THE EMBRYOLOGY OF THE COHO SALMON, ONCORHYNCHUS KISUTCH (WALBAUM)

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ABSTRACT

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By

Ibrahim H. Zeitoun

This survey was an attempt to complete our knowledge about the life history of the coho salmon, Oncorhynchus kisutch (Walbaum). Its early development was typically teleostean. A prolonged period of a discoidal-type cleavage followed fertilization. By the fourth day of incubation the blastula stage was completed and on the closure of the blastoporal lips most of the primordia organ systems were established. The peculiar features of teleosts were attained following the formation of the yolk sac. Hatching occurred after about forty days under continuously flowing water with a constant temperature of 10 $\pm$ 1 C. Prior to hatching the twisting of the heart and the formation of the branchial arches were observed, both of which are characteristic of primitive vertebrates. The discoidal cleavage, the development of the finfold, and the pharyngeal extension pushing the ventral mouth forward prove the higher phylogenetic position of the coho among teleosts.

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TABLE OF CONTENTS

LIST OF FIGURES

INTRODUCTION

Oncorhynchus kisutch (Walbaum) is a member of the family salmonidae. It has several common names: silver salmon, coho salmon, or jack salmon. It is an anadromous fish usually found along the Pacific coast of North America extending from the Coronado Islands, Mexico, north to Alaska and south on the Asiatic side to the Japanese coast. It prefers to inhabit cool, clear waters (Shapovalov and Taft, 1954). Recently fishery biologists of the United States succeeded in introducing this species in the Great Lakes.

The spawning season in the rivers and streams lasts from September to November. Other cohos are reported to spawn in January and some as late as March (Walden, 1964). The adults move upstream and the female constructs a nest or redd in the gravel of shallow waters where she deposits her eggs which are immediately fertilized by her male mate's milt. The parents then die and are washed downstream.

The adults show a secondary sexual dimorphism prior to and during the spawning season. In the sea they are silver in color on both sides with small dark spots on their backs. In the spawning season, however, the female is dark in color while the male becomes reddish and its jaws are elongated, the lower jaw

forming the kype. The smolts usually migrate to the sea after one year and after two years in the sea they attain sexual maturity and return to the river to spawn and die. Some males, known as jacks or grilse, mature and return to spawn after only one year in the ocean (Shapovalov and Taft, 1954). Jones (1966) reported that the average weight of the coho, O. kisutch, in southern waters ranges from five to ten or twelve pounds and slightly higher in British Columbia. Shapovalov and Taft (1954) stated that the males in the spawning season are slightly larger than the females and the mean length of the male is 64.7 cm and that of the female is 63.8 cm.

There is little and scattered information about the embryology of teleosts, although it is important to any fishery biologist who deals with the life history or production of fish. This information is necessary to the study of ontogenetic and phylogenetic relationships. Balfour (1885), McEwen (1953), and Balinsky (1966) described the embryology of vertebrates in general, with a poor treatment of teleosts. Price (1934a, 1934b, 1935) made a thorough study of the whitefish Coregonus clupeaformis. Battle (1940) worked on the early stages of the development of goldfish, Carassius auratus, from the time of fertilization to hatching. Armstrong and Child (1956) made extensive observations on the normal early development of the mummichog Fundulus heteroclitus. Recently Oris (1968) made a comprehensive survey of the embryology of the English sole, Parophyrys vetulus, briefly treating the early development

of each organ, using a few photomicrographs.

In regard to the work which has been done on the salmoninae, Riddle (1917) described the early stages of the development of chinook salmon Oncorhynchus tschawytscha from the time of fertilization to the gastrula stage. Wales (1941) described the embryology of steelhead trout without dealing with the early cell cleavage, which has been recently described by Knight (1963). Battle (1944) made an extensive description of the embryology of the Atlantic salmon, Salmo salar, dealing with many organs during early development. The present work presents a survey on the embryology of the coho salmon, O. kisutch, in order to fill the gap in its life history.

MATERIALS AND METHODS

The eggs used in this survey were obtained from the Platte River Station of the Michigan Department of Natural Resources, Benzie County, Michigan. Approximately three thousand eggs were collected on October 31, 1969 from two females. The eggs were placed in a plastic bowl filled with enough river water to cover them. The milt was then stripped from three healthy males over the eggs. The contents were stirred for three minutes to allow fertilization. The excess milt was washed away by several changes of the natural water, and the eggs were placed in big jars filled to the neck with stream water. The eggs were brought back to Michigan State University where they were placed in a Heath Fish Incubation Cabinet, Model 1B-16,* which was combined with a temperature regulator tank and flowing water regulator. In this way, the eggs were continuously subjected to well-aerated and constant temperature flowing water. The constant temperature used in this work was 10 $\pm$ 1 C.

Two groups of the fertilized eggs were collected at each sampling time, ten eggs in each group. The samples

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*Available through Heath Tecna Corporation, 19819 94th Avenue South, Kent, Washington.

were taken at one-hour intervals from the time of fertilization to the 24-hour stage, then one sample every six hours until the third day of incubation, and one-day interval samples through the whole period of incubation until hatching.

The first group of fertilized eggs was fixed in Bouin's solution while the second group was fixed in Smith's solution. The Smith solution was replaced by Kahle's fixative after the eyed stage. The above mentioned killing and fixative solutions are recommended by Guyer (1936), since they fix satisfactorily and cause much less distortion and shrinkage to the germ tissues. Two methods of dehydration were used, the alcohol and xylol method was usually applied to the whole mount and the transverse series. Also the aniline method, using toluene as a clearing agent, was used before the infiltration step of the embryo. Two series have been prepared in this study, one of whole mounts and one of transverse sections at different stages of development.

The embryos were embedded in Fisher Tissuemat 52.5 ± 0.5 C, and sectioned at 5 μ and 8 μ, the majority being cut at 8 μ and mounted by the water-albumen method. The whole mount embryos were stained with alum cochineal solution and because of the large size of the coho salmon's eggs the embryos were taken off the yolk mass. The transverse sections were stained with Delafield's hematoxylin using eosin as a counter stain. Canada balsam was the mounting substance used in both sets.

