TEXT-BOOK OF EMBRYOLOGY

MACMILLAN AND CO., Limited
LONDON · BOMBAY · CALCUTTA · MADRAS
MELBOURNE

THE MACMILLAN COMPANY
NEW YORK · BOSTON · CHICAGO
DALLAS · SAN FRANCISCO

THE MACMILLAN CO. OF CANADA, Ltd.
TORONTO

TEXT-BOOK

OF

EMBRYOLOGY

VOL. II

VERTEBRATA
WITH THE EXCEPTION OF MAMMALIA

BY

J. GRAHAM KERR

REGIUS PROFESSOR OF ZOOLOGY IN THE UNIVERSITY OF GLASGOW

MACMILLAN AND CO., LIMITED
ST. MARTIN'S STREET, LONDON
1919

TO

THE MEMORY OF

THREE CAMBRIDGE NATURALISTS

CHARLES DARWIN, M.A., CHRIST'S COLLEGE

FRANCIS BALFOUR, M.A., TRINITY COLLEGE

ADAM SEDGWICK, M.A., TRINITY COLLEGE

PREFACE

THE object of this volume is to sketch in its main outlines the science of Vertebrate Embryology as disclosed by the study of the non-mammalian vertebrates. It is not meant as a work of reference as regards details. The facts of embryology are dealt with as illustrating general principles: large masses of data which have no particular bearing, in the present condition of knowledge, are deliberately omitted.

It is believed that a volume upon the lines indicated is greatly needed—not merely for students intending to specialize in vertebrate morphology but also for students of medicine who desire to know something of the framework of morphological principles which serves to unite together the detailed facts of anatomy. The science of embryology, in fact the science of animal morphology as a whole, has suffered much through the patient but undiscriminating accumulation of masses of mere descriptive detail which have tended to obscure general principles and incidentally to smother interest in one of the most fascinating of sciences. It is hoped that the student who reads through portions of this book will have at least his suspicions aroused that behind the dull facts of anatomical structure there lies a very charming and living philosophy.

It has again been one of the misfortunes of vertebrate embryology that its teaching has been dominated in great part by general ideas based upon insufficient data. In an evolutionary science like morphology the real fundamental principles are to be elicited by enquiry into the more archaic types of existing animal life. But the material for the earlier embryological investigations was chosen not for its archaicism but rather for purely practical reasons such as accessibility or ease of investigation. It follows that at the present time when we have knowledge of the more archaic subdivisions of the vertebrata not accessible to the early builders of the science, it is necessary to regard the historical foundations of vertebrate embryology rather critically in the light of the fuller knowledge of to-day. In essaying the writing of this volume I have been fortunate in having at my disposal—for the first time in the history of embryology—developmental material of all three genera of Dipnoi as well as of *Polypterus*—in addition to the more accessible material of the other relatively archaic groups constituted by the Elasmobranchs, Actinopterygian Ganoids, and Urodele Amphibians. This has rendered possible an all-round survey of the chief problems of vertebrate embryology which would otherwise have been quite impossible.

As already indicated I do not intend this volume as a work of reference on the details of vertebrate embryology: that rôle is fulfilled by the wonderful and indispensable *Handbuch* edited by O. Hertwig—of which incidentally I have made constant use and to which I must express my sincere acknowledgments. Nor do I attempt to give full historical accounts of the development of various parts of the subject. The literature lists are merely guides to point the way to the student who desires to extend his reading to original papers. The dates given in these lists are as a rule the dates given on the title-page of the complete volume, and are merely to facilitate finding the particular paper: they must not be taken as giving the actual date of publication of the individual memoir.

I have to express my grateful thanks to various friends. As regards the first three chapters I had the benefit of the wise counsel of Mr. Walter Heape, who unfortunately however found himself compelled by the exigencies of war work to withdraw from the Editorship.

Various chapters have benefited by the help and advice of my friend and colleague, Dr. W. E. Agar. The entire volume has been read in proof by Mr. James Chumley and Dr. Monica Taylor, to both of whom I am deeply indebted. To Dr. John Love and to Dr. Jane Robertson I am indebted for helpful criticism in regard to special sections of the book.

The illustrations which form a marked feature of the volume I owe for the most part to the artistic skill, combined with high scientific accuracy, of Mr. Kirkpatrick Maxwell. Apart from the completely original figures it will be noticed that there are many which have been worked up from illustrations in original papers, but which are practically new figures. In all such cases, however, I have thought it only right to make due acknowledgment of the author of the original figure.

For permission to borrow particular text-book figures I have to thank Professor Frank R. Lillie, Mr. John Murray, Messrs. Masson & Cie, and Messrs. Macmillan. The present unfortunate circumstances of international strife call for a special acknowledgment of the generous way in which Professor Alf. Greil entrusted to me the originals of his valuable unpublished figures illustrating the development of the heart in the bird. They are reproduced on pages 384 and 385.

I have included the name of Charles Darwin in the dedication of this volume to emphasize the fact that Embryology is primarily a branch of synthetic evolutionary science. While the fashion of the day in evolutionary research favours rather experimental research into the phenomena of inheritance and more or less speculative enquiry into the ultimate mechanism of inheritance or into the possible causes of evolutionary change—morphology, and more especially embryology, is steadily at work all the while, mapping out the paths along which the evolution of organisms and their constituent organs has taken place. Working away in comparative seclusion, unadvertised, and for the most part unnoticed, embryology is thus building up an important part of the framework of what will be the permanent edifice of evolutionary science.

J. GRAHAM KERR.

February 3, 1919.

CONTENTS

CHAPTER I

CHAPTER II

CHAPTER III

CHAPTER IV

CHAPTER V

CHAPTER VI

CHAPTER VII

CHAPTER VIII

CHAPTER IX

CHAPTER X

CHAPTER XI

APPENDIX

CHAPTER I

SEGMENTATION, GASTRULATION, AND THE FORMATION OF THE GERM LAYERS

THE Vertebrate begins its individual existence in the form of a single cell, the **Zygote** or fertilized egg, which in turn originates in the process of fertilization by the fusion or conjugation of two gametes.[1] Of these the microgamete or spermatozoon, derived from the male parent, is of relatively insignificant bulk as compared with the macrogamete or unfertilized egg. As a consequence the more obvious features of the Zygote, such as shape, size, and so on, are simply taken over from the macrogamete—in other words, they are of maternal origin. Such maternal features may remain obvious for some time during early stages of development, so long in fact as the maternal protoplasm remains predominant in bulk as compared with that elaborated under the control of the Zygote nucleus, but it seems unnecessary to assume that this fact has the important bearing upon questions connected with Heredity which has been claimed for it by some workers on Invertebrates.

The Zygote is a typical **cell**, composed, so far as its living substance is concerned, of cytoplasm and nucleus, the cytoplasm containing a lesser or greater amount of food-material or yolk. In shape it is in the vast majority of cases approximately spherical. In the Myxinoids it is elongated, almost sausage-shaped, and in a certain number of cases, for example *Amia*, its shape is literally "oval."

The macrogamete—and therefore the Zygote—differs much in size in different Vertebrates, ranging from about ·1 mm. in diameter in *Amphioxus* to as much as 85 mm., or more, in the case of the African Ostrich. In some of the Sharks the size of the Zygote is also very great and this was doubtless the case too, with that of such extinct birds as *Aepyornis*.[2] Such relatively huge Zygotes are of

---

[1] A general account of the processes of gametogenesis and fertilization has already been given in Vol. I. and they are not further dealt with in this volume.

[2] Assuming that the Zygote of *Aepyornis* bore the same ratio in size to its protective envelopes as does that of the Ostrich it would measure about 160 mm. in diameter.

interest as being in bulk the largest single cells known in either the Animal or the Vegetable Kingdom.

The adjoining list based upon data obtained by various observers —M'Intosh and Masterman, Bashford Dean, Boulenger, Budgett, Bles, Semon, Salensky and others—brings out in more detail the differences in the size of the Zygote amongst the lower Vertebrates.

APPROXIMATE DIAMETER OF EGG (IN MILLIMETRES) OF VARIOUS FISHES AND AMPHIBIANS

Amphioxus ·1.
Petromyzon 1.
Bdellostoma 14 – 29 × 7 – 10·5.
Pristiurus melanostomus 16.
Acanthias 35 – 40.
Japanese Lamnid ? (Doflein) 220.
Torpedo ocellata 20 – 25.
Polypterus 1·1.
Acipenser 2 – 2·8.
Lepidosteus 3.
Amia 2·5 – 3 × 2 – 2·5.
Boccus labrax 1·4.
Mullus surmuletus ·9.
Cottus scorpius 1·75.
Trigla gurnardus 1·5.
Agonus cataphractus 1·8.
Trachinus vipera 1·3.
Scomber scombrus 1·2.
Gobius minutus 1 – 1·4.
Cyclopterus lumpus 2·5.
Anarrhichas lupus 5·5 – 6.
Pholis gunnellus 1·7.
Gadus collaris 1·4.
G. aeglefinus 1·4.
G. virens 1·1.
Motella mustela ·7.
Brosmius brosme 1·3.
Ammodytes lanceolatus ·76.
Hippoglossus vulgaris 3 – 3·8.
Bothus maximus 1.
Pleuronectes platessa 1·8.
P. microcephalus 1·3.
P. limanda ·75.
Solea vulgaris 1·2.
Clupea harengus ·9 – 1.
C. sprattus ·1.
Ceratodus 2·8.
Protopterus 3·5 – 4.
Lepidosiren 6·5 – 7.
Axolotl 2.
Salamandra maculosa 4.
Triton 1·8.
Necturus 6.
Hypogeophis alternans 4 – 5.
H. rostratus 7 – 8.
Xenopus laevis 1·5.
Pipa 6 – 7.
Alytes obstetricans 3 – 5.
Pelobates fuscus 1·5.
Bufo lentiginosus 1.
Hyla goeldii 4.
Nototrema fissipes 10.
Paludicola fuscomaculata 1.
Engystoma ovale 1·25.
Cornufer salomonis 5.
Rhacophorus reinwardtii 3.
Rana temporaria 2.
R. opisthodon 6 – 10.

Within the limits of a single genus different species may show marked differences in the size of their eggs, *e.g.* the Teleostean fish *Arius australis* has eggs a little over 3 mm. in diameter (Semon) while in the case of *A. boekii* they measure over 10 mm. in diameter and in *A. commersonii* as much as 18 mm.

Even within the limits of a single species quite measurable, though less conspicuous, differences in size exist between the eggs of different females, and the same holds also, though to a far less extent, for the individual eggs laid by a single female.

The differences in size which have just been alluded to are correlated with the fact that the egg of the Vertebrate carries in its cytoplasm a less or greater amount of reserve food-material or **yolk**. The presence of a readily available supply of food within the egg carries with it the immense advantage of freeing the young animal, during the early stages of its development, from the need of having to fend for itself. And, correlated with this, the necessity of developing more or less complicated adaptive features to fit it for survival as an independent free-living creature in these early stages is avoided.

The yolk consists occasionally of fluid but more usually of rounded or sub-angular granules of highly nutritious material. The yolk granules are frequently of a characteristic colour, yellow or salmon colour or greenish, and these impart their colour to the egg as a whole. Where however the yolk becomes very finely subdivided we find, as in the case of coloured glass ground into powder, that the characteristic colour is replaced by white. This fine subdivision of the yolk with its accompanying white colour is commonly found in parts of the egg where metabolism is particularly active, for example those portions in which active growth or cell division is about to take place, the fine subdivision making the yolk readily assimilable and so available for metabolic needs. Where the yolk is comparatively small in amount, as in *Amphioxus* (Fig. 1, A), it may be distributed nearly equally throughout the egg substance; in other words there is an approach

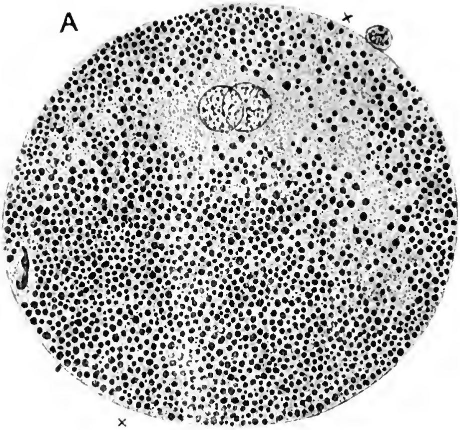
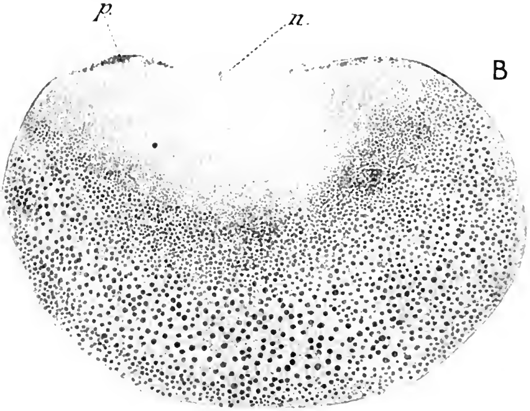


Fig. 1.

A.—Section through egg of *Amphioxus*. After Cerfontaine, 1906.)

The small crosses mark the position of apical and abapical poles. Near the apical pole is seen the second polar body adherent to the surface of the egg. The egg- and sperm-nuclei have not yet fused, and are seen in close proximity to one another.



B.—Section through egg of *Polypterus*, showing more marked tendency for the yolk granules to concentrate towards the abapical pole (Telolecithal condition).

n, nucleus. *p*, pigment.

The yolk granules are indicated in both figures by dark dots.

to the **isolecithal** condition: but as a rule in the Vertebrate the yolk is large in amount and is concentrated towards the lower or abapical pole of the egg, the protoplasm towards the upper or apical pole being comparatively poor in yolk (**Telolecithal** condition).

This segregation of the dead yolk and the living protoplasm towards opposite poles of the egg is well seen in the relatively huge egg of the bird where the protoplasm is concentrated in a **germinal disc** containing practically no yolk and forming a cap at the apical pole of an enormous mass of yolk practically free from protoplasm.

It has already been indicated that the egg may have a characteristic coloration due to the colour of the yolk. Such yolk coloration may be looked upon as accidental and without any special biological significance in itself. Many eggs on the other hand especially amongst the Ganoid fishes and the Amphibians are given a dark colour by the presence within them of brownish-black pigments belonging to the melanin group. Such pigment appears to be of definite biological significance, providing as it does an opaque coat which protects the living protoplasm from the harmful influence of light. Eggs in which it occurs develop, as a rule, under conditions where they are exposed to intense daylight. The eggs of ordinary Frogs and Toads for example which are surrounded by clear transparent jelly have a well-developed pigment coat. On the other hand in the case of Frogs and Toads whose eggs are surrounded by light-proof foam (see Chapter VIII.) or are deposited in burrows underground they are commonly without pigment.

In all probability this deposition of melanin pigment in the superficial protoplasm of the egg (normally in its *upper* portion) is to be interpreted as having been originally a direct reaction to the influence of light, the metabolism being so affected as to bring about the formation of this particular iron-containing excretory pigment.

It may be objected that the pigment is produced before the egg is laid (*e.g.* the Common Frog) and therefore before it is exposed to the action of light, but as a matter of fact the body-wall of the adult is by no means opaque to light rays and even while still in the ovary the eggs are exposed to the influence of faint light. If we may take it, as seems probable, that the influence of natural selection has gradually developed in such cases the particular type of sensitiveness to light which leads to the formation of melanin, on account of its protective value, then there is nothing surprising in the developing of this sensitiveness at earlier and earlier periods until at last it has resulted in the pigmentation of the still intra-ovarian egg in response to the feeble light rays which penetrate the body-wall.

The other possible explanation of this precocious pigment formation is that the production of the pigment though originally taking place as a direct reaction to light in the laid egg, has become so engrained in the constitution of the species that it now comes about even in the absence of the original stimulus. The objection to this explanation is that it postulates the inheritance of an "acquired character," and that is unfortunately not justified by our knowledge so far as it goes at present.

SEGMENTATION

The first important steps in the evolution of the unicellular Zygote into the multicellular adult are seen in the process of **Segmentation** which is, in fact, a process of mitotic cell division showing special peculiarities in different groups of the Vertebrata. During this process there appear in succession on the surface of the egg grooves which gradually deepen and eventually divide the egg incompletely or completely into distinct segments or **Blastomeres**. Before entering into the details of this process it will be convenient to describe it in outline and define the various technical terms used in its description.

The first phase of segmentation is commonly marked by the appearance of a superficial groove which may conveniently be designated by the letter *a*, passing through both the upper and lower poles of the egg. Such a groove or furrow is termed **meridional**, as it marks a great circle on the surface of the egg corresponding to a meridian of longitude on a terrestrial globe. The single nucleus of the Zygote meanwhile divides by mitosis — a daughter nucleus passing into each hemisphere. From the known facts of fertilization we have reason to believe that the Zygote nucleus contains exactly equivalent amounts of chromatin from each of the two parents. In the process of mitosis this maternal and paternal chromatin is again shared equally between the two daughter nuclei.

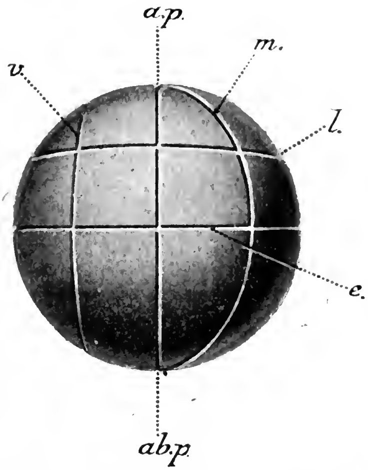


Fig. 2.—Diagram to illustrate technical terms used in describing the process of segmentation.

a.p, apical pole; *ab.p*, abapical pole; *e*, equatorial furrow; *l*, latitudinal furrow; *m*, meridional furrow; *v*, vertical furrow.

The first meridional furrow gradually deepens so that the egg becomes completely divided into two blastomeres or segments each representing a hemisphere of the Zygote. A second meridional furrow (β) now appears in a plane perpendicular to that of the first and by the deepening of this the egg becomes divided into four equal blastomeres.

The next furrow to appear may be one running round the equator of the egg (**equatorial**). In eggs, however, which are not absolutely isolecithal—and this holds for all the lower Vertebrates—

the third furrow appears, not at the equator but at a level nearer the apical pole, and is termed a **latitudinal** furrow, corresponding as it does with a parallel of latitude upon a terrestrial globe. The distance of this furrow from the equator, its degree of latitude so to speak, is roughly proportional to the degree of telolecithality of the particular egg, suggesting that the volume of living protoplasm may be roughly equivalent in amount upon the two sides of the division plane to which this furrow gives rise.

When this third division is completed the egg consists of eight blastomeres, the four on the apical side of the division plane being smaller (**micromeres**) than those on the abapical side (**macromeres**).

The next furrows to appear are two in number and in the simplest condition they are meridional, bisecting the angles between the two first furrows. More frequently however those furrows instead of traversing the pole of the egg are discontinuous at this point and each is displaced somewhat so as to join the first or second meridional furrow at a less or greater distance away from the pole. To such a furrow we apply the term **vertical** (Fig. 2, *v.*, cf. also Figs. 14 and 16, C).

It is as a rule noticeable that meridional or vertical furrows tend to become apparent first in their portions nearest to the upper or apical pole of the egg, their lower ends gradually extending downwards towards the abapical pole. This phenomenon appears to be due to the retarding influence of the dead and inert yolk. The proportion of this to the living protoplasm becomes greater and greater as the distance from the apical pole is greater, and in correlation with this the retarding effect becomes more and more pronounced.

After segmentation has reached the stage indicated its further progress tends to become irregular. New furrows make their appearance—latitudinal, and vertical or meridional—and the surface of the egg takes on the appearance of a mosaic-work, while its substance becomes cleaved or split apart into corresponding blastomeres as the superficial furrows gradually deepen into slits.

At somewhere about this period there begins a new type of mitotic division in which the individual blastomere becomes split in a direction parallel to a plane tangential to its outer surface, so that it divides into an outer blastomere visible in surface view and an inner one concealed in the interior of the egg.

With the further progress of segmentation the blastomeres divide over and over again, so that eventually the egg is converted into a very large number of small cellular elements. The rapidity with which the cells divide bears a rough inverse relation to the richness of their contents in yolk. Dead inert yolk tends to cause the cell to lag behind in the process of division, and the result of this in telolecithal eggs is that the difference in size between micromeres and macromeres becomes more and more marked as segmentation goes on—the lower and more richly yolked segments tending to lag, in their mitotic division, more and more behind the less yolky upper elements. This inequality is found at its maximum in the large eggs of Elasmobranchs, Reptiles, and Birds, where the main mass of the egg has its proportion of protoplasm reduced so nearly to vanishing point that it does not divide at all. It is only a small portion of the egg in the neighbourhood of the apical pole that is rich enough in protoplasm to carry out the process of segmentation into separate cells. This is known as the **germinal disc** or, later on, when it has segmented into a mass of cells, **blastoderm.** An egg of such a type, showing partial or incomplete segmentation, is termed **meroblastic** in contrast with the more primitive **holoblastic** type in which the egg segments as a whole.

The blastomeres into which the egg divides being composed of protoplasm—a somewhat viscous fluid—tend under the physical laws of surface tension to assume a spherical shape except when flattened by pressure against their neighbours. There thus exist normally chinks between the blastomeres filled with watery fluid.

As the process of segmentation proceeds this intercellular fluid increases in amount and the process normally culminates in the stage known as the **blastula.** The blastula consists of a more or less spherical mass of cells surrounding a relatively considerable volume of fluid which is for the most part no longer distributed in small chinks but collected together into a large space—the **blastocoele** or segmentation cavity.

In the simplest case, that of *Amphioxus*, the wall of the blastula is composed of a single layer of cells—the cells towards one pole being larger and containing fine granules of yolk or food material. In holoblastic Vertebrates above Fishes it is however, as a rule, no longer composed of a single layer, the roof of the segmentation cavity being frequently composed of two layers while the floor is composed of a thick mass of large heavily yolk-laden cells.

The details of the segmentation process may now be followed out as it occurs in the various types of lower Vertebrates.

AMPHIOXUS

Amphioxus is, of all the lower Vertebrates, that in which developmental processes are least interfered with by the presence of yolk, and for this reason the phenomena shown during its segmentation must form the basis for the comparative study of the corresponding phenomena in the Vertebrata in general.

The process of segmentation in *Amphioxus* was described first in two works which are now amongst the classics of morphological science: the first by A. Kowalevsky (1867) and the second by B. Hatschek (1881).

The process begins (Fig. 3) with the appearance of a depression of the surface in the region of the apical pole. This depression takes an elongated groove-like form and extends outwards at each end until finally it forms a wide meridional valley encircling the entire egg (Fig. 3, B). This valley gradually deepens dividing the egg into two halves. Finally after about 5 minutes from the commencement of the process the protoplasmic bridge connecting the two halves snaps across and the egg is now completely divided into two blastomeres, each of which assumes a spherical shape in response to surface tension. The two blastomeres become slightly flattened where they are in contact *i.e.* in the plane of the first meridional furrow (Fig. 3, C). The future course of development shows that this plane corresponds to the sagittal plane of the embryo (Cerfontaine, 1906): in other words the two blastomeres represent the right and left halves of the developing individual.

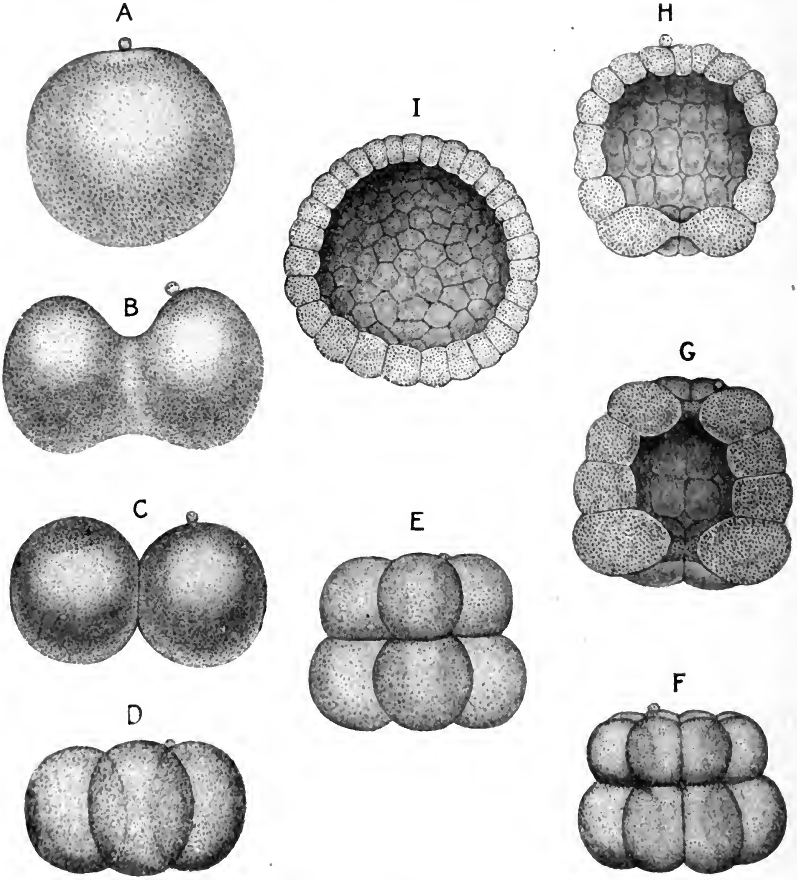


FIG. 3. Illustrating the process of segmentation of the egg of *Amphioxus*. (After Hatschek, 1881.)

The apical pole is above in each figure. The second polar body is seen in proximity to it.

After a resting period of about an hour a second meridional furrow develops in a manner similar to the first and in a plane perpendicular to the plane of the first furrow. This gradually deepens and each hemisphere becomes divided into two blastomeres, each of which as before assumes a spherical shape and then becomes flattened out slightly against the other. Of the four blastomeres which are now present two, shown by subsequent development to be anterodorsal in position, are according to Cerfontaine normally smaller than the other two.

The two meridional furrows (α and β) are followed after an interval of about a quarter of an hour by a latitudinal furrow slightly above the equator and this divides each of the four segments into two. The egg now consists of eight blastomeres—four smaller micromeres on the apical side of the latitudinal division plane, and four larger macromeres upon its abapical side. Each micromere lies, according to Hatschek, exactly over the corresponding macromere so that the apical side of the egg as seen from above looks like A in Fig. 4.

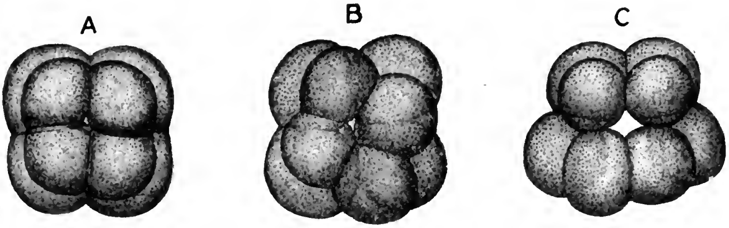


FIG. 4.—Apical view of Amphioxus eggs at the eight-blastomere stage. (After E. B. Wilson, 1893.)

A, "Radial" type; B, "Spiral" type; and C, "Bilateral" type.

Wilson (1893), followed by Samassa (1898), has however drawn attention to the fact that in a considerable proportion of cases the cap of four micromeres is, as seen from above, rotated in a clockwise direction through an angle varying from 0° to 45° (Fig. 4, B) thus conforming to Wilson's "spiral" type of segmentation or cleavage. Again in a still smaller percentage of eggs at this stage the blastomeres are arranged according to Wilson's "bilateral" type (Fig. 4, C) the eight blastomeres being arranged symmetrically on each side of the first division-plane but either two or all four macromeres being displaced outwards somewhat from this plane.

Fourth division. After another short interval (less than a quarter of an hour) a new set of furrows appear bisecting each of the already existing blastomeres so that the embryo comes to consist of sixteen blastomeres arranged in two tiers, eight micromeres above and eight macromeres below (Fig. 3, F). Hatschek described this fourth set of furrows as being meridional (Fig. 3, F) while according to Cerfontaine (1906) the division planes are when first indicated meridional in position but become displaced somewhat so as to be in the case of the micromeres perpendicular to the first (sagittal) division-plane or in that of the macromeres slightly oblique.

Fifth division. Each blastomere divides again,[1] the smaller blastomeres towards the apical pole dividing rather earlier than the others, and the result is that there are now thirty-two blastomeres in all, arranged in eight meridional rows of four cells each, the cell at the lower (abapical) end being decidedly larger than the others. Between these four large elements a wide opening is present (Fig. 3, G) leading into a space which made its appearance as a little chink between the blastomeres of the four-cell stage but which has since then increased greatly in size. This space in the interior of the egg is the blastocoele or segmentation cavity.

From this period onwards the segmentation process becomes less regular. There has already shown itself a tendency for the larger blastomeres towards the lower pole to lag behind somewhat. And the arrangement of the blastomeres becomes less regular as they become smaller and fit more closely together. In particular the bilateral symmetry in the arrangement of the blastomeres which is conspicuous in most of the eggs during the earlier stages (Cerfontaine) ceases to be apparent.

To summarize the later phases of segmentation it may simply be said that the blastomeres go on dividing, the segmentation cavity increases in size, its communication with the exterior closes up, and there is formed eventually a blastula of approximately spherical shape. The wall of the blastula is composed of a single layer of cells those towards the apical pole being smaller and less rich in yolk than those on the opposite side (Fig. 3, I).

RANA

In the case of the Frog we have an egg in which as compared with that of *Amphioxus* there is present a much greater proportional amount of yolk and which in consequence serves well to illustrate the nature of the influence of yolk upon segmentation.

The process of segmentation begins with the appearance, in the region of the apical pole, of a small dimple on the surface of the egg which gradually lengthens out to form the first meridional furrow (α). The furrow gradually extends downwards over the surface of the egg (Fig. 5, A) and becomes completed by reaching the lower pole after about an hour and a quarter.[2] It also extends inwards from the surface and finally bisects the egg into two hemispheres.

The second furrow (β) is also meridional and is in a plane

---

[1] As there are marked discrepancies between the accounts given by different observers we may take it as probable that there is considerable variability in the details of segmentation about this stage.

[2] See, however, later for caution in reference to the time factor in development.

perpendicular to that of the first. It appears about three-quarters of an hour after the latter and, like it, extends downwards and inwards so that the egg becomes divided into four approximately equal segments.

The third furrow is latitudinal in position being situated (Fig. 5, C) roughly about 20° above the equator. It extends inwards and the egg is now converted into eight blastomeres, four micromeres towards the apical pole and four macromeres towards the lower pole.

Closer study of these first three cleavages in the case of the Frog brings out a number of important points. It will be noticed in Fig. 5 that the circular area of the egg-surface which is free from pigment is placed somewhat eccentrically so that at one edge it approaches the equator of the egg much more nearly than it does at the opposite edge. It will be noticed further that the egg as judged by the distribution of pigment is arranged symmetrically about the plane of the first furrow. This furrow seems to correspond, under normal conditions, with the sagittal plane of the embryo, and therefore the two hemispheres separated by the first furrow correspond to the right and left halves of the embryo. The study of later stages will bring out the fact that the point in the boundary of the unpigmented portion which lies nearest to the equator marks what will become the posterior end of the embryo.

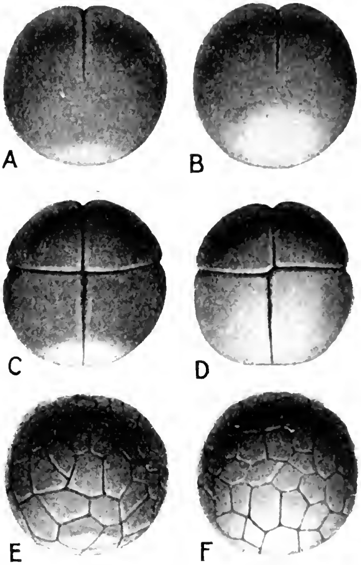


FIG. 5.—Illustrating segmentation of Frog's egg. (After Schultze, 1899.)

From the time of appearance of the third furrow onwards wide differences occur between different eggs. Occasionally one may be found in which matters proceed with diagrammatic regularity. Two new meridional furrows appear intersecting the angle between α and β and like the latter they gradually extend downwards, halving each of the existing blastomeres and thus giving rise to sixteen blastomeres—in two tiers of eight, micromeres above, macromeres below. Then a latitudinal furrow appears dividing the micromeres, and later a similar furrow dividing the macromeres; so that there are now four tiers of eight blastomeres each.

Commonly however there is no such regularity either in the arrangement or in the time of appearance of the furrows. The meridional furrows in particular tend to be replaced by vertical furrows which intersect α or β at some distance from the poles. As regards the variation in order of development of the various furrows a good idea will be got from Fig. 6.

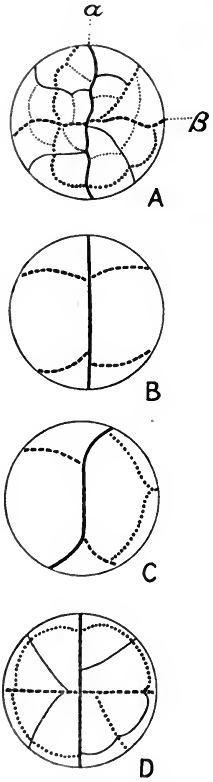


FIG. 6.—Illustrating the variation in the order of appearance of the first cleavage furrows in *Rana palustris*. (After Jordan and Eycleshymer, 1894.)

The sequence, in time, of the appearance of the furrows is indicated as follows:—1, ━━━━ ; 2, ╍╍╍╍ ; 3, ········ ; 4, ──── ; 5, .

Whatever be the case with the divisions immediately succeeding the eight cell stage, from now onwards there is little regularity. All that can be said is that each individual blastomere goes on dividing over and over again, the length of time elapsing between successive divisions bearing a rough relation to the amount of yolk present in the particular blastomere.

Already at the third cleavage the eight blastomeres have a distinct chink—the commencing blastocoele—between their inner ends and as segmentation goes on this space becomes larger. The thirty-two-cell stage is a blastula which in a meridional section (Fig. 7, A) is seen to correspond in its general character with the blastula of *Amphioxus* but to differ from it in three features: (1) it is of larger size, (2) it is composed of fewer cells and (3) the difference in size between the less richly yolked cells towards the apical pole and the more heavily yolked cells towards the opposite pole is more marked.

As development proceeds a farther difference becomes apparent. In the various mitotic divisions during the preceding phases of segmentation the axis of the spindle has been arranged more or less tangentially but now divisions begin to take place in which the spindle axes are arranged radially and the division-planes tangentially. When this happens one of the two resulting daughter cells is nearer the centre, the other nearer to the surface of the blastula and the effect of repeated divisions of this type is that the blastula-wall loses its original character of being composed only of a single layer of cells and becomes several cells thick (Fig. 7, B).

ELASMOBRANCHS

The egg of any ordinary Elasmobranch such as a Dogfish, Skate, or Torpedo, illustrates the type of segmentation that takes place in an egg in which the proportion of yolk present approaches the maximum. In this case the zygote nucleus commonly undergoes two mitotic divisions before there is any external symptom of segmentation of the cytoplasm. Usually a single furrow makes its appearance first, incising the surface of the germinal disc but not extending to its periphery (Fig. 8, A). Occasionally a second regular furrow makes its appearance intersecting the first at right angles and it is a curious point that it is sometimes this second furrow which corresponds to the first nuclear division.

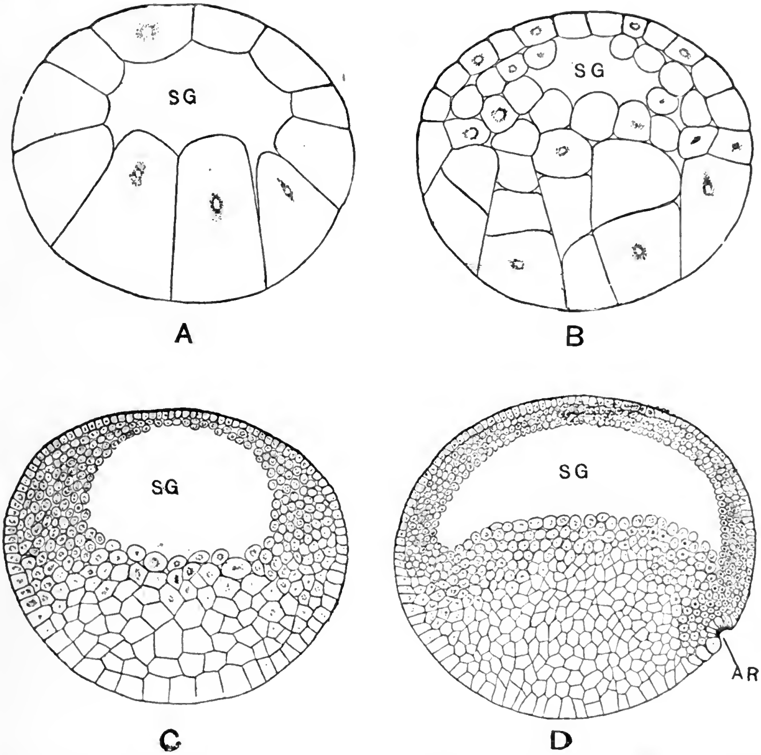


Fig. 7.—Vertical (meridional) sections through blastulae of Frog. (From Morgan, 1897.) *AR*, commencing invagination; *SG*, segmentation cavity.

These first two furrows apparently represent the first two meridional furrows of the holoblastic egg though in the Elasmobranch the first to appear may be either α or β. More usually, in place of a second regular furrow developing, irregular branches of the first furrow, or even independent furrows, appear and an arrangement of somewhat radiating furrows is brought about which gradually becomes converted into a network (Fig. 8, B, C, D).

It should be noticed in regard to these segmentation furrows that the first latitudinal furrow cannot be identified in the Elasmobranch and further that the study of sections shows that the furrows sometimes cut into the germinal disc obliquely instead of being perpendicular to the egg surface.

The nuclei of the blastoderm divide synchronously and after four divisions have taken place, when there are sixteen nuclei in place of the original single zygote nucleus, the segmentation furrows (Fig. 8, C) form a network dividing up the blastoderm into smaller central and larger peripheral blastomeres. These blastomeres are, however, not completely isolated from one another but are still in continuity at their bases, and, in the case of the peripheral blastomeres, at their outer ends.

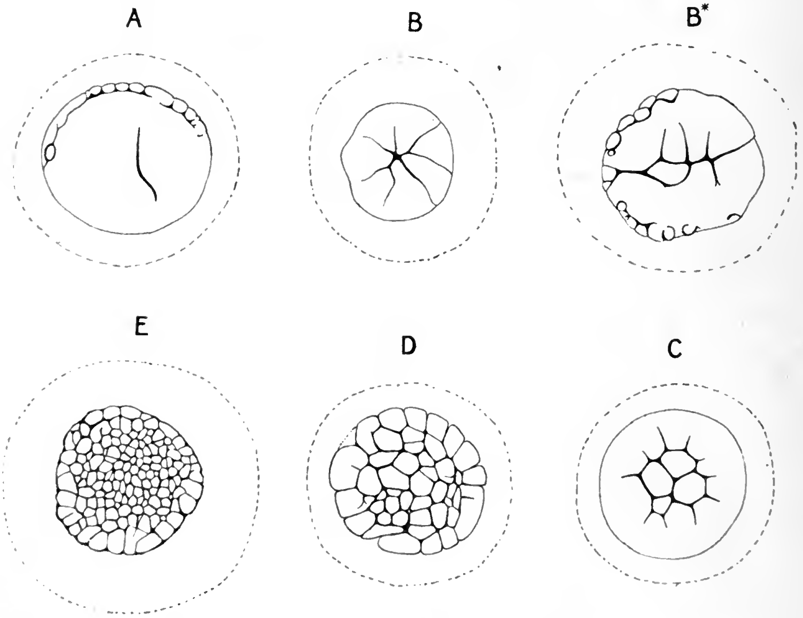


FIG. 8.—Surface views of the blastoderm of Elasmobranchs illustrating the process of segmentation. (After Rückert, 1899.)

A, B, C, *Torpedo*; B*, *Pristiurus*; D, E, *Scyllium*. Fig. B* shows an abortive segmentation which may often be observed transitorily round the margin of the germinal disc [Merocyte segments of Ruckert].

Up to the fifth mitotic division the axes of the mitotic spindles have been approximately parallel to the surface but now blastomeres begin to divide with their spindle axes perpendicular to the surface so that a set of superficial segments becomes separated off. Beneath these fluid accumulates intercellularly and a segmentation cavity arises (Fig. 9, B).

During the sixth division some of the blastomeres forming the floor of the segmentation cavity become separated off from the underlying, unsegmented, yolk (Fig. 9, C) and in surface view the blastoderm assumes the appearance shown in Fig 8, D.

Up to and including the seventh division mitosis takes place practically synchronously throughout the blastoderm. In *Torpedo* Rückert (1899) found that even in the ninth division the majority of the nuclei still divided synchronously and that in some eggs the same was the case with the tenth division but in any case approxi-

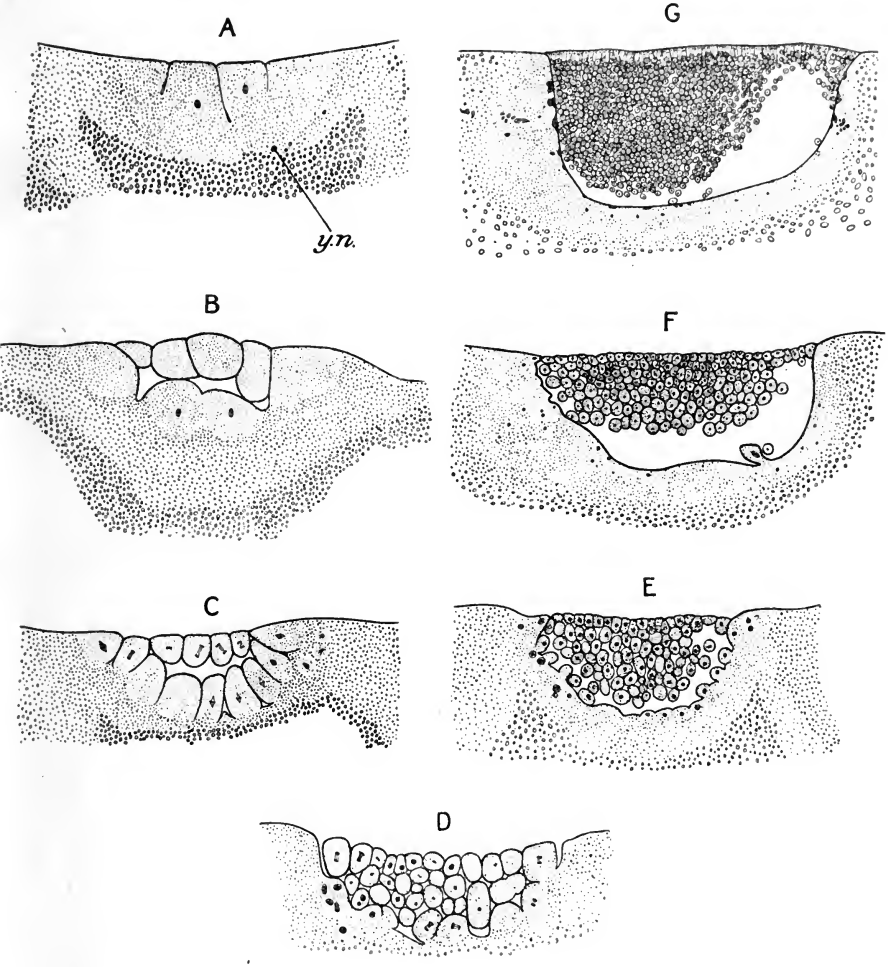


Fig. 9.—Vertical sections through Elasmobranch blastoderms illustrating the process of segmentation. (After Rückert, 1899.)

A, C, D, E, *Torpedo*; B, *Scyllium*; F, G, *Pristiurus*. E, F, and G are sagittal sections with posterior edge of blastoderm to the right.

mately about this period individual nuclei lag behind others and the regular rhythm becomes lost.

This rhythm of nuclear division is of interest in relation to the size of the individual blastomeres. It is often noticeable in an Elasmobranch blastoderm that the blastomeres are somewhat smaller in what is shown by later development to be its posterior half *i.e.* the half next the side on which the embryonic rudiment makes its appearance later. It would be natural to suppose that the smaller size of the blastomeres is due to their having gone through a greater number of divisions but this explanation is rendered less satisfactory by the synchronism of the mitotic divisions. Apparently the inequality is at least to some extent due to the zygote nucleus, and, later on, the first segmentation furrows, being not quite central in position in the germinal disc but situated slightly towards its posterior edge (Rückert).

The stage up to which mitosis remains synchronous varies amongst individuals of one species and *a fortiori* amongst those of different species and genera. Thus in *Pristiurus* it is, commonly, regular only up to the fifth mitosis according to Rückert.

While segmentation has been proceeding, important changes have been taking place in the segmentation cavity. About the time of the seventh division the rounded inner blastomeres fill up most of the cavity so that it becomes reduced to chinks between the individual blastomeres. These chinks are filled with fluid secreted by the egg substance, and in the yolk beneath the blastoderm the activity of this process of secretion is indicated by the appearance of fluid vacuoles.

As development goes on the amount of fluid increases greatly and about the tenth division it begins to collect especially between the blastoderm and the yolk, forming the "germ cavity" of Rückert (Fig. 9, D, E, F). This cavity is best marked towards the posterior side of the blastoderm and in ground-plan is crescentic in shape. It varies greatly in its degree of development in different individuals.

Whether it is advisable to use a separate name for this cavity is very questionable. When a broad view is taken of the relations of blastomeres and segmentation cavity in the Elasmobranchs these seem to be similar in kind to those which hold in the case of the Lung fishes. In these fishes, as will be shown later, the blastomeres which originally formed the floor of the segmentation cavity become later on shifted in position towards its roof but the resultant change in the topographical relations and form of the segmentation cavity would clearly afford no valid reason for giving it a new name.

The Yolk Syncytium.—The layer of substance immediately underlying the blastoderm and segmentation- or germ-cavity is distinguished from the main mass of yolk upon which it in turn rests by the finer grained character of its yolk granules, and by its greater richness in protoplasm. This layer shows no division into cells and is therefore termed the yolk-syncytium[1] (H. Virchow: Rückert's term "merocytes" is synonymous). The marginal portion round the edge of the blastoderm is sometimes termed the germ-wall.

Functionally the yolk-syncytium is apparently concerned especially with the digestion and assimilation of the yolk. Scattered about in it are nuclei, often of enormous size and irregular form. Concerning the origin and fate of these nuclei much discussion has raged and the matter cannot yet be regarded as satisfactorily settled. The question is complicated by the fact that, as shown by Rückert (1890), polyspermy appears to be a normal occurrence in Elasmobranchs. In addition to the single microgamete which takes part in the formation of the zygote-nucleus a variable number of extra spermatozoa make their way into the egg and give rise to accessory sperm-nuclei. Where such sperm-nuclei are situated in the coarse yolk they apparently soon degenerate but when, on the other hand, they are within the protoplasm of the germ-disc they remain during the early stages of development in a living and apparently healthy condition, even undergoing mitosis synchronously with the nuclei derived from the zygote-nucleus up to the fourth or even fifth or sixth division in the case of *Torpedo.*

The importance of this fact should be noted in connexion with our ideas of the reciprocal physiological relations of nucleus and cytoplasm. It is fully recognized that the nucleus governs and controls cell metabolism: it is not always so fully recognized that conversely the cytoplasm exerts an important influence over the nucleus. Clearly the fact that the accessory sperm-nuclei "keep step" in their mitotic divisions with the embryonic nuclei must be due to some influence exerted on the former nuclei through the cytoplasm. It should, in fact, never be forgotten that cytoplasm and nucleus are merely locally specialized portions of the same common living substance or protoplasm.

At first the accessory sperm-nuclei are clearly distinguishable in the germinal disc from the true embryonic nuclei by their smaller size and reduced (**haploid**) number of chromosomes. After the zygote-nucleus has undergone two mitoses however—or even before the second mitosis—the accessory sperm-nuclei wander—or become transported by cytoplasmic movements—outside the limits of the germ-disc. They continue their mitotic rhythm for a time so that, for example, at the 8-nuclear stage of the blastoderm they may be seen in groups of eight lying in the yolk-syncytium. During early stages of segmentation numerous such obviously accessory sperm-nuclei may be seen in the syncytium but as time goes on the nuclei

---

[1] Although Haeckel originally defined the term syncytium (*Die Kalkschwämme*, Bd. I. p. 161) as a protoplasmic mass *formed by the fusion of originally separate cells* the word has come into such general use for a multinucleate mass of protoplasm which shows no subdivision into cells, whatever its origin may have been, that there seems no serious objection to the use of the term yolk-syncytium as suggested by Virchow.

of the syncytium are seen to be of a different character. They are now of enormous size and of peculiar lobed appearance. The lobing becomes more complex as time goes on and appears to be due to incomplete and irregular attempts at amitotic division.

The discussions, alluded to above, have centred round the mode of origin of these highly characteristic giant nuclei. Balfour, who first described them (1874), did not express any opinion as to their origin. Rückert in his first paper (1885) on Elasmobranch development looked on them simply as specialized embryonic nuclei and gave the masses of protoplasm in which they are embedded the name "merocytes." Latterly however Rückert, after his discovery of polyspermy in Elasmobranchs, has taken the view that the yolk-nuclei are really the accessory sperm-nuclei before alluded to which have altered their character in correlation with the altered environment in which they find themselves after leaving the germinal disc.

In spite of Rückert's more recent observations and conclusions, and in spite of their being supported by Samassa, Beard and others, it must, I think, be admitted that the sperm-origin of the yolk-nuclei is by no means demonstrated. And all general considerations are in favour of Rückert's earlier view being the correct one, namely that the nuclei of the yolk-syncytium are genetically of the same order as the ordinary embryonic nuclei. Such general considerations render it extremely improbable that accessory gamete nuclei should really play an important physiological part in the developing embryo: it is far more probable that such nuclei simply persist for a time, undergo mitosis a few times and then degenerate and disappear.

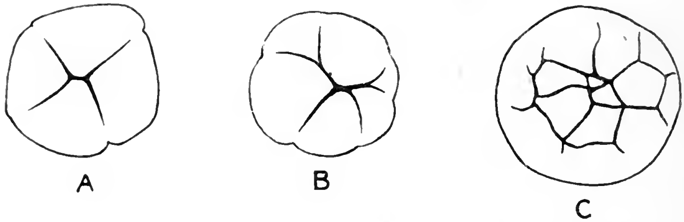


FIG. 10.—Views of the segmenting germinal disc of *Bdellostoma stouti*. (After Bashford Dean, 1899.)

The variations in the process of segmentation are well illustrated by the three cases just described and it will be convenient now to summarize the general characteristics of the process in the various remaining groups.

LAMPREYS.—In the Lamprey the phenomena of segmentation agree closely with those observed in the frog and need not be further described.

MYXINOIDS.—In the Myxinoids the somewhat sausage-shaped egg is heavily yolked and possesses a germinal disc situated close to one pole. A few segmentation stages of *Bdellostoma* (Fig. 10) have been described by Bashford Dean (1899) and as might be expected the segmentation is meroblastic. Apparently the first two furrows (α and β) have the normal meridional arrangement the specimen figured by Dean (Fig. 10, A) showing a displacement at the intersection of the two furrows. These latter do not reach the edge of the germinal disc. The third set of furrows (Fig. 10, B) appear to be vertical and in the next stage figured (Fig. 10, C) the furrows have become joined up to form an irregular network which still barely reaches the edge of the blastoderm.

CROSSOPTERYGIANS. — Our knowledge rests entirely on the observations of Budgett (Graham Kerr, 1907). These, necessarily fragmentary, observations suffice to show that the process of segmentation is of great interest. In the earliest stage observed, but not figured, by Budgett the egg was "segmenting in four equal portions, the constrictions being deeper than in the frog." A second egg (Fig. 11, A and B) is in the eight-blastomere stage. The blastomeres are practically equal in size and it may be inferred with considerable probability that in *Polypterus* two meridional furrows are succeeded by a latitudinal one which is very nearly equatorial. The nearness of the latitudinal furrow to the equator is remarkable in view of the fact that the egg of *Polypterus*, as shown by the study of sections (Fig. 1, B, p. 3), is not by any means nearly isolecithal.

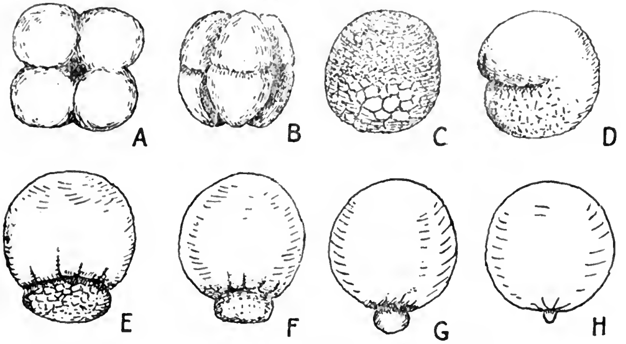


FIG. 11.—Segmentation and gastrulation in *Polypterus*. (Drawings by Budgett. Graham Kerr, 1907.)

A, represents a view of the apical pole: the remaining figures are side views.

ACTINOPTERYGIANS.—The typical Teleost is characterized by the fact that its richly yolked eggs show a more complete segregation of protoplasm and yolk than do those of any other Vertebrata. In correlation with this the segmentation is here the most markedly meroblastic in character. These features suggest that in the ancestral Teleost the yolk was large in quantity and that the egg as a whole was of great size. Amongst present-day Teleosts however it is only, comparatively speaking, a few forms mostly inhabiting fresh water, which produce eggs of very large size *e.g. Gymnarchus niloticus* (Budgett, 1901; Assheton, 1907) where they measure about 10 mm. in diameter, or the Salmon or Trout where they measure from 4 to 5 mm.

The majority of fishes produce eggs in enormous numbers, amounting in some cases to several millions, and correlated with this the size of the individual egg has become much reduced. The average diameter of a Teleostean egg may be taken as about 1 mm. In an egg of this size segmentation of so markedly meroblastic a character would be puzzling except on the hypothesis that the meroblastic condition had arisen in ancestral forms in which the eggs were much larger.

The larger part of the egg consists of a spherical mass of practically pure yolk. On the surface of this is a thin layer of protoplasm containing droplets of oil, and this layer of protoplasm is more or less distinctly thickened in the region of the apical pole to form a germinal disc in which is contained the nucleus. Irregular prolongations of the superficial protoplasm may sometimes, especially in immature eggs, be traced inwards into the substance of the yolk.

A characteristic feature of many teleosts is the tendency for the yolk to assume a liquid form. This is particularly marked in many pelagic eggs where it is not merely liquefied but runs together at the time of spawning or of fertilization to form a sphere of glassy transparency. There may further be, interspersed amongst the ordinary yolk, droplets of oily looking fluid often with a distinctive colour. These may unite into a few droplets or into a single larger drop forming a conspicuous, often coloured, sphere in the midst of the ordinary yolk. The colour and size of such drops frequently afford an easy means of recognizing the species to which a particular egg belongs. They may also have a characteristic position and may be surrounded by a special condensation of protoplasm or, on the other hand, they may simply float freely in the main mass of fluid yolk. Although these droplets may, as already indicated, exhibit peculiarities characteristic of particular species they do not seem to give indications of genetic affinity in regard to genera or larger groups: nor do they show any definite relation to the conditions, pelagic or otherwise, under which the egg develops (Prince, 1886).

The yolk of teleosts is also characterized by a diminution of its specific gravity which causes the egg to assume a reversed position with the apical pole below, and which further, in the case of a vast number of marine fishes, causes the egg as a whole to float freely suspended in the sea water.

Seeing that the Teleostei as a group is above all characterized by specialization for a swimming existence, independent of a solid substratum, we are perhaps justified in assuming that the freely floating pelagic mode of development above mentioned was originally present throughout the group. The **demersal** type of development, where the eggs are deposited on the solid substratum, would then be regarded as a secondary reversion to, rather than a persistence of, a pre-teleostean habit. Possibly the reversed position of the egg is to be regarded as a means of protecting its more sensitive apical portion from injury by contact with the surface film of the water in which it floats.

When fertilization takes place the most conspicuous immediate result is the onset of a gradual concentration of the protoplasm in the germinal disc—the disc becoming at the same time more heaped up, its vertical diameter increasing and its horizontal diminishing.

The segmentation of the germinal disc in teleostean fishes is usually of a very regular and characteristic kind. It is illustrated as seen in surface view by Fig. 12. The germinal disc lengthens out into an elliptical shape. The first furrow to appear (A) is meridional and occupies the shorter diameter of the ellipse. The second furrow is also meridional and in a plane perpendicular to that of the first. The third and fourth sets of furrows (B, C, D) are vertical and they become arranged so as to be practically parallel to the first and second, with the result that the blastoderm as seen in surface view assumes a very characteristic arrangement of sixteen segments arranged in four rows (Fig. 12, D).

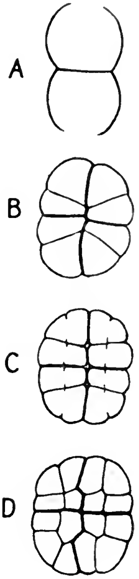


Fig. 12. — Segmentation in the blastoderm of a teleostean fish (*Serranus atrarius*) as seen in surface view. (After Wilson, 1891.)

The internal phenomena of segmentation may be described from what occurs in the Trout (Kopsch, 1911). In the first place it has to be noted that the early furrows do not extend right through the substance of the germinal disc but leave a continuous basal stratum of protoplasm next the yolk. The blastoderm assumes a two-layered condition by the 3rd and 4th furrows curving round in their deeper portions so as to intersect the preceding division-planes which were throughout perpendicular to the surface (Fig. 13, B). Up to the 16-cell stage all the segments remain connected by broad protoplasmic bridges apart from the continuous basal layer of protoplasm which connects the deepest cells together.

In the 32-cell stage (Fig. 13, C) the cells of the superficial layer have become completely isolated while the deep cells are still connected together. With the next division the blastoderm becomes three layered, the cells of the intermediate layer being derived some from the superficial, some from the deep layer, as is shown by the evidence of broad bridges of protoplasm which persist here and there between sister cells.

With the next division (128-cell stage, Fig. 13, D) the four-layer condition is reached, the cells of the basal layer being still connected by a thick continuous stratum of protoplasm. By this time it is found that the nuclear divisions of the basal layer are clearly lagging behind those of the other layers. As segmentation proceeds further the continuous basal sheet of protoplasm decreases relatively in thickness. For a time (Fig. 13, E) bulgings of its upper surface indicate that it is giving off cells into the overlying layer but as the thinning process goes on these become less and less numerous.

H. Virchow distinguished three zones in the basal sheet of protoplasm—marginal, intermediate and central, although the latter is not quite central but situated rather towards the posterior or embryonic edge of the blastoderm. The intermediate zone is marked off from the others by the fact that the thinning process has there progressed farther.

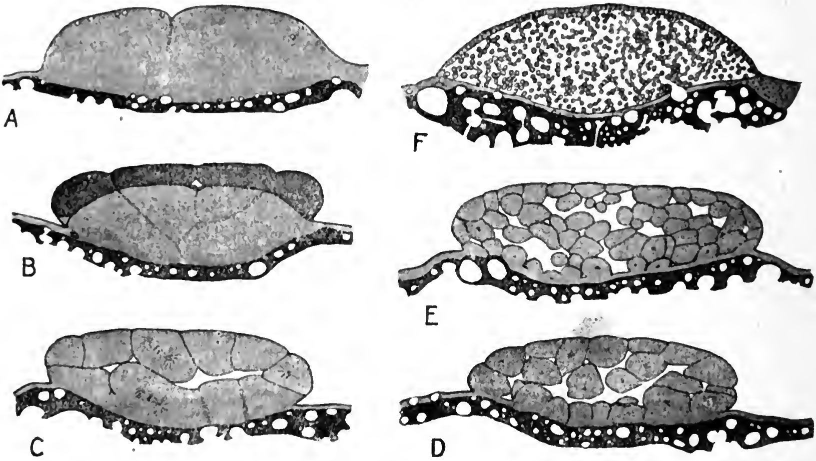


FIG. 13.—Vertical sections through the blastoderm of a Teleost (*Salmo fario*) illustrating the process of segmentation. (After Kopsch, 1911.)

A, end of second division. Section perpendicular to plane of first furrow which is therefore seen cut across. B, commencement of fourth division. Plane of section as in A. The division surfaces of the third division are seen to curve inwards so as to meet the first division surface. As a result the latter has become distorted and no longer forms a plane. C, middle of sixth division. D, beginning of eighth division. E, beginning of tenth division. F, 62-hour blastoderm. (The dark portion at the top of Fig. B represents the free surface bounding the second furrow: the dark tone at the lower edge of each figure represents yolk.)

Up to about the twelfth division the nuclei all through the blastoderm divide practically synchronously except those of the basal layer which as already indicated lag behind. Soon after this however (from about the 41st hour—Kopsch) the divisions become irregular.

The basal layer becomes the yolk-syncytium: the cell limits visible on its upper side become obliterated and it becomes more and more flattened out. Although its nuclei undergo repeated mitosis there is no longer any budding off of cells, the nuclei simply lying within the substance of the syncytium.

As they increase in number nuclei from the central and marginal regions spread into the intermediate zone, which up to now contained very few nuclei, while others pass outwards into the peripheral protoplasm (**Periblast**—Agassiz and Whitman, 1885) lying outside the limits of the blastoderm.

Towards the end of the second day the syncytial nuclei begin to increase markedly in size and they begin to undergo abnormal multipolar mitoses. During the third day they complete the assumption of these peculiarities which are characteristic of the nuclei of a yolk-syncytium—enormous size, curiously lobed appearance, and the tendency for the lobes to become nipped off irregularly so as to give rise to groups of small nuclei.

During these later stages of segmentation the blastoderm becomes flattened somewhat and instead of bulging out over its attached base all round, its surface passes into the extrablastodermic surface by a slope very much as it did before segmentation began (Fig. 13, F).

ACTINOPTERYGIAN GANOIDS.—The ganoid fishes are of special embryological importance because, so far as actinopterygians are concerned, they appear to be the least modified descendants of those ancestral forms from which the Teleostean fishes have been evolved. Study of their developmental phenomena is desirable in order to see to what extent they throw light upon the peculiarities of development which characterize the Teleostean fishes. It will be necessary therefore to review the segmentation processes so far as they are known in each of the three types—the Sturgeon, *Amia* and *Lepidosteus.*

The only Sturgeons of which anything is known regarding their early development are the common sturgeons of the genus *Acipenser. Polyodon, Psephurus* and *Scaphirhynchus* are so far completely unknown, though it is highly desirable that their development should be investigated.

In both *Acipenser ruthenus* (Salensky, 1878) and *A. sturio* (Bashford Dean, 1895) the segmentation (Fig. 14, A) is of the same general type. The unsegmented egg measures about 2 mm. in diameter in the Sterlet (*A. ruthenus*), about 2·8 mm. in the Sturgeon (*A. sturio*). The lower part of the egg contains coarse yolk granules while in the region of the apical pole it is richer in protoplasm and the yolk is more finely granular. The first furrow (*a*) is meridional, appearing first at the apical pole and gradually spreading downwards and at the same time cutting more deeply into the yolk. The second furrow (β) is similar and at right angles to the first. The third set of furrows seem to be typically vertical (A 2) but they show much variation and may be practically meridional or may show a tendency to be latitudinal. The next set of furrows again vary between vertical and latitudinal and from now onwards there is no apparent regularity in the segmenting of the various blastomeres. There eventually results a blastula (A 5) the upper portion of which, forming rather less than a hemisphere, is composed of micromeres while the lower part is composed of large, richly yolked, macromeres.

In *Amia* the "oval"-shaped egg measures about 2·5-3 mm. by

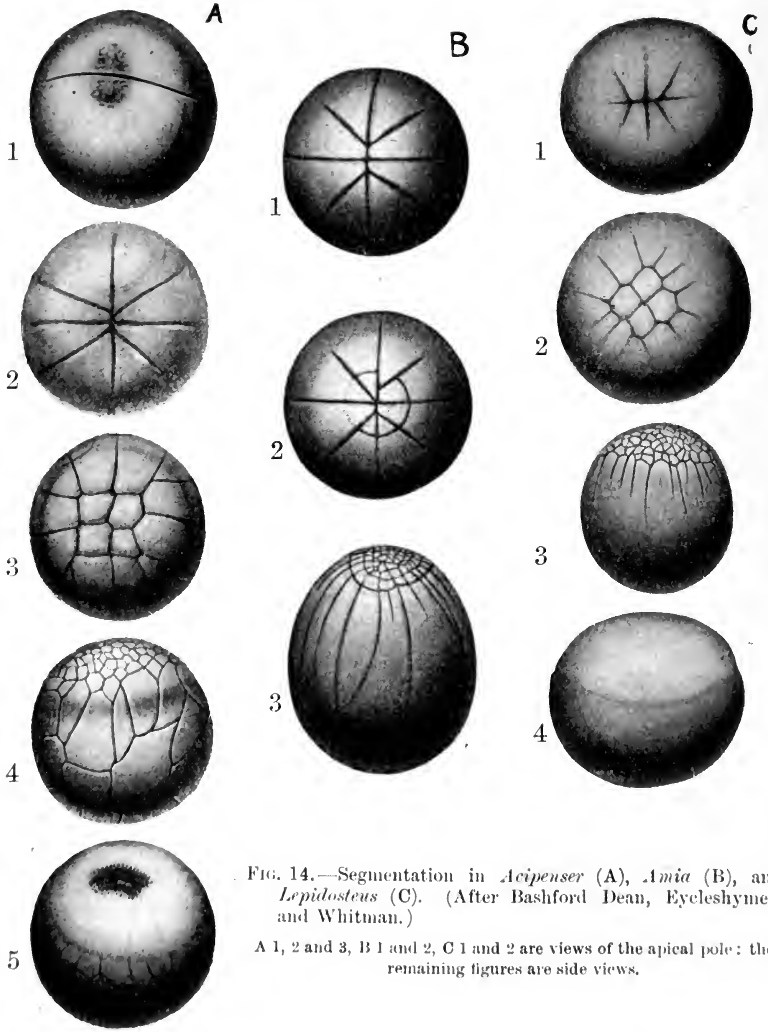


FIG. 14.—Segmentation in *Acipenser* (A), *Amia* (B), and *Lepidosteus* (C). (After Bashford Dean, Eycleshymer, and Whitman.)

A 1, 2 and 3, B 1 and 2, C 1 and 2 are views of the apical pole: the remaining figures are side views.

about 2-2·5 mm. In the region of the apical pole which lies at the end of the long axis is a cap-shaped portion richer in protoplasm—an approach in fact to a germinal disc—while the rest of the egg is rich in dark grayish brown yolk.

The segmentation (Fig. 14, B) begins, about an hour and a half after fertilization, with the successive appearance, at the apical pole, of two meridional furrows (α and β) which gradually sweep downwards to the opposite pole of the egg. Before they reach it, four vertical furrows make their appearance, commencing at a point on furrow α not far from the pole and gradually extending downwards over the lower part of the egg (Fig. 14, B 1).

Before these vertical furrows reach the lower pole a new furrow —latitudinal—develops a short distance from the apical pole, marking off a polar group of eight micromeres (Fig. 14, B 2). At the next division these divide into a superficial and a deep segment (the former being separate—the latter continuous with the yolky mass beneath) while the macromeres divide by vertical furrows.

Next an irregular latitudinal furrow develops below the previously existing one, by which a new micromere is segmented off from the upper end of each macromere.

Lepidosteus.—The ellipsoidal or "oval" egg measures about 3·5 mm. by 3·2 mm. and has a cap of protoplasm with fine grained yolk at its apical pole.

Segmentation (Fig. 14, C) in its early stage is like that of *Amia* except that the furrows are more sluggish in spreading downwards over the egg-surface. They never in fact reach much beyond the equator; in other words, in the case of *Lepidosteus,* the lower hemisphere of the egg does not normally segment at all. The egg therefore has advanced beyond the condition seen in *Amia* and has become meroblastic.

In the later stages of segmentation the region of the upper pole is occupied by a lenticular mass of blastomeres which may be termed the blastoderm, and this is bounded at its lower edge and over its lower surface by a set of elements which remain in continuity with the yolk. Later on the divisions between these elements tend to disappear and their place becomes occupied by a "yolk-syncytium" containing numerous nuclei.

To summarize then, we have exemplified by the three ganoids *Acipenser, Amia* and *Lepidosteus,* three steps in evolutionary change, associated with an increasing degree of telolecithality, from the holoblastic type of egg met with in Lampreys or Amphibians or Crossopterygians to the meroblastic type as it exists in modern Teleosts.

LUNG-FISHES.—The early stages of segmentation have been observed in two out of the three still existing lung-fishes—*Ceratodus* (Semon, 1893) and *Lepidosiren* (Graham Kerr, 1900).

In the case of *Ceratodus* the egg measures about 3 mm. in diameter and is pigmented in the neighbourhood of the apical pole. The first two furrows (Fig. 15, A 2 and 3) are meridional and at right angles to one another. Each appears first at the apical pole and extends downwards with varying rapidity. The third set of furrows are vertical and make their appearance usually before the second meridional furrow (β) has reached the lower pole. The egg becomes thus divided into eight practically equal blastomeres.

A latitudinal furrow then develops about 45° above the equator, so that the egg now consists of eight micromeres round the apical pole and eight macromeres.

After this stage segmentation usually becomes irregular although sometimes two additional latitudinal furrows make their appearance in succession so that the egg consists of four tiers each of eight blastomeres. Eventually, as segmentation proceeds, a blastula is formed of the type shown in Fig. 15, A 7.

The segmentation cavity first appears about the time of the fourth cleavage as a small chink. It rapidly expands and in the blastula figured (Fig. 15, A 7) it is of large size.

In *Lepidosiren* (Fig. 15, B) the egg measures usually between 6·5 and 7 mm. in diameter. It is free from pigment in correlation with the fact that it develops in a burrow shaded from the action of light. In the region of the apical pole is a whitish cap in which the yolk is in very minute particles while elsewhere it is in large coarse granules.

The first two furrows (Fig. 15, B 2 and 3) are meridional and at right angles to one another. The third set of furrows (Fig. 15, B 4) are vertical though occa-

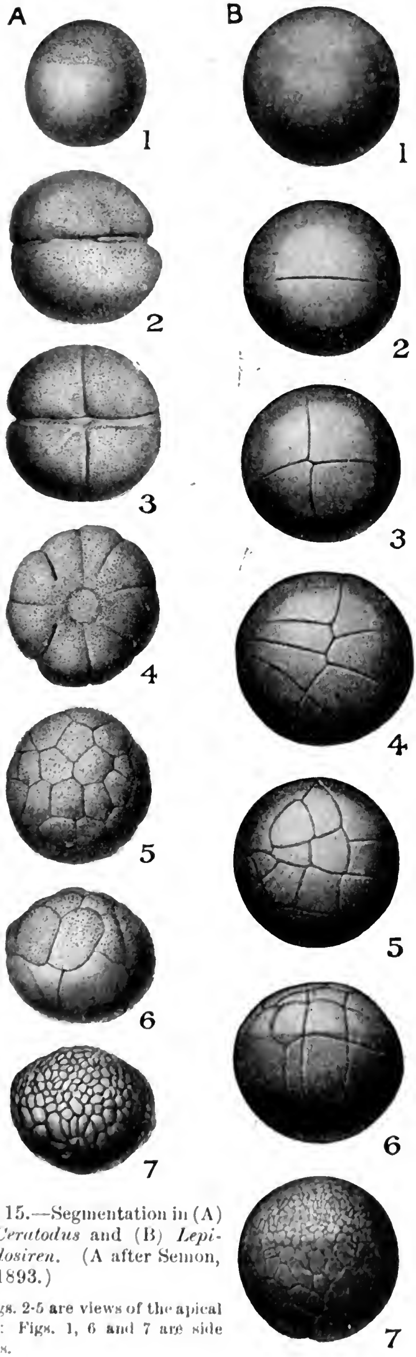


FIG. 15.—Segmentation in (A) *Ceratodus* and (B) *Lepidosiren*. (A after Semon, 1893.)

Figs. 2-5 are views of the apical pole: Figs. 1, 6 and 7 are side views.

sionally one or other of them may become latitudinal. The various meridional and vertical furrows gradually extend downwards towards the lower pole of the egg in the order of their appearance, and during the earlier stages the lower hemisphere of the egg possesses only such furrows (Fig. 15, B 6).

As the blastomeres go on segmenting there is produced eventually a blastula with an upper hemisphere of small cells which appear white because of the finely subdivided condition of their yolk and a lower hemisphere of larger more yolky elements (Fig. 15, B 7).

Already at the stage when the egg is divided into four segments a space develops between the blastomeres. As segmentation goes on the micromeres tend to round themselves off, leaving wide chinks between containing fluid. By the blastula stage the fluid has collected together into a spacious segmentation cavity which is visible in the whole egg as a dark shadow in its upper hemisphere. At first the cavity is rounded and is roofed in by a single layer of cells but later it spreads out, takes a planoconvex form and its roof comes to be composed of two layers of closely apposed cells.

AMPHIBIA.—The Amphibia are in the matter of segmentation the most interesting and important group of the vertebrata, for in no other group does there exist so much variety in the proportional amount of yolk present in the egg. Much work still remains to be done in regard to this group in the way of detailed study of the process of segmentation in its relation to the amount and concentration of the yolk.

As already indicated the extent of the influence which the yolk exerts in retarding the living activities of the protoplasm, such as growth and division, bears a rough relation to its proportional amount. As regards the majority of cases this may be said to vary directly with the size of the egg. The largest eggs are as a general rule the most richly yolked. But the rule is by no means an invariable one that the influence on the segmentation is directly proportional to the total amount of yolk in the egg as a whole. For a smaller egg, containing a smaller amount of yolk, may yet have that yolk more concentrated in one region so as to produce there a more intense retarding influence—as is the case naturally in many small Teleostean eggs or as may be demonstrated experimentally by concentrating the yolk artificially through the action of centrifugal force. O. Hertwig was able by centrifugalizing frogs' eggs and so causing the yolk to become concentrated in the abapical hemisphere, to bring about a complete cessation of cleavage in that hemisphere so that the egg thus assumed a meroblastic character.

The variety in the size of the egg within the limits of the group Amphibia has already been indicated by the table on page 2. The process of segmentation agrees in the main with what has been described for the frog but there is much variation in detail. The variations have to do both with the position of the furrows and with their appearance in point of time. One gets a good idea of the general tendency of variation by studying numerous eggs of a single species, for example in the case of *Rana palustris* Jordan and Eycleshymer (1894) found amongst other variations in the mode of appearance of the first furrows, those illustrated in Fig. 6 (p. 12). And similar differences occur between the eggs of different species.

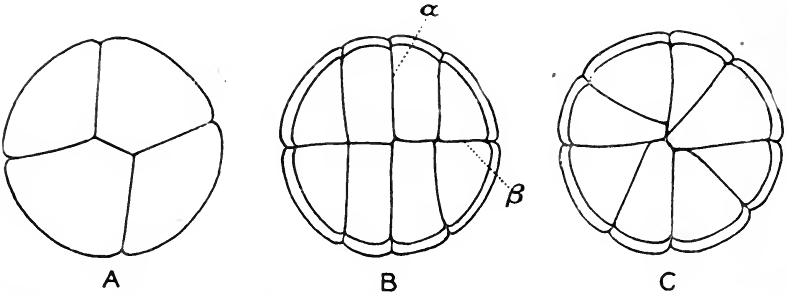


Fig. 16.—Variations in topographical relations of early segmentation furrows in the egg of *Rana temporaria*. (A, B after Morgan, 1897; C after Jenkinson, 1913.)

The figure in each case represents a view of the apical pole of the egg.

As regards difference in position of the furrows two of the commonest variations are the following. At the four-blastomere stage two blastomeres may be pressed outwards from the apical pole as in Fig. 16, A. Again meridional furrows may be replaced by vertical furrows as in Fig. 16, B and C.

As regards variations in time these are chiefly associated with the retarding of segmentation in the lower yolk-laden segments. This reaches its maximum, so far as Amphibians are concerned, in the Gymnophiona, where segmentation spreads so slowly into the lower parts of the egg that during what are ordinarily called the segmentation stages the yolk remains completely uncleaved. It would in fact be concluded from an inspection of these stages alone that the egg is a meroblastic one. Examination of later (gastrulation) stages however shows that the yolk does eventually segment although tardily.

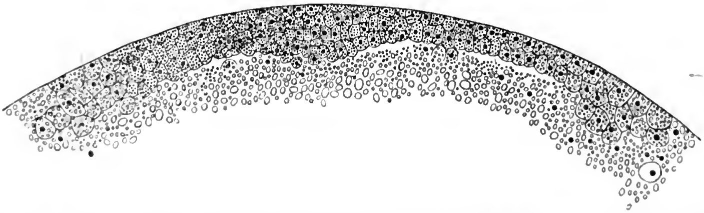


Fig. 17.—Vertical section through apical portion of egg of *Ichthyophis* at an advanced stage of segmentation. (After P. and F. Sarasin.)

Upon the whole it seems to be the case that the Urodele egg segments more slowly—during at least the first stages—than does that of the Anura. It may be said also that, on the whole, eggs with a large mass of yolk show a tendency for the first latitudinal furrows to be nearer the apical pole, so that the micromeres which they cut off are relatively smaller. Also it seems to be the case that in the lower, more yolky, parts of the egg the latitudinal furrows are retarded to a particularly great extent, so that in such heavily yolked eggs there is frequently visible a preponderance of vertical and meridional furrows in the lower parts of the egg.

ELASMOBRANCHII.—Of the more typically meroblastic vertebrates the Elasmobranchs call for little in the way of further remarks. The general features of their segmentation have already been described (p. 12).

The eggs of all Elasmobranchs hitherto investigated are of large size and undergo a meroblastic segmentation. Up to the present time no Elasmobranch has been discovered in which the eggs are small and holoblastic, though it is quite possible that such forms exist. It need hardly be said that if they do the study of their embryology will be of extraordinary importance as it will be of the greatest help in enabling us to disentangle those developmental phenomena of Elasmobranchs which are primitive from those which are merely secondary modifications due to the accumulation of yolk.

SAUROPSIDA.—The Sauropsida, like the Elasmobranchs, possess large and richly yolked eggs with a meroblastic segmentation, but the process of segregation of yolk and protoplasm has not been carried to such an extreme as in Elasmobranchs, not to mention Teleosts. A germinal disc is present but this still contains a considerable amount of yolk and at its periphery passes by much more gradual transitions into the main mass of yolk. Further in the more primitive Reptiles the blastoderm frequently occupies a much larger proportion of the whole egg than it does in the Elasmobranch.

The general features of segmentation resemble those of Elasmobranchs though the earliest phases depart in many cases less than they do in Elasmobranchs from what is seen in holoblastic eggs. Thus the process may commence with the appearance of a meridional furrow followed by a second at right angles to it and then by two pairs of vertical furrows very much as in an actinopterygian ganoid (Fig. 14, B and C).

This is seen most clearly in the less specialized egg of Reptiles. Even in the Reptile however the process is liable to become irregular at an early stage by the reduction of particular furrows or their irregular orientation. In the Birds (Patterson, 1910) the irregularity is still more marked and even the third set of furrows may no longer be clearly recognizable.

As in the case of other bulky and heavily yolked eggs polyspermy appears to occur normally and an abortive accessory segmentation may make its appearance round the accessory sperm-nuclei. As in the Elasmobranch (Fig. 8, B*) this is only a transient phenomenon the accessory furrows flattening out and disappearing as the accessory sperm-nuclei degenerate.

Again as in the Elasmobranch a yolk-syncytium is developed beneath and around the segmented portion of the blastoderm.

A marked difference between the Sauropsidan[1] and the Elasmobranch type of egg at a fairly advanced stage of segmentation becomes apparent on comparing them with the corresponding stages of eggs of a less markedly telolecithal character (*e.g.* Fig. 14). It is seen that the blastoderm in an Elasmobranch such as that shown in Fig. 8 D, E corresponds to the mass of micromeres of the holoblastic egg, while in the Sauropsidan it corresponds to the mass of micromeres together with the apical ends of the large macromeres.

This is really an expression of the fact that in the Sauropsidan the germinal disc extends outwards into the main yolk, and shades off gradually into it. The result is that the segmentation process in the outer portion of the blastoderm is delayed by the presence of yolk precisely in the same way as in the lower portion of the holoblastic egg.

GASTRULATION

The segmentation process is in the more primitive Vertebrates, as in many other groups of the Metazoa, succeeded by a process of gastrulation, in which the blastula becomes converted into a **gastrula** *i.e.* a type of embryo consisting of the two primary cell-layers, **ectoderm** and **endoderm**, enclosing a cavity, the **archenteron**, which corresponds morphologically with the coelenteron of the Coelenterate and which opens freely to the exterior.

While the process of gastrulation is fairly clear in the most primitive vertebrates it becomes less and less so in the more highly modified members of the group until finally in the Amniota it becomes completely obscured. To facilitate the understanding of the modifications which the process of gastrulation undergoes it will be well to study it first as it occurs in three of the more primitive Vertebrates namely *Amphioxus, Polypterus,* and *Lepidosiren.*

(1) AMPHIOXUS.—The blastula of *Amphioxus* is composed of a single layer of cells, those towards the apical pole being smaller, those on the opposite side being larger and containing in their cytoplasm larger and more numerous granules of yolk. The process of gastrulation is ushered in by the large-celled portion of the blastula-wall becoming flattened as shown in Fig. 18 A. The portion of the flattened area which, as shown by later stages, is anterior in position develops a slight depression (Fig. 18, B) which gradually deepens and at the same time spreads backwards (Fig. 18, C, D, E, F, G) until the large-celled portion of the embryo is completely invaginated within the small-celled portion and the two-layered gastrula stage is attained.

---

[1] See Chapter X., Segmentation of Fowl's egg, F, G, H.

Had the process been merely as stated the result would have been a gastrula with a very wide mouth. But along with the process of involution there takes place an active growth of the lip or rim of the gastrula. This growth is especially active anteriorly as is shown by the fact that mitotic figures are most numerous in this region and become less and less frequent towards the posterior part of the rim. The result is that the original mouth of the gastrula — the **protostoma** — becomes gradually encroached upon by the gastrular lip — the encroachment being most marked anteriorly so that the opening becomes not merely diminished in size but also appears to shift its position towards what will become the posterior end of the embryo. It will be noticed that what really happens is not a process of shifting of the opening as a whole, but rather the persistence of the hinder portion of the opening while its anterior portion has disappeared. Such a remnant of the original protostoma may conveniently be known by the special term **blastopore**.

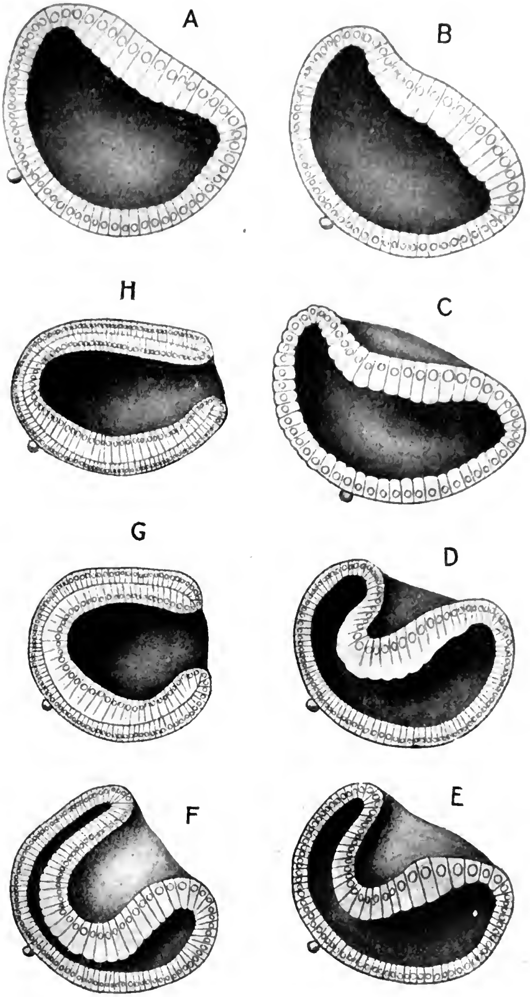


FIG. 18.—Illustrating the process of gastrulation in *Amphioxus* as described by Cerfontaine. The second polar body marks the neighbourhood of the apical pole. The individual figures are viewed from what is seen later on to be the left side of the Amphioxus, the dorsal side being above and the head end pointing towards the left side of the page.

Expressed somewhat differently—the cavity of the gastrula has become roofed in by a process of **overgrowth** on the part of its lip. The most conspicuous factor in this process consists of backgrowth of the anterior portion of the lip while the growth of the lateral portions inwards towards the mesial plane (so as to narrow the opening from side to side) is less and less active the greater the distance from the anterior end, until finally, in the extreme posterior portion of the lip, such growth as takes place is relatively inconspicuous.

In the process of gastrulation in *Amphioxus* there are then two distinct processes at work (1) a process of **invagination** or involution of the large-celled portion of the wall of the blastula and (2) a process of overgrowth, most pronounced in the case of that portion of the gastrular lip which is originally anterior. By the agency of (1) there are established the two primary cell-layers — ectoderm and endoderm while by the agency of (2) there is formed the dorsal wall of the embryo with its potential later developments such as central nervous system and notochord.

It will be noticed that the originally anterior portion of the gastrular rim, when it has completed its backgrowth, lies above, dorsal to, the now greatly diminished gastrular opening or blastopore. Consequently it comes to be spoken of as the **dorsal lip** of the blastopore.

Although, strictly speaking, the terms endoderm and ectoderm are expressive of topographical relation and their use is permissible only after the one layer has become at least partially invaginated within the other, yet it should be carefully borne in mind that these two primary layers have already become distinctly differentiated from one another during the blastula stage, long before the process of invagination begins. One might indeed go farther and say that endodermal characteristics, *e.g.* richness in yolk, have already made themselves apparent in the abapical portion of the egg even before segmentation begins.

This fact is of far-reaching importance as we shall find in other Vertebrates that the histological characteristics of ectoderm and endoderm become apparent not merely before the actual process of gastrulation takes place but, it may be, completely independently of that process.

(2) Polypterus.—In *Polypterus* an early stage in the process of gastrulation (Fig. 19, B) shows a well-marked groove encircling the egg a short distance on the abapical side of the equator. This groove marks the line along which involution of the egg-surface is taking place and its adapical lip represents the lip of the gastrula. It is to be concluded from a still earlier stage observed and drawn by Budgett (Fig. 19, A) that the involution groove appears first in the region corresponding to the anterior lip of the gastrula of *Amphioxus* and gradually becomes extended at its two ends until complete. This is of importance as betraying a tendency for the invaginative activity to be accentuated in this portion of the gastrular lip and diminished elsewhere.

The fact that the yolk portion of the blastula consists not of a single layer of cells as in *Amphioxus* but of a solid bulky mass forming a large proportion of the whole blastula, renders it physically impossible for the yolk hemisphere to be involuted bodily into the interior of the apical hemisphere. As a consequence we find in *Polypterus* that the process of involution is replaced to a greater extent than in *Amphioxus* by overgrowth, the gastrular lip growing over the mass of yolk-cells as seen in Fig. 19, C, D and E. As this process of overgrowth continues the projecting **yolk-plug**—the mass of yolk-cells not yet enclosed—gradually diminishes in size and eventually disappears completely in the now narrow blastopore. As yet material is not available to show definitely whether the overgrowth is more active in what corresponds to the anterior portion of the gastrular lip of *Amphioxus* but the probability is in favour of this being the case and the figures are orientated on the assumption that this is so.

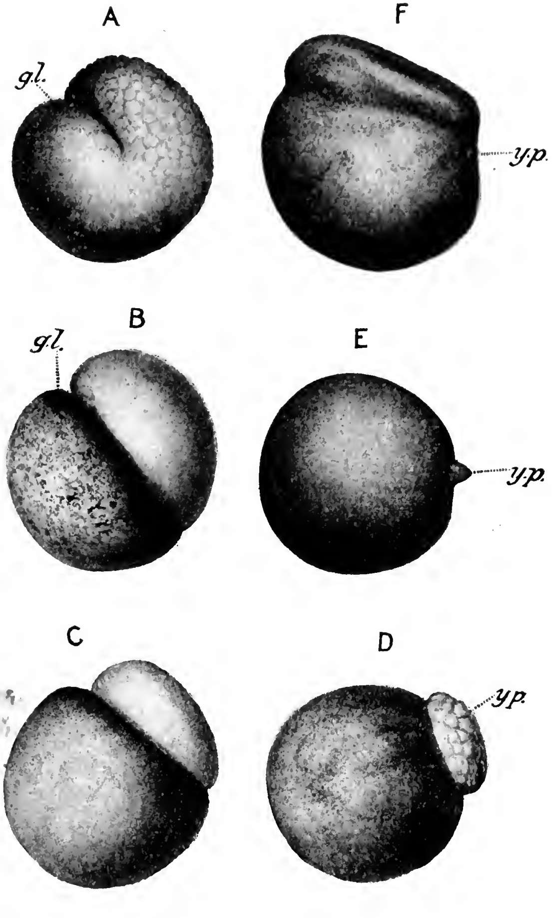


Fig. 19.—Illustrating process of gastrulation in *Polypterus*. (Figs. A, C, E, F after drawings by Budgett.)

g.l, gastrular lip; *y.p*, yolk-plug. The egg is in each case viewed from the left side and has the dorsal side above. The original apical pole is directed downwards and towards the left side of the page as in the preceding figure of *Amphioxus*.

The two features to be specially noted in the gastrulation of *Polypterus* as compared with that of *Amphioxus* are (1) the accentuation of the process of overgrowth and the reduction of the process of involution and (2) the tendency, in early stages at least, for the invaginative activity to be diminished along the region corresponding to the posterior part of the gastrular rim of *Amphioxus.*

(3) LEPIDOSIREN.—The first sign of gastrulation is afforded by the appearance, a short distance to the abapical side of the equator, of a latitudinally arranged row of small dimples or depressions of the surface which soon become joined up to form a continuous invagination-groove. This (Fig. 20, A and B) may extend through about one-third of the circumference of the egg but in contrast with what happens in *Polypterus* the groove, instead of increasing in length, becomes shorter, flattening out and disappearing at its two ends.

The final stage is seen in Fig. 20, E, where the gastrular lip is short, and curved into a crescent, forming the dorsal boundary of the blastopore.

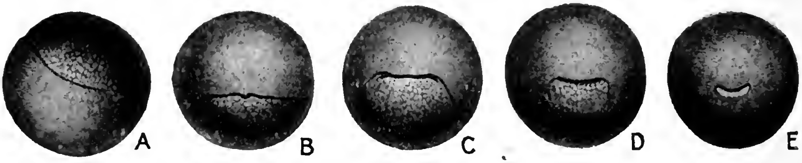


FIG. 20.—Illustrating the process of gastrulation in *Lepidosiren.*

Fig. A is a side view, the egg being orientated so as to correspond with the figures of *Amphioxus* and *Polypterus*, the large-celled yolky abapical portion of the egg being above and towards the right hand. Figs. B to E are views looking directly at the gastrular rim (dorsal lip of the blastopore), or in the case of E directly at the completed blastopore. Consequently, as the gastrular rim is during these phases of development not stationary, the views B to E are not orientated morphologically in exactly the same way.

At this stage the large yolk-cells with their conspicuous salmon colour have been completely covered in by small cells—a condition that has been brought about through the agency of two distinct factors (1) the process of overgrowth with which we have already become familiar and (2) a new process to which the name **delamination** is given.

As is shown by the sections drawn in Fig. 21, the yolky or abapical portion of the blastula-wall is in *Lepidosiren,* as it was in *Polypterus,* far too bulky to be involuted bodily as was the case in *Amphioxus.* Again the enclosing of the yolky mass by the gastrular lip growing over it as in *Polypterus* is rendered impossible by the fact that the gastrular lip is normally here never completed to form an entire circle. It is, as has been explained, restricted to a comparatively small linear extent.

This small persisting portion of gastrular lip probably does advance over the surface of the yolk by a process of overgrowth, giving rise in this way, just as in *Amphioxus,* to what will become the dorsal wall of the embryo with its central nervous system and notochord. That this is the case seems to be indicated by sagittal sections through eggs cut in celloidin while still contained within the egg-shell. In these the gastrular lip has a distinct wedge shape this being apparently impressed upon it as it pushes its way between the egg-shell and the surface of the yolk. Corroborative evidence is afforded by the numerous mitotic figures found throughout the

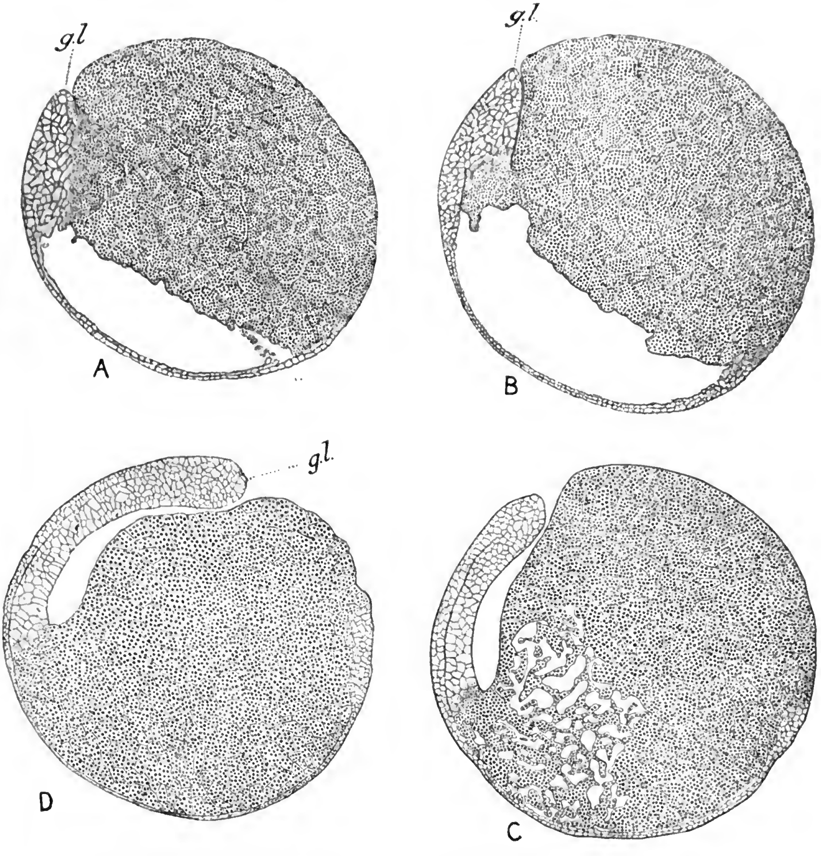


Fig. 21.—Sagittal sections illustrating gastrulation in *Lepidosiren*.

g.l, gastrular lip.

thickness of the archenteric roof which indicate that it is undergoing rapid growth.

It will be gathered, however, from a consideration of Fig. 20 that those parts of the margin of the small-celled region of the egg's surface which are not involuted to form a groove, must also advance over the surface of the yolk, for these parts of the margin form at first (Fig. 20, A and B) practically a great circle of the egg, while in subsequent stages (C and D) they form a curve of gradually diminishing radius.

The method by which the small-celled area extends is shown clearly in Fig. 22, A, where it is seen that portions of large yolk-cells adjacent to the small-celled area become split off as small cells which are added to that area. It is to this splitting-off process that the name delamination is applied.

It is clear, then, that in the gastrulation of *Lepidosiren* three processes are at work (1) Involution of the surface—this is conspicuous in the first stages (see Fig. 22, B and C), (2) Overgrowth by the gastrular lip—the "dorsal" lip as it is commonly termed from its ultimate position, and (3) Delamination. These same three factors are at work in the gastrulation of the lower Vertebrates in general, and a clear realizing of their nature is necessary to a comprehension

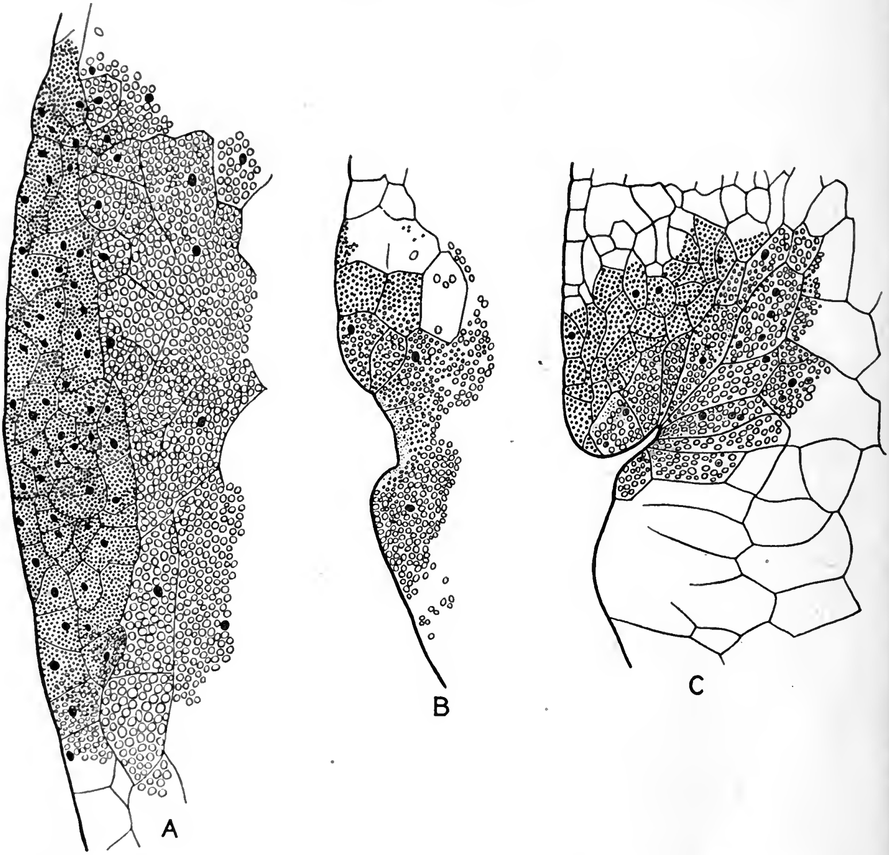


FIG. 22.—Portions of sagittal sections of *Lepidosiren* egg during early stages of gastrulation.

A, showing process of delamination. The small-celled ectoderm is seen on the left: it is becoming extended by the addition, to its lower edge, of cells split off from the yolk-cells. The latter are recognizable by their larger size and by the larger size of the yolk-granules with which their cytoplasm is laden. B and C showing involution of the surface along the invagination-groove.

of the superficially very different gastrulation-phenomena observable in the various groups.

With regard to the first of these processes, involution of the surface, it must be clearly understood that such appearances as that depicted in Fig. 21, B, point indubitably to the occurrence of a true process of invagination or involution of the surface of the egg. It is necessary to emphasize this as some embryologists are sceptical as to the occurrence of true invagination and believe that a more important part in the formation of the archenteric cavity is played by a mere cleavage, or splitting apart, of the cells which are to form the roof and the floor respectively. Brachet (1903), indeed, goes the length of stating in regard to *Lepidosiren* and *Protopterus*, with only the data published by myself before him, that "the first trace of the archenteron is due to a cleavage, the result of which is the formation of a slit"—a statement which is certainly not justified.

On the other hand it should also be borne in mind that there is no difficulty *a priori* in the way of admitting that portions of enteric cavity may come to arise secondarily by a process of splitting in the midst of a solid mass of endoderm or yolk-cells. This type of modification in the embryonic development of organs which were originally formed by invagination or evagination is one which occurs quite frequently. Numerous examples of it are mentioned in the course of this volume.

Before closing this account of the gastrulation of *Lepidosiren* attention should be drawn to a remarkable and important phenomenon which has been observed in both *Lepidosiren* and *Protopterus*. During the early stages of gastrulation, while the segmentation cavity is widely patent, the small blastomeres in the neighbourhood of its abapical side are seen, where not distorted by pressure from their neighbours, to be approximately spherical in shape. Elementary physics teaches that this is an indication that they are isolated masses—that their protoplasmic substance is not continuous from cell to cell. In a later stage (Fig. 21, C) however the blastomeres in the region of the segmentation cavity do become continuous with their neighbours and form a coarse reticulum traversing the cavity, the fluid contents of which now fill the meshes of the network.

The importance of the phenomenon described lies in the fact that here we have an actual case, clearly demonstrable, of isolated embryonic elements fusing to form a syncytial reticulum—a type of process which may probably, as will be indicated later, play an important part in the development of the Vertebrate nervous system.

GASTRULATION IN THE VARIOUS GROUPS OF ANAMNIA

LAMPREYS.—The Lamprey (*Petromyzon fluviatilis*) shows in its gastrulation (Fig. 23) an intermediate condition between that of *Amphioxus* and that of the more heavily yolked holoblastic forms. The abapical portion of the blastula is yolk-laden and thickened, though not to the extent seen in *Polypterus.* The segmentation cavity remains, therefore, relatively capacious so as to permit of a considerable amount of invagination of the yolk mass into it.

The gastrulation process so far as can be judged seems to consist mainly if not entirely as in *Amphioxus* of (1) invagination and (2) overgrowth, only in this case the relative importance of the former has been lessened and that of the latter increased.

AMPHIBIA.—It will be convenient to consider first the gastrulation-phenomena as seen in the common frog (*Rana temporaria*), this animal having been more exhaustively studied than has any other Amphibian.

The first sign of the onset of gastrulation is the appearance of a short latitudinal linear involution (Fig. 24, *a*) of the surface of the blastula considerably on the abapical side of the equator (about 25°, Kopsch) and just at the boundary of the large-celled region. It appears on that side of the egg on which the blastula roof is commonly rather thinner than it is elsewhere. This involution groove, as seen in a surface view of the egg, extends laterally and as it does so assumes a crescentic curvature (Fig. 24, *b*). The extension of the groove in length continues while its radius of curvature diminishes until finally it forms a closed circle (Fig. 24, *b*, *c*, *d*).

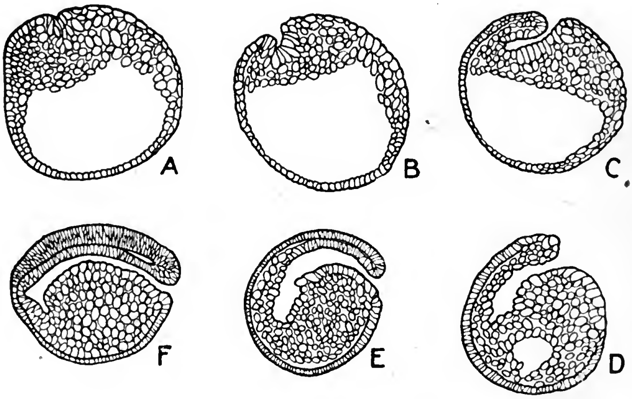


FIG. 23.—Gastrulation in *Petromyzon*: based on Goette's figures (1890).

The individual sections are orientated in the same way as the corresponding sections in Figs. 18 and 21.

The groove at its first appearance lies close to the boundary between the small cells of the apical region—characterized in the frog by their dense black pigment—and the large pale-yellow yolk-cells. During subsequent stages the groove continues to mark the boundary between the two types of cell, so that in the last stage mentioned when the groove forms a complete circle the mass of almost white yolk-cells within it (yolk-plug) stands out in striking contrast with the coal-black cells covering the rest of the egg surface.

As will be gathered from an inspection of Fig. 24 the gradual covering in of the yolk-cells takes place in an eccentric fashion. On the side opposite to that on which the original involution groove makes its appearance there is comparatively little displacement of the boundary between large cells and small, while on the side where the groove is the displacement is relatively great—from *a* to *d* in the diagram. Intermediate points of the boundary between large and small cells are displaced more or less according to their greater or less proximity to the point of original involution.

As regards the method by which the yolk-cells become covered in, it would appear that the "dorsal" lip of the groove advances over the yolk by a process of overgrowth, while at those parts of the boundary where there is no invagination-groove the process is one of delamination. The growth of the dorsal lip is clearly indicated by the outline of the yolk-plug in sagittal sections which indicates distortion by pressure from the dorsal lip.

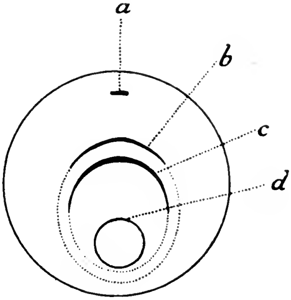


FIG. 24.—Diagram to illustrate overgrowth by dorsal lip of blastopore in the Frog. (After Morgan, 1897.)

The lines *a*, *b*, *c*, *d* represent the involution groove at successive stages of development.

It will be realized from what has already been said that the outer lip of the circular groove (Fig. 24, *d*) is simply the rim of the gastrula-mouth or protostoma and that the preceding stages are above all characterized by this rim being incomplete. In other words the activity concerned in the involution of the gastrular rim is accentuated at one point (*a*) while it is suppressed to such an extent elsewhere as only to become apparent at a comparatively late stage when the edge of the small-celled region has already spread to a great extent over the yolk-cells by a process of delamination.

It will also be realized that it is not strictly accurate to speak of the circular area bounded by small cells as representing the gastrula-mouth until it is completely enclosed by the gastrular rim.

The internal changes which accompany the phenomena just described are illustrated by the sagittal sections shown in Fig. 25. In C the portion of the involution groove which first appeared has become much deepened and runs for some distance parallel to the surface as the archenteric cavity. It is bounded superficially by a completed portion of gastrular wall showing the two primary cell layers, ectoderm and endoderm.

Some of the yolk-cells round the margin of the segmentation cavity are frequently to be seen, though not in the section figured, to be spreading along the inner surface of its roof, towards the point which was the apical pole of the blastula.

In the later stages the overgrowth by the gastrular lip, accompanied, no doubt, by a certain amount of involution though this is difficult to determine with certainty, has proceeded much

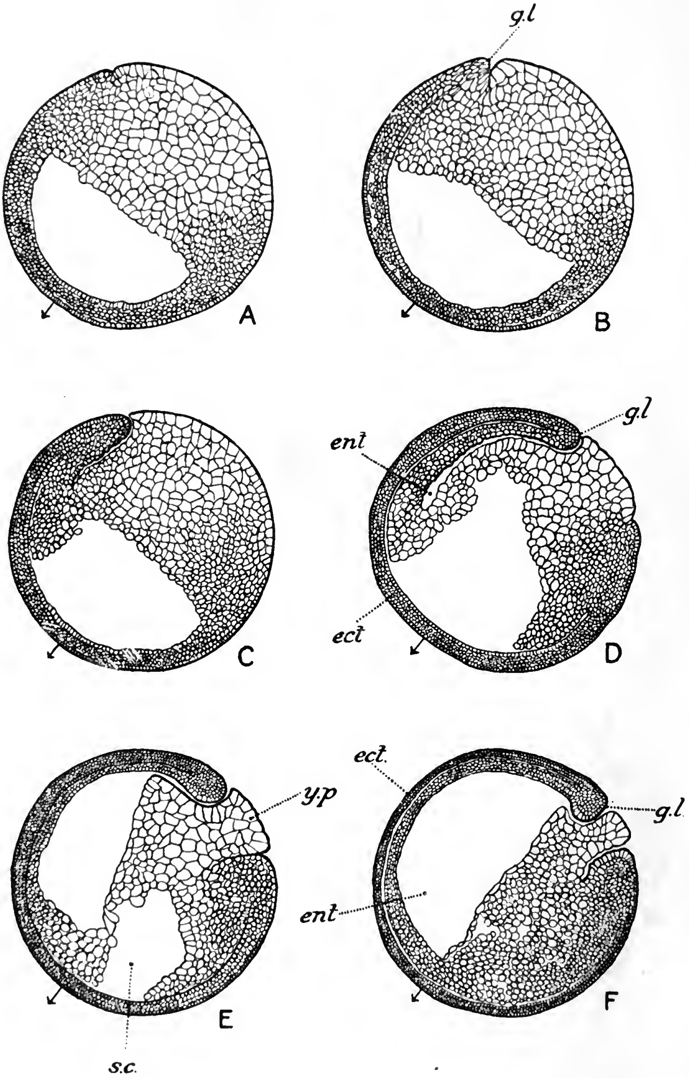


Fig. 25.—Sagittal sections through the egg of *Rana temporaria* illustrating the process of gastrulation. (After Jenkinson, 1913.)

ect, ectoderm; *ent*, archenteric cavity; *g.l*, gastrular lip; *s.c*, segmentation cavity; *y.p*, yolk-plug. The arrow indicates the original apical pole.

further so that the archenteron is much deeper. The spreading of the yolk-cells along the roof of the segmentation cavity, already alluded to, has in these later stages sometimes proceeded so far that that cavity is nearly completely walled in by yolk-cells.

While the archenteric cavity increases in volume the segmentation cavity becomes gradually reduced. Normally the latter cavity goes on shrinking until it is finally obliterated but according to O. Schultze (1887) a certain proportion of eggs show a variation from the normal which appears to be of importance for the interpretation of what happens regularly in certain other groups. As seen in Fig. 25, E, the layer of yolk-cells which separates the archenteron from the segmentation cavity is liable to become extremely thin, and Schultze believes that in certain cases this thin partition breaks down and disappears, so that the archenteric and segmentation cavities are thrown into one. What appears at first sight to be the archenteric cavity of subsequent stages would in such cases be really a complex consisting of the true archenteron fused with the remains of the segmentation cavity.

If these observations are to be depended upon, they are of very special interest. For, if the confluence of archenteric and segmentation cavity really occurs as an occasional variation in such Amphibians as the Frog, this may be taken as a foreshadowing of the similar phenomenon which has become a normal characteristic of the development of many of the higher Vertebrates.

It must however be borne in mind that there exists a dangerous source of possible errors of observation, which it is difficult to guard against, namely that when an egg of the stage of Fig. 25, E, is transferred from one fluid to another, as in the ordinary technical processes which precede section-cutting, violent diffusion currents are set up between the fluid in the segmentation cavity on the one hand and that in the archenteron on the other, and these currents must be very liable to cause rupture in the intervening partition, even when in life this is quite continuous.

As gastrulation nears its end the circle formed by the gastrular lip becomes gradually smaller. Finally its lateral edges come together so that it takes the form of a short longitudinally placed slit, the remains of the yolk-plug at the same time passing out of sight. The gastrula is now complete.

As regards the subsequent fate of the slit-like blastopore it may be mentioned that, for the most part, it becomes obliterated by fusion of its two lips. The portions at its two ends, however, remain open as two pores of which the more anterior becomes the **neurenteric canal** while the posterior becomes, either directly or after temporary obliteration, the **anus**.

The process of gastrulation in the majority of Anurous and Urodele Amphibians pursues a course similar in its main features to that of the frog. Detailed studies of the process are, however, urgently needed in those Amphibians which have particularly small eggs and in which therefore gastrulation is less modified by the presence of yolk.

Gymnophiona.—There are certain Amphibians in which the telolecithal condition of the egg is so pronounced as to lead to a condition nearly approaching the meroblastic. Any such forms occurring either amongst the Dipnoi or the Amphibia must necessarily be of great importance owing to the fact that these groups are less far removed than are any other Vertebrates, from the line of descent of the Amniota and that, in consequence, the study of their development may be expected to throw light upon features occurring in the meroblastic eggs of Amniotes.

Amongst Amphibians of this type the Gymnophiona alone have been subjected to careful study (P. and F. Sarasin, 1887–1893; Brauer, 1897). The following summary of the main features in their gastrulation processes is based on Brauer's description.

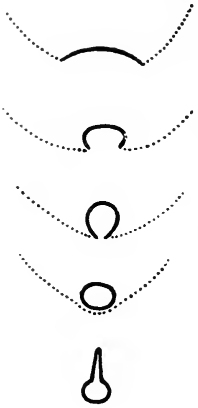


Fig. 26. — Successive stages of gastrular lip in *Hypogeophis* as seen in plan. (After Brauer, 1897.)

The egg of *Hypogeophis* shows at the period preceding gastrulation a round patch of micromeres, or blastoderm, covering $\frac{1}{5}-\frac{1}{6}$ of the surface of the egg in the neighbourhood of its apical pole. Gastrulation commences with the posterior edge of the blastoderm losing its forward curvature and becoming curved backwards (Fig. 26), the curved part of the edge becoming sharply demarcated by the formation of a slight cleft-like invagination of the egg-surface—which is deepest in its centre and shallower towards its extremities. In front of this invagination the superficial (ectodermal) cells of the blastoderm take on a distinctly columnar form. The edges of the blastoderm apart from the line of invagination are in the meanwhile gradually spreading outwards over the yolk. As shown in Fig. 27, A, the cells (*g.l*) forming the anterior wall of the invagination are columnar in form, and the fine-grained character of their yolk makes their general appearance resemble that of the ectoderm cells. This is, however, to be taken, not as meaning that they really are of ectodermal nature but rather merely as an indication of active metabolism associated with active growth. The invagination-groove gradually, by backgrowth and ingrowth of its lateral portions, assumes a more pronounced backward curvature (Fig. 26) taking first the shape of a crescent, later of a horseshoe and finally of a closed ring.

The central part of the groove almost from the beginning increases rapidly in depth so as to form a narrow cavity—the archenteron—which extends forwards. The roof of this cavity is formed of cells agreeing in their fine-grained protoplasm with those of the ectoderm, while its floor on the other hand is composed of cells which in their coarse-grained character resemble rather the yolk-cells.

In front of the archenteron are the irregular remains of the segmentation cavity and a communication becomes established between the two cavities so that they form a continuous space—the broader front part of which is derived from the segmentation cavity, the narrower posterior part from the true archenteron (Fig. 27, C

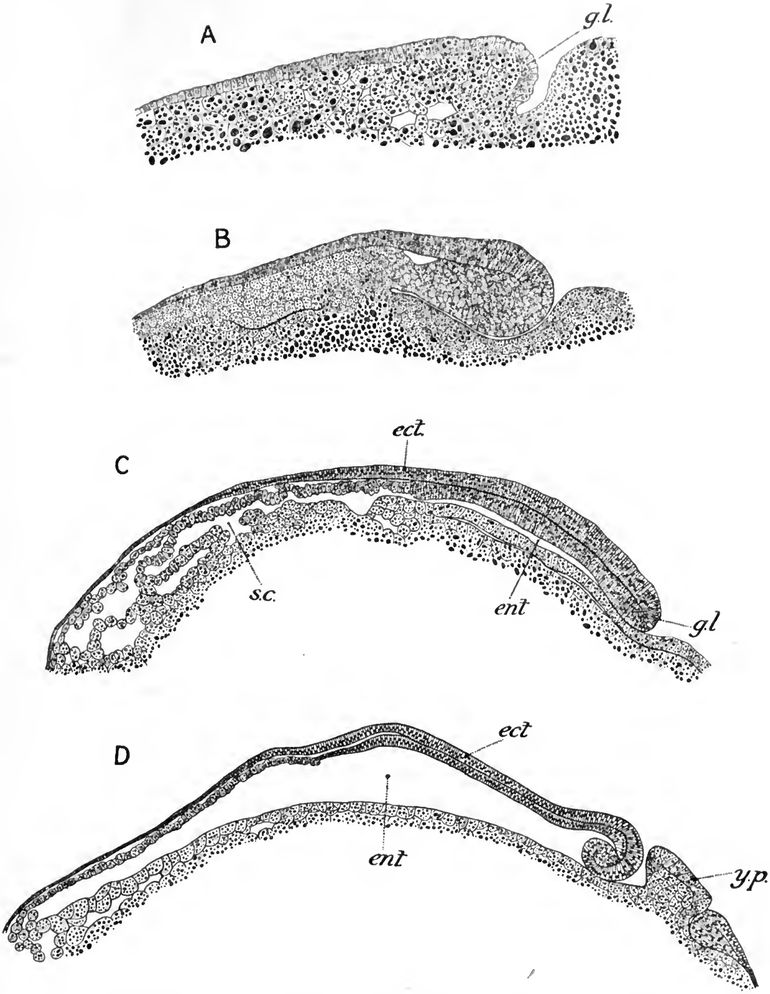


Fig. 27.—Sagittal sections illustrating the process of gastrulation in *Hypogeophis*. (After Brauer, 1897.)

ect, ectoderm; *ent*, archenteric cavity; *g.l*, gastrular lip; *s.c*, segmentation cavity.

and D). The two sections of the cavity remain for a time clearly distinguishable by the character of the cells which form the roof—those of the archenteric portion being composed of fine-grained protoplasm like that of the ectoderm while those of the portion derived from the segmentation cavity are typical yolk-cells.

At a stage when the involution groove forms a nearly complete circle a sagittal section presents the appearance shown in Fig. 27, C. The ectoderm is thick and columnar posteriorly, but in front and laterally it thins out into a cubical epithelium which has extended over the whole surface of the egg with the exception of a small area behind the gastrular rim. In the roof of the enteric cavity the boundary between the archenteric portion formed by overgrowth (and probably involution) and that formed from yolk-cells is marked by an abrupt change in the character of the cells which at once become less tall and less columnar. Farther in still the yolk-section of the roof shows marked irregularities of its inner surface and its cells assume a more rounded form. The anterior limit of the blastocoelic portion of the enteric cavity is not, as yet, clearly defined.

The last section figured (Fig. 27, D) is taken from an egg in which the gastrulation lip forms a complete ring. Consequently the section shows a conspicuous yolk-plug (*y.p*) within the gastrular lip which, it will be noted, has developed a covering of small fine-grained cells over its surface. The inrolling of the gastrular lip visible in the section indicates that the enteric roof is growing actively in length though Brauer does not make it clear to what extent the formation of the archenteron is due to this and to what extent to actual involution. Naturally it would be very difficult if not impossible to decide this point definitely without experiments on the living egg. The gastrular opening gradually decreases in diameter (the yolk-plug disappearing from view as it does so) and eventually it closes from before backwards by its lateral lips coming together (Fig. 26); its posterior part however remains open as the anus.

In the foregoing description is given merely a summary of those features in the gastrulation of *Hypogeophis* which appear to be of importance in relation to the corresponding phenomena of the Amniota: amongst these may be specially mentioned the process of constriction of the gastrular opening, and the double origin of the enteric cavity from archenteron and blastocoele, only its hinder portion being derived from archenteron.

Another important feature not specifically alluded to in the text but which is indicated clearly by Fig. 26 is that during the process of gastrulation the boundary of the small-celled area is sweeping onwards over the egg's surface. It does this probably by a process of delamination as in *Lepidosiren*. The important point to notice, however, is that the small-celled boundary is not blocked in its extension onwards by the gastrular lip. The yolk-plug becomes covered with small cells and after the ends of the rim have met so as to form a complete circle the small-celled region still spreads onwards, so that the slit-like blastopore of later stages lies well within the margin of the small-celled area. Thus were development modified by the slurring over of the early stages of the invagination-groove so that this only became apparent at the period when it had assumed the form of a longitudinal slit, it would at the time of its first appearance be situated well within the small-celled area instead of at its hinder margin. The importance of this consideration will become manifest later on in connexion with the interpretation of the developmental phenomena of the Amniota.

ELASMOBRANCHII.—The egg of the Elasmobranch at the time immediately preceding gastrulation differs from the blastula of the ordinary Amphibian or Lung-fish in its much greater size. The small-celled or micromeric apical portion of the blastula is represented here by a relatively small mass of cells—the blastoderm—in the region of the apical pole while the large-celled portion is represented by the yolk. This latter is composed, practically, of a mass of yolk granules, the protoplasmic matrix being reduced almost to vanishing-point. As in the eggs previously described, the micromeric portion gradually spreads round and encloses the yolk and here again we find the same three factors at work—involution, overgrowth and delamination.

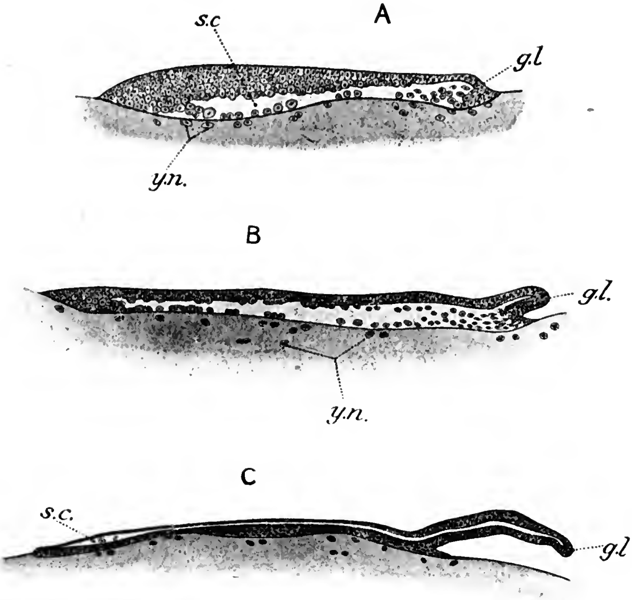


FIG. 28.—Sagittal sections through Elasmobranch blastoderms (*Torpedo*) illustrating the process of gastrulation. (After Ziegler, 1902.)

g.l, gastrular lip; *s.c*, segmentation cavity; *y.n*, yolk nuclei.

The first step in the gastrulation process consists in the involution of the surface along the posterior edge of the blastoderm. This involution groove spreads outwards on each side until it may extend along $\frac{1}{3}$ to $\frac{1}{2}$ the circumference of the blastoderm. The blastoderm is meanwhile spreading outwards all round and, as it does so, the central part of the groove becomes deepened to form a tubular cavity, the archenteron, which runs forwards from the mid-posterior margin. In the roofing in of this archenteron it is apparently a process of overgrowth which plays the main part—but along the rest of the blastoderm margin the process of overgrowth appears to die away and its place is taken by delamination very much as was the case in *Lepidosiren*. This is shown by the fact that the invagination-groove, which, as already remarked, extends outwards on each side for some distance, never deepens to any considerable extent except in its middle part.

In the region in front of the archenteron the deeper or lower layer cells of the blastoderm increase greatly in number and spread forwards so as gradually to fill up the segmentation cavity. The remains of the latter persist longest near the anterior margin and the ectoderm covering the last remnant of the segmentation cavity commonly projects as a small but conspicuous elevation above the general surface of the blastoderm.

These lower cells eventually take on a mesenchymatous character for the most part. Those lying next the yolk-syncytium however give rise to a definite epithelium, known as the yolk epithelium. Some of them are said to penetrate actually into the yolk where their nuclei assume the characters of the nuclei of the yolk-syncytium. The floor of the archenteron is formed by the yolk epithelium which is continuous round the inner, or anterior, end of the archenteric cavity with the endoderm of its roof.

ACTINOPTERYGII.—It is unfortunate that in the more familiar Actinopterygians belonging to the group Teleostei—of which it is so easy to obtain developmental material—the phenomena of gastrulation are obscure and their investigation is impeded by technical difficulties in the way of making satisfactory sections. We shall therefore confine ourselves to indicating in a few words the more conspicuous features of the process.

On the whole the features of gastrulation closely resemble those met with in Elasmobranchs—a resemblance which however we are not justified in regarding otherwise than as a phenomenon of convergence, seeing that the general evidence of morphology points to the ancestors of the Teleosts being much more closely related to the holoblastic Ganoids than to the existing Elasmobranchs. A characteristic feature to be noted is that here, as will be found to be the case in various mammals, the superficial cells of the blastoderm become much flattened and form a thin protective covering layer which takes no part in the development of the embryo.

When gastrulation is commencing the posterior margin of the blastoderm presents in longitudinal vertical sections the appearance of being turned inwards to form the two primary layers. There is no actual patent archenteric cavity though the inflected portion clearly represents the archenteric roof, the floor being apparently represented by the underlying syncytial layer.

The growth in length of the archenteric roof seems to be brought about mainly by a process of overgrowth similar to that met with in other forms.

A point of special interest is that the posterior portion of the archenteric roof, in the neighbourhood of what will become later the mesial plane, is without the distinct demarcation between ectoderm and endoderm which is present elsewhere. This continuity of the two primary cell layers apparently represents what is known in the Amniota as the primitive streak—a structure of great morphological interest which will be discussed later on (Goronowitsch, 1885; Jablonowski, 1898).

While these processes are in progress the margin of the blastoderm elsewhere is also advancing over the surface of the yolk so as gradually to enclose it. This enclosure of the yolk clearly corresponds to what we have seen in other cases but it is difficult to be quite certain as to how far it takes place by actual delamination and how far this has been replaced by a secondary independent growth.

It is only when the exposed surface of yolk becomes reduced to a small round patch that the cell-margin bounding it shows inflection all round so as to justify us in speaking of a blastopore.

In the surviving Ganoid members of the group Actinopterygii we find that the process of gastrulation, as is the case with other characteristics, repeats conditions which are probably to be looked on as ancestral. The gastrulation clearly belongs to the same general type as that of Lampreys, Amphibians, and Lung-fishes. That of *Acipenser* (Salensky, Bashford Dean, 1895) seems more nearly to resemble that of *Polypterus*, and that of *Amia* (Bashford Dean, 1896) and more especially *Lepidosteus* (Bashford Dean, 1895) to point towards the mode of gastrulation found in the modern Teleosts.

GASTRULATION IN AMNIOTA

In comparing the process of gastrulation in the Amphibians and Lung-fishes with that in *Amphioxus* or *Polypterus* we have seen that there is a tendency for the greater part of the gastrular rim either to become completely obsolete or to be, at least, greatly delayed in its appearance, for example in the frog the greater part of the gastrular rim makes its appearance only in a comparatively late stage in the process of gastrulation.

In the Amniota we find that this tendency has gone further. It is only in the lowest group—the Reptilia—that an undoubted gastrular lip is clearly recognizable. In the two remaining groups, the Birds and Mammals, there is no convincing evidence that it has not completely disappeared from development.

Reptiles.—In a Reptilian egg before the commencement of gastrulation the apical portion is covered by a blastoderm consisting of a superficial layer of flattened ectoderm cells and, underneath this, rounded lower layer cells which are separated by interstices containing fluid.

In the centre of the blastoderm (Fig. 29, A) an area, circular or elliptical or pear-shaped with its narrow end posterior, becomes distinguishable from the rest of the blastoderm by its slightly greater opacity. The area in question is known as the **embryonic shield** (*e.s*), and its opacity is due to its ectoderm being thickened, the individual cells having taken on a columnar form.

Either enclosed within or projecting beyond the posterior outline of the embryonic shield (Fig. 29, B) is a small area in which there is no layer of columnar ectoderm sharply marked off from the lower cells. This forms the **primitive plate** (Fig. 31, *p.p*). The boundary of the embryonic shield gradually spreads outwards and the primitive plate comes to be, if it is not already, enclosed within it.

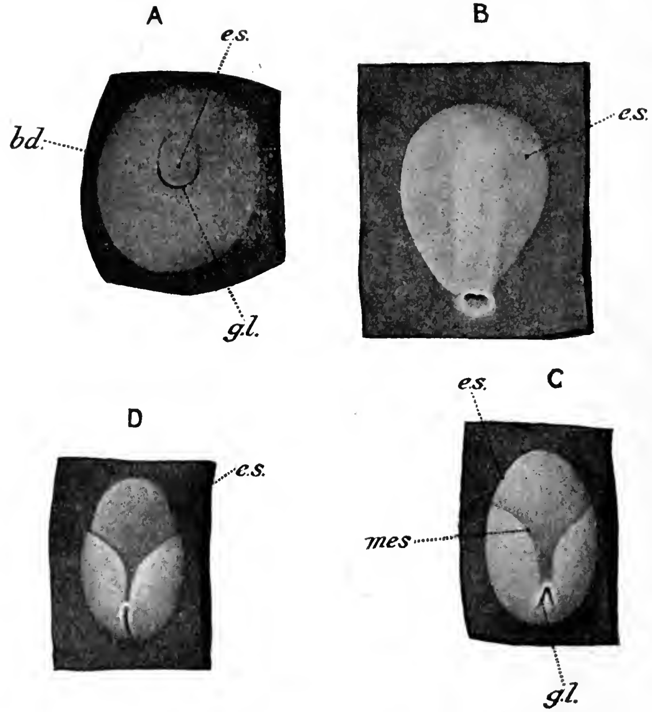


Fig. 29.—Illustrating gastrulation in the Gecko (*Platydactylus*). (After Will, 1892.)

A, showing complete blastoderm with the embryonic shield in the centre. This is bounded behind by the gastrular rim, precociously developed in this specimen. B, embryonic shield of specimen at the stage in which the archenteric floor is breaking down. C, embryonic shield at later stage where gastrular rim is bent back into a Λ-shape bounding the yolk-plug: the outline of the mesoderm sheet is seen on each side. D, embryonic shield showing stage at which the gastrular lips have come together so as to bound a longitudinal slit. *b.d*, edge of blastoderm; *e.s*, embryonic shield; *g.l*, gastrular lip; *mes*, limit of mesoderm.

Within the limits of the primitive plate the surface of the egg now becomes involuted to form a groove bounded anteriorly by a lip which from its correspondence with what we have seen in lower forms, more especially in the Gymnophiona, is clearly to be recognized as the gastrular lip. This lip gradually shifts backwards and, as it does so, undergoes alterations in shape, which differ somewhat in different species and even in different individuals of the same species but which in their main features are illustrated by Fig. 30. In its later stages the lip becomes bent or curved backwards so as to have the shape of a ∩ or a Λ (Figs. 29, C, and 30).

Considerable variation occurs between different individuals in the time of the first appearance of the gastrular lip, and in the Gecko *Platydactylus* Will (1892) observed a correlated variation in shape. Where it appeared relatively early, the involution had the form of an elongated crescentic groove, while in cases where its appearance was delayed the involution formed a shorter and more rounded opening.

As in other cases the central part of the invagination groove becomes deepened to form a cavity which is clearly homologous with the main part of the archenteric cavity in, say, a frog. This cavity starts by passing directly inwards, perpendicular to the egg-surface, but it soon bends forwards and runs parallel to the surface (Fig. 31, C and D). The cavity just mentioned (Fig. 31, D, *ent.*) being an archenteron the layer of cells lining it corresponds to that which in the lower forms is called endoderm. It is therefore misleading to replace this by any other name: to emphasize the fact that they line the true archenteric cavity it may be advisable to speak of the cells in question as the *archenteric* endoderm in spite of the clumsiness of the expression.

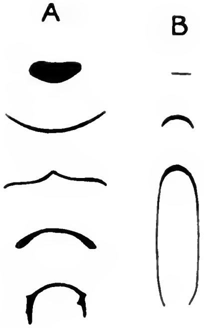


FIG. 30. — Successive stages of gastrular rim or lip as seen in surface view. A, *Chelonia*, (Mitsukuri, 1896). B, *Platydactylus*, (Will, 1892).

In the meantime the lower layer cells immediately underlying the ectoderm assume a flattened form and become joined together by their edges to form a definite epithelium which may conveniently be termed the **secondary endoderm** (Fig. 31, C and D, *end'*). The more deeply situated cells underlying the secondary endoderm remain spherical and are separated by wide spaces forming a segmentation or subgerminal cavity. These deeper spherical cells have their numbers constantly reinforced by additional cells which are apparently budded off from the underlying yolk-mass.

The floor of the archenteron becomes closely apposed to the secondary endoderm immediately beneath it (Fig. 31, D). The two cell-layers fuse, irregular perforations develop in the membrane formed by their fusion, and the result is that the archenteron is thrown into communication with the "subgerminal" cavity (Fig. 31, E). Shreds of the partition persist for some time but eventually the two spaces form a perfectly continuous cavity just as happened with archenteric and segmentation cavity in the Gymnophiona.

The portion of the primitive plate which is embraced by the horse-shoe shaped gastrular lip corresponds to the yolk-plug of amphibians and in some cases too (*Lacerta*—Will) it becomes completely enclosed, the tips of the horse-shoe curving inwards and

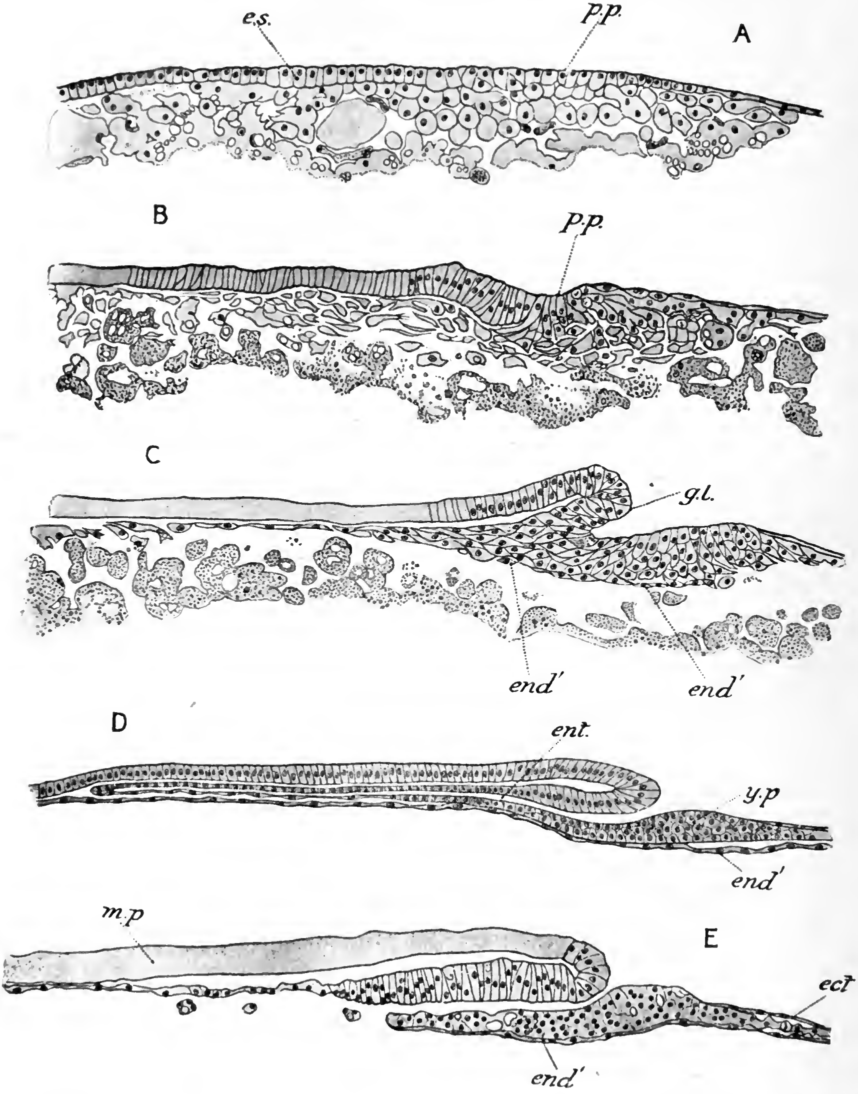


FIG. 31.—Sagittal sections through early stages of *Platydactylus*. (After Will, 1892.)

A is of the stage shown in Fig. 29. A; E is of the stage of Fig. 29, B; B, C, and D are of intermediate stages. *e.s*, embryonic shield; *ect*, ectoderm; *end′*, secondary endoderm; *ent*, archenteric cavity; *g.l*, gastrular lip; *m.p*, thickened ectoderm which will give rise later to the central nervous system (medullary plate); *p.p*, primitive plate; *y.p*, yolk-plug.

meeting to form a closed ellipse. The yolk-plug differs from that of amphibians merely in its being elliptical in outline instead of circular.

During these changes the anterior or dorsal part of the gastrular lip grows actively backwards over the surface of the yolk-plug, the portion of yolk-plug which is covered over in this way becoming added to the floor of the archenteron and its superficial cells becoming converted into archenteric endoderm.

The last phase in the closing of the gastrular opening consists in its lateral walls approaching the mesial plane so that the opening assumes the form of a longitudinal slit (Fig. 29, D). Part of this slit persists for some time as a neurenteric canal—a communication between the enteric cavity and the cavity of the neural groove or tube (Fig. 32, C)—while a portion farther back seems to be represented by the anus although in this case the patent opening disappears temporarily so that no absolute continuity can be traced. In the region where the lips have undergone fusion there persists for a time complete continuity between the different cell-layers. The study of sections shows this continuity to be precisely the same as that which occurs in the primitive streak of Birds and Mammals (Fig. 32, B, D, E), and we have thus suggested a clue to the meaning of that otherwise enigmatical structure.

