heart development in Trichogaster trichopterus, another Anabantidae, as is found in M. opercularis. The heart develops first as a straight tube extending down along the anterior margin of the yolk sac. This tube is not along the mid-line as it is in other teleosts, but is laterad, under the left eye. Later the heart differentiates into an atrium and a ventricle. The anterior section

(ventricle) moves ventrad and posteriad; simultaneously, the posterior section (atrium) moves anteriorly and dorsally. In final position the atrium is in dorsal juxtaposition to the ventricle and overlaps the posterior curvature of the ventricle.

The ear seems to develop in a method distinct from the usual teleost. Unlike most teleosts in which the ear develops from a placode of ectodermal origin, the otocyst of M. operc-ularis originates as an organization of head mesenchyme, parallel to the myelencephalon.

The ear develops first as a solid cone later becoming a hollow, thin-walled, single chamber. Still later the ear may be seen as a two- then three-chambered structure, consisting of endolymphatic area, sacculus, and utricle. The maculae of the ear connect directly to the lateral line system which in M. opercularis is spotty and not continuous as is found in other teleosts. The character of spotty lateral line system is found throughout the Anabantidae. The lateral line system may also be found anterior to the ears in the region of the eyes.

In overall development M. opercularis follows the patterns similar to T. trichopterus. However M. opercularis has a slightly slower rate of development, especially up to hatching time. TABLE 1 shows a comparison of T. trichopterus, Fundulus heteroclitus, and M. opercularis. It may be noted that a variance of sequence of events occurs (those hourly markings listed in parenthesis), especially for the air bladder development, mouth opening, yolk absorption, and hatching time.

SUMMARY

The purpose of this study has been to describe the development of the Paradise Fish, Macropodus opercularis Linnaeus (Perciformes: Anabantidae), from cleavage to five days post hatching. Approximately one hundred and fifty embryos were examined. In the section Organology, the brain, eye, ear, notochord, heart, and gut were discussed in detail from the time and nature of origin to the condition as found five days post hatching. A summary, in intervals of five hours, was presented to show the overall picture of development. Included in that summary were events of structures in addition to those presented in the section Organology. A table was presented showing a comparison of certain features of the development of Fundulus heteroclitus, Trichogaster trichopterus, and Macropodus opercularis.

The patterns of development were discussed and compared to other teleosts. As a result of this study it was found that:

1. For the most part M. opercularis followed the typical teleostean pattern of development, except as noted below.
2. The heart develops under the left eye along the anterior margin of the yolk sac. This is

similar to that reported by Ingersoll (1951) for the Blue Gourami, Trichogaster trichopterus.

3. The ear develops from the head mesenchyme lateral to the brain rather than from a ectodermal placode.

4. The gut develops first in the posterior region then differentiates cephalad.

It was also learned that:

1. No true bone exists in the skeletal system up to five days of development post hatching at 80°F.

2. The hatching time was 35-37 (36) hours after fertilization at 80°F.

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LEGEND TO FIGURES

anal papilla	an pp	iris	ir
anal plug	an pl	Kupffer's vesicle	K v
anus	an	lateral finbud	lf
apical flexure	a f	lateral ventricle	lv
atrio-ventricular valve	a v	lens	ln
atrium	a	lips	l
cartilage	c	liver	li
cornea	co	liver diverticulum	li d
decussation	d	maculae	ma
dorsal finbud	d f	mouth region	mo re
ear	e	neural keel	n k
endolymphatic area	en a	neurocoel	nc
epiphysis	ep	notochord	no
esophagus	es	nuchal flexure	n f
eye	ey	operculum	o
gill arches	g a	optic bud	op b
glomerulae	gl	optic chiasma	op ch
gut	g	optic cups	op c
heart	h	optic lobes	op l
hindgut	hg	optic nerve	op n
infundibulum	i	optic stalk	op s
inner nuclear layer	i n l	optic vesicle	op v

otic ganglion . . . . . o g
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pharangeal wings . . . . ph w
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piliform layer . . . . . p l
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retina . . . . . r
retractor lentis . . . . r l
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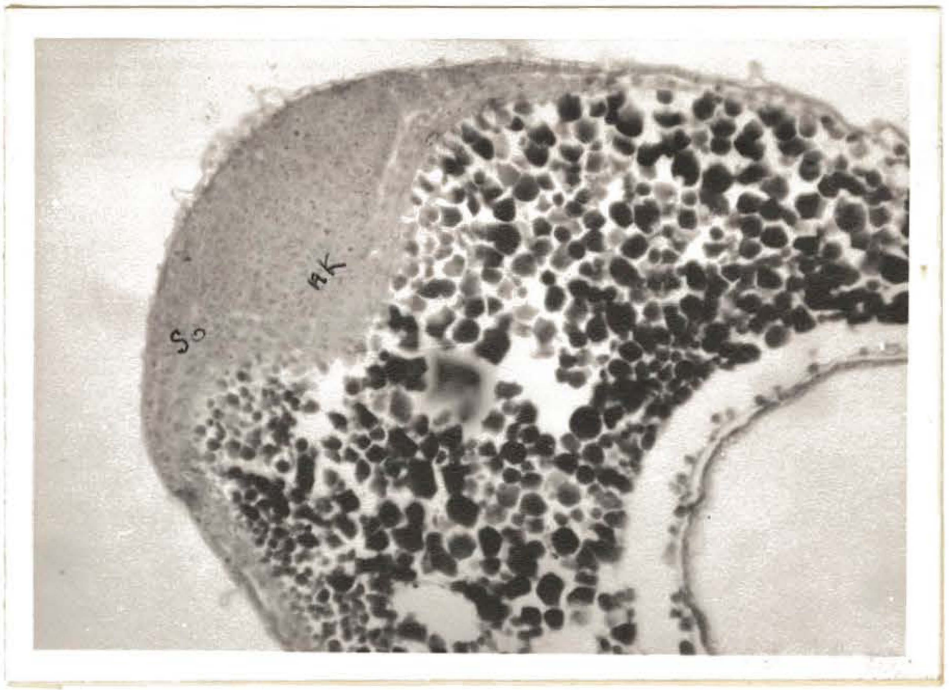
spinal cord . . . . sp c
stomach . . . . . st
swim bladder . . . . sw b
truncus arteriosus . t a
ventricle v
white matter . . . . w m
Wolffian ducts . . . W d

Fig. 1 Age 13 hours

An early crossectional view of the neural keel showing the undifferentiated somites. 200X.

Fig. 2 Age 21 hours

An early crossectional view of the lateral ventricles and the telencephalon showing the thickness of the walls of the telencephalon. 400X.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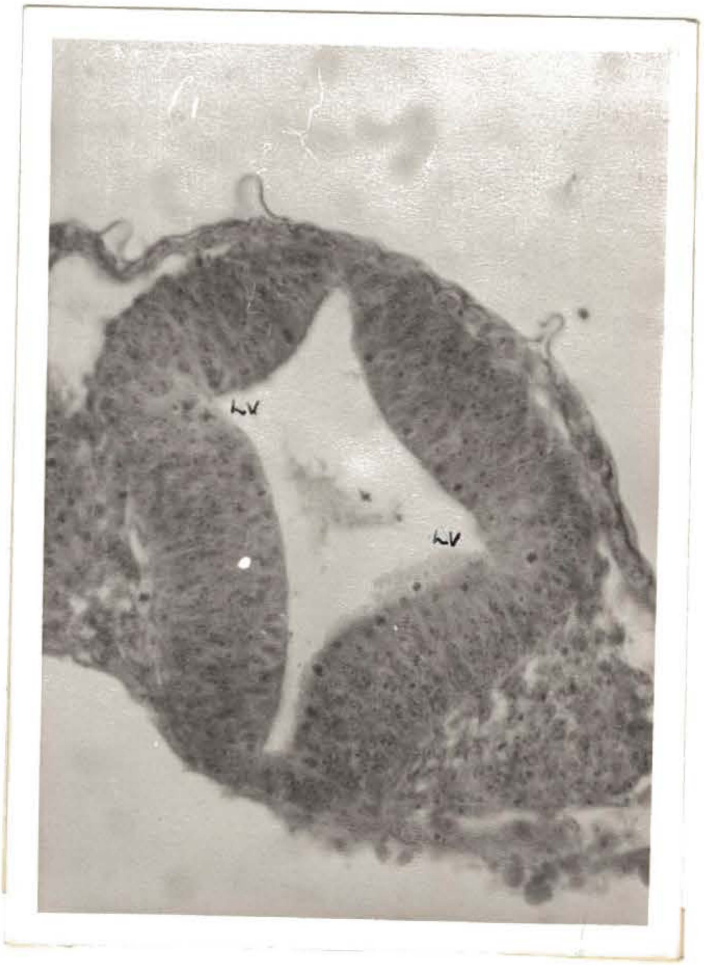
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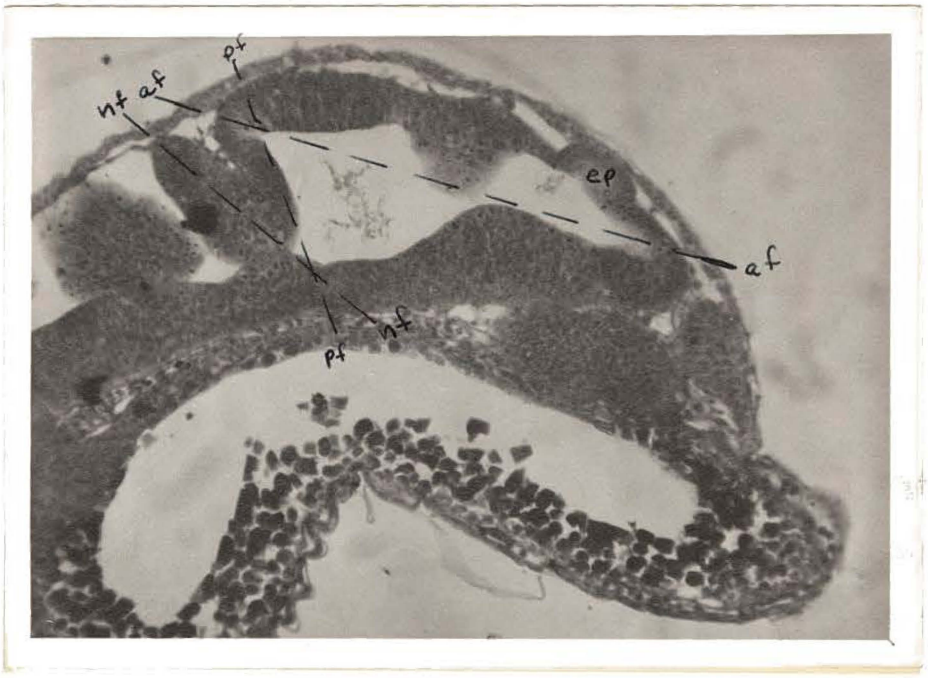
Fig. 3 Age 23 hours

A parasagittal view of the brain showing the epiphysis, forebrain, and flexures of the brain. 200X.

Fig. 4 Age 26 hours

A crossectional view of the brain showing the infundibulum, epiphysis, open neurocoel, optic cup deep, and lens free. 200X.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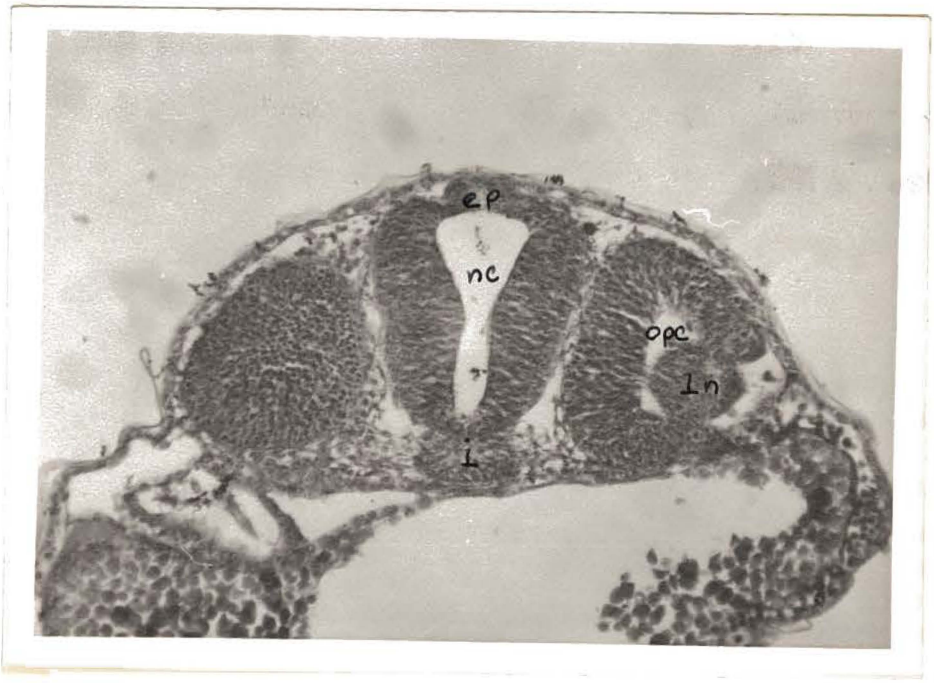
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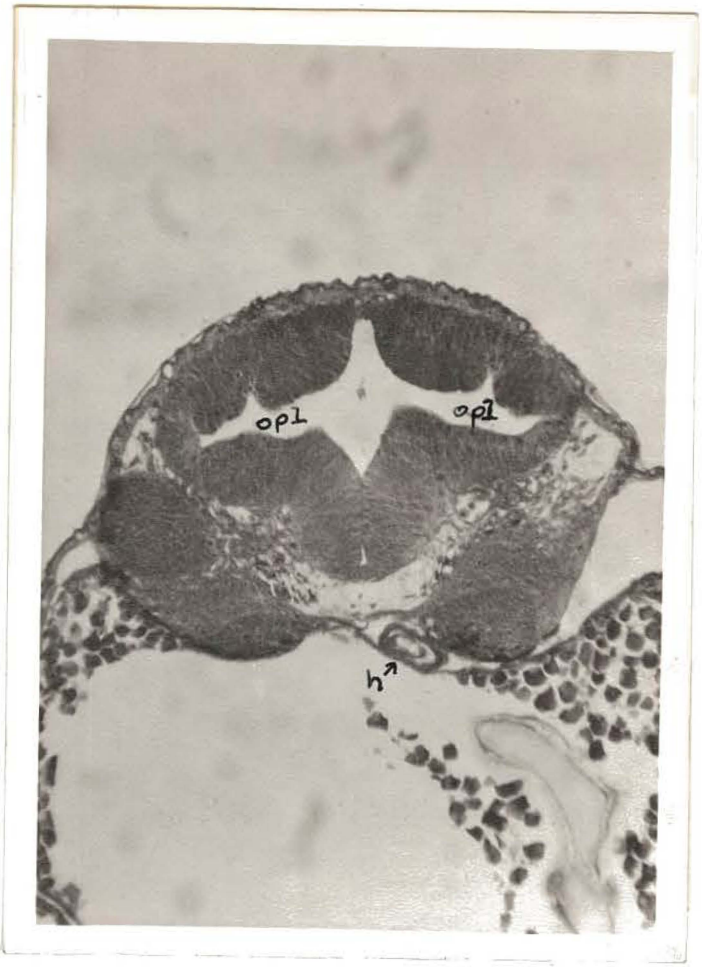
Fig. 5 Age 28 hours

A view of the brain showing the optic lobes hollow and an early view of the heart. 200X.

Fig. 6 Age 60 hours

A late view of the brain showing the optic lobes as mere slits, the operculum, and white matter dorsal and ventral in the brain. 100X.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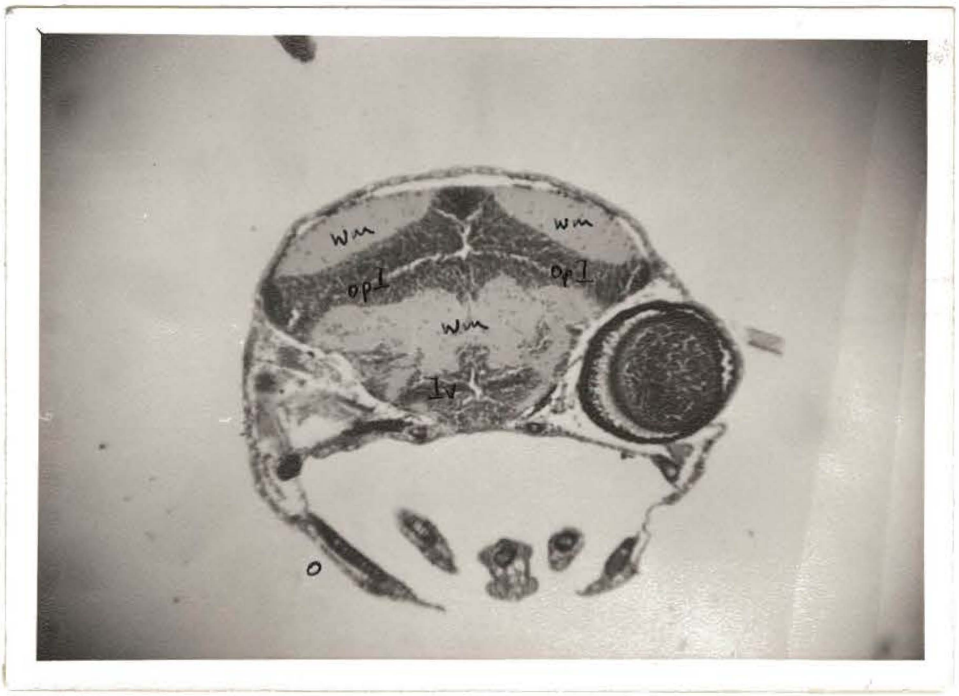
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Fig. 7 Age 90 hours

A late crossectional view of the brain showing the white matter dorsal and ventral, neurocoel as a mere slit, operculum, gill arches, and the eye. 100X.