Observations were made at 10X, 20X, 45X, and 125X. Some

photographs of the embryos were made with a Nikon AFM-M-35 camera and Olympus microscope; others were taken with a Leitz-Wetzlar microscope equipped with a 35 mm Leitz camera.

The hatching occurred in approximately forty days at a water temperature of 10 ± 1 C. This period varies with the temperature of the water (Embody, 1934). No serious fungous infection appeared during the incubation, and the total mortality rate of the eggs was approximately twelve percent. The greatest rate of mortality occurred during the tender period of development and hatching. The high percentage of dead eggs may be due to the handling during the sensitive period and to incomplete development of the hatched embryos.

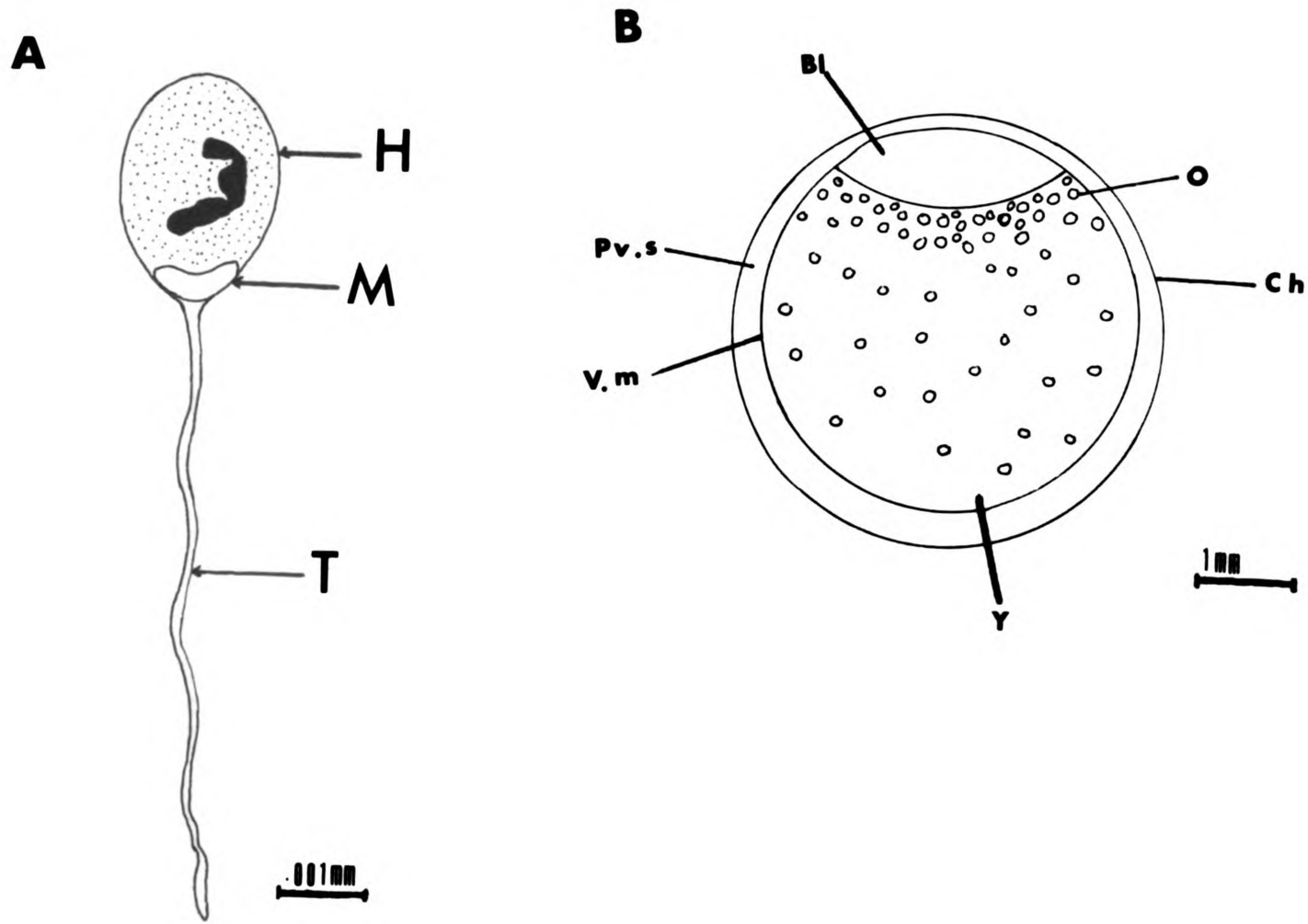
DEVELOPMENT

Description of fertilized eggs and the sperm

The fertilized egg of the coho salmon, Oncorhynchus kisutch, is spherical, light yellow in color, and has a diameter ranging between 5-5.5 mm (Figure 1,B). The yolk occupies the largest part of the egg. It is semifluid when the egg is fresh. At the animal pole the blastodisc is mounted over the yolk sphere and is continuous with the plasma membrane (or vitelline membrane). The chorion or zona radiata, which is a protective membrane secreted in the female ovary by the follicular cells, forms the outer membrane or shell of the egg. In between the two egg membranes there is the perivitelline space, which is filled with fluid. Many oil droplets are distributed through the yolk, but there is a conspicuous concentration of them at the animal pole. Riddle (1917) suggested that they are nutrient materials for the developing embryo. The oil globules regulate the specific gravity of the egg during development (Richards, 1931).

The milt is a whitish, colloidal fluid containing a huge number of spermatozoa. The sperm is minute, slender, and has a length of approximately .01 mm (Figure 1,A).

Figure 1.-A, Diagram of the sperm of the coho salmon; B, Diagram of the fertilized egg. Bl., blastodisc; Ch., chorion; H., head; M., middlepiece; O., oil globule; Pv.s., perivitelline space; T., tail; V.m., vitelline membrane; Y., yolk.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Early segmentation stages

The segmentation and development in the teleost's eggs are restricted to the blastodisc; it is a discoidal-type of cleavage. After fertilization the blastodisc is formed by the streaming of the cytoplasm toward the animal pole, where the egg nucleus rests, forming a patch of protoplasm over the yolk sphere (Solberg, 1938). The regular rounded germ disc is attained at the fourth-hour stage, at which it bulges slightly over the yolk level.