It will have been gathered that the archenteric cavity has become greatly reduced in importance in the Reptile as compared with the more primitive vertebrates. It has become much reduced in relative size,[1] and it soon loses its individuality, becoming merged with the irregular segmentation spaces lying beneath the blastoderm. Correlated with this we can no longer speak of direct conversion of the archenteric cavity into the enteron or alimentary canal, except to a trifling extent. The latter arises, for the most part, as will be shown later, in a quite different manner from the secondary endoderm.

BIRDS.—In the Reptile, as compared with one of the more primitive anamnia, the main peculiarity of the gastrulation process lies in the fact that the cavity which opens to the exterior by the blastopore is normally of double origin, only its posterior portion being derived from archenteron. Consequently the layer of endoderm which lines it is only to a comparatively small extent derived from the archenteric lining, the much greater anterior part being formed from elements of independent origin.

In the Amniota above Reptiles the replacement of archenteric by secondary endoderm has gone still further, inasmuch as the formation of an archenteron has in them either completely disappeared from development, or at the least is reduced to a faint vestige, and the endoderm is therefore entirely secondary.

---

[1] In some forms, such as *Lacerta*, the archenteric portion of the enteron appears to be for a time much shorter relatively than in others (e.g. *Platydactylus*) but this is corrected later on by active overgrowth on the part of the dorsal lip (Will, 1895).

As regards the Birds, which of the higher Amniotes alone concern us in this volume, there is complete agreement that they are to be looked on as highly-specialized descendants of Reptilian ancestors. It follows therefore that their developmental phenomena should be considered in relation to the corresponding phenomena in Reptiles.

Leaving out of account certain vague phenomena which have been interpreted, in the present writer's opinion unjustifiably, as reminiscences of gastrulation processes (see Chapter X.), the formation of a gastrular lip seems to have

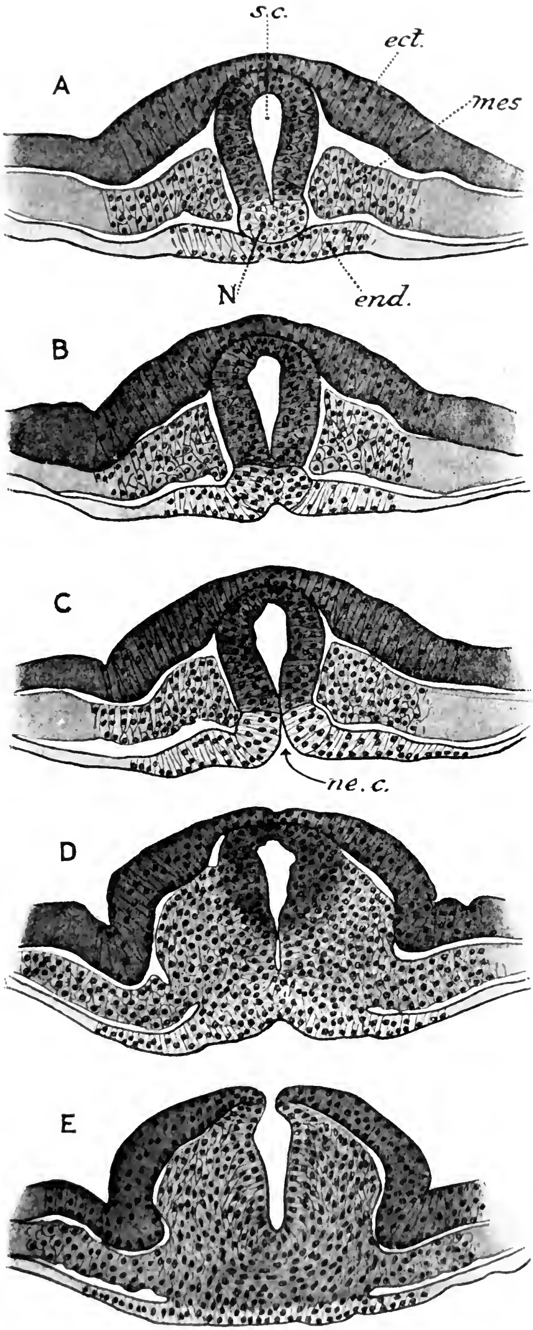


FIG. 32.—Transverse sections through region of neurenteric canal of *Chelonia* embryo with about 16 segments. (After Mitsukuri, 1896.)

The mid-dorsal ectoderm has become covered in to form the neural tube (*s.c.*) as will be described in Chap. II. Fig. C shows the neurenteric canal opening upwards through this, while Figs. B, D and E, taken from sections anterior and posterior to the neurenteric opening, show the continuity of tissue from ectoderm to endoderm which is a characteristic feature of a primitive streak. *ect*, ectoderm; *end*, endoderm; *mes*, mesoderm; *N*, notochord; *ne.c*, neurenteric canal; *s.c*, spinal cord.

been eliminated entirely from ontogenetic development in Birds. What *is* conspicuous is a well-marked **primitive streak** which makes its appearance in the posterior half of the blastoderm along what will be the axial line of the body of the embryo (see Chap. X.). A groove develops along the surface of the streak—the **primitive groove.**

Histologically the primitive streak is, in its early stages, a line of proliferation from the inner surface of the ectoderm, the blastoderm being composed only of the two primary layers at the time of its appearance. That the ectoderm alone is responsible for the first appearance of the primitive streak, a point difficult to make absolutely certain by ordinary observation, appears to be demonstrated by the study of an abnormal 36-hour embryo Peawit (*Vanellus cristatus*) described by Röthig (1907) in which the endoderm was completely absent while ectoderm and primitive streak were quite normal.

An inspection of blastoderms at successive periods in development shows the primitive streak lying always behind the medullary folds (cf. Fig. 227, Chap. X.), and it might therefore be readily assumed that the embryonic body develops entirely in front of the primitive streak. That this is not so is clearly shown by experiments (Kopsch, 1902) in which a scar is made with a hot needle about the front end of the primitive streak during an early stage in its development. If the egg is carefully sealed up again it may go on with its development, and in such a case the scar is found later on to be situated not near the hind end of the embryo but well forward in the head region.

What apparently happens is that the primitive streak grows actively in length with the general growth of the blastoderm but that all the while it is becoming correspondingly shortened at its headward end. As a matter of fact its anterior end becomes gradually converted from before backwards into notochord and the adjoining parts of the mesoderm. The front part, which is undergoing this change, loses its connexion with the ectoderm while it becomes on the other hand continuous with the endoderm and is reinforced by proliferation from it: it then forms what is known as the **Head process.**

The point of special morphological importance to notice about the primitive streak is its continuity with the two primary cell-layers. Throughout the greater part of its length it is continuous with ectoderm, in its front half with both ectoderm and endoderm, and in its forward prolongation—the head process—with endoderm. Correlated with this is the further fact that in some cases (Tern, Goose, Duck, Wagtail, *Melopsittacus*) the tissue of the primitive streak is traversed by a typical neurenteric canal.

Taking these various features into consideration it is impossible to avoid the conclusion that the primitive streak represents the line of coalescence of the gastrular lips just as it actually is in

Reptiles, and that the neurenteric canal represents a persisting portion of a once slit-like gastrula mouth which is otherwise obliterated.

ORIGIN OF THE MESODERM

General Remarks.—Already during the process of segmentation the differentiation of the two primary cell-layers commences—the superficial cells towards the apical pole dividing more actively, being smaller, and being less laden with food-yolk and thus establishing a character of their own as ectodermal cells. The full establishment of the primary layers however is only consummated during the process of gastrulation when the ectoderm comes by the various processes already described to enclose the remaining cells the (archenteric) endoderm.

The establishment of the two primary layers is followed immediately (indeed the two processes frequently overlap) by the development of the intermediate cell-layer—the **mesoderm**—which will in the adult form the great mass of the body—all in fact except the epidermis and its derivatives on the one hand and the enteric epithelium and its derivatives on the other.

The problem of the evolutionary history of the mesoderm of Vertebrates is one upon which there is little agreement. Anything of the nature of elaborate and detailed treatment of the subject would be out of place in a textbook of moderate size and a short sketch such as the following is necessarily coloured by the general morphological views of the writer. While the views set forth in the following paragraphs seem to the author to fit most satisfactorily the facts so far as these are established beyond reasonable doubt there are other embryologists who would give an account differing considerably from that given here.

To the present writer it seems of importance in endeavouring to arrive at reliable general conclusions from the facts of observation to bear in mind particularly the risk of reaching erroneous conclusions through basing arguments upon phenomena observed in the head region or tail region of the embryo. Intense **cephalization**, *i.e.* intense structural modification of the anterior region of the body, to form a **head**, is admittedly one of the fundamental characters of the phylum Vertebrata. In this modification the mesoderm has been deeply involved so that there is always a considerable weight of probability against conditions observed in the head region being primitive. Again the tail region is also intensely modified, as is indicated *e.g.* by the transient appearance within it of a vestigial portion of alimentary canal with surrounding body-cavity. Here again then, though not to the same extent as in the head-region, suspicion rests upon the primitiveness of all phenomena of development peculiar to this region of the body.

It is advisable then, for these reasons, to exercise great caution in making use of any developmental phenomena except those observed in typical trunk segments as a basis for speculations upon the evolutionary origin of the mesoderm.

It has, further, to be borne in mind that observations upon the development of the mesoderm in its early stages have to be made by the method of serial sections, and that in the interpretation of such sections the liability to error becomes greatly increased if the sections are not exactly in one of the three following sets of planes—(1) transverse to the morphological axis, (2) "horizontal," and (3) parallel to the sagittal plane. This type of technical difficulty is in many Vertebrate embryos most marked in the head and tail regions.

For these reasons it seems safest, in considering generally the ontogenetic development and the probable evolutionary history of the mesoderm, to ignore all observations except those made on typical trunk segments between the level of the otocyst in front and of the anus behind. This will accordingly be done in what follows.

It is agreed by the majority of students of Vertebrate embryology that the most nearly primitive condition of the mesoderm known to occur in the embryos of Vertebrates is that seen in *Amphioxus*, where it consists for a time of a series of endodermal pockets, converted later into closed sacs, upon each side of the body (Fig. 34, B).

It appears fully justifiable to conclude that both of the stages mentioned represent ancestral conditions in the evolution of the Vertebrate mesoderm. The excretory organs of the Vertebrate, in the form of paired segmentally arranged tubes, afford in themselves strong evidence that at one time the Vertebrate coelome was in the form of isolated segmentally arranged chambers.

In the case of *Amphioxus* the segmented character of the mesoderm persists only dorsally, the ventral portions of the successive segments becoming fused together so as to give rise to a continuous unsegmented splanchnocoele or peritoneal cavity.

In the Craniata the smallest departure from the condition in *Amphioxus* is seen in such comparatively primitive forms as Lampreys, Crossopterygians and Lung-fishes. In these a solid continuous mesoderm rudiment becomes split off from the endoderm on each side, remaining for some time continuous laterally with the endoderm (Fig. 40, B, C, p. 65). In the outer or lateral part of this mesoderm rudiment the segmentation, which even in *Amphioxus* was only temporary, never makes its appearance. The dorsal portion does segment but the segment is a solid block of cells in which a cavity only appears later on. It is fairly clear that these mesoderm segments, except for the fact that they are continuous in their ventral portions and that they are at first solid (a modification of development which is very common in hollow organs), agree closely with the segments of *Amphioxus* and that they are homologous structures merely somewhat modified from the primitive condition met with in *Amphioxus*.

In endeavouring to institute a more precise comparison of the mesoderm segment in its earliest stage, in the typical Vertebrate, with that of *Amphioxus*, the way is found to be blocked by a secondary adhesion (or absence of separation!) having come about between the mesoderm segment and the endoderm from which it has arisen.

The young mesoderm pouch of *Amphioxus* is attached to the endoderm at its base—*i.e.* its ventral end. Its homologue in the more typical Vertebrate, on the other hand, is continuous with the endoderm in two different regions, one ventral and one dorsal. This is illustrated by such a diagrammatic section as that shown in Fig. 33, B, in which the solid mass of mesoderm on each side, indicated by the medium tone, is continuous with the mass of endoderm or yolk-cells at the points *a* and *b*. The question is, which of these two points is to be interpreted as representing the root of the mesoderm pocket in *Amphioxus*? Clearly only one of them can represent this and the other region of continuity must represent a secondary fusion of mesoderm with endoderm.

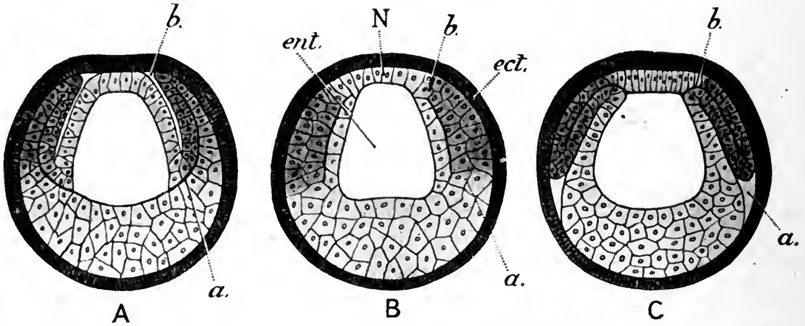


Fig. 33.—Diagram illustrating (B) the origin of mesoderm from endoderm in an Amphibian, and (A and C) the two methods of correlating it with the mode of mesoderm formation in *Amphioxus*.

a, *b*, see text; *ect*, ectoderm; *ent*, enteric cavity; *N*, notochordal rudiment.

The majority of embryologists, following O. Hertwig (1882), believe that the dorsally situated region of continuity marked *b* is the primary connexion as is illustrated by Fig. 33, C. On this view the mesoderm segment of the Vertebrate springs from the endoderm at a point about the level of the notochord, it grows downwards on each side of the alimentary canal and eventually its tip meets the tip of its fellow of the other side of the body in the mid-ventral line. In this view, again, the continuity which can sometimes be shown to exist between mesoderm and endoderm at the point *a* would be regarded as secondary and without evolutionary significance.

If however due weight be accorded to what is observed in the development of the lower holoblastic vertebrates it seems more reasonable to the present writer to conclude that the more ventrally situated connexion, that marked *a*, is the primitive one and that the more dorsally situated, *b*, is the secondary acquirement (Fig. 33, A).

Upon the former hypothesis the extension of the mesoderm laterally by delamination from the endoderm, which does certainly occur in some forms (see below), would be an inexplicable mystery. On the second hypothesis on the other hand this splitting off of mesoderm would be comparable with a gradual deepening of the angle which bounds the mesodermal pocket of *Amphioxus* on its mesial side (see Fig. 34, B). The dorsal attachment is, on this view, to be regarded as a secondary fusion between mesoderm and endoderm. In the higher vertebrates this region becomes the seat of active cell proliferation which plays a great part in the production of mesoderm.

After these general remarks we may proceed to consider shortly the details of the early development of the mesoderm in a few examples of the lower Vertebrata.

AMPHIOXUS.—In *Amphioxus* the development of mesoderm begins with the formation of a longitudinal fold or outpushing of the endoderm on each side of the mid-dorsal line (Fig. 34, A, *mes*). In this way there is formed on each side an upwardly-projecting groove or gutter, the narrow cavity of which is a prolongation of the archenteric cavity (Fig. 34, B). Constrictions appear now in the wall of this gutter which divide it up into successive segments — the constrictions developing in order from the head-end backwards. The groove or fold is in this way converted into a series of pockets the coelomic or **enterocoelic pouches**. The cavity of these pouches except in the case of the first two usually becomes for a time practically obliterated by the outer and inner walls coming into contact. Finally the communication between pouch and archenteron becomes closed and the pouch

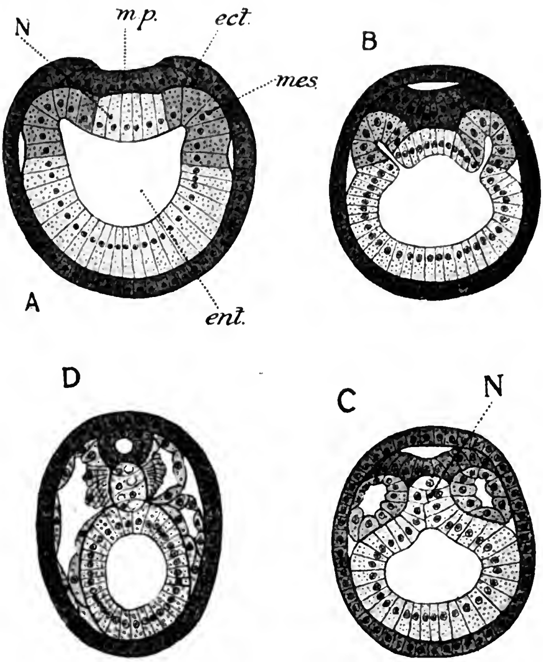


FIG. 34.—Transverse sections of young *Amphioxus* illustrating the origin of the mesoderm. (After Hatschek, 1881.)

ect, ectoderm ; *ent*, enteric cavity ; *m.p*, medullary plate ; *mes*, mesoderm ; *N*, notochordal rudiment.

itself becomes nipped off from the remainder of the archenteron (Fig. 34, C).

The original archenteron is now replaced by a main portion, the

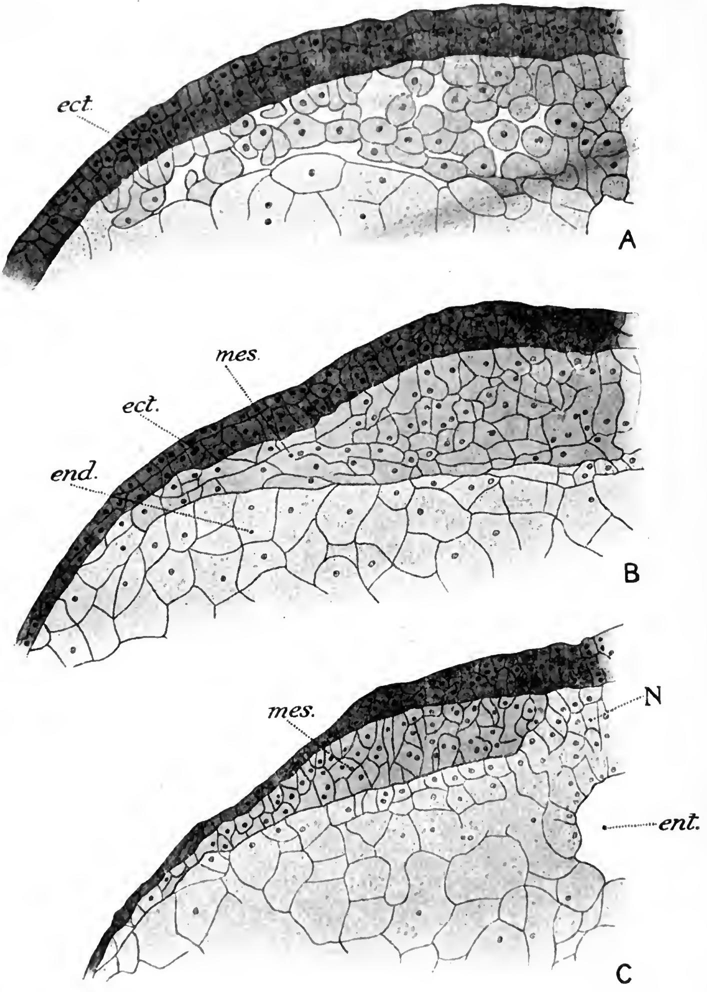


FIG. 35.—Transverse sections through embryos of *Lepidosiren* to illustrate the origin of the mesoderm.

A, stage 12; B and C, stage 14. *ect*, ectoderm; *end*, endoderm; *ent*, enteric cavity; *mes*, mesoderm; *N*, notochordal rudiment.

enteron, the wall of which—the definitive endoderm—will eventually become the lining epithelium of the alimentary canal, and, lying dorsal to this on each side, a series of closed sacs, or practically solid blocks (their outer and inner walls being in contact). These sacs or blocks are the **mesoderm segments** and their cavities are the segmentally arranged rudiments of the **coelome**. The subsequent fate of the mesoderm segments will be traced later (Chap. IV.).

Of the lower holoblastic forms amongst the Vertebrata in the stricter sense we will consider first *Lepidosiren*, in which, owing to the large size of the cell-elements, the details of mesoderm formation are particularly clear and unmistakable.

The mode of origin of the mesoderm which occurs in *Lepidosiren* is illustrated by Fig. 35. The section shown in Fig. 35, A is taken from an egg of the same age as that figured on p. 35 (Fig. 21, C) in illustration of the disappearance of the segmentation cavity. Immediately below the ectoderm is a mass of rounded blastomeres with intervening chinks—remnants of the segmentation cavity: towards the mesial plane the blastomeres are more closely packed together. The small blastomeres in question are clearly distinguished by their finely-grained yolk from the large yolk-cells with their coarsely-grained yolk which form the bulk of the egg. The mass of small blastomeres is destined to give rise laterally to the mesoderm and mesially to the notochord. It must clearly be borne in mind that the mass is composed simply of small blastomeres and that it passes at its outer margin without any break into the ordinary yolk-cells.

As development goes on, the mass of small elements becomes compacted together (Fig. 35, B), the chinks between the cells disappearing. At the same time the boundary between them and the yolk-cells becomes more definite, so as to delimit more clearly the mesoderm rudiment (*mes*) from the definitive endoderm.

Fig. 35, C is taken from an egg of the same age but here the mesoderm rudiment has become limited also on its mesial side by a split which marks it off from the notochord (*N*).

At a somewhat later stage, the mesoderm mass on each side becomes divided into segments by splits, transverse to the axis of the body, which make their appearance at regular intervals from before backwards, but it is to be noted that in *Lepidosiren* (as in all Vertebrates except *Amphioxus*) this splitting of the mesoderm is confined to its dorsal portions. There is thus produced along each side of the body a series of incomplete mesoderm segments[1] which pass at their lower or ventral ends into an unsegmented sheet of "lateral"

---

[1] Such incomplete mesoderm segments as are described above occur in all the typical vertebrates and are known by various names such as mesoblastic somites, protovertebrae, myotomes. These names are in various degrees erroneous or misleading. The word somite means a complete body segment and it is not allowable to apply it to a single organ. The name protovertebra dates from the days in which these structures were supposed to be the embryonic vertebrae, which they are now known not to be. Of the three terms mentioned myotome is the least objectionable as at least the greater part of the segmented portions of mesoderm become definite myotomes later on. On the whole however it seems most convenient to retain the expression mesoderm segment, the word segment not being necessarily used in the precisely defined way in which such a purely technical morphological term as "somite" must be used.

mesoderm. This latter gradually spreads ventralwards by delamination from the large yolk-cells and eventually the mesoderm sheets on the two sides become continued into one another ventrally.

As will be noticed there are no coelomic spaces within the mesoderm rudiments at these early stages: they arise secondarily later on.

If we review the above-described stages in the early development of the mesoderm segment in *Lepidosiren*, in which, as already indicated, the large size of the cell-elements ensures unusual freedom from the danger of errors of observation, we see that the last described stage is clearly in agreement with the hypothesis that it is a repetition of the stage in *Amphioxus* when the mesoderm existed in the form of a series of enterocoelic pouches on each side. The only conspicuous difference is that, whereas in *Amphioxus* these were actual pouches, here they are solid blocks of cells in which a cavity only makes its appearance at a later stage of development. That this difference is in no way a serious one will become apparent to the reader as he realizes that it is one of the commonest modifications of developmental phenomena, when yolk is abundant, that primitively hollow organs develop in the embryo from solid rudiments and only form their cavity secondarily.

It may be accepted then with confidence that the solid mesoderm segments of *Lepidosiren* at the stage indicated, continuous ventrally with the endoderm, represent the enterocoelic pouches of *Amphioxus* modified in correlation with the abundance of yolk.

The first stages in the development of the mesoderm of *Lepidosiren* are obviously very different from what are found in *Amphioxus* and the differences here also we may justifiably attribute to the immense thickening of the endodermal wall of the archenteron correlated with the storing up of a large amount of yolk in its cells.

In the other groups of holoblastic vertebrates the main features in the early development of the mesoderm agree with those just described for *Lepidosiren*. In all of them the archenteron is provided with a thick wall of heavily yolked endoderm cells, those forming the roof or dorsal part of the wall being smaller and provided with finer yolk-granules. Out of this smaller-celled mass the mesoderm segments become carved by the development of splits very much in the same way as in *Lepidosiren* (cf. Fig. 40, B—*Petromyzon*).

Amongst these groups the Amphibia call for a little further consideration.

In the frog a split develops on each side which separates the roof of the archenteric cavity into two layers, an inner layer, one cell thick, of definitive endoderm and an outer, two cells thick for the most part, the mesoderm. This split is seen in Fig. 36 which represents a section, transverse to the axis of the archenteron, through an egg with large yolk-plug. The split in this section terminates below at about the level of the floor of the archenteric cavity while above it stops short of the level of the notochord.

A little later a split at its dorsal end demarcates the mesoderm rudiment from the notochord. The mesoderm rudiment, forming now a broad band on each side of the embryo, becomes divided into segments by splits which cut it across and a condition is reached corresponding closely with that already described for *Lepidosiren* where the mesoderm consists of a series of solid segments on each side continuous ventrally with the mass of yolk cells forming the main part of the endoderm.

As in *Lepidosiren* the ventral unsegmented part of the mesoderm becomes prolonged ventrally by the extension downwards of the split between it and the endoderm. In other words the mesoderm extends ventrally by a process of delamination from the endoderm.

In the anterior part of the body the sheet of mesoderm becomes split off completely from the yolky endoderm before it quite reaches the mid-ventral line so that the sheets belonging to the two sides are discontinuous ventrally but in the hinder region the two splits meet ventrally so as to give rise to a sheet of mesoderm continuous across the middle line. Under ordinary circumstances the mesoderm sheets in the anterior region grow ventrally and eventually fuse with one another (as will be described later) while in the posterior region this fusion is anticipated by the two lateral rudiments being continuous from the beginning.

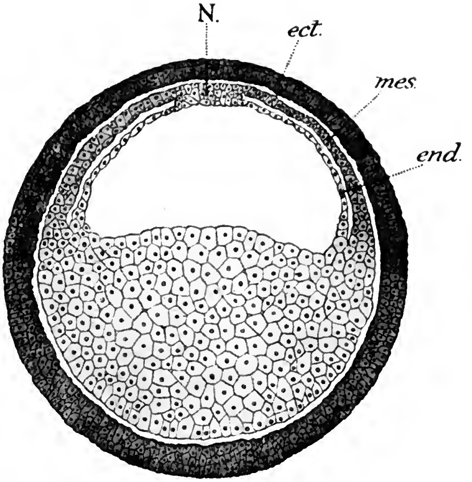


FIG. 36.—Transverse section through an embryo of *Rana* illustrating the origin of the mesoderm. (After Schwink, 1889.)

ect, ectoderm; *end*, endoderm; *mes*, mesoderm; *N*, notochord.

So far everything seems fairly simple, but it now remains to allude to certain peculiarities which have done much to obscure the clear understanding of the method of mesoderm formation and which are especially important for the proper comprehension of the first formation of mesoderm in the meroblastic vertebrates.

The peculiarities in question are to be seen in the hinder part of the trunk region. In this region the split which separates off mesoderm from endoderm remains for a time incomplete at a point just external to the notochord. Each segment therefore remains for a time continuous with the endoderm at this point. The level of these junctions of mesoderm and endoderm is marked by a longitudinal groove of the inner surface of the wall of the archenteron so that where the junction exists the archenteric cavity may be said to project slightly into it. The cells at this point develop pigment in their protoplasm (Fig. 37, B); they frequently show mitotic figures and appear to be actively proliferating, cells being added at this point to the mesoderm.

The peculiarities which have just been described, and which occur in various amphibians, have important bearings in two different directions. In the first place they form an important part of the basis for O. Hertwig's hypothesis of mesoderm formation in the Vertebrata, the junctions, which have just been described, between endoderm and mesoderm being interpreted by him as representing the original stalks of the mesoderm segments as they occur in *Amphioxus*. As already indicated there do not appear to the writer to be sufficient reasons for regarding these connexions as primitive rather than those more ventrally situated. The balance of probability appears rather to favour the view that of the two connexions it is the ventral one which is the persistent original one, and that it is the dorsal which is to be interpreted as due to secondary fusion.

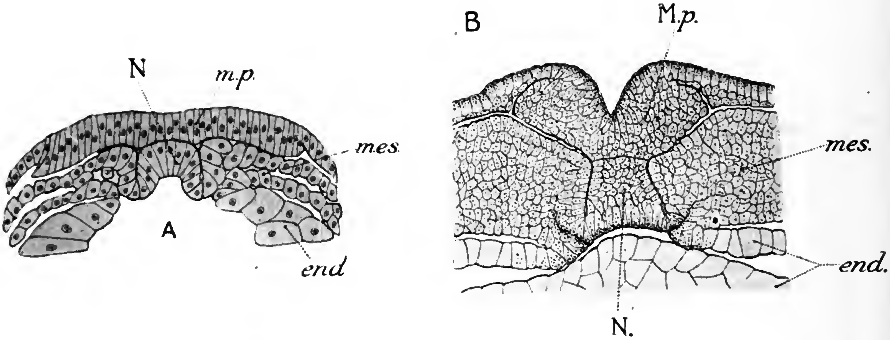


FIG. 37.—Transverse sections through embryos of (A) *Triton* and (B) *Rana temporaria* showing continuity of endoderm and mesoderm on each side of the notochord. (After O. Hertwig, 1882 and 1883.)

end, endoderm; *m.p*, medullary plate; *mes*, mesoderm; *N*, notochordal rudiment.

The second bearing is at least equally important. It rests on the occurrence of active cell-proliferation on each side of the notochordal rudiment. For in some of the meroblastic vertebrates (Amniota)—correlated with the more and more complete segregation of yolk from protoplasm—this zone of proliferation becomes apparently the main source of the mesoderm.

ELASMOBRANCHII.—In the Elasmobranch, while there are still traces of formation of mesoderm by a process of delamination from the main mass of endoderm or yolk (Fig. 38, A), a more conspicuous mode of formation is provided by active proliferation of the endoderm cells along the inner and outer edges of the sheet of mesoderm.

In early stages and in the anterior part of the embryo this proliferation process may alone be in evidence, so that in place of a broad continuous sheet of mesoderm there are found two narrow strips—one (Fig. 38, C, *mes′*) arising from the endoderm just external to the notochordal region and the other (*mes″*) arising from the endoderm peripherally. The two strips are known respectively as the axial (Rückert; or Gastral, Rabl) and the peripheral mesoderm (Rückert; or Peristomal mesoderm, Rabl).

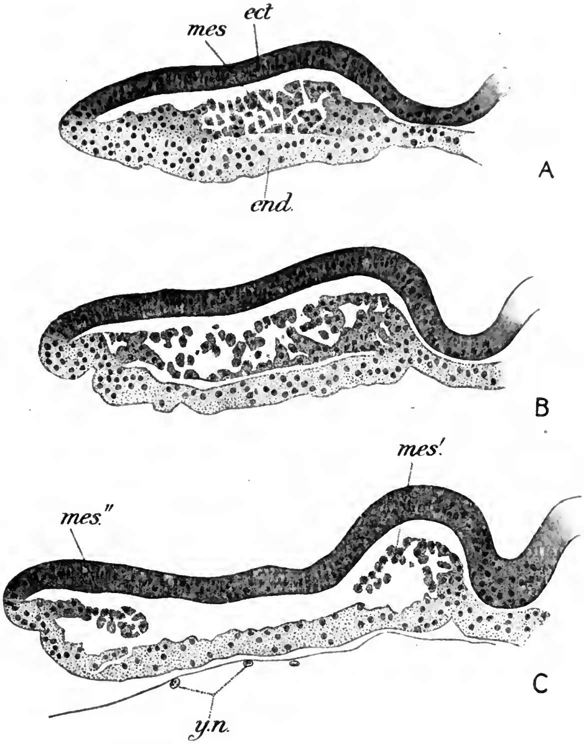


FIG. 38.—Three transverse sections through an embryo of *Pristiurus* (Stage B, Balfour), illustrating the origin of the mesoderm. (After C. Rabl, 1889.)

Section A, through the posterior half of the embryo; B, through the middle; C, through the anterior half. *ect*, ectoderm; *end*, endoderm; *mes*, mesoderm; *y.n.* yolk nuclei.

Much discussion has centred round this double origin of the mesoderm and attempts have been made to distinguish axial and peripheral mesoderm in holoblastic forms including even *Amphioxus*, thus infringing one of the chief canons of embryological science—that developmental phenomena in the higher forms are to be explained by those of the lower and not vice versa.

In the formation of axial mesoderm we recognize a zone of fusion of mesoderm and endoderm accompanied by proliferation of mesoderm entirely analogous with that which occurs in Amphibians but which had not yet made its appearance in lower holoblastic forms.

Whether it is justifiable to regard the outer zone of mesoderm formation in the Elasmobranch as equivalent to the region of delamination (a process which necessarily involves cell-proliferation) is doubtful. It is indeed doubtful to what extent there is justification for drawing any morphological distinction between axial and peripheral mesoderm, seeing that the two regions of proliferation are on the protostoma hypothesis morphologically closely related to one another (see Chap. IX.).

If we look at the matter from the point of view of physiology rather than of morphology we may probably recognize in the active formation of axial mesoderm an expression of the general tendency in the meroblastic egg for all processes of growth and cell proliferation to become concentrated towards the mesial plane dorsally and to slacken off peripherally and ventrally.

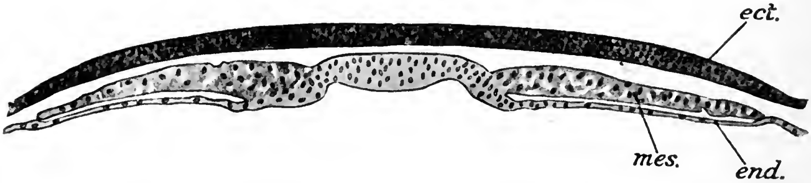


FIG. 39.—Transverse section through the blastoderm of a snake (*Tropidonotus*) illustrating the origin of the mesoderm. (After O. Hertwig, 1906.)

ect, ectoderm ; *end*, endoderm ; *mes*, mesoderm.

REPTILES.—According to the view taken in this book the mesoderm in the holoblastic Craniates at one period spread outwards by a process of delamination from the yolk-laden endoderm.

In the Amphibians we have seen that a new source of addition to the mesoderm had made its appearance in the form of a zone of proliferation on each side of the notochord, in which region cells are budded off into the mesoderm.

In the Reptiles—admittedly descendants from Amphibian-like ancestors—in correlation with the concentration of developmental activity towards the mid-dorsal line brought about by the accumulation of the yolk ventrally, this parachordal source of mesoderm has become predominant while the lateral source has become greatly reduced.

In Fig. 39 is represented the typical mode of mesoderm formation as seen in a transverse section through the trunk region of a reptilian embryo. The mesoderm is seen to be spreading out as a wing of cells towards either side from the notochordal or primitive streak region between the two primary cell-layers.

BIRDS.—In the Birds also the method of first mesoderm formation appears to be closely comparable with that of Reptiles and Amphibians. Here, at the time when the mesoderm begins to make its appearance, the position of the notochord is occupied by the primitive streak. The mesoderm forms a loose sheet of irregularly shaped cells spreading out on each side and added to from two distinct sources: on its inner side by proliferation from the primitive streak and on its outer side by delamination from the endoderm of the germ wall.

It will facilitate comprehension of the evolutionary changes which the writer believes to have taken place in the mode of development of the mesoderm within the phylum Vertebrata if the main steps are summarized in a diagram. In Fig. 40, A shows the primitive condition where the mesoderm segments are in the form of enterocoelic pockets (*Amphioxus*). In B, with increasing amount of yolk, the hollow pocket is represented by a solid block in which the cavity will develop secondarily (*Petromyzon*). In C the condition is similar but the dorsal portion of the embryonic body is more flattened out, the bulk of the yolky endoderm over which it is spread having

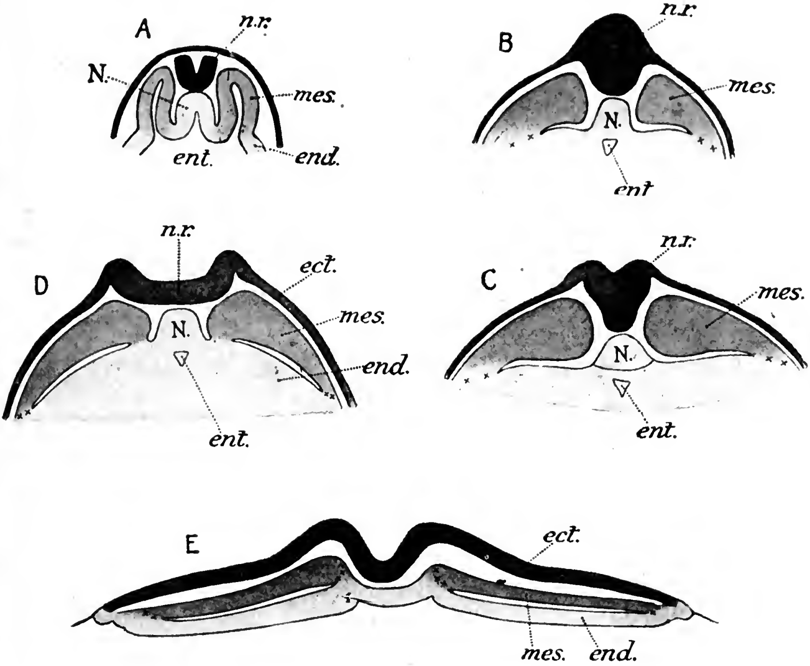


Fig. 40.—Semi-diagrammatic transverse sections through the embryos of various vertebrates to illustrate the origin of the mesoderm.

A, *Amphioxus*; B, *Petromyzon*; C, *Lepidosiren*; D, Amphibian; E, Elasmobranch. *ect*, ectoderm; *end*, endoderm; *ent*, enteric cavity; *mes*, mesoderm; *N*, notochord; *n.r*, neural rudiments. The small crosses indicate regions in which active extension of the mesoderm is taking place.

become greater (Lung-fish). In D the secondary continuity of the mesoderm with the endoderm just outside the notochord is present and proliferation of mesoderm cells has commenced in this region (Amphibia). Finally, in E, with the very great increase in the bulk of the yolk, the dorsal part of the embryo is still more flattened out, and the addition to the mesoderm by proliferation of endoderm cells into it close to the notochord has now become conspicuous (Elasmobranch).

THE MESENCHYME

The fate of the mesoderm whose origin has just been traced is to give rise directly to the peritoneal epithelium which lines the body cavity and covers the organs lying within it, and also to the muscular system. Indirectly it, however, also plays a great part in the formation of what is known as the mesenchyme.

Whereas for a time the Vertebrate body is composed of compact masses or layers of cells, it is a general characteristic that, as development goes on, individual cells detach themselves and wander away through the body, multiplying by fission accompanied by mitosis, and behaving in fact very much as if they were independent organisms. In the course of the many generations of these cells which arise during the process of individual development, they become divided into various strains which show marked differentiation for the performance of different functions.

Some retain a relatively primitive amoeboid form and undertake such functions as the transport of food material, the absorption of moribund tissues in regions where shrinkage in volume or atrophy is taking place, and the ingestion and destruction of attacking organisms such as disease germs. Some, their protoplasm laden with insoluble excretory products as a result of their active metabolism, wander towards the light and settle down near the surface of the body as pigment cells or chromatophores which serve on the one hand to protect the underlying tissues from the light and upon the other to give distinctive coloration to the animal. Others again settle down in an abundant jelly-like intercellular matrix to form **connective** or packing **tissue**, which in turn shows evolution in various directions in accordance more particularly with different developments of the intercellular matrix. Of special importance are these types in which the matrix becomes hard and rigid so as to form skeletal tissues such as bone and cartilage.

Another important strain of these cells is characterized by the fluidity of the matrix and the independence of the individual cells which float in it. This liquid connective tissue forms the **blood** which, pumped through an elaborate system of vessels, serves on the one hand for the transport of food and oxygen to the tissues, and on the other for carrying away the waste products of metabolism to the special excretory organs the duty of which is finally to remove these harmful substances.

The sum of these amoeboid cells, which proceed along the various evolutionary paths above indicated, were, by O. Hertwig, given the name **Mesenchyme**—to distinguish them from the **mesothelium**, or mesoderm in the restricted sense, in which the cells remain in the form of continuous layers or epithelia.

The original mesenchyme cells arise by emigration from the pre-existing cell layers. Possibly all three layers give rise to mesenchyme cells. It is the mesoderm however which does so most conspicuously. In an Elasmobranch embryo, for example, active budding off of mesenchyme cells is seen over large areas of the somatic mesoderm and similarly from the inner surface of the splanchnic mesoderm. Most active of all is the production of mesenchyme cells from the splanchnic mesoderm near the lower end of the mesoderm segment, where the proliferating mesenchyme cells may form a conspicuous mass projecting towards the mesial plane and termed the sclerotome.[1] The special consideration of the sclerotome and of the mesenchyme in general will come most conveniently after the other derivatives of the mesoderm (Chaps. IV., V., VI.).

---

[1] The use of the word sclerotome in this restricted sense has come to be practically universal in embryological literature and is therefore followed in this volume. The word was invented by Goodsir and defined by him, at the British Association meeting in 1856, as meaning a segment of the supporting tissue or framework (whether "fibrous" or cartilaginous or osseous) in a segmented animal.

LITERATURE

Agassiz and Whitman. Mem. Mus. Comp. Zool., xiv, **1885** and **1889.**
Assheton. The Work of John Samuel Budgett. Cambridge, **1907.**
Balfour. Quart. Journ. Micr. Sci., N.S. xiv, **1874.**
Brachet. Arch. Biol., xix, **1903.**
Brauer. Zool. Jahrbücher (Anat. Abt.), x, **1897.**
Budgett. Trans. Zool. Soc. London, xv, **1901.**
Cerfontaine. Arch. de Biol., xxii, **1906.**
Dean, Bashford. Journ. Morph., xi, **1895.**
Dean, Bashford. Quart. Journ. Micr. Sci., xxxviii, **1896.**
Dean, Bashford. Kupffers Festschrift, **1899.**
Goette. Abhandl. zur Entwickelungsgesch. d. Tiere, v, **1890.**
Goodsir. Edinburgh New Philosophical Journal, v, **1857.**
Goronowitsch. Morph. Jahrb. x, **1885.**
Hatschek. Arb. zool. Inst. Univ. Wien, iv, **1881.**
Hertwig, O. Jenaische Zeitschrift, xv and xvi, **1882** and **1883.**
Hertwig, O. Handbuch d. Entwicklungslehre, Jena, **1906.**
Jablonowski. Anat. Anzeiger, xiv, **1898.**
Jenkinson. Vertebrate Embryology. Oxford, **1913.**
Jordan and Eycleshymer. Journ. Morph., ix, **1894.**
Kerr, Graham. Phil. Trans., B, cxcii, **1900.**
Kerr, Graham. The Work of John Samuel Budgett. Cambridge, **1907.**
Kopsch. Internat. Monatsschrift f. Anat. u. Phys., xix, **1902.**
Kopsch. Arch. mikr. Anat., lxxviii, **1911.**
Kowalevsky. Mém. Acad. Sci. St. Pétersbourg, Ser. 7, xi, **1867.**
Mitsukuri. Journ. Coll. Sci. Tokyo, x, **1896.**
Morgan. The Development of the Frog's Egg. New York, **1897.**
Patterson. Journ. Morph., xxi, **1910.**
Prince. Ann. Mag. Nat. Hist., Fifth Series, xviii, **1886.**
Rabl, C. Morph. Jahrb., xv, **1889.**
Röthig. Arch. mikr. Anat., lxx, **1907.**

Rückert. SB. Ges. Morph. Phys. Münch., **1885**.
Rückert. SB. Ges. Morph. Phys. Münch., **1890**.
Rückert. Kupffers Festschrift, **1899**.
Salensky. Memoirs of the Society of Naturalists of Kasan, vii, pt. 3, **1878**. (In Russian. A French translation of the more important parts will be found in Arch. de Biologie, ii, 1881.)
Samassa. Arch. Entwickelungsmechanik, vii, **1898**.
Sarasin, P. and F. Ergebnisse naturwiss. Forsch. auf Ceylon. Wiesbaden, **1887–1893**.
Schultze, O. Köllikers Festschrift. Leipzig, **1887**.
Schultze, O. Arch. mikr. Anat., lv, **1899**.
Schwink. Die Entwicklung des mittleren Keimblattes und der Chorda dorsalis der Amphibien. Munich, **1889**.
Semon. Zoologische Forschungsreisen, i. Jena, **1893**.
Will. Zoolog. Jahrbücher (Anat.), vi, **1892**.
Will. SB. Berl. Akad., **1895**.
Wilson, E. B. Journ. Morph., viii, **1893**.
Wilson, H. V. Bull. U.S. Fish-Commission, ix, **1891**.
Ziegler. Entwickelungsgesch. d. niederen Wirbeltiere, Jena, **1902**.

CHAPTER II

THE SKIN AND ITS DERIVATIVES

THE skin of the vertebrate consists of the **epidermis**—the persistent and less or more modified ectoderm—resting upon the superficial layer of mesenchyme—the **dermis**—which in the higher forms becomes strengthened by the formation of numerous tough interlacing fibres.

In studying the development of the skin in the various types of vertebrate we find that the ectoderm undergoes characteristic modifications to fit it for the carrying out of special functions. In the fishes it becomes converted into a highly glandular mechanism concerned with the production of slippery mucus for the diminution of what the naval architect calls "skin-friction," in other words the friction between the surface of the body and the water in contact with it. Local or general specializations of this glandular apparatus lead to the development of cement organs by the secretion of which the young animal is able to attach itself to solid supports, to the production of digestive ferments by which the eggshell is softened or, in the case of the portion of ectoderm which lines the buccal cavity, the digestion of the food initiated, or to the production of poisonous defensive or offensive secretions. In the case of the terrestrial amphibians the glandular apparatus serves to keep the skin moist, while in the Birds it develops arrangements for oiling the feathers.

Again the ectoderm develops important protective functions. It becomes hardened and toughened to give mechanical protection: it becomes more or less loaded with opaque pigment to prevent the penetration of light rays, while in those highest vertebrates, in which, correlated with intensely active metabolism, the body is kept at a higher temperature than its surroundings, the superficial horny layer becomes as it were frayed out into a fluffy coating of feathers or hair which with its entangled air retards loss of heat from the surface of the body.

Finally the ectoderm forms the great mechanism for the reception of impressions from the external world. It develops sensory cells which may become crowded together to form organs of special sense while from its deeper layers arise the main portions of the central nervous system.

THE EPIDERMIS

The ectoderm covering the surface of the embryo becomes converted, normally, into the epidermis of the fully developed individual. Very usually the embryonic ectoderm consists of two layers of cells, the lower layer composed of actively living cells, the superficial of flattened plate-like protective cells. This outer layer has been termed by Krause the **periderm**: its superficial protoplasm is commonly hardened to form a **cuticle** in the strict sense of the term. Normally it plays no active part in development and is shed at an early period.

The deep layer of the ectoderm on the other hand is active. Its cells multiply so that it becomes several layers thick: the outer layers become cornified to form the horny stratum of the epidermis while the deeper cells, composed of active living protoplasm, form the stratum of Malpighi.

The outer layer of ectoderm cells may be for a time ciliated. This is well seen in young Amphibian embryos (Assheton, 1896). In *Rana temporaria* the 6-mm. embryo possesses ciliated cells scattered thickly over its surface, the movement of the cilia being such as to drive a current of water tailwards over the surface of the embryo. When the external gills develop, a specially strong ciliary current sweeps backwards over them and it is noteworthy that this current passes over the olfactory organ *en route* to the external gills so that the olfactory organ possibly plays an important part in testing the quality of the water going to the respiratory organs. The ciliary apparatus is sufficiently powerful at the stage in question to cause an embryo of this stage when laid on the bottom of a flat glass vessel to slide along at the rate of a millimetre in from four to seven seconds. As development proceeds the ciliation becomes less and less prominent and in a 20-mm. tadpole it has almost disappeared except on the surface of the tail which remains richly ciliated until the time of metamorphosis. This persistence of the tail cilia is doubtless correlated with the fact that the skin of the tail plays an important part in the process of respiration.

HORNY DEVELOPMENTS OF THE EPIDERMIS

Scales.—In many terrestrial Vertebrates the horny layer of the epidermis becomes so thickened and hardened as to become practically rigid. In such cases the flexibility of the skin as a whole is retained by the thickened areas of epidermis being separated from one another by lines along which thickening does not take place. The thickened portions now form epidermal scales of the type seen in Reptiles. They may take the form of simple rounded projecting bosses or tubercles as in Chameleons, or they may be flattened horny plates arranged edge to edge—as in Chelonians or as on the ventral side of the body in Crocodiles or the dorsal surface of the head in Snakes and Lizards—or, finally, they may overlap like slates on a roof as is the case on the bodies of Lizards and Snakes. Occasionally, as in certain Lizards, individual scales may become greatly thickened and assume a conical spike-like form.

The individual scale arises in development (Fig. 41) as a slight elevation of the surface beneath which the dermal connective tissue is somewhat concentrated. The epidermis covering the projection develops a well-marked cuticle. As development goes on the epidermis increases much in thickness and the cells of the outer layers become entirely cornified so as to form a horny plate or scale—supported by the underlying tough condensed portion of the dermis.

It will be borne in mind that such typical reptilian scales have to be sharply distinguished from the morphologically quite different scales developed in the dermis in fishes. The ordinary reptilian scales serve mainly to protect the body from mechanical violence and from desiccation.

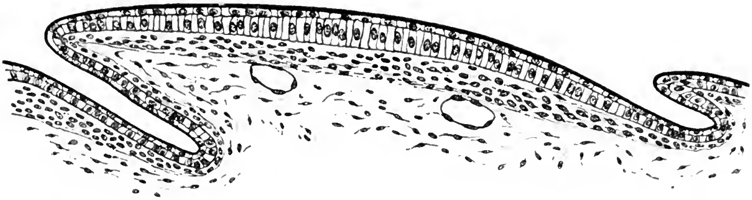


FIG. 41.—Early stage in the development of the scale of a snake as seen in a longitudinal section perpendicular to the surface of the skin.

Feathers.—In the homoiothermic Birds, where the body is kept at a constant temperature usually higher than that of the surrounding atmosphere, the scales have become for the most part replaced by fluffy feathers which with the air entangled in their interstices form an admirable non-conducting envelope to retard the loss of heat by radiation, or convection, from the surface of the body.

The rudiment of the feather begins (Fig. 42, A) as a slight thickening of the epidermis resting upon somewhat condensed dermis. The rudiment in fact differs little from that of a normal scale. The rudiment comes to project backwards (B) and then increases in length (C), projecting freely tailwards while its now relatively narrow base of attachment becomes sunk below the general surface into a pit or **follicle**.

The rudiment now consists of a core of dermis surrounded by thick epidermis. The epidermis becomes incised along its axial surface by deep longitudinal grooves which divide its deeper portions into longitudinally arranged masses (Fig. 42, D, *b*), the rudimentary **barbs**, while leaving the superficial portion as a continuous sheath (*sh.*). The grooves in question do not reach to the base of the rudiment—the unincised basal portion forming the quill of the feather. The horny sheath becomes strongly cornified and then breaks open and the longitudinal thickenings of the epidermis, now also strongly cornified, break away from the sparse cornified dermal tissue of the axis and form the fluffy barbs of the down feather.

In the basal quill portion of the feather the epidermis immediately covering the outer end of the axial dermal tissue or **pulp** forms a thin strongly cornified superficial layer which separates off as a septum cutting across the cavity of the quill. This process being repeated periodically gives rise to a series of horny caps fitting one over the other, in the interior of the quill (Fig. 42, H, *c*).

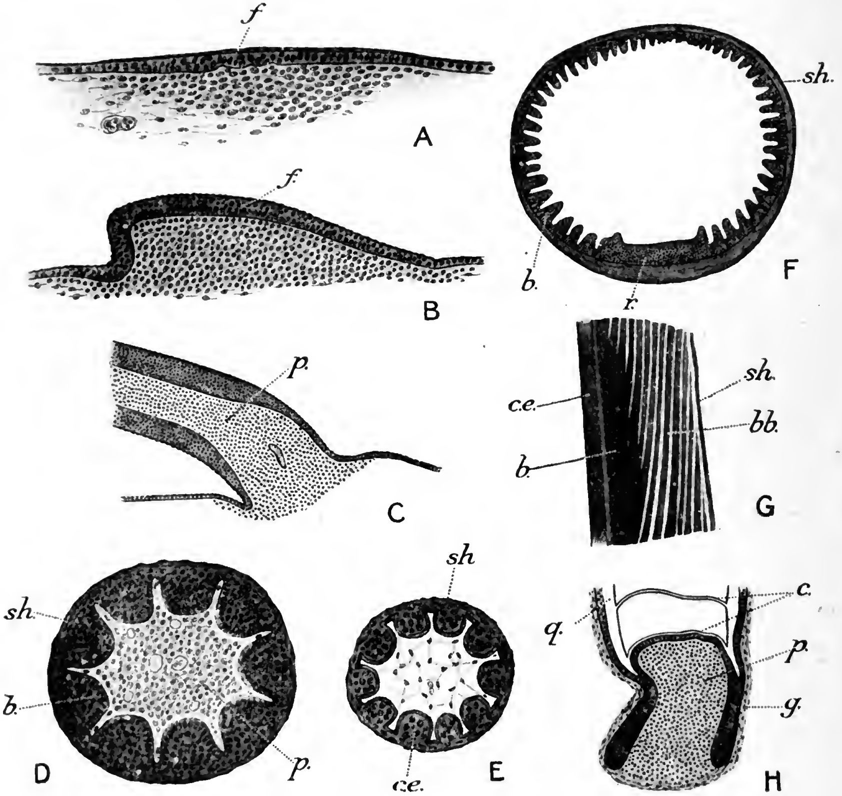


Fig. 42.—Illustrating the development of feathers. (After Davies, 1889.)

A, B, C, longitudinal sections; D, E, F, transverse sections (D, E, down feather; F, flight feather); G, longitudinal section through barb rudiment showing developing barbules; H, longitudinal section through base of feather. *b*, barb; *bb*, barbule; *c*, horny septa; *c.e*, layer of cylindrical epithelium; *f*, feather rudiment; *g*, germinal region; *p*, pulp; *q*, quill; *r*, rachis; *sh*, sheath.

The flat feathers, found as contour feathers arranged in patches over the general surface and as Remiges and Rectrices in the wings and tail, originate from the basal portions of down feathers which undergo a great increase in length. The basal part of the rudiment in this case increases much in diameter. The epidermis here again becomes incised on its inner surface to form barb rudiments. These however are much more numerous (Fig. 42, F) than in the typical down feather and, further, instead of being arranged strictly longitudinally they are arranged somewhat spirally, starting from a continuous epidermal thickening (*r*) which runs along the outer side of the feather rudiment. This thickening is the rudimentary **rachis** or shaft and the barb rudiments run from it spirally round the feather rudiment until their tips meet along its inner side.

The feather is thus in early stages curled into a cylindrical form round the central dermis or pulp—the whole being enclosed in a continuous sheath which disintegrates sooner or later setting free the elastic barbs and allowing them to flatten out to form the **vexillum** or vane.

As is well known the barbs are united together in the fully-developed feather into a functionally continuous web, through the agency of the **barbules** which project from the two sides of the barbs much as the barbs do from the rachis. The mode of origin of the barbules is seen in a longitudinal radial section through a barb such as that shown in Fig. 42, G, where the outer portion of the barb rudiment is seen to be splitting up into barbules (*bb*) while its inner portion remains continuous to form the definitive barb (*b*).

Traced downwards, towards the base of the feather, the rachis increases in width so as to extend round the whole periphery of the feather rudiment. Its outer layer assumes a translucent character and forms the cylindrical *quill* (**calamus**), the basal end of which becomes somewhat narrowed, bounding the **umbilicus**, the opening through which the dermal pulp extends up into the interior of the quill. The pulp of the feather undergoes a gradual shrinkage leaving behind it the series of cornified caps (H, *c*) formed on its apical surface as already mentioned and which eventually lie loose within the quill.

The lips of the umbilicus are continued (Fig. 42, H) into a deep rim of uncornified epidermis (*g*). This with the dermal papilla projecting into the feather base remains inactive until the period of moulting when it springs into activity, grows rapidly, and becomes converted into a new feather which pushes the old one out and takes its place.

The scales which frequently occur upon the legs and feet of birds are probably not, as might at first sight be supposed, to be looked upon as having persisted from the Reptilian condition. They frequently bear feathers in the young condition and are probably secondary developments replacing an earlier feathery covering.

In view of the convincing evidence offered by comparative anatomy and palaeontology we are compelled to believe that Birds have been evolved out of Reptile-like ancestors. Accepting this view and having regard further to the fact that Reptiles are typically covered with a coating of scales, we may safely also accept the view that feathers are to be looked upon as highly specialized and modified scales.

While the mode of development of the feather fully substantiates this hypothesis, perhaps the most interesting point that emerges from its study is that the successive sets of feathers—the down feathers of the nestling, and the annual or other sets of feathers in the adult—are not to be looked on, as has been customary, as successive series of independent individual feathers. On the contrary the down feather and the definitive feathers, which succeed it in the series of moults, are all simply portions of a single greatly elongated and basally growing structure—the first down feather being its tip, and the succeeding feathers being successive portions of it. The moult consists not in the shedding of the whole feather but merely in the breaking off of its projecting portion.

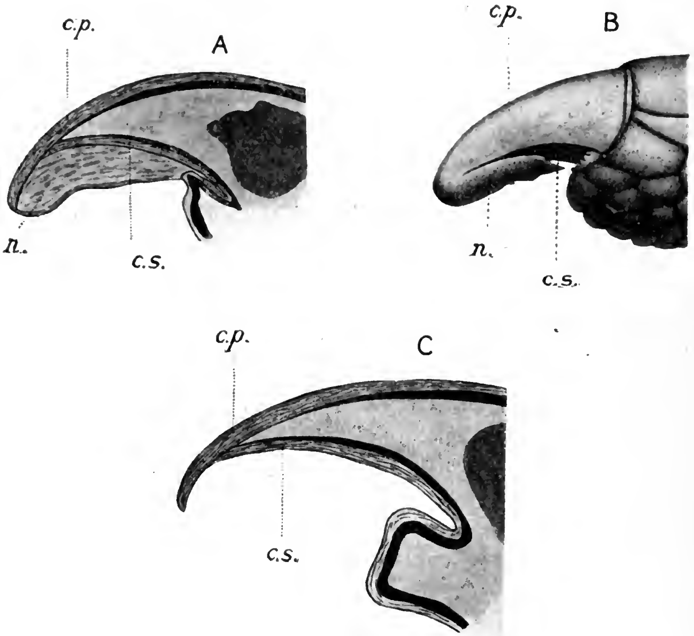


FIG. 43.—Illustrating the neonychium or claw-pad in the developing Bird. (From Agar, 1909.)

A, median longitudinal section through the claw of a chick of 19 days' incubation. B, claw of a chick taken in the act of hatching. The neonychium is seen beginning to break away from the rest of the claw. C, section similar to A, but from a chick 12 hours after hatching. *c.p*, claw-plate; *c.s*, sole of claw; *n*, claw-pad (neonychium).

Claws, which make their first appearance in Anura (*Xenopus*), arise as special developments of the horny layer ensheathing the tip of the digit. To produce and retain a sharp edge or point by differential wear the claw is normally of denser consistency and harder on the dorsal side and laterally, forming the "claw-plate" (Boas) (Fig. 43, C, *c.p*), while on the ventral side it forms the softer "sole" of the claw (Boas) (Fig. 43, C, *c.s*).

Neonychia or Claw-pads.—To the embryo of an Amniotic Vertebrate, enclosed within its delicate membranes, the possession of sharp claws on the digits would obviously be a source of considerable danger during the later stages of development when the embryo moves its limbs, because of the liability of such structures to tear the foetal membranes. This danger is obviated by a beautiful adaptive arrangement which has been described by Agar (1909).

In the embryo, the concavity on the lower side of the claw is completely filled up by a soft rounded pad or cushion (Fig. 43, A, *n*) formed by a thickening of the horny layer of the epidermis superficial to the sole of the claw. Agar has given the name Neonychium to this structure. In addition to mammals, which do not concern us here, Agar has studied these claw-pads in the Fowl and in the Lizard *Tejus* and there can be no doubt that the expanded claw-tips observed by Rathke (1866), Voeltzkow (1899) and Goeldi (1900) in Crocodilian (Fig. 44) embryos are the same structures and it seems probable that they will be found to occur in claw-bearing Amniotic Vertebrates generally.

The neonychia are purely foetal structures which become detached soon after hatching (Fig. 43, B and C) leaving behind the functional claw.

FIG. 44.—Right pectoral limb of an embryo Crocodile about two months after oviposition, showing the hoof-like neonychia. (After Voeltzkow, 1899.)

Jaws and Oral Combs of Anuran larvae.—Amongst the most interesting developments of the horny layer are the jaws and oral combs of frog tadpoles. The buccal opening is bounded by an upper and lower horny jaw, and external to and roughly parallel with these are rows of little horny denticles which form the oral combs and are used for fraying out the food. The number and arrangement of these rows of denticles—"upper labial" and "lower labial"—differs in different Anura and they afford useful characters for the identification of tadpoles (see Boulenger, 1897).

The horny jaw is composed simply of a row of denticles so closely apposed as to be in contact. The terminal functional portion of each denticle is seen in longitudinal section (Fig. 45, A and B) to be composed of a series of hollow cones of hard horny material which closely ensheath one another. The terminal cone as it undergoes wear and tear eventually drops off, its function being taken over by the cone which it previously ensheathed.

These cones form simply the terminal members of a series which extends inwards in the form of a curved column nearly to the inner surface of the epidermis. Only the terminal members are strongly cornified, the other members showing less and less cornification until at a little distance down the series the cone is seen to be composed of unmodified protoplasm containing at one side, near its base, a nucleus. The cone is in fact simply a cornified cell. Traced towards the base of the column the cells are seen to be composed of more granular protoplasm and to have not yet developed a hollow, while the extreme base of the column is formed by an initial cell of comparatively small size and flattened shape.

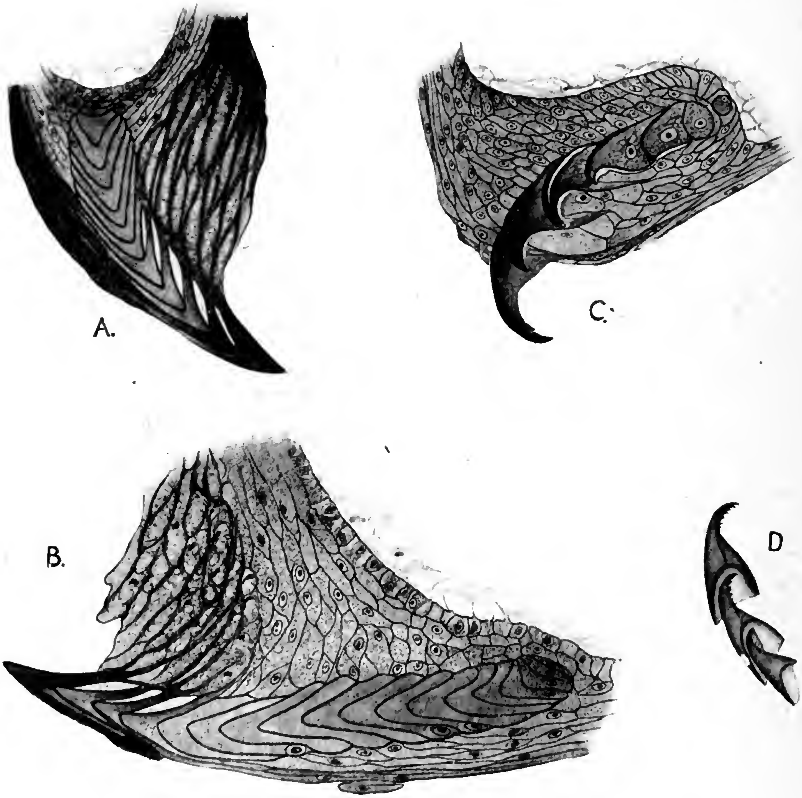


FIG. 45.—Illustrating the development of the larval teeth of Tadpoles.

A, B, C, *Paludicola fuscomaculata*; D, *Rana temporaria*. (D after Gutzeit, 1889.)

The whole column is seen, then, to be composed of a sequence of conical teeth forming a replacement series, each tooth being a single cornified cell.

The jaw, composed of a closely set row of such columns, is supported by the neighbouring parts of the epidermis which also undergo a certain amount of cornification. Thus just internal to the jaw is a cushion-like mass of large slightly cornified cells which forms an efficient backing to it (cf. Fig. 45, A and B) while external to the jaw the surface of the epidermis is composed of flattened much cornified cells (Fig. 45, A).

The oral combs consist of a pallisade-like arrangement of similar denticles which however in this case are not in contact. Fig. 45, C, shows a longitudinal section through the posterior labial comb of *Paludicola.* Here again we see a succession-column of epidermal cells commencing with a small initial cell near the inner surface of the epidermis. From the initial cell outwards the cells increase in size, become gradually cornified and each one fits closely into the base of the next one which becomes more and more deeply excavated as the tip is approached.

Two conspicuous differences distinguish the denticle of the oral comb from that of the jaw: (1) instead of being regularly conical in shape it is claw-shaped with serrated edges (Fig. 45, D) the tip being recurved, and (2) the hollow base of the cornified cell is not entirely occupied by its successor in the series: it also accommodates an indifferent cell of the epidermis (supporting cell of Gutzeit) which bulges into it.

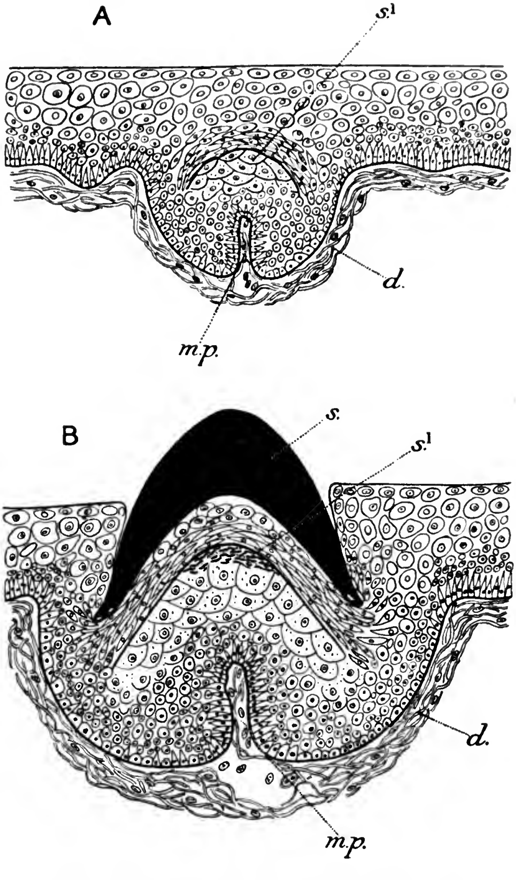


FIG. 46.—Vertical section through lingual spine of *Petromyzon.* (After Warren, 1902.)

A, earlier, B, later stage. *d*, dermis; *m.p*, mesodermal papilla; *s*, functional spine; *s*¹, spine rudiment.

I have described the development of these interesting structures as they occur in a South American tadpole (*Paludicola*)[1] but the description fits quite well the mode of development as it occurs in Tadpoles generally (Keiffer, Gutzeit), the differences between different species and genera, though of systematic importance, being differences in detail such as shape and arrangement of the individual teeth of the comb.

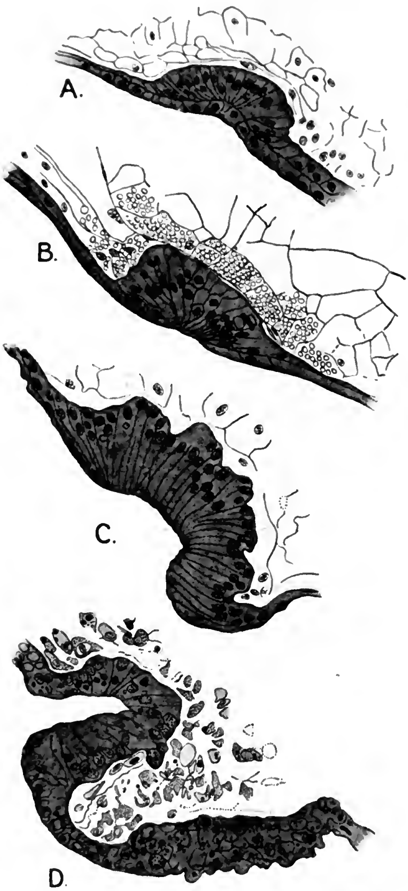


Fig. 47.—Illustrating the development of the cement-organ of *Lepidosiren* as seen in sagittal sections.

A, stage 23; B, stage 25; C, stage 31; D, stage 35. In A the rudiment of the cement-organ is seen to be a thickening of the deep layer of the ectoderm; in B and C the superficial layer has disappeared over the thickened glandular area; in D the organ is commencing to shrivel and crowds of phagocytes are collected in its neighbourhood.

"Teeth" of Cyclostomes.—The horny teeth of cyclostomatous fishes, though they would naturally fall to be treated in the next chapter, situated as they are within the buccal cavity, may conveniently be considered now owing to their resemblance—on a much larger scale and with multicellular structure—to the horny denticles of the tadpole.

The tooth-like spines of the cyclostome are cones of highly cornified epidermal cells. Each tooth develops in the substance of the epidermis (Fig. 46, A) being strikingly like a hair-rudiment during early stages. Successional spines develop beneath the bases of the functional ones as shown in Fig. 46.

GLANDULAR DEVELOPMENTS OF THE EPIDERMIS.—In the Anamnia it is usually the case that scattered cells of the epidermis take on a glandular function and serve to form a slimy secretion which amongst other functions serves to diminish the "skin-friction" which is the main resistance to movement through water. Such unicellular glands may become collected together to form multicellular glands. Of these the most conspicuous examples are found, outside the Mammalia, in the Lung-fishes and Amphibians—where they form the flask-glands and the cement-organs.

---

[1] Probably *P. fuscomaculata* according to Boulenger.

The **flask-glands** of Lung-fishes and Amphibians develop in the first instance as solid local proliferations of the deep layers of the epidermis which grow down into the subjacent connective tissue of the dermis and form a lumen by secondary excavation. The fully developed flask-gland is ensheathed in a coat of smooth muscle-fibres and it is an interesting fact that these are believed to be developed from the ectodermal cells of the gland-rudiment.

Cement-organs of apparently ectodermal origin occur in two out of the three surviving lung-fishes—*Protopterus* and *Lepidosiren*—and form conspicuous features during late embryonic and larval life (see Fig. 200, F, Chap. VII.).

In an embryo of *Lepidosiren* three days before hatching the cement-organ forms a crescentic structure stretching across the mid-ventral line with its concavity forwards, just behind the position in which the mouth will appear later. About stages 32-34 the organ is at its maximum development and forms a large prominently projecting structure ventrally below the opercular region. Towards the end of larval life the cement-organ commences to atrophy, the process being furthered by its invasion by crowds of phagocytes, and in a short time the organ has completely disappeared.

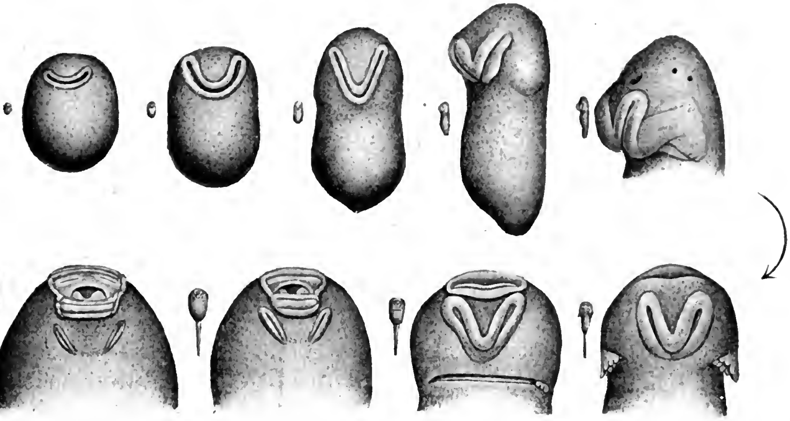


FIG. 48.—Embryos and larvae of *Bufo vulgaris* to show the cement-organ upon the ventral surface. (After Thiele, 1887.)

The cement-organ is a derivative of the deep layer of the epidermis. It commences as a slight thickening of this layer (Fig. 47, A) the cells assuming a tall columnar form. These columnar cells become the secretory cells while the superficial layer of the ectoderm breaks down over them so as to expose their outer ends (Fig. 47, B and C). It is to be noted that there is no trace whatever of any connexion of this cement-organ with the endoderm: it is ontogenetically a purely ectodermal structure (see, however, p. 181).

Cement-organs of very similar appearance are found in the larvae of many Anura. In this case they appear very precociously in development, being indeed in some cases the first definite organs to become visible on the surface of the embryo. Fig. 48 illustrates the development of the cement-organ in the common toad (*Bufo vulgaris*) from the time of its first appearance up to the time of its atrophy. The organ is seen to be at first crescentic as in *Lepidosiren* then to become V-shaped and finally to become paired by the atrophy of its median portion.

When at the height of its development, the cement-organ shows characteristic differences in form and position in different species of Anura and is consequently of use in identifying the species of Tadpoles.

The general appearance of the Anuran cement-organ as observed in sections is illustrated by Fig. 49. The glandular layer is commonly said to belong to the superficial layer of the ectoderm but this does not seem by any means certain.

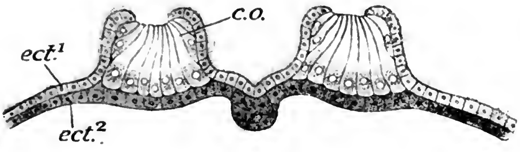


FIG. 49.—Section through the cement-organ of a Frog Tadpole (*Rana temporaria*) 8 mm. in length. (From Assheton, 1896.)

c.o, cement-organ ; *ect*¹, superficial layer of ectoderm ; *ect*², deep layer of ectoderm.

Pigment of the Skin.—One of the conspicuous features of the skin in the majority of Vertebrates is the fact that it is coloured by the deposition within it of excretory matter in the form of pigments. This is of physiological importance to the organism in two different ways, firstly in that it gives to the particular species its characteristic coloration, and secondly it serves to protect the underlying living tissues from the harmful influence of light rays.

A certain amount of pigment may be formed within the protoplasm of the ectoderm cells. For example in frog tadpoles of about an inch in length, numerous fine granules of melanin are crowded together near the surface of the outer layer of ectoderm cells, just beneath the cuticular superficial layer.

But by far the most important part of the pigmentary system of Vertebrates consists of mesenchyme cells with pigment-laden cytoplasm which are positively heliotropic during life and creeping by the extrusion of slender pseudopodia, like those of Foraminifera, crowd together immediately beneath the ectoderm and form there a light-proof layer, some of them even wandering into the substance of the ectoderm between its constituent cells.[1]

The chromatophores, during the process of development, commonly become specialized in different directions so that in the fully developed

---

[1] The interpretation of the branched chromatophores as mesenchymatous in origin appears to the author to accord best with observation but it should be mentioned that some regard them as modified ectoderm cells, as for instance Winkler (1910).

condition several distinct types may be recognized. Thus in *Lepidosiren* the most abundant type of chromatophore is characterized by the stout projections of the cell-body which carry the finer pseudopodia and by the somewhat brownish pigment granules. A less abundant type has long slender, less richly branched and often varicose pseudopodia with dense black and opaque pigment granules. Still another type of chromatophore has its protoplasm charged with bright yellow pigment.

The melanin pigments are probably to be looked upon as waste products of cell metabolism. They are iron-containing pigments and during at least the later periods of development their production is commonly associated with the breaking down of that other great iron-containing pigment—haemoglobin.

Their production is also related to the degree of activity of the cell metabolism. Thus, in the male Lepidosiren, at the close of the breeding season, when the moribund remains of the richly vascular respiratory projections of the hind limb are being devoured by crowds of voracious phagocytes, there takes place an active formation of melanin.

Again melanin apparently tends particularly to be produced when the cell metabolism of comparatively unspecialized cells is interfered with by the prolonged action of light-rays. Thus as already indicated the layer of protoplasm in the egg which is turned towards the light frequently develops melanin granules. Again in developing eyes it commonly holds that comparatively unspecialized mesenchyme cells wandering into the zone of exposure to the light deposit melanin granules in their cytoplasm.

Cells then which become chromatophores may be regarded as cells which are specially sensitive to light-stimulus and whose metabolism is liable to be so modified thereby as to produce pigment.

Although it is reasonable to suppose that melanin-formation is primarily related to the influence of light it must not be forgotten that, as indicated in the preceding chapter, the actual laying down of pigment in the case of species where it has become a specific character may take place under circumstances in which the light-stimulus is incomparably more feeble than that which probably originally brought about pigmentation in the course of phylogenetic evolution, as *e.g.* in the case of the ovarian egg of the frog. That pigment-formation during individual development still remains linked up with exposure to light is shown by the frequency of the unpigmented condition in Anuran larvae which develop in water rendered opaque by fine clay held in suspension (Wenig, 1913).

To illustrate the dependence of pigment-formation upon light during individual development the case of young flatfish (Pleuronectidae) is sometimes quoted, where the shading of the upper and the illumination of the lower surface during development brings about a reversal of the ordinary colouring (Cunningham, 1893). It is possible however that this reversal of colouring is due merely to the strongly heliotropic tendencies of the chromatophores which lead them to migrate actively towards the illuminated side and there to remain.

The chromatophores of Vertebrates often display their sensitiveness to light very markedly by movement reactions. Such are well seen in the young stages of many fishes and Amphibians. In the young Lepidosiren for example the chromatophores during the day have their pseudopodia extended in all directions and their bodies flattened out into a plate-like form so that they constitute a light-proof coat giving a rich purplish-black effect. At dusk the pseudopodia become slowly withdrawn so that a few hours after darkness has set in the chromatophores have shrunk into minute spheres so wide apart as to have no influence on the general colouring. The young fish is then practically colourless except for the large yellow chromatophores here and there which remain expanded.

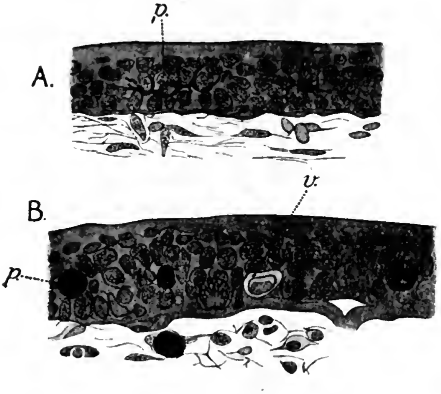


FIG. 50.—Section through epidermis of *Lepidosiren* larvae.

A, fixed under conditions of dull daylight; B, under conditions of darkness during the night. (The chromatophores in the dermis, which crowd together under the inner surface of the epidermis, are not shown in A.) *p*, chromatophore; *v*, blood-vessel.

During the course of development in many fishes, anurous Amphibians, and a few Reptiles such as the chameleon, the comparatively simple reactions to light such as have just been indicated develop into reactions of a much more complex type in which the central nervous system is involved. Research into the development of these more complex reactions is highly desirable for at present little is known regarding them.

NERVOUS SYSTEM

The nervous system, which has to do with the receiving of, and the reacting towards, impressions from the outer world, appears to have arisen in evolution, as might have been expected, from the outer layer or ectoderm. The first steps in the evolution of the Vertebrate nervous system are not within the scope of direct observation but the view is probably correct that it arose from a diffuse subepidermal network or plexus of the type still persisting in some of the more primitive invertebrates. In the development of the Vertebrate embryo the main parts of the nervous system may still be seen to take their origin from the ectoderm.

ORIGIN OF THE CENTRAL NERVOUS SYSTEM.—The first obvious trace of the central nervous system consists of a thickening of the ectoderm of the dorsal surface of the embryo. This thickening extends forwards from the anus, or from a point slightly behind the anus, to the head region, and is termed the **medullary plate**. The thickening of the medullary plate is due primarily to the ectoderm cells, or where two layers are present, to the deep-layer cells of the ectoderm taking on a tall columnar form.

There is also growth in area of the medullary plate and this, in conjunction with the binding down of the medullary plate along the line of the notochord and primitive streak, causes it to become curved from side to side so as to form a gutter or groove—the **medullary groove**. The, usually conspicuous, lips of this groove are known as the **medullary folds**.

As the medullary plate keeps on increasing in width it bulges downwards and laterally into the surrounding mesenchyme and assumes the form of a longitudinally placed tube with a slit along its dorsal wall representing the original opening of the groove.

Finally the lips of this slit grow towards one another and undergo fusion, so as completely to close in the **neural tube**, which now separates off from the ectoderm of the outer surface. The closing in of the neural tube commonly commences in the hinder head region and spreads from this point forwards and backwards.

An interesting modification of this normal mode of origin of the neural tube is found in the case of Lampreys, *Lepidosiren* and many of the teleostomatous fishes. In this modification of the typical process the increase in bulk of the medullary plate leads to its growing downwards into the underlying tissue as a solid keel. In the middle of this at first solid rudiment a cavity appears secondarily either by the development of a fine intercellular split or by the cells along the axis breaking down. The cavity so formed gradually dilates and eventually there is a neural tube agreeing with that of normal forms.

The neural tube which has originated in the way described is the rudiment of the central nervous system, its anterior portion becoming relatively enlarged to form the **brain** while the remainder forms the **spinal cord**.

The central nervous system during the period of its development gradually attains to a condition of the greatest complexity and all that will be attempted here is to give an outline sketch of the more conspicuous changes which take place in its general form and in the arrangement of its parts without going into minute detail.

SPINAL CORD.—The spinal cord remains throughout life in the form of a tube the lumen of the tube becoming relatively insignificant while the walls become greatly thickened especially laterally. The relatively small size of the lumen (**central canal**) is not, as a rule, due merely to its retaining its embryonic dimensions while the walls of the tube are growing in thickness. On the contrary actual occlusion of part of the lumen takes place in the great majority of the lower Vertebrates. The side walls of the tube approach one another so as to convert the rounded lumen into a vertical slit and finally they come into contact and fuse so as completely to obliterate the cavity except in its ventral portion which remains open as the definitive central canal.

In the case of the Bird—in which the process has been worked out in detail (see Ramón y Cajal, 1909) the increase in thickness of the wall of the neural tube is due primarily to the cells composing it taking on a tall columnar form—the individual cell extending right from the central canal to the outer surface. The cell-body becomes very attenuated, with a marked dilatation containing the nucleus. The nuclei become necessarily situated at different levels and this in an ordinary transverse section obscures the fact that the wall is still composed only of a single layer of cells.

With subsequent development the cells become differentiated into those which are actually nervous and those which remain relatively indifferent and fulfil a mainly supporting function. The latter continue for a considerable period to traverse the whole thickness of the wall. They increase greatly in length: their form becomes more and more attenuated the greater part of their length becoming practically filamentous with small irregular projections and varicosities, while the portion nearer the central canal, in the course of which the nucleus is embedded, remains somewhat stouter.

The presence of such supporting cells traversing the whole thickness of the wall is only temporary: in later stages they are replaced by the greatly branched **neuroglia** cells. While many authors have taken the view that these latter are to be regarded as immigrant mesenchyme cells—a view that has weighty general considerations in its favour—Ramón y Cajal and others have adduced strong evidence to show that they are simply the original supporting cells which have withdrawn, or lost, their internal and external portions and assumed a complicated branched form.

In addition to the comparatively indifferent supporting cells which have just been mentioned there are present in the wall of the neural tube the numerous elements which are destined to become actual neurones or nerve-cells. Such embryonic nerve-cells have been termed by His **neuroblasts** in contradistinction to the non-nervous elements or **spongioblasts**.

At first isodiametric these cells like their neighbours take on a tall columnar shape stretching throughout the thickness of the wall: their terminal portions become more and more attenuated and they present a spindle-like (bipolar) appearance. Later their shape becomes pearlike the stalk being prolonged into a nerve fibre (**neurite, axon**) while finally the development of branched projections (**dendrites**) brings about the definitive multipolar condition.

These developing neurones lie in the spaces between the indifferent cells and from an early stage (third day in the case of the fowl embryo) the use of appropriate methods reveals the presence of neurofibrils in their protoplasm. The tail-like prolongation of the neurone which forms the neurite or axon is still believed by the great majority of workers to arise as an actual outgrowth of the cell body as was taught by His. Others regard the appearances upon which this belief is based as being probably deceptive, as will be explained later.

The longitudinal axons of the spinal cord are concentrated in its outer layers forming the " white substance " of the early anatomists. This makes its appearance as a rule in the more primitive Vertebrates as a continuous layer and in the higher forms as a sharply separated dorsal and ventral portion upon each side.

The enclosure of the axons in the insulating medullary sheaths commences only within a few days of the end of incubation, in the case of the bird, and at similarly advanced stages of development in other Vertebrates. The sheath is generally believed to be secreted by the protoplasm of the axon. Its formation tends to take place approximately synchronously in all the axons belonging to a particular group. This fact, in conjunction with the use of specific stains for the insulating substance, facilitates the mapping out of the various groups of axons.

The spinal cord, like the rest of the central nervous system, becomes invaded during ontogeny by immigrant mesenchymatous tissue. This provides the capillary network which traverses the nervous tissue, in addition doubtless to many other elements of a less conspicuous kind.

A curious detail which is noticed in studying sections of developing spinal cord (or brain) is that the active cell-multiplication is confined to the layer next the central cavity, in other words to what was originally the superficial region of the ectoderm. This is in striking contrast with the general ectoderm of the surface of the body where cell-multiplication is confined to the deep (Malpighian) layer.

BRAIN.—The anterior portion of the neural tube becomes enlarged and dilated to form the brain and this gradually becomes so modelled as to present the various regions seen in the brain of the adult. The general course of this process will first be sketched as it occurs in *Lepidosiren* one of the lower gnathostomatous Vertebrates in which the egg is holoblastic.

DIFFERENTIATION OF THE MAIN REGIONS OF THE BRAIN IN LEPIDOSIREN.—The brain rudiment becomes apparent as a slight enlargement of the neural tube. The first sign of differentiation is the appearance of a constriction marking off the primitive fore-brain or **cerebrum** (archencephalon of Kupffer) from the primitive hind-brain or **rhombencephalon**. As development goes on this boundary becomes specially marked ventrally where the floor of the brain bulges upwards into the cavity as a transverse fold[1] (see Figs. 52 and 53, *f*).

---

[1] This fold may in other vertebrates make its appearance before the medullary groove is covered in. This is shown clearly in *Polypterus*—Fig. 80, B, p. 146.

The side wall of the hind-brain now comes to project outwards as a prominent knob while the side wall of the fore-brain also bulges outwards—the bulging in this case being the rudiment of the **cerebral hemisphere** (Fig. 51, A, *c.H*).

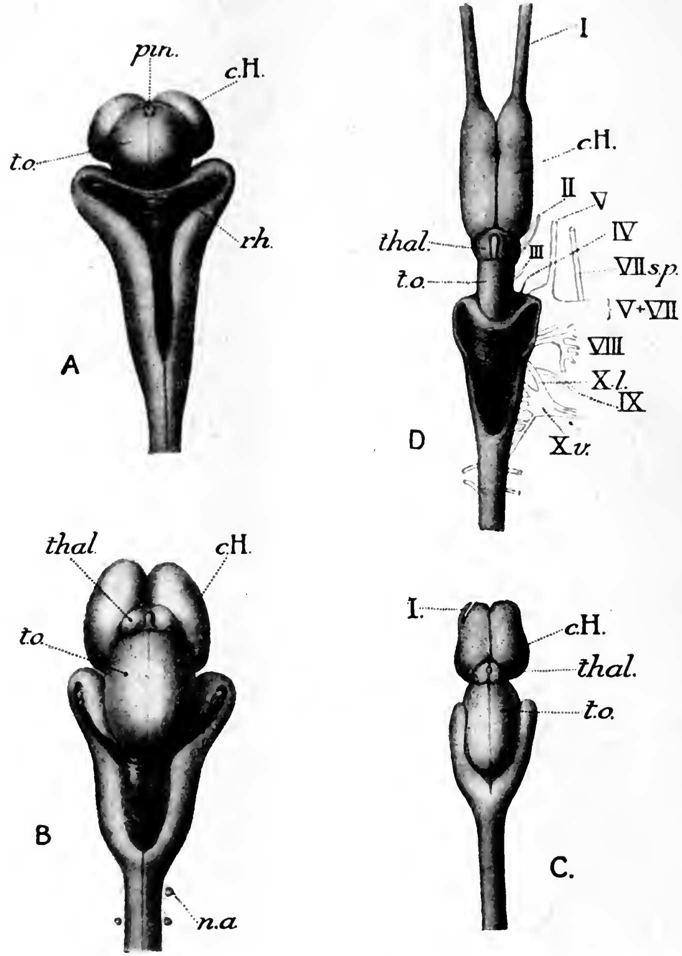


Fig. 51.—Brain of young Lepidosiren as seen from the dorsal side.

A, stage 31+; B, stage 35−; C, stage 38; D, adult. *c.H*, cerebral hemisphere; *n.a*, neural arch; *pin*, pineal body; *rh.*, rhombencephalon; *thal*, thalamencephalon; *t.o*, *tectum opticum*. I, II, etc., cranial nerves. [Figs. A and B are more highly magnified than C, and Fig. C than D.]

The portion of the primitive fore-brain lying just in front of the transverse fold of the brain-floor is the **infundibulum**. Farther forwards the inner surface of the brain-floor forms a transverse groove bounded behind and in front by a slightly projecting ridge—the rudiments of the **optic chiasma** and of the **anterior commissure** respectively (Fig. 53, *ch*, *a.c*).

About stage 31 a little pocket-like diverticulum of the roof of the primitive fore-brain makes its appearance (Fig. 53, D, *pin*). This is the **pineal body** and its appearance is of topographical importance as serving to demarcate the primitive fore-brain roof into thalamencephalic and mesencephalic portions.

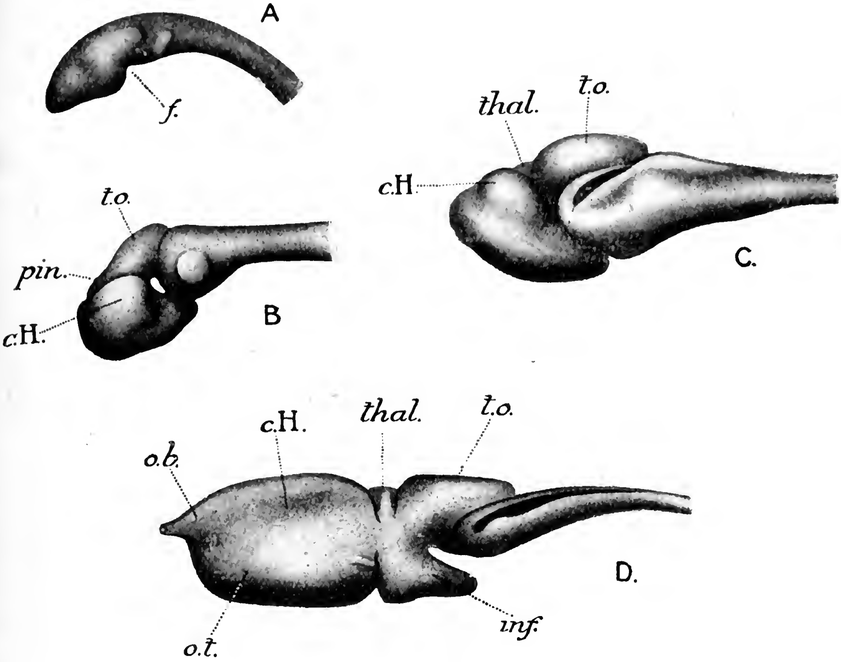


Fig. 52.—Brain of young Lepidosiren as seen from the left side.

A, stage 26; B, stage 31; C, stage 35 – ; D, stage 39. *c.H*, cerebral hemisphere; *f*, primitive fold of brain-floor; *inf*, infundibulum; *o.b*, olfactory bulb; *o.t*, olfactory tubercle; *pin*, pineal body; *thal*, thalamencephalon; *t.o*, *tectum opticum*.

The lateral bulgings of the fore-brain have become more prominent and now project forwards beyond the limit of the rest of the fore-brain. In the mesial plane between the two hemispheres there projects upwards and forwards a little pocket of the anterior wall of the fore-brain. This is the rudiment of an organ of unknown significance—the **paraphysis** (Fig. 53, D, *par*).

Soon after the appearance of the pineal body the roof of the primitive fore-brain becomes divided into a posterior portion belonging to the **mesencephalon** and an anterior portion belonging to the **thalamencephalon** (Fig. 51, B, *t.o*, and *thal*). As development goes

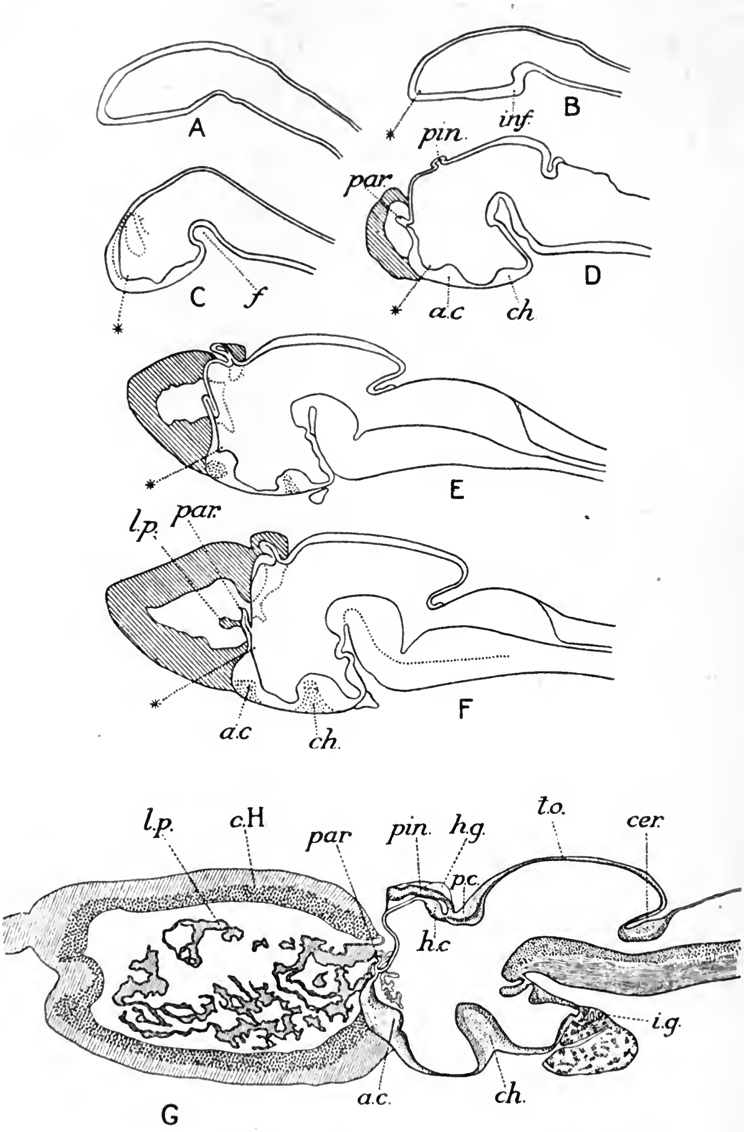


FIG. 53.—Camera tracings of sagittal sections through the brain of *Lepidosiren* at successive periods of development.

Figs. A to F are drawn under the same magnification, Fig. G under a lower magnification. A, stage 25; B, stage 28; C, stage 29 +; D, stage 31; E, stage 35; F, stage 38; G, adult in second year. *a.c*, anterior commissure; *cer*, cerebellum; *c.H*, cerebral hemisphere; *ch*, optic chiasma; *f*, primitive fold of brain-floor; *h.g*, ganglion habenulae; *h.c*, habenular (superior) commissure; *i.g*, infundibular gland; *inf*, infundibulum; *par*, paraphysis; *pin*, pineal body; *p.c*, posterior commissure; *l.p*, choroid plexus of lateral ventricle; *t.o*, *tectum opticum*; * originally anterior end of brain-floor. Structures occurring not in the sagittal plane but in sections parallel to but some distance from it, are shaded with oblique lines.

on the constriction between thalamencephalon and mesencephalon becomes more marked. The roof of the former remains thin and membranous, forming the cushion-like **dorsal sac** upon which the pineal body rests. The roof of the mesencephalon becomes slightly thickened on each side of the mesial plane forming the **tectum opticum** but correlated with the small size of the eyes in *Lepidosiren* the thickening never becomes so great as to produce projecting optic lobes such as are formed in most Vertebrates.

In the hind-brain region the greater part of the roof, covering in the fourth ventricle, becomes thin and membranous. Across the anterior boundary of the hind-brain the roof does not undergo this secondary process of thinning but persists as a transverse thickened band—the rudiment of the **cerebellum**.

SUBSEQUENT DEVELOPMENT OF THE BRAIN REGIONS

RHOMBENCEPHALON or Hind-Brain.—The hind-brain, correlated perhaps with the fact that it contains nerve-centres of supreme importance to life, develops precociously and reaches a relatively enormous size during early stages (Fig. 51, A, *rh*). The bulging inwards which marks its anterior limit is doubtless to be regarded as an expression of the active growth in length of its floor during these early stages.

During later stages of development it forms a conspicuous projecting restiform body on each side reaching forwards nearly to the anterior limit of the mesencephalon but this becomes again less and less prominent as the adult condition is approached. The cerebellum retains through life its primitive condition as a simple transverse thickening of the hind-brain roof.

MESENCEPHALON.—The roof, as already indicated, becomes thickened somewhat on each side (tectum opticum) but not to such an extent as to bulge outwards and form optic lobes. Close to its anterior limit a conspicuous bridge of transversely-running nerve-fibres makes its appearance at a late stage of development. This is the **posterior commissure**—an important brain landmark (Fig. 53, G, *p.c*).

THALAMENCEPHALON.—The side wall of the thalamencephalon becomes greatly thickened to form the **optic thalamus** which bounds on each side the slit-like third ventricle. The roof becomes for the most part thin and membranous forming the dorsal sac. On either side of the pineal body however it becomes greatly thickened to form the **habenular ganglion**. As these ganglia develop a bridge of transverse nerve-fibres makes its appearance uniting them—the superior or, better, **habenular commissure**.

The pineal body as development goes on enlarges somewhat and assumes a carrot shape. Its lumen becomes obliterated posteriorly so that it no longer opens into the third ventricle. The anterior isolated part of the cavity becomes eventually almost filled with granular material produced by the breaking down of the epithelial lining.

The paraphysis forms for a time a conspicuous tube passing upwards and forwards in the space between the two hemispheres and ending blindly. In later stages of development it undergoes a relative reduction in size, and becomes irregularly twisted and mixed up with the choroid plexus of the ventricles.

On either side of the paraphysis and just dorsal and posterior to its base, the wall of the brain becomes involuted into the third ventricle, the involuted portion being thin and membranous and enclosing an ingrowth of blood-vessels. This vascular ingrowth represents a structure which in most Vertebrates is continuous across the mesial plane with its fellow so as to form an unpaired structure the **velum transversum**. This is regarded by most writers on the brain as an important landmark in brain topography.

On the floor of the thalamencephalon the optic chiasma and the anterior commissure form prominent bulgings into the ventricle. Each develops nerve-fibres in its substance, connected in the one case with the organs of vision and in the other with the cerebral hemispheres, especially those portions devoted to the sense of smell.

In front of the optic chiasma lies a deep **optic recess** which is prolonged outwards by an outgrowth of the side wall of the brain, the optic outgrowth, which gives rise to a great part of the eye and will be described later. Behind the chiasma is the infundibulum, the tip of which at a late stage in development (about stage 38) sprouts out into narrow tubular diverticula. These increase in length, wind hither and thither, and partially penetrate into the substance of the pituitary body which lies immediately beneath. The epithelium of these tubular diverticula assumes a glandular appearance and together they constitute the "infundibular gland"—often called the "nervous portion of the pituitary body."

The series of sagittal sections in Fig. 53 is of interest from its bearing upon a question which has excited some discussion, namely as to what point in the fully developed brain of the vertebrate corresponds to the morphologically anterior end of the brain rudiment in earlier stages of development. It has been held by many morphologists, such as von Baer, His, Sedgwick, that the tip of the infundibulum represents the anterior end of the primitive brain, the present condition having been brought about by the anterior portion of the brain becoming bent upon itself into a retort shape. As will be seen by an inspection of the figures the brain of *Lepidosiren* lends no support to this idea. On the contrary the tip of the infundibulum clearly corresponds to a point close to the letter A of Fig. 53, A. On the other hand, equally clearly the anterior tip (*) of the brain-floor of an early stage such as that shown in Fig. 53, B is represented in the adult by a point well up on the anterior wall of the thalamencephalon (**lamina terminalis**) and just ventral to the root of the paraphysis.

Cerebral Hemispheres.—The hemispheres arise as bulgings of the side wall of the fore-brain. As development goes on they increase in size and grow first dorsalwards and later on forwards until in the adult they are relatively very large. This increase in size is associated with a corresponding growth in the thickness of the wall of the hemisphere—except at its hinder end next the thalamencephalon. Here the inner wall of the hemisphere facing the thalamencephalon remains relatively thin.

About stage 35 a small rounded portion of this thin part of the hemisphere wall bulges into its cavity—the **lateral ventricle**. This ingrowth contains a vascular loop and is the rudiment of the choroid plexus of the hemisphere or lateral plexus. The plexus grows rapidly into the ventricular cavity, forming an irregular crumpled lamina which in the adult attains to great size and complexity traversing the whole lateral ventricle (Fig. 53, F and G, *l.p*). No doubt this, by diffusion between the blood in its vessels and the fluid in the lateral ventricle, helps to provide for the nutritive and respiratory needs of the hemisphere wall.

During the later development of the hemisphere its walls become differentiated into regions in the manner described by Elliot Smith (1908). Most important from the point of view of general vertebrate morphology is the fact that a distinct **cortex** is developed in the form of a layer of ganglion-cells traversing the roof of the hemisphere parallel to its surface, and at about one-third of the distance from the surface to the ventricular cavity. This cortex extends on the one hand just on to the mesial face of the hemisphere and on the other to a point rather more than one-third of the distance from dorsal to ventral edge on the outer face of the hemisphere.

Of this cortical formation, which constitutes the **archipallium**, the mesial portion corresponds to the **hippocampus** of higher vertebrates, and the outer portion to the **pyriform lobe**. The **neopallium** which in the higher forms becomes interposed between these does not appear yet to have become distinctly recognizable in *Lepidosiren*.

Less important from the point of view of general morphology but more conspicuous in their structural expression are certain changes which take place in relation to the olfactory apparatus.

The portion of hemisphere wall to which the first cranial nerve is attached—the **olfactory bulb**—is at first simply part of the lateral wall of the hemisphere but as development proceeds it is found to take the form of a sort of cap lying on the dorsal side or roof of the hemisphere at about the middle of its length as viewed from above. This change in position is brought about by an enormous hypertrophy of the portion of the ventral wall of the hemisphere which lies in front of the optic chiasma—the **olfactory tubercle**.

Later on, from stage 38, the portion of hemisphere roof lying posterior to the olfactory bulb undergoes active growth in length with the result that the bulb is gradually carried forwards and eventually comes to lie right at the anterior end of the hemisphere (Fig. 52, D, *o.b*). At the same time the bulb comes to form a definite hollow projection of the brain surface immediately dorsal to the still greatly enlarged olfactory tubercle (*o.t*).

DIFFERENTIATION OF THE BRAIN REGIONS IN ACANTHIAS.—The development of the brain of Elasmobranchs has been worked out by Kupffer (1906) for *Acanthias* and his account has been made use of in writing the following short summary.

Figures of the early stages of the medullary plate as seen in surface view are given in Chap. XI. The medullary plate projects forwards from the posterior boundary of the blastoderm and is raised well above the general surface. As it increases in length its lateral edges become raised up so that the portion on each side slopes inwards and downwards into a kind of valley. Each half of the medullary plate extends back into one of the "caudal lobes" which with growth in length come to project freely beyond the edge of the blastoderm.

Another result of the increase in length is that the anterior end of the medullary plate comes to project freely forwards over the blastoderm forming a head-fold. Each side of the medullary plate arches inwards towards the mesial plane and the whole becomes converted into a neural tube in a perfectly normal fashion.

As in the case of *Lepidosiren*, the first sign of differentiation of the brain into its parts is a division into primitive fore-brain (Archencephalon) and hind-brain. The demarcation is again most distinct ventrally where the brain-floor bulges into the ventricle (Fig. 54, B) as a prominent fold. Later on this fold spreads upwards on each side to the dorsal surface forming the rhombo-mesencephalic fissure which marks off the mid-brain from the hind-brain. It is only at a later stage in development that the mesencephalon becomes marked off by a constriction from the anterior portion of the archencephalon which forms the thalamencephalon.

It is of interest to compare sagittal sections through the brain of the Elasmobranch with the corresponding sections already described for the holoblastic lung-fish. Neglecting small differences in detail there is seen to be a striking difference between the two brains—most marked in the middle stages figured—in relation to the longitudinal axis. In Fig. 54, C the Elasmobranch brain is seen to be as a whole strongly curved in a ventral direction: it shows a high degree of "cerebral flexure." The corresponding stage of the Dipnoan brain is on the other hand almost straight, the superficial appearance of curvature being due mainly to the prominent fold of its floor which projects up into the cavity at the level of the mid-brain.

This cerebral flexure, which is especially conspicuous not only in the brain of the Elasmobranch but also in the other types of Brain (Mammalian and Avian) that were first investigated developmentally, has played a large part in discussions on brain morphology. Thus the idea, already alluded to, that the tip of the infundibulum is the morphologically anterior end of the brain rests upon it.

That this idea is unsound seems clearly to be indicated by such series of sections as that shown in Fig. 53. As a matter of fact it seems probable that very pronounced flexure of the brain, such as is seen in the developing Elasmobranch, is to be regarded as a secondary

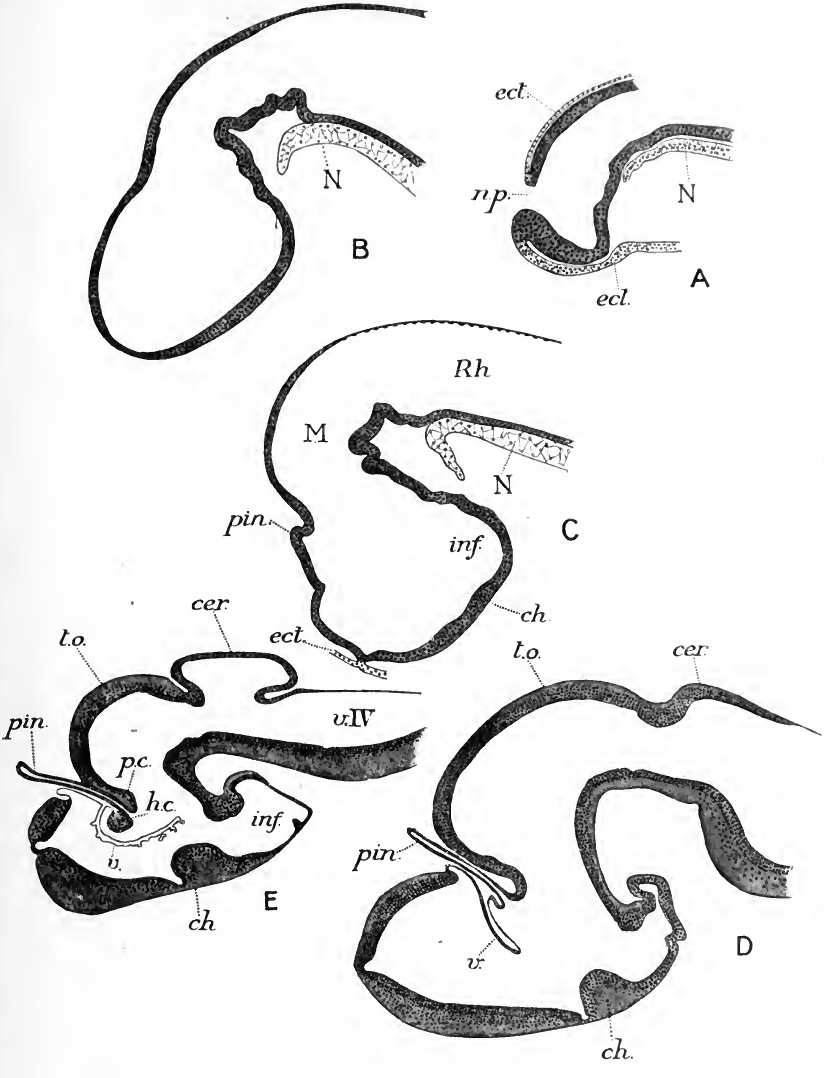


FIG. 54.—Sagittal sections through the brain of *Acanthias*. (After Kupffer.)

A, 3·3 mm. embryo; B, 7-8 mm.; C, 10 mm.; D, 27 mm.; E, 70 mm. *cer*, cerebellum; *ch*, optic chiasma; *ect*, external ectoderm; *h.c*, habenular commissure; *inf*, infundibulum; *M*, cavity of mesencephalon; *N*, notochord; *n.p*, anterior neuropore; *p.c*, posterior commissure; *pin*, pineal organ; *Rh*, cavity of rhombencephalon; *t.o*, *tectum opticum*; *v*, *velum transversum*; *v.IV*, fourth ventricle.

result of the heavily yolked condition of the egg. As a result of the concentration of yolk towards the ventral side in such heavily yolked Vertebrates the processes of growth are retarded upon that side. But it is clear that retardation of growth in length on the ventral side as compared with the dorsal would bring about a flexure towards the ventral side. That the cerebral flexure is due rather to such a general cause than to any inherent peculiarity in the brain itself is supported by the fact that the notochord is also strongly flexed (see Fig. 54, C).

As a consequence of these considerations, we are inclined to take the view that the phenomenon of cerebral flexure is of much less fundamental morphological significance than is commonly supposed.

Comparing the later stages figured for *Acanthias* with those of *Lepidosiren,* it will be seen that the brain shows the same elements although these differ in their relative size and other features in the two cases. Thus the cerebellum of the sharks—correlated with the active and complex movements of these fishes—becomes much more developed. It grows greatly in anteroposterior extent and that causes it to bulge outwards as shown in Fig. 54, E (*cer*).

The pineal body is slender and elongated in form: the velum forms a conspicuous infolding of the thalamencephalic roof continuous across the mesial plane.

The wall of the anterior portion of the primitive fore-brain undergoes a fairly uniform increase in thickness throughout with the exception of a transverse band just in front of the velum which becomes thin and membranous. This portion of the brain increases somewhat in transverse diameter so that it is broad in shape as seen from above, but there is no definite bulging in its side wall to form a distinct hemisphere. The material that would ordinarily go to form the hemispheres remains here in the thickness of the wall.

The olfactory bulb arises as a slight projection from the side wall of the fore-brain, but as development proceeds and the olfactory organ becomes removed from the brain by the interposition of mesenchyme the olfactory bulb remains in contact with the olfactory organ, its attachment to the brain becoming drawn out into a more or less elongated stalk the **olfactory peduncle** or **olfactory tract.**

The salient features in the establishment of the topography of the Vertebrate brain have been illustrated in outline in the sketch which has just been given. It would be beyond the scope of this work to make any attempt to fill in the picture in detail but it is necessary to recall a few points which are of interest to morphologists apart from specialists in neurology.

It should in the first place be borne clearly in mind that the brain—like indeed the whole of the nervous system (see below, p. 118) is to be looked upon as a fundamentally continuous structure. The parts which compose the adult brain—medulla, cerebellum, mesencephalon and so on—are not to be regarded as constituent units which go to build up the complete brain, but rather as specialized portions of a once homogeneous whole. The process of specialization has probably been linked up more particularly with the processes of localization or centralization of particular functions in particular brain regions. When this has come about, increase in the number of ganglion-cells devoted to the particular function will cause an increase in bulk of that portion of the brain in which they are situated and it will assume definite characteristics of its own.

The first step in the development of such a brain region consists in the mere thickening of the brain wall but with still greater increase in the number of cellular elements involved mere increase in thickness becomes insufficient for their accommodation and an increase in area comes about in addition. This necessarily causes a bulging of the particular part of the brain wall and some of the most characteristic differences between the brains of different types of Vertebrate depend upon whether the bulging takes place outwards or inwards.

Thus in the majority of Vertebrates the cerebellum bulges outwards as has been indicated in the case of *Acanthias.* In Teleostean fishes on the other hand this is the case with only the hinder part of the cerebellum: its anterior portion in these fishes bulges downwards and forwards underneath the roof of the mesencephalon forming the well-known **valvula cerebelli.** In the more primitive ganoid fishes on the other hand such as *Polypterus* (Graham Kerr, 1907) the hind portion of the cerebellum also grows inwards, so as to form a structure projecting back into the fourth ventricle in just the same fashion as the valvula cerebelli projects forwards.

A somewhat similar difference appears to be present in the case of the hemispheres. These originate in most subdivisions of the Vertebrata as paired bulgings of the wall of the primitive fore-brain, and the present writer agrees with Studnička (1896) in feeling compelled to accept on this ground the view taught by many of the older morphologists such as von Baer, Reichert and Goethe that the hemispheres are to be looked on as fundamentally paired structures, rather than the view, more fashionable of recent years, which regards the portion of the primitive brain lying in front of the velum and optic recess as forming together with the hemisphere region an unpaired complex (Telencephalon—His). The more complete knowledge that we now possess regarding the development of the brain in the more primitive Vertebrates with holoblastic eggs, seems to the writer to make it clear that the reasons which have led to a departure from the older view can no longer be regarded as adequate. We take it then that the hemispheres are fundamentally paired projections of the side wall of the primitive fore-brain. Physiologically they are probably to be regarded as portions of the brain wall which have become specially enlarged in relation with the sense of smell, just as are the optic outgrowths in relation with the sense of sight.

Now whereas in the majority of Vertebrates the hemispheres bulge outwards, in the more primitive Teleostomes (*e.g. Polypterus,* Graham Kerr, 1907) they bulge inwards. In the typical Teleosts what apparently corresponds to the hemisphere forms simply a solid mass projecting into the cavity of the fore-brain, the structure which is usually and probably erroneously spoken of as the *corpus striatum* in these fishes.

A part of the brain which is of very special morphological interest is the thalamencephalon—which persists with singularly little change throughout the series of Vertebrates. Even in *Amphioxus* sagittal sections through the front end of the neural tube present appearances which vividly suggest the thalamencephalon of the more typical Vertebrates (Kupffer) and raise the question whether—as is probable enough on other grounds—the head region in *Amphioxus* is degenerate and once possessed a brain.

Amongst the structures connected with the thalamencephalon special interest attaches to the pineal body.[1] So far this has been alluded to merely as a comparatively simple diverticulum of the thalamencephalic roof. In the majority of Vertebrates it remains comparatively uncomplicated and its main function appears to be that of forming a peculiar internal secretion.

In two sets of Vertebrates—the Lampreys on the one hand, and *Sphenodon* and many Lizards on the other—there becomes developed in relation to it an organ possessing a close resemblance to an eye, of the "camera" type, possessing a retina and in some cases a lens. The organ appears to be functional as the tissues overlying it are commonly free from pigment and its retinal cells on exposure to light show a change of position in their pigment granules similar to what is commonly found in visual organs. Though functional it does not follow that the organ serves for the detection of what we call light: it may be that its sensitiveness is rather towards radiant energy of other wave-lengths than that included within the range of the visible spectrum.

There is a general tendency amongst those who have carried out researches upon the pineal eye to regard the eyelike condition as a relatively archaic condition of the pineal organ—a tendency which is encouraged by the evidence of palaeontology that certain ancient Tetrapods of the palaeozoic and mesozoic periods possessed a highly developed pineal organ—the skulls of these animals possessing a relatively huge parietal foramen, corresponding with the foramen in the roof of the skull of modern lizards in which the pineal eye lies embedded.

The evidence of embryology indicates that the most archaic condition of the pineal organ was a simple diverticulum of the brain roof projecting towards the skin on the dorsal surface of the head. There is no clue whatever as to the original meaning of this diverticulum. But we do know from the study of invertebrates that where tissue rich in nerve-elements comes to be exposed to light there is

---

[1] An admirable account of the structure and development of this region of the brain by Studnička will be found in Oppel (1905).

frequently shown a well-marked tendency to the evolution of eye-like structure. In Molluscs for example we find eyes developing on the edge of the mantle (*Pecten*), round the tips of the siphons (*Cardium* sp.), on the dorsal surface of the body (independently in *Chiton* and *Oncidium*)—and similar instances might be quoted from other groups.

Bearing such facts in mind one is compelled to acknowledge the possibility, if not probability, of such a projecting piece of nervous tissue as the pineal diverticulum, lying close under the surface of the head on its dorsal side, in the position where light stimulus would be most pronounced, developing *secondarily* in some cases into an organ of the nature of an eye.

Discovered by Leydig (1872), its structure investigated by Spencer (1886) and other workers, the development of the pineal eye has formed the subject of a number of excellent researches. It will be convenient to take as an example that of the common lizards of the genus *Lacerta* (Novikoff, 1910).

The first indication of the organ appears in embryos of about 3 mm. in length in the form of a thickening of the thalamencephalic roof, in the region of the mesial plane, and divided by a transverse furrow on its outer surface into a smaller anterior and a larger posterior portion. This thickened part of the brain roof comes to bulge outwards and forms a prominent projection (Fig. 55, A) the groove dividing it externally into anterior and posterior portions being still visible though less distinct.

The projecting pocket now grows forwards parallel to, and in close contact with, the brain roof (Fig. 55, B), its forwardly projecting portion becoming constricted off from the rest. The constriction in question deepens and the anterior portion (*parapineal* body) becomes nipped off to form a completely closed vesicle (Fig. 55, C)—the rudiment of the eye. As the external ectoderm recedes from the brain roof, with the increase in the amount of mesenchyme between the two, the parapineal vesicle remains close to the ectoderm and consequently retreats from the brain surface (Fig. 55, D).

The eye is now seen to be connected with the brain wall by a distinct optic nerve which, in full accordance with the view taken in this book with regard to nerve-trunks in general, is merely a primary bridge which already existed (Novikoff) at a time when the eye vesicle and the brain roof were still in immediate contact and which simply became extended in length as the gap between eye and brain became greater and greater (Fig. 56, *p.n*). Nerve-fibres develop in this optic nerve which pass at their cerebral end into the habenular commissure. Transverse sections through a 9-mm. embryo show that the fibres on entering the commissure bend away to the right, passing eventually to the right habenular ganglion. In this connexion with the right habenular ganglion *Lacerta* resembles the other lizard *Iguana tuberculata* (Klinckowström, 1894) but curiously differs from *Sphenodon* where according to Dendy (1899) the connexion is with the left habenular ganglion.

Meanwhile the wall of the vesicle lying next the outer skin

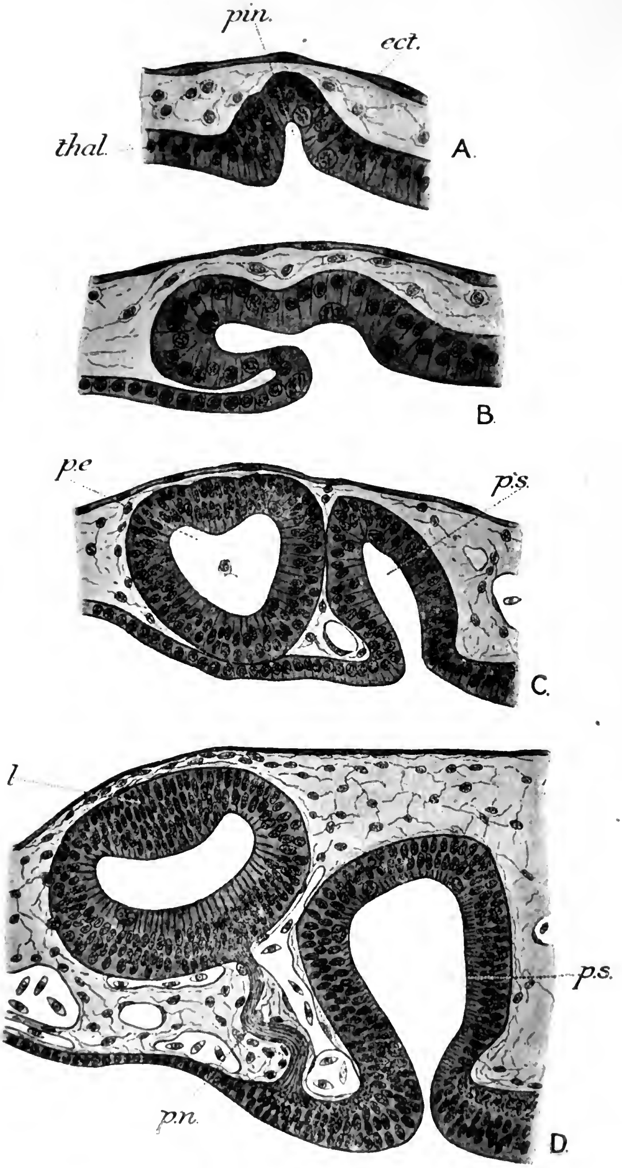


FIG. 55.—Sagittal sections through the pineal organ of embryos of *Lacerta*. (After Novikoff, 1910.)

A, *L. vivipara*, 3 mm.; B, ditto, 4 mm.; C, *L. muralis*, 6 mm.; D, *L. vivipara*, 9 mm. *ect*, external ectoderm; *l*, lens; *p.e*, pineal eye; *p.n*, pineal nerve; *p.s*, pineal stalk; *pin*, pineal outgrowth; *thal*, roof of thalamencephalon.

assumes a lenticular form, its cells becoming much elongated though remaining in a single layer. This lenticular thickening occasionally becomes lost during development—a fact which may be taken as forming a piece of evidence in favour of the view that the eye, at the present time, is in a retrogressive phase of evolution.

Those parts of the vesicle wall which do not take part in the formation of the lens undergo histogenetic changes into retinal tissue. The cells undergo differentiation in two directions. The one set become pigment cells—tall columnar cells which traverse the whole thickness of the retina and have their nuclei towards the basal or outer end and which develop dark melanin granules in their protoplasm.

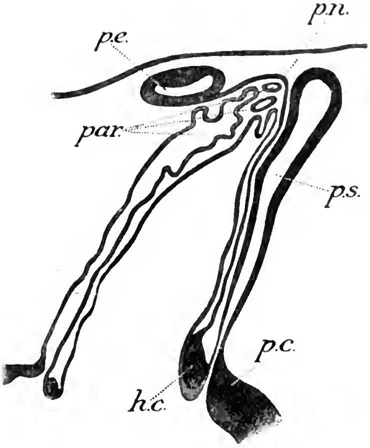


Fig. 56.—*Lacerta muralis*, 25 mm., sagittal section through roof of Thalamencephalon. (After Novikoff.)

h.c, habenular commissure; *p.c*, posterior commissure; *p.e*, pineal eye; *p.n*, pineal nerve; *p.s*, pineal stalk; *par*, paraphysis.

Interspersed with these are the percipient cells, shorter in form, their basal ends not reaching the outer surface, and carrying at their inner ends cilium-like structures which project into the cavity of the vesicle. The idea that these projections correspond physiologically to rods appears to be negatived by the fact that they occur also on the inner ends of the cells forming the lens.

At their basal ends these cells are continued into nerve-fibres, which form a distinct layer internal to the nuclei of the pigment cells and are eventually continuous physiologically with the fibres of the pineal nerve. Scattered amongst, and in the neighbourhood of, this fibrous layer ganglion-cells are present: they are about the first definite elements to become recognizable during the histogenesis of the retina and appear first close to the point of attachment of the optic nerve.

The cavity of the pineal eye is kept distended by a clear substance, the vitreous body, and this is colonized by a certain number of cells (see Fig. 55, C) which are most probably to be regarded as immigrant mesenchyme cells.

In *Sphenodon*, the sole survivor of the only other existing group of Reptiles in which a pineal eye is present, the development of the organ according to Dendy (1899), who has worked it out in detail, agrees with that of *Lacerta* in its main features.

In the Lampreys, also, somewhat eye-like developments occur in the pineal region. In the adult two vesicles—a dorsal ("pineal") and a ventral ("parapineal")—are found overlying the roof of the thalamencephalon. In each of these the lower wall shows histological characteristics of retinal tissue and each is in continuity with the brain—in the case of the parapineal organ directly and in the case of the pineal by an elongated stalk containing nerve-fibres.

The parapineal organ lies in some cases (*Geotria*—Dendy, 1907) slightly to the left of the pineal and its nerve-fibres have been traced into the left habenular ganglion while those of the pineal organ have been traced to the right habenular ganglion. In neither case does the outer wall of the vesicle show any signs of thickening to form a lens—so that neither organ can form an image—but the overlying tissue is comparatively transparent so that diffuse light stimulus can reach it.

According to Studnička the two organs develop as evaginations of the brain roof one (parapineal) in front of the other. The parapineal evagination soon loses its lumen and becomes solid and it is noteworthy that at first it is continuous on each side with the habenular ganglion of that side. Later on it becomes by differential growth shifted far forwards, away from the region of the habenular ganglia, and it loses its connexion with the right ganglion while it remains connected by nerve-fibres with the left.

The two questions of special interest which present themselves in regard to the pineal and parapineal organs are (1) were they originally ocular in structure and function and (2) were they paired or unpaired?

(1) It is obvious that the presence of an eye-like pineal or parapineal organ in certain Reptiles and in Lampreys, and of a large parietal foramen in the skull of various extinct Vertebrates suggests the possibility of these organs having had the form of visual sense organs in the ancestral Vertebrate. Against this however must be set the fact that in all other Vertebrates than those mentioned, including such relatively archaic forms as Elasmobranchs, Crossopterygians, Dipnoans and Urodeles, there is no trace whatever of eye structure.

It seems highly improbable that a well-developed visual organ once present on the dorsal side of the head in the ancestors of Vertebrates should have disappeared without leaving a trace in all the varied groups, with their very different modes of life, outside the limits of the Lampreys and Reptiles. To the present writer it does not appear that the evidence, so far as it exists at present, is anything like convincing that the pineal eye is an ancestral feature of Vertebrates in general rather than a mere secondary development.

(2) Various recent investigators of the pineal organs are inclined to look upon them as being originally paired structures, the pineal organ in the strict sense being the right-hand member of the pair and the parapineal organ the left. This is perhaps most clearly suggested by the Lampreys in which the parapineal organ is connected by nerve-fibres with the left habenular ganglion and the

pineal organ with the right (though also with the posterior commissure). Again in various Vertebrates (Teleostomatous fishes—Hill, 1894: Amphibians, Birds—Cameron, 1903, 1904) the parapineal organ is in early stages slightly to the left of the pineal organ.

On the whole it does not appear to the present writer that the evidence is sufficient to make the view probable. In the Lampreys the connexion of the parapineal body with only the left habenular ganglion appears, as indicated above, to be secondary: it is originally connected with both right and left. Again, to turn to the Reptilia, the eye is in *Sphenodon* connected with the left habenular ganglion and in the Lacertilia with the right, although it seems perfectly clear from the figures given by Dendy and Novikoff respectively that the eye is morphologically the same organ in the two types mentioned. Were it not so we should be driven to the position that of a pair of pineal eyes originally present one has disappeared entirely in *Sphenodon* and the other has disappeared equally completely in the lizards. The improbability from a physiological point of view of this having happened need not be accentuated.

Here again, then, there seems to be up to the present no sufficient reason for departing from the view that pineal and parapineal organs were primitively median in position, one in front of the other. As to their original significance we have no obvious clue: the absence of convincing evidence that they were originally eyes does not of course preclude the possibility of their having been originally some kind of sense organ.

NEUROMERY.—It has been noticed in various Vertebrates, particularly Elasmobranchs, Amphibians and Birds, that the neural rudiment while still in the form of an open plate is sometimes divided up by numerous and regular transverse markings. Whether this appearance of segmentation is caused simply by the active growth in length of the medullary plate or whether on the other hand it has some deeper significance has not been conclusively determined. The name **neuromere** has been given to the apparent segments. That these are really primitive morphological segments as is believed by many and as is implied in the termination "-mere" seems improbable.

The existence of a clearly marked segmentation of the nervous system where it occurs—in the phyla Annelida and Arthropoda—is brought about by the concentration of ganglion-cells in serially repeated masses, in correlation with the presence of serially repeated appendages (parapodia in Polychaeta), and there is no sufficient evidence to show that such were ever present in the ancestral Vertebrate. The fact that the longitudinal muscles are divided into myotomes would not be sufficient by itself to account for the external form of the central nervous system being segmented, for in that case the segmentation would be still clearly marked in the many fishes where the myotomes remain practically unmodified.

During later stages, after the neural tube has become closed in, "neuromeres" are particularly conspicuous in the brain region.

They are sometimes very distinct in the hind-brain of fowl embryos of about the fourth day (see Fig. 236 in Chap. X.). It is however an outstanding characteristic of the head region as compared with the trunk that the segmentation of its mesoderm has become blurred and in great part obliterated. It is under those circumstances improbable that a primitive segmentation of the central nervous system, which is in its nature linked up to a segmentation of mesodermal structures, should have remained particularly distinct in a region where the mesodermal segmentation itself has become particularly obscure.

The appearances mentioned seem to be adequately explained by the active growth of the developing brain within its confined space, combined with the presence round it of mesodermal tissue with vestigial segmentation. It will be noticed in the figure referred to above that the dividing lines between the "neuromeres" are spaced out at exactly the levels where we should expect to see boundaries of mesoderm segments were the existing series prolonged forwards. Segments are no longer visible in this region but there is, as will appear later, convincing evidence that the series of segments did formerly extend through this region now occupied by continuous mesenchyme.

It may well be that the individuality of the segments, no longer visible as such, is still expressed by a difference in consistency of the mesenchyme, sufficient to mould by its varying resistance the actively growing hind-brain as it presses against it.

DEVELOPMENT OF THE PERIPHERAL NERVES.—The development of the peripheral nerves of Vertebrates has been the subject of a large amount of investigation, partly on account of its intrinsic interest and partly on account of its bearing upon physiology and pathology. In spite of the labours of numerous investigators the problem—for we may take it that the mode of development is *fundamentally* the same throughout—has not yet by any means been satisfactorily decided.

While bearing in mind the undesirability of making use of modern facts merely to support, or to undermine, old hypotheses, it will be convenient to approach the question by stating shortly the three prevalent views as to the main features of the development of peripheral nerve-trunks neglecting differences in detail. For shortness these three views may be termed after their most prominent supporters (1) the **His** view, (2) the **Balfour** view and (3) the **Hensen** view.

THE **HIS** VIEW (Outgrowth theory).—This hypothesis may be said to have been founded by Kupffer in the embryological portion of Bidder and Kupffer's work (1857) on the spinal cord. As however Kupffer later on gave up the view, in favour of that of Balfour, the hypothesis now under consideration is commonly associated with the name of His, who played the main part in building up the theory and who fully deserves to be regarded as its principal founder.

It is to be noted in passing that Kupffer's original observations were made upon Mammals and those of His (1868) upon the Fowl. In other words, in both cases the embryos were such, in regard both to the minute size of their cell elements and to their high position in the Vertebrate scale, as to be unsuited to provide a reliable basis for the generalization that has been built upon them.

The His view as expounded by one of its most distinguished supporters S. Ramón y Cajal (1909) may be summarized as follows, the case of the *motor* nerves being taken for the sake of simplicity. Each motor nerve-fibre arises as an outgrowth from a neuroblast, or young ganglion-cell, lying within the spinal cord. The fibre sprouts out from the neuroblast, makes its way to the surface of the spinal cord, perforates that surface, and proceeds to grow freely through the mesenchyme. The free end of the fibre forms a "cone of growth," commonly shaped somewhat like a grain of barley and with a pointed end. This "cone of growth" shows an active amoeboid movement, by which it insinuates itself through the interstices of the mesenchyme. Sometimes it may be seen to flatten or mould itself against obstacles in its path.

In the Fowl these processes take place during the third and fourth days. Eventually (about the fifth day, in the Fowl, in most cases) the growing nerve-fibres reach their destination and become joined up to the muscle cells which form their definitive end-organs.

The essential feature of the His view is that the nerve-fibre (which already shows the characteristic specific reactions of a nerve-fibre, *e.g.* on impregnation with silver salts, and is therefore not merely a strand of undifferentiated protoplasm) sprouts out from the central nervous system and grows through the intervening mesenchyme with a free end until it becomes joined up *secondarily* with the end-organ.

The His view at the present day rests upon a large body of observed facts. In studying the embryology of almost any Vertebrate it is easy to find in sections what appear to be freely ending nerve-fibres sprouting out from the spinal cord. Some of the most beautiful preparations of this kind have been made by Ramón y Cajal and others by the use of silver impregnation methods.

Perhaps the most striking evidence, which has recently been adduced in favour of the His view, has been obtained by experimental methods, especially by Harrison (1908, 1910). In one set of experiments which have been regarded as particularly convincing Harrison removed small pieces of embryonic spinal cord from Frog embryos at a period just before that at which the motor nerves became visible, and was able, by using ordinary antiseptic precautions, to keep them in a living condition for relatively prolonged periods mounted in a drop of sterile lymph under a coverslip upon a slide. The lymph soon clotted and held the piece of spinal cord in position. Harrison now observed in many cases little projections making their appearance from the pieces of spinal cord which he identified as rudimentary motor nerves.

Any possible doubt as to the correctness of this identification was removed by Burrows (1911) repeating the work on the chick and obtaining the specific staining reaction of neuro-fibrils in the structures in question. These nerve-rudiments when kept alive under the conditions mentioned were observed to increase rapidly in length, the rate of growth being in one case as high as 56μ per hour. The end of the rudiment (Fig. 57) was somewhat enlarged and projected into fine protoplasmic tags which showed active amoeboid movement. It is this amoeboid protoplasm at the free end of the fibre which, in Harrison's belief, is the active agent in the extension of the nerve-fibre.

As to the method by which it is, in the actual body, guided along the proper path to its destination, Harrison does not commit himself, but he appears to have a leaning towards the view held by Ramón y Cajal that it is mainly a matter of chemiotaxy.

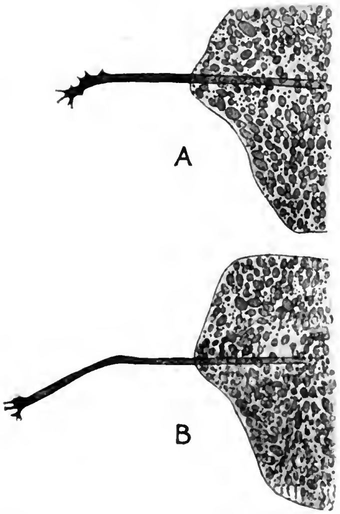


FIG. 57.—View of outgrowing nerve-fibre. Both figures are drawn from the same live preparation, B twenty-five minutes later than A. (After Harrison, 1908.)

In the words of their author (1908) these "experiments place the outgrowth theory of His upon the firmest possible basis,—that of direct observation. The attractive idea of Hensen must be abandoned as untenable."

It should be added that the His theory fits in very well with current views in physiology and pathology—in particular with the fashionable neurone doctrine, according to which the cellular units which compose the nervous system are not in organic continuity with one another. Obviously this hypothesis and the outgrowth hypothesis, according to which the nerve-fibre is for a time separated by a gap from its end-organ, lend one another mutual support.[1]

---

[1] It must be borne in mind, however, that the histological basis of the neurone theory is not universally admitted to be beyond suspicion. Its main foundation consists of observations by the Golgi and similar methods of metallic impregnation. In preparations made in this way single cellular units are frequently picked out without the reaction taking place in neighbouring units arranged in series with them. A ganglion-cell A with its axon and terminal branches stands out deep black in the preparation, while the ganglion-cell B, next to it in the series, shows no reaction. Such an observation obviously suggests discontinuity.