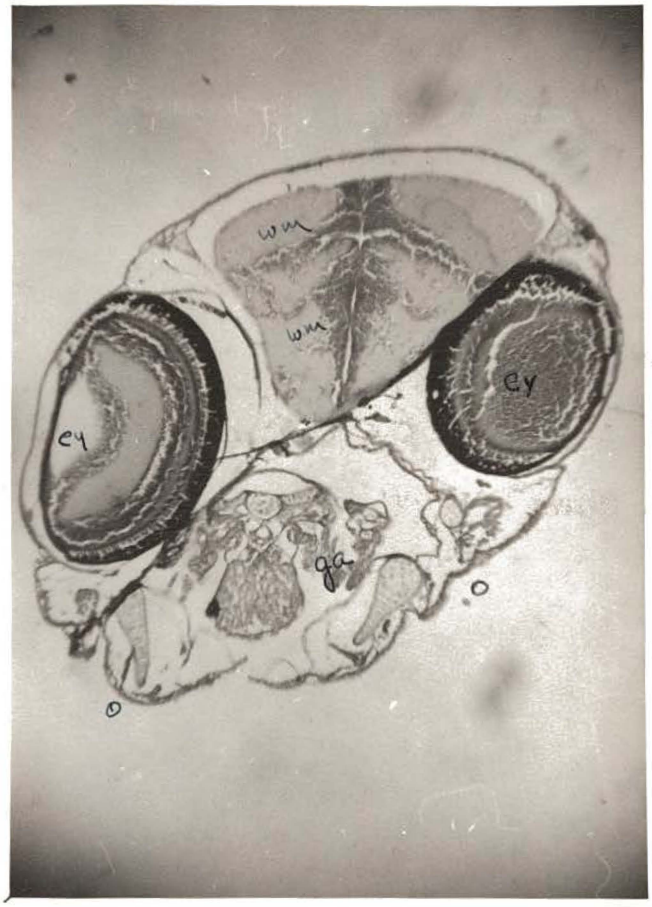
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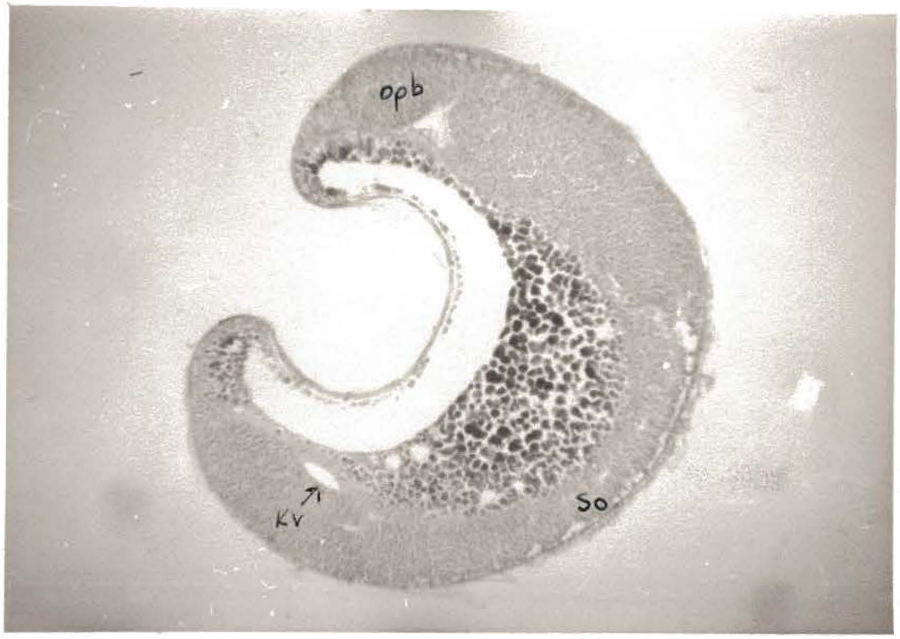
Fig. 8 Age 12 hours

A sagittal view of the very early eye as an optic bud, brain solid, somites, and Kupffer's vesicle. 100X.

Fig. 9 Age 14 hours

An early crossectional view of the eye as an optic vesicle, and the early cavitation of the brain.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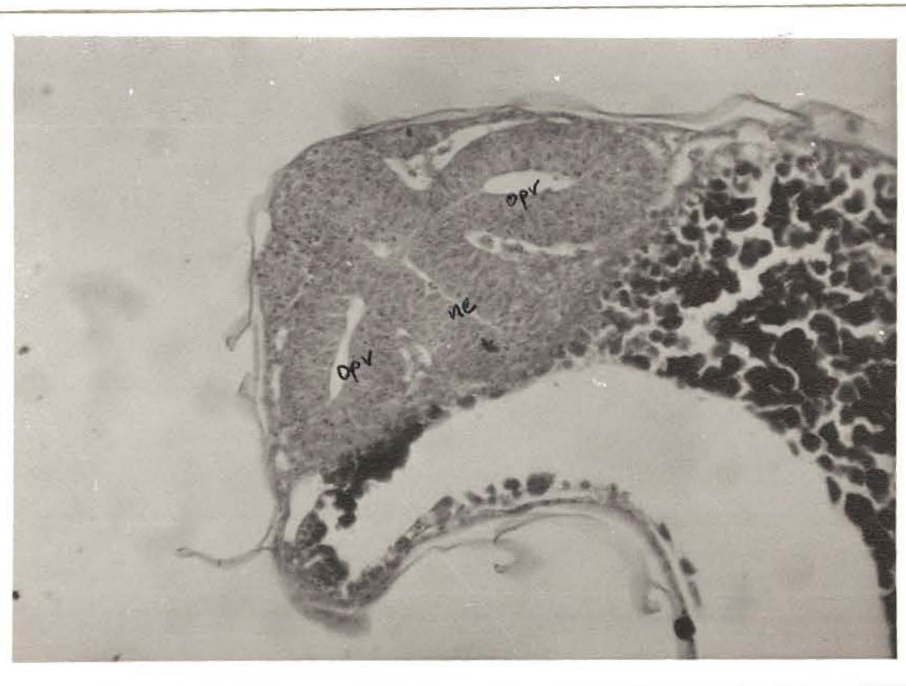
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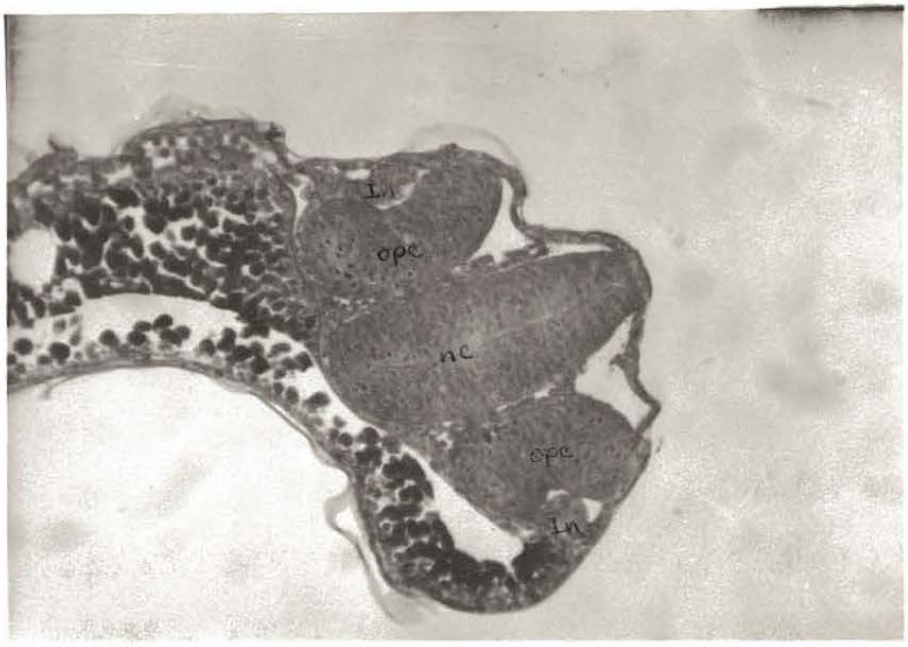
Fig. 10 Age 19 hours

A crossectional view of the eye with the optic cups indented and the lens as an attached placode; also it shows the brain with the verticle slit (Cavitation) initiating and the general ellipsoidal shape of the brain. 200X.

Fig. 11 Age 22 hours

An early crossectional view of the optic stalk. 200X.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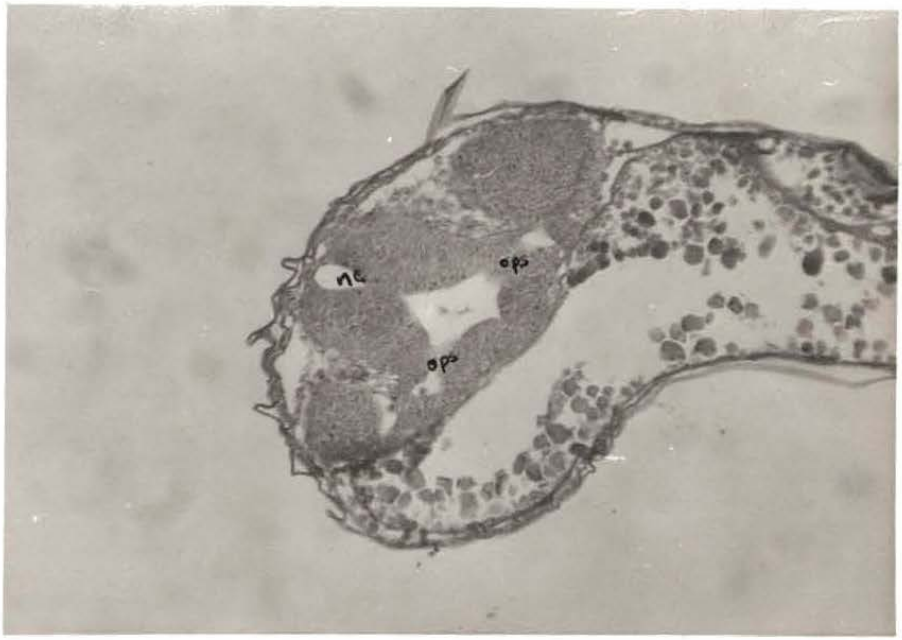
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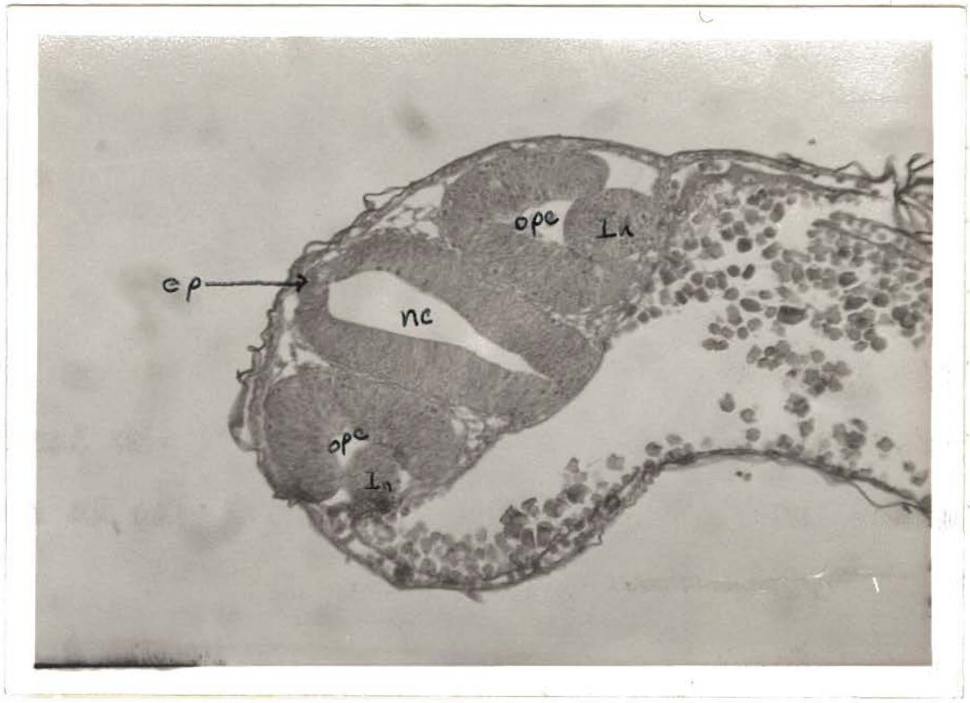
Fig. 12 Age 22 hours

A crossectional view of the eye showing the lens attached, optic cup deep and curved. Also it shows the open neurocoel and epiphysis. 200X.

Fig. 13 Age 33 hours

An enlarged crossectional view of the eye showing the pigment as it initially appears in the sclera, the lens capsulated and laminated, the optic cup deep and bent, and the retina a single homogenous layer. 400X.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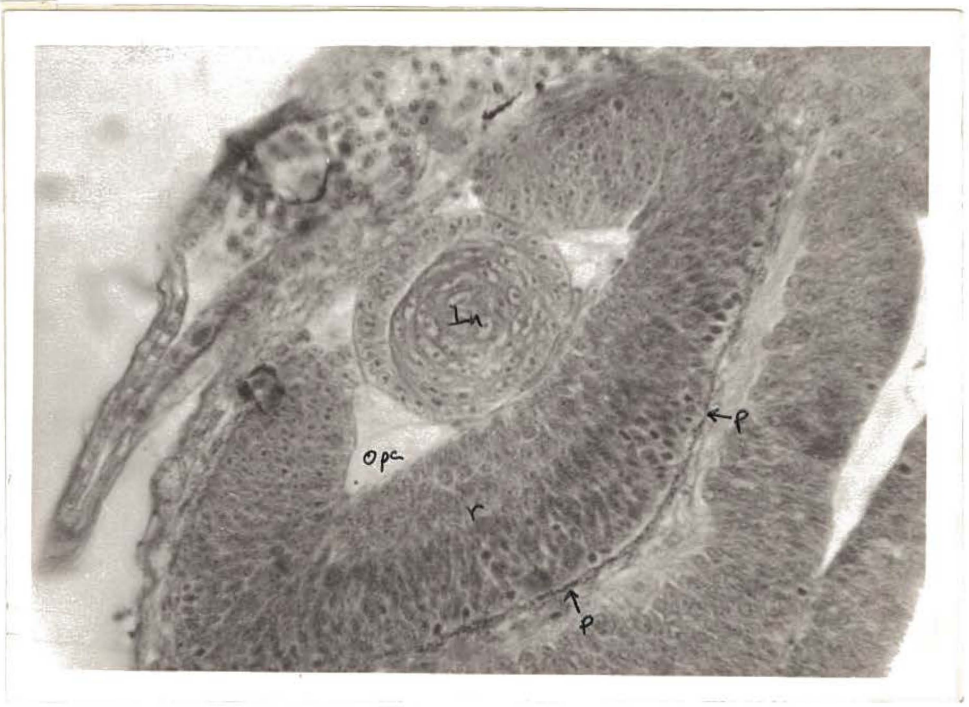
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Fig. 14 Age 33 hours

A crossectional view of the eyes showing the piliform layer, retractor lentis, the four layers of the eye, white matter lateral in the forebrain and ventral in the midbrain, and pigment in the sclera. 100X.

Fig. 15 Age 57 hours

An enlarged crossectional view of the eye showing the rods and cones lengthening, pigment in the sclera, and the four layers of the retina. 400X.