The splitting of the one-cell germ disc to two cells commenced at about the ninth hour. At this stage a slight lengthening of the blastodisc can be noticed, and the first cleavage plane is meridional on the blastodisc and does not extend deeply through it, but a basal cytoplasmic sheet is left. The splitting occurred on the short axis of the blastodisc, giving rise to two equal blastomeres (Figure 2,A).

Within two hours of the first cleavage the second furrow of cleavage occurred at a right angle to the first along the long axis of the blastodisc, and the result of cleavage was four equal blastomeres (Figure 2,B).

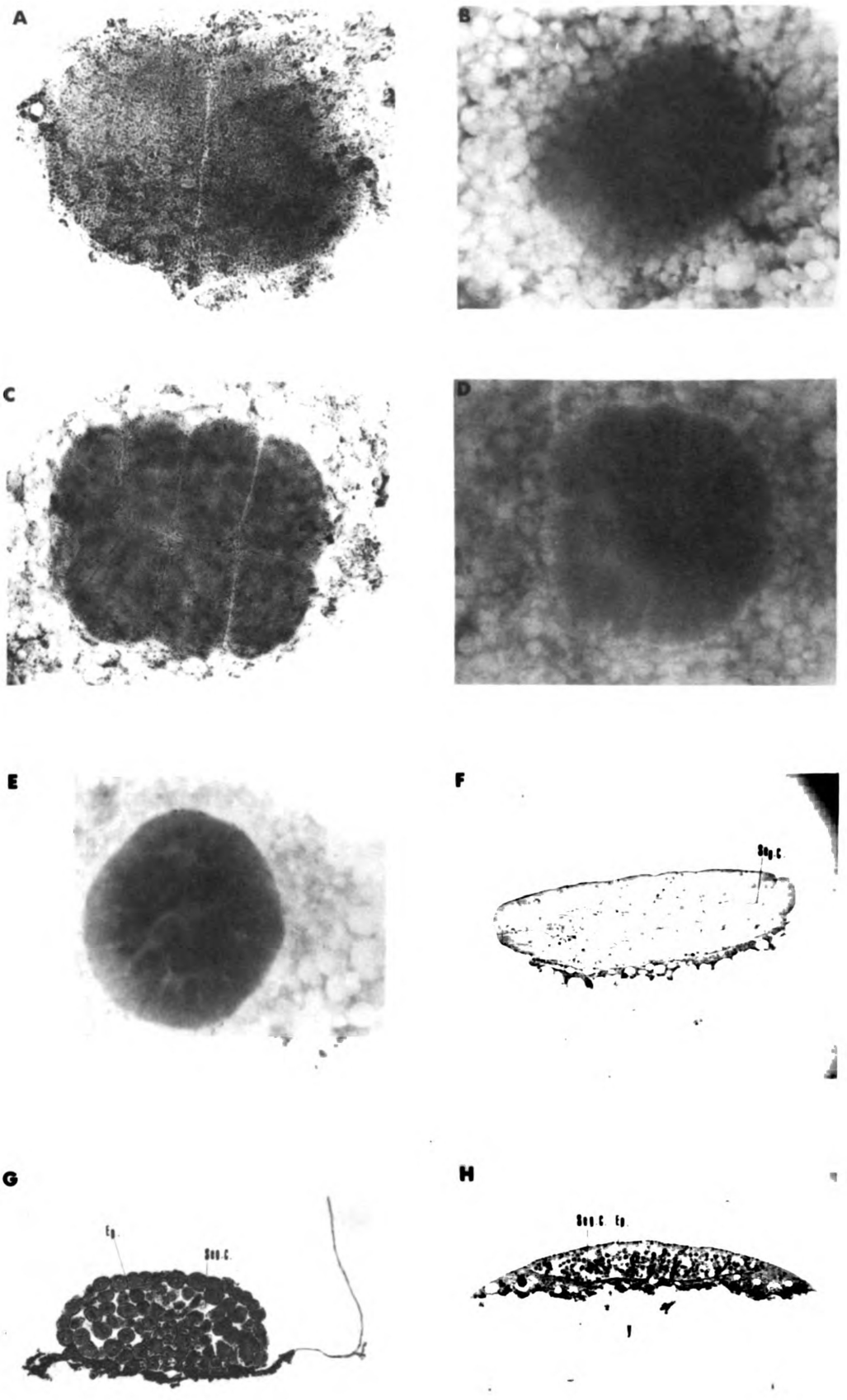
Prior to the third cleavage the blastodisc showed more lengthening along the second plane of cleavage. The third cleavage occurred fourteen hours after fertilization (Figure 2,C). It was a latitudinal division parallel to the second plane and the resulting eight blastomeres are arranged in two rows, four cells each. The furrows following the first cleavage are

deeper and extend through the protoplasm, completely dividing it.

The sixteen-cell stage appeared in eighteen hours. The blastodisc in this stage retains the rounded pattern and starts to raise from the yolk beneath it (Figure 2,D). Multiple segmentation gives the two-layered 32-cell stage at 22 hours, and the three-layered 64-cell stage at 24 hours (Figure 2,F), with more layers of cells added as development proceeds. In the following stages the cells become smaller and it is difficult to count them and to trace the cleavage planes, which start to be irregular. The blastodisc in these stages is properly called the blastoderm.

