THE EARLY DEVELOPMENT OF THE CHONDROCRANIUM OF *SALMO SALAR*.

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ABSTRACT.

150 *S. salar* embryos were studied by van Wijhe's technique. 14 stages, from 3 weeks before hatching to 10 weeks after hatching, were used. Results: The parachordal and trabecula arise independently. No separate anterior centre of chondrification was found for the parachordal as described by Stöhr. It may sometimes arise in this way, but is probably exceptional. The hyomandibula and symplectic arise separately and later join. Meckel's cartilage arises independently. The otic capsule first appears in the region where the hyomandibula will become attached to it lateral to the anterior end of the parachordal. The quadrate arises independently and about 5 weeks later the upper jaw develops from it as an outgrowth, the pterygoid process. The stylohyals, ceratohyals, hypohyals and basihyal arise as separate cartilages. The stylohyals are the last of the group to appear. The ceratobranchials and hypobranchials arise as separate cartilages. The fifth branchial arch has no hypobranchials and its ceratobranchials remain relatively poorly developed. Four epibranchials and all the pharyngobranchials studied (the first three) arise as separate cartilages. The copula is a single median structure usually divided into two segments. The first three branchial arches are associated with the anterior of these, the fourth and fifth branchial arches with the posterior. The taenia marginalis arises independently and finally joins the otic capsule and ethmoid plate. The epiphysial bar grows medially from the taenia to fuse with its fellow from the other side, and thickens to form the tectum which roofs over the anterior part of the skull. The ethmoid plate forms medially by a union of the anterior ends of the trabeculae. The interorbital septum grows up from the centre and a lamina orbitonasalis from each side. The former joins the median process, while the latter joins the lateral process of the taenia. To show the rate of cartilage development morphologically 3 curves were made from the drawings, by plotting against time: (1) the number of new cartilages appearing each day; (2) the total area of cartilage in the head; and (3) the ratio of cartilage area to head area. All showed a falling off in cartilage development at the time of hatching. A possible explanation for this cessation of development is that energy which was being used in building new material has been diverted to the arduous task of hatching.

INTRODUCTION.

No detailed account of the skull development of *Salmo salar* has been given since Parker[1] described the early stages. His work was reviewed and enlarged upon by Stöhr[2] who,

[1]Parker.   *Phil.Trans. Roy. Soc.* **163**, 95, (1873).

[2]Stöhr.   *Festschr. der J. Max. Wurzburg*, **2**, pp. 73-93, (1882).

however, added very few illustrations. Gaupp[3] summarized existing knowledge and included drawings of three models. His is the most recent work on early development although the later development of the skull is well known. This paper reports the results of a re-examination of the early development attempting to clear up the following points, none of which are adequately dealt with in any preceding publication: the origin and progressive development of the cartilages; the manner in which the cartilages unite; the relationship of the developing visceral arches.

MATERIALS AND METHODS.

Several batches of eggs were obtained two days after fertilization from the Government Fish Hatchery in Bedford, N. S., and were kept in a tank of running water at a constant temperature of approximately 5°C. The eggs hatched from 41 to 60 days after fertilization, depending on the temperature of the water. Each week, or more frequently in younger stages, 10-15 eggs or embryos were removed from the tank for fixation in Bouin's fluid. After hardening, and removal of the yolk sac (and shell where necessary), they were stained by van Wijhe's[4] method. Van Wijhe's stain contains 1% hydrochloric acid, which destroys most of the tissues leaving the cartilage to stand out clearly in blue. Benzyl benzoate, which was adopted as a clearing fluid and permanent preservative, has marked advantages over other agents. It clears with exceptional speed and effectiveness, making the stained cartilaginous parts stand out with great clarity, and it prevents brittleness, thereby permitting easy slicing with a razor blade when necessary. A complete set of whole mounts was obtained using this method.

Every embryo in each sample was examined, in order to be sure that the one used in the figure was typical. When any difference was noted, examples of each have been drawn.

[3]Gaupp. Hertwig's *Handbuch der Entwicklungslehre der Wirbeltiere*, Verlay von Gustav Fischer, Jena, **906**, p. 660.

[4]Lee. "*The Microtomist's Vade-mecum*," P. Blakiston's Son & Co., Philadelphia, **1928**, p. 920.

Early attempts to make drawings with the aid of a camera lucida were abandoned in favour of the following method which was easier and more accurate. A squared eye-piece micrometer was inserted into the microscope and calibrated with a stage micrometer. Drawing paper was squared to correspond at a magnification of 40. When the embryos were examined under the microscope, they appeared with the eye-piece squares superimposed. The embryos could then be drawn to fit into the corresponding squares on the paper. To facilitate drawing and to observe the cartilaginous structures more clearly, median sagittal sections were made of the heads of the larger embryos and the eyes were removed. Hand sections were made in a few cases, separating the visceral arches from the neurocranium. These sliced embryos were compared carefully with intact specimens.

DESCRIPTION.

The following is a description of the significant stages in the development of the chondrocranium. The nomenclature follows closely that used by de Beer[5] in a similar paper on the development in the trout. It has been found simpler to describe the developmental condition in terms of the number of days before and after hatching, rather than with reference to the number of days after fertilization.

Fig. I. Embryo H1A. 21 days before hatching. This is the earliest specimen in which cartilage shows. The first structures to appear are paired cylindrical rods, the parachordals, which arise in the posterior region of the head on either side of the notochord. There is a dense enlarged region at the posterior ends which will later give rise to the occipital arches. Each of the parachordals arises from a single centre of chondrification. None of the specimens observed showed a separate anterior centre of chondrification, as was described by Stöhr[2]. De Beer[5] does not find any separate anterior centre in the trout and he suggests that Stöhr's specimens may have been exceptions.