The possible fallacy in these observations lies in the fact that the stain used is not a true stain in the ordinary sense of the word but merely a precipitation of metal upon

The His view is concerned primarily with the actual functional nerve-fibres. As regards the primitive sheath (Gray Sheath of Schwann), in which these fibres are enclosed, the His view regards it as being formed by mesenchyme cells which apply themselves to, and spread out over, the surface of the originally naked nerve-fibre.

(2) THE **BALFOUR** VIEW (Cell-chain theory).—While Schwann (1839) long ago described the multicellular structure of nerve-trunks in the foetuses of mammals, it was F. M. Balfour (1876) who really founded the view that the nerve-trunk arises in development from a chain of cells. Balfour found in Elasmobranch embryos that the nerve-trunk was represented by a chain of cells in early stages (Fig. 58, *v.r*), and similar observations have been made by subsequent observers. According to this view the whole nerve-trunk is multicellular in origin, the cells not only forming the sheath of the nerve-trunk but also giving rise to the nerve-fibrils which come into existence traversing the cellular strand from end to end.

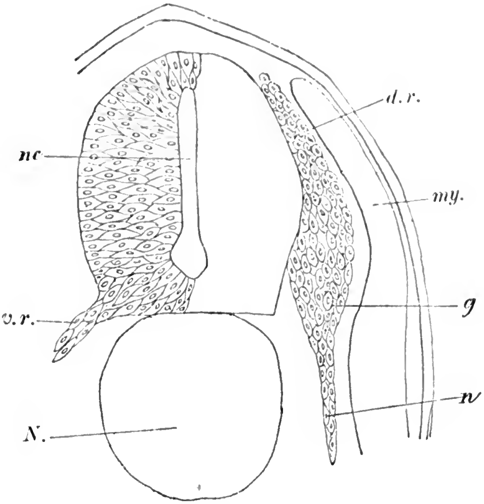


FIG. 58.—Section through the dorsal part of the trunk of a Torpedo embryo. (From Balfour's *Embryology*.)

d.r, dorsal root; *g*, spinal ganglion; *my*, myotome; *N*, notochord; *n*, nerve-trunk; *nc*, cavity of spinal cord; *v.r*, ventral root.

On the question of the origin of the cells which constitute the nerve-rudiment opinions vary. Most supporters of this view have regarded them as having emigrated from the spinal cord (*e.g.* Balfour, van Wijhe, Dohrn): while others (Kölliker) have looked on them as mesenchymatous in nature. Sedgwick took this latter view and as he regarded the mesenchyme as a continuous syncytium, the bridges connecting the cells being primitive—persisting from the

---

the surface of the cell and its processes. We know from the recognized unreliability of the method that the occurrence, or not, of this precipitation is liable to be decided by extremely delicate chemical differences. We know further that the axis cylinder, however it arose in development, is morphologically and physiologically a prolongation of the cell-body (ganglion-cell), and therefore that its metabolism is under the control of the nucleus of that cell-body. The individuality of the cell and its prolongation, due to the metabolic control by its own special nucleus, is probably quite enough, in itself, to account for a chemical character of its surface sufficiently different from that of its neighbours to influence the precipitation without there being, as the neurone theory assumes, any absolute discontinuity.

incomplete separation of the cells during the processes of segmentation and cell division—the view in his hands came to approach the next view to be mentioned that of Hensen.

(3) THE **HENSEN** VIEW (Primitive continuity theory).—This view has found its strength in general physiological considerations rather than in convincing facts of observation. According to Hensen (1864, 1868, 1903) the nerve which connects centre with end-organ is a primary connexion which has been there from the beginning. It existed first as a simple bridge of protoplasm, such bridges being present between the various cells of the body owing to the fact that the processes of segmentation and cell division are not complete so as to lead to absolute isolation of the cells or segments from one another. According to this view the growth in length of a nerve-trunk is simply the extension of a pre-existing bridge, as the organs at its two extremities —centre and end-organ — are pushed apart from one another during the course of development.

Hensen figures in his papers (Fig. 59) what he takes to be such nerve-rudiments, in the form of numerous fine filamentous structures passing across the space between spinal cord and myotome. There is however no evidence to show that these filaments have anything to do with nerve-trunks. Although for this reason it is impossible to accept the main observational basis of Hensen's view, that does not necessarily invalidate the physiological considerations which may be held to give an *a priori* probability to the correctness of his general theory.

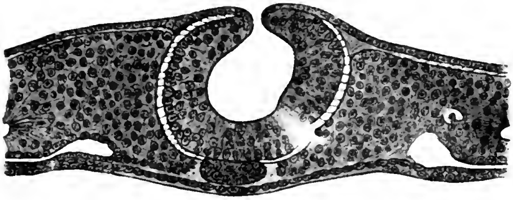


FIG. 59.—Illustrating Hensen's view of the origin of peripheral nerves. The section is taken from a 9-day Rabbit embryo, and passes through the trunk region. (After Hensen, 1903.)

The three views which have been outlined above were fashioned by their respective authors long ago as embryological science goes. Since then new facts have become known which have to be taken into account when considering their acceptability as working hypotheses at the present time. Some of these facts will now be touched upon.

DEVELOPMENT OF PERIPHERAL NERVE-TRUNKS IN LEPIDOSIREN

It is obvious enough from the diversity of statements by skilled observers that the investigation of the method of development of the peripheral nerves in Vertebrates is beset by technical difficulties and resulting liability to error. In such a case it is of special importance to choose for investigation types of animal in which this liability to error is reduced to its narrowest limits. Such an animal should be, on the one hand, comparatively archaic—it should belong to one of the relatively more primitive groups of Vertebrates—and, on the other hand, its histological texture should be as coarse as possible, its cell elements being of large size.

Amongst Vertebrates investigated up to the present time in regard to nerve-development *Lepidosiren* (Graham Kerr, 1904) is unrivalled in its combination of these qualifications and a summary will now be given of the main features which have been made out from the study of the development of the motor nerves in this animal. It will be convenient to commence with the fully formed nerve-trunk and then work backwards towards the earlier and more obscure stages.

Fig. 61 represents a portion of nerve-trunk from a fully developed larva of stage 34. The nerve-trunk consists of a cylindrical bundle of nerve-fibrils, dotted over the surface of which are the numerous large nuclei of the protoplasmic sheath. The sheath itself is so thin as to be practically invisible even under a high-power immersion objective except in the angle close to a nucleus where it is distinctly visible.

Fig. 60, D is taken from a larva ten days after hatching. At this stage the nerve-trunk, when examined superficially, has the appearance of a thick strand of protoplasm containing numerous nuclei or a chain of cells. Careful examination of well-fixed and well-stained specimens shows however that this conspicuous mass of protoplasm is really only the sheath, the true nerve-trunk (*n*) being visible traversing it from end to end. Scattered about in the thick sheath of this stage there are still to be seen granules of yolk (black in the figure) which have not yet been used up.

Fig. 60, C is taken from a larva at the time of hatching. At this stage the nerve-trunk is a well-developed bundle of nerve-fibrils, just as in the later stages, but throughout the greater part of its length it is devoid of a sheath of protoplasm. In the section figured the sheath is visible as a mass of nucleated and heavily yolked protoplasm enclosing a portion of the nerve-trunk towards its outer end. This mass of protoplasm is obviously just a condensed part of the general mesenchyme which is scattered about in the form of irregular heavily yolked masses throughout the spaces between the main organ systems.

The mass in question is identical with the rest of this mesenchyme in its various features and every here and there it is continued into it without a break. The section figured shows the whole length of the motor nerve-trunk from the ventral root to the myoblasts or muscle cells which form the myotome. Towards its outer end the trunk is seen to break up into numerous diverging strands which are directly continuous with the protoplasm of the myoblasts (see below, p. 204).

Fig. 60, B is taken from an embryo about three days before hatching. At this stage the myotome has barely commenced to

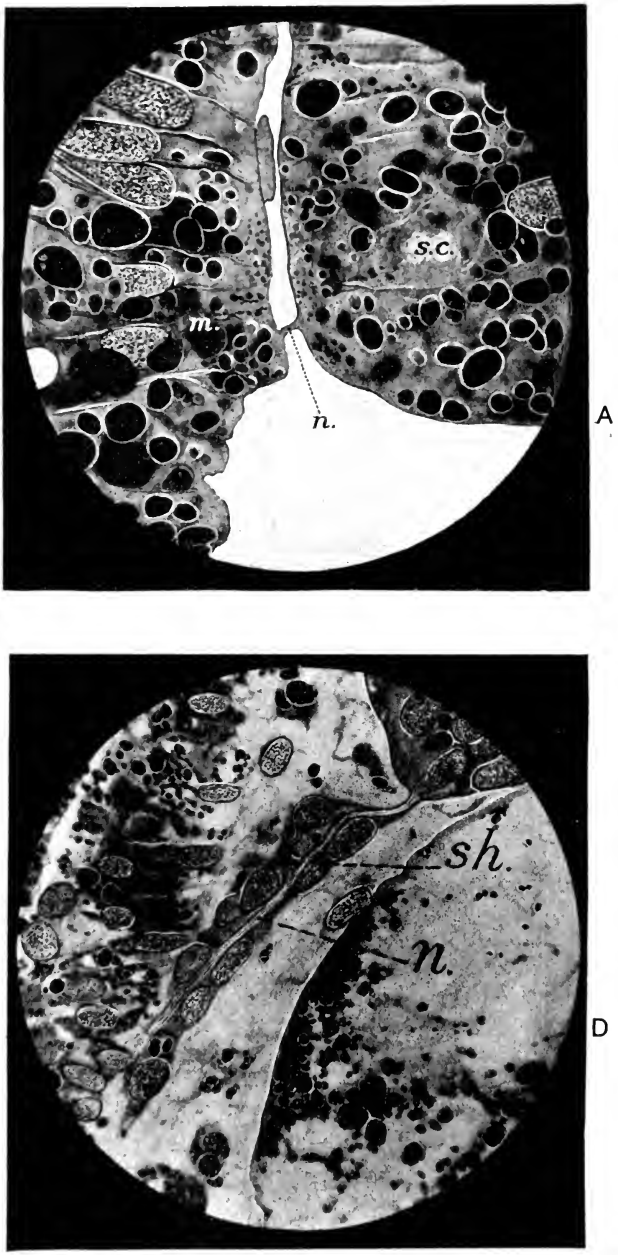


FIG. 60.—Portions of transverse sections through young Lepidosirens to illustrate the development of the spinal nerves (ventral roots).

A, stage 24; D, stage 29 +. *m*, myotome; *n*, nerve-trunk; *s.c*, spinal cord; *sh*, sheath.

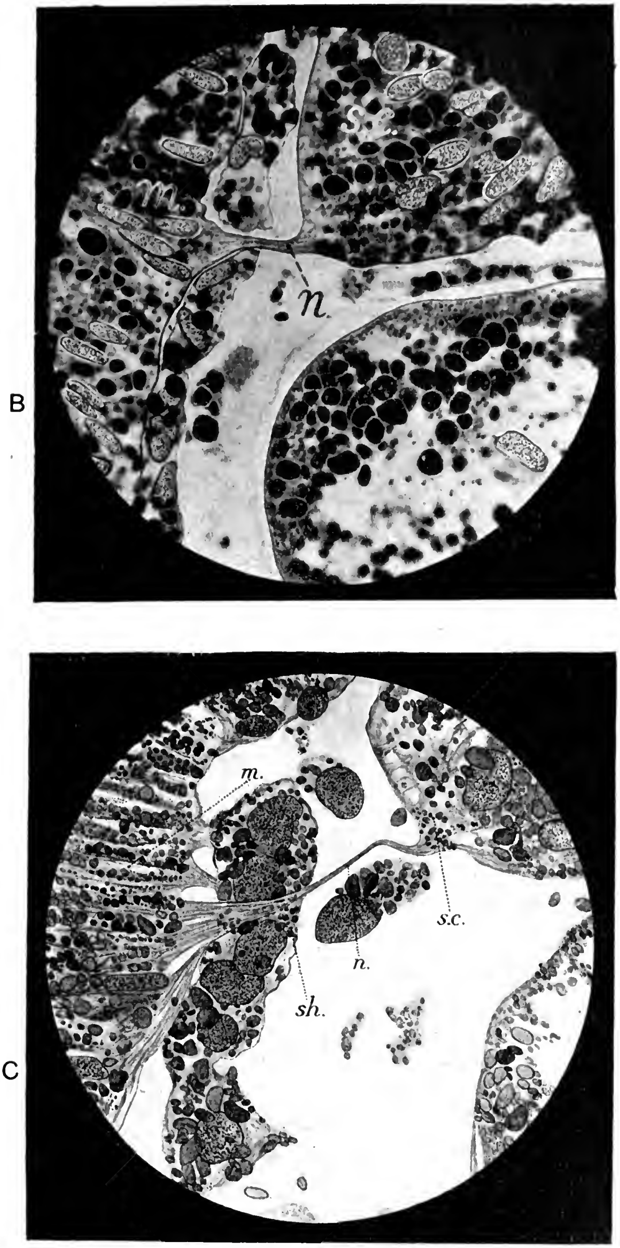


Fig. 60A.—Portions of transverse sections through young Lepidosirens to illustrate the development of the spinal nerves (ventral roots).

B, stage 25; C, stage 27. *m*, myotome; *n*, nerve-trunk; *s.c*, spinal cord; *sh*, sheath recede from the spinal cord, but yet each motor nerve is already present as a distinctly fibrillated trunk bridging across the narrow gap between spinal cord and myotome. A few mesenchyme cells have wandered into the gap but they have not yet begun to concentrate round the nerve-trunk.

Fig. 60, A is taken from an embryo of stage 24 at a time when myotome and spinal cord are still in close contact with one another. In specimens which were extended in one plane under normal salt solution while still alive and subjected to the action of the fixing agent in that position, it is found that the myotome is frequently pulled slightly away from the spinal cord (as in the specimen figured) and in such cases it is found that the nerve-trunk already exists in the form of a bridge of soft granular protoplasm (*n*) without any trace of fibrillation, connecting spinal cord and myotome. That these bridges are really the nerve-trunks is indicated by their occurrence one to each myotome, apart from the fact that a continuous series of stages have been observed between them and the fully developed nerve-trunks.

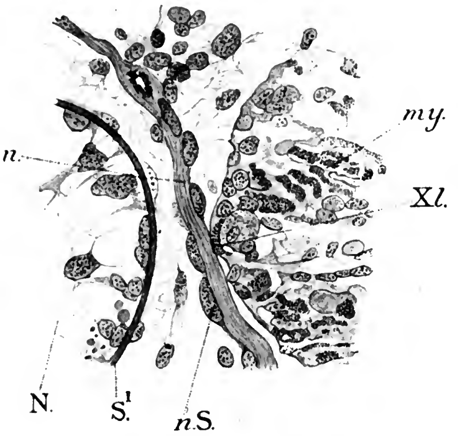


Fig. 61.—Part of transverse section of *Lepidosiren* (stage 34), showing a portion of nerve-trunk.

my, myotome; *N*, notochord; *n*, nerve-trunk; *n.S*, nucleus of nerve sheath; S^1, primary sheath of notochord; *Xl*, lateral branch of vagus.

In summing up we may take the various stages in their proper chronological sequence.

(1) The nerve-trunk is already present as a protoplasmic bridge at a period so early in development that spinal cord and myotome are still in contact with one another.

(2) As the embryo grows and the myotome recedes from the spinal cord this protoplasmic bridge increases in length and becomes fibrillated.

(3) As the nerve-trunk lengthens amoeboid masses of mesenchymatous protoplasm collect round it and gradually spread out over its length to form the protoplasmic sheath.

In stages later than those figured the sheath protoplasm insinuates itself in amongst the nerve-fibrils of the trunk, dividing them up into bundles or **nerve-fibres**. As the myotome resolves itself into the various muscles of the adult each piece retains its primitive nerve-strand, drawing it with it, as it becomes pushed about by the processes of differential growth, as its own special nerve.

It should be mentioned that the most important point in the above description—the existence of the motor trunk in the form of a bridge of protoplasm between myotome and spinal cord at a time when they are still in close proximity—has been confirmed for another very primitive group of Gnathostomes, the Elasmobranchs (Paton, 1907), as is shown in Fig. 62.

It will now be convenient to review the facts just described for *Lepidosiren* in relation to the general theory of nerve-development.

(1) It is clear in the first place that the His view is put out of court, seeing that before there is any development of nerve-fibrils the motor nerve-trunk already exists in the form of a bridge of protoplasm connecting spinal cord and myotome.

(2) It is equally clear that the Balfour view is inapplicable: the nerve-rudiment cannot in early stages by any possibility be regarded as a chain of cells, seeing that its total length is greatly less than the diameter of a single cell-nucleus.

(3) While the nerve-rudiment forms a primary connexion between spinal cord and myotome, in the sense that it is in existence before these organs begin to recede from one another, there is no evidence by which the connexion can be traced back to intercellular bridges or **plasmodesms** (Strasburger, 1901) of early, *e.g.* segmentation, stages in the development of the egg, as would be the case according to Hensen's theory.

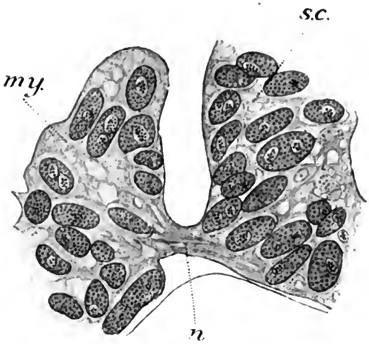


FIG. 62.—Part of transverse section through a 4·5 mm. embryo of *Pristiurus*, showing the motor nerve-trunk rudiment. (After Stewart Paton, 1907.)

my, myotome; *n*, nerve-trunk; *s.c*, spinal cord.

(4) The primitive protoplasmic bridge gradually becomes fibrillated but there is no means of determining with any degree of certainty how these fibrils are developed.

It is suggested[1] that the development of the actual nerve-fibril is simply the gradual coming into view of a pathway produced by the repeated passage of nerve impulses over a given route.

It is clear from the study of the simpler organisms that one of the most ancient properties of living protoplasm is that of the transmission of impulses through its substance. Although nothing is really known as to the precise nature of living impulses it is reasonable to suppose that they involve changes in the distribution of energy analogous to those involved in the passage of an electric disturbance. If this be the case their passage between two points will be determined by the relative potential, and the route along which the impulse passes will be that of least resistance. If the conductivity of the

---

[1] Graham Kerr, 1904. It has been pointed out that similar suggestions in regard to the nervous system in general were made long ago by Herbert Spencer.

living substance were uniform the path would be a straight line joining the two points: if the conductivity were not uniform on the other hand the path would be diverted along routes of high conductivity where the total resistance would be at its minimum. Looking at matters from such a point of view we should regard a motor ganglion-cell at the moment of functioning as a centre of high potential and its muscle ending as of low potential, while a sensory cell at the moment of its functioning would be a centre of high potential and the central termination of its nerve-fibre as at relatively low potential.

In early stages of evolution, whether phylogenetic or ontogenetic, we may take it that vital impulses flitted hither and thither in an indefinite manner within the living substance and that one of the features of progressive evolution has been the gradual more and more precise definition of the pathways of particular types of impulse, as well as of the transmitting and receiving centres between which they pass.

We may then regard the appearance of neuro-fibrils within the protoplasmic rudiment of the nerve-trunk as the coming into view of tracks, along which, owing to their high conductivity, nerve impulses are repeatedly passing.[1] It may be that as each successive passer-by causes a jungle pathway to become more clearly defined so each passing impulse makes the way easier for its successors, and makes it less likely for them to stray into the surrounding substance.

The special physiological meaning of the differentiation of the fibril would simply be the increase of its conductivity—possibly towards one specific type of impulse—but correlated with this are optical and staining peculiarities which, though unessential in themselves, make the fibril recognizable to the eye as a definite structure.[2]

The nerve-trunk in *Lepidosiren* is seen to be at first naked and later on to acquire a sheath formed by concentration of mesenchyme round it. This sheath is at first richly laden with nutriment in the form of yolk granules but these are gradually used up as the nerve-trunk goes on with its development, the products of digestion of the yolk being doubtless passed on to the developing nerve-trunk. This as well as the marked increase in the number of nuclei in the sheath seem to indicate that the main rôle of the sheath is to look after the nutritional needs of the nerve-trunk.[3]

We have dealt, so far, only with the motor nerve-trunks. In regard to the general method of development of sensory nerves, there

---

[1] Paton (1907) shows that impulses are actually transmitted across the protoplasmic bridge at a very early stage in the case of Elasmobranchs.

[2] The hypothesis here outlined in connexion with the embryonic development of nerve fits in well also with certain of the phenomena observed in the regeneration of nerves which have been severed and joined together again [see *Trans. Roy. Soc. Edin.*, xli, p. 126, also Mott, Halliburton and Edmunds in *Proc. Roy. Soc.*, B, vol. 78].

[3] The medullary sheath of nerve-fibres is non-cellular and appears to be produced by the secretory activity of the protoplasm of the axon.

exists the same divergence of opinion as in the case of the motor nerves, and in endeavouring to decide which view has upon its side the balance of probability, it is well to bear in mind similar conditions to those alluded to on p. 106. Bearing these in mind, it is of interest to notice that in *Lepidosiren* (Elliot Smith, 1908) the process of development of the olfactory nerve takes place along exactly similar lines to that of the motor trunks. And it is significant that, in the opinion of those well qualified to judge (Retzius, Golgi, Ramón y Cajal, van Gehuchten, Kölliker, Elliot Smith), this nerve has advanced less from the primitive condition than has any other nerve, and in its general arrangements has undergone extraordinarily little complication during ontogeny.

Already at a time when the olfactory organ has not yet commenced to recede from the wall of the hemisphere the olfactory nerve exists as a stout protoplasmic bridge (Fig. 63, *I*) which gradually increases in length as the olfactory organ recedes from the hemisphere. This observation seems to indicate clearly that the mode of development of the sensory nerve-trunk is fundamentally the same as that of the motor: that it develops out of a preexisting protoplasmic bridge between centre and end-organ.

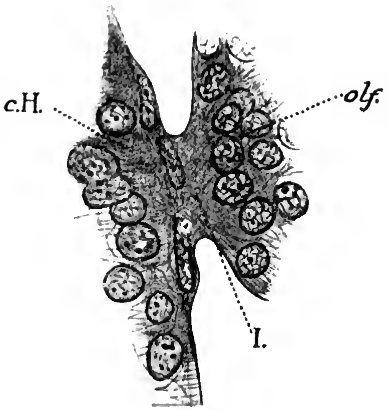


FIG. 63.—An early stage of the olfactory nerve of *Lepidosiren*. (From Elliot Smith, 1908.)

c.H, lateral wall of hemisphere; *olf*, olfactory organ; *I*, olfactory nerve. The nuclei seen in the region where the olfactory nerve enters the hemisphere belong to the olfactory bulb.

REMARKS UPON THE GENERAL PROBLEM OF NERVE DEVELOPMENT

It will be admitted by most Zoologists that we are justified in believing that the process of nerve-development is probably fundamentally the same throughout the Animal Kingdom. It will also be clear, even from the short and imperfect statement which has been given here, that the detailed study of the phenomena of nerve-development has led, in the minds of different observers, to widely divergent conclusions as to the exact nature of the process. The subject is one to the discussion of which we may devote with advantage some further space. It is in itself of great embryological and physiological interest. It presents many problems still unsolved. And it may be taken as a type of biological controversy with which it will be to the student's advantage to become acquainted.

In approaching the question from the present-day standpoint it appears impossible to get round the fact that in two of the most archaic groups of Vertebrates (Elasmobranchii and Dipnoi) the motor nerve-trunk is already present as a protoplasmic bridge at a time when myotome and spinal cord have not yet commenced to recede from one another. It does not seem possible to explain the appearances recorded in these cases by any conceivable errors of observation. But if such bridges exist in these relatively archaic groups, the balance of probability is entirely on the side of their representing the primitive mode of development of nerve-trunks in general, and of a fundamentally similar mode of development occurring in other Vertebrates though possibly in a modified and less distinct form.

On the other hand appearances of the kind which led to the original formulation of the His view, and which are still adduced in its support, and which are easily observed in series of sections through almost any type of Vertebrate embryo — nerve-trunks passing out from the spinal cord and ending freely amongst the mesenchyme—are peculiarly apt to be misleading.

Such a misleading appearance is produced sometimes by comparatively simple causes—by breakage of the nerve-trunk or by the nerve-trunk passing away out of the plane of a section and being unrecognizable when cut *transversely* in a neighbouring section. In other cases the appearance of a freely ending nerve-trunk is due to the portion of nerve-trunk which has received its protoplasmic sheath being distinctly visible in a stained section, while the delicate peripheral portion which is still naked is practically unrecognizable. On account of such liability to misinterpretation a very large proportion of the observational evidence which supports the His view is open to suspicion.

A physiological difficulty which has been raised against accepting the His view is that involved in the idea that the free end of the growing fibre tracks down and finds its appropriate end-organ. It is pointed out that it never makes a mistake—never becomes joined up to a wrong end cell. And yet, if it be the case that nerve-fibres do grow outwards with free ends in the way involved in the His theory, certain experimental results show that such fibres do possess a very decided power of making mistakes. This is brought out clearly by the beautiful experiments of Braus (1905).

In the experiments in question Braus made use of the method, invented by Born and developed by Harrison, Spemann and others, in which portions of one amphibian embryo are grafted upon the body of another, when the grafted portion ("parasite"—Braus) proceeds to develop as part of the individual ("autosite"—Braus) upon which it has been grafted.

In the experiments which are most important in their bearing on the point now under discussion the early rudiment of the pectoral limb was grafted upon a host in the region of the head. In this position the rudiment went on developing into a perfectly normal limb containing a normal arrangement of the limb nerves. Now the implanted limb in such a case (Fig. 64) is situated in a region innervated by the facial nerve and the study of sections showed that the nerves in the implanted limb were continuous centrally with branches of the facial nerve.

If we attempt to interpret this experiment on the outgrowth view we find ourselves compelled to admit that the facial fibres concerned made the serious mistake of growing into a limb rudiment and then continued on their mistaken course until finally they established the muscular connexions normal for the nerves of such a limb. Braus repeated this type of experiment in a number of cases and there appears to be no question as to the accuracy of his observations. If accurate, however, they provide a formidable, if not unsurmountable, difficulty for the outgrowth view—a difficulty which is by no means got rid of by the suggestion (Harrison, 1908) that after arriving in the limb the nerves are "merely guided in their growth by the structures present in the transplanted part."

FIG. 64.—Young Toad (*Bombinator*) on which an additional limb has been grafted in the head region. (After Braus, 1905.)

A similar difficulty is seen in post-embryonic nerve-development in the fact well known to surgeons that functional continuity can be established between the cut central stump of one nerve (*e.g.* spinal accessory) and the severed peripheral portion of another (*e.g.* facial).

And so again in the development of anastomoses between peripheral nerves such as the well-known "dialyneury" of Gasteropod molluscs, or the short circuiting of the left pulmonary nerve over the dorsal side of the oesophagus which has come about in the evolution of the Crossopterygians and Lung-fishes.

All such cases present great if not insuperable difficulties to the His view.

Again much of the evidence which is brought to the support of the His view is seen when looked at critically to be less convincing than it appears to be at first sight. Thus for example with the experiments of Harrison already described, which are regarded by their author as settling the whole question. Their true value will become more apparent if we bring Harrison's results into correlation with the results described above for *Lepidosiren.*

As has already been shown, in this animal the motor nerve-trunk is represented at an early stage by a bridge of soft fragile protoplasm. These bridges require a very favourable object and very careful technique for their detection, and it is clear that one could not expect to see them in comparatively coarse preparations made by excising a piece of living unfixed spinal cord. There is therefore no guarantee that such protoplasmic nerve-rudiments were not already present in the pieces of spinal cord investigated by Harrison.

Let it be assumed that such an experiment is repeated upon *Lepidosiren* with a small piece of spinal cord rudiment with the protoplasmic bridge attached to it (Fig. 65, A). The piece of spinal cord is well supplied with food material in the form of yolk and, if kept under suitable conditions, it would go on developing. So also might the protoplasmic bridge, for every one agrees that the metabolic control of the motor nerve is exercised by the central ganglion-cell nuclei within the spinal cord. If this happened and the process went on quite normally we should get in succession stages such as those shown in B and C of Fig. 65.

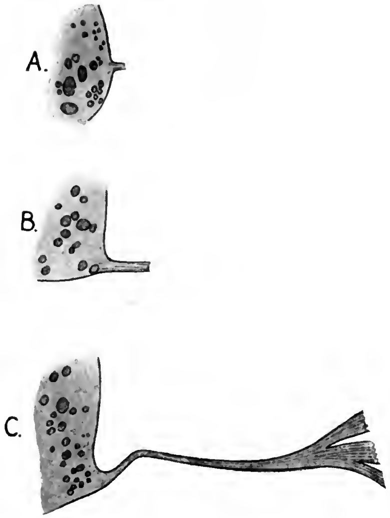


FIG. 65.—Drawings taken from the same preparations as those illustrated in Fig. 60, showing a piece of spinal cord with the developing motor nerve but ignoring the myotome which is in the actual embryo continuous with the outer end of the nerve.

Now these would be interpreted by Harrison presumably as demonstrating the outgrowth view, whereas all that they really show is that, given suitable conditions, the motor nerve increases in length—a fact which of course is obvious. What is needed as a demonstration of the His view is not merely to show that a nerve-trunk increases in length but to show (1) that it normally has a free end and (2) that it grows within the body at a greater rate than the tissues in which it is embedded, so that there is brought about a differential movement in which the free end pushes its way through the tissues surrounding it. This has not been shown by Harrison's experiments nor could it possibly be shown by this type of experiment. In *Lepidosiren* the study of sections shows as has already been pointed out that, although the motor nerve-trunk grows actively in length with the increase in bulk of the body, at no period from the earliest stage figured has it a free end; it is throughout connected with its end-organ.[1]

In a word, it appears to the present writer that what are commonly regarded as the most convincing pieces of evidence in favour of the His view are by no means convincing.

Views resembling that of His in that they also involve an out-

---

[1] The actively moving pseudopodium-like tags which Harrison observed at the end of his outgrowing nerve-trunk are believed by the present writer to be mesenchymatous in their nature—possibly shreds of sheath protoplasm. It is a general feature of embryonic mesenchyme that its protoplasm shows active amoeboid movement.

growth of the motor trunk from the spinal cord, but differing from it in the essential feature that the outgrowth is simply protoplasmic and not fibrillar, have been enunciated by some modern workers such as Dohrn and Held. Dohrn (1888) describes the motor nerve-rudiment as arising by a "plasmatic outflow from the neural tube" but Paton later on finds that at the stage referred to by Dohrn the nerve-rudiment is already continuous at its outer end with the protoplasm of the myotome.

Held (1909) also regards the motor trunk as arising by outgrowth from the spinal cord at a time when the myotome is still comparatively close to it. It has to be borne in mind in interpreting such sections as Held figures that there is more liability to error in demonstrating the absence of continuity than in demonstrating its presence, owing to the extremely fragile character of the nerve-trunks during early stages in development and their consequent liability to rupture during the ordinary processes of preparation which precede section-cutting.

It is sometimes said that the difficulty attaching to the His view involved in the idea of the nerve-fibre tracking down its own particular end-organ disappears if the view is taken that the outgrowth takes place at a stage so early as that indicated by Dohrn and Held. But as a matter of fact this involves, as indicated, a distinct departure from the view enunciated by His according to which not merely undifferentiated protoplasm but definite fibrillated trunks grow out from the spinal cord. Further if, as Held believes, the individual fibrils grow out in the substance of the protoplasmic outgrowth each one has still to seek out the particular portion of the myotome which will eventually be converted into its own proper muscle-cell—a view which, looking to the comparatively undifferentiated condition of the myotome cells at these early stages, is even more difficult to comprehend physiologically than the outgrowth towards a specialized muscle.

The embryological evidence upon which the His view rests is seen, when submitted to critical examination, to be unconvincing. The same is the case with the observational evidence upon which the Balfour view rests. The nuclei and cell-bodies which commonly give a multicellular appearance to the nerve-rudiment are quite reasonably interpretable as sheath-cells, *i.e.* mesenchyme elements which have collected round and it may be migrated into the, at first noncellular, nerve-trunk.

In *Lepidosiren*, with its coarse and heavily yolk-laden mesenchyme, it is comparatively easy to distinguish such elements from the actual nerve-trunk embedded in them, but in most Vertebrates this criterion is not available and there is no certain means of distinguishing in ordinary microscopic preparations the protoplasm of the nerve-trunk from that of the sheath-cells.

The primitive protoplasmic bridge described in 1902 for *Lepidosiren* as representing the motor trunk at a time when myotome and spinal cord have not yet commenced to move apart, confirmed later in the case of the motor trunks of Elasmobranchs by Paton, and in the case of the olfactory nerve of various Vertebrates by Elliot Smith and others, seems to rest upon a secure basis of observation. It is difficult, therefore, to avoid the expectation that the progress of future research will show such a primitive protoplasmic bridge between centre and end-organ to be the normal forerunner of nerves in general.

But, if this be so, we are faced by the question as to the actual mode of origin of such bridges and here we pass into a region where direct observation is either impossible or unreliable. Those who accept Hensen's views in their entirety would look upon them as representing intercellular connexions persisting from the earliest segmentation stages. Reasons have already been given (p. 37) for disbelieving in the persistence of such bridges between the cells of the segmenting egg. The connexion appears certainly to arise at some later period—but exactly when seems to be a question incapable of answer by direct observation.

When considering these general problems regarding the nervous system it should be borne in mind that the nervous system has for its main purpose the keeping of the various parts of the body linked together into an organic whole, in spite of their increasing differentiation and specialization. It has for its function the providing of exquisitely specialized pathways by which the living impulses can traverse the whole length of a relatively immense body at least as readily as they originally did the minute blob of ancestral protoplasm.

Bearing in mind this primary consideration will cause one to reflect that the evidence must be overwhelming before one is justified in believing that this organ system, whose most striking functional feature is continuity, has come in the course of evolution to be characterized by the structural discontinuity involved in the neurone theory of adult structure, or in the outgrowth theory of ontogenetic development.

Again it is important to bear in mind the high degree of probability attached to the view, originated long ago by O. and R. Hertwig (1878), that the nervous system of the higher metazoa, including Vertebrates, has been evolved out of a subepithelial nervous network of the kind still seen in some of the more lowly organized groups such as Coelenterata and Echinodermata. We may suppose that such a plexus was present in the far back ancestors of Vertebrates over the basal surface of both ectoderm and endoderm cells (as in modern Actinians, Havet, 1901) and that nerve-trunks became evolved as local condensations of such a network, just as we still see in the nerve-strands of a Medusa or a Starfish.

In this connexion, it is of interest to note that according to the protostoma theory of the fundamental structure of the vertebrate body, which will be found stated later, in Chapter IX., the points represented by the two ends of the motor trunk were originally in close proximity, and a condensed strand of the network joining the two points would naturally be left as a bridge when they became separated by the deepening of the cleft between mesoderm and endoderm (cf. Fig. 66).

To sum up, in regard to the mode of development of the nerve-trunks, it seems reasonable in the present state of our knowledge

(1) to reject definitely that portion of the Hensen view which looks on the protoplasmic bridges as having persisted from the commencement of segmentation,

(2) to regard the His view of free-ending fibrillated outgrowth as non-proven and for various reasons improbable,

(3) to believe that the nerve-trunk already exists as a protoplasmic bridge between centre and end-organ at a period when these are still in immediate contact, even although this has up to the present been definitely shown by actual observation only in a few peculiarly favourable instances,

(4) to leave the exact period at which the protoplasmic bridges come into existence an absolutely open question as being beyond the limit of reliable observation,

(5) as regards the sheath of Schwann, to accept the view that it is derived from mesenchyme.

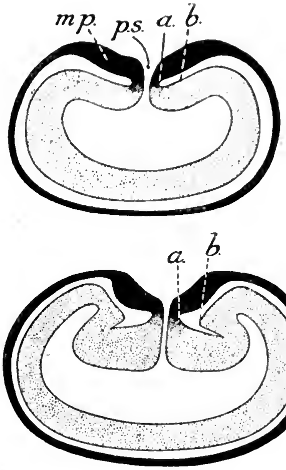


FIG. 66.—Illustrating the structure of a hypothetical primitive Vertebrate at a time when the protostoma was still open. In the lower figure an enterocoelic pocket, the rudiment of a mesoderm segment, is becoming demarcated from the rest of the endoderm by the downward spreading of a split between the points *a* and *b*. In the earlier stage shown in the upper figure this split has not yet begun to develop, and the points *a* and *b* are seen in close proximity to one another on the outer surface of the endoderm.

m.p, medullary plate; *p.s*, protostoma.

It will be noticed that little has been said so far regarding the mode of development of the actual neurofibrillae. Their origin is indeed unverifiable by direct observation, with any certainty, owing to their minute size. They appear to spread outwards from the centre, and Held interprets this appearance by a kind of His theory on a minute scale, holding that each fibril grows out with a free end through the protoplasmic bridge. On the other hand if it be the case as suggested on p. 112 that the fibrils simply represent the specialized paths of nerve impulses there would be nothing surprising in their becoming visible first in the neighbourhood of the ganglion-cell from which the impulses start and from which also is exercised control over the metabolism of the nerve-trunk. Were this the case we should get appearances which would closely simulate growth of freely ending fibrils—centrifugal in the case of motor nerves and centripetal in the case of sensory nerves.

This view as to the meaning of the fibrils bridges over a good many of the difficulties in the way of accepting the outgrowth view, either as regards the individual fibrils or the nerve-trunk as a whole. Thus the secondary establishment of anastomoses between peripheral nerves becomes less surprising if it be the case that undifferentiated protoplasm is liable to develop nerve-fibrils as a reaction to the passage of nerve impulses through it, for wherever there are nerves there must be a certain amount of leakage of the particular form of energy which constitutes the nerve impulse.

So also with the joining up of the central and peripheral ends of a severed nerve or of the central stump of one nerve with the peripheral portion of another. In such cases we should assume that indifferent protoplasm accumulating between the cut ends gradually becomes fibrillated in response to the passage of more or less imperfect impulses through it, the newly developed portions of fibril being necessarily, from their mode of formation, continuous with both central and peripheral fibrils, leading respectively to the "high-potential" and the "low-potential" end of the nerve-fibre.

Again it is known that a mass of embryonic ganglionic tissue implanted into some abnormal portion of another individual may establish nervous connexions with the surrounding tissue. On the outgrowth hypothesis this demonstrates "error" on the part of the outgrowing fibres: on the functional view it simply involves the gradual differentiation of paths along which impulses spread outwards from the high potential ganglion-cells into the low potential surrounding tissue.

On the whole, the present writer believes that this view, that the formation of nerve-fibrils is a response to functional activity, is at the present time the most plausible working hypothesis and also the one which is most likely to lead to fruitful research. Before leaving the subject it may be well to emphasize the fact that the solution of this general problem of nerve-development is to be sought in the study of Vertebrates of large-celled coarse histological texture, combined with a low degree of specialization of general structure. No amount of observations upon small-celled highly specialized Vertebrates will ever lead to a really convincing solution with the methods now at our disposal.

Finally we would once more emphasize the fact that the kernel of the problem seems to centre round the origin of the fibrillae. Do they or do they not develop in a pre-existing bridge of protoplasm? Assuming that they do, the possibility of such bridges dating back to the period of segmentation seems to be definitely excluded. The question at what precise moment they do become established seems to be of minor importance.

While the present writer is inclined to believe that the junction is already in existence while end-organ is still in close apposition to the central nervous system there is no difficulty in principle in the way of admitting that the bridge may in certain cases be formed somewhat later, as Dohrn describes, provided always that the gap to be bridged over is small and the bridge itself protoplasmic and not fibrillar. It is probably along such lines that we may look for a reconciliation between the supporters of His (the outgrowth view) and those who believe in the protoplasmic bridge view but it will involve dropping what are essential features in the outgrowth view as enunciated by His himself—(1) that the outgrowth arises at a time when the end-organ has already retreated to a considerable distance from the nerve-centre and (2) that the outgrowth is already fibrillated during the outgrowing process and before it is united to its end-organs.

SPINAL GANGLIA AND DORSAL ROOTS.—As has already been indicated, the central nervous system of the Vertebrate consists in its most primitive condition of a specialized area of the ectoderm of the dorsal surface. It is further very characteristic of the Vertebrate that those ganglion-cells which belong especially to the sensory fibres have become concentrated into segmentally arranged clumps towards the margin of the nervous plate and have eventually come to lie outside the limits of the actual plate, or tube into which the plate becomes converted. These little detached pieces of the central nervous system are the ganglia of the dorsal roots or the **spinal ganglia**.[1]

During actual ontogeny the ganglion rudiments in some cases (*e.g.* Birds, Fig. 67, A) become distinctly apparent while the spinal cord is still in the form of an open medullary plate. They appear in the form of a continuous proliferation from the inner surface of the ectoderm in the angle between the medullary plate and the external ectoderm. In such cases the two rudiments become carried in towards one another, as the edges of the medullary plate curve inwards to form the neural tube, and undergo fusion across the mesial plane. There is thus formed a median unpaired plate or tract of cells lying just over the roof of the neural tube and between it and the external ectoderm. This is known as the **neural crest** (Marshall).

More usually the ganglionic rudiment makes its first appearance after the closure of the neural tube and in such cases the paired stage of the rudiment is slurred over, the neural crest being formed by proliferation of the roof of the neural tube. This is well seen in the case of Elasmobranchs (Fig. 67, B, C).

However it originated, the plate-like neural crest splits into two

---

[1] There is reason to believe that this is an instance of a widespread tendency in evolution for groups of ganglion-cells to undergo gradual shifting towards the direction from which their most frequent afferent impulses come. In other words there is a tendency to shorten the afferent path by shifting the cell-body. This principle of **neurobiotaxis** has been developed by Ariëns-Kappers in his various papers (*e.g.* 1913). It is particularly conspicuous in the changes which have come about in the position of the ganglionic centres of the various cranial nerves within the brain in the different groups of Vertebrates.

lateral halves and then grows outwards on each side opposite each myotome, each outgrowth representing a single spinal ganglion. Eventually these break apart but in some of the more primitive Vertebrates the intervening portions of neural crest persist for a time in the form of a distinct longitudinal commissure (Fig. 68) linking up the series of spinal ganglia to one another (Elasmobranchii —Balfour: Dipnoi).

The mode of development of the fibres forming the dorsal root, whether by outgrowth from the ganglion-cells of the spinal ganglion or by differentiation of an already existing protoplasmic bridge, comes under the general controversy as to nerve-development and need not be specially discussed.

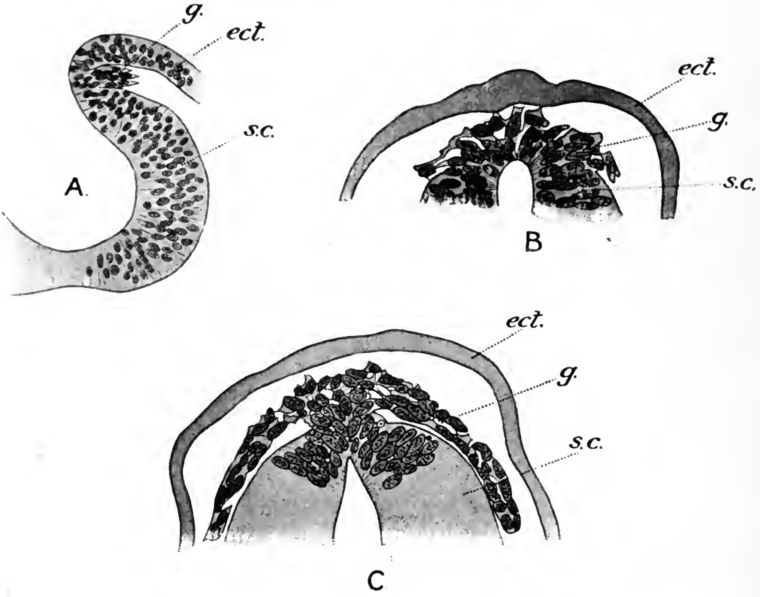


Fig. 67.—Illustrating the mode of origin of the spinal ganglia.

A, fowl embryo with four mesoderm segments (after Neumayr, 1906); B and C, *Torpedo* 4 mm. embryo (after Dohrn, 1902); *ect*, ectoderm; *g*, rudiment of ganglion; *s.c*, spinal cord.

Cranial Nerves.—The development of the cranial nerves has been investigated by many workers and an immense amount of detailed observation has been accumulated. There is however great discrepancy in detail between the results obtained by different workers, and much of the observation seems to be perilously near the limit of probable error. Consequently the material seems hardly ripe for treatment in a text-book of a general kind and nothing of the sort will be attempted here beyond noting one or two points of particular importance.[1]

---

[1] A modern account of the development of cranial nerves will be found in Neumayr (1906).

In the first place we find in the head region as in the trunk a tendency for the nerve-fibres to come off from the central nervous system in segmentally arranged clumps, and for the motor fibres to be situated more ventrally and the sensory more dorsally. In the head region however the dorsal root becomes reinforced by a large mass of motor fibres which have become shifted dorsalwards and incorporated with it.

A neural crest develops resembling that of the trunk and in the Birds it can be seen similarly to have a paired origin, arising before the complete closure of the medullary tube. This neural crest of the brain region forms an anterior prolongation of that in the trunk: it is quite continuous with the latter, it develops outgrowths similarly, and the intervening portions here also persist for a time as a longitudinal commissure. A number of the most important cranial nerves are simply prolongations of the outgrowths in question—**V, VII, VIII, IX** and **X.**

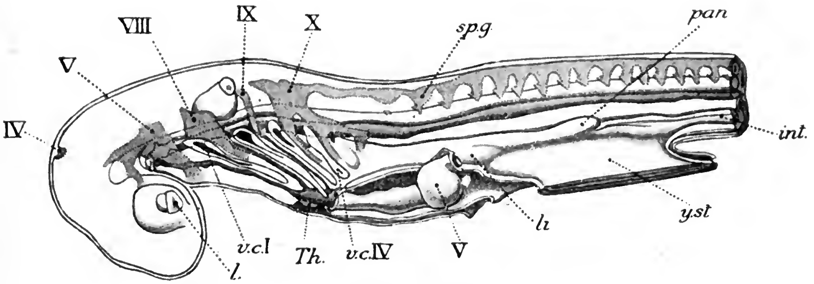


FIG. 68.—*Acanthias*, stage 23, 9 mm. long, showing ganglia of cranial and spinal nerves. (After Scammon, 1911.)

int, intestine; *l*, lens; *li*, liver; *pan*, pancreas; *sp.g*, ganglia of spinal nerves; *Th*, thyroid; *V*, ventricle; *v.c*, visceral cleft; *y.st*, yolk-stalk; IV, V, etc., ganglia of cranial nerves.

A conspicuous feature in the development of the cranial nerves is that in portions of their length they receive components directly from localized thickenings **(placodes)** of the ectoderm (Kupffer, Beard) a possible reminiscence of the time when nerve-trunks became evolved out of a plexus in direct relation to the external ectoderm.

I. The Olfactory nerve is unrivalled amongst all the sensory nerves of the Vertebrates as a subject for investigation on account of its large size, its short uncomplicated course and its retention of comparatively primitive conditions even in the adult. Research should therefore be specially concentrated upon its mode of development.

In the case of *Lepidosiren*, as already indicated, Elliot Smith has shown that the olfactory nerve is simply a drawn-out primary connexion between brain and olfactory organ, already present at a period before these organs have begun to move apart.

In other vertebrates (Elasmobranchs—Holm, Teleosts, Amphibians

—Cameron and Milligan) there is evidence that the same mode of development holds. One of the important points to be settled is whether the nuclei which are seen scattered about in the young nerve-trunk and which give it a syncytial appearance are not really immigrant sheath nuclei. The conditions in *Lepidosiren* where it is easy to distinguish the heavily yolked sheath protoplasm make it appear probable that this will be found to be the case.

II. The optic nerve is not a true peripheral nerve comparable with the other cranial nerves but simply a narrow isthmus or stalk connecting the main brain with its outlying portion which forms the retina. Its development is mentioned in the description of the eye.

III, **IV** and **VI.** The oculomotor, pathetic and abducent nerves appear to agree exactly in their main developmental features with ordinary motor nerves of the trunk (Neal, 1914).

It is not proposed to say anything here regarding the topography of the cranial nerves but some points regarding it will be touched upon later on in connexion with the segmentation of the head.

SYMPATHETIC.—The sympathetic ganglia, as was first shown by Balfour (1878) for Elasmobranchs, are derived directly from the spinal (or cranial) nerves. In its earliest recognizable stage the ganglion forms a swelling on the course of the nerve just ventral to and continuous with the spinal ganglion. With further development the ganglion bulges more and more pronouncedly towards the mesial plane at about the level of the dorsal aorta. The nerve-trunk in this region now splits longitudinally and the ganglion becomes shifted farther towards the mesial plane, lying immediately over the posterior cardinal vein and remaining connected by a slender bridge —the **ramus communicans**—with the spinal nerve from which it has become split off.

In Sauropsida the sympathetic ganglia arise in similar fashion. In Amphibia and Sauropsida, where the sympathetic ganglia are in the adult connected by a longitudinal trunk, this latter is said to arise secondarily, the ganglia being at first quite separate. In view, however, of the difficulty of detecting such nerve-trunks in early stages of development it will be well not to dismiss altogether the possibility that the ganglia are after all in continuity from the beginning.

From the basis of the sympathetic system so laid down extensions apparently sprout out into the various tissues which are eventually innervated by this system—but again there has to be admitted great possibility of error. The problem of the mode of development of these obscure portions of the nervous system will probably only be satisfactorily settled after we know with certainty the processes at work in the development of the main nerve-trunks and ganglia.

THE ORGANS OF SPECIAL SENSE.—We may take it that in the early stages of the evolution of the nervous system, while this system was still a diffuse network, there were already present scattered sensory cells—cells specialized for the reception of impressions from without. Local concentrations of such sensory cells and their further specialization for the better perception of some particular type of stimulus has led to the evolution of the various organs of special sense.

The special sense organs of the vertebrate fall into two categories —(I.) the organ of vision, perhaps the oldest organ of special sense, which is developed within the limits of the central nervous system and (II.) the other organs of special sense which have probably arisen more recently from the sense cells of the skin outside the limits of the central nervous system.

As the organs belonging to the second category have evolved less far from the primitive condition they will be considered first. They appear to have become specialized functionally in two different directions, those in the neighbourhood of the mouth for the appreciation of differences in chemical composition—the organs of taste and smell—and those on other parts of the body surface for the appreciation of vibrations of the surrounding medium—the lateral line organs and the organ of hearing.

OLFACTORY ORGAN.—The olfactory organ arises in the form of a localized thickening of the ectoderm on each side towards the anterior end of the head. Later this thickened ectoderm becomes depressed below the general surface so that it assumes a saucer- and later a cup-shape; its external opening eventually becomes comparatively narrow.

In many of the Elasmobranch fishes the olfactory organ retains throughout life the condition of a simple inpushing of the skin opening to the exterior on the ventral side of the snout. In many Vertebrates on the other hand characteristic changes come about in the relations of the external opening. These will best be understood by considering first what happens in the lung-fish *Protopterus* as shown by Fig. 69. In A and B the olfactory organ is visible as a little rounded dimple on each side. In C the dimple has become a deep groove running obliquely from before backwards and outwards. It is further seen that this groove is becoming involved in the sinking in of the skin to form the buccal cavity. In D and E the groove has become a deep slit narrow in its middle part and dilated at its two ends. Finally, in the stage represented in F, the margins of the narrow part of the slit have undergone complete fusion so that the continuous slit of the preceding stage is now represented merely by its terminal portions which form two widely separated rounded openings—the anterior and posterior **nares** (*olf*^{1} and *olf*^{2}).

Turning to the other Vertebrates we find various divergences from this simplest mode of origin of the external and internal nares as seen in *Protopterus* and *Ceratodus*. In the Actinopterygian fishes the phenomena are quite similar to those described, only here differential growth leads to the gradual shifting of the olfactory organ and its openings from the ventral side of the snout up to its dorsal side. The result is a topographical reversal of the positions of anterior and posterior naris: the morphologically posterior naris coming to lie in front of that which was originally anterior.

In the Amphibian and Amniote the upper lip, which completes the buccal cavity in front, is situated *between* the anterior and posterior narial openings, so that the latter opens into the buccal cavity

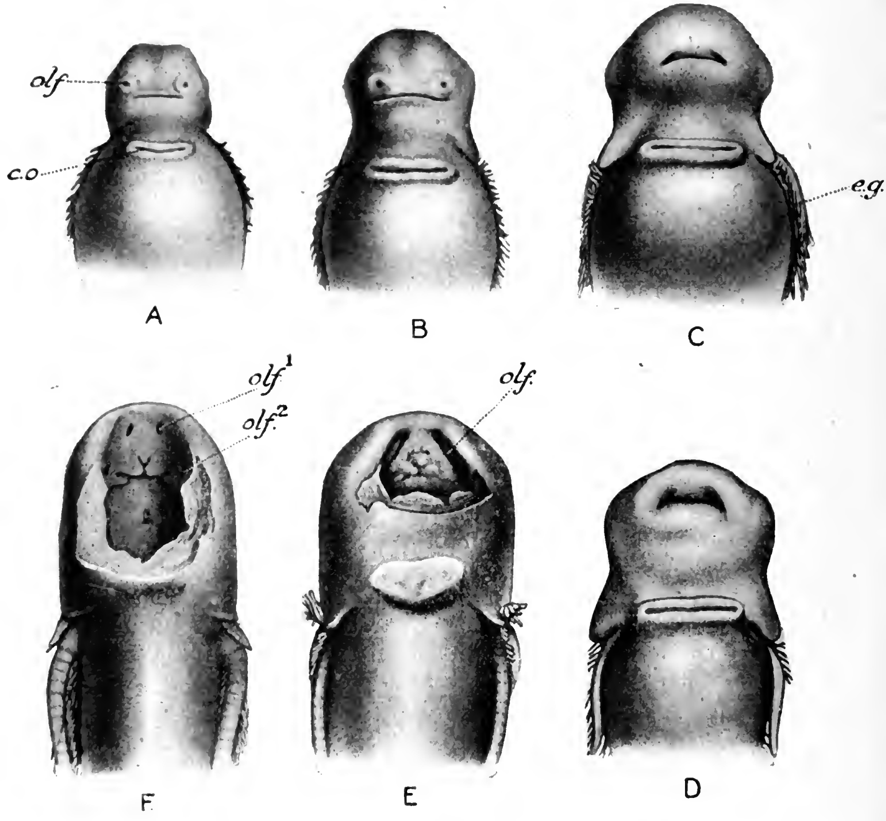


Fig. 69.—Ventral views of head region of larva of *Protopterus* at stages 31 (A), 32 (B), 34 (C), 34 (D), 35 (E), and 36 – (F), to illustrate the development of the olfactory organ.

c.o, cement organ; *e.g*, external gill; *olf*, olfactory organ; *olf*^{1}, anterior ("external") naris; *olf*^{2}, posterior ("internal") naris. In C the curved line running across the ventral side of the head is the posterior margin of the mouth: the darkly shaded grooves passing inwards and forwards from its outer ends are the olfactory rudiments.

(**internal naris**), while the former remains outside (**external naris**). In the developing Amniote embryo (cf. Fowl, Chap. X.) the general arrangements, while essentially the same as those of *Protopterus*, are somewhat obscured by the modelling of the face region. The ridge which forms the upper lip, or anterior boundary of the buccal cavity, is cut across by the olfactory slit, here a wide and deep cleft, into a median portion (**median nasal process**) and a lateral portion (**maxillary process**).

The ridge bounding the olfactory involution on its outer side remains for a time separated by a distinct groove from the maxillary process but as the latter grows forwards it obliterates this groove as well as the superficial portion of the cleft which separates it from the median nasal process, the deep portion of the cleft remaining as a definitive canal leading from olfactory organ to buccal cavity.

In Amphibians, as in *Lepidosiren* among the lung-fishes, the posterior naris is frequently formed as a secondary perforation which breaks through from the posterior portion of the olfactory organ into the part of the buccal cavity lined by "endoderm." This is a secondary modification of a type which will be discussed in the next chapter in the description of the development of the buccal cavity in these forms.

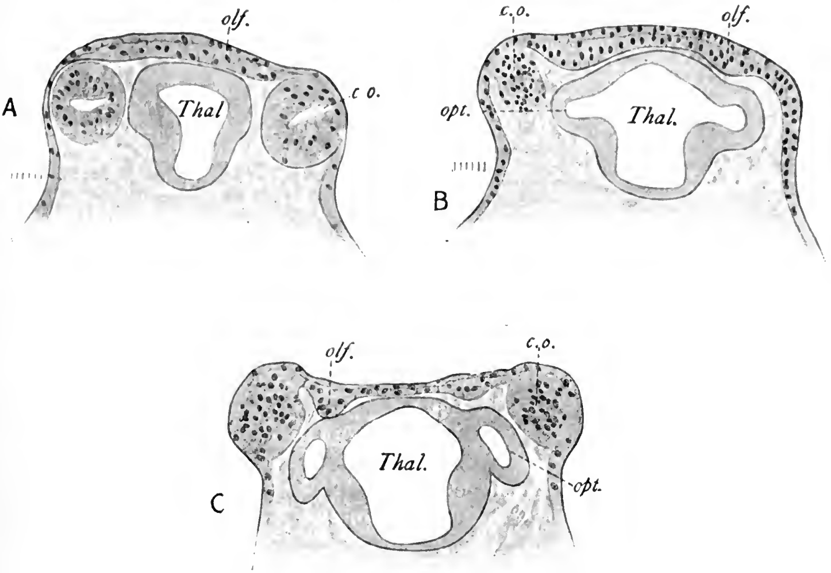


FIG. 70.—Horizontal sections through the olfactory organs of *Polypterus* of stages 25 (A), 26 (B), and 27 (C).

c.o, cement organ; *olf*, olfactory rudiment; *opt*, optic stalk; *Thal*, cavity of thalamencephalon.

The first rudiment of the olfactory organ has been described as a thickening of the ectoderm. As in the case of other nervous or glandular developments of the ectoderm the superficial layer (Fig. 70) takes no part in its formation. Commonly it degenerates and disappears over the actual olfactory epithelium. Again, as frequently happens in the development of primitively hollow organs, the rudiment may be for a time solid, forming a simple solid downgrowth in which a cavity makes its appearance secondarily, the actual involution of the surface being delayed or reduced or absent (Fig. 71).

Sometimes, as is well shown in the case of *Polypterus* (Fig. 70, A), the olfactory thickenings are at first continuous across the mesial plane and this fact, taken in consideration with the fact that in the Lampreys the olfactory organ of the adult is unpaired, obviously suggests the possibility that the olfactory organ of Vertebrates in general was originally unpaired (Kupffer). Though this must be admitted as a possibility the evidence does not appear to be sufficient to give the idea probability in view of the fundamentally paired character of the portions of the brain associated with the olfactory organ, even in the case of the cyclostomes where the organ as a whole has an unpaired appearance.

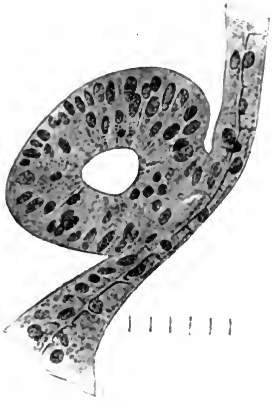


FIG. 71.—Longitudinal vertical section through *Polypterus* (stages 28-29), showing the olfactory rudiment as a thickening of the deep layer of the ectoderm in which a cavity has developed secondarily.

After the olfactory involution has become definitely established it undergoes various complications of form, differing in detail in the various groups but consisting for the most part of bulgings outwards on the part of the lining epithelium so as to bring about an increase in its area. In the Elasmobranch these outgrowths take the form of parallel grooves which gradually become converted into deep slits separated by thin partitions — the Schneiderian folds. In Crossopterygians instead of numerous folds with free edges complete septa are formed which radiate out from an axis formed by the olfactory nerve and divide the cavity as seen in transverse section into distinct chambers, the lining of which in turn forms deep folds. In the higher forms the outgrowths of the olfactory lining are fewer in number and the projections left between them form the **turbinals** which have characteristic arrangements in the different groups.

Amongst the Reptiles a conspicuous development of the olfactory apparatus is the **Organ of Jacobson**. This arises as a pocket-like outgrowth of the lining epithelium, on its mesial side and near its ventral edge, which becomes gradually constricted off from the olfactory organ and opens into the buccal cavity in the region of the posterior nares. In Chelonians, Crocodiles and Birds this organ has disappeared except for a possible vestige in the form of a transient bulging of the olfactory lining.

A diverticulum which may correspond to Jacobson's organ makes its appearance in Lung-fishes and Urodeles but in this case it becomes gradually displaced outwards until it lies external to the olfactory cavity.

A curious, possibly adaptive, arrangement has been noticed in late developmental stages of certain Sauropsida, where for a time the external nares are plugged by a proliferation of ectoderm (*Apteryx*—T. J. Parker, *Sphenodon*—Dendy). Such temporary obliteration of a channel at a period of development where it is unnecessary or harmful is a phenomenon which occurs fairly frequently: examples of it will be met with later in connexion with the alimentary canal and the excretory organs.

OTOCYST.—The Vertebrate possesses a pair of otocysts situated one on each side of the hind-brain. Each arises in the typical fashion familiar in the invertebrates, by a sensory portion of ectoderm becoming depressed below the surface of the skin and eventually isolated as a closed vesicle. As in the invertebrates certain of the lining cells of the otocyst secrete otolithic masses of Calcium carbonate.

The otocyst of the Vertebrate however shows two developments which do not occur amongst the invertebrates. Firstly, in connexion with the primitive function of the organ, that of balancing, the wall of the growing otocyst becomes moulded into the three **semicircular canals** which are arranged in planes at right angles to one another. These canals have for their function the analysis of any rotatory movement into its components in these three planes. And secondly a special region of the otocyst wall becomes specialized in connexion with a new sense—that of hearing—and grows out into a curved horn-like pocket, the **lagena,** which may become greatly enlarged and spirally coiled, in Vertebrates in which the sense of hearing is very acute, forming the organ known as the **cochlea.**

The development of the otocyst may be described as it occurs in the Fowl (Röthig and Brugsch, 1902). The otocyst begins to make its appearance during the second day of incubation as a thickened area of ectoderm on each side of the hind-brain. This thickened area becomes depressed below the general surface, forming a saucer-shaped depression which gradually deepens till it forms a deep pit. The lips of this pit, especially dorsally, grow inwards so as to constrict the opening which is finally completely obliterated, the original open depression being thus converted into a closed, somewhat pear-shaped, sac the **otocyst.** The wall of the otocyst remains for a time continuous through a solid bridge with the outer ectoderm (Fig. 72, A) but as a rule this bridge persists merely for a very short time and only a small cellular tag attached to the otocyst remains to mark its position.

As development goes on the otocyst increases in size by growth of its wall and this growth is especially marked ventrally and laterally with the result that the point which was originally connected to the ectoderm becomes displaced so as to be situated on the mesial side of the otocyst. This portion of the otocyst wall now comes to project upwards as a distinct pocket-like outgrowth the **recess** (Fig. 72, B, *r*). External to this a wider bulging of the wall foreshadows the anterior vertical semicircular canal (*a.v*) and a little later a similar bulging more ventral in position—the horizontal canal (*h.c*). The lagena also is foreshadowed by a slight downward bulging of the floor of the otocyst.

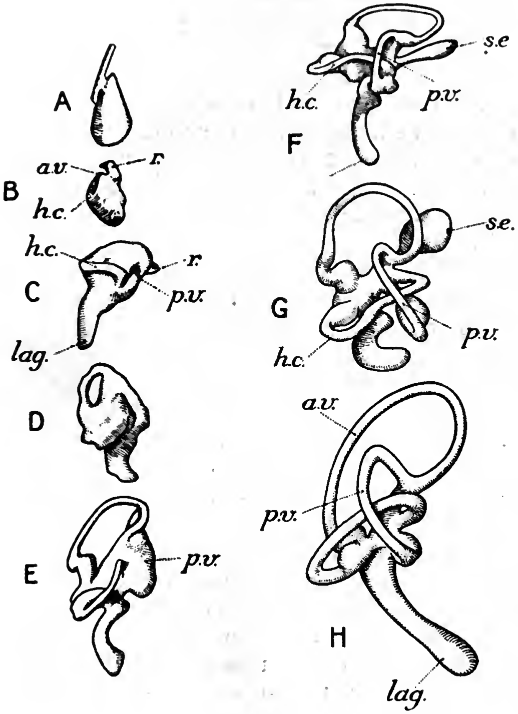


FIG. 72.—Illustrating the development of the otocyst in the Fowl. (A-G after Röthig and Brugsch, 1902 ; H after Retzius.)

A and B, early stages ; C, towards end of seventh day ; D, towards end of eighth day ; E, five days ; F, towards end of ninth day ; G, towards end of twelfth day ; H, adult. A is a view from in front, B from behind, while C-H represent the left otocyst as seen from the left side. *a.v*, anterior vertical canal ; *h.c*, horizontal canal ; *lag*, lagena ; *p.v*, posterior vertical canal ; *r*, recess ; *s.e*, endolymphatic duct.

With further development the posterior vertical canal rudiment appears also as a bulging of the otocyst wall continuous with that which will form the anterior canal (Fig. 72, C-F, *p.v*). The three canal rudiments come to project more and more prominently, the recess assumes the tubular shape of the **endolymphatic duct** and the lower portion of the otocyst (**saccule**) with its projecting lagenar pocket and endolymphatic duct becomes more sharply marked off from the rest of the otocyst (**utricle**). The pouch-like rudiments of the semicircular canals, as they come to project more freely from the utricle, assume a flattened form and finally the central portion of the wall on each side bulges inwards and fuses with that on the other.

In this way the central portion of the cavity of each pouch becomes obliterated while the persisting peripheral part takes the form of a curved tube—the definitive canal. At first the space subtended by the canal is traversed by a continuous septum formed out of the fused walls but this soon disappears leaving the canal as a freely projecting arch which opens into the utricle at each end. The **ampulla** appears at an early stage as a dilatation of the canal rudiment at one end.

As will already have been gathered, the three canal rudiments do not appear synchronously—the anterior vertical appearing first, then the horizontal and finally the posterior vertical. The same order is followed in subsequent development the anterior vertical canal keeping ahead of the other two—probably a foreshadowing of the greater importance of this canal, parallel to the sagittal plane, in the function of flight.

It should be noticed that the posterior vertical canal assumes its position at right angles to the planes of the other two secondarily. At first its rudiment is continuous with and almost in the same plane as the anterior canal but as it assumes the tubular form it swings outwards and forwards slipping, as it does so, over the horizontal canal in the way indicated in Fig. 72, F, G, H.

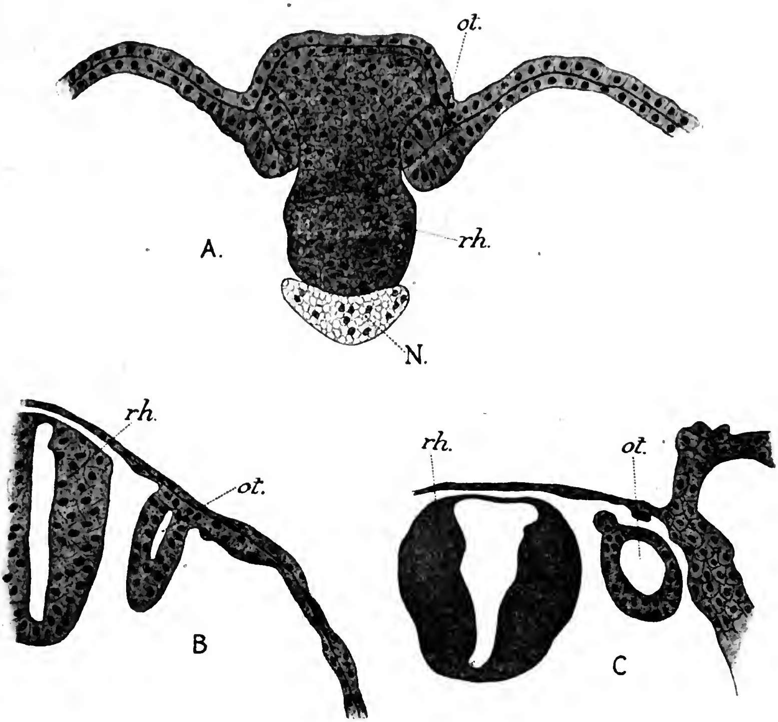


Fig. 73.—Development of the otocyst in Lung-fishes as seen in transverse sections. (From drawings by M. C. Cairney.)

A, *Lepidosiren*, stage 21; B, *Protopterus*, stage 23; C, *Lepidosiren*, stage 28. *N*, notochord; *ot*, otocyst; *rh*, rhombencephalon. In Fig. C the rudiment of the endolymphatic duct is visible as an outgrowth from the otocyst wall dorsally and mesially.

The otocyst of Vertebrates in general develops along similar lines to those described for the Fowl while presenting modifications in detail. The sensory epithelium is a development of the deep layer of the ectoderm and in cases where the ectoderm is distinctly two layered during the early stages of otocyst development the superficial layer is seen to pass over the rudiment unaltered (Fig. 73, A). In Lung-fishes the cavity of the otocyst appears secondarily in the midst of an apparently solid downgrowth of the deep layer of the ectoderm (Fig. 73, B) but the examination of earlier stages (Fig. 73, A) shows that here also there is an actual involution of the deep layer although there is at first no patent cavity.

In the Elasmobranchs the otocyst retains throughout life its connexion with the exterior, the connexion becoming drawn out into a slender tube. In Birds the recess, and therefore the endolymphatic duct, represents the remains of this original connexion, but curiously enough in certain other Vertebrates *e.g.* Lung-fishes (Fig. 73, C) the last connexion of otocyst with external ectoderm lies lateral of and somewhat anterior to the endolymphatic duct which latter here develops as an independent outgrowth of the otocyst wall. This is to be looked on as a secondary modification of the more primitive arrangement seen in Elasmobranchs. The structure named endolymphatic duct in Teleosts also arises as a secondary outgrowth of the otocyst wall.

The endolymphatic duct or recess commonly persists in the adult as a conspicuous blindly ending diverticulum of the otocyst wall. In Lung-fishes and Amphibians its wall proliferates actively giving rise to projections which in the Lung-fishes and some Urodeles, *e.g.* the Axolotl, meet to form an irregular sac over the roof of the fourth ventricle. In the Anura the irregular thin-walled sac formed in this way spreads forwards and also laterally until it becomes continuous ventrally so as completely to surround the hind-brain. An unpaired prolongation of this sac extends tailwards immediately dorsal to the spinal cord within the vertebral canal. Paired outgrowths of this extend outwards along with each spinal nerve and expand at their ends round the spinal ganglia to form the **calcareous bodies** so conspicuous in the adult frog. The whole system of outgrowths is conspicuous in the adult from the white otolithic particles in its interior. The vertebral portions become eventually broken up into a network of irregular tubes which is interpenetrated by a network of capillaries (Coggi, 1889).

Somewhat similar outgrowths of the endolymphatic duct make their appearance in Sauropsida although in this case they do not undergo the wide extension that they do in the Anura. In the Geckos however they do become extended so as to form a large superficially placed irregularly lobed sac which covers over a great part of the neck region close under the skin (Wiedersheim).

LATERAL LINE ORGANS.—These sense-buds (**neuromasts**), which are found arranged in rows on the head and body of fishes and aquatic amphibians, take their origin in linear thickenings of the deep layer of the ectoderm which spread along the surface of the head and body and eventually become segmented up into separate pieces. In correlation with the function of these organs, which apparently is to detect slow vibrations in the water and is therefore closely allied to hearing, it is of interest to notice that the ectodermal rudiment from which they arise appears to be in some cases continuous at first with that which gives rise to the otocyst.

The sense organs are in correlation with their origin at first placed superficially but as development goes on they in most cases become depressed beneath the surface either in isolated pits or in continuous grooves. The latter may in turn remain open or may become covered in to form tubes except where at intervals openings remain leading to the exterior. This is the condition which is reached in the adults of the majority of fishes.

The lateral line sense organs being correlated with an aquatic habit commonly degenerate on the assumption of a terrestrial existence. Various Anura however which remain purely aquatic after metamorphosis retain their full equipment of lateral line organs.

ORGAN OF PINKUS.—In *Lepidosiren* and *Protopterus* a peculiar organ of special sense lies deeply embedded in the tissue on each side of the head in close contact with the wall of the auditory capsule. This organ, discovered in *Protopterus* by Pinkus, has been shown by Agar (1906) to be developed from the ectodermal ingrowth which forms the outer end of the spiracular rudiment.

EYE.—As the eye develops in the same general manner, differing only in detail, in the different subdivisions of the Vertebrata it will be convenient to describe first its development in the Fowl—the Vertebrate of which it is easiest to obtain material for practical study.

The first obvious rudiment of the eye consists of a projection of the side wall of the thalamencephalon which juts out at right angles to the axis of the body and gives a characteristic hammer-shape to the fore-brain region (see Fig. 231, Chap. X.). A transverse section across the head near its front end in a chick about the middle of the second day of incubation shows (Fig. 234, D) the thalamencephalon extending out on each side as the **optic outgrowth.**

As development goes on and mesenchyme accumulates between the brain-wall and the ectoderm the proximal part of each optic outgrowth becomes constricted, from above downwards, to form a relatively narrow **optic stalk** (Fig. 74, A, B, D, *o.s*). The optic outgrowth is closely apposed against the inner surface of the external ectoderm and a slight thickening of the latter soon becomes apparent just where it is in contact with the surface of the optic outgrowth in (Fig. 74, B, *l*). This thickening is the first rudiment of the **lens.** The lens-rudiment gradually becomes sunk below the general surface to form a saucer- and later a cup-shaped depression. As the rudiment becomes involuted in this way, the outer wall of the optic outgrowth also becomes invaginated to form a cup-like structure—the **optic cup** (Fig. 74, B and C). The cup-like lens-rudiment becomes gradually con-

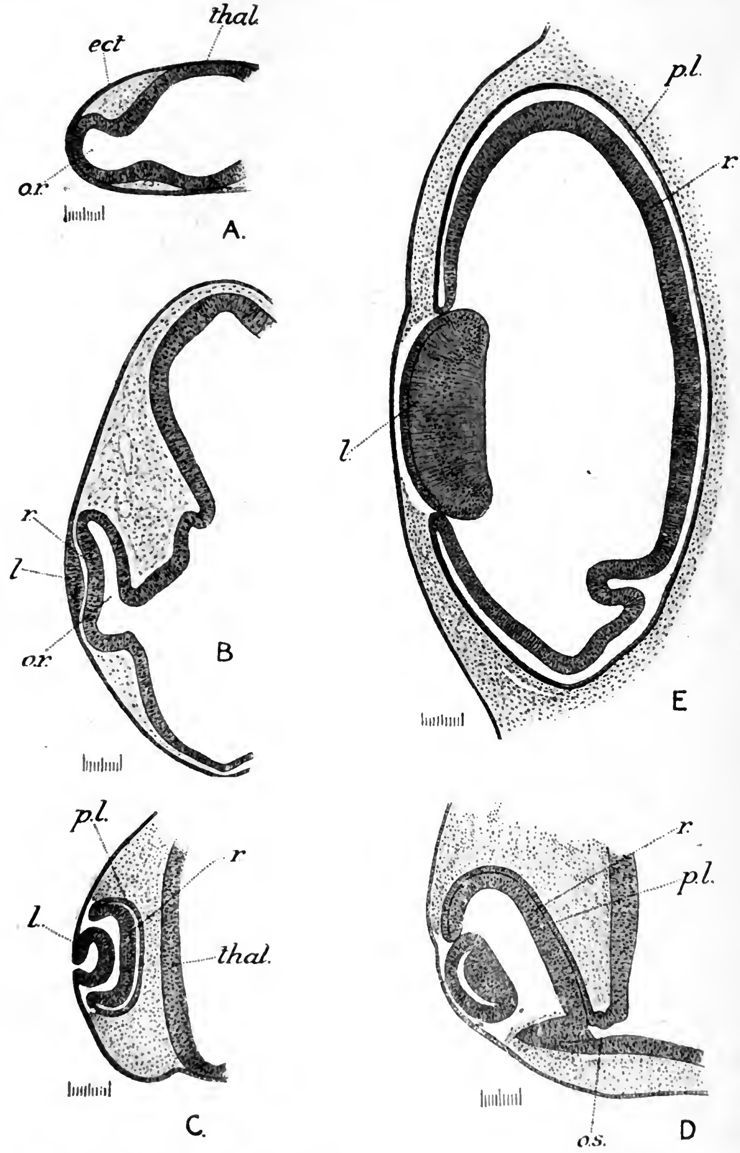


Fig. 74.—Development of the eye as seen in transverse sections of Fowl embryos.

A, latter half of second day of incubation; B, end of second day; C, 2½ days; D, 3 days; E, latter half of fifth day. *ect*, external ectoderm; *l*, lens; *o.r*, rudiment of eye; *o.s*, optic stalk; *p.l*, pigment layer of retina; *r*, retina; *thal*, wall of thalamencephalon.

stricted off and finally completely separated from the outer ectoderm (Fig. 74, C and D).

In the meantime a marked difference becomes apparent between the two layers forming the wall of the optic cup. The layer next the cavity of the cup becomes greatly thickened its cells becoming tall and columnar: it forms the rudiment of the visual layer or **retina** in the strict sense. The outer layer of the cup-wall on the other hand degenerates, it becomes thinner and later it deposits melanin pigment in its cells. It forms the **pigment-layer** of the retina.

The invagination of one wall of the optic outgrowth within the other is not confined to that portion of the outgrowth in proximity to the external ectoderm as might be supposed from the description so far. The invagination involves also the ventral wall of the rudiment towards its outer end and for some distance along the optic stalk. The result is that the wall of the optic cup is interrupted by a gap ventrally the **choroid fissure**[1]—and that the optic stalk for some distance from the optic cup has a deep groove along its ventral side.

The cavity of the optic cup, as is the case with cavities generally in the embryonic body, becomes filled with clear fluid secreted into it by the surrounding cells. This fluid becomes jelly-like later on and forms the basis of the **vitreous body**.

As development goes on the eye increases greatly in size and assumes a spherical shape, the lens blocking up its opening towards the skin and the choroid fissure becoming obliterated by its lips coming together. The site of the fissure remains apparent for some time owing to the formation of pigment in the pigment-layer being delayed in its immediate neighbourhood.

As the eye increases in size the Retina for a time grows more actively than the rest so as to be thrown into wrinkles (Fig. 74, E). The lens which was a hollow vesicle becomes solid its cavity being filled up by a great thickening of its deep wall, the cells of which grow out into a tall columnar form (Fig. 74, D and E).

The essential parts of the eye as an optical instrument have now been laid down—the lens for the production of an image, the retinal wall of the optic cup for the reception of that image and the conversion of its light waves into nerve impulses, and the optic stalk which will become the optic nerve for the transmission of these impulses to the brain. To these essential parts there are added various accessory structures developed from mesenchymatous cells which accumulate round the parts of the eye already formed. In particular there is formed a protective capsule of tough connective tissue—the **sclerotic** with its transparent portion the **cornea**, covered externally by the ectoderm forming the corneal

---

[1] The term "choroid" fissure is in reality misleading, having been adopted when the fissure was interpreted as a cleft in the choroid, in the days before the existence of the pigment-layer of the retina was recognized.

epithelium—and a highly vascular and deeply pigmented **choroid** between the sclerotic and the pigment-layer of the retina.

As development proceeds watery fluid collects in the space between the lens and the cornea. Round its periphery this space is bounded internally by the inturned edge of the optic cup which overlaps the lens. For a considerable distance out from the lens, as far as the **ora serrata**, this portion of the wall of the optic cup does not undergo differentiation into functional retinal tissue. Its marginal portion covered on its outer surface by a prolongation of choroid becomes the **iris**. Here the inner "retinal" layer develops pigment and becomes in other respects like the outer layer. The outer layer itself gives rise to the radial and circular muscle fibres of the iris—a fact of morphological interest to be grouped along with the development of muscle fibres by the ectoderm of flask-glands referred to on a previous page. Outside the periphery of the iris the retinal layer remains comparatively undifferentiated as an unpigmented layer of columnar epithelium. It lies internal to a special development of choroid which forms the ciliary body and in which are developed the muscles of accommodation.

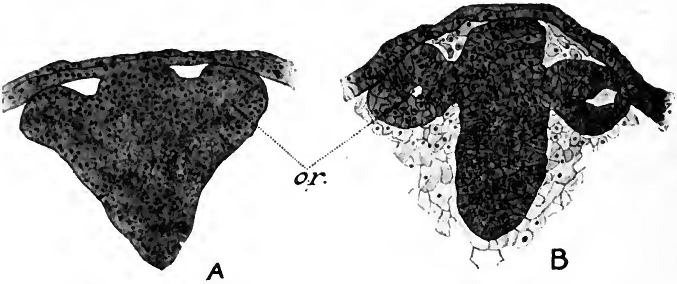


FIG. 75.—Transverse sections through the brain of *Lepidosiren*, showing the solid optic rudiments (*o.r*).

A, stage 21; B, stage 23.

The mode of origin of the eye as a whole and of its component parts as seen in the Fowl embryo having been sketched in outline, it will now be convenient to take the various parts in turn and note further features of interest without restricting consideration merely to the Bird.

In the first place it is to be noted that the rudiment of the eye is at first solid in those Vertebrates in which the central nervous system is solid at the time of its appearance, the cavity of the optic rudiment developing secondarily (Fig. 75, A, B, *o.r*). As this secondary cavity increases in size both in the optic rudiment and in the brain a condition is gradually reached like that already described and the further development is on normal lines.

Retina.—The fully-developed retina—which it will be remembered is morphologically a specialized portion of the brain-wall —is an organ of extreme complexity. Its structure even in the adult is by no means completely worked out, and our knowledge of the details of its histogenesis is most imperfect. The most conspicuous feature is the great increase in thickness, the retinal cells becoming slender and columnar in form. Later on the nuclei are seen to become arranged in layers, this being an expression of the fact that the cells are also becoming specialized into layers of different structure and function. The details of development of these are almost completely unknown and there is here an interesting field for investigation.

The layer of visual or percipient cells lies on the proximal side of the retina, and their rods and cones—the special parts of the visual cells which are believed to have the function of converting light waves into nerve impulses—are at the ends of the cells which point *away from* the lens. To reach these rods and cones the light rays have therefore to traverse the whole thickness of the retina. This remarkable arrangement of the retina, precisely the opposite of what we should expect, is one of the characteristic features of Vertebrates. Its morphological significance is however at once made clear by a consideration of the main facts of development of the eye as already outlined. These, in fact, show that what becomes the proximal surface of the retina, *i.e.* the surface which faces away from the lens, was originally part of the *inner* surface of the brain rudiment and therefore of the *outer* surface of the ectoderm before it became involuted to form the brain.

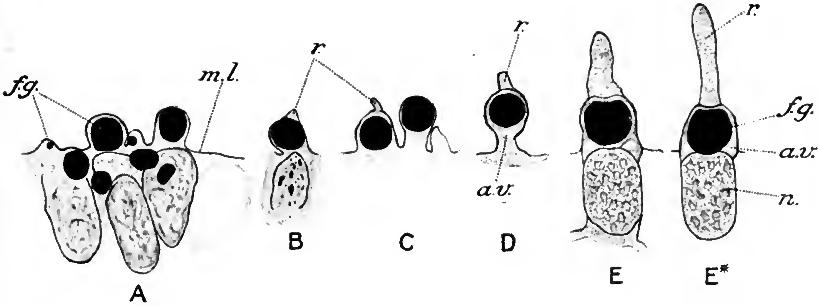


Fig. 76.—Illustrating the development of the rods in *Lepidosiren*. The upper side of the figures represent the side turned away from the lens.

A, B, C, D from stage 35; E, fully developed visual cell at stage 38, fixed during exposure to light; E*, similar element killed in the dark. *a.v*, annular vacuole; *f.g*, fatty globules stained black by osmic acid; *m.l*, external limiting membrane; *n*, nucleus of visual cell; *r*, rod.

The visual cells develop therefore on what was originally a part of the outer surface of the body and their rods point in a direction which was originally outwards. The mode in which the rods develop is illustrated by Fig. 76 which is taken from *Lepidosiren* (Graham

Kerr, 1902). Similar observations have been subsequently made in the case of Amphibians.

The first obvious step in the specialization of the visual cell is the appearance of a fatty globule in its protoplasm. The end of the cell turned away from the lens now grows out into a projection and pushes back the fine cuticular limiting membrane (**external limiting membrane**) which has developed over this surface of the retina, into a little pocket. The oil globule which gradually increases in size passes into this pocket (Fig. 76, A, *f.g*) and lies in it ensheathed in protoplasm. The protoplasm now becomes heaped up into a little conical protuberance (Fig. 76, B, *r*) which is the rudiment of the rod. At first the limiting membrane is distinct over the surface of the rod but gradually, as the latter assumes a cylindrical shape, its protoplasm takes on a clear structureless appearance throughout: it apparently becomes in fact converted into cuticular material. This cylinder of cuticular material increases in size, assumes a characteristic appearance with alternating discs of dimmer and more transparent material as seen in the fixed specimen and the rod is complete (Fig. 76, E and E*).

The rods complete their development sooner or later according as they are nearer or farther away from the optical axis of the eye and their time of development shows great variation in different individuals. The cones in those Vertebrates in which cones are present are merely specialized rods.

LENS.—The lens shows in its early stages, in various groups of Vertebrates, departures from the normal condition as described for the Bird, of exactly similar kinds to those seen in the development of the otocyst. In particular, the lens tends to develop out of a solid downgrowth of the deep layer of the ectoderm. This is well seen in Elasmobranchs (Fig. 77, A-E) where a rounded solid lens-rudiment is formed by proliferation of the ectoderm, this rudiment becoming isolated and developing a cavity secondarily. It is of interest to notice that even here a slight dipping down of the external surface into the lens rudiment is apparent for a time (Fig. 77, B).

In Amphibians, Lung-fishes and Teleostomatous fishes the lens arises in a manner intermediate between what occurs in Elasmobranchs and what occurs in Sauropsida. In the forms mentioned the lens arises as a downgrowth of the deep layer of the ectoderm (Fig. 77, F-I) and in some cases this downgrowth is simply an invagination of this layer, the only difference from the Sauropsidan condition being that here the opening of the invagination is closed by the superficial layer being continued across it (Fig. 77, J, K).

As regards the later stages in the development of the lens all that need be said is that it undergoes an enormous increase in size—by absorbing nourishment from its surroundings, for it has no blood-vessels—the cells of the deep wall becoming greatly elongated and taking on a clear glassy appearance, while the superficial wall remains as a layer of cubical epithelial cells over the outer surface of the lens.

Sclerotic, Cornea, Choroid.—These portions of the eyeball are gradually differentiated out of mesenchyme which becomes concentrated round the primary parts of the eye. In the case of the cornea the first stage in the developmental process consists in the accumulation between lens and ectoderm of a clear jelly-like secretion continuous, and identical in character, with that which fills the optic cup.

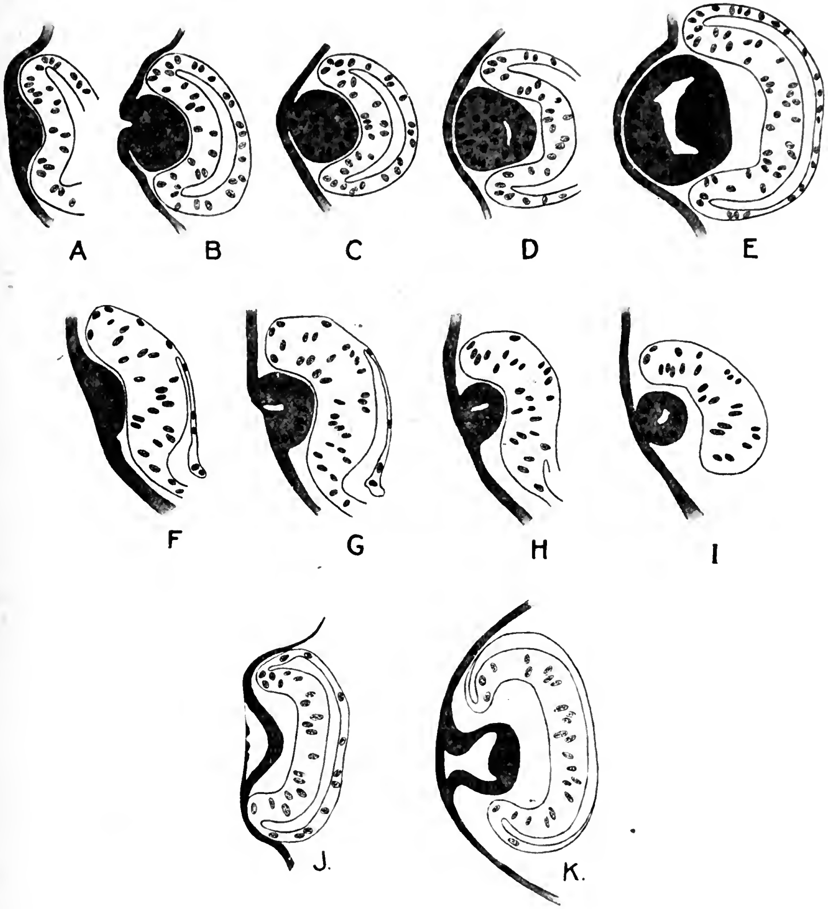


Fig. 77.—Variations in the early stages of the development of the Lens.
A-E, *Pristiurus*; F-I, *Siredon* (after Rabl, 1898); J, K *Phyllomedusa* (after Budgett, 1899).

As development goes on (Knape, 1909), a thin layer of this jelly-like material, about midway between the lens and the ectoderm, becomes condensed to form the rudiment of **Descemet's membrane.** Amoeboid cells from the mesenchyme round the optic cup creep along the deep surface of Descemet's membrane and there settle down to form a single layer of flattened cells. On the deep side of this **corneal endothelium** a split gradually develops in the jelly-like matrix: this contains a watery secretion (**aqueous humor**) and becomes the **anterior chamber** of the eye. The portion of matrix lying superficial to Descemet's membrane becomes colonized by cells from all round its margin. It forms the main portion of the cornea, while a thin layer lying next the ectoderm remains uncolonized and gives rise to **Bowman's membrane.**

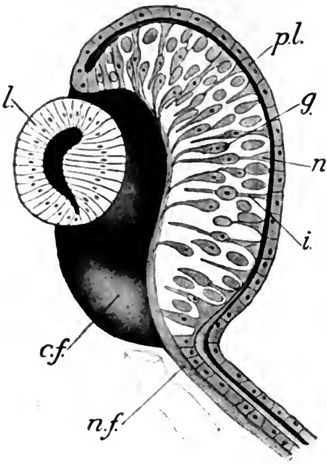


Fig. 78.—Semidiagrammatic figure of the bisected eye of a Vertebrate embryo (*Rana* 8 mm.) to show the course of the optic nerve-fibres (after Assheton, 1892).

c.f, Wall of choroid fissure; *g*, ganglion-cell; *i*, indifferent, supporting, cell; *l*, lens; *n.f*, nerve-fibres: *p.l*, pigment layer of retina; *n*, percipient cell.

Vitreous Body.—The cavity of the optic cup is from the beginning filled with clear fluid which keeps it distended and there is no apparent reason to assume that this arises otherwise than by the same method as holds with the eyes of many invertebrates *i.e.* as a secretion of the surrounding retinal cells. The fluid gradually acquires the jelly-like consistency characteristic of the fully-formed vitreous body.

Amoebocytes wander at a comparatively early period into the vitreous rudiment — in the Fowl embryo about the third day—and at a later period a continuous mass of mesenchyme tissue projects into it through the choroid fissure. This mass of mesenchyme develops a network of blood-vessels continuous with those of the surrounding tissue. In the more primitive Vertebrates this mesenchymatous mass reaches no great development but in the Teleostei and the Sauropsida, the most highly specialized groups amongst the non-mammalian Vertebrates, it does so and persists throughout life, as the **falciform process** with its muscle-fibres for the purpose of accommodation in the one case (Teleostei), and the highly vascular, and probably mainly nutritive, **pecten** in the other (Sauropsida).

Optic Nerve.—As already indicated the optic nerve is not strictly speaking a peripheral nerve at all. It is a slender drawn-out portion of the brain analogous with the olfactory tract of a teleostean fish, connecting the main portion of the brain with the small highly specialized portion which has become converted into the optic cup. Its function being a conducting one the main mass of this stalk-like portion of the brain is composed of white substance or nerve-fibres.

These fibres instead of passing outwards over the rim of the cup all round, as they possibly did originally, have become crowded together during the course of evolution into a single large bundle on the ventral side of the cup. In accordance with the general principle of economy of tissue this bundle of nerve-fibres has become sunk into a deep notch in the wall of the cup—the choroid fissure—so that it passes directly to the optic stalk.

While this is probably a correct statement as regards phylogenetic evolution, matters are somewhat simplified in the development of the individual, inasmuch as the choroid fissure is brought about not by the notching of the already formed cup rim but by the rim ceasing to develop at the site of the choroid fissure while it grows actively everywhere else.

As regards the development of the actual nerve-fibres, all that need be said is that they first make their appearance in the wall of the optic stalk ventrally and that they increase rapidly in number, passing between the epithelial cells of the stalk, loosening them out, and causing them in great part, if not entirely, to degenerate. The individual fibres certainly for the most part become differentiated in a centripetal direction *i.e.* from the retina towards the brain, but whether this means that they are actually sprouting out from ganglion-cells of the retina as is generally believed, or on the other hand that their fibrils are simply becoming differentiated centripetally within a continuous pre-existing protoplasmic connexion, remains to be demonstrated.

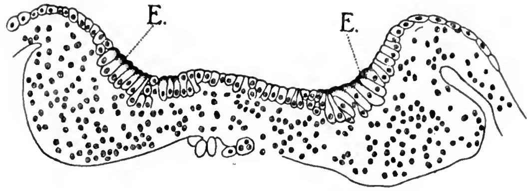


FIG. 79.—Transverse section through the still open neural plate of *Rana palustris* near its anterior end, showing the position of the optic rudiments (*E*) already marked out by the formation of pigment (after Eycleshymer, 1895).

EMBRYOLOGY AND THE EVOLUTIONARY ORIGIN OF THE EYE.—The peculiar reversed position of the Vertebrate retina may perhaps be taken as an indication that that organ had already come into existence, though no doubt in a very simple form, at a stage in Vertebrate phylogeny when the central nervous system had not yet sunk down below the surface. It is therefore of interest that in certain Vertebrates the rudiment of the retina does actually become apparent during embryonic development at a period when the medullary plate is not yet closed in. Thus Eycleshymer (1895) has described in *Rana palustris* and in *Amblystoma* how a patch of pigment appears for a time on the surface of the medullary plate (see Fig. 79) in the position which will later on form the optic outgrowth.

Although we are perhaps justified in believing that the eye of existing Vertebrates was already present as a patch of epithelium sensitive to light in the far back evolutionary period when the forerunner of the central nervous system was still a portion of the outer surface of the body, we do not, in the present writer's opinion, appear to be justified in connecting up the eye of typical Vertebrates with the "eyes" of *Amphioxus* or of Tunicates. It seems more probable that the eyes of these highly specialized creatures are organs which have developed independently within their own groups.

PITUITARY BODY.—To be included amongst the derivatives of the ectoderm is that enigmatical organ the **pituitary body** (" Hypophysis cerebri "). This — the " anterior lobe " of the pituitary body in mammalian anatomy—arises normally as an ingrowing pocket of ectoderm on the ventral side of the head, situated as a rule close to the hinder limit of the stomodaeum but in the case of the Cyclostomes just outside its anterior boundary. This pituitary involution extends inwards beneath the infundibulum. In the Cyclostomata it retains its original form of a tube communicating with the exterior, but in the gnathostomatous Vertebrates its outer end becomes gradually constricted into a narrow duct which in the great majority of cases becomes finally obliterated, so that the organ now forms a closed sac lying immediately underneath the infundibulum. The wall of the sac sprouts out into numerous tubular projections between which develops highly vascular mesenchyme, providing the rich blood supply necessary to the definitive function of the organ as a ductless gland.

As regards variations in the development of the pituitary involution: it may arise as a *solid* ingrowth of ectoderm (Lung-fishes, Amphibians); it may be two-lobed (Teleosts) or three-lobed (Lacertilia) in its early stages; its external opening may become secondarily displaced up on to the dorsal side of the head (Lampreys); its inner end may come to open secondarily into the pharynx (Myxinoids). As already indicated the wall of the infundibulum in the Gnathostomata comes into intimate relation with the pituitary body in the restricted sense, forming the so-called posterior lobe, or cerebral portion, or nervous portion, of the pituitary body.

LITERATURE

Agar. Anat. Anzeiger, xxviii, **1906.**

Agar. Anat. Anzeiger, xxxv, **1910.**

Assheton. Quart. Journ. Micr. Sci., xxxiv, **1892.**

Assheton. Quart. Journ. Micr. Sci., xxxviii, **1896.**

Balfour. Phil. Trans. Roy. Soc., clxvi, **1876.**

Balfour. Monograph on the development of Elasmobranch Fishes. London, **1878.**

Bidder und Kupffer. Untersuchungen über die Textur des Rückenmarks und die Entwickelung seiner Formelemente. Leipzig, **1857.**

Boulenger. Tailless Batrachians of Europe. Ray Society, **1897.**

Braus. Anat. Anzeiger, xxvi, **1905.**

Budgett. Quart. Journ. Micr. Sci., xlii, **1899.**

Burrows. Journ. Experim. Zool., x, **1911.**

Cameron. Proc. Roy. Soc. Edinb., xxiv, **1903.**

Cameron. Proc. Roy. Soc. Edinb., xxv, **1904.**

Coggi. Atti Accad. Lincei, Anno 286, Ser. iv, Mem. Class. Sci. fis., vi, **1889.**

Cunningham and MacMunn. Phil. Trans. Roy. Soc., B, clxxxiv, **1893.**

Davies. Morph. Jahrb., xv, **1889.**

Dendy. Quart. Journ. Micr. Sci., xlii, **1899.**

Dendy. Quart. Journ. Micr. Sci., li, **1907.**

Dendy. Phil. Trans. Roy. Soc., B, cci, **1910.**

Dohrn. Mitt. zool. Sta. Neapel, viii, **1888.**

Dohrn. Mitt. zool. Sta. Neapel, xv, **1902.**

Eycleshymer. Journ. Morph., x, **1895.**

Goeldi. Zoolog. Anzeiger, xxiii, **1900.**

Gutzeit. Zeitschr. wiss. Zool., xlix, **1889.**

Harrison. Anat. Record, ii, **1908.**

Harrison. Journ. Experim. Zool., ix, **1910.**

Havet. La Cellule, xviii, **1901.**

Held. Die Entwicklung des Nervengewebes bei den Wirbeltieren. Leipzig, **1909.**

Hensen. Virchows Archiv, xxxi, **1864.**

Hensen. Arch. mikr. Anat., iv, **1868.**

Hensen. Die Entwicklungsmechanik der Nervenbahnen der Säugetiere. Kiel and Leipzig, **1903.**

Hertwig, O. and R. Das Nervensystem und die Sinnesorgane der Medusen. Leipzig, **1878.**

Hill. Journ. Morph., ix, **1894.**

His. Untersuchungen über die erste Anlage des Wirbelthierleibes. Die erste Entwickelung des Hühnchens im Ei. Leipzig, **1868.**

Kappers, Ariëns. Report XVIIth International Congress of Medicine. London, **1913.**

Keiffer. Arch. Biol., ix, **1889.**

Kerr, Graham. Quart. Journ. Micr. Sci., xlvi, **1902.**

Kerr, Graham. Trans. Roy. Soc. Edinb., xli, **1904.**

Kerr, Graham. The Work of John Samuel Budgett. Cambridge, **1907.**

Klinckowström. Zool. Jahrb. (Anat.), vii, **1894.**

Knape. Anat. Anzeiger, xxxiv, **1909.**

Kupffer. Hertwigs Handbuch der Entwicklungslehre. **1906.**

Leydig. Die in Deutschland lebenden Arten der Saurier. Tübingen, **1872.**

Neal. Journ. Morph., xxv, **1914.**

Neumayr. Hertwigs Handbuch der Entwicklungslehre. **1906.**

Novikoff. Zeitschr. wiss. Zool., xcvi, **1910.**

Oppel. Lehrbuch der vergleichenden mikroskopischen Anatomie der Wirbeltiere. Jena, **1905.**

Paton. Mitt. zool. Sta. Neapel, xviii, **1907.**

Rabl, C. Zeitschr. wiss. Zool., lxiii, **1898.**

Rathke. Untersuchungen über die Entwickelung und den Körperbau der Krokodile. Braunschweig, **1866.**

Ramón y Cajal. Histologie du système nerveux de l'homme et des Vertébrés. Paris, **1909.**

Retzius. Proc. Roy. Soc., B, lxxx, **1908.**

Röthig and Brugsch. Arch. mikr. Anat., lix, **1902.**

Scammon. Keibels Normentafeln zur Entwicklungsgeschichte, xii, **1911.**

Schwann. Mikroskopische Untersuchungen über die Übereinstimmung in der Struktur und dem Wachsthum der Thiere und Pflanzen. Berlin, **1839.**

Smith, Elliot. Anat. Anzeiger, xxxiii, **1908.**

Spencer, Baldwin. Quart. Journ. Micr. Sci., xxvii, **1886.**

Strasburger. Jahrb. wiss. Botanik, xxxvi, **1901.**

Studnička. S.-B. Böhmisch. Gesellsch., xv, **1896.**

Thiele. Zeitschr. wiss. Zool., xlvi, **1887.**

Voeltzkow. Abh. Senckenberg. naturf. Gesellsch., xxvi, **1899.**

Warren. Quart. Journ. Micr. Sci., xlv, **1902.**

Wenig. Anat. Anzeiger, xliii, **1913.**

Winkler. Arch. Entw. Mech., xxix, **1910.**

CHAPTER III

THE ALIMENTARY CANAL

THE alimentary canal or **enteron** of the Vertebrate consists of a tube passing from the mouth to the anus. The wall of this tube is known technically as the **splanchnopleure** in contradistinction to the **somatopleure** or body-wall (Balfour). It consists of an inner lining epithelium, the endoderm, ensheathed in a complex coating of mesoderm—the **splanchnic** mesoderm—consisting of connective tissue, blood-vessels, lymphatics, nerves, and coelomic or peritoneal epithelium.

As is commonly the case in other metazoa the endodermal lining is in the Vertebrate more or less encroached upon at the oral and anal ends of the tube by the spreading inwards of ectoderm. The parts of the tube which come thus to be lined with ectoderm are known as **stomodaeum** and **proctodaeum** (Lankester, 1876) while the intervening region lined by endoderm is known as the **mesenteron**. In the Vertebrata there is very slight development of proctodaeum but an important section of the buccal cavity is, as will be seen later, stomodaeal in its nature.

It is also customary in embryological writings to use the somewhat loose expression foregut for the anterior portion of the alimentary canal (reaching back to the pylorus or to the opening of the bile-duct), which in the meroblastic vertebrates becomes differentiated off from the yolk-sac comparatively early in development.

A good idea of the blocking out of the main regions of the alimentary canal in one of the lower vertebrates is got by inspecting sagittal sections of embryos and larvae at different stages of development such as those shown in Fig. 80. From the gastrula stage (A) on to the stage illustrated in Fig. 80, C, the endoderm forms a simple sac with its opening posterior (anus) and with its ventral wall greatly thickened owing to the fact that its cells contain the main store of yolk. From the stage of Fig. 80, D onwards the foregut (*f.g*) becomes gradually constricted off in a tailward direction from the mass of yolk, while at the opposite end of the body, correlated with the outgrowth of the posterior trunk region of the embryo and the backward shifting of the anus, the yolky mass becomes extended into the form of a thick-walled tube—the rudiment of the intestine (*ent*). From the stage of Fig. 80, E, onwards active growth of the true tail or postanal region is taking place, and it is noteworthy that, during this process, the endoderm retains for a considerable time its continuity with the mass of actively growing undifferentiated tissue at the tip of the tail and becomes drawn out into a cylindrical **postanal gut** (*pa.g*). This remains conspicuous for a time but eventually disintegrates and disappears completely. The main mass of yolk-cells, forming the ventral wall of the middle part of the enteron, gradually shrinks in volume, as the yolk is absorbed and carried off by the circulating blood for distribution to the growing and developing tissues of the body, and eventually the gut wall is no thicker in this region than it is elsewhere.

BUCCAL CAVITY.—The alimentary canal of the adult Vertebrate commences with the buccal cavity which is in part at least—as shown by the presence within it of placoid and glandular elements corresponding with those of the skin—stomodaeal in its nature. The stomodaeum however is not as a rule developed, as is so usually the case in the Invertebrates, from a simple involution of the ectoderm forming a depression of the surface below the general level. It arises rather by the walling in of an area on the ventral side of the head through the development of ridge-shaped outgrowths. These ridges may be termed respectively the **maxillary ridge** and the **mandibular ridge** accordingly as they give rise later on to the upper or to the lower jaw.

The roof of the buccal cavity, or at least its anterior portion, is simply to be looked on as part of the primitive ventral surface of the head, delimited by the maxillary ridge on either side. The floor, on the other hand, represents the mandibular ridge (Fig. 80, H, *m.r*) which has grown forwards in a direction parallel to the roof. The inner wall of the buccal cavity is in close contact with the anterior extremity of the endodermal alimentary tube but for a time the two cavities remain separated by a thin membranous diaphragm made up of the apposed layers of ectoderm and endoderm. This may conveniently be termed the **velar membrane** as the organ known as the velum in *Amphioxus* or *Petromyzon* consists simply of the remains of this membrane.

The formation of the anterior, stomodaeal, portion of the buccal cavity is seen in its simplest form in some of the lower holoblastic Vertebrates such as Crossopterygians, Lung-fishes or Urodele amphibians.

In Fig. 69, D (p. 126) in the case of *Protopterus*, or in Fig. 100 (p. 178) in the case of *Polypterus* (see also Fig. 80, H), what will become later the anterior part of the buccal roof is seen to be simply a portion of the ventral surface of the head, bounded behind by the transverse mandibular ridge—the rudiment of the lower jaw—and on each side by the longitudinal maxillary ridge.

As is well shown in the figure of *Polypterus*, and as is also the case with *Protopterus*, the buccal roof in front, that is to say in the neighbourhood of the mesial plane, passes without interruption into the external skin: in other words the maxillary ridge is not continued to the mesial plane so as to meet its fellow. In later stages the roof of the mouth would be hidden in a view from the ventral side owing to the forward growth of the lower jaw.

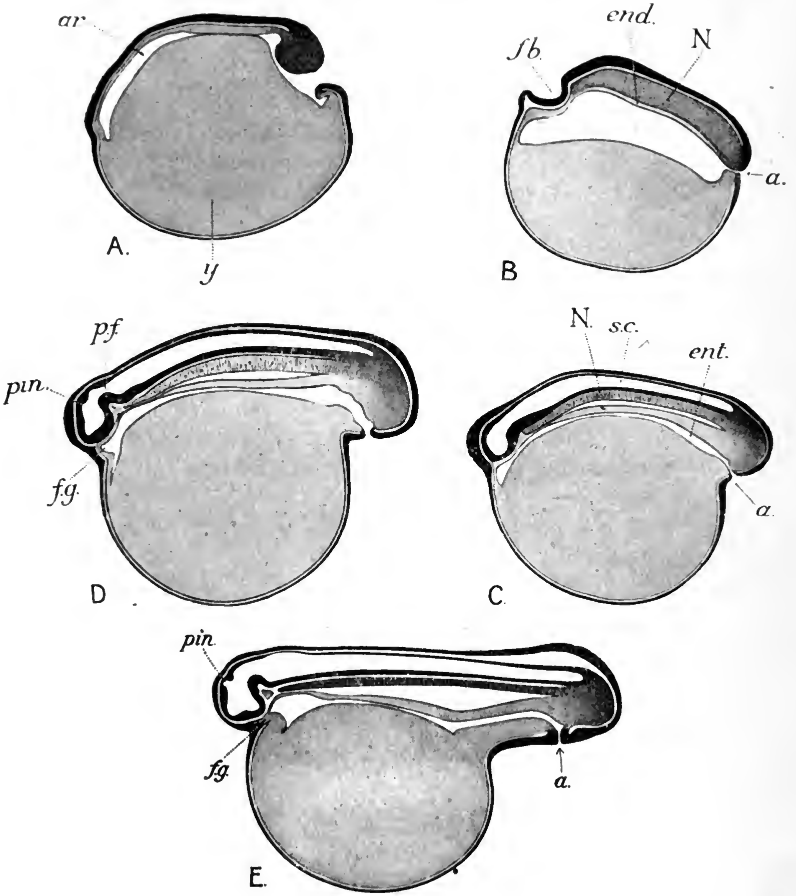


FIG. 80.—Sagittal sections through *Polypterus*.

A, stage 14; B, stage 17; C, stage 20; D, stage 23; E, stage 24 +. *a*, anus; *ar*, archenteron; *end*, endoderm; *ent*, enteric cavity; *f.b*, cavity of forebrain; *f.g*, foregut; *N*, notochord; *p.f*, primary fold of brain floor; *pin*, pineal rudiment; *s.c*, cavity of spinal cord; *y*, yolk.

The anterior portion of the buccal cavity in Urodele Amphibians arises in a manner essentially similar to that described above.

In the Gymnophiona and the Amniota a characteristic modification of the mouth margin is brought about by the fact that, as already mentioned, the maxillary ridge is cut across by the olfactory groove and so divided into the outer maxillary process and the inner median nasal process, the latter of which is continuous with its fellow across the mesial plane, forming with it the so-called **fronto-nasal process** (see Chap. X.).

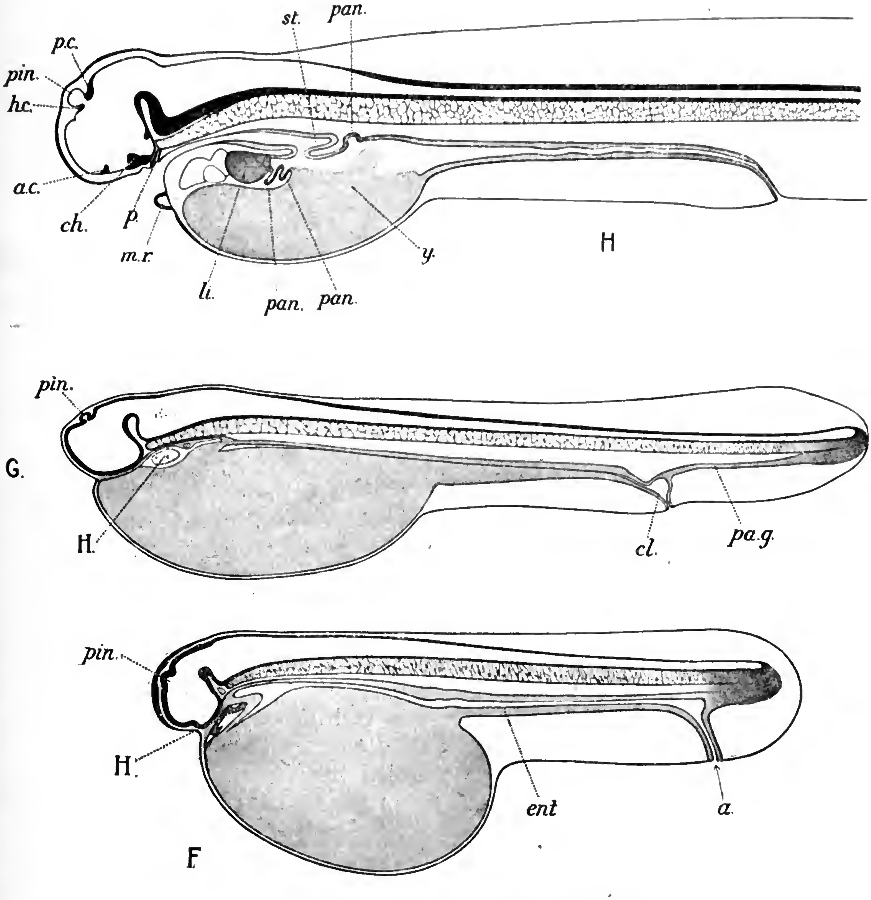


Fig. 80A.—Sagittal sections through *Polypterus*.

F, stage 26; G, stage 29; H, stage 32. *a*, anus; *a.c*, anterior commissure; *ch*, optic chiasma; *l*, cloaca; *ent*, enteric cavity; *H*, heart; *h.c*, habenular commissure; *li*, liver; *m.r*, mandibular ridge; *p*, pituitary involution; *p.c*, posterior commissure; *pa.g*, postanal gut; *pan*, pancreatic rudiment; *pin*, pineal rudiment; *st*, stomach; *y*, yolk.

It is of interest to notice that in various Vertebrates the buccal opening is at first elongated in an antero-posterior direction instead of from side to side. Such is the case with *Scyllium* (Sedgwick, see Fig. 81) and *Torpedo* amongst Elasmobranchs. In these cases the slit-like mouth is bounded on each side by a longitudinal ridge.

Later on each ridge becomes sharply bent, about the middle of its length, in such a way as to give the buccal opening a rhomboidal shape and at the same time to mark off the ridge into a maxillary portion in front and a mandibular portion behind. In Anura a somewhat similar arrangement is found.

"ENDODERMAL" SECTION OF BUCCAL CAVITY.—The fully developed buccal cavity has incorporated in it a posterior portion—varying in relative extent in different Vertebrates—which is derived not from the ectoderm but from the anterior portion of the "endodermal" enteric rudiment. The simplest way in which this portion becomes added to the anterior portion is seen in those Vertebrates in which the anterior part of the enteric cavity is patent throughout development. In this case the velar membrane simply ruptures—its remnants soon becoming absorbed—and the stomodaeal cavity is thrown into open communication with the enteric cavity. This is the case in certain Anura (*Rana*) and in Amniota.

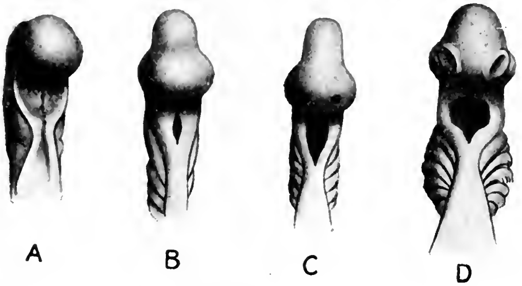


FIG. 81.—Ventral view of head region of embryos of *Scyllium canicula*. (After Sedgwick, 1892.)

A, 7-8 mm.; B, slightly more advanced than A; C, 11-12 mm.; D, 16 mm.

In many Vertebrates no velar membrane is present, owing to the fact that the foregut either becomes solid for a time (*Polypterus*, Fig. 80, D-G) or is so at the beginning (Teleostei, Urodela, *Lepidosiren* and *Protopterus*). In such cases the peripheral layer of the yolky foregut rudiment gradually assumes an epithelial character and the yolk along its middle breaks down, so that a cavity arises—continuous with the stomodaeal cavity and forming the hinder section of the definitive buccal cavity. The proportion which this posterior portion bears to the anterior section derived directly from the outer surface is very different in different groups. It apparently attains its maximum in Teleosts where it forms practically the whole of the buccal cavity.

Points of critical importance to the germ-layer theory are raised in this connexion by the fact that teeth, organs belonging originally to the outer surface, are developed in this posterior region of the buccal cavity from yolky "endoderm." This is well seen in a Urodele, or a lung-fish such as *Lepidosiren* or *Protopterus* (Fig. 82). The attempt is made to get round this difficulty by assuming that the layer of epithelium which makes its appearance over the surface of the buccal rudiment, and in relation with which the teeth develop, is really an ingrowth from the ectoderm.

It is, as a matter of fact, quite continuous with the ectoderm,

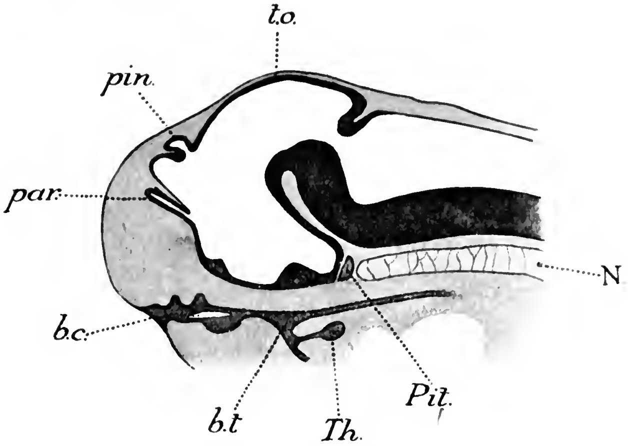


Fig. 82.—Sagittal section through head region of a Protopterus larva (Stage 33).

b.c, buccal cavity; *b.t*, anterior boundary of tongue; *N*, notochord; *pin*, pineal body; *par*, paraphysis; *Pit*, pituitary body; *Th*, thyroid rudiment; *t.o*, *tectum opticum*. The position of dental rudiments is indicated by the two upward projections of the dorsal wall of the buccal cavity.

but examination of carefully prepared celloidin sections (Fig. 83) shows that at its inner end the epithelium passes by imperceptible

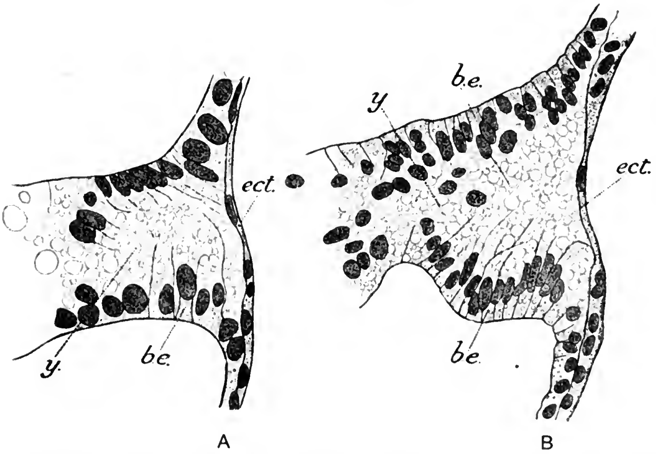


Fig. 83.—Sagittal sections through the region of the buccal cavity of (A) *Lepidosiren*, stage 30, and (B) *Amblystoma*, 7·5 mm. in length.

b.e, buccal epithelium; *ect*, ectoderm; *y*, solid mass of yolk-cells in position of buccal cavity.

gradations into the ordinary yolky endoderm, with no trace of the sharply defined edge which it would possess were it a layer of ectoderm pushing its way inwards. It extends inwards simply by a process of delamination from the yolky "endoderm."

The real lesson to be learnt from these cases is that the characters of one germ-layer are liable to spread over its boundary into territory belonging to another layer or, in other words, that the territories of the various layers are liable to be separated by an indefinite debatable zone rather than by a mathematically sharp line. It follows that the apparent position of an organ-rudiment in relation to such a boundary is not necessarily to be taken as giving any definitive proof as to which of the two cell-layers that organ belongs to.

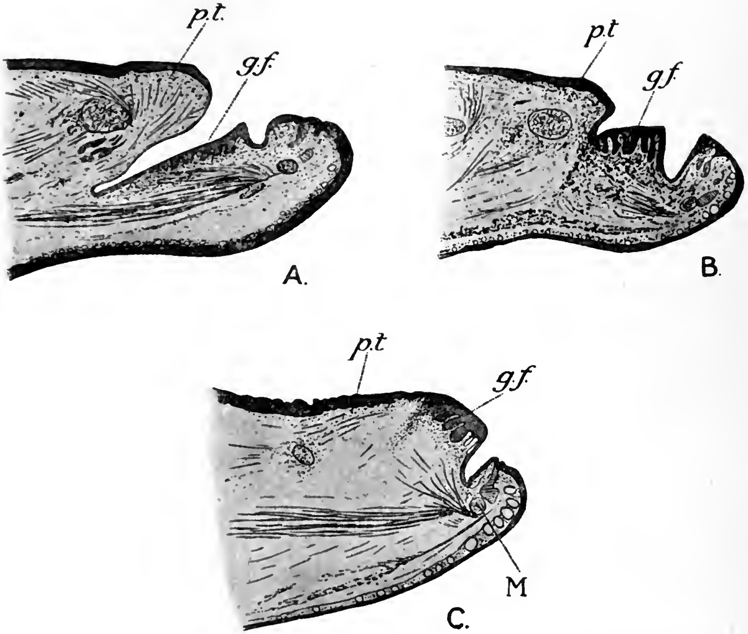


FIG. 84.—Sagittal sections illustrating the development of the tongue in Urodeles. A and B, *Triton*; C, *Salamandra* (after Kallius, 1901); *g.f*, gland field; *M*, mandibular arch; *p.t*, primary tongue.

THE TONGUE.—The tongue is a portion of the buccal floor which becomes demarcated off from the rest by a split formed by a downgrowth of the lining epithelium of the mouth. Its mode of development is well illustrated by what happens in Urodele Amphibians as described by Kallius. Here there develops first a **primary tongue**, ensheathing the anterior and ventral portion of the hyoid arch (Fig. 84, *p.t*), which becomes marked off, except at its hinder end, by a deep groove in the floor of the mouth.

A horseshoe-shaped thickening of the buccal epithelium now develops external to, and parallel with, the groove bounding the primary tongue, and consequently lying on the floor of the mouth between the primary tongue and the lower jaw. The thyroid involution is situated between this thickening and the tip of the tongue.

The ectodermal thickening develops numerous glands, each originating as a solid ectodermal down-growth, and is known as the **gland-field.** Externally it is bounded by a shallow groove. Later on the cleft or groove separating the gland-field from the primary tongue becomes obliterated by fusion of its walls, and the gland-field becomes raised up in a dorsal direction (Fig. 84, B) the tongue-tip shrinking backwards so that eventually the demarcation between primary tongue and gland-field disappears (Fig. 84, C). Meanwhile the groove bounding the gland-field externally becomes deepened. It forms the outer limit of the **definitive tongue** which is thus a compound structure, its tip and edges developed from the original gland-field, its postero-median part from the primary tongue.

In the fishes the tongue remains non-muscular and non-glandular: it is simply the primary tongue. In the Axolotl the tongue appears also to be a primary tongue, the gland-field making a transient appearance as a rudiment but eventually undergoing atrophy (Kallius).

In the Amniota the tongue is, as in the terrestrial Urodeles, a compound structure, the primary tongue rudiment becoming fused with an elevation of the floor of the mouth lying in front of the Thyroid rudiment. This elevation, called by His the *tuberculum impar,* represents morphologically the gland-field of the Urodeles.

The tongue of Cyclostomes is remarkable for its complexity: it has complex muscular and skeletal arrangements and on its surface it develops the horny spines which function as teeth and simulate teeth in their appearance. In *Bdellostoma* the tongue develops as a cushion-like swelling of the floor of the mouth at an early period while the velar membrane is still intact. In *Petromyzon,* on the other hand, it does not develop until the time of metamorphosis.

It has already been shown how the olfactory organs come to communicate with the buccal cavity by the posterior nares. In the Amniota these become sunk into a recess in the roof of the mouth and in the higher Reptiles, as in the Mammals, this recess becomes shut off from the buccal cavity by a horizontal shelf which grows in from the side and meets its fellow to form the palate. How this has come about in evolution is illustrated by the three Lizards shown in Fig. 85.

In ontogeny the mode of origin may be similar, the palatine outgrowths meeting and fusing with one another in the middle line (Crocodiles) or, as happens more usually, a median ridge or septum extends backwards from between the primitive posterior nares and the palatine processes meet and fuse with its ventral edge. In the two cases the physiological result is the same—the shunting backwards of the communication between olfactory and buccal cavities, a process which reaches its extreme in the Crocodilia where the palate extends back to about the level of the glottis.

STOMODAEAL GLANDS.—Whereas in the majority of Fishes the stomodaeal lining possesses only isolated gland-cells, in the air-breathers on the other hand there are developed definite multicellular glands. These originate as a rule from solid down-growths of the lining epithelium which develop a cavity secondarily. In Urodeles there is, as already mentioned, a special aggregation of these glands forming the gland-field in front of the tongue, while a single gland of considerable size develops from the roof of the mouth in the region between the olfactory sacs (Intermaxillary or internasal gland).

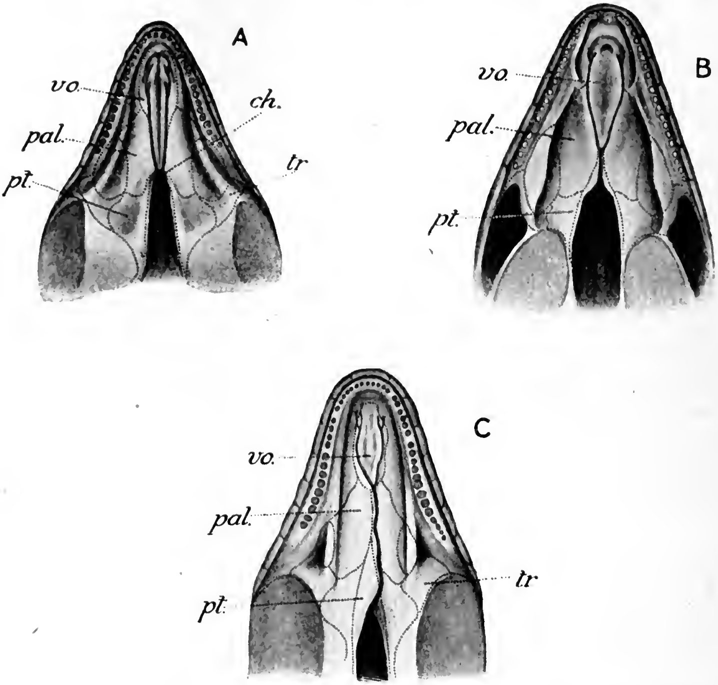


FIG. 85.—View of the roof of the mouth in three species of Lizard (A, *Egernia Kingii* ; B, *Mabuia quinquetaeniata* ; C, *Lygosoma rufescens*), illustrating the shifting back of the communication between nose and mouth. (After Voeltzkow, 1899.)

ch, recess into which primitive posterior nares open ; *pal*, palatine ; *pt*, pterygoid ; *tr*, transverse bone ; *vo*, vomer.

In terrestrial Reptiles glands are present in numbers on the roof of the mouth (Palatine), beneath the tongue on each side of the middle line (Sublingual) and along the edge of the mouth just external to the row of teeth (Labial). The poison glands are specialized and enlarged labial glands of the upper jaw except in

Heloderma where they are the enormously enlarged sublingual glands.

Similar localized developments of the buccal glands occur in Birds and some of them may reach a great size as, for example, the enormous sublingual glands of the Woodpeckers.

PHARYNX.—The part of the alimentary canal which follows immediately behind the buccal cavity is highly characteristic from the fact that in Vertebrates it is concerned with the function of breathing. The special organs which are developed to carry out this respiratory function fall into two groups one represented by the **Lung**—adapted for respiratory exchange with the atmosphere, the other by the **Gills**—adapted for respiratory exchange with gases in solution in the water. As the balance of probability is in favour of the latter being the more archaic they will here be considered first.

The gills are seen in their most typical and familiar form in the various groups of Fishes where there is present upon each side of the pharyngeal region a series of **visceral clefts**—slit-like openings leading from the pharyngeal cavity to the exterior—separated from one another by masses of solid tissue known as the **visceral arches** or **gill septa**. The walls of the clefts are highly vascular and their surface is commonly raised into conspicuous plate-like projections—the **respiratory lamellae** — which serve to increase the area of respiratory tissue.

In the most archaic arrangement, seen in Elasmobranch fishes, the front lip of each cleft, except the first, is prolonged backwards to form a small valvular flap overlapping the external opening. In the Holocephali, Teleostomi, and Dipnoi the anterior one of these flaps, that projecting back from the hyoid arch, becomes greatly enlarged to form the **operculum** which overlaps not merely one but the whole series of clefts lying behind it. Correlated with this the outer portion of each succeeding septum, which in the Elasmobranch gave origin to its valvular flap, has disappeared, leaving only the portion lying next the pharyngeal cavity.

The cleft lying in front of the hyoid arch—the **spiracle**—is usually modified, its respiratory tissue having been reduced and even its opening being diminished in size or completely absent, but its general relations in the adult are such as to permit of no doubt as to its serial homology with the clefts behind it.

Most usually there are on each side six clefts—a spiracle and five **branchial clefts**—but there is reason to believe that there was a greater number present in primitive Vertebrates—seeing that the number of persistent clefts becomes on the whole less as one ascends the vertebrate scale and that here and there among the more archaic forms a greater number than the usual is found (*Bdellostoma* up to 14, *Notidanus cinereus* 7, *N. griseus* 6).

In a few of the more archaic Vertebrates there develop during larval life, in addition to the visceral clefts with their respiratory lamellae or **internal gills**, respiratory organs of another type—the **external gills**. As there is some reason to believe that these are more ancient organs than the gill-clefts they will here be considered first although they are much less familiar than the clefts with their internal gills. The branchial organs will therefore be considered in the following order: (I.) External gills, (II.) Visceral clefts, (III.) Internal gills.

(I.) EXTERNAL GILLS.—The true external gills are organs which are commonly confounded with the ordinary or internal gills developed in the walls of the gill-clefts. They appear however to be quite independent of these in their origin and they would probably have attracted more attention and interest than they have done had it not been for the fact that they occur in their typical form in only three subdivisions of the Vertebrates (Crossopterygii, Dipnoi, Amphibia) and that two out of these three groups comprise animals of extreme rarity, the developmental stages of which have not been generally accessible to embryologists.

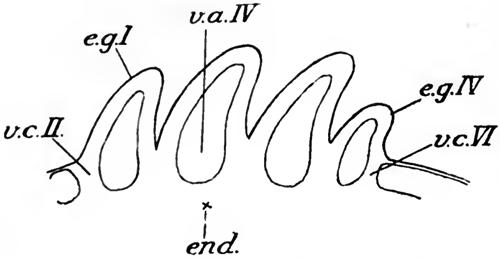


FIG. 86.—Diagrammatic longitudinal section through the early rudiments of the external gills of *Lepidosiren* (Stage 25).

e.g, external gill; *end*, endoderm; *v.a*, visceral arch; *v.c*, visceral cleft rudiment.

The typical External gill is a projection from the surface of the body on the outer side of a visceral arch. It consists of a core of mesenchyme with a covering of ectoderm; it is traversed by a vascular loop consisting of the main aortic arch which passes out to its tip and then doubles back; and it commonly has a pinnate form, paired projections growing out so as to increase its respiratory surface. It is provided with muscles by means of which the possessor is able to flick it sharply backwards so as to renew the water in contact with it.

The external gill as a rule is without any special skeletal support but in the larval Polypterus a short rod of cartilage projects into its base, and in the extinct Dolichosoma of the Gas coal of Bohemia there was apparently present a well-developed segmented skeleton within the substance of the external gills.

The external gill develops as an outgrowth from the tissue of the visceral arch at a period at which the clefts are not yet perforated. It arises as a bulging of the surface (Fig. 86) and in the author's opinion the endoderm of the cleft rudiments takes no part in its formation. At the same time it is only right to state that the prevalent opinion in the past has been different. The outer surface of the visceral arch in the region where the external gill will develop is covered by a layer of cells thicker than the neighbouring ectoderm, and in some cases this thickened portion of the ectoderm shows in its deeper portions a rich deposit of yolk, so as to look exactly like the yolk-laden endoderm. Greil explains this appearance by supposing that true endoderm cells actually spread outwards and replace the deep layer of the ectoderm, so that the external gill-rudiment would be partly endodermal in its nature. There is however no definite evidence of any such process taking place and the present writer would interpret the appearances as meaning simply that the ectoderm covering the external gill-rudiment becomes thickened, and stores up a supply of yolk in its deeper layers, as a physiological preparation for the active processes of growth which are about to take place as the external gill rapidly increases in length. In this he agrees with Marcus (1908).

The general appearance of the developing external gills is well seen in *Hypogeophis* (Fig. 87) or in *Lepidosiren* (Fig. 200). In *Lepidosiren* there are present four upon each side of the body. At first the four are quite independent of one another but as development goes on they become raised upon a common base so as to give the appearance of a single organ with four branches (Fig. 200, B-E).

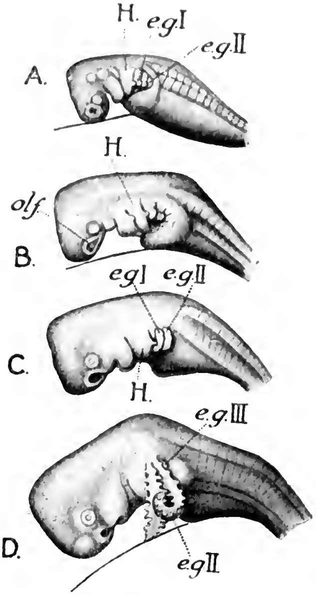


Fig. 87.—Hypogeophis embryos showing development of the external gills. (After Brauer, 1899.)

e.g, external gill; *H*, hyoid arch; *olf*, olfactory organ. The rounded knobs seen projecting in B from the hyoid arch, and also from the mandibular arch in front of it, are possibly external gill rudiments which do not go on with their development.

The distribution of true external gills amongst the main groups of Vertebrates is shown in the following table:

Visceral Arch.	I. Mandibular.	II. Hyoid.	III. First Branchial.	IV. Second Branchial.	V. Third Branchial.	VI. Fourth Branchial.
Elasmobranchii . .						
Crossopterygii . .		×				
Dipnoi			×	×	×	×
Amphibia . . .	*v.**	*v.*	×	×	×	
Amniota . . .						

* *v* = Vestigial.

In those animals in which they are well developed the external gills are for a time the main functional breathing organs. They are richly vascular and the renewal of the water in contact with their surface is provided for by a well-developed muscular mechanism by which they are sharply flicked from time to time, or, in early stages, by rich ciliation of their surface as in the Frog (Assheton, 1896) or *Cryptobranchus* (Smith, 1912). They are as a rule merely temporary organs. As the respiratory function comes to be sufficiently performed by other organs their circulation becomes sluggish, their tissues moribund. They become invaded by leucocytes and eventually undergo complete atrophy. In *Protopterus* distinct vestiges persist for a prolonged period while in various Urodeles they remain functional throughout life.

The external gills, highly vascular and projecting freely into the surrounding medium, present tempting objects for attack by other organisms. They are therefore extremely liable to injury, and correlated with this they present a high power of regeneration. In correlation also with the same fact we find that they tend to be eliminated from development in certain members of groups which are as a whole characterized by their presence. Such is the case in the Amphibia where they are characteristic of the group in general but where in particular cases they are reduced (*Hyla arborea*) or completely absent (*Bombinator*) although we must believe they were present in the ancestors of these forms.

This tendency for the external gills to become eliminated from development in the process of evolution raises the interesting morphological question: were External Gills at any period more widely distributed amongst Vertebrates than they are at present? And, if so, are their vestigial representatives still to be found in any cases where they no longer develop as functional respiratory organs?

This interesting problem, which offers an inviting field for research, has not yet had sufficient attention devoted to it. Even if it were the case that external gills once existed in the ancestors of forms in which they are no longer present as functional organs there is always the possibility if not probability that their disappearance has been so complete as to leave no observable trace. Nevertheless such vestiges might persist and are worth looking for.

Under these circumstances it is of interest to note that already certain structures are known which are interpretable as vestiges of once-present external gills. Thus in Gymnophiona what appear to be transient rudiments of mandibular and hyoidean external gills make their appearance during development (Fig. 87, B). Again in the case of the Mandibular and Hyoid arches of Urodeles, on which no functional external gills develop, Drüner (1901) has found what appear to be vestiges of the muscles of external gills. Again in the larvae of various Urodeles there occurs in connexion with each mandibular arch a curious styliform projection known as the **balancer**, from the fact that the larva balances itself upon them as upon a pair of limbs (Fig. 88, *b*). Each of these has a vascular loop within it and it in fact appears to be the modified external gill of the mandibular arch which has lost its respiratory and taken on a supporting function.