The periblast layer, which is continuous with the plasma membrane and surrounds the blastoderm, began to be distinguishable at the 32-cell stage around the edge of the blastodisc, and as development proceeded it spread inward beneath the germ disc, forming the subblastodermic periblast. The periblast is responsible for the digestion of the yolk for the growing blastoderm (Balinsky, 1966). The external cells of the blastoderm are closely attached while those of the internal layers are loosely joined together, creating intercellular spaces which form the beginning of the segmentation cavity (Figure 2,F). As division proceeds the uprising of the blastoderm increases and the segmentation cavity lies with the blastodermal layers above and the periblast layer below. The latter stage is called the blastula stage (36 hours, Figure 2,G). The blastula

Figure 2.-Early cleavage stages of the coho salmon. A, The first cleavage. Two cells at nine hours, 44X; B, Four-cell stage at eleven hours, 40X; C, Eight-cell stage at fourteen hours, 44X; D, Sixteen-cell stage at eighteen hours, 40X; E, 32-cell stage at 22 hours, 40X; F, Transverse section of the 32-cell stage, showing the beginning of the segmentation cavity; G, Transverse section of the early blastula, 36 hours; H, Transverse section of the late blastula, showing the flattening of the blastoderm over the yolk. Seg.C., segmentation cavity; Ep., epidermal layer.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Seg C.
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Seg C. Ep.

stage of the coho salmon, O. kisutch, lasts a relatively long period, during which the blastoderm is flattened over the yolk without any increase in the area it rests upon. The blastoderm is composed of several layers of cells, usually called the epiblast (Figure 2,H).

Gastrulation

At about the 84-hour stage the germ ring is clearly observed as a result of the accumulation of the blastoderm cells around its edge (Figure 3,A). This cell accumulation is presumably due to the more rapid divisions of the marginal cells than those in the middle of the epiblast. The gastrulation actually starts at the 96th hour of incubation. The cells at one edge of the germ ring begin to involute and spread over the yolk (Figure 3,B). The involuted layer is the hypoblast of the embryo. The subgerminal cavity lies between the epiblast and the hypoblast. It is known that the involution of the epiblast occurs from the posterior margin of the germ ring which is also the dorsal blastoporal lip (McEwen, 1953).

Along with the involution the epiboly begins and the anterior and the lateral margins of the germ ring commence to extend over the yolk sphere, attempting to close the blastopore and forming the yolk sac. The dorsal lip of the blastopore shows only a slight movement during epiboly.

On the sixth day of incubation the embryonic shield first appeared by the convergence of the blastodermal cells on the dorsal blastoporal lip. The embryonic shield coincides with

the long axis of the future embryo and the embryo axis corresponds to the second plane of early cleavage (McEwen, 1953).

As development proceeds the embryonic shield increases in size. The extra-embryonic ectoderm spreads around the yolk and closes the blastopore after approximately eleven days of incubation.

The early embryo

As the inflected cells move toward the anterior end of the embryo, the extra-embryonic blastoderm moves around the yolk to the opposite side and the embryonic shield increases in size and thickness (Figure 3,C).

On the seventh day of incubation the primary organ rudiments are distinguishable, the endoderm exists just above the periblast, the mesoderm which is derived partly from the epiblast and partly from the hypoblast, occupies the mid-region of the embryo and the uppermost layer is the ectoderm. Along the midline of the embryo the ectoderm shows a distinguishable internal concentration of cells representing the ectoderm neural cells, and just beneath it the mesodermal axial cells of the notochord can be observed (Figure 3,D).

A day later the extra-embryonic ectoderm covers one-fourth of the yolk and at the same time the embryonic shield increases in length. The medullary keel is observed, especially at the anterior end of the embryonic shield, which will form the brain. The thinner middle and posterior portions of the neural keel represent the rudimentary spinal cord. The

optic anlagen arise as lateral swellings from the prosencephalon and just behind them four masses of cells arise on both sides of the neural keel, but they do not distinguish the mesencephalon from the rhombencephalon. The notochord becomes visible and extends from the posterior margin of the brain region to the anterior end of the undifferentiated tail knob. In sections the notochord appears as a circular, hollow and narrow axial structure beneath the neural ectoderm. The mesodermal tissues are condensed as lateral plates along the notochord margins, and eleven pairs of mesodermal somites are visible externally.

The ninth day the blastoderm spreads and covers approximately half of the yolk sphere; the embryo is better seen rising over the yolk with twenty pairs of mesodermal somites. The embryo increases in length particularly toward the caudal end, which is thinner than the anterior end of the embryo. The neural keel is enlarged and grows deeper than the preceding stage and the eye stalk can be detected as a means of communication between the forebrain and the eye anlagen. The auditory placodes, from which the internal ear vesicles develop, appear as two solid masses of cells on the inner surface layer of the epidermis on both sides of the hindbrain. The pectoral fin buds can be observed as minute, external lateral projections on both sides of the embryo just behind the ear placodes.

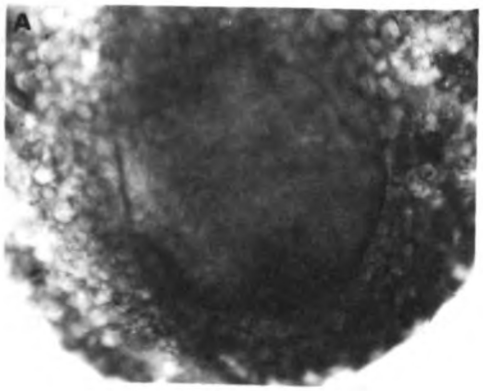
At the ten-day stage the extra-embryonic ectoderm spreads over three-quarters of the yolk mass. The epiboly movement

is more active on the ventral blastoporal lip and it gradually decreases along the ventral lips toward the dorsal lip where it shows only a slow posterior movement. In this stage the caudal knob changes to form a thick and definite round structure and the finfold appears as a very narrow ectodermal outgrowth, which starts dorsally from the posterior end of the head region, moves around the caudal knob and advances ventrally to about the middle of the trunk region (Figure 3,E). The thickening of the ectodermal neural keel increases especially in the brain region (Figure 3,F). The brain begins to differentiate into its primary three vesicles: the prosencephalon or forebrain, the mesencephalon or midbrain, and the rhombencephalon or hindbrain. The olfactory placodes are distinguishable in the form of two thickenings of the epidermis on either side of the forebrain and in front of the eye vesicles which have been invaginated inward and transformed into a double-walled, cuplike structure (Figure 3,F). The eye lens anlagen are developed as two thickenings from the inner epidermal walls which correspond to each eye cup, and the optic cup is still in connection with the prosencephalon through the optic stalk.