[5]de Beer. *Quart. Journ. Micr. Sci.* **71**, 259, (1927).
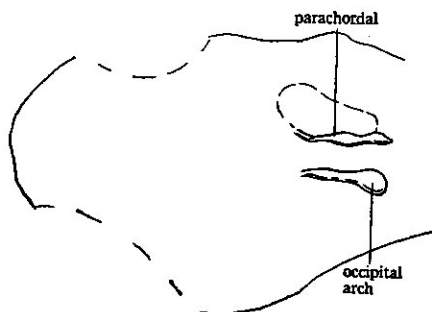
Fig. I.   Embryo H 1A, dorsal view (21 days before hatching).
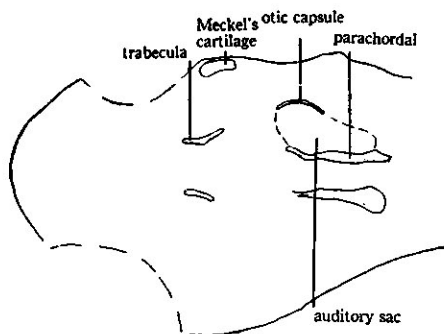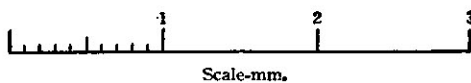
Fig. II.   Embryo H 1B, dorsal view (21 days before hatching).

Scale-mm.

Figures I. to XX. are drawn to the above scale.

Fig. II. Embryo H1B. 21 days before hatching. The parachordals have not grown any larger but they are more thoroughly chondrified. Several new cartilages appear. The trabeculae arise as small rods, anterior to and slightly more ventral than the parachordals. They are curved outward around the future hypophysial fenestra. Meckel's cartilage may be distinguished as a knob, at the outer posterior border of the mandibular arch. A small portion of the otic capsule has progressed to procartilage.

Fig. III (a and b). Embryo H3A. 21 days before hatching. The trabeculae have lengthened, and in lateral view, the posterior ends are on a level with the parachordals, though still distinctly separated from them. The occipital arches are arising at the posterior ends of the parachordals. The visceral arches also show considerable development. Meckel's cartilage is more dense, and above it the quadrate and symplectic appear, one behind the other. The hyomandibula and otic capsule arise close together, lateral to the anterior end of the parachordal.

Fig. IV. Embryo H3B. 21 days before hatching. The trabeculae have joined the anterior ends of the parachordals, thus forming two supporting rods extending under the brain. Three new visceral arches have appeared. The ceratohyals originate as short paired rods posterior to Meckel's cartilages. Behind them, the ceratobranchials of the first and second branchial arches appear.

Fig. V. Embryo IA. 14 days before hatching. Meckel's cartilages have extended well forward into the mandibular arch, but they do not meet. The ceratohyals are longer and more thoroughly chondrified; median to the anterior ends two small cartilages, the hypohyals, have arisen independently. The first two ceratobranchials have elongated, and behind them the ceratobranchials of the third and fourth branchials appear. The cartilage of the quadrate is more dense and the hyomandibula and symplectic are joined by a thin layer of cartilage.
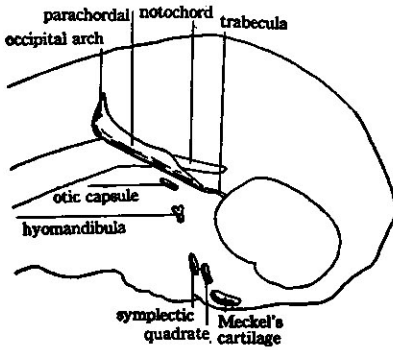
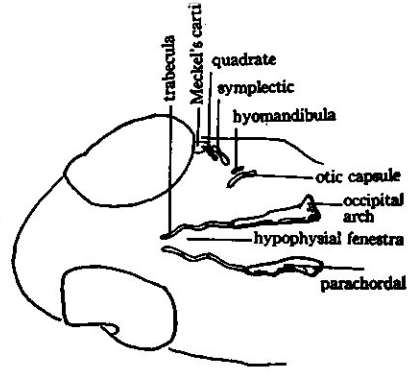Fig. III (a). Embryo H 3A, lateral view (21 days before hatching).

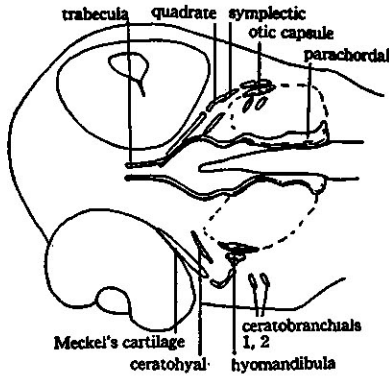Fig. III (b). Embryo H 3A, dorsal view (21 days before hatching).

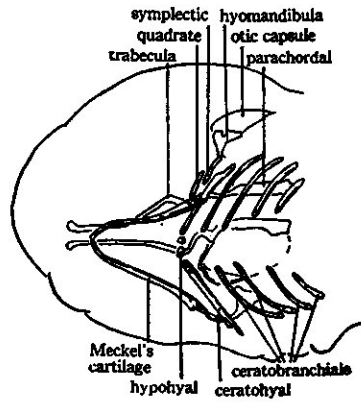Fig. IV. Embryo H 3B, dorsal view (21 days before hatching).

Fig. V. Embryo I A. ventral view (14 days before hatching).

Fig. VI. Embryo IB. 14 days before hatching. The trabeculae have extended forward almost to the front of the head, at the same time widening at the anterior ends. The otic capsules have been joined to the parachordals, anteriorly by the anterior basicranial commissure, and posteriorly by the posterior basicranial commissure. The enclosed space is the basicranial fenestra. The occipital arches have grown upward considerably, leaving a space between them and the otic capsules, which will later be closed off as the jugular foramina. The visceral arches are little changed. The connection between the hyomandibula and symplectic is complete and the copula appears as a small median cartilage directly behind the hypohyals.
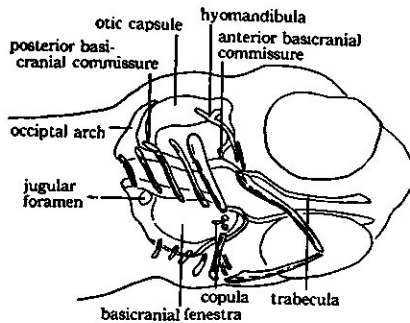
Fig. VI.   Embryo I B, ventral view (14 days before hatching).