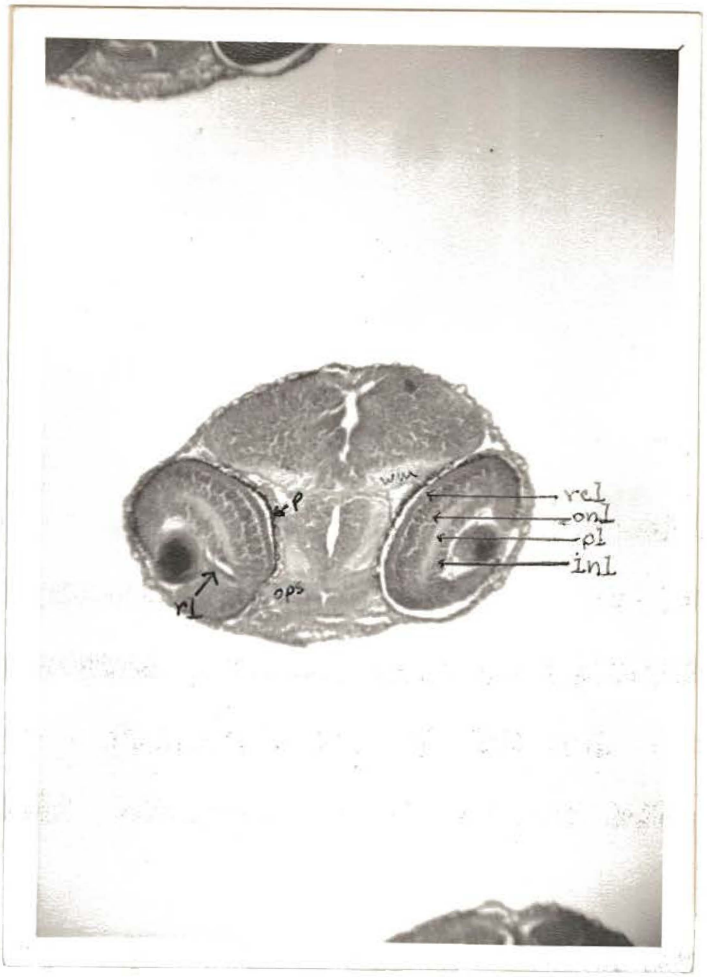
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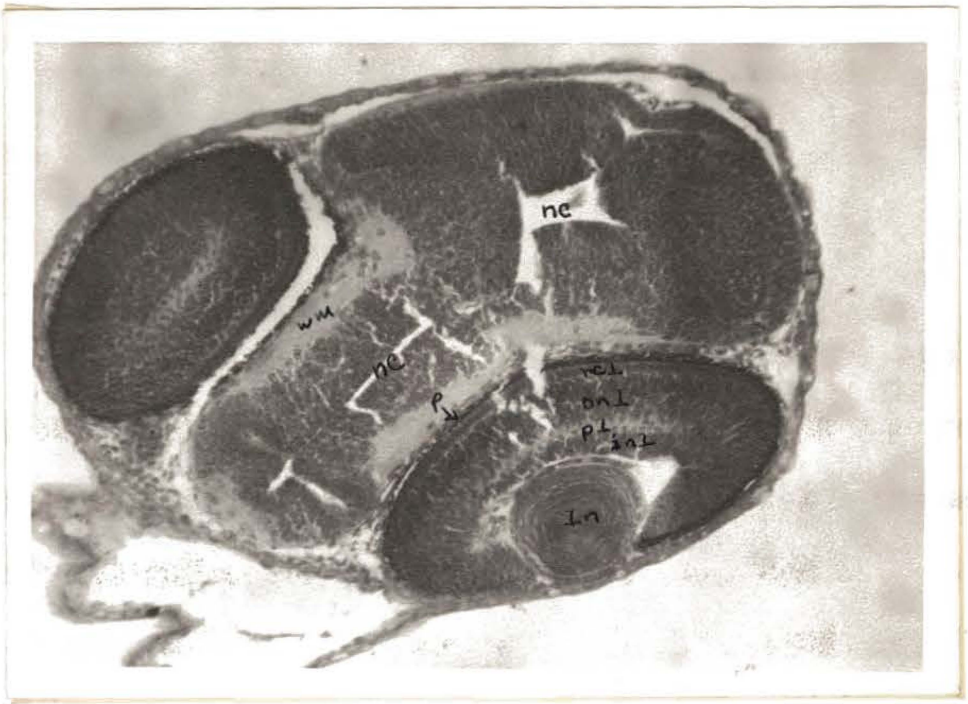
Fig. 16 Age 58 hours

A crossectional view of the brain and eyes showing the lens with a characteristic loose appearance, the four layers of the retina, pigment in the sclera, white matter lateral in the diencephalon. 200X.

Fig. 17 Age 60 hours

A crossectional view of the eye showing the rods and cones completing their lengthening, pigment starting to invade the rod and cone layer, the four layers of the retina, iris pigmented, and white matter ventral, lateral and dorsal. 200X.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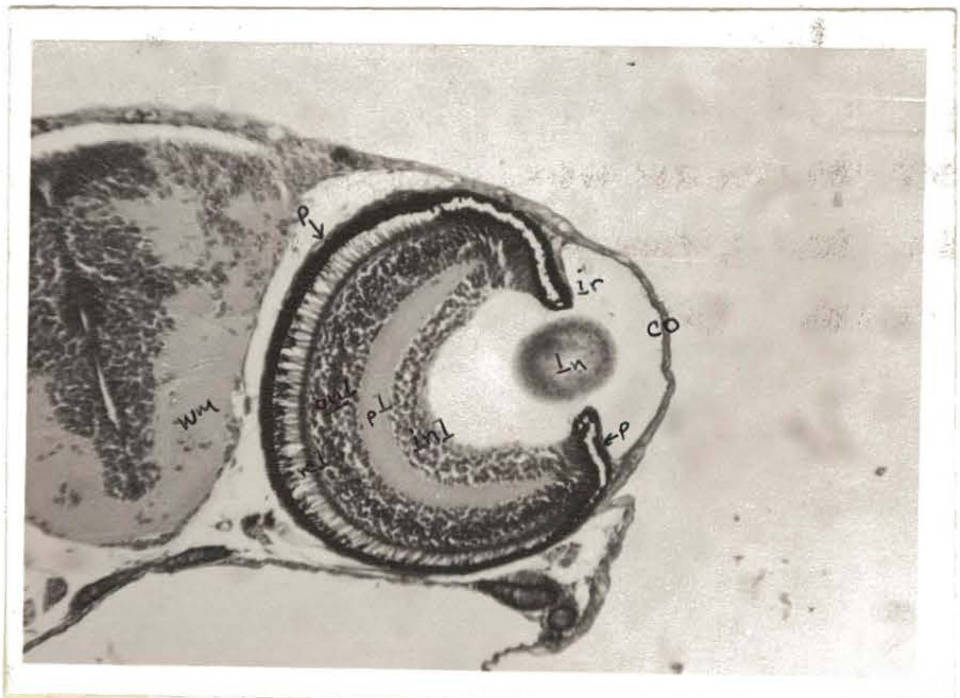
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Fig. 18 Age 60 hours

A crossectional view of the brain and eyes showing the bulge of the cornea, white matter ventral, lateral, and dorsal, the neurocoel as a mere slit, and the pharynx. 100X.

Fig. 19 Age 84 hours

A late crossectional view of the brain and eyes showing the optic nerve complete, optic chiasma, retractor lentis, white matter dorsal, optic lobes closed completely, the four layers of the retina, pigment in the sclera, mouth region, rods and cones lengthening, and grey matter only along the neurocoel. 100X.

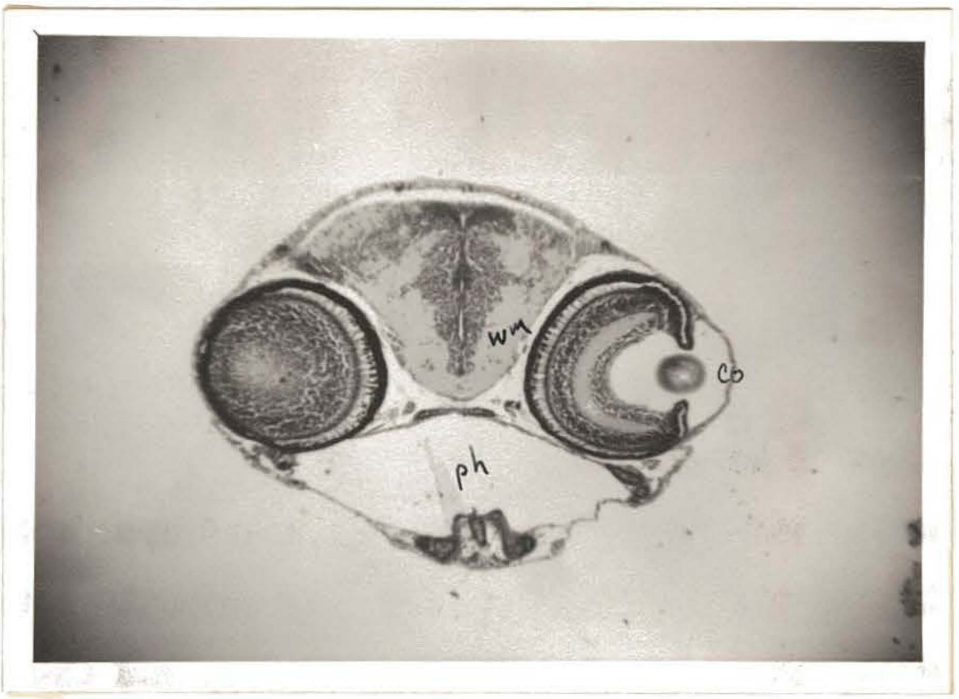
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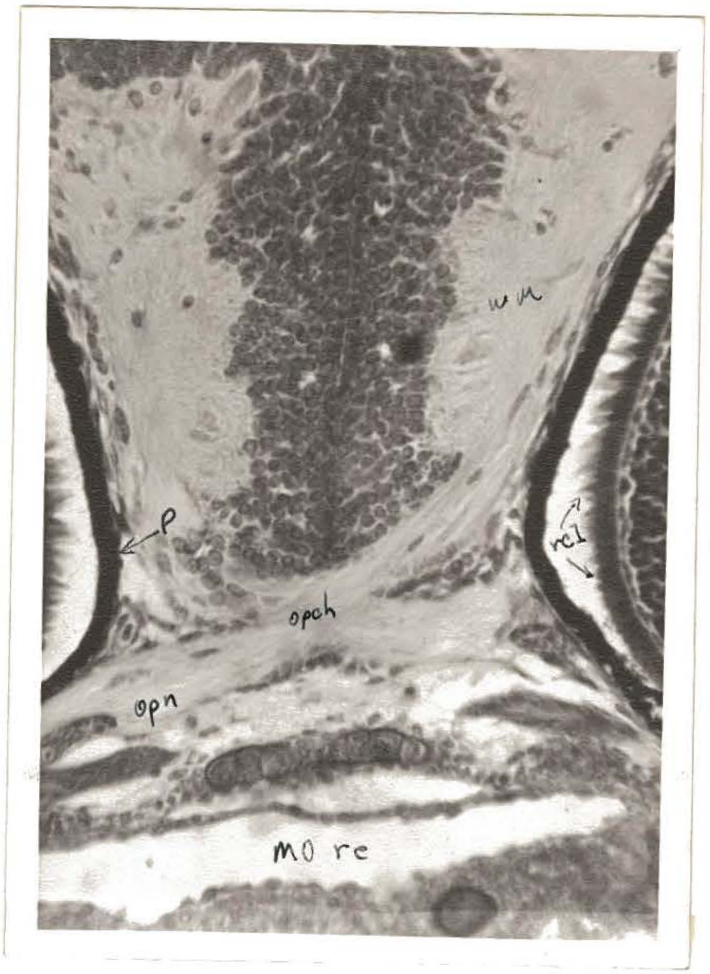
Fig. 20 Age 84 hours

An enlargement of Fig. 19 showing the optic chiasma, (the decussation may be seen upon close examination) and grey matter surrounding the neurocoel. 400X.

Fig. 21 Age 84 hours

An enlargement of Fig. 19 showing the retractor lentis, optic chiasma, four layers of the retina, lengthening of the rods and cones, pigment in the sclera, optic nerve complete, and grey matter surrounding the neurocoels. 200X.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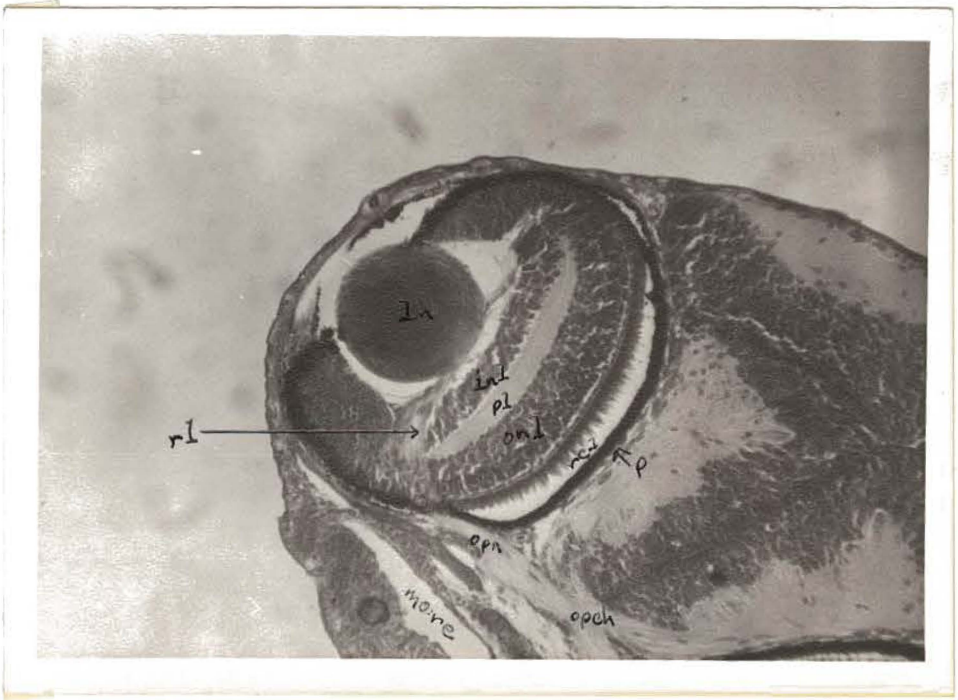
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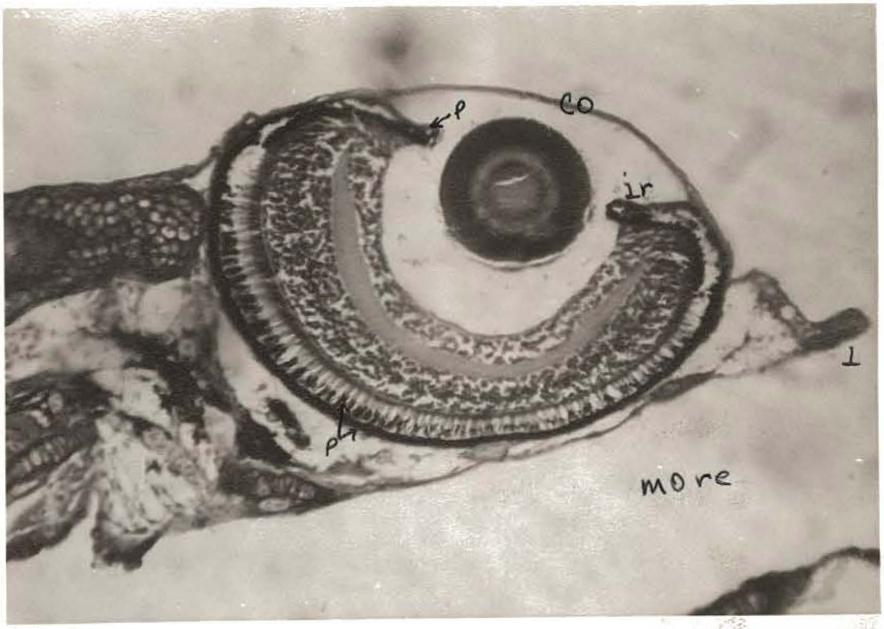
Fig. 22 Age 90 hours

A sagittal view of the eye showing the pigment covering one-half of the rod and cone layer, slight bulge of the cornea, iris pigmented, four layers of the retina, rods and cones long, mouth open, and the lips. 200X.