While external gills occur within three main subdivisions of the Vertebrates, namely Teleostomatous fishes (Crossopterygians—the most archaic of existing Teleostomes), Lung-fishes, and Amphibians, there are two main groups—Elasmobranchs and Amniotes—in which they are conspicuous by their absence. Having regard to the tendency of the organs in question to disappear (as in the cases already alluded to amongst the Amphibia) their absence in a special group would not in any case constitute strong evidence that they were never present in the ancestors of that group. As it happens however there is in the two groups mentioned a definite cause which seems quite competent to account for the disappearance of external gills, namely the development of a new organ — the yolk-sac with its highly developed vitelline network of blood-vessels—which in addition to its primitive function must necessarily also function as a very efficient organ of respiratory exchange and so render any pre-existing respiratory organ no longer necessary.

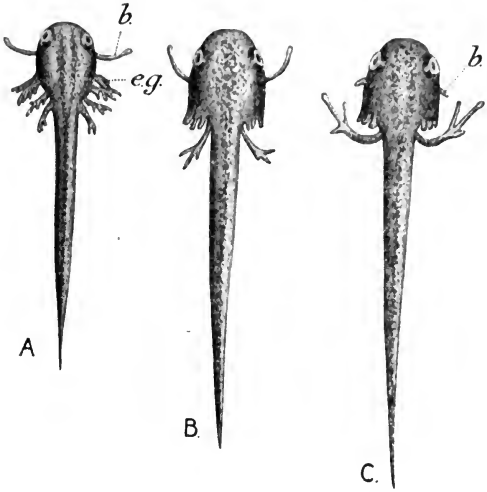


Fig. 88.—Three stages in larval development of a newt (*Triton taeniatus*) as seen from above. (After Egert, 1913.)

b, balancer; *e.g*, external gill of first branchial arch. In Fig. A what looks like a posterior external gill is the pectoral limb. In Figs. B and C the external gills have been cut away leaving only their basal stumps.

Taking into consideration the presence of external gills in three archaic groups of Vertebrates it seems to the present writer to be clearly indicated that these organs are a very ancient characteristic of the Vertebrate phylum. The only alternative indeed is to regard them as having become evolved independently in the three groups in which they occur. It is difficult to accept this as in any way probable having regard to the similar morphological relations of the organs in question.

It might be suggested that somewhere on the course of a large blood-vessel, such as an aortic arch, would be a most natural place for the development of a new respiratory organ. Such a suggestion however is entirely fallacious for simple physical reasons: for new breathing organs will tend to become evolved not on the course of a large vessel where the quantitative relation of surface to volume in the blood-vessel is at its minimum but rather where there is present a rich superficial network of capillaries, in which the ratio in question is at its maximum.

(II.) VISCERAL CLEFTS.—The visceral clefts develop in what appears to be the most archaic method in Lampreys and Elasmobranchs where each arises as a lateral pocket (**visceral pouch**) of the pharyngeal wall which meets and fuses with a, much shallower, ingrowth of the ectoderm, the apposed portion of endoderm and ectoderm breaking down so as to bring about a free communication between pharynx and exterior. Each cleft thus consists of a, usually much larger, inner portion lined with endoderm and an outer portion lined with ectoderm.

The most frequent type of modification of this probably primitive mode of cleft development is that so usually met with in the development of hollow organs, namely that the cleft-rudiment, instead of being a hollow pouch from the beginning, is for a time in the form of a solid lamina of endoderm, which only at a later period develops a cavity in its interior and becomes an open cleft. This modification is found in Teleostomatous fishes, Lung-fishes and Amphibians.

In the young Elasmobranch the gill-clefts are at first long slits traversing the whole dorsi-ventral extent of the lateral wall of the pharynx. Each septum or arch grows back at its outer edge to form a valvular flap overlapping the cleft next behind it. In most cases this backgrowth fuses with the next septum at its dorsal and ventral ends so as to reduce the external opening of the cleft to a comparatively small dorsi-ventral extent.

In all Gnathostomes, excepting the typical Elasmobranchs but including the Holocephali, the hyoidean backgrowth becomes greatly enlarged to form the operculum which overlaps the whole series of clefts behind it. Correlated with this the outer portions of the subsequent septa with their backgrowths become reduced. In these cases we frequently find a marked tendency for the edge of the opercular backgrowth to become fused with the body so as to restrict the size of the opening behind it. Thus in the Eel the opercular opening becomes reduced to a small persistent ventral portion, while in *Symbranchus* the same holds but in this case the two openings have fused together to form a small ventrally placed median pore.

A similar condition to this occurs in the tadpole of *Discoglossus* while in other Anura the persisting opening is displaced to the left side. Finally in Amniotes the fusion of opercular margin with body-wall takes place along its whole extent so that the branchial region becomes completely enclosed (see Chap. X.).

SPIRACLE.—The spiracle or hyomandibular cleft always shows a considerable amount of modification. In Elasmobranchs its dorsal portion alone becomes perforate, although fusion of the pouch with the ectoderm takes place throughout its whole dorsi-ventral extent.

Respiratory lamellae develop only on its anterior wall and these, as development proceeds, become vestigial forming the **pseudobranch.**

In Teleostean fishes the spiracular pouch (Fig. 89 A, *vc.* I) flattens out and disappears (Goette) so that the pseudobranch (*ps.*) on its anterior wall comes to lie on the inner face of the base of the operculum and appears to belong to the second cleft (Fig. 89, B). In Lung-fishes the solid endodermal rudiment never becomes perforate. It becomes gradually reduced during development while its outer ectodermal portion becomes, as already indicated, converted into a special sense-organ.

In Anurous Amphibians and in the Amniota the distal portion of the cleft rudiment becomes greatly dilated to form the tympanic cavity, while the proximal part forms the relatively narrow Eustachian tube.

Just as the varying condition of the spiracle indicates a tendency for this cleft to undergo reduction so a similar but still more marked tendency exists for the gill clefts to become reduced at the other (posterior) end of the series. This is illustrated in the first place by the reduction in the number of functional clefts seen in passing from the lower Vertebrates to the higher. It is also frequently manifested in developmental stages. Thus amongst the Amphibia we find that in the Gymnophiona (*Hypogeophis,* Marcus) a rudimentary 7th cleft makes its appearance though it never reaches the ectoderm, while the 6th is open for a time. In Urodeles a 6th rudiment appears and is for a time connected with the ectoderm but does not become perforate, while in Anura this cleft appears only as a small and transient rudiment which never reaches the ectoderm.

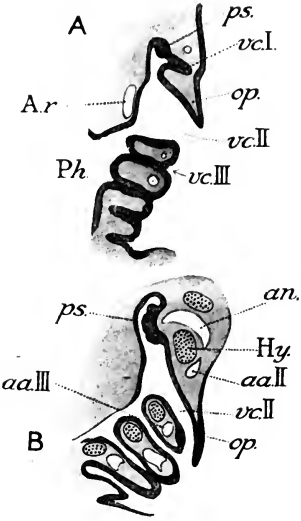


FIG. 89.—Horizontal sections through Salmon embryos explaining position of pseudobranch on inner surface of operculum. (After Goette, 1901.)

A.r, aortic root; *aa,* aortic arch; *an,* anastomotic vessel connecting aortic arches I and II; *Hy,* hyoid arch; *op,* operculum; *Ph,* cavity of pharynx; *ps,* pseudobranch; *vc,* visceral cleft.

(III.) INTERNAL GILLS.—The internal gills or respiratory lamellae arise as ridge-like or, at first, finger-like projections of the cleft lining. The chief matter of dispute regarding their development has been the question whether they belong to the endodermal or the ectodermal portion of the cleft lining. In cases where, as frequently happens, the lamellae begin to develop after the cleft is completely formed, the appearances are sometimes in favour of the one sometimes in favour of the other interpretation. Goette (1901) in fact goes the length of regarding the lamellae as being of endodermal origin in the case of the spiracle and ectodermal in the case of the succeeding clefts, so that the spiracular pseudobranch would on a strict interpretation of the germ-layer theory not be serially homologous with the other gills.

In the present writer's opinion, as already indicated, such observations upon the first origin of organs which develop in the region of the blurred boundary between two layers are not to be taken as affording evidence of any serious importance in regard to the morphological nature of such organs. Greater weight however seems due to evidence obtained from cases where the first traces of gill lamellae are visible at a period before the bounding membrane of the cleft is ruptured, when the cleft consists still of two distinct pouches—one ectodermal, the other endodermal—separated by a still complete partition. Such is the case in *Acipenser* and Goette shows that in this case the lamella-rudiments arise outside the partition from what is undoubtedly an ectodermal surface (see Fig. 90, *g.l*).

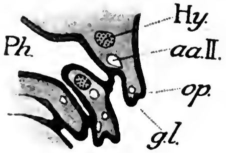


Fig. 90.—Horizontal section through branchial region of young Acipenser showing the ectodermal origin of the gill lamellae. (After Goette, 1901.)

aa, aortic arch; *g.l*, rudiment of gill lamella; *Hy*, hyoid arch; *op*, operculum; *Ph*, cavity of pharynx.

The same discussion extends to the general lining of the cleft—as to how much of the lining of the adult cleft is ectodermal and how much endodermal. Goette and Moroff (1902) hold that only the portion of the cleft in the immediate neighbourhood of its pharyngeal opening is to be regarded as endodermal, all the rest being ectodermal. But here again in view of the blurred character of the boundary between the two layers it seems hardly profitable to speculate on the matter.

In certain fishes the gill-lamellae are for a time prolonged outwards into long threads which project through the cleft opening into the surrounding fluid. Such is the case in the embryos of Elasmobranchs, in which it is only the lamellae upon the posterior face of each arch that become prolonged, those on the anterior face not projecting beyond the edge of the septum. Eventually the projecting part of the filament disappears while its attached basal portion becomes the definitive lamella. In a few Teleosts a similar temporary modification of the lamellae takes place—perhaps the best example being *Gymnarchus* (Budgett, 1901; Assheton, 1907. See Fig. 199).

Evolutionary History of the Branchial Respiratory Organs.—As regards the early evolutionary history of these branchial respiratory organs one very generally accepted view looks upon the visceral clefts as being the most primitive, the internal gills as having developed next, and the external gills as being due to secondary extension of respiratory tissue outwards from the clefts. It seems however, bearing in mind what we now know regarding the development and distribution of external gills, at least equally if not more probable that the evolution of these organs has been in the opposite direction.

On this latter hypothesis the external gills would be regarded as the primitive respiratory organs, inherited probably from pre-vertebrate ancestral forms. The evolution of clefts between their bases would be explicable as an arrangement for pumping water over the surface of the external gills, while it could be readily understood that the respiratory tissue would then tend to spread inwards along the lining of the clefts, where it would be both advantageously situated for carrying out its breathing function and, at the same time, protected from the dangers to which external gills are exposed. The development of respiratory lamellae to increase the area of this respiratory tissue on the wall of the cleft would be a further and natural development.

The chief difficulty in the way of accepting this as a working hypothesis lies in the existence of animals admittedly near the base of the Vertebrate scale—such as *Amphioxus* and the Cyclostomata—in which there are no external gills and no vascular yolk-sac to account for their disappearance. This difficulty is undoubtedly a serious one but on the whole the present writer is inclined to think the difficulty is not so great as to justify the immediate rejection of the hypothesis: it becomes less formidable when it is borne in mind that the forms mentioned although evidently archaic in some of their characteristics bear in others equally convincing evidence of high specialization.

LUNG.—In all the groups of Gnathostomata excepting the Elasmobranch fishes the pharyngeal wall develops a great outgrowth which, as will become apparent later, is to be looked upon as homologous throughout the series and as primarily respiratory in its function—the **lung**. The lung appears in its most familiar and typical form in the tetrapod Vertebrates and its development in these will accordingly be considered first.

Here in an early stage of its development the lung is in the form of a pocket of the pharyngeal floor projecting downwards in the mid-ventral line. This pocket commonly makes its first appearance as a longitudinal groove or gutter in the floor of the pharynx at about the level of the last visceral cleft. The groove becomes constricted off from behind forwards, so as to form a blindly ending pocket communicating in front with the pharyngeal cavity by a narrow opening—the **glottis**—and extending back immediately ventral to the pharynx. The blind end of the pocket grows actively tailwards and becomes deeply bilobed—the two lobes becoming respectively the right and left lung, while the unpaired portion connecting them with the glottis becomes the **trachea** or **pneumatic duct**.

While the lung passes in its early history through stages corresponding on the whole with those described there are differences in detail in different groups--the most conspicuous of these variations being, as is so often the case in the development of hollow organs, that the rudiment is at first solid and the cavity appears secondarily in its interior. This is the case in various anurous amphibians and in *Lepidosiren* and *Protopterus*.

It has been indicated that the lung is primarily a ventrally placed pocket of the pharyngeal wall, that is to say its wall is a portion of splanchnopleure. It follows that the cavity of the lung is lined by endoderm while its outer layers (connective tissue, blood-vessels, muscles, etc.) are composed of splanchnic mesoderm.

As regards the further development of the lung, the main steps are concerned with its respiratory function and have to do with the increase of the respiratory surface. In such an animal as the Newt, where the lung retains a relatively primitive condition, the endodermal lining grows equally as the organ increases in size, so that even in the adult the lung has the form of a simple sac with smooth endodermal lining. In a Frog or a Lizard, however, growth activity is specially marked at particular spots so that at these spots the endoderm forms outward bulgings into the covering of splanchnic mesoderm.

In these Sauropsida in which the pulmonary apparatus reaches its highest degree of evolution (Tortoises, Turtles, Crocodiles and Birds, in an ascending series) these pockets of the endodermal lining become more and more extensive, and more and more complicated, so as to give rise to a thick spongy mass, which forms the bulk of the lung, surrounding the now relatively small clear central space. The latter, forming as it does an apparent continuation of the bronchus or paired portion of the trachea, is spoken of as the **intrapulmonary bronchus**. Further the respiratory function becomes concentrated towards the terminal portions of the pockets, their proximal portions forming simply conducting channels—branches of the intrapulmonary bronchus.

In the Chameleons, towards the end of development, a number of the endoderm outgrowths bulge out beyond the general level of the surface of the lung upon its ventral side. These persist in the adult as large diverticula which when the animal blows itself out are inflated with air. In the embryos of Birds similar outgrowths make their appearance, four from each lung, but in this case as development goes on the outgrowths continue to increase in size and form the characteristic **air-sacs** of the adult bird.

THE LUNG OF BIRDS.—As the Birds, in correlation with the intensely active metabolism as indicated *e.g.* by their high body temperature, stand pre-eminent amongst Vertebrates in the high stage of evolution which has been reached by their lung, the ontogenetic development of this organ will be followed out in a little more detail (Moser, 1902; Juillet, 1912).

In the Fowl the pulmonary diverticulum of the pharyngeal floor makes its appearance about the beginning of the third day. By the end of this day the rudiment is bifurcated at its hind end, each lobe being the rudiment of a lung in the restricted sense and containing a prolongation of the enteric cavity lined by tall columnar endoderm cells. Outside the endoderm is a thick layer of mesenchyme and this in turn is covered by columnar coelomic epithelium.

The endoderm-lined cavity is destined to become the main intrapulmonary bronchus—the **mesobronchus**. This remains unbranched until the fifth day when its endoderm begins to bulge out, near the point where it enters the lung, to form the first **entobronchus**. During further development a series of three other entobronchial outgrowths sprout out from the external surface of the mesobronchus close behind the first outgrowth. The four entobronchi so arising are closely contiguous and form a longitudinal row (Fig. 92, E1-E4).

A set of similar outgrowths make their appearance spaced out along the mesial side of the mesobronchus posterior to the entobronchi: these are the rudiments of the **ectobronchi**. A third set of outgrowths on the lateral side of the mesobronchus are the rudiments of the small secondary lateral bronchi (Campana). Of these sets of outgrowths the first and second are the most important and they are arranged in a slightly spiral row along the wall of the mesobronchus.

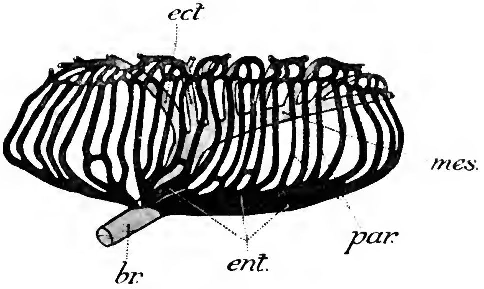


Fig. 91.—Diagram illustrating the arrangement of the main air-passages in the lung of the Fowl as seen from the mesial plane. (After Juillet, 1912.)

br, bronchus; *ect*, ectobronchi; *ent*, entobronchi; *mes*, mesobronchus; *par*, parabronchi.

The mesobronchus, as it grows in length, assumes a somewhat ∿-shaped curvature, by which the group of ectobronchi are carried towards the dorsal face of the lung while the entobronchi are nearer the ventral surface (cf. Fig. 91). Both ectobronchi and entobronchi grow rapidly parallel with and close to the surface of the lung-rudiment. They soon produce secondary branches as projections of their walls and these secondary branches increase greatly in length traversing the substance of the lung at first close to its median surface and, later, deep down in its substance as well—the entobronchial branches growing in a dorsal and the ectobranchial in a ventral direction.

The two sets of branches as their tips approach one another are seen to alternate in position (Fig. 91). When they have approached closely each branch bifurcates and its two tips become closely apposed to the two tips belonging to the other series which lie closest to them. About the thirteenth day these apposed tips become completely fused and their cavities continuous so that there is now established a series of channels running in a dorsiventral direction through the substance of the lung and communicating dorsally with the ectobronchi and ventrally with the entobronchi. The channels in question are termed **parabronchi** (Fig. 91, *par*). These are embedded in an abundant matrix of mesenchyme which from about the tenth day becomes divided up into more or less prismatic masses each having in its axis an individual parabronchus—the prisms being delimited from one another by the development of intervening blood-vessels. The mesenchyme which constitutes the inner portion of this sheath round each parabronchus becomes later replaced by a layer of smooth muscle fibres.

At about the same period as the fusion of the parabronchial tips takes place, the wall of the parabronchus begins to grow out into numerous little pockets arranged in radiating fashion. These extend outwards, perforating the muscular sheath, and at a short distance from the parabronchus divide into branches which in turn elongate and become the air-capillaries of the fully developed lung. Judging from adult structure it would appear that the tips of these fuse with others to form the continuous air-capillaries so that the latter would be formed much in the same way as the parabronchi but it has not been possible, so far, to demonstrate this by actual observation.

The essential features of the development of the Bird's lung as above outlined may be summed up in the statement that in this type of lung the diverticula of the intrapulmonary bronchus, which in other Vertebrates end blindly, become here joined together tip to tip to form continuous tubular channels. To allow this arrangement to function efficiently an apparatus is needed to force the air through the system of respiratory tubes: such an apparatus is provided by the air-sacs.

AIR-SACS.—The ventral part of the lung-rudiment is for a time formed of a thick mass of mesenchymatous tissue which has been termed by Bertelli the **primary diaphragm**, from the fact that it becomes continuous along its lateral margin with the side wall of the splanchnocoele, so as to form a kind of floor separating off the lung from the splanchnocoele which lies ventral to it. The air-sacs arise as outgrowths of the bronchial cavities and are on each side four in number: the first or most anterior giving rise to the cervical sac, the second by bifurcation to interclavicular and anterior thoracic sacs, the third to the posterior thoracic and the fourth to the abdominal sac. The rudiments sprout out into the substance of the primary diaphragm and become greatly distended within it, bulging out ventrally amongst the viscera so that the ventral layer of the diaphragm becomes stretched out into a thin membranous wall delimiting the cavity of the air-sac on its ventral side. The dorsal part of the primary diaphragm, lying above the air-sacs, persists as the floor of the lung or **secondary diaphragm** (ornithic diaphragm of Bertelli, pulmonary aponeurosis of Huxley).

The air-sac rudiments sprout out (Fig. 92) from the main pulmonary cavities—the cervical from the first entobronchus, the interclavicular and anterior thoracic jointly from the third entobronchus, the posterior thoracic and the abdominal from the mesobronchus. Later on additional secondary communications between the air-sac cavity and the pulmonary cavities are established (except in the case of the cervical air-sac) by means of the **recurrent bronchi** of Juillet. These arise in the ordinary fowl about the tenth day of incubation in the form of outgrowths of the wall of the air-sac either near its tip (interclavicular and anterior thoracic) or just before it emerges through the general surface of the lung (posterior thoracic and abdominal) as shown in Fig. 92.

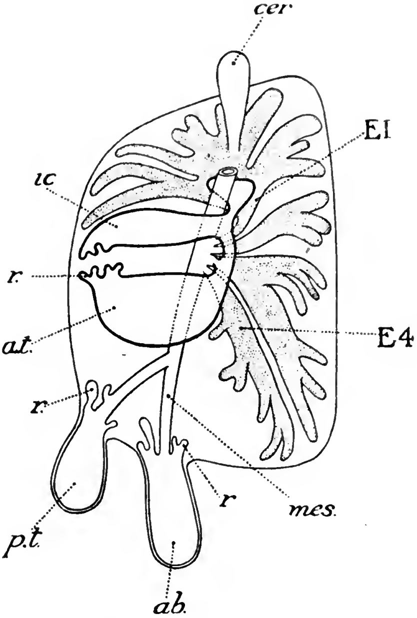


Fig. 92.—Diagrammatic view of the right lung of a Fowl embryo of the tenth day as seen from the ventral side, illustrating the origin of the air-sacs. (After Juillet, 1912.) The four entobronchi are shaded.

ab, abdominal air-sac; *at*, anterior thoracic air-sac; *cer*, cervical air-sac; *E*1 and *E*4, first and fourth entobronchi; *ic*, interclavicular air-sac; *mes*, mesobronchus; *p.t*, posterior thoracic air-sac; *r*, recurrent bronchus.

These outgrowths burrow into the superficial layer of the lung, branch and become joined up, in a manner the details of which have not yet been worked out, with the system of parabronchi. The communications are visible in suitable preparations of the adult lung as groups of openings, each group leading into the lung from the appropriate air-sac—those of the interclavicular and anterior thoracic lying towards the lateral edge of the ventral surface of the lung, about the level of the attachment of the bronchus, and those of the posterior thoracic and abdominal sacs lying near the hind end of the lung, close to the direct opening between it and the corresponding air-sac.

It would appear that the function of these recurrent channels is to conduct the air forced out of the air-sacs in the expiratory effort through the system of air-capillaries, the muscular coat of the parabronchi doubtless playing an important part in directing the passage of the air through the system of air-capillaries rather than through the parabronchi themselves.

The formation of the air-sacs does not exhaust the remarkable proliferative powers of the wall of the lung in Birds. Further outgrowths arise from the walls of the air-sacs which burrow through the neighbouring tissue, even through bone, taking the place of the marrow and rendering the bones pneumatic. Such outgrowths may extend even into the terminal phalanges of the digits. They may also extend in amongst the connective tissue of the skin or between the muscles.[1]

THE LUNG IN FISHES

In the typical fishes or Teleostei, which of all Vertebrates are the most highly specialized in adaptation to a purely swimming habit, one of the most characteristic organs is the swim-bladder or **air-bladder**. In its most highly developed form (in the **physoclistic** Teleosts) this consists of a closed sac, lying above—dorsal to—the splanchnocoele and filled with gas containing a large proportion of oxygen. Special developments of the lining epithelium provide a mechanism whereby the amount of gas in the organ can be increased by a process of secretion or diminished by a process of absorption.

This mechanism, which is under the control of the nervous system, has for its main function the keeping the body of the fish at the same constant specific gravity as the water in which it is swimming — counteracting changes in its specific gravity which would otherwise result from variations of pressure due to change of depth, or from variations in volume of gas produced by fermentative processes in the alimentary canal. The air-bladder with its compensating mechanism keeps the fish precisely at the specific gravity of the surrounding medium so as to obviate the expenditure of muscular effort in order to keep at one depth such as is necessary in the case of a shark or other fish unprovided with an air-bladder.

The air-bladder arises in development as an outgrowth of the wall of the alimentary canal behind the region of the gill-clefts. This burrows its way backwards dorsal to the splanchnocoele and eventually attains to a large size. Its tubular connexion with the alimentary canal (pneumatic duct) becomes constricted across and severed so that the organ is completely isolated from the alimentary canal. In a good many cases however—namely, in the **physostomatous** Teleosts—the duct persists and remains patent throughout life.

In many fishes the dorsal wall of the air-bladder bulges out in a headward direction (Fig. 93) forming a diverticulum which may reach a great size so that in the adult the organ has the appearance of being composed of two segments marked off from one another by a constriction (Fig. 93, B), the pneumatic duct communicating with the hinder one of the two. The constriction may become accentuated

---

[1] Since the above account was written a full and well-illustrated description of the development of the Fowl's lung has been published by Locy and Larsell (*Amer. Journ. Anat.*, xix and xx, 1916). These authors' results amplify and in the main confirm those of Juillet.

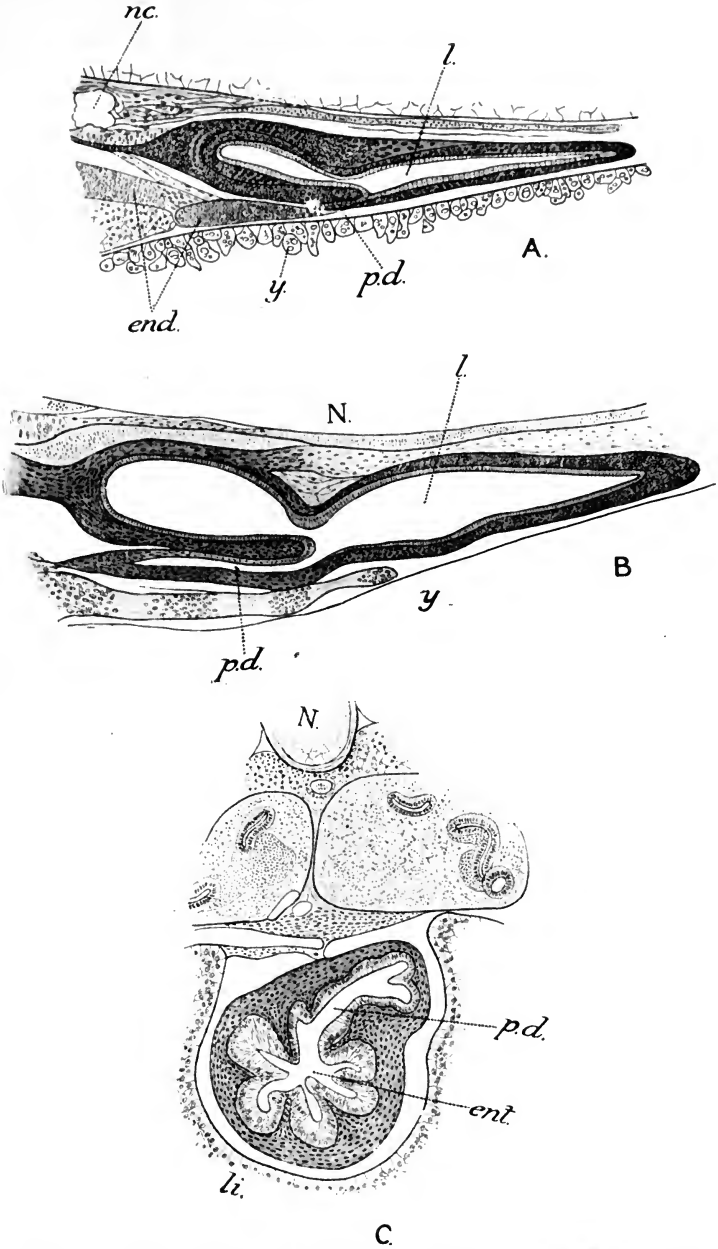


FIG. 93.—Development of the air-bladder of a Teleost. (After Moser, 1904.)

A, *Rhodeus*, 5 mm., longitudinal section; B, *Rhodeus*, 6 mm., longitudinal section; C, *Rhodeus*, 7 mm., transverse section, showing small pouch-like outgrowth of pneumatic duct; *end*, endoderm; *ent*, enteric cavity; *l*, air-bladder; *li*, liver; *N*, notochord; *nc*, pronephric chamber; *p.d*, pneumatic duct *y*, yolk.

to form a kind of diaphragm perforated in its centre and capable of being thrown into vibration by air being forced from one chamber into the other so as to function as a sound-producing organ (*e.g.* Gurnards). Other outgrowths may develop: thus for example in many Siluroids numerous branched projections are formed along each side of the air-bladder.

The air-bladder rudiment is at its first appearance in some cases approximately dorsal in position (*Salmo*). In *Rhodeus* Moser (1904) has shown that the diverticulum is at first on the right side of the alimentary canal. The same observer found that during the early stages of development of the air-bladder the portion of alimentary canal from which it springs undergoes a process of rotation about its long axis in such a direction that a point on its dorsal surface is carried towards the left side.

Although the actual development has been worked out only in a few cases, we may infer safely from the adult relations (Rowntree, 1903) that the amount of this rotation differs greatly in different members of the group Teleostei. Thus in Siluroids and Cyprinodonts the glottis or pharyngeal opening is in the adult still to the right of the mesial plane; in others such as the genera *Osmerus, Clupea, Chirocentrus* it is practically median; in still others such as Mormyrids, Characinids, Gymnotids and Cyprinids it has passed the mesial plane so as to lie upon its left side, while in the case of the Characinids *Macrodon, Erythrinus* and *Lebiasina* the glottis has come to be completely lateral on the left side. This rotation of the gut in the region of the glottis is of much morphological importance as will be shown later.

In the young *Rhodeus*, 7 mm. in length, Moser finds that a well-marked diverticulum from the pneumatic duct is present (Fig. 93, C). Later on it gradually disappears. A similar diverticulum occurs in *Salmo* and in the Carp, and in all probability in numerous other Teleosts: its morphological significance will be discussed later.

ACTINOPTERYGIAN GANOIDS.—In these fishes the development of the air-bladder takes place on similar lines to that described for Teleosts. In *Amia* the additional detail has been made out that the rudiment is at first in the form of a longitudinally placed groove which becomes constricted off from the alimentary canal from behind forwards just as frequently happens in the case of the typical lung-rudiment of air-breathing Vertebrates (Bashford Dean, 1896; Piper, 1902). A rotation of the section of alimentary canal in the region of the glottis takes place similar to that which occurs in the Teleost.

LUNG-FISHES.—In the adult *Ceratodus* an organ occurs which is equally lung and air-bladder. It forms an unpaired sac lying dorsal to the splanchnocoele just like a typical air-bladder, but the pneumatic duct, instead of opening directly into the alimentary canal dorsally, passes round the right side and opens by a ventrally placed glottis. In *Lepidosiren* and *Protopterus* the general arrangement is the same except that here the organ is deeply bilobed: a right and a left lung or air-bladder occupying the place of the single organ of *Ceratodus*.

The meaning of the ventral position of the glottis in these Lung-fishes, and, in fact, the morphological nature of the whole organ, is demonstrated by the examination of early stages in development. In these the organ is found to be a perfectly typical *lung*-rudiment (Fig. 94, B)—a mid-ventral projection from the pharyngeal floor of precisely the same kind as that found in tetrapodous vertebrates (C).[1]

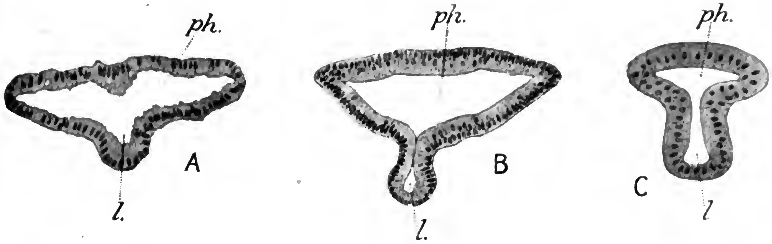


FIG. 94.—Transverse sections through the endoderm of the pharynx showing an early stage in the development of the lung.

A, *Polypterus*, B, *Ceratodus*, and C, *Bombinator* (C after Goette, 1875). *l*, lung-rudiment; *ph*, pharynx.

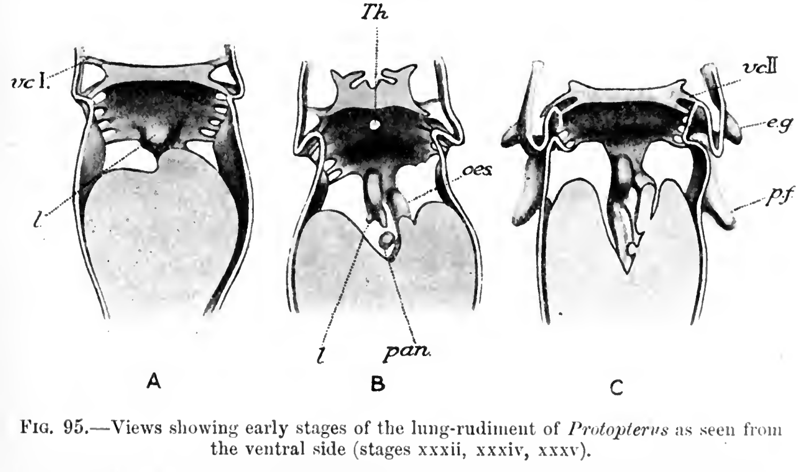


FIG. 95.—Views showing early stages of the lung-rudiment of *Protopterus* as seen from the ventral side (stages xxxii, xxxiv, xxxv).

e.g, external gill; *l*, lung; *oes*, oesophagus; *pan*, dorsal pancreas; *p.f*, pectoral limb; *Th*, thyroid; *v.c*, visceral cleft rudiment. (Cut surfaces are indicated by uniform light tone.)

Subsequent stages are illustrated by Figs. 95 and 96. The lung rudiment at first a rounded knob (Fig. 95, A) grows backwards and soon becomes bilobed (B). The figure does not bring out one important fact namely that the lung-rudiment as it grows backwards

---

[1] The projection is at first *solid* in the case of *Lepidosiren* and *Protopterus*.

twists upon itself, in such a way that points upon its ventral surface would move towards the embryo's right side. (In other words the lung-rudiment rotates about its long axis in a counter-clockwise direction as seen from behind, its front end remaining fixed.) The two lobes are the right and the left lung-rudiment but on account of the rotation just mentioned which extends through more than 180° the left lobe at this stage represents what was originally the right side of the rudiment.

The two lungs of *Lepidosiren* or *Protopterus* are thus reversed in

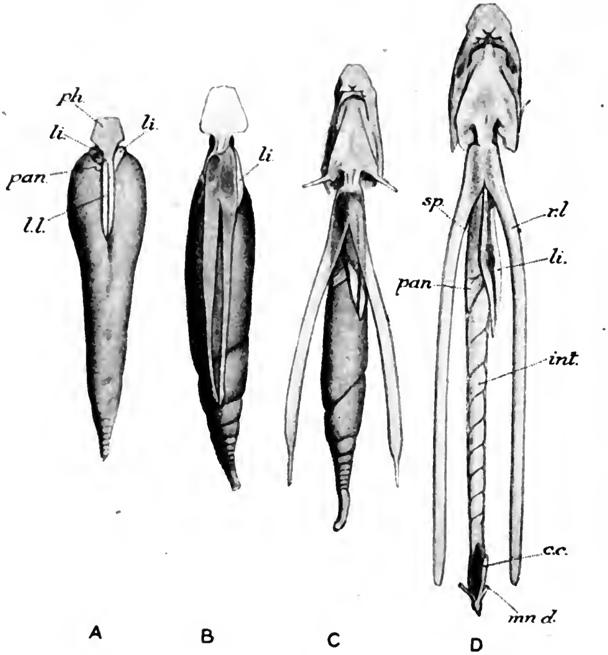


FIG. 96.—Dissections of mid-gut of *Lepidosiren* at stages 32 (A), 35 (B), 36 (C), and 37 (D), showing the modelling of the intestine and also the later stages in the development of the lungs. Seen from the dorsal side.

c.c, cloacal caecum; *int*, intestine; *l.l*, left lung; *li*, liver; *mn.d*, Wolffian duct; *pan*, pancreas; *ph*, pharynx; *r.l*, right lung; *sp*, spleen.

position—the right lung of these forms being homologous with the left of other Vertebrates. An important detail is that in early stages the original right lung, *i.e.* the definitive left, is decidedly larger than its fellow (Fig. 95, B). In later stages this inequality disappears, the smaller lung overtaking the other in its growth (Fig. 96).

In the case of most individuals the lungs assume their dorsal position simply by growing directly tailwards, the oesophagus being pushed out of the way towards the left side (Graham Kerr, 1910). In certain specimens however, which doubtless in this respect retain the archaic mode of development, the lung-rudiment (Fig. 97, *l*) describes a spiral curve round the oesophagus so that the bifurcated

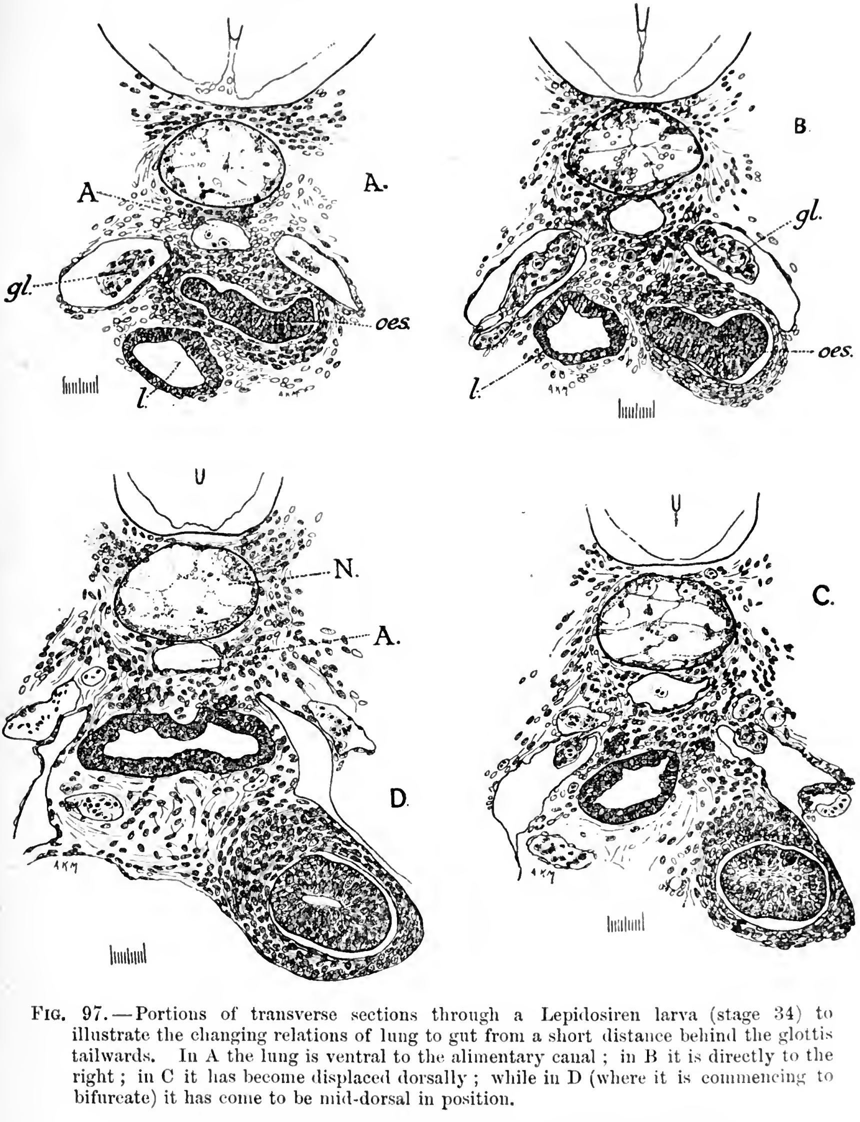


Fig. 97.—Portions of transverse sections through a Lepidosiren larva (stage 34) to illustrate the changing relations of lung to gut from a short distance behind the glottis tailwards. In A the lung is ventral to the alimentary canal ; in B it is directly to the right ; in C it has become displaced dorsally ; while in D (where it is commencing to bifurcate) it has come to be mid-dorsal in position.

A, aorta ; *gl*, glomerulus of pronephros ; *l*, lung ; *N*, notochord ; *oes*, oesophagus.

hinder end of the rudiment, which will give rise to the lungs in the restricted sense, comes to lie dorsal to the alimentary canal.

The lungs continue their tailward growth in the substance of the dorsal mesentery (Fig. 97, D) but eventually the portion of this mesentery containing the lung and dorsal to it becomes greatly thickened from side to side and finally merges completely in the roof of the splanchnocoele, so that in the adult condition the lungs lie completely outside the body-cavity—between it and the vertebral column.

In *Ceratodus* (Gregg Wilson, 1901; Neumayr, 1904) the lung is at first, as in the other two lung-fishes, ventral in position (Fig. 94, B) but in this case the originally left lung, which in *Lepidosiren* and *Protopterus* is for a time during development reduced in size, seems to have disappeared almost entirely, being represented only by a small and transient rudiment. Further detailed studies of the early stages in the development of the lung of *Ceratodus* are much needed to make clear the origin and fate of this vestigial left lung. But it seems clear from what is already known that the monopneumatic condition of *Ceratodus* has come about in evolution through the suppression of the originally left lung.

As the lung completes its development, its cavity becomes encroached upon by two median longitudinal ridge-like ingrowths, one dorsal and the other ventral. It used to be supposed that these marked an incipient division of the lung into a right and a left half so as to bring about the condition seen in *Lepidosiren* or *Protopterus*—the monopneumatic condition being supposed to be the more nearly primitive. It will have been gathered from what has been said that this point of view is no longer tenable and that the monopneumatic condition of *Ceratodus* is to be looked on as secondary and not primary.

CROSSOPTERYGIANS.—Of the two surviving examples of the Crossopterygian ganoids—the most archaic existing members of the Ganoid-Teleostean stem—a few stages in the development of the lung have been investigated in *Polypterus* (Graham Kerr, 1907). In the earliest stage observed the lung-rudiment was in the form of a mid-ventral groove formed by an outgrowth of the pharyngeal lining (Fig. 94, A, *l*). This groove becomes deeper and towards its posterior end widens out ventrally so as to have a ⊥-shape in transverse section.

Posteriorly the lung-rudiment grows back into a pair of horn-like projections—the rudiments of the right and left lung. These extend backwards in the connective tissue of the splanchnopleure and they very soon show a marked inequality in their rate of growth the left lagging behind the right. As growth goes on this inequality becomes more and more marked, so that in a larva of about 30 mm. in length the right lung extended right back to the cloaca while the left projected back only about 3 mm. behind the glottis.

In these later stages another important feature is to be noticed, one which is correlated with the fact that the air-filled lung necessarily acts as a float in an aquatic animal. This feature is that the lung tends to assume a position symmetrical about the median plane. Thus in the anterior region where both lungs are present they are situated laterally, balancing one another, while farther back where only the right lung is present this shifts towards the mesial plane until it is symmetrical about that plane, lying in the dorsal mesentery (Fig. 98, A and B).

EVOLUTION OF THE AIR-BLADDER.—The facts that have been enunciated above, with regard to the development of the lung in Dipnoan and Crossopterygian fishes, are of much morphological interest. When pieced together with what has been said regarding the development of the air-bladder of Teleostean fishes they afford data from which the evolutionary history of the Teleostean air-bladder can be traced out with a high degree of probability. That history may be stated in a few words to have probably been as follows:

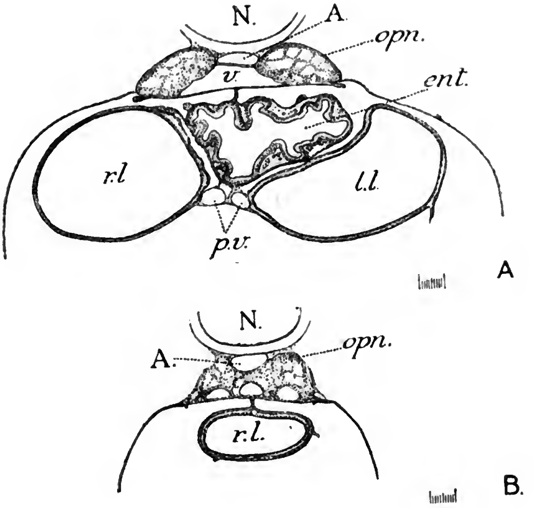


FIG. 98.—Sections through the lungs of a larva of *Polypterus* 30 mm. in length.

A, more anterior; B, more posterior; *A*, aorta; *ent*, enteron; *l.l*, left lung; *N*, notochord; *opn*, opisthonephros; *p.v*, pulmonary veins; *r.l*, right lung; *v*, interrenal vein.

1. The primitive condition was that of a lung, communicating with the pharynx by a ventrally placed glottis—for we have seen that the embryonic rudiment of the organ in the most archaic forms possessing it is a typical lung-rudiment.

2. The organ became bilobed, growing back into a right lung and a left lung.

3. In the forms which took to a purely swimming existence, and became specialized in the direction of adaptation to this, there came about an asymmetry of the lungs, the right lung increasing and the left lung diminishing. Why this should have happened is not yet absolutely certain: it may probably have been in adaptation to active movements of lateral flexure, for we see the same thing taking place in Gymnophiona, Snakes and Snake-like Lizards. That it has been the right rather than the left lung which has increased in size, is probably correlated with the rotation of this region of the alimentary canal in a counter-clockwise direction as seen from behind (see p. 168) which would tend to interfere more with the circulation through the left lung than with that through the right, by lengthening the course of the left pulmonary artery. Steps

in the development of this asymmetry are seen in *Polypterus* and in the Lung-fishes.

4. In purely aquatic creatures the dictates of adaptation would naturally cause the air-filled lung to assume a dorsal position. An initial phase of this is repeated in *Polypterus* where the right lung has become dorsal and median in its hinder portion. In the Lung-fishes a further step is taken—the whole of the lung becoming dorsal except the pneumatic duct which still remains to mark out the path by which the lung moved dorsalwards round the right side of the alimentary canal.

That the movement dorsalwards was round the *right* side was no doubt due to the right lung being predominant and the left reduced in size. In the case of *Ceratodus* the predominance of the original right lung has been retained, the other being completely obsolete except for a short period during development. In *Lepidosiren* and *Protopterus*, on the other hand, the lopsidedness disappears, the original left lung regaining during ontogeny its primitive equality in size with its fellow.

5. In the Actinopterygians—those fishes which show the highest degree of evolution in adaptation to a swimming mode of life—the lung has in the course of its evolution passed through similar stages to those exemplified by *Polypterus* and *Ceratodus*. Here again only the original right lung persists as the air-bladder, the vestige of the left lung being possibly represented by the little diverticulum found by Moser upon the pneumatic duct in early stages of development.[1] In the Actinopterygians a further step onwards has been made in that the glottis has assumed a dorsal position. This is fully explicable by the rotation which this part of the gut has undergone, aided no doubt by the principle of economy of tissue which would tend to bring about a shortening of the unnecessarily long pneumatic duct. In some cases there still persist vestiges of the ancient cellular respiratory lining of the swim-bladder (*e.g. Lebiasina, Erythrinus*).

6. Finally in the Physoclistic forms—the most highly specialized of all—the swim-bladder has become completely isolated from the gut, its respiratory function has gone and it subserves a mainly hydrostatic function.

The outline given above represents a scheme of evolution which in the light of modern research has a high degree of probability. Of course as in all such evolutionary speculations there exist details which are still difficult to explain. While most of the facts of comparative anatomy fit in well with it, some do not—such as, for example, the nerve-supply and the blood-supply of the air-bladder of *Amia*—but it may be anticipated with considerable confidence that these difficulties will be lessened or disappear with the progress of research.

---

[1] See p. 168. This matter affords an interesting subject for further research.

DERIVATIVES OF PHARYNGEAL WALL OTHER THAN THE RESPIRATORY ORGANS

THYROID.—The Thyroid gland arises as a mid-ventral outgrowth of the pharyngeal or buccal floor about the level of the Hyoid arch. In those Vertebrates in which the pharyngeal rudiment is solid at this stage the thyroid outgrowth is also solid at its first appearance (Fig. 99, A, *Th*) and develops its cavity secondarily by cytolysis.

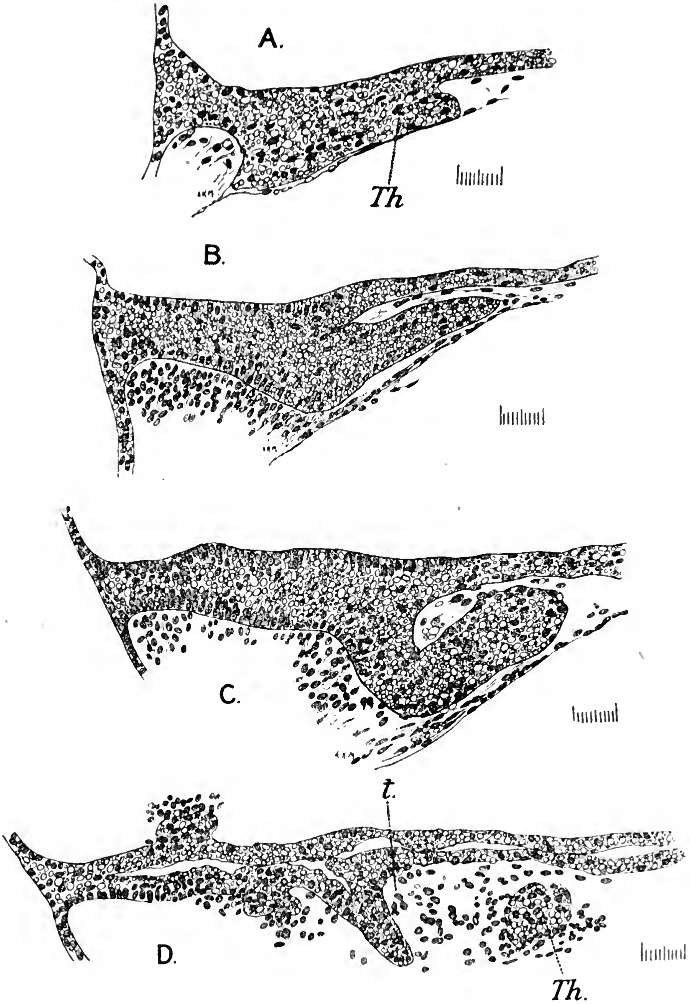


FIG. 99.—Sagittal sections through anterior portion of alimentary canal of *Lepidosiren* illustrating the development of the Thyroid.

A, B, C from specimens of stage 30; D, stage 31; *Th*, thyroid; *t*, tongue.

The Thyroid becomes gradually constricted off from the pharynx (Fig. 99, B and C) remaining for a time connected by a narrow stalk or duct with the pharyngeal or rather buccal floor just in front of the primary tongue (see Fig. 82, p. 149). This stalk of attachment becomes nipped across and the thyroid forms a mass (Fig. 99, D) or vesicle rounded in form or somewhat elongated in an antero-posterior direction lying in the mid-ventral line beneath the pharynx and just in front of the ventral aorta.

The originally simple vesicle undergoes a process of sprouting and division by which it becomes converted into a mass of rounded vesicles, each possessing a wall composed of a single layer of cubical epithelial cells and separated from its neighbours by highly vascular mesenchyme which penetrates in between the vesicles to form the stroma of the organ.

During later development the Thyroid undergoes characteristic changes of form in different subdivisions of the Vertebrata. Thus in Teleosts it frequently assumes a more or less diffuse character, the follicles being distributed in the neighbourhood of the ventral aorta and roots of the afferent branchial vessels. In the Amphibia and Amniota the organ becomes deeply constricted into two laterally placed lobes which may remain connected or may become separated, so that it assumes a paired character as happens in Amphibians and Birds.

With the processes of differential growth involved in the development of the neck, the thyroid may undergo considerable displacement from its point of origin. Thus in adult Lizards it lies across the trachea well forwards from its hind end while in other reptiles and in birds it lies farther back close to the roots of the great arteries.

It is now generally accepted that the clue to the phylogenetic history of the Thyroid is afforded by its development in *Petromyzon* (W. Müller, 1871). Here there develops a mid-ventral outgrowth of the pharyngeal floor, forming a short gutter in the branchial region, the lining of which is composed partly of glandular cells which secrete a sticky mucus and partly of cells which bear powerful flagella. Morphologically this gutter is the same as the endostyle of *Amphioxus* and during larval life its function is also similar: it appears to be in fact simply a shortened up endostyle. The slit-like pharyngeal opening becomes gradually reduced in length till it forms merely a small pore.

At the time of metamorphosis the pore becomes obliterated so that the organ becomes a closed vesicle underlying the pharynx. This vesicle divides up into a number of small vesicles and its mucous secretion accumulates in their interior as a colloid substance like that of the Thyroid vesicles of the Gnathostomata. In a word, the endostyle of the Ammocoetes stage becomes the Thyroid of the adult, and there seems no reason to doubt that the same has happened in phylogeny and that the thyroid of the Vertebrate is simply the modern representative of the endostyle of the protochordate ancestor.

An interesting feature is that while the physiological importance of the thyroid in the modern Vertebrate is that of a ductless gland for the production of internal secretion to be absorbed by the blood, it still goes on producing the mucous material used by the far back protochordate ancestor for entangling food particles, though that substance is no longer, owing to the disappearance of the duct, discharged into the pharyngeal cavity.

BRANCHIAL BUDS.—There make their appearance in the developing Vertebrate a series of bud-like proliferations of the endodermal epithelium of the branchial clefts which may be known as branchial buds. They appear at the upper and lower angles of the clefts and the series shows its fullest development in the Lampreys, where buds develop at the dorsal and ventral angles of all the clefts. In the majority of fishes investigated they have been found to appear at the dorsal angles of all the clefts except the first; in Urodele Amphibians at the dorsal angle of all clefts and at the ventral angle of II., III. and IV.; in Anura at the dorsal ends of I. and II. and at the ventral ends of II.-V.; in *Lacerta* at the dorsal ends of I.-III. and the ventral ends of III. and IV.; in *Gallus* at dorsal and ventral ends of III. and IV.

The morphological significance of these organs is still completely obscure. Physiologically some of them appear to be of importance during the later stages of development preceding sexual maturity inasmuch as they give rise to that often bulky organ the **Thymus**. This arises by the fusion together of more or fewer of the dorsal buds, the others undergoing no further development. Thus in *Lepidosiren* (Bryce, 1906) dorsal buds III. and IV. develop into thymus while II. and V. undergo no further development: in *Ceratodus* (Greil, 1913) II., III. and IV. give rise to Thymus while V. and VI. do not develop further: in *Hypogeophis* II., III., IV. and V. give rise to Thymus while rudiments on I. and VI. atrophy.

In regard to the much discussed histogenesis of the thymus all that need be said here is that the originally solid epithelial rudiment becomes in the course of development loosened out into a sparse reticulum interpenetrated by mesenchyme richly traversed by blood-vessels and crowded with leucocytes.

The ventral buds, where they occur, become constricted off from the branchial epithelium forming simple rounded masses of epithelial cells (Amphibians) or they may be subdivided up by intrusive connective tissue into solid portions (Reptiles) or hollow vesicles (Birds). The small organs so formed are termed by their discoverer Maurer epithelial bodies: their physiological significance is quite unknown.

There normally develops in the Vertebrate either on both sides or only on the left side a small pouch-like diverticulum of the pharyngeal wall close to the ventral edge of the last gill cleft, whatever the number of this be in the morphological series. The diverticulum becomes separated from the pharynx and commonly gives rise to numerous rounded vesicles somewhat resembling those of the thyroid in appearance. The organ thus formed was named by van Bemmelen who discovered it in Elasmobranchs—suprapericardial body—while Maurer has termed it the postbranchial body. Nothing is definitely known regarding either its function or its evolutionary history, though it is sometimes regarded as representing a vestigial last gill-pouch. A curious point is the tendency of the organ to unilateral development as it makes its appearance only upon the left side in a large number of cases (*Acanthias*, *Lepidosiren* and *Protopterus*—see Fig. 109, B—most Urodeles, some Lizards).

CEMENT ORGANS OF TELEOSTOMATOUS FISHES.—It has long been known that the larvae of Actinopterygian ganoids possess cement-organs on the head in front of the mouth. Balfour (1881) wrote of this as "a very primitive Vertebrate organ, which has disappeared in the adult state of almost all the Vertebrata; but it is probable that further investigations will show that the Teleostei, and especially the Siluroids, are not without traces of a similar structure."

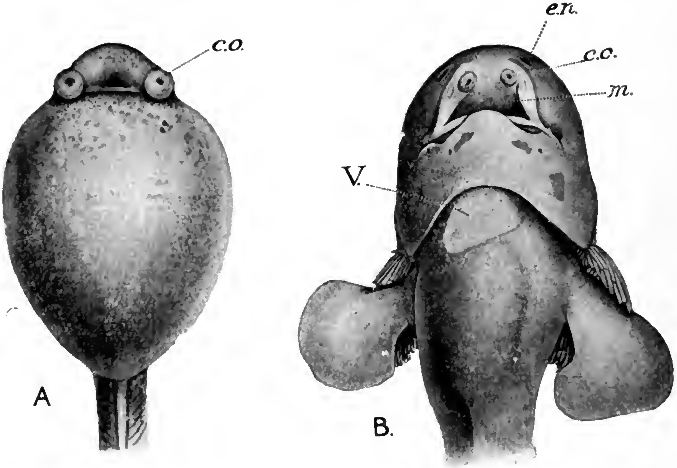


FIG. 100.—Ventral views of Polypterus larva to show the cement-organs.

A, Stage 30; B, Stage 33; *c.o*, cement-organ; *e.n*, olfactory organ; *m*, mouth; *V*, ventricle of heart.

The organs in question were generally regarded as being developed from a thickening of the ectoderm. Miss Phelps (1899) first stated that they originated from endoderm (*Amia*) and the present writer, at the time ignorant of her work, was greatly surprised to find himself forced to this same conclusion by the examination of Budgett's material of *Polypterus*.

The cement-organ of *Polypterus* (Graham Kerr, 1906 and 1907), when at the height of its development, forms a stout cylindrical structure with a deep hollow at its free end, projecting from the head on each side as shown in Figs. 100, A, and 197, C, *c.o.* A longitudinal section through the centre of the organ at about this stage (stage 26, Fig. 101, E) shows that the organ is covered by the ordinary 2-layered ectoderm. Round the lip of the opening at its free end, the superficial layer of ectoderm stops, while the deep layer seems to dip down as a deep involution to form the secretory epithelium (*c.o*) which lines the cavity. All the appearances seem to point to the secretory epithelium being ectodermal in its nature. How deceptive these appearances are will be gathered from an inspection of Fig. 101, A-E.

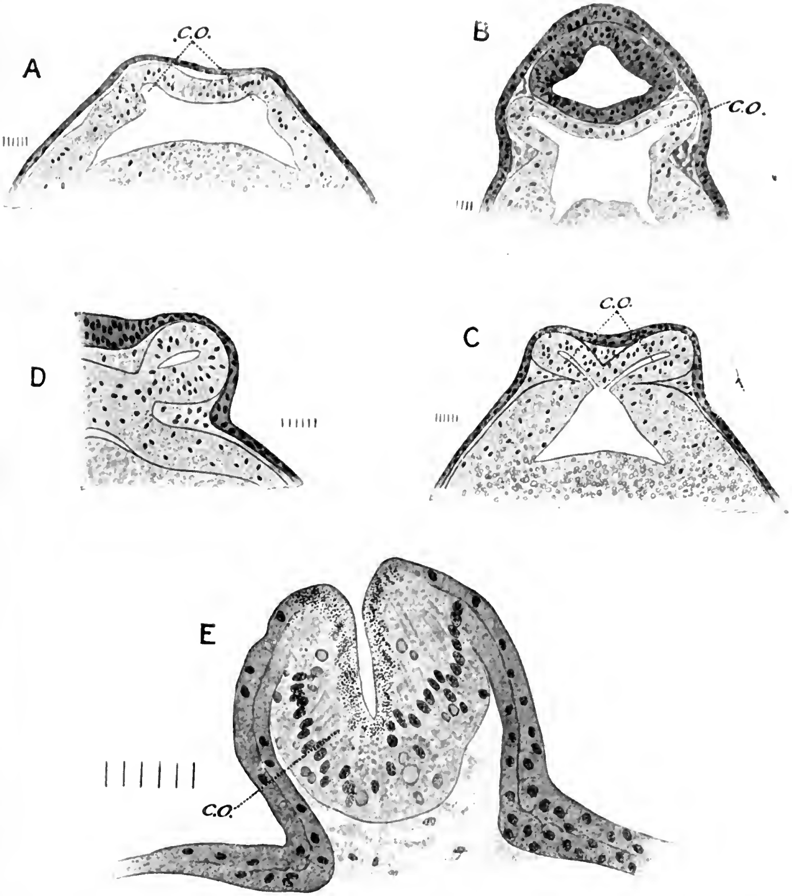


Fig. 101.—Illustrating the development of the cement-organ of *Polypterus*. B represents part of a transverse section, the other figures portions of horizontal sections.

A and B, stage 20; C, stage 23; D, stage 24; E, stage 26. *c.o*, cement-organ. The darker tone indicates ectoderm.

The first rudiment of the organ is seen to be a simple pocket-like outgrowth of the gut-wall (A, *c.o*): this becomes more and more prominent (B, C): it becomes gradually constricted off at its base from the gut-wall, its cavity becoming isolated first (D). Finally it separates completely from the main endoderm and its outer end undergoes fusion with the deep layer of the ectoderm. Its cavity then opens to the exterior and the fully functional condition is reached—the endodermal origin of the secretory lining being for a time betrayed by the conspicuous persistent yolk granules in its cells.

It will be noted that the exposed side of the secretory epithelium, that on which the secretion is extruded, is that which originally faced inwards towards the lumen of the alimentary canal. In other words the direction in which the extrusion takes place is morphologically the same as that of any other part of the glandular lining of the gut-wall.

As is the case in other forms the cement-organ is a transient, purely larval, structure. About stage 31 (Fig. 197, D) degeneration commences: the gland shrivels up, the gland-cells becoming more slender and dark pigment making its appearance in their interior, the epithelium becomes penetrated by ingrowing blood-vessels, its cell-boundaries become indistinct. The process of atrophy goes on rapidly and by stage 36 (Fig. 197, F) the organ has completely disappeared.

An interesting variation from the normal course of development is found in specimens in which the cement-organ rudiments are more or less approximated to one another. This variation reaches its maximum in occasional individuals in which they are completely fused and form an unpaired structure, continuous across the mesial plane.

In the actinopterygian Ganoids the cement-organ develops along the same general lines as those just indicated. In the Sturgeons the development has been worked out recently by Sawadsky (1911) in *Acipenser ruthenus.* Here the organ forms a rounded projection, very much in the same position as that of *Polypterus,* but in this case each becomes divided by a groove so as to form two rounded knobs. These knobs eventually grow out to form the tactile barbels of the adult, the secretory epithelium being carried out on the surface of the barbel as it grows.

The secretory epithelium is here also endodermal, its rudiment being the gut-wall immediately dorsal to the position in which the mouth will develop later and being continuous across the mesial plane. The unpaired condition which occurs in *Polypterus* as a variation is thus normal in the case of the sturgeon. As the head increases in length the secretory epithelium becomes carried out on its ventral surface, looking just as if it were the thickened ectoderm of this surface. Finally the paired condition comes about, the lateral parts of the secretory epithelium coming to be supported by the knob-like projections already mentioned.

Amia is of special interest in regard to its cement-organs as it was in this form that their endodermal origin was first announced.[1] The organs are for a time in the form of a pair of rounded knobs, one on each side, but these take on a crescentic shape so that together they form a circular wall, interrupted anteriorly and posteriorly. Each organ contains a pocket-like projection of the gut-wall which takes on a somewhat sausage-like form in correlation with the curved shape of the organ as a whole. This endodermal sac separates from the main endoderm and becomes constricted across, so as to form a curved row of closed vesicles from six to ten in number. Each vesicle fuses with the ectoderm and develops an opening to the exterior so that it takes on the appearance of a cup at first deep and narrow, later shallow and wider, its lining continuous with the deep layer of the ectoderm.

When the larva reaches a length of 13-14 mm. it makes less use of its cement-organ and the latter commences to degenerate, sinking beneath the surface with which, however, it remains connected by a narrow tubular channel. By about the 20 mm. stage this has disappeared and soon there is no trace of the organ to be found even in sections.

In *Lepidosteus* the organ appears to be similar while in the other ganoids its development still remains to be worked out.

These cement-organs are of special interest and importance for more than one reason. In the first place they are of importance in revealing a quite unexpected pitfall in the way of the investigator trained to have implicit faith in the germ-layer theory, for they show how a particular organ may become transferred from one germ-layer to another even though not belonging to the transitional zone where the two layers are continuous. A very common modification of ontogenetic development consists in the slurring over or even omission of particular stages in early development. Were this to happen in the case of the early stages in the development of the cement-organ say of *Polypterus*, it is easy to see that the organ might have every appearance of being purely ectodermal in its nature, although it is, as a matter of fact, endodermal.

It appears to the present writer quite possible, if not probable, that this modification has actually come about in the Dipnoi and Amphibians, and that the cement-organs of these groups, although they develop from the ectoderm in those forms which have been investigated (p. 79), are really homologous with the cement-organs of the Teleostomi, their endodermal stage having been eliminated from ontogenetic development. Further investigations are needed in the Amphibia—to see whether no trace exists, in any member of the group, of an original connexion with the endoderm.

As regards the original nature of these organs it is impossible to arrive at any certain conclusion. Arising as they do in the form

---

[1] Phelps (1899). The actual discovery seems to have been made by Reighard. Cf. Reighard and Phelps (1908).

of endodermal pockets, they obviously recall gill pouches on the one hand and coelenteric pouches on the other. Their position suggests a pair of premandibular gill pouches: their function, that of forming an excretion (cement), perhaps indicates rather coelomic affinities and the present writer suggested (1906) their possible correspondence with premandibular head cavities of other Vertebrates. Reighard and Phelps (1908) homologize them with the anterior pair of head-cavities of Elasmobranchs while van Wijhe (1914) supports a homology with the ciliated organ of *Amphioxus*.

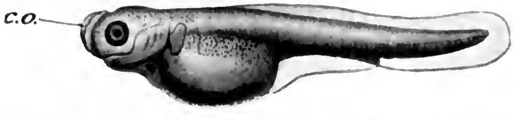


Fig. 102.—Larva of *Sarcodaces odöe*. (After Budgett, 1901.) c.o, cement-organ.

Altogether these cement-organs are very interesting and puzzling structures and would well repay further investigation. A thorough comparative study should be made of their development in the archaic Crossopterygians and of their possible homologues in Elasmobranchs.

Little is known regarding cement-organs in Teleosts, though it is probable they will be found to occur in various tropical freshwater fishes. Budgett (1901) found a large cement-organ on the head of the larva of the Characinid *Sarcodaces odöe* (Fig. 102, *c.o*). In a larva believed to be that of the Mormyrid *Hyperopisus bebe* he found six well-marked cement glands on the head which in this case secrete fine threads by which the larva hangs suspended in the water until the yolk is used up (Fig. 103). *Heterotis* and *Gymnarchus* also possess similar organs—very small in the latter case (Assheton, 1907).

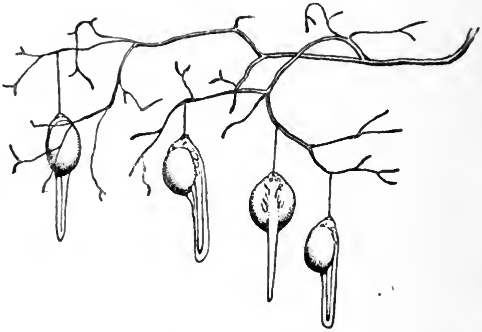


Fig. 103.—Teleostean larvae, supposed to be those of *Hyperopisus bebe*, suspended from the rootlets in the nest. (From Budgett, 1901.)

The organs in these various fishes present the appearance of being ectodermal thickenings: we have as yet no information as to whether, as may be suspected, they really originate from the endoderm.

Digestive Tract.—The respiratory region of the alimentary canal is succeeded by the true digestive tract and this shows more or less pronounced differentiation into successive portions—

oesophagus, stomach, intestine and its subdivisions, cloaca. In correlation with the digestive and assimilative function of the intestinal endoderm this serves during early stages as the favourite storehouse of food-yolk, and the concentration of yolk in the abapical portion of the unsegmented egg is to be looked on as a foreshadowing of the fact that this portion of the egg will later become the endoderm.

In the holoblastic Vertebrates the mass of heavily yolked endoderm cells becomes, as it were, modelled into a tubular shape by the reciprocal activity of endoderm and splanchnic mesoderm; the rudiment so formed undergoing active growth in length and differentiation of structure while the yolk is being assimilated.

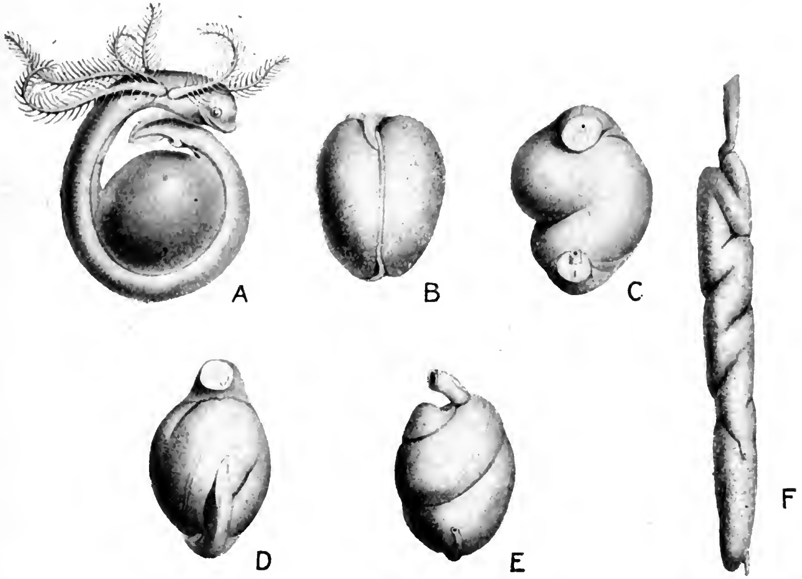


Fig. 104.—Illustrating the modelling of the yolk in *Ichthyophis*. (After Sarasins, 1889.)

A and B illustrate the same stage, B representing a view from the dorsal side. The small-celled epithelial portion of the gut-wall is seen passing down the centre of Fig. B. C, D, and E represent later stages drawn from the ventral side; F (7 cm. embryo) ventro-lateral view from the right side.

In the two most archaic groups of holoblastic gnathostomes, the Crossopterygians and the Lung-fishes, a feature of special interest is the development of the spiral valve. In *Lepidosiren*, as is indicated by Figs. 105 and 106, this takes its origin by the solid mass of yolk-laden endoderm becoming modelled into a right-handed spiral coil — the deep incision which separates successive turns of the spiral being filled up by ingrowing mesenchyme belonging to the splanchnic mesoderm. There can be little doubt that this is a secondarily modified mode of development, but nevertheless it is probable that the spiral coiling of the endodermal rudiment is to be explained as a repetition of an ancestral condition in which the intestine as a whole was long and spirally coiled.

An important feature of such a spiral coiling of the gut rudiment is that it would necessarily tend to bring about a twisting of the alimentary canal just in front of the spirally coiled portion in a counter-clockwise direction as seen from behind, *i.e.* a movement in which points on the ventral side of the alimentary canal would become shifted towards the right side. As already indicated such a twisting of this region of the alimentary canal actually does take

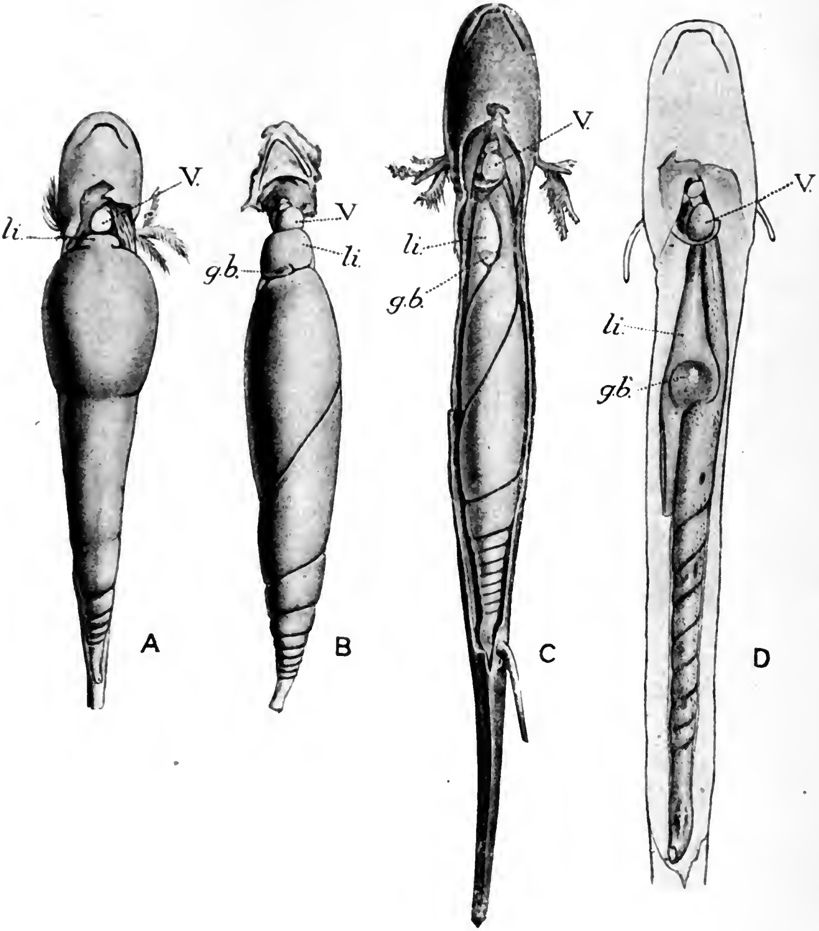


FIG. 105.—Dissections of young Lepidosirens of stages 32 (A), 35 (B), 36 (C), and 37 (D), from the ventral side to show the modelling of the intestine.

g.b, gall-bladder; *li*, liver; *V*, ventricle.

place in development causing the lung rudiment to shift dorsally round the right side of the alimentary canal.

In the more richly yolked Vertebrates the ventral portions of the gut-wall are more and more clogged up with yolk and this results in a greater and greater concentration of developmental activity in the dorsal wall. This is clearly indicated by transverse sections through the developing gut of Vertebrates which though rich in yolk are still holoblastic. Such sections (Fig. 107) show the dorsal wall of the gut to consist of small active cells arranged as a columnar epithelium, while the side walls and floor consist of large comparatively inert yolk-laden elements. It is only as development goes on, and as the yolk is consumed, that the epithelial small-celled character gradually spreads ventrally.

In the actually meroblastic Vertebrates, the heavily yolked portions of the primitive gut-wall never undergo segmentation at all, unless possibly as regards a thin superficial layer. They remain as a continuous mass of yolk, round which the epithelium gradually spreads. In this case the formation of all the important organs of the alimentary canal is concentrated in the dorsal portion which becomes gradually folded off from the main mass of the yolk. This folding-off process takes place most actively in the anterior region, so as to form the tubular fore-gut, and also posteriorly, the intermediate portion re-

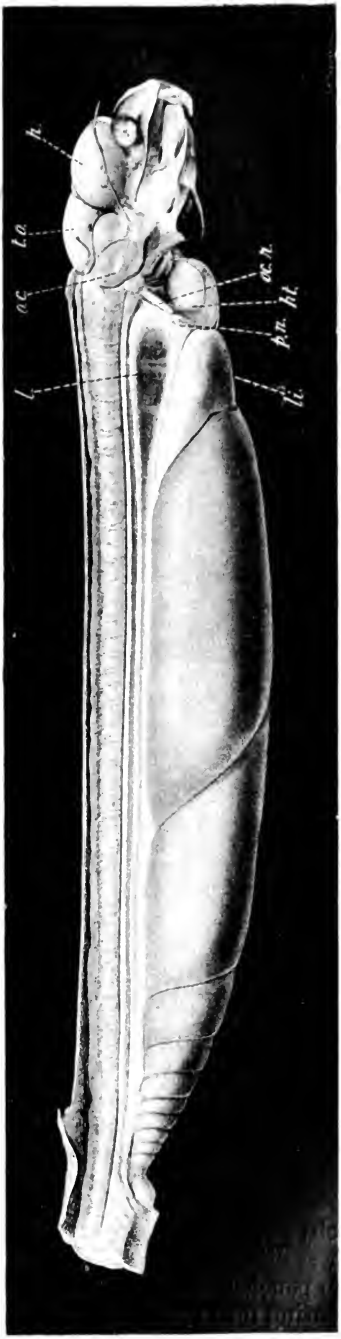


FIG. 106.—Dissection of Lepidosiren larva of stage 35.

h, hemisphere; *ht*, heart; *l*, lung; *li*, liver; *o.c*, auditory capsule; *oc.r*, occipital rib; *p.n*, pronephros; *t.o*, tectum opticum.

maining for a time as a longitudinal groove opening ventrally towards the yolk. As the lips of this groove gradually coalesce at each end the communication between the gut cavity and the yolk becomes gradually narrowed down to the tubular cavity of the **yolk-stalk** situated at first behind the liver but later becoming shifted forwards by differential growth. Eventually this becomes obliterated and the definitive alimentary canal becomes completely isolated from what remains of the yolk. In many Teleostean fishes this isolation takes place at a very early stage in development.

The alimentary canal is, in correlation with its digestive function, necessarily a highly glandular organ. Primitively the secretory functions are carried out by unicellular glands, scattered about amongst the other epithelial cells of the endoderm, but in the Vertebrates, as in all the more complex Metazoa, special concentrations of gland cells and of secretory activity take place in localized portions of the enteric wall. Each of these specially glandular patches undergoes a great increase in its area, which causes it to bulge outwards as a simple or much subdivided and complicated pocket, forming a distinct glandular appendage of the alimentary canal.

FIG. 107.—Transverse section through hind portion of intestine of a larva of *Ichthyophis*. (After Sarasins, 1889.) The stage of development was that shown in Fig. 104, F. The sheath of splanchnic mesoderm is omitted.

LIVER. — Of these glandular appendages, in the case of Vertebrates, the most ancient appears to be the liver, which is already present in *Amphioxus*. In this animal the liver originates in ontogeny (Hammar, 1893) as a pocket-like outgrowth of the alimentary canal wall on its ventral side and slightly posterior to the hind end of the pharynx. Apart from increase in size and relative narrowing of its base of attachment the liver in *Amphioxus* undergoes no further complication but retains its extraordinarily primitive pouch-like condition throughout life.

In the holoblastic Craniates the liver arises similarly as a ventral projection of the alimentary canal wall. This shows the customary modifications in correlation with the presence of yolk, arising in some cases in the more primitive fashion as a hollow pocket (Lampreys, many Amphibians, *Ceratodus*), in others (many Amphibians, *Lepidosiren* and *Protopterus*) as a solid knob of yolk-laden cells (Fig. 105, *li*). This grows rapidly in size, as it uses up its food-yolk, and becomes constricted off from the main mass of yolk by ingrowing mesenchyme, until its attachment becomes narrowed down to a slender stalk—the rudiment of the **bile-duct**.

The pouch-like rudiment of the liver undergoes an active process of sprouting into numerous secondary pockets, each of which becomes greatly elongated and branched, and gives the gland a tubular character. This character may be retained throughout life (Lampreys) but normally the tubules undergo anastomosis so as to form a network of trabeculae. While this is to be regarded as the primitive mode of development of the tubules it is to be noted that they more usually in actual fact show the modification of development which we have learned to associate with the presence of yolk, being at first solid and taking their origin not by a process of outgrowth but rather by a process of modelling by ingrowing mesenchyme.

In the meroblastic Vertebrates also the liver may be described as originating from a mid-ventral outpushing of the enteric wall. Variations occur in detail, in correlation with the varying relations of the hepatic portion of enteric wall to the fore-gut and yolk-sac. If this part of the gut-wall has already been folded off from the yolk-sac and incorporated in the fore-gut, then the early stages of development of the liver diverticulum pursue their normal course. If, on the other hand, it still forms part of the yolk-sac wall, the hepatic rudiment makes its appearance as a projection from this, and it may be in its first beginnings paired, its two halves separated by the longitudinal slit by which the cavities of the definitive gut and the yolk-sac are still continuous.

ELASMOBRANCHII.—The hepatic diverticulum at an early stage bulges out to form a conspicuous outgrowth on each side anteriorly—the rudiments of the right and left lobes of the liver. The median portion between these becomes in its anterior region converted into secretory tissue while its posterior part becomes the bile-duct, with its dilatation the gall-bladder.

In *Acanthias* (Scammon, 1913) the first rudiment of the liver, which makes its appearance at a time when this region of the enteron is not yet floored in but opens freely into the subjacent yolk-sac, is distinctly paired. In view of the unpaired condition in *Amphioxus* and the holoblastic Craniates there can be little doubt that this condition in *Acanthias* is a secondary modification as indicated above. Secondary pockets soon make their appearance on the wall of the secretory portion of the rudiment, and grow actively into elongated and much-branched tubules. These fuse together secondarily to form the network characteristic of the fully developed liver. This network is bathed by the blood of the vitelline veins (see Chap. VI.).

After the embryo (*Acanthias*) has reached a length of 25-28 mm. the walls of the tubules, or trabeculae of the network, increase greatly in thickness so that both their own cavities and the intervening blood-spaces become relatively reduced and the organ assumes the compact definitive condition.

Whereas the tubules become throughout the greater part of their extent secretory in function the proximal portions, each common to a group of tubular branches, function merely as ducts. These communicate with the main bile-duct formed from the posterior and median portion of the rudiment. The gall bladder originates as a bulging of the floor of the bile-duct towards its anterior end.

The formation of the posterior and longer section of the bile-duct, which will be extrahepatic in the adult, lags in its development behind the anterior portions of the rudiment. Such differences in the time of appearance of different parts of the hepatic apparatus—liver, gall-bladder, bile-duct—are to be looked on as mere secondary modifications of development,—the primitive condition being that of a simple pocket of the gut-wall such as persists in *Amphioxus*.

SAUROPSIDA.—The hepatic apparatus here again makes its appearance as a longitudinally situated pocket of the morphologically ventral wall of the gut. In birds this is situated at first on the anterior wall of the yolk-stalk (Fig. 108, A). The diverticulum grows actively into an anterior (dorsal) and a posterior (ventral) pocket (Fig. 108, C, *li.* 1 and *li.* 2) while the intervening portion becomes flattened out and incorporated in the gut-wall.

There thus come to be two distinct liver-rudiments an anterior and a posterior. Of these each sprouts out at its end into irregular projections which eventually fuse and form a spongy mass, surrounding the cavity of the ductus venosus, and having in its meshes blood-spaces which

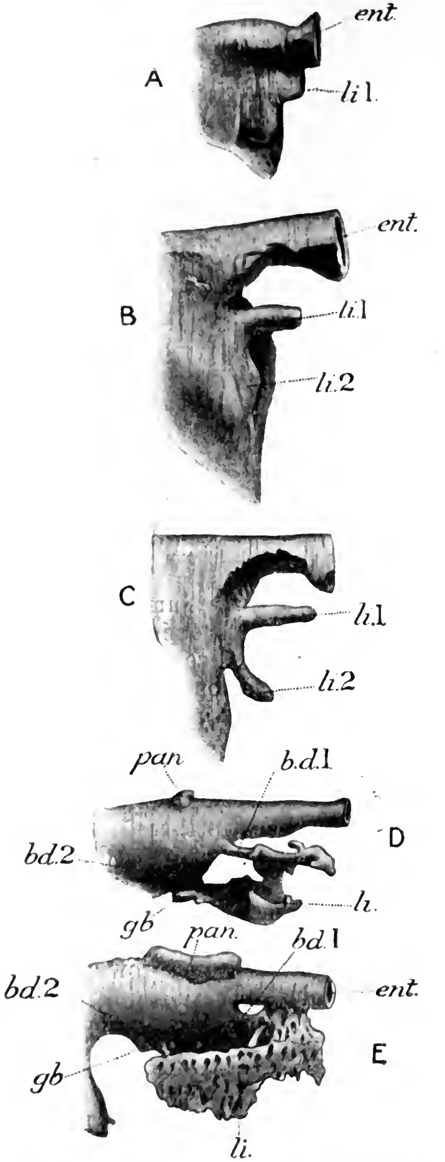


FIG. 108.—Illustrating early development of the liver in Birds.

A, 47-hour chick; B, 52-hour chick; C, 50-hour chick (after Brouha, 1898); D, fourth-day chick; E, 7 mm. embryo of the Roseate Tern—*Sterna paradisiaca*—(after Hammar, 1897). bd^1, rudiment of anterior ("left") bile-duct; bd^2, posterior ("right") bile-duct; *ent*, cavity of fore-gut; *gb*, rudiment of gall-bladder; *li.* 1 and 2, anterior and posterior liver-rudiments; *pan*, dorsal rudiment of pancreas.

communicate with the just-mentioned vessel. This spongy mass, the trabeculae of which are at first solid and only secondarily develop a lumen, forms the secretory portion of the liver, while the proximal portions of the outgrowths persist as the two conspicuous bile-ducts of the adult bird (Fig. 108, D, E, *bd.* 1 and *bd.* 2). In such birds as possess a gall-bladder this is formed by a dilatation close to the point of junction of the posterior bile-duct with the gut-wall (Fig. 108, D, E, *gb*).

PANCREAS.—The pancreas, though in the adult a single structure, arises typically from three distinct rudiments, each of which is at first a simple pocket-like outgrowth of the splanchnopleure. One of the rudiments (cf. Fig. 80, H) is situated dorsally a little posterior to the stomach, the other two, which appear somewhat later, are ventral and arise as outpushings of the hepatic diverticulum in the region of the bile-duct. The ventral pancreatic rudiments are commonly paired, arising one on the right and one on the left of the bile-duct.

The three rudiments increase in size, secretory tubules sprout out from them and the two ventral rudiments become carried in a dorsalward direction, up the right side, by the rotation which the gut undergoes in this region (see p. 168). The right ventral rudiment comes in contact with the dorsal rudiment and fusion takes place—all three rudiments forming a single organ the three-fold origin of which is indicated by its three communications with the alimentary canal.

Such may be considered the typical mode of development of the pancreas, but important variations in detail occur in the different groups. In Cyclostomes and Elasmobranchs only the dorsal pancreas is known to occur. Its development in the former group requires further investigation. In Elasmobranchs it arises as a longitudinal groove of the enteric wall dorsally and a little posterior to the opening of the bile-duct. It becomes constricted off from before backwards and in accordance with the rotation of the alimentary canal it becomes shifted to the left side and ends up by being ventral.

In Crossopterygians the three typical rudiments appear (Fig. 80, H) but their development has not been followed in detail. Eventually the pancreatic complex extends forwards beneath the liver and completely fuses with it forming a thick layer over its ventral surface in the region near the opening of the bile-duct.

In Actinopterygian Ganoids also (Piper, 1902; Nicolas, 1904), the pancreatic complex derived from the original three rudiments becomes fused with the substance of the liver, only its posterior dorsal portion remaining extrahepatic. The main duct of the pancreas is the persistent stalk of the right ventral rudiment which opens into the gall-bladder formed by the dilated terminal part of the bile-duct. Of the two other pancreatic ducts the left ventral apparently atrophies entirely, while the dorsal is said in the case of *Amia* to disappear but in the Sterlet (*Acipenser ruthenus*) to persist.

In Teleosts the early development agrees closely with that of the ganoids, only a doubt exists whether the definitive pancreatic duct (**Duct of Wirsung**) may not be formed by a fusion of the two ventral ducts rather than by the persistent right duct alone. During later stages great differences arise between different members of the group. In some (*Silurus, Esox*) the complex forms a single compact gland, in others (*Scomber, Cyprinus*) it becomes divided into a number of independent lobes, in others, including the majority of the more familiar Teleosts, it becomes greatly branched and is diffused in the substance of the dorsal mesentery while in still others (Labridae, *Syngnathus*) the condition resembles that of the ganoids a large part of the organ being intrahepatic (Laguesse, 1894).

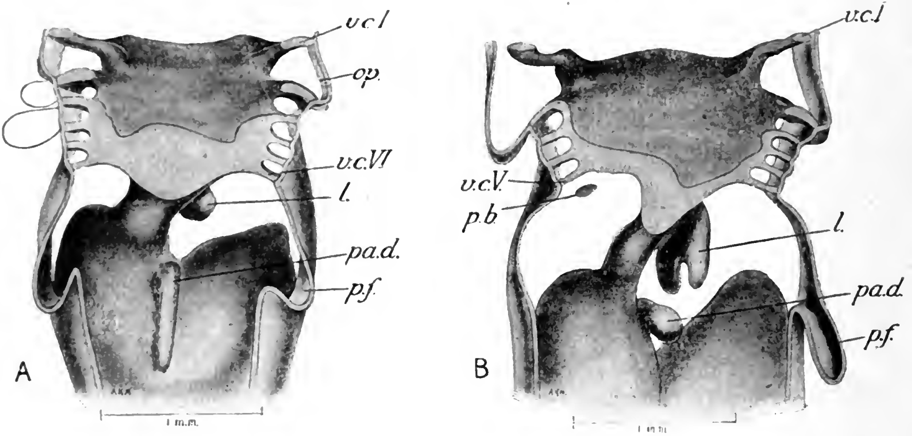


Fig. 109.—Dorsal view showing rudiments of dorsal pancreas and lung in larvae of *Protopterus* (stages 32 and 34).

l, lung; *op*, operculum; *pa.d*, dorsal pancreas; *p.b*, postbranchial body; *p.f*, pectoral limb; *v.c*, visceral cleft rudiment.

In Lung-fishes the three typical rudiments make their appearance. In *Protopterus* the dorsal rudiment (see Fig. 109, A, *pa.d*) is a solid outgrowth (hollow in *Lepidosiren*) from the gut-wall, usually rounded in form but occasionally elongated in an antero-posterior direction as in the specimen figured (Fig. 109, A). The attachment to the gut becomes rapidly constricted to a narrow stalk and a cavity develops in the interior of the rudiment. The ventral rudiments appear a little later, as solid projections one on each side of the attachment of the bile-duct to the gut. The two ventral rudiments, as they increase in size, meet and fuse dorsal to the bile-duct, and later on the dorsal surface of the right ventral rudiment comes in contact and fuses with the ventral surface of the dorsal rudiment. The stalks of the three rudiments remain as three ducts, the two ventral opening just posterior (original right rudiment) and anterior (original left) respectively to the opening of the bile-duct, while the dorsal opening is situated at the extremity of the spout-like pyloric valve.

The general course of development in *Lepidosiren* is similar and in both it is characteristic that the pancreas never bulges beyond the mesodermal coating of the splanchnopleure. It remains embedded throughout life in the gut-wall and is consequently not noticeable in an ordinary dissection.

In *Ceratodus* (Neumayr, 1904) the development of the pancreas is similar though here the left ventral rudiment, which in *Protopterus* is smaller in size than the right, remains rudimentary.

The Amphibia are of special interest from the fact that it was a member of this group (*Bombinator*) in which Goette (1875) first observed the origin of the pancreas from three separate rudiments. Goeppert (1891) was able to extend the observation to various other Amphibians, both Urodele and Anuran, and to show that in Urodeles the dorsal rudiment retains its duct, opening just behind the pylorus, while in the Anura this duct disappears. In both cases the ducts of the two ventral rudiments undergo fusion to form a duct of Wirsung which opens into the bile-duct.

In Reptiles (*Lacerta*—Brachet, 1896) the right ventral and the dorsal rudiments fuse to form the definitive pancreas, the left ventral atrophying (cf. Lung-fishes). According to Brachet the duct of the dorsal rudiment does not disappear but fuses with that of the right ventral to form the definitive pancreatic duct.

Birds show three rudiments which undergo fusion into a complex in the normal fashion, all three ducts remaining functional and conspicuous in the adult. Suppression of the left ventral rudiment occurs as an occasional variation.

The observed facts of development of the Pancreas clearly justify the conclusion that this organ of the modern Vertebrate has arisen in the course of evolution from three originally separate diverticula of the glandular enteric wall—a pair arising from the hepatic pouch and the third from the dorsal wall. The precise localization of the rudiments at comparatively distant points of the enteric wall point to the probability that the nature of the secretion was originally different in the case of the ventral pancreas from that of the dorsal.

Pyloric Caeca.—The caeca which are present in the pyloric region in many actinopterygian fishes arise as simple outgrowths of the gut-wall. The interesting suggestion has been made (Taylor, 1913) that the simple circle of these caeca, which is apparently their most primitive arrangement, corresponds morphologically with the curious valve found in various fishes (*Amia*, Lung-fishes, *Symbranchus*, *Anguilla*, etc.) in which the pyloric end of the stomach is prolonged back into a kind of spout which is ensheathed by the anterior end of the intestine. The circular prolongation forward of the intestinal cavity round the gastric spout might clearly give rise to a circle of pyloric caeca simply by subdivision into a number of separate portions each of which continued to open into the gut cavity at its hinder end.

Rectal Gland.—This organ, which occurs in Elasmobranchs, arises as a simple pocket-like outgrowth of the gut-wall. The superficially similar caecum of Lung-fishes will be dealt with in connexion with the renal organs.

Cloaca.—In the more archaic Vertebrates the ducts of the excretory organs open into the terminal part of the intestine which is thus a cloaca. It is believed by many that the excretory ducts originally opened at the hind end of the trunk independently of the alimentary canal and it is natural to suppose that the openings of the ducts have become gradually shifted first into close proximity to the anus and finally on to the lining wall of the alimentary canal. This again suggests that the cloaca may really be a proctodaeum—that the skin has been involuted to form its lining and that with this involution the renal openings have also been carried inwards.

Unfortunately the facts of ontogenetic development do not so far as can be seen at present fit this simple and attractive hypothesis. The cloaca is, except for a small portion close to its opening, of purely endodermal origin—the renal ducts open on what is part of the primary enteric wall. A suggested explanation of this fact differing from that mentioned above will be found in the chapter dealing with the renal organs.

A cloaca seems always to be developed though in some cases (*e.g.* Teleostean fishes) it flattens out and disappears later so that the renal organs and the gut come to have independent external openings.

The **bursa Fabricii,** a conspicuous glandular appendage of the dorsal wall of the cloaca in young birds, has usually been regarded as proctodaeal in its origin but it is now known to arise in ontogeny from vacuolar spaces in a solid projection from the cloacal rudiment, dorsal to the stalk of the allantois (Wenckebach, 1888) and would therefore appear to belong to the mesenteron rather than to the proctodaeum.

The anal opening of the Vertebrate, as may have been gathered from Chap. II., is to be regarded as representing morphologically a portion of the gastrular mouth or protostoma. In a large number of Vertebrates however the opening arises in ontogeny not in this way but rather as a secondary perforation, although even in such cases the perforation arises in the line of the closed protostoma.

Temporary Occlusion of the Alimentary Canal.—The alimentary canal is, in correlation with its function, a hollow tube. In a large number of Vertebrates, however, there are more or less extended periods of development during which the cavity is completely absent, either throughout the length of the canal or in certain portions.

In its simplest condition this occurs as a special case of the temporary absence of lumen so frequently found in the development of eventually hollow organs from a richly yolk-laden rudiment. An idea of how it has come about will be got from an inspection of the various stages of the development of the alimentary canal of *Polypterus* as shown in Fig. 80 on p. 146. During early stages the archenteric cavity is seen to be widely patent throughout, except that there is no mouth opening. During the later stages of development, immediately prior to the canal becoming functional, its walls throughout the region between the fore-gut and the cloaca become closely apposed, so as almost entirely to obliterate the cavity. Later on the walls recede from one another and the lumen becomes again patent.

It would obviously be merely a slight accentuation of this modification of development for the cavity to be completely obliterated for a time. A still further modification would be brought about by the omission altogether of the original hollow stage from the ontogenetic record. This actually occurs in the case of the fore-gut in those Vertebrates in which this region of the enteric rudiment is yolk-laden: where, on the other hand, the yolk is practically completely concentrated in the mid-gut region as in meroblastic Vertebrates it does not occur as a rule.

The most striking temporary occlusions of the alimentary canal during development have to do with its terminal apertures. Thus there is not a single existing Vertebrate, so far as is known, in which the mouth opening persists from the gastrular stage, or in which even any connexion has so far been traced between the definitive mouth opening and the protostoma. In every case, even in *Amphioxus,* the mouth opening develops comparatively late as a secondary perforation. This modification of development is in the present writer's opinion to be attributed to the entire dependence of members of the Vertebrate phylum upon food-yolk during early stages of their development, the need for a functional mouth having thus disappeared.

The anteroposterior extent of this occlusion of the alimentary canal in the region of the oral opening differs in different subdivisions of the phylum. It may include a large part of the stomodaeal as well as the endodermal portion of the buccal cavity as in the Lung-fishes (p. 148) but more usually it is confined to the boundary between the two, *i.e.* to the site of the original mouth opening the closely apposed ectoderm and endoderm being at this level continuous across the site of the future opening as the velar membrane (p. 145). The secondary perforation by which the alimentary canal comes to communicate with the exterior at its front end is in the case of some larval Vertebrates (*e.g. Lepidosiren*) closely correlated with the commencement of pharyngeal respiration but where the development is embryonic it commonly still takes place long before the existence of any obvious functional need (*e.g.* Chick, fourth day). At its hinder end the archenteron is, as has been shown in Chap. I., widely open to the exterior in all the lower Vertebrates during early stages and in various cases this opening can be traced either into direct continuity, or into less direct but still clear relationship, with the anal opening. The explanation of this lesser degree of modification of the development of the anal opening as compared with the mouth may probably be associated with the less accentuated delay in the functional need for this opening. At stages long before ingestion or inspiration takes place by the mouth, the formation of waste products during the digestion of the yolk necessitates an outlet from the enteric canal at its hinder end. Where obliteration does take place during still earlier stages this is probably correlated with the fact that the need of the opening is still non-existent.

It is of interest to notice that obliteration of the anal opening which is of a directly adaptive significance may take place at a later stage. Thus in *Lepidosiren* during about the first two weeks of larval life, when large numbers of practically motionless larvae are lying crowded together in the nest, the anal opening, which had been continuously patent in earlier stages, is closed, so as to prevent the poisonous excretory products from finding their way out. So also in the case of the Elasmobranch embryo enclosed within its egg-shell. In the Amniota the perforation of the anus is delayed to a relatively late period doubtless for a similar reason.

It is characteristic of the phylum Vertebrata that the anal opening no longer occupies its primitive position at the extreme end of the body but has become shifted forwards along the ventral side. This shifting has probably come about with increased specialization for swimming by lateral flexure of the body, the withdrawal of the alimentary canal with its surrounding splanchnocoelic cavity from the hinder portion of the body, leaving the space they occupied free for increased development of the lateral muscles. This shifting forwards of the anus, leading to the differentiation of a distinct postanal or tail region, has occurred in all Vertebrates, least markedly in the more archaic groups. It reaches its maximum in some members of that group of Vertebrates which is above all others highly specialized for active swimming, the Teleostei, in some families of which the anus has actually assumed a jugular position.

During the actual ontogeny of the Vertebrate the process by which the anus comes to occupy a position more or less distant from the tip of the tail region is somewhat modified from that which probably occurred during phyletic evolution. We do not find that the anus remains at the tip of the tail during the growth in length and that it then gradually shifts forwards along the ventral side. What happens is that the opening at an early stage assumes a ventral position and that the tail region proceeds to sprout out dorsal to it. The process will be understood from an inspection of Fig. 80 (p. 146). In B the anus is at the hinder end, in C it has assumed a ventral position being overhung by the bulging tail rudiment, in D, E, F, G the tail rudiment is seen to be extending actively past the position of the anus, the specially actively growing tissues being indicated by the darker shading.

In Fig. 80, G, a feature is well shown which occurs in the embryos of most Vertebrates — the postanal gut (*pa.g*). It was shown in Chap. I. how a connexion—the neurenteric canal—existed in some Vertebrates between the cavity of the enteron and that of the neural rudiment at their posterior ends. Here, in the postanal gut, we have such a connexion still persisting in a drawn-out form though, as in the present case, it may be a solid strand of yolky cells and not a hollow tube. The postanal gut is a purely transitory structure which at a relatively early period of development disintegrates completely.

In endeavouring to determine the morphological significance of the postanal gut it is necessary to bear in mind that the Vertebrate in early stages develops from before backwards and that the growth in length by the addition of new segments takes place at its hinder end where there is a mass of actively growing embryonic tissue forming a kind of "growing point." The tissue of this, although to the eye quite undifferentiated, contains the elements which form all the various tissues such as nerve cord, notochord, myotomes, alimentary canal, etc. As growth goes on these gradually become differentiated out, the differentiation always proceeding from before backwards. If we now look at such a young Vertebrate as that shown in Fig. 80, G, we see the typical Vertebrate structure, including alimentary canal (*pa.g*) extending right back practically to the tip of the tail: it is only at the extreme tip that the various organs merge together into undifferentiated embryonic tissue. The only striking peculiarity is that the communication of the alimentary canal with the exterior, the anus, is not in the midst of the growing tissue of the tip, as it would be, for example, in a young Chaetopod worm, but well forwards on the ventral side.

This peculiarity, in the writer's opinion, finds its explanation in the development from before backwards already alluded to. The appearance of the anus at a point relatively far forwards means that it and the organs related to it such as the excretory ducts complete their development at an earlier period of time. As it is of functional importance that the organs in question should do so, in contradistinction to the purely motor arrangements farther back, we see a physiological reason why evolution should have brought about a development of the anal opening in its anterior position from the beginning, and the elimination of those stages in which it was situated farther back.

As regards the phyletic evolution of this part of the enteron, we may sum up probabilities as follows: that the alimentary canal with its surrounding splanchnocoele originally extended to the hind end of the body: that the anal opening came to be shifted on to the ventral wall of the canal: that it then underwent a gradual shifting forwards along the ventral side: that as it did so the now postanal portion with its splanchnocoele gradually atrophied the position they occupied becoming filled mainly with muscle.

LITERATURE

Assheton. Quart. Journ. Micr. Sci., xxxviii, 1896.
Assheton. The Work of J. S. Budgett. Cambridge, 1907.
Balfour. Comparative Embryology, ii, 1881.
Brachet. Journ. de l'Anat. et de la Physiologie, xxxii, 1896.
Brauer. Zool. Jahrb. (Anat.), xii, 1899.
Brouha. Journ. de l'Anat. et de la Phys., xxxiv, 1898.
Bryce. Journ. Anat. and Phys., xl, 1906.
Budgett. Trans. Zool. Soc. Lond., xvi, 1901.
Dean, Bashford. Zool. Jahrb. (Syst.), ix, 1896.
Drüner. Zool. Jahrb. (Anat.), xv, 1901.
Egert. Zool. Anzeiger, xlii, 1913.
Goeppert. Morph. Jahrb., xvii, 1891.
Goeppert. Morph. Jahrb., xx, 1893.
Goette. Entwicklungsgeschichte der Unke. Leipzig, 1875.
Goette. Zeitschr. wiss. Zool., lxix, 1901.
Greil. Semons Forschungsreisen in Australien, i. Jena, 1913.
Hammar. Arch. f. Anat. u. Entwicklungsgesch., 1893.
Hammar. Anat. Anzeiger, xiii, 1897.
Juillet. Arch. zool. expér. [5], ix, 1912.
Kallius. Anat. Hefte (Arb.), xvi, 1901.
Kerr, Graham. Proc. Roy. Phys. Soc. Edin., xvi, 1906.
Kerr, Graham. The Work of J. S. Budgett. Cambridge, 1907.
Kerr, Graham. Quart. Journ. Micr. Sci., liv, 1910.
Laguesse. Journ. de l'Anat. et de la Phys., xxx, 1894.
Lankester, E. Ray. Quart. Journ. Micr. Sci., xvi, 1876.
Marcus. Arch. f. mikr. Anat., lxxi, 1908.
Moroff. Arch. f. mikr. Anat., lx, 1902.
Moser. Arch. f. mikr. Anat., lx, 1902.
Moser. Arch. f. mikr. Anat., lxiii, 1904.
Müller, W. Jenaische Zeitschrift, vi, 1871.
Neumayr. Semons Forschungsreisen in Australien, i, 1904.
Nicolas. Arch. Biol., xx, 1904.
Phelps. Science, N.S. ix, 1899.
Piper. Arch. f. Anat. und Entwicklungsgesch., Suppl. Bd., 1902.
Piper. Verh. Anat. Ges., Halle, 1902.
Reighard and Phelps. Journ. Morph., xix, 1908.
Rowntree. Trans. Linn. Soc. Lond., (2) ix. 1903.
Sarasin, P. and F. Ergebnisse naturwiss. Forschungen auf Ceylon, ii, 3. Wiesbaden, 1889.
Sawadsky. Anat. Anzeiger, xl, 1911.
Scammon. Amer. Journ. Anat., xiv, 1913.
Sedgwick. Quart. Journ. Micr. Sci., xxxiii, 1892.
Smith. Journ. Morph., xxiii, 1912.
Taylor. Quart. Journ. Micr. Sci., lix, 1913.
Voeltzkow. Abh. Senck. Ges., xxvi, 1899.
Wenckebach. Ontwikkeling en de bouw der bursa Fabricii. Proefschrift. Leiden, 1888.
Wijhe, van. Verhand. Konink. Akad. Wetensch. Amsterdam, Tweede Sectie, Deel xviii, 1914.
Wilson, Gregg. Proc. Roy. Phys. Soc. Edin., xiv, 1901.

CHAPTER IV

THE COELOMIC ORGANS

INTRODUCTION.—The mesoderm of *Amphioxus* consists in an early stage, as already indicated (p. 57), of a row of closed sacs arranged serially one behind the other upon each side of the body. At this time the coelome of *Amphioxus* is in the extremely archaic condition of a series of metamerically arranged paired compartments — a condition resembling that of the less modified forms of Annelids. The coelomic sacs gradually spread in a ventral direction until they meet. For a time after this happens the sacs of opposite sides of the body remain separated by a longitudinal partition the ventral mesentery. Similarly the apposed posterior and anterior walls of neighbouring sacs belonging to the same side of the body, form thin membranous septa like those of Annelids.

A highly characteristic difference from the Annelid arrangement begins to show itself a little before hatching in the ventral portion of the body, in as much as the transverse septa break down and disappear thus converting what was hitherto a chambered coelome in this region into a continuous space. There is no obvious reason why this loss of segmentation of the ventral portion of the mesoderm has come about in evolution. A general characteristic, however, of the phylum Vertebrata is the loading up of the ventral part of the endoderm with yolk and it may well have been that the loss of the mesoderm septa ventrally arose in correlation with the presence of a greater amount of yolk in the ancestral condition than exists in the present-day Amphioxus.

A further striking difference between the Vertebrate and the Annelid is expressed in the extent to which the coelomic wall gives rise to muscular tissue. In the Annelid practically the whole extent both of the somatic layer lining the body-wall and the splanchnic layer covering the gut gives rise to muscular tissue. In *Amphioxus* however, and the same holds for Vertebrates in general, the ventral portion of the somatic mesoderm, the portion which loses its segmental character—loses also its capacity for producing muscle.

On the other hand the dorsal portion of the mesoderm, which retains its segmentation, retains also, and to an accentuated degree, its muscle-forming capacity. It separates off from the ventral or splanchnocoelic portion of the mesoderm in the form of a series of segmentally arranged sacs—the **myotomes**—and the wall of these gives rise to almost the whole of the muscular system. The myotomes are at first, from their mode of origin, restricted to the dorsal side of the body, but as development goes on active growth of their ventral portions takes place and they extend downwards, overlapping and covering in the splanchnocoelic mesoderm right down to the mid-ventral line. In this way a muscular body-wall is provided for the ventral region of the body in which the original muscle-producing capacity of the somatic mesoderm had disappeared.

The evolutionary origin of this curious secondary muscularization of the ventral body-wall of the Vertebrate is unexplained but the suggestion may be hazarded that it was associated with the loss of segmentation of the ventral splanchnocoelic mesoderm, the primitive mode of movement of the Vertebrate—by waves of lateral flexure—being only able to utilize longitudinal muscles divided into segments. We may take it that the splanchnocoelic muscular layer, as it lost its segmentation, would become less efficient for purposes of movement, and that, correlated with this, its territory would then tend to be encroached on by the still segmented, and therefore more efficient, dorsal portion of the muscular layer until eventually it came to be replaced completely by it.

As a result of the developmental processes which have just been indicated the mesoderm of *Amphioxus,* which for a time consisted of a metameric series of paired sacs, is now represented by (1) the segmentally arranged myotomes and (2) the unsegmented splanchnocoelic lining. To these a third element becomes added in the form of a pocket-like outgrowth from the myotome wall close to its lower end (Fig. 144, A, *scl*, p. 285). This grows first towards the mesial plane and then dorsally, insinuating itself into the space between myotome on the one hand and notochord and spinal cord on the other, until it occupies practically the whole of that space right up to the mid-dorsal line. This pocket-like diverticulum is the **sclerotome** (p. 286).

In the typical Vertebrate a fourth derivative of the mesoderm segment is of importance: it takes the form of a connexion which persists for some time between the myotome and the splanchnocoelic mesoderm as a narrow stalk or isthmus. This—the **protovertebral stalk** or **nephrotome** (Rückert, 1888) with its cavity the **nephrocoele**—is of great importance from its relation to the nephridial organs but its existence has not up to the present been demonstrated in *Amphioxus.*

We will now proceed to trace out the subsequent fate of these various derivatives of the primitive mesoderm segments.

Coelomic Cavities.—The only portions of the coelomic cavities which remain patent are the nephrocoeles (which will be dealt with later on) and the splanchnocoele or peritoneal cavity.

It may be taken as probable that the body-cavity of the ancestral Vertebrate was divided up into segmentally arranged compartments by transverse septa, and into a right and left half by a sagittally placed partition supporting the alimentary canal and forming the dorsal and ventral mesentery; in other words that the general arrangement was like that of a primitive Annelid worm. This seems to be indicated by the mode of development of the mesoderm in *Amphioxus*.

In Vertebrates above *Amphioxus* the segmented condition of the splanchnocoele has disappeared even from development.[1] The sagittally placed mesentery on the other hand still appears in ontogeny in the form of the partition remaining between the edges of the lateral mesoderm as they approach one another on the ventral and on the dorsal sides of the alimentary canal respectively. In correlation with the great increase in length, and consequent coiling, of the alimentary canal of the Vertebrates—a condition which probably existed even in the ancestors of those gnathostomes in which the alimentary canal is now short (p. 184)—the ventral mesentery disappears at an early stage of development throughout that portion of its extent which lies on the tailward side of the liver.

The dorsal mesentery on the other hand persists throughout life, serving as a bridge to carry the complicated connexions of the gut wall with the vascular and nervous systems, although perforations may appear in it, more or less extensive in different groups of Vertebrates. The complicated foldings and frillings which the dorsal mesentery undergoes, owing to its enteric edge having to keep pace with the increase in length of the gut, are of interest mainly to specialists in the anatomy of particular groups and need not be dealt with here.

In the fishes, in which the lung performs an important hydrostatic function, that organ grows back in the substance of the dorsal mesentery, and in accordance with its tendency to assume a more and more dorsal position, the portion of mesentery lying above it may become incorporated in the dorsal wall of the splanchnocoele, the result being that the lung in the adult now lies entirely dorsal to and beyond the limits of the body-cavity (Dipnoi,[2] Actinopterygii).

Apart from its primary segmentation, the splanchnocoele shows a tendency for special portions to become secondarily separated off from the main cavity. The most important case of this occurs at

---

[1] While it has to be granted that the splanchnocoele of the Vertebrates represents the ventral portion of the coelome which has lost its segmentation, care must be taken not to assume that this loss of segmentation has necessarily extended dorsalwards to precisely the same level in all Vertebrates. Like other anatomical boundaries the dorsal limit of the splanchnocoele is doubtless fluctuating and vague. It is therefore wise not to attach too great importance to the exact position of the first rudiment of an organ which develops in one case on the dorsal and in another on the ventral side of the boundary between segmented and unsegmented mesoderm such as for example the gonad (p. 270).

[2] Cf. Graham Kerr, 1910.

the hinder end of the heart where there exists on each side a broad bridge by which the duct of Cuvier passes from the somatopleure to the sinus venosus. This bridge becomes extended headwards and dorsally on each side of the oesophagus until it meets the dorsal wall of the splanchnocoele thus forming with the oesophagus a floor separating the anterior portion of the splanchnocoele into two cavities, one dorsal and one ventral, each opening posteriorly into the main splanchnocoele. Of these two cavities the dorsal becomes completely obliterated by fusion of its splanchnic (oesophageal) and somatic walls from before tailwards. The ventral one on the other hand roofed in by the oesophagus persists as the **pericardiac cavity**.

The communication of this posteriorly with the main splanchnocoele is obstructed in the middle by the flattened headward surface of the liver which is embedded in the distended ventral mesentery, while laterally the communication is for a time open. As development goes on however the opening on each side becomes obliterated by an ingrowth from the somatopleure which spreads downwards from the bridge of tissue containing the duct of Cuvier and the free edge of which meets and fuses with the mesoderm covering the headward surface of the liver. The pericardiac cavity comes in this way to be bounded posteriorly by a complete wall of tissue a large part of which consists simply of the mesodermal sheath of the liver. As the body of the embryo increases in diameter this wall of tissue keeps pace with it as does also the liver. The latter organ however in subsequent growth of its anterior or headward surface does not keep growing in continuity with the substance of the septum but becomes separated from it by a deep cleft, the region of continuity between liver and septum becoming thus restricted to a small area dorsal and close to the mesial plane. Similarly the region of continuity between the headward face of the septum and the wall of the sinus venosus which is at first of relatively considerable dorsiventral extent becomes reduced to a narrow bridge of tissue.

In the Elasmobranchs the isolation of pericardiac cavity from the main splanchnocoele is only temporary. A median pocket-like extension of the pericardiac cavity spreads tailwards immediately dorsal to the sinus venosus in the substance of the mesodermal sheath covering the ventral surface of the oesophagus. This develops on each side a communication with the main cavity of the splanchnocoele which persists throughout life as a crescentic slit on the ventral surface of the oesophagus (Hochstetter, 1900). This secondary communication between pericardiac coelome and splanchnocoele is known as the **pericardioperitoneal canal**.

In Myxinoids, throughout life, and in *Petromyzon*, during the larval period, the rudiment of the wall separating pericardiac from splanchnocoelic cavity remains in the form of a simple bridge enclosing the duct of Cuvier so that the two cavities are in wide communication with one another.

In the Amphibia and Amniota the pericardiac cavity becomes telescoped back into the general peritoneal cavity, its hinder wall becoming extended so as to form a thin membranous bag enclosing the heart and separating it from the other viscera.

Apart from the walling off of the pericardiac from the main peritoneal cavity there is found in the case of the Amniota a well-marked tendency for the latter cavity to undergo further subdivision, special portions becoming more or less completely walled in by secondary fusions taking place between apposed portions of the peritoneal lining.

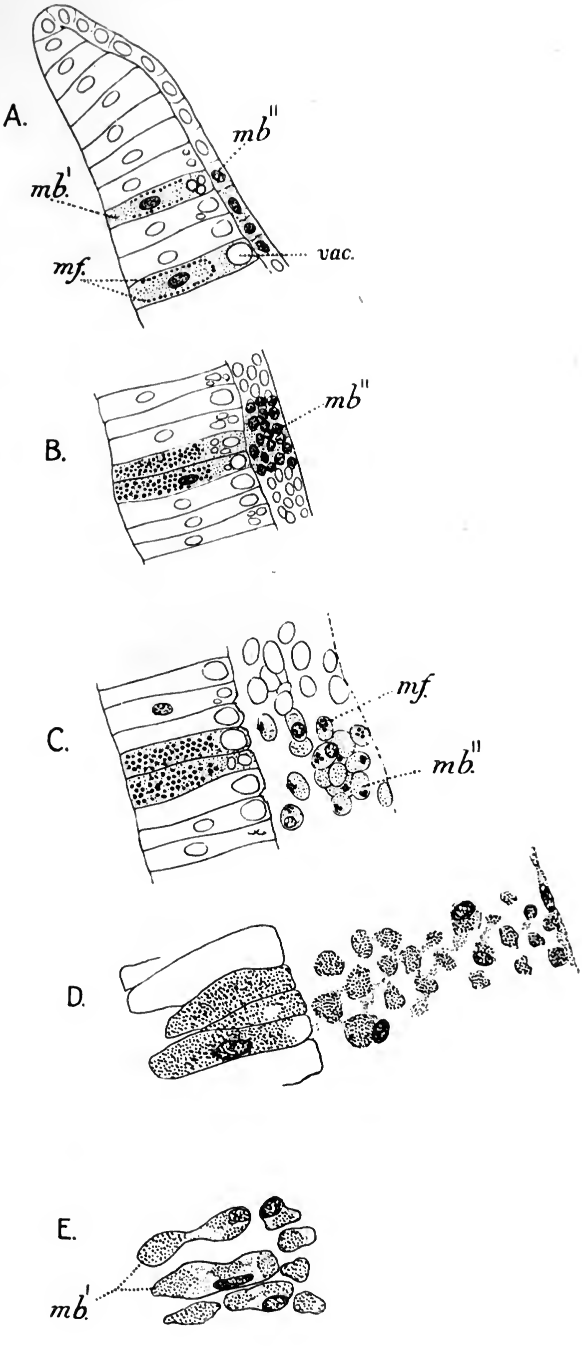


Fig. 110.—Differentiation of the myotome as seen in transverse sections of Lepidosiren larvae.

A, stage 30; B, stage 31 +; C, stage 32; D, stage 35 –; E, dividing myoblasts of inner wall from stage 36. *mb'*, myoblasts of inner wall; *mb''*, myoblasts of outer wall; *mf*, contractile fibrils; *vac*, vacuole. The contractile fibrils cut across are shown as distinct black dots.

For example in Birds[1] the mesodermal coating of the lung upon its ventral side becomes continuous (1) with that lining the body-wall laterally so as to enclose the portion of splanchnocoele dorsal to the lung as a **pleural cavity**, and (2) with that covering the surface of the liver, forming a ventral pulmonary ligament which serves to wall in a pulmo-hepatic recess lying between it and the mesentery. A third connexion, the origin of which is associated with the development of the abdominal air-sacs, forms the thin post-hepatic septum which stretches from the ventral surface of the lungs obliquely downwards and backwards to the ventral body-wall.

Amongst Reptiles somewhat similar arrangements exist, differing in detail in the different groups.

THE MYOTOMES.—The developmental changes by which, in a gnathostomatous Vertebrate, the myotomes become converted into masses of muscle-fibres are excellently shown by *Lepidosiren* in which the cellular elements are particularly large and distinct. In this animal the myotome is at first solid, but later on develops a small cavity or myocoele by the breaking down of its central cells. This myocoele soon becomes obliterated by its inner and outer walls coming together. The cells of the inner wall assume a more regular shape, taking the form of large parallelepipedal cells (Fig. 110, A, *mb′*), flattened dorsiventrally and stretching in an anteroposterior direction throughout the whole length of the myotome. The nuclei of these large cells—**myoblasts** or myoepithelial cells—divide, mitotically, so that they assume a syncytial character. Their protoplasm develops a longitudinally fibrillated appearance and presently distinct cross-striped contractile fibrils (*mf*) make their appearance in the protoplasm—each fibril running through the whole length of the myoblast or in other words from end to end of the myotome. The contractile fibrils, which as seen in a transverse section are arranged in a ⊃ -shaped pattern (Fig. 110, A, *mf*), become more and more numerous and soon fill up the inner two-thirds of the myoblast almost entirely, there remaining only a relatively small amount of perifibrillar protoplasm between them (Figs. 110, B, and 111).

The outer end of the myoblast does not for some time develop any contractile fibrils but there appear in its protoplasm large vacuoles (*vac*) which form a broad clear band in horizontal sections—of much use as a landmark to indicate the outer limit of the inner wall of the myotome. The cells of the outer wall of the myotome take the form of elongated cylinders stretching throughout the length of the myotome and in their protoplasm longitudinal fibrils make their appearance as in the case of the inner wall myoblasts (Figs. 110, C; 111, B). The longitudinal fibrils become fused at their ends with connective-tissue septa formed by mesenchyme cells which wander in between consecutive myotomes.

---

[1] For a well-illustrated account of the complicated arrangements in detail see Poole (1909).

Some such mesenchyme cells also penetrate into the substance of the myotome and settle down there to form connective tissue. The cylindrical myoblasts of the outer wall undergo active multiplication (Fig. 110, C) so that it comes to be greatly thickened, composed of many layers of muscle-cylinders—those towards the outer surface going on dividing actively while those further in towards the mesial plane increase much in size as they develop more and more fibrils in their interior.

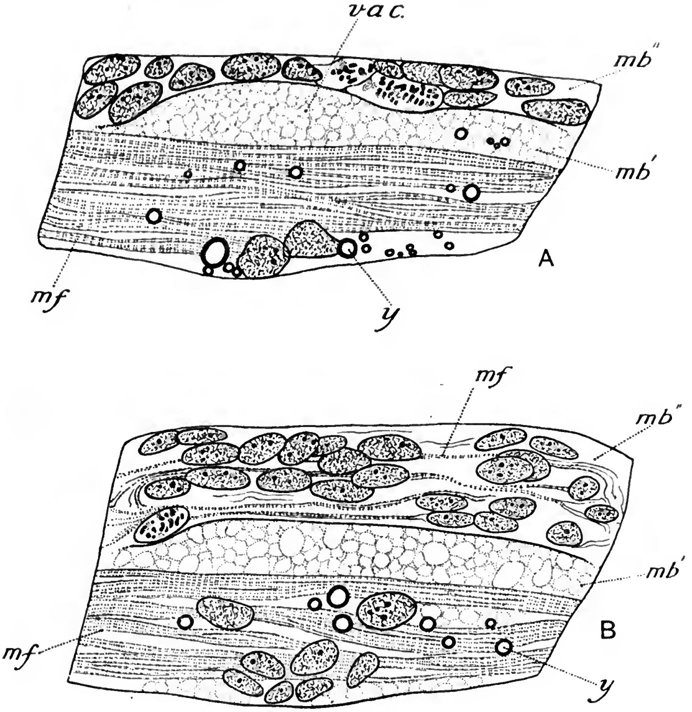


FIG. 111.—Differentiation of the myotome as seen in horizontal sections of Lepidosiren larvae.

A, stage 31; B, stage 31+. *mb'*, myoblast of inner wall; *mb''*, myoblasts of outer wall: *mf*, contractile fibrils; *vac*, vacuoles; *y*, yolk.

As the outer wall of the myotome continues to increase in thickness the myoblasts of the inner wall become relatively more and more insignificant. Eventually they divide up into muscle-cylinders like those of the outer wall so that it is no longer possible to distinguish the inner wall portion of the myotome from the outer wall part. The muscle-cylinders become the muscle-fibres of the adult, the undifferentiated protoplasm between the fibrils persisting as the **sarcoplasm** the superficial layer of which may be somewhat condensed to form the **sarcolemma**.

A point to be noticed, of much morphological interest, is that the inner wall myoblasts of *Lepidosiren* are for a time (Fig. 110, A) in the form of typical myoepithelial cells such as are familiar in some of the lowest invertebrates. They are, as indicated in Chap. II., in continuity with the central nervous system by a protoplasmic tail-like extension of the cell-body closely resembling that which occurs in Nematode worms (Fig. 112). The peripheral portion of this remains as a mass of granular protoplasm on the surface of the muscle-fibre—the **motor end-plate**. The latter is therefore to be regarded as a portion of the muscle-cell which retains its protoplasmic condition rather than as a portion of the nerve-fibre.

The mode of conversion of the embryonic myotome into the muscle-segment has been described as it occurs in *Lepidosiren* because of the two special safeguards against error which exist in that animal, (1) the large size of the histological units and (2) the fact that the boundary between outer and inner walls of the myotomes is marked by a clear and unmistakable landmark in the form of the vacuolar zone constituted by the outer portions of the inner wall myoblasts. It now remains to indicate shortly the more important differences in detail which are to be found in descriptions of the process as observed in others of the lower Vertebrates.

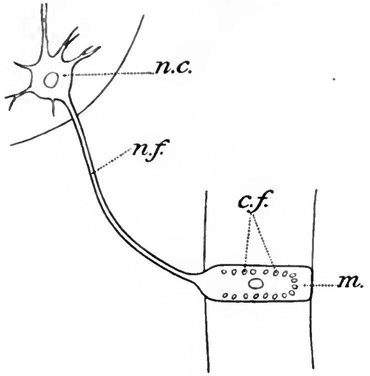


FIG. 112.—Diagram of a motor ganglion-cell in the spinal cord continuous through the substance of a nerve-fibre with a muscle-cell in the myotome.

c.f, contractile fibrils in myoepithelial cell; *m*, myoepithelial cell; *n.c*, ganglion-cell; *n.f*, motor nerve-fibre.

The chief of these concerns the fate of the outer wall of the embryonic myotome. In *Lepidosiren* as has been stated the outer wall gives rise to muscle. In the case of Elasmobranchs and Ganoids, Balfour stated explicitly that the outer wall of the myotome similarly takes part in the development of muscle. Many authorities (Hertwig, Rabl, Maurer), however, deny that this is the case: according to them the outer wall plays no part in muscle-formation: it simply breaks up into amoeboid cells which contribute to the dermal mesenchyme. Hence these investigators term the outer wall of the myotome the "Cutis-layer." In the case of the Sturgeon, Maurer corroborates Balfour's statement that the myotome is composed of two layers of muscle-elements but according to him the outer layer is simply budded off from the inner and does not represent the original outer wall of the myotome as Balfour supposed.

In the Amniota the myotome in early stages is almost square as seen in a transverse section practically the whole of the wall next the endoderm representing the sclerotome. Cells proliferating from this invade the myocoele and completely fill it up. It is only in later stages that the myotome becomes extended into the normal plate-like form by active growth at its inner (dorsal) and outer edges. Of the two walls of this stage the inner admittedly becomes converted into muscle-cylinders. The outer becomes loosened out into a mass of irregularly shaped cells and these are commonly believed to give rise to dermis. In view of what happens in *Lepidosiren,* where accuracy of observation is so much more easily attained to, it seems advisable not to accept this as absolutely certain.

At the same time it may be allowed that there is no *a priori* difficulty in the way of admitting that portions of myotome which in one type of Vertebrate give rise to muscle, may in another have ceased to do so, for, as already indicated, a quite similar process of concentration of muscle-development in a localized portion of somatic mesoderm is a fundamental characteristic of the whole Vertebrate phylum.

The series of paired myotomes, each composed of a mass of longitudinal muscle-fibres traversing it from end to end, forms the material out of which is formed the, often extremely complicated, system of voluntary muscles of the adult Vertebrate. The various myotomes as they increase in size become divided up into it may be numerous pieces and these are pushed hither and thither by processes of differential growth until the arrangement of the numerous adult muscles contrasts greatly with the simple longitudinal arrangement of the original myotomes. During the various displacements which it undergoes the individual muscle or fragment of myotome remains in organic connexion with its nerve-centre by means of its motor nerve and the course of these nerves in the adult frequently gives an important clue to the developmental migrations of the particular muscles.

No attempt will be made here to follow out the evolution of the complicated muscular arrangements of the adult beyond a short sketch of the method in which the muscles of the fins or limbs originate.

The median fin is simply the extension of the body in the median plane and we should therefore naturally expect it to be muscularized by prolongations of the myotomes growing into it. The actual process is clearly illustrated in Fig. 113. In A a muscle-bud is seen to be projecting from the end of each myotome where a median fin is developing—the upper group of buds belonging to the dorsal fin, the lower to the anal. The buds diminish in size towards each end of the series and in the case of the dorsal fin, towards its anterior end, there are a considerable number of abortive buds which never come to anything. The muscle-buds grow into the fin fold and then become cut off from the main part of the myotome to form the muscles of the fin as is shown in B.

The paired fins or limbs become muscularized by very similar, segmentally arranged, buds and it is necessary from the outset to bear in mind that this similarity need have no deeper significance than that the paired fins also necessarily obtain their muscularization from the segmentally arranged myotomes. The process as it occurs in the pelvic fin of the shark *Spinax* is illustrated by Fig. 114. In the 20 mm. embryo (A) the fin rudiment is seen as a longitudinal ridge and a series of myotomes in the neighbourhood of this ridge are seen each to be forming at its lower edge two projecting muscle-buds. These sprout out into the limb rudiment, assume an elongated form (B) and then become separated off from the myotome (C). Each bud now splits into two layers a dorsal and a ventral and each of these undergoes histological differentiation and becomes a bundle of muscle-fibres—one of the radial muscles of the fin: so that four radial muscles are derived from each myotome, a dorsal and a ventral from each of the two original buds. Such is the process in its main outlines.

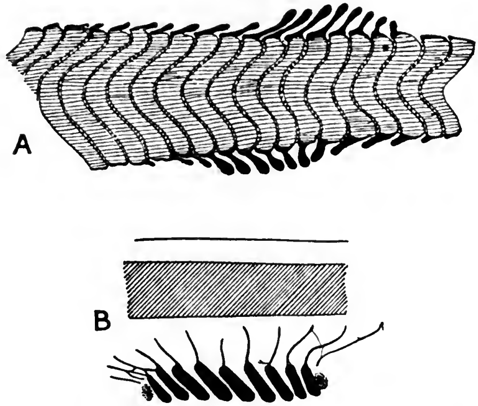


FIG. 113.—Muscularization of median fin in *Lepidosteus*. (After Schmalhausen, 1912.)

A, 13 mm.; B, 21 mm. The muscle-buds and, in the lower figure, the nerves connected with them are shown in black.

The existence of a disturbing complication of this simple scheme is indicated by the adult arrangements, in as much as it can be shown that a single motor spinal nerve (*i.e.* the nerve belonging to a single myotome) is related to more than the four radial muscles to which alone we should expect it to be related were the account which has just been given complete. This discrepancy is brought out particularly clearly by physiological experiments. Careful stimulation of a single spinal nerve very commonly causes three consecutive (dorsal or ventral) radial muscles to contract instead of only two, and in some cases apparently a still greater number. This seems clearly to indicate that the end-organs, in other words the muscle-fibres, belonging to a particular motor nerve or myotome are in the adult not strictly confined within the limits of the two pairs of radial muscles corresponding to that motor nerve or myotome.

To those who believe in the organic continuity of muscle-cell and nerve-fibre from an extremely early stage of development the idea obviously suggests itself that a shifting of some of the constituents from one muscle-bud into its neighbours takes place during development. According to Mollier (1893) and Braus (1899) such a process actually occurs. Broad anastomoses or bridges make their appearance connecting the various radial muscle rudiments with their neighbours near their proximal ends. These connecting bridges persist for a short time and then disappear. According to the authors mentioned they are the expression of a cellular interchange taking place between neighbouring muscle rudiments.

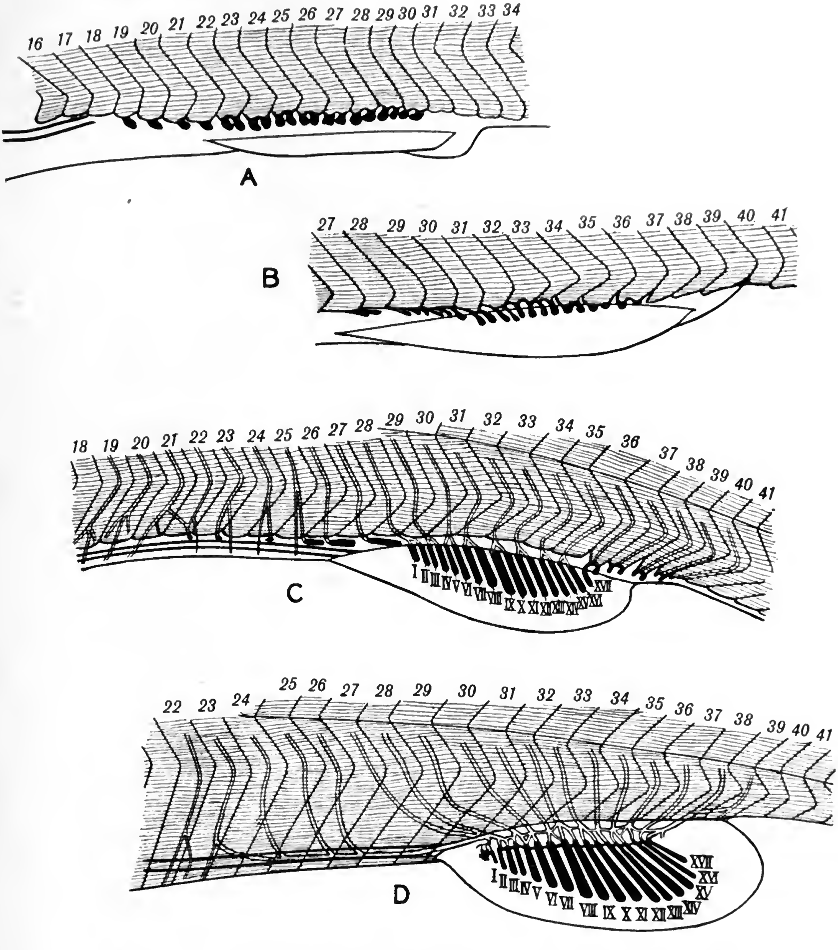


Fig. 114.—Illustrating the muscularization of the pelvic fin in *Spinax*. (After Braus, 1899.)

A, 20 mm. (70 mesoderm segts.); B, 25 mm. (74 m.s.); C. 26 mm.; D, 32 mm. The myotomes are indicated by Arabic numerals. The muscle-buds are shown in black, those within the fin rudiment being numbered with Roman numerals. The nerve-trunks are shown with double contour.

E. Müller (1911) believes the connecting bridges in the case of *Acanthias* to be special developments of a syncytial network which lies between the buds from the commencement: he fails to find in this animal any evidence of shifting of muscle-cells along the bridges. The matter appears to stand in need of further investigation.

Already within the group of Elasmobranchs we find modification of the typical mode of muscularization of the fins outlined above. In the case of the most anteriorly placed muscle-bud of the pectoral fin of *Spinax* the bud resolves itself into its constituent cells which separate before giving rise to muscle-cells. Again at the anterior and posterior limits of the pectoral limb musculature in *Pristiurus* and *Torpedo* the compact stage of the muscle-bud is eliminated entirely and the cells which muscularize the fin are budded off separately from the myotome, wandering from their place of origin into the limb rudiment and there settling down (Braus).

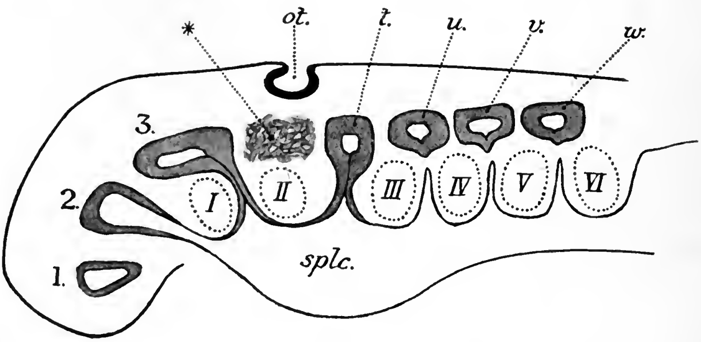


Fig. 115.—Diagram to illustrate the arrangement of mesoderm segments in the head-region of a young Elasmobranch embryo. (From a drawing by Agar.)

ot, otocyst; *splc*, splanchnocoele; *t*, *u*, *v*, *w*, occipital myotomes; 1, 2, 3, anterior myotomes; I, II, etc., visceral clefts; *, "Fourth" myotome of van Wijhe.

Amongst Vertebrates outside the group of Elasmobranchs such modification appears to be the rule. Thus in *Acipenser* and apparently in *Lacerta* typical muscle-buds arise singly from the myotomes concerned. In Teleosts Harrison finds muscle-buds in the pelvic fin but a diffused origin in the pectoral. In Lung-fishes and Amphibians the origin seems to be again diffuse and the same appears to be the case in Birds.

Mesoderm of the Head-Region.—There are two important characteristics of the head-region of the Vertebrate ultimately connected with the muscular system: (1) loss of flexibility, associated with the evolution of brain and skull and (2) special muscularity of the wall of the alimentary canal, associated with the presence of important movable skeletal structures enclosed in the substance of the visceral arches. These peculiarities find their expression (1) in the tendency to suppression of the myotomes of the head-region and (2) in the retention, to a greater extent than in the trunk, of the muscle-forming capacity of that part of the mesoderm which lies ventral to the myotomes.