On the closure of the blastopore (11 days) the changes become rapid. The rudimentary eye lens consists of a rounded and undifferentiated mass of cells which is still in contact with the inner wall of the epithelium. The retinal wall of the optic cup is thickened and its inner cells can be seen as fusiform in shape. The auditory vesicles are enlarged, forming

a small, hollow structure in the hindbrain region and still are attached to the epidermal dorsal wall of the body. The brain has increased in thickness and the hindbrain splits into two divisions, the metencephalon or cerebellum, and the myelencephalon or medulla oblongata. The medulla oblongata shows a series of segmentations before its entrance in the spinal cord. The midbrain remains the thinner part of the brain, though its lateral and ventral floors undergo thickening. The divisions of the forebrain are not clearly seen in the whole mounts, but in the transverse sections the telencephalon or cerebrum can be detected as well as the diencephalon. The infundibulum arises as a slight depression from the diencephalon floor. There are 36 pairs of mesodermal somites. The heart primordium is marked clearly in this stage by the concentration of the mesodermal cells along the midline of the ventral side of the body just behind the eye vesicles and above the peri-blast. The notochord does not show a noticeable lengthening, but it increases slightly in diameter and it is still hollow with no signs of vacuolation. The finfold is prominent and in the transverse sections it appears as a ridge of two separated epithelial layers, each one is one cell in thickness. The Kupffer's vesicle is distinguishable below the caudal region as a small, round invagination of the yolk. McEwen (1953) has concluded that it has a neural function. The pectoral fin buds project slightly laterally just behind the auditory anlagen. In this stage the endoderm under the mesoderm, as well as the

Figure 3.-The early embryonic stages. A, The germ ring and the early embryonic shield, 44X; B, Transverse section showing the beginning of involution; C, The early embryo showing the optic anlagen and the caudal knob after six days of incubation, 20X; D, Transverse section of the trunk region of the seven-day embryo, showing the primary organ rudiments; E, The structure of the embryo just before the closure of the blastopore at ten days, 20X; F, Head region of the same embryo showing the ectodermal neural keel and the sensory organ rudiments. end., endoderm; Ep., epiblast; ep., epidermis; Hyp., hypoblast; mes., mesoderm; n.k., neural keel; neur., neural cells; not., notochord.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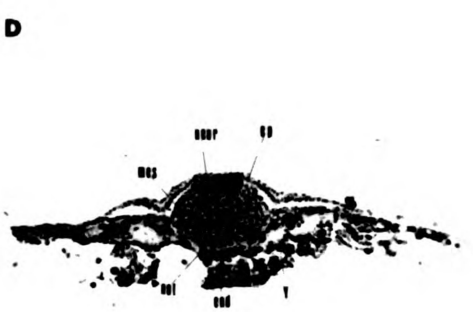
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notochord, forms a simple tube which is easily observed in the transverse sections and represents the pharynx in the head region.

The late embryo

(i) Stages from 13 to 20 days of incubation

As development proceeds, the embryo increases in size and the caudal region begins to curl over the yolk sac. The embryo lies over a slightly thickened part of the extra-embryonic tissue. The head in these stages shows torsion over the yolk. The notochord has increased in diameter and extends to the caudal tip, and the vacuolations commence during the end of this period. The brain is greatly increased in size, particularly the hindbrain region, where the metencephalon and the myelencephalon increase in width. The expansion of the hindbrain (rhombecephalon) cavity gives rise to the fourth ventricle, which is clearly seen from the dorsal view (Figure 4,C). The spinal cord at the end of this period has started to appear in the caudal region as a separate cord lying over the notochord. The olfactory placodes in the antero-lateral region of the head have invaginated, thus forming the olfactory sacs, and their naris opens exteriorly. Each eye cup gives rise to a definite outer, thin iris and an inner retina proper. The pigmentation of the eyes begins after this differentiation by the invasion of the chromatophores within the retinal wall. The eye lens is separated completely from the corresponding ectodermal wall and lies within the eye cup

cavity. The lens shows further differentiation, its outer margin transformed to the lens epithelium and the cells of the inner bulk are elongated and transformed to the lens fibers (Figure 4,B). The gut can be seen from the ventral view of the embryo as a simple tube extending to the posterior end of the trunk region. In the pharyngeal region and just behind the auditory vesicles, the ectoderm walls form a series of grooves, each one corresponding to one of the endodermal gill pouches of the pharynx. The pectoral fin buds are enlarged and were observed by the naked eye as small, lateral extensions immediately behind the pharyngeal region of the embryo (Figure 4,A). There are 64 pairs of mesodermal somites. The coelomic mesoderm shows more differentiation. The endocardium forms a single tube extending ventrally below the head region and its venosus portion extends forward. The pronephric ducts and tubules can be traced in the transverse sections as lateral tubes from the dorsal portion of the coelomic mesoderm (Figure 4,D).

(ii) Stages from 21 to 27 days

The development of the gill arches is the important feature in these stages; they are developed by the perforation of the ectoderm wall of the gill grooves, a process described in the preceding period. Later on the hyoid arch extends backward from its external surface in order to cover the gill buds behind. From the dorsal view, the mandibular arch is completely hidden, and the hyoid arch is attached to it, forming the

hyomandibular arch, while the other gill arches are exposed. The dorsal aorta is distinguishable and can be traced along the body to the caudal region, below which the caudal vein runs. The vitelline vein is evident and is directly connected to the heart. It is filled with oval blood cells containing an apparent nucleus. The cells located on the notochord rim become thicker and form the notochord sheath and their protoplasmic strands form a netlike structure, filling the notochord center. The notochord vacuolation is now complete in the entire notochord. The eyes are heavily pigmented but the retinal wall undergoes more differentiation with the appearance of the sclera. The head is darker in color, due to the small, scattered spots of melanophores on its dorsal region (Figure 4,E). The myotomes are enlarged, the myosepta are definite, and the myofibrils are observed in both the whole and the transverse specimens (Figure 4,F). The gut rests ventrally and extends along the midline of the body cavity, and in these stages is observed to be lifted off the yolk. Below the intestine the subintestinal vein is seen in the transverse sections. The tail through these stages has increased in both length and in width since it is completely flattened over the yolk and the dorsal and the ventral margins of the finfold lie on both sides of the body.