Fig. VII (a and b). Embryo 6. 14 days before hatching. The size has increased, and the prootic process, a slight prominence at the anterior border of the base of the otic capsule, is noted.

Fig. VIII (a and b). Embryo 7. 7 days before hatching. The neurocranium has advanced noticeably. Just in front of the hypophysial fenestra, the trabeculae are closing together. The prootic process is more prominent and another process is growing out from the anterior basicranial commissure to

meet it, the postpalatine process.   The otic capsule is more
dense and the cartilage is beginning to grow up the sides.
There is as yet no roof, although a roll of cartilage has grown
up the dorsal border at each side.   The semicircular canals
appear within the otic capsule.   The notch in the hyomandi-
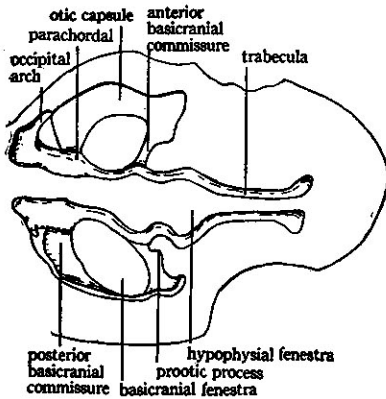bular has closed over forming a foramen for the facial nerve

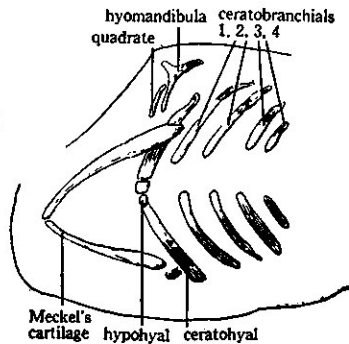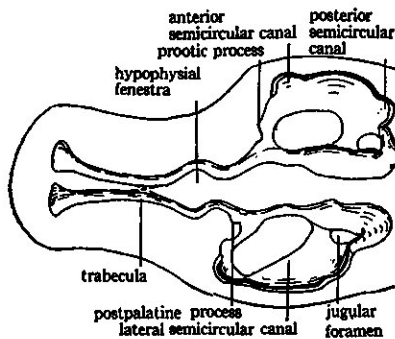Fig. VII (a).   Embryo 6, dorsal
view (14 days before hatching).

Fig. VII (b).   Embryo 6, ventral
view (14 days before hatching).

Fig. VIII (a).   Embryo 7, dorsal
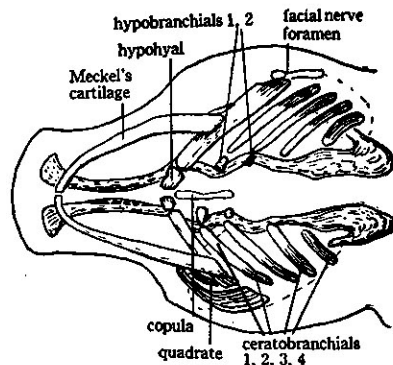view (7 days before hatching).

Fig. VIII (b). Embryo 7, ventral
view (7 days before hatching).

Fig. IX. Embryo 8. Time of hatching. (Hatching extends over three weeks. This embryo was taken at the mid-period). The trabeculae have joined at the anterior ends to form the ethmoid plate and a narrow bridge has formed between them in front of the hypophysial fenestra, the trabecula communis. The developing otic capsule may be seen by the figure to have extended about half way up each side with two
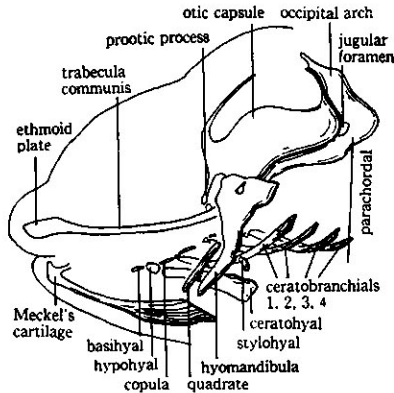

Fig. IX. Embryo 8, lateral view (time of hatching).

thin bars extending above to form the beginning of the roof. Several new cartilages have been added to the visceral arches. The stylohyal appears between the junction of the hyomandibula and symplectic and the posterior end of the ceratohyal. The basihyal is an unpaired median cartilage in front of the hypohyals. The copula extends in the mid-line to the level of the third branchial arch and the hypohyals of the first three branchial arches have arisen between the copula and the ceratobranchials.
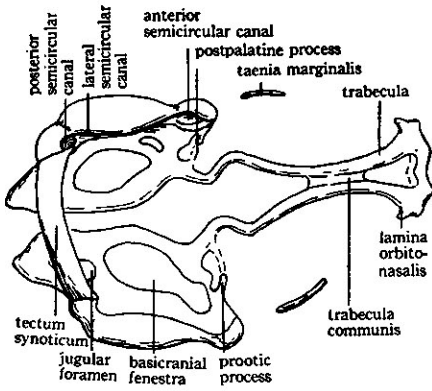
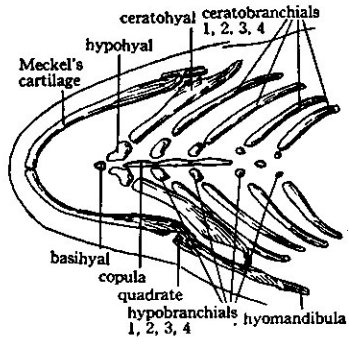Fig. X (a).   Embryo 9, dorsal view
(7 days after hatching)

Fig. X (b).   Embryo 9, ventral view
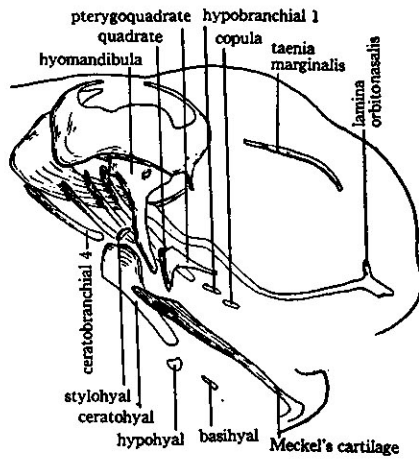(7 days after hatching)

Fig. X (c).   Embryo 9, lateral view (7 days after hatching).