Fig. 23 Age 108 hours

A crossectional view of the eyes and brain showing the entrance of the optic nerve to the retina a brain, the optic nerve in part between the eye and brain, the gill arches, pitment in the sclera, the four layers of the retina and white matter dorsal in the brain. 100X.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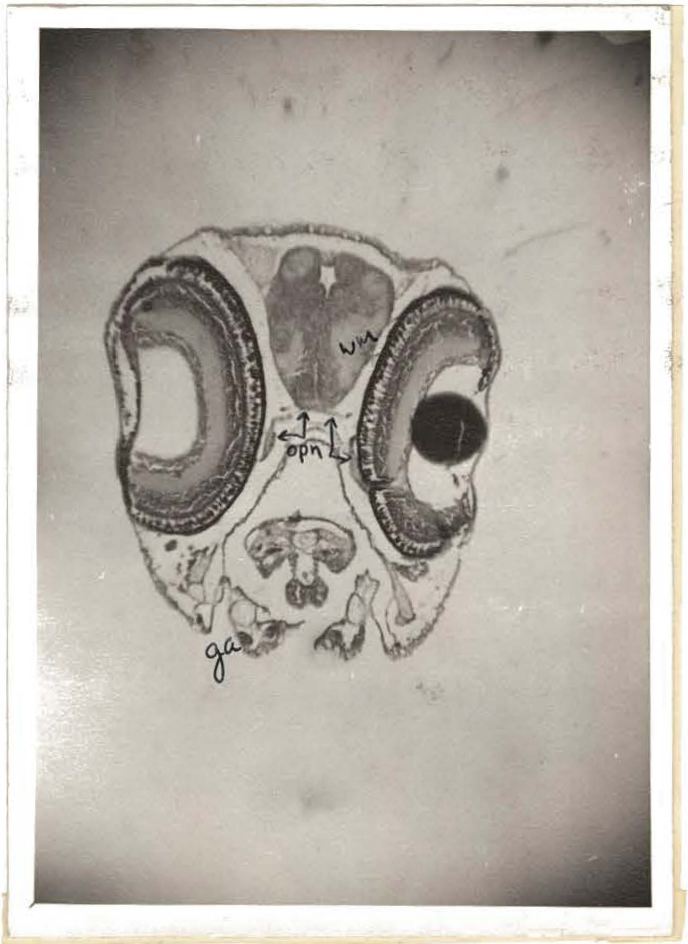
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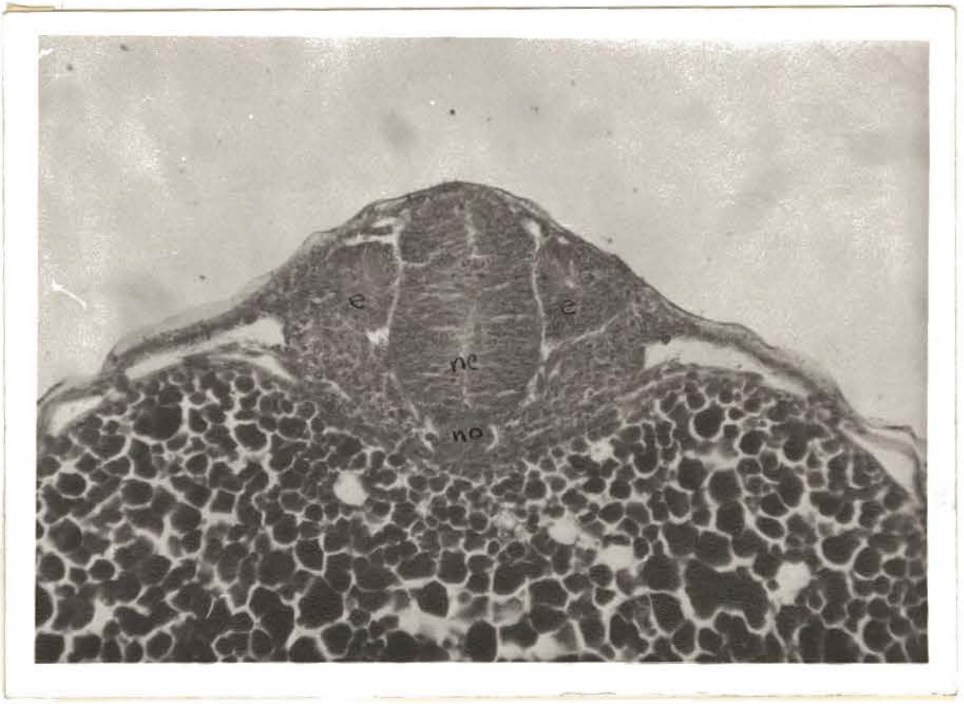
Fig. 24 Age 16 hours

A crossectional view of the head showing the ear initially, the general ellipsoidal shape of the brain, head mesenchyme, the initiating slit of the brain, and the notochord as a small solid rod. 200X.

Fig. 25 Age 48 hours

A crossectional view through the myelencephalon at the level of the ears showing the ears as a single chamber, maculae, the pharynx open as a slit, white matter ventral in the brain, notochord small and vacuolated, and the neurocoel closing (early). 200X.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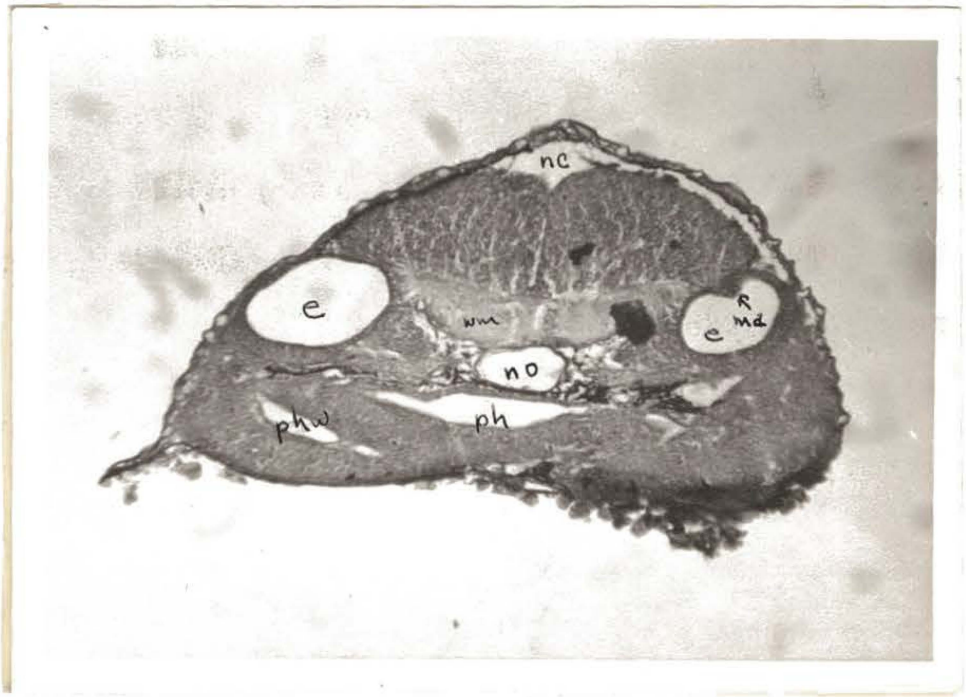
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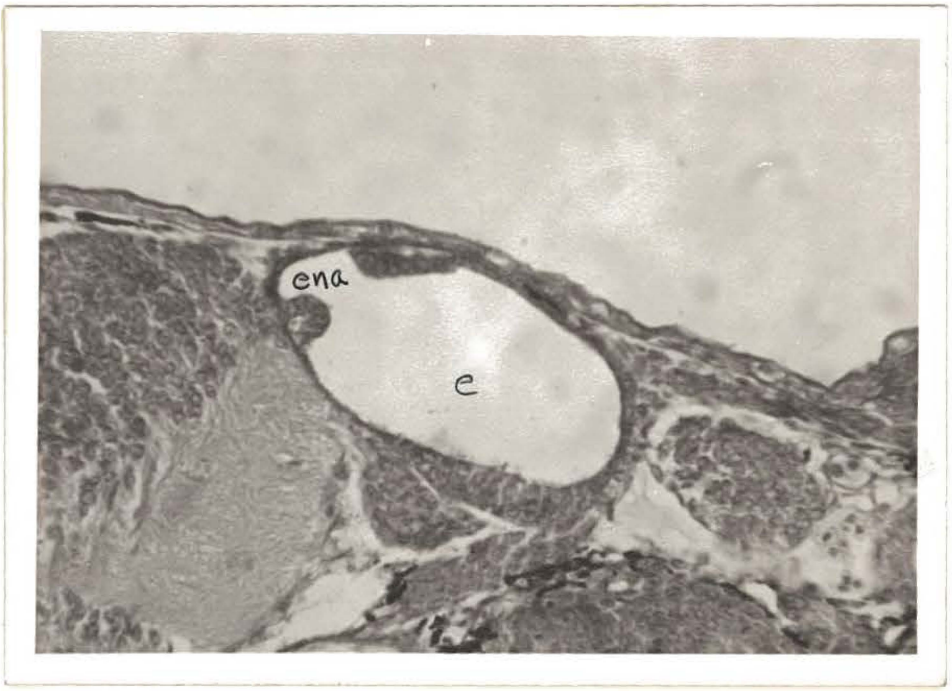
Fig. 26 Age 54 hours

An enlarged crossectional view of the ear showing the first shelf beginning to form and an otic ganglion. 400X.

Fig. 27 Age 57 hours

An enlarged crossectional view of the ear showing the first shelf completed, thereby making the ear a two chambered structure, also delimiting the endolymphatic area. 400X.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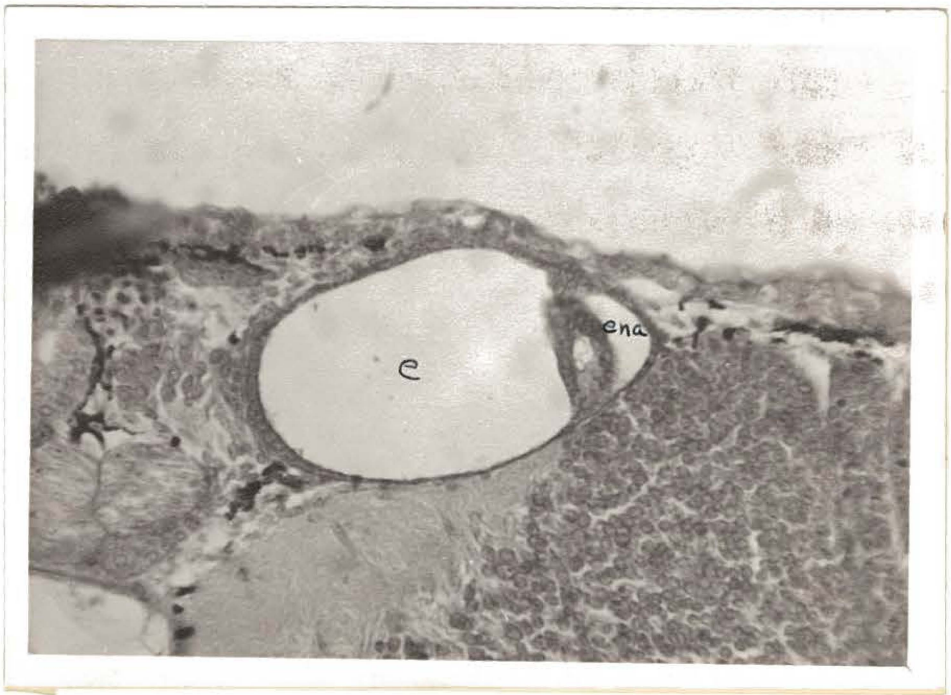
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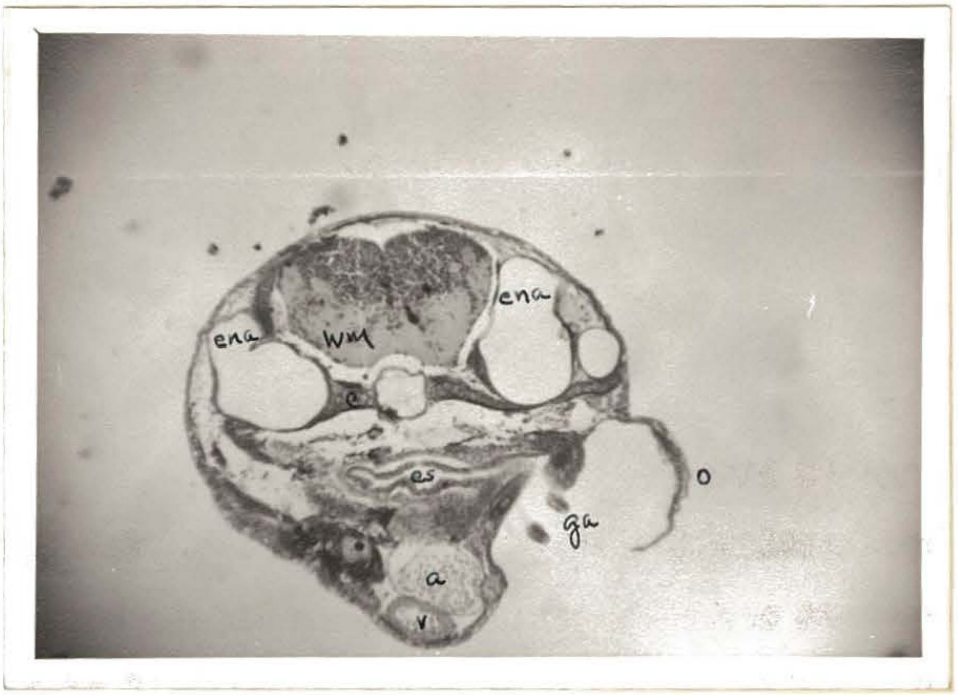
Fig. 28 Age 60 hours

A crossectional view of the ear as a three-chambered structure and also showing the cartilage base of the brain, white matter encompassing one-half of the brain, the open esophagus (anterior portion), the atrium dorsal to the ventricle, gill arches, operculum, and endolymphatic area. 100X.

Fig. 29 Age 90 hours

A late crossectional view of the ear as a three-chambered structure, also showing white matter dominant, the atrium dorsal to the ventricle, esophagus open, and operculum complete. 100X.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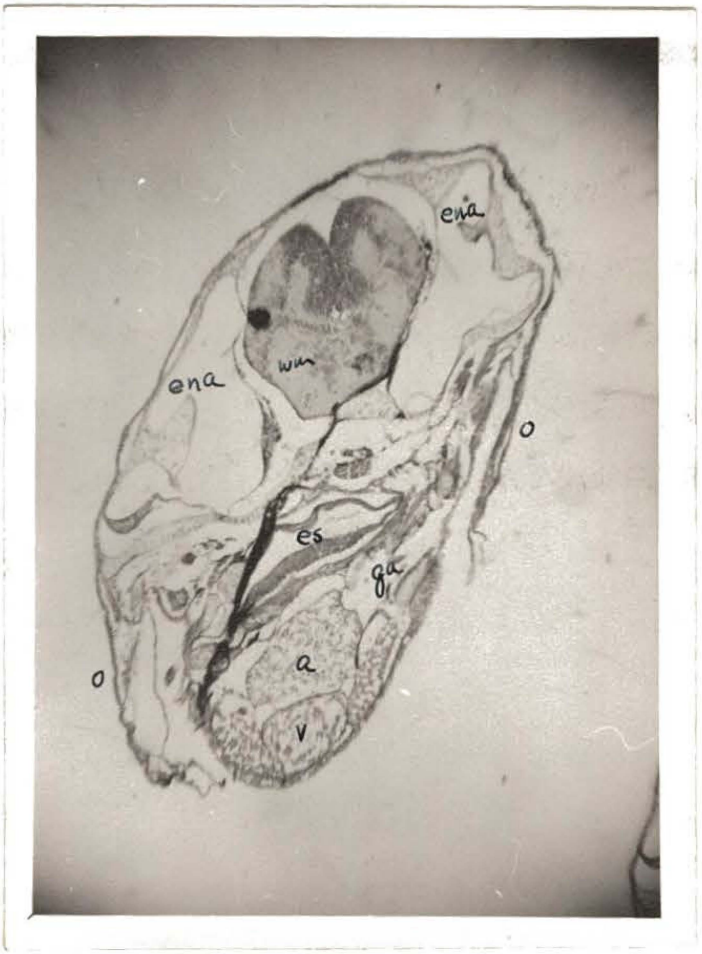
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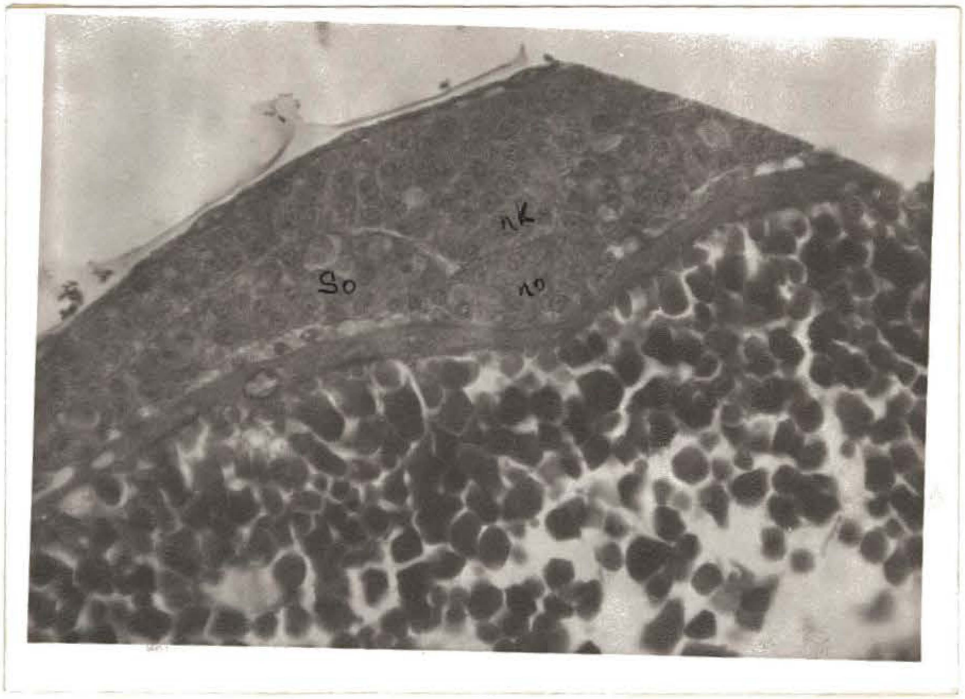
Fig. 30 Age 14 hours

A crossectional view of the early notochord and also the neural keel and undifferentiated somites. 400X.