The mesoderm of the head-region shows the least amount of modification posteriorly where its relation to the mesoderm of the trunk is still clear. In the occipital region—the region between the otocyst and the occipital arch, which may be taken as the hinder limit of the skull—we find a series of typical ("occipital"—Fürbringer) myotomes, the mesoderm ventral to which takes part in the lining of the splanchnocoele just as in the trunk-region. This series of occipital myotomes seems clearly to be undergoing a process of reduction. It is largest in such comparatively primitive forms as Elasmobranchs. Again during ontogenetic development the series commonly shows progressive reduction. In *Spinax* for example seven occipital myotomes make their appearance, but as development goes on the anterior three (*t*, *u*, *v*)[1] break up and disappear, the fourth (*w*) does so incompletely, while the last three (*x*, *y*, *z*) develop into definite muscle-segments though of small size. As each anterior myotome disappears those behind it become shifted forwards so that its place becomes occupied by the myotome originally behind it in the series. It will be realized that there is thus introduced a serious source of possible error which has to be carefully borne in mind in observations on the development of the occipital region where the identification and correct reference of individual myotomes to their place in the series is of importance.

Anteriorly the series of occipital myotomes is prolonged forwards past the otocyst by a mass of mesoderm (* in Fig. 115) which was regarded by van Wijhe (1883)—who may be said to have laid the foundations of modern work upon the segmentation of the mesoderm of the head—as the equivalent of a single ("fourth") myotome. It has already been indicated that the series of occipital myotomes is undergoing reduction from its front end backwards and it seems on the whole more probable that van Wijhe's "fourth" myotome in the Gnathostomata is to be interpreted not as a single myotome but rather as the degenerate remnant of a series of myotomes. The number of myotomes originally present in this series does not appear to be capable of decision with any degree of certainty. Possibly it was very considerable and Froriep finds even in ontogeny (*Torpedo*) that during early stages (Stage "D" of Balfour) as many as six distinct segments are recognizable in the region in question—in other words that the series of myotomes commences not with *t* but with *n*, the anterior members of the series disappearing in turn as development proceeds. A point of

---

[1] The hind end of the series—the occipital arch—being taken as a fixed point while the front end varies, Fürbringer has introduced the convenient method of designating the individual occipital myotomes (or their nerves) by the terminal letters of the alphabet—the last being *z*, the one next in front *y*, and so on. The myotomes behind the occipital arch are counted as belonging to the trunk and are designated by numerals 1, 2 and so on (cf. Fig. 220).

interest is that the anterior limit of this series of recognizable segments agrees approximately with the anterior end of the definitive notochord.

In front of the "fourth" myotome of van Wijhe we find what appear to be fairly typical third and second myotomes, each continuous ventrally with the wall of the pericardiac portion of the splanchnocoele. Of these myotome III gives rise to the External Rectus muscle and II to the Superior Oblique. At the front end of the series we have the first or premandibular or oculomotor myotome, peculiar in that it is fused with its fellow across the mesial plane and that it no longer shows any connexion with the splanchnocoelic mesoderm. It gives rise to the four eye-muscles supplied by the Third cranial nerve—the Superior, Internal, and Inferior Rectus, and the Inferior Oblique.

We have so far dealt only with the myotomes but the lateral or splanchnocoelic mesoderm is also continued well forwards into the head-region. Its more ventral portion forms the lining of the pericardiac cavity, while its more dorsal portion becomes traversed by the visceral pouches or clefts. The splanchnocoelic mesoderm ventral to myotomes II and III comes to form a stalk-like connexion between the myotome and the pericardiac wall (Fig. 115). This stalk is hollow in the case of myotome II and lies in the mandibular arch: in the case of myotome III it is solid and lies in the hyoid arch. In both cases the wall of the stalk gives rise to the muscular apparatus of the particular arch—in the one case the masticatory muscles and in the other the hyoidean musculature which is destined to attain to such a development in the mammals as the musculature of the face.

The splanchnocoelic mesoderm corresponding to the myotomic mass behind myotome III (* in Fig. 115) is said to give rise to the musculature of the branchial arches. As the myotomic mass in question shrivels up during development, and the occipital myotomes move forwards to take its place, these myotomes come to overlie the splanchnocoelic mesoderm which gives rise to the branchial muscles. Consequently as will be realized the position of myotomes *t*, *u*, and *v* in relation to clefts III, IV, and V as shown in Fig. 115 is secondary, the myotomes having moved forwards before the formation of these clefts.

The above sketch has dealt with the cephalic mesoderm of Elasmobranchs but a similar scheme of development with minor variations in detail holds for other Vertebrates. Upon the whole it may be said that with upward progress in the evolution of the Vertebrata the segmentation of the mesoderm in the hinder part of the head becomes more and more obscured. Right up to the highest forms however traces of it persist. In Fowl embryos of about the third day of incubation the series of obvious myotomes may often be seen to be prolonged forward (see Fig. 236) by faintly visible blocks agreeing in size and exactly in series with the myotomes.

These blocks may be indistinguishable in ordinary thin sections but quite distinct in stained preparations of the whole embryo. It will require strong evidence to justify the refusal to give them the interpretation that at once suggests itself—that these slight condensations of the mesenchyme are as it were the ghostly remnants of once existing myotomes which in Birds have ceased to become functional.

An important side issue of their presence to be borne in mind is that the slightly greater resistance of the more condensed portions of mesenchyme must necessarily exercise pressure upon the soft surface of the rapidly growing brain and produce a modelling of its surface which may be adequate to explain at least some of these appearances of segmentation of the brain-region which are included under the term neuromery.

The blocks in question extend well forwards—in the specimen figured (Fig. 236) there are four distinguishable anterior to the middle of the otocyst and they may be taken as additional evidence in favour of there being not one but a number of myotomes represented in the region of van Wijhe's "fourth" myotome.

It is of interest to note that in the Lampreys the blurring of the segments immediately posterior to the third of van Wijhe's series seems not yet to have come about and there is an undoubted simple "fourth" myotome (Koltzoff, 1901). We may justifiably associate this with the low degree of cephalization in these creatures which has involved a persistence of, or more probably a reversion to, an apparently archaic condition of this myotome and its immediate successors in the series.

The relations of segments I, II and III to the eye-muscles have been worked out in a number of Elasmobranchs and similar conditions have been described in Reptiles and Birds. Our knowledge of the holoblastic Vertebrates in this respect is still fragmentary. In the case of *Lepidosiren* and *Protopterus* the eye-muscles develop out of compact masses of mesenchyme in which it is impossible to recognize definite segments (Agar, 1907) while on the other hand in *Ceratodus* (Gregory, 1905) these segments make their appearance much as in Elasmobranchs.

Before leaving this part of the subject it should be pointed out that not all morphologists are convinced that segments I, II and III are actually serially homologous with the undoubted mesoderm segments or myotomes of the trunk-region: the blurring of the mesoderm arrangements between them and the admitted myotomes, and more especially their late appearance in ontogeny, at a time when the anterior members of the occipital series have already degenerated, are brought as evidence against the more generally accepted view. The present writer does not feel inclined to attach great weight to these objections. (1) The break or blurring of the series immediately behind III seems adequately explained by the disappearance of functional muscles in this region and (2) the relatively late appearance of myotomes I to III is explicable by the fact that the functional muscles derived from them are connected with the eyeball an organ which becomes complete and functional only at a relatively late period of development.

HYPOBRANCHIAL OR HYPOGLOSSAL MUSCULATURE.—In addition to the musculature already indicated the Vertebrate head possesses on its ventral side a system of hypobranchial muscles which have the appearance of a prolongation forwards of the longitudinal muscles of the ventral body-wall. This hypobranchial musculature as a matter of fact does arise in ontogeny as a prolongation forwards of the anterior trunk and occipital myotomes, as is well shown by *Lepidosiren* or *Protopterus* (Agar, 1907).

About stage 29 the ventral ends of myotomes *y*, *z* and 1 are seen to be growing out at their ventral ends into a long slender prolongation (Fig. 116). These processes grow outwards in front of the pronephros and undergo complete fusion at their tips. The fused apical portion *c.h* soon separates from the parent myotomes and grows forwards, on each side of the pericardiac cavity, until it reaches the hyoid arch. It now spreads ventrally until it meets its fellow below the pericardiac cavity. The common mass so formed becomes converted into a sheet of longitudinal muscle-fibres, attached posteriorly to the shoulder girdle and anteriorly for the most part to the hyoid arch (coracohyoid muscle, Fig. 117, *cor. hy*), the branchial arches being reduced in the fishes in question. As the muscle goes on with its development the anterior boundary of the portion belonging to myotome 1 becomes marked by a connective-tissue intersection, while in some specimens a similar intersection appears to demarcate *y* from *z*.

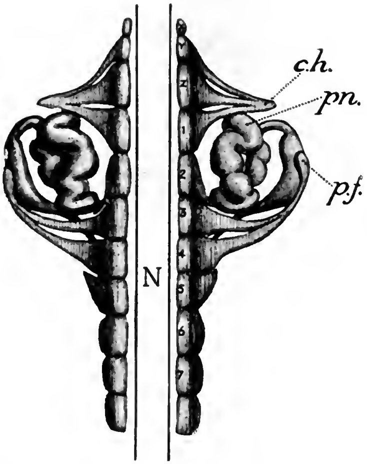


FIG. 116.—Dorsal view of anterior myotomes of a Protopterus of stage 29. (After Agar, 1907.)

c.h, coracohyoid muscle; *N*, notochord; *p.f*, muscle-bud to pectoral limb; *pn*, pronephros.

In other Vertebrates the hypobranchial or hypoglossal musculature appears to originate in the same way—difference occurring only in the number of myotomes which take part. Five appears to be the most usual number (*Scyllium*, Corning: Teleosts, Harrison).

ELECTRICAL ORGANS.—The conspicuous sign of a muscle becoming active is that it changes its shape: an inconspicuous accompaniment of this change of shape is the production of a slight electrical disturbance. In the case of most electrical organs we have to do with portions of the muscular system in which the function of contraction has been reduced to a subsidiary rôle or abolished entirely, while the production of electrical disturbance has become predominant. We have here an excellent example of the principle of "substitution of functions," which is constantly at work during evolution, the previously predominant functions of organs becoming subsidiary or falling into abeyance and being replaced by functions which were previously subsidiary.

The development of the electrical organ can be conveniently studied in the case of the Skate (*Raia*) of which the most complete description has been given by Ewart (1888, 1889, 1892). In this animal the electric organ forms an irregularly spindle-shaped body which lies embedded in the lateral muscles on each side of the tail region. It varies in size in different species and is distinguishable to the naked eye from the muscle by its more gelatinous appearance.

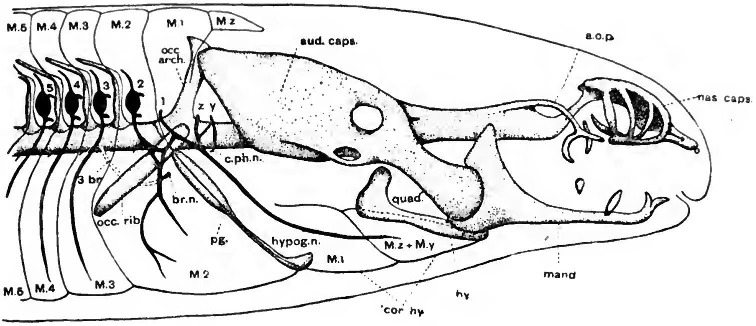


FIG. 117.—Side view of skull and myotomes of *Lepidosiren*, stage 38. (From Agar, 1907.)

Cartilage dotted, myotomes indicated by outlines, nerves black. *a.o.p*, antorbital process; *aud. caps*, auditory capsule; *br.n*, brachial nerve; *c.ph.n*, nerve to dorsal portion of constrictor of pharynx; *cor. hy*, coracohyoid muscles; *hy*, hyoid arch; *hypog.n*, hypoglossal nerve; *M.y*, *M.z*, *M.*1, *M.*2, etc., myotomes; *mand*, Meckel's cartilage; *nas. caps*, nasal capsule; *occ. arch*, occipital arch; *occ. rib*, occipital rib; *pg*, pectoral girdle; *quad*, quadrate; *y*, *z*, nerves; 1, 2, 3, etc., spinal nerves; 3 *br*, branch from 3 to brachial nerve.

On examining transverse sections through the tail it is seen that the electric organ occupies the place of the middle one of five superimposed portions into which the muscle is divided. And this clearly suggests, as Babuchin first pointed out, that the electric organ is morphologically part of the muscular system. That this is actually so is placed beyond dispute by the facts of development. In an embryo of *R. batis* about 7 cm. in length the position of the future electric organ is indicated by a slight modification of the muscle fibres, inasmuch as some of these (Fig. 118, B) show a tendency to assume the shape of a club, the anterior end of the fibre being slightly thickened. In contact with this thickened end is a mass of protoplasm crowded with nuclei. This represents the motor endplate which has assumed a terminal position.

In a slightly older embryo (Fig. 118, C) the club-shaped fibre of the preceding stage has become further modified, the anterior end

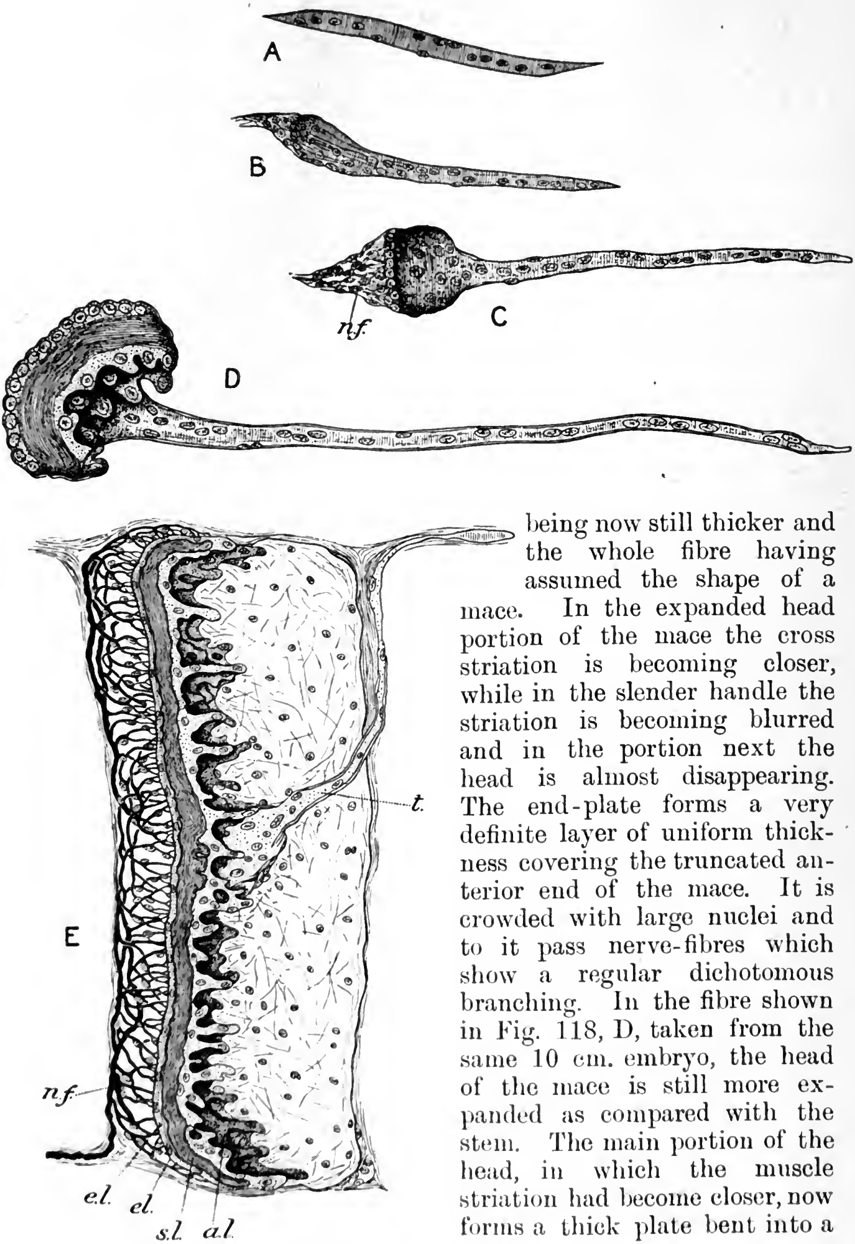


FIG. 118.—Development of the electric organ of *Raia batis*. (After Ewart, 1888.)

A and B from an embryo slightly over 7 cm. in length; C and D from an embryo about 10 cm. in length; E, from a specimen about 67 cm. in length. *a.l*, alveolar layer; *e.l*, electric layer; *el*, electrolemma; *n.f*, nerve-fibres; *s.l*, striated layer; *t*, vestigial remains of muscle-fibre.

being now still thicker and the whole fibre having assumed the shape of a mace. In the expanded head portion of the mace the cross striation is becoming closer, while in the slender handle the striation is becoming blurred and in the portion next the head is almost disappearing. The end-plate forms a very definite layer of uniform thickness covering the truncated anterior end of the mace. It is crowded with large nuclei and to it pass nerve-fibres which show a regular dichotomous branching. In the fibre shown in Fig. 118, D, taken from the same 10 cm. embryo, the head of the mace is still more expanded as compared with the stem. The main portion of the head, in which the muscle striation had become closer, now forms a thick plate bent into a saucer shape with its concavity posterior and composed of numerous closely packed lamellae. It forms what is termed in the fully developed organ the **striated layer**. On its anterior face the striated layer is covered by the end-plate, now known as the **electric layer,** while its posterior face is covered by a thick layer of richly nucleated protoplasm which, from the deeply pitted character of its posterior surface, is known as the **alveolar layer**. From this passes backwards the main part of the muscle-fibre which shows symptoms of degeneration especially in the portion next the alveolar layer where it becomes vacuolated. Whether the alveolar layer represents, as seems probable, a localized thickening of the sarcolemma is not clear from the descriptions.

In the fully developed condition (Fig. 118, E) the muscle-fibre has become converted into the functional **electroplax** (Dahlgren, 1908) or electrical unit. What was the head of the mace in earlier stages is now expanded to form a broad thin circular disc, lying perpendicular to the long axis of the body—the stem of the mace having degenerated into an apparently insignificant and functionless vestige (Fig. 118, E) or having disappeared entirely. The electroplax is formed of the striated layer which is almost flat except round its edge where it is bent in a tailward direction. It is completely ensheathed in syncytial protoplasm, that on its posterior face forming the alveolar layer, probably nutritive in function, that on its anterior face forming the electric layer. Into the latter there pass the numerous end-twigs of the nerve-fibres, the superficial (*i.e.* headward) layer showing a characteristic fibrillation of the protoplasm in a direction perpendicular to the surface (nervous layer—Ewart) in contrast to the deeper portion in which the protoplasm is granular and nucleated (nuclear layer—Ewart). The tail-like vestige of the posterior portion of the muscle-fibre is directly continuous with the striated layer. With the latter it represents the contractile portion of the original muscle-fibre, while the ensheathing protoplasm whether electric layer, or alveolar layer, or sheath of the tail-like vestige, is probably to be regarded as representing the superficial portion of the sarcoplasm.

As the muscle-fibres pass through the above-described modifications, the connective tissue between them increases in quantity and becomes condensed between the electroplaxes in such a way that each electroplax becomes enclosed in a disc-shaped compartment. The walls of this fit close to the electroplax round its edge while, on the other hand, the anterior and posterior walls are separated, especially the latter, by a wide space from the face of the electroplax. This space is occupied by connective tissue with sparsely scattered cells and a jelly-like appearance. That on the anterior side is traversed by the very numerous nerve-fibres which branching dichotomously pass towards the electric layer, while that on the posterior side is traversed by blood-vessels.

During the earlier stages of development the electric organ increases in size, partly by the adding on to it of new electroplaxes formed at its periphery, but the marked growth which takes place in the organ later on is due to actual growth of the individual units which form it. Thus comparing a skate of 180 cm. length with one of 45 cm. the individual electroplaxes are found to have increased in size practically in the same proportion as has the body as a whole.

The above description deals with the development of the electric organ as it takes place in *Raia batis*. In other species of skate the process appears to be similar as regards its main features, but it is interesting to notice that the relative expansion of the front end of the muscle-fibre to form the electroplax is much less pronounced in certain species than is the case in *R. batis*.

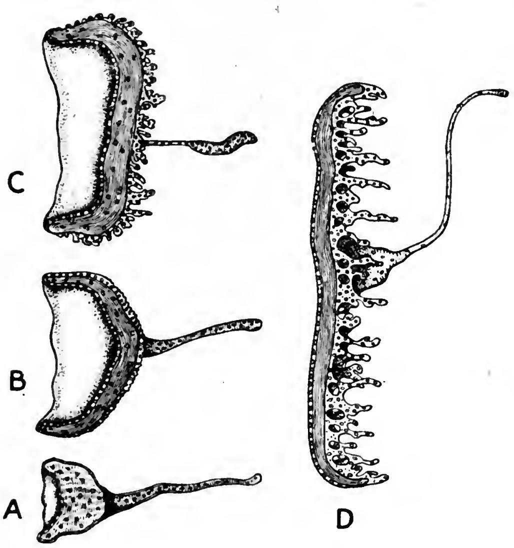


FIG. 119.—Illustrating the adult condition of the electroplax in (A) *Raia radiata*, (B) *R. circularis*, (C) *R. fullonica*, and (D) *R. batis*. (After Ewart, 1892.)

Of the species so far investigated *R. radiata* shows the least advanced stage of evolution. In this species (Fig. 119, A) the electroplax is, as in various other species (*e.g. R. circularis* and *R. fullonica*, Fig. 119), in the form of a cup rather than a disc. In *R. radiata* the wall of the cup is very thick and retains throughout life only slightly modified muscle structure. The electric layer is relatively feebly developed, the thick alveolar layer is represented by hardly modified sarcolemma and the tail is only comparatively slightly degenerate.

The skate has been taken as the basis for the description of the development of the electric organ since the phenomena concerned have been particularly clearly worked out in this fish. In the Torpedoes the electric organ develops from muscles in the region of the visceral arches by very similar stages. As regards the electric organs of Teleosts our knowledge is still very insufficient. In Mormyrids and in *Gymnotus* they are clearly modified portions of the lateral muscles as in the skate; in *Astroscopus* (Dahlgren) they are believed to be derived from eye-muscles; while in *Malopterurus* though generally believed to be modified skin glands they are believed by Dahlgren and Kepner (1908) to be more probably of muscular origin.

Lateral Mesoderm.—The lateral mesoderm forms the lining of the splanchnocoele. Its superficial layer persists throughout life as the coelomic or **peritoneal epithelium**, while its deep surface produces by proliferation abundant mesenchyme cells which form a connective-tissue backing to the epithelium. The development of muscle-fibres, which is so characteristic a feature of the coelomic lining in the dorsal or myotomic region, is here to a great extent suppressed, this portion of the mesoderm no longer playing any part in the muscularization of the body-wall. It still however takes place in restricted areas, smooth or striped muscle-fibres being developed in those portions of the mesoderm which invest particular organs such as heart and blood-vessels, alimentary canal with its appendages, oviduct. The development of the musculature of the heart will more suitably be treated in the chapter dealing with the vascular system. As regards the muscles of the gut-wall we have little detailed knowledge, what there is being related mainly to the musculature of the skeletal elements contained in the visceral arches.

In the larva of *Lepidosiren* the important point has been established by Agar (1907) that the sheath of muscle which forms the constrictor of the pharynx is of double origin, its ventral and larger portion being a development of the splanchnic mesoderm covering the pharynx, while its dorsal portion arises as an outgrowth from one (*y*) or more of the occipital myotomes. The fact that muscular tissue derived from myotomes may join the splanchnic muscle to form part of the muscular sheath of the alimentary canal is of importance (1) by impressing upon us that an apparently homogeneous muscular apparatus may really be heterogeneous —muscularized from two quite distinct sources, and (2) by indicating the possibility of splanchnic musculature being replaced by myotomic or conversely. Obviously the muscular sheath just mentioned might, by reduction of one or other of its component parts, become purely myotomic or purely splanchnic. As will be gathered later (Chap. VII.) the point is an important one from its bearing upon the discussion of certain problems of morphology.

Renal Organs. — In triploblastic Metazoa the function of excreting nitrogenous waste products is commonly carried out by tubular organs to which Lankester (1877) gave the name **nephridia**. Under this term were included the excretory tubes of Chaetopods, Molluscs, Rotifers, Trematodes, Turbellarians and Vertebrates. Subsequent research soon brought to light an important structural difference as regards the inner ends of these nephridial tubes in different groups of animals. In certain groups the tube possesses at its inner end an open funnel or nephrostome[1] which leads from the coelome into the cavity of the tubule, while in other groups the inner end of the tubule is without any coelomic funnel but is on the other hand provided with an arrangement of flame-

---

[1] Goodrich terms such funnels "coelomostomes" and uses the word nephrostome in a special restricted sense.

cells. The appreciation of this difference gave rise, not unnaturally, to a suspicion — which would now appear to be unfounded—that under Lankester's name nephridium were included excretory tubes of two morphologically distinct types and the use of the word nephridium was often restricted to the one of these types in which the coelomic funnel was present.

Later researches brought out the fact that in some cases—certain Polychaete worms—the excretory tube may possess both flame-cells and coelomic funnel. And finally the hypothesis was developed—by Meyer and especially Goodrich (1895) — that the nephridial tube and the coelomic funnel were originally quite distinct organs with separate openings to the exterior. On this view the primitive excretory tube or protonephridium (Goodrich) was provided with flame-cells at its inner end, while apart altogether from it and opening independently to the exterior was the coelomic funnel which formed the primitive exit for the reproductive cells. In the course of evolution there came about a fusion of the two structures, the coelomic funnel becoming as it were grafted on to the nephridium and in many cases shifted up the wall of the tubule right to its inner end. Such a compound organ (Nephromixium — Goodrich) might retain for a time both flame-cells and coelomic funnel—as in the Polychaetes alluded to above—or the flame-cells might, as is more usual, disappear leaving an excretory tube possessing at its inner end a coelomic funnel which shows no trace of its morphologically independent origin. To support the hypothesis which has just been outlined there is brought in the evidence of embryology which testifies (see Vol. I. p. 158) that the main part of the excretory tube is developed as an ingrowth of the ectoderm, while the coelomic funnel arises as an outgrowth of the mesoderm.

This hypothesis has met with very general acceptance not merely with regard to the excretory organs of Annelids alone but also as a theory of the morphology of excretory tubes in general. As, however, the writer of this volume takes up a somewhat different standpoint it will now be necessary to state shortly what that standpoint is.

The word nephridium will be used in the original sense as meaning an excretory tube whether possessing flame-cells or a coelomic funnel at its inner end.

Physiologically the open funnel and the flame-cell appear to be associated primarily with two different sets of spaces. The funnel is associated with coelomic spaces and it serves to transmit to the exterior the products of the lining of such spaces—fluid, excretory, or reproductive. The flame-cell is associated rather with the meshes of the mesenchymatous spongework: it serves to filter off from these spaces watery fluid containing excretory salts in solution. The activity of the "flame" is in direct relation to the pressure of fluid within these spaces: if the pressure is lowered by making a minute puncture in the body-wall the movement at once ceases—to commence again when pressure is restored. This association of flame-cells with the spaces of the mesenchyme is seen in the more lowly forms in which they occur and it is therefore justifiable to regard it as primitive in spite of the exceptional cases in which flame-cells occur in coelomic cavities.

The question of the relative antiquity in evolution of coelomic funnel and flame-cell is one which cannot be decided with certainty, depending as it does in turn on the unsolved question as to whether coelome or mesenchyme was evolved first. Looking to the occurrence of mesenchyme in Coelenterates (*e.g.* the Alcyonarians), in animals in which there is not as yet any closed-off coelome, the balance of probability seems to be on the whole in favour of the flame-cell having originated first, in other words in favour of the original nephridium being of the type called by Goodrich protonephridium. The fact that existing excretory tubes of this type arise from the ectoderm is also an argument for its antiquity, as it seems natural to suppose that primitively excretory products were got rid of at the outer surface of the body.

In the primitive ancestral form the genital cells, formed by the lining of the enterocoelic pouches, would reach the exterior through the protostoma or primitive mouth but as evolution proceeded and the coelenteric pouches became separated from the enteron to form a closed coelome[1] another mode of exit would have to be evolved. The natural mode of such exit would be by rupture of the coelomic wall at its weakest spot. Such weak spots would be provided at points where the cavity of the nephridial tube came into proximity with that of the coelome. At such points rupture would take place and the tendency would be for such a temporary rupture, at the time of maturity of the genital cells, to be replaced by a permanent[2] opening from coelome into nephridium. This permanent opening would be the coelomic funnel (coelomostome, nephrostome in the original sense).

The coelomic funnel, though originally developed to transmit the genital cells, would necessarily also serve as an exit for superfluous coelomic fluid, and the fluid so transmitted would necessarily serve incidentally to flush out excretory matters passed into the lumen of the tube by the activity of its walls and would thus fulfil the function originally fulfilled by the fluid drawn in by the flame-cell. The function of the flame-cells being in this way otherwise provided for they would tend to disappear.

The nephridial tube thus came to transmit (1) the reproductive cells and (2) the highly poisonous excretory products. There is, as it appears to the writer, ample evidence that under such circum-

---

[1] The argument involves as will be seen the assumption that the coelome was in its evolutionary origin enterocoelic. This assumption appears to be justified by the numerous cases in which the coelome so arises in ontogeny.

[2] An actual case where such a temporary rupture, brought about at parturition, has come to be a fixed character of the species and develops independently of mechanical rupture, is seen in the "median vagina" of certain Marsupials.

stances the tendency of subsequent evolution would be to separate from one another the paths to the exterior of the genital cells and of the poisonous excretory products respectively. It might fairly be anticipated for physiological reasons that there would be such a tendency but that it actually exists is demonstrated by the facts of comparative anatomy and embryology. Over and over again we find cases where such separation has undoubtedly come about. For example in the evolution of the Gasteropoda the right nephridium has lost its excretory function and come to be merely a genital duct. In Vertebrates there are several familiar examples of parts of the renal system which have to do with transmitting genital cells becoming separated off from those which retain a renal function.[1]

The present writer then believes the balance of probability to be in favour of the evolutionary origin of the type of nephridial tube commonly met with in coelomate animals, possessing a coelomic funnel or nephrostome at its inner end, having come about in the manner outlined above. The essential difference between the view here outlined and that developed by Goodrich is that it rejects the idea that evolution has brought about a more and more intimate connexion between originally independent genital funnel and nephridial tube as opposed to physiological probability. On the contrary it regards the funnel as having opened into the tube at the time of its first appearance, the progress of subsequent evolution having been in the direction of separating genital funnel and nephridial tube and not of uniting them. Even in the case of Polychaete worms the arguments against interpreting the anatomical arrangements in different genera as illustrating evolutionary sequence in the *reverse* order to that believed in by Goodrich seem unconvincing and insufficient to counterbalance the weight of physiological probability.

In the case of a tube leading from the coelome to the exterior the two ends are almost of necessity mesodermal and ectodermal in their nature respectively. Consequently, the fact that the "nephromixium" has such a twofold origin in ontogeny does not appear to the present writer to constitute evidence of any particular weight that it actually arose in phylogeny by the fusion of two pre-existing independent organs. As regards the proportion derived from the two layers the probability would be that the specially excretory portion was originally ectodermal—excretory products being naturally got rid of by the outer surface—and that the portion specially concerned with the getting rid of coelomic products would be mesodermal—arising as a bulging of the coelomic lining.

Accepting as a working hypothesis that the nephridial system of tubes with their nephrostomes arose in the manner outlined above, it is important to bear in mind how greatly the system would be influenced in its subsequent evolution by the establishment of

---

[1] *E.g.* the separation of the Müllerian duct from the kidney system or the separation of the renal collecting tubes from the Wolffian duct.

circulating mesenchyme or blood. This would render possible the shortening up of the nephridial tubes and the more definite localization of the excretory tissue. Whereas the original flame-cell type of excretory apparatus was diffuse—the flame-cells being scattered throughout the mesenchyme sponge-work—as it is still to be seen in the more lowly organized forms—it would now become compact, the waste-products being brought to it by the movements of the circulating blood.

NEPHRIDIAL ORGANS OF VERTEBRATES.—Before passing on to the details of development of the renal organs in Vertebrates it is necessary to notice one or two points of general importance regarding the morphology of these organs within this particular phylum, and also to define precisely the sense in which certain technical terms will be used.

In the first place the kidneys or renal organs of Vertebrates are built of tubules each of which is a nephridium according to the original definition of the term.

The conclusion, already arrived at, that the ancestral Vertebrate possessed a completely segmented coelome, carries with it the further conclusion that in all probability a pair of nephridial tubes originally opened to the exterior from each segment. A characteristic feature however of the Vertebrates (with the exception of *Amphioxus*) is that the nephridia open not directly to the exterior on the surface of each segment as in a typical Annelid but into a longitudinal duct which passes back along each side of the body and communicates at its hind end with the cloaca. The whole series of nephridial tubes on each side of the body is known as the **archinephros**[1] and the duct as the **archinephric duct.**

In the embryos of Vertebrates development takes place from the head end backwards. We should therefore expect the nephridial tubules to appear in regular sequence from before backwards. It is however highly characteristic of the Vertebrate that the tubules, instead of developing in this regular sequence, develop in three batches one behind the other—an anterior, a middle, and a posterior. These constitute respectively the **pronephros, mesonephros,** and **metanephros** (Lankester, 1877). In many of the lower Vertebrates there is no separation between mesonephros and metanephros, the two forming a continuous structure which acts as the functional kidney. Such a type of renal organ consisting of the series of tubules corresponding to mesonephros together with metanephros may conveniently be termed the **opisthonephros.**[2]

Of the four types of kidney just mentioned the first—the pronephros—forms the functional kidney in *larval* Vertebrates. It is well seen in the larvae of Crossopterygians, Actinopterygians, Lungfishes, and Amphibians, while, as Sedgwick first pointed out, it is

---

[1] Archinephron, Lankester (1877). Price's term Holonephros is also frequently used in the same sense.

[2] In analogy with the use of the word opisthosoma in the group Arachnida.

reduced in forms with richly yolked eggs where the development is not larval. Its reduction or disappearance in the last-mentioned forms may probably be due to the facilities afforded for getting rid of excretory products by simple diffusion from the blood circulating on the surface of the yolk-sac into the surrounding medium.

The opisthonephros forms the functional kidney in the adults of most if not all anamniotic Vertebrates.

Distinct mesonephros and metanephros are found in the Amniota —the mesonephros being functional during the later embryonic period and in the Reptiles during the first few months after hatching, while the metanephros forms the definitive kidney of the adult, its excretory activities being reinforced during the first few months in the case of Reptiles by the still functional mesonephros.

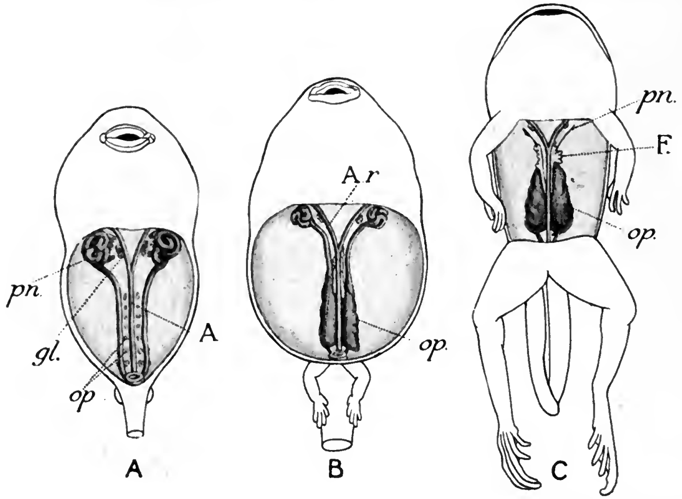


FIG. 120.—Renal organs of the Frog (*Rana temporaria*) as seen from the ventral side after the ventral wall of the splanchnocoele and the portion of the alimentary canal contained within it have been removed. (After Marshall, 1893.)

A, 12 mm. tadpole; B, 40 mm. tadpole; C, frog at time of metamorphosis. *A*, dorsal aorta; *A.r*, aortic root; *F*, fatty body; *gl*, glomerulus; *op*, opisthonephros; *pn*, pronephros.

THE PRONEPHROS.—A typical functional pronephros is well seen in a frog tadpole of about half an inch in length (Fig. 120, A, *pn*). It consists of a massive organ lying dorsal to the anterior portion of the splanchnocoele on each side. It consists mainly of a much convoluted tube the anterior portion of the archinephric duct, and into this there open three segmentally arranged pronephric tubules also, except the anterior one, much coiled and twisted. While the organ as a whole is retroperitoneal, *i.e.* outside the coelomic lining, there exists an opening leading from the splanchnocoele into each tubule —the **nephrostome** (Fig. 121, *ns*). Duct and tubules are lined with cubical, or almost columnar, epithelium and in the neighbourhood of the nephrostome the cells become pigmented and carry powerful flagella. At the lip of the nephrostome the lining epithelium of the tubule is continued into the flattened epithelium lining the splanchnocoele which is richly ciliated in its immediate neighbourhood.

The archinephric duct is continued back from the pronephros along the coelomic roof to open at its hinder end into the cloaca.

The pronephros has very characteristic relations to the blood-vascular system. The tubules serve to transmit to the exterior the fluid secreted by the coelomic epithelium and a patch of this epithelium, lying on the roof of the splanchnocoele at its mesial side and facing the nephrostomes, has its secretory activity much exaggerated. This specially secretory epithelium has its area increased by bulging into the splanchnocoele, the bulging portion enclosing a vascular skein connected with the aortic root. This bulging structure is known as the **glomerulus** (Figs. 120 and 121, *gl*). The anterior convoluted part of the archinephric duct and the tubules opening into it have other relations to the vascular system, for their surface is bathed by the blood of the posterior cardinal sinus which forms a system of irregular spaces between them. This double relation to the blood-system is doubtless correlated with the double function of the organ. It serves in the first place to get rid of watery fluid secreted by the coelomic epithelium, and with this function the glomerulus with its aortic blood supply is concerned; secondly it has to extract poisonous waste products from the circulating blood, and this is done by the wall of the tubule acting on the venous blood which bathes its surface.

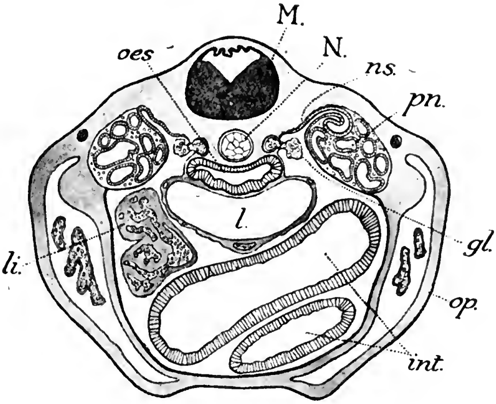


Fig. 121.—Transverse section through a 12 mm. Tadpole at the level of the pronephros. (After Marshall, 1893.)

gl, glomerulus; *int*, intestine; *l*, lung; *li*, liver; *M*, medulla oblongata; *N*, notochord; *ns*, nephrostome; *oes*, oesophagus; *op*, operculum; *pn*, pronephros.

Development of the Pronephros in Hypogeophis.—*Hypogeophis*, a member of the Gymnophiona, will be taken as an example of the mode of development of the pronephros for the following reasons:

(1) In this as in other Amphibians the pronephros still becomes an actively functional organ. Consequently the probabilities are in favour of its developmental processes having departed less from the

ancestral methods than in the case of those Vertebrates (*e.g.* Elasmobranchii, Amniota) in which the organ is modified to the extent of being reduced to a functionless rudiment.

(2) Its histological texture is comparatively coarse and the general structural arrangements in the embryo are so distinct as to eliminate to a great extent risk of observational errors.

(3) Its development has formed the subject of a particularly careful and complete investigation (Brauer, 1902).

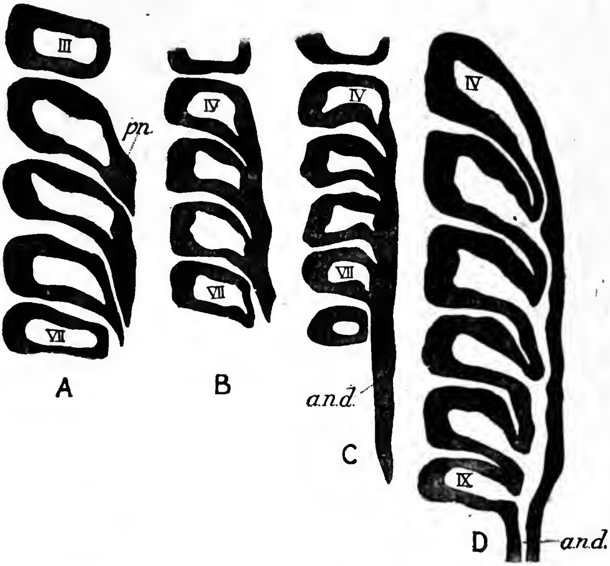


Fig. 122.—Early stages in the development of the pronephros of *Hypogeophis*. Each figure represents a longitudinal section, so arranged as to pass outwards through the nephrotomes, cutting them across, and viewed from the dorsal side. (After Brauer, 1902, slightly simplified.)

A, from an embryo with 15 mesoderm segments; B, 12 segments; C, 16 segments; D, 27 segments. *a.n.d*, archinephric duct; *pn*, pronephric tubule. The Roman figures are placed in the nephrocoeles.

The first signs of the pronephros make their appearance—in embryos with about 9 or 10 mesoderm segments—in the form of bulgings outwards of the outer or somatic wall of the nephrotome of segments IV and V. These outward bulgings are the rudiments of the pronephric tubules. A third soon appears in segment VI (Fig. 122, A, cf. also Fig. 123, A, *pn*). The three rudiments grow actively in length pushing their way tailwards along the body just external to the nephrotomes. They come to be in close contact and presently fuse to form a rod-like structure (Fig. 122, B) which continues to extend backwards towards the tail and becomes tubular through developing a cavity secondarily in its interior. This, at first solid, rod-like structure (Fig. 122, C, *a.n.d*) is the rudiment of the archinephric duct, which thus owes its origin to the fusion

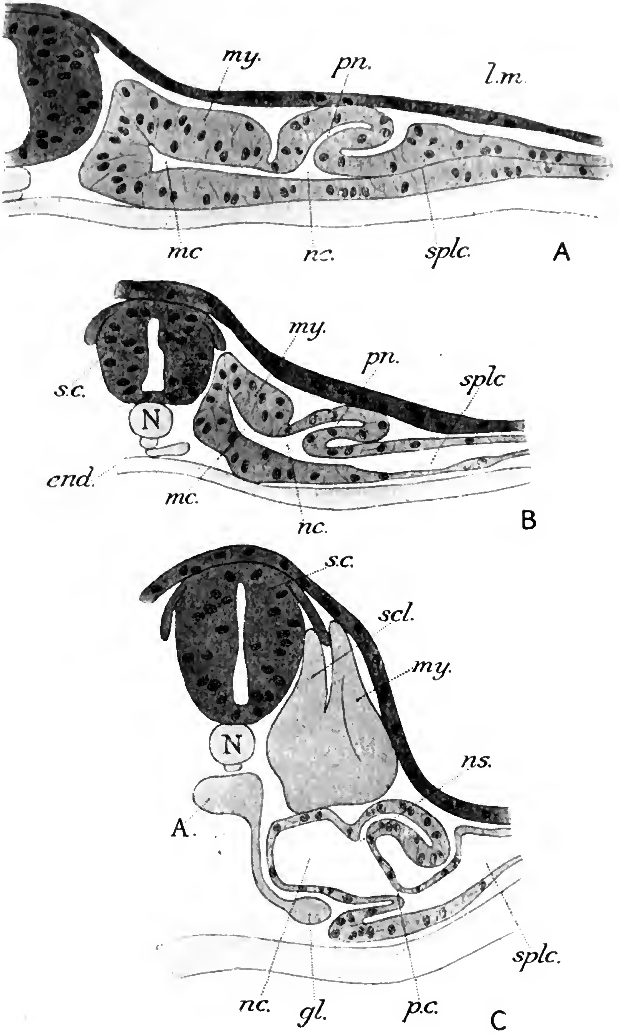


Fig. 123.—Development of pronephros of *Hypogeophis* as seen in transverse sections. (After Brauer, 1902.)

A, embryo with 22 segments; B, with 29 segments; C, with 44 segments. *A*, dorsal aorta; *end*, endoderm; *gl*, glomerulus; *l.m*, lateral mesoderm; *mc*, myocoele; *my*, myotome; *N*, notochord; *nc*, nephrocoele; *ns*, nephrostome; *p.c*, peritoneal canal; *pn*, pronephric tubule; *s.c*, spinal cord; *scl*, sclerotome; *splc*, splanchnocoele.

together of the outer ends of the tubule rudiments belonging to segments IV, V, and VI.

Additional tubule rudiments to the number of about 8 arise in order behind those first laid down. They arise in exactly the same way as the first (Fig. 122, B, VII) but as the archinephric duct rudiment has already grown past their point of origin they become joined on to the duct by their outer ends undergoing *secondary* fusion with it. Each tubule rudiment grows actively in length so that it eventually forms a much-coiled tube connecting the nephrocoele or coelomic cavity of the nephrotome with that of the duct.

In the meantime the nephrotome is undergoing important changes apart from the tubule rudiment. Its cavity, the nephrocoele, from being a mere slit with its floor and roof in contact, becomes widely dilated and it becomes cut off from the dorsal part of the segment which forms the myotome and sclerotome (Fig. 123, C). The nephrotome also becomes gradually constricted off from the lateral mesoderm but in this case the separation either never becomes completed (Fig. 123, C, *p.c*) or if it does so, is merely temporary—communication being soon re-established at the point where the constriction took place. The nephrocoele is thus, even in the fully developed pronephros, in open communication with the splanchnocoele by a more or less narrow channel the **peritoneal canal** (Fig. 123, C, *p.c*), the splanchnocoelic end of which forms the **peritoneal funnel.**

As the nephrotome and tubule go on with their development there arise characteristic relations with the blood-vascular system. An intersegmental branch from the dorsal aorta passes to each nephrotome, causing its floor to bulge into the nephrocoele (Fig. 123, C, *gl*) and form a conspicuous projection—the glomerulus—which later on fills up most of the nephrocoelic space. From the glomerulus the vessel passes (as the *vas efferens*) into a network of blood-spaces lying between the coils of the tubule and belonging to the posterior cardinal vein.

The fully formed pronephros of *Hypogeophis* is composed of about a dozen segmentally arranged units each developed in the way described. It is to be noted however, that the last three of these units never become fully developed and further that behind the last as well as in front of the first unit of the functional pronephros each segment has its typical nephrotome though this never proceeds with its development. In other words the pronephros of *Hypogeophis* possesses at its anterior and posterior ends a number of units more or less reduced or vestigial.

It is also of interest to notice certain variations which occur in connexion with the relations of the tubule to the nephrotome. In what may be termed the typical arrangement the nephrostome opens from the dilated part of the nephrocoele (Fig. 124, A). Frequently however it has become shifted on to the constricted peritoneal canal (Fig. 124, B). When it does this there are apt to arise very misleading appearances as illustrated by the accompanying figure, whereby on the one hand the tubule appears to lead directly from the splanchnocoele, the chamber containing the glomerulus appearing to be a side branch (Fig. 124, C), or on the other hand the pronephric chamber appears to form the dilated end of the tubule while the peritoneal canal appears to form a side branch (Fig. 124, D).

In connexion with what has been said it is important that the student should get clear in his mind from the beginning (1) that the cavity into which the glomerulus projects (known as the cavity of the Malpighian body in the more highly evolved types of kidney) is simply a more or less completely separated off portion of the coelome (nephrocoele) and that neither it nor the peritoneal canal is to be regarded as a portion of the tubule, and (2) that the actual tubule commences at the nephrostome or opening leading into it from the nephrocoele. The word nephrostome throughout morphology means an opening leading from coelome into nephridium. It is necessary to accentuate this because in many embryological writings the term nephrostome, or nephrostomal canal, is applied to the peritoneal canal which is not an opening leading from coelome into nephridium but simply a communication between the splanchnocoelic and the nephrocoelic portions of the coelome. A clear appreciation of these points is of help in facilitating the comprehension of a difficult chapter in Vertebrate morphology.

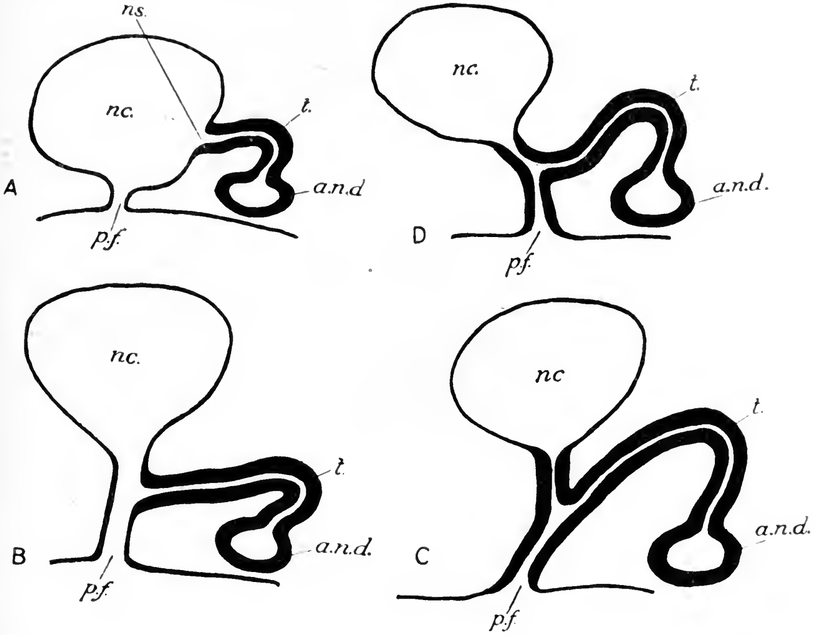


Fig. 124.—Illustrating variations in the relations of nephrocoele, tubule and peritoneal canal in the pronephros of *Hypogeophis.*

a.n.d, archinephric duct; *nc*, nephrocoele; *ns*, nephrostome; *p.f*, peritoneal funnel; *t*, tubule.

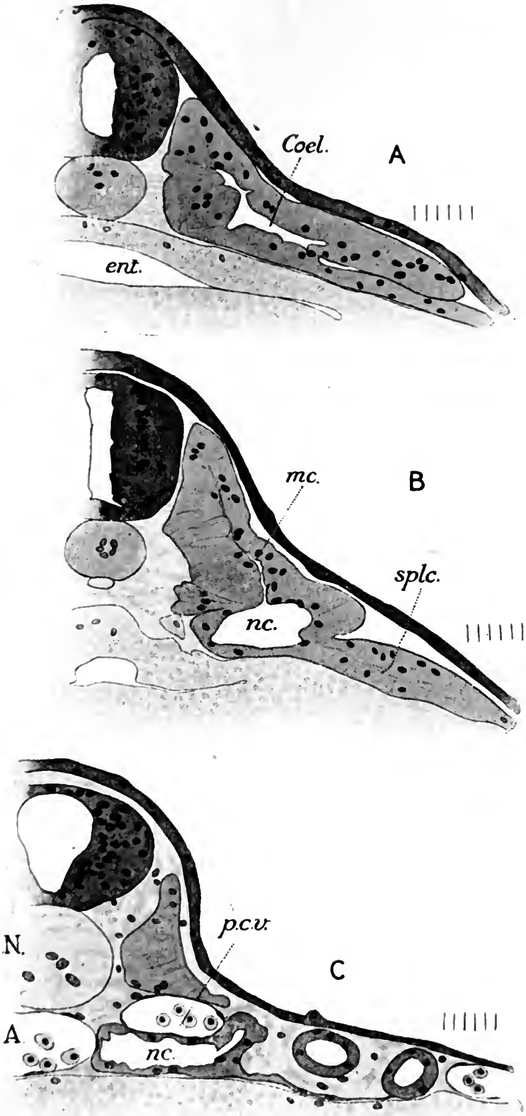


FIG. 125.—Transverse sections of *Polypterus*—stages 20, 23, and 28—passing through the rudiment of nephrostome B, which is seen projecting outwards from the wall of the nephrocoele.

A, dorsal aorta ; *Coel*, coelome ; *ent*, enteric cavity ; *mc*, myocoele ; *N*, notochord ; *nc.* nephrocoele ; *p.c.v*, posterior cardinal vein ; *splc*, split representing splanchnocoele.

In other Vertebrates possessing a functional pronephros the appearances seen in early stages are readily reconcilable with those described above for *Hypogeophis*, and we may take it that, apart from variations in detail, this represents the normal mode of development of the organ.

CROSSOPTERYGII. — In Crossopterygians, so far at least as *Polypterus* — the only member of the group investigated— is concerned, the first rudiments of the pronephric tubules are in the form of projections which pass outwards and backwards from the external side of each of the anterior nephrotomes (Fig. 125, A). The number of these tubule rudiments presumably varies, seven being seen in one specimen and nine in another. Apparently the tubule rudiments become fused at their outer ends to form a solid mass—the rudiment of the archinephric duct. In the stages shown in Fig. 126, A and B, five tubule rudiments are seen passing at their outer ends into the duct rudiment. As development goes on however the tubules belonging to nephrotomes I, III, and IV, those labelled A, C and D in the figure, become reduced in size and finally

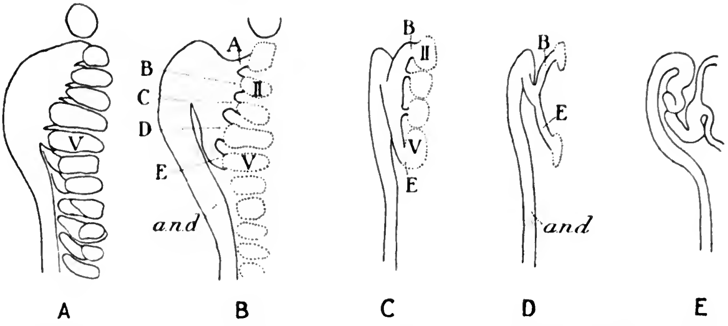


Fig. 126.—Dorsal view of pronephros of *Polypterus* at stages 20, 23, 24+, 25 and *about* 28.

a.n.d, archinephric duct. The tubule rudiments are indicated by letters, the nephrotomes by Roman numerals.

disappear, while B and E on the other hand increase in length and become the functional tubules. The anterior end of the archinephric duct becomes gradually modelled out of the solid rudiment already referred to in the way indicated in the figure.

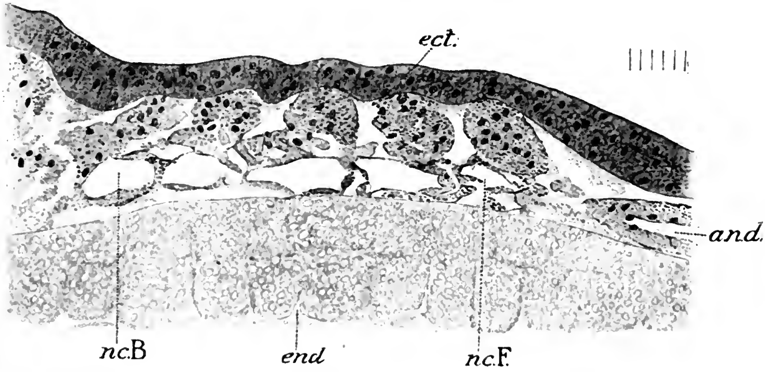


Fig. 127.—Part of a longitudinal vertical section through the series of nephrocoeles in the pronephros of *Polypterus*—stage 24+.

a.n.d, archinephric duct; *ect*, ectoderm; *end*, endoderm; *nc.B*, nephrocoele "B"; *nc.F*, nephrocoele "F."

After it has assumed its definitive tubular form this front part of the archinephric duct commences to grow actively in length, it becomes thrown into complicated coils and forms a large fraction of the entire bulk of the pronephros in its early functional stages.

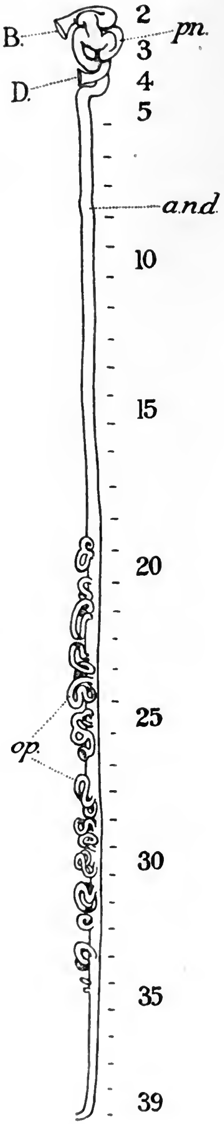


Fig. 128.—Renal organs of the right side of a Protopterus larva of stage 34. (From a reconstruction by M. Robertson.)

a.n.d, archinephric duct; *op*, opisthonephric tubules; *pn*, pronephros. The capital letters indicate nephrostomes and the figures metotic * mesoderm segments.

In its later functional stages the pronephros reaches relatively enormous bulk, occupying the whole thickness of the body-wall, but in these later stages the two tubules become much elongated and coiled as well as the duct itself.

The nephrocoeles belonging to the various nephrotomes which develop tubules form a series of closed cavities lying in a row one behind the other (Fig. 127, *nc.B*, *nc.F*). They are for a long time, in *Polypterus*, the only coelomic spaces which are widely open (Fig. 125, B). As development goes on the nephrocoeles connected with the functional tubules (B and E) become more and more dilated, their wall becoming thinner as they do so, and the floor bulging into the cavity to form the glomerulus. Eventually the cavity of the nephrocoele becomes continued ventrally, a split spreading downwards to form the splanchnocoele, into which the nephrocoele opens freely. The portions of splanchnic mesoderm to which the glomeruli are attached, *i.e.* the floors of the original nephrocoeles, become folded in towards one another, as the splanchnocoelic cavity dilates, to form the dorsal mesentery so that the glomeruli are eventually borne by the mesentery one on each side.

Meanwhile the nephrocoeles belonging to the tubules which atrophy gradually shrink up and disappear, and as they do so the two large functional nephrocoeles increasing still more in size meet and their cavities as well as their glomeruli become continuous. No definite constrictions (peritoneal canals) are formed between nephrocoeles and splanchnocoele, unless possibly during late stages, but the dorsal portion of the more posterior nephrocoele becomes cut off from the splanchnocoele by another method—the free edge of the glomerulus coming to fuse with the somatopleure so as to form a floor to the nephrocoele.

---

* "Metotic" = posterior to the otocyst.

This posterior nephrocoele is still in wide communication with the splanchnocoele indirectly by way of the anterior nephrocoele.

Dipnoi.—In *Lepidosiren* and *Protopterus*[1] the fully functional pronephros of the larva possesses usually two tubules (Fig. 128, B and D). These are the surviving members of a series of tubule rudiments extending through at least the anterior 4-7 segments but probably extending much further back. The tubules which become fully developed are normally "B" and "D" *i.e.* those corresponding to the second and fourth mesoderm segments. Thus the second tubule does not correspond with the second tubule in the fully developed pronephros of *Polypterus*. The tubules appear to originate (cf. Fig. 129, A, B) as in *Hypogeophis* except that the outgrowths from the nephrotomes are solid as in *Polypterus* and such is the case also with the archinephric duct rudiment.

The nephrocoeles of the two main pronephric tubules undergo fusion as in *Polypterus* so as to form a large pronephric chamber on each side. This is continuous with the pericardiac portion of the splanchnocoele and the two glomeruli as usual become fused together to form a compound glomerulus.[2] In *Lepidosiren* the fusion of the pronephric chambers takes place before the appearance of the glomerular rudiments. These appear first on the floor of the continuous cavity (Fig. 129, C, *gl*) and very soon undergo fusion themselves. By differential growth the root of attachment of the glomerulus becomes gradually shifted towards the mesial plane and dorsally (Fig. 129, D and E) so that it comes to hang down into the pronephric chamber or nephrocoele from a point in close proximity to the dorsal aorta.

The pronephric chambers are at first perfectly continuous with the splanchnocoele which spreads outwards from them. Later on the pronephros becomes greatly enlarged and bulges across the splanchnocoele until it comes in contact with the mesodermal sheath of the oesophagus. Fusion then takes place (at the point marked with * * in Fig. 129, E) between the surfaces in contact so that the glomerulus comes to be enclosed in a secondary pronephric chamber, which however remains freely open to the splanchnocoele at its hinder end. The glomerulus becomes firmly slung diagonally across this chamber by its tip undergoing fusion on the ventrolateral side of the chamber with the mesoderm investing the pronephros.

In *Ceratodus* (Semon, 1901) the pronephros probably develops in a manner similar to that described in the case of the other two Lung-fishes. The organ in its first stage is a solid projection of the mesoderm the appearance in section being similar to that figured for *Lepidosiren*. The portions of the rudiment corresponding to the individual tubules are in such close apposition as to be at first indistinguishable (as is often the case in the other two Lung-fishes):

---

[1] A large part of the investigations upon which this account is based were carried out by Miss Muriel Robertson in the University of Glasgow during 1904.

[2] The word **glomus** is often used for such a compound glomerulus.

it is only when nephrocoeles begin to appear (in the regions of the fifth and sixth segments) that the segmented nature of the rudiment

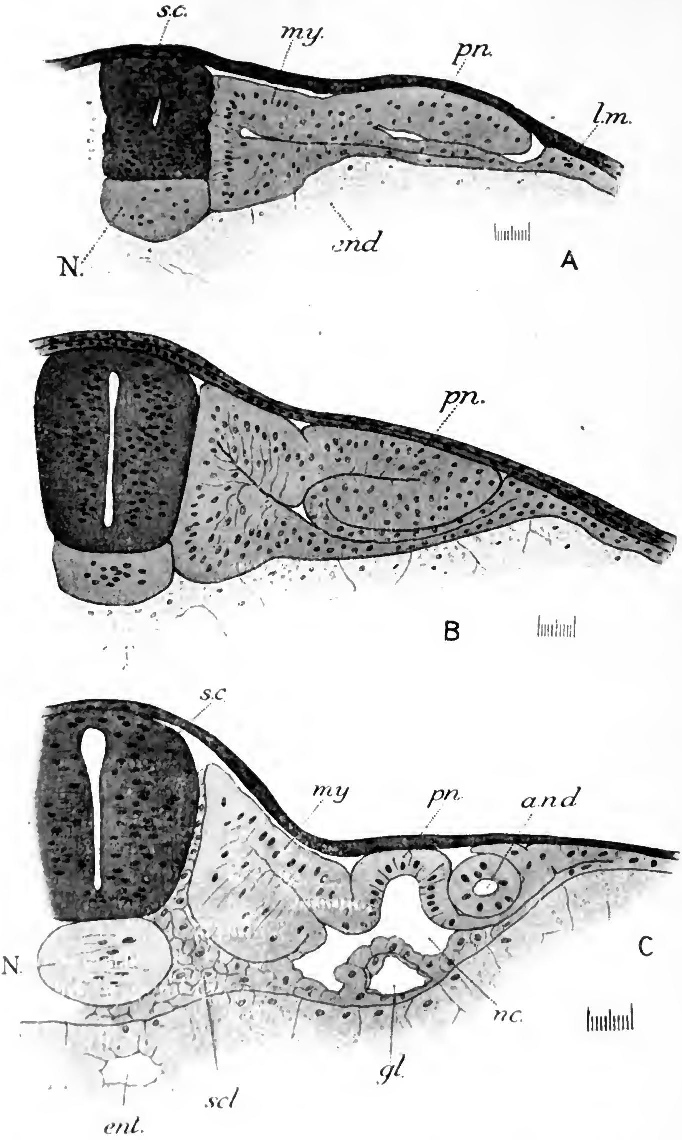


Fig. 129.—Development of the pronephros in *Lepidosiren* as shown in transverse sections.

A, stage 21; B, stage 21; C, stage 24+. *a.n.d*, archinephric duct; *end*, endoderm; *ent*, enteric cavity; *gl*, glomerulus; *l.m*, lateral mesoderm; *my*, myotome; *N*, notochord; *nc*, nephrocoele; *pn* pronephric tubule; *s.c*, spinal cord; *scl*, sclerotome.

becomes apparent. The fully functional pronephros has two tubules on each side, corresponding to the segments above mentioned: it may be presumed that these are the survivors of a once greater number, though there is no record of other rudiments having been actually observed.

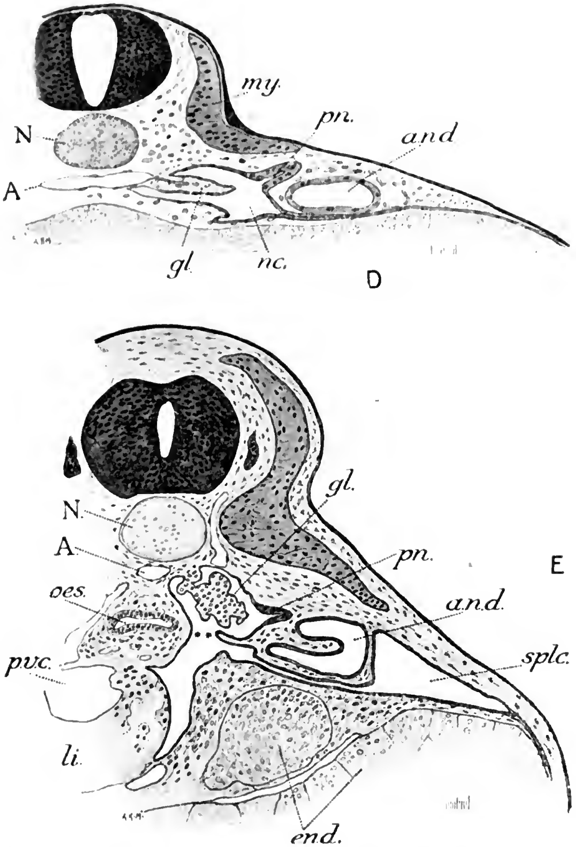


Fig. 129a.—Development of the pronephros in *Lepidosiren* as shown in transverse sections.

D, stage 30; E, stage 31+. *A*, dorsal aorta; *a.n.d*, archinephric duct; *end*, endoderm; *gl*, glomerulus; *li*, liver; *my*, myotome; *N*, notochord; *nc*, nephrocoele; *oes*, oesophagus; *pn*, pronephric tubule; *p.v.c*, posterior vena cava; *splc*, splanchnocoele.

Actinopterygii.—The acquirement of a thorough knowledge of the development in the more primitive members of the group—the ganoids—is an essential preliminary to the proper comprehension of the development of the more highly evolved Teleosts but unfortunately our knowledge of renal development in the ganoids is still far from complete.

The tubule rudiments appear to arise in normal fashion, as outgrowths of the lateral wall of the nephrotome. These outgrowths show the familiar variation of being sometimes hollow sometimes solid. Thus in *Amia* according to Felix (1904) the anterior three rudiments are hollow pockets while those farther back are at first solid.

Tubule rudiments make their appearance from segment III to segment XIII but here as elsewhere only relatively few of these complete their development and are to be found in the pronephros at the height of its functional activity. Thus in a six-day Acipenser larva Jungersen found six functional tubules while in *Amia* Felix finds only a single tubule functional. In the latter case the tubule opens from a large pronephric chamber apparently formed by the fusion of at least three nephrocoeles. The tubule belongs originally to the most anterior of these and corresponding to it there is present a single open peritoneal canal. Later on this becomes replaced functionally by another peritoneal canal situated farther back. In *Lepidosteus* the functional pronephros has at least three tubules each with its nephrocoele (Felix, 1904).

As in the case of the Lung-fishes the dorsal part of the splanchnocoele in the pronephric region becomes floored in by the approximation of the mesial surface of the pronephros to the lateral surface of the oesophagus (cf. Fig. 129A, E) so as to form a secondary pronephric chamber. In *Lepidosteus* this forms a widely patent cavity with which the first nephrocoele becomes completely merged and which remains ventrally in continuity with the main splanchnocoele by a narrow richly ciliated tubular channel. In *Acipenser* the first nephrocoele undergoes a similar modification while the remaining five are fused with one another but isolated from the splanchnocoele.

TELEOSTEI.—The development of the renal organs has been worked out in detail in the case of the genus *Salmo* by Felix (1897). In this case the myotomes are already separate from the more ventrally situated portions of the mesoderm at a very early stage. The first rudiments of the pronephros are in the form of a series of somewhat conical, segmentally arranged, solid projections from the median edge of the lateral mesoderm towards the mesial plane. These projections—five in number (segments 3-7) in a 26-day Trout—are probably to be regarded as nephrotomes which have been precociously separated from the myotomes, if indeed they ever were continuous. These five nephrotomes soon come into intimate contact so as to be no longer distinguishable. They now together form a continuous mass of mesoderm the so-called pronephric fold. The dorsal and outer portion of this mass becomes nipped off to form the anterior portion of the archinephric duct except at one point where a connecting isthmus remains to form a tubule. The mesial portion of the mass becomes the wall of the single pronephric chamber.

The whole mass is at first solid, the cavity of duct, tubule, and pronephric chamber, developing secondarily.

The cavity of the pronephric chamber is for a time continuous with the split-like splanchnocoele, but it soon becomes constricted off from it and forms a completely closed cavity. Bearing in mind the segmented condition of the pronephric rudiment in its first stage of development and the process of fusion of successive nephrocoeles which takes place in Ganoids, we may conclude that the pronephric chamber of the Teleost probably represents a number of nephrocoeles fused together. The single pronephric tubule is very possibly the same member of the series as that which occurs in *Amia* although this has not yet been actually determined.

A remarkable peculiarity found within the group Teleostei is that in a few genera (*e.g. Fierasfer, Zoarces, Lepadogaster*) the pronephros retains its renal function throughout life (cf. Guitel, 1901, 1902).

AMPHIBIA.—In Amphibians other than Gymnophiona the pronephric rudiment first becomes visible as a solid swelling of the somatic mesoderm at the level of the anterior mesoderm segments (*Rana* segments 2-9, *Triton alpestris* 1-6, Mollier). Though at first no segmentation is to be detected by the ordinary methods of observation in this swelling it is reasonable to interpret it as representing morphologically a series of closely apposed or fused nephrotomal projections like those of *Hypogeophis*. This pronephric rudiment gradually becomes demarcated off from the rest of the mesoderm by a split which becomes apparent on its ventral side at first laterally and then spreads inwards.

The rudiment now forms a thick flap (cf. Lung-fishes, Fig. 129, A and B) hanging down on the outer side of the mesoderm, and continuous with the somatic mesoderm along its dorsal and median edge. Segmentally arranged coelomic splits make their appearance along the line of attachment of the pronephric flap and these we may interpret as incipient nephrocoeles. The split already mentioned as demarcating the pronephric rudiment ventrally spreads round its median edge, so as to detach it completely from the (nephrotomic) mesoderm except at segmentally arranged points where a connecting isthmus remains as the nephrostomal end of a tubule. The pronephric rudiment now undergoes a kind of modelling process similar to that occurring in Crossopterygians and Lung-fishes, its outer portion being gradually cut off from behind forwards so as to form the archinephric duct, while the part nearer the mesial plane forms the recurrent portion of the duct with the tubules connected with it.

The whole rudiment is at first solid. The earliest coelomic spaces to appear are the nephrocoeles and from these split-like extensions spread outwards in each tubule rudiment, while towards the outer margin of the rudiment the continuous longitudinal cavity of the archinephric duct develops.

Of the tubule rudiments, as usual, only a few become functional—

in Anura commonly 3, in Urodeles commonly 2 (in *Amphiuma* 3 according to Field). Probably here as elsewhere the number is really a variable one. As the tubules develop they show active increase in length so that they become much coiled and the same applies to the part of the archinephric duct lying in the pronephric region.

It is only when they first appear that the nephrocoeles show a segmental arrangement: later on they become merged in the general splanchnocoele. Along the inner wall of the dorsal portion of this cavity, *i.e.* the portion which represents the fused nephrocoeles, the glomerulus develops as a continuous laterally projecting fold of splanchnic mesoderm. Usually the portion of the body cavity containing the glomerulus becomes for a time incompletely shut off from the rest to form a secondary pronephric chamber as in Lungfishes, the mesoderm covering the lungs undergoing fusion with that covering the bulging surface of the pronephros. The secondary pronephric chamber may in turn be subdivided by the edge of the glomerulus fusing with the mesoderm covering the pronephros.

MEROBLASTIC VERTEBRATES.—As a rule, in the Meroblastic Vertebrates the pronephros never becomes a functional organ, and correlated with this it shows a reduction in its structure. Possibly, as already indicated, this may be due to the presence of the large yolk-sac with highly vascular surface in contact with the external medium, which will facilitate the getting rid of excretory material by diffusion outwards.

ELASMOBRANCHII.—In Elasmobranchs the ventral ends of certain of the anterior mesoderm segments, usually commencing with segment VII, become dilated to form vesicular cavities (van Wijhe, 1889) which are probably to be interpreted as nephrocoeles. The tubule rudiments appear as thickenings of the somatic wall of these nephrocoeles which grow outwards and being in close apposition form at their outer ends, apparently by fusion, a solid continuous pronephric swelling. The tubule rudiments make their appearance in sequence from before backwards.

Different workers vary in their statements as to the number of rudiments in different forms [*Scyllium*, 5—Rückert, 3—van Wijhe; *Pristiurus*, 5—Rückert, 4—Rabl, 3—van Wijhe; *Raia clavata*, 5—van Wijhe; *R. alba*, 8—Rabl; *Torpedo*, 7—Rückert (Fig. 130)] from which we may conclude safely that the number of tubule rudiments is very liable to variation both as between different species and different individuals. This variability may be taken in correlation with the fact, observed by van Wijhe, that in *Pristiurus* dilated nephrocoeles made their appearance from segment I to segment XIV, gradually diminishing in size towards the end of the series, although tubule rudiments appeared only in 3 segments. Both phenomena indicate that the pronephros in Elasmobranchs as in other groups has undergone reduction from a once much greater anteroposterior extension.

In a comparatively late stage the tubule rudiments develop their lumen. The pronephric swelling extends backwards into the archinephric duct. No glomerulus develops but segmentally arranged branches of the dorsal aorta appear on the right side corresponding in number and degree of development with the pronephric tubules. These give rise either one of them (Rückert, van Wijhe) or by fusion together (Rabl) to the root of the vitelline artery but are termed by Rabl pronephric arteries.

The pronephros undergoes rapid degeneration and eventually nothing is left of it but the coelomic funnel of the Müllerian duct (see below).

Sauropsida.—In the fowl pronephric tubule rudiments develop to the number of about 12, in the form of solid outgrowths of the somatic mesoderm at the level of the nephrotomes although, except in the case of the most anterior, the mesoderm is not yet segmented at this level at the time when the rudiments appear. In at least some cases segmental dilatations of the otherwise split-like coelome occur opposite the tubule rudiments and are no doubt to be interpreted as the nephrocoeles of the corresponding tubules. The first tubule rudiment makes its appearance in embryos with 8 or 9 segments, its position showing considerable variation (usually segment 4, 5 or 6).

The successive tubules appear in rapid succession—almost synchronously. At about the 19-segment stage the myotomes become separated from the nephrotomes, the latter remaining in continuity with the lateral mesoderm and their cavities (nephrocoeles) with the splanchnocoele. About the same stage the backwardly projecting tip of each tubule rudiment undergoes fusion with its successor in the series and thus gives rise to a continuous longitudinal rod-like structure — the rudiment of the archinephric duct (Felix, 1904). The archinephric duct in its anterior portion thus would appear to develop in a manner essentially the same as that found in the Gymnophiona and so many other of the lower vertebrates.

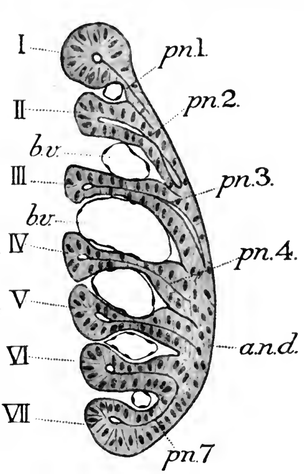


Fig. 130.—Horizontal section through rudiment of right pronephros of *Torpedo*. (After Rückert, 1888.)

a.n.d, archinephric duct; *b.v*, blood-vessel; *pn.*1, etc., pronephric tubules. The nephrotomes are numbered with Roman numerals.

The hinder end of each tubule rudiment, as well as the archinephric duct itself, is at first solid. The definitive lumen makes its appearance (about 20-segment stage) secondarily in the form of discontinuous chinks which gradually become continuous and spread backwards.

Pronephric glomeruli develop in the Bird though at a late stage when the pronephros is already degenerating. They were discovered first by Balfour and Sedgwick (1878) in the Fowl where they vary in number from about 3 to about 7. They may, as so commonly occurs in pronephric glomeruli, undergo a less or greater amount of fusion with one another and also with the anterior glomeruli of the opisthonephros. The whole pronephros in the Bird undergoes rapid atrophy and by the sixth day of incubation has usually in the Fowl completely disappeared except the glomeruli which may still be detected for a day or two longer.

In the Reptiles also a rudimentary pronephros makes its appearance but degenerates without becoming functional. The nephrotomes or protovertebral stalks, at first solid, develop a patent cavity or nephrocoele. In a varying number of segments (in Lizards 6-8, commencing with segment V) pronephric tubule rudiments develop as outgrowths of the somatic wall of the nephrotome after the ordinary fashion and fuse together at their outer ends to form the archinephric duct.

THE ARCHINEPHRIC DUCT.—As has already been indicated, it is characteristic of the Vertebrate that its nephridial tubes no longer open directly to the exterior, but that, on the contrary, they open into a longitudinal duct on each side—the archinephric duct—which in turn opens into the alimentary canal towards its hinder end. The first steps in the evolution of the archinephric duct have passed beyond our ken and to decide as to how they came about we have to balance probabilities on a basis of somewhat scanty embryological and anatomical data. Two obvious possibilities present themselves —(1) that the row of segmentally arranged nephridial openings came to be sunk beneath the general surface in a longitudinal groove and that this groove became covered in to form a longitudinal duct, and (2) that the external opening of each tubule became shifted backwards so as to open into its successor in the series and so give rise first to a common opening with it and later to a common longitudinal duct (Fig. 131) in the way exemplified by the posterior kidney collecting tubes of male Elasmobranchs. On which side the balance of probability lies will be apparent on considering the developmental facts so far as they are known to us at present.

It will be recalled that in *Hypogeophis*, according to Brauer, the anterior portion of the archinephric duct arises by a number of pronephric tubule rudiments bending tailwards at their outer ends and undergoing fusion together. The fused portion forms the duct rudiment and it proceeds to extend backwards by independent growth until eventually it reaches and fuses with the wall of the cloaca. It is only a small portion of the duct close to its anterior end which is formed by the direct fusion of tubule rudiments—the tubules farther back growing out and fusing secondarily with the already formed duct.

If we turn to other Vertebrates we find considerable evidence for believing that *Hypogeophis* presents to us a mode of development of the archinephric duct which is relatively primitive. In a number of Vertebrates there appear to be distinct traces of the formation of the front end of the archinephric duct by fusion of the outer ends of tubule rudiments in a manner essentially the same as that which holds for *Hypogeophis*. As will have been gathered from the preceding pages this is the case with such different groups of Vertebrates as Elasmobranchs, Crossopterygians, Lung-fishes, Reptiles and Birds.

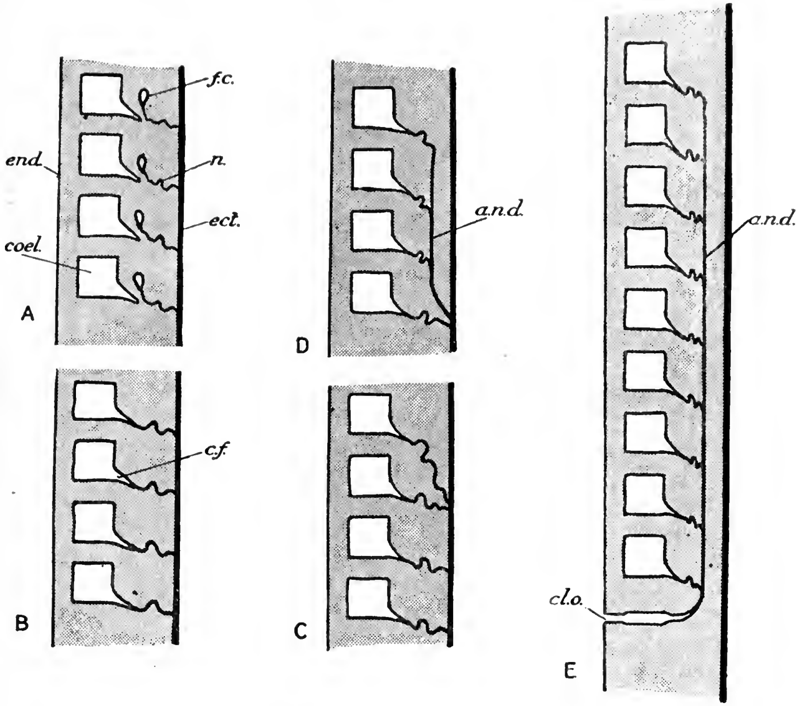


FIG. 131.—Diagram illustrating a possible mode of evolution of the archinephric duct.

A, the coelomic compartments are bulging towards the nephridial tubes; B, the compartments have come to open into the nephridial tubes and the flame-cells have disappeared; C, D, the external openings of the nephridia are becoming shifted backwards so as to give rise to the archinephric duct; E, the archinephric duct is completely formed and communicates with the enteron through one of the segments retaining, or reverting to, its primitive enterocoelic connexion. *a.n.d*, archinephric duct; *c.f*, coelomic funnel; *coel*, coelomic cavity; *cl.o*, cloacal opening of archinephric duct; *ect*, ectoderm; *end*, endoderm; *f.c*, flame-cell; *n*, nephridial tube.

If we are justified in looking upon this mode of formation of the duct in ontogeny as relatively primitive, it obviously affords strong support to the second of the two above-mentioned hypotheses as to the evolutionary origin of the archinephric duct: the bending back of the tubule rudiments would then be interpretable as a developmental reminiscence of the backward shifting of their external openings which took place during phylogeny.

The independent backgrowth of the remainder of the duct in *Hypogeophis* is probably to be regarded as a case of accelerated or precocious development to allow the anterior tubules to become functional at an early stage of development before those farther back have developed.

As regards the ontogenetic development of the main part of the duct in other Vertebrates we find the most divergent statements and it seems clear that this divergence can only be explained by the actual facts not being always the same.

In the Sauropsida it is admitted that the main part of the duct is formed as in *Hypogeophis* by independent backgrowth. Amongst the Anamnia the same is said to be the case in Elasmobranchs by Balfour and by Rabl, and in *Alytes* according to Gasser, but other authors describe two other methods of formation as occurring.

The first of these is found in Elasmobranchs according to van Wijhe, Beard, Rückert and others. According to these investigators, the archinephric duct makes its first appearance as a longitudinal ridge-like thickening projecting inwards from the ectoderm. This becomes split off as a solid ectodermal rod which develops a cavity secondarily and forms the archinephric duct. Such a mode of development would be of great morphological interest as it would lend decided support to the view that the archinephric duct originated in evolution as an ectodermal groove—it being a common ontogenetic modification that what is morphologically a groove develops ontogenetically in the form of a solid ridge-like ingrowth. It has however to be borne in mind that there exists a serious source of possible error in making observations upon the archinephric duct in early stages. The duct lies between ectoderm and somatic mesoderm—the two cell-layers mentioned fitting close round it. During the various processes to which the embryo is subjected preparatory to being cut into sections the ectoderm usually separates slightly from the mesoderm, and the archinephric duct tends to adhere firmly to one or other of these layers. This is the case more particularly at its tip, where it is pushing the ectoderm and mesoderm apart as it grows back and is therefore in particularly intimate contact with them. It is exceedingly difficult in studying sections to distinguish with certainty between such intimate contact and actual organic continuity. In cases where the hinder part of the duct is adherent to the ectoderm an appearance is produced which simulates closely a development by splitting off from the ectoderm.

As a matter of fact C. Rabl's very careful investigations (1896) fail to confirm the ectodermal origin of the duct in Elasmobranchs and upon the whole in the writer's opinion there does not appear to be any longer justification for accepting it as actually occurring.

The other mode, by which the extension of the archinephric duct backwards has been described as taking place in the Anamnia, is that the duct becomes split off from the underlying somatic mesoderm. It is necessary again to bear in mind the caution expressed above but making full allowance for this it seems impossible to escape the admission that in many forms (*Petromyzon*, Lung-fishes, most Amphibians, Teleosts and probably actinopterygian Ganoids) the duct is prolonged backwards by a process of this kind.

It being accepted that in a number of Anamnia a large part of the archinephric duct arises in development by being split off from the mesoderm, we are faced by the problem how this mode of development is to be correlated with the mode of development by fusion of the outer ends of tubule rudiments. It may be suggested that what has happened is that the development has been accelerated—as often happens—by skipping over the early stages. The mode of development in question may have been derived from the more primitive mode by the omission of the separate tubule stage and the passage at once to the stage in which the tubule ends are fused into a continuous structure.

In some cases however the primitive mode of development has undergone a further modification. This is exemplified by *Polypterus* (Graham Kerr, 1907) where the hinder portion of the duct appears to be formed by bodily conversion of the series of nephrotomes. These are not segmented but form a continuous structure which becomes converted directly into the archinephric duct.

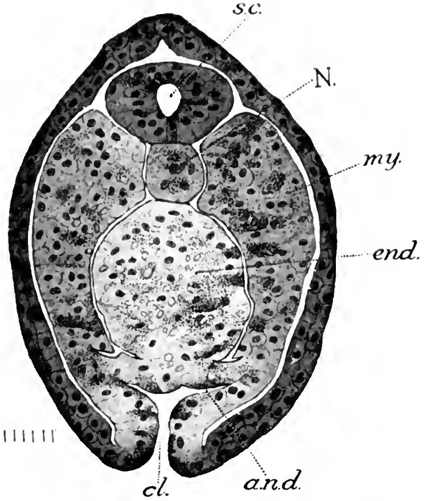


FIG. 132.—Transverse section through *Polypterus* of stage 23 at level of cloacal opening.

a.n.d, opening of archinephric duct into cloaca; *cl*, opening of cloaca to exterior; *end*, alimentary canal rudiment; *my*, myotome; *N*, notochord; *s.c*, spinal cord.

In whichever way the archinephric duct completes its extension backwards, it eventually comes to open into the cloaca. This is, in the great majority of Vertebrates, described as coming about by fusion of the previously freely-ending tip of the duct with the cloacal wall. It is obvious that such a process cannot correspond with what happened during evolution as the duct must have had its posterior aperture throughout in order to perform its function.

It is possible that a clue to the evolutionary origin of the communication between archinephric duct and alimentary canal is given by *Polypterus*. It has already been mentioned that in this animal the hinder part of the archinephric duct arises by bodily conversion of the row of fused nephrotomes. Fig. 132 shows that the opening of archinephric duct into the alimentary canal presents a striking resemblance to the primitive communication of mesoderm segment with enteron, and it is suggested that it actually is this primitive communication which has remained patent while in all the other segments it has disappeared.

The two archinephric ducts open at first separately into the cloaca, one on each side. In some groups of Vertebrates however their terminal portions become gradually approximated and eventually fused together into an unpaired dorsal vesicle which may undergo various modifications. In Elasmobranchs it forms the **urinogenital sinus** which bulges forwards and on each side becomes prolonged into the **sperm-sac**. In Lung-fishes it forms the cloacal caecum: in Teleostei the urinary bladder.

It is noteworthy that in the adult Lung-fish the communication of the kidney ducts with the caecum is close to the posterior opening of the latter, so that a small amount of shifting would cause these ducts to open into the cloaca independently of the caecum.

This suggests a possible evolutionary origin of the **allantois**. It is conceivable that a caecum similar to that of Lung-fishes arose by a fusion of the terminal portions of the kidney ducts *ventral*, instead of dorsal, to the alimentary canal and that the ducts then came to be emancipated from the caecum which remained as a ventral diverticulum of the cloaca to form the allantois. We have no definite evidence as to the evolutionary origin of the allantois and it is well to bear in mind the possibility here indicated in addition to the simpler and perhaps more probable hypothesis that the allantois was from the beginning simply a bulging outwards of the ventral cloacal wall as it is actually in ontogenetic development.

DEGENERATION OF THE PRONEPHROS.—The rôle of the pronephros as the functional renal organ is usually confined to comparatively early stages in development and at the end of this period, when its function is being taken over by the opisthonephros, the pronephros commences to undergo characteristic degenerative processes which normally culminate in its almost complete disappearance.

In the frog (Marshall and Bles, 1890) these processes become apparent in the tadpole of about 20 mm. in length. The archinephric duct becomes more or less obstructed behind the pronephros and as fluid continues for a time to pass into the tubules the latter become greatly distended in places, their lining cells assuming a cloudy appearance, the cell boundaries becoming indistinct and their inner surfaces losing their smooth outline and becoming ragged. The whole organ shrinks in size, becomes invaded by leucocytes, the nephrostomes close, one after the other, and by the end of the first year the whole organ with the adjacent portion of the archinephric duct has practically disappeared.

MÜLLERIAN DUCT.—Throughout the series of gnathostomatous Vertebrates, with the exception of the teleostomatous fishes, the oviducts are admittedly homologous. They—the Müllerian ducts—are above all characterized by the fact that they open freely into the splanchnocoele at their anterior end by an open funnel (*ostium tubae*). There exists in some of the more archaic fishes what appears to be distinct evidence that the Müllerian duct has been evolved out of the tubules and duct of the pronephros and it will therefore be convenient to consider this evidence now.

The Elasmobranchs are the fishes in question. In *Torpedo* (Rückert, 1888) as the pronephros degenerates its tubules become reduced to the three hindermost. Of these three the two posterior degenerate while the other—tubule E—persists and its enlarged nephrostome becomes the coelomic funnel of the Müllerian duct. Other workers (*e.g.* van Wijhe and Rabl), working on other Elasmobranchs (*Pristiurus*), trace back the coelomic funnel of the Müllerian duct also to an opening derived from the pronephros and nephrostomal in its nature, but they believe the opening to be formed not by the persistence of a single enlarged nephrostome but rather by the fusion of three or four nephrostomes together. That it is morphologically a single nephrostome is however rendered more probable by what we now know regarding the development of the pronephros in those of the more archaic fishes in which it develops as a functional organ. It will be recalled, for example, how in *Polypterus* tubule E (like B) becomes enlarged as compared with A, C, and D. A pronephric tubule enlarged in this manner in correlation with purely excretory needs would provide an obviously adequate beginning for the evolution of a funnel for the transmission of the eggs like that at the front end of the Müllerian duct.

While the funnel of the Müllerian duct is nephrostomal in origin the main part of the duct is developed in the Elasmobranchs (Semper, 1875; Balfour, 1878) from the archinephric duct. The latter undergoes a process of splitting from before backwards into a dorsal and a ventral tube, the latter being at first a solid thickening of the ventral wall of the archinephric duct. Of the two tubes so formed the ventral is continuous with the pronephric funnel, while the dorsal carries the openings of the kidney tubules farther back in the series: the former becomes the Müllerian duct, the latter persists as the functional duct of the opisthonephros (Fig. 133, C, *W.d*).

This mode of development is satisfactorily explained by the assumption that the relatively archaic fishes in which it occurs are repeating the process by which the Müllerian duct arose in evolution. Such a splitting of an originally common duct into two, so as to separate the routes by which two different products reach the exterior, is probably of frequent occurrence in evolution. Good examples are seen in the splitting of the common genital duct of hermaphrodite gasteropods (*e.g.* the ordinary snails) to form a separate oviduct and vas deferens. It appears then justifiable to accept as a working hypothesis that the Müllerian duct arose in evolution by being split off from the archinephric duct and that its coelomic funnel is a persistent pronephric funnel.

Turning to Vertebrates other than Elasmobranchs, well-marked differences are found to exist between the phenomena as described for different groups and even for members of the same group by different observers. While some of these may be due to observations being pushed to within the limits of probable error it is impossible to avoid the conclusion that great differences do actually exist in the details of development of the Müllerian duct.

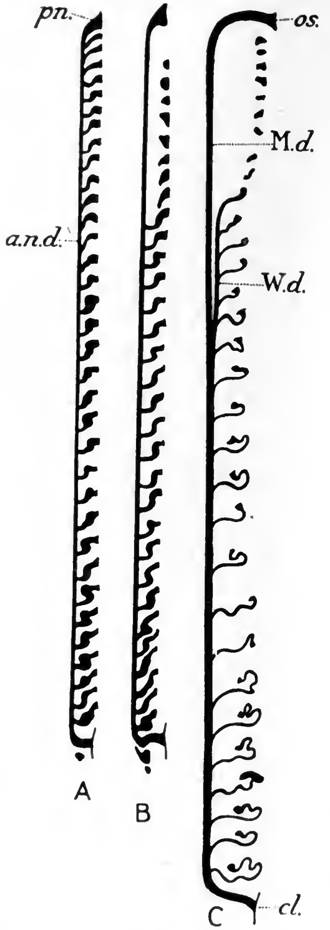


Fig. 133.—Arrangement of archinephric duct, etc., in embryos of *Pristiurus*. (Based on Rabl's figures.)

A, male 17 mm.; B, female 19 mm.; C, female 27 mm. *a.n.d*, archinephric duct; *cl*, cloaca; *M.d*, Müllerian duct; *os*, coelomic opening of Müllerian duct; *pn*, pronephric nephrostome; *W.d*, duct of opisthonephros.

It is possible on general embryological principles to arrive at an idea of the kind of variations which might be expected to show themselves from the supposedly primitive mode of development.

I. The Müllerian duct might continue to arise in an unmodified manner by splitting from the archinephric duct, its funnel being a persisting nephrostome.

II. In correlation with the fact that the one derivative of the archinephric duct (duct of the opisthonephros) is required to be functional at a very early period, while the other (Müllerian duct) does not function until adult life, there would be a tendency for the two ducts no longer to keep exactly abreast in their development but to become separated, the Wolffian duct developing relatively earlier, the Müllerian relatively later. To enable this to take place, the primitive stage in which the two ducts were still one would tend to be more and more curtailed until it was eventually eliminated and the two ducts were independent from the beginning.

III. The independently arising Müllerian duct might retain the mode of extension backwards by intrinsic growth, eventually reaching and fusing with the wall of the cloaca.

IV. Its separation from the somatic mesoderm might take place relatively later than its extension backwards so that it would arise in development comparatively late, as a longitudinal ridge or fold gradually separating off from the mesoderm from before backwards.

A survey of the phenomena as described for the various subdivisions of the Vertebrata shows that as a rule they may be fitted without difficulty into one or other of these types of modification.

Thus in the Amphibia some, especially of the older observers, described the extension of the Müllerian duct as taking place by splitting off from the archinephric duct as in Elasmobranchs, others as being due to independent intrinsic growth, still others as a process of folding or splitting off from the splanchnocoelic epithelium. One of the most careful modern accounts (H. Rabl, 1904) based upon the phenomena observed in the relatively primitive Urodela (*Salamandra*) states that the funnel is the persisting "second" nephrostome of the pronephros and that the portion of duct behind this arises as a thickening of the coelomic epithelium—the cells first assuming a columnar shape, then becoming arranged in several layers to form a ridge projecting into the subjacent connective tissue, and finally becoming split off as a solid rod. Only the anterior portion of the Müllerian duct is formed in this way, the rod-like rudiment so formed proceeding to grow back independently to form the hinder part of the duct.

In Reptiles and Birds the ostium is described as originating as a pit in the coelomic epithelium, which we may look on as a delayed and modified nephrostome, and the extension backwards as taking place by independent growth. The evagination of the epithelium to form the pit is, as is usual in such cases, preceded by the epithelium becoming somewhat thickened.

The mode of origin of the Müllerian duct has not yet been worked out in detail in the Ganoids and Lung-fishes. In ordinary fishes (Teleostei) the conditions are peculiar and will be dealt with along with the development of the ovary.

As regards the further development of the Müllerian duct, it should be noted that its completion and opening into the cloaca is commonly delayed till a comparatively late stage—often till a period but shortly before sexual maturity.

Though primarily retroperitoneal the Müllerian duct comes, with increasing growth, to bulge into the splanchnocoele, pushing inwards the peritoneal lining which comes to surround it as a sheath containing muscles, blood-vessels, etc. Its lining epithelium becomes glandular and specialized to minister to the nutritive and protective needs of the egg in ways which differ in the different groups.

Various modifications make their appearance in later stages. Very frequently the coelomic opening becomes shifted by the addition to the tube of a secondary extension formed from the peritoneal lining. In Elasmobranchs this shifting is towards the mesial plane and except in a few species leads to complete fusion so as to form a single median opening for the two oviducts. Again the hinder ends of the Müllerian ducts are in many cases approximated and they too may fuse to form a terminal unpaired portion.

In the case of the Birds the right oviduct lags behind in development from about the eighth day of incubation; it never opens into the cloaca, and it persists in the adult as a functionless vestige.

There is considerable probability that the **genital pores**—paired openings leading from the hinder end of the splanchnocoele directly into the urinogenital sinus (Cyclostomata) and through which the gametes pass out—are to be looked on as Müllerian ducts in the last stage of reduction, the whole duct having disappeared except its hinder opening. Whether there is any evidence bearing on this in their ontogeny is not yet known.

The Müllerian duct goes through the early stages of development in the male as well as in the female. It usually however never opens into the cloaca and it soon becomes reduced to a vestige. This may persist to a greater or less extent as an individual variation or as a normal characteristic, *e.g.* in the male Elasmobranch or Lung-fish well-marked vestiges remain in the adult, and so, still more markedly, in some of the Amphibia such as the Bufonidae and some of the Gymnophiona.

OPISTHONEPHROS.—Here again Brauer's excellent account of the development in *Hypogeophis* (1902) may be taken as a basis of our description. The opisthonephros in this amphibian is composed of segmentally arranged units extending from segment 24 to segment 100. Each unit is identical in composition with those of the pronephros, consisting of a tubule and a chamber (Malpighian body) containing a glomerulus and communicating with the splanchnocoele by a peritoneal canal. As in the case of the pronephros, each unit arises in development from the nephrotome or protovertebral stalk, the tubule rudiment being in the form of a diverticulum of the lateral or somatic wall of the nephrotome, the blind end of which comes in contact and fuses with the wall of the duct secondarily. Again as in the case of the pronephros, the nephrotome becomes completely separated from the myotome. It also becomes constricted off from the splanchnocoelic mesoderm, incompletely in some cases, a narrow communication — the peritoneal canal — remaining open between the nephrocoele and the splanchnocoele, but more usually completely. In this latter event a new peritoneal canal is developed secondarily in place of that which has been obliterated, a diverticulum growing out from the wall of the nephrotome which meets and fuses with the splanchnocoelic epithelium.

There are differences in detail between the development of pronephros and opisthonephros, *e.g.* the tubule rudiment makes its appearance relatively later in the case of the latter—at a period after the nephrotome has become constricted off from the splanchnocoelic mesoderm. A further difference lies in the fact that there takes place in the opisthonephros a great increase in the number of its tubules—secondary, tertiary, etc. tubules being added to those of the original series. These arise in characteristic fashion. An outgrowth arises from the posteromedian portion of the nephrotome and becomes constricted off as a small round vesicle with thick wall, composed of tall epithelial cells, and a small lumen. This is a **secondary nephrotome.** It remains for a time without change but eventually behaves very much as the original (primary) nephrotome, one wall becoming invaginated to form a glomerulus, and pocket-like outgrowths giving rise, one to a tubule rudiment, the other to a peritoneal canal. An important difference in detail is seen in the behaviour of the duct, which sends out a tubular projection of considerable length to meet the secondary tubule. This outgrowth arises from the duct some distance behind the point where the primary tubule opens into it.

The secondary nephrotome in turn buds off a tertiary nephrotome which again behaves as before and its tubule is met by a projection from near the tip of the outgrowth of the duct which has already developed in relation to the secondary nephrotome. Consequently secondary and tertiary tubules open into the archinephric duct by a common **collecting-tube** formed of this outgrowth.

Apparently new generations of subsequent nephrotomes may go on being formed in a similar fashion each from the preceding one until there may be as many as eight in a single segment, all of them, except the primary, opening into a common collecting-tube.

The degree of development reached by the opisthonephric units is different in different parts of its length. They attain full development in the manner above described in the region of segments 50-100. In the region in front of this (segts. 30-50) the secondary nephrotomes and their derivatives never become functional and their rudiments degenerate. Still further forward (segts. 24-29) even the primary units as a rule degenerate without completing their development.

Apart from differences in detail it is clear that the primary units of the opisthonephros present the most striking resemblance to those of the pronephros and the evidence that they are serially homologous seems convincing.

Normally there is a gap of a few segments between the hind end of the pronephros (segt. 15) and the front end of the opisthonephros (segt. 24) but Brauer found that even in these segments there makes its appearance a distinct nephrotome, with the vestige of a glomerulus, although it does not proceed with its development. Consequently the units of pronephros and opisthonephros (primary) are to be regarded as members of a once continuous series. That this series once extended back beyond the present limits of the opisthonephros is indicated by the fact that distinct nephrotomes are present in segments 101-104, but as was the case in the intermediate zone between pronephros and opisthonephros these do not proceed to develop tubules.

Elasmobranchii.—It will be convenient now to consider shortly the development of the opisthonephros in the Elasmobranch fishes as they have provided the material for a large proportion of the most important work dealing with the morphology of the Vertebrate kidney. It was in the opisthonephros of Elasmobranchs that Sedgwick (1880) made his classical discovery — which forms the foundation on which our present-day knowledge rests — that the nephridial tube of the Vertebrate is a development of the coelomic wall, of that part of it which we now call nephrotome or protovertebral stalk. Since the date of Sedgwick's work the opisthonephros of Elasmobranchs has formed the subject of detailed studies by Rückert, Rabl, van Wijhe, and other well-known investigators.

Owing to the lower end of the myotome in these fishes becoming displaced in a lateral direction, through the accumulation of mesenchyme between it and the mesial plane, the protovertebral stalk becomes rotated outwards so as to assume a nearly horizontal position (Fig. 134, A, *nt*), the originally dorsal end of the stalk becoming now external, and the originally external side coming to be ventral. The duct (*a.n.d*) thus comes to lie ventral to the nephrotomes instead of being on their outer side as was the case originally. The nephrotomes become isolated from the myotomes by their ends next the myotomes breaking up into mesenchyme. The result is that the nephrotomes now form a series of blindly ending pocket-like projections of the coelomic epithelium which curve outwards dorsal to the duct.

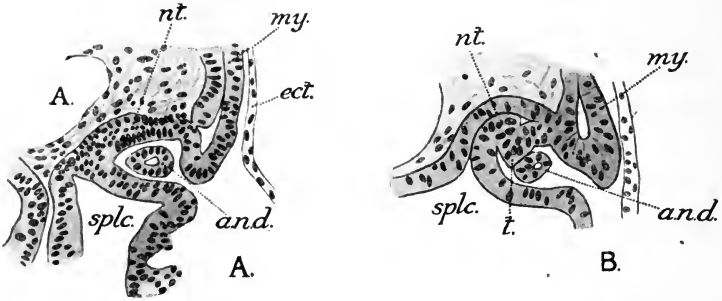


FIG. 134.—Origin of opisthonephric tubule in Elasmobranchs. A, *Pristiurus* (after C. Rabl, 1896); B, variation observed in *Torpedo* (after Rückert, 1888).

A, dorsal aorta; *a.n.d*, archinephric duct; *ect*, ectoderm; *my*, myotome; *nt*, nephrotome; *splc*, splanchnocoele; *t*, tubule rudiment.

Each pocket has an epithelial wall and it is noticeable that the somatic portion of the wall is markedly thicker than the splanchnic, the cells of the former being taller and more columnar in shape. As development goes on it is found that the thicker more columnar celled portion of the wall of the pocket extends for some distance on to its dorsal wall, and this is interpreted by Rückert and Rabl as meaning that the somatic epithelium is spreading inwards towards the mesial plane, replacing splanchnic epithelium as it does so. In view of what we know regarding the development of other groups it seems more reasonable to explain the appearance as being expressive of outward growth on the part of the somatic wall of the nephrotome—the terminal portion becoming the tubule rudiment. Rückert (1888) figures a remarkably interesting variation which he came across in *Torpedo* in which the separation of nephrotome from myotome had been delayed. In this (Fig. 134, B) the nephrotome still forms a distinct stalk continuous with the myotome and the tubule rudiment is visible as a pocket-like projection of its somatic wall, agreeing exactly with the assumedly primitive type of tubule rudiment as it occurs in the pronephros of one of the lower holoblastic Vertebrates. To correlate this specimen with the normal condition all that is necessary is to imagine the portion of the stalk next the myotome to have disappeared by becoming resolved into mesenchyme. The rest of the stalk together with the tubule rudiment would then remain as a curved blindly ending pocket[1] the tip of which would represent the tip of the tubule rudiment. This curved pocket-like structure increases in length, its tip comes into contact with, and later fuses with, the dorsal wall of the duct and it is in this way converted into a short tube opening at its inner end into the splanchnocoele and at its outer into the duct. The tubular structure so arising does not retain its simple tubular shape but undergoes the series of changes shown in Fig. 135. Its cavity dilates in the middle to form the definitive nephrocoele, the cavity of the Malpighian body (*m.b*); its splanchnocoelic end becomes relatively narrow to form the peritoneal canal (*p.c*)[2]: its outer end becomes also relatively narrow and it is this outer portion which undergoes an immense increase in length and becomes the functional tubule.

Opisthonephric rudiments appear in the fashion above indicated throughout the greater part of the length of the body where the splanchnocoele is present. They commence behind the pronephros (about the 8th or 9th segment) and extend back to the cloaca or a few segments posterior to it. In the latter case the postcloacal rudiments do not come to anything. Their occurrence is to be looked on as a reminiscence of a period when the alimentary canal and splanchnocoele extended farther tailwards. It is to be noted also that the group of tubules at the front end which subserve a genital function in the male similarly appear only as transient rudiments in the female.

The portions of the opisthonephros which perform an active renal function increase much in bulk and this, as elsewhere, is brought about not merely by the great increase in length of the individual tubules, but also by the addition of numerous new tubules, each with its Malpighian body etc., of the second, third and so on, order. Probably (Balfour, 1878) these arise by a process of budding of the nephrotomes of a similar type to that which occurs in *Hypo-*

---

[1] Care should be taken to avoid the not uncommon error of referring to the whole of this structure as the "tubule-rudiment."

[2] Attention has already been drawn (p. 227) to the undesirability of applying the misleading adjective "nephrostomal" to this canal.

geophis though in the case of the Elasmobranchs the details seem to be more obscure and the descriptions are conflicting.

The male Elasmobranch is an excellent example of a Vertebrate in which the nephridial system is responsible for the function of conveying to the exterior both the renal excretory materials and the reproductive cells and we find a well-marked tendency to separate the routes of those two products towards the exterior. This separation is brought about by the shifting backwards of the openings of the collecting-tubes of the posterior, purely renal, part of the opisthonephros, so that instead of being spaced out along the course of the duct they come to be coincident with its opening into the urinogenital sinus. This backward shifting is most pronounced, and it also makes its appearance earliest in ontogeny, in the most anterior of the tubules in question. It is accompanied by a fusion together of the terminal parts of the collecting-tubes into a continuous ridge-like projection of the dorsal wall of the duct, in which the individual lumina are for a time greatly reduced or even completely obliterated. Eventually, as a rule, the ridge splits up and the terminal parts of the collecting-tubes regain their individuality—forming a group of distinct tubes, varying in number in different forms from about 4 (*Spinax*) to about 15 (*Acanthias*), and converging so as to open close together into the urinogenital sinus. In some cases the splitting apart is not complete and more or fewer of the tubes may be united together to form a longitudinal "ureter."

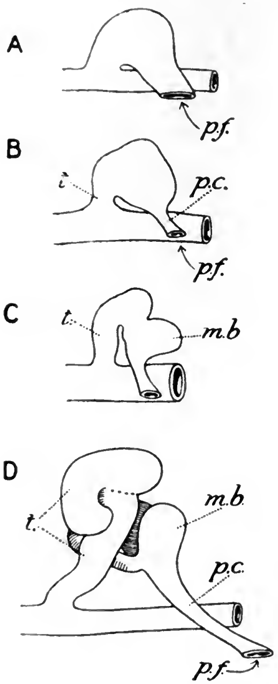


Fig. 135.—Illustrating the later development of a segmental unit of the opisthonephros in male Pristiurus. The figure is in each case a view from the mesial side. (After C. Rabl, 1896.)

A, 15th unit of 17 mm. embryo; B, 15th unit of 22·5 mm.; C, 15th unit of 25·3 mm.; D, 25th unit from same embryo as C. *m.b*, Malpighian body; *p.c*, peritoneal canal; *p.f*, peritoneal funnel; *t*, tubule.

The mode of origin of the Malpighian body—the definitive condition of the nephrotome—from which each opisthonephric tubule leads has already been indicated. It is for a time rounded in form (Fig. 135) but eventually one portion of its wall—varying greatly in position—comes to bulge inwards to form the glomerulus containing a loop of blood-vessel.

The peritoneal canal during development lengthens out considerably (Fig. 135, D) and becomes narrower. This narrowing is most marked in the posterior third of the opisthonephros and in this we see what is probably the expression of a general tendency for the portion of coelome containing the glomerulus to become more and more completely isolated from the main splanchnocoele as the renal unit becomes more and more highly evolved. Eventually, in the adult of the majority of Elasmobranchs, the peritoneal canal becomes completely obliterated, but in a considerable number of others[1] this happens, if at all, only towards the anterior and posterior ends of the opisthonephros so that the greater part of the organ retains open peritoneal funnels throughout life. Bles (1897) has made the interesting suggestion that there is a physiological correlation between the persistence of open peritoneal funnels and the absence of **abdominal pores** — secondary perforations of the wall of the splanchnocoele in the neighbourhood of the anus which make their appearance, at a late period of development, in various Elasmobranchs and other Vertebrates.

URODELA.—The third type of development of the opisthonephros amongst the more primitive Vertebrates is found in the Amphibians, especially in the Urodeles. The excellent account given by Fürbringer (1877) still forms a thoroughly adequate basis for the description.

The Amphibians possess, as has already been shown, a large and highly developed pronephros amply sufficient for their excretory needs during early periods of development. In correlation with this there is marked delay in the development of the opisthonephros, the myotomes having already become separated and their stalks or nephrotomes breaking up into mesenchyme before the opisthonephric units make their appearance. The rudiments of these units—the nephrotomes—become reconstituted in the midst of the mesenchyme as solid cellular strands which may retain their metameric arrangement (*Amphiuma* — Field, 1891; anterior segments in *Triton, Amblystoma,* etc.) but usually have completely lost it. Each of these nephrotome rudiments is a solid strand of cells which curves outwards dorsal to the duct. In the anterior region where, as is specially clear in *Triton,* the inner end of the strand is for a time continuous with the lining of the splanchnocoele, the general arrangement is clearly the same as that of the Elasmobranch (cf. Fig. 136, A, with Fig. 134). The splanchnocoelic end of the nephrotome disappears for a time while the main portion develops a cavity in its interior and becomes converted into a vesicle with epithelial wall lying immediately dorsal to the duct (Fig. 136, B). This vesicle becomes elongated in a mediolateral direction (? by active growth of its outer wall) and then assumes a characteristic curvature first ∩- and then ∽-like in shape (Fig. 136, C). The mesial end of the ∽ gives rise to the Malpighian body, the remainder to the actual tubule, its outer end undergoing fusion with the wall of the duct (Fig. 136, D). The tubule grows rapidly in length and is forced into complicated

---

[1] E.g. *Cestracion philippi, Rhina squatina, Scyllium canicula, S. stellare, Pristiurus melanostomus, Spinax niger, Acanthias vulgaris, Scymnus lichia*—(Bles, 1897).

coils and windings as it does so (Fig. 136, E) while the Malpighian body dilates and its dorsal wall becomes invaginated to form the glomerulus.

As a rule the primitive continuity of nephrotome with the splanchnocoelic lining disappears in the Amphibian as already indicated, but it becomes re-established by a peritoneal canal developing secondarily (Fig. 136, D) as an outgrowth, arising in Urodeles usually from the neck of the Malpighian body and in Anura from a point farther down the apparent tubule, which grows towards and fuses with a thickening of the coelomic epithelium. Such displacements of the communication between Malpighian coelome and splanchnocoele are probably of a similar nature to those mentioned in the case of the pronephros of *Hypogeophis* (see p. 226).

In those parts of the opisthonephros which are actively renal in function, *i.e.* the hinder portion in Urodeles and the greater part of the whole length in Anura, there takes place great increase in bulk, associated with the development of generations of subsequent tubules. Such secondary, tertiary, etc. tubules make their appearance amongst the mesenchyme in the form of cellular strands which resemble closely — both in their original appearance and in the series of changes which they pass through —those from which the primary elements arise (Fig. 136, F, t^3). Eventually the secondary tubule comes to open into the primary tubule, the terminal section of which thus forms a collecting-tube common to both, while the tertiary tubule similarly comes to open into

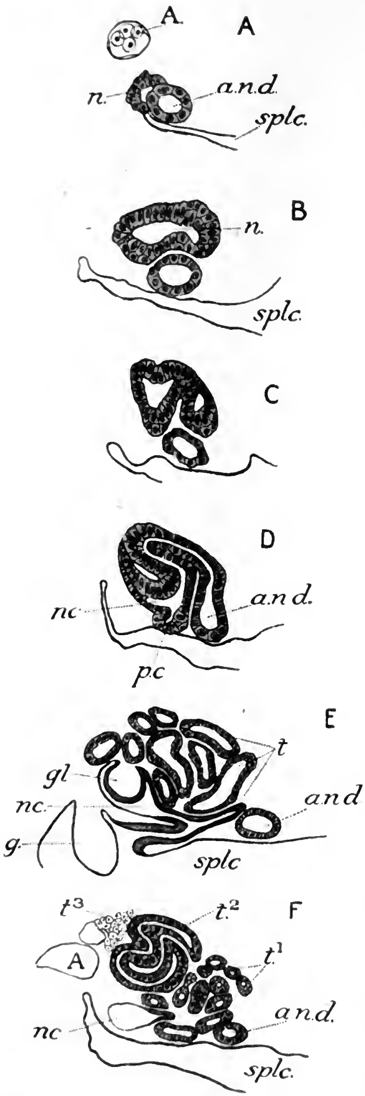


FIG. 136.—Transverse sections showing various stages in the development of the opisthonephros. (After Fürbringer, 1877.)

A, *Triton alpestris*; B, *Salamandra maculata*, 14 mm.; C, D, *Salamandra maculata*, 17 mm.; E, *Salamandra maculata*, 21 mm.; F, *Salamandra maculata*, 25 mm. *A*, dorsal aorta; *a.n.d*, archinephric duct; *g*, gonad; *gl*, glomerulus; *n*, nephrotome; *nc*, nephrocoele; *p.c*, rudiment of peritoneal canal; *splc*, splanchnocoele; *t*, tubule; t^1, t^2, t^3, primary, secondary, and tertiary tubule rudiments.

the secondary. As the various generations of tubules go on with their development, undergoing the same histological differentiation and increasing enormously in length, they become inextricably mixed up together to form the compact fully developed opisthonephros of the adult.

Eventually, in the Urodele, the duct is slightly displaced outwards so as to leave a distinct gap between it and the opisthonephros across which pass the terminal parts of the collecting-tubes. In the male Urodele the openings of these become, as a rule, shifted backwards to the hind end of the duct as in Elasmobranchs.

The Amphibia alone among tetrapod Vertebrates retain the relatively primitive feature of possessing open peritoneal funnels in the adult, and they can be excellently demonstrated with their actively moving flagella by examining the slender anterior portion of the excised and still living kidney of a female Urodele in normal salt solution under the microscope. In the anterior genital portion of the opisthonephros of the male they as a general rule (not in *Spelerpes,* Spengel) remain however obliterated.

In the Anura (Nussbaum, 1886) the peritoneal canals at an early stage of larval life lose their connexion with the Malpighian body or tubule and establish a secondary connexion with the blood spaces between the tubules, thus affording a route by which the fluid in the splanchnocoele is returned to the blood, analogous to that provided by the lymphatic system in higher Vertebrates.

AMNIOTA.—In the Amniota the opisthonephros of the Fishes and Amphibians is represented by the mesonephros and metanephros—and it will be convenient to consider the mesonephros first.

MESONEPHROS OF BIRDS.—As has already been pointed out one of the marked differences between *Amphioxus* and the Craniata is that in the latter segmentation is no longer apparent at any stage of development in the ventral or splanchnocoelic region of the mesoderm. The Amniota show a further accentuation of this difference inasmuch as the loss of mesodermal segmentation has extended so far towards the dorsal side as to involve the region of the nephrotomes. In the early embryo of the bird the nephrotomic part of the mesoderm has the form of an unsegmented mass—the **intermediate cell-mass**—showing more or less distinct traces of being composed of a somatic and a splanchnic layer continuous with the corresponding layers of the splanchnocoelic mesoderm and of the myotome. Although the intermediate cell-mass no longer consists of discrete nephrotomes, traces of its primitive segmental nature persist in its connexions with the segmentally arranged myotomes and in the fact that its connexion with the lateral mesoderm is not continuous in a longitudinal direction.

As regards the mode of origin of the actual mesonephric units differences exist, as was shown long ago by Sedgwick (1880), which are of much interest owing to the fact that the less modified mode of development found at the front end of the series is readily correlated with that which is found in the Anamnia, while the more highly modified mode of development occurring posteriorly is equally readily correlated with what happens in the metanephros of the Amniota.

In the anterior region (approximately segments 12-15) the intermediate cell-mass is compact, recognizably two layered, and the split which separates the two layers may be obviously continuous with the splanchnocoele (Fig. 137, A). It separates at an early stage from the myotome, but it remains continuous at intervals with the lateral mesoderm. The intermediate cell-mass becomes closely apposed to and very soon directly continuous with the duct by a narrow isthmus in each segment—the tubule rudiment (Fig. 137, A). Ventrally, *i.e.* near its junction with the splanchnocoele, the split between the two layers of the nephrotome dilates and forms a definite nephrocoele which opens into the splanchnocoele by a wide peritoneal canal (Fig. 137, B, *p.c*). The tubule rudiment develops a lumen leading from nephrocoele into duct,[1] and the dorsal wall of the nephrocoele becomes

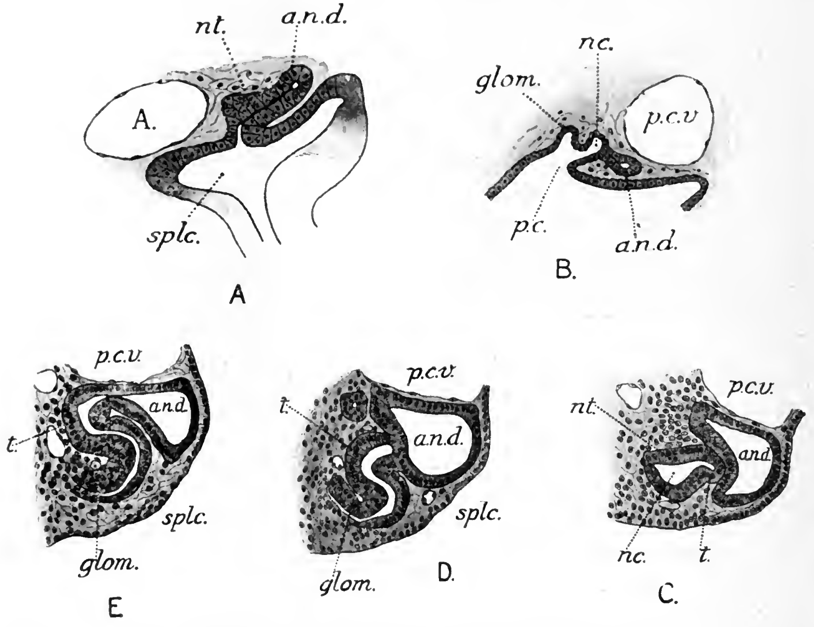


FIG. 137.—Sections illustrating the development of the mesonephros in Birds. (A and B, after Sedgwick, 1881 ; C, D, E, after Schreiner, 1902).

A, 22-segment chick at level of the 15th segment; B, 34-segment chick at level of 13th or 14th segment (combined from two sections); C, 45-segment duck at level of 29th segment; D, 45-segment duck at level of 25th segment; E, 45-segment duck at level of 24th segment. *A*, dorsal aorta; *a.n.d*, archinephric duct; *glom*, glomerulus; *nc*, nephrocoele; *nt*, nephrotome; *p.c*, peritoneal canal; *p.c.v*, posterior cardinal vein; *splc*, splanchnocoele; *t*, tubule rudiment.

---

[1] The opisthonephric duct in the Amniota is known as the mesonephric or **Wolffian duct** as its function is restricted to draining the mesonephros or "Wolffian body."

invaginated into the cavity to form a glomerulus (*glom*) which may become much enlarged so as to extend right out into the splanchnocoele.

As the process of development is traced back into the second region of the mesonephros (stretching approximately from segment 16 to 19 or 20) a distinct modification becomes apparent. The intermediate cell-mass in this region becomes loosened out into mesenchyme, and amongst this loose tissue what may be termed the definitive nephrotomes make their appearance secondarily in the form of roundish condensations of cell elements. Each of these becomes more and more sharply marked off from the surrounding mesenchyme, its cells assume a radial arrangement, and presently a small rounded cavity appears in the centre. This cavity dilates and the result is a hollow vesicle with a wall composed of a single layer of cells—the definitive nephrotome.

In the third, hinder, region of the mesonephros, extending from about segment 20 or 21 backwards, the process in the Fowl, though not in the Duck, has undergone the further modification that the intermediate cell-mass is from an early period completely isolated from the peritoneal epithelium. The peritoneal canals have here completely disappeared, except for faint vestiges, the cells of the peritoneal epithelium still showing here and there traces of the same arrangement as they have further forwards where they are passing into a peritoneal canal. Apart from this separation from the peritoneal lining, the process is similar to that already described. Here also the intermediate cell-mass becomes separated out into loose mesenchyme in which the definitive nephrotomes make their appearance secondarily.

An important feature in the above described processes of development is the obliteration of the primitive segmentation of the nephrotome region. When the definitive nephrotomes become visible, and so bring into view the metameric segmentation of the mesonephros, a further modification becomes apparent in that the mesonephric segments, except towards the front end of the series, are more crowded together than are the primitive mesoderm segments as represented by the myotomes (Sedgwick, 1880). Thus in the Duck Schreiner (1902) found in the region of myotome XX, 4 or 5 mesonephric rudiments, in that of myotome XXV—7, in that of XXVI—9, in that of XXVII as many as 13.

As development proceeds, the mesonephric elements become still more crowded together inasmuch as from segment 21 or 22 backwards "subsequent" nephrotomes make their appearance in the mesenchyme. These closely resemble in appearance the primary nephrotomes, with which they are at first in close proximity if not in actual continuity, and they develop in succession one over the other, each series forming a vertical row over its primary nephrotome. The number of subsequent tubules is greatest posteriorly where there are commonly four to a segment.

The later development of the individual nephrotome of the mesonephros takes place in the Birds along lines exactly similar to what takes place in lower forms such as the Amphibia. The tubule rudiment originates as an, at first solid but later pocket-like, outgrowth of the lateral wall of the nephrotome (Fig. 137, C, *t*). The tip of this presses against the mesial wall of the duct and, as the tubule grows in length, fusion takes place and the lumina of duct, tubule rudiment and nephrotome—which together form a characteristic ∾-shaped structure as seen in a transverse section—become continuous (Fig. 137, D and E). The portion of the ∾ nearest the mesial plane represents the nephrotome in the strict sense, *i.e.* the forerunner of the Malpighian body, and has assumed a watch-glass shape, its dorsal wall being involuted into the cavity as the rudiment of the glomerulus (*glom*).

The further development of the mesonephric unit, which need not be followed out in detail in this book, consists in (1) the immense growth in length of the tubule, which leads to its becoming inextricably intertwined with its neighbours, (2) the histological differentiation of its wall, and (3) the differentiation of the Malpighian body.

It should be mentioned that where the tubules are much crowded together they do not all establish a communication with the duct in the typical manner above described. Some, even of the primary tubules, come to open into neighbouring tubules. In the case of the subsequent tubules, some open into the duct in the typical fashion, others open into neighbouring tubules, while the majority become connected with pocket-like outgrowths from the duct. These outgrowths are greatly developed in some birds (cf. Duck, Fig. 138, B), becoming much elongated and taking the form of branched collecting-tubes into each of which open a series of subsequent tubules (cf. *Hypogeophis*), the whole condition distinctly foreshadowing arrangements presently to be mentioned in the metanephros.

The mesonephros acts as the renal organ only for a short period during the early stages of development. In the Fowl it begins to develop about the end of the second day of incubation, it reaches its maximum about the 7th or 8th day, and almost immediately thereafter begins to show signs of degeneration as the renal function becomes concentrated in the metanephros. The mesonephros never completely disappears though it ceases to be of any importance as a renal organ: its persistence is correlated with the fact that this portion of the opisthonephros has already in the forerunners of the Amniota important functions connected with reproduction. Its modification in relation to these functions will be gone into later.

METANEPHROS OF BIRDS.—The continuous mass of mesenchymatous tissue representing the nephrotomes or protovertebral stalks does not cease at the hinder limit of the mesonephros at segment XXX: it is continued on through segments XXXI, XXXII, XXXIII and XXXIV to the level of the opening of the duct into the cloaca. The nephrotomal tissue in the segments mentioned remains for a time passive but eventually it gives rise to the definitive nephrotomes of the metanephros. The metanephros is therefore ontogenetically as was indicated long ago by Sedgwick (1880) in its origin simply a tailward continuation of the mesonephros. In the terminology used in this book it consists of the greatly enlarged posterior segment or segments of the opisthonephros. The development of the metanephros is inaugurated by the appearance of the rudiment of the **ureter** or metanephric duct. This arises as an outgrowth (Fig. 138, B, *ur*) from the dorsal wall of the mesonephric duct near its posterior end. The outgrowth extends in a dorsal direction and then spreads out at its tip, projecting very slightly tailwards but growing much more actively in a headward direction along the outer side of the hinder or metanephric portion of the nephrotomal mesenchyme. This latter becomes secondarily (about the end of the fifth day) marked off by a distinct break from the mesonephric portion. About the same time the dorsal wall of the actively growing ureter begins to develop pocket-like outgrowths (Fig. 138, D). These increase in length, branch repeatedly, especially the hinder ones, and become collecting-tubes. As this takes place the nephrotomal mesenchyme becomes condensed into small portions, one of which ensheaths the growing tip of each branch of the collecting-tubes. In these terminal caps of mesenchyme definitive nephrotomes gradually come into view, similar to those of the mesonephros. In other words the definitive nephrotome is at first a mere rounded cellular mass. This develops a lumen and, as the latter dilates, assumes a vesicular form, and finally the actual tubule makes its

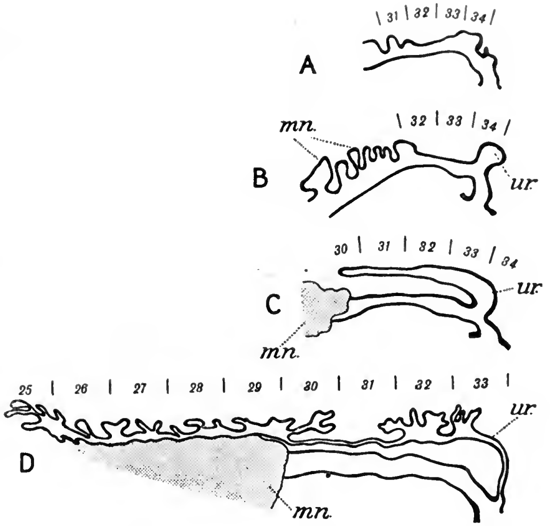


Fig. 138.—Reconstructed outlines of hind end of mesonephric duct and ureter in Bird embryos as seen from the left side. (After Schreiner, 1902.)

A, duck embryo with 48 segments; B, duck embryo with 50 segments; C, duck embryo, 10·75 mm.; D, fowl embryo, 13·5 mm. *mn*, mesonephros; *ur*, ureter. The Arabic numerals indicate the position of the mesoderm segments.

appearance as an outgrowth which fuses secondarily with the tip of the collecting tube. The Malpighian bodies begin about the ninth day to develop their special characteristics in a manner similar to those of the mesonephros. An important point to notice is that the metanephros differentiates from behind forwards instead of in the opposite direction as does the mesonephros.

About 24 hours (the exact time varies greatly) after the first appearance of the ureter the part of the mesonephric duct between it and the cloaca becomes incorporated in the cloaca so that mesonephric duct and ureter come to have independent openings into the cloacal cavity.

As the metanephros goes on with its development it comes to be situated in great part dorsal to the mesonephros but it will be understood that this topographical relationship is secondary. At first it is completely posterior to the mesonephros. Even the ureter is in its first stage localized about segment XXXIV (Fig. 138, B) and its extension forward as far as segment XXV or even farther is purely secondary. It will be noticed in Fig. 138 how exactly the ureter in its first beginnings resembles one of the pocket-like outgrowths of the duct which in the mesonephric region develop into collecting-tubes, and it seems scarcely possible to avoid the conclusion that the metanephros of the Fowl is simply the enormously hypertrophied nephridial apparatus of a single segment, the ureter being a greatly elongated collecting-tube with an immense number of subsequent tubules opening into it.

OPISTHONEPHROS IN OTHER GROUPS OF VERTEBRATES

CROSSOPTERYGII.—Our knowledge of the early stages of development is still fragmentary being based upon three specimens of *Polypterus* (stages 32, 33 and 36) obtained by Budgett (Graham Kerr, 1907).

In the youngest of these stages a number of the opisthonephric units have made their appearance in the form of rounded cell masses arranged segmentally in the mesenchyme ventral to the myotomes, those which are best developed possessing a distinct lumen.

In the specimen of stage 33 these rudiments have become elongated forming thick curved masses, one end of which is closely applied to, or even fused with, the dorsal wall of the duct. The lumen is restricted to the end farthest from the duct, which represents the definitive nephrotome, while the part which extends towards the duct—the tubule rudiment—is solid.

In the 30-mm. larva described by Budgett (1902) the opisthonephros commences about 4 segments behind the pronephros and stretches through about 39 segments with from two to five Malpighian bodies and tubules in each segment. On the right side of the body 18 of the Malpighian bodies—roughly one to each segment —communicated with the splanchnocoele by a nearly straight peritoneal canal. The earlier developmental material does not suffice to show definitely whether or not, as is probable, this peritoneal canal is a secondary connexion with the peritoneal epithelium. The peritoneal funnels exist only for a time during larval life: in specimens 90 mm. in length they had disappeared. In *Calamichthys* (Lebedinsky, 1895) the peritoneal canals have been found still persisting in a larva of 15 cm.

ACTINOPTERYGIAN GANOIDS.—Here again the definitive nephrotomes appear as solid masses of cells arranged segmentally. The gap separating them from the pronephros is in the more primitive Sturgeons about 3 or 4 segments, in the more highly evolved *Amia* 16 or 17 (Jungersen, 1893–4). The rudiment grows in length, develops a lumen secondarily, joins on to the duct by its lateral end while its mesial end dilates to form the Malpighian body—all in the usual fashion. At a late period—after the Malpighian bodies have already assumed their characteristic features—they develop peritoneal canals as outgrowths from their walls which meet and fuse with the peritoneal epithelium secondarily. Later on the peritoneal canals become again obliterated and appear to be absent in the adult except in the case of *Amia.*

TELEOSTEI.—In the Teleostean fishes, as is indeed the case to a certain extent in all the members of the Teleostomi, the opisthonephros is delayed in development in correlation with the prolonged functioning of the pronephros. According to Felix (1897) in the Trout the first opisthonephric units or definitive nephrotomes begin to make their appearance about 70 days after fertilization as rounded clumps of cells, in the centre of which a small lumen appears. These lie immediately dorsal to the duct, in the connective tissue trabeculae which at this stage of development traverse the cavity of the interrenal vein. These rudiments appear first about the middle third of the duct and gradually spread backwards, those in front being segmental in position while those farther back are no longer segmental and fuse together into irregular masses. Each rudiment grows actively in length and goes through the usual series of changes before joining up to the duct.

To the primary units just described are added secondary and tertiary units. These develop exactly as do the primary except that in the case of the tertiary set the tubule may fuse either with the duct directly or with an already developed tubule.

As the tubules increase enormously in length they become inextricably entangled together extending even across the median plane so that the substance of the two kidneys becomes continuous through the interrenal trabeculae. It is further characteristic of the teleostean kidney that there takes place in it a great development of round-celled pseudolymphoid (Felix) tissue. This forms a packing tissue between the tubules and appears to be formed by proliferation from the walls of the interrenal venous spaces.

The opisthonephros extends back for a short distance behind the cloaca. This postcloacal portion drains into a pocket-like prolongation which grows back from the duct, usually on the right side only.

A remarkable peculiarity has been observed in certain Teleosts (*Lepadogaster*, Guitel, 1901) in which, correlated with the persistence of the enlarged glomerulus of the pronephros, the Malpighian bodies of the opisthonephros have, at least in the adult, completely disappeared.

DIPNOI.—In the Lung-fishes the development of the opisthonephros closely resembles that in Amphibia. In *Lepidosiren* and *Protopterus* the units appear as rounded, at first solid, masses independent alike of myotome and of peritoneal epithelium. In *Protopterus* they commence about segment 14-18 but in some specimens they appear to be represented by slight condensations of mesenchyme right forwards as far as the hind limit of the pronephros. The rudiments extend back to about segment 36 *i.e.* to about one segment in front of the cloaca. They are roughly segmental in position and remain so during the greater part of larval life. Each rudiment gives rise to a typical Malpighian body and a tubule which joins on to the duct secondarily. There does not appear to be any trace of peritoneal canals developed although they are for a time present in *Ceratodus.*

The development of the primary units is followed by the development of subsequent ones but the origin of these has not so far been worked out.

In *Protopterus,* though not in *Lepidosiren,* the hinder ends of the kidneys become continuous across the mesial plane and this fused portion becomes gradually marked off conspicuously by its pale colour the cortical region of the paired kidneys becoming crowded with amoebocytes laden with melanin which settle down there and give it a coal-black appearance.

REPTILIA.—In the Reptiles we find, as we should expect, that the process of development follows upon the whole the same lines as in Birds but at the same time shows various features in which the condition remains more primitive. Thus in *Lacerta* Schreiner (1902) finds that, except in the hinder portion of the opisthonephros, the units arise directly from typical nephrotomes or protovertebral stalks. These become isolated from the peritoneal mesoderm and then from the myotome. Each develops a lumen and becomes vesicular and its lateral wall gives rise to an outgrowth which becomes the tubule rudiment and fuses with the duct. No peritoneal canals are developed, though vestiges of these may appear—a vestigial peritoneal funnel appearing as a slight projection from the splanchnocoele into the ventral end of the nephrotome (*Lacerta*), or the latter remaining for a time connected with the peritoneal lining by a solid stalk representing the peritoneal canal (*Anguis*).

In the posterior segments the nephrotomes are no longer distinct: they form a continuous mass of mesenchyme stretching uninterruptedly from segment to segment. In this, cellular condensations occur which give rise to definitive nephrotomes and these also are no longer strictly segmental, there being about 2 to each segment from segment 25 to 30.

The definitive nephrotomes pursue the normal course of development. The first to appear are towards the ventral edge of the nephrotomal tissue but later other subsequent units appear in succession more dorsally.

GENERAL MORPHOLOGY OF THE RENAL ORGANS OF VERTEBRATES.—The main problem connected with the morphology of the renal organs is that which deals with the serial homology of its constituent elements. Lankester (1877) clearly implied this homology when defining his terms archinephros etc. while, looking at the matter from a more strictly embryological standpoint, Sedgwick (1881) formulated the view that pronephros, mesonephros and metanephros are simply successive portions of a single elongated ancestral excretory organ possessing a duct and segmentally arranged, serially homologous, tubules.