Fig. X (a, b and c). Embryo 9. 7 days after hatching.
Two important structures appear in this stage; the taenia
marginalis and the pterygoid process of the quadrate. The
taenia marginalis arises anterior to and slightly below the dorsal
edge of the otic capsule. The cartilage will grow backward
and join the otic capsule and forward to fuse with the ethmoid
plate. The roof of the otic capsule has begun, and a narrow
bridge of cartilage joins the occipital arches dorsally, com-
pleting the foramen magnum. The bridge represents a part
of the tectum synoticum which will form the roof of the pos-
terior parts of the brain. The semicircular canals are plainly
visible through the otic capsule. The otic capsule and occi-
pital arch have joined dorsally closing the jugular foramen.
The trabecula communis has extended farther forward between
the trabeculae toward the ethmoid plate. Upgrowths are
developing on the posterior lateral borders of the ethmoid
plate, the laminae orbitonasales. They will fuse with lateral
downgrowths from the taeniae marginales. The pterygoid
process of the quadrate, the future upper jaw, also makes its
appearance at this time. It extends only a short distance
forward as yet. The copula has reached the level of the
fourth ceratobranchial and the hypobranchial of the fourth
branchial arch has arisen.

Fig. XI. Embryo 10. 14 days after hatching. The speci-
men is considerably larger than the previous one. The taeniae
marginales have lengthened at both ends; posteriorly they
approach the otic capsules and anteriorly both are produced
into two processes, one lateral, the other median. The lateral
process grows down to meet the lamina orbitonasalis; the
median process will eventually fuse with an unpaired median
upgrowth from the anterior border of the ethmoid plate, the
interorbital septum. In the region of the otic capsule, the
prootic process and the postpalatine process have joined.
The connection is called the lateral commissure and forms a
foramen for the exit of the jugular vein and the hyomandi-
bular nerve. The sides of the otic capsule are almost complete
and through the walls the anterior, lateral, and posterior

semicircular canals are visible. The pterygoquadrate has extended farther forward. The ceratobranchial of the fifth branchial arch appears as a short rod.

Fig. XII (a, b and c). Embryo 11. 21 days after hatching. The taeniae marginales have joined the otic capsules posteriorly; anteriorly, the lateral processes have fused with the laminae orbitonasales. A thickening appears on the inner side of the middle of each taenia marginalis. These will grow together to form the epiphysial bar. The trabecula communis has fused with the ethmoid plate. Under the ethmoid plate an anterior centre of chondrification arises from the pterygoquadrate which is joined to the rest of the pterygoid process by a thin thread of cartilage. The anterior end of the pterygoid exhibits an ethmopalatine and a rostropalatine articulation.

Fig. XIII. Embryo 12. 28 days after hatching. The epiphysial bar has formed between the taeniae marginales and is thickening to form the tectum, the roof of the anterior part of the skull. The interorbital septum has fused with the median processes of the taeniae marginales. The sides of the otic capsules are now complete. The pterygoquadrate reaches well forward to the level of the ethmoid plate. The epibranchials of the first and second branchial arches have appeared between the parachordals and the posterior ends of the ceratobranchials.

Fig. XIV (a, b and c). Embryo 13. 35 days after hatching. The otic capsule is partially roofed with cartilage and the tectum synoticum is wider. The basicranial and jugular foramina are smaller and the foramen enclosed by the lateral commissure is becoming secondarily divided into two. The visceral arches have grown more substantial. Five ceratobranchials, four hypobranchials, three epibranchials and only one pharyngobranchial are present. The copula extends down the middle in two segments; one for the first three branchial arches, and one for the last two. The side view of this specimen shows clearly the relation of the cartilages.
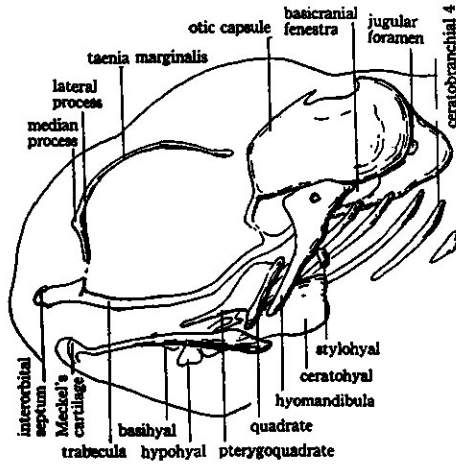
Fig. XI.   Embryo 10, lateral view (14 days after hatching).
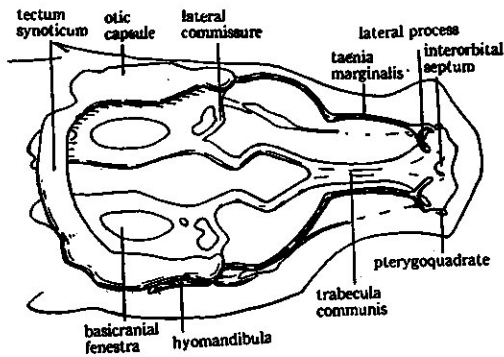

Fig. XII (a).   Embryo 11, dorsal view (21 days after hatching).
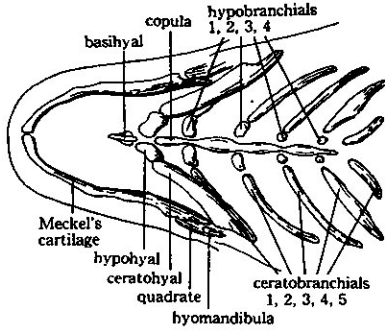
Fig. XII (b).   Embryo 11, ventral view (21 days after hatching).
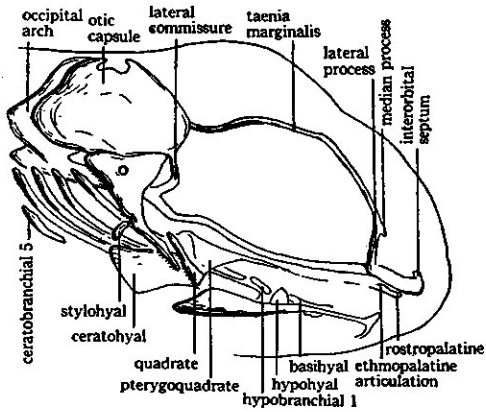

