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Title	The Embryological Study of Fishes. Ⅰ. General observation on the early development of the dog-salmon, Oncorhynchus keta WALBAUM
Author(s)	SAITO, Saburo
Citation	Journal of the Faculty of Agriculture, Hokkaido Imperial University, 48(3), 267-289
Issue Date	1950-09-30
Doc URL	https://hdl.handle.net/2115/12747
Type	departmental bulletin paper
File Information	48(3)_p267-289.pdf

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The Embryological Study of Fishes. I. General Observation on the Early Development of the Dog-Salmon, *Oncorhynchus keta* WALBAUM.

By

SABURO SAITO

(With 8 Plates and 2 Text-figures)

Introduction

Hitherto many experiments on the vitality of eggs of fishes have been carried out physiologically by many writers and it has been confirmed that the resistance of the egg to the mechanical treatment and various chemicals varies with the developmental stages. In general, the "eyeing period" is recognized to be the strongest in vitality. There should be an intimate connection between the developmental degree of organs and the vitality of the egg. However nothing has been done toward this problem. The writer has intended to explain the problem from the embryological point of view. Before going further, it is a pleasure to record here my gratitude to Prof. Dr. T. INUKAI for his suggestion of the problem. My sincere thanks are also due to the members of the Hokkaido Salmon-Hatchery, Mrrs. K. SHINAGAWA, S. SANO and others who have supported the materials to me in this work.

I. Material and method

The material employed in this study is the eggs of Chum (Dog-Salmon), *Oncorhynchus keta* WALBAUM, which occurs frequently in northern waters including Hokkaido, north eastern districts of Main-Land of Japan, Saghalien, Kuriles, Primoskaya and Kamtchatika.

In Hokkaido the salmon appears as an anadromous fish during the period from October to around the middle of February for spawning. About two

[Jour. Facul. Agr., Hokkaido Univ., Sapporo, Vol. XLVIII, Pt. 3, April, 1950]

thousand eggs were obtained, which were artificially inseminated in the Chitose Salmon-Hatchery.

They were incubated in the tank of the laboratory with the temperature of 11—11.5°C. On the 32 day the egg developed to the "eyeing period" and on the 62nd—63rd day hatched out. The BOUIN's fixing reagent (Picric-acid, Formalin and Glacial acetic acid) was employed. About 40 eggs were fixed at once on each day. After fixing, the eggs were transfered into 80% alcohol and then the blastoderm including the embryo was removed carefully from the yolk using the needle. The embryo was sectioned in 10 μ in thickness by means of paraffin method and stained with Acid Haematoxylin after EHRLICH. The total embryo at each stage stained with EHRLICH's Acid Haematoxylin was also prepared.

II. Observation

The developmental stages were divided so far observed into two; namely a period from the insemination to the development of three principal layers, the ecto-, meso- and endo-derms and a later period until the so-called "eyeing period" characterized with the differentiation of the retinal and tapetal layers of eye.

In the first period the egg is very week against the environmental stimuli while at the end of the second it is resistant to the treatment in the hatchery.

A. The first period

The eggs taken from the fish which comes up the stream for spawning are measured from 4.5 mm. to 6 mm. in diameter without regard to the degree of ripening. The outermost vitelline membrane of the egg is consisted of two layers. The outer layer is thin, 6 μ in thickness, very transparent, and laminated and perforated by many very fine canals which are revealed in optic section under the microscope as numerous fine radiating striae. This is of a typical character of a zona radiata proposed by W. WALDEYER. The inner layer is homogeneous in structure and is so-called "Swelling layer" because of the swelling character in water. The boundary between two layers is indistinct. There is a single minute funnel shaped opening in the membrane at the animal pole, the micropyle, which is much larger in size than the radiating striae of zona radiata.

In the mature egg, the yolk granules are involved in the mesh-work of protoplasm which is continued with that of the cortical layer from which the germ disk is derieved. The immature egg is distinguished from the

mature one by the character of yolk granules which are transparent and dark yellowish tint.

The protoplasm which surrounds the germ disk is uniformly granulated, and of a yellowish or pale amber tint. The disk is darker in colour and rather opaque on account of its granular walls. The germ disk is always found on the upper pole in the mature egg.

The segmentation of salmon egg shows a partial type of the meroblastic ova. The germ disk just after the fertilization has a discoidal form, with the blunted or rounded off periphery. At about 20 hours after the insemination most of the eggs show 2-cell stage, while some attain 4-cell stage. The blastoderm at about 70 hours (Pl. I, Figs. la, b) upheaves on the egg surface. being constituted with the irregular cell mass. The blastoderm spreads gradually, while the outer layer of the cell mass takes a regular arrangement (Fig. 3 ; *ec*). The segmentation cavity appears distinctly in the mass (Fig. 3 ; *se*). Then, the blastoderm is thickened at one side by the accumlation of cell mass (Fig. 2). At this time the blastoderm assumes a scalene-triangular form in its section across the thickened part. The axis of triangle corresponds to the longitudinal axis of the future embryo.

There are seen several free nuclei in the yolk adjoining the blastoderm (Pl. I, Fig. lb ; *yc*). They are modified blastomeres lacking the cytoplasm, which are proliferated at the late cleavage stage into the yolk abjoining the blastoderm and undergo imperfect cell division. As the development proceeds, a considerable number of these nuclei become visible. The yolk spheres surrounding them take liquified appearance, and at length a layer continuous with the blastoderm assume a rigorous distinctness of syncytium (*ys*), so-called "yolk hypoblast" or "periblast".

So far as the salmon egg is concerned C. K. HOFFMANN's opinion (1880) is wrong as he affirmed that at the moment of the fertilization the female pronucleus presents a caryokinetic phenomenon and is divided into two one of which blends with the male pronucleus to form the first segmentation nucleus, while the other one becomes the origin of all periblastic nuclei in *Scorpaena, Juris, Crenilabrus, Heliasis, Fierasfar, Syngnathus, Hyppocampus* and *Gobius*.

The segmentation cavity so far formed is not distinct. In this regard the writer is not wholly in accord with the opinion of KLEIN (1876) and RYDER (1882). KLEIN insists the segmentation cavity is formed by the elevation of the blastoderm at one side, so it has no contact with the periblast layer lying just below it.

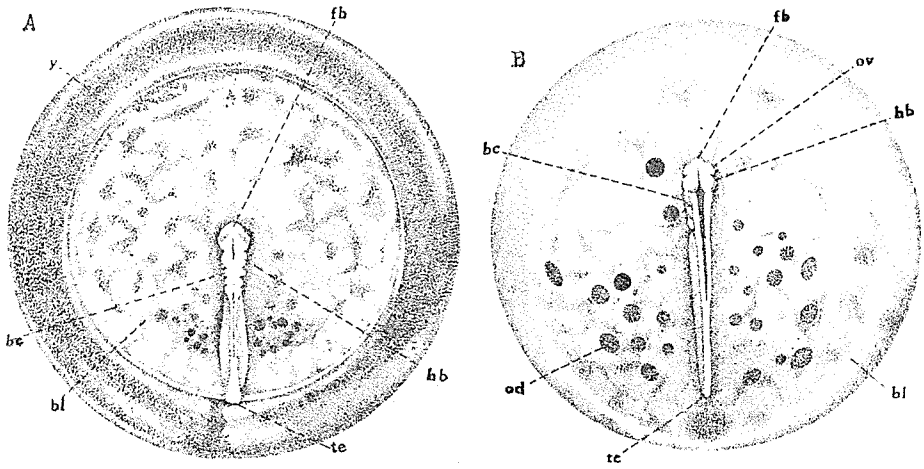
The blastoderm extends over the yolk surface by slow degrees, its elevation lowering gradually (Fig. 3). The blastomeres of the blastoderm are

divided into two groups. The cells of the upper layer become columnar and the remaining blastomeres form a irregular mass in the deep. The former is the ectoderm and the latter is called as the lower layer. The segmentation cavity exists between the yolk and the lower cell layer.

At about a week of incubation an important change takes place at the thickest part of the blastoderm, the gastr lation occuring (Figs. 4. 5). At first, the ectoderm of the thickest end of the blastoderm is inflected as a small arc, and connected with the lower layer cells which assume a columnar form becoming the endoderm (Fig. 5 ; *ed*). The cavity between the endoderm and the yolk mass is the anlage of the archenteron, inflected part consequently holds a blastopore under it. The dorsal lip of the blastopore corresponds to the embryonic rim (*er*).

The above process is the true gastrulation as HAECKEL reported (1857). The delamination as RYDER (1882) showed in the cod development is not seen. RYDER described that the ectoderm or his "sensory layer" differentiates from the underlying cells at the head end of the embryonic shield and advances towards the tail end of the embryo. In his observation the stage of the gastrulation is likely to be missed.

At the time in which the gastrulation occurs there is no external sign of the embryo in the blastoderm. After a while, the caudal swelling is formed



Text-fig. 1. Surface view of blastoderm ; A is an egg on the about 12th day, showing the embryo which lies on one of the radii of the blastoderm, and the length of the brain as compaired to the spinal cord is very great. B is a blstoderm with an embryo on the 18th day, showing the growth of blastoderm over the tail end of embryo and the appearance of rudiments of gills. The black circular dots are oil drops.

bc, gill ; *bl*, blastoderm ; *fb*, fore-brain ; *hb*, hind-brain ; *mb*, mid-brain ; *od*, oil drop ; *te*, tail end.

to become the embryo which grows from the rim towards the center of the blastodermic area. The other part of the area spreads widely over the yolk (Text-fig. 1). Until about the 12th day, the posterior end of the embryo lies on the edge of the spreading blastoderm and the head in the center, the longitudinal body axis of embryo corresponding to the radius of the blastoderm circle. The margin of the blastoderm spreads very fast while the growth of the embryo does not match with it and so the head is no longer found in the center of the blastoderm.