In discussing this archinephros theory it is necessary to bear in mind the following points:—

(1) The names pronephros, mesonephros and metanephros according to their original definitions signify three sets of renal structures forming a succession along the length of the body in a tailward direction:—(*a*) an anterior or headward set, (*b*) a middle set and (*c*) a posterior or tailward set respectively. It is inadmissible by the terms of the original definition to use them in any other sense and to do so is bound to lead to confusion.

(2) In addition to the anteroposterior series of renal units there may develop a sequence of elements within the same body segment—*i.e.* the development of the primary unit may be followed by the production of a series of subsequent units, secondary, tertiary, quaternary and so on, probably derived originally from the primary nephrotome by a process of budding. The extent to which such subsequent units may develop differs greatly in different animals and in different segments. In the pronephric region there are commonly none, in the opisthonephros of *Hypogeophis* there may be as many as twenty in a segment, while it is possible that the metanephros of the Bird is to be looked on as a gigantic mass of subsequent tubules belonging to a single segment.

It is obvious that in comparing renal elements of different parts of the series care must be taken that the comparisons are made between elements of the same order, and it is further obvious that a danger to be guarded against is involved in the theoretical possibility of the suppression of the tubules of one order—say the primary tubules—in some particular region.

The comparison of mesonephros with pronephros involves then these two fundamental questions:—

(1) Does the mesonephros contain a set of units of the same order as those of the pronephros?—*i.e.* in this case *primary* elements and

(2) Are these elements serially homologous throughout the length of pronephros and mesonephros?

From the facts of development as stated earlier in this chapter it is clear what the answer to these two questions must be. It has been shown that in *Hypogeophis* and other forms the first tubule to appear in each segment of the opisthonephros arises as a direct outgrowth from the nephrotome exactly in the same way as the pronephric tubule: it is clearly then a primary tubule, and its Malpighian body, arising directly from the main part of the stalk, is also primary. The evidence then seems conclusive that in *Hypogeophis* the pronephric and opisthonephric tubules form a homologous series, and naturally if this is true of *Hypogeophis* it is, in all probability, true of other Vertebrates.

Yet the view has been strongly advocated and is still held by many morphologists that there is no precise homology between the units which build up pronephros and opisthonephros. Rückert, van Wijhe, Field, Semon, Boveri, Felix, have been among the more important protagonists of this view. They have brought forward such arguments as the following:—

(1) While the pronephric tubule arises as an outgrowth of somatic mesoderm, the mesonephric is derived partly from somatic and partly from splanchnic.

(2) The pronephric tubules arise relatively early and in continuity with the archinephric duct, the mesonephric tubules arise much later and in discontinuity with the duct.

(3) The glomerulus of the pronephros is unsegmented and lies in the general splanchnocoele: that of the mesonephros is segmental and lies in a special chamber the cavity of the Malpighian body.

These arguments however do not appear any longer to have the weight which formerly attached to them.

(1) The evidence of *Hypogeophis* that opisthonephric tubules arise as outgrowths of the somatic wall of the nephrotome just as do the pronephric tubules seems quite convincing.

(2) In *Hypogeophis* all the pronephric tubules except the first three join up to the duct secondarily precisely as do the opisthonephric tubules. Further the precocious completion of the archinephric duct is a physiological necessity, in view of the early functioning of the pronephric tubules, and this in turn involves as a necessary consequence that the tubules behind those which first function become joined to it secondarily.

(3) The glomerulus of the pronephros *is* segmental and the pronephric chambers are also segmental at first in some of the more archaic forms and the unsegmented condition is purely secondary.

Another line of argument is directed not against the view that pronephros and mesonephros are built up of serially homologous units but rather against the strict homology of the functional parts of these units. Thus it is stated that in the region of the pronephros in addition to the main tubules there occur rudiments of other tubules which resemble more closely those of the mesonephros and similarly that in the region of the mesonephros, in addition to the ordinary tubules, there occur vestiges of another set of tubules resembling more closely those of the pronephros. Consequently, of the set of potential tubules (primary, secondary etc.) which is repeated in each segment, it is not the corresponding member which becomes the functional or main tubule in the pronephric and opisthonephric regions respectively. To the present writer the various observations which have been brought to support this argument do not appear to be anything like so convincing as the very clear evidence afforded by *Hypogeophis* and he consequently holds that in the present state of our knowledge there is no adequate reason to refuse to accept the precise homology of the first-appearing ("primary") tubules of the opisthonephros with those of the pronephros.

The idea of the primitive continuity between mesonephros and metanephros is less open to attack than that between the pronephros and the anterior (mesonephric) portion of the opisthonephros. Apart from the evidence of embryology we find in various of the lower vertebrates (Elasmobranchs, Urodeles) an elongated opisthonephros in the adult which shows in the clearest possible manner an incipient stage in the differentiation of the organ, into an anterior genital region and a posterior renal region, of precisely the same kind as we believe to have taken place in the Amniota.

Further we have seen that in actual ontogeny the tubules of mesonephros and metanephros arise from an at first perfectly continuous mass of nephrotomal mesenchyme. As regards the minor problem whether one or more primary tubules still persist in the metanephros among its immense mass of subsequent tubules there is, as yet, no adequate evidence.

Accepting then the idea of the archinephros as a sound theory of the primitive condition of the renal system of Vertebrates we may sketch out the probable course of the modifications which have come about in its development somewhat as follows.

Primitively its tubules developed—in accordance with the development of the body-segments generally—in regular sequence from before backwards.

The disappearance of segmentation in the ventral portion of the coelome enabled the early-formed tubules—those towards the head end—to drain the whole length of the splanchnocoele. Correlated with this these tubules became greatly enlarged and their efficiency greatly increased.

This high development of the anterior tubules to drain the whole splanchnocoele enabled them to cope with the entire excretory needs of the developing animal for a prolonged period and the tubules behind them in the series being unnecessary were either delayed in their appearance or ceased entirely to develop.

Thus a gap arose separating off the precociously developed tubules as the pronephros. Within the pronephros itself there was a tendency for functional activity to become specially marked in certain tubules these becoming enlarged in comparison with the others. The increase in size of pronephric tubules was accompanied by increase in the size of their glomeruli, which consequently came into contact and fused together.

As the pronephric tubules drained the whole splanchnocoele the peritoneal canal leading to their nephrocoeles became wider and wider until at last they ceased to be marked off from the rest of the splanchnocoele.

The opisthonephric tubules—the renal functions being still for a time undertaken by the pronephros—developed in regular sequence from before backwards. With the acquisition of new outlets for fluid in the splanchnocoele, such as abdominal pores, or connexions with lymphatic or blood vessels, the peritoneal canals leading from it into the nephrocoeles (Malpighian bodies), in which the secretion of coelomic fluid was specially concentrated, became gradually reduced and finally disappeared, there being no longer any physiological need for them.

Within the series of opisthonephric tubules, the excretory function became more and more concentrated in the segments nearest the cloacal opening. In these segments the opisthonephros increased in bulk owing to the specially active budding processes which gave rise to successive generations of subsequent (secondary, tertiary, quaternary and so on) tubules.

The final stage in this process was reached in the Birds, where renal activity became concentrated in a single segment close to the cloacal opening. In this segment an immense hypertrophy of the opisthonephric elements took place, successive generations of tubules being added on in front. Thus the opisthonephric mass belonging to this segment came to extend headwards dorsal to the anterior portion of the opisthonephros (mesonephros) and became the definitive kidney or metanephros.

ORIGIN OF THE NEPHRIDIAL DUCTS.—As already pointed out the nephridial tubes in craniate Vertebrates open primitively into a longitudinal archinephric duct—the presence of this duct being the most conspicuous feature which differentiates the renal system in Vertebrates from the presumably ancestral condition as exemplified by Annelids, where the tubules open separately upon the external surface.

Two possible ways in which this duct may have originated in evolution have already been indicated and it has also been indicated that on the whole the balance of probability seems to be in favour of the view that it came into being through the backward shifting of the external opening of each tubule till it became coincident with the next behind it.

Those who take this view usually assume that the archinephric duct originally opened posteriorly upon the outer surface of the body and that its opening became secondarily shifted into the cloaca. But as already pointed out there is no embryological support for this view. Everywhere the archinephric opening is at first within the endodermal part of the alimentary canal and this suggests that the communication of duct with cloaca has come about in some other way. The evidence of *Polypterus* suggests as already indicated that the opening into the cloaca represents the persistent primitive communication of a mesoderm segment with the enteron. It is quite conceivable that a secondary communication between archinephric duct and gut may have come about in this way, in correlation with the pronephric part of the archinephros reaching the actively functional condition at a period when the mesoderm segment at the level of the anus had not yet been completely separated from the endoderm. Once this secondary opening was established it would be a natural consequence for the post-anal portion of the nephridial system to atrophy and disappear.

The hypothesis indicated in this description derives the nephridial apparatus of the Vertebrata from an ancestral condition resembling that characteristic of Annelids — the main difference being that in the Vertebrates the nephridial tubes open into a longitudinal duct which at its hinder end communicates with the alimentary canal. It is of great interest then to find even within the group of the Annelida clear expressions of the tendency for the nephridial tubes to open into such a duct. The best marked case of this known up to the present appears to be that of the Earthworm *Allolobophora antipae* described by Rosa (1906). Here (Fig. 139) in the posterior portion of the body the nephridial tubes lead into a longitudinal duct which fusing posteriorly with its fellow opens into the alimentary canal on its dorsal side and near its posterior end. In other words in this particular case an arrangement precisely like that of the vertebrate has been evolved out of an ancestral condition in which segmentally placed nephridial tubes opened independently upon the outer surface.

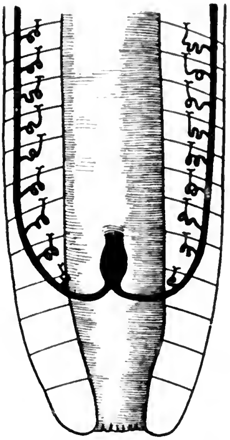


Fig. 139. — Diagram of the posterior end of the body of *Allolobophora antipae* as seen from the dorsal side to show the relations of the renal organs (shown in black) according to Rosa (1906).

In regard to the origin of the typical metanephric duct or ureter as seen for example in a Bird there are two obvious possibilities. If the metanephros represents a number of nephridial segments its special duct may have originated by such steps as are represented by the adult condition in male Urodeles and male Elasmobranchs *i.e.* by the openings of the collecting-tubes into the original duct becoming displaced backwards. Or on the other hand if the metanephros represents the greatly enlarged tubule system of a single segment the ureter would probably have arisen simply by the enlargement of the collecting-tube of that segment. When one studies the facts of development as now known (see p. 258 and especially Fig. 138) the balance of probability appears to be decidedly in favour of the second of these hypotheses representing the method by which the ureter has actually arisen in phylogeny.

THE GONAD.—The great mass of the cells which constitute the body of a Vertebrate or any other of the higher Metazoa are specialized for the performance of particular functions in the ordinary life of the individual, and, correlated with this specialization, such cells have lost the power of giving rise to reproductive cells or gametes. The main mass of the body constituted of such specialized cells is known technically as the **soma.** At one or more points in the body there remain however patches of cells which have not undergone this specialization for ordinary vital functions and which retain the power of giving rise under favourable circumstances to gametes. The sum total of such cells constitute the **gonad.** The word gonad is commonly used in a loose sense as an equivalent of ovary or testis but it should be borne in mind that each of these organs contains a large proportion of immigrant tissues —connective tissue, blood, nerves and so on—which are strictly speaking part of the soma.

The problem of greatest general importance attaching to the development of the gonad of Vertebrates is that which concerns the origin of the cells (**gonocytes**) which constitute it.

And the interest of this question rests especially on the fact that in certain invertebrates the germ-cells have been traced back to blastomeres specially set apart at early stages of segmentation. All the probabilities seem to indicate that such a process if it occurs in the animal kingdom at all, is of a fundamental character and that indications of the same process may be confidently looked for in other groups.

The most however that we seem to be justified in asserting to be definitely established for Vertebrates is that genital cells are derived from the mesoderm of the coelomic wall. Apart from the actual facts of observation such development of gonocytes from coelomic lining fits in well with general morphological ideas. It is clear that we must believe that in the simplest diploblastic ancestor of the Vertebrates the gonocytes were derived from epithelial cells. It is also clear that, on the view that the Coelomata passed through an Actinozoan-like stage during their evolution, we must regard it as probable that during that stage the gonocytes were situated, as in existing Actinozoa, in the endodermal epithelium lining the pockets between the mesenteries—an epithelium which, on that view, is represented by the endoderm of the enterocoelic pouch of an Amphioxus embryo and by its derivative the coelomic mesoderm of an adult Amphioxus or other Vertebrate.

In *Amphioxus* the gonad of the adult shows special peculiarities which mark it off from all other Vertebrates. Bearing in mind however that the general arrangement of the mesoderm of the adult Amphioxus, which also shows striking peculiarities, is preceded by a condition in ontogeny which there is reason to regard as more nearly primitive than occurs in any other Vertebrate—the possibility at once suggests itself that this may also be the case with the gonad. Consequently it becomes important to enquire what are the early conditions of the gonad in *Amphioxus* and whether it is reasonable to interpret the conditions in the more typical vertebrates as being modifications of those illustrated by *Amphioxus.*

The earliest so far recognized stage of the gonad (Boveri, 1892; Zarnik, 1904) consists of a thickened portion of coelomic epithelium at the ventral end of the mesoderm segment *i.e.* in the region where at an earlier stage the segmented part of the mesoderm was continuous with the portion which loses its segmentation. The thickening lies close to the headward boundary of the segment and within its ventral angle. As the segment has already become nipped off from the lateral mesoderm it is not possible to say from actual observation that the thickening belongs to the splanchnic rather than the somatic wall though this is probable from the condition in the more typical vertebrates. The genital thickening is repeated over a number of segments (from about the 9th or 10th to about the 34th or 35th—Zarnik).

There are then three important points to be gathered from the study of the origin of the gonad in *Amphioxus*:—

(1) It arises as a thickening of coelomic epithelium *i.e.* it shows the mode of origin characteristic of coelomate animals in general,

(2) It arises close to the boundary of segmented and unsegmented mesoderm, and

(3) It arises on the dorsal side of that boundary.

In the more typical Vertebrates the ovary or testis first becomes clearly recognizable as a rule in the form of a longitudinal ridge—the **genital ridge**—which runs along the dorsal wall of the splanchnocoele on each side, at a varying distance from the line of attachment of the dorsal mesentery, and projects into the splanchnocoelic cavity. The genital ridge commonly extends over a greater antero-posterior extent than does the functional gonad later on—*e.g.* in the Salmon of the 185th day it extends from about the level of the fourth trunk myotome back to behind the anus (Felix). The restricted portion of the ridge which is destined to develop into functional ovary or testis is termed by Felix the gonal portion to distinguish it from the portions in front (progonal) and behind (epigonal) which remain sterile.

The relatively great anteroposterior extent of the gonad during early stages in its development is probably to be regarded, along with the greatly elongated condition in the adult of some of the more archaic Vertebrates, as evidence that at one period of evolution the gonad extended throughout the whole length of the splanchnocoele.

As development goes on the genital ridge increases in depth and is now termed the **genital fold.** This is composed of peritoneal epithelium covering a supporting and, later on, vascular core of mesenchymatous connective tissue.

The rudiment of the actual gonad in the strict sense consists of a thickening of the peritoneal epithelium covering the genital fold—the **germinal epithelium.** This thickened germinal epithelium may extend over both mesial and lateral surfaces of the genital fold as in most Amphibians, Reptiles and Birds or it may be restricted to its lateral (*Ichthyophis* ♀, most Teleosts) or median (Elasmobranchs except in very early stages, *Ichthyophis* ♂) surface.

Of the more primitive holoblastic Vertebrates the Amphibia are the only group on which detailed observations on the origin of the gonad have been recorded. We shall accordingly summarize the early stages in the development of ovary and testis in this group and where possible in its more primitive subdivision the Urodela.

Fig. 140 illustrates the earliest stages of the gonad so far identified in Urodeles, as described by Schapitz (1912) for the Axolotl. Fig. A is taken from an embryo in which the protovertebral stalk or nephrotome is not yet completely restricted off from the myotome. On its outer side is seen the rudiment of the archinephric duct. The stalk is continuous ventrally with the lateral or splanchnocoelic mesoderm. In its inner portion certain of its cells (*e.g.* the two adjoining cells in the figure in which the nucleus is shown in a darker tone) are beginning to show recognizable indications of nuclear and cytoplasmic features which are characteristic of the gonad later on. It will be borne in mind that the wall of the protovertebral stalk is morphologically part of the coelomic wall to which therefore these gonad cells also belong. In the sections shown in B and C the mass of cells showing these peculiarities has become more and more distinctly marked off from the lateral mesoderm (*mes*) and may now be spoken of definitely as the gonad. In the stage illustrated by D the lateral mesoderm is seen to be spreading inwards towards the mesial plane ventral to the gonad and it is beginning to show here and there a distinct split separating its somatic and splanchnic layers. In the later stages (E, F, G) this split becomes a patent cavity—the splanchnocoele (*splc*)—and the gonad is seen to lie on the dorso-lateral side of this, separated from the actual cavity by the somatic layer of peritoneal epithelium. In the last stage figured (G) the gonad is causing a slight bulging of the peritoneal lining into the splanchnocoele: this bulging is the incipient genital ridge (*g.r*).

During the earlier of the stages illustrated the gonocytes gradually acquire the superficial histological characters of germ-cells. The cell-body is larger than that of the other cells, it remains full of yolk particles, and in the spaces between the latter are to be seen fine granules of dark pigment. The nucleus is elongated or lobed in shape, the chromatin distributed in fine particles so that the nucleus as a whole stains less deeply than do the nuclei of other cells, and large round nucleoli are present, frequently corresponding in number with the lobes of the nucleus.

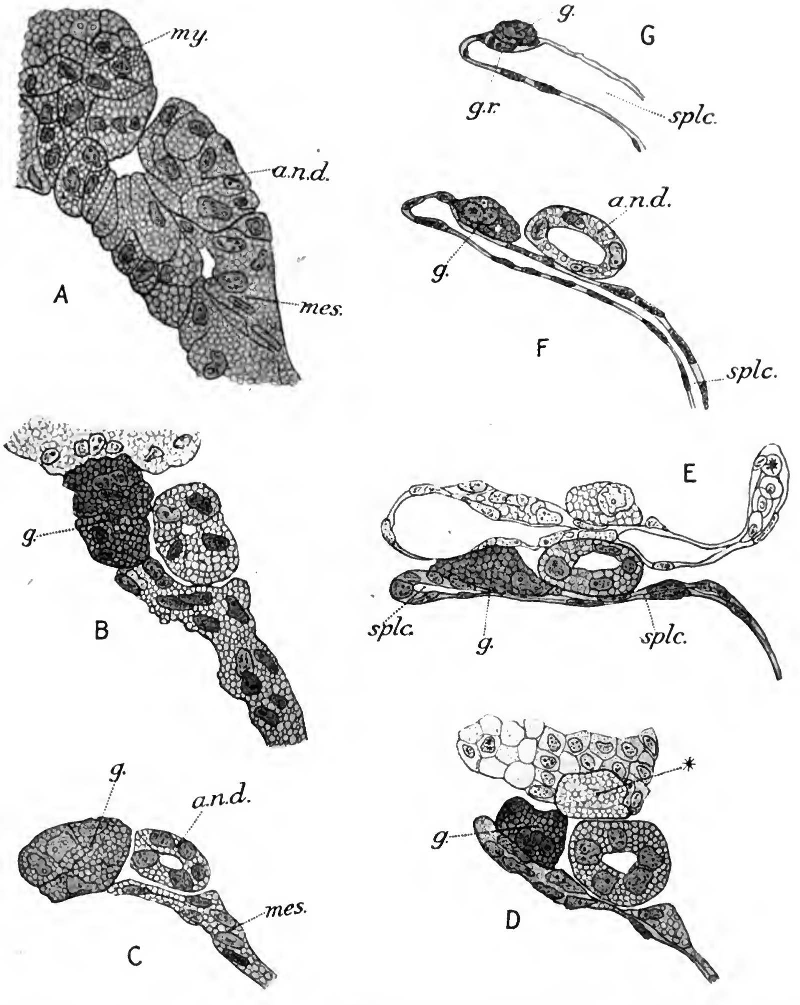


Fig. 140.—Origin of the gonad in *Amblystoma*. (After Schapitz, 1912.) A, 7-8 days after fertilization; B, 10 days; C, 12 days; D, 18 days; E, 17-18 days; F, 19 days; G, just after hatching.

a.n.d, archinephric duct; *g*, gonad; *g.r*, genital ridge; *mes*, lateral mesoderm; *my*, myotome; *splc*, splanchnocoele. At * in Fig. D is seen one of the heavily yolked cells which are interpreted by some as accessory gonocytes.

The embryonic gonad during the stages which have been described is not as a rule continuous from end to end. On the contrary it consists of isolated pieces and these in many cases show distinct traces of metameric arrangement, the pieces being directly opposite the mesoderm segments. The discontinuity becomes less marked in the later stages but even in an 18-day embryo Schapitz found the gonad still consisting on one side of the body of metamerically arranged blocks while on the other it had become a continuous strand, except for a single small isolated piece posteriorly.

From what has been said it seems clear that the gonad of the Urodela is a derivative of the coelomic wall lying close to the boundary between the segmented and the unsegmented (lateral) portions of the mesoderm. As in early stages it consists of blocks with a roughly segmental arrangement it would appear to lie on the dorsal or nephrotomal side of the boundary mentioned. There is no apparent reason for declining to interpret this early segmented stage of the gonad as a persistent trace of a primitive segmental arrangement like that of *Amphioxus*.

The tendency for the segmented condition to disappear in the typical Vertebrates is adequately explained by the gradual dorsalward encroachment of the unsegmented splanchnocoele. The boundary between segmented and unsegmented (lateral) mesoderm has altered much in position during the course of evolution, and there is no adequate reason to suppose that this boundary is not still a fluctuating one and if it is so we may expect varying traces of the original segmented condition to present themselves during development.

The gonad has been described as being paired throughout but it may be mentioned that various observers have noticed an unpaired condition at one or other period during the early stages of development. This appears to be adequately interpreted as a secondary fusion similar to that occurring between the right and left opisthonephros in a Teleost or in *Protopterus* rather than as a primary condition.

We have traced back the gonad to its first recognized beginnings in one of the relatively primitive holoblastic Vertebrates. Before passing on to its farther development it has to be noticed that there exists a considerable volume of evidence pointing to the existence of additional germ-cells which arise independently of the coelomic lining and some of which migrate into the germinal epithelium and may give rise eventually to functional gametes. It is not proposed to describe this evidence as it has not as yet, in the present writer's opinion, reached the stage of being convincing. It does not appear to have been satisfactorily demonstrated that the supposed extra gonocytes are really gonocytes at all rather than somatic cells. What is needed to provide such a demonstration is a careful study by skilled cytologists of the nuclear features of these cells, to determine whether there are any definite nuclear characters (such as Boveri discovered to be present in the gonocytes of *Ascaris megalocephala*) showing them to be beyond doubt gonocytes and affording a means of tracking them down in their supposed migration. Mere shape and staining capacity of the nucleus as a whole, or presence of nucleoli, do not seem sufficiently definite characters as these are probably directly related to volume and metabolic activity of the cell. Cytoplasmic features—of which much use is made in this connexion—such as richness in yolk or roundness in shape are also unreliable. As regards the first of these, the study of the development of embryos rich in yolk brings out clearly the fact that the cells in particular tissues do not, by any means, all keep pace with one another in their developmental processes. Individual cells lag behind, and one of the commonest characteristics of such cells is that the yolk stored up in their cytoplasm remains unaffected for some time after that in the neighbouring cells has been completely used up. Obviously in such a case richness in yolk, even when occurring along with greater size due to less active division, does not constitute evidence of any weight as regards difference in morphological nature between the heavily yolked cell and those round about it. Again there is reason to believe that yolk may be stored up secondarily in particular cells or portions of tissue of a developing embryo as a preparation for future needs quite apart from the actual germ-cells.

As regards approximation to a spherical shape, it should be remembered that there is a usual tendency for irregularly shaped or branching cells, such as those of ordinary mesenchyme, to assume temporarily a rounded form at the period during and about mitosis. Such cells are apt to assume an appearance misleadingly like that of young germ-cells.

The various features above indicated occurring together are sufficient to give a characteristic appearance to the cells in the main gonad but they form hardly definite enough criteria to prove that cells elsewhere are germ-cells in face of the strong probability that the whole mass of germ-cells in the body are of a common origin.

GENITAL RIDGE AND GENITAL FOLD.—The genital ridge was left as a slight bulging inwards of the peritoneal epithelium covering in the gonocytes. As development goes on the ridge becomes converted into a prominent fold — the genital fold. The peritoneal epithelium at first passes continuously over the surface of the strand of gonocytes but soon a change comes about in their relative positions the gonocytes coming to be incorporated in the thickness of the epithelium which may now therefore be spoken of as germinal epithelium. The gonocytes are to be seen first along the free edge of the fold (Fig. 141, A) and this during subsequent development swells out greatly and forms the functional ovary or testis, while the proximal portion acts merely for suspensory purposes. The gonocytes increase in number by mitotic division but are also reinforced from small apparently indifferent cells lying between them (Fig. 141, C, gc^1). We may take it that these small cells are in all probability to be interpreted as cells of the original gonad which have lagged behind in development, though it is naturally difficult from mere observation to make certain that they are not ordinary peritoneal cells. At a particular stage in development (between 26 and 33 mm. in

Rana temporaria—Bouin) the total number of gonocytes in the gonad undergoes a remarkable reduction, *e.g.* from between 200 and 250 in a frog tadpole of 26 mm. to between 37 and 46 in a tadpole of 33 mm. (Bouin). During this process individual gonocytes

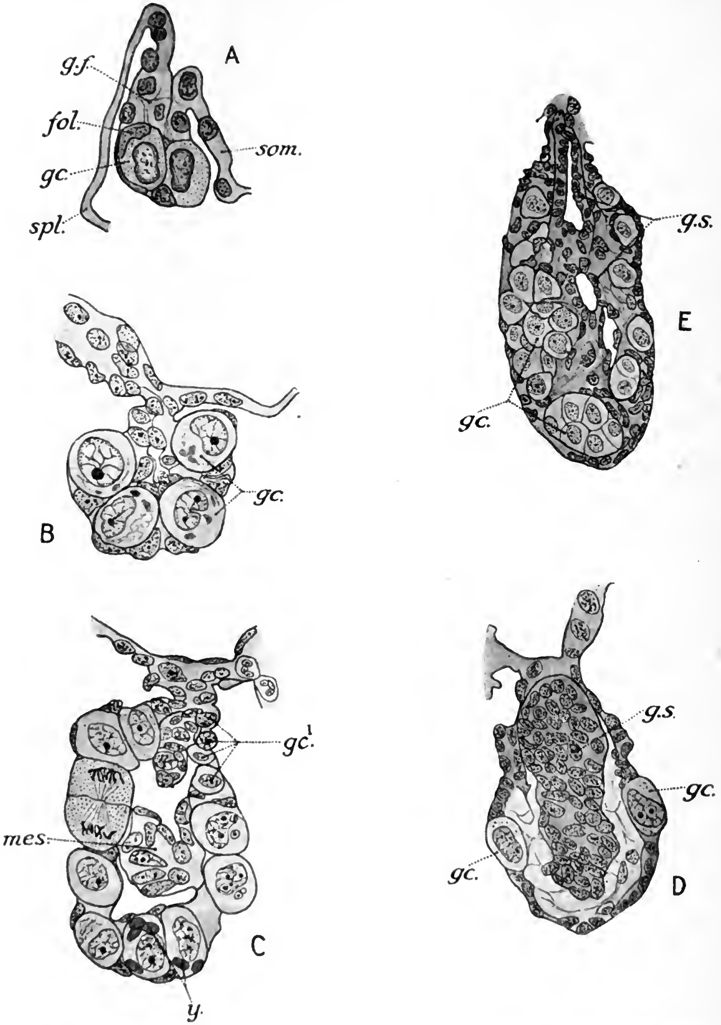


FIG. 141.—Development of the gonad in Amphibia as seen in transverse sections. (A, after Schapitz, 1912; B–E, after Bouin, 1901.)

A, larva of Axolotl (*Amblystoma mexicana*); B, *Rana temporaria*, 20 mm. tadpole; C, 24 mm. tadpole; D, 33 mm. tadpole; E, 35 mm. tadpole with hind legs completely developed. *fol*, nucleus of follicle-cell; *g.f*, genital fold; *g.s*, genital strand; *gc*, gonocyte; *gc*¹, transitional stages showing conversion of apparently indifferent cells into gonocytes; *mes*, mesenchyme; *som*, somatic mesoderm; *spl*, splanchnic mesoderm; *y*, yolk.

degenerate and in many cases they appear to be shed into the splanchnocoele leaving behind them the spaces or follicles in which they lay walled in by indifferent cells. The meaning of this phenomenon is obscure but a suggestion is made regarding it below.

A period of active mitotic division of the gonocytes now sets in which leads to the formation of solid masses of gonocytes projecting down into the interior of the young genital gland (Fig. 141, E). These gonocytes are the ancestral **oogonia** or **spermatogonia** as the case may be.

Up till now the genital fold has been spoken of as if it were merely a fold of the coelomic epithelium. As a matter of fact the fold very soon becomes invaded along its attached edge by immigrant mesenchyme cells forming a solid connective tissue core or framework which serves both a supporting and later, when it develops blood-vessels, a nutritive function to the developing germ-cells. The penetration of nests of gonocytes into this in the form of solid downgrowths of the germinal epithelium may be interpreted as representing an ancestral increase in area of the fertile portions of the germinal epithelium — the increase being originally brought about by the formation of hollow downgrowths into the vascular stroma, and these downgrowths having secondarily lost their cavities. The otherwise mysterious degeneration and shedding of large numbers of gonocytes already referred to may probably be regarded as a means of providing room for the localized parts of germinal epithelium which undergo this active proliferation.

Urinogenital Network.—A characteristic feature of the Vertebrate is the set of tubular channels—**vasa efferentia**—which in most of its subdivisions connects the testis with the opisthonephros and is frequently to be recognized in more or less vestigial form in the female sex as well.

Apart from variations in detail, these channels may be said to pass from the cavity of the testis to the cavities of the Malpighian bodies of the opisthonephros. They are clearly recognizable during early stages of development as solid strands of cells lying within the genital fold (Fig. 141, D, *gs*), the cavity in their interior developing secondarily. As regards their first origin the majority of observers state that they are first recognizable at their renal ends and have therefore interpreted them as outgrowths from the coelomic epithelium of the Malpighian body, *i.e.* from the nephrotome. Other observers seeing them make their appearance gradually in the core of the genital fold and reaching the Malpighian body secondarily regard them as differentiating *in situ* from the mesenchyme, while still others have adduced evidence in favour of the cells which give rise to the strands being budded off from the peritoneal epithelium close to the attached base of the genital fold. The disparity between the statements of different observers is most reasonably to be attributed to actual variation in the mode of development. It may be assumed that originally the connexions of the genital portion of the peritoneal epithelium with the peritoneal funnels, or with the nephrostomes, were in the form of open ciliated grooves or gutters on the surface of the peritoneum, that later on these became closed in to form tubular channels, and that in actual ontogenetic development in the modern amphibia the development from the coelomic epithelium has become obscured except for traces now at one end now at the other.

At their distal ends the cell-strands in the male can be traced gradually farther and farther into the genital fold until they come into immediate relationship with the cell-nests of gonocytes. In the female of Urodela and Anura the strands do not spread so far into the genital fold, nor are they, even in early stages, so well developed as in the male.

The **fatty body** is developmentally simply a portion of the genital fold which becomes specialized as a store-house of fat. In Anura it is the progonal portion which undergoes this differentiation while in Urodeles and Gymnophiona the rudiment of the fatty body is continued backwards as a ridge along the mesial face of the genital fold throughout its extent.

The fat is stored in the connective tissue of the organ, the fat cells being usually interpreted as immigrant mesenchyme cells which have invaded the rudiment by its base of attachment. It has also been suggested that these fat cells are peritoneal in their origin (Abramowicz, 1913)—a suggestion of obvious interest in view of the general tendency in the animal kingdom for potential germ-cells to undergo degeneration in order to provide nourishment for the germ-cells which become functional.

TESTIS.—The development of the functional testis out of the genital fold is seen in peculiarly simple and diagrammatic form in the Gymnophiona. Here the strands of the urinogenital network, as they sprout into the interior of the testis, anastomose together along its axis so as to form a central canal—around which, embedded in the stroma of the organ, lie the rounded nests of gonocytes. Fusion takes place between each gonocyte-nest and the wall of the central canal and then each nest develops a cavity in its own interior and becomes a hollow ampulla opening into the canal at its inner end.

Various modifications of this simple scheme are to be found. In Gymnophiona themselves ampulla-formation becomes suppressed except in localized regions between successive vasa efferentia, so that intervening portions of the testis are sterile and form merely thin tubular connexions between the bead-like fertile portions. Again the ampullae vary in shape: they may be elongated and tubular (*Discoglossus*) or, as in the majority of cases, flattened against one another by pressure. The "axial" canal again may lie close to the surface: it may become greatly branched, as in most Urodeles, or may form a complicated network as in most Anura.

OVARY.—In the differentiation of the ovary (Bouin, 1901) the most important points to be noted are the following. As regards the germinal epithelium the most conspicuous feature is of course the immense increase in size, accompanied by the storing up of yolk in the cytoplasm, exhibited by those gonocytes which are to become functional eggs. Synchronously the indifferent cells of the germinal epithelium in their neighbourhood become converted into follicular cells, having for their main function the ministering to the metabolic needs of the growing egg-cells. The intervening portions of germinal epithelium, which do not undergo this modification, retain their germinal character and provide successive batches of eggs in successive breeding seasons.

The cellular strands of the urinogenital network assume, as in the male, a tubular form, their wall becoming a cubical epithelium. As in the male the ovarian ends of these channels come into close relation with one another and fuse to form a central canal. In the Anura the fusion together to form an axial cavity appears to be less complete than in the male, a number of isolated central cavities being formed one behind the other. Fusions which take place later lead merely to reduction in the number of these central spaces (in *Rana* from about 12-15 down to about 5-7—Bouin). With further development, and as the functional egg-cells increase in size, the epithelial walls of these spaces become thin and flattened. Eventually they become pressed together and the cavity is reduced to a mere slit. The portions of the tubes lying nearer to the attachment of the ovary become vestigial.

The presence of these axial cavities in the ovary, homologous with the central canal into which the microgametes are shed in the male, is of great morphological interest. It suggests the possibility that at one time the eggs were shed into this central space and therefore that the condition now holding in the Vertebrata, where the eggs are shed into the open splanchnocoele, is to be interpreted as a reversion to, rather than a persistence of, the primitive method of shedding the eggs.

Leaving on one side the elaboration of histological detail which is not dealt with in this volume, the development of ovary and testis shows in its main features great uniformity throughout the gnathostomatous Vertebrates, and may therefore be dismissed with a few general remarks. Everywhere we see the gonad consisting at an early stage of a localized patch of coelomic epithelium in contact with nutritive and supporting mesenchyme: everywhere we see this coming to project into the splanchnocoele as a more or less prominent ridge or fold.

A conspicuous feature is the widespread tendency towards increased localization of the actual functional areas of the germinal epithelium. This is seen on a small scale in the development of cell-nests of gonocytes separated by indifferent or sterile portions: it is seen again in the restriction of fertility to a relatively small antero-posterior part of the genital fold, long progonal and epigonal portions becoming sterile: it is seen again even in the actual differentiated testis where a considerable length towards the posterior end may lose its fertility and assume a merely conducting function,[1] or where such sterile portions may be repeated at regular intervals throughout the length of the testis (Gymnophiona).

This concentration of the activity of the gonad may affect its bilateral symmetry. In Elasmobranchs both ovaries may be present and functional (*Laemargus*, *Notidanus griseus*), or one may be functionally inactive (*Centrophorus*, *Trygon*), or as happens in the majority, one, usually the left, fails to complete its development and is reduced to a more or less insignificant vestige. A similar reduction of one ovary takes place in many Teleosts. In the Birds the right ovary ceases its development at an early period and soon disappears entirely in the majority of individuals, although exceptions are of comparatively frequent occurrence.

OVARY AND OVIDUCT OF TELEOSTOMATOUS FISHES.—In the most archaic of existing teleostomatous Fishes—the Crossopterygian ganoids—the ovary is in the form of a typical genital fold which sheds its eggs into the splanchnocoele, from which in turn they pass out by a Müllerian duct. Consequently we may take it, in the absence of convincing evidence to the contrary, that the ancestral condition of the ovary and oviduct in the teleostomatous Fishes did not differ from that in other primitive gnathostomes such as Elasmobranchs, Dipnoans, or Urodeles. A peculiarity however of the Crossopterygian oviduct as compared with that of the other groups mentioned is seen in the reduction of its glandular activity, and this reduction—which finds its physiological expression in the reduction of tertiary egg envelopes—probably gives a clue to the subsequent evolutionary history of the oviduct within the group Teleostomi, in the majority of which the whole Müllerian duct has apparently been reduced to the verge of complete disappearance.

As regards the ovary itself there has come about secondarily—perhaps in correlation with increase in number and diminution in size of the eggs—a condition in which the eggs are not set free in the general splanchnocoele but are shed into an ovarian cavity, the wall of which is in complete continuity with that of the oviduct. The ovarian cavity is formed by the walling in of the portion of splanchnocoele which lies along the fertile (usually lateral) face of the ovary. The precise method of enclosure differs in detail in different Teleosts. In some (*e.g. Perca*, *Gasterosteus*, *Acerina*, *Zoarces*) the ovigerous surface of the genital fold becomes invaginated, or overgrown by flaps which eventually meet and undergo fusion (Fig. 142, A): in others (*e.g.* Cyprinoids) the free edge of the genital fold meets and undergoes fusion with the wall of the splanchnocoele (Fig. 142, B).

Which of these two types of development is the more nearly primitive cannot be stated with certainty but the balance of

---

[1] *Lepidosiren* and *Protopterus* (Graham Kerr, 1901); *Polypterus* and probably Teleosts (see below).

probability seems on the whole in favour of the first mentioned, for the formation of folds or grooves of the fertile surface of the genital fold, so as to give increased area, is a very usual phenomenon, and the formation of a single longitudinal groove would readily lead to the first-mentioned condition. On the other hand the replacement of this condition by the second is also readily understandable.

The ovary passes without a break into the oviduct which is simply the posterior sterile portion of the genital ridge in which a cavity develops secondarily—not always continuously—from before backwards. The oviduct differs greatly in length in different Teleosts: in some (*Zoarces, Cyclopterus*) the ovary itself may stretch right back to the genital pore.

Although the above description fits in with the normal conditions, there are various Teleosts in which the processes of fusion connected with the ovary do not take place and in which the ovary remains as a genital fold hanging down into the splanchnocoele, e.g. in the case of the Salmon fusion of the ovarian edge with the body wall takes place anteriorly for a short distance and again in the posterior sterile region, but the greater part of the fertile region of the ovary hangs free. In such cases the eggs are shed into the splanchnocoele and pass to the exterior by genital pores (compare Cyclostomata, p. 246).

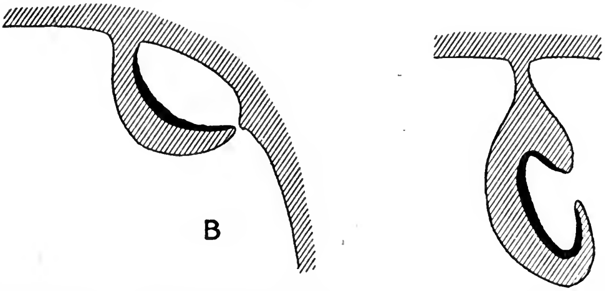


FIG. 142.—Diagram illustrating the conversion of the genital fold into a closed ovary in the Teleostean fishes.

Unfortunately we are still in almost complete ignorance regarding the development of ovary and oviducts in the Ganoids. From the little we do know it would appear that in *Lepidosteus* (Balfour & Parker, 1882) the ovary becomes enclosed in the same manner as in Cyprinoids (Fig. 142, B). Posteriorly it is continuous with the oviduct as in Teleosts generally. In the other Ganoids the ovary retains the form of a genital fold hanging down into the splanchnocoele while the oviduct is provided anteriorly with a coelomic funnel. The position of this funnel, far removed from the front end of the splanchnocoele, is sometimes used as an argument against the homology of this opening with the ostium of a true Müllerian duct, but such an argument carries little weight as we know from the higher vertebrates that the ostium of an undoubted Müllerian duct is liable to undergo secondary shifting into such a position. Again the fact that the opening lies on the mesial side of the ovary is adduced as an argument in the same sense but in this case we have definite embryological evidence from *Polypterus* (Budgett, 1902) that this position is secondary, the early rudiment of the duct lying external to the ovary and immediately ventral to the Wolffian duct as is the case with a typical Müllerian duct. Consequently there is no sufficient reason to doubt that these oviducts with open ostia in ganoids are really Müllerian ducts.

PHYLOGENY OF TELEOSTEAN OVIDUCT.—The facts of development show clearly that the main part of the Teleostean oviduct is of the same morphological nature as the ovary with which it is continuous. It arises from the hinder part of the primitive ovary which has become sterile and assumed a merely conducting function.

The main difficulty connected with the morphology of the organ is that of accounting for the joining up of the part of the oviduct of ovarian origin with the cloaca or exterior. Balfour suggested that this had come about by the oviduct becoming fused with the lips of the "abdominal pores." As an objection to this was adduced the observation by Hyrtl that in *Mormyrus* abdominal pores exist along with oviducts. This objection disappears, however, if we remember that in Balfour's time there were confused together under the same name two different types of aperture—true abdominal pores and genital pores. Substituting genital pores for abdominal Balfour's view seems still the most feasible. The probability seems to be that the main steps in the evolution of the Teleostean oviduct were as follows :—

(1) The primitive oviduct or Müllerian duct underwent gradual atrophy becoming gradually shorter[1] until eventually nothing was left but its external opening—the genital pore. This process would doubtless be correlated with the loss of its glandular function and this in turn may have been connected either with the adoption of pelagic spawning, in which special tertiary investments for the eggs were no longer required, or with a special development of primary envelopes within the group. A stage was thus reached which is represented by the condition in *Salmo*. Of course we do not know whether *Salmo* has retained this condition or has reverted to it : the latter is more probable.

(2) The portion of splanchnocoele along the ovigerous surface became enclosed so as to form a cavity which served to conduct the shed ova back into the neighbourhood of the genital pore. Anteriorly the ovarian surface abutting on this cavity remained fertile, while posteriorly it became sterile, so that the posterior portion of the cavity performed merely a conducting function (oviduct).

(3) The lips bounding the posterior end of the oviduct from

---

[1] We may see early stages in this process illustrated by the Ganoids *Amia* and *Acipenser*.

being merely in proximity to the genital pore came to be completely fused with the edges of the latter opening which consequently became the oviducal aperture.

URINOGENITAL CONNEXION.—We have already summarized for the Amphibia the course of development of the urinogenital network —the system of tubes or *vasa efferentia* which connect testis and kidney and which serve as the outlet for the sperm. It is now necessary to glance at some points in the general morphology of this system of tubes. It has already been indicated that at their genital end the tubes become merged together in the axial cavity of the testis. The latter we must regard as morphologically an isolated portion of splanchnocoele into which the spermatozoa are shed although it is no longer traceable to splanchnocoele in actual ontogeny. It has also been suggested that the tubular channels were probably originally open grooves of the peritoneal lining which became converted into closed tubes as the gonad became isolated from the main splanchnocoele.

The vasa efferentia frequently show a tendency, more or less pronounced, to anastomose together into a network. In the Amphibia it is a very general though not invariable rule that anastomosis takes place close to the edge of the kidney, forming the longitudinal "marginal canal" which is conspicuous in most Amphibians. A similar marginal canal is formed in Elasmobranchs.

In taking a general view of the system of vasa efferentia we find that one of its characteristics is, as in the case of the gonad itself, a tendency to increased localization of its functional portions. Thus during ontogeny in Amphibians the vasa efferentia towards the hinder end of the series become blocked and non-functional, or disappear entirely, leaving only those at the anterior end functional. This process reaches its limit in such forms as *Alytes* or *Discoglossus* where only two or a single member of the series persist.

Similarly in Elasmobranchs the number of functional vasa efferentia becomes reduced to a few at the anterior end of the series (*Centrophorus* 9, *Scyllium* 6, *Acanthias* 4-6, *Pristiurus* 3, *Mustelus* 2-3, *Raia* 1). The same happens in Amniota.

In the Dipnoi on the other hand the localization takes place at the hind end of the series, the functional vasa efferentia being reduced to about half-a-dozen (*Lepidosiren*) or to a single one (*Protopterus*).

Another phenomenon which makes its appearance is the simplification and shortening of the route by which the spermatozoa pass from the vasa efferentia towards the exterior. Primitively the vas efferens opens into an otherwise normal Malpighian body containing its glomerulus and continued into a functional renal tubule. This condition may persist (*Rana esculenta*, *Bufo*), or the glomerulus may disappear (*R. temporaria*), or finally the whole Malpighian body and its tubule may be shortened and widened and converted into a simple tubular continuation of the vas efferens towards the opisthonephric

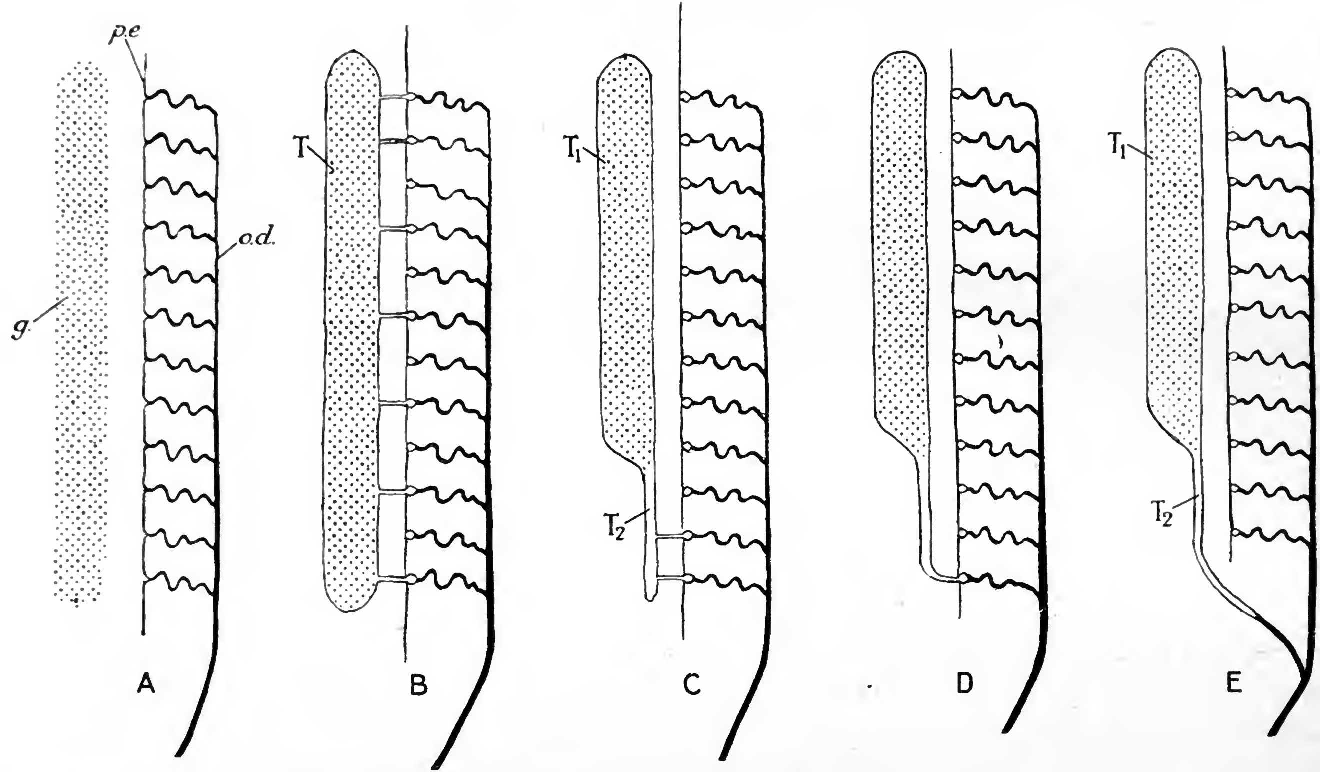


FIG. 143.—Diagram illustrating the evolution of the genital duct of the male Teleost.

A, condition in which the sperms were shed into the splanchnocoele and passed outwards through the nephrostomes and tubules of the opisthonephros; B, testis shut off from splanchnocoele and communicating with the cavities of certain Malpighian bodies scattered throughout the length of kidney and testis (exemplified by *Acipenser*, etc.); C, testis divided into functional (T_1) and sterile (T_2) portions and vasa efferentia reduced to a few in hinder portion of T_2 (exemplified by *Lepidosiren*); D, vasa efferentia reduced to a single one at hind end of T_2 (exemplified by *Protopterus*); E, the communication between the hind end of T_2 and the opisthonephric duct has become simplified (exemplified by *Polypterus* or young Teleost). *g*, gonad (portion of lining of coelome); *o.d*, duct of opisthonephros; *p.e*, peritoneal lining with nephrostomes opening through it.

duct (*Discoglossus, Alytes,* anterior vasa efferentia of *Bombinator*). In *Alytes* the single vas efferens with its continuation becomes completely emancipated from the kidney tissue and lies in the adult some distance from the anterior end of the kidney.

The same phenomenon is seen in Elasmobranchs and Amniotes where the opisthonephric tubules connected with the vasa efferentia never reach the length and complicated convolution of the normal tubule and the Malpighian bodies either degenerate (*Scyllium, Pristiurus,* Birds) or are eliminated entirely from ontogeny (Skates).

On the other hand the simplification of the route from testis to Wolffian duct may come about in a different fashion, as is seen in *Amia,* where the opening of the vas efferens has become shifted from the Malpighian body down the course of the tubule, in some cases till it has come to open directly into the duct.

A careful study of the method by which these various modifications come about during ontogeny is greatly needed.

Application of the general principles outlined above seems to afford a probable explanation of the remarkable arrangement in Teleostean fishes, where the testis is continued back into a special sperm duct which opens to the exterior near the opening of the kidney duct (Fig. 143).

The presence of a urinogenital network along the whole length of the testis in Ganoids (*Acipenser, Lepidosteus*) justifies the assumption that the ancestral Teleost possessed this primitive arrangement of the network. In the case of *Polypterus* the testis is continued back as a duct which opens into the urinogenital sinus formed by the hinder ends of the Wolffian ducts. The duct, however, is not, except near its termination, a simple tube but contains a network of cavities continuous with those of the testis. It is in fact not a simple duct but a portion of the testis which has become sterile.

Similarly in the case of various typical Teleosts it has been shown (Jungersen, 1889) that the duct is formed by the hinder part of the genital ridge, that it contains for a time a network of cavities continuous with those of the functional testis—that it is in other words the modified and sterile hinder portion of the testis—and, finally, that posteriorly it opens into the Wolffian duct.

Now the method by which the condition met with in *Polypterus* or in the young Teleost has arisen is probably indicated by what has happened in the two Lung-fishes *Lepidosiren* and *Protopterus.* In the former the testicular network is reduced to the extent that only about half a dozen vasa efferentia persist at the hind end of the series. In *Protopterus* these are still further reduced to a single vas efferens which passes from the hinder end of the sterile portion of the testis—" sperm duct " of the older descriptions—into the hind end of the kidney and communicates with the Wolffian duct through the hindermost kidney tubules.

The only further step needed from the condition exemplified by *Protopterus* to that of *Polypterus* or the young Teleost is that the communication between the sterile or duct portion of the testis and the Wolffian duct should come to be by a direct tubular prolongation of the vas efferens instead of by tortuous kidney tubules. That such a shortening up and simplification of the channel from testis to Wolffian duct does actually tend to come about in evolution is demonstrated by the precisely similar series of modifications which have occurred in the Anura at the front end of the urinogenital network. In these not only has the series of vasa efferentia become reduced to a single (anterior) member in such a form as *Discoglossus* but the kidney tubule interpolated between it and the Wolffian duct has become shortened and widened, so that there exists simply a single tube leading from the testis and continued at its other end into the Wolffian duct.

Giving consideration to these various points it appears to be justifiable to relate the probable evolutionary history of the sperm duct of the Teleost in the following terms:—

I. Primitively the elongated testis communicated with the Wolffian duct by way of (*a*) a series of vasa efferentia distributed along its length, and (*b*) the tubules of the opisthonephros.

II. The posterior portion of the testis became sterile and functioned merely as a reservoir and duct.

III. The vasa efferentia became reduced to those connected with this sterile region and finally to the hindermost one of these.

IV. The channel formed by this together with the kidney tubules into which the spermatozoa passed from it became shortened and widened until it reached the condition of a simple tube leading from the hind end of the testis into the hind end of the Wolffian duct.

V. The final stage was reached by the opening of this tube into the Wolffian duct becoming shifted back until its opening to the exterior came to be independent.

SUPRARENAL ORGANS.—The organ familiar to students of the Amniota and especially of the Mammalia under the name Suprarenal or Adrenal is now generally recognized as being not a single organ but an organic complex formed by the union of two originally separate elements—the medullary substance and the cortical substance. These two elements arise quite independently in ontogeny, the medullary substance being derived from the sympathetic ganglia, while the cortical substance arises in the form of a number of thickenings of coelomic epithelium on the roof of the splanchnocoele between the two kidneys. That this independence in early stages of ontogeny is a repetition of a condition which occurred during phylogeny is indicated by the fact that in Fishes the two elements are still independent. The names medullary and cortical substance, referring as they do to a topographical relation which occurs only in mammals, are obviously unsuitable from the point of view of comparative morphology and it is becoming customary to apply other more characteristic names. The medullary substance in mammals and what corresponds with it in other Vertebrates has a very characteristic chemical or physical reaction, in that it takes on a deep yellow or brown colour when treated with salts of chromic acid. Hence it is convenient, and usual, to apply to it a name expressive of this reaction—such as **Chromophile** (Stilling), Chromaffine (Kohn) or Phaeochrome (Poll).

The cortical tissue has also characteristic features—in particular the fact that its cytoplasm contains numerous granules of lipoid or fat-like substance, soluble in Ether, Xylol, etc., staining deeply with various Aniline stains, and giving the characteristic black with Osmic Acid. For masses of this tissue the name **Interrenal** organ may be used (Balfour) which although a topographical term like cortical substance has the advantage of being correct for vertebrates in general during at least the early stages of their development.

Of the more primitive groups of gnathostomatous Vertebrates only the Elasmobranchs and the Amphibians have been studied carefully in regard to the development of these organs and we shall consequently use them as illustrating the general mode of development which, with variations in detail, holds throughout the groups dealt with in this volume.

ELASMOBRANCHII.[1]—The Interrenal organs are here interrenal in position through life, forming either one (Sharks) or a pair (Skates and Rays) of elongated bodies lying in the region of the mesial plane and extending for some distance opposite the hinder part of the opisthonephros.

In *Scyllium* (Poll) the interrenal makes its first appearance (7 mm. embryo) in the form of a number of irregularly distributed thickenings of the splanchnic mesoderm in the region of the root of the mesentery, just ventral to the dorsal aorta. The possibility of metameric arrangement in the very earliest stages does not seem to be absolutely excluded but there is no evidence of this so far. The rudiments are most numerous in the genital region but they occur as far forwards as the hind end of the pronephros and back as far as the cloaca. The rudiments of the two sides, projecting towards the median plane, meet and become continuous, and as antero-posterior fusion also comes about, the rudiment takes the form (10 mm. embryo) of a cellular rod lying beneath the dorsal aorta and above the mesenteric root, and for a time still continuous with the splanchnic mesoderm which gave it origin. For a time there is close apposition, amounting to apparent continuity of tissue, between this rod and the opisthonephric nephrotomes lying on either side of it, but it is doubtful whether any special morphological significance is to be attached to this. In embryos of 16-28 mm. in length the interrenal organ gradually becomes separated in a tailward direction both from the coelomic epithelium and the nephrotomes, and assumes its definitive form.

Only the tailward part of the series of original rudiments

---

[1] The best general account of the development of the Suprarenal organs is that by Poll (1905).

completes its development in the way described. The whole series extends through about 25 segments but of these only about the posterior half take part in the formation of the interrenal rod: the anterior ones either atrophy completely or develop into small accessory interrenals.

The chromophile organs of the Elasmobranch (Swale Vincent, 1897) are small, rounded, segmentally arranged bodies lying ventral to the intercostal arteries—the anterior few on either side forming a continuous structure which was regarded by the earlier workers as an accessory heart (Duvernoy). These bodies are, as Balfour showed (1878), derivatives of the sympathetic ganglia. In a Scyllium of about 53 mm. the lateral part of the ganglion rudiment begins to show differentiation from the rest, its cells being relatively smaller than those which are destined to become ganglion-cells, and their protoplasm not only staining more deeply with ordinary stains but also developing the characteristic chromic acid reaction. In the Scyllium of 90 mm. the chromophile organ has assumed its definitive rounded form. Intrusive connective tissue forms a sparse stroma and capsule and in the former a capillary network is present. The series of segmentally arranged chromophile masses undergoes much modification in subsequent development—some, particularly at the ends of the series, aborting, others undergoing fusion. The details vary in different genera, the result being a striking variety in the adult arrangements in the various members of the group.

Amphibia.—Brauer (1902) in his work on the renal organs of *Hypogeophis* gives a clear account of the development of the suprarenals.

The interrenals appear as in Elasmobranchs in the form of cellular proliferations of the coelomic epithelium, in this case a little external to the root of the mesentery. These proliferations are paired and segmental in their arrangement, and extend from the region of the pronephros to that of the cloaca. The cellular buds become constricted off from the coelomic epithelium and lie above it as rounded masses embedded in the mesenchyme. As the two posterior cardinal veins approach and fuse the interrenal buds become displaced upwards so as to lie between the cardinal vein and the dorsal aorta. As development goes on processes of fusion take place between the rudiments more especially anteriorly where they come to form an unpaired elongated mass lying below the dorsal aorta and for the most part dorsal to the posterior vena cava (*i.e.* the fused posterior cardinals) but here and there passing laterally round the vein to its ventral surface or even piercing it—the fusion between the two cardinals having been obstructed at such points. In the posterior half of the organ the several rudiments retain their distinctness and lie on the ventral face of the opisthonephros.

The chromophile bodies develop as in the Elasmobranchs from split off portions of the sympathetic ganglion rudiments. These become shifted in a ventral direction round the dorsal aorta and take up their position in intimate contact with the interrenal bodies, lying in the posterior paired region of the interrenal on its mesial face, elsewhere dorsal or lateral or even completely surrounding the interrenal. The chromophile bodies stand out with great distinctness from the interrenal by their fine grained deeply-staining protoplasm and their larger nuclei.

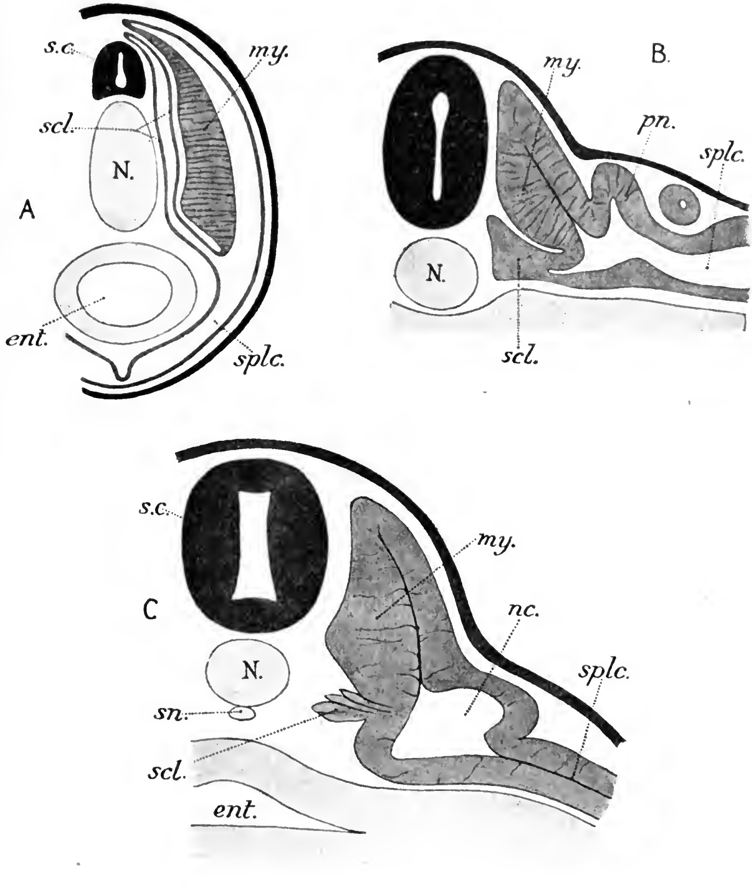


FIG. 144.—Illustrating origin of the sclerotome as seen in transverse sections through young stages of

A, *Amphioxus* (for the sake of clearness the spaces have been exaggerated, and the ventral portion of the diagram is taken from a rather younger stage than the dorsal); B, *Lepidosiren*, stage 24; C, *Polypterus*, stage 23. *ent*, enteric cavity; *my*, myotome; *N*, notochord; *nc*, nephrocoele; *pn*, pronephros; *s.c*, spinal cord; *scl*, sclerotome; *sn*, subnotochordal rod; *splc*, splanchnocoele.

We thus see that in the Amphibia the originally separate interrenal and chromophile bodies become during the course of development associated together to form a suprarenal complex of the type seen in the higher Vertebrates. Incidentally the unsuitability of the terms medullary and cortical is accentuated, for here when one of the elements comes to surround the other it is the chromophile which does so — precisely the opposite to what happens in the Mammalia.

THE SCLEROTOME.—In *Amphioxus* the sclerotome (Fig. 144 A, *scl*) arises as a pocket-like diverticulum of the splanchnic mesoderm just ventral to the myotome. It grows inwards and dorsalwards, pushing its way between the notochord and spinal cord on the one hand and the myotome on the other, until it reaches the mid-dorsal line where it meets its fellow of the opposite side. The epithelial walls of the sclerotome finally break up into mesenchyme—amoeboid connective tissue cells. The cells derived in this way from the outer wall of the sclerotome apply themselves to the mesial face of the myotome, penetrating in between its muscle cells and forming septa of connective tissue between adjacent myotomes, while those derived from the inner wall go to form packing tissue in the interstices round spinal cord and notochord. Over the spinal cord this packing tissue forms a tough protective roof. During this resolution of the sclerotomes into mesenchyme all trace of the original segmental character of the sclerotomes disappears.

It is customary — although the present writer regards it as questionable whether this is wholly justified—to regard the mode of origin of the sclerotome seen in the developing Amphioxus as representing the primitive mode of development. Upon this assumption we may describe what takes place in the typical Vertebrates as follows. The breaking up of the sclerotome into mesenchyme tends to take place at earlier and earlier periods of development—the diverticulum stage becoming more and more transient and eventually disappearing completely so that sclerotome formation comes to be represented merely by a very active proliferation of mesenchyme cells from the splanchnic surface of the mesoderm ventral to the myotome (cf. Fig. 144 C, *scl*).

It must not be supposed that the whole of the connective tissue in the body is necessarily derived from the sclerotome. On the contrary it would appear that other regions of the mesoderm also give rise to mesenchyme cells. Thus the inner surface of the splanchnic mesoderm of the gut-wall would appear to give rise to the connective tissue of this region, and the whole of the splanchnocoelic mesoderm of the postanal region apparently becomes resolved into mesenchyme.

On the whole perhaps the safest position to take up is that of regarding the power of forming mesenchyme as a general property of the mesoderm, and of regarding the sclerotome merely as expressing a localized concentration of this power, rather than as being the representative of some primitive pocket-like diverticulum of unknown function.

LITERATURE

Abramowicz. Morph. Jahrb., xlvii, **1913.**
Agar. Trans. Roy. Soc. Edin., xlv, **1907.**
Balfour. Monograph on the development of Elasmobranch Fishes. London, **1878.**
Balfour and Parker. Phil. Trans. Roy. Soc., clxxiii, **1882.**
Balfour and Sedgwick. Proc. Roy. Soc., xxvii, **1878.**
Bles. Proc. Roy. Soc., lxii, **1897.**
Bouin. Arch. de Biol., xvii, **1901.**
Boveri. Anat. Anz., vii, **1892.**
Brauer. Zool. Jahrb. (Anat.), xvi, **1902.**
Braus. Morph. Jahrb., xxvii, **1899.**
Budgett. Trans. Zool. Soc. Lond., xvi, **1902.**
Dahlgren and Kepner. Animal Histology. New York, **1908.**
Ewart. Phil. Trans. Roy. Soc., 179 B, **1888.**
Ewart. Phil. Trans. Roy. Soc., 179 B, **1889.**
Ewart. Phil. Trans. Roy. Soc., 183 B, **1892.**
Felix. Anat. Hefte (Arb.), viii, **1897.**
Felix. Hertwigs Handbuch der Entwicklungslehre, iii, **1904.**
Field. Bull. Mus. Comp. Zool. Harvard, xxi, **1891.**
Fürbringer. Entwick. der Amphibienniere. Heidelberg, **1877.**
(See also Morph. Jahrb., iv, **1878.**)
Goodrich. Quart. Journ. Micr. Sci., xxxvii, **1895.**
Guitel. Bull. Soc. Sci. et Méd. de l'Ouest, x, **1901**, and xi, **1902.**
Gregory. Semons Forschungsreisen. i, **1905.**
Hochstetter. Morph. Jahrb., xxix, **1900.**
Jungersen. Arb. zool.-zoot. Inst. Würzburg, ix, **1889.**
Jungersen. Zool. Anzeiger, xvi and xvii, **1893, 1894.**
Kerr, Graham. Proc. Zool. Soc. Lond., **1901.**
Kerr, Graham. The Work of John Samuel Budgett. Cambridge, **1907.**
Kerr, Graham. Quart. Journ. Micr. Sci., liv, **1910.**
Koltzoff. Bull. Soc. Imp. Nat. Moscow, xv, **1901.**
Lankester. Quart. Journ. Micr. Sci., xvii, **1877.**
Lebedinsky. Arch. mikr. Anat., xliv, **1895.**
Marshall. Vertebrate Embryology. London, **1893.**
Marshall and Bles. Studies Biol. Lab. Owens College, ii, **1890.**
Mollier. Anat. Hefte (Arb.), iii, **1893.**
Müller, E. Anat. Hefte (Arb.), xliii, **1911.**
Nussbaum. Arch. mikr. Anat., xxvii, **1886.**
Poll. Hertwigs Handbuch der Entwicklungslehre, iii, **1905.**
Poole. Proc. Zool. Soc. Lond., **1909.**
Rabl, C. Morph. Jahrb., xxiv, **1896.**
Rabl, H. Arch. mikr. Anat., lxiv, **1904.**
Rosa. Archivio zoologico, iii, **1906.**
Rückert. Arch. f. Anat. u. Entwick., **1888.**
Schapitz. Arch. mikr. Anat., lxxix, **1912.**
Schmalhausen. Zeitschr. wiss. Zool., C, **1912.**
Schreiner. Zeitschr. wiss. Zool., lxxi, **1902.**
Sedgwick. Quart. Journ. Micr. Sci., xx, **1880.**
Sedgwick. Quart. Journ. Micr. Sci., xxi, **1881.**
Semon. Keibels Normentafeln. iii, **1901.**
(Also in Zool. Anzeiger, xxiv, **1901.**)
Semper. Arb. zool.-zoot. Inst. Würzburg, ii, **1875.**
Vincent, Swale. Trans. Zool. Soc. Lond., xiv, **1897.**
Wijhe, van. Über die Mesodermsegmente und die Entwicklung der Nerven des Selachierkopfes. Verhand. Akad. Wet. Amsterdam, xxii, **1883.** (Reprinted, Groningen, 1915).
Wijhe, van. Arch. mikr. Anat., xxxiii, **1889.**
Zarnik. Zool. Jahrb. (Anat.), xxi, **1904.**

CHAPTER V

THE SKELETON

THE skeletal tissues of the animal body show a variety which is at first sight quite bewildering. Closer scrutiny however reveals certain general principles which are at work. In a very restricted set of cases we see that the supporting structure consists of a row or rod of cells which is rendered stiff through the individual cells being blown out or distended with fluid. Such **turgor** of cells is a far less conspicuous feature in the animal kingdom than it is in the vegetable. It is well seen in the axial row of endoderm cells which supports the tentacles of the Hydrozoa. In the Vertebrate it is seen in the notochord.

Far more usually the support is given by a definite supporting substance with such physical qualities as rigidity, tensile strength, elasticity, as may be required in the particular case.

These supporting substances of the animal body again show the greatest variety in their morphological nature but they may all be classed between two extremes — in one of which the supporting substance consists clearly of modified cells or portions of cells and in the other of dead intercellular substance. Examples of the former are seen in the remarkable phagocytic organs of nematode worms where an enormous cell becomes developed into an immensely complicated branched structure of stiff horny consistency upon the terminal twigs of which are perched innumerable minute blobs of phagocytic protoplasm. A good example of the second type is seen in the skeleton of ordinary coral—a mass of hard calcareous material lying clearly outside the limits of the living cells.

It is necessary to emphasize the fact, which is frequently lost sight of, that the differences between these two types are superficial rather than fundamental. They are merely the extremes of a series and are connected up by innumerable intermediate conditions. The skeleton of an Arthropod such as a Lobster is in the early stages of its development simply the stiffened and hardened outer layer of the cytoplasm of the ectoderm cells, while in its latest stage, immediately before it is shed, it has become a thick layer of dense chitinous and calcified non-living substance lying outside the limits of the living protoplasm.

Non-living material "secreted" by cells consists no less of modified cytoplasm, although here the cytoplasm so modified does not form a continuous mass and retain its original position in regard to the rest of the cell body. It arises commonly as isolated droplets or particles which may secondarily run together within the body of the cell or, without this happening to any obvious extent, are extruded from it, passing on to a free cell surface or into the intercellular matrix. The process can be followed by observation, naturally, only in cases where the secretion runs together into discrete droplets or particles sufficiently large to be visible under high powers of the microscope, as commonly happens in gland-cells. Far more frequently the extruded particles are so small—possibly molecular—as completely to elude observation. Such is the case where the intercellular substance undergoes skeletal modification: all that can be observed is simply a gradual transformation in the physical and chemical characters of the matrix, due in some cases to a gradual change in the metabolic activities of the cells which inhabit it, in others to the immigration into it of cell-colonists of a new type.

The supporting skeleton of the Vertebrate is an **endoskeleton**;[1] it is developed not on the outer surface of the body but within its substance. In this it contrasts with the skeleton of the Arthropod or Mollusc which is exoskeletal—consisting of thickened and stiffened cuticle. In the case of the most ancient skeletal structure in the Vertebrate body—the notochord—the stiff supporting character is due to the individual cells being distended by fluid secreted in their interior but as a rule in other skeletal tissues the stiffness is given not by the cells but by the intercellular matrix.

THE NOTOCHORD AND ITS SHEATHS.—A comparative study of the Vertebrate skeleton shows that it illustrates three phases of evolutionary progress (1) the notochordal phase, (2) the cartilaginous or chondral phase and (3) the bony or osseous phase. Of these the primitive is indisputably the first. It is a phase which is passed through during ontogeny in all Vertebrates and it remains permanent throughout life in *Amphioxus.*

The notochord is in its origin a rod of cells split off from the endoderm along its mid-dorsal line. This is seen in all the lower Vertebrates. In some of the more primitive members of the group the notochordal rudiment is for a time deeply grooved on its lower side, so as to form an inverted gutter along the middle of the enteric roof, and it may well be that this is to be regarded as the primitive mode of formation of the organ.

The notochord becomes constricted off as a cylindrical rod extending along the dorsal side of the alimentary canal from a point just behind the tip of the infundibulum to the tip of the tail. The

---

[1] It is regrettable that the term exoskeleton has crept into use by writers on Vertebrate anatomy for structures such as fish-scales. As will be seen later these are really endoskeletal, even the enamel being developed on the *inner* surface of the epidermis.

individual cells develop in their cytoplasm fluid vacuoles which increase in size and become confluent until at last the cell takes the form of a comparatively thin layer of protoplasm surrounding an enormous vacuole and containing embedded in its substance at one point the nucleus. The turgescent condition of the cells inflated with fluid gives them the firmness which enables the notochord to carry out its function as a supporting structure.

The inflation of the cells with fluid carries with it another result namely a great increase in size of the individual cells. This in turn causes a great increase in size of the notochord as a whole, showing itself particularly by increase in diameter but also by increase in length. The latter is not able to take place with perfect freedom and the result is that the individual cells tend to be compressed into the form of transverse discs.

The notochordal rudiment at an early stage becomes covered by a thin, elastic, highly refracting, cuticular formation known as the **primary sheath** of the notochord ("*elastica externa*"). After the formation of this the superficial layer of the notochord soon resumes its cuticle-forming activity but now in a somewhat modified form—a **secondary** or "fibrous" sheath, thicker and more jelly-like in appearance, being produced internal to the primary sheath. In Cyclostomes and Sturgeons this secondary sheath remains throughout life without conspicuous change beyond increase of thickness and the assumption of a tough fibrous character and is physiologically the most important part of the axial skeleton of the trunk region.

The superficial layer of notochordal cells, lying in immediate contact with the inner surface of the secondary sheath, do not as a rule undergo the process of vacuolation which affects the inner cells. They remain as a layer of compact protoplasmic cells known as the **notochordal epithelium**.

In some Vertebrates (Dipnoi, Agar, 1906) a short stretch of notochord, from the tip backwards, degenerates within its primary sheath at an early stage, breaking up into loose mesenchyme. As the notochord behind the degenerated portion grows in length its front end is pushed forwards so as to re-occupy the vacated portion of primary sheath. The extent to which this process takes place throughout the Vertebrata in general, and also its meaning, are deserving of further enquiry.

HYPOCHORD (Subnotochordal Rod).—In the anamniotic Vertebrates there is formed what is apparently an accessory notochord lying ventral to the true notochord and hence known as the **Hypochord** or Subnotochordal rod. This organ (see Gibson, 1910) arises after the notochord and in an entirely similar manner, *i.e.* as a longitudinal rod of cells split off from the endoderm in the mid-dorsal line and sometimes possessing a distinct groove along its lower surface facing the enteric cavity. On its surface it normally develops a primary sheath precisely like that of the notochord.

We may be sure, from its wide distribution amongst the more primitive Vertebrates (Lampreys, Elasmobranchs, Teleostomes, Dipnoi, Amphibia) and the early stage of development at which it appears, that the hypochord is an organ of great antiquity in the Vertebrate stem, but we have no definite knowledge of its ancestral significance. The fact that it does not occur in *Amphioxus* has rendered possible the suggestion that it represents the longitudinal groove which in this animal runs along the mid-dorsal line of the pharyngeal wall. But this idea is negatived by the fact that the hypochord extends right back to the tail and is not merely a pharyngeal organ, and the probability seems to be that it has come down from a period in evolution long before the appearance of *Amphioxus*. It is perhaps simplest to regard it merely as an accessory notochord.

Whereas the true notochord plays an important physiological rôle—as the main part of the axial skeleton during early stages, and as the foundation for the vertebral column of later stages—the hypochord has no such justification for its persistence. It lasts only for a short time and eventually breaks up and completely disappears.

In the Amniota there is no typical hypochord developed but it is possible that a thickening of the mid-dorsal endoderm which is frequently found in the pharyngeal region (*e.g.* in the Second day Fowl embryo) may represent a last vestige of it.

SKELETAL DEVELOPMENTS OF THE CONNECTIVE TISSUE

Whereas the notochord is derived directly from the endoderm, the cartilaginous and bony components of the skeleton on the other hand are modifications of the mesenchyme or connective tissue, which forms a considerable proportion of the entire bulk of a typical vertebrate.

Connective tissue in its least specialized form may be seen in practically any late vertebrate embryo as a reticulum or spongework—a syncytial framework—of much-branched cells, the processes of which are continuous from one cell to another, while the meshes are occupied by a clear fluid or jelly-like matrix. Masses of this tissue form a kind of packing between and around the various epithelia of the body, while it also, in the form of discrete wandering cells, actually invades the epithelial tissues and colonizes them. Such immigrant elements are found for example between the muscle-fibres, in the substance of the central nervous system, and even frequently between the epithelial cells of the epidermis.

The primitive or embryonic connective tissue undergoes gradual differentiation in accordance with the physiological rôle which it has to play in different localities. This differentiation finds expression in such superficial features as shape and arrangement of the individual cells and more fundamentally in the peculiarities in metabolism which lead to its storing up particular substances in its protoplasm

—pigment of chromatophores, fat of the cells of adipose tissue—or again in the influence exerted by the metabolic activity of the cell upon the character of the matrix. This matrix is commonly described as intercellular, which is quite correct, but the important point is not the question whether it is inter- or intra-cellular but the fact that it is in immediate relation to, and under the influence of, the living protoplasm of the cell. The portion of matrix in contiguity with one of the irregularly shaped connective-tissue cells is comparable with an intracellular vacuole the outer wall of which has thinned out and disappeared. The matrix has been formed by the breaking down of living substance and it seems merely a matter of phraseology whether we speak of it as modified protoplasm or as dead "formed" material.

The most familiar differentiation of the matrix of connective tissue consists in the development within it of thin tough fibres, characterized by the physical property that they soften and dissolve, yielding gelatin, under the action of boiling water, and that they become further toughened by the action of tanning agents. These fibres run indiscriminately in all directions or, in the more specialized conditions, are definitely orientated, as in the case of **tendon** where they are parallel and arranged in longitudinal strands, or of **aponeuroses** where they are arranged in thin layers, those of one layer perpendicular to those of the next. Other portions of the matrix take the form of **elastic fibres**—characterized by their elasticity, by their connexion together to form a network, by their being much less easily affected by boiling water, and by their not yielding gelatin.

The amount of matrix present differs greatly in different localities. It may be reduced to a very small amount—to a mere demarcating line—between closely fitting plate-like cells, as in the case of the endothelium covering the surface of a tendon, or it may be large in amount and comparatively rigid as in the case of the two great skeletal tissues **cartilage** and **bone**.

Cartilaginous or Chondral Skeleton.—The cartilage is characterized by its cells taking on a rounded form and becoming separated by an abundant semitransparent, elastic, chondrin-containing matrix. The process of chondrification becomes apparent first of all in the somewhat dense packing tissue ("skeletogenous layer")[1] immediately surrounding the notochord. This connective tissue becomes locally modified to form little blocks of cartilage known as the arch-elements (**arcualia**—Gadow, 1895), lying just outside the primary sheath and arranged in four longitudinal rows, two dorsal composed of the rudiments of the **neural** arches, two ventral—the rudiments of the **haemal** arches. These arch-elements are apparently in the primitive condition duplicated in each segment, *i.e.* within the limits of a myotome or sclerotome there are situated two pairs of neural and two pairs of haemal arch-elements.

---

[1] The term **prochondral** is applied to the young cartilage in its early stages before the characteristic intercellular matrix makes its appearance.

There now takes place in two of the more primitive groups of Vertebrates—the Elasmobranchii (including the Holocephali) and the Dipnoi—a remarkable process whereby the secondary sheath of the notochord becomes converted into a sheath of cartilage. Certain of the cartilage cells in the arch rudiment take on an amoeboid character and burrowing their way through the primary sheath, apparently by the help of a digestive ferment, invade the secondary sheath (Fig. 145, *m.c*). Continuing their migration they become distributed equally throughout the whole substance of the secondary sheath, including those portions in the head region which will later on form part of the cranium. The immigrant cells finally settle down in the substance of the secondary sheath and the latter becomes a cylinder of cartilage.

It is important, with an eye to the evolution of the vertebral column in Vertebrates higher in the scale, to bear in mind that this invasion of the secondary sheath by immigrant cartilage cells takes place at four points in the transverse plane, corresponding to the bases of the four arch rudiments, and that this arrangement is repeated twice within the limits of one segment owing to the arch rudiments being so repeated. Consequently if we suppose the colonization of the secondary sheath to be restricted to the neighbourhood of the transverse plane in which the arch rudiments are situated the result would be the formation of *two* rings of cartilage within the limits of a single segment.

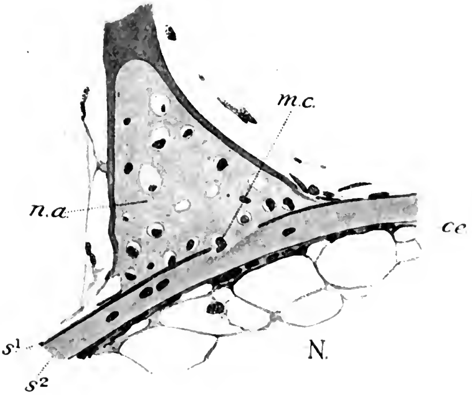


Fig. 145. — Part of a transverse section through a Lepidosiren of stage 38, traversing one of the neural arch rudiments.

c.e, notochordal epithelium; *m.c*, migrating cartilage cell; *N*, notochord; *n.a*, neural arch; s^1, primary sheath; s^2, secondary sheath.

In the case of Lung-fishes and Holocephali the chondrified secondary sheath undergoes no further modification but in typical Elasmobranchs it becomes divided up into segments, which form the centra or bodies of the vertebrae, in the manner to be described later on. In this process the originally uniformly flexible notochord with its sheaths becomes replaced physiologically by a series of rigid masses, flexibility being given to the whole by the presence of the intervening joints. As this jointed condition of the vertebral column originated in evolution at a time when the longitudinal muscles of the body were already divided into myotomes, we may take it as probable, for obvious mechanical reasons, that the rigid skeletal masses arose in a position alternating with the muscle segments. The individual vertebral centra were in other words from the beginning intersegmental in position in relation to the general body metamerism.

In sketching out in somewhat greater detail the further development of the vertebral column the assumption will be again made use of, as it was in dealing with the mesoderm segments, that the trunk region has in all probability departed least from the primitive condition, and the facts quoted will in the main be taken from this region of the body.

The student who goes on to peruse original memoirs will notice that this rule is by no means always accepted. Some writers will be found to assume that the caudal region is more nearly primitive, and, in accordance with this assumption, to interpret the phenomena observed in the trunk vertebrae by those observed in the caudal, instead of *vice versa*.

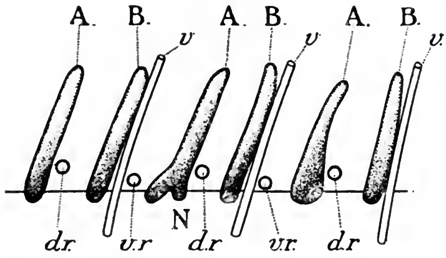


FIG. 146.—Arrangement of dorsal arch-elements in hinder trunk region of a *Petromyzon* larva 95 mm. in length. (After Schauinsland, 1906.)

A, anterior, B, posterior neural arch-elements; *d.r*, dorsal root of spinal nerve; *N*, surface of notochord; *v*, intersegmental blood-vessel; *v.r*, ventral root of spinal nerve.

In this connexion it must be borne in mind that the Vertebrate is above all things essentially a coelomate animal. No one doubts that whatever the common ancestor of the Vertebrates was like it was at least coelomate. And most morphologists would admit further that the weight of evidence indicates that in this ancestor the splanchnocoele extended throughout the greater part of its length and that the existence of a considerable stretch of body towards the hind end devoid of splanchnocoele (*i.e.* a tail region) is secondary. But if the caudal region has in this way undergone profound secondary modification of its structure it is clear that it is not in this region of the body that we should expect to find persisting primitive modes of development of the axial skeleton.

It is now necessary to follow out the fate of the arch-elements. As already mentioned the primitive arrangement of these appears to have been two pairs to each segment, above and below, so that corresponding with each myotome there were, on each side, two neural elements an anterior (A) and a posterior (B), and two haemal elements an anterior (*a*) and a posterior (*b*).

NEURAL ARCHES.[1]—Apparently the most nearly primitive arrange-

---

[1] In writing these sections on the vertebral column much use has been made of Schauinsland's descriptions (1906) to which the student is referred for a more detailed account than is here possible.

ment of the arches is that which occurs in the hinder trunk region of the Lamprey (Fig. 146). In this animal, as is well known, the dorsal (sensory) and ventral (motor) nerve-roots are still separate and are spaced out alternating with one another at approximately equal distances along the sides of the spinal cord. The dorsal arch elements alternate, in their turn, with the nerve-roots, so that there are, on each side, an anterior (A) and a posterior (B) neural arch-element within the limits of a single myotome.[1] It should be noticed particularly that of these the anterior is situated between the sensory and the motor nerve-root belonging to the segment. This suggests a possible explanation of the later evolutionary history of these cartilages (A) which in the typical Fishes tend very usually to become reduced in size, even to the point of disappearance. It may be that this reduction in size is connected with the fact that in the Fishes, as indeed in all gnathostomatous vertebrates, the two nerve-roots have become approximated together to form a common sensori-motor spinal nerve. On the other hand this explanation would leave untouched the fact that a similar reduction in size may occur in the corresponding ventral or haemal arches.

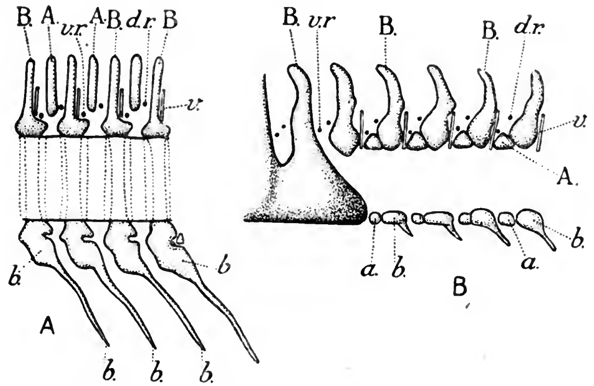


FIG. 147.—A, arrangement of arch-elements in mid-trunk region of a Carcharias embryo 85 mm. in length; B, *do.* in anterior region of a Sturgeon (*Acipenser huso*) 36 mm. in length. (After Schauinsland, 1906.)

A, anterior neural arch-element; *B*, posterior *do.*; *a*, anterior haemal arch-element; *b*, posterior *do.*; *d.r*, sensory nerve-root; *v*, blood-vessel; *v.r*, motor nerve-root.

The reduction in size of the "A" elements, which is of so frequent occurrence amongst the typical fishes, is well shown in Figs. 147, A and B, which are based upon Schauinsland's reconstructions. This marked reduction is by no means of universal occurrence. The two common Dog-fish—*Scyllium* and *Acanthias*—are familiar examples of fishes in which the "A" elements

---

[1] In the anterior trunk region the arrangement is apt to be modified—the intersegmental vessel, which forms the anterior limit of the segment, coming to lie on the tailward side of the A cartilage of that segment (Schauinsland).

("intercalary pieces," "interdorsals") remain nearly as well developed in the adult as the "B" elements.[1]

In Lung-fishes (here and there) and in Urodele amphibians the "A" pieces can still be recognized (cf. Fig. 148); they have also been observed in the embryos of various Reptiles. In this case they usually lose their individuality at an early period, becoming completely merged in the definitive neural arch formed by the "B" elements lying next to them on their headward side, but in some cases, *e.g.* in the tail region of *Lacerta*, they have been found to persist as discrete structures even in the adult, forming a vestigial second neural arch behind the main arch.

The neural elements become prolonged dorsally and meet so as to form a complete neural arch and the apex of this becomes prolonged as an unpaired piece in the mesial plane to form the **neural spine**. The complete neural arch formed in this way frequently becomes segmented up into separate pieces of cartilage. The arcualia in such cases become each divided into a larger basal (basidorsal—B, interdorsal—A, Gadow) and a smaller apical (supradorsal) portion. The spine may segment into three superimposed rod-like portions.

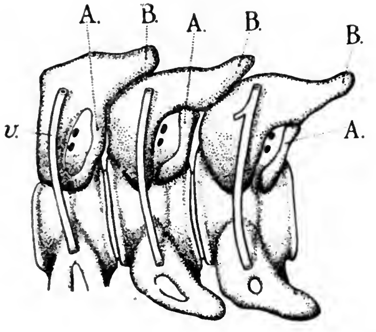


FIG. 148.—Arrangement of arch-elements in anterior caudal region of a Siredon 50 mm. in length. (After Schauinsland, 1906.)

Reference letters as in Fig. 147.

HAEMAL ARCHES. — In the Cyclostomes typical haemal arches are absent, although possibly vestiges of them are represented by a continuous ridge of cartilage occurring in the tail region of *Petromyzon* where the neural arches have also been reduced to a similar continuous ridge (Schneider).

Of haemal arch elements there were apparently primitively two pairs to a segment just as in the case of the neural arches. This seems to be clearly indicated by *Callorhynchus* (Fig. 149). It is also well shown in the young Sturgeon (Fig. 147, B) where the anterior element (*a*) in each segment has undergone reduction in size exactly as was the case with the corresponding neural element (A). A similar condition is found in many Elasmobranchs, though not in all, the "*a*" elements being in some cases apparently completely absent.

---

[1] The examination of one of these Dog-fishes brings out another point of general importance namely that the arch-element as it increases in size is apt to spread round a nerve-root in its neighbourhood. The result is that in the adult the nerve-roots may pass out, not between the arch-elements, but through them. The lesson to be learnt from this is that the topographical relation of skeletal elements to nerve-trunks is not to be taken as infallible evidence as to the primitive situation of such elements.

In various Fishes, for example *Laemargus* and *Amia* (also in some Amphibia, see Fig. 153, B), the haemal arch-element in the trunk region segments into two pieces—one of which carries the rib and may become shifted dorsally, while the other becomes displaced in a ventral direction. The ventral pieces come to form projections downwards from the centrum of the vertebra on each side of the aorta and have been termed "aortic supports." They may be termed **haemal processes** as they appear to be homologous with the knob-like structures bearing this name which are to be seen in the caudal region of *e.g. Laemargus*, projecting inwards from the haemal arch into the tendinous septum which underlies the caudal aorta.

In the caudal region the haemal arch-elements are commonly much longer than in the trunk. They bend round to meet one another and are prolonged into a **haemal spine.** These features are associated with the extension of the body in a dorsal and ventral direction correlated with the use of this region of the body for the purposes of movement.

Towards the head end of the series it not uncommonly happens in Cartilaginous fishes that the haemal arch-element becomes broadened out over the surface of the notochord indicating the beginnings of the evolution of perichordal centra (see below). This is well shown by *Callorhynchus* (Fig. 149) where incipient centra are distinctly seen, formed by the much-enlarged and fused haemal arch-elements (*a* and *b*).

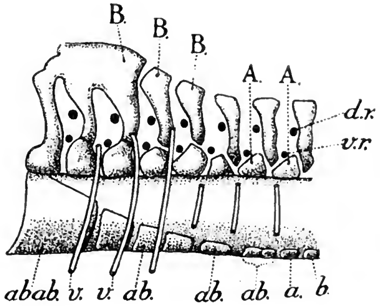


FIG. 149.—Portion of vertebral column of a 10 cm. embryo of *Callorhynchus* a few segments posterior to the hind end of the skull. (After Schauinsland, 1906.)

Reference letters as in Fig. 147.

In the air-breathing vertebrates there are no longer double sets of complete haemal arch-elements but a distinct trace of this condition is seen in such a Urodele Amphibian as *Siredon* (see Fig. 148) where a large perforation through the haemal arch element, traversed by the intersegmental blood-vessel, betokens its double origin.

A characteristic feature of the Amniota is that the haemal arch (the cartilaginous forerunner of the "chevron-bone") tends to become displaced forwards so as to assume an intervertebral position or even to become fused with the vertebra lying in front (*Anguis*—Goette).

VERTEBRAL CENTRA.—Except in the case of Cyclostomes, Holocephali and Lung-fishes, the elastic notochord becomes replaced physiologically during development by the series of vertebral centra. In the various subdivisions of the Vertebrata we find two distinct methods by which vertebral centra are produced (1) by the segmentation of the cartilaginous secondary sheath (**sheath centra**; chorda centra—Gadow) and (2) by the enlargement of the bases of the arch-elements which grow round the notochord and give rise to centra outside the primary sheath—(**perichordal centra**; arch centra—Gadow).

SHEATH CENTRA are seen in Elasmobranchs. In the region which will develop into a centrum the chondrified secondary sheath becomes thickened so as to bulge inwards and constrict the notochord (Fig. 150). A more deeply staining "middle zone" soon becomes distinguishable in this thickened part of the secondary sheath (Fig. 150, *m.z*) having a shape something like that of a dice-box, its central part lying much nearer to the axis of the notochord than do its two extremities. This middle zone becomes the main part of the wall of the amphicoelous centrum, its substance becoming usually strengthened by the calcification of its intercellular matrix.

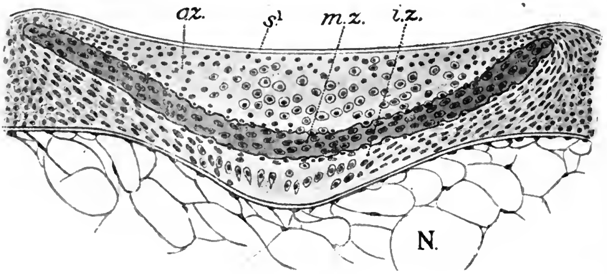


FIG. 150.—Part of sagittal section through the secondary sheath of a Scyllium of 61 mm. total length showing an early stage in the development of a centrum. (After C. Rabl, 1893.)

i.z, inner zone; *m.z*, middle zone; *o.z*, outer zone; *N*, notochord; s^1, primary sheath.

The inner zone (Fig. 150, *i.z*) may grow in thickness so as to cause greater and greater constriction of the notochord. This process attains to its maximum in the Skates where it extends inwards to the axis and causes the formation of thick septa which divide the notochord into isolated intervertebral fragments. More usually however the inner zone does not undergo this increase, it tends to become absorbed at each end and forms simply a ring of cartilage in the centre of the vertebra.

The outer zone in many Elasmobranchs becomes calcified in parts: the calcified regions often showing a regular arrangement *e.g.* concentric cylindrical shells or radiating septa.

PERICHORDAL CENTRA.—As a matter of fact the purely chordacentrous condition is merely a temporary one in the Elasmobranch, as in later stages of development the sheath centra become surrounded by a layer of cartilage provided by the bases of the arch-elements, which spread over the surface of the sheath centrum (Goette, 1878).[1] This outer layer of cartilage may undergo calcification later and become continuous anteriorly and posteriorly with the edge of the calcified middle zone. Meanwhile the primary sheath of the notochord is liable to disappear so that there is no obvious clue left to the independent origin of the portions of the vertebral body derived from the sheath and the arch-elements respectively.

In the course of the further evolution of the vertebral body this outer layer of perichordal origin, which in an ordinary Elasmobranch like *Scyllium* or *Acanthias* serves merely to reinforce the sheath-centrum, is destined to become all-important while the sheath portion is destined to disappear. A step in this direction has already been made by the Rays where (*Torpedo*—Schauinsland) the secondary sheath remains thin and where the primary sheath soon disappears so as to bring about complete fusion between the thin sheath layer of cartilage and the much thicker external mass derived from the arches.

In those Teleostomatous fishes which possess centra the spreading of the bases of the *cartilaginous* arch-elements round the notochord is commonly not marked: possibly this is correlated with the precocious development of the bony centrum.

In the Urodele Amphibians the vertebral bodies develop in the manner illustrated by Fig. 151. A series of ring-shaped cartilages ("intervertebral cartilage," Gegenbaur: Fig. 151, A, *c*) make their appearance round the notochord in mid-segmental positions. These rings gradually extend for a considerable distance in a headward and tailward direction, immediately superficial to the notochordal sheath, and between it and a thin, tubular, segmented sheath of bone (non-cellular) which has already made its appearance (Fig. 151, A and B, *b*). The intervertebral cartilage also increases considerably in thickness, bulging out between the adjacent somewhat expanded ends of the bony tubes already mentioned. In various of the more primitive Urodeles the vertebral bodies practically remain in this condition, flexibility being given to the vertebral column as a whole by the intervertebral cartilages interposed between the rigid bony segments. In the more highly developed Urodeles on the other hand there is a tendency to form an opisthocoelous joint, *i.e.* a joint concave on its tailward and convex on its headward side (Fig. 151, C). This may find expression merely in a softening of the cartilage along what would be the surface of the joint, or a layer of the cartilage may be liquified so that the intervertebral cartilage is completely divided across into a smaller concave anterior part and a larger convex posterior part fitting together by regular articular surfaces, forming in other words a completely developed joint.

---

[1] In *Petromyzon* a few of the most anterior neural arches (*e.g.* 4th and 5th in an old specimen of *P. fluviatilis*) have expanded bases which spread ventrally almost completely round the notochord so as to form a kind of arch centrum which carries rib-like projections laterally (Schauinsland, 1906).

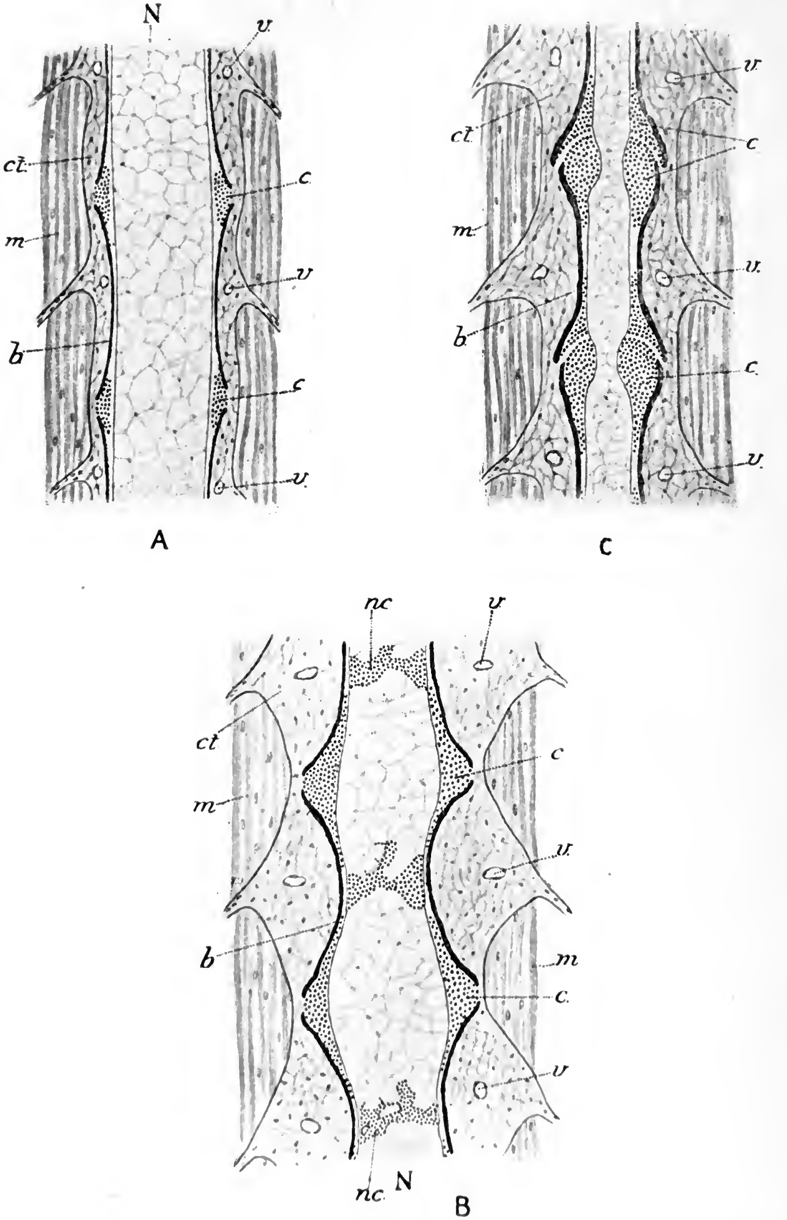


Fig. 151.—Illustrating the development of the vertebral centre in Urodeles as seen in horizontal sections. (Based on figures by Schauinsland, 1906.)

A, *Salamandra*, 22 mm.; B, *Siredon*, 50 mm.; C, *Triton*, 16 mm.; *b*, bone; *c*, cartilage; *ct*, connective tissue; *m*, myotome; *N*, notochord; *nc*, notochordal cartilage; *v*, blood-vessel.

The notochord becomes more or less constricted by the ingrowth of the intervertebral or joint cartilage which pushes the sheath in front of it. Besides this constriction of the whole notochord with its sheath the substance of the notochord becomes eventually, sometimes at a relatively late stage of development, interrupted by the development of *intra*vertebral cartilage which may form a complete cartilaginous partition across the notochord at about the middle of each vertebra (Fig. 151, B, *nc*). The origin of this cartilage is disputed. Some (Lwoff, Zykoff, Gadow) derive it from immigrant cartilage cells which have penetrated through the notochordal sheath from outside, while others (Gegenbaur, Field, Ebner, Klaatsch, Schauinsland) believe it to originate by the metamorphosis of actual notochordal cells, probably cells of the notochordal epithelium. In spite of a possibly greater volume of evidence supporting the latter view it is difficult to avoid the impression that the former has in its favour the balance of *a priori* probability.

The Reptiles are commonly regarded as the least specialized of the three subdivisions of the Amniota and it may therefore be convenient to let them form the basis of our description. Schauinsland's work may be referred to for more minute detail.

The sclerotome tissue grows actively and comes to be specially concentrated immediately round the notochord to form the **perichordal layer**. This layer is at first—in accordance with its origin from the sclerotomes—segmented (Fig. 152, A, *scl*) but the original segmentation soon disappears so that it forms a perfectly continuous investment to the notochord. A secondary segmentation now becomes visible in as much as the perichordal layer is decidedly thicker in a position corresponding to the middle of each original segment. These thickenings mark it off into a series of reel-shaped pieces each of which is a primary vertebral body (Fig. 152, B and C, *p.v.b*). It will be understood that the hinder half of each primary vertebral body is derived from the front half of a sclerotome while the front half of the same primary vertebral body is derived from the posterior half of the next sclerotome in a headward direction.

In other words each primary vertebral body is formed from the adjoining halves of two original segments, and as a result of this the primary vertebral bodies necessarily alternate in position with the myotomes, each myotome running from about the level of the middle of one primary vertebral body to a level about the middle of the next in the series (Fig. 152, B).

The portions of the sclerotomes lying outside the perichordal layer undergo fusion also. This outer part of the sclerotome bulges out between the myotomes while it extends dorsalwards so as to arch over the spinal cord. It is in the wall of the tunnel so formed that the neural arch-elements make their appearance while the sclerotome tissue ventral to them takes part in the formation of the body of the definitive vertebra. The superficial part of the vertebral body arising in this way from sclerotome tissue outside the perichordal layer

(Fig. 152, C, *S*) is best developed laterally (*Sphenodon*) though it extends as a thinner layer over both the dorsal and ventral sides of the perichordal layer. Eventually chondrification takes place and the vertebral body, derived partly from perichordal and partly from sclerotome tissue lying outside and continuous with the neural arch portion, becomes converted into a mass of cartilage in which the only clue to its compound origin is the somewhat flattened shape of the cartilage cells in the inner part derived from the perichordal layer (Fig. 152, C, *p.v.b*).

During the development of the vertebral centra the notochord becomes constricted across much as in Urodeles. A complete septum of notochordal cartilage is formed across the middle of each vertebra in *Sphenodon* and in the Lacertilia. In the ordinary Lizards this appears to arise as a ring-shaped ingrowth of cartilage which constricts the notochord, pushing the primary sheath in front of it (Gadow, 1897) while in *Sphenodon* and also in the Geckos the cartilage makes its appearance internal to the notochordal sheath (Howes and Swinnerton, 1901). It may be suspected that in the latter case immigrant cartilage cells have made their way through the notochordal sheath though this has not so far been demonstrated.

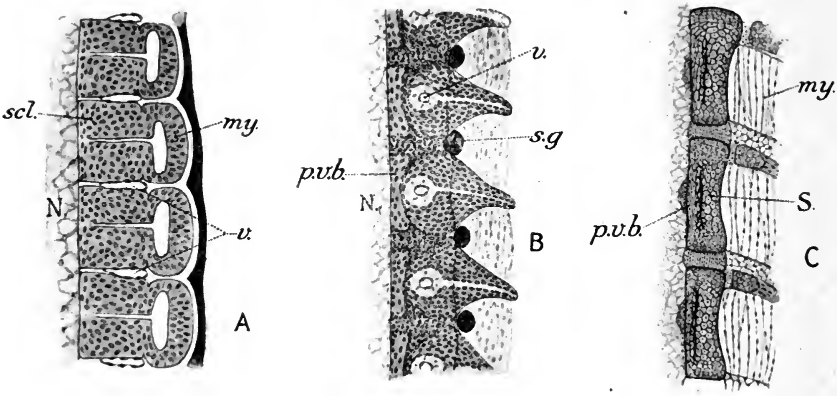


FIG. 152.—Diagram illustrating the mode of development of the vertebral centra in a Reptile as seen in horizontal sections. (Based mainly on Schauinsland's figures of *Sphenodon*, 1906.)

my, myotome; *N*, notochord; *p.v.b*, primary vertebral body; *S*, superficial portion of centrum arising outside perichordal layer; *s.g*, spinal ganglion; *scl*, sclerotome; *v*, blood-vessel. In comparing the segmental relations of A and B the intersegmental blood-vessels (*v*) form useful landmarks.

RIBS.—The ribs are long cartilaginous projections from the vertebrae which run outwards and ventrally in the substance of the myosepta and serve to support and strengthen the wall of the splanchnocoele. As Goette (1878, 1879) first showed, there are included under the name "ribs" two morphologically different structures, which may be distinguished by the names dorsal or upper ribs and ventral or lower ribs.

In *Polypterus* both sets of ribs are well developed—the dorsal ones larger towards the head, the ventral larger towards the tail. In other vertebrates the rule is that only one set is developed, though the other may be represented by more or less distinct rudiments or vestiges. Thus in Actinopterygian ganoids, Teleosts and Dipnoans the ribs are ventral ribs while in Elasmobranchs, Amphibians and Amniotes they are dorsal ribs.[1]

Both types are associated with the myosepta but whereas the dorsal ribs lie at the level of the horizontal septum which divides the lateral musculature into a dorsal and a ventral half, the ventral ribs, on the other hand, lie along the peritoneal edge of the myoseptum where it abuts on the lining of the splanchnocoele.

Probably both sets of ribs are to be interpreted morphologically as outgrowths from the vertebrae and the balance of evidence appears to favour the view that both are fundamentally outgrowths from the series of haemal arch-elements.

Ventral Ribs.—This is clearly the case with the ventral ribs which are simply the ventral prolongations of the haemal arch-elements, frequently jointed off from the basal stump of the arch (**transverse process**) by the conversion of a thin layer of the cartilage into fibrillar material. In the skeleton of a Lung-fish, a Crossopterygian, or an Actinopterygian the ribs are seen to form a perfectly continuous series with the haemal arches of the tail region.

Dorsal Ribs.—The nature of the dorsal ribs tends to be obscured by the fact that their point of attachment to the vertebra shows much variation *e.g.* they may appear to arise not from the haemal but from the neural arch. That we have to do here with a secondary shifting in a dorsal direction is indicated by various considerations. Amongst the Rays it can sometimes be seen that the ribs towards the head end of the series become more and more displaced dorsally, so that they come to project from the neural arch. Then it will be remembered that in various fishes the haemal arch-element becomes divided into a ventral part (haemal process) and a dorsal part which latter carries the rib and may undergo a considerable displacement in a dorsal direction.

In Urodele Amphibians Goeppert has shown that the apparent attachment of the rib to the neural arch has come about in a somewhat complicated fashion as illustrated by Fig. 153. The most nearly primitive condition is that shown in the larva of such a perennibranchiate form as *Necturus* (Fig. 153, A). Here the haemal arch-element (*h.a*) sends off a strong outgrowth (*r.b*), the "rib-bearer," which passes in a dorsal direction closely applied to the

---

[1] Distinct traces of dorsal ribs occur in various Teleosts, *e.g.* Salmonids and Clupeids. The numerous little bones found in the myosepta of various Teleosts in addition to the true ribs are probably to be looked on as independent and secondarily developed "tendon bones."

surface of the neural arch, from which however it is marked off by a thin fenestrated layer of bone (*b*). It will be seen from the diagram that the bases of the neural and haemal arches and the base of the rib-bearer enclose a space through which runs the vertebral artery (*v.a*). The haemal arch-element passes horizontally outwards beyond the base of the rib-bearer and the rib itself forms merely a prolongation of the haemal arch-element, becoming segmented off from its proximal portion ("transverse process") by the development of an intercalary zone of fibrillar joint tissue. A little way out from its base the rib grows out into a projection which is directed dorsally and towards the median plane. This dorsal process is prolonged into a ligament which is attached at its end to a mass of bony tissue developed on the outer side of the rib-bearer and indicated in the diagram by the diagonal shading.

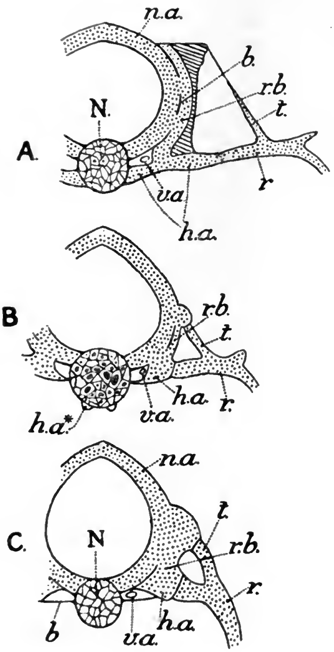


FIG. 153.—Illustrating the attachment of rib to vertebra in the Urodela according to Goeppert (1896).

A, trunk vertebra of *Necturus* larva; B, trunk vertebra of *Salamandra maculosa* larva; C, trunk vertebra of *Triton alpestris* larva. *b*, bone; *h.a*, haemal arch-element; *h.a**, haemal process; *N*, notochord; *n.a*, neural arch; *r*, rib; *r.b*, rib-bearer; *t*, dorsal process of rib; *v.a*, vertebral artery.

[The diagonally shaded portion of A represents bone.]

In the larva of *Salamandra maculosa* the condition is found which is illustrated by Fig. 153, B. The most important difference from the condition seen in *Necturus* is that the basal part of the haemal arch-element has become greatly reduced, and is now attached to the notochordal sheath merely by a thin thread of bone. The rib-bearer grows out from the haemal arch-element as before but it is shorter and is more completely fused with the neural arch. The dorsal process of the rib has increased in strength and now extends right to the dorsal end of the rib-bearer and is firmly attached to it, so that the rib has assumed an obvious double-headed character with a practically equally strong dorsal and ventral attachment through the substance of the rib-bearer to the neural arch.

Finally in the larva of *Triton alpestris* the condition is found which is illustrated by Fig. 153, C. The original basal part of the haemal arch-element which lay ventral to the vertebral artery has disappeared, so far as cartilage is concerned, its place being taken by a thin thread of bone. The rib-bearer is shorter and stouter than in *Salamandra* and its fusion with the neural arch still more complete. The double-headed rib has all the appearance now of simply articulating with a massive projection of the outer side of the neural arch: its original connexion with the haemal arch would never be suspected.

The question naturally arises whether in other Amphibians in which the transverse process and rib projects from the neural arch, the dorsalward shifting has come about in the same manner as is apparently the case in Urodeles. The probabilities appear to be against this. In the remaining two groups of Amphibia—the Anura and the Gymnophiona—the transverse process, though it springs from the neural arch, lies still ventral to the vertebral artery, which suggests that there has taken place here a simple shifting dorsalward of the whole of the haemal arch carrying the rib, including its basal portion.

In the case of the Amniota, Schöne (1902) has carefully investigated the development of Reptiles and has failed to find anything corresponding to the rib-bearer of Urodeles. In all probability here as in Anura there has taken place a simple dorsal movement of the rib and transverse process.

The Amniote rib appears to arise generally in continuity with the anterior half vertebra (*a*) *i.e.* from material derived from the posterior half of the sclerotome. In the case of *Sphenodon* (Schauinsland) the sacral and usually the caudal ribs, on the other hand, appear to contain material derived from both halves of the vertebra, the ribs being in these regions much broader than they are elsewhere and marked by a longitudinal groove indicating their double origin. In the last vertebrae of the tail these may give rise to two separate transverse processes attached to each side of the vertebra tipped each one by a small rib-rudiment.

The uncinate processes on the ribs of certain Reptiles and Birds arise as independent centres of chondrification. They may later on ossify and fuse completely with the rib (most Birds) or they may never show complete fusion (*Apteryx, Sphenodon*). In *Sphenodon* they become simply calcified without undergoing true ossification (Schauinsland).

STERNUM.—The sternum of the Amniota arises typically by the fusion together of the ventral ends of a number of the anterior rib-rudiments into a continuous plate on each side. The two lateral plates so formed undergo fusion across the mesial plane to form the definitive unpaired sternum, a plate of cartilage still continuous with the ribs. Eventually the sternum becomes segmented off from the ribs and may become calcified by the deposition of limy particles in the intercellular matrix (Reptiles) or replaced by bone (Birds).

In Amphibia also the sternum arises by the fusion together of two longitudinal bands of cartilage but no connexion can be traced between these and the ribs. This peculiarity, as compared with the Amniota, is apparently to be correlated with the comparatively short extension of the ribs in a ventral direction which is characteristic of this group of Vertebrates. In the Fishes the sternum has not yet made its appearance.

SKULL.—The skull is a mass of condensed and strengthened mesenchyme serving essentially to support and protect the organs of the head. It protects the brain and sense organs: and it forms a support and framework for the masticatory and other apparatus connected with the mouth and pharynx. In correlation with this its characteristics in detail are secondary to characters of the brain and other organs.

The skeletonization of the mesenchyme does not take place continuously but commences in irregular patches which gradually spread and eventually join together. Though there is frequently considerable agreement between different Vertebrates in the position of the centres of skeleton formation in the head there are in other cases equally well-marked variations between forms known to be phylogenetically closely related. It is as a rule impossible to say definitely whether or not the first appearance of skeleton at particular points is of phylogenetic significance or is on the other hand related merely to existing arrangements of the adult.

Under the circumstances all that will be attempted here is a short sketch of the general features of cranial development without entering at all into minute detail. For a full and detailed description reference should be made to the admirable work of Gaupp (1906).

As has already been indicated there is a marked tendency for the arch - elements to undergo fusion towards the head end, the axial skeleton being necessarily rigid instead of flexible in the brain region. Eventually towards the front end of the series both neural arches and vertebral centra become completely fused together to form part of the skull.

The skull consists in its simplest form primarily of a **chondrocranium**—a trough of cartilage, the cavity of which is occupied by the brain and more or less open on its dorsal side. Somewhere about the middle of the floor of the chondrocranium there exists a recess in which rests the infundibulum of the brain, and the portion of cranial floor lying behind this is distinguished by having the notochord embedded in it—this organ having its anterior limit just behind the tip of the infundibulum. We are thus brought into touch with a deep-seated distinction between the posterior or **epichordal** (chordal—Kölliker) region of the cranium and the anterior or **prechordal** (Kölliker). We are probably justified in regarding the epichordal region of the cranium as being morphologically a metamorphosed portion of vertebral column in which the processes of fusion, already indicated as frequently occurring in the anterior region, have attained to their maximum. As will be explained later the process of incorporation of a few vertebrae (the number varying in different groups) into the hinder end of the cranium can still be observed in ontogeny and it is probable that during the contemporary evolution of some of the lower Vertebrates (Elasmobranchs, Sturgeons) there is still going on a process of spreading backwards of the hinder limits of the skull with the incorporation into it of additional vertebrae.

As regards the evolutionary origin of the prechordal part of the cranium we have so far no clue.

The primary cartilaginous cranium does not remain by itself in any Vertebrate. There become inseparably fused with it the cartilaginous capsules which surround and protect the nose (**olfactory capsule**) and the ear (**otic** or **auditory capsule**). Cartilage may also develop in the sclerotic of the eyeball but owing to mobility of the eyeball being necessitated by its having to be turned towards the direction from which impressions are received, the cartilage in this case does not undergo fusion with the chondrocranium.

There are also associated with the cranium, and more or less closely connected with it, the series of hoop-like cartilages of the **visceral arches**[1] and finally in many of the subdivisions of the Vertebrata important bony elements become added on to the chondrocranium. The description of the development of the skull will therefore fall naturally into three sections: (1) The Chondrocranium including the sense capsules, (2) The Visceral Arches, and (3) The Bony Skull.

THE CHONDROCRANIUM.—The chondrocranium shows many differences in detail in the various groups of Vertebrates. The epichordal portion commonly makes its appearance as a pair of rods of cartilage—the **parachordal** cartilages—lying one on each side of the front part of the notochord. The prechordal portion similarly takes its origin in a pair of **trabeculae** which lie dorsal to the buccal cavity on each side of the infundibulum. Important differences are seen in the relations of these primary cranial cartilages in different members of the Vertebrata. Thus in Elasmobranchs, Ganoids, Teleosts, Reptiles and Birds the trabeculae are at first quite isolated from the parachordals while in Lampreys, Amphibians and Lungfishes they are continued at their hind ends into the parachordals (Sewertzoff).

Again in many Vertebrates, apparently in correlation with the great development of the eyes in early developmental stages, the cranial cavity no longer extends forwards between the eyes. Its walls have come together to form an interorbital septum and foreshadowing this, the trabeculae are closely approximated or even fused in the median line. Gaupp applies the term **tropibasic** to such a type of cranium and contrasts it with the **platybasic** type in which

---

[1] In the neighbourhood of the margin of the mouth there frequently develop in the lower Vertebrates (Fishes and Amphibians) isolated pieces of cartilage (**labial cartilages**). These are sometimes termed the precranial skeleton, and various speculations have been made as to their possible evolutionary significance. Up to the present there appears to be no convincing evidence that they are other than mere secondary developments and consequently they will not be referred to further in this book.

the cranial cavity still extends forwards between the eyes and the trabeculae retain their primitive parallel position some distance apart. The skull in actinopterygian Ganoids, Teleosts, Amniotes and certain Elasmobranchs develops after the tropibasic type while in Amphibians, Lung-fishes, Crossopterygians and some Elasmobranchs it retains the platybasic condition.

As the platybasic type of cranium is admittedly the more primitive we shall deal with it first and will take as our example the cranium of the Lung-fishes *Lepidosiren* and *Protopterus* as described by Agar (1906).

DEVELOPMENT OF CHONDROCRANIUM IN *LEPIDOSIREN* AND *PROTOPTERUS*.—The first rudiments of cranium become apparent about stage 31 in the form of a longitudinally situated condensation of mesenchyme—the rudiment of the trabecula—lying upon each side beneath the thalamencephalon and mesencephalon. At its front end the trabecula rapidly extends dorsalwards to form a vertical plate of prochondral tissue lying against the side wall of the thalamencephalon, and terminating in front against the optic nerve. The dorsal portion of this plate, just internal to the deep ophthalmic nerve, is the **orbito-temporal process** (Fig. 155, A, *o.t*). From the outer surface of the trabecula, just in front of the main portion of the trigeminal nerve, there projects outwards a horizontal shelf of cartilage (Fig. 155, A, *g.r*). This is the rudiment of the portion of cranium which contains the ganglia belonging to the Trigeminal and Facial nerves (Gasserian recess, Bridge). The cranial rudiment becomes prolonged backwards, the backward prolongation representing the **parachordal** cartilage of meroblastic Vertebrates.

This parachordal rudiment lies on each side of the front portion of the notochord but, unlike what is more usual in other Vertebrates, it is separated from the notochord by a considerable space (Fig. 154, A). The cranial rudiment so far described gradually becomes chondrified. About this time there appears a condensation of mesenchyme round the outer side of the otocyst: this is the outer wall of the auditory capsule (Fig. 154, A, *a.c*). A little later than the stage mentioned a knob of cartilage begins to develop on each side of the notochord at the level of the septum between metotic myotomes III and IV. This is an enlarged and precociously developed neural arch which, becoming, as will be seen presently, incorporated in the skull, is known as the **occipital arch**. The base of this spreads forwards along the dorsolateral surface of the notochord to form the **occipital plate** (Figs. 154 and 155, B, *o.p*).

By stage 34 the chondrocranium has reached the condition shown in Figs. 154 and 155, B. The trabeculo-parachordal cartilage has spread outwards and has become continuous with the rudiment of the auditory capsule so that the greater part of the lateral portion of the definitive chondrocranium is now laid down in cartilage. The two trabeculae have extended forwards, converging towards one another and passing in front into an unpaired mass of cartilage lying between the olfactory organs—the **internasal septum** (*in.s*).

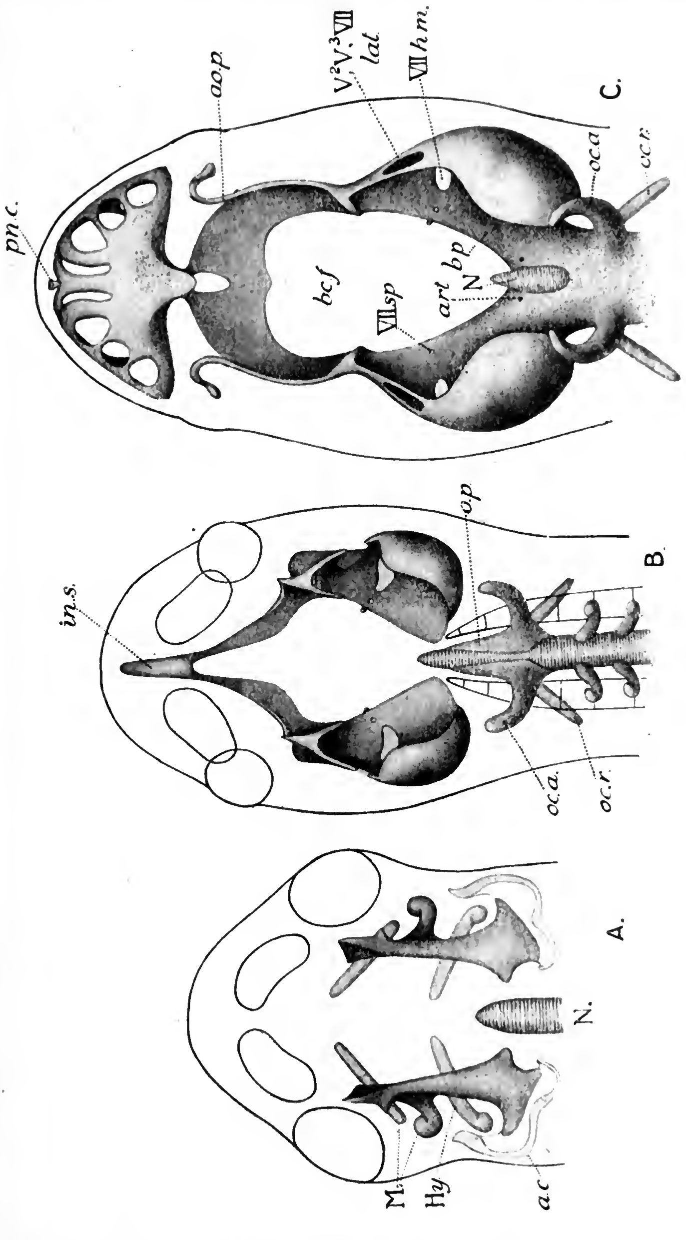


FIG. 154.—Illustrating the development of the chondrocranium in Lung-fishes. (After Agar, 1906.)

A, *Protopterus*, stage 31; B, *Lepidosiren*, stage 34; C, *Lepidosiren*, stage 36 +. *a.c*, auditory capsule; *ao.p*, antorbital process; *art*, foramen for artery; *bc.f*, basicranial fontanelle; *b.p*, basilar plate; *Hy*, hyoid arch; *in.s*, internasal septum; *M*, mandibular arch; *N*, notochord; *oc.a*, occipital arch; *oc.r*, occipital rib; *o.p*, occipital plate; *pn.c*, prenasal cartilage; V^2, V^3, VII *lat*, foramen for exit of maxillary and mandibular divisions of trigeminal nerve, together with buccal and superficial ophthalmic branches of Facial and communicating branch between Facial and Vagus; VII *hm*, foramen for hyomandibular branch of Facial; VII *sp*, foramen for superior palatine branch of Facial.

The floor of the cranium has made little progress, its position being, for the most part, occupied by a large basicranial fontanelle.

Posteriorly the occipital plates have spread forwards but are still separated by a distinct space from the true chondrocranium. The precociously developed occipital arches (*oc.a*) have reached a large size and the pair of corresponding ribs—the **occipital ribs** (*oc.r*)—which are so characteristic a feature of the Dipnoan skull have also made their appearance.

In the next stage figured (Fig. 154, C) the occipital plates have become continuous with the parachordal cartilages forming a broad **basilar plate** (*b.p*) in which is embedded the notochord, except its tip which is still to be seen projecting freely into the basicranial fontanelle but which later on disappears. The side wall is extending dorsalwards and has enclosed the roots of the trigeminal and facial nerves, forming the outer wall of the Gasserian recess. Further forwards the front part of the basicranial fontanelle has become in great part obliterated by cartilage continuous laterally with the anterior extensions of the trabeculae. The floor of the cranium is still deficient except anteriorly and posteriorly. Laterally a long **antorbital process** (*ao.p*) has grown out from the dorsal edge of the trabecula, passing forwards into the upper lip.

The olfactory organ has by this stage become enclosed in a characteristic **olfactory capsule**. From the anterior end of the internasal septum a horn-like outgrowth spreads outwards and backwards to form the ventral edge of the capsule, meeting posteriorly an independently developed **subnasal cartilage** (Fig. 155, C, *s.n.c*). This horn-like cartilage is met by four cartilaginous outgrowths from the dorsal side of the internasal septum which arch forwards and outwards over the olfactory organ. The roof of the olfactory capsule owing to this mode of origin has a characteristic fenestrated appearance. Between and in front of the olfactory capsules the internasal septum comes to project forwards slightly as the **prenasal cartilage** (Fig. 154, C, *pn.c*).[1]

In the last stage figured (Fig. 155, C) the chondrocranium has reached practically the condition of the adult. The occipital arches have extended dorsally so as to fuse, on the one hand, with one another to form the median supraoccipital ridge, and, on the other, with the auditory capsule. A horizontal shelf of cartilage has grown outwards from the side wall of the cranium and quadrate cartilage (see below) enclosing a space (*P.O*) in which lies Pinkus's organ (see p. 133). The dorsal portion of the internasal septum extends backwards slightly as the **mesethmoid** cartilage (*me*).

---

[1] When investigating the development of *Lepidosiren* in South America in 1896 I was struck by the fact that badly macerated skeletons of about this stage, with the lower jaw and other cartilaginous arches detached, as is commonly the case, and with the olfactory capsule frayed out at its edge, presented a remarkable resemblance to the remains of the curious little "lamprey" *Palaeospondylus* described by Traquair (1893). The resemblance was such as to leave little doubt in my mind that *Palaeospondylus* is really a Dipnoan—either larval or an adult form of small size and primitive structure. This conclusion is supported independently by the investigations of W. and I. Sollas (1903) who conclude that *Palaeospondylus* is an Amphibian. Any one without practical knowledge of young Lung fishes would quite naturally suppose their imperfect remains to be those of young Amphibians.

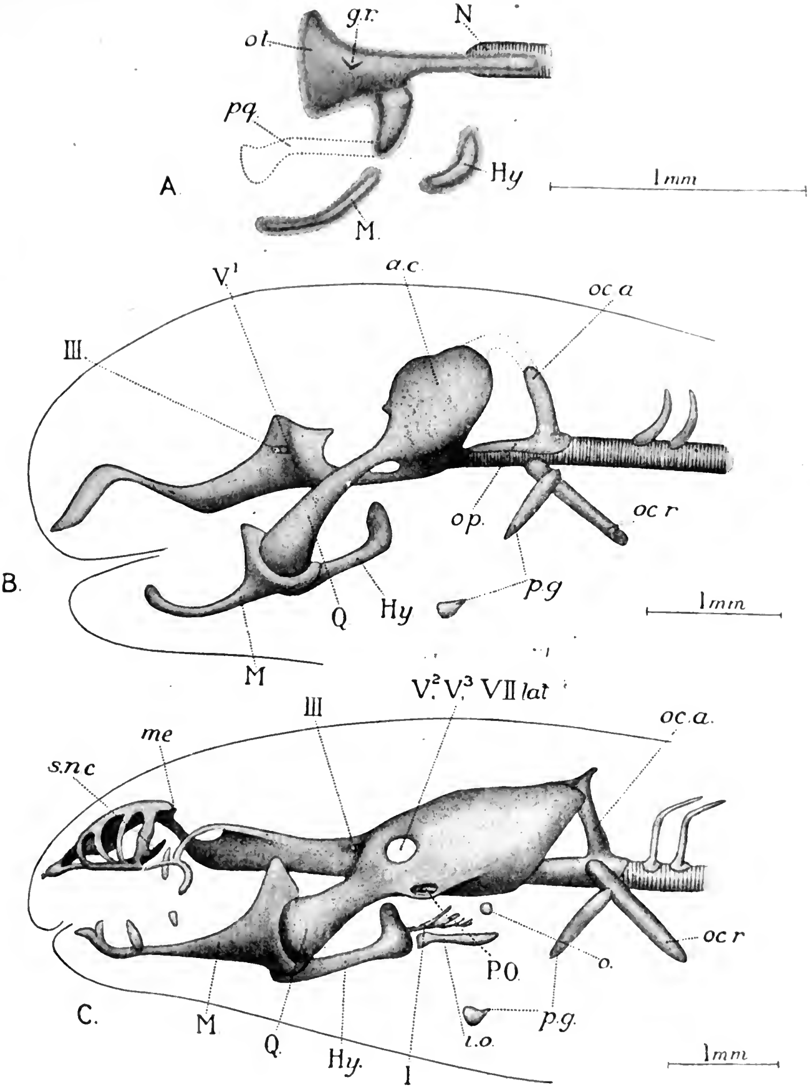


Fig. 155.—Illustrating the development of the chondrocranium in Lung-fishes. (After Agar, 1906.)

A, *Protopterus*, stage 31; B, *Lepidosiren*, stage 34; C, *Lepidosiren*, stage 38. *a.c*, auditory capsule; *g.r*, floor of Gasserian recess; *Hy*, hyoid arch; *i.o*, interopercular; *me*, mesethmoid; *M*, mandibular arch; *N*, notochord; *o*, opercular; *oc.a*, occipital arch; *oc.r*, occipital rib; *o.p*, occipital plate; *o.t*, orbito-temporal; *p.g*, pectoral girdle; *P.O.* position of Pinkus's organ; *p.q*, palatoquadrate; *Q*, quadrate; *s.n.c*, subnasal cartilage; I, first branchial arch; III, foramen for Oculomotor; V, VII, foramina for Trigeminal and Facial.

In the case of the young Protopterus the cartilage goes on extending considerably with growth. In the region of the auditory capsule it spreads dorsalwards and reaches the middle line so as completely to roof in the cranial cavity at this level. Further forwards in the region of the Gasserian recess the side wall of the cranium also extends dorsalwards, though in this region there remains a wide deficiency in the cartilaginous roof.

In *Lepidosiren* the increase in cartilage is less marked, in fact the chondrocranium of the adult remains in many respects in the same condition as that of the larval Protopterus.

The Dipnoan chondrocranium obviously belongs to that type in which the primary basal cartilages of the skull are continuous on each side—almost or quite from the beginning, the separation into distinct trabecular and parachordal portions, if visible at all, being confined to a very brief period, and in which the cranial cavity extends forwards between the orbits (platybasic type). Seeing that a similar type of chondrocranium occurs in the majority of the holoblastic lower Vertebrates the probability is that it represents a more nearly primitive condition than the tropibasic type, with parachordal separate from trabecula, which is more usual in the meroblastic vertebrates.

DEVELOPMENT OF THE CHONDROCRANIUM IN ELASMOBRANCHS.—As the chondrocranium has for its main function the support and protection of the brain, and develops in close relation with it, it will be of interest to compare with the development of the Dipnoan cranium that of one of the meroblastic Vertebrates in which the brain is modified in early stages by a greatly developed cerebral flexure. The Elasmobranchs are admittedly the most nearly primitive of such forms and may therefore most suitably be taken as the example.

Here (*Pristiurus* and *Acanthias*—Sewertzoff, 1899) the first indications of skull development make their appearance about stage 25 (see Chap. XI.), as a concentration of mesenchyme on each side of the notochord about the level of the otocyst. This spreads, headwards and tailwards, as a parachordal strand of prochondral tissue, extending anteriorly as far as the Facial nerve and continuous posteriorly with the rudiment of the vertebral column. As the parachordal plate takes definite form it develops in its occipital portion segmentally arranged rounded swellings which project dorsally between the nerve-roots and correspond in position with the intermuscular septa.

The prochondral parachordal plates gradually become chondrified and at the same time they extend outwards and dorsalwards to form the basilar plate. As they do so the metameric projections flatten out and disappear in the anterior portion while posteriorly they become more pronounced, growing up dorsally between the nerve-roots and finally meeting over the roots so as to enclose them in distinct foramina. In the region behind the definitive skull the swellings in question do not fuse but develop into discrete arch-elements. The last of the swellings to be included in the skull would appear to be, as a rule, in Sharks and Dog-fish that between metotic myotomes 7 and 8 (Braus, 1899) though in all probability variation occurs as between different genera and species and possibly even between individuals of the same species.

The trabeculae are strikingly different in their relations during early stages from those of the Dipnoan. Instead of being continuous with the parachordals they are at first separated from them by a wide gap in which appears on each side a small nodule of cartilage the **Polar cartilage** (van Wijhe, 1905). Further the long axes of trabeculae and parachordals instead of being in line are practically at right angles to one another. It is probable that both of these peculiarities are to be associated with the greatly developed cerebral flexure. The fore-brain has as already described been bent downwards into a kind of retort shape, the floor of the thalamencephalon coming to face in a tailward direction. As the thalamencephalic floor has undergone this displacement the trabeculae have been carried with it, so as to assume a practically dorsiventral direction, and this same movement has probably brought about the severance of their original continuity with the parachordals. It will be noticed from Fig. 156 that the displacement has gone even further than has been indicated so far, for the trabecula has been translated bodily in a tailward direction so that it lies in a plane considerably posterior to the level of the anterior end of the parachordals.

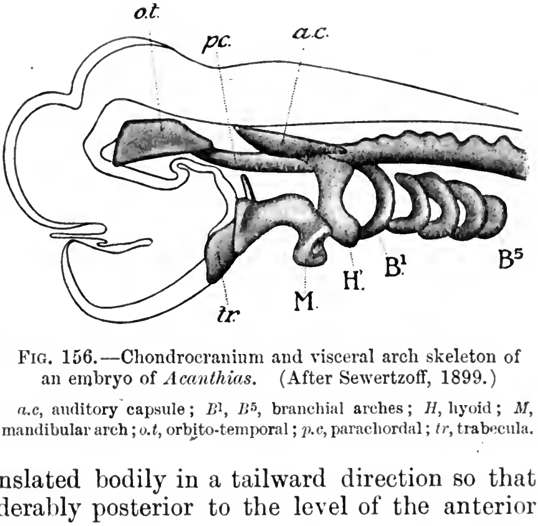


FIG. 156.—Chondrocranium and visceral arch skeleton of an embryo of *Acanthias*. (After Sewertzoff, 1899.)

a.c, auditory capsule; B^1, B^5, branchial arches; *H*, hyoid; *M*, mandibular arch; *o.t*, orbito-temporal; *p.c*, parachordal; *tr*, trabecula.

At the front end of the parachordal a plate of cartilage (Fig. 156, *o.t*) develops in the side wall of the cranial cavity corresponding generally with the orbito-temporal plate of the Lung-fish (Alisphenoid plate, Sewertzoff; Sphenolateral, Gaupp). This is described by van Wijhe as being an outgrowth from the parachordal, while Sewertzoff states that it is at first distinct.

The auditory capsule originates according to Sewertzoff as a simple outgrowth from the parachordal which gradually spreads outwards and dorsalwards round the otocyst, while according to van Wijhe the first trace of cartilage is on the outer side of the otocyst and is independent.