Fig. 31 Age 16 hours

A crossectional view of the posterior end of the spinal cord as it touches Kupffer's vesicle. Somites are also visible. 200X.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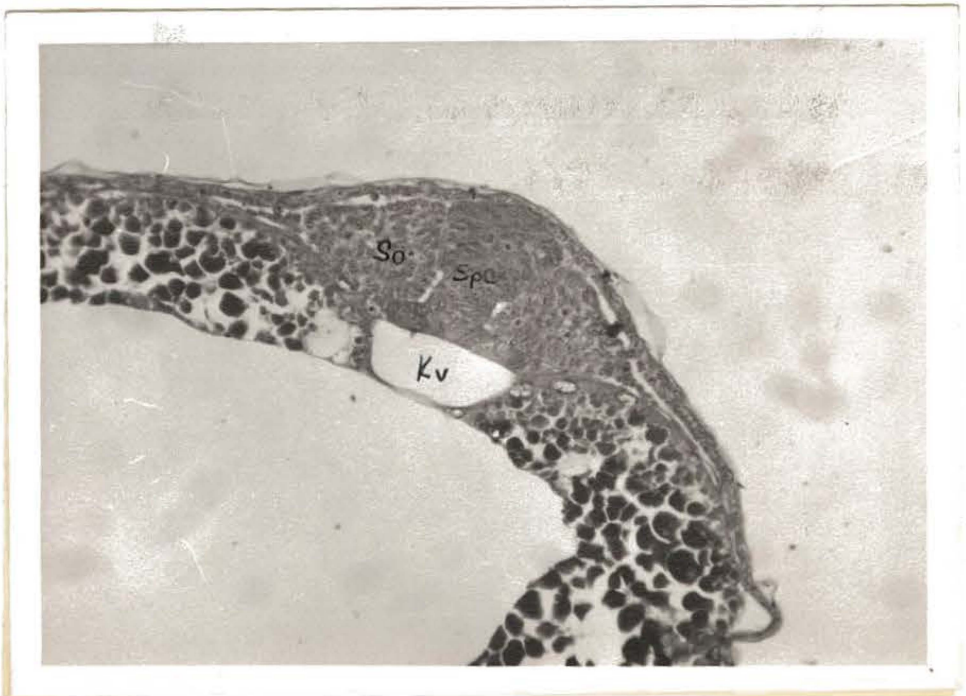
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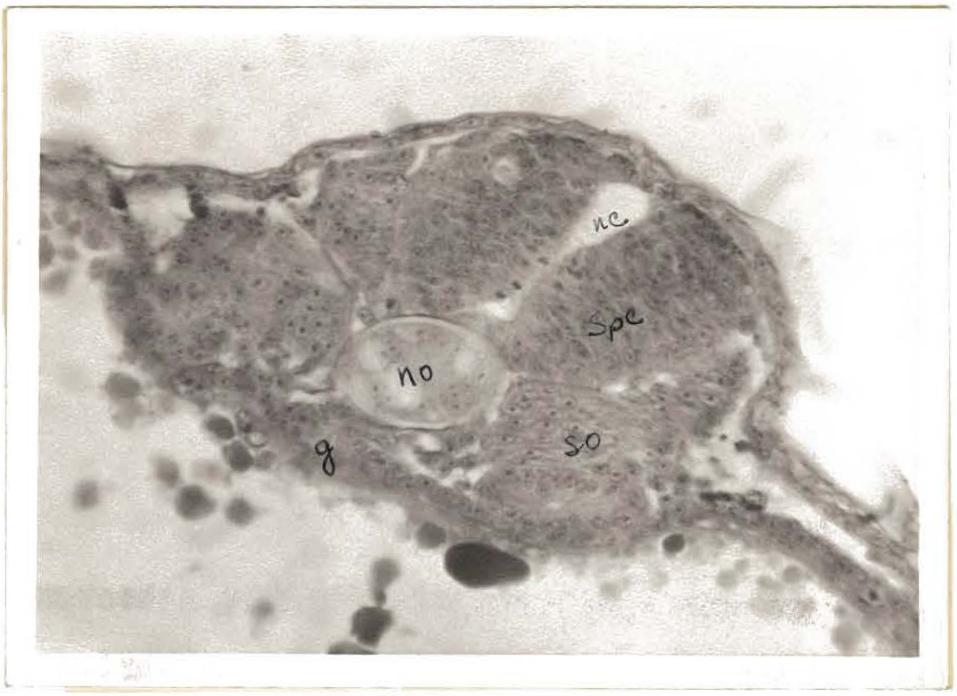
Fig. 32 Age 23 hours

An enlarged crossectional view of the notochord as it begins to vacuolate, the early gut, posterior section of the spinal cord, and differentiated somites. 400X.

Fig. 33 Age 25 hours

An enlarged crossectional view of the notochord as it appears a few hours later than in Fig. 32; the notochord is nearly vacuolated. 400X.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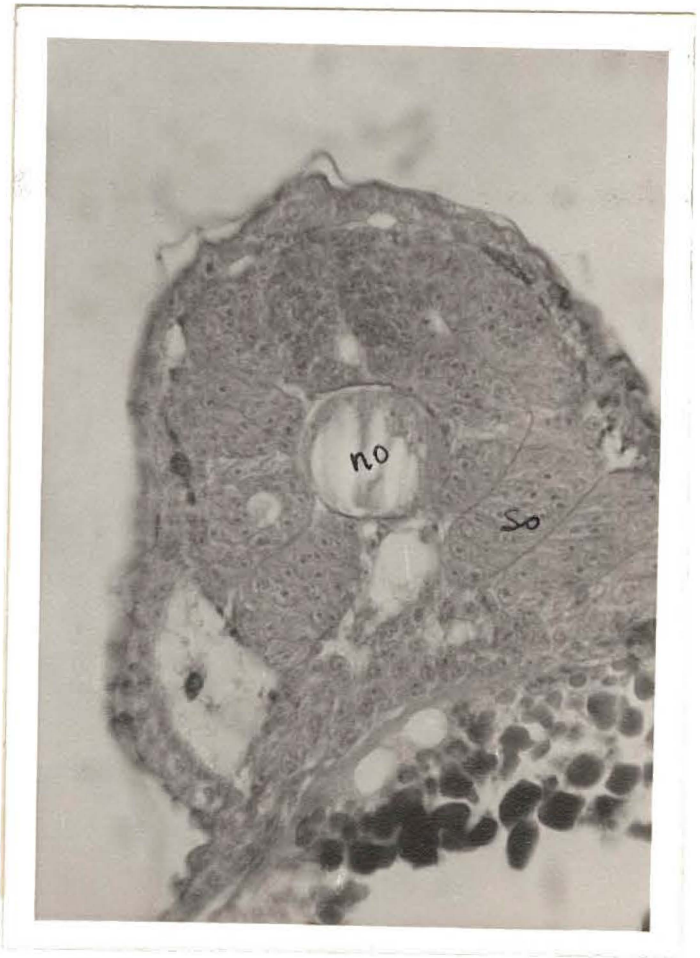
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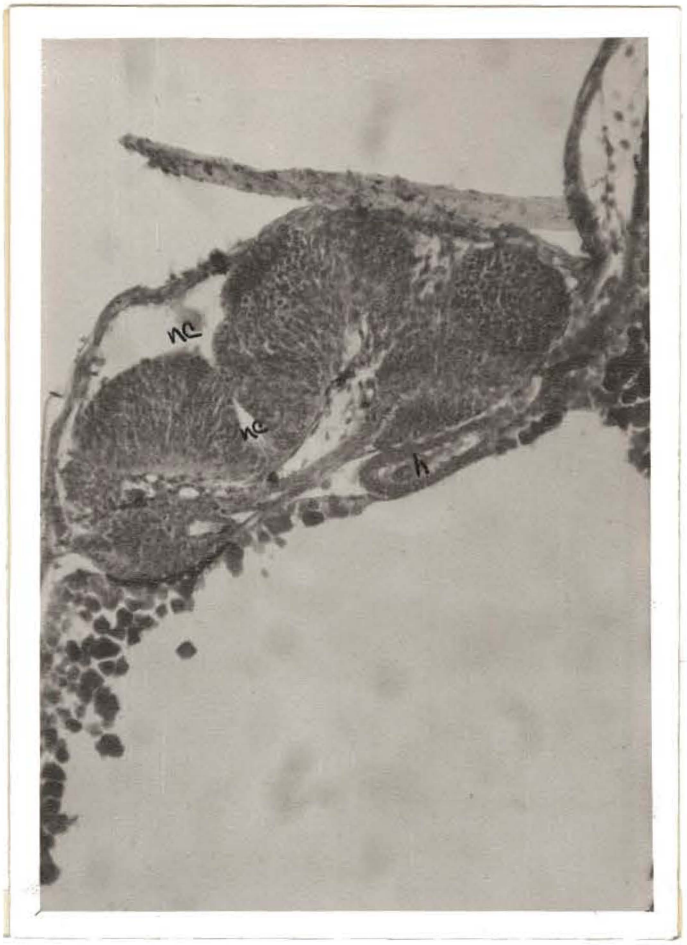
Fig. 34 Age 28 hours

A crossectional view of the early heart beneath the mid brain. 200X.

Fig. 35 Age 40 hours

A crossectional view of the heart showing the atrio-ventricular valve, atrium, ventricle, ear as a single chamber, otic ganglia, and white matter as it appears in the first stages in the brain. 100X.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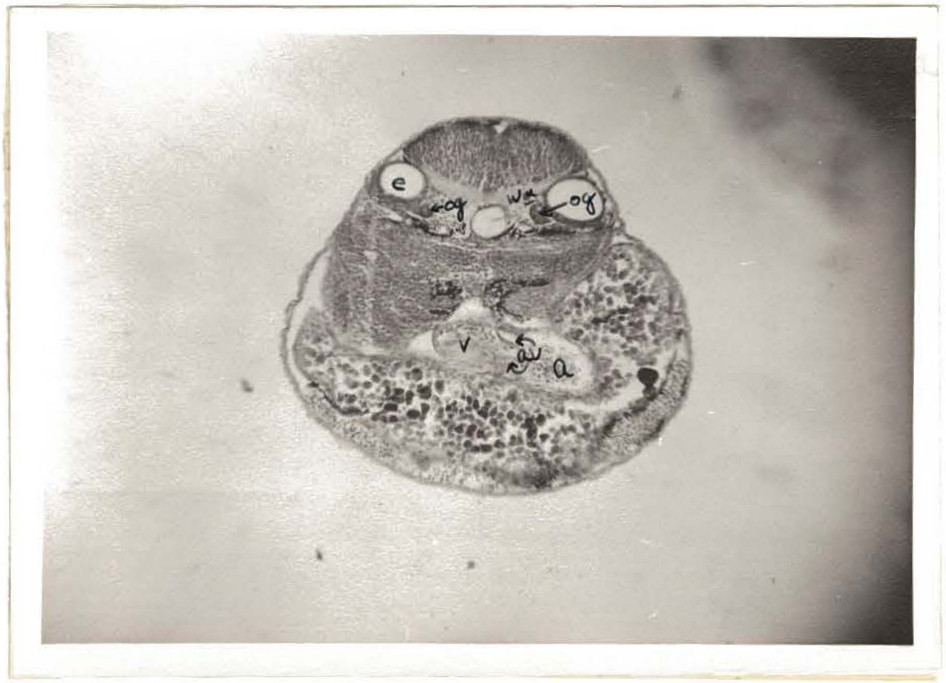
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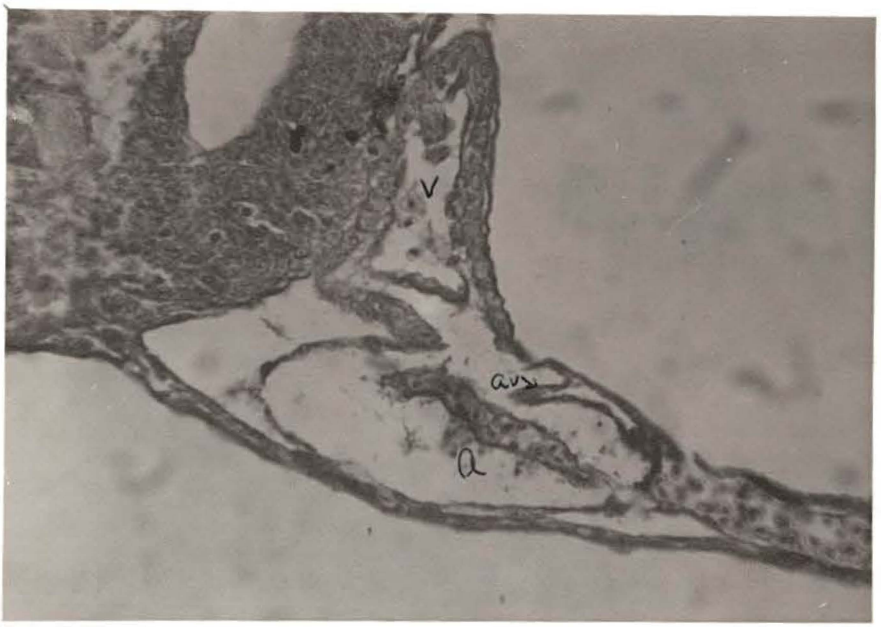
Fig. 36 Age 51 hours

A crossectional view of the heart as it appears in linear arrangement. Note the atrio-ventricular valves. 400X.

Fig. 37 Age 64 hours

A crossectional view of the heart in the initial stages of moving into the "S" shape, also showing the first shelf in the ear, endolymphatic area, cartilage base, white matter encompassing one-half of the brain, and the open pharynx. 100X.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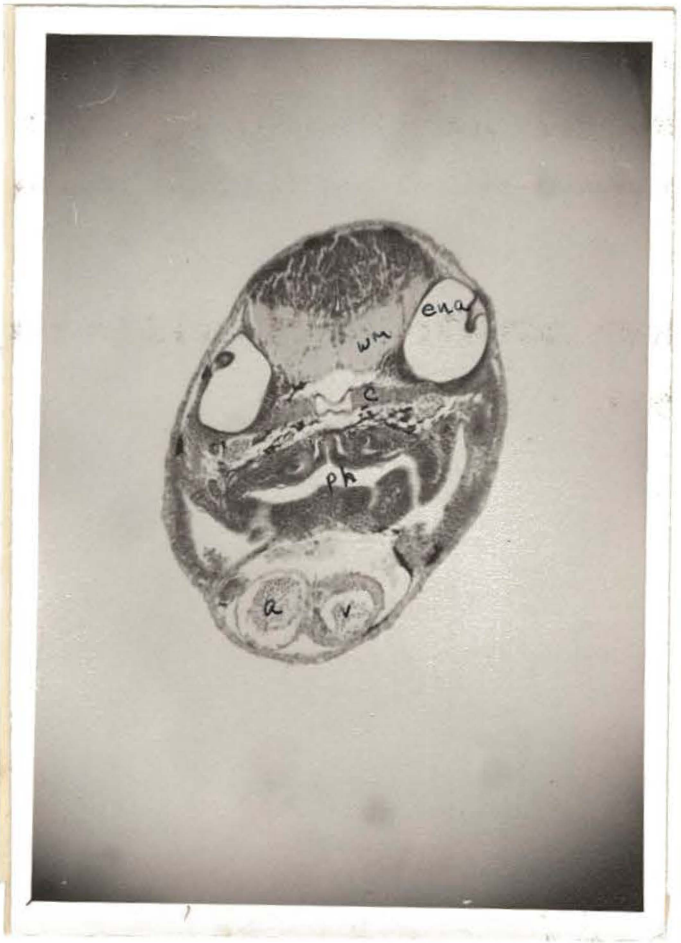
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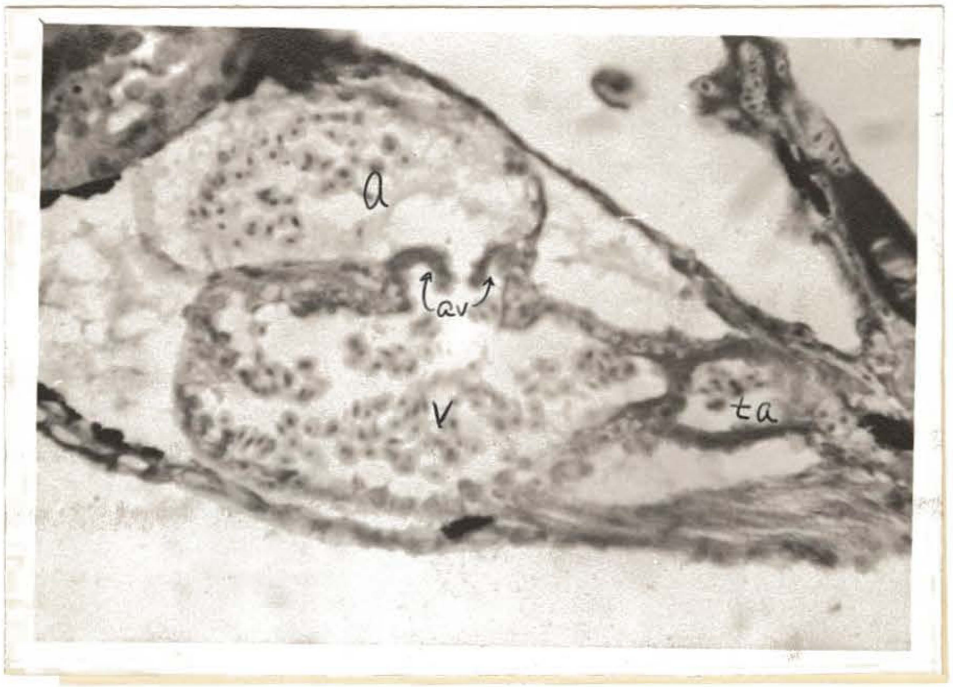
Fig. 38 Age 96 hours

An enlarged late sagittal view of the heart showing the positional relationships of the truncus arteriosus, ventricle, atrium, truncus arteriosus-atrial valve, atrio-ventricular valve, and the atrium covering one-half of the ventricle. 400X.