As seen from the surface the axis striation of the embryo disappears. Then the anterior part is enlarged as the cephalic region, which is divided later into three distinctly separate lobes, nemely the fore-, mid- and hind-brains.

With the external differentiation of the embryo the differentiation of lower cells into the endoderm proceeds along the axis of the embryo. On the 9th day when the embryo is clearly distinguished from the extra-embryonal part in the surface view, the cell mass is composed of two thick layers of cells in section (Pl. I, Fig. 5). As the differentiation of the endoderm advances, loose cell mass, the mesodermal cells, appears between the endoderm and ectoderm at either side of the embryo. From this stage the difference between the embryonal part and the extra-embryonal part becomes distinct. The former is composed of three strata of cells, ecto-, meso- and endo-derms while the latter is represented by almost a single layer of cells. One more cell component exists in this stage, imbedding in the yolk layer under the blastoderm. It has, no doubt, the same origin with the blastomeres, but takes no part in constituting the embryo. This is the periderm or yolk cells which serves the nutrition of the embryo.

B. The second period

During this period, the three principal layers, ecto-, meso- and endo-derms, differentiates respectively into various organs. In order to avoid the intricacy, the fate of each three layers may be dealt with separately.

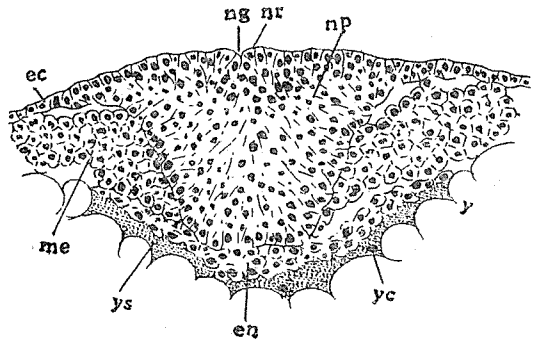
1. Development of ectodermal organs

The development of the brain and spinal cord presents some peculiar feature as seen in general Teleostean development (Pl. I, Figs. 6a-c) in which the medullary canal develops at a relatively late stage, or after the neural cord has been split off distinctly from the ectoderm layer (Pl. IV, Fig. 9). At the late stage of gastrula, the ectoderm thickens perceptibly along the median longitudinal axis of the embryo by the rapid partial cell division of

the ectoderm, and then the medullary plate is differentiated. The medullary plate continues to become thicker as the development proceeds, and then begins to make a ridge on the under side of the blastoderm, pushing aside the mesoderm cells.

The next event of the development of the medullary plate is the separation of it from the ectoderm forming a neural cord (Pl. III, Figs. 8a-d). On the 12th day, the anterior part of the plate assumes a form of a solid cord of cells by the longitudinal vertical thickening of the ectoderm (Pl. I, Fig. 6). The separation of the nerve cord from the ectoderm generally proceeds from anterior to posterior. On about the 14th day, the medullary canal does not yet develop (Pl. II, Figs, 7a-f). The posterior part remains for a while a little flat, assuming "neural plate" (Pl. II, Fig. 7f).

As above stated, the neural cord at first is absolutely solid or strand of cells which takes later a compressed form. The existence of a medullary groove or a furrow in the sense in which we know it in the embryo of Amphibians is found with difficulty. However, as shown in the Text-figure 2 sometimes a depression in the median dorsal line is seen and this is to be regarded as the medullary groove. The writer hesitates to decide the problem through the present observation only.



Text-fig. 2. Cross section through the anterior part of an embryo 11 days old, showing a depression which may be regarded as the neural groove. X 150
ec, ectoderm ; *en*, endoderm ; *me*, mesoderm ; *ng*, neural groove ; *np*, neural cord ; *nr*, neural ridge ; *y*, yolk ; *yc*, yolk cell ; *ys*, syncytium.

The blastoderm continues to spread over the surface of the yolk by so-called "epiboly". The rim of the blastoderm shown in Text-figure 1A is moved progressively toward the naked pole of the yolk in order to entirely. Before the closure of the rim at the opposite pole of the embryo the "yolk blastopore" of J. A. RYDER is remained for a short time.

The upheaval of the fore or cephalic end of the embryo results at the same time the protuberance of the anterior part from the blastoderm. At about 2 weeks of incubation, paired optic vesicles or rudiments of the eye appear (Pl. III, Fig. 8a and Text-fig. 1B). They develop at first as the thickened lateral lobe of the fore-brain (Fig. 8a), but the attaching stalk is gradually pushed down to the bottom of the brain (Compare Fig. 9a with Fig. 8a). The new position in which the stalk arises from the fore-brain is

the future position whence the optic nerve starts.

During the early stage, the optic vesicle is a solid mass of cells. However, as the development proceeds, at about two weeks, a slit-like lumen appears within the mass somewhat oblique to the plane of the blastoderm in section (Fig. 8a). Now a depressed double-walled optic vesicle is placed forwardly oblique to the axis of the embryo. The bottom of the lumen is connected with the process from the cavity of the fore-brain forming the eye-stalk. At this time the oblique position of the vesicle is changed to become vertical to the plane of blastoderm and parallel with that of the medullary cavity. Afterwards the outer layer (*pl*) is transformed into the retina, while the inner layer (*tl*) which becomes thin the tapetal covered by a pigmented layer. The cetoderm close to the optic vesicle thickens (Pl. IV, Fig. 9b ; *el*), and invaginates inwards converting into the eye-lens (Pl. V, Fig. 10a and Pl. VI, Fig. 12b and Pl. VII, Fig. 13a). The distal part of the optic vesicle is depressed corresponding to the invagination of the lens and assumes a cup-form, "optic cup". At first, the opening of the optic cup directs obliquely downwards (on the 24th day) (Pl. VII, Fig. 13b). The closing of the opening is effected chiefly by the growth of the above rim, and gradually an eye ball is formed. With the further development the lens is enclosed in the ball, the wall of which becomes thinner (Pl. VIII, Fig. 14c).

At the early stage of development of the eye the anlage of the auditory organ is differentiated from the ectoderm at the side of the hind-brain as a pair of miuute involutions. The rudiments begin to develop at about the 15th day. At first they are apparently solid (Pl. III, Figs. 8b, c). On the 18th day they become thick-walled ovoidal vesicles with a very small cavity (Pl. IV, Figs. 9f, g). This internal cavity gradually increases in size, while the outer wall becomes gradually thinner (Compare Pl. V, Fig. 10c with Pl. VIII, Figs. 14e, f). The vesicle is separated gradually from the surface ectoderm. It has an elongated blind ending beneath the ectoderm (Pl. VIII, Figs. 14e, f). On the base of the vesicle ganglion cells aggregate (Pl. VI, Fig. 12h and Pl. VII, Fig. 13f). The vesicle elongates somewhat antero-posteriorly, and produces a long "aqueductus vestibuli" at the dorsal end (Pl. VIII, Fig. 14f). The auditory organ remains for a considerable period in such a simple state.

According to J. A. RYDER (1882), the auditory vesicles of the cod contain lymph fluid at the early stage and on the 15th day two otoliths, asterisk and sagitta, appear in the vesicle. As regards the development of otoliths, no observation is available in the present stage of development.

The origin of the olfactory or nasal organs appears as the involution of the ectoderm like the auditory, developing, however, in a later period. The

rudiment is seen as a pair of small thickened patches of the ectoderm under the fore-brain, just anterior to the optic vesicle. This is on about the 24th day (Pl. VII, Fig. 13a ; *olf*). In consequence of the growth and the change of the place of various organs as above cited, the nasal involutions are displaced downwards, so as to locate above the point where the mouth will open. They are at first simple thickenings of the ectoderm (Pl. VII, Fig. 13a). By the 31st day each thickened patch becomes involuted as a thick saccular depression continuous with the ectoderm (Pl. VIII, Fig. 14c). As the development proceeds, the olfactory sacs are covered with the epithelium which is called "Schneiderian membrane". During the post-larval stage the epithelium of the olfactory vesicle many folds on the floor which has a radial arrangement connected with the nerve ending. The change of the position and the length of the olfactory nerve occurs. The olfactory nerve is at first extremely short, and it is believed to be originated primarily from the upper hinder portion of the neural cord destined to form the cerebrum as called by A. M. MARSHALL (1879) "neural crest". In relatively late stage transverse sections show the roots of the olfactory nerve arising from the sides of the cerebral lobes and passing to the nasal pits.

We return again to the change of the central nervous system, especially the development of the brain from the medullary plate. The differentiation of the cerebro-spinal axis into two parts, the brain and the spinal cord, occurs at the early stage of the neural cord without the medullary canal (Pl. I, Figs. 6a-c). The change of the spinal cord in the further development is simple ; the differentiation of the wall of the central canal to the grey matter takes place. On the contrary, the differentiation of the various parts of the brain is more complex than that of the spinal cord.

The first appearance of the brain region is marked by the widening of the medullary plate. On the 14th day, the brain has the form of a pentagonal mass in section (Pl. II, Figs. 7a, b). and its anterior bluntly pointed extremity is the rudiment of the cerebrum. On the 15th day it becomes a laterally compressed rectangular mass and the embryo upheaves over the egg surface conspicuously at the part of the brain (Pl. III, Figs. 8a-c).

By this time a distinct constriction at the anterior part of the cord just behind the eye (Text-fig. 1A). On about the 18th day the medullary canal appears for the first time (Pl. IV, Figs. 9a-h). In a later period the second constriction appears a little behind the former and marks off the cerebellum and myelencephalon which transforms into the medulla oblongata (on about the 22nd day) (Pl. VI, Fig. 12d). In the anterior part of the brain the third constriction separates the fore-brain or cerebrum from the mid-brain. By the 24th day the cerebral regions develop and the first, second, third and fourth

vesicles or cerebral cavities are formed (Pl. VII, Figs. 13a-g). The brain is compressed laterally characteristic to the early stage of Teleostean development. As described by F. M. BALFOUR (1885), at first the length of the brain as compared to the spinal cord is very great.