(iii) Stages from 28 to 34 days

The gradual increase in the embryo's size is accompanied by a slight decrease in the yolk sphere. The opercle, which started to grow posteriorly in the previous period, covers the

first gill arch by the 28th day and the second gill arch by the 34th day. The notochord increases in diameter and extends from its anterior end to the midbrain region and posteriorly to the tip of the tail where it and the neural cord curve up (Figure 4,D). The brain shows enlargement, especially in its middle vesicle, which starts to overlap all the other parts of the brain. The sense organs, the eyes, the auditory vesicles, and the nostrils have enlarged and show more differentiation. The cells of the optic cup have been arranged in several layers representing the choroid, and the retinal and ganglionic layers of the retina proper; the optic stalk is transformed into the optic nerve and enters the floor of the diencephalon. The lens fibers which occupy the inner portion of the eye lens sphere are more condensed. The olfactory pits move dorsally and their epithelium is surrounded by mesenchyme cells, which also surround the auditory vesicles, later giving rise to cartilage and producing the olfactory and auditory capsules, respectively. The transverse sections show that a sheet of cartilage beneath the floor of the brain has developed as a part of the chondrocranium. The eye cups undergo a similar change, forming the eye capsules. The liver makes its first appearance during this period as a ventral pouch coming from the gut in the body cavity and the intestine ends at approximately the posterior end of the trunk, marking the location of the anus. The ventricle of the heart is well-defined and the atrium is demarcated by the vitelline vein which enters the

Figure 4.-Stages of the late embryo. A, Whole mount of the twenty-day embryo, showing the pectoral fin buds and the beginning of pigmentation of the eyes, 20X; B, Transverse section of the eye region of the same embryo showing the olfactory pits and the eyes; C, Transverse section in the hind-brain region showing the hollow notochord and the heart; D, Transverse section of the anterior trunk region; E, 24-day embryo showing the position of the vitelline vein, 6.5X; F, Transverse section in the caudal region of the same embryo; G, The undifferentiated caudal finfold, 125X. d.f.f., dorsal finfold; e.c., eye cup; e.l., eye lens; f.b., forebrain; H., heart; m.b., midbrain; n.k., neural keel; n.r., nose rudiment; p.neph., pronephric duct; v.f.f., ventral finfold.


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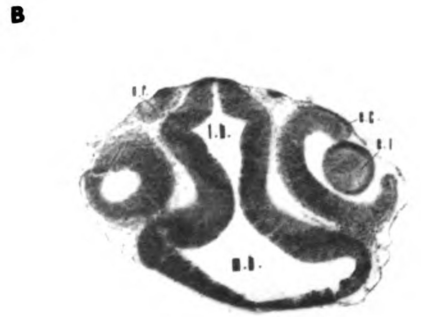
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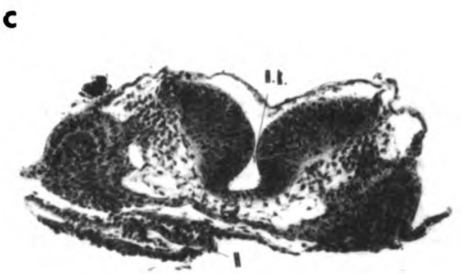
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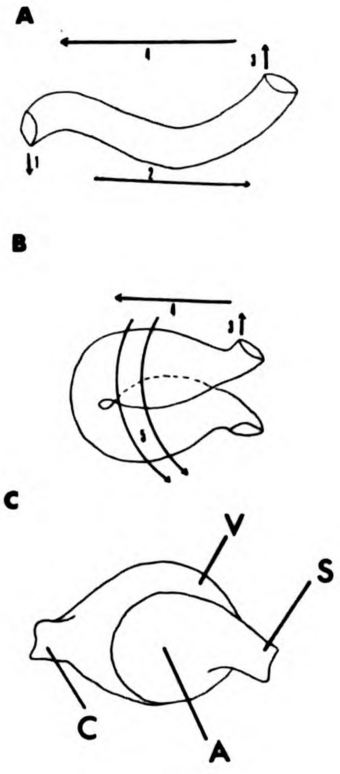
sinus venosus (Figure 5,E and F). The rudiment of the lower jaw is inferior to the forebrain and lies over the yolk. The pigmentation spots are increased and the dorsum of the head is mottled (Figure 5,D).

(iv) Stages from 35 days to hatching (40 days)

During this period the heart shows two important changes. First the twisting, which may have begun during the final days of the previous period, is now completed. The anterior or the venous portion of the endocardial tube has inflected and grown backwards. Its posterior or arterial end has grown upward and forward and in the meantime, the heart has coiled to the left as seen in diagrams A, B, and C of Figure 5. The heart appears as an oval structure with a distinct ventricle and atrium. Secondly, the heart moves back to lie just behind the gill arches. The heart and the blood vessels are filled with erythrocytes. Semicircular canals of inner ear are distinct and the ear capsules are formed. The eyes are prominent and heavily pigmented so that they hide the eye lens (Figure 6,B). The movement of the pupils of the eyes is observed, and the anterior part of the head shows a slight extension, forming the snout. The pharynx advances, pushing the mouth cavity forward and the four pairs of gill arches are completely covered with the opercle, forming the branchial chamber. By focusing on the gill buds one can see the development of the gill papillae on both surfaces of each gill bud, marking the rudimentary gill rakers and filaments. The lower jaw is well-