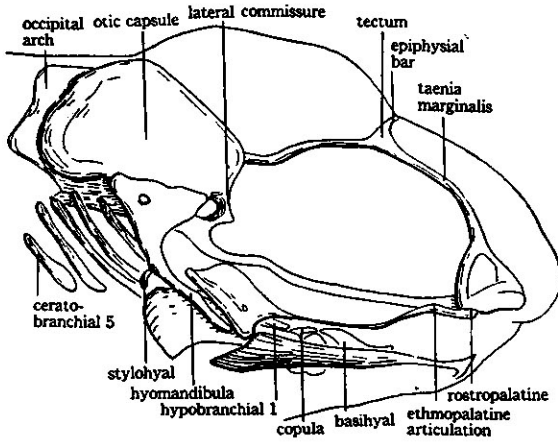Fig. XII (c).   Embryo 11, lateral view (21 days after hatching.)

Fig. XIII. Embryo 12, lateral view (28 days after hatching).
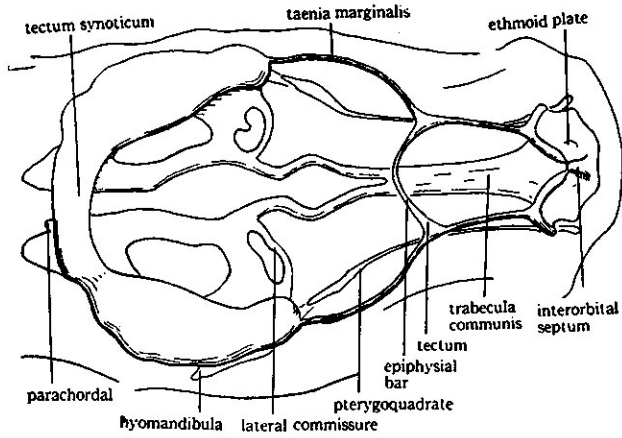

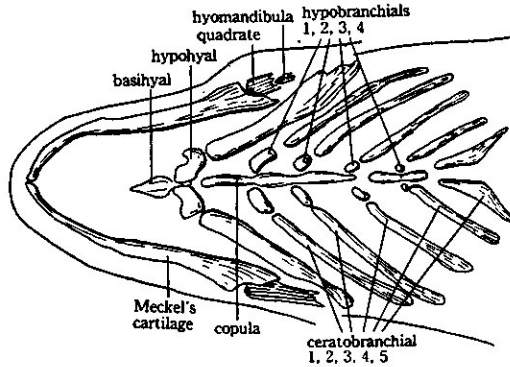Fig. XIV (a). Embryo 13, dorsal view (35 days after hatching).

Fig. XIV (b).   Embryo 13, ventral view (35 days after hatching).
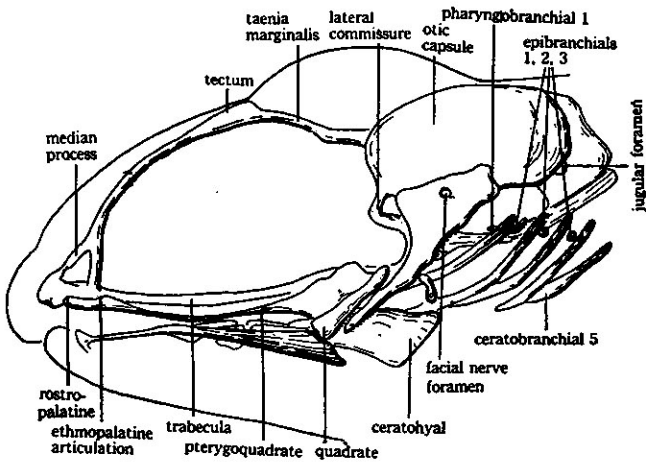
Fig. XIV (c).   Embryo 13, lateral view (35 days after hatching).

Fig. XV. Embryo 14. 41 days after hatching. The whole head of this specimen has become elongated and flattened, a characteristic which persists in older embryos. It also presents several developments among the foramina. A list provided by Gaupp[8] of the nerves and vessels which exist from the various foramina has been incorporated into figure XV. The foramina and nerves are:—

1. The jugular foramen, between the occipital arch and the otic capsule which provides an exit for the vagus nerve.

2. The foramen enclosed by the lateral commissure and the anterior basicranial fenestra which is completely divided into two. The jugular vein emerges through the antero-lateral foramen; the hyomandibular nerve of VII through the median one.

3. The basicranial fenestra which is situated in the floor of the otic capsule. Through it the glossopharyngeal nerve emerges.

4. A foramen in the hyomandibula providing an exit for the facial nerve.

Gaupp's figure which shows the foramen enclosed by the lateral commissure, completely divided into two at this stage, has been confirmed. De Beer[5] finds only a single foramen in the trout. A diagram (figure XX) has been made using Gaupp's model. The cartilages are more developed than any stage described here and the foramina for nerves and vessels show clearly. Two foramina were found in the hyomandibula of two specimens out of five examined at this stage. A possible explanation is that the facial nerve divides into two before leaving the skull. The pharyngobranchials of the second and third branchial arches have appeared.