The cranial flexure or the curvature of the brain which is very striking in the embryos of other types such as Elasmobranchs, Amphibia and birds is not remarkable in this case. Some authors like J. A. RYDER say the flexure of the encephalon in the Teleostean embryo is almost inconsiderable. However, in the present material there is a certain bend, the forebrain overlapping the mid-brain in spite of the great thickness of the wall.

The origin of the mouth in the Teleostean embryo has been worked out with difficulty. According to DOHRN the mouth of the young fish of *Alosa* develops from the anterior part of the mesenteron growing out at first as two narrow, pointed, horizontal clefts which break through the ectoderm at two points before the head grows out. The writer could not assure the development of the stomodaeum in the embryo in which the head projected prominently over the egg surface (Pl. VIII). On the development of the stomodaeum, the writer agrees with RYDER who insisted the stomodaeum is observed with difficulty. So far as the present observation is concerned the development of the oral tract of endoderm near the future mouth is a little pronounced as early as the 20th day of incubation (Pl. V, Fig. 10d). On the other hand, the proctodaeum develops clearly in an early period. In the embryo on the 20th day the proctodaeal depression of the ectoderm appears, with its wall continues with the neural cord (Pl. V, Figs. 10g-i and Fig. 11).

2. Development of mesodermal organs

At first the mesoderm is a simple loose cell mass situating between the ecto- and endo-derms. As the development proceeds, it becomes gradually denser and by the great development of the ventral keel of the neural cord, it comes to lie on the either side of it. The first evidence of the mesodermic mass appears on about the 11th day (Pl. I, Fig. 6 and Text-fig. 2), and the somites develop by the transverse segmentation of the lateral plate (on about the 15th day) (Pl. II, Figs. 7b, c). As a rule, they are segmented off in succession from anterior towards the body end, but the most anterior somite which develops just behind the auditory vesicle, is differentiated later than those on the thoracic portion (See Figs. 7b, c in Pl. II and Figs. 8b, c in Pl. III).

A little after the differentiation of the brain and the spinal cord, two

pairs of somites are distinguishable on the body of the embryo. They are at first triangular in shape in the cross section and one of side of it closely attachs to the wall of the neural cord. In the posterior portion of the embryo the mesodermic cells remain undeveloped (Pl. II, Figs. 7d-f). The somites in the caudal region, which are formed after the caudal projection is marked, have at first a different form from those on the trunk, showing the crescent shape in transverse section. Though at the anterior part of the body the neural cord, notochord and the mesodermic elements are identified clearly (Pl. VI, Fig. 12s), at the tip of the tail, however, in the early stage, the whole of these structures are absolutely blended and lost in the apical cell mass.

Prior to the outgrowth of the tail, the body increases very notably in thickness and the somites begin to assume the crescent form (Pl. IV, Figs. 9f-h). With the growth of the embryo, the somites increases in volume, increases both in length and width being taken account. In the later stage, the form of somites changes, the anterior somites assuming >-shaped form as viewed from the side. This change of the form proceeds backwards, being more marked at the anterior end than at the posterior in the early stage. Finally they develop each other ; that is, the hinder beveled margin of one somite, now called as "muscle segment", covers the anterior margin of the succeeding one. At this stage, the lower lateral plate, or the somatopleure extends for a considerable distance over the yolk sac and the lower splanchnopleural layer is inserted between the endoderm and somatopleural layer along with the growth of the somatopleure.

At about the time in which the tail begins to bud out and the muscular somites of the anterior body are formed, the formation of a longitudinal fold is observed in the somatic layer of the peritoneum, being splitted off gradually as a pair of longitudinal canals, on either side of the body (Pl. IV, Fig. 9k). This is the segmental duct. The canal does not close anteriorly (Pl. V, Fig. 10e), but remains open to the body cavity, thus giving rise to a funnel equivalent to the pronephric funnels of Petromyzon and Myxine.

In the embryo on the 18th day, the anterior end of the segmental duct is found extending forward close to the auditory vesicles (Pl. V, Figs. 10i, j). Traced backwards, the segmental duct of an embryo at about the 28th day opens into the extreme hinder part of the intestine (Pl. VII, Fig. 13s).

The segmental duct is a simple. straight cylindrical canal throughout, except the anterior extremity. The wall of it is composed of a single layer of cells. The anterior extremity convolves more or less to form the pronephros. The mesonephric glomeruli develop along the segmental canal a little behind the region of pectoral fins extending to the allantoic vesicles or urinary

bladder.

The observation on the development of the vascular system of fishes are very scant. The earliest anlage of the heart is shown as two semicircular solid masses of large mesodermal cells at either side of the neural cord in a section through the auditory vesicles (on the 15th day) (Pl. III, Fig. 8c). As the development advances they become two spherical masses, and move gradually posteriorly (Pl. IV, Fig. 9i). With the further development, two rudiments approach gradually to each other and soon coalesce into a solid cell mass at the median line of the body. Next, a cavity appears in the cell-mass. According to F. M. BALFOUR (1885) in Teleostei the heart is formed as in birds and mammals by the coalescence of two anlages, each of which makes a tube before the formation of the heart. As stated above, in the present material, the heart cavity arises after the coalescence of two rudiments (Pl. IV, Fig. 9i ; Pl. V, Fig. 10h). The wall of the heart is formed primally with two layers. The outer layer is thick but at first is incomplete in form lacking the dorsal side (Pl. VI, Fig. 12g ; *oht*), and the inner lamina, the epithelioid lining of the heart is composed of delicate flattened cells (*iht*).

By about one month, the differentiation of the venosus sinus is marked off as a dilated anterior end, and the development of ventricles and bulbous aortae occurs at a considerable later period. The blood cells are observed already on the 24th day of development (Pl. VII, Figs. 13p-s).

3. Development of endodermal organs

The notochord and the mid-gut or intestine have an intimate connection with each other in their development. The notochord begins to differentiate at the end of the gastrulation, on about the 10th day. At the stage of the medullary plate, a cord of the columnar cells which are the same morphologically with endoderm-cells, is seen in contact with the median axis of the medullary plate. This special cell-cord gives rise to the notochord. The separation is already effected through the greater part of the embryo on about the 11th day (Pl. I, Figs. 6b, c), and extends from behind the mid-brain to the posterior extremity, in which it mixed completely with the components of the mesoderm and endoderm (Pl. II, Figs. 7e, f ; Pl. III, Figs. 8f, g).

In the next stage, the notochord presents the characteristic feature of the rigid tissue. In the notochord so differentiated the constituting cells are not distinguishable from the other cells of endoderm. Before the medullary canal formation they are elongated in shape, arranging irregularly but they are soon arranged parallel with each other (Pl. I, Figs. 6b, c ; Pl. IV, Fig. 9k).

By the time of the appearance of the medullary canal in the neural cord, the component cells of the notochord become again round in form (Pl. II, Figs. 7c, d ; Pl. III, Figs. 8c-e ; Pl. IV, Figs. 9g-j). As the development advances, a large vacuole appears in the cytoplasm, and later one or more others are added to the first. These vecuoles gradually increase in size and push the nucleus to one side. The boundary of each cell becomes obscurely, while the outline of the notochord is clearly marked (Pl. V, Figs. 10e, f). Meanwhile some vacuoles in each cell are broken and combined to form one large vacuole which increases in size so as to occupy the greater part in the cell pushing aside the protoplasm. The scanty protoplasm in the cell is now made of continuous strands and bridges.

By the time of the formation of the tail, the posterior extremity of the notochord appears on the dorsal surface of the tail end, to which the neural cord does not extend (Pl. VI, Fig. 12t).

The development of the intestine is peculiar in many respects ; first, its solid and depressed form in the early stage as mentioned above, and second, the mode of formation of the oral part which develops from behind forwards as there appear no marked oral invagination of the ectoderm or a stomodaeum. Thirdly the intestinal canal comes out as a lumen in the endoderm mass instead of the invagination process.

As in the other vertebrates the intestine is developed from the endoderm. In the embryo of about the 15th day it is still a solid band of cells underlying the notochord (Pl. II, Figs. 7d-f). At first, its anterior extremity does not reach to the anterior end of the head (Pl. II, Fig. 7a) and the posterior extremity is lost in the invaginated blastoderm cells (Pl. II, Figs. 7e, f). The relation of the hinder end of the intestine to the neural cord exhibits a feature homologous with that observed in *Amphioxus* in which the neural canal continues at its posterior extremity directly with the intestine making the neurentric canal perhaps because of a very short post-anal length.

In the embryo on the 18th day of development, the intestine makes a very notable differentiation. In the anterior portion it has a slight lumen (Pl. IV, Ffg. 9h). On the 22nd day, a canal is formed almost throughout the entire length (Pl. VI, Figs. 12j-q), except the region in front of the pectoral fin-fold (Fig. 12i), in which a depressed solid mass still exists. The intestine is clearly separated from the notochord at the anterior part by the insertion of the mesodermic elements. The feature of the separation is seen obviously in the hinder part of the embryo. On either side of the anterior depressed region of the intestine the hyomandibular cleft appears, behind which six other gill-clefts develop one after another closing with each other. They show the nature of the narrow lateral outgrowth from the side of the

solid intestine, not breaking out through the skin (Pl. V, Fig. 10c ; Pl. VI. Fig. 12i ; Pl. VII, Figs. 13f-i).