The cartilage belonging to the various elements which have been mentioned spreads outwards from each till they form a continuous trough-like chondrocranium. The trabeculae become continuous with one another first towards their morphologically anterior ends—a vacuity persisting for a time beneath the infundibulum. As might be inferred from the study of Fig. 54 (p. 93), which shows how the floor of the thalamencephalon gradually assumes its definitive horizontal position, the trabecular portion of the cranial floor *pari passu* swings forwards and comes to be more nearly in line with the parachordal portion. The displacement of the trabeculae which we have associated with the exaggerated cerebral flexure is then a temporary phenomenon which tends to become corrected during subsequent development. The cartilage formed by the fusion of the anterior ends of the trabeculae becomes prolonged forwards between the olfactory organs as a rod of cartilage, the **rostral cartilage,** which represents the ventral edge of the internasal septum.

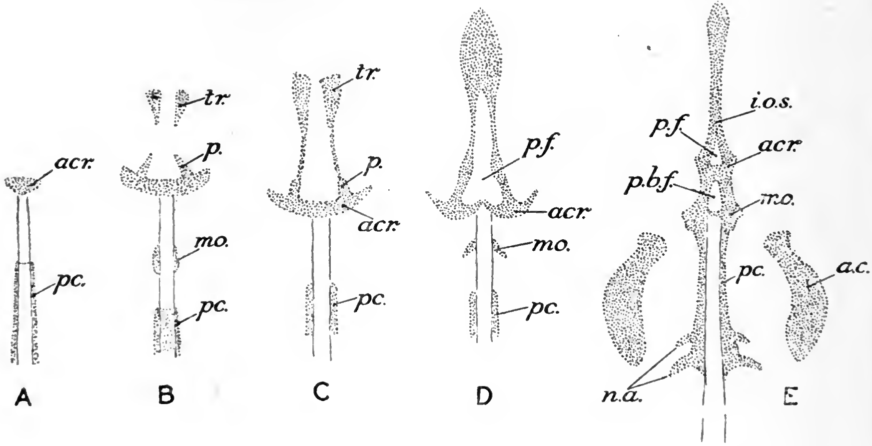


Fig. 157.—Diagrams illustrating the early development of the chondrocranium of Birds. (Based on figures by Sonies, 1907.)

A, Chick, 11 mm.; B, Duck, 13 mm.; C, Duck, 15 mm.; D, Duck, 14 mm.; E, Chick, 12 mm. *a.c*, auditory capsule; *acr*, acrochordal cartilage; *i.o.s*, interorbital septum; *mo*, mesotic cartilage; *n.a*, neural arch; *p*, polar cartilage; *p.f*, pituitary foramen; *p.b.f*, posterior basicranial fontanelle; *p.c*, parachordal; *tr*, trabecula.

The outer wall of the olfactory capsule appears as an, at first independent, piece of cartilage on the anterolateral side of the olfactory organ which gradually spreads round the organ in question and becomes continuous with the rest of the cranium.

It is unnecessary to follow out in detail the modelling of the definitive cranium but it should be noticed that the cranial cavity gradually becomes roofed in by the upgrowth of its side walls, and that, in some cases at least (*Pristiurus*), this roofing-in process becomes completed first in the region between the auditory capsules. This fact is of interest when correlated with the persistence of this portion of the chondrocranial roof in *Protopterus*—suggesting that this is probably the most archaic portion of the cranial roof of the Vertebrate. At the same time the possibility must not be lost sight of that instead of being of ancestral significance this feature may be associated merely with particular activity of cartilage-formation in the region of the otocyst, connected with the need of protecting that superficially placed organ of sense.

Development of the Chondrocranium in Birds.—According to Sonies (1907) the first cartilage to make its appearance is an unpaired plate arranged in a frontal plane and surrounding the notochord at its highest point in the cerebral or mesencephalic flexure. Sonies terms this (Fig. 157, *acr*) the **acrochordal** cartilage and states that it makes its appearance in the 5-day embryo of the chick. What appears to correspond to it in *Apteryx* is described by T. J. Parker as the prochordal cartilage, though in this case it lies quite anterior to the notochord. Very soon after the acrochordal cartilage, the parachordal makes its appearance—ensheathing the notochord. As this is thickest laterally and very thin ventrally and especially dorsally (where indeed it may be absent) it presents when viewed as a transparency from the dorsal or ventral side a misleading paired appearance. In *Apteryx* however the parachordals have apparently retained the actual paired condition.

For a time the parachordal and acrochordal cartilages are separated by a wide gap but later (11-12 mm.) this becomes filled in by the development of the paired elongated **mesotic** (basiotic) cartilages (Fig. 157, B, *mo*). In the Duck these are at first independent, but in the Chick they appear to be, even at the time of their first appearance as cartilage, continuous with the parachordals. Extending forwards they become continuous with the acrochordal, bounding upon their mesial side a space in which no cartilage is present—the **posterior basicranial fontanelle** (Fig. 157, E, *p.b.f*). Postero-externally the mesotic cartilage fits round the lagena, forming the rudiment of the cochlear part of the auditory capsule.

The parachordal cartilage spreads out on each side forming the basilar plate of cartilage and in embryos of about 7 days (13-14 mm.) two pairs of neural arch-elements make their appearance as lateral projections near its posterior end (Fig. 157, E, *n.a*)—the posterior, situated between the Hypoglossal and the First Cervical nerve, developing first. In the Kestrel (*Tinnunculus alaudarius*) Suschkin (1899) found three such occipital arches (Fig. 158, *n.a*) and Gaupp looks upon this as probably the typical number for Birds.

The acrochordal spreads out and forms a transversely situated plate of cartilage.

The trabeculae appear in the chick embryo of about 11 mm. as paired parallel rods of cartilage underlying the fore-brain. Posteriorly each passes into a swelling lying lateral to the pituitary body and as in the Duck and Starling (*Sturnus*) this forms at first an independent piece Sonies terms it the polar cartilage. Even in the Duck embryo this polar cartilage (Fig. 157, C, *p*) becomes very soon continuous with the trabecula in front and with the acrochordal cartilage behind. The connective tissue between the anterior ends of the trabeculae gradually chondrifies in continuity with them in both Chick and Duck (Sonies). In the Kestrel Suschkin found a, for a time independent, **intertrabecular** plate of cartilage in this position (Fig. 158, *itr*). This intertrabecular tract of cartilage serves to bound anteriorly the fontanelle (Fig. 157, D, *p.f*) in which the pituitary body lies and through which pass the two internal carotid arteries. Posteriorly this fontanelle is demarcated from the posterior basicranial fontanelle by the acrochordal cartilage later the posterior boundary of the *sella turcica.* It appears to be characteristic of Birds that this *dorsum sellae* undergoes a considerable amount of reduction during later development.

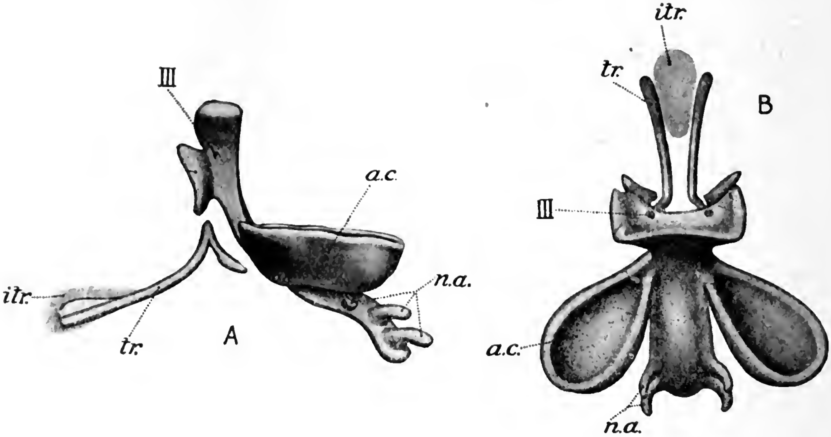


FIG. 158.—Early stage in the development of the chondrocranium of the Kestrel (*Tinnunculus alaudarius*). A, side view; B, dorsal view. (After Suschkin, 1899.)

a.c, auditory capsule; *itr*, intertrabecular cartilage; *n.a*, neural arches; *tr*, trabecula; III, foramen for oculomotor nerve.

In Chick embryos of about 12 mm. a patch of cartilage has made its appearance external to the otocyst between the lateral and the superior (anterior) semicircular canals which gradually spreads forming the external wall of the auditory capsule and closely moulded to the surface of the canals. This **periotic** cartilage (Fig. 157, E, *a.c*) is for a time separated by a wide gap from the basilar plate but this gap gradually becomes more and more encroached upon until reduced to a narrow fissure through which cranial nerves IX, X, and XI find their exit. Apart from this fissure the basal and periotic cartilages become continuous. As the wall of the auditory capsule extends dorsally it remains incomplete at two points where perforated by the Facial and Auditory nerves.

The roof of the chondrocranium is represented by a quite inconsiderable *tectum synoticum*, which originates as a pair of at first separate cartilaginous rods (Chick ·21 mm.). These very soon become continuous with one another and with the auditory capsule.

DEVELOPMENT OF CHONDROCRANIUM IN GENERAL.—The three examples of chondrocranial development which have been dealt with will suffice to give a general idea of the process with its variations. A survey of the known facts in Vertebrates generally shows that the first rudiments of the chondrocranium consist of paired elongated pieces of cartilage (preceded by prochondral tissue) lying on each side of the mesial plane and on the morphologically ventral side of the brain. These rudiments are divisible into a (para)chordal portion lying at the side of the notochord, and a prechordal portion lying anterior to this. A break in the continuity of the cartilage frequently occurs somewhere about the limit between these regions and this had led to the regarding of the portions so separated—trabecula in front and parachordal behind—as being fundamentally distinct morphological elements. As a matter of fact the break, when it does occur, appears to vary in position: thus in *Petromyzon* the "trabeculae" extend back for some distance beyond the tip of the notochord, so that their hinder parts are parachordal in position. In many cases the break is visible only for a very short period, while in others (*Lepidosiren*) there is complete continuity between trabecula and parachordal. On the whole it appears justifiable in the present state of our knowledge to regard the break in continuity between trabecula and parachordal not as marking a demarcation between two originally distinct morphological elements but rather as a secondary solution of continuity correlated with exaggerated cerebral (mesencephalic) flexure.

The parachordal cartilage in the case of the Elasmobranchs passed backwards by perfectly insensible gradations into the cartilage of the vertebral column. In that portion (occipital region) which lies between the hinder limit of the definitive cranium and the vagus nerve there appear for a time evidences of segmentation, corresponding with that of the vertebral column, and it is therefore justifiable to regard this portion of the parachordal cartilage as representing a region of fused vertebrae. In the anterior or mesotic portion there are no visible metameric swellings but, as the relations to the notochord are otherwise identical, it is difficult to refuse a homology in this case which is granted in the case of the hinder portion. Here again, then, we should be inclined to regard the distinction between the mesotic and the occipital portions of the parachordal as merely a secondary differentiation in what was once a continuous structure or series of structures: in other words we should regard the whole of the parachordal region of the cranium as representing a modified portion of vertebral column which has been absorbed into the cranium.

The foundations then of the vertebrate chondrocranium are laid in the form of paired basal cartilages which are eventually continuous throughout parachordal and trabecular regions but which may for a time consist of separate portions lying one in front of the other. As chondrification spreads from each of these primary elements, they become united together in a continuous plate of cartilage, forming the floor of the chondrocranium. From this in turn chondrification spreads upwards to form the side walls and roof, and forwards into the ethmoid and nasal regions.

To the brain-case so formed there become added the protective capsules of the olfactory organ and otocyst. As each of these organs is a development of the external skin, we may assume with a considerable degree of probability that their cartilaginous capsules were originally independent of the cranium.

Any repetition however of this completely independent stage of the sense-capsules in question has apparently become obliterated from ontogenetic development. Portions of the sense capsule may arise from separate centres of chondrification *e.g.* in the case of the auditory capsule the first rudiment may be in the form of an independent patch of cartilage in the region of the lateral semicircular canal. Even in such cases however the inner portion of the capsule develops in continuity with the chondrocranium. Again the Dipnoan arrangement, where the otic capsule is without any wall upon its mesial side so that it takes the form merely of a bulging of the lateral cranial wall, is to be looked upon as secondary.

Skeleton of the Visceral Arches.—The anterior portion of the alimentary canal forms a tube leading from the mouth back underneath the cranium, its lateral walls perforated, and therefore weakened, by the visceral clefts. The coelomic space being no longer present in this region somatopleure and splanchnopleure are in continuity, a continuous mass of mesenchyme extending from ectoderm to endoderm. This mass of tissue is divided by the clefts into the series of visceral arches and each of these is characteristically strengthened by a tract of tissue in its interior undergoing condensation and chondrification to form half-hoop shaped cartilaginous arches. These arches are named according to the mesenchymatous arch in which they lie—**Mandibular** (I), **Hyoid** (II) and **First branchial** (III), **Second branchial** (IV) and so on.

The skeletal branchial arches differ in number in different vertebrates, just as do the corresponding mesenchymatous arches (see p. 153). In the Lamprey,[1] which probably in this respect shows the most nearly primitive arrangement, the two half-hoops of a pair become continuous with one another ventrally. In the gill-breathing fishes the hoop typically becomes divided by joints into four segments on each side, with a median ventral copula—no doubt an adaptive arrangement to facilitate the movements of respiration. Where branchial respiration is reduced the arch has reverted to its primitive un-

---

[1] There is in the writer's opinion no sufficient evidence to doubt that the visceral skeleton of Cyclostomes is homologous with that of Gnathostomes.

segmented condition (*Lepidosiren*, Amniota) and no trace of segmentation appears during ontogeny.

In Elasmobranchs (Dohrn, 1884) chondrification begins on each side and then spreads dorsally and ventrally. Segmentation takes place first into a dorsal and ventral half and later each of these segments again. The gill rays develop independently of the hoop and only come into contact with it later.

The hyoid arch corresponds closely with the branchial arches in its mode of development.

The arches so far dealt with—branchial and hyoid—having to do primarily with the function of branchial respiration show their typical development in Fishes. With the disappearance of this function they become degenerate. This degeneration makes itself manifest in (1) reduction of segmentation, (2) tendency to fusion between successive arches and (3) reduction in number from behind forwards.

Thus in a Newt four cartilaginous branchial arches make their appearance but they are for a considerable period continuous dorsally and ventrally with their neighbours in the series, and they develop only one joint upon each side *i.e.* the half-hoop consists of two segments instead of four. In a Lizard only two cartilaginous branchial arches make their appearance, and in a Bird only one.

The hyoid and the anterior branchial arches have probably been saved from complete disappearance in the higher Vertebrate by the fact that they have taken on important functions in connexion with the tongue and have become specialized in accordance therewith. Thus in the case of the frog tadpole there is found, when the branchial apparatus is at the height of its development, a continuous cartilaginous hyobranchial skeleton, in which may be recognized parts corresponding to hyoid arches, copula between these, and 4 pairs of branchial arches continuous ventrally. At the time of metamorphosis this becomes greatly modified to give the adult condition (Gaupp, 1894): the mid-ventral portions become greatly expanded to form a flattened plate—the so-called "body of the hyoid": the hyoid arch becomes an elongated slender rod which serves to suspend the apparatus from the skull: the branchial arches disappear except the ventral end of the second which persists as a stump ("Postero-median process").

MANDIBULAR ARCH.—The usually accepted idea of the mandibular arch is to regard it as a half-hoop shaped cartilage resembling the other arches, to which is added a forwardly projecting outgrowth—the **palato-pterygoid bar**—which forms the primitive upper jaw skeleton. In actual ontogeny there is always a less or greater amount of departure from this general scheme.

In the Amphibians and Lung-fishes the hoop-like character of the main portion of the arch has been most completely retained. Here (Fig. 155, A) the arch develops on each side as a curved bar of cartilage—a mid-ventral copula having been detected in certain cases. The cartilage soon becomes completely continuous at its upper end with the chondrocranium and its dorsal end becomes segmented off, as the **palato-quadrate** cartilage, from the larger ventral portion — **Meckel's cartilage**—which forms the primitive skeleton of the lower jaw.

In the animals mentioned the lower jaw remains throughout life connected with the cranium through the dorsal portion of the original arch. This must be looked on as in all probability the primitive mode of attachment of lower jaw to skull and such skulls may therefore be termed **protostylic.**[1]

Both in Lung-fishes and Urodele amphibians the palato-pterygoid process is much reduced. In Urodeles it makes its appearance only at a late stage of development and is of comparatively small size. In *Lepidosiren* and *Protopterus* it has become eliminated almost entirely from development, being represented for a short time by a slight condensation of tissue which never becomes chondrified. This is probably to be interpreted as a modification of development induced by the precocious development of the bony skeleton of the upper jaw which in the forms mentioned replaces functionally the originally cartilaginous skeleton.

The Elasmobranch fishes do not exhibit this reduction of the palato-pterygoid bar for this becomes the functional upper jaw. On the other hand an important modification of development has taken place in correlation with the fact that in these fishes the original dorsal end of the mandibular arch has lost its primitive function of suspending the jaw, this function having been taken over by the enlarged dorsal end of the hyoid arch (**Hyostylic** type of skull). In correlation with this the portion of the mandibular arch lying above the pterygoid outgrowth is, all through development, greatly reduced. It is apparently represented by the prespiracular cartilage, which develops comparatively late.

The mandibular arch makes its appearance in *Acanthias* (Sewertzoff, 1899) as a **C**-shaped rod of cartilage lying in the rim of the buccal opening on each side (Figs. 156 and 159). The lower half of this segments off as Meckel's cartilage, while the upper half, which develops from behind forwards, clearly represents the pterygo-quadrate bar. This becomes continuous with and later articulated towards its anterior end with the trabecula—a doubtless secondary connexion with the cranium seeing (1) that it arises from the anterior and later developed portion of the palato-pterygoid outgrowth and (2) that in primitive sharks such as *Notidanus*, in Lung-fishes, and in Urodele amphibians, the attachment of mandibular arch to skull is further back in the auditory region—in fact in the region of the original dorsal end of the mandibular arch.

In the lower Vertebrates apart from those mentioned the development of the cartilaginous mandibular arch takes place on

---

[1] Graham Kerr, 1908. Attention is drawn in this paper to the need of an additional term to designate the more primitive type of so-called autostylic skull. A similar suggestion had, however, already been made by Gregory (1904).

similar lines. In the Reptiles and also in Birds the palato-pterygoid outgrowth is again reduced in size—in correlation with the fact that in the Tetrapod Vertebrates the tooth-bearing function of the original upper jaw or palato-pterygoid bar has been taken over by the secondary upper jaw composed of bones such as the maxilla and premaxilla.

BONY OR OSSEOUS SKELETON

Bone, like cartilage, is a modified connective tissue. In its typical form it differs from cartilage in the facts, that its matrix yields on being boiled a larger proportion of gelatine, that the matrix is rendered rigid by being strongly calcified, and that the cytoplasm projects as slender branching processes which ramify through the matrix and are commonly continued into those of other cells. Many different varieties of bony tissue exist. In ordinary bone the cell elements are completely surrounded by the calcified matrix. On the other hand some of the cells may have the main part of their cell-body outside the calcified mass, only a slender prolongation being surrounded by it (Bones of *Lepidosteus* and *Amia*). Or this peculiarity may apply to all the cells (Dentine of higher Vertebrates) or finally no cells or parts of cells are enclosed within the hard matrix—as is often the case in early stages of development and as occurs in the adult condition in many Teleostean fishes.

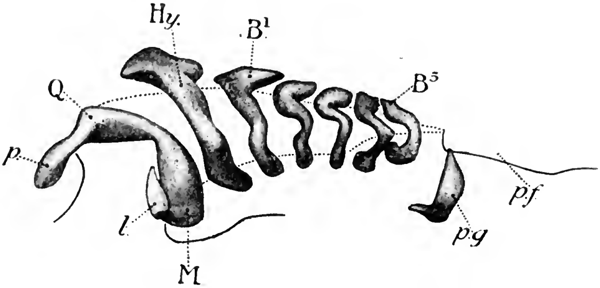


FIG. 159.—Skeleton of visceral arches and pectoral girdle of 20·5 mm. embryo of *Spinax*. (After Braus, 1906.)

*B*1, *B*5, branchial arches; *Hy*, hyoid; *l*, labial cartilage; *M*, mandibular arch; *p*, palato-pterygoid bar; *p.f*, rudiment of pectoral fin; *p.g*, pectoral girdle; *Q*, knob for attachment to trabecular region of skull.

Probably the most archaic type of bony skeleton in existing Vertebrates is seen in the **Placoid scales** of the Elasmobranchs and consequently the mode of development of these will logically fall to be considered first.

The appearance of the scale is foreshadowed by a localized condensation of the dermal connective tissue immediately beneath the epidermis. Presently this begins to bulge upwards like a dome into the epidermis. The epidermal cells immediately bounding this little dermal elevation take on a columnar shape: they constitute the **enamel epithelium** (Fig. 160, *e*). The (dermal) cells on the surface of the dome or papilla, immediately underlying the enamel epithelium, also become distinct and form a definite layer of **odontoblasts**.

The hard substance of the scale makes its appearance as a cone of **dentine** fitting over the surface of the dermal papilla and in turn ensheathed by the enamel epithelium. The dentine cone, which usually becomes directed tailwards, gradually thickens, encroaching upon the dermal papilla or **pulp** which it surrounds. It lies immediately outside the odontoblasts and as it increases in thickness the outer portion of some of the odontoblasts persists as a fine thread of cytoplasm extending out through the substance of the dentine, so that when dried the dentine is seen to be traversed by innumerable fine slightly diverging canals each of which contained a protoplasmic thread.

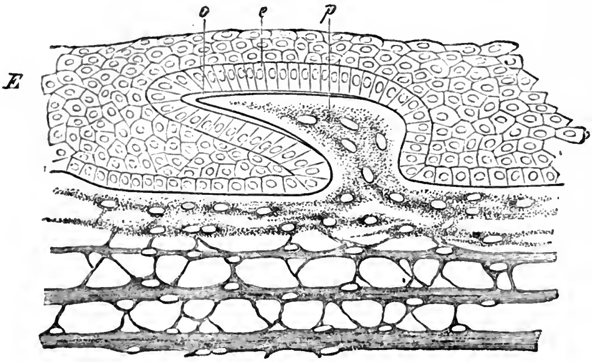


FIG. 160.—Longitudinal vertical section through the skin of an embryonic Shark to show a developing placoid scale. (From Balfour's *Embryology*: figure by Gegenbaur after Hertwig, 1874.)

E, epidermis; *e*, enamel epithelium; *o*, enamel; *p*, dermal papilla.

The hard material of the dentine is commonly regarded as calcified matrix but there is evidence which points rather to its being formed of modified cell cytoplasm. This point will be returned to in connexion with the development of the teeth.

Towards the surface of the cone the calcified substance changes its character. It becomes extremely hard (consisting of very dense calcium carbonate), transparent and highly refracting, and the terminal branches of the tubules within it are reduced to an extreme degree of fineness. This outer layer is commonly known as **enamel**. It may be comparatively thick, or on the other hand it may be extremely thin as for example in the scales of *Acanthias* except the enlarged spine-like scales in front of the dorsal fins, on the anterior face of which it is well developed. The enamel is in turn covered on its surface by an extremely thin membrane-like layer—the enamel cuticle—and Huxley (1859) made out the important point that this is continuous with the basement membrane of the epidermis outside the limit of the scale rudiment.

In the teeth of the higher animals, which as will be seen later are simply modified placoid scales, the enamel is sharply marked off from the dentine and it is usual to regard it as of totally different origin namely as a kind of cuticular formation by the inner ends of the enamel epithelial cells. The chief reasons for this view are the sharp differences in appearance and composition from the dentine in these higher Vertebrates, and the fact that the cells of the enamel epithelium undergo a shortening as the enamel layer thickens—as if the inner ends of the epithelial cells were undergoing conversion into enamel from within outwards.

It is however curiously difficult to find evidence sufficiently convincing to justify the almost universal acceptance of this idea even as regards the higher Vertebrates. And in the case of the Fishes the evidence—such as the location below the basement membrane and the frequently quite gradual transition between the so-called enamel and the dentine—strongly supports the idea that the former is simply a modification of the outer layer of the dentine.

The basal edge of the cone of dentine comes to spread outwards all round parallel to the surface of the skin as irregular trabeculae forming a strong **basal plate** by which the scale is firmly fixed in the dermis. This basal plate is usually of homogeneous appearance but its substance shows a gradual transition to the typical dentine of the spine, and in the case of *Callorhynchus* (Schauinsland, 1903) the basal plate as a whole shows, just as it does in the ancient fossil Coelolepids, dentinal structure. There seems then no reason to doubt that the basal plate is in its nature closely allied to dentine or in other words that it is bone in the broad sense of the term.

TEETH.—A section across the jaw of an ordinary Dog-fish is sufficient to demonstrate the important morphological fact of the homology of the teeth and the placoid elements of the skin. Teeth are simply placoid elements belonging to that portion of the outer skin which is carried inwards to form the stomodaeum. Or conversely the spines of the placoid scales are simply teeth which have not been carried inwards into the stomodaeum. In accordance with this the placoid scales were long ago (1849) named, by Williamson, **dermal teeth**. The demonstration of the homology in detail will be found in a classical paper by O. Hertwig (1874).

The lining of the buccal cavity being morphologically part of the outer skin the probability is that originally teeth or placoid elements were distributed equally all over it. But in the evolution of the Vertebrata there has clearly taken place a restriction of the teeth to particular parts of the lining where they can be most effective. In some of the lower fishes (many Elasmobranchs, *e.g. Acanthias*) teeth of a simple character, practically unmodified placoid elements, are still to be found scattered over the roof of the buccal cavity and even extending back into the pharynx. Unfortunately the development of these has not been worked out in detail. In Teleostean fishes however a very simple type of tooth development has been described *e.g.* in the Pike (*Esox lucius*). The teeth are here no longer scattered equally over the buccal lining; they are restricted to the dentary, maxilla, vomer, palatine, and the inner surface of the visceral arches. The teeth on the roof of the mouth arise as simple conical dermal papillae which project into the epidermis and develop enamel, dentine, and an irregular trabecular bony base on the same general lines as described above for the typical placoid element.

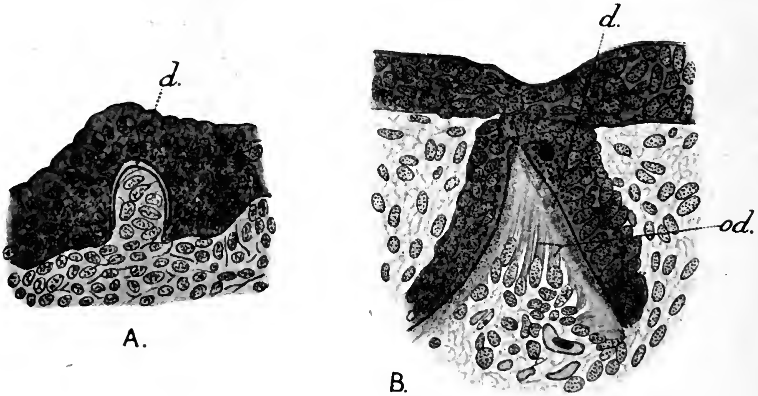


FIG. 161.—Early stage in the development of the tooth in (A) *Ceratodus* and (B) *Lepidosiren*. (A after Semon, 1899.)

d, dentine; *od*, odontoblasts.

Relatively primitive conditions are found again in the Dipnoi and Amphibia (Urodela and Gymnophiona) in the latter of which the teeth may be very widely distributed *e.g.* on premaxilla, maxilla, vomer, palatine, pterygoid, parasphenoid (*Spelerpes*), as well as on the dentary and occasionally on the splenial. In the simplest cases the tooth originates as a simple conical or rounded dermal papilla which projects upwards into the ectoderm (cf. *Ceratodus*, Fig. 161, A) but in other cases an onward step has been made and the "ectoderm" in the region where the papilla develops tends to grow down below the general level of the ectoderm into the underlying connective tissue of the dermis (cf. *Lepidosiren*, Fig. 161, B).

In the Amniota this tendency becomes more pronounced, the ectoderm covering the tooth-germ not merely projecting downwards into the underlying mesenchyme but becoming constricted off from the rest of the ectoderm so as to remain connected with it only by a narrow stalk or isthmus.

The tooth is built up of precisely the same elements as the placoid scale—dentine, enamel and basal plate. Its modifications are such as to make it more efficient for its special purpose. The projecting spine becomes exaggerated to form the functional part of the tooth: it remains conical, or it becomes a flattened blade with plain or serrated edge, or it becomes a low flattened crushing plate.

To secure greater strength the pulp may become traversed by hard trabeculae (vaso-dentine).

Regarding each of the three elements mentioned above there is a certain amount of controversy. As regards the dentine there is the question of its origin—whether it is to be regarded as calcified matrix or as modified cytoplasm. The evidence of *Lepidosiren*—which on account of the size of its cell elements is always of weight in such questions—seems very clearly on the side of the latter view. As shown in Fig. 161, B, the cytoplasm of the odontoblast passes uninterruptedly into the calcified dentine, the spaces between the odontoblasts on the other hand dying away as the dentine is approached. But if in a relatively archaic creature like *Lepidosiren* the main part of the dentine is undoubtedly modified cytoplasm this at once raises a strong presumption in favour of the same being the case in the higher Vertebrates even if it be not actually obvious.

Again as regards the enamel it is taught practically universally that it is formed after the manner of an internal cuticle by the cells of the enamel epithelium. This idea has come down to us from the days of the early investigators who devoted themselves especially to the investigation of Man and those Vertebrates most closely allied to him. In those days the structure of the lower animals was interpreted according to the data obtained from Man and his allies. The whole outlook was the opposite of that which holds in these evolutionary days when the accepted principle of all morphological work is to interpret the higher and more complex animals by data obtained from those lower in the evolutionary scale. Applying this principle to the case of the teeth of the most archaic Vertebrates we see in the Elasmobranch fishes that the outermost layers of the dentine develop the special modifications already alluded to—extreme denseness and hardness, transparency and high refraction, reduction of the proportion of organic material, reduction of the tubular cavities. Here the enamel is undoubtedly modified dentine.

But if this be so there are only two alternatives open to us in interpreting the enamel of the higher Vertebrates. It is either to be regarded as a further stage in the differentiation of the outer layer of dentine or it is to be regarded as something quite new, a new substance formed by the enamel epithelium. This latter is the generally accepted view and in accordance with it the hard layer on the teeth of fishes was given by Williamson the name

Ganoine to distinguish it from the true enamel of the higher Vertebrates.

As regards the basal plate the main question at issue is the evolutionary one whether or not the view of Gegenbaur should be accepted that these basal plates constitute the first phase in the evolution of the bony skeleton. This question will more suitably be discussed in connexion with the bony skeleton in general.

Lastly questions of general interest are raised by these exceptional cases where the developing tooth cannot be traced into immediate relationship with the ectoderm. In *Lepidosiren* and *Protopterus* as well as in the Urodele Amphibians portions of the lining of the buccal cavity which give rise to the teeth have the appearance of being derived in the embryo from endoderm. Again in Teleostean fishes teeth are developed far back in the pharyngeal region, in other words in a portion of the alimentary canal which is lined with endoderm.

Such cases obviously cause serious trouble to those who apply the germ layer theory rigidly. They explain them by supposing that there takes place in development an actual spreading inwards of ectoderm over the surfaces on which teeth will develop. As indicated in Chapter III. in dealing with the buccal lining of Urodeles and Lung-fishes the writer of this volume believes that the evidence adduced so far of the ingrowth required by this explanation is not to be relied upon. He would rather explain such cases as due to the more or less broad debatable zone between the ectoderm and endoderm, the influence of one layer being liable to spread into the other and there being no sharp line the position in regard to which decides definitely to which layer a particular organ belongs.

EGG-TOOTH OF REPTILIA.—In the embryos of Reptiles there appears a precociously developed "egg-tooth" at the tip of the upper jaw which has for its function the rupture of the egg-shell. In Geckos there are a pair of these present, attached to the premaxilla close to the mesial plane. In other Reptiles the left egg-tooth appears only as a transient rudiment and the functional (right) tooth takes up a practically median position so that it appears to be unpaired. It is of interest that this holds also for snakes in which there are no definitive teeth in the premaxillary region (Röse, 1894).

POISON FANG OF VIPERIDAE.—The poison fang of the Viperidae is highly specialized for the injection of poison, its pulp being traversed by a longitudinal tube, composed of dentine and attached to the outer wall of the tooth along its anterior face. The inner tube—or poison canal—passes at each end into an open groove the openings so formed serving for entrance and exit of the poison respectively. The main features of the development are illustrated by the transverse sections shown in Fig. 163 (p. 329). The poison canal makes its appearance as a longitudinal infolding of the dentine, the ectoderm of the tooth-germ seeming to push the dentine in before it to form a groove (Fig. 163, 7). The groove deepens and its lips meet (6) so as to convert it into a tube. From its mode of formation this tube is at first filled with ectoderm of the tooth-germ. Eventually however this ectoderm disintegrates and leaves an open tubular cavity (2).

Up till this stage the tooth is still enclosed in the ectodermal germ which has increased much in size (2) but eventually this ectodermal mass also disintegrates with the exception of its outermost layer, so as to give rise to the cavity of the sheath in which the functional tooth is contained. As the tooth becomes functional this cavity comes to communicate with the duct of the poison-gland so that it receives the poisonous secretion, and owing to the poison-canal retaining the form of an open groove towards the basal end of the tooth it in turn receives the poison from the cavity of the sheath.

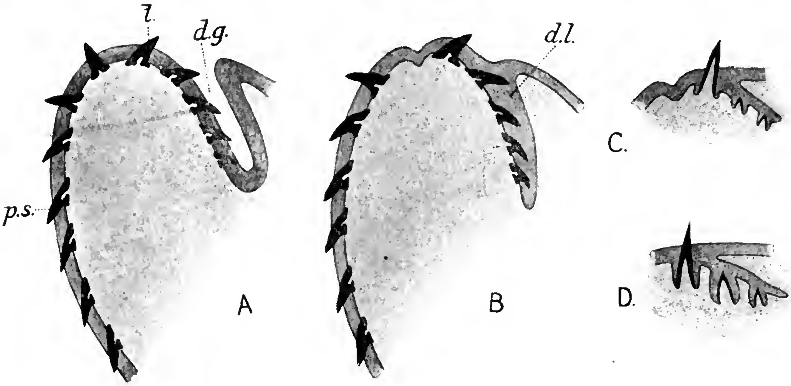


FIG. 162.—Diagram illustrating tooth succession in an Elasmobranch (A, primitive, B, existing condition); an Amphibian (C); and a Reptile (*Lacerta*) (D).

d.g, dental groove; *d.l*, dental lamina; *p.s*, placoid scale; *t*, tooth.

Enamel is present as a thin layer towards the point of the fang: traced towards the base it passes into a simple fine cuticle-like basal membrane.

SUCCESSION OF TEETH.—In cases where the teeth have become restricted to special areas, and more particularly in cases where from their shape and from the habits of their owner they are liable to be broken off or damaged by wear or otherwise, it is usual to find a special arrangement for the replacement of the lost or injured teeth by new ones. Such an arrangement is seen in its simplest form in an ordinary Dog-fish or Shark (Fig. 162, A and B). The portion of skin—ectoderm with its strong and fibrous underlying dermis—to which the tooth-bases are attached is gradually, by processes of differential growth, caused to shift its position in an outward direction over the edge of the jaw which supports it. This is brought about by the skin undergoing a continual slow process of absorption or atrophy along the outer margin of the jaw (about the point marked by a slight indentation of the surface in Fig. 162, B), while there takes place a compensating process of growth, or formation of new skin, along the inner margin of the jaw, near the bottom of a deep groove (Fig. 162, A, *d.g*). The young skin arising in this region, like young skin elsewhere, produces placoid elements and it is these which, growing older as they gradually move forward over the jaw surface, become the functional teeth. Under normal circumstances the rate of outward progress is such that by the time the tooth is becoming inefficient through wear it gets into the absorptive region and is shed.

It should be mentioned that, to make it more easily intelligible, the above account has been simplified in one important detail. The replacement groove is as a matter of fact in the Elasmobranchs mentioned no longer an open groove. Its walls have become fused together, so as to obliterate its cavity and form a solid lamina of ectoderm which dips down into the mesenchyme round the boundary of the mouth, just within the jaw, as shown in Fig. 162, B.

In Amphibia the general arrangements for replacement of the teeth are similar to those of Elasmobranchs, as may be gathered from Fig. 162, C, but an advance beyond the Elasmobranch condition is found here in that the functional tooth is firmly fused to the jaw. It remains stationary throughout its period of active functioning and it is only at the end of that period, when it is lost either by being broken off or by a process of natural shedding accompanied by resorption, that the next replacement tooth in the series moves up to take its place.

In Reptiles (Fig. 162, D) again the general arrangement is similar except that economizing of material has now taken place, the dental lamina being relatively reduced in bulk between the tooth-germs, so that the latter project prominently from the lamina instead of being embedded in its substance as was the case in the Amphibian.

Amongst the Lizards certain modifications occur which are of importance as foreshadowing arrangements which occur in Mammals. Thus it may happen (*Iguana*, Leche, 1893) that the first generation of teeth to be formed never become functional but disappear before hatching. Again the replacement mechanism may become reduced in the anterior part of the series (*Agama*—Carlsson, 1896; *Chamaeleo*—Röse, 1893). In the Chameleon the ordinary replacement mechanism is no longer functional except at the extreme hind end of the jaw, where alone new teeth are produced.

The large poison-fangs of poisonous snakes are peculiarly liable to injury and we find, as might be expected, that the replacement mechanism is in their case particularly well developed. The dental lamina (Fig. 163, *d.l*), which is very extensive and thinned down to such an extent as to become perforated by numerous openings in its more superficial and no longer active portions, is curved scrollwise upon itself and upon its concave surface develops tooth-germs in rapid succession, as many as ten being visible at one time in the ordinary Viper. As the tooth-germs develop and approach the surface they take up a position in two rows (3, 5, and 2, 4, in Fig. 163).

The maxilla, which carries the functional fang, has two bases of attachment for teeth, an inner and an outer, and these are made use of alternately—a functional tooth with external attachment being succeeded by one with internal and conversely.

The replacement takes place approximately synchronously in the two maxillae a pair of teeth attached to the right-hand bases of attachment of the two maxillae (in other words attached to the outer base of attachment on the right maxilla and to the inner base of attachment on the left maxilla) being replaced by a pair attached to the left-hand bases of attachment (inner on right maxilla, outer on left maxilla). In consequence of this arrangement the individual teeth of a functional pair are the same distance from one another as their predecessors and their successors.

In this modification of the primitive linear arrangement of the replacement teeth we have doubtless a mechanism to secure more rapid succession, and in this connexion it is of interest to notice that the replacement of the functional tooth is not dependent upon its having already suffered injury or become worn out but takes place at regular intervals (about six weeks in the case of the European Viper, Kathariner) while the snake is leading an active life.

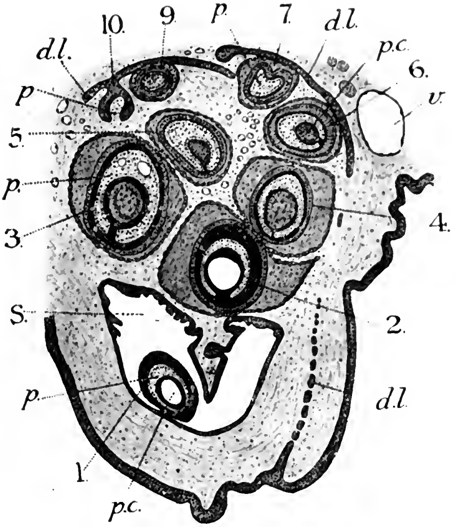


Fig. 163.—Part of transverse section through upper jaw of a young Viper. (After Röse, 1894.)

The developing teeth are numbered in order of sequence. No. 8 is not shown. Dark tone=ectoderm; pale tone=mesenchyme. Dentine is shown in black. The enamel which is present as a thin sheath over the apical end of the tooth is not shown. In 7 the poison canal is seen as an open groove along the side of the tooth, filled with ectoderm; in 6 this has become converted into a tube (*p.c*) still filled with ectoderm cells; in 2 the cells have degenerated, leaving a clear lumen; in 1 all of the ectoderm external to the tooth except its outermost layer has broken down to form the cavity of the tooth-sheath (*S*). The functional tooth (1) in the section here figured belongs to the outer series, to which 3 and 5 also belong. *d.l*, dental lamina; *p*, pulp; *p.c*, poison canal; *S*, tooth-sheath; *v*, blood-vessel.

In the Crocodiles the dental lamina becomes broken up into a network, and finally reduced to a strand of tissue running longitudinally along the jaw, slightly to the inner side of the tooth-bases. A succession of tooth-germs are produced from this strand, each one lying to the inner side of a functional tooth. As the successional tooth develops it causes absorption of the inner wall of the functional tooth, and gradually comes to lie within the base of the latter. Finally the old tooth is shed and its successor remains in its place.

In toothed Birds, so far as is known (*Hesperornis,* Marsh, 1880), the tooth replacement seems to have taken place in the same way as in Crocodiles. In modern birds a slight transitory ectodermal thickening has been interpreted as the vestige of a dental lamina (*e.g.* Terns—Röse, 1892; Carlsson, 1896) but the evidence is not convincing. Careful researches in this direction in the less highly specialized birds are highly desirable.

TOOTH-PLATES.—In many of the lower Vertebrates instead of, or in addition to, teeth of the ordinary conical shape adapted for piercing, there are present massive plate-like structures adapted for crushing. Large tooth-plates of this kind were conspicuous structures in many of the extinct fishes and lower Tetrapoda. Amongst living Vertebrates they are exemplified by many of the Skates and Rays, by the Holocephali and by the Dipnoi. Embryological study has shown that these plate-like teeth may arise in either of two possible ways.

In the Skates and in the Holocephali the tooth-plate is a single much enlarged and flattened tooth. In *Callorhynchus* Schauinsland (1903) describes how the tooth-plate originates in a widespreading dental papilla of a depressed dome shape. The outer layer of this develops a cap of dentine in the ordinary way. Below and continuous with this there develops a trabecular spongework of calcified tissue which shows a transition from ordinary dentine in its superficial parts to a tissue closely resembling normal bone except that the cell-bodies remain superficial, only their branching processes becoming embedded in the calcified material. As development goes on the individual trabeculae become thicker and more numerous and the intervening meshes filled with ordinary vascular mesenchyme become less and less conspicuous until the tooth as a whole assumes its definitive strong and massive character. No typical enamel seems to be formed but Schauinsland points out that the enamel epithelium seems to exercise a distinct modifying influence over the superficial layer of dentine which becomes hard and glassy (Vitrodentine) wherever it is in contact with the enamel epithelium.

In the case of Lung-fishes Semon (1899) has given a beautiful demonstration, which I can fully confirm, that the tooth-plates originate not by the enlargement and modification of single teeth but by the fusion of a number of originally separate denticles. The evidence of Palaeontology it may be mentioned is in complete agreement with the embryological evidence furnished by *Ceratodus* on this point and we may take the latter as a particularly good example of the recapitulation of phylogenetic evolution during the development of the individual.

The originally separate denticles develop as already explained (p. 324) in typical placoid fashion, giving rise to little hollow cones of dentine. Trabeculae of bony tissue ("trabecular dentine," or "pulp dentine") spread inwards from the bases of these cones through the underlying mesenchyme, so as to join up the various denticles by a loose calcified spongework. As development goes on the trabeculae of this thicken, the pulp-filled meshes become pro-

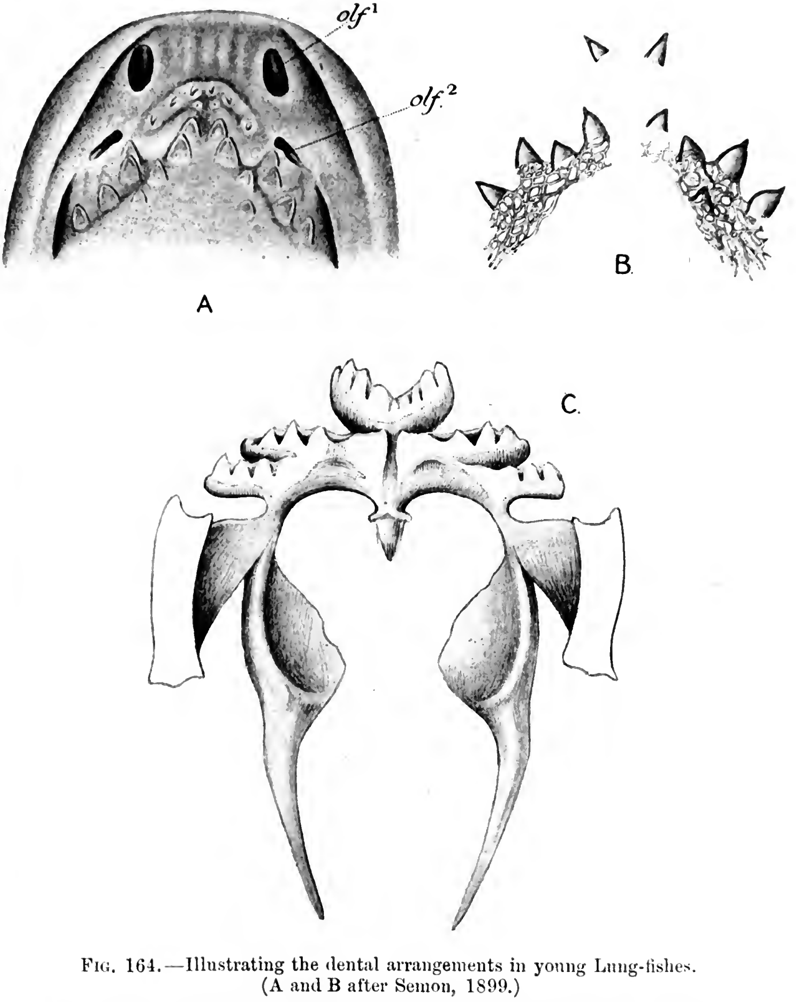


Fig. 164.—Illustrating the dental arrangements in young Lung-fishes. (A and B after Semon, 1899.)

A, roof of mouth of a Ceratodus of stage 48, showing the separate conical teeth; B, teeth of roof of mouth from a slightly younger specimen (stage 46) after the soft tissues have been cleared away by the action of dilute alkali; C, *Lepidosiren*, macerated upper jaw of young specimen, showing the pointed cusps still present on the tooth-plates; olf^1, anterior naris; olf^2, posterior naris.

portionally reduced, and the trabecular mass becomes the compact substance of the adult tooth. In the functional tooth the tips of the original denticles have completely disappeared.

In *Lepidosiren* and *Protopterus* the separate denticle phase of development is not so distinct as in *Ceratodus* but a reminiscence of it is seen in the pointed cusps which are present on the teeth in early stages (Fig. 164, C).

THE BONES IN GENERAL.—The view is now accepted by many morphologists, following Hertwig and Gegenbaur, that the true bony skeleton has come about in evolution by the spreading inwards of bone-forming activity from the skin, where it arose in association with the coating of placoid scales which occurs in the lowest Gnathostomata. The probability of this view being correct is rendered apparent by a survey of the phenomena of development of some of the bones in the lower Vertebrates. Both in Lung-fishes and in Amphibians the bones of the skull which carry teeth are found to arise in development in the form of more or less trabecular bony tissue which spreads outwards from the tooth-bases in the same way as has already been described as occurring in the development of the compound tooth in Lung-fishes (Fig. 164).

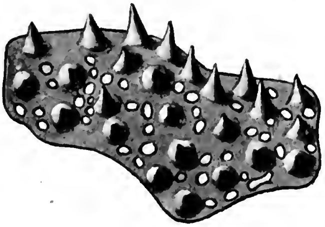


FIG. 165.—Vomer of a 2·5 cm. larva of Axolotl (×45). (After O. Hertwig, 1874*.)

O. Hertwig (1874*) found for example that the vomer, palatine and opercular of Urodele Amphibians are developed in this way, forming perforated bony plates studded with conical teeth (Fig. 165). In the case of dentary, maxilla, and premaxilla, part of the bone arises in exactly the same way, while part on the other hand spreads through the mesenchyme without having teeth on its surface. It is to be noted that these bones at first, as frequently happens in the development of bony tissue, have no cells actually enclosed in the calcified substance. Later on the teeth in some cases disappear, leaving behind merely the basal plate of bone which gradually increases in thickness. On turning to the Anura it is found that the bony trabeculae develop precociously and form the basal plate of bone while the teeth belonging to it are delayed in their appearance and may even be omitted.

The embryology of the Amphibia then teaches us (1) that typical bones may be developed from the basal trabeculae connected with placoid elements and (2) that a secondary modification may arise in which the tooth formation is delayed or suppressed, the trabecular basal plate simply developing by itself and becoming converted into the definitive bone.

The facts as narrated by Hertwig for Amphibia do not stand alone. On the contrary an exactly similar mode of development is seen in the "membrane" bones of the roof of the mouth in Teleosts, and in the tooth-bearing bones of Lung-fishes. Again there are present minute enamel-tipped teeth scattered over the surface of the dermal bony plates of Crossopterygians and various Siluroid Teleosts such as *Loricaria, Hypostoma, Callichthys.*

Such facts as those just enumerated seem to justify the acceptance, as a working hypothesis, of the view that at least the more superficially placed dermal bones of the Vertebrata have actually arisen in the course of evolution from the basal trabeculae or plates connected with placoid scales.

Admitting this a further question presents itself. What was the evolutionary origin of the more deeply situated masses of bony tissue, for example those which replace cartilage? Has the tissue which gives rise to these gradually been infected with bone-forming activity which has spread inwards from the skin? Or has this bone-forming power in the deeper tissues arisen independently? It is in this connexion extremely instructive to study the gradual spreading of the irregular shreds of bony material from the tooth-base of a Lepidosiren. They gradually spread onwards through the connective-tissue matrix like crystals forming in a fluid, and there is no apparent reason why such spreading should not continue for relatively great distances, provided the necessary pathway of connective tissue is present. It appears in fact thoroughly reasonable to regard the deeper portions of the bony skeleton, like the more superficial, as having arisen in evolution by the spreading inwards of bone-forming activity from the skin.

In considering this important morphological problem, the origin of the bony skeleton, it must be borne in mind that the all-important fact, which far outweighs all other evidence available, is that in the Elasmobranchii, the group of gnathostomatous Vertebrates which is admittedly the most archaic, the placoid scales are the only elements of the osseous skeleton which have as yet made their appearance. There is no suggestion that the ancestors of existing Elasmobranchs ever possessed a bony skeleton apart from the placoid scales. Consequently in the Vertebrate groups which have been evolved subsequently to the Elasmobranchs the bony tissue must either be a further development of the bony basal plates of the placoid scales, or else a new independent development. If the former view is shown to have in its favour a reasonable degree of probability we are bound to accept it as our working hypothesis until a better is suggested, for it alone of the two views mentioned is really constructive, the other offers no explanation but merely the negation of an explanation. In the opinion of the present writer the reasonable degree of probability has been amply demonstrated by the facts which have been quoted.

It is also necessary to avoid attaching too great importance to the differences in detail which have arisen in the evolutionary history of bony tissue under different circumstances. Such differences may become conspicuous and highly characteristic — for example the difference in relation to the calcified material—whether the cell elements are completely surrounded by it as in the ordinary bone of the higher Vertebrates, or have merely a prolongation of the cell-body embedded in it as in ordinary dentine.

Such differences in detail may be of great interest in themselves. For example the bony tissue forming the scales of *Lepidosteus* is characterized by the fact that some of the bone-cells show the dentinal characteristic that the main part of the cell-body lies on the surface of the calcified material and only a prolongation of it is enclosed within the hard substance. Now Goodrich (1913) has made out the important fact that this peculiarity is not confined to the scales but extends to the whole of the bony skeleton. Such a fact is obviously a strong additional evidence of intimate evolutionary relationship between the scales and the rest of the bony skeleton.

Again such detailed differences may raise interesting problems, for example whether the "ordinary bone" type or the dentinal type (as is perhaps probable) is the more primitive type of bony tissue.

Interest in such details must not be allowed to obscure the main conception of bony tissue as contrasted with cartilaginous, or the problem of its evolutionary origin. As regards that origin we seem justified in believing that bone formation has during the evolution of the Vertebrata spread from the dermis—from the neighbourhood of the placoid scale bases—into the deeper tissues and so given rise to the deeper portions of the bony skeleton. On the other hand we do not appear to be justified in regarding the evolution of the deeper parts of the skeleton as being due to a sinking downwards of actual individual placoid elements. Nor, in the author's opinion, is there reliable evidence, so far, bearing on the further problem whether or not the first scleroblasts or bone-forming cells of the Vertebrata were immigrants from the ectoderm. This view, which was supported by Gegenbaur, has a considerable amount of *a priori* probability in its favour in view of the facts of skeleton formation in the lower invertebrates.

It is no longer possible in the present state of knowledge to classify bones, as did the older workers, simply into two sharply defined sets—membrane bones and cartilage bones. The most that we can do is to recognize various stages in the process of shifting inwards from the skin, from which as already indicated they probably arose in the early stages of their evolution.

Firstly we have the most primitive type which may be termed **dental bones**, which are superficial in position and which still are connected at one period or other with teeth. Typical examples are the bones already referred to in the roof of the mouth in Amphibians.

A second category consists of bony plates which have lost their tooth structures and have sunk down to a deeper level. These frequently become applied to the surface of the cartilaginous skeleton, remaining separated however from the cartilage by a layer of unmodified connective tissue. Such may be termed **investment bones** (Allostoses, Gaupp).

Finally a third category of bones are the **substitution bones** (corresponding roughly to the old group of cartilage bones; Autostoses, Gaupp). In these the formation of bone has spread into the connective tissue in immediate contact with the cartilage, and as the tissue is formed, room for it is made by the destruction of the previously existing cartilage, which it therefore comes to replace.

While it is convenient to recognize these three types of bone development, and probably justifiable to interpret them as representing successive steps in the evolution of bone, it must not be supposed that they are absolutely distinct: intermediate forms occur frequently and a single bone of the adult may arise during ontogeny in part according to one type and in part according to another.

Bony tissue being rigid and inextensible, it is essential to the functions of movement and growth, that it should not be continuous throughout the body. It consequently takes the form of separate bones, the junctions between which are specialized either for movement, or for addition of new bony tissue at their margins. Each bone arises by the spreading outwards of bony tissue from one or more centres of ossification. The study of the arrangement and homology of the various bones constitutes an important part of the science of Comparative Anatomy—particularly important for the reason that it is the bony skeleton alone which is as a rule preserved in the fossil remains of Vertebrates belonging to past phases of Evolution.

It should be borne in mind that a single bony plate in such a part of the skeleton as the skull may represent ossification which has spread out irregularly from the bases of a large number of the original placoid elements. In view of this it will be realized that great caution must be exercised in homologizing apparently similar bones in different groups of the lower Vertebrates. Thus the same name—implying homology—is commonly given to similar bones in the skull of a Crossopterygian, an Actinopterygian, and a Lung-fish or Amphibian. There is no guarantee of any precise homology in such cases and the student should be on his guard against taking very seriously the nomenclature of such bones as expressing exact and well-determined homology.

Fish Scales.—In the Fishes, that is in those Gnathostomata in which the skin has not yet become specialized for Respiration (Amphibians), or for protection against desiccation (Reptiles), or for diminishing loss of heat (Birds and Mammals), there is commonly present a coating of dermal bones which most usually take the form of **scales**. Such scales are in the most general terms simply plates of bone in one or other of its varieties. The development of what is probably the most primitive type—the placoid scale—has already been dealt with. It need only be added that individual scales, interspersed regularly amongst the others, pause in their development, and only proceed with the process when room is provided by the already developed scales becoming spaced out during the growth of the body.

While the placoid scale is simply an individual dermal tooth the ganoid scales as seen in the surviving *Polypterus* or *Lepidosteus* are on the other hand tooth-plates, numerous minute denticles being associated with each scale. In these fishes also there is a certain amount of independence between the dermal plate of bone and the actual denticles which are at first quite separate from it (Nickerson, 1893; Goodrich, 1908). The reduction in size of the dental cones and the loss of their attachment to the bony plate are steps towards their complete disappearance which has been reached in the scales of ordinary Teleostean fishes.

In the ganoid scale of *Polypterus* or *Lepidosteus* the protective power of the bony plate has been greatly increased by its superficial layers undergoing modification of an analogous kind to that of the superficial layers of the dentine cone in the teeth of fishes. This portion of the scale is extremely dense, hard and enamel-like and is without cells embedded in it. Like the corresponding layer in the tooth of a fish it is commonly known by Williamson's name Ganoine. The advisability of using this name, rather than enamel, rests mainly upon the assumption that enamel is a substance fundamentally different, derived from a different cell-layer, from bone or dentine. If it be the case however that enamel is merely the superficial layer of dentine which has undergone secondary modification then there seems no particular harm in adhering to the custom—until recent years quite general—of using the word enamel for the superficial layer of the ganoid scale.

Ganoid scales are still comparatively thick and bulky structures but in the typical Teleosts the scales have become very thin plates of bone so modified as to be very tough and flexible, and overlapping like slates on a roof so as to be able to slide over one another during flexure of the body. This overlapping has been rendered possible owing to the surface of the scale being no longer inseparably linked to the ectoderm by the development of teeth. The scale is developed in the thickness of the dermis and it is only at its posterior edge, if anywhere, that it is even in early stages connected with the epidermis.

Vestiges of dermal denticles have been described in Teleostean fishes and these deserve fuller investigation. Marett Tims (1906) describes a stage in *Gadus* in which the scale consists of separate platelets each with a tooth-like spine projecting from it, while in such South American Siluroids as *Callichthys* and *Loricaria* the plates of the bony cuirasse bear numerous small spines which appear to be typically tooth-like in structure.

The scale is a plate of bone immersed in the dermis and it therefore naturally grows by the addition of new bone all over its surface. In the ganoid scale the quality of the bone differs on the inner and outer surfaces, that formed on the outer surface being the enamel or ganoine already referred to. In the highly evolved Teleost, where the scale has increased in area at the expense of thickness, the addition of new bone on the flat inner and outer surfaces of the scale is relatively small in amount as compared with that round the edges.

In accordance with variations from time to time in the metabolic activity concerned in the production of the new bone the latter tends to show variations in rapidity of growth, density and other characters, and consequently to show a more or less distinct layered arrangement. Where there are periodic variations in the metabolism of the fish—associated it may be with sexual activity or with food supply or with changes in the physical environment (*e.g.* seasonal changes of temperature)—these variations may be duly chronicled in the contemporary layers of the scales. Such scale records are often particularly distinct and easily observed in the scales of the Teleostei owing to their thin flat character and the predominance of growth at their edges.

The development of the Cycloid scales of Dipnoi has not been investigated in detail. So far as the main features of their development are concerned they apparently resemble the scales of Teleosts. Like them they are, except at their posterior edge, deeply embedded in the dermis. On their outer surface they are prolonged into numerous, often recurved, spines which in all probability represent true denticles although they have lost their primitive relation to the epidermis.

BONY VERTEBRAL COLUMN.—In all gnathostomatous Vertebrates, except the Elasmobranchs (including Holocephali) and Sturgeons, the vertebral column becomes in great part bony. The process of ossification is found in its first beginnings in the Lung-fishes, where the arches become ensheathed in bone.

In the bony Ganoids and Teleosts ossification usually commences in the connective tissue bounding the surface of the arcualia, the first shreds of bone being completely cell-less. From the arches the bone spreads over the surface of the chordal sheath (in *Amia* it develops here first—Schauinsland) to form the rudiment of the bony centrum. In *Coregonus* it is stated (Albrecht, 1902) that for a time two bony rings can be distinguished round each centrum (cf. variation in *Amia* mentioned below on p. 339). From the thin superficial sheath of bone an irregular spongework of bony trabeculae spreads outwards and forms the bulky centrum of the definitive vertebra. As this process goes on the basal portions of the cartilaginous arches become surrounded by bone and may persist as four tracts of cartilage running outwards through the bony centrum (*e.g.* *Esox*—Pike). Most usually the arches become completely bony: the original bony sheath covering their surface becomes perforated on its median side by invading vascular connective tissue which destroys the cartilage and deposits bone in its place.

The neural spine even when segmented in the cartilaginous condition becomes ensheathed in a continuous layer of bone.

In the case of Teleosts the cartilaginous stage of the haemal arches is frequently completely eliminated from development, the arches being laid down as bone in the connective tissue.

In the Urodele Amphibia bone makes its appearance as a cell-less sheath round the surface of the centrum, which gradually increases in thickness and becomes cellular, enclosing connective-tissue cells, and also spreads over the surface of the arcualia. The cartilage becomes gradually absorbed and replaced by the bone.

In *Sphenodon*, which may be taken as an example of the more primitive Reptiles, a bony sheath similarly develops round the centrum, but according to Schauinsland it consists at first of a distinct dorsal and ventral half. The bony tissue of the dorsal portion spreads upwards so as to enclose the bases of the neural arch-elements but the main portion of the arch-element on each side becomes enclosed in an independent bony sheath of its own. This latter appears first on the outer side of the cartilaginous arch and may persist as a separate bony element for a long period, even throughout life in the Crocodiles and various other Reptiles.

Bone formation also spreads inwards into the substance of the cartilaginous centrum along what possibly corresponds to the boundary between the primary centrum and the chondrified tissue external to it (Fig. 152, p. 302). Thus arises a deep-seated centre of active bone formation.

From these various centres ossification spreads, the cartilage being gradually supplanted by bone. Not the whole of the bone so deposited is permanent: a great part of that lying outside the primary centrum becomes again absorbed, leaving a superficial tract connected with the more central portion only by sparse bony trabeculae, the meshes being occupied by intrusive connective tissue.

An interesting adaptive feature is found in the tail region of certain Reptiles (Lizards, *Sphenodon*) which enables the possessor to break off its tail suddenly when seized by an enemy. In these animals the halves of the centrum derived from successive sclerotomes have reverted to a condition of incomplete fusion—the ossification being more or less interrupted in the plane of contact of the two successive sclerotomes by a transverse septum of cartilage. As at the same time the corresponding connective-tissue septum between consecutive myotomes remains weaker than usual a violent contraction of the caudal muscles is able to tear across both cartilaginous and connective-tissue septum and break off the distal portion of the tail.

In the case of the Birds it would appear that the main centre of ossification of the centrum corresponds to the deep-seated one in *Sphenodon*, the superficial bone-forming activity being much reduced. A characteristic feature of the Birds, associated primarily with their peculiar respiratory movements, is the extensive fusion which takes place between the vertebrae of the trunk region.

THE COMPOSITION OF THE DEFINITIVE VERTEBRA.—A fascinating but difficult chapter in Vertebrate morphology is that which deals with the composition of the definitive vertebra. We have already, in describing the development of the cartilaginous vertebral column, mentioned the elements which go to build it up—neural, haemal, and central. The difficulties of interpretation arise from the fact that great variety shows itself in the ultimate fate of these elements and in the manner in which they undergo fusion with their neighbours. This can perhaps best be illustrated by the case of *Amia* as described by Schauinsland. Here in some cases two amphicoelous centra (α and β) are developed corresponding to a single segment, each one carrying its pair of neural and pair of haemal elements, those attached to the anterior centrum (*A* and *a*) being relatively small, those on the posterior centrum (*B* and *b*) on the other hand well developed. Variations from this diagrammatic arrangement are found in different parts of the body.

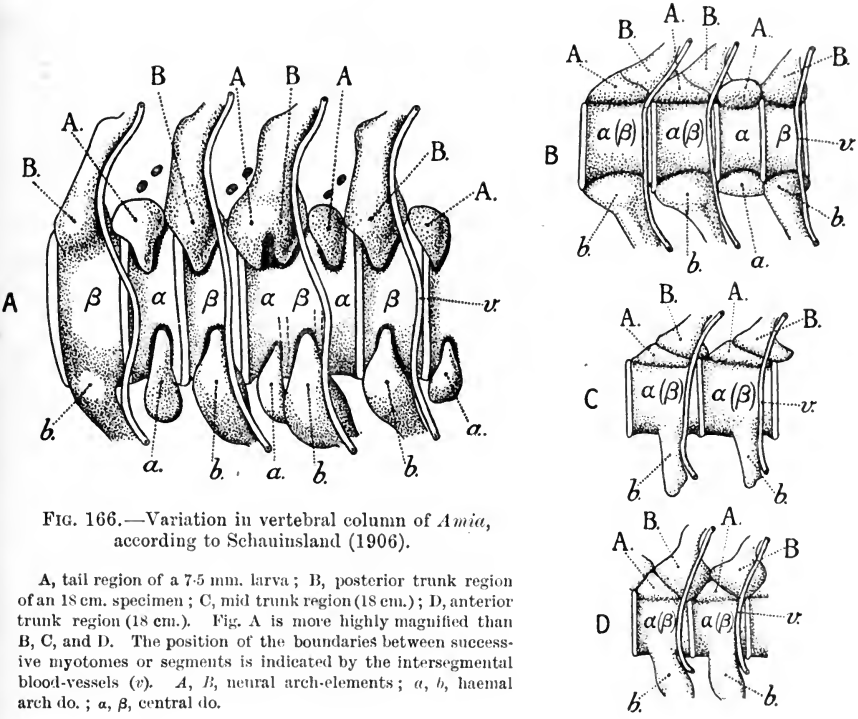


FIG. 166.—Variation in vertebral column of *Amia*, according to Schauinsland (1906).

A, tail region of a 7·5 mm. larva; B, posterior trunk region of an 18 cm. specimen; C, mid trunk region (18 cm.); D, anterior trunk region (18 cm.). Fig. A is more highly magnified than B, C, and D. The position of the boundaries between successive myotomes or segments is indicated by the intersegmental blood-vessels (*v*). *A*, *B*, neural arch-elements; *a*, *b*, haemal arch do.; α, β, central do.

In the tail region (Fig. 166, A) the original condition frequently persists although in aged individuals the arch-elements (*A*, *a*) of the anterior vertebra of the segment are liable to become completely overgrown and hidden by bone. On the other hand there frequently takes place fusion between adjacent centra so that compound centra are produced. Most usually in this case it is the two centra (α, β) of one segment which undergo fusion but in some cases the posterior centrum of one segment fuses with the anterior centrum of the next so that the resulting compound vertebral centrum ($\beta\ \alpha$) belongs to two successive segments. Again in some cases as exemplified by the specimen figured (Fig. 166, A) three successive centra may undergo fusion.

Towards the front end of the tail and throughout the trunk region the two centra of one segment undergo fusion but apparently the hinder centrum has undergone reduction in size with the result that its neural arch-element (B) becomes displaced on to the top of the smaller anterior element (A) (Fig. 166, B, C).

Towards the extreme front end of the trunk the neural element B becomes practically intervertebral in position (Fig. 166, D). It is to be noticed that in these cases, where the element B has been displaced, the bony splints which develop on its surface never spread downwards from it, so that it remains throughout life without any continuity of structure with the rest of the vertebra.

Towards the tip of the tail there is apparently no regularity, all kinds of fusions and modifications of the various elements taking place.

To sum up we see in *Amia* two complete potential vertebrae corresponding to each segment,[1] each with its central, its neural and its haemal elements.

The two vertebrae of a segment may be represented by the formulae $\begin{array}{c}A\\\hline\alpha\\\hline a\end{array}$ and $\begin{array}{c}B\\\hline\beta\\\hline b\end{array}$ where α and β are the centra, A and B the neural arch-elements and a and b the haemal arch-elements. In the trunk region the ordinary compound vertebra may be represented thus $\begin{array}{c}AB\\\hline\alpha\beta\\\hline ab\end{array}$ but in occasional cases fusions take place so as to produce vertebrae of the type $\begin{array}{c}BA\\\hline\beta\alpha\\\hline ba\end{array}$ or of the type $\begin{array}{c}ABA\\\hline\alpha\beta\alpha\\\hline aba\end{array}$ or $\begin{array}{c}ABAB\\\hline\alpha\beta\alpha\beta\\\hline abab\end{array}$. Such a fusion as that shown in the last formula produces a vertebra with a very long body upon which may persist four sets of neural and four sets of haemal elements.

The great range of variation seen in *Amia* from the presumedly original condition, even in different regions of the vertebral column of one individual, emphasizes the need of much caution in the laying down of general principles regarding the composition of the definitive vertebra. It seems justifiable to admit the two pairs of neural and two pairs of haemal arch-elements into the general

---

[1] Diplospondylous condition—von Jhering.

scheme of a vertebral segment in addition to the central element. But a difficulty is at once raised by the not infrequent appearance (as in *Amia* and many Elasmobranchs) of two centra within the limits of a segment. To get over this it has been suggested that primitively there were actually present two complete vertebrae, each with centrum, neural arch and haemal arch, within the limits of a single segment (dispondylous or diplospondylous condition). Physiological considerations however support the probability of there having been primitively a single vertebral centrum, extending from about the middle of one pair of myotomes to about the middle of the next pair. If it is borne in mind that the material of the anterior and posterior halves of the centrum is derived from two independent sources—successive pairs of arch-elements or of sclerotomes—it will seem reasonable to explain the occasional duplicity as due primarily to absence of the complete fusion which normally comes about between the halves of the centrum derived from the two sources, the two halves proceeding with their development independently. Conversely a more complete fusion, extending over a number of these potential half-vertebrae instead of merely two of them, would lead to cases of elongated definitive vertebrae carrying a number of arches.

BONY SKULL.—The caution expressed on p. 335 is especially necessary in connexion with the bones of the skull. Here we have a department of morphology which took shape in the early days of that science. The efforts of the older anatomists were devoted to the working out of homologies between the bones of different groups of Vertebrates and individual bones were given the same name—they were decided to be homologous—mainly on the basis of similarity of relations in the adult animal, with only the most slender basis of either palaeontological or embryological knowledge. The consequent uncertainty as to the precise homology of similarly named bones in different groups of Vertebrates makes it in the author's opinion impossible to write a satisfactory account without treating each of the main groups in detail by itself. As to do this would require more space than is available he would refer readers who desire such information to Gaupp's volume and the literature there cited and will confine himself here to a very brief sketch.

In the lowest Gnathostomata, as represented at the present day by the Elasmobranch fishes, the skull retains throughout life its cartilaginous character, the bony tissue being confined to the placoid elements of the skin. In the other groups of Gnathostomata the purely cartilaginous condition is temporary, the cartilaginous skull becoming strengthened and in places, though never entirely, replaced by bone.

In the floor of the chondrocranium there make their appearance a mid-ventral row of replacement bones the **basi-occipital**, the **basisphenoid,** and the **presphenoid.** Laterally to each of these elements the cartilage becomes replaced by a pair of bones known respectively as the **exoccipitals**, the **alisphenoids** and the **orbitosphenoids.** In the occipital region the cartilaginous roof gives way to a replacement bone—the **supraoccipital**—but in the corresponding region further forwards the cartilage either does not develop or if it does develop never becomes replaced by bone.[1]

In addition to the three groups of replacement bones mentioned, the cartilage of the sensory capsules gives way to replacement bones. In the wall of the auditory capsule there develops anteriorly a **prootic** and in the remaining part of the capsule wall there develop other ossifications differing in number in different groups—as many as four in the case of Teleostean fishes — **epiotic, opisthotic, sphenotic** and **pterotic.** The wall of the olfactory capsule similarly becomes replaced by **ethmoid** bones—varying much in the different groups. The wall of the eyeball in a few cases develops a ring of flattened replacement bones in the substance of the sclerotic.

In addition to the replacement bones already indicated there develop others in connexion with the visceral arches. Thus we find the upper portion of the mandibular arch becoming replaced by the **quadrate**, the upper portion of the lower jaw by the **articular** and the palato-pterygoid outgrowth by **palatine** and **pterygoid** elements which however exhibit great differences in their mode of development. The segments of the hyoid and branchial arches become replaced by various **hyal** and **branchial** bones in the bony fishes.

The bony skull is completed by numerous more superficially placed bones—some belonging to the ordinary investment type, others retaining their dental character. The cranial roof immediately in front of the occipital region is typically covered by a pair of **parietal** bones; in front of these are a pair of **frontals.** In the Amniota particularly are developed such additional elements as **squamosal, postfrontal, jugal.** The Ethmoidal region develops such bones as **nasal, prefrontal, lachrymal, septomaxillary.**

In the region of the buccal cavity we find a particularly rich development of investment and dental bones. The function of upper jaw, originally exercised by the palato-pterygoid bar, becomes taken over by new bones—the **maxilla** and **premaxilla**—lying external to it, the palato-pterygoid bar becoming shunted inwards except its hinder quadrate portion and no longer forming the margin of the buccal cavity. Behind the maxilla or jugal there may develop a **quadrato-jugal**: in the region of the palato-pterygoid parts of the palatine and pterygoid are of this origin and so also are the **vomer** and **parasphenoid.**

Just as the primitive upper jaw becomes replaced functionally by bones of superficial origin, so also with the lower jaw—the original Meckel's cartilage becoming ensheathed by **splenial** and **dentary** (the latter taking on the tooth-bearing function) with such other bones as **angular, supra-angular,** and **coronoid.** In

---

[1] The **pleuroccipital** bone of the Dipnoi arises as a close investment of the occipital arch (Agar, 1906).

the region of the Hyoid arch the Teleostomatous fishes with their greatly developed operculum develop a series of **opercular** bones.

Of these various bones mentioned in relation with the buccal cavity and pharynx the majority show more or less distinct evidence of their dental origin [Premaxilla, Maxilla, Dentary, Palatine, Pterygoid, Vomer, Parasphenoid, Opercular—cf. Hertwig, 1874*]. In some cases this may be apparent only in part of the bone, the rest developing as an ordinary investment bone, while in a few cases bone which is in one part of the investing type may in another part present all the features of a replacement bone.

The student should recognize that the ossification of the skull, though differing greatly in degree in different Vertebrates, is never complete. In the adult Elasmobranch the cranium is entirely cartilaginous, bone being confined to the placoid scales: in a Sturgeon there is still a well-developed chondrocranium but the surface of the head is covered with large bony plates: in such a Teleost as the Salmon the chondrocranium also persists to a great extent but extensive tracts of the cartilage are replaced by bone while the superficial plates of bone are now in much more intimate relations to the surface of the cartilage: in such a Teleost as a Cod again the cartilage is reduced in the adult to such an extent as to be quite inconspicuous. It never however completely disappears and the macerated skull of a Vertebrate as seen in an osteological or palaeontological collection is imperfect, being without parts which may be of great morphological significance.

AUDITORY SKELETON.—In those Tetrapoda in which a tympanic membrane is present the vibrations of this membrane are transmitted through the tympanic cavity to a movable portion of the wall of the auditory capsule by a special arrangement of skeletal structures. These reach their highest development in the auditory ossicles of the Mammalia which have attracted much attention from students of mammalian anatomy and have been the centre of much controversy as to their phylogenetic origin. In the non-mammalian Vertebrates the two outer members of the chain of ossicles—the malleus and incus—have not yet made their appearance so that we are only concerned in this volume with the inner or stapedial portion which is represented in the Sauropsida and most of the Anura by the **columella auris.** It will be convenient to study the development of this in the case of the Lacertilia in which it has been recently investigated by Versluys (1903), Cords (1909) and Goodrich (1915).

It will be recalled that the tympanic cavity is the dilated outer end of the spiracular or hyomandibular gill pouch, and the Eustachian tube is the inner or pharyngeal portion of this pouch. The pouch is for a time open to the exterior, forming an ordinary spiracular cleft, bounded in front by the mandibular and behind by the hyoid arch. In the hyoid arch is situated the main branch (hyomandibular) of the Facial nerve and from this, near its dorsal end, there comes off a branch—the **chorda tympani**—which runs in a ventral direction behind the cleft to its lower limit and then curves forwards beneath the cleft towards the region of the lower jaw and floor of the buccal cavity.

The external opening of the spiracular cleft gradually closes, from below upwards, as is usual with this cleft, a stage being passed through in which only the dorsal end of the cleft is open—precisely as in the adult of an ordinary Elasmobranch. As the lower limit of the opening gradually shifts dorsalwards the chorda tympani remains in close relation with it so that the portion of the nerve on the ventral side of the opening assumes a more and more dorsal position. Eventually even the dorsal vestige of the cleft closes so that the spiracle has now reverted to the condition of a pouch. Owing to the shifting in position of the chorda tympani as it followed the retreating lower edge of the spiracular opening this nerve now passes forwards dorsal to the main portion of the pouch, instead of entirely ventral to it as it did originally.

The dilatation of the outer end of the pouch to form the tympanic cavity is brought about mainly by active growth of the lower portion of its posterior wall. This bulges outwards and spreads forwards and dorsalwards beneath the epidermis, from which however it remains for a time separated by a considerable thickness of mesenchyme. Later on this thins out relatively so that the three layers bounding the tympanic cavity on its outer side—endoderm, mesenchyme, ectoderm—form a thin membrane—the **tympanic membrane.** As the tympanic dilatation goes on expanding in a dorsal and anterior direction the chorda tympani becomes displaced in front of it still further from its original position.

In the mesenchyme of the hyoid arch there takes place a gradual condensation to form the rudiment of the skeletal arch. The lower and main portion of this condensation becomes the cartilage of the definitive main cornu of the hyoid. Its dorsal portion also becomes converted into cartilage, taking the form of a stout rod the inner ("stapedial") end of which fits into the **fenestra ovalis**—a vacuity in the wall of the auditory capsule—while its outer portion ("**extra-columella**," Gadow) extends outwards towards the skin, embedded in the mesenchyme of the posterior wall of the spiracular pouch or tympanic cavity. Finally the lining of this cavity grows actively dorsally and ventrally to the columella so that it bulges backwards both above and below the columella. The pockets of tympanic lining so formed meet round the columella and fuse together so that the columella, instead of being embedded in the hind wall of the cavity, now passes right through it, enclosed in a delicate sheath of mesenchyme covered with endoderm. The extension of the tympanic cavity backwards past the columella causes an extension of the tympanic membrane in the same direction so that the point at which the tip of the extra-columella reaches the skin, instead of being situated behind the tympanic membrane as it was originally, comes to be about the centre of that membrane.

The general mode of development of the columella and the cavities associated with it as seen in *Lacerta* appears to be typical of the Sauropsida in general. It is now necessary to refer to a few additional details.

The inner end of the columella (**stapes**) fits into the fenestra ovalis. It is for a time, during prochondral or cartilaginous stages or both, continuous with the wall of the auditory capsule and is probably to be interpreted as a portion of this wall which has become separate and movable.

Chondrification of the columella commences in the Lacertilia from three centres according to Versluys and it is to be noted that the separation between extra-columella and stapes arises secondarily within the region of cartilage which develops from the innermost centre.

In Birds an interesting variation has been discovered (Goodrich, 1915) in the relations of the chorda tympani. In the Duck these are normal, agreeing with what has been described for *Lacerta*. In the ordinary Fowl and the Turkey however the stage in which the chorda tympani is posterior to the hyomandibular cleft is omitted from development. Even in early stages it is found to pass in front of the pouch or cleft.

This is one of those cases which emphasizes the need of caution in regarding the course of a nerve as a necessarily deciding factor in discussions as to the morphological nature of particular organs. Position in regard to a particular nerve-trunk often affords us most valuable evidence regarding the primitive position of an organ. Here, however, we have it impressed upon us that we must never rely absolutely upon such a piece of evidence taken by itself. Were we to do so in this case we should be led into the absurdity of concluding that the tympanic cavity of the Turkey is not homologous with that of the Duck.

As a matter of fact nerve-trunks do not always form impassable barriers to the evolutionary change in position of organs. A skeletal structure may spread round a nerve-trunk (*e.g.* neural arches of Dog-fish) and becoming absorbed behind it may come to be transposed entirely past the nerve. In the case of the chorda tympani and the tympanic cavity it is clear that the nerve lay primitively behind and below the cavity and we may probably take it that, in accordance with the general principle that nerve-trunks tend to shorten and so economize material, in the course of evolution it became shifted dorsalwards through the mesenchymatous middle layer of the outer wall of the tympanic cavity before it became thin and membranous, so as eventually to lie completely dorsal and anterior to the tympanic membrane.

Incidentally the variation from normal development occurring in the Turkey and Fowl is one of those cases apparently impossible to explain on the outgrowth theory of nerve-development, but readily understandable on the view of nerve-development supported in

Chapter II., according to which new nerve-paths may arise in response to the short circuiting of nerve-impulses.

A tympanic cavity with membrane and columella occurs in many of the Anura—as for example the ordinary Frogs and Toads (*Rana*, *Bufo*)—while in others such as the genera *Bombinator* and *Atelopus* ("*Phryniscus*") it is absent. In the Urodele Amphibians the tympanic cavity and membrane have not yet made their appearance. The inner end of the columella is however represented by a movable plate of cartilage fitted into the fenestra and in various genera the extra-columellar portion is represented by a rod-like outgrowth from this. The former apparently develops from the auditory capsule while regarding the latter there is much difference of opinion as to the extent of its relation to the cartilage of the hyoid arch. The disagreement between different observers probably means that there are actual differences between different genera of Amphibia. This is quite what is to be expected, for whenever we find a single organ of which part is derived from one embryonic source and part from another the proportion contributed by the two sources is liable to vary, so that in one case it may be the portion derived from the one source which is conspicuous and in another case that derived from the other.

The **tympanic ring** within which the tympanic membrane is stretched arises in the form of an outgrowth from the rudiment of the quadrate, *i.e.* from the upper portion of the skeleton of the mandibular arch. This outgrowth separates off and grows round the outer end of the hyomandibular pouch in the form of a crescent the two horns of which eventually meet to form a complete ring.

Skeleton of the Median or Unpaired Fins.—The median fin, thin and membranous as it is in its most highly evolved condition, is supported by characteristic skeletal arrangements. Into these two distinct elements enter, one mesial represented by rays of cartilage or bone (radials), the other superficial and of dermal origin.

The mesial fin-rays are frequently in close relation to the neural and haemal arches and it is reasonable to suppose that in the process of evolution, as the hind end of the body became extended in a dorsal and ventral direction, so as to attain to the flattened form conducive to efficiency in propelling the body, the neural and haemal spines underwent a corresponding extension for the purposes of support. This view is corroborated by the existing Dipnoi in which the mesial fin remains a comparatively slightly differentiated extension of the body dorsally and ventrally and in which the mesial supporting elements are simply the prolonged neural and haemal spines, each secondarily subdivided into three segments. The same is the case in Fishes generally so far as the ventral portion of the caudal fin is concerned in which the mesial supports develop also for the most part as typical haemal spines.

The mesial supports of the dorsal portion of the median fin on the contrary do not in Fishes generally show this relation to the vertebral arches. They arise, *e.g.* in Elasmobranchs, in ontogeny as independent rods of cartilage without definite relation to the metamerism of the body and later on become segmented into three pieces. In those cases, so far as they have been investigated, in which the radial elements are connected with a continuous basal plate of cartilage, this latter appears to arise in ontogeny as a continuous plate, though there is no reason to doubt that it arose in phylogeny by the fusion together of the basal portions of originally separate rays.

This want of correspondence of the mesial elements of the dorsal fin skeleton with the vertebrae is probably sufficiently explained as a secondary result of the prolonged working of the general principles which have governed the evolution of the median fin and which find their expression in the tendencies (1) of the continuous fin to become specially developed at particular points and to die away in the intervening spaces, (2) of the resulting separate fins to have their base of attachment to the body shortened and (3) of these fins to be situated on the body at the points where they are mechanically most effective.

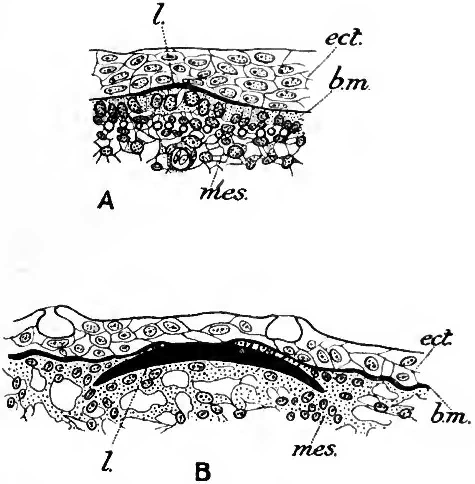


Fig. 167.—Two successive stages in the development of the lepidotrichia of *Salmo*.

A, Salmon (after Harrison, 1893); B, Trout (after Goodrich, 1904). *b.m*, basement membrane; *ect*, ectoderm; *l*, lepidotrichial rudiment; *mes*, mesenchyme of dermis.

Dermal Supports of Median Fins.—The median fins being primarily mere extensions of the body in the vertical plane it would only be reasonable to expect that they would show traces of skeletal elements comparable with the placoid elements or their derivatives characteristic of the rest of the surface. And in fact the dermal skeletal supports of the median fins can, some of them, be clearly recognized as homologous with scales, while in others although this may no longer be recognizable their origin is found to be closely associated with the basement membrane as was the case with the dermal teeth.

It will be convenient to consider first of all the dermal skeletal elements in which the direct relation to scales is most clear. Such are the bony fin-rays of Crossopterygian and Actinopterygian fishes. In an ordinary Teleost (Fig. 167) the fin-rays of this type (**lepidotrichia**, Goodrich) appear in their earliest stage, as shown by

Harrison (1893), in the form of a localized thickening of the basement membrane underlying the ectoderm. This thickening becomes more and more marked and eventually separates off round its edges in the manner shown in Fig. 167, B, mesenchyme insinuating itself all round between the ray and the basement membrane, so that the former eventually lies free from the basement membrane (or in some cases still connected with it by narrow bridges) deep down in the mesenchyme. The ray soon becomes calcified. New layers are deposited on its inner and outer surfaces, mesenchyme cells become included within its substance and it becomes a plate of ordinary bone. The rays are elongated structures which develop from the fin base towards the tip. They often become jointed, either by calcification being interrupted at intervals (Goodrich) or by a secondary solution of continuity (Harrison).

Rays are formed in the manner described on each surface of the thin membranous fin. The rays of opposite sides correspond exactly in position and become later on fused across the mesial plane so as to form a single unpaired ray whose paired origin is indicated only by its forking at its inner end to embrace the tip of the median radial, the process of fusion between the two elements not taking place at this proximal end. In the dorsal and anal fins and in the ventral part of the caudal fin the lepidotrichial fin-rays correspond segmentally with the true median skeletal elements the tips of which they embrace as indicated above.

In the more primitive Teleostomes the identity in nature of these fin-rays with the scales which cover the rest of the body is still more obvious. In *Polypterus* and *Lepidosteus* they develop a coating of ganoine and even bear distinct small denticles on their surface. It is also interesting to notice that in the anal fin and ventral part of the caudal fin of *Polypterus* the fin-rays at their proximal ends merely pass in beneath the edges of the body scales and do not take on any relation to the true median skeletal elements. The palaeontological fact may be recalled in passing that in some of the extinct fishes a perfect gradation can be traced between the fin-rays and typical body scales (see Goodrich, 1904).

In addition to the fin-rays just described, the homology of which with scales may be taken as well established, it is very usual to find another type of fin-ray in which this homology is not so obvious. This is exemplified by the horny fin-rays (see Goodrich, 1904) which occur in the fins of Elasmobranchs (including Holocephali), in the "adipose" fin of Salmonids and Siluroids, and towards the margins of the fins generally in adult Ganoids and Teleosts. These horny fin-rays develop either as thickenings of the basement membrane or at least in immediate contact with it. Mesenchyme cells insinuate themselves between the ray and the basement membrane as the ray separates off. The ray gradually becomes farther removed from the basement membrane and mesenchyme cells collecting round it deposit fresh layers on its surface so that its diameter becomes increased. In the fins of Elasmobranchs and in the adipose fins of Teleosts these horny fin-rays become much elongated, but ordinarily in the Teleostome they become relatively shortened during development, apparently being absorbed at their proximal ends while they grow at their distal ends, so that in the fully developed fin they form merely a marginal fringe—the individual horny rays being concentrated about the ends of the lepidotrichia.

The fin-rays in question have the main feature in common with the lepidotrichia that they arise in very close relation to the basement membrane and later on separate from it and sink into the underlying mesenchyme. It is probably allowable to look upon them as being of the same nature morphologically as the lepidotrichia but as having evolved still farther from the primitive scale-like condition. In Lung-fishes horny fin-rays occur of somewhat intermediate character in the form of slender parallel rods irregularly jointed and branched distally. In the later stages of their development these rays are apt to assume a bony character, becoming strongly calcified and enclosing branched bone corpuscles in their substance. The early development of these rays has not, so far, been worked out in *Ceratodus* in which they are best developed. In *Protopterus* Goodrich found them in early stages in at least very close proximity to the basement membrane.

We may probably look upon the dermal fin-rays of fishes as belonging to one morphological category, representing structures of originally placoid nature which have sunk down into the mesenchyme and degenerated, or—to use a preferable expression—become specialized, into more or less horny structures. The earliest stage in this process we should see in the lepidotrichia of Teleostomatous fishes where the scaly nature is still quite clear. The fin-rays of Lung-fishes would represent a farther stage, in which the scale homology is no longer clear, and the horny rays of Elasmobranchs and Teleostomes would represent the final stage, in which all trace of the original nature had disappeared except the origin in close association with the basement membrane.

The lepidotrichia of the Teleost and its horny fin-rays would represent different generations, the lepidotrichia being a later generation which have, as it were, had less time for modification. In this connexion it should be mentioned that the horny fin-rays of the Elasmobranch do not all belong to one generation. Their production from the basement membrane may go on for a long period so that instead of a single layer a thick mass of rays may be developed.

SKELETON OF THE PAIRED LIMBS

I. Fins.—To be consistent with the plan adopted in this book we should commence with the development of the paired fin in its most nearly primitive existing form. Before this can be done it is necessary to decide which is the most nearly primitive of the various forms of paired fin met with in surviving fishes. The present writer takes the view that undoubtedly the most primitive type of paired fin known to occur in existing Vertebrates is the paddle-like limb of *Ceratodus.* Physiologically this type of fin is as clumsy and archaic an organ in comparison with the paired fin of a Shark or Actinopterygian fish as is the most primitive type of savage's paddle compared with a racing oar. Further we know from the data of palaeontology that the *Ceratodus* type of limb is of great antiquity and that it was a common type of fin amongst the more ancient Sharks and Ganoids as well as amongst the Lung-fishes. There are only two possible explanations of its occurrence in the three groups mentioned. Either (1) it is an archaic type of fin inherited from the common ancestors of those groups or (2) it has been evolved independently in the three groups. The latter explanation seems very improbable—for such a type of organ would become evolved independently in different groups, only if it were physiologically very efficient. But it seems quite impossible with knowledge of the structure of the limb of *Ceratodus* and its use in the living animal to regard it as an organ of great locomotor efficiency. Apart from this consideration we have the historical fact that this type of fin has vanished away entirely in the two successful types of fish, the Sharks and the Teleostomes, and has persisted unchanged only in one of the surviving Lung-fishes. There seems then no escaping the first of the two possible conclusions mentioned above, that the paddle-like fin of *Ceratodus* and the ancient Lung-fishes, Ganoids and Sharks is a common heritage from the ancestral group out of which these fishes evolved and is therefore the most archaic of the known types of paired fin.

DEVELOPMENT OF THE PECTORAL LIMB SKELETON IN CERATODUS.—The skeleton of the limb makes its first appearance (Semon, 1898) about stage 45 (Fig. 201) as a rod-like condensation of connective tissue along the axis of the limb which tapers gradually towards the apex. Histological differentiation, by which this rod-like structure passes through a prochondral into a completely chondrified condition, proceeds from the base towards the apex. While in the prochondral condition the rod is a continuous structure, chondrification takes place from separate centres, with the result that the rod becomes converted into a series of blocks of cartilage, each separated from its neighbours by a thin layer of unchondrified tissue. The basal block, lying within the body wall and spreading in a ventral direction, is the rudiment of the pectoral girdle; the rest of the series forms the axis of the limb.

The lateral rays make their appearance later, the development again proceeding from base towards apex, and those on the preaxial or definitively dorsal side preceding those on the postaxial or definitively ventral. Each ray spreads out from the prochondral tissue between two segments of the axis and it is noteworthy that rays develop from the first (proximal) of these intersegmental joints although in the course of further development these, doubtless to give greater mobility to the fin, disappear. In the details of its development each ray repeats that of the main axis.

In addition to the normal rays, which are attached to the axis at the level of the intersegmental spaces, occasional rays make their appearance opposite the segments themselves. According to Semon these sprout out from the thin superficial layer of the axial tissue which like that between the segments persists for long in an unchondrified condition. These extra rays are most frequent on the postaxial side of the limb which in the pectoral limb becomes ventral, in the case of the pelvic limb dorsal (see Chap. VII.).

Growth of the fin and of its enclosed skeleton continues for a long period—even after the adult condition is attained. As regards the skeleton this continued process of growth takes place by two methods (1) by a simple continuation of the extension at the apex, and (2) by the already formed elements of the cartilaginous skeleton, axial or radial, continuing their individual growth in size.

As the definitive condition of the skeleton is reached, the intersegmental tissue chondrifies, towards the apex forming soft hyaline cartilage with a sparse matrix and towards the body taking the form of fibro-cartilage. Towards the base of the limb this fibro-cartilage develops many fluid-filled cavities so as to assume an almost spongy character and in this way give greater mobility. This is specially marked at the junction with the shoulder girdle and between the first and second segments of the limb-axis and in these two cases the apposed surfaces of hyaline cartilage are curved and concentric so as to afford a distinct development in the direction of a true ball and socket joint.

The details of development of the pelvic limb skeleton apparently agree with those of the pectoral. In this case also the girdle arises in the form of two originally separate halves.

ELASMOBRANCHII.—The earliest stage in the development of the pectoral limb and its girdle in Elasmobranchs is that described by Ruge and by Braus (1904) in *Spinax* where there exists a condensation of connective tissue in the form of a curved rod on each side of the body close under the skin, in the position shown in Fig. 159, *p.g* (p. 321). This forms the rudiment of the pectoral girdle. From it there grows outwards a short projection into the limb-rudiment which as it is clearly homologous with the axial cartilage of *Ceratodus* we may call by the same name. The girdle rudiment increases in length both dorsally and ventrally, and ventrally the two rudiments come to be in apposition. On each side a tract of cartilage now develops in the prochondral rudiment: the two cartilaginous rudiments show a similar dorsal and ventral extension and presently they also come into apposition ventrally and form a continuous structure across the mid-ventral line.

In other Elasmobranchs (see Mollier, 1894) the conditions appear to be similar on the whole to those described in *Spinax*.

The prochondral rudiment of the axial cartilage extends out into the limb-rudiment, forming a broad plate which tapers off posteriorly (Fig. 168). The condition is in its essentials the same as that in *Ceratodus* except that here the axial rudiment is laid back along the side of the body. The prochondral fin-rays arise, as in *Ceratodus*, in the form of outgrowths from the axial element. These are restricted to the outer (preaxial) side of the limb. They develop in *Spinax* (Braus) in series from before backwards except that anteriorly, in the region which will give rise to mesopterygium and propterygium, a few rays develop in the opposite sequence from behind forwards.

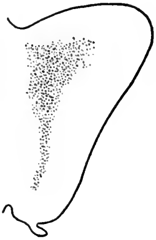


FIG. 168.—Section through pectoral fin of *Torpedo* embryo, parallel to surface of fin. (After Mollier, 1894.)

The prochondral rudiment of the skeleton is shaded.

The chondrification of the limb skeleton appears to take place in *Mustelus* and *Torpedo* continuously but in *Spinax* it sets in first in the axial portion and then in the rays in the same succession as they first appear. The separate segments of the rays in *Spinax* also develop in succession as separate centres of chondrification.

To understand the morphological relations of these early stages it is advisable to refer back to the paddle type of limb as it exists in the ancient sharks of the genus *Pleuracanthus.* Here (Fig. 169, B) we find a limb resembling generally that of *Ceratodus* but differing from it in two conspicuous details. (1) The skeletal axis has become relatively larger and clumsier, its original elements having probably undergone extensive processes of fusion both with one another, as shown by the fact that the cartilages of the axis are in places less numerous than the lateral rays, and also with the basal portions of these lateral rays. (2) The rays on the postaxial side of the limb are much reduced in number, only a few persisting towards the limb apex.

The tendency of the postaxial rays to disappear in these archaic sharks (and the same tendency is seen in Lung-fishes) justifies us in believing that the external side of the pectoral limb-axis in the young Elasmobranch is morphologically *preaxial.* This conclusion raises the interesting question—Are there any vestiges of postaxial rays to be found in existing Elasmobranchs? This question has to be answered in the affirmative. In *Centrophorus* (Fig. 169, C) Braus finds a number of postaxial rays near the tip of the fin in a late stage of development; in *Spinax* at least one similar piece of cartilage occurs; and even in the adults of various Sharks Gegenbaur and Bunge found similar vestiges. As vestigial organs are notoriously variable more extended investigations into the occurrence of such vestigial postaxial rays are very desirable. They should be carried out on as many different species of Shark as possible and on as large as possible a number of individual specimens of each species.

The skeleton of the pelvic girdle arises in a manner similar in its main features to that of the pectoral girdle. It is however characteristic of Elasmobranchs (except Holocephali) that the portion of the girdle dorsal to the attachment of the limb undergoes atrophy in later stages of development. As in the pectoral fin an axial cartilage appears with fin rays sprouting from its external side. Here also a separate cartilage develops anteriorly with a few rays attached to it but it is doubtful whether it is justifiable to homologize this in detail either with the propterygium or the mesopterygium of the pectoral fin.

The cartilaginous skeleton of the clasper arises in continuity with the rest of the fin skeleton and appears to consist of the tip of the limb axis with possibly a few modified rays. The claw-like structures are simply modified placoid scales.

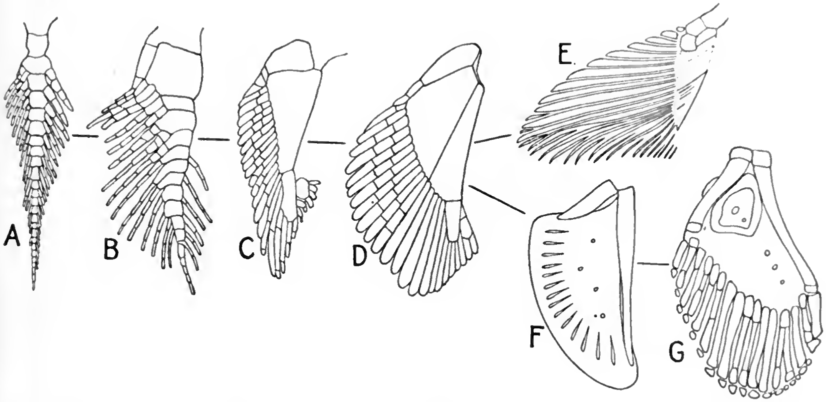


Fig. 169.—Pectoral fin skeletons of: A, *Ceratodus* (Semon); B, *Pleuracanthus* (Fritsch); C, *Centrophorus* embryo (Braus); D, *Acanthias* (Gegenbaur); E, *Cladoselache* (Bashford Dean); F, *Polypterus* larva (Budgett); G, *Polypterus* (Wiedersheim).

The outer or preaxial side of the limb is to the left, except in A.

Teleostomi.—As regards *Polypterus*, commonly regarded as the most archaic of existing Teleostomes, our knowledge of the development of the limb skeleton is fragmentary. In the larva of stage 36 (Fig. 197) the skeleton of the pectoral limb is in the form of a thin lamina of cartilage with small irregularly scattered perforations. This is connected with a shoulder girdle rudiment consisting of a simple curved rod of cartilage. In the 30 mm. larva described by Budgett (1902) the girdle has become shortened into a compact block of cartilage and the cartilaginous plate lying within the limb itself has become thickened along its anterior and mesial edges. These thickened portions are separating off to form the rod-like "propterygium

gium" and "metapterygium" (Fig. 169, F). The main portion of the plate is becoming split up towards its margin by a series of slits into a number of radiating pieces which represent the separate radii of the fully developed fin (Fig. 169, G). The close correspondence between the fin skeleton at this stage of its development and the fin skeleton of a shark is obvious from Fig. 169.

In Actinopterygians the pectoral limb skeleton is in the prochondral stage a continuous mass, of which the anterior and mesial part separates off to form the pectoral girdle, while the distal portion spreads outwards to form the rays.

The pelvic limb skeleton shows a fundamentally similar origin from a continuous prochondral rudiment, but here the skeletal rudiment appears first in the projecting limb and only secondarily spreads within the body wall into the region of the pelvic girdle. It appears to the present writer that no special weight need be attached to such cases where the skeleton develops earlier in the limb than in the body wall: they are probably to be regarded simply as special cases of the frequent tendency for highly specialized organs to be laid down precociously in development.

II. LIMBS OF THE TETRAPODA.—In the Amphibia also the pectoral girdle and the skeleton of the limb itself are foreshadowed by a single condensation of mesenchyme which extends in a dorsal and ventral direction to form the girdle and out into the limb to form its skeleton. No general rule can be given as to the relative time of development of the various parts. In *Bombinator* according to Goette the girdle rudiment appears first and the limb skeleton sprouts from it: in *Proteus* according to Wiedersheim the limb skeleton appears first and the girdle later. In the girdle rudiment the dorsal or scapular portion becomes apparent first. Chondrification takes place separately in the girdle and the limb, the joint remaining unchondrified.

The cartilaginous pectoral girdle of the Amphibian, as of other quadrupeds, takes on the form of a λ upon each side of the body—the three branches of the λ being known as **scapular, coracoid** and **precoracoid** portions respectively and the glenoid articulation for the limb being situated at the meeting point of the three portions. As the two ventral branches of the λ are in some cases continuous with one another at their tips through a strong membrane, it seems not improbable that they had originally the form of a continuous flattened plate of cartilage, of which the central portion has now disappeared, leaving the thickened marginal parts as precoracoid (anterior) and coracoid (posterior) respectively. On this view the epicoracoid when present would represent the persisting thickened ventral margin of the primitive girdle.

In actual ontogeny the three branches spread gradually outwards from the original rudiment, while the epicoracoid when present is formed by the coracoid spreading forwards at its ventral end and fusing with the end of the precoracoid. The two lateral halves of the girdle come to overlap one another in the mid-ventral line and in the case of the higher Anura complete fusion takes place.

Amongst the Reptilia the first rudiment of the pectoral limb skeleton has been investigated by Mollier (1895) and found to consist of a condensation of mesenchyme in the glenoid region corresponding partly to the glenoid portion of the girdle and partly to the basal portion of the limb skeleton—the two being thus again continuous at first. The girdle portion of the rudiment spreads ventrally to form the coracoid region, then dorsally to form the scapular. The chondrification of the various parts takes place in the order of their appearance.

In Chelonians the girdle takes on the typical λ-shaped form with a more or less pronounced projection from the lower end of the coracoid forwards towards the lower end of the precoracoid which apparently represents the epicoracoid of Amphibians. In *Sphenodon* and in Lizards on the other hand the ventral portion of the cartilaginous girdle consists of a flattened plate which may become perforated by several foramina. Whether this flattened ventral portion corresponds to coracoid and precoracoid is doubtful. It seems on the whole more probable (Goette) that the precoracoid has disappeared in these forms owing to its functional replacement by the clavicle, a process seen in its incipient form in Anura. This view is supported by the occurrence of a distinct strand of condensed connective tissue in the position where the precoracoid should be though in this case it does not become chondrified but becomes replaced by bone (clavicle) at a later stage.

In Birds the girdle forms a simple curved rod without any bifurcation into coracoid and precoracoid portions ventrally.

Each lateral half of the pelvic girdle of quadrupeds is, like the pectoral girdle, typically of a λ-shape, the three limbs being known here as **ilium** (dorsal, more correctly **iliac bone** or **iliac cartilage**), **pubis** (anterior) and **ischium** (posterior). The frequency with which the pubis and ischium are continuous at their ventral ends suggests that here also they represent the persisting thickened marginal parts of a once flattened plate-like ventral portion of the girdle.

As in the case of the pectoral girdle the three processes are formed by simple spreading outwards from the original rudiment. In Amphibia chondrification takes place apparently from a single centre on each side (*Triton*, Bunge, 1880) giving rise to a pair of longitudinal plates of cartilage which meet ventrally.

In Reptiles each half of the pelvic girdle passes through the typical λ-shape. The ventral end of the pubis, like that of the ischium, meets its fellow across the mid-ventral plane forming a symphysis. In some cases, *e.g.* *Sphenodon* and certain Chelonia, the pubic symphysis becomes connected up with that of the ischia by a longitudinal bar of cartilage. In the Crocodiles the pubic portion of the girdle becomes eventually segmented off at its dorsal end from the rest of the girdle.

In Birds the pelvic girdle during the prochondral stage passes through the ʌ-shape, the right and left halves distinct from one another and each at first continuous with the skeleton of the limb. Pubis, ilium and ischium usually chondrify separately but in many cases (*e.g.* in the Common Fowl usually) ilium and ischium may become chondrified in continuity, and less frequently all three elements chondrify in continuity. A highly characteristic feature of the avian pelvis is that the pubis swings in a tailward direction about its attached dorsal end until it assumes a position parallel with that of the pubis. In the pelvis shown in Fig. 170, B, this rotation is just commencing.

The **Cheiropterygium** (Huxley), or skeleton of the limb in Amphibia and Amniota, consists of three distinct portions corresponding respectively to the Upper Arm or Thigh, the Forearm or Leg, and the Hand or Foot. As these portions are looked upon as homologous in the fore and hind limbs it is convenient to have a morphological name for the corresponding parts of the two sets of limbs, and such names have been proposed by Emery and Haeckel — **Stylopodium,** Zeugopodium or **Zygopodium,** and **Autopodium.** In the autopodium there may further be recognized Basipodium (carpus or tarsus), Metapodium (metacarpus or metatarsus) and Acropodium (phalanges).

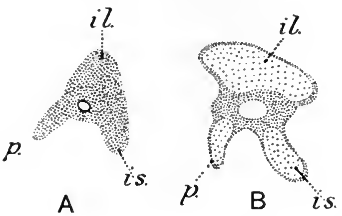


FIG. 170.—Side view of pelvis of Bird embryos. (After Mehnert, 1888.)

A, *Podicipes*; B, *Larus*. *il*, iliac region; *is*, ischium; *p*, pubis.

The limb skeleton is typically at first quite continuous. A rod-shaped condensation of mesenchyme appears first in the limb stump —the rudiment of the stylopodium (femur or humerus)—and as the limb grows this spreads outwards, bifurcating as it does so to form the rudiments of the zygopodial skeleton: with further growth the two limbs of this unite distally to form the rudiment of the autopodial skeleton. Chondrification takes place from the base of the limb outwards, each separate element of the adult making its appearance as a separate chondrification centre.

The skeleton of the autopodium originates in a flattened plate-like extension of the prochondral zygopodial skeleton. In this the various carpal or tarsal elements make their appearance as separate centres of chondrification. It seems unnecessary in a general text-book like the present to go into the great variations in detail which are found amongst the various tetrapods in regard to the skeleton of carpus and tarsus. It need only be said that the striking variations found in different groups from the schematic arrangement, such as is illustrated by Fig. 171, seem to have been brought about by enlargement or reduction of individual elements, or the fusion together of originally separate elements.

From the plate-like rudiment of carpus or tarsus there spread out radiating extensions normally five in number to form the skeleton of the digits. In the Amniota these appear practically synchronously although in Amphibians there is a tendency for them to develop in regular sequence according to the number of the digit (Rabl, 1901). In the substance of these the phalanges make their appearance as discrete chondrifications.

In the Birds the loss of individuality of the digits involved in the conversion of the tip of the pectoral limb into a rigid support for the flight feathers has been accompanied by processes of reduction and fusion of the orginal elements. In the prochondral stage five digits are laid down but only II, III and IV proceed with their development. Of these metacarpal II becomes reduced to a small stump projecting from III: metacarpals III and IV become fused with one another at both ends: and the three distal carpals become fused with the metacarpals to form the carpo-metacarpus characteristic of the Bird.

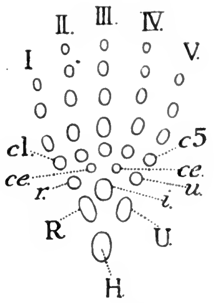


FIG. 171. — Cartilaginous elements which develop in the fore limb of *Emys*: the figure is combined from several stages. (After Mehnert, 1897.)

ce, centrale; *c* 1-5, distal carpals; *i*, intermedium; *H*, humerus; *R*, radius; *r*, radiale; *U*, ulna; *u*, ulnare.

In the hind limb of Birds there are also laid down prochondral rudiments of the five digits and again I and V become reduced although not so completely as in the fore limb. V reaches the stage of a small metacarpal nodule of cartilage which however soon disappears. Metacarpals II, III and IV fuse with one another and with a cartilage which represents the distal row of tarsals to form the characteristic tarso-metatarsus. Metatarsal I disappears except in its distal portion. And finally the two proximal carpals which are visible for a time fuse with the end of the tibia to form the tibio-tarsus.

BONY SKELETON OF THE LIMBS. PECTORAL GIRDLE.—In the Sturgeons the original cartilaginous pectoral girdle persists, lying close under the skin of the posterior branchial region. Plates of bone corresponding exactly with those on the rest of the skin develop superficial to the girdle and serve to reinforce it. Of these bony plates there are two principal ones on each side, one in the region of the glenoid surface the **cleithrum** (Gegenbaur) and one extending ventrally to meet its fellow—the **clavicle.** In existing Crossopterygians where the evolution of the bony skeleton has reached a higher level than in the Sturgeons the same two bony elements develop but here the original shoulder girdle—its function being to a great extent taken over by the cleithrum—becomes relatively reduced in size. It lies on the inner surface of the cleithrum and its cartilage gives place in part to two replacement bones—the **scapula** dorsal, and the **coracoid** ventral. It is to be noted also that the cleithrum sinks more deeply into the tissues while the clavicle remains superficial.

In the higher bony fishes—Ganoids and Teleosts—the conditions are very similar to those of *Polypterus*—the primitive shoulder girdle being small and usually becoming replaced in great part by bone (scapula and coracoid) and the main supporting function being exercised by the independently developed cleithrum.

In the Dipnoi more nearly primitive conditions are retained as the original cartilaginous girdle remains well developed throughout life and retains its continuity with its fellow ventrally. Cleithrum and clavicle however are also developed and they show a higher condition in that they are developed in intimate contact with the surface of the cartilaginous girdle, the clavicle ensheathing the anterior face of the coracoid portion.

In Amphibians the scapula becomes replaced incompletely or completely by bone which spreads dorsalwards from the region of the glenoid articulation. The coracoid may remain cartilaginous (most Urodeles) or become replaced by bone. The precoracoid also tends to be strengthened by the formation of bone. In the common frog (*Rana*) and Toad (*Bufo*) the bone ("clavicle") is in the form of a splint lying along the anterior side of the precoracoid and originating in the connective tissue some little distance from the cartilage. In other cases the bony tissue completely surrounds and to a great extent invades and replaces the cartilage. We may infer with considerable probability that the bone in question was originally in phylogeny a "membrane" bone and that becoming more and more intimately related to the precoracoid cartilage it has in the latter form become more or less completely a "cartilage" bone—a good example of the type of evidence which has led morphologists to minimize the importance of the distinction between these two types of bone.

In the Amniota scapula and coracoid are replaced nearly or quite completely by bone. A clavicle like that of Amphibians develops in relation to the precoracoid in Reptiles except Crocodiles. In Birds what appears to be the same element (**furcula**) is widely separated from the coracoid, probably for mechanical reasons connected with flight, while a separate centre of ossification appears at the apposed ventral ends of the two bones. In Reptiles a somewhat similar element—the episternum—makes its appearance and is continued tailwards along the mid-ventral surface of the sternum and it is possible that in Birds the ossification lying between the ventral ends of the clavicles represents the anterior segmented off portion of this and the keel of the sternum the rest.

Pelvic Girdle.—The cartilaginous pelvic girdle becomes replaced by bone less or more completely without receiving any reinforcement from investing bones. The iliac, pubic and ischial portions ossify each from its own centre except in Amphibia where the pubic region remains cartilaginous.

In bony Teleostomatous fishes each half of the pelvic girdle becomes replaced by a plate of bone the morphological nature of which has been much discussed. Detailed studies of its development in a variety of different teleosts and in the more primitive ganoids are much needed.

LITERATURE

Agar. Trans. Roy. Soc. Edin., xlv, **1906.**

Albrecht, A. Entwickelung des Achsenskelettes der Teleostier. (Diss.) Strassburg, **1902.**

Braus. Morph. Jahrb., xxvii, **1899.**

Braus. Haeckelfestschrift (Jenaer Denkschriften, xi), **1904.**

Braus. Hertwigs Handbuch der Entwicklungslehre, iii, **1906.**

Budgett. Trans. Zool. Soc. Lond., xvi, **1902.**

Bunge. Entwickelungsgeschichte des Beckengürtels der Amphibien, Reptilien und Vögel. (Diss.) Dorpat, **1880.**

Carlsson. Anat. Anzeiger, xi, **1896.**

Carlsson. Anat. Anzeiger, xii, **1896*.**

Cords. Anat. Hefte (Arb.), xxxviii, **1909.**

Dohrn. Mitt. Zool. Stat. Neapel, v, **1884.**

Gadow. Phil. Trans. Roy. Soc., B, clxxxvi, **1895.**

Gadow. Phil. Trans. Roy. Soc., B, clxxxvii, **1897.**

Gaupp. Schwalbes Morph. Arbeiten, iii, **1894.**

Gaupp. Hertwigs Handbuch der Entwicklungslehre, iii, **1906.**

Gibson. Anat. Anzeiger, xxxv, **1910.**

Goeppert. Gegenbaurs Festschrift. Leipzig, **1896.**

Goette. Arch. mikr. Anat., xv, **1878.**

Goette. Arch. mikr. Anat., xvi, **1879.**

Goodrich. Quart. Journ. Micr. Sci., xlvii, **1904.**

Goodrich. Proc. Zool. Soc. Lond., **1908.**

Goodrich. Proc. Zool. Soc. Lond., **1913.**

Goodrich. Quart. Journ. Micr. Sci., lxi, **1915.**

Gregory. Biol. Bull., vii, **1904.**

Harrison. Arch. mikr. Anat., xlii, **1893.**

Hertwig, O. Jenaische Zeitschr., viii, **1874.**

Hertwig, O. Arch. mikr. Anat., xi, Suppl., **1874*.**

Howes and Swinnerton. Trans. Zool. Soc. Lond., xvi, **1901.**

Huxley. Todd's Cyclopaedia of Anatomy and Physiology, v, **1859.**

Kerr, Graham. Proc. Roy. Phys. Soc. Edin., xvii, **1908.**

Leche. Anat. Anzeiger, viii, **1893.**

Marsh. Odontornithes. Washington, **1880.**

Mehnert. Morph. Jahrb., xiii, **1888.**

Mehnert. Schwalbes Morph. Arbeiten, vii, **1897.**

Mollier. Anat. Hefte (Arb.), iii, **1894.**

Mollier. Anat. Hefte (Arb.), v, **1895.**

Nickerson. Bull. Mus. Comp. Zool. Harvard, xxiv, **1893.**

Rabl, C. Morph. Jahrb., xix, **1893.**

Rabl, C. Zeitschr. wiss. Zool., lxx, **1901.**

Röse. Anat. Anzeiger, vii, **1892.**

Röse. Anat. Anzeiger, viii, **1893.**

Röse. Anat. Anzeiger, ix, **1894.**

Schauinsland. Zoologica, xxxix, **1903.**

Schauinsland. Hertwigs Handbuch der Entwickelungslehre, iii, **1906.**

Schöne. Morph. Jahrb., xxxi, **1902.**

Semon. Forschungsreisen in Australien, i. Jena, **1893–1913.**

Sewertzoff. Kupffers Festschrift. Jena, **1899.**

Sollas, W. and I. Phil. Trans. Roy. Soc., B, cxcvi, **1903.**

Sonies. Petrus Camper, iv, 4, **1907.**

Suschkin. Nouv. Mém. Soc. des Naturalistes de Moscou, xvi, **1899.**

Tims, Marett. Quart. Journ. Micr. Sci., xlix, **1906.**

Traquair. Proc. Roy. Phys. Soc. Edin., xii, **1893.**

Versluys. Zool. Jahrb. (Anat.), xix, **1903.**

Wijhe, van. Comptes rendus 6$^{\text{me}}$ Congrès internat. Zool. 1904, Berne, **1905.**

Williamson. Phil Trans. Roy. Soc., cxl, **1849.**

CHAPTER VI

VASCULAR SYSTEM

As has already been indicated the vascular system of the animal body consists of strands of highly specialized mesenchyme—the cells (**corpuscles**) along the axes of the strands being detached from one another and floating freely in a fluid intercellular substance (**plasma**), while the superficial cells are united together to form the walls of tubular channels—the **vessels.** The vessel walls are provided with a coating of muscle-fibres and this muscular coat becomes greatly thickened and specialized at one or more points to form hearts which serve as pumps to force the blood through the system of vessels.

The fundamental plan of the Vertebrate vascular system appears to have been like that of an Annelid worm, with two main longitudinal blood-vessels, situated respectively one on the neural side of the alimentary canal and one on the side opposite to this, connected together by a series of half-hoop shaped vessels encircling the alimentary canal laterally. In the Vertebrate the longitudinal vessel on the neural side of the alimentary canal is the **dorsal aorta** and in it the blood runs in a tailward direction. The longitudinal vessel on the other (ventral) side of the alimentary canal develops the **heart** on its course: its precardiac portion is the **ventral aorta,** its postcardiac the **subintestinal vein.** In this ventral vessel the blood passes in a headward direction. Half-hoop shaped vessels lying in front of the heart and connecting ventral aorta and dorsal aorta are the **aortic arches.**

ORIGIN OF THE HEART AND VESSELS IN THE HOLOBLASTIC VERTEBRATES.—Amongst holoblastic Vertebrates the first steps in the development of the vessels have been investigated in the Newt (*Triton*) by Mollier (1906) and his account will here be followed.

In an embryo with six mesoderm segments the lateral sheets of mesoderm have met ventrally except in the region of the liver where they terminate in a free edge. This free edge is thickened and the thickening extends back along the mid-ventral line towards the cloaca as the rudiment of the subintestinal vein — the entire thickening having thus a Y-shape (Fig. 173, A).

At a stage with twelve segments this Y-shaped vascular rudiment is continued forwards as a couple of strands of cells, lying on each side on the inner surface of the splanchnic mesoderm and apparently derived from it. These are destined to give rise in their hinder portions to the two vitelline veins and in their anterior region to the first rudiments of the heart (Fig. 172, A, *enc*).

At a stage with fifteen segments the paired strands of cells have assumed a disposition like that shown in Fig. 173, B. They approach one another as the mesoderm extends downwards and presently fuse across the mesial plane (Fig. 172, B and C), the fused portion being the rudiment of the heart while the two anterior limbs represent the first (mandibular) pair of aortic arches and the two posterior limbs the vitelline veins. The heart rudiment is at first extremely short in an antero-posterior direction being much broader than it is long. This is correlated with the shortness of the foregut. As the latter lengthens the heart rudiment keeps pace with it, and becomes elongated (Fig. 173, D). As it does so the tissue within the rudiment becomes loosened and takes the form of a syncytial network with wide meshes.

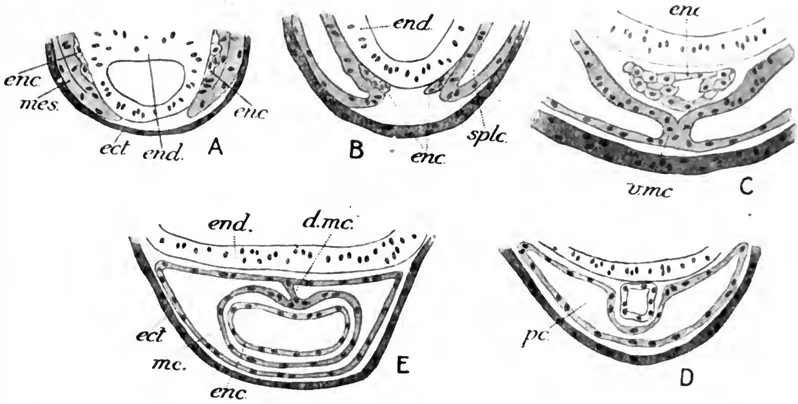


FIG. 172.—Ventral portions of transverse sections of young Amphibians to illustrate the development of the heart. (Based on figures by Mollier, 1906.)

A, B, D, E, *Triton* (A twelve segments, B sixteen do., D twenty do., E twenty-six do.); C, *Rana*. *d.mc*, dorsal mesocardium; *ect*, ectoderm; *enc*, endocardium; *end*, endoderm; *mc*, myocardium; *mes*, mesoderm; *pc*, pericardiac cavity; *v.mc*, ventral mesocardium.

In the meantime the mesoderm on each side, now containing a wide coelomic (pericardiac) space, has grown down to the mesial plane ventral to the heart, so as to give rise to a **ventral mesocardium** which however only persists for a short time (Fig. 172, C and D, *v.mc*). About this same period fluid begins to collect in the interstices between the cells of the subintestinal strand, with the result that some of the cells in its interior assume a spherical form and are recognizable as embryonic blood corpuscles. Mollier notes that about this stage the subintestinal strand comes into extremely close relation to the yolk-cells, there being in places apparently complete continuity between the two—hence the conclusion on the part of observers who did not study earlier stages that the vascular strand was actually derived directly from the endoderm.

About the stage with sixteen to eighteen segments the rudiments of the **Duct of Cuvier** and dorsal aorta become apparent, in the form of cells at first scattered and later joined into strands. The aorta cells anteriorly often show connexions with the sclerotomes and Mollier admits that some of them may actually be derived from the sclerotomes (see p. 364) though he considers that the main source of origin is the upper angle of the lateral mesoderm.

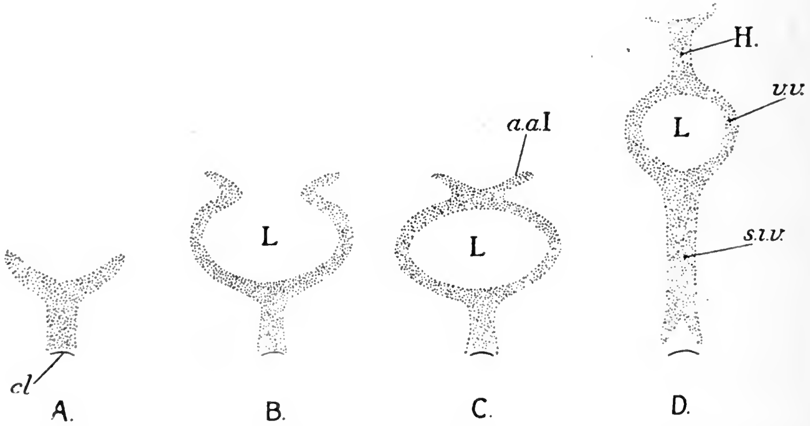


FIG. 173.—Rough diagram to illustrate the form of the early rudiments of heart, vitelline veins, and subintestinal vein in *Triton* as seen in plan. A, six mesoderm segment stage; B, fifteen segments; C, eighteen segments; D, twenty segments.

*a.a.*1, mandibular aortic arch; *cl*, position of cloaca; *H*, heart; *L*, position of liver; *s.i.v*, subintestinal vein; *v.v*, vitelline vein.

At the stage with twenty segments a network of fine channels has appeared over the surface of the yolk, between it and the mesoderm, foreshadowing the **vitelline network** of blood-vessels. The subintestinal strand has become still looser in texture and prolongations may be found passing from it inwards amongst the yolk-cells. The heart has now attained the form of a straight tube the protoplasmic strands in its interior disappearing while its superficial cells take on an endothelial character, and are recognizable as the **endocardium.** The splanchnic mesoderm has become closely moulded round it ventrally and laterally (Fig. 172, D) forming the rudiment of the **myocardium** and the latter begins to show contractions causing slight movement of the fluid contents of the heart.

By the twenty-seven segment stage the anterior limbs of the subintestinal strand have become definite (**vitelline**) veins with well-defined lumen filled with fluid in which spherical young corpuscles float freely. The large flat cells forming the wall are probably simply the modified superficial cells of the strand though Mollier thinks these may be reinforced by additional mesoderm cells from without. The vitelline veins are continued in front into the posterior venous limbs of the heart and the heart itself is seen in transverse sections (Fig. 172, E) to be now completely enclosed in myocardium, the inner wall of the pericardiac space having become moulded right over its dorsal side. Where the two sheets of mesoderm, one from each side, have met dorsal to the heart there still persists a septum—the **dorsal mesocardium** (Fig. 172, E, *d.mc*) which serves to sling up the heart to the ventral side of the foregut.

The dorsal aorta is at this stage particularly instructive. Posteriorly it is represented by scattered cells, lateral in position, thus betraying their lateral origin. Further forwards these have approached the mesial plane and form a pair of cellular strands. Further forwards still—in the region of the first eight segments—these have become still more nearly mesial in position and over part of their extent have undergone actual fusion to form the unpaired aortic rudiment.

About this stage the dorsal aortic rudiment is connected up to the vitelline network by a series of segmentally arranged vessels (segments 5-17) which had made their appearance about the twenty-segment stage as segmentally arranged strands of cells.

The rudiments of the ducts of Cuvier make their appearance even earlier than that of the dorsal aorta, in the form of cells derived according to Mollier from the somatic mesoderm at the cranial side of the pronephros. These rudiments develop extensions in a headward and in a tailward direction to form the **cardinal veins.** The vessels of the head region develop *in situ* from the mesenchyme and the same may probably be said of the smaller vessels generally.

The Crossopterygian fish *Polypterus* (Graham Kerr, 1907) is, apart from its generally archaic character, particularly suitable for the study of the first beginnings of the vascular system owing to the fact that the long axis of the embryonic body is straight, so that horizontal as well as sagittal sections may be made passing through practically the whole length of the dorsal aorta during its early stages, when in its hinder portion it has not yet taken definite form.

The first conspicuous stage in the development of the dorsal aorta consists in the collecting together of irregular multinucleate masses of yolky protoplasm in a row beneath the hypochord (Fig. 174, A). Vacuolar spaces develop in these masses and foreshadow the aortic cavity. The masses of protoplasm become more closely aggregated into a cylindrical shape while the vacuolar spaces increase enormously in size and eventually flow together to form the continuous aortic cavity. In the specimen figured in

Fig. 174, B, the cavity was perfectly continuous towards the head end, while posteriorly it was still in the form of isolated vacuoles.

The cells which form the rudiment of the dorsal aorta are from their coarsely-yolked character clearly derived ultimately from the primitive endoderm, but the question remains whether they are derived from the definitive endoderm directly or through the intermediary of the mesoderm. Such a section as that shown in Fig. 175 indicates that the latter is the case. The definitive endoderm shows a perfectly sharply defined surface, clearly marked off from the aortic rudiment, while the mesoderm of the sclerotome on the other hand is continuous with the aortic rudiment. We may say therefore with high probability that the aortic cells are derived from the sclerotome.

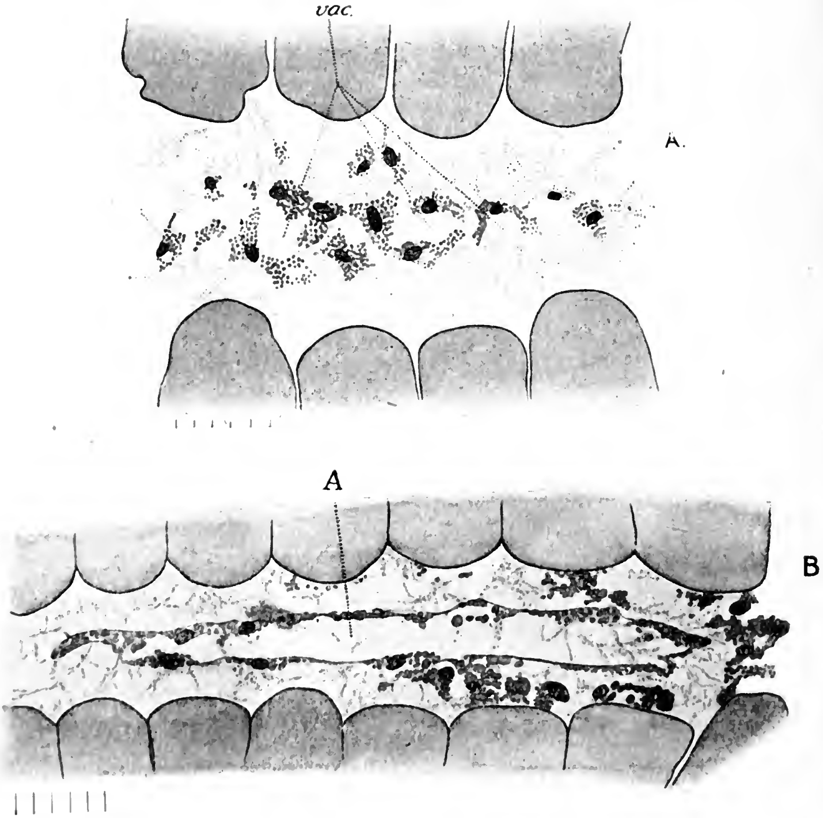


FIG. 174.—Portions of horizontal sections through *Polypterus* larvae of stages 21 + (A) and 25 (B) showing the rudiment of the aorta in longitudinal section.

A, aortic rudiment; *vac*, vacuoles.

A remarkable feature has been noticed in the development of the dorsal aorta of *Polypterus* which requires further investigation both in that genus and in any other Vertebrates in which it may be found to occur. In *Polypterus* in the stage immediately preceding that in which the aortic cells collect together the position of the future aorta is distinctly marked out by the arrangement of the delicate reticulum that is visible connecting up the various organ-rudiments of the larva. This reticulum is usually regarded as an artifact caused by the action of the fixing and preserving solutions upon the albuminous substances contained in the fluids of the embryonic body but the fact that it becomes arranged in this peculiar fashion to foreshadow the future aorta at once raises the question whether it is not really a reticulum of living substance.

The aortic cavity in *Polypterus* has been seen to originate by the fusion of intracellular vacuoles. The cavity is filled with clear fluid and this condition persists even after the main channels of the vascular system are laid down. The blood is at first simply fluid or plasma without corpuscles. This plasmatic condition may persist even after circulation has commenced and the heart propels through the vessels simply the clear cell-less fluid. Here we find repeated in ontogeny an extremely archaic condition of the circulation. The plasma becomes peopled with corpuscles comparatively suddenly.

The portions of vessel wall lying external to the lining endothelium appear to arise from mesenchyme cells.

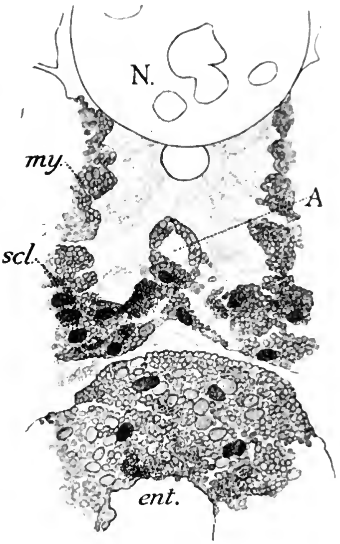


FIG. 175.—Portion of transverse section through *Polypterus* of stage 25 showing the relations of the aortic rudiment (*A*) to the sclerotome (*scl*).

ent, enteric cavity ; *my*, myotome ; *N*, notochord.

SOURCE OF THE CORPUSCLES.—The blood corpuscles are to be looked on, broadly speaking, as mesenchyme cells which have lost their connexion with their neighbours and float free in the plasma. Their precise sources in ontogeny appear to be various:—

(1) They can frequently be seen in process of being budded off by the wall of the embryonic blood-vessel into its cavity.

(2) In other cases the vessel with its contents is seen to arise as a solid mass of cells, those at the periphery becoming the wall (endothelium) of the vessel rudiment while those more deeply placed round themselves off, becoming separated by chinks containing fluid, and develop into corpuscles. This may be regarded as a

modification of (1) brought about by a hurrying on of the development of the corpuscles.

(3) In still other cases the cells of the mesenchyme reticulum in certain localities *e.g.* in the spaces between the tubules of the pronephros draw in their processes, round themselves off and are carried away in the blood stream as primitive corpuscles.

This last mode of origin may account for the fact that the corpuscles have been observed to make their appearance suddenly in large numbers in the circulating blood. The sudden setting free of large numbers of corpuscles is possibly due to an epidemic of mitosis in the mesenchyme cells, as it is well known that the onset of the mitotic process frequently induces a retraction of the processes of the cell-body and its assumption of an approximately spherical shape.

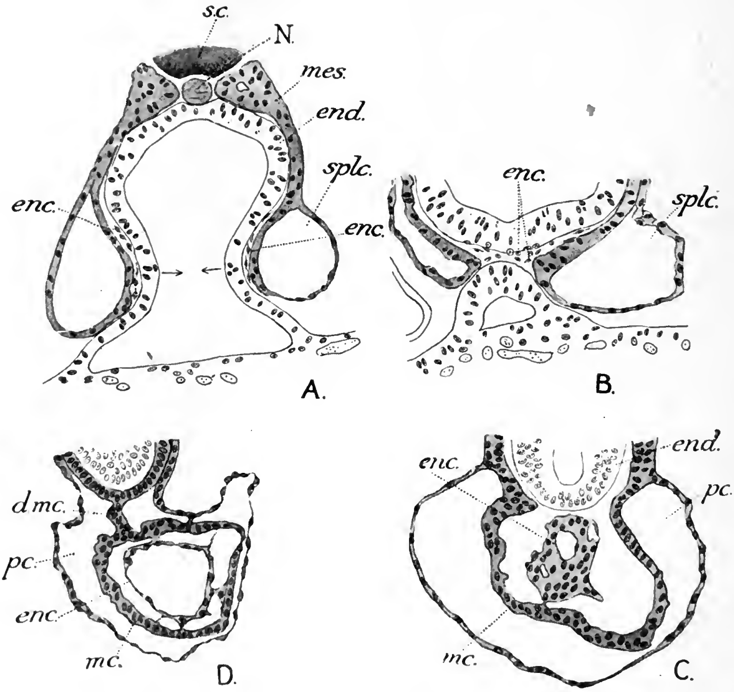


FIG. 176.—Portions of transverse sections through Elasmobranch embryos illustrating the origin of the heart.

A, *Torpedo,* stage with one gill cleft; B, *Torpedo,* stage with two gill clefts; C, *Pristiurus,* stage with twenty-five segments; D, *Pristiurus,* stage with forty segments. (After figures by Rückert (1888) and Mollier (1906).) *d.mc,* dorsal mesocardium; *enc,* endocardium; *end,* endoderm of foregut; *mc,* myocardium; *mes,* mesoderm; *N,* notochord; *pc,* pericardiac cavity; *s.c,* spinal cord; *splc,* splanchnocoele.

It is not proposed to trace out in this volume the further development of the blood corpuscles—how the originally similar indifferent corpuscles become differentiated with further development into specialized strains—Erythrocytes or Red corpuscles and the various types of Leucocytes; for an account of this in *Lepidosiren* the student may be referred to the beautiful memoir by Bryce (1905).

The mode of origin of the vascular system in the holoblastic vertebrates in general seems to resemble in its main lines that described above. The chief question that has given rise to discussion is one which in the opinion of the present writer resolves itself very much into a question of mere verbal expression—namely whether it is more correct to state that the first rudiments of the vascular system (or parts of them) are derived from mesoderm or from endoderm. When it is borne in mind that the mesoderm of the Vertebrate is essentially a derivative of the endoderm, it will be realized that the question is of minor importance whether or not special portions, such as rudiments of the vascular system in particular cases, lag behind the main mesoderm in their separation from the endoderm, so as to originate from the latter directly instead of from the already differentiated mesoderm.

ORIGIN OF THE HEART IN MEROBLASTIC VERTEBRATES.—The heart in its earliest stages shows in meroblastic vertebrates generally a set of conditions quite similar to those met with in holoblastic forms. In Elasmobranchs (Fig. 176, A) the first obvious rudiment of the heart is in the form of a number of cells of irregular shape which make their appearance on each side of the foregut, between it and the splanchnic mesoderm, from which latter they are apparently derived. As the foregut separates off from the endoderm of the yolk the irregular row of these cells shifts downwards and towards the mesial plane, so as to form a single elongated group underlying the foregut[1] (Fig. 176, B). The individual cells unite together and form a syncytial mass containing vacuolar spaces (Fig. 176, C, *enc*). Finally (Fig. 176, D) this elongated mass assumes a tubular form, its vacuoles coalescing and increasing in volume to form a wide cavity, while the protoplasm becomes thinner and forms the endothelial wall. The myocardium (*mc*) comes to surround the endothelial tube in exactly the way described for holoblastic forms.