Fig. 39 Age 252 hours

A late sagittal view of the heart showing the positional relationships as listed in Fig. 38 as well as the obvious difference in wall thickness of the three chambers (atrium, ventricle, and truncus arteriosus). The atrium now covers all of the ventricle. 200X.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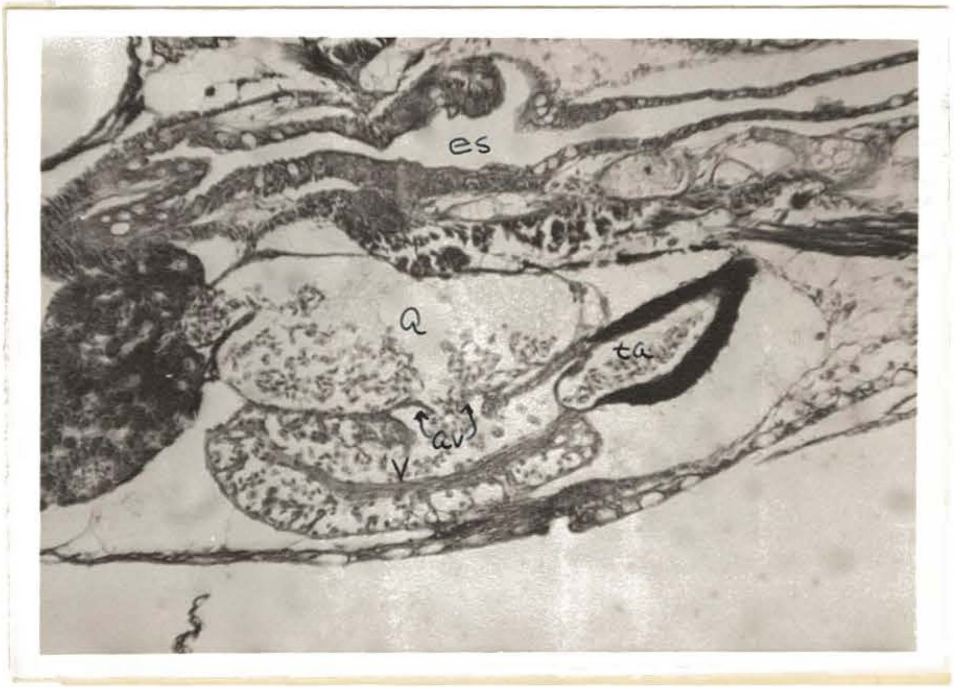
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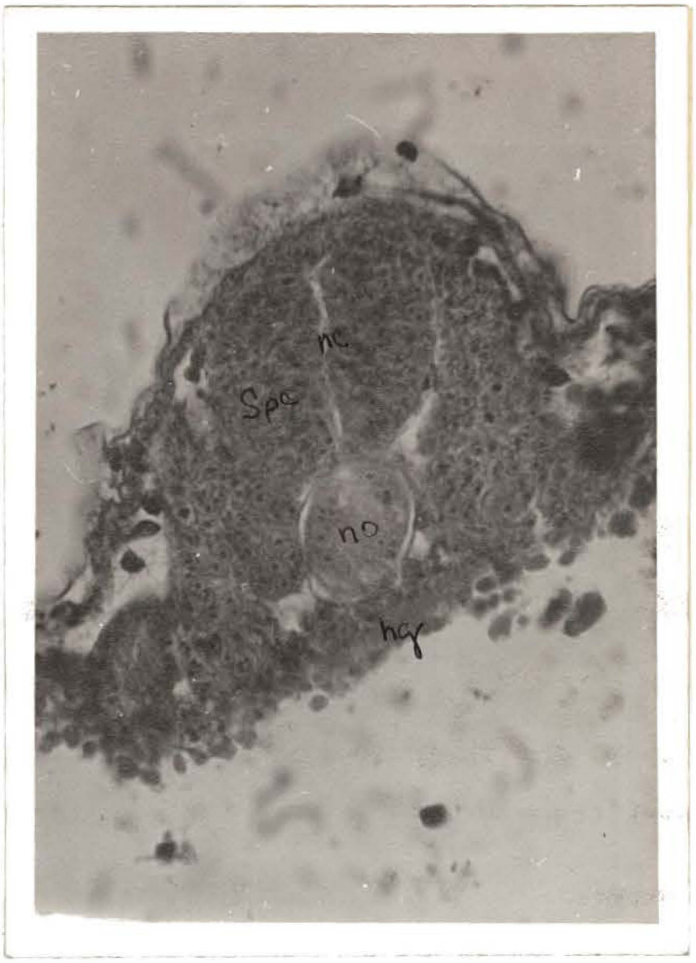
Fig. 40 Age 21 hours

A crossectional view of the hindgut as it appears initially, also showing the spinal cord with the neurocoel just opening and the notochord as a solid, large, round disc (in cross section). 400X.

Fig. 41 Age 24 hours

A crossectional view of the hindgut showing the condition of the hindgut and midgut at early development. Also showing the spinal cord with the neurocoel well open and the notochord beginning to vacuolate. 400X.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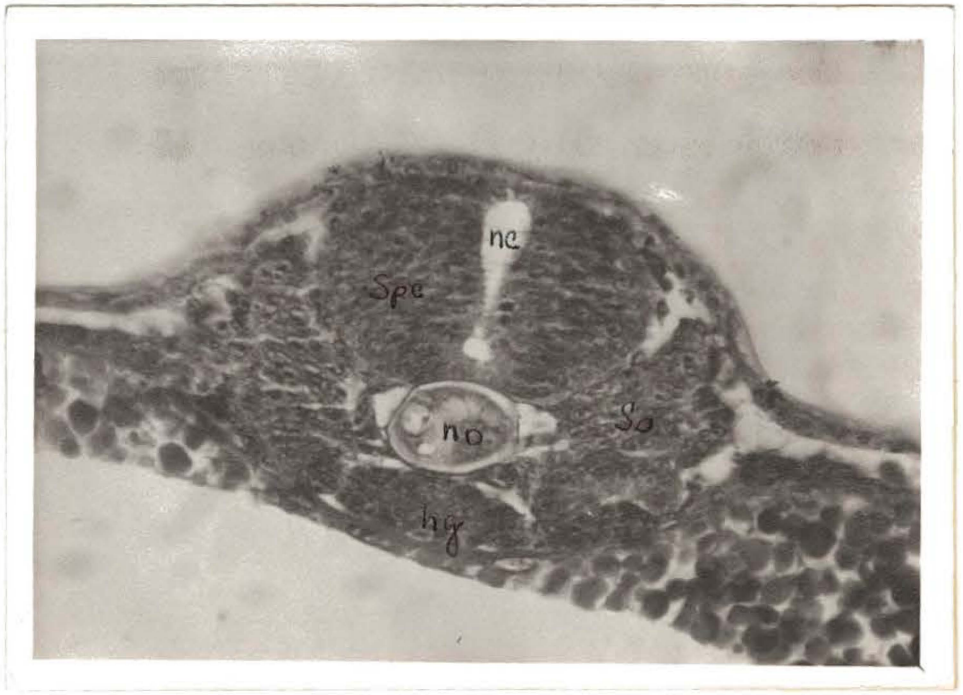
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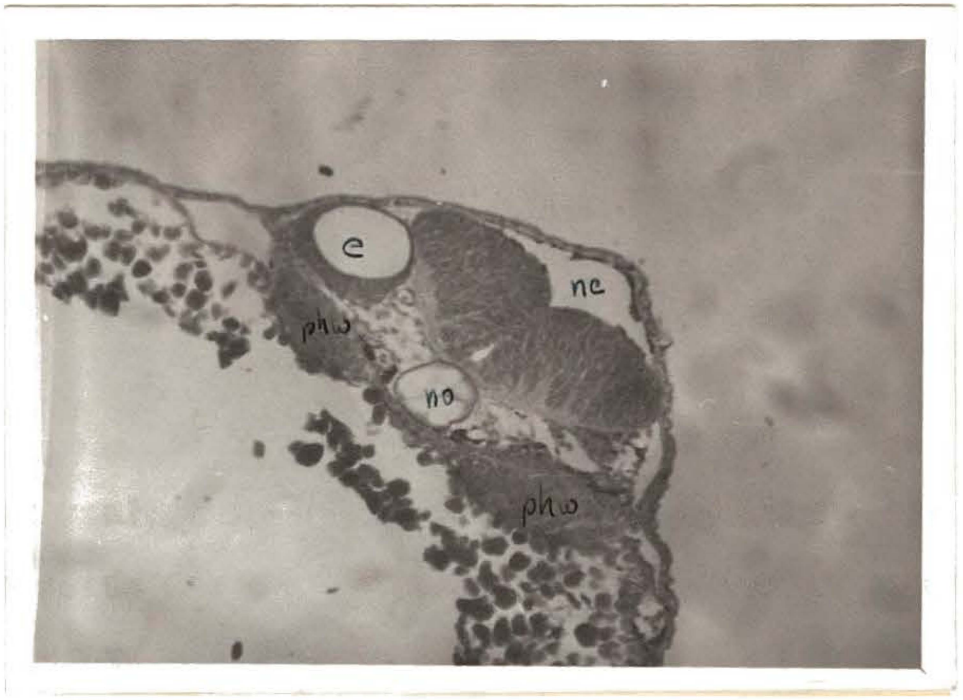
Fig. 42 Age 31 hours

A crossectional view of the pharyngeal wings as they appear as solid wings, also showing the white matter as it appears in its initial stages in the brain, the ear as it thins dorsally, and the notochord composed of 6-7 cells. 200X.

Fig. 43 Age 31 hours

An enlarged crossectional view of the hindgut showing the condition of the hindgut and midgut as they first open. The notochord is large (same diameter as the spinal cord); the spinal cord is hollow; the neurocoels are closing; white matter rings the spinal cord, and the somites are differentiated. 400X.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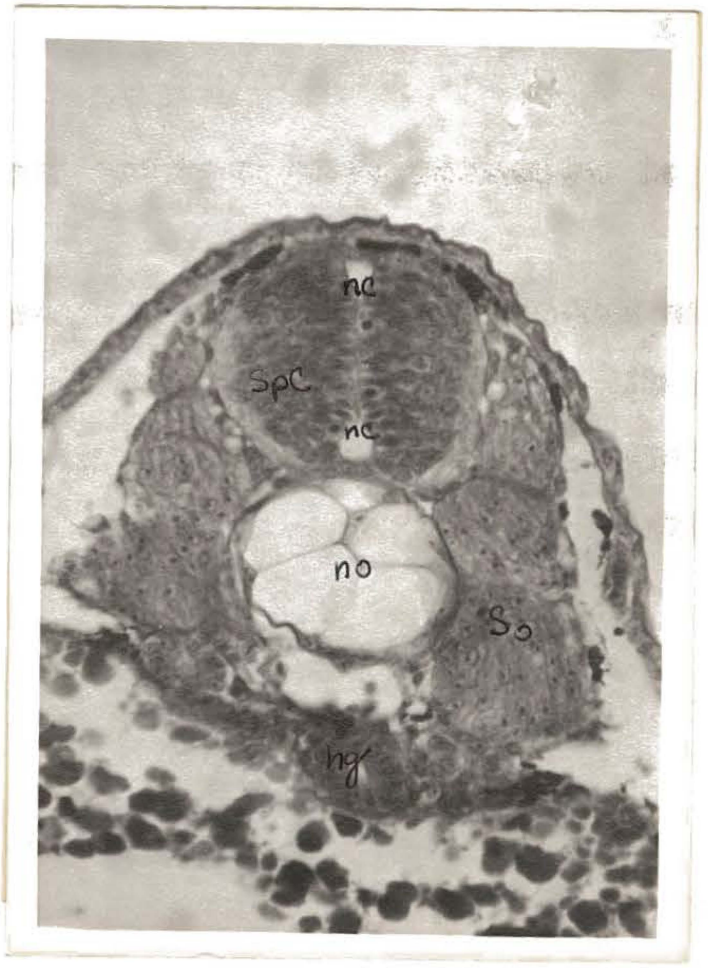
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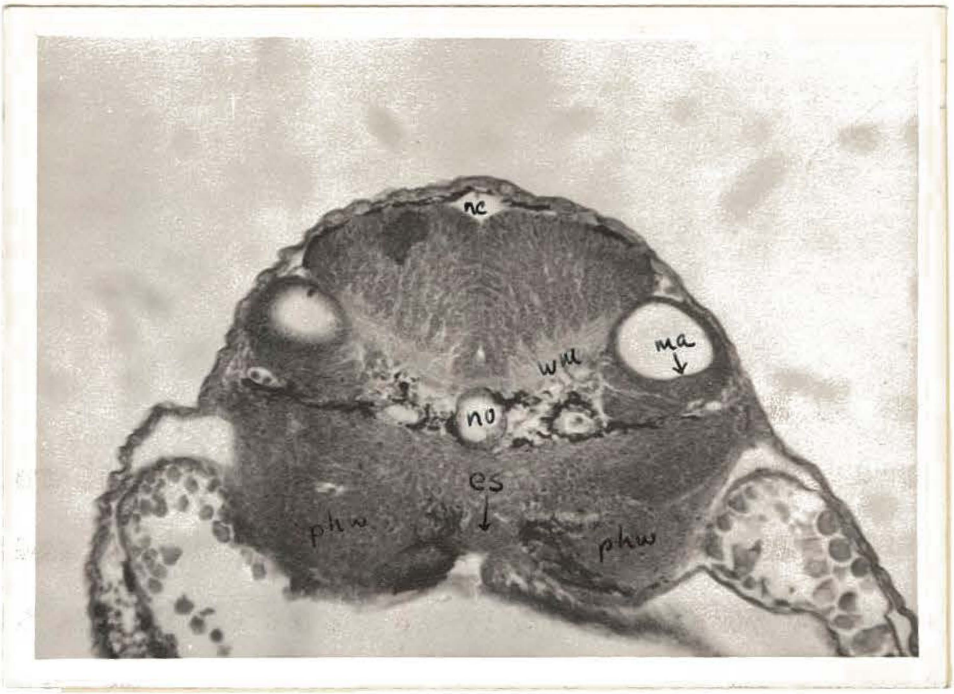
Fig. 44 Age 35 hours

A crossectional view showing the positional relationship of the pharangeal wings (posterior region) and the esophagus (anterior region). Also shown are the ear thinning, maculae, notochord small, and vacuolated, white matter ventral in the brain, neurocoels closing, and otic ganglia. 200X.

Fig. 45 Age 35 hours

Figures 45, 46, 47 are a sequence of crossections of the posterior portion of the hindgut taken from the same slide, each view in order. Figure 45 is the most anterior section showing the "anal plug" as it fills the hindgut. Also shown are the Wolffian ducts, spinal cord, notochord (of four cells), and the dorsal finbud. 200X.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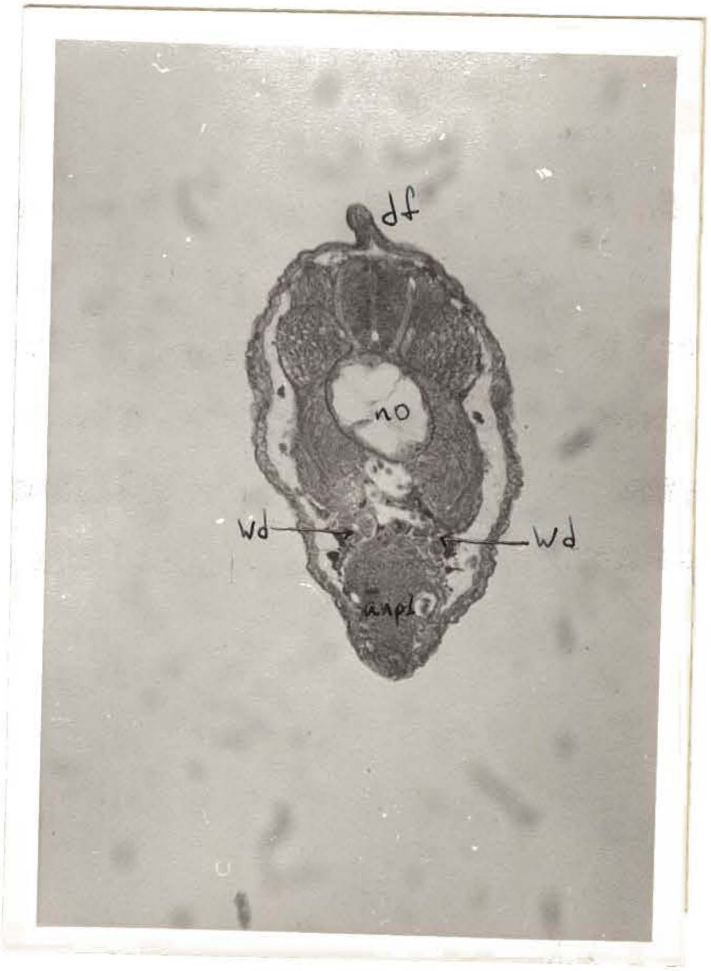
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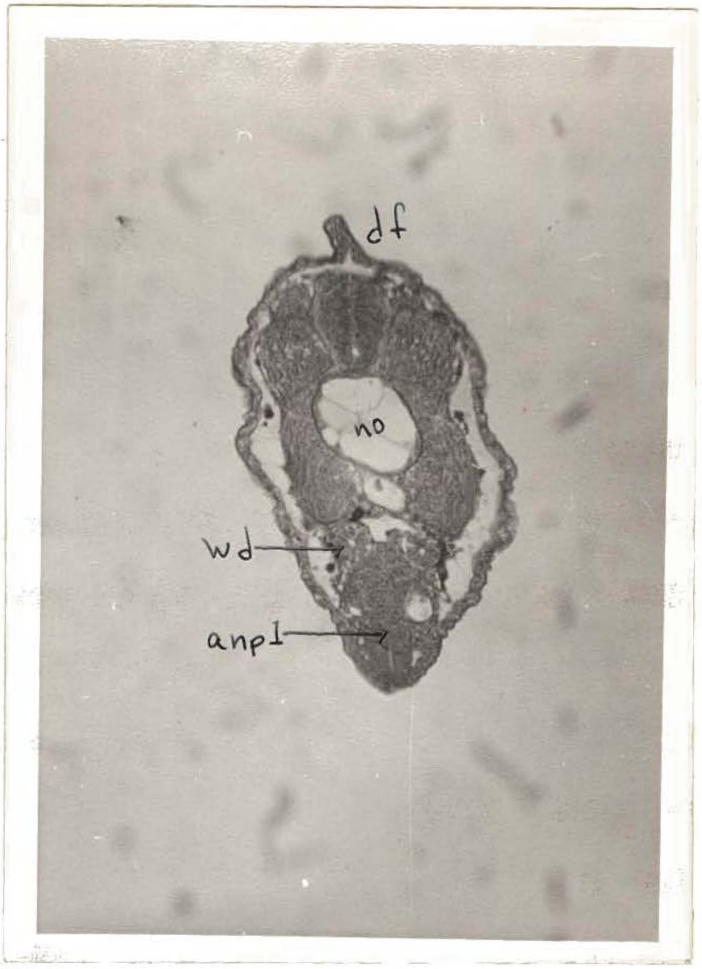
Fig. 46 Age 35 hours

See Figs 45 and 47. A crossectional view of the posterior termination of the hindgut as it enters the cloacal cavity, also the "anal plug" in relation to the anal opening. The same structures as listed in Fig. 45 may be seen here. 200X.