The differentiation of the liver occurs also as a solid outgrowth from the ventral wall of the intestine in the region of the pectoral fin shortly after the developemnt of the gill-cleft.

4. Development of fins

Of the paired of fins, the pectoral or anterior pair is the first develop and the ventral or pelvic pair does not appear until the time of hatching. The first sign of the pectoral fin appears as a slight longitudinal elevation of the skin on either side of the body a little behind the auditory vesicle (Pl. VI, Fig. 12j). It starts as a very low hardly noticeable ectodermal fold and elongates without expanding its base. The margin of the fold becomes thinner at its distal end, while the basal part thickens by the migration of mesodermal cells.

Of the unpaired fins, the dorsal and caudal fins are developed as a single median dorsal fold of the epithelium, and later the both are separated from each other by means of a partial atrophy in the fold (Pl. VI, Fig. 12q ; Pl. VII, Figs. 13m, t). With regard to the development of the caudal fin AL. AGASSIZ (1877) observed in the flounder embryo that the anlage of the fin appears at first symmetrically or nearly so on the ventral plane around the end of the body, but soon the development of the ventral part with its rays exceeds the dorsal. At the same time the posterior part of the notochord bends upwards in the tail. In Cyprinid fish, *Gnathopogon elongatus caerulescens* (SAUVAGE) as studied by M. NAKAMURA (1949), both the dorsal and caudal fins differentiate from the natatory fold of the skin after hatching. In the present material, however, on the 22nd day of development, the posterior termination of the notochord bends upwards in the tail. There is found no trace of the natatory fold (Pl. VI, Fig. 12t). Consequently, the development of the epithelial natatory fold of the body end attains asymmetry from the outset, differing from the case of AGASSIZ. It appears at first on the dorsal side alone and the ventral side is formed a little later (on about the 24th day) (Pl. VII, Fig. 13t).

III. Discussion

The constitution of the egg membrane of other kinds of fish species, for the instance, the cod, shad, white fish, sculpin etc. is quite different from the salmon egg having no zona radiata. The difference of colour with

the degree of the ripening has been also reported in other species. This is effected by the change of the character of the plasm enveloping the germinal vesicle.

The origin of the yolk cell or the periblast of osseous fishes has been studied by many workers such as E. KLEIN (1876), C. K. HOFFMANN (1883), J. A. RYDER (1885), H. F. ZIEGLER (1896) on *Scorpaena, Salmo salar, Julis, Gadus, Coregonus, Crenilabrus* and *Fieraster*. According to HOFFMANN who studied on *Scorpaena* and *Julis*, one of the nuclei resulted from the first cleavage becomes the archiblast and the other becomes the periblast. The former makes blastomeres and the latter develops into the yolk cells or periblasts. So far as the present material is concerned, the differentiation occurs at the later stage of segmentation.

On the fate of the yolk cell, there are two different points of view. Some ones insist that they are only a transient existence, while the others are of the opinion that they become ordinary cells at a later period with the protoplasm surrounding them. It is affirmed that they functionate as the provisional blood during the development. However, the opinion that they may become blood corpuscles is improbable. RYDER (1882) is wrong as he states that the yolk cell would be formed the blood corpuscle at the moment when the archiblast and periblast differentiate. It is quite clear that the blood cells are differentiated at the later period from the mesoblast without any connection with the yolk cells.

Special attention has been paid with regard to the origin of the segmentation cavity. Some writers such as HAECKEL reported that it is formed as the result of elevation of the blastoderm losing the contact with the yolk cell layer below it. In the present observation the segmentation cavity is unquestionably originated directly from the cleavage cavity.

In general, the embryos of most of the vertebrates, the development of the brain and spinal cord takes place at first as the ectodermal furrow which deepens, while its sides join in the middle line folding inwards making a canal throughout the whole length. As is special to Teleostean embryos, the development of the neural element in the salmon appears at first as a solid cord or a strand of cells and the neural canal develops in relatively later stage in which the neural cord is splitted off from the epidermal layer overlying it.

The cranial flexure is less marked in Teleostei as in Cyclostomata, Ganoidei and Amphibia while it is pronounced in Elasmobranchii, Reptilia, Aves and Mammalia.

The development of the stomodaeum in the salmon embryo occurs later than the proctodaeum without exception. The opening of the mouth

appears generally before hatching. However, the mandible is of a very diminutive structure in Teleostean embryos even after hatching, and it finishes development at some days after hatching in the Cyprinid fish. The first sign of the proctodaeum appears as a depression of the ectoderm at the base of the tail-bud in a very early stage, in which the enteron is still a solid band of endodermal cells.

The development of the notochord in Teleostean embryo has offered a disputed question for a long time to the embryologists. Some authors hold that it is derieved from the ventral edge of the neural keel by delamination, and splitted off from before backwards as a cord of cells. Others, however, stand on the opinion that it develops from the median longitudinal part of the endoderm underlying the neural cord before the endoderm acquires a feature of intestine. According to other authors, the mesoderm in the mid-dorsal line is thickened and then closely fused with the layer of the endoderm beneath it, making the beginning of the notochord. The present author thinks that the difference of opinions according to the author comes not from the difference of the observation of the fact but from the uncertain terminology concerning the place of development. In fact, the original place whence the notochord, endoderm, and mesoderm develop is called by many authors carelessly as endoderm. This is not the endoderm in a strict sense but the primitive cell mass, some of which gives rise to the endoderm in future.

There have been reported two types of development of the unpaired fins in osseous fishes. The first type is seen in the cod embryo ; the anlage of the fin develops at the posterior part of the body as a single filament of which the dorsal part is continued directly to the ventral around the tail end. The dorsal as well as the ventral fin is separated from the tail fin by means of the partial atrophy of the embryonal anlage. The second type is shown in the present case as explained before, the anlage appearing both on the dorsal side and on the ventral side separately. The anlage of the ventral fin develops independently with the other unpaired fins. In general, the period of the development of rays in the fin seems to differ with different genera ; that is, in *Salmo* and *Oncorhynchus* they appear at an early stage, and in others such as *Alosa, Gadus, Pomolobus,* they do not develop even after the formation of the fan-shaped tail. Of the paired fins, the breast fins or pectorals develop earlier than the ventrals or pelvic fins in all Teleost.

The fact that the early renal organs are formed in different regions in the embryos according to different genera is an interest subject. According to RYDER (1882), in *Gambusia* genus it exists quite anteriorly, and is pressed

forwards against the auditory vesicles, while, in the present material the renal organs are developed from a little behind the pectoral fins

IV Summary

The essence of the present study is summarized as the following.

1. The periblastic cells are developed from the components of the blastoderm proper. They are modified blastomeres, that is, they are the nuclei proliferated at the late cleavage period from the blastoderm into the yolk and undergo the imperfect cell division without accompanying the cytoplasm.

2. Without doubt, the yolk cells are of great importance in neurishing the embryo and never take part in the formation of the germinal layers; in other words, they assume the röle of provisional blood. Some author's view that the yolk cells become blood corpuscles, is impossible to affirm, for the blood cells are differentiated from the mesoblast without regard to the yolk cells.

3. The true gastrulation process is seen, that is, the posterior end of the blastoderm is inflected as a small arc at first. Then, as the inflected part grows forwards the lower layer cells continuing with the surface of blastoderm assume a columnar form and arrange regularly, and thus the endoderm is differentiated. The writer cannot accept the opinion that the endoderm is produced only through a true delamination.

4. The development of the central nervous system presents some very remarkable peculialities, that is, it develops at first as a quite solid cord of ectodermal cells and the medullary canal appears in the relatively late stage. The medullary groove and the medullary fold of the other forms of Vertebrata are not seen in the development of the fish. It is a simple thickening of the median axis of the ectodermic part.

5. In the most cases of Vertebrata, though the anlage of the sense organs such as the optic vesicle, nasal organ and auditory organ arise first as the involution of the ectoderm, those of fishes appear as solid ingrowths in which the cavity develops later respectively. The optic and auditory organs begin to develop at nearly the same stage (on about the 15th day), and the olfactory organ appears later (on about the 24th day).

6. The proctodaeal depression of the ectoderm appears at an early period (on the 20th day), while the stomodaeum develops at the later period. The latter is not observed in the "eyeing period".

7. The earliest evidence of the heart is shown as two semispherical masses of mesoblast. They become soon two spherical masses and gradually

approach with each other in order to form a single solid cell mass on the median line of the body. Later the cavity appears in the mass. The present result on the heart development is quite different from that of some authors in which the heart in Teleostei is formed as in birds and mammals by the coalescence of two tubular anlage.

8. The intestine is differentiated at first as a solid and depressed band of endodermal cells, in which a lumen makes appearance. The appearance of the lumen of the intestine is not brought about by a process of invagination as seen in the other vertebrate forms, but by the separation or retreat of cells.

9. The epitherial natatory fold, which gives rise to the dorsal and caudal fins respectively appears at first at the dorsal side alone. The period of appearance of it at the ventral side comes a slightly later, and so at first the fin is asymmetrically formed differing from the result observed by AL. AGASSIZ on the flounder.

This study was aided by a grant from the Foundation for the Scientific Research of the Department of Education.
Contribution No. 115 from Fish. Inst., Agr. Fac., Hokkaido University.