Fig. XVI (a, b and c). Embryo 15. 48 days after hatching. The neurocranium is more solid. The taeniae marginales and the tectum synoticum have enlarged. The ethmoid region is well developed. In the side view (a) the mouth is wide open showing very clearly the relations of the visceral arches.
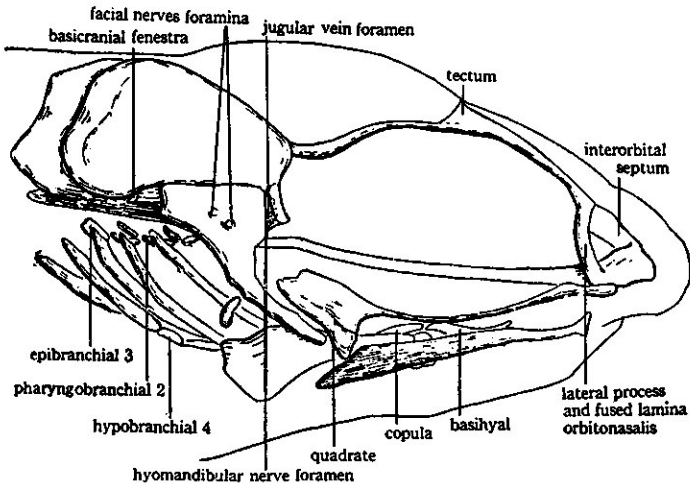
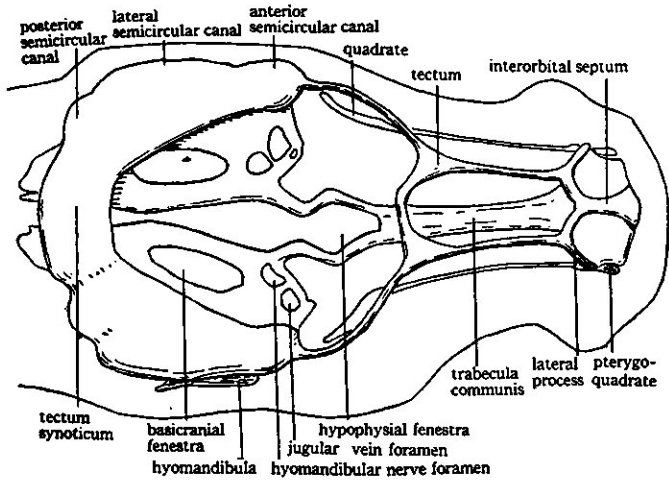Fig. XV.   Embryo 14, lateral view (42 days after hatching).

Fig. XVI (a).   Embryo 15, dorsal view (48 days after hatching).
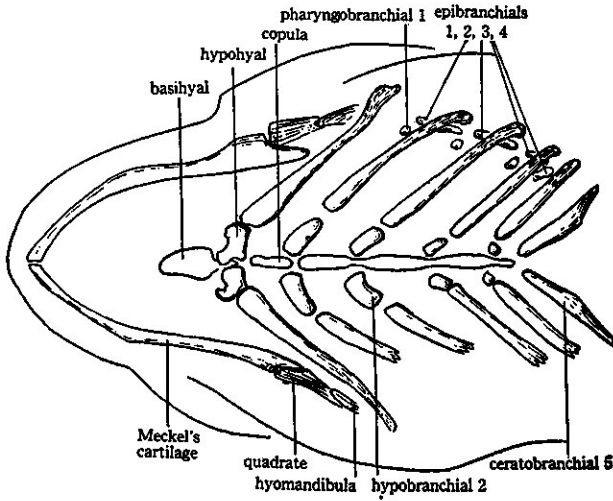
Fig. XVI (b).   Embryo 15, ventral view (48 days after hatching).
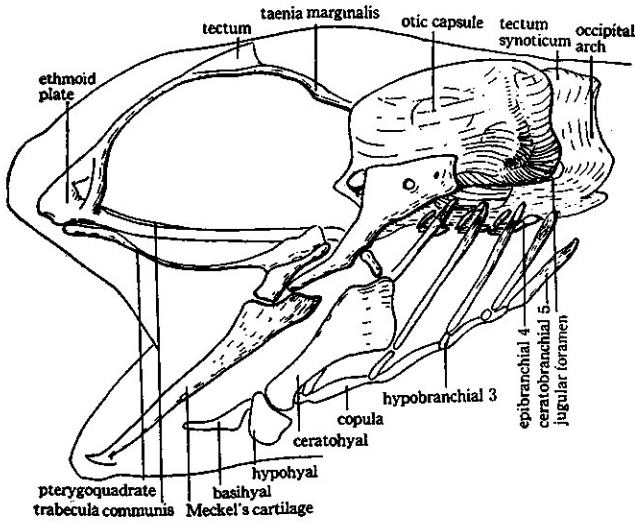

Fig. XVI (c).   Embryo 15, lateral view (48 days after hatching).
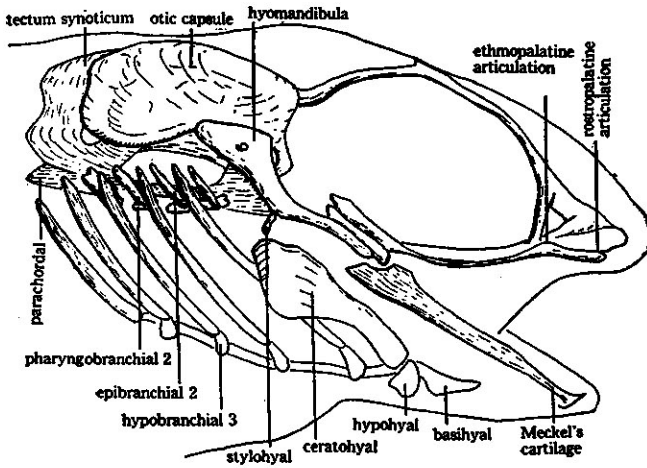
Fig. XVII.   Embryo 16, lateral view (55 days after hatching).
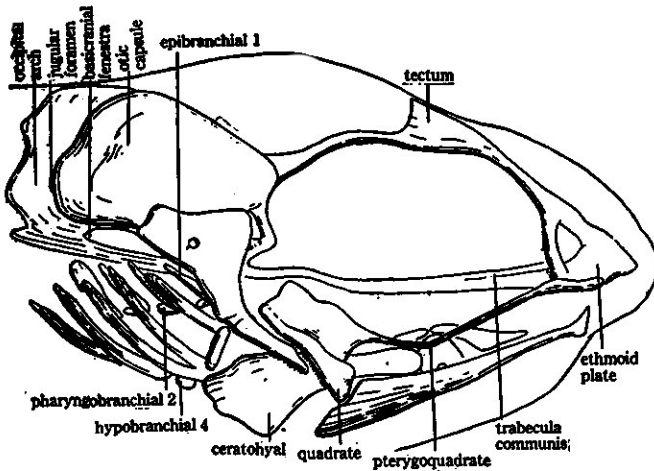

Fig. XVIII.   Embryo 17, lateral view (62 days after hatching).