Figure 5.-The development of the heart. A,B,C, Diagrams showing the twisting of the heart, the arrows indicate the order of the looping; D, Thirty-day embryo showing the darkly pigmented body, 6.5X; E, Transverse section of the anterior trunk region of the same embryo; F, Dorsal view of the looping heart and the connection of the sinus venosus with the vitelline vein, 125X; G, Transverse section of the 35-day embryo showing the muscled ventricle of the heart. A., atrium; C., conus arteriosus; H., heart; S., sinus venosus; V., ventricle; v.v., vitelline vein.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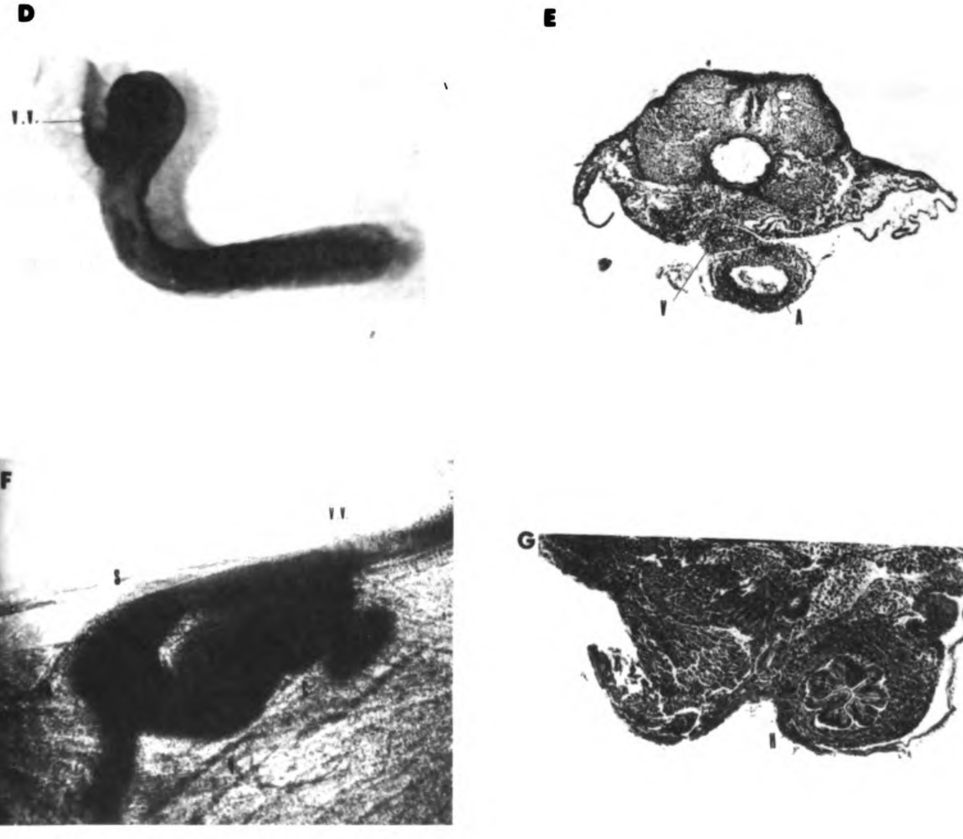

D
E
F
G

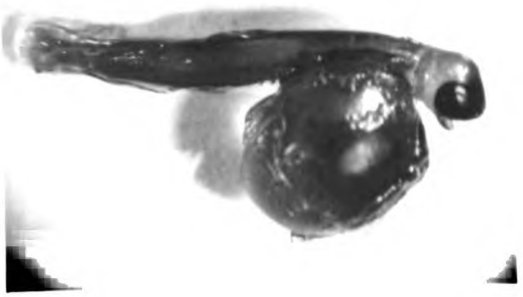
developed but it is immobile and is supported by cartilagenous elements (Figure 6,D). The somitic movements are distinguishable. The rudimentary dorsal, adipose, and anal fins can be seen as thickening parts of the disintegrating finfold. The pectorals are thin folds in which the supporting rays develop. The embryo just before hatching forms almost a complete circle over the yolk, with a loosely attached head and detached tail.

The first signs of hatching occurred after approximately 36 days of incubation, but all the hatched larvae died during or shortly after hatching. This may be due to some defect in the hatching enzymes or to an earlier faulty development which interrupted the normal processes of hatching (Battle, 1944). But Riddle (1917) stated that the mortality of the hatching chinook salmon, O. tshawytscha, may be due to certain physiological changes which did not occur in the chorion prior to hatching. Bishai (1962) said that the yolk-sac disease is one of the causes of the high mortality of the alevins. The yolk-sac disease is caused by the rough handling of the eggs during incubation or the malfunction of the thyroid gland. A period of intensive hatching occurred in the 39th and 40th days. Most of the fish emerged from the chorion by the tail while the remaining ones emerged headfirst. Then the alevin underwent an active lashing movement by which it freed itself from the chorion. Riddle (1917) mentioned that the pectoral fins move just before hatching, piercing the chorion. After breaking away the alevin lies on its side for a period of rest, and

does not move except when disturbed. The just-hatched alevin is sixteen millimeters in length, scaleless, and shows a coordinated movement of the opercle and lower jaw as a part of the respiratory process, which is mostly accomplished by the vascular system covering the yolk (Figure 6,A). Rays in the caudal fin are faintly seen with some pigment cells along them (Figure 6,E). The abdomen is attached to the yolk sac, the weight of which is responsible for the lashing movement of the body, although the muscular system of the body is well-developed. At the end of 28 days the alevin has almost absorbed the yolk and enters the larval stage, being ready to feed on external food.

Figure 6.-The hatched embryo. A, The newly-hatched embryo showing the lower jaw and the vascular system around the yolk sac, 6.5X; B, Transverse section of the eye region showing the differentiated retinal wall and the eye lens; C, Transverse section of the trunk region just behind the anus; D, Transverse section of the oral region, showing the lower and upper jaws; E, The faint caudal fin rays and the upcurved notochord, 125X; F, Transverse section of the trunk region just in front of the anus. d.a., dorsal aorta; e.l., eye lens; e.r., eye retinal wall; int., intestine; l.j., lower jaw; my., myotome.