Explanation of the plates
Abbreviations

af, anal fin ; *aht*, rudiment of heart ; *al*, archenteron ; *ao*, aorta ; *aun*, ganglion of auditory nerve ; *auv*, auditory vesicle ; *av*, aqueductus vestibuli.
ba, branchial arch ; *be*, branchial cleft ; *bl*, blastopore.
cb, cerebellum or epencephalon ; *ch*, notochord ; *co*, body cavity or coelom.
df, dorsal fin ; *dwa*, dorsal wall of archenteron.
ec, ectoderm ; *el*, eye-lens ; *en*, endoderm ; *enr*, enteron ; *ep*, epidermic layer of ectoderm ; *er*, embryonic rim ; *es*, eye-stalk.
fb, fore-brain or prosencephalon ; *fv*, facial vessel.
hb, hind-brain or metencephalon.
ien, protruded endoderm, dorsal part of which will form the notochord ; *iht*, epithelioid layer of heart.
le, lower layer cell; *lmp*, lateral plate of mesoderm.
m, micropyle ; *mb*, mid-brain ; *me*, mesoderm ; *mes*, mesodermic somite ; *mob*, medulla oblongata or myelencephalon ; *mp*, muscle plate formed of large cells.
nc, neural cord ; *ng*, neural groove ; *nk*, neural canal ; *np*, neural plate ; *nr*, neural ridge.
oc, optic cup ; *oht*, muscular wall of heart ; *olp*, olfactory pit ; *op*, optic vesicle ; *os*, anterior end of segmental duct ; *ov*, opthalmic vessel.
pd, proctodaeum ; *pf*, pectoral fin.
r, zona radiata ; *rl*, retinal layer.
sc, segmentation cavity ; *sd*, stomodaeum ; *sdp*, segmental duct ; *sop*, somatopleure ; *sp*, splanchnopleure.
tf, tail fin ; *tl*, tapetal layer.
vf, ventral fin.
y, yolk granules ; *yc*, yolk cell ; *ys*, syncytium of yolk.

Plate I

Fig. 1a. Side view of a blastoderm about 70 hours old, showing the elevation of the blastoderm on the egg surface. X 50

Fig. 1b. Section of a blastoderm in the same stage as it of fig. 1a, showing that its right part has become somewhat thicker than the opposite part. X 100

Fig. 2. Section of a blastoderm 100 hours of age. One side is thicker than the other, so that it presents a scalene triangular form. X 150

Fig. 3. Transverse section of a blastoderm a little further advanced than the stage of fig. 2. The blastomeres of an outer layer arrange regularly. X 100

Fig. 4. Longitudinal section of a blastoderm at the gastrulation stage. The ectoderm is inflected at a marginal portion of the blastoderm. The archenteron is formed of the endoderm at the dorsal wall and its ventral wall is consisted of the yolk. X 150

Fig. 5. Longitudinal section of a blastoderm on the 9th day, further advanced than it shown in fig. 4. The left thickened portion clearly displays two principal embryonic layers. The segmentation cavity is seen still at right side, extending to the thickened rim of the blastoderm. X 100

Figs. 6a-c. A series of transverse sections through the anterior part of the rudimentary nervous cord of an embryo on the 12th day. Fig. 6a shows the strong thickening of the ectoderm pushing the mesoderm, from which the cerebrum must be differentiated. Fig. 6b is a section

through the anterior end of the developing notochord. X 150

Plate II

Figs. 7a-f. Six serial transversal sections of an embryo on the 14th day. In all figures, the neural canal has not come still into view, and the neural cord has not been splitted off from the epidermal layer. At the anterior part the notochord separates from the endoderm proper (Figs. 7b-d), and the mesoderm differentiates into the somite and the lateral plate (Fig. 7c). While, at the posterior part the mesodermic somite has not been differentiated and the medullary plate is continuous with the endoderm which develops into the alimentary tract (Figs. 7e, f). Fig. 7e corresponds to the figure showing the neurentric canal in the embryo of other animals such as the bird, and fig. 7f is a section immediately behind the opening of the neurentric passage, showing the invagination for the proctodaeum. X 150

Plate III

Figs, 8a-g. Serial cross sections of an embryo on the 15th day. The anterior part of the neural cord separates from the epidermal layer of the ectoderm (Figs. 8a, b), but at the posterior part it has not been yet splitted off (Figs. 8c-g). The eye vesicle appears as the lateral growth of the fore-brain, which is somewhat oblique to the axis of brain (Fig. 8a). The rudiment of the auditory vesicle comes into view as a solid thickening of the ectoderm (Figs. 8b, c). The mesoderm differentiates into the somite and the lateral plate at the anterior part of the body, and the lateral plate spilitts off into the splanchno- and somato-pleures, and consequently, there appears a coelomic cavity (Figs. 8b, c). Moreover, a part of the splanchnopleure at the either side of the neural cord differentiates into the rudiment of the heart (Fig. 8c, *aht*). The axial portion of the endoderm is separated off as the notochord at the anterior body (Figs. 8c-e), but at the posterior part, at where the alimentary tract has been formed, it is invaginated to form the notochord, and its posterior end blends into the neural cord. X 150

Plate IV

Figs. 9a-k. A series of transverse sections of an embryo on the 18th day. The optic vesicle is carried downwards by the growth of the fore-brain, and at the same time it takes nearly vertical position (Fig. 9a). The ectoderm against the vesicle thichens as the lens of eye, and the vesicle itself becomes a depressed double walled optic cup (Fig. 9b). The thick walled ovoidal auditory vesicle with a small cavity appears (Fig. 9f). The neural cord is separated from the epidermal layer and the canal has come into view (Figs. 9a-g) with the exception of the posterior part (Figs. 9h-k). The endoderm converges into a tubular enteron (Figs. 9g, h). The notochord is differentiated through the entire length of the body (Figs. 9f-k), but it at the posterior part does not take yet the essential feature of the rigid tissue (Fig. 9k). The segmental duct appears between the somite and the lateral plate (Fig. 9i). X 150

Plate V

Figs. 10a-j. A series of transverse sections of an embryo on the 20th day. Figs. e and f are more slightly magnified representation (X 200) than the others (X 150). There occurs the differentiation of the hindbrain (Fig. 10c; *hb*). The ectodermal thickening for the

eye-lens is carried in the deepened optic cup (Fig. 10a ; *el*). The auditory vesicle remains still a thick-walled ovoidal feature (Fig. 10c ; *auv*). The axis of the endodermal layer converted into the notochord (*ch*) and its posterior end continues to the ectodermal roof of the proctodaeum (Fig. 10g) which wholly is fused with the posterior extremity of the neural cord (Fig. 10h). The outline of the notochord shows the rigid appearance (Figs. 10e, f).

Fig. 11. A diagrammatic figure of a longitudinal section of the posterior part of an embryo, showing the relation of various organs with each other. The axial endoderm is wholly converted into the notochord (*ch*), which is bended into the ectoderm forming the roof of the proctodaeum (*pd*) at the posterior end. On the other hand, the neural cord (*ne*) and the endoderm (*ed*) which has a fate to develop into the enteron, are continued with this ectodermal roof of the proctodaeum.

Plate VI

Figs. 12a-t. Serial transverse sections of an embryo on the 22nd day. A constriction on the hind-brain separates the hind-brain into the cerebellum and medulla oblongata (Fig. 12d). To the inner side of the auditory vesicle the ganglion of the auditory nerve closely applies (Fig. 12h). The somites in the caudal region are crecentic, and they on either side crasp the notochord, neural cord and a mesoblastic band of cells (Fig. 12s). The masculr dorsal wall of the heart is incomplete. The posterior extremity of the notochord bends upwards, extending to the body surface (Fig. 12t). The intestinal canal is not seen in the front region of the pectoral fin which appears its first sigh as the elevation of the thickened ectoderm. X 110

Plate VII

Figs. 13a-u. A series of transverse sections of an embryo on the 24th day. The cerebral region still retains the laterally compressed form that is a character of the early development of the teleostean brain. The eye lens is separated off from the epidermal layer and falls in the optic cup. The ganglion of the auditory nerve is seen (Fig. 13f). The rudiments of the olfactory organs appear as a pair of small thickened patches of the ectoderm on the under side of the fore-brain. The posterior end of the segmental duct opens into the hinder part of the intestine (13s). The blood cells are observed in the blood vessels (Figs. 13p-s). The unpaired dorsal fin appears as a median fold of the epithelium. The head projects prominently over the egg surface. X 110

Plate VIII

Figs. 14a-g. Serial cross sections of an embryo on the 31st day. The rim of the optic cup is reflected inwards and more fully covers the lens, becoming thinner. The internal cavity of the auditory vesicle increases in size, but its wall becoms thinner, and it removes inwards remaining contacted with the body surface by an elongated duct ending blindly close beneath the skin. The ectodermal thickened patch of the olfactory organ involutes as a thick saccular depression continuous at its border with the skin. X 110