Fig. 47 Age 35 hours

See Figs. 45 and 46. A crossectional view of the posterior termination of the hindgut as it forms the cloacal cavity. The anal papilla may be seen to be open to the external. All of the structures as listed in Fig. 45 may also be seen here. 200X.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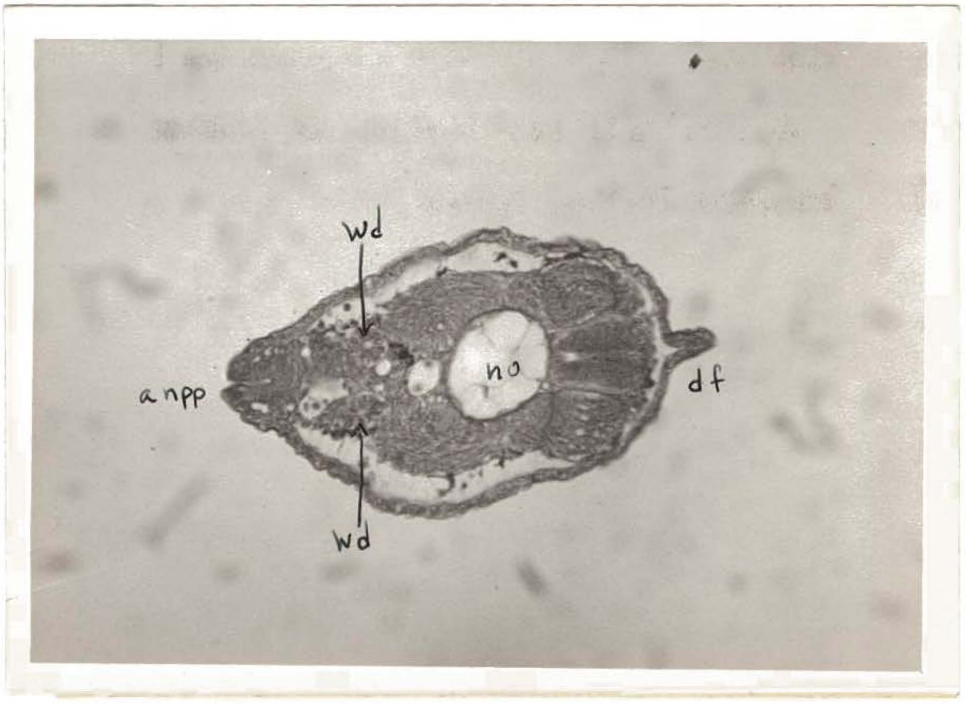
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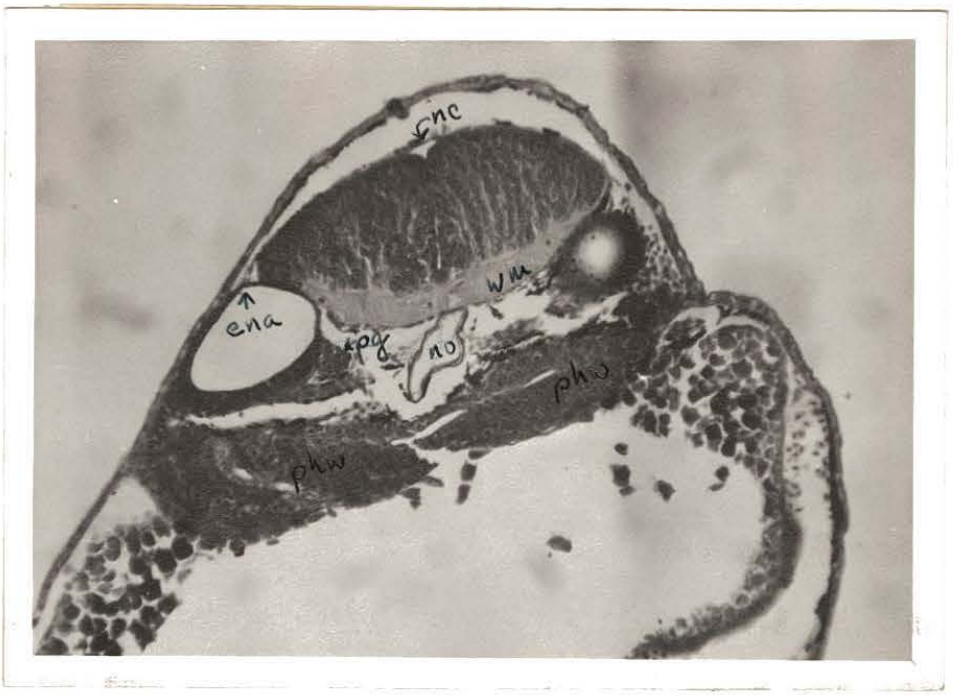
Fig. 48 Age 39 hours

A crossectional view of the pharynx showing the lateral slits and also the otic ganglia, white matter ventral in the brain, neurocoel nearly closed, the ear thinning, and the endolymphatic area.

Fig. 49 Age 46 hours

A crossectional view of the esophagus as it appears as a solid rod. Also shown are the glomerulae, Wolffian ducts, pigment in the brain, notochord vacuolated, and lateral finbuds. 100X.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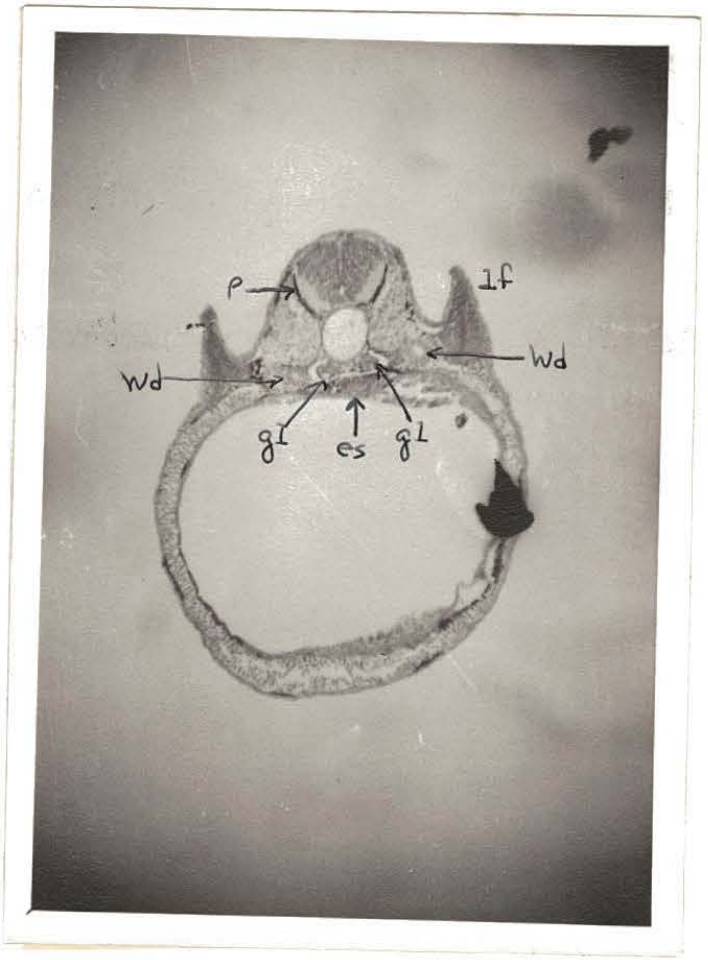
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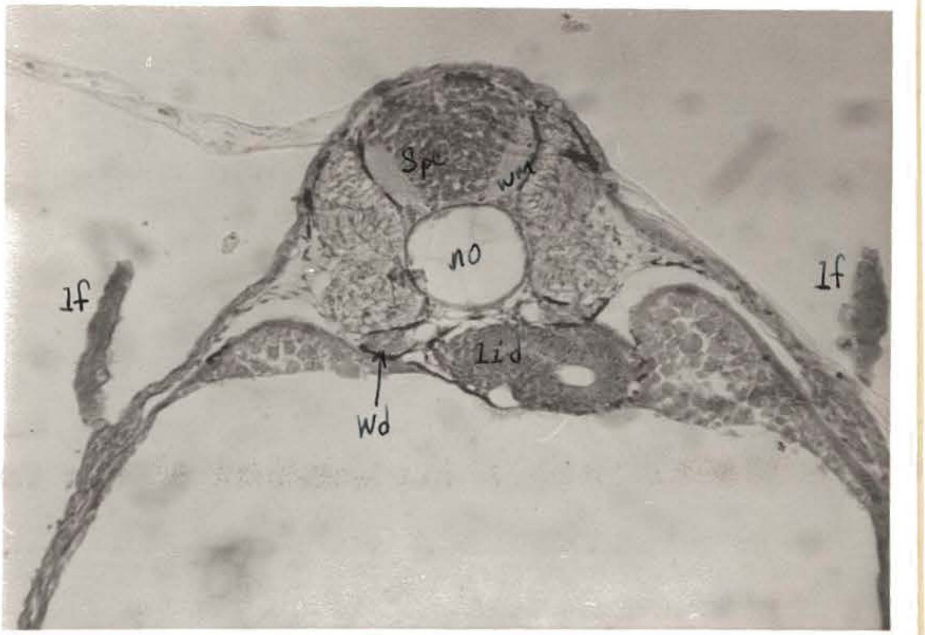
Fig. 50 Age 48 hours

A crossectional view of the junction of the foregut and midgut showing the liver diverticulum, notochord composed of four cells, spinal cord with lateral white matter, lateral finbuds, and Wolffian ducts. 200X.

Fig. 51 Age 48 hours

A sagittal view of the midgut and hindgut showing the partitions. The somites show clearly the metemeration of the muscles. 200X.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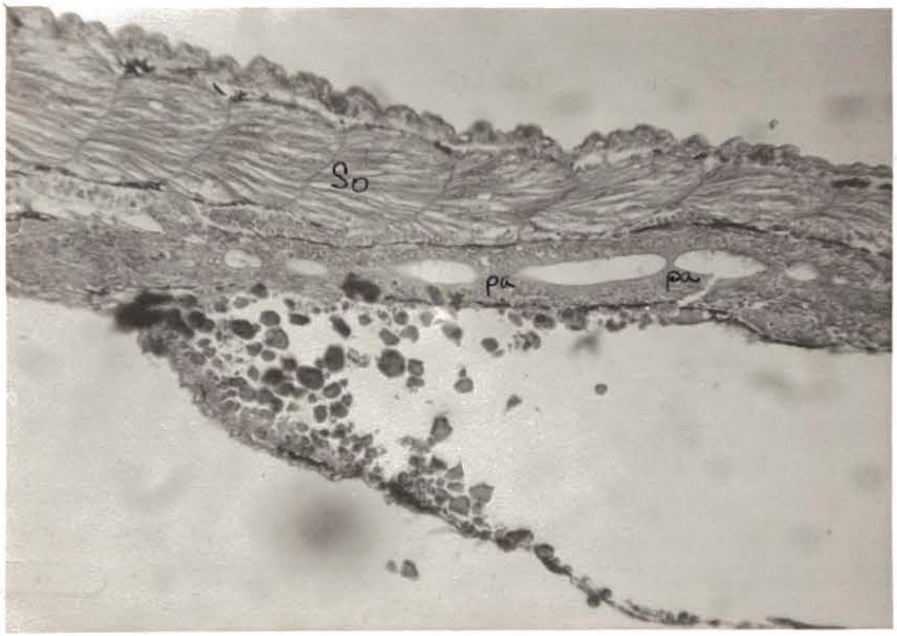
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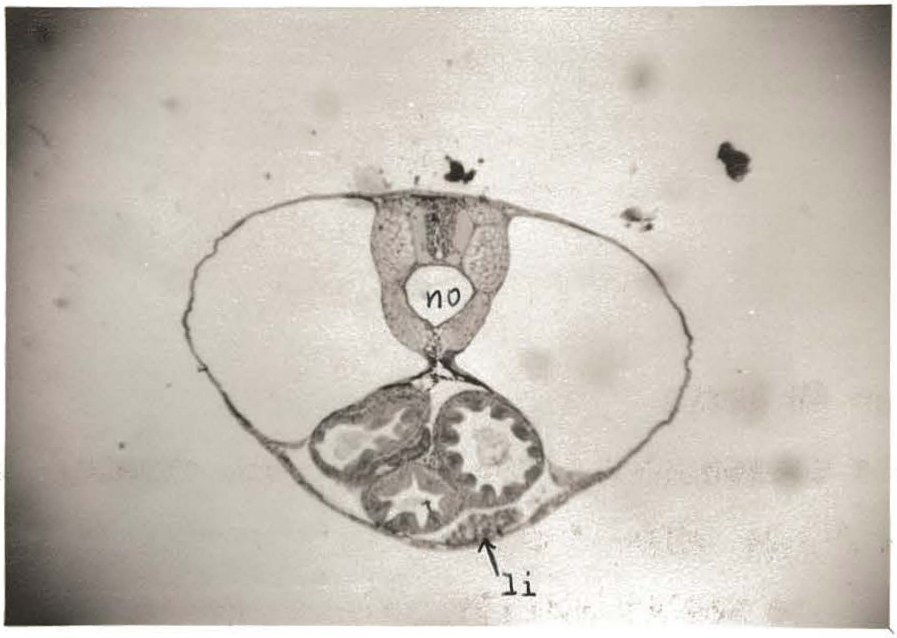
Fig. 52 Age 60 hours

A late crossectional view of the gut showing the result of coiling. Also visible are the liver, spinal cord with white matter covering the entire lateral surface, somites, and notochord composed of three cells. 100X.