Fig. XVII. Embryo 16. 55 days after hatching. This embryo has been drawn on an angle from the ventral side resulting in a distortion which makes the pterygoquadrate appear to be slightly above the trabecula. The visceral arches are clear and the fourth epibranchial and third pharyngobranchial are present. The nasal capsule is becoming closed over with cartilage from the anterior edge of the ethmoid plate. The ethmopalatine and the rostropalatine articulations are very well marked on the anterior extremity of the pterygoid process.

Fig. XVIII. Embryo 17. 2 days after hatching. This specimen shows the tectum closing over between the eyes. The cartilage is still thinner in the region where the hyomandibula and symplectic have fused. The pharyngobranchials and epibranchials are larger.

Fig. XIX. Embryo 18. 9 days after hatching. This was the final stage studied. No new structures appear but those already there have enlarged. It will be noted that there are two foramina in the hyomandibula of this specimen also. The visceral structures are clear.

THE RATE OF CARTILAGE FORMATION.

It is obvious that yolk cannot be visibly turned into cartilage, or anything else, in an embryo without an accompanying chemical alteration, capable of detection by suitable tests. Very little is known about the origin of cartilage even in adult mammals and Miyazoki[6] is only now perfecting a technique for this sort of investigation. If it were possible to furnish from the morphological side figures showing the rate of cartilage formation in an embryo at various stages and to set them up against chemical analyses of corresponding material, an attack on the cartilage problem from a new angle could be made. Complete chemical analyses of salmon embryos are lacking, but from the material in this paper the necessary morphological data may be compiled.

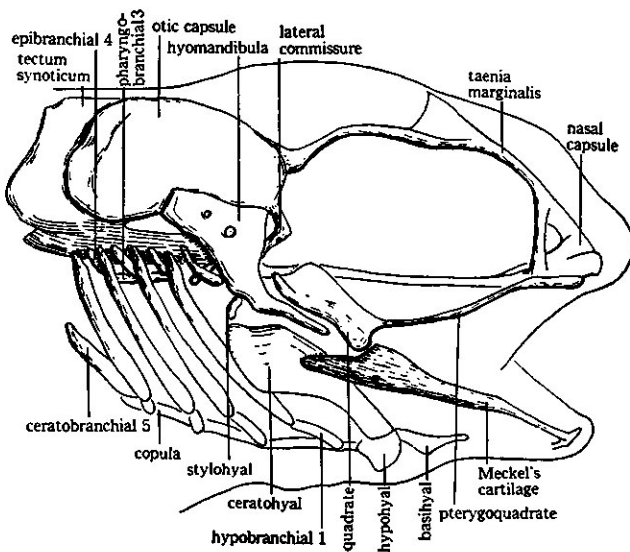[6]Miyazoki. *Journ. Biochem,* **20,** 211, (1934).

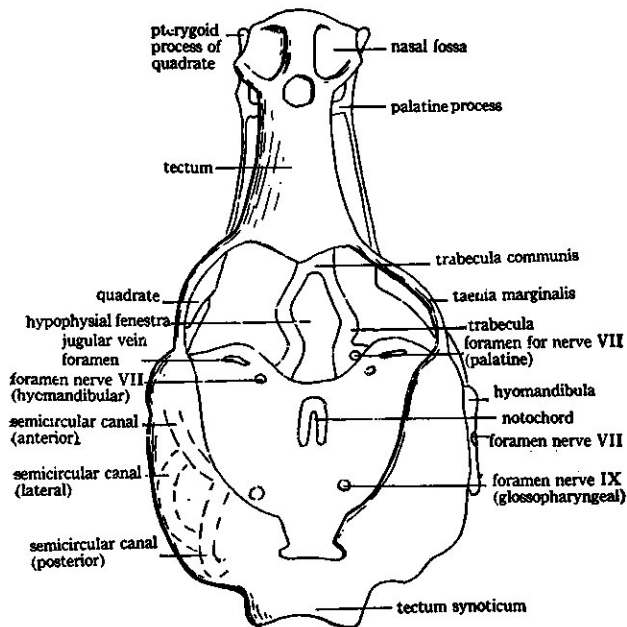Fig. XIX.   Embryo 18, lateral view (69 days after hatching).

Fig. XX.   Diagram to show exit of nerves and blood vessels
(After Gaupp).

Three quantitative uses have been made of the drawings. No one of them is entirely free from objection: taken together however, they permit, by their agreement with one another, conclusions and suggestions.
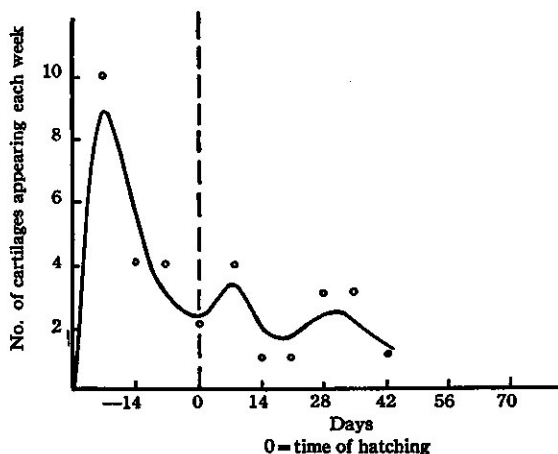

Fig. XXI.