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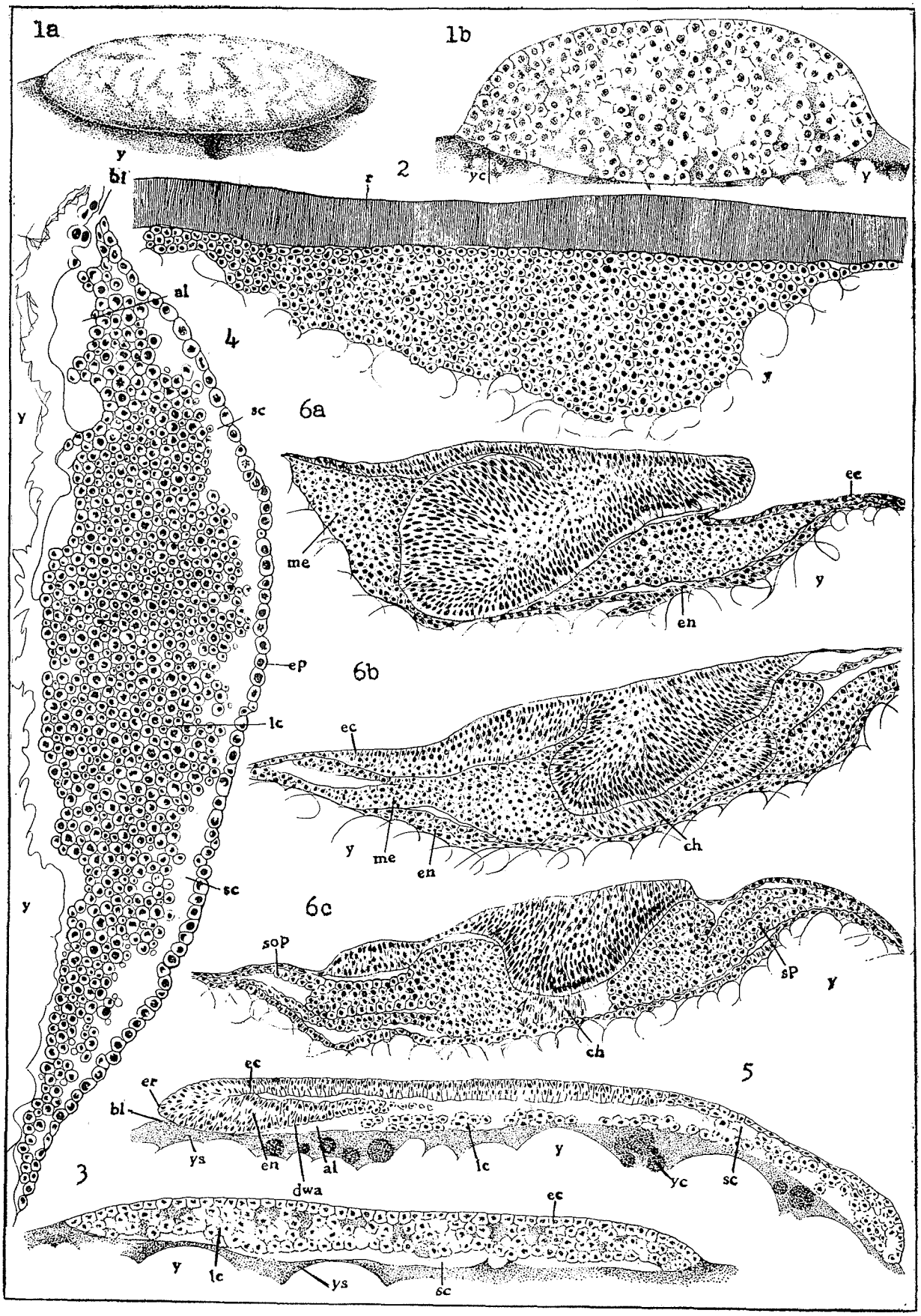
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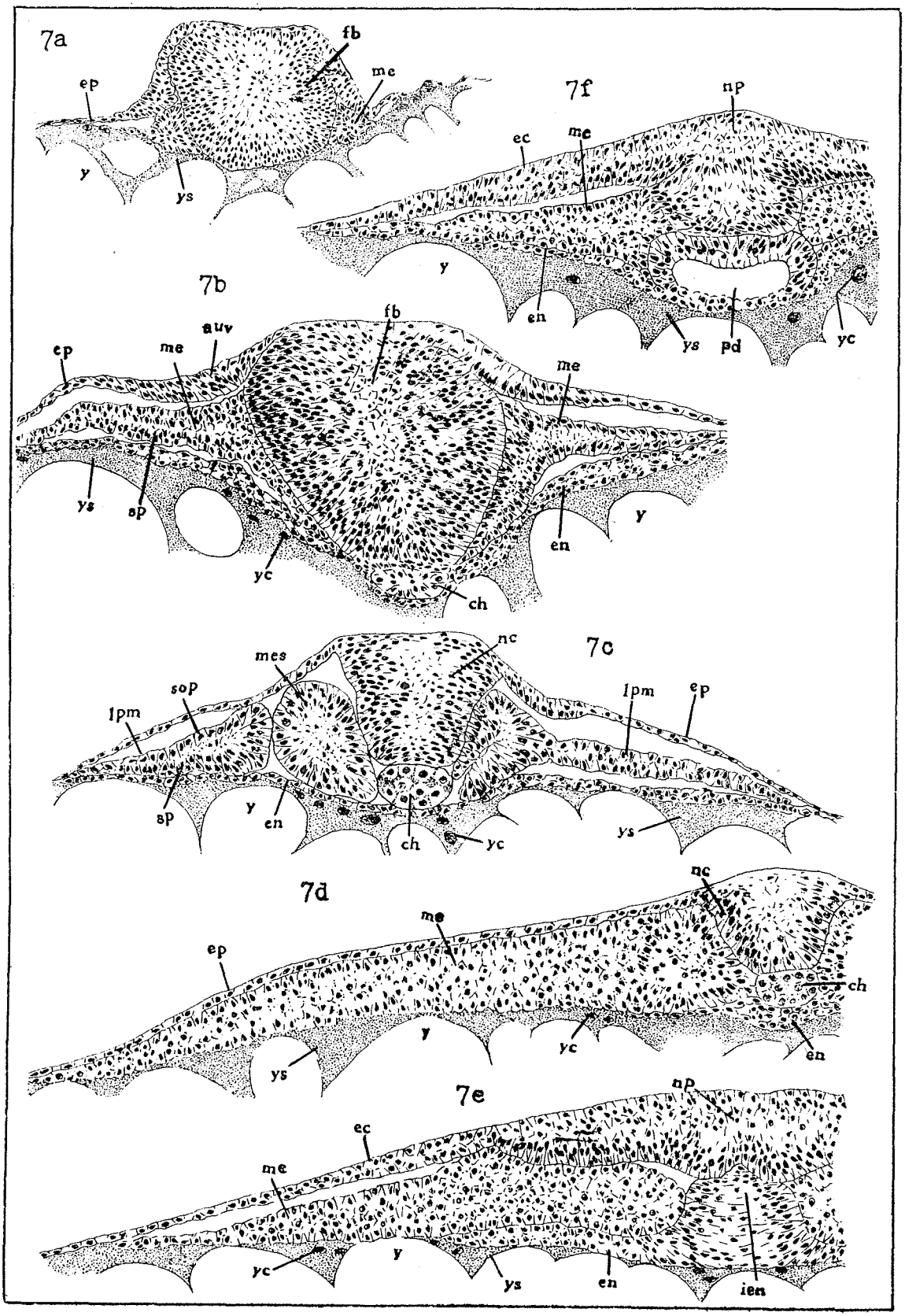
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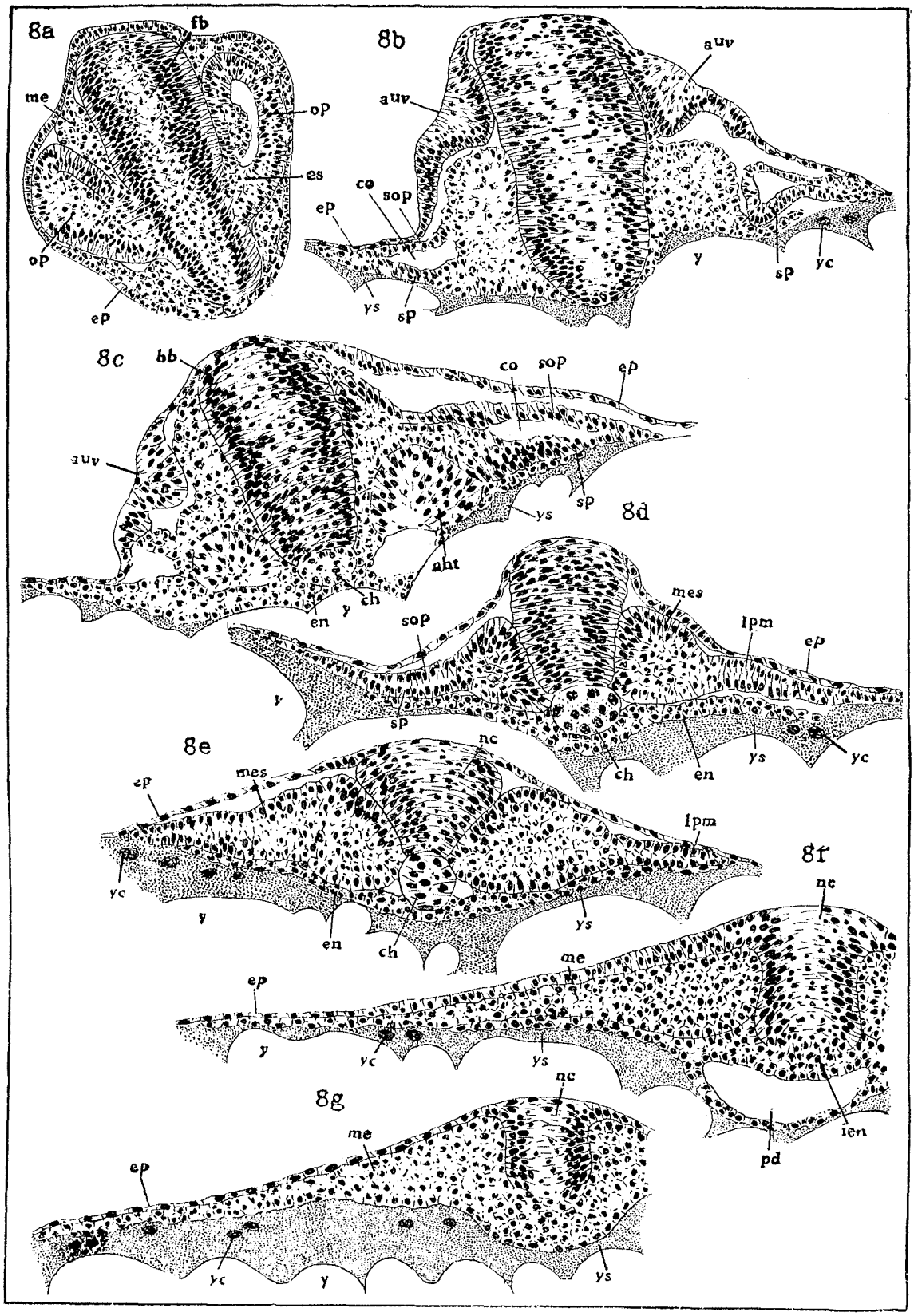