Fig. 53 Age 60 hours

An enlarged crossectional view of Fig. 54 showing the villous appearance of the esophagus. 400X.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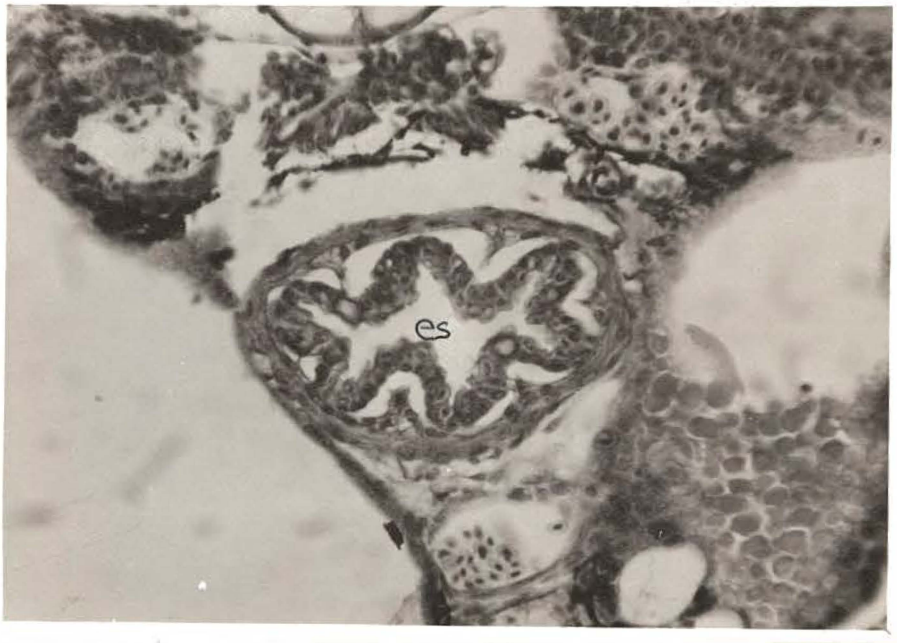
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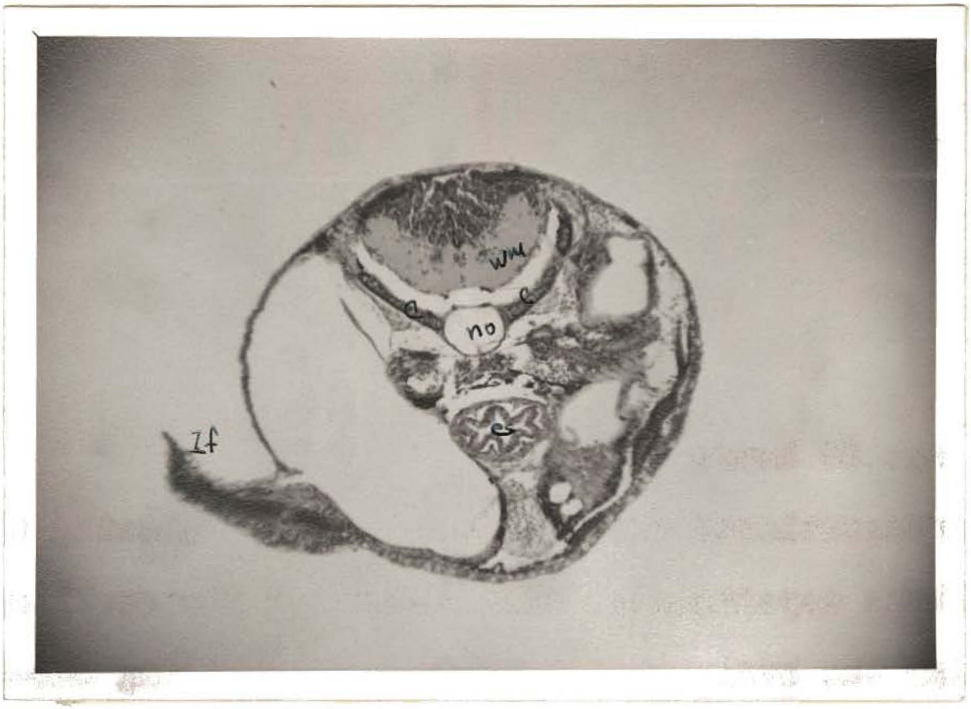
Fig. 54 Age 60 hours

A crossectional view of the esophagus showing the villous appearance. Also shown are the cartilage base under the brain, white matter encompassing one-half of the brain surface area, notochord composed of three cells, the posterior part of the ear, and the lateral finbud. 100X.

Fig. 55 Age 69 hours

A sagittal view of the gut (nearly the entire gut is shown) showing the distension of the stomach. Also shown are the entire esophagus, gill arches, anus, liver, ear with part of a shelf showing and cartilage under the brain. (The brain is bent due to the anterior end of the embryo being here bent ventrally). 100X.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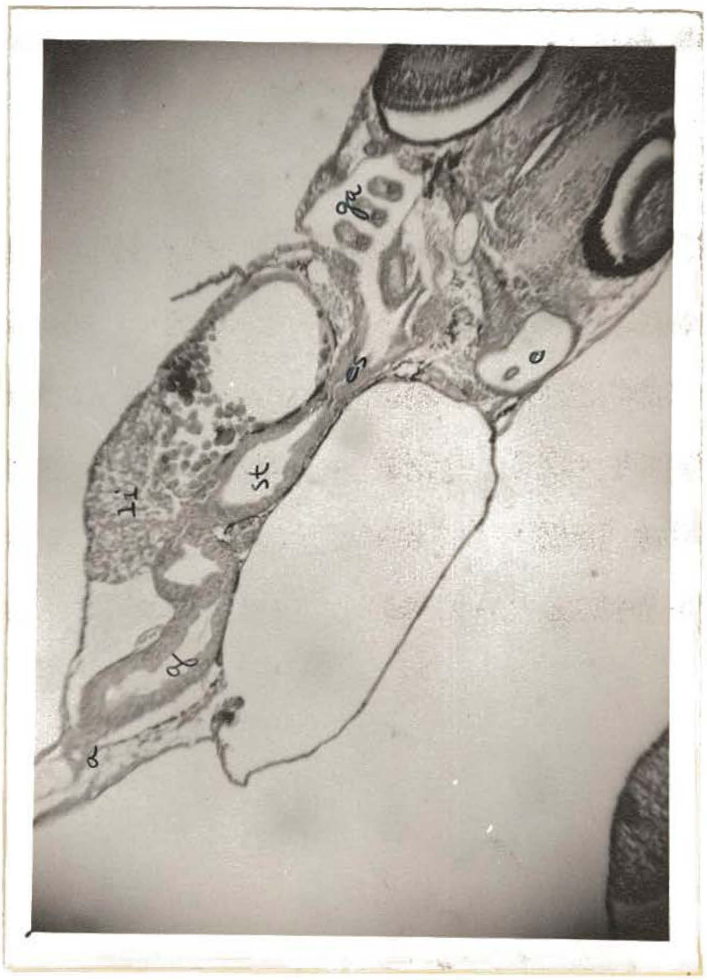
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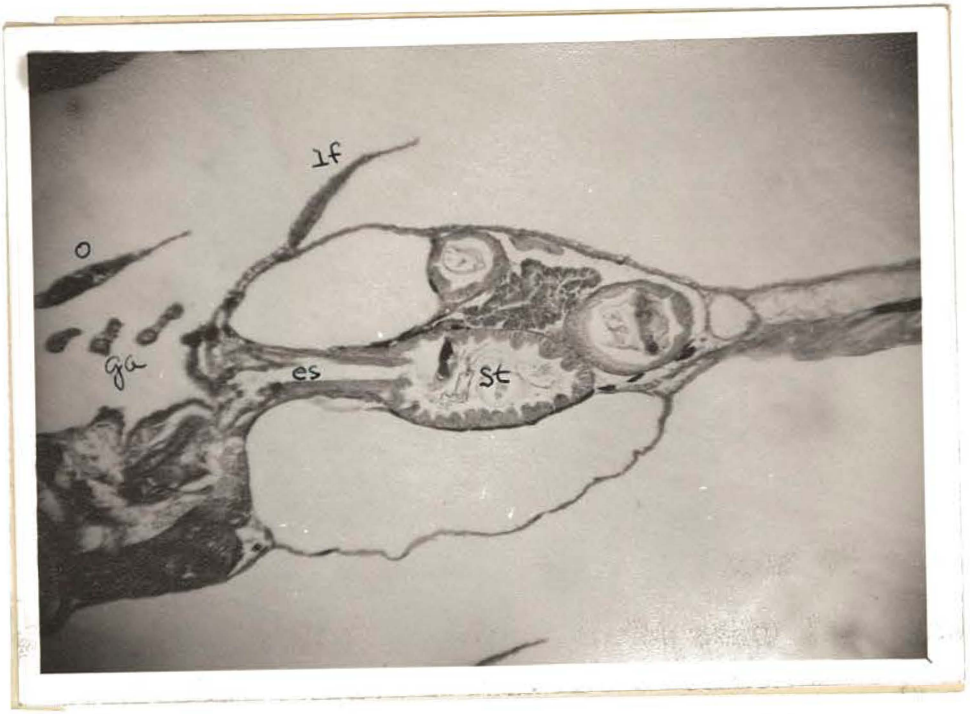
Fig. 56 Age 90 hours

A sagittal view of the gut showing the entire esophagus and the distension of the stomach. Also shown are the coils of the gut, liver, gill arches, operculum, and lateral finbud. 100X.

Fig. 57 Age 96 hours

A crossectional view of the gut showing the distension of the stomach. Also shown are the coils of the gut, liver, lateral finbud, swim bladder, spinal cord, and notochord composed of four cells. 100X.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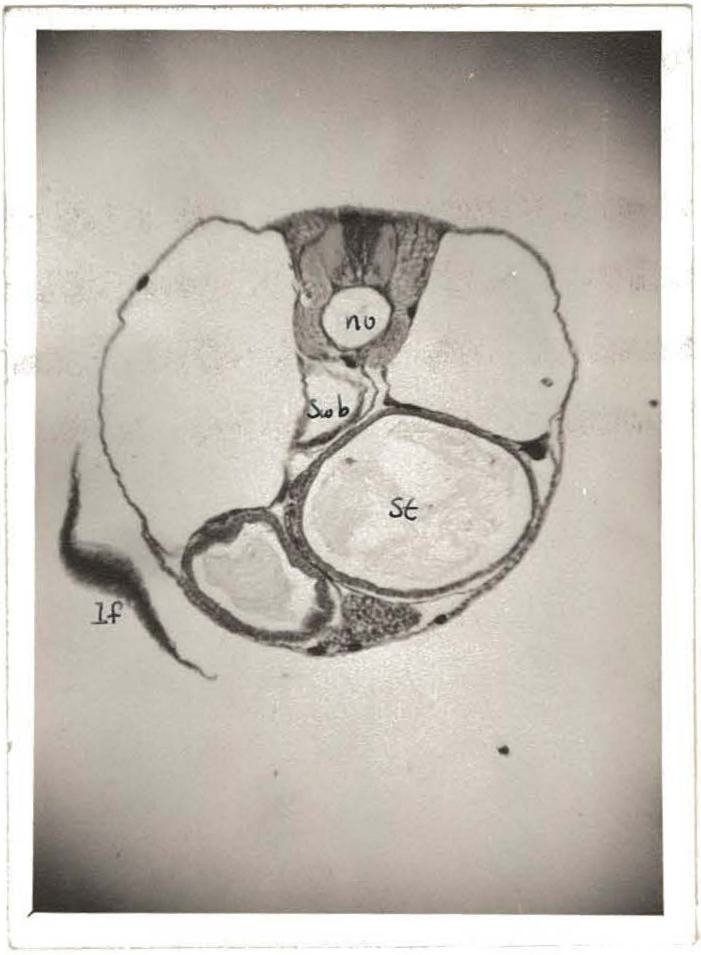
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THE EMBRYOLOGY OF THE PARADISE FISH, *MACROPODUS OPERCULARIS* LINNAEUS

An Abstract

of a Thesis Presented to

the Department of Zoology and Entomology

Brigham Young University

Provo, Utah

In Partial Fulfillment

of the Requirements for the Degree

Masters of Science

by

Lewis M. Mulkay

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INTRODUCTION

The purpose of this study was to describe the normal embryological development of the Paradise Fish, Macropodus opercularis, Linnaeus. It was undertaken to learn the ages at which various structures and systems appeared and to determine whether or not the typical pattern of teleostean development was followed. Allen (1951) used M. opercularis for his investigation of anomalies caused by x-irradiation. In order to understand more fully the anomalies produced by x-rays in this species, it was felt that a more detailed knowledge of the normal development should be known.

The natural habitats for the Paradise Fish are the rice paddies, small rivers, and streams of China (Innes, 1955). Its economic importance is probably as a mosquito control in those areas, since its size is too small for it to be of value as a food source. The ease of laboratory breeding and the great number of offspring produced (up to 1200 eggs per breeding) make the Paradise Fish a convenient organism for studies in both experimental and descriptive embryology.

According to Berg (1947) the Paradise Fish is a member of the order Perciformes, family Anabantidae (ex parte Labyrinthici). Anabantidae are characterised by having a complex mass of labyrinths in the auditory area. These

labyrinths function to trap and store air which the fish gulps into its mouth. The air then moves out over the gills and out through the operculum. This extra respiratory process helps the Paradise Fish to live in the warm, muddy waters of its native rice paddies in spite of the low oxygen content found there.

The literature pertaining to Macropodus opercularis deals primarily with taxonomy. There are a few studies of particular structures such as: chromatophores (Dalton and Goodrich, 1937), air-breathing organs (Das, 1927), color patterns (Goodrich and Smith, 1937), respiratory labyrinths (Ito, 1950), chloride secreting cells (Liu, 1942), and hydrostatic apparatus (Peters, 1946). No detailed study on the normal embryology has been found in literature at this time. Only superficial studies relating to the natural history features, gross anatomical observations and notes on development can be found (Boulart, 1872 and Pouchet, 1872). Dr. Hans M. Peters (1956) of the University of Tuebingen, Tuebingen, Germany, referred to work to be published about Macropodus opercularis, but thus far it has not been found in the literature by this author.

METHODS AND MATERIALS

The fish used for the present study were kept in three gallon and twenty gallon aquaria. The temperature was maintained between 24° and 28°C. Some of the newly hatched embryos were retained to observe their gross development. These were first fed infusoria and powdered lettuce. The powdered lettuce served primarily as a food for bacteria, which in turn served as food for infusorial organisms, and it was the infusoria that served as the first food for the fry as they developed. When the fry were old enough, they were fed brine shrimp and ground liver.

After the eggs and embryos were collected, they were placed in Bouin's solution. The specimens were then progressed stepwise, through a series of alcohols to dehydrate them (Brauer, 1955). Cedal-oil was used instead of the usual clearing agent, Xylol, in order to prevent hardening of the yolk material. Following clearing, the embryos were embedded in paraffin wax, mounted on wooden blocks, and sectioned at four to six microns. They were then stained with haematoxylin and counterstained with eosin (Brauer, 1955).

Approximately one hundred and fifty embryos in varying stages of development were examined with an Olympus binocular compound microscope at magnifications of 40, 100, 200, and 400

diameters. Illumination was provided by a Bausch and Lomb model PR27 illuminator that was modified by various blue and neutral filters.

In order to illustrate appropriate developmental stages, certain sections were photographed with a Zeiss-Ikon camera using Kodak, Panatomix X, FX135 film. Various exposure times were used depending upon the magnification used and the intensity desired.

ORGANOLOGY

This section includes a description of the normal stages of development of the major organs, presented in unit form for each organ discussed. The sequence of development of each organ is described from initial appearance or organisation to the stage of development as found at four or five days of age. For the most part this includes the major changes in each of the organs discussed. Not every organ of M. operacularis is discussed nor is any one discussed completely. The organs are taken up in the following order: brain, eye, ear, notochord, heart, and gut.

The Brain

The brain is one of the first definite structures to be recognised in the developing embryo. It progresses from a primitive neural keel, through the formation of a large ellipsoidal rod, and on to a sequence of first opening to form the neurocoels, then a subsequent closing of the neurocoels. As viewed in cross section the shape of the brain changes from a flat, wedge-shaped keel to an ellipsoidal rod, to a triangular adult brain.

The neural keel is formed of ectoderm on the surface of the blastodisc, laying along the long body axis. At 11 hours (Fig. 1) it is shallow but wide in cross section (5-8 cells

deep). At the same time that the neural keel appears, Kupffer's Vesicle appears in the posterior region (Figs. 8, 31). At 12 hours the neural keel is narrower and deeper (12-15 cells deep). By 16 hours the brain has changed from a keel to a solid ellipsoidal structure anteriorly, the anlage of the forebrain (Fig. 3). The posterior region remains in the form of a keel-shaped structure.

The forebrain is a solid structure at 16 hours, as described above. The process of cavitation of the forebrain is initiated at 19 hours, when a vertical slit appears along the midline (Figs. 9, 10, 24). At 20 hours the infundibulum appears at the base of the diencephalon (Fig. 4) and the brain is flexed (Fig. 3), expressing the pontine, apical, and nuchal flexures. The optic stalk (diencephalon) is hollow at 23 hours (Fig. 11). By 25 hours the epiphysis (Fig. 4) is formed at a point dorsal to the pontine flexure (diencephalon). at 35 hours white matter is found in the lateral areas of the telencephalon and diencephalon.

The midbrain follows somewhat the same time pattern as the forebrain. It is a solid ellipsoid at 16 hours (Fig. 25) and is opened by cavitation by 19 hours when a vertical slit appears along the midline. The mesencephalon develops a wide cavity by 20 hours, forming the third ventricle (Fig. 3). The optic lobes (Fig. 2) form by cavitation of the mesencephalon by 21 hours. By 25 hours the cavity of the optic lobes widen considerably but are narrow again by 28 hours. Also at 28 hours the floor of the mesencephalon is very thick, and

ABSTRACT

The development of Macropodus opercularis Linnaeus, (Perciformes: Anabantidae), is described from cleavage to five days post hatching. For the most part M. opercularis follows the pattern of typical teleostean development except as noted below:

1. The heart develops under the left eye along the anterior margin of the yolk sac. This is similar to that reported by Ingersoll (1951) for the Blue Gourami, Trichogaster trichopterus.
2. The ear develops from the head mesenchyme alongside the brain rather than from a placode.
3. The gut develops first in the posterior region then differentiates cephalad.

It was also learned that:

1. No true bone exists in the skeletal system up to five days of development post hatching at 80°F.
2. The hatching time was 35-37 (36) hours after fertilization at 80°F.