In the Sauropsida again the phenomena are similar. To take as an example the Fowl: about the stage when two or three segments are formed isolated cardiac cells begin to appear on each side between the endoderm and splanchnic mesoderm. These cells increase in number and form a longitudinal tract on each side, the two converging and meeting anteriorly. As the foregut becomes constricted off the two cardiac strands come together from before backwards in front of the yolk-stalk though even at the eight-segment stage they are not completely fused. The endothelial rudiment now forms a loose syncytial spongework with large meshes containing

---

[1] Rückert (1888) believes them to be reinforced by cells derived from the ventral endoderm of the foregut.

fluid. The myocardium develops as in the other forms already mentioned.

ORIGIN OF THE PERIPHERAL BLOOD-VESSELS IN THE MEROBLASTIC VERTEBRATES.—In those Vertebrates which have meroblastic eggs the concentration of yolk in the highly modified ventral endoderm accentuates the need of an efficient transport system by which this food material may be taken up and conveyed to the yolkless and actively developing parts of the embryo. In accordance with this we find that such vertebrates show a precocious development of a rich network of blood-vessels over the surface of the endoderm or yolk and this vitelline network affords admirable material for the study of the earliest stages in the development of peripheral blood-vessels.

It will be convenient to consider in some little detail the early development of the vitelline network in the Fowl, material for the study of which is easily obtainable and which further has been worked out in detail by numerous investigators. Rückert has furnished an excellent modern account (1906) and upon it the following description is based: for fuller detail reference must be made to the original.

The first signs of blood-vessel formation make their appearance extremely early, at a stage when the primitive streak is present but hardly any trace, or only a small stump, of the so-called head-process at its front end. The mesoderm has at this stage spread slightly beyond the edge of the pellucid area, especially posteriorly. Round its posterior edge the mesoderm assumes a mottled appearance owing to the development in it of small cell condensations—the first trace of the **blood-islands** as they were called by Pander. These blood-islands are sometimes arranged in two separate series one on each side but more usually they form a U-shaped arrangement parallel and in close proximity to the posterior limit of the mesoderm.

In a slightly later stage the mottled area containing blood-islands — the **vascular area** — is of a U-shape, extending through about the posterior half of the extent of the mesoderm. The blood-islands are less conspicuous in front, gradually fading away, as they do also on the side next the primitive streak. They are most strongly marked towards the external margin of the mesoderm and here they, as well as the whole sheet of mesoderm, are being added to by delamination from the endoderm of the germ-wall. The blood-islands, the first rudiments of blood-vessels, are simply thickenings and condensations of the mesoderm. They at first have the appearance in sections of occupying its whole thickness but later it is seen that each blood-island is roofed over by a layer of unmodified mesoderm—demarcated apparently by a simple process of splitting-off of the superficial layer of cells.

As development goes on, the area of vascular rudiments spreads inwards into regions of the mesoderm which have been for some time completely separated from the endoderm by a well-marked split.

Blood-islands developing in such regions are therefore clearly derivatives of the mesoderm and there is no possibility of the endoderm playing a direct part in their formation as might be the case peripherally in the region of the germ-wall.

The vascular rudiments become joined up by strands of cells to form a network and this network gradually spreads inwards, its extension being brought about by a progressive differentiation *in situ* from the mesoderm: there is no actual sprouting inwards of the already formed strands of the network as is suggested sometimes by the study of whole blastoderms and as was once supposed to take place.

Of the network of cell strands which traverses the rudiment of the vascular area the bulkier portions give rise to masses of blood corpuscles surrounded by an endothelial wall, the more attenuated portions to endothelial tubes without any corpuscles in their interior. In the former case the superficial layer of the cells forming the blood-island becomes raised up from the main mass of cells, fluid accumulating beneath it in spaces which are at first isolated but later become continuous. The flattened cells which are raised up represent the endothelial wall while the main mass of cells left behind represent developing corpuscles. It is to be noted that the endothelial wall separates from the mass of corpuscles first below (*i.e.* on the side towards the yolk) and laterally, so that after fluid has accumulated in the rudimentary vessel the mass of corpuscles still remains attached to its, as yet undifferentiated, roof. The narrower strands between the main blood-islands and also all those in the pellucid area, except sometimes a few near its posterior end, give rise simply to endothelial tubes containing fluid plasma. As the circulation begins the masses of embryonic corpuscles gradually break up, first in the region of the sinus terminalis,[1] the individual corpuscles being whirled away by the current and carried to the heart and thence through the circulation.

The origin of the vitelline network has also been investigated in Elasmobranchs (especially *Torpedo*) by numerous workers. It agrees in its main features with what occurs in the Fowl.

As regards the peripheral vessels in general, of the Vertebrata, we may say that they take their origin as chinks within the mesenchyme filled with a clear fluid secretion (plasma). These chinks are at their first appearance in some cases clearly intercellular while in others they at first have the appearance of intracellular vacuoles. As has already been pointed out in dealing with connective tissue (p. 292) this difference though at first sight impressive loses most of its apparent importance when regarded critically. In this particular case the protoplasmic masses in which the vacuoles appear are as a rule multinucleate and it is clearly impossible to draw a sharp line of morphological distinction between spaces in such masses with

---

[1] The topography of the vascular area will be found illustrated later in the special chapter on the development of the Fowl.

partially broken down walls, and the ordinary intercellular spaces of syncytial embryonic connective tissue.

GENERAL CONSIDERATIONS REGARDING THE MORPHOLOGY OF THE VERTEBRATE HEART.—It may be regarded as a primitive characteristic of blood-vessels that their walls are contractile, peristaltic waves of contraction serving to propel the blood in their cavities. It is usually the case however in the more complex animals that this contractility becomes concentrated in one or more localized portions of the vessels, known as hearts, in which the vessel becomes much enlarged and its muscular coating thickened and rhythmically contractile.

In the craniate Vertebrates there is one heart present and it represents an enlarged portion of the ventral vessel in the region immediately behind the gills. During ontogeny the heart still repeats the archaic evolutionary phase in which it was tubular in character. As development goes on the primitive heart, or cardiac tube, shows rapid increase in size within the pericardiac chamber of the coelome in which it lies. This chamber is relatively small in size and in the lower, fish-like, Vertebrates is bounded by rigid unyielding walls. The confined nature of this space in which the heart has been evolved has, by imposing restrictions upon it during its increase in size, exercised a profound influence upon the modelling of the vertebrate heart. It is therefore desirable to have a clear idea of the general relations of the heart to the pericardiac cavity, during its increase in size, before attempting to study its development in detail in the various groups of Vertebrates.

The portion of vessel originally included between the anterior and posterior limits of the pericardiac cavity will be referred to here as the **primitive heart** or **cardiac tube.** As development proceeds the increase in size of the primitive heart reveals itself in (1) increase in length and (2) increase in diameter.

(1) As regards the former, the cardiac tube is at its posterior and anterior ends—where it enters and leaves the pericardiac cavity respectively—firmly embedded in the tissues of the pericardiac wall. These ends being consequently in the lower, fish-like, vertebrates rigidly fixed in position, it has of necessity come about that the cardiac tube, while in the course of evolution it has increased in length, has lost its original straight form and has been thrown into a system of bends or kinks which have had an important influence upon the structure of the fully evolved heart. This bending process is repeated, though with obscuring of some of its detail, during ontogeny and it is an interesting morphological problem to endeavour to unravel the details of the process from the data of comparative anatomy and embryology.

Apparently the primary flexure of the cardiac tube is represented by a simple loop or bulging towards the right side of the body, which is visible in the embryos of most Vertebrates during early stages of heart development.

With increasing growth in length of the cardiac tube this simple curvature becomes converted into a double flexure the heart taking on a S-shape. Of the two curves which make up the S one which has its concave side towards the head represents the original loop, while the other which is convex towards the head has developed in the portion of cardiac tube lying posterior to the primary loop. Of these two curves the one last mentioned, that which is morphologically posterior, is in an approximately vertical plane. The anterior or primary curve on the other hand shows much variation in position in different Vertebrates. While on the whole it still bulges towards the right side, as did the primary loop, the portion of it formed by the originally headward section of the tube comes in many cases to lie ventral to the other limb of the curve. In other cases this, originally anterior, portion of the tube lies for a time dorsal to the other, as is the case in *Salamandra*. The difference will be appreciated by comparing the relative positions of *c* and *V* in Figs. 184, A, and 178.

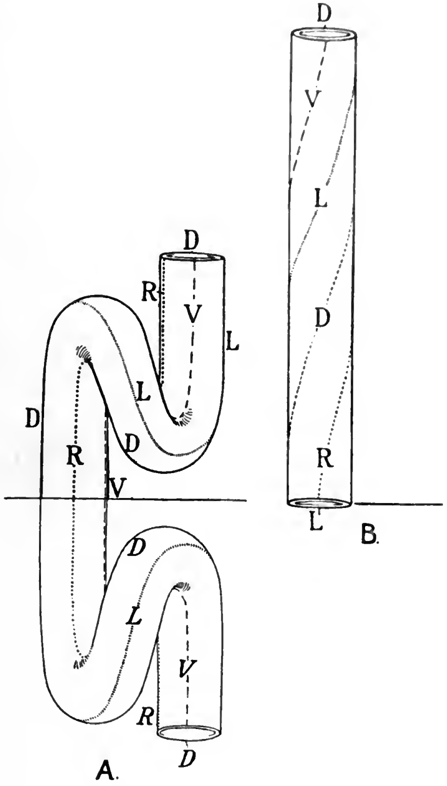


FIG. 177.—A, diagram to illustrate the flexure of the cardiac tube in the adult *Lepidosiren*, as seen from the ventral side. The portion of the diagram above the horizontal line represents the conus: the portion below the horizontal line would represent the rest of the heart on the assumption that this portion of the cardiac tube possesses a similar curvature to that of the conus. Longitudinal lines drawn along the tube mark the originally dorsal (*D*), ventral (*V*), right (*R*), and left (*L*).

B shows the spiral twisting produced by straightening out a tube possessing the same flexure as the conus portion in diagram A.

Of all the lower vertebrates in which the pericardiac space is still bounded by rigid inextensible walls it is the group of Lung-fishes that shows the heart at the highest level of evolution. And in correlation with this fact we find that in these fishes the kinking of the cardiac tube attains its maximum. In a fully developed Lepidosiren (see below, pp. 376-378) the anterior portion of the cardiac tube (the "conus arteriosus") has developed a further flexure in addition to those already described. The nature of this flexure is shown in the upper portion of Fig. 177, A, which represents diagrammatically the conus of *Lepidosiren* as seen from the ventral side.

The extreme anterior end of the tube, being fixed firmly at its exit from the pericardiac cavity, retains its primitive morphological position: its originally dorsal side is actually dorsal.

Traced backwards the tube is seen to become sharply bent upon itself in a headward direction, in such a way that the side of the tube which was originally on the left side comes to be ventral, as is indicated by the finely dotted line *L* in the figure. Tracing the tube onwards a second sharp flexure is found and the tube resumes its antero-posterior direction. This second flexure involves a complete reversal of the tube. Its originally right-hand edge (indicated by the coarsely dotted line *R*), which had come to be dorsal as a result of the first flexure, is now ventral.

The changes in the position of the tube caused by the two flexures may be summed up by saying that the half of the tube which was originally dorsal, and which remains dorsal at its anterior or headward end, has come to be situated on the right side at the posterior or ventricular end of the portion of the tube now under consideration (conus). Similarly the half of the tube which at the headward end is ventral, has come to be at the ventricular end on the left side.

The lower half of the diagram represents the portion of cardiac tube which gives rise to the main part of the heart and it is to a certain extent hypothetical, inasmuch as it does not rest on a complete series of observations, but it is clear that the morphologically right side of the cardiac tube, which is topographically ventral in the middle part of the figure, has to get back to its original right-hand position at the hinder end of the cardiac tube (which like the front end is firmly fixed in position), and it is reasonable to infer that the flexure of this portion, which gives rise to the atrium and ventricle, would be found, were its unravelling possible, to be symmetrical with the anterior flexure already dealt with.

It is interesting to take such a model as that represented in Fig. 177 and subject the conus portion to a process of straightening out—such as would happen in nature if the conus were to shrink in length, its anterior and posterior ends remaining fixed. The result is shown in Fig. 177, B. The conus assumes a twisting in a right-handed spiral through three right angles. In the Amniota it will be found that the representatives of the conus of the Lung-fish—the roots of the great arteries, pulmonary and systemic—as they pass headwards from the ventricular part of the heart, twist round one another in just such a spiral.

(2) As regards the increase in diameter of the cardiac tube, it is characteristic that this does not take place equally throughout. At

certain levels the increase in diameter is much less pronounced than it is elsewhere, with the result that the tube appears to be constricted at these points while it bulges out between them. This development of a series of dilated portions of the heart-tube is the first step in its segmentation into a series of chambers. Of these chambers there are typically in the lower vertebrates four—**sinus venosus, atrium, ventricle** and **conus arteriosus.**

Allusion must be made in passing to an unfortunate confusion of nomenclature which is apt to prove a stumbling-block in the way of the student who is trying to get his ideas clear regarding the morphology of the heart. The name conus arteriosus was first used, so far as the comparative anatomy of the lower Vertebrates is concerned, by Gegenbaur (1866) who used it to designate the structure lying between ventricle and ventral aorta in Elasmobranchs and Ganoids, and characterized by its possessing a muscular, rhythmically contractile wall and by its containing longitudinal rows of pocket valves. The name was introduced in order to accentuate the supposedly fundamental difference, already suggested by Johannes Müller (1845), between the structure in question and the **bulbus arteriosus** of Teleostean fishes. This latter is not provided with striped muscle in its wall, it is not rhythmically contractile: in other words it does not form *physiologically* a part of the heart. Objections, and quite valid ones, have been raised against the use of Gegenbaur's name from the side of Human anatomy, it being pointed out that the "conus" of the lower fishes corresponds rather with the "bulbus" of the human heart. Human anatomists working at the embryology of the vertebrate heart in consequence commonly use the name *bulbus cordis* for the part of the heart under discussion. Gegenbaur's name however has come to be so universally used by comparative anatomists in reference to the heart of the lower vertebrates as to indicate the desirability of using it in a work on comparative morphology such as this. It will be understood then that the name conus arteriosus is used in this volume as equivalent to what is by many writers termed bulbus cordis,[1] without prejudice however to the question whether or not Gegenbaur was justified in his belief that conus arteriosus and bulbus arteriosus are fundamentally distinct structures.

ELASMOBRANCHII.—The Elasmobranch heart passes through the typical early stages, first as a straight tube (see Chap. XI.), then as a tube which bulges towards the right side, and then as a tube with the characteristic S-shaped double flexure already alluded to (Fig. 178). The three limbs of the S during further development become converted into (1) atrium with sinus venosus, (2) ventricle, and (3) conus arteriosus. The well-marked constriction which demarcates atrium from ventricle forms the **auricular canal.** The progress

---

[1] I avoid in this book using the term *truncus arteriosus* as it is unnecessary and is liable to cause confusion owing to the want of precision with which it is commonly used.

towards the condition of the fully developed heart is marked mainly by the increase in relative size of the atrium and ventricle. Whereas however the increase in the size of the atrium takes the form mainly of a mere process of dilatation, that of the ventricle is accompanied by a much more marked thickening of its wall. This is brought about by the inner surface of the myocardium forming numerous projections into the lumen which, becoming more and more pronounced and interlacing and fusing with one another, form eventually a spongework and encroach considerably on the ventricular cavity round its periphery. The endocardium fits closely over the surface of each of these myocardiac trabeculae.

The physiological meaning of the formation of the trabeculae during the evolution of the ventricle probably lies in the fact that a bundle of muscle which has for its function the pulling together of the ventricular wall can carry out this function more efficiently if it runs straight between its two ends, in other words if it is in the position of a chord to the curve of the ventricular wall rather than simply a portion of that curve.

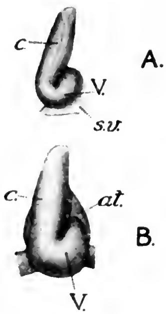


Fig. 178.—Two stages in the development of the heart of *Acanthias* seen from the ventral side. (After Hochstetter, 1906.)

at, atrium; *c*, conus arteriosus; *s.v*, sinus venosus; *V*, ventricle.

Attention must now be directed to a very characteristic and important proliferation of the endocardiac cells which makes itself apparent in particular regions. In the conus such proliferation takes place along the course of four longitudinal lines, giving rise to cells which lie in the space between endocardium and myocardium. As this proliferation goes on the endocardium is eventually made to bulge into the lumen as four prominent **endocardiac ridges.** In *Acanthias* (Gegenbaur, 1894), one of the four ridges—that which is ventral in position—is reduced in size.

In the auricular canal similar endocardiac proliferations take place, one upon the headward and one upon the tailward wall respectively of the canal. Here also each causes a prominent bulging of the endocardium into the cavity—the **atrioventricular cushion** (anterior and posterior).

Both the ridges of the conus and the atrioventricular cushions constitute a valvular apparatus in that, by the contraction of the myocardium lying outside them, they can be jammed together so as to occlude the lumen into which they project. In both cases, as development goes on, they undergo metamorphosis into a purely mechanical and automatic valvular apparatus. In the conus each ridge becomes excavated into a number of pocket valves ("semilunar" valves), the cavities of which open in a headward direction. Greil and others explain these cavities as being produced simply by the backward pressure of the blood but it is advisable to regard such simple mechanical explanations of developmental phenomena with suspicion. Similarly the atrioventricular cushions become excavated on their ventricular side and form the two atrioventricular valves of the adult. A pair of laterally placed valves also develop at the opening of sinus into atrium but according to extant descriptions (Röse, 1890) arise merely as infoldings of the heart wall.

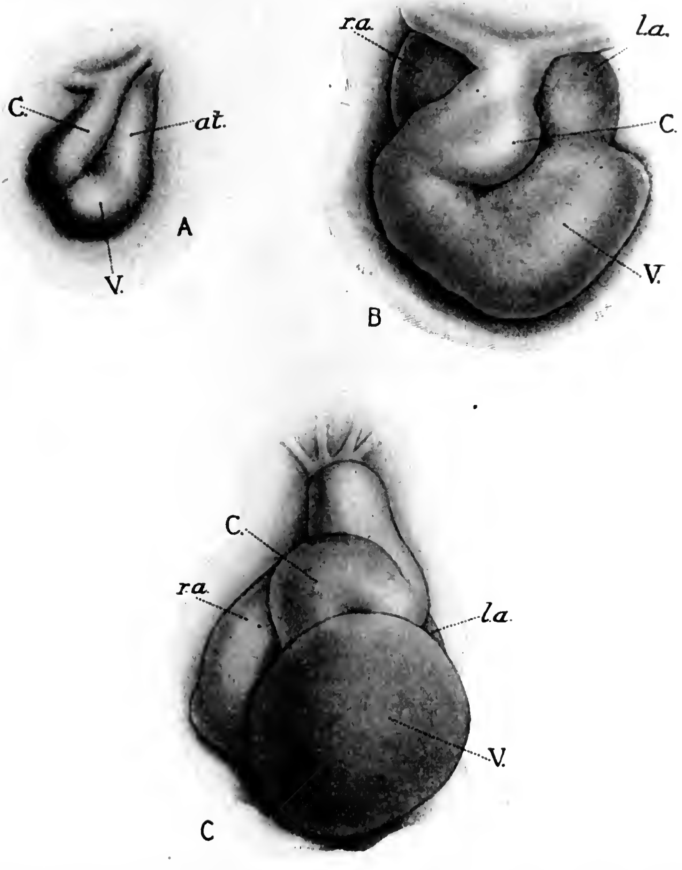


Fig. 179.—Views of the heart of *Lepidosiren* as seen from the morphologically ventral side. (B and C after J. Robertson, 1913.)

A, stage 32; B, stage 31; C, stage 35. *at*, atrium; *C*, conus arteriosus; *l.a*, left auricle; *r.a*, right auricle; *V*, ventricle.

Dipnoi.—The development of the dipnoan heart has formed the subject of a careful and exhaustive study by Robertson (1913) and her account of the development of the heart in *Lepidosiren* forms the basis of the following description.

The first peculiarity to be noticed in comparison with the heart of the Elasmobranch is correlated with the fact that the head and anterior trunk region of the embryo are bent downwards and closely applied to the surface of the yolk. As a consequence, the pericardiac space is reduced to a flat chink, the point of entrance and the point of exit of the cardiac tube being in close proximity to one another at its upper end. The result is that the cardiac tube as it grows in length assumes the form of a flattened loop, first V-shaped (Fig. 179, A) and later ϑ-shaped, the apex of the loop being directed in a ventral direction, and the originally posterior (tailward) limb of the loop (*at*) coming to lie to the left of the anterior limb (C). The cardiac tube becomes demarcated into the same set of chambers as in the Elasmobranch—sinus venosus, atrium formed from the posterior limb of the cardiac loop, ventricle formed from the apical portion of the loop, and conus formed from the greater part of the anterior limb. The dilatation of the walls of the several chambers is not uniform. In the case of the atrial wall the dilatation is most marked dorsally and more especially laterally: the posterior wall on the other hand lags behind in its growth and the result is that the sinu-atrial and atrioventricular openings remain comparatively close to one another (compare adult condition as shown in Fig. 180). Similarly in the case of the ventricular portion of the heart the increase in size is mainly on the ventral and lateral sides, the dorsal wall lagging behind so that the communication between atrium and ventricle and that between ventricle and conus also remain in close proximity (cf. again Fig. 180).

After the demarcation of the chambers there come about two important changes in the general form of the heart—the first is the assumption of bilateral symmetry on the part of the ventricle, correlated with a rotation of the ventral side of the ventricle towards the animal's left side. The other consists of a very marked increase in the length of the conus which, owing to the fixation of its two ends, is made to assume the characteristic double flexure already described and illustrated (see also Fig. 179, C).

DEVELOPMENT OF SEPTA IN THE HEART.—By far the most important feature of the Dipnoan heart, as compared with that of the Elasmobranch, is that now, for the first time, there comes about that separation of the heart into an arterial and a venous half, which is so characteristic of the higher Vertebrates. In *Lepidosiren* this separation is inaugurated at a relatively early stage of development (stage 27)—at a time when the cardiac tube, as yet, shows no indication of a division into chambers—by proliferation of the cells of the endocardium on its outer surface. This takes place along a line which passes along the posterior wall of the U-shaped heart from the left side of the sinu-atrial opening, through the auricular canal down towards the apex of the cardiac loop. As this proliferation goes on it causes the endocardium to bulge into the lumen so as to form a prominent ridge traversing the hinder wall of atrium and ventricle. It is of morphological interest to notice that this **atrioventricular ridge** extends from its dorsal end not directly ventralwards but towards the animal's right side, so that, if the ridge in question be taken as marking an originally longitudinal line along the wall of the cardiac tube, it indicates that this part of the cardiac tube has undergone a process of twisting like that of a left-handed screw, in other words a twisting of the kind which might be expected on the hypothesis that the flexure of the atrioventricular portion of the heart was originally that suggested in the discussion on p. 372.

A second endothelial proliferation takes place along the atrial wall facing that on which the atrioventricular ridge has developed. The projection formed in this way grows towards and fuses with the atrioventricular ridge to form the **atrial septum** which divides the atrium into a larger right and a smaller left **auricle.** Owing to the left-handed position of the atrioventricular ridge at its dorsal end the sinus opens into the right auricle. The pulmonary vein as it develops comes to open on the left face of the septum, *i.e.* into the left auricle.

The ventricle becomes similarly divided into a right and a left chamber[1] the foundation of the septum consisting of the atrioventricular ridge already mentioned. In this case however Robertson does not describe any endocardiac proliferation *vis-à-vis* to the ridge but says the septum is completed by muscular trabeculae growing towards and eventually fusing with the edge of the ridge.

The conus arteriosus is characterized by the development of a series of longitudinal endocardiac ridges similar in nature to those of Elasmobranchs. A conspicuous difference in detail is that each ridge is markedly discontinuous, the portions situated in the anterior and in the posterior section of the conus developing independently. We may take it that the ridges were primitively in the Vertebrata longitudinal and continuous and the secondary discontinuity visible in Lung-fishes and also in the higher Vertebrates may be associated with two probable causes: (1) interference with the development of the middle region of the conus by the flexure into which it is thrown, and (2) the tendency, as seen in Elasmobranchs and Ganoids, for the terminal members of the longitudinal rows of valves to become enlarged relatively to the rest. There can be no doubt that the longitudinal ridges as we see them in the Lung-fishes and the higher Vertebrates are revertive rather than persistent primitive features. In other words the ancestors of these Vertebrates passed through the phase of evolution in which each ridge had become converted into a row of pocket valves. This seems clearly indicated by the fact that the latter condition holds in modern Elasmobranchs and primitive Ganoids. But if so then the tendency for the terminal valves of the row to be specially developed in that earlier phase of evolution may show itself

---

[1] The separation does not normally become quite complete in the adult either in the case of atrium or ventricle.

on reversion to the ridge condition in more or less great suppression or diminution of the middle portion of the ridge.

In the anterior section of the conus four longitudinal ridges develop, situated respectively on the right-hand side (1), dorsally (2), on the left-hand side (3), and ventrally (4). This anterior end of the conus retains as already explained its primitive position and we shall therefore always refer to the four ridges according to the position they have in this undisturbed portion of the conus as Right, Dorsal, Left and Ventral respectively, the adverb morphologically being understood before the adjective in each case. The right and left ridges make their appearance first and they alone become prominent, forming thin shelf-like structures which project right in to the centre of the cavity so that their edges overlap. For a short distance at the extreme anterior end they become fused together so as to form a continuous septum. The left ridge is comparatively short, tapering off posteriorly, but the right extends back through the anterior and middle section of the conus. At the point of flexure between middle and posterior sections there is a break during early stages but later on the ridge becomes continuous with a portion of ridge which projects from the ventral wall of the posterior section of the conus. There is no reason to doubt that this is really part of the same morphological structure as that with which it is in line in the anterior section of the conus and we shall therefore term it the posterior portion of the right ridge. The whole of this right ridge forms what is often called the **spiral valve** of the conus.

The dorsal and ventral ridges of the anterior section of the conus are later than the lateral ridges in making their appearance and soon disappear again. The ventral ridge is especially feebly developed.

In the posterior section of the conus the right ridge—now ventral in position—is alone well developed. The other three appear as rudiments, they are at no time prominent and they become resolved into vestigial pocket valves which may still be detected in the adults. This latter fact justifies the conclusion already reached that during the ancestral history of the Lung-fishes a stage was passed through during which the conus was provided with longitudinal rows of functional pocket valves, in other words that the primitive ridges seen in the conus of the modern Lung-fish are revertive rather than persistent.

VALVES OF THE HEART.—The sinu-auricular opening is guarded on the right side by a valve. This develops out of the inpushed fold of the cardiac wall in the constriction between sinus and atrium. The atrioventricular opening is guarded by a highly characteristic bevelled plug (Fig. 180, *AV.p*) which when the ventricle contracts is pulled downwards so as completely to occlude the opening. Developmentally this plug arises as a thickening of the atrioventricular ridge. This ridge, which, as already indicated, forms the common foundation of auricular and ventricular septum, traverses the auricular canal, projecting into it from behind so as to give the atrioventricular opening a horse-shoe shape. It is the part which lies above (dorsal to) the opening which becomes thickened and eventually assumes a cartilaginous character to form the plug. The plug is to be regarded as the homologue of the posterior atrioventricular cushion of Elasmobranchs but Robertson failed to find any trace of an anterior cushion.

The conus in the completely developed state is characterized by the absence of the functional valvular apparatus found in its homologue in other Vertebrates. On the one hand the endocardiac ridges, functional in the young Elasmobranch or Ganoid, are no longer in a condition to fulfil their original function and on the other the pocket valves are here vestigial.

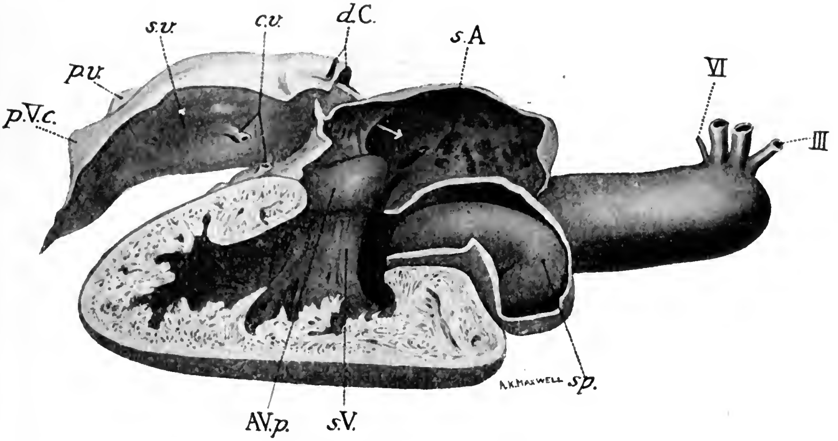


FIG. 180.—Heart of an adult Lepidosiren with the right side removed. (After J. Robertson, 1913.)

AV.p, atrioventricular plug; *c.v*, coronary vein (cut); *d.C*, ducts of Cuvier; *p.v*, pulmonary vein; *p.V.c*, posterior vena cava at its opening into the sinus venosus; *s.A*, atrial septum; *s.V*, ventricular septum; *s.v*, sinus venosus (its opening into the right auricle indicated by an arrow); *sp*, spiral valve; III, VI, aortic arches cut near their ventral ends.

MUSCULARIZATION OF THE HEART.—Bud-like projections from the myocardium grow into the cavity of the heart, meet together and in the ventricle form a muscular spongework of the same type as that seen in Elasmobranchs. In the case of the ventricle numerous trabeculae arising in this way converge upon the free edge of the atrioventricular ridge and become continuous with it. As development goes on this spongy mass of trabeculae undergoes condensation and acquires the solid character of the fully developed septum. The septum is continuous dorsally with the atrioventricular plug and it forms a muscular apparatus by which the plug is pulled down so as to fit into and close the opening. The myocardium of the auricular canal and of the conus never forms a spongework but remains as a compact layer of muscle. In the case of the conus this muscular coat is very feebly developed in the middle and cephalic portions—in which fact we may probably recognize a degenerative feature seeing that in the Elasmobranch the conus musculature is well developed right to its front end. The meshes of the ventricular spongework, as development goes on, come to spread somewhat round the auricular canal and round the ventricular end of the conus, so that each of these structures has the appearance of being drawn into the ventricular cavity.

Sauropsida.—The most exhaustive account of the development of a Sauropsidan heart is that dealing with *Lacerta* by Greil (1903) and upon it the following description is based. In its early stages the heart passes through the familiar tubular form and becomes bent, first bulging in a simple curve towards the right and then assuming a double S-like curvature just as in the Elasmobranch. About stage 17-18 the constriction of the heart into sinus venosus, atrium, ventricle and conus becomes apparent—the three last mentioned chambers bulging outwards between the grooves which limit them. The atrial portion does not in these early stages take up the purely dorsal position seen in the Elasmobranch or Lung-fish but remains for a time well to the left.

The conus, in its early stages, much reduced in relative size as compared with that of the Elasmobranch, undergoes a marked increase in length, which causes it to assume a bayonet-shaped curvature in which we may see a reminiscence of the sharp double flexure seen in the conus of the Lung-fish. In the Lizard however this curvature of the conus is merely temporary. As development goes on the increase in length of the conus instead of being more pronounced than that of the heart as a whole becomes less so with the result that between stages 21 and 26 the anterior flexure of the conus becomes pulled out and replaced by a right-handed spiral twist.

Development of Septa.—The septation of the heart is inaugurated by the appearance of localized proliferation of the endocardiac lining. In the auricular canal, which runs in an antero-posterior direction rather than dorsi-ventrally, owing to the atrium lying anterior to the ventricle instead of dorsal to it as was the case in the Lung-fish, there develop two endocardiac cushions, one dorsal (posterior), the other ventral (anterior). Of these the ventral or anterior one which was not apparent in *Lepidosiren* is well developed and is continued as an endocardiac ridge round the anterior (headward) wall of the atrium on to its roof (compare Fig. 183, C, *at.s*). As development goes on this projects more and more prominently into the cavity of the atrium and forms the main part of the septum between the two auricles. By about stage 26 it has grown half-way across the atrial cavity, and by about stage 29 it reaches the auricular canal. While in a sense the atrial septum is now complete it is not so physiologically as secondary perforations have made their appearance in the septum so as to keep the two auricular cavities in free communication. The two endocardiac cushions of the auricular canal become joined together by a bridge of endocardiac tissue which forms the free edge of the auricular septum. This is followed by a complete fusion taking place between the middle parts of the two cushions, so that the atrioventricular opening becomes completely divided into a larger right and a smaller left portion. The, at first, thick mass of tissue which separates these two openings becomes gradually converted into a thin plate, situated in the plane separating atrium from ventricle, and therefore perpendicular to the plane of the atrial septum. This plate is divided sagittally into a right and a left half by its line of attachment to the septum. The free edge of each half is concave and projects freely into the corresponding auriculoventricular opening—forming the mesial or septal valve of that opening.

In the meantime a new endocardiac cushion develops on what were the right and left sides of the auricular canal. These also become thin flaps and form the lateral auriculoventricular valves. It will be noticed that there have developed round the original atrioventricular opening *four* proliferations of endocardium—the same number as was found in the conus of the Lung-fish and as will be found in the conus of the Amniota, thus supporting the idea that there are four longitudinal endocardiac ridges potentially present throughout the cardiac tube of the higher Vertebrates though they may become actually apparent only in the conus region.

In the Lizard the atrioventricular ridge, which was so conspicuous in the Lung-fish, has practically become reduced to the portion lying within the auricular canal—the dorsal (posterior) endocardiac cushion. The ventricle is undivided.

The conus on the other hand undergoes a complete and somewhat complicated process of septation. This is inaugurated by localized proliferation of the endocardium to form longitudinal ridges. As in the Lung-fish these arise discontinuously there being distinct anterior (headward) and posterior rudiments. Anteriorly the normal four ridges develop, the dorsal and ventral appearing in this case at an earlier stage (17 or 18) than the lateral ones. Towards the ventricular end two ridges first make their appearance in a dorsal (ridge B, Greil) and ventral situation (A, Greil) respectively. Of these the ventral one becomes eventually continuous with the right-hand anterior rudiment. It clearly corresponds with the similarly situated ridge in the hinder portion of the conus of *Lepidosiren* and like it is to be interpreted as the hinder portion of the morphologically right-hand ridge. The ridge *vis-à-vis* to that just mentioned, here dorsal in position, would similarly represent the hinder portion of the morphologically left-hand ridge. Later on a small and transient ridge (C, Greil) makes its appearance on the right-hand wall of this hinder portion of the conus and this would represent the hinder portion of the morphologically dorsal ridge.

As development goes on the wall of the conus becomes changed in histological character, its striped muscles become replaced by smooth and in general it takes on the ordinary features of arterial wall so that it resembles a portion of the ventral aorta rather than of the heart. As may be seen in a living embryo this histological change is accompanied by a physiological one, for the rhythmic contractions of the heart are seen now to extend forwards as far as the anterior limit of the striped muscle but no farther. Altogether the superficial appearance is just as if the ventral aorta ("truncus arteriosus") were extending backwards at the expense of the conus, and the word truncus is frequently used to include the whole as far back as the limit for the time being of the smooth non-striated muscular wall. It must however not be forgotten that in the strict morphological sense all that part of the heart is conus which corresponds to the conus of *Lepidosiren*. The special criterion which identifies it is the appearance of double flexure or the resultant spiral coiling during its development. The muscular coating, so characteristic a feature in the vertebrates below the Amniota, is associated with a definite type of functional activity: in the Amniota that type of functional activity has disappeared and with it the characteristic type of wall.

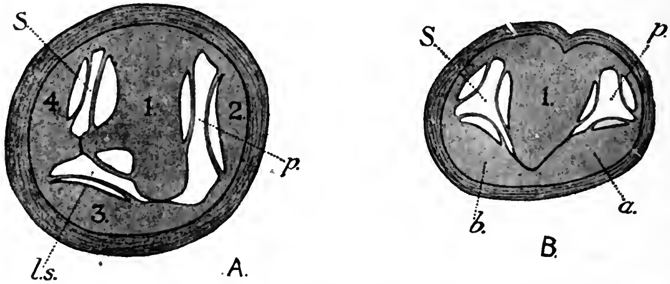


FIG. 181.—Diagrammatic transverse sections through conus of *Lacerta* (A) and *Gallus* (B) to show the endocardiac ridges and the pocket valves.

1, morphologically right ridge; 2, dorsal; 3, left; and 4, ventral. *a* and *b*, problematical ridges discussed in text; *p*, pulmonary cavity; *S*, main systemic; *l.s*, left systemic cavity.

The ventral aorta is in its hinder portion, where it becomes continuous with the front end of the conus, divided[1] into a dorsal (pulmonary) and a ventral (aortic) cavity by a horizontal septum and this is prolonged backwards along the wall of the conus by the right and left ridges. Of these the right is very large, it projects across the lumen and gradually fuses with the left ridge (Fig. 181, A). This ridge (3) is low and double and it is with its dorsal portion, *i.e.* the portion next the dorsal ridge, that the fusion takes place. By the spreading backwards of this process of fusion of the right and left ridges the horizontal septum of the ventral aorta becomes prolonged back as a septum in the conus—no longer horizontal

---

[1] See below, p. 393.

however but spirally twisted owing to the twisting of the conus already mentioned. Parallel with and preparatory to this process of fusion the distal ridge rudiments spread backwards, pursuing a spiral course and thus making evident the spiral twisting of the conus as a whole. It is interesting to notice that the line of insertion of the ridges, and therefore of the septum formed by their fusion, becomes marked on the outer surface of the conus by a distinct incision—a preliminary step towards the complete splitting of the conus in the plane of the septum which takes place in Birds and in Mammals.

In addition to the dividing of the cavity of the conus into a pulmonary and an aortic portion in the manner just described there takes place also, in the Lizard, a splitting of the aortic portion into two parts, corresponding to the right and left halves into which the systemic portion of the ventral aorta is divided.[1] The septum separating these becomes prolonged backwards at its hinder end, on the one hand, into the ventral ridge of the conus (Fig. 181, A, 4) and on the other into a quite similar ridge developed upon the surface of the septum which separates the aortic from the pulmonary (Fig. 181, A, 1). These two ridges facing one another across the aortic cavity gradually extend backwards and undergo fusion just as in the other case so as to form a complete septum dividing the aortic cavity into two (*S* and *l.s*).

In this way then the original conus becomes replaced by a set of three tubes twisted spirally round one another, forming the roots of the two systemic aortae and of the pulmonary artery, still however enclosed in a common wall.

VALVES OF THE HEART.—The right and left valves which guard the opening from sinus into atrium are formed simply by the exaggeration of the fold of the cardiac wall which delimits these two chambers from one another. The origin of the auriculoventricular valves has already been described. The pocket valves of the systemic aortae and pulmonary artery are derived from the endocardiac ridges of the conus as in the Elasmobranch. According to Langer (1894) the outer valve in each of the three vessels (pulmonary artery, left systemic aorta, right systemic aorta) are derived from the Dorsal, Left and Ventral endocardiac ridges (2, 3 and 4) respectively, while the inner valve in all three is derived from the hypertrophied Right ridge (1), which with its outgrowth takes part in the formation of all three vessels (Fig. 181, A).

The question as to whether or not the pocket valve is formed from the extreme ventricular end of the conus ridge, or whether on the other hand a considerable portion of this end of the conus with its contained ridges becomes incorporated in the ventricle as maintained by Langer and Greil does not appear to the present writer to be satisfactorily settled. It is advisable that the point should be re-investigated upon abundant material.

GALLUS.—(Figs. 182 and 183.) The most detailed investigations

---

[1] See below, p. 393.

of the development of the Bird's heart are those of Greil, of which unfortunately there is available so far only the abstract given by Hochstetter (1906). As we should expect from the close genetic relationship between Birds and Reptiles there is a close correspondence between the general features of the development of the heart in the two cases. It will suffice then to draw attention to the more important points in which the development of the Fowl's heart has been found to differ from that of *Lacerta*. So far as external form is concerned the most striking difference is that the sinus venosus loses its identity as a distinct chamber of the heart. It becomes as it were incorporated in the right auricle, all except its left portion which persists as the cardiac end of the left duct of Cuvier or anterior Vena Cava.

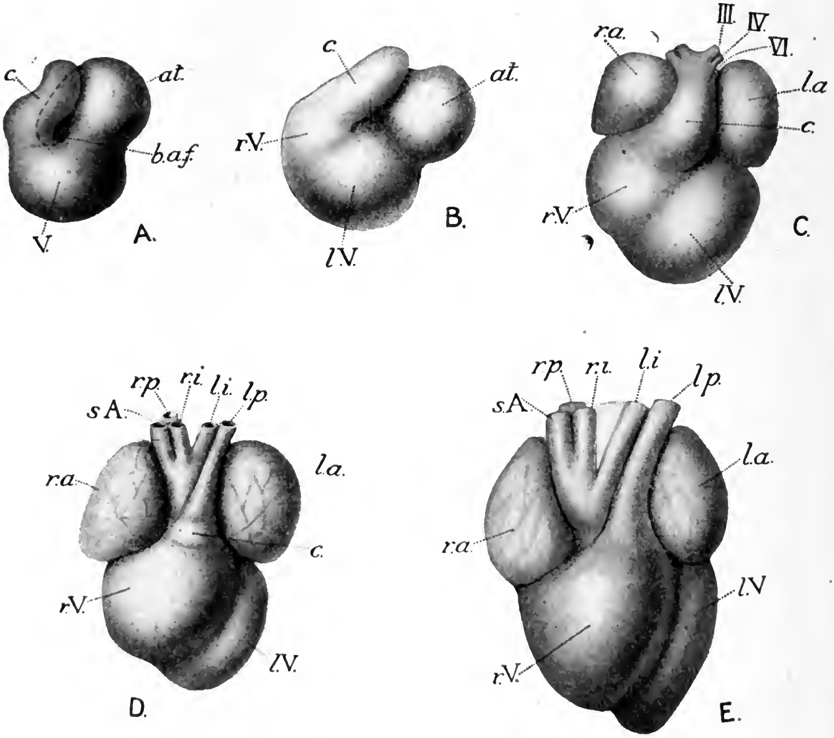


FIG. 182.—Illustrating the development of the heart in the fowl. (After original drawings by Greil.)

at, atrium; *b.a.f*, bulbo-auricular fold; *c*, conus; *l.a*, left auricle; *l.i*, left innominate artery; *l.p*, left pulmonary; *l.V*, left ventricle; *r.a*, right auricle; *r.i*, right innominate artery; *r.p*, right pulmonary; *r.V*, right ventricle; *s.A*, systemic aorta.

An important advance upon the condition in *Lacerta* is found in the division of the ventricular part of the heart into a right and a left ventricle. This division comes about in a somewhat complicated fashion the chief points in which, judging from Greil and Hochstetter's descriptions and figures, appear to be as follows. The ventricular portion of the cardiac tube is at an early stage encroached upon by the deep ("bulbo-auricular") fold which separates the conus

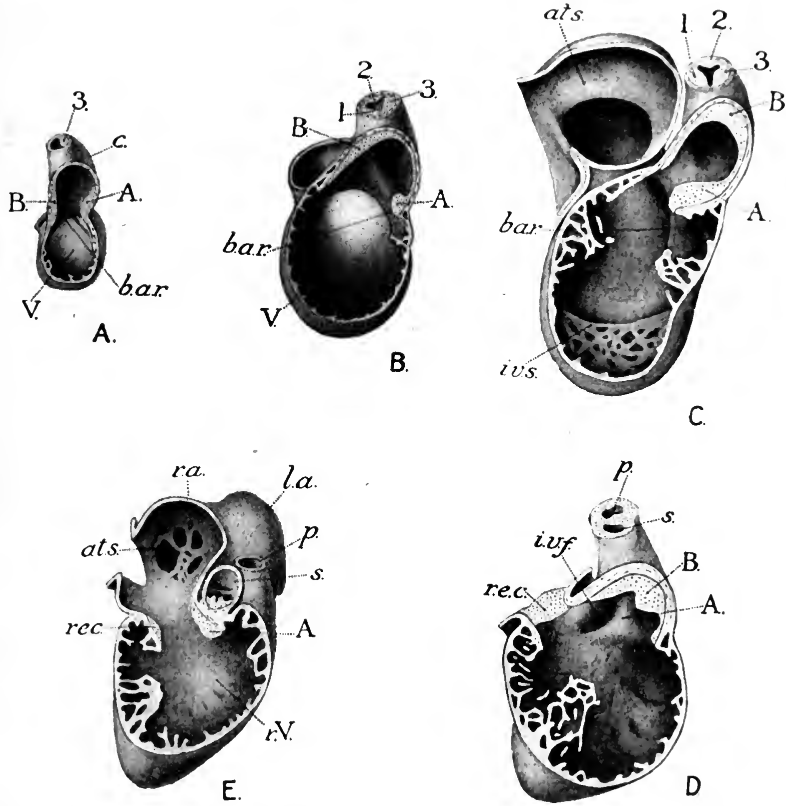


FIG. 183.—Stages in the development of the heart of the Fowl, viewed from the right side. The right wall of the heart has been removed in each case. (After original drawings by Greil.)

A, *B*, proximal ends of ridges of conus; *at.s*, atrial septum; *b.a.r*, bulbo-auricular ridge; *c*, conus arteriosus; *i.v.f*, interventricular foramen; *i.v.s*, trabecular portion of ventricular septum; *l.a*, left auricle; *p*, pulmonary artery; *r.a*, right auricle; *r.e.c*, cut surface of endocardiac tissue of atrio-ventricular opening; *r.V*, cavity of right ventricle; *s*, systemic aorta; *V*, ventricle; 1, 2, 3, conus ridges.

from the atrial part of the heart (Fig. 182, A, *b.a.f*). The encroachment of this fold gives the ventricular part of the tube a squat U-shape. As the ventricle dilates the extent of the encroachment becomes reduced and the fold may now be called the bulbo-auricular ridge of the heart-lining (Fig. 183, A, *b.a.r*). During the fourth day this becomes extended tailwards along the ventral wall of the ventricle (Fig. 183, B, C, *b.a.r*) the ridge and its extension forming the rudiment of the anterior portion of the ventricular septum. The remaining and larger portion of the septum arises otherwise, from a local exaggeration of the muscular trabeculae which in the Bird, as in lower forms, sprout into the ventricular cavity so as to convert the peripheral portion of that cavity into a sponge-work. This sponge-work becomes exaggerated in the prominence and thickness of its trabeculae along a plane marked on the external surface of the heart by a distinct groove—the **interventricular groove** (Fig. 182, C). This trabecular part of the septum (Fig. 183, C, *i.v.s*) is at first loose and spongy but it gradually becomes condensed, at first along its thickened free edge, and loses its spongy character. It gradually extends forwards and becomes continuous on the one hand with the bulbo-auricular portion and on the other with the septum of the auricular canal. The ventricular cavity is now divided into a right and a left chamber except at its anterior end where there remains an interventricular foramen (Fig. 183, D, *i.v.f*). It will be realized that but for the presence of this foramen the blood could not circulate, as the only means of exit from the ventricle—the opening leading into the conus—lies completely on one side (right) of the original bulbo-auricular fold and, therefore, of the ventricular septum of which the fold in question forms a part. As a matter of fact this interventricular foramen never disappears, though it loses its right to that name, for it becomes continued as a groove over the surface of the mass of endocardiac tissue lying between it and the conus. Eventually this groove becomes overgrown by its edges and converted into a tubular channel, continuous on the one hand—through the original interventricular foramen—with the cavity of the left ventricle and on the other with the systemic or aortic cavity of the conus. This tubular channel persists in the adult condition—as the communication between left ventricle and systemic aorta.

Finally, before leaving the interventricular septum, it has to be mentioned that dorsally (Fig. 183, D) it becomes continuous with the bridge of endocardiac tissue which divides the atrioventricular opening into a right and left half, the ventricular side of this bridge growing out to meet the trabecular part of the septum. The atrial side of the bridge is continuous with the atrial septum, which develops here as in *Lacerta*, and the result is that the main part of the heart is now divided into two halves, the left auricle opening into the left ventricle and the right auricle opening into the right ventricle (Fig. 183, E). It is to be noted however that secondary perforations appear in the atrial septum (Fig. 183, E, *at.s*)—so as to allow the systemic blood which enters the right auricle from the sinus venosus to reach the left auricle, and through it the left ventricle, without having to traverse the pulmonary circulation during the period before the lungs are functional.

It will be noticed in Figs. 182, C and D that in the later stages of its development the ventricular portion of the heart undergoes a certain amount of rotation, the right ventricle becoming displaced somewhat towards the left side, ventrally to the left ventricle. The result of this is to undo to a small extent the spiral twist of the conus.

The conus of the Fowl develops typical endocardiac ridges, here however only three in number, and the individual ridges retain a more nearly primitive condition in that in early stages they are not so completely divided into two distinct rudiments, while in later stages two of the three are obviously continuous. One of these ridges is clearly the morphologically Right. Here again it is much enlarged (Fig. 181, B, 1) and grows right across the cavity to form a complete septum between the pulmonary (*p*) and systemic (*S*) portions of the cavity.

Regarding the identity of the two other ridges there is some doubt. They are identified by Greil as the Dorsal (2) and Left (3) while the Ventral (4) is supposed to have disappeared. It appears to the present writer however that the possibility should be considered whether they do not together represent the Left ridge, with which in *Lacerta* the free edge of the enlarged Right ridge comes in contact and which in the latter animal shows an incipient division into two parts by a longitudinal groove.

The septum formed by the enlarged Right ridge follows a spiral course, its line of insertion being indicated by a spiral groove on the outer surface of the conus. In the Bird this groove gradually deepens into a slit which splits the septum into two halves and as a consequence divides the conus into two separate vessels which course spirally round one another—the roots of the pulmonary arteries and the systemic aorta respectively. No vestige of a septum subdividing the aortic cavity has so far been described.

VALVES.—Pulmonary artery and systemic aorta are each provided with three pocket-valves at their ventricular end. These arise in the manner indicated in Fig. 181, B. Each vessel receives a valve split off from the enlarged Right ridge. The pulmonary and the systemic cavities receive further a valve split off from the endocardiac thickenings marked *a* and *b* respectively. Following Greil these would be attributed to the Dorsal and Left ridges, while accepting the alternative interpretation suggested above they would both be referred to the morphologically Left ridge. There remains a third pocket-valve in each cavity. That in the systemic cavity no doubt represents the otherwise missing Ventral ridge, while if *a* and *b* together represent the Left ridge then the third pocket-valve of the pulmonary cavity would represent the Dorsal ridge. As there is no reason to doubt the reliability of a pocket-valve as evidence of a once existing endocardiac ridge we should be driven—if we reject the explanation here suggested—to assume the former existence of an additional ridge between the Right and the Dorsal and there seems no justification otherwise for doing this.

The pocket-valves are stated not to develop at the extreme hinder limit of the ridges, the septum stretching back beyond them to become continuous with the interventricular septum.

In the right auriculoventricular opening the inner or septal valve is not developed, the ventricular septum fusing with what in *Lacerta* becomes converted into the valve in question.

The main features of heart development having been illustrated from these three different groups, Elasmobranchii, Dipnoi and Sauropsida, it will be convenient now to indicate the more important peculiarities which have been detected in other groups of the lower Vertebrates. It should be understood however that in the case of several of these, such as Cyclostomes, Ganoids and even Amphibians, apart from Urodeles, our knowledge is still fragmentary.

In *Polypterus* (Graham Kerr, 1907) the cardiac tube when in the form of a loop shows a similar displacement to that which occurs in *Lepidosiren*—the lower end of the loop being pushed forwards in front of the yolk. In this case however the displacement has gone farther than in the Lung-fish so that the cardiac loop is completely inverted—its apex being directed forwards, while the ventral aorta passes off in a tailward direction. A similar displacement occurs in Teleosts.

The conus of Ganoid fishes shows the usual endocardiac ridges which become converted into longitudinal rows of pocket-valves as in Elasmobranchs. In *Polypterus* these ridges are six in number, alternate ones being much reduced in size, with the result that in the adult three rows of large pocket-valves alternate with three rows of small ones. In all these fishes the endocardiac ridges and their resultant rows of pocket-valves run straight along the conus and there is no reason to doubt that this is the primitive condition. To determine the primitive number of the ridges in Fishes more research is needed although there is little doubt that four was the number present in the primitive Tetrapods.

In the Teleostean fishes we find in place of the conus arteriosus the structure known as the aortic bulb. As already indicated (p. 373) this is distinguished from the typical conus by well-marked histological and physiological differences. And it is frequently regarded as being morphologically a part not of the heart but of the ventral aorta.

If however we take, as we are probably justified in doing, the point of exit from the pericardiac cavity as being relatively fixed and as marking the headward limit of the cardiac tube or primitive heart, then it becomes clear that the aortic bulb, lying as it does within the pericardiac cavity, is really a portion of the primitive cardiac tube and of that part of it which lay between the ventricle and the ventral aorta—in other words the conus arteriosus. What has happened in the evolution of the Teleostean heart is in all probability entirely analogous with what has taken place in the

Amniota, namely that the conus arteriosus has gradually lost its power of rhythmic contraction while *pari passu* its myocardiac coating of striped muscle has degenerated and its primitive histological characteristics have been replaced by others resembling more closely those of the ventral aorta.

During ontogeny it would appear from Hoyer's work on *Salmo* (1900) that the conus in the embryo possesses the characteristic features—a layer of striated muscle in its wall, and longitudinal ridges (two in number) projecting into its lumen—and differs from that of an Elasmobranch merely in the fact that these features do not extend throughout the whole of the distance between the ventricle and the anterior limit of the pericardiac space, but only through about the posterior half of that distance. In the adult the two ridges are represented by the two pocket-valves.

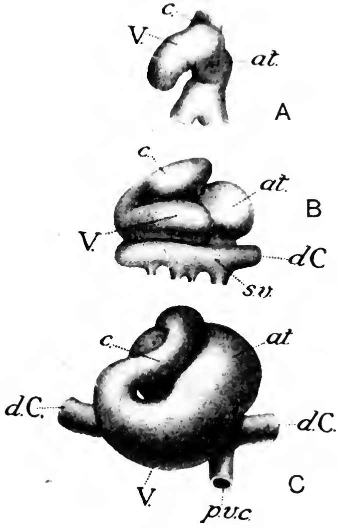


Fig. 184.—Views of developing heart of *Salamandra* as seen from the ventral side. (After Hochstetter, 1906.)

at, atrium; *c*, conus arteriosus; *d.C*, duct of Cuvier; *p.v.c*, posterior vena cava; *s.v*, sinus venosus; *V*, ventricle.

In Urodele amphibians (*Salamandra*—Hochstetter, 1906) the heart during the period when it is in the form of a tube with an S-like curvature is conspicuously different in appearance from that of the Vertebrates already described, owing to the fact that the two curves of the S lie in different planes from those which they occupy elsewhere. The morphologically posterior or tailward curve lies here nearly in the horizontal plane while the anterior curve lies in a nearly vertical plane—the limb of the curve which will become conus lying dorsal to the ventricular portion, so that it is hidden in a view of the heart from the ventral side (Fig. 184, A). Special interest is lent to this curvature of the atrioventricular portion of the cardiac tube by the fact that it reproduces accurately the type of curvature which we inferred as being present in this portion of the vertebrate heart in our general discussion of its morphology (compare Fig. 184, A, with lower portion of Fig. 177).

As development proceeds the left-hand end of the ventricular portion, *i.e.* its actually headward end, swings ventrally and tailwards so that the long axis of this portion of the heart comes to be perpendicular to the sagittal plane of the body (Fig. 184, B). As the ventricular part of the heart shifts backwards the conus becomes visible in a view from the ventral side. The backward shifting continues until the ventricle comes to lie ventral to the sinus (Fig. 184, C) instead of well in front of it as it did originally. The ventricle is now on the tailward side of the atrium which bulges out on each side, its ventral wall fitting closely to the dorsal side of the conus. The sinus becomes marked off from the atrium by a constriction, which deepens most markedly on the right side so that the sinu-atrial opening becomes displaced towards the left. It is to be noted that during these stages in development the portion of the ventricular wall lying on its concave (anterior) side undergoes relatively very slight increase in size. The result is that the ventricle bulges in a tailward direction and the openings by which it communicates with atrium and conus respectively remain relatively closely approximated together.

The atrial septum arises as a ridge or fold of endocardium which, as in the Sauropsida, projects into the lumen from the anterior (headward) wall. The concave free edge of this grows towards the atrioventricular opening while its base of attachment spreads, on the one hand ventrally until it becomes continuous with the anterior atrioventricular cushion, and on the other dorsally and backwards, to the left of the sinus opening and to the right of the pulmonary vein opening, till it becomes continuous with the posterior (tailward) atrioventricular cushion. The conspicuous openings in the atrial septum known to exist in adult Urodeles though not in Anura are secondary perforations.

The conus develops discontinuous ridges. Of these there are four anterior rudiments of which the Dorsal and Ventral develop first and the Right and Left later. Of posterior rudiments only three have been described but that the missing one is at least sometimes present is shown by the adult arrangements of the resulting pocket-valves in different Amphibians. Thus four pocket-valves have been observed in the posterior circle in specimens of *Siren, Necturus,* the Axolotl, and *Salamandra* (Boas). In the anterior circle cases are known of one of the four valves being reduced in size (*Siren,* Axolotl, *Triton, Salamandra*), vestigial (*Pipa*), or gone entirely (*Proteus, Rana*). Other cases occur in which an additional valve makes its appearance through one of the original ones becoming split (*Necturus*). All these variations in the pocket-valves of the adult are of importance in relation to the embryonic ridges which the valves represent. Details will be found in Boas (1882).

Of the anterior rudiments the right-hand ridge (1) is prolonged backwards as the spiral fold which projects across the lumen and divides it imperfectly into an aortic and a pulmonary cavity. In some cases the spiral fold apparently makes an abortive attempt to pursue the course of development which it went through in the ancestral fish-like form, as it segments up into a row of little knobs each of which, we may take it, represents a pocket-valve (*Triton punctatus, T. cristatus*). In other cases (*Necturus, Coecilia*) the spiral fold has in the adult completely disappeared (Boas, 1882).

The hearts of Reptiles in general agree closely in their developmental features with that of *Lacerta*. The most important variations are seen in the Crocodiles (Hochstetter, 1906*). The conus here is of interest in that it still repeats with particular clearness the sharp double flexure seen in the Dipnoan (Fig. 185).

The ventricular portion of the heart becomes completely divided into a right and left ventricle by a septum which is formed for the most part from trabecular projections of the myocardium but in part also from the endocardiac bridge which divides the atrioventricular opening as in the Bird. This septum becomes quite complete, the interventricular foramen which exists for a time closing up and the interventricular septum becoming continuous with the aortic septum of the conus. This is rendered a possible physiological arrangement in the crocodile by the fact that here the opening from the ventricle into that cavity of the conus which is continuous with the right systemic aorta, is farther to the left than in Lizards, while on the other hand the right atrioventricular opening is farther to the right. The result is that the opening of the right systemic aorta and the left auriculoventricular opening lie to the left of the septum, while the openings of the left aorta and pulmonary artery together with the right auriculoventricular opening lie to the right of the septum. Such differences in the position of the ventricular openings of the great arteries are regarded by Hochstetter as due to varying degrees of incorporation of the obliquely placed conus into the ventricle.

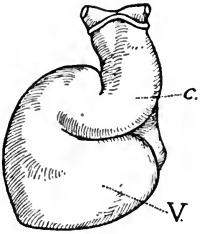


FIG. 185.—Heart of embryonic Crocodile. (After Hochstetter, 1906*.)

c, conus arteriosus; *V*, ventricle.

The **foramen of Panizza**, that remarkable communication which exists in the crocodile between the two systemic aortae close to their point of exit from the ventricles, arises as a secondary perforation of the aortic septum comparatively late in development just before the closing of the last remains of the interventricular foramen.

The splitting up of the conus into independent vessels remains as a rule in the Reptiles, as in *Lacerta*, in an incipient condition, indicated merely by a slight grooving of the surface. In Ophidia however the superficial groove becomes so deepened as to split the conus completely into an independent aortic and pulmonary root as in Birds.

Passing in review the broad features of heart development in the lower Vertebrates the following general principles seem to emerge:—

(1) The primitive heart or primitive cardiac tube is that portion of the ventral or subintestinal vessel included within the limits of the pericardiac coelome.

(2) Annular segments in the wall of this tube lag behind in increase of diameter so that the tube becomes constricted into a

series of chambers—sinus venosus, atrium, ventricle and conus arteriosus—the originally peristaltic waves of contraction tending to become reduced at each constriction so that the chambers come to contract in series.

(3) The primitive valvular apparatus consists of a series of longitudinal ridges. These are best marked in the conus where they were originally at least four in number. The presence of six in *Polypterus* and the occurrence of more than four rows of valves in various other relatively primitive Ganoids and Elasmobranchs suggests that the number may have been greater. Individual ridges may in various forms become reduced or disappear, while in other cases apparently an increase in number may take place. The ridges show very generally a tendency to develop from two distinct rudiments, an anterior one and a posterior one, but the continuity of the rows of valves in the lower fishes indicates the probability that this discontinuity is secondary. In the portions of the heart behind the conus there is no complete series of ridges like those in the conus though it is possible that vestiges of such ridges are represented by the endocardiac cushions and septal rudiments.

(4) Using the ridges of the conus as marking morphologically longitudinal lines it is seen that the conus has in the Lung-fishes undergone a double flexure of such a kind as to produce when straightened out a right-handed twist of the conus through approximately three right angles.

(5) In tetrapodous Vertebrates the conus shows a similar right-handed twist and this is adequately explained by the relative reduction in length which the conus has undergone if it be assumed that there was an ancestral condition resembling that of the existing Dipnoan.

(6) From the Lung-fishes upwards the originally right-hand ridge of the conus becomes hypertrophied and, either alone or by fusion with its *vis-à-vis*, forms a longitudinal septum dividing the cavity of the conus into two parts—pulmonary and aortic—twisted spirally round one another.

(7) Correlated with this process is a tendency for the wall of the conus to lose its striped muscle and its rhythmic contractility. This process takes place from before backwards and the portion which has suffered this change, and the wall of which has assumed the ordinary arterial character, is in common usage included under the name truncus arteriosus.

(8) The atrioventricular part of the cardiac tube undergoes a double flexure similar in nature to that seen in the conus of the Lung-fish but of such a kind as would if straightened out give a left-handed twist.

(9) The valves and septa of this part of the heart originate in the endocardiac cushions and in *Lepidosiren* atrial septum and ventricular septum are at first continuous with one another.

(10) In the evolution of the valves of the heart there has come

about a substitution of automatically acting pocket- or flap-valves for the original endocardiac ridge or cushions which for their functioning were dependent upon a complex and fallible neuromuscular mechanism.

Arterial System.—The conus arteriosus is primitively prolonged forwards into the ventral aorta which gives off on each side the series of aortic arches. The most important evolutionary change which the originally simple tubular ventral aorta undergoes is a process of splitting whereby the stream of blood to the lungs is separated from that to the tissues generally. This splitting is seen for the first time in the Lung-fishes. Here a dorsal portion of the cavity is separated off, continuous behind with the pulmonary cavity of the conus and ending blindly in front by its floor meeting its roof. The anterior termination of this cavity is just in front of the point of origin of aortic arch V, and this arch along with arch VI, owing to the fact that they branch off from the ventral aorta somewhat dorsally, open from the cavity in question. The horizontal partition which forms the floor of this dorsal pulmonary cavity begins in ontogeny as a rudimentary ingrowth on each side between the origins of arches IV and V. The two rudiments grow back, fuse, and form a horizontal partition continuous at its hinder end with the Right and Left ridges of the conus which, as already explained, form the floor of its pulmonary cavity.

A similar splitting off of a dorsal, pulmonary, part of the ventral aorta occurs in air-breathing Vertebrates in general.

As regards the remaining, or systemic, portion of the ventral aorta, the main point to notice is its tendency to split into two separate halves in its anterior portion, a process correlated probably with economy of material, allowing as it does the origins of the aortic arches to be displaced outwards so as to shorten these arches. This splitting or bifurcation of the ventral aorta spreads backwards for a variable distance, commonly to about the level of aortic arch III or IV.

In the Reptiles there comes about an independent splitting into two lateral halves of the aortic cavity posterior to the region of the bifurcation just alluded to. A vertical septum grows backwards from the anterior lip of the opening into aortic arch IV of the left side and becomes continuous with the septum separating the two systemic cavities of the conus.

When this aortic septum is formed the ventral aorta in its hinder portion contains three cavities—a dorsal, pulmonary, leading to aortic arches V and VI of both sides, a left ventral leading to aortic arch IV of the left side, and a right ventral leading to aortic arch IV of the right side together with the paired anterior part of the ventral aorta and the aortic arches springing from them. Each of the three cavities is continuous behind with the corresponding cavity of the conus. In the majority of Reptiles (not in *Lacerta*) the separation of these cavities is followed by splitting of the septa between them so that the ventral aorta is resolved into three distinct vessels forming portions of the common pulmonary artery and of the right and left systemic aortae respectively.

THE AORTIC ARCHES AND THEIR DERIVATIVES.—From the ventral aorta there are given off on each side a series of half-hoop-shaped aortic arches which pass in a dorsal direction, between successive visceral clefts, to open eventually into the dorsal aorta. In the region where it receives the aortic arches the dorsal aorta is frequently paired (forming the **aortic roots**) either temporarily or throughout life. This assumption of the paired condition may not improbably be of similar significance to that of the ventral aorta *i.e.* have to do merely with the economizing of material. In any case the precise extent of the paired condition does not appear to be of any great morphological importance.

As regards the aortic arches themselves the following general features are to be noted: (1) that they develop in order of position from before backwards in agreement with the general principle of development of the vertebrate body and (2) that individual arches tend to become reduced in size in correlation with diminution of functional activity. Thus the mandibular and hyoid arches having lost or at least greatly diminished their respiratory activity even in the lower fishes we find a corresponding disappearance or reduction of their aortic arches.

An important point to notice is that where the particular visceral arch carries a true external gill (Crossopterygians, *Lepidosiren* and *Protopterus*, Urodele and some other Amphibians) the aortic arch passes out as a loop into the external gill. The aortic arch is in fact in these forms during early stages, before the gill-clefts are perforated, *the* vessel of the external gill. Such relations on the part of vessels of the fundamental morphological importance of the aortic arches are not to be dismissed lightly as modern adaptive modifications. They appear to indicate that the archaic function of the aortic arch was to supply the external gill with blood.

As the external gill ceases to function a short circuit is formed at its base, through which the blood passes directly to the dorsal part of the arch without traversing the external gill. In Urodeles, according to Maurer, the short-circuiting vessel sprouts downwards from the dorsal limb of the arch but in *Lepidosiren* Robertson finds it arising simply by the enlargement of pre-existing chinks. Thus the definitive aortic arch, in those cases in which an external gill is for a time present, includes a portion secondarily intercalated in its course and derived from the short-circuiting vessel.

In the typical fishes, where respiration is carried on by the wall of the gill-cleft, there becomes intercalated in the course of the aortic arch a respiratory network of capillaries, so that the arch is divided into a distinct ventral (afferent) and dorsal (efferent) portion. In such a fish as *Lepidosiren*, where the respiratory activity of the gills is comparatively small, the aortic arch can be traced throughout as a distinct channel; while in the Amniota, where the walls of the gill-clefts have completely lost their respiratory function, the respiratory network never develops at all.

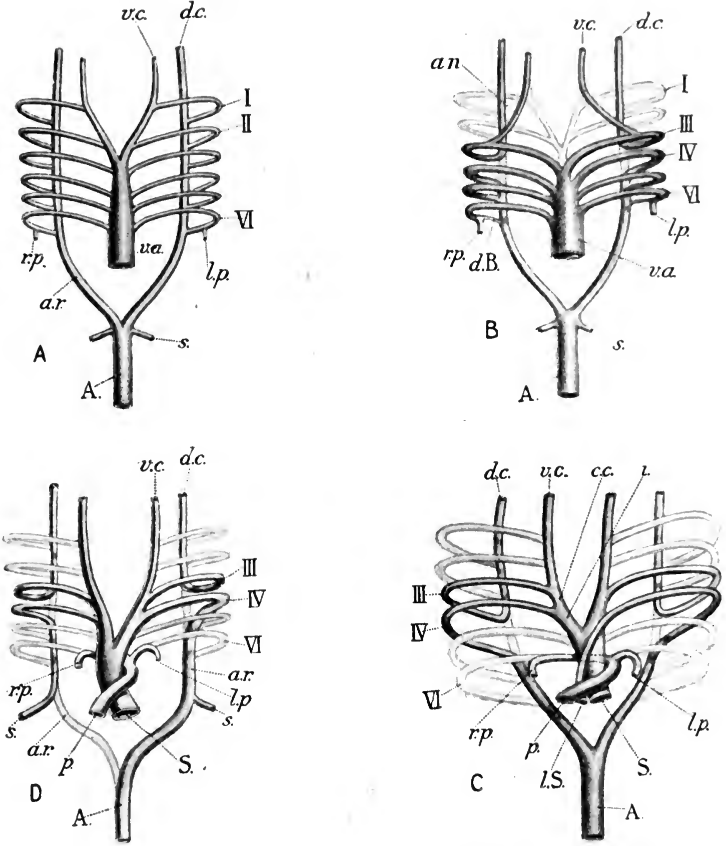


FIG. 186.—Scheme of aortic arches and their derivatives as seen from the ventral side. The parts of the original scheme which disappear during development are shown in pale tone.

A, complete unmodified series of aortic arches; B, arrangement in a Urodele; C, in a Reptile; D, in a Mammal. *A*, dorsal aorta; *a.r*, aortic root; *an*, anastomotic vessel; *c.c*, common carotid; *d.B*, duct of Botallus; *d.c*, dorsal (internal) carotid; *i*, innominate artery; *l.p*, left pulmonary artery; *l.s*, left systemic; *p*, pulmonary; *r.p*, right pulmonary; *S*, systemic aorta; *s*, subclavian artery; *v.a*, ventral aorta; *v.c*, ventral (external) carotid; I, II, etc., aortic arches.

The longitudinal vessels with which the aortic arches are connected are prolonged forwards as the **carotid arteries** which supply the head with blood. The ventral aorta is prolonged forwards as the ventral, or external, carotid while the prolongation forwards of the aortic root forms the dorsal, or internal, carotid. Of the alternative names dorsal and ventral (Mackay, 1889) are to be preferred to internal and external, for the latter though in common use are less precise.

During the development of the young individual there is laid down a general scheme of aortic arches and associated vessels agreeing with that just described, and the processes of modification whereby there becomes evolved out of this the complicated and very different arrangement of the great arteries of the adult afford material for one of the most fascinating chapters in vertebrate embryology. The general lines of these processes are best illustrated by an outline of what happens in the group Reptilia.

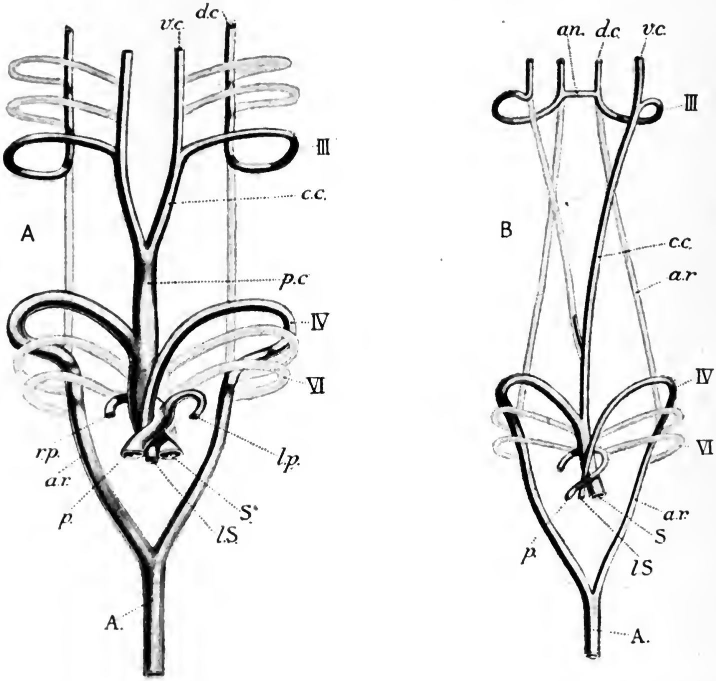


Fig. 187.—Illustrating modification of the carotid arteries, correlated with elongation of the neck region.

A, Varanid Lizard; B, Grass-snake (*Tropidonotus*); *p.c*, primary carotid. (Other letters as in Fig. 186.)

The arrangement which the main arteries assume in adult Reptiles shows much variety. The relation which the adult arrangement in the more important Reptilian types bears to the primitive scheme may be gathered from an inspection of Figs. 186, C, 187, A, B, and 187A, C.[1] Through the conspicuous differences in

---

[1] It must be remembered that in the actual animal the various bends and turns of the vessels tend to become straightened out. For example arch III becomes simply a portion of a straight internal carotid artery. In the diagrams the original curvature of the arches is retained for the sake of clearness.

detail there can be seen general agreement in the fate of various aortic arches and of other parts of the primitive arterial scheme. Thus the ventral aorta is continued forwards to form the paired ventral ("external") carotid arteries (*v.c*) while the aortic roots similarly extend forwards as the dorsal ("internal") carotids (*d.c*). Aortic arches I and II disappear. Arch III persists as the root of the dorsal carotid while the portion of ventral aorta behind it, when paired, is the **common carotid** (*c.c*). Arch IV is the Systemic arch which sends the blood to the hinder portions of the aortic roots (*a.r*) and thence to the dorsal aorta (*A*). Arch V is reduced, appearing only as inconspicuous and transient vestiges during development. Of arch VI the proximal portion becomes the root of the pulmonary artery (*r.p* and *l.p*) while its dorsal portion disappears.

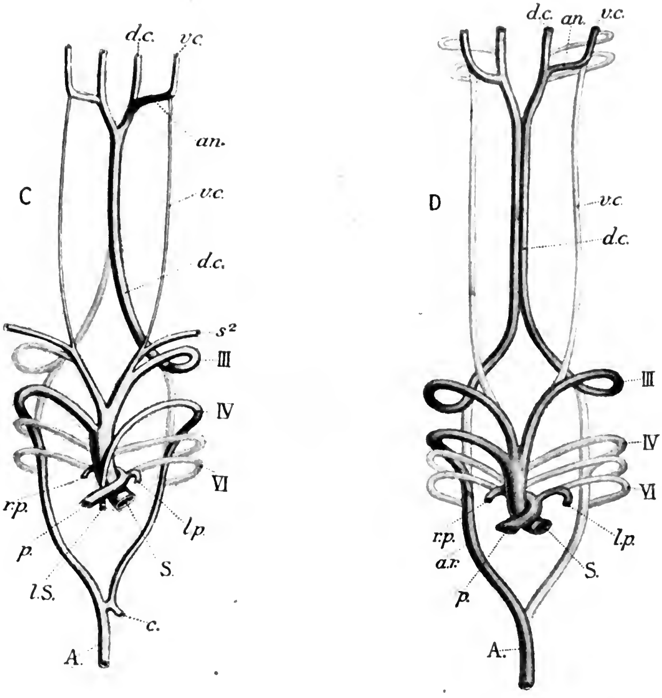


Fig. 187A.—Illustrating modification of the carotid arteries, correlated with elongation of the neck region.

C, Crocodile; D, Bird; *c*, coeliac artery; *s*², secondary subclavian.
(Other letters as in Fig. 186.)

The chief differences in detail are as follows: those affecting the carotids will be given more fully later on.

In Lizards and Chelonians the dorsal part of arch VI persists as a **duct of Botallus** forming a connexion between the pulmonary artery and the aortic root just as is shown in Fig. 186, B (*d.B*) for the Urodele amphibian. In other cases it may persist as a ligamentous vestige, as is the case on the left side in *Tropidonotus*.

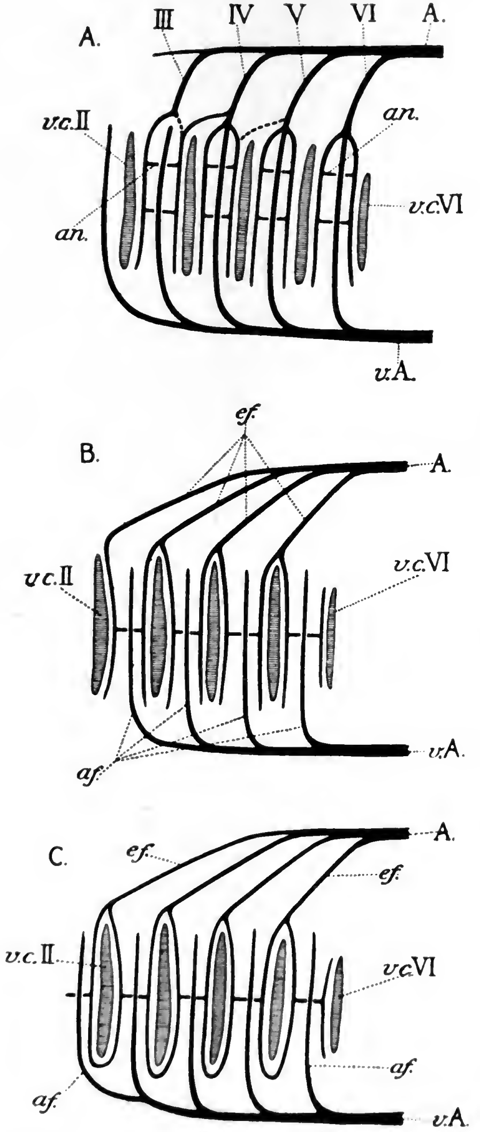


FIG. 188.—Illustrating the modification of aortic arches III-VI during ontogeny in *Scyllium*, according to Dohrn.

A, Dorsal aorta; *af*, afferent branchial; *an*, anastomotic vessel; *ef*, efferent branchial; *v.A*, ventral aorta; *v.c*, visceral clefts; III, IV, V, VI, aortic arches.

As a rule the portion of aortic root lying between arches III and IV disappears during development but in most Lizards (not in Chameleons and Monitors) it persists in the adult, so that in a dissection arches III and IV appear to run into one another peripherally.

In Monitors (Varanidae), in correlation with the elongation of the neck, arches III and IV become widely separated from one another and the intervening portion of ventral aorta shows a corresponding lengthening both in its paired (common carotid) and its unpaired (**primary carotid**) portions.

In Birds (Fig. 187A, D) arch IV completely disappears on the left side and with it the portion of the left aortic root lying posterior to it. Consequently in the adult Bird there is only a single systemic aortic arch and it passes down the right side of the body.

ELASMOBRANCHII.—In the Ichthyopsida, as we should expect, the departures from the primitive scheme are less pronounced:

they are mainly in details. In the Elasmobranchs perhaps the most conspicuous of these is to be seen in the relations of the efferent vessels, each of which emerges, not from an ordinary aortic arch traversing a gill septum, but from a vascular loop surrounding a gill-cleft, the general arrangement being that shown in Fig. 188, C. According to Dohrn (1886) this arrangement comes about in the following way. As the walls of the clefts form lamellae and develop respiratory activity, two branches grow downwards, one anterior and one posterior, from the dorsal end of each aortic arch (Fig. 188, A, VI). These branches become connected together by cross bridges as shown in the figure (*an*). The aortic arch now undergoes reduction and eventually becomes obliterated, just ventral to the point where the two branches are given off (Fig. 188, A, arch III), so that the arch is now divided into two distinct parts—a ventral afferent and a dorsal efferent — the latter prolonged ventralwards into the two branches. Of these the posterior branch of each pair becomes somewhat reduced in size, it develops a secondary connexion at its upper end with the efferent vessel next behind and loses its connexion with its original efferent vessel. The result is that, after this has happened, each efferent vessel again possesses two branches at its ventral end but these, instead of passing into the same gill septum, pass into two adjoining septa one in front of and one behind the intervening cleft (Fig. 188, B). Eventually the ventral ends of each pair of branches become joined so that the cleft is now surrounded by a complete efferent loop—the loops of successive clefts being connected together by a single persisting anastomotic vessel (Fig. 188, C).

In *Chlamydoselachus* the modification of the aortic arches just described does not take place.

The number of aortic arches corresponds with that of the visceral arches and is normally six.

In correlation with the presence of the pseudobranch on the posterior face of the mandibular arch in Elasmobranchs the first aortic arch in these fishes is well developed but its primitive relations with the arterial scheme become much obscured owing primarily to the development of large new afferent and efferent channels connected with the pseudobranch, which carries in its train the reduction of both the ventral and the dorsal portions of the original aortic arch.

In the case of the second aortic arch, correlated with the fact that the anterior face of this visceral arch has lost its respiratory function, there is developed only a single, posterior, efferent downgrowth instead of two as is the case with the arches farther back. A wide anastomosis between this and the first aortic arch just below the spiracle provides the secondary afferent vessel to the pseudobranch which as already mentioned supplants the primitive afferent vessel formed by the ventral portion of the first arch.

Cyclostomata.—In the Lamprey it should be noted that according to Dohrn (1888) an aortic arch corresponding to aortic arch I of Gnathostomata makes its appearance and then disappears again. In Myxinoids the most important feature is that in them the number of aortic arches reaches its maximum for Craniata—up to 14 in *Bdellostoma*.

Crossopterygii.—Our knowledge is in this case very incomplete. The chief peculiarity (Graham Kerr, 1907) is that, correlated with the large size of the external gill belonging to arch II, which forms the sole respiratory organ during early stages of larval life, aortic arch II makes its appearance relatively early and the development of the other aortic arches is postponed. Distinct vestiges of aortic arch I make their appearance. The succeeding aortic arches remain small for a prolonged period. Aortic arch VI becomes much enlarged in its ventral part in correlation with the fact that it supplies the pulmonary artery.

Actinopterygii.—In the Teleostean fishes and in the Ganoids that approach most nearly to them (*Lepidosteus*—F. W. Müller, 1897; *Amia*—Allis, 1900) complicated changes, which need not be detailed, take place in arches I and II in relation with the blood supply of the pseudobranch which in these fishes (p. 159) comes to lie on the inner surface of the operculum.

Dipnoi.—In *Lepidosiren* (Robertson, 1913) aortic arches I and II never become complete well-developed vessels: they are vestigial and their ventral portions do not appear to develop at all. The remaining four aortic arches are well developed, each passes out into an external gill and in each an intercalary piece becomes developed to short-circuit the blood-stream at the time the external gills atrophy. In *Ceratodus* and *Protopterus* efferent downgrowths make their appearance as in Elasmobranchs but they remain connected with the dorsal portion of their own aortic arch and do not undergo fusion at their ventral ends so that the condition in the adult departs less from the primitive than it does in the Elasmobranch.

Amphibia.—In Urodela the arrangement of aortic arches (Fig. 186, B) closely resembles that in Lung-fishes. Arches I and II are reduced: the latter in fact is according to Maurer (1888) no longer to be detected at all in the case of *Triton*. Arches III, IV and V are prolonged outwards into external gills and in each case a short-circuiting piece becomes intercalated as the external gills lose their functional activity. According to Maurer the intercalary portion makes its appearance as a downgrowth from the dorsal or efferent limb of the aortic arch but this may perhaps be doubted in view of the fact that in Lung-fishes the corresponding piece of vessel develops by the widening out of pre-existing chinks (Robertson, 1913).

Amniota.—Arch IV along with the aortic root into which it passes forms the main systemic aorta on each side. That of the left side is connected, in correlation with the spiral twist of the conus and its derivatives, with the *right* side of the ventricular cavity in proximity to the opening of the pulmonary artery. With the separation of the two ventricles the arch in question remains connected with the right ventricle. With increasing efficiency of the pulmonary circulation the venous blood of the right ventricle would be drawn off more and more to the pulmonary artery and, correlated with this, we find in those Sauropsida in which metabolism is most active and respiration most efficient that this fourth arch on the left side with its aortic root disappears completely during development, leaving only the single right-hand arch and root to form the proximal part of the systemic aorta (Birds, Fig. 187A, D).

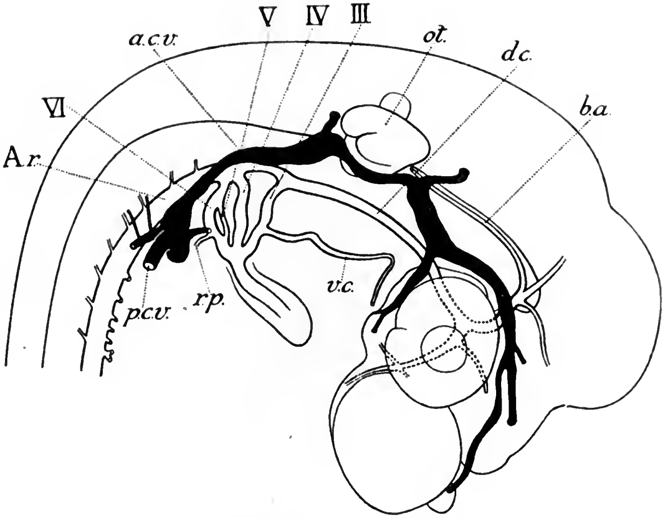


FIG. 189.—Blood-vessels of Crocodile of stage 55-56. (After Hochstetter, 1906*.) Arteries are shown in outline, veins black.

a.c.v, anterior cardinal vein; *A.r*, aortic root; *b.a*, basilar artery; *d.c*, dorsal carotid; *ot*, otocyst; *p.c.v*, posterior cardinal vein; *r.p*, right pulmonary artery; *v.c*, ventral carotid; III-VI, aortic arches.

Arch V, in the Amniota, appears only transiently and so greatly reduced in size as to have completely escaped the notice of the earlier investigators. Hence in Rathke's classical scheme of the aortic arches which is given in the older text-books only five arches are shown, the posterior one being called the fifth. With our present-day knowledge of the homology of the lungs of Amniota with those of Crossopterygians and Lung-fishes, such a scheme is clearly erroneous, as it would involve the pulmonary artery, which is certainly the same vessel throughout, taking its origin in the Amniotes from the fifth and in the Ichthyopsida from the sixth aortic arch. So without any special embryological data we should be justified in believing that in the Amniota an aortic arch has disappeared in front of the last one. The persisting vestiges of this fifth arch, which are now known to occur commonly in the embryos of the Amniota, were detected first by van Bemmelen (1886) in Reptiles and Birds. A good example of such a vestigial fifth arch is seen in the embryo of the Crocodile (Fig. 189).

The cause of the reduction of this fifth aortic arch is probably to be recognized in the fact that it receives its blood from the pulmonary cavity of the conus and of the ventral aorta (Graham Kerr, 1907*). As a consequence of this, during the evolution of the lungs as the main organs of respiration a larger and larger proportion of the blood in the cavity mentioned has become drawn off to the lungs, leaving less and less for arch V, with the natural result that the latter has become reduced to the verge of disappearance.

Before leaving the subject of the aortic arches it is necessary to point out that their diagrammatic arrangement as shown in Figs. 186 and 187 is commonly much obscured in the adult. For during development there occur not merely the disappearance of large portions of the original scheme of arches and the straightening out of the unnecessary curves, but also other complications. The chief of these are due to the longitudinal vessels—ventral aorta or aortic roots—lagging behind in their growth in length. This leads, according to the position in which it takes place, to the crowding together of the ventral or the dorsal ends of consecutive aortic arches, and their mere approximation may be succeeded by actual fusion so that two or more arches may come to have a common root emerging from the ventral aorta, or a common terminal portion opening into the aortic root.

PULMONARY ARTERY.—The pulmonary artery makes its first appearance in Crossopterygian fishes (*Polypterus*) as a branch from arch VI towards its ventral end which passes to the lung and adjoining parts of the pharyngeal wall. Throughout the series of lung-breathing Vertebrates it develops similarly as an outgrowth of the sixth aortic arch. A result of the main blood-stream of this arch passing off into the pulmonary branch is that the dorsal part of the arch lying beyond the point of origin of the pulmonary artery becomes as a rule reduced in size, forming the duct of Botallus. Except in the case of certain Reptiles (p. 397) the duct of Botallus becomes in normal individuals of the Amniota completely obliterated soon after birth.

In the Lung-fishes the point of origin of the pulmonary artery is displaced up to the dorsal end of arch VI where it is fused with arch V. Thus arch V is able to carry blood directly to the pulmonary artery and correlated with this it does not undergo the reduction in size which has taken place in the Amniota.

In *Lepidosiren* and *Protopterus* an important development of the pulmonary artery takes place inasmuch as its area of distribution extends on to the lung belonging morphologically to the other side of the body. Thus the right pulmonary artery comes to supply the dorsal side of both lungs, and the left artery the ventral side of both lungs. Each lung in other words receives a supply of blood from *both* pulmonary arteries and this illustrates an initial step towards the condition in *Amia* where the right and left sides of the air-bladder—the homologue of the right lung—are supplied with blood directly by a typical right and left pulmonary artery. In the Actinopterygian fishes apart from *Amia* the pulmonary artery has disappeared entirely from development and the air-bladder receives its blood-supply by secondary connexions with the dorsal aorta and its branches.

CAROTID ARTERIES.—As has already been indicated the great longitudinal arteries—ventral and dorsal aortae—are prolonged forwards into the region of the head as the carotid arteries—ventral and dorsal. Of these the latter, receiving as it does, in the case of the more primitive Vertebrates, blood which has been oxygenated by passing through the gills, becomes the more important and is responsible for supplying blood to the brain.

The ventral carotids are found from the Lung-fishes onwards—perhaps in correlation with the reduction of the first two aortic arches. They are known commonly under the name external carotid (= lingual artery of Amphibia) and supply blood to the ventral side of the head, though cases are known amongst animals no longer having functional gills (certain Mammals) in which they take over the blood-supply of the brain also.

It will be convenient to consider first the carotids as they occur in the development of the Amniota. The simplest condition is found in an ordinary Lizard (*Lacerta*) where they are seen as apparent prolongations forwards of the aortic root and of the ventral aorta respectively. This simple arrangement becomes in other Amniota modified during the course of development in different ways, of which the following are the chief. In various Lizards *e.g.* Chameleons and Monitors (and the same holds for the great majority of Amniota —Fig. 187, A) the portion of aortic root between aortic arches III and IV disappears during development, the consequence being that arch III comes to form the posterior portion of the internal carotid artery, becoming drawn out in the process of growth so as to be in line with the front part of that artery derived from the aortic root. The paired part of the ventral aorta from which the third arch was given off now becomes the common carotid artery (*c.c*). The unpaired portion of the ventral aorta, from which the common carotids spring, is known as the primary carotid and in the long-necked monitors this becomes much elongated, the growth in length of the neck taking place in the region between aortic arches III and IV (Fig. 187, A).

In the European Grass-snake (*Tropidonotus*, Fig. 187, B) an anastomosis is formed between the two internal carotids just behind the head (Fig. 187, B, *an*) and, correlated with this, the right common carotid as a rule disappears except for a small branch at its hinder end which supplies the Thyroid gland (O'Donoghue, 1912). The blood-supply of the head-region therefore passes to it entirely by the persisting left common carotid (*c.c*). In Snakes other than *Tropidonotus* considerable variety exists in the condition of the common carotids. Thus amongst the Boidae the two arteries may remain of approximately equal size (*Boa*) or on the other hand the left may be reduced (*Python*).

In Chelonians and Crocodiles (Fig. 187A, C) the growth in length of the neck takes place in the region in front of arch III so that here it is the portions of the carotid arteries in front of this level which undergo elongation. In both of these groups an anastomosis forms in the head-region between dorsal and ventral carotids and, correlated with this, the main blood-stream tends to pass to the head by the dorsal carotids, the ventral vessels becoming to a less (Crocodiles) or greater extent (Chelonians) reduced in size (van Bemmelen, 1887; Mackay, 1889). In the Crocodiles a still further modification takes place, inasmuch as the two dorsal carotids become for a considerable part of their length fused together into a single vessel, and following upon this aortic arch III of the right side atrophies, so that here as in *Tropidonotus,* though for a different reason, the main blood-supply of the head comes from the left side.

In the Birds the condition closely resembles that of the Crocodile. Here also the ventral carotids become reduced—in this case to the point of complete disappearance—in the neck region as a consequence of an anastomosis with the dorsal carotids in the head. Here also the enlarged dorsal carotids approach one another on the ventral side of the vertebral column. In those birds which depart least from the primitive condition in this respect (Ostrich, Emu, Casuari, Tinamus, Penguins, Divers, Gulls, Plovers, Snipe, Rails and their allies, Fowls, Pigeons, Ducks, Ibises, Storks, Herons, Cormorants and some Gannets, Birds of Prey, Parrots, Hornbills, Motmots, Goatsuckers) the two definitive carotids merely lie in proximity to one another. In many birds however they become fused together into a single vessel over a great part of their length and in such a case there may be no further modification (certain Herons such as the common Bittern, some Cockatoos, some Gannets), or, as is the general rule, this fusion is followed by the disappearance of the third aortic arch of the right side just as was the case in the Crocodiles (*Rhea*, *Apteryx*, Grebes, Quails, some Cockatoos, Capitonidae, Toucans, Hoopoe, Meropidae, Trogons, Woodpeckers, most Swifts, Humming birds and Passerine birds). In a few cases on the other hand it is the third aortic arch of the left side which becomes reduced to a small vestige (Flamingo) or disappears entirely (*Eupodotis* — the African Bustard).

Amongst the anamniotic Vertebrates typical dorsal and ventral carotids are present in Amphibians and Lung-fishes. The arrangement in Urodeles is illustrated by Fig. 186, B: the chief peculiarity to be noted is that the posterior portion of the external carotid (*v.c*) has disappeared in the adult, the persisting anterior portion receiving its blood through a new anastomotic channel (*an*) from the third aortic arch. The posterior portion of the "external carotid" or "lingual artery" of the adult Amphibian is really constituted by this new development.

The connexion of this newly developed portion of vessel with arch III is just at the point where the short circuit is formed between the afferent and efferent parts of the arch, and in Lungfishes (*Lepidosiren*—Robertson, 1913) the blood-supply for the external carotid comes to it, for a time during early stages, from the dorsal end of arch III or from the aortic root, through what seems to be a precocious development of this same short-circuiting channel. In this case the vessel in question is at first simply continued from its dorsal origin forwards into the external carotid: it is only later that it communicates with the ventral or afferent end of arch III so as on the one hand to form the short circuit, and on the other to permit the blood to pass to the external carotid from the ventral aorta.

In the more typical fishes the ventral carotid is not yet present. The dorsal carotids of the two sides develop an anastomotic connexion beneath the base of the skull so as to form with the aortic roots a complete "cephalic circle" which shows characteristic differences in different Teleostean fishes (Ridewood, 1899).

INTERSEGMENTAL ARTERIES OF THE BODY-WALL.—The dorsal aorta gives off on its dorsal side paired arteries which run out into the body wall between the myotomes. One of the most important features of this series of intersegmental vessels is that for a time during early stages of development they provide the blood-supply to the limb rudiments.

The main artery of the fore-limb—the **subclavian** artery—appears during the stages in question to be simply a prolongation into the limb rudiment from one of these intersegmental arteries—not necessarily the same artery of the series in different types of Vertebrate, or even in different developmental stages of the same Vertebrate. Thus in *Lacerta* it is said to be the seventh intersegmental artery (van Bemmelen, Hochstetter, 1906) which becomes the subclavian artery and in the Fowl the fifteenth (eighteenth or nineteenth if the three or four intersegmental vessels in the head-region are included—Hochstetter, 1890). In the Duck, Rabl (1907) found that during the fifth day of incubation the subclavian varies from the eighteenth to the twenty-first intersegmental artery and that in some cases two or even three such vessels may pass out into the limb rudiment at one time.

Probably we may take it that the general principle at work is this—that the limb, as it became shifted along the side of the body in the course of evolution, received its blood-supply from successive intersegmental arteries as it came to be opposite to them, and that during ontogeny there takes place an imperfect repetition of this process.

The fact that the pectoral limb is supplied with blood by an intersegmental artery raises the question whether or not this is to be regarded as the primitive mode of blood-supply. For if this question be answered in the affirmative we should be confronted with an important point which would have to be borne in mind in all speculations as to the evolutionary origin of the limbs of Vertebrates. As a matter of fact, however, recent investigations tend to answer this question in the negative.

In the Chick (Evans, 1909) the limb rudiment in its earliest stages is traversed by an irregular network of blood spaces and this receives its blood-supply directly from the dorsal aorta by a number of slender channels—it may be as many as ten or eleven on the right side where they are commonly most numerous. These vessels are scattered irregularly over an antero-posterior extent of from three to five mesoderm segments and they take their origin from the dorsal aorta quite independently of and considerably ventral to the intersegmental arteries. As development proceeds a few of these supply channels—those which happen to be most nearly intersegmental in position—become relatively larger and finally a single one, at about the level of the eighteenth intersegmental artery, becomes especially enlarged and carries the main stream of blood to the limb rudiment while the others gradually diminish in size and eventually disappear. The persisting enlarged vessel becomes the subclavian artery and secondarily its origin from the aorta becomes displaced in a dorsal direction until eventually it arises by a common root along with the intersegmental artery of which it now appears to form a branch.

In the Duck similar observations have been made so we are probably justified in stating that in the earliest stages of ontogenetic development the blood-supply of the pectoral limb is not metameric, and that the relation with the intersegmental artery observed in slightly later stages is a secondary acquirement.

In most vertebrates the subclavian artery which arises in the manner above described (**primary** subclavian) persists throughout life. In Birds however a cross connexion develops between the primary subclavian just at the base of the limb, and the ventral end of the third aortic arch. This cross connexion gradually increases in size while the proximal part of the primary subclavian, arising from the aortic root, becomes correspondingly reduced and eventually disappears entirely. The result is that the permanent artery of the fore-limb in the adult branches off, not from the dorsal aortic root but from the definitive (*i.e.* morphologically "internal") carotid, close to its hinder end. A similar substitution takes place in Chelonians and Crocodiles (Fig. 187A, C) and this is the explanation, first given by Mackay (1889), of the otherwise puzzling fact that in certain Vertebrates the subclavian artery passes out ventrally to the vagus nerve ("secondary subclavian") instead of dorsally as it does normally.

The **iliac artery** to the hind limb has also been traced back in at least Lizards and Birds to the series of intersegmental arteries but here again it would appear from later investigations that the definitive artery is merely a surviving and enlarged representative of a number of original supply vessels (Evans, 1909).

Representatives of the series of intersegmental arteries are recognizable in various Vertebrates in the hinder part of the head-region. Thus in *Lacerta* (van Bemmelen, Hochstetter, 1906) three have been detected in the head-region. Of these the first two disappear while the third becomes prolonged forwards immediately beneath the brain to become continuous with the internal carotid at the level of the mid-brain. These prolongations fuse together in the mid-line and form the basilar artery.

A similar basilar artery continuous with the internal carotids is of common occurrence in Vertebrates though the relations of its paired fore-runner to the series of intersegmental arteries differ in different forms.

The vertebral artery of Sauropsida is in its origin intimately related to the intersegmental arteries. Thus in *Lacerta* the intersegmental vessels posterior to the subclavian become connected together by a longitudinal anastomotic vessel, which persists and forms the cervical portion of the vertebral artery. In Snakes the two similarly-arising vessels apparently usually undergo fusion together in their anterior portion while farther back the unpaired condition is reached by the disappearance of the rudiment on the right side (Hochstetter, 1906).

The arterial blood-supply of the urino-genital organs is also generally provided by branches from the intersegmental arteries of the embryo.

MESENTERIC ARTERIES.—The digestive tract receives a varying number of branches from the dorsal aorta which may be at first paired and undergo fusion secondarily or may be unpaired from the beginning. In those vertebrates which have a bulky yolk-sac a pair of these are precociously developed as **vitelline arteries**. In some cases there is a remarkable relation between the chief mesenteric artery (**coeliac**) and the pronephros. Thus in *Lepidosiren* a connexion becomes established between the blood-spaces of the right pronephros and those of the gut wall, and the branch of the dorsal aorta which supplies the right pronephros persists as the root of the definitive coeliac artery. In such Teleostean fishes as the Trout the connexion with the pronephric arterial supply is only temporary, a new anastomotic channel arising farther back between dorsal aorta and mesenteric artery which remains as the definitive root of the latter vessel.

VENOUS SYSTEM.—As an example of the development of the venous system in a holoblastic Vertebrate we will take that of *Lepidosiren* (Robertson, 1913).[1]

---

[1] For comparison with *Lepidosiren*, an account of the development of the vascular system of *Ceratodus* will be found in Kellicott (1905).

The first part of the venous system (Fig. 190, A)—indeed of the vascular system—to take definite form consists of the two **vitelline veins**, which pass tailwards over the anterior surface of the yolk (stage 24). Anteriorly they become conjoined to form the heart while posteriorly they are continued into the rudiments of the vitelline network (stage 24-25). The appearance of the vitelline veins is followed almost immediately by the development of a longitudinal venous channel on each side anteriorly, superficial to the aortic arches —the **anterior cardinal** vein. At its hinder end the anterior cardinal is continued into a set of venous spaces in the region of the pronephros (**pronephric sinus**) and onwards behind this as the **posterior cardinal** vein.

The pronephric sinus is continued along its outer edge, by a number of channels, into the vitelline network of venous spaces, lying in the splanchnic mesoderm over the surface of the yolk. In this network a conspicuous channel becomes apparent, leading from the anterior end of the pronephric sinus outwards to the vitelline vein, and so, by way of the anterior part of the vitelline vein, to the heart. This vessel so constituted, which at first makes a wide sweep over the lateral surface of the yolk, is the **Duct of Cuvier** (Fig. 190, b, *d.C*). As development goes on the Ducts of Cuvier become greatly shortened and at the same time widened until eventually they form in the adult very short wide channels for the conveyance of the blood from the cardinal veins into the sinus venosus (see Fig. 190, b and c and d).

The posterior cardinal vein on each side appears about stage 24 in the form of spaces along the course of the archinephric duct which become joined up so as to form two longitudinal vessels running parallel to the duct, one on its mediodorsal the other on its ventrolateral side, the two vessels being joined round the duct by numerous anastomoses (Fig. 190, b and c, *p.c.v*).

These posterior cardinal veins accompany the archinephric ducts throughout their length and just in front of the cloaca are joined by the hind ends of the bifurcated subintestinal vein (see below) and of the dorsal aorta. The vessel formed on each side by the union of these three elements is continued back past the cloaca and unites with its fellow of the opposite side to form a vessel lying immediately beneath the post-anal gut. This vessel is to be interpreted morphologically as a post-anal portion of the subintestinal vein and as will be shown later it is destined to become the caudal vein of the adult.

It will now be convenient to trace out the subsequent fate of what may be called the **dorsal venous system**, consisting primarily of the anterior and posterior cardinal veins.

Posterior Cardinal Veins.—The posterior cardinal vein was left in the form of a pair of vessels, an inner and an outer, lying close to the archinephric duct and connected together by numerous anastomoses. As the opisthonephros develops between these channels they consequently come, over a considerable part of their length, to be

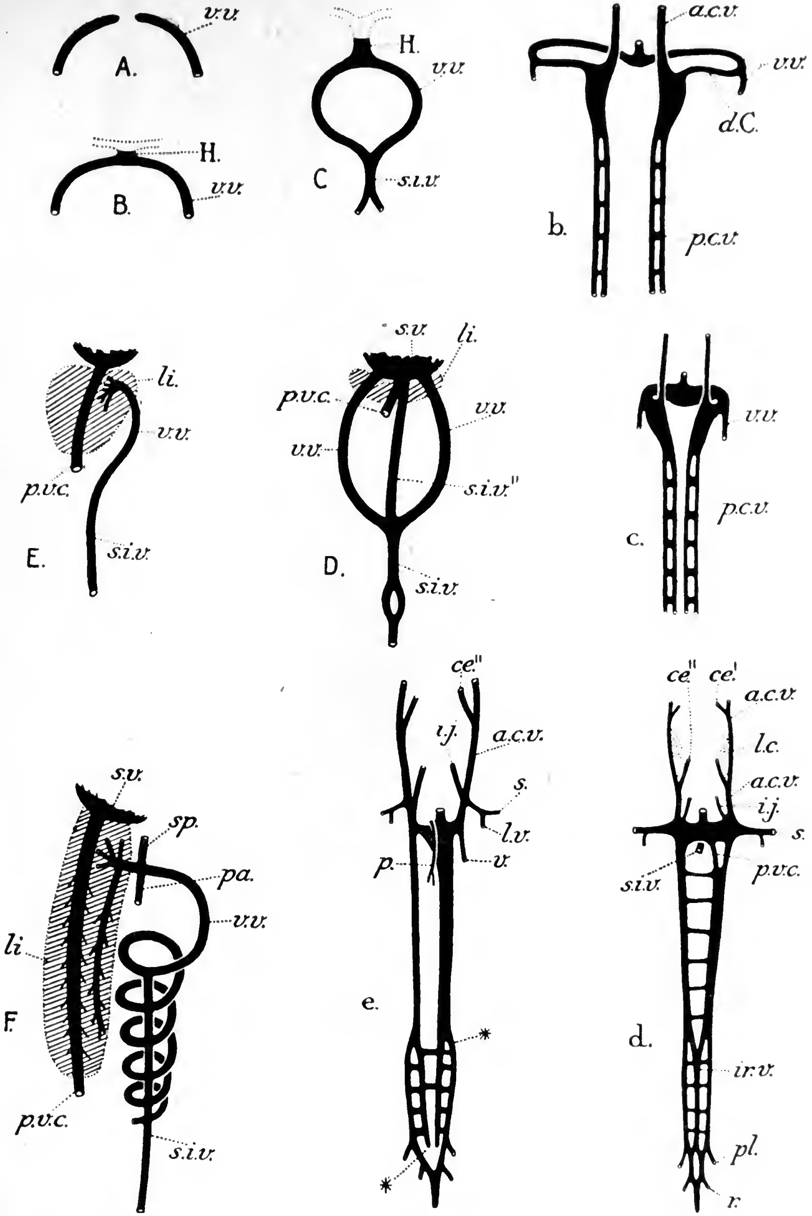


FIG. 190.—Development of venous system of *Lepidosiren*. (After Robertson, 1913.)

A-F, ventral portion of venous system as viewed from the ventral side; b-e, dorsal portion as viewed from the dorsal side. A, stage 24; B, 25; C, 30; D, 32; E, 35; F, young adult; b, 27-30; c, 30-31; d, 31+; e, young adult. *a.c.v*, anterior cardinal vein; *ce′* and *ce″*, anterior and posterior cerebral veins; *d.C*, duct of Cuvier; *H*, heart; *i.j*, inferior jugular; *ir.v*, inter-renal vein; *l.c*, lateral cephalic vein; *l.v*, lateral cutaneous vein; *li*, liver; *p*, pulmonary vein; *pa*, vein from pancreas; *p.c.v*, posterior cardinal; *pl*, pelvic vein; *p.v.c*, posterior vena cava; *r*, rectal vein; *s*, subclavian vein; *s.i.v* and *s.i.v″*, subintestinal vein; *s.v*, sinus venosus; *sp*, splenic vein; *v*, vestigial front end of right posterior cardinal; *v.v*, vitelline veins; * points at which vessels become discontinuous.

separated from one another by a considerable space in which the renal organ lies. As this happens the inner components of the two posterior cardinals become approximated and eventually undergo fusion with one another to form an **inter-renal** vein (Fig. 190, d, *ir.v*). In *Lepidosiren* this fusion is only temporary and the two components again recede from one another—remaining, however, connected by a small number of anastomotic vessels (Fig. 190, e). There now takes place a severance of the continuity of the inner component at its hinder end (Fig. 190, e,*), and a little later a similar severance of the outer component at its front end. The physiological result of these interruptions of continuity is that the blood from the caudal region now reaches the opisthonephros entirely by way of the outer component, it then passes through the substance of the kidney and is drained away entirely by the inner component. In other words the outer component and its backward continuation has now become the **renal portal** vein.

While these changes are going on in the opisthonephric region of the posterior cardinal the portion of that vein in front of the opisthonephros takes on the form of a single channel, the original inner component becoming enlarged while the outer component becomes reduced and eventually disappears. The right and left posterior cardinal veins in this region in front of the opisthonephros become connected by numerous transverse vessels (Fig. 190, d). A short circuit now becomes established by which the blood from the right posterior cardinal can pass direct to the sinus venosus through the substance of the liver (Fig. 190, d, *p.v.c*).

This short-circuiting channel is of great morphological importance. It constitutes the intrahepatic or headward section of the **posterior** ("inferior") **vena cava**, which in the higher vertebrates becomes the largest vein in the body. Its appearance here is followed by two important results. (1) The main blood-stream from the kidney region tends to pass to the heart, more and more by this direct channel, which in correlation with this becomes larger and larger. (2) The portion of right posterior cardinal vein lying behind its junction with the intrahepatic channel becomes correspondingly enlarged.

These two components together constitute the definitive posterior vena cava, in which vessel therefore we recognize two fundamentally distinct portions, an anterior or intrahepatic, and a posterior or cardinal. A secondary result of the diversion of the blood-stream from the right posterior cardinal vein through the hepatic component is that the anterior portion of the former vein, lying in front of the junction of the two components, becomes relatively reduced in size. It loses its continuity with the rest of the right posterior cardinal and persists as the small vein shown at *v* in Fig. 190, e.

An important clue to the origin of the posterior vena cava in phylogeny is given by the condition seen in the adults of existing Lung-fishes. The opisthonephros in these vertebrates retains its primitive elongated form, extending far forwards in the splanchnocoele, and the front end of the right opisthonephros is in immediate apposition to the tip of the liver which is also situated dorsally and on the right side. It is no doubt the approximation of the tips of these two organs, still persisting in the adult Dipnoan, which paved the way for the establishment of direct continuity between their vascular networks and the consequent short-circuiting of the renal blood through the hepatic vein into the heart (Graham Kerr, 1910).

The ladder-like connexions between right and left posterior cardinals in the region anterior to the inter-renal vein gradually disappear in turn from before backwards (Fig. 190, d and e).

Anterior Cardinal Veins.—Apart from relatively less important details, the chief change which comes about in regard to the anterior cardinal is the diversion of the blood-stream about the level of the otocyst into a more laterally placed channel called the **lateral cephalic vein**, which eventually becomes intercalated in the course of the anterior cardinal, and to all appearance forms simply a portion of that vein (Fig. 190, d, *l.c*). The anterior cardinal vein at first passes back along the side of the head region (following the course shown by the dotted outline in Fig. 190, d), ventral to the otocyst and internal to the posterior cranial nerves, to join the front end of the pronephric sinus. Presently (stage 30) a branch of the anterior cardinal vein makes its appearance and extends backwards external to the ganglion of the seventh cranial nerve, bending inwards and rejoining the anterior cardinal between the eighth and ninth cranial nerves. A little later (stage 31) the vessel forming the outer side of this loop becomes prolonged back and forms a second loop external to the ninth cranial nerve and rejoining the main vessel between nerves IX and X. Finally (stage 31 +) a similar extension backwards occurs external to the vagus, rejoining the anterior cardinal vein just behind it. The lateral cephalic vein develops from the outer portions of these three vascular loops, the development of each of the three segments being followed by the atrophy of the corresponding section of the original anterior cardinal, except in the case of the most posterior section which persists as a short wide vein opening along with the posterior cerebral vein (*ce″*) into the definitive anterior cardinal.

Ventral Venous System.—In addition to the dorsally placed cardinal veins there exist certain important veins situated more ventrally and developed in relation with the vitelline veins. The vitelline veins spread backwards on each side as a wide vessel consisting of an enlarged channel of the vitelline network which covers the whole surface of the yolk. Posteriorly they unite in the mid-ventral line to form a very short **subintestinal vein** in front of the anus (Fig. 190, C, *s.i.v*). Later on the space bounded by the two vitelline veins becomes bisected by a median ventral vein which looks like a prolongation forwards of the subintestinal vein and is known by the same name (Fig. 190, D, *s.i.v''*). It will be realized that this anterior section of the subintestinal vein is developmentally of a different nature from the posterior portion for it is formed by a short-circuiting of the blood-stream through the vitelline network, while the posterior portion represents rather the conjoined hinder ends of the paired vitelline veins (Fig. 190, C and D). This difference in development is no doubt purely secondary and we may take it that the later condition, where the subintestinal vein is continuous right forwards to the heart, represents the really primitive condition of this vein in evolution. The continuity of the subintestinal vein at its front end with the heart is brought about in ontogeny through blood-sinuses which make their appearance in the liver (stage 31).

The right vitelline vein and the anterior, secondarily formed, portion of the subintestinal vein now gradually disappear (about stages 32 - 35). The left vitelline vein ceases to form a continuous channel over the surface of the liver to the heart, so that the blood in it is forced to traverse the system of blood sinuses within the liver. In other words the whole of the blood which streams forwards in the subintestinal vein is diverted along the persisting left vitelline vein into the network of blood spaces in the liver. Subintestinal vein and left vitelline vein have thus come to constitute the **hepatic portal** vein. The latter becomes complicated by a branch sprouting out from the front end of its subintestinal portion. This branch spreads round the alimentary canal along the line of the spiral valve, fusing with the subintestinal vein at each point of intersection (Fig. 190, F). As the liver increases in length a special supply channel from the portal vein lengthens out along its left side, giving off numerous branches into the liver substance (Fig. 190, F). From this the blood drains by numerous efferent vessels into the intrahepatic portion of the posterior vena cava.

Caudal Vein.—The post-anal portion of the subintestinal vein was left (p. 408) at a stage when its anterior bifurcated portion was continuous on each side not only with the pre-anal portion of the same vein but also with the dorsal aorta and the posterior cardinal. As development goes on the first two of these connexions disappear so that the post-anal subintestinal vein is now continuous anteriorly only with the posterior cardinal. With the atrophy of the post-anal gut, it comes to lie immediately beneath the caudal portion of the dorsal aorta and is now known as the **caudal vein**, its anterior forked portion forming the hinder ends of the renal portals.

The veins which have been described constitute the main trunks of the venous system; in the later stages of development a number of other important vessels appear which are indicated in the diagrams. The **subclavian** vein (Fig. 190, d, *s*) appears about stage 31 + leading from the pectoral limb into the pronephric sinus. As this sinus atrophies the point of opening of the subclavian vein comes to be situated on the anterior cardinal vein (Fig. 190, e, *s*). From the subclavian vein a small **lateral cutaneous** vein (*l.v*) passes back in the body-wall. An **inferior jugular** vein (*i.j*) passes back from the head-region, lateral to the pericardiac cavity, and opens into the anterior cardinal close to its hind end and on its ventral side. In later stages the point of junction of the inferior jugular with the anterior cardinal comes to be shifted relatively forwards, as was the case with the subclavian. Farther forwards the anterior cardinal is joined by an anterior and a posterior cerebral vein (*ce′* and *ce″*) from the inside of the head. At the hind end of the system **rectal** (*r*) and **pelvic** (*pl*) veins open into the renal portal. The former appear to be the persistent remains of the anastomotic branches which in early stages connected the hind end of the subintestinal vein with the posterior cardinal.

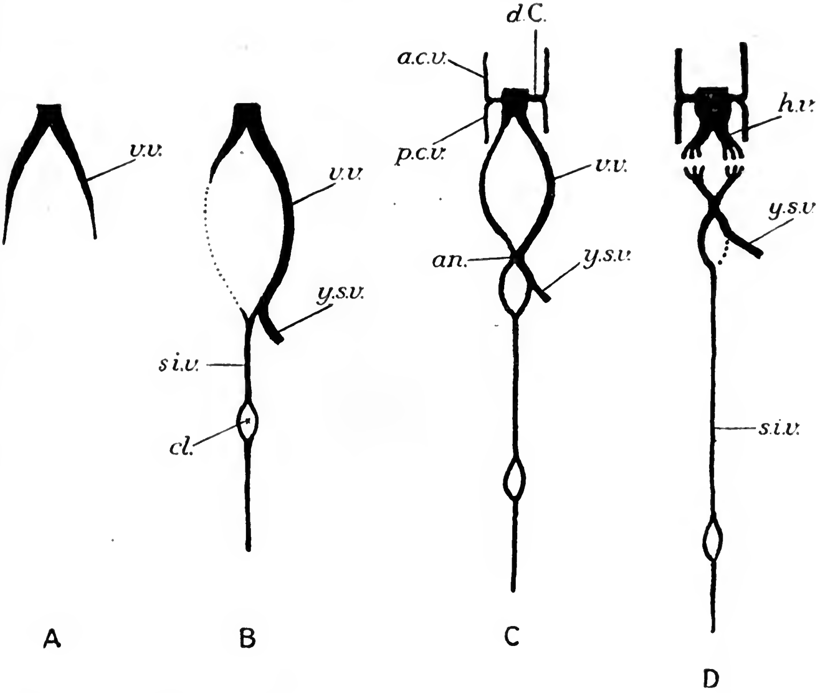


FIG. 191.—Diagrams illustrating early stages in the development of the venous system of Elasmobranchs according to Rabl (1892) and Hochstetter (1906).

a.c.v, anterior cardinal vein; *an*, fused portion of vitelline veins behind liver; *cl*, position of cloaca; *d.C*, duct of Cuvier; *h.v*, hepatic vein; *p.c.v*, posterior cardinal vein; *s.i.v*, subintestinal vein; *v.v*, vitelline vein; *y.s.v*, main vein from yolk sac.

ELASMOBRANCHII.—It is instructive to compare with the development of the venous system in a holoblastic vertebrate the corresponding phenomena as they occur in the Elasmobranchs, the lowest of the meroblastic gnathostomes. An inspection of Fig. 191 brings out the most conspicuous difference, one that could be foretold a priori, namely that, in agreement with the general principle that animals with a large supply of yolk tend to show a precocious development of the vessels on the surface of the yolk, the vitelline veins and their derivatives make their appearance relatively earlier as compared with the cardinals than they do in *Lepidosiren*.

Here again the first of the main trunks to make their appearance are the vitelline veins, which by their fusion anteriorly form the heart (Fig. 191, A, *v.v*). The most conspicuous difference in the next subsequent stages is that the two veins are for a considerable period strongly asymmetrical (Fig. 191, B), the right becoming greatly reduced, and only the left being commonly traceable back into the subintestinal vein (*Pristiurus*—Rabl, 1892; *Acanthias*—Hoffmann, 1893). There can be no reasonable doubt that this is to be looked upon as a secondary modification of a symmetrical condition of the two veins as seen in *Lepidosiren*, and probably the impelling factor which has brought about the modification is to be recognized in the fact that the main channel for draining away the blood from the surface of the yolk-sac is situated on the left side and opens into the left vitelline vein (Fig. 191, *y.s.v*).

The unpaired mesial prolongation forwards of the subintestinal vein between the two vitelline veins which was so conspicuous in *Lepidosiren* has not been noticed in Elasmobranchs.

Both vitelline veins break up into a network as they traverse the liver, the parts of the veins in front of this network persisting as the **hepatic veins** of the adult (Fig. 191, D, *h.v*). The portion of right vitelline vein behind this network is for a time much reduced, the left alone serving to supply the network with blood. Immediately behind the liver, and in front of the yolk-sac vein, the two vitelline veins are fused together for a short distance into a single vessel (Fig. 191, C, *an*), and the same is the case again from about the level of the pancreas backwards where they form the subintestinal vein, though it should be noticed that there still persist during early stages of the development of the subintestinal vein in Elasmobranchs distinct traces of the paired condition, the vein consisting of two parallel components situated on each side of the mid-ventral line of the intestine. These components soon become connected together by numerous anastomoses and eventually fuse completely to form a median vessel except where this is prevented by the presence of the cloaca.

The main vein from the yolk-sac (*y.s.v*) joins the left vitelline vein in the region in front of the pancreas and presently the portion of left vitelline vein behind the opening of the yolk-sac vein disappears. It thus comes about that the blood from the subintestinal vein passes forwards to the liver entirely through the right vitelline vein—in contrast with *Lepidosiren* where it did so by the left vitelline vein.

Cardinal veins and duct of Cuvier make their appearance, the chief difference from *Lepidosiren* being the comparative shortness of the duct of Cuvier which does not take the wide sweep over the surface of the yolk that it does in *Lepidosiren* during early stages. This difference is related to the fact that here the first rudiment of the duct of Cuvier opens into a portion of the vitelline vein which has fused with its fellow to form the hind end of the cardiac tube, while in *Lepidosiren* it opens much farther back, the result being that in *Lepidosiren* a considerable stretch of free vitelline vein becomes incorporated in the definitive duct of Cuvier (compare Figs. 191, C and 190, b).

As in *Lepidosiren* the caudal vein becomes continuous with the posterior cardinals and loses its continuity with the pre-anal portion of the subintestinal vein. The inter-renal vein however develops here simply as a forward extension of the caudal vein according to Rabl. A number of anastomotic vessels connect up the inter-renal vein with the "posterior cardinal"—the equivalent of the external component of this vein in *Lepidosiren.* The posterior cardinal now becomes obliterated behind the anterior one of these anastomotic vessels while the inter-renal becomes separated off from the caudal vein so that the whole blood-stream from the latter has to pass through the kidneys to reach the inter-renal. The latter vein splits into a pair of vessels eventually, thereby revealing that the inter-renal vein here is homologous with that of *Lepidosiren* in spite of its different—no doubt secondarily modified—mode of development.

The anterior cardinal vein here again becomes in part replaced by a lateral cephalic vein.

In the lower vertebrates in general we may recognize the same main trunks as occur in Dipnoi and Elasmobranchs, with differences in detail. The following account gives an outline sketch of the development of the venous system in the various groups, the outline being filled in more fully in the case of *Polypterus* on account of the very archaic character of this fish.

CYCLOSTOMATA.—In the Lamprey, according to Goette (1890), the pair of vitelline veins appear first, spreading backwards on either side of the liver rudiment and meeting behind in the unpaired and much dilated subintestinal vein (Fig. 192, A). The vitelline veins break up into a network in the liver but on the left side the post-hepatic section of vitelline vein disappears, so that the hepatic portal vein is formed by the subintestinal and right vitelline vein (Fig. 192, B and C)—somewhat as in the Elasmobranch, and unlike *Lepidosiren* where it is the right vein which disappears. The vein of the "spiral valve" of the intestine arises comparatively late, at the time of metamorphosis according to Goette, and on the opposite side of the gut from that on which the subintestinal vein lies. This latter is no longer ventral but high up on the right side, owing to a rotation which the gut has undergone.

The anterior and posterior cardinal veins present the peculiarity that they open at first separately into the vitelline veins. Later they become fused together to form the duct of Cuvier. Eventually the left duct of Cuvier disappears completely (Fig. 192, C) the blood from the left cardinals passing to the right side by a new anastomotic vessel which develops ventral to the dorsal aorta (Fig. 192, C, *an*)—an arrangement presenting a remarkable analogy with what happens in certain Mammals.

CROSSOPTERYGII.—In these archaic Teleostomes the main features of the development of the venous system have been investigated in *Polypterus*—the less specialized of the two surviving genera (Graham Kerr, 1907).

In the earliest stage described there is a well-developed subintestinal vein which in front breaks up into a vitelline network.

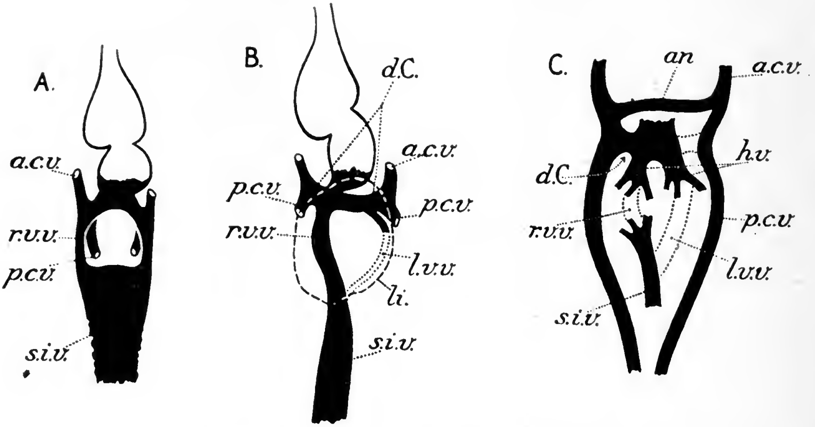


FIG. 192.—Development of veins in *Petromyzon* as seen from the ventral side. (After Goette, 1890.)

a.c.v, anterior cardinal vein; *an*, anastomotic vein; *d.C*, duct of Cuvier; *h.v*, hepatic veins; *li*, outline of liver; *l.v.v*, left vitelline vein; *p.c.v*, posterior cardinal vein; *r.v.v*, right vitelline vein; *s.i.v*, subintestinal vein. Portions of the venous trunks which disappear during ontogeny are shown by dotted outlines; the outline of the liver is shown in B by an interrupted line.

This drains into a pair of lateral vitelline veins which unite in front to form the heart. Posteriorly the subintestinal vein bifurcates to pass on each side of the cloaca and then joins again to form the post-anal subintestinal vein. In its double cloacal portion, and also in front of this, wide communications pass between the subintestinal vein and the posterior cardinals. The ultimate fate of the subintestinal vein differs in its pre-cloacal and post-cloacal portions. The former loses its identity in that it becomes entirely resolved into portions of the vitelline network. The post-cloacal portion becomes converted into the caudal vein in the normal fashion as already described for *Lepidosiren*. It has already been mentioned that wide communications were established between the paired cloacal portion of the subintestinal vein and the posterior cardinals. With the breaking up of the pre-cloacal part of the vein the main blood-stream passes through these communications into the posterior cardinals and the latter take on the appearance of a direct forward prolongation of the caudal vein.

Dorsally there develops on each side a cardinal trunk which swells out into a great irregular sinus (*pn*) in the region of the pronephros. On its outer side branches pass from the pronephric sinus into the general vitelline network. In this network a specially wide channel develops, starting from the pronephric sinus and sweeping outwards over the yolk to join the lateral vitelline vein and so reach the heart. This channel, which becomes gradually more and more sharply defined, is the duct of Cuvier (Fig. 193, A, *d.C*). The pronephric sinus is continued backwards into the posterior cardinal vein. This is at first distinct from its fellow but at an early stage fuses with it to form a median inter-renal vein (Fig. 193, B, *ir*)—the fusion being foreshadowed by the development of anastomotic connexions between the two veins while still separated from one another by a distinct space (Fig. 193, A, *an*). Towards the cloaca the posterior cardinals taper off and are connected by irregular anastomotic channels with the subintestinal vein, as already mentioned, and also with the dorsal aorta. Eventually, as already indicated, the inter-renal vein and the caudal vein form a continuous vessel.

During the later stages a striking asymmetry becomes apparent in the anterior, unfused, portions of the posterior cardinals—the left becoming greatly reduced as compared with the right (Fig. 193, D). The main blood-stream thus passes forwards on the right side, and upon this side a special direct channel develops on the ventral side of the pronephros, through which the blood-stream is able to reach the duct of Cuvier without passing through the tangle of pronephric tubules. The asymmetry affects also the ducts of Cuvier—showing itself first in that of the left side becoming relatively shorter than its fellow, which retains for a time its wide sweep over the surface of the yolk (Fig. 193, B and C, *d.C*). Eventually it too becomes shortened and its calibre becomes considerably greater than that of the left side (Fig. 193, D).

From the pronephric sinus a branch (*l.v*) develops about stage 30 which passes dorsalwards and then backwards beneath the lateral line nerve—the lateral cutaneous vein. This, a large vessel about stage 33, becomes reduced to an insignificant vestige later on.

The anterior cardinal vein runs along the side of the head region, passing through the angle on the ventromesial side of the otocyst, between the latter and the brain-wall. At its front end the vein dilates into a large sinus, which gives off irregular branches to the mesoderm of the head. At an early stage an anastomotic channel makes its appearance on the outer side of the otocyst continuous anteriorly and posteriorly with the anterior cardinal. When this channel has been established (Fig. 193, A, *l.c*) the blood-stream from the head divides in front of the otocyst and passes backwards, part

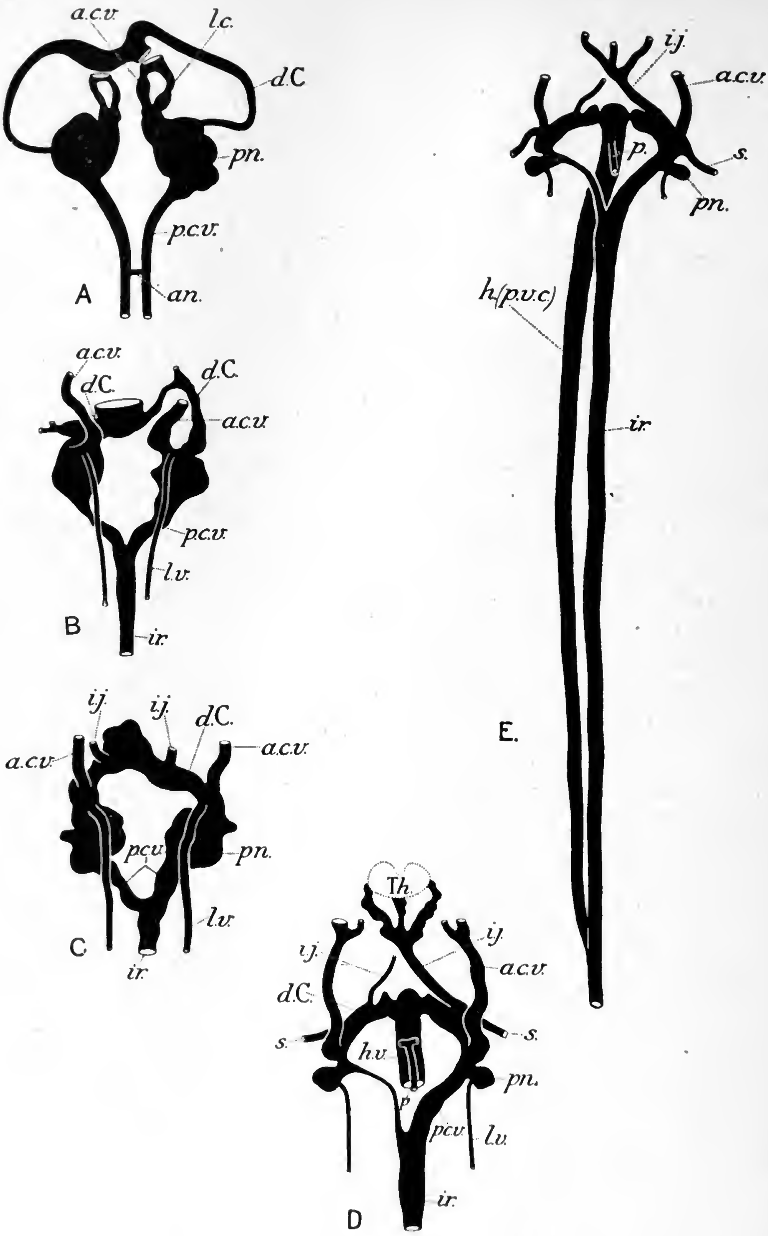


Fig. 193.—Development of dorsal venous system of *Polypterus*, as seen from the dorsal side.

Stages 27-29 (A); 31 (B); 33 (C); 30 mm. larva (D and E). *a.c.v*, anterior cardinal vein; *an*, anastomotic vein; *d.C*, duct of Cuvier; *h.v*, hepatic vein; *h.(p.v.c)*, posterior vena cava; *i.j*, inferior jugular; *ir*, inter-renal vein; *l.c*, lateral cephalic; *l.v*, lateral cutaneous; *p*, pulmonary vein; *p.c.v*, posterior cardinal; *pn*, pronephric sinus; *s*, subclavian vein; *Th*, thyroid.

on its outer and part on its inner side. Eventually the inner channel becomes constricted across and finally completely severed at its hinder end so that the whole blood-stream passes back by what was the outer channel. The inner channel persists as a small vein which opens at its front end into the definitive anterior cardinal. We see then that here, as in the Lung-fish or the Elasmobranch, the "anterior cardinal" vein of later stages has intercalated in its length a segment of lateral cephalic vein.

An inferior jugular vein on each side (*i.j*) drains the blood from the ventral side of the head into the duct of Cuvier, and as development goes on these become asymmetrical, the left becoming greatly reduced while the right forms a large vessel, trifid at its front end where it receives the blood from the thyroid (Fig. 193, D, *Th*).

The liver rudiment, at first a portion of the general yolk-mass, is at this stage supplied with blood by the portion of the general vitelline network which extends over it. The portal vein develops simply as an enlarged channel of the network on the left side of the hepatic rudiment. There is no information as to whether this is really the persisting left lateral vitelline vein as we might expect: nor is it known whether the hepatic vein is derived from the prehepatic portion of this same vein.

An important feature is that there becomes established an anastomosis between the blood-vessels of the liver at the hinder end of that organ and the inter-renal vein, so that a portion of the inter-renal blood-stream becomes short-circuited direct to the heart through the hepatic vein, which in correlation with this forms a wide channel throughout the length of the liver (Fig. 193, E, *h.*(*p.v.c*)). This enlarged hepatic vein is of morphological importance as it is clearly the equivalent of a posterior (or "inferior") vena cava. In the Crossopterygian this vessel takes a step in evolution beyond the condition in Lung-fishes, inasmuch as its posterior portion becomes denuded of liver substance, so that it runs for a considerable distance through the splanchnocoele as a naked vessel.

Another important vein which makes its first appearance in the Crossopterygian is the pulmonary vein. The hepatic vein, which towards its headward end lies on the dorsal side of the liver, comes into close contact with the pharyngeal floor in the region of the glottis, and venous spaces developing in the mesoderm sheath of the pharynx come to open into it. These are apparently the forerunners of the pulmonary vein. In the 30-mm. larva, as in the adult, there is a main pulmonary vein which still opens into the hepatic vein on its dorsal side (Fig. 193, D, *p*). In addition to this main pulmonary vein, formed by the fusion of two branches coming from the ventral side of the two lungs, there is a small accessory vein coming from the dorsal side of the root of each lung and from the adjoining parts of the pharyngeal wall. These also open into the hepatic vein just in front, and on each side, of the opening of the main vein.

TELEOSTEI.—Amongst the Teleostean fishes we find the same venous trunks laid down as in the Lung-fish or Elasmobranch. There is within the group considerable variability but the variations are as a rule derivable from a primitive type like that of *Lepidosiren*. Thus in *Salmo* the subintestinal vein bifurcates in front into the two paired vitelline veins while in numerous other Teleosts (*Esox, Belone, Syngnathus, Hippocampus, Gobius*) it passes forwards into a median unpaired vitelline vein. Each of these conditions is obviously derivable from that illustrated by *Lepidosiren*, by the disappearance, on the one hand, of the median, and, on the other, of the paired vitelline veins.

AMPHIBIA.—In *Salamandra* (Choronshitzky, 1900) two lateral vitelline veins are described, the right comparatively small in size. Behind the liver rudiment they lie close together near the mid-ventral line and passing forwards they diverge, passing one on each side of the liver rudiment to unite in front of it and form the hind end of the heart. The two veins undergo fusion behind the liver to form the subintestinal vein and in front of the point of fusion the right vein disappears so that, as in the Lung-fish, all the blood passes to the heart round the left side of the liver. The **mesenteric** vein develops as a branch of the right vitelline vein close to its front end and after the disappearance of the greater part of the right vitelline vein the mesenteric is seen replacing it as the right limb of a horseshoe-shaped arrangement of veins which embraces the diver rudiment from in front. The portion of the vitelline veins in front of the mesenteric breaks up into the hepatic network. The vitelline vein shrinks to an inconspicuous vestige while the mesenteric becomes relatively large and forms the hepatic portal of the adult.

The posterior cardinal veins (Hochstetter, 1888) run alongside the archinephric ducts, which they more or less surround, to the region of the pronephros where each dilates to form a large pronephric sinus. In the opisthonephric region the vein forms two main channels an inner and an outer (Fig. 194, B, *op*), the former eventually undergoing fusion with its fellow to form an inter-renal vein which becomes later the renal portion of the posterior vena cava. The outer channel, as in the Lung-fish, becomes continuous with the caudal vein to form the renal portal, while at the front end of the opisthonephros it loses its connexion with the