Plate III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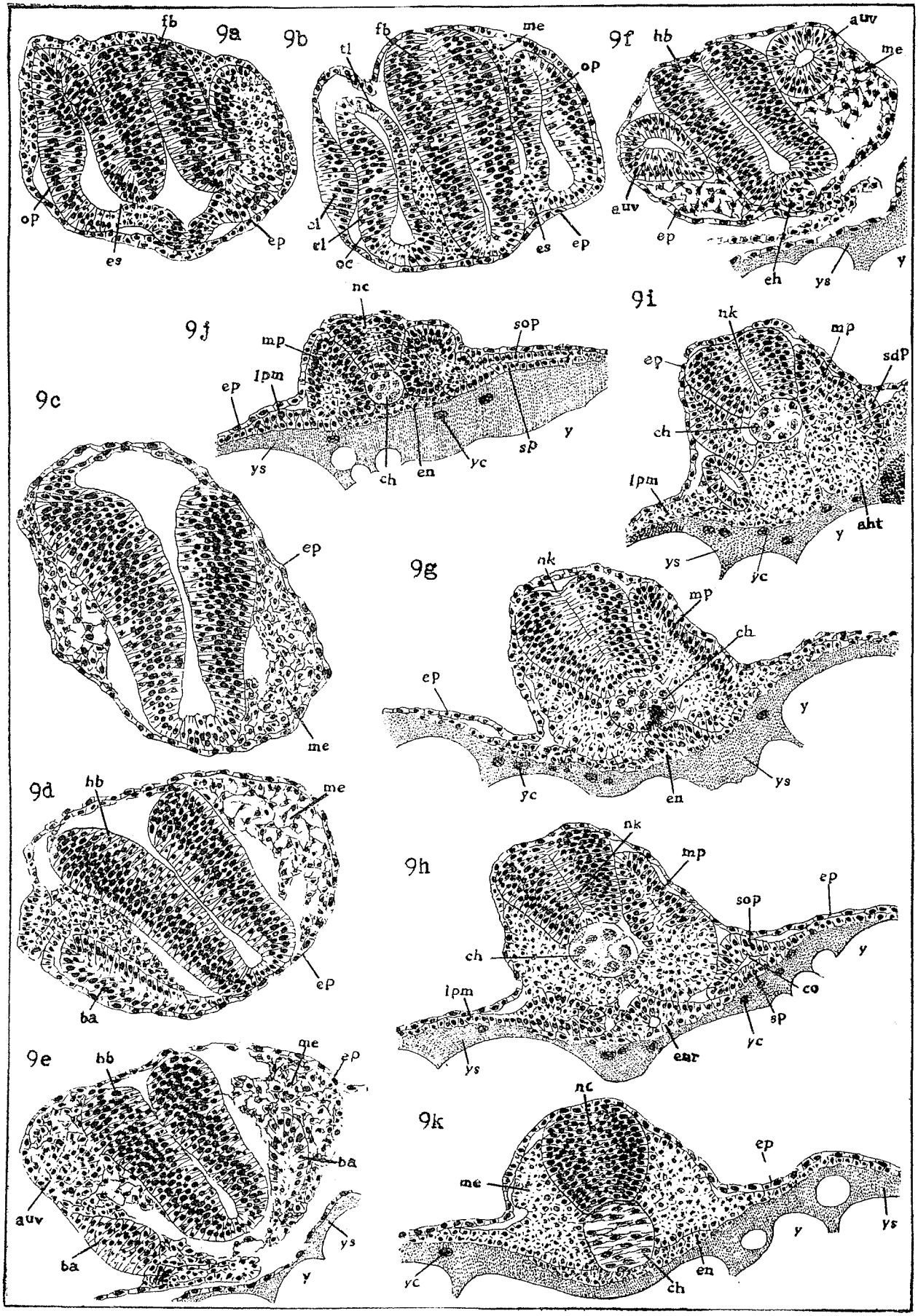
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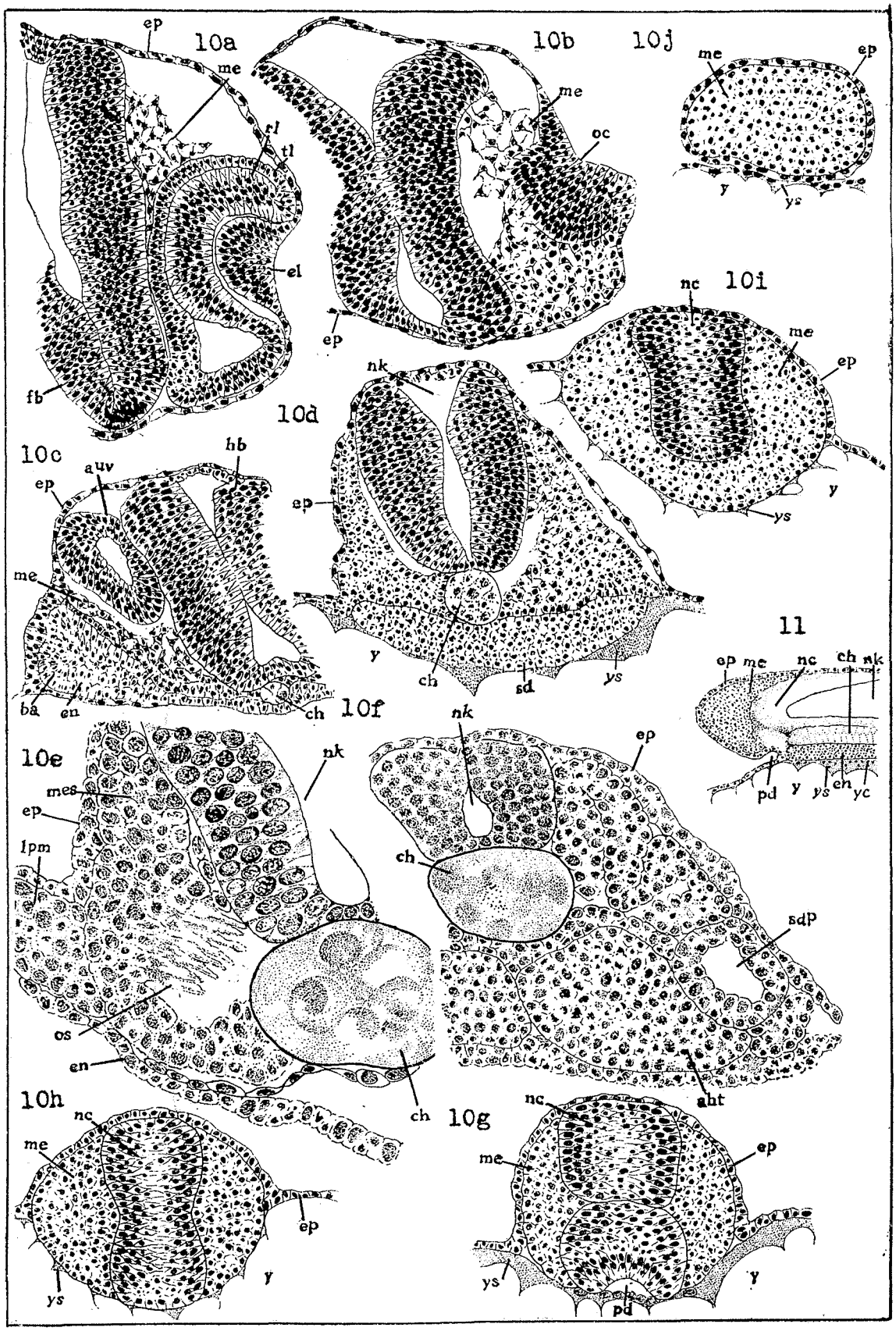
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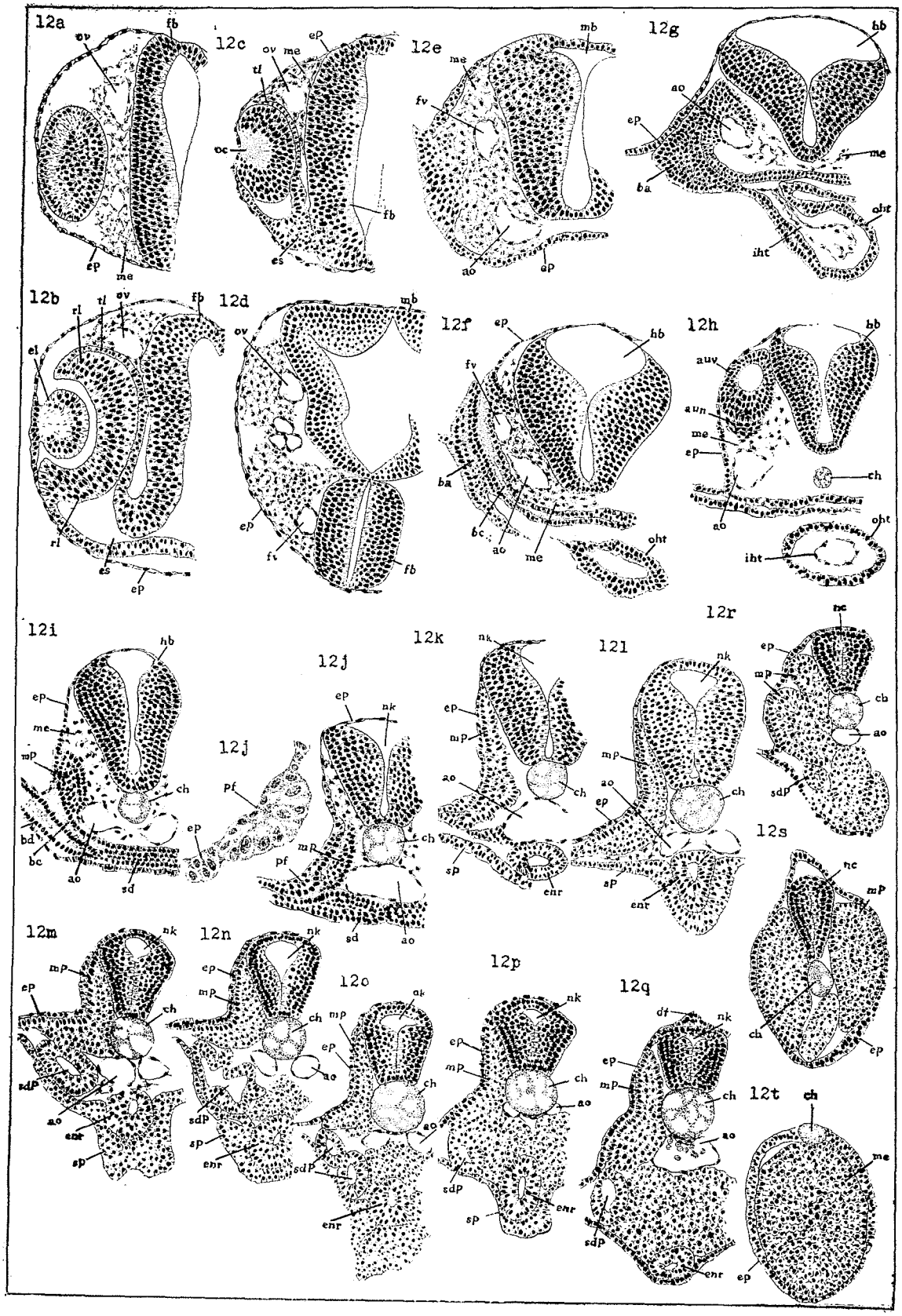
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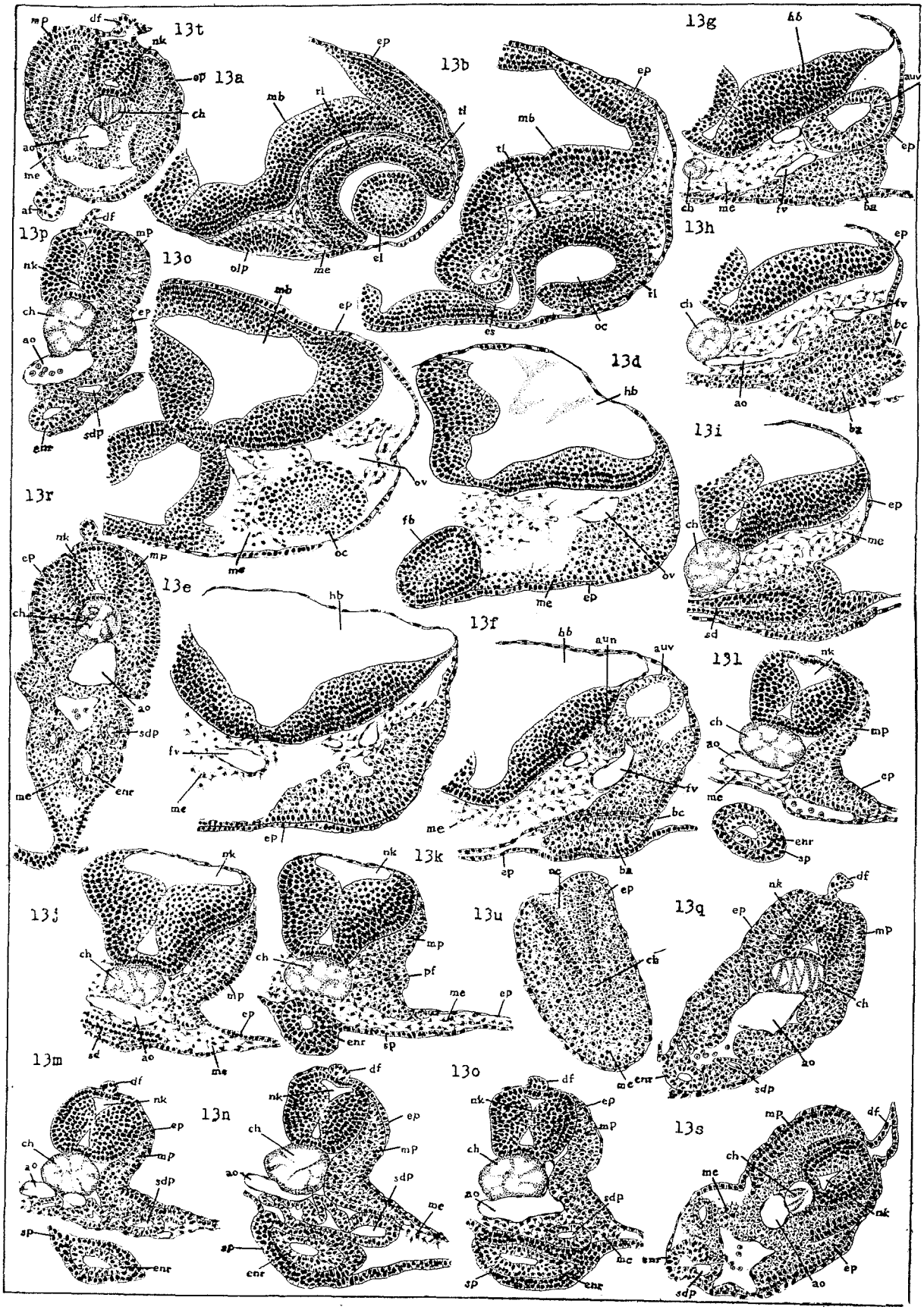
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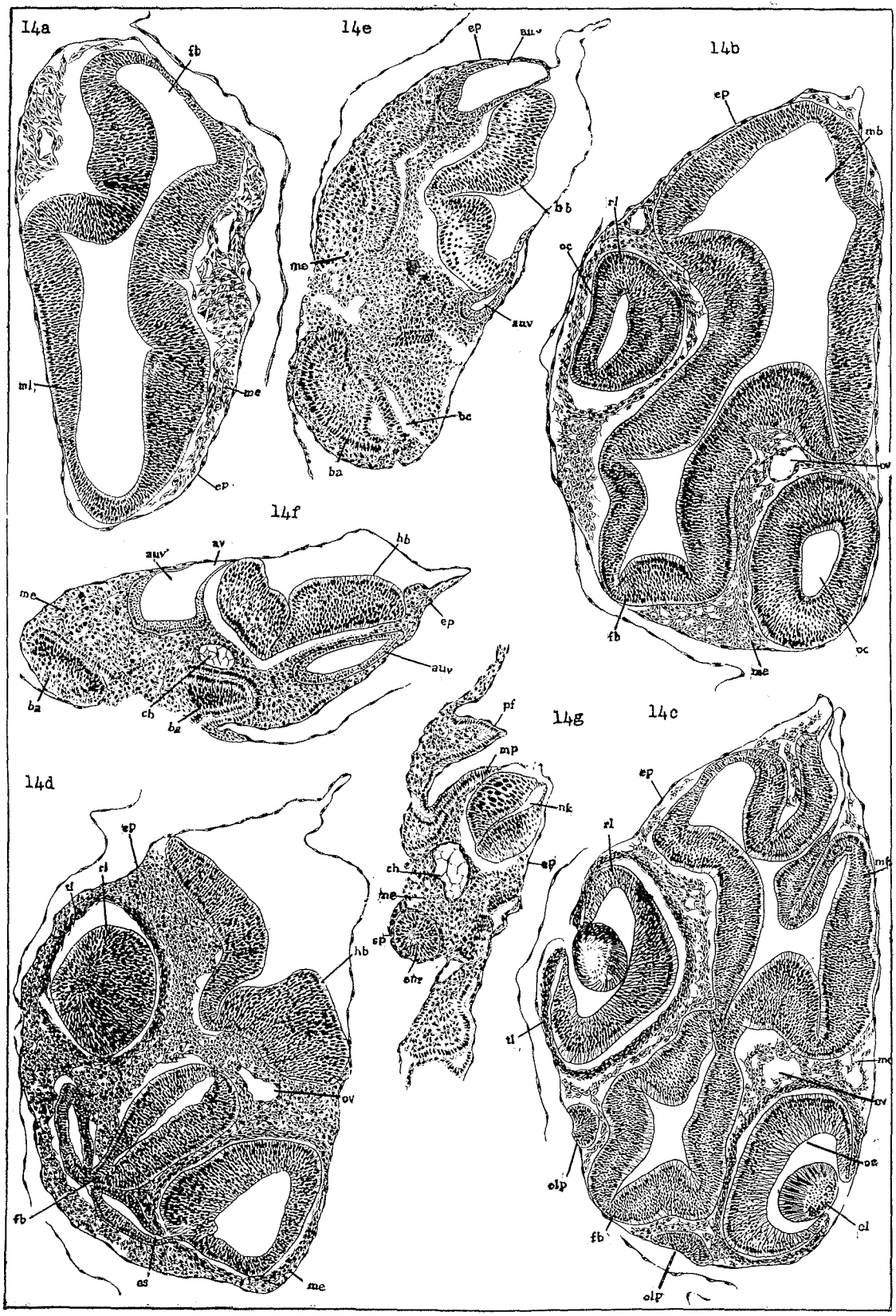
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