Figure XXI shows a curve made by plotting the number of new cartilages appearing each day against time. This curve shows a sharp rise during the fourth week before hatching when cartilage first originates. The number of new cartilages appearing each week falls off until hatching. A sudden rise occurs the week after hatching followed by a drop, after which the number increases again. The maximum number of cartilages, 33, is reached 6 weeks after hatching. The curve illustrates three points: first, that there is an irregularity at hatching; second, that the effects of hatching are felt for three subsequent weeks; third, that there is a subsequent resumption of the interrupted embryological processes. The sudden rise after hatching may be due to the release of the perivitelline fluid and hatching enzymes which have been retarding development previous to hatching.

The principal sources of error are that considerable differences exist in the sizes of cartilage pieces even on their first appearance, and that no account is taken of growth subsequent to initial appearance. The first of these difficulties is made less serious by the large number of cartilages involved, 33 in all.
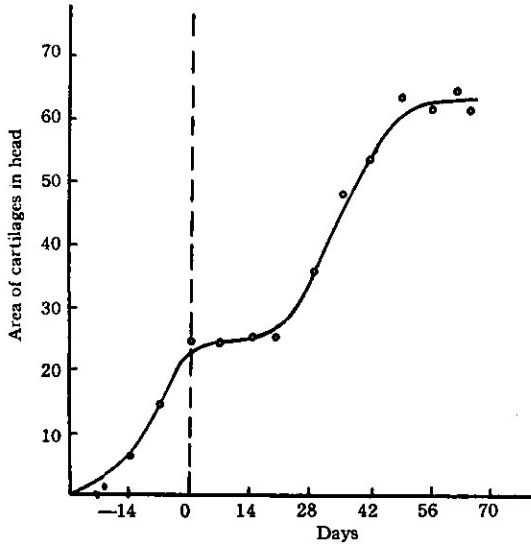

Fig. XXII

Figure XXII has been made by plotting against time the total area of cartilage seen on one side of the head. On drawings of the lateral views of various embryos, the area in square centimeters of each cartilage was measured with the aid of a planimeter, and the total added up. The curve shows several interesting points. It will be noted that there is a rapid development of cartilage from the origin, three weeks before hatching, up to the time of hatching. At the time of hatching development ceases abruptly and the curve flattens out almost to a straight line for three weeks, after which it rises again rapidly until it reaches a maximum about sixty days after hatching, where it remains. The results supple-

ment and confirm figure XXI and are not subject to its errors of method. They fail, however, to show the relationship of cartilage area to total head, obviously a point of importance.

This latter defect has been eliminated in the curve of figure XXIII, which shows the ratio

$$\frac{\text{total cartilage area}}{\text{head area}}$$

plotted against time. It shows a rise up to the time of hatching a, sudden drop and then a rise again. The explanation for the drop after hatching, since the cartilage area is known from figure XXII to be a constant at this time, is that the head continues to increase in size for about 2 weeks after the time of hatching when its growth stops for a short time.
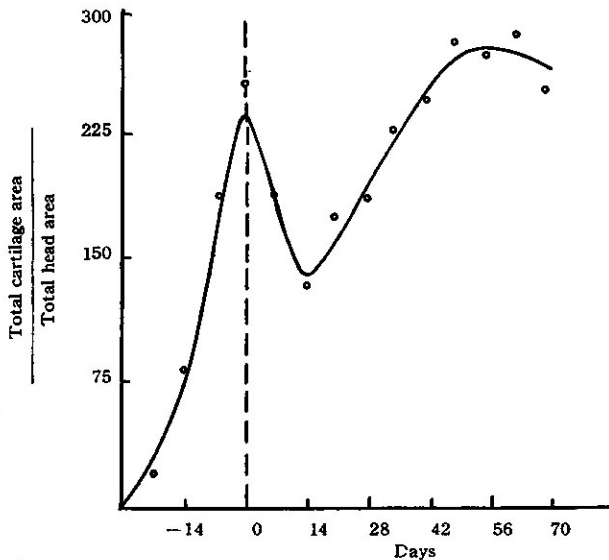
Fig. XXIII.

The reason for presenting figures XXII and XXIII in terms of area rather than volume is that the volume of an area curving over the top of an embryonic head cannot be accurately estimated either from a drawing or from the animal itself. The

fact that all the cartilages of the head are about the same thickness minimizes the error inherent in the method as used. The total area of the head was measured from a lateral view in the same way as the cartilage areas, the back of the head being considered as a perpendicular line drawn at the posterior end of the parachordal.

There are several possible explanations for the slackening off of cartilage development at hatching. A great many essential changes are taking place at this critical period in the life of the salmon. Most of the available energy of the embryo is turned to the effort of hatching to produce, for instance, the characteristic shell digesting enzymes. This could well result in a pause in the building of new structures. The act of hatching demands a great deal of movement on the part of the embryo which uses much energy. This movement and its utilization of energy continues in the free swimming larva. A new process is brought into being by the act of hatching, respiration by means of gills, another demand to cut down the amount of energy available for building new tissues. All of these features together at the time of hatching could cause the arrest in cartilage formation. Furthermore, it was found that not only did the cartilages already present stop growing, but also that few new ones made their appearance during this critical period. After three weeks, the embryo has recovered from the effects of hatching and has readjusted itself to the new mode of respiration; then cartilage development proceeds again.

The chemical explanation for this behaviour of cartilage will be interesting. Cartilage is known to be a mucoprotein, chondroitin sulphuric acid, but little else is known about its composition or how it is formed. Steudel and Osato[7] analyzed the shell of the herring egg and found no mucoprotein in the shell. They suggest that mucoprotein may be found in the jelly surrounding the shell. While the shell has never been seriously considered as a source of embryonic building material,

[7]Steudel & Osato. *Hoppe-Seyler's Zeitschrift fur physiologische Chemie,* **127**, 220, (1923).

the possibility should not be overlooked. Hayes[8], working on the chemical changes in the salmon embryo, has found the curve for fat to correspond closely to that shown in figure XXIII. It may be that when more is known about the chemistry of mucoprotein these facts may be correlated.

I am very grateful to Dr. F. R. Hayes, under whose direction this research has been carried on, and to Dr. D. Pelluet for her assistance and many suggestions.

[8]Hayes. *Journ. Biochem.* **24**, 3, 735, (1930).