A



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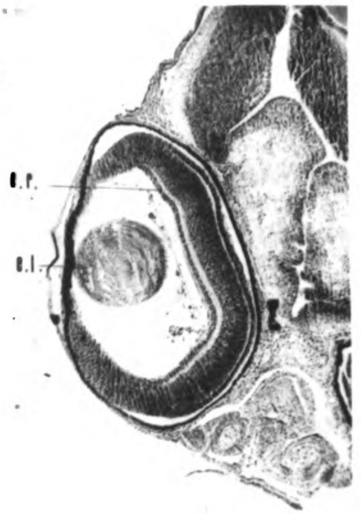

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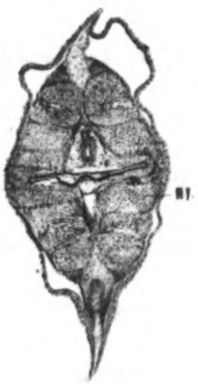

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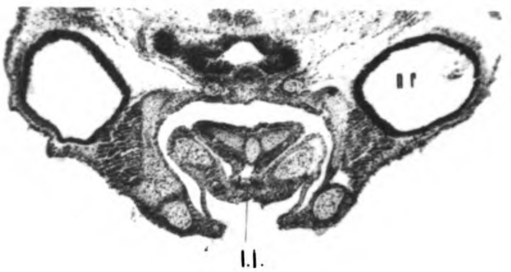

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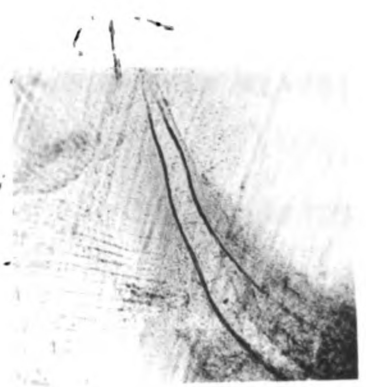

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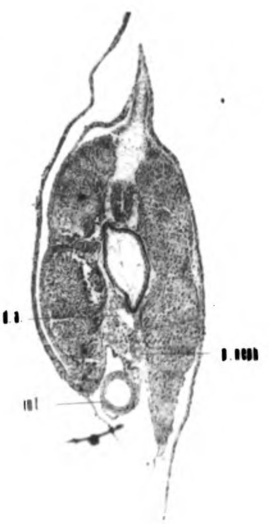

DISCUSSION

It is obvious that the early stages of development of the coho salmon, Oncorhynchus kisutch, are typically teleostean. Hatching occurred after forty days of incubation at a temperature of 10 ± 1 C, or after the embryo was subjected to 720 thermal units. The thermal units required for the whitefish is 325 (Price, 1935), the Atlantic salmon requires 470 T.U. (Battle, 1944), and the steelhead trout needs 573.9 T.U. (Wales, 1941). This indicates that the coho salmon embryo has a relatively long period of incubation. According to Embody (1934), the incubation period varies with the temperature. The similarity of the coho's cleavage with that of other teleosts deviates with the slight lengthening of the blastodisc, which was observed in the first cleavage stage of the coho eggs. Also the swimming bladder was not seen during the embryological stages of the coho. Both results are unlike those observed in the development of other teleosts. This reveals that the development of the swimming bladder may take place after hatching.

The concentration of oil globules at the animal pole and around the blastodisc prior to the initial segmentation is of physiological significance since the globules are composed of phospholipids and lipoproteins and are absorbed by the embryo

from the beginning of development until the gastrular overgrowth (Smith, 1957). The periblast, which was seen beneath the blastoderm and in contact with the yolk does not participate in the formation of the embryo but it may serve in breaking down the yolk for the growing embryo (Balinsky, 1966).

It was found that at the closure of the blastoporal lips most of the primordia of the organ systems had been established and could be traced easily, such as the development of the brain and the establishment of its three main vesicles, the forebrain, the midbrain, and the hindbrain. The establishment of the sensory organs was accompanied by the differentiation of the retinal wall of the eye cups, the appearance of the nostrils, and the development of the internal ears. The gut as an endodermal organ, and its well-developed pharynx, was also found.

Following the disappearance of the yolk plug the embryological features peculiar to teleosts were apparent. The subdivisions of the brain and their nerve connections with the sensory organs were easily observed. The differentiation of both the cells of the retinal wall forming the four retinal and ganglionic layers, and the eye lens forming the lens epithelium and lens fibers were clearly seen. Another of these features was the development of the infundibulum. The gill arches, which are partly endodermal and partly ectodermal, were distinguishable, as was the development of the operculum as an external extension from the hyomandibular arch. Pectoral fins appeared as lateral, fleshy extensions. The fish attained

the complete hatching number of somites which is 64 pairs at the twenty-day stage and in the following stages the somites underwent enlargement and the myofibrils and myosepta were well-defined.

The establishment of the heart from the ventral portion of the coelomic mesoderm, the pronephric ducts developing from its dorsal portion, and the twisting of the heart and its migration to the pharyngeal region are all characteristic of primitive vertebrates. The discoidal cleavage of the blastodisc, the finfold as a primitive ectodermal outgrowth giving rise to the unpaired fins of the alevin, and the pharyngeal extension pushing the ventral mouth forward, prove the higher phylogenetic position of this species among fishes, since these structures can be found in the primitive teleosts.

SUMMARY

Approximately 3,000 coho salmon, O. kisutch, eggs were obtained from the Platte River Station of the Michigan Department of Natural Resources. The eggs were placed in a Heath Fish Incubation Cabinet supplied with continuous flowing water with a constant temperature of 10 ± 1 C. The eggs were fixed in Bouin's and Smith's solutions; the latter was replaced by Kahle's solution after the sensitive period of the embryo. Staining was done with alum cochineal for the whole mounts and with Delafield's hematoxylin and eosin for the transverse sections, which have been cut at 5 μ and 8 μ. Photographs were selected from both series from the initial cleavage through hatching. The coho salmon, O. kisutch, embryology is typically teleostean. The blastula stage was completely formed by the fourth day of incubation and in the sixth day the gastrulation began by the involution, epiboly, and convergence of the blastoderm cells. At the end of the gastrulation, after eleven days, the yolk sac was formed and most of the rudimentary organs were distinguishable. Prior to hatching the organ systems showed a high differentiation and more development. Hatching began on the 36th day and intensive hatching occurred on the 39th and 40th days of incubation. The yolk sac was completely

absorbed 28 days after hatching and the alevin was then ready to feed on external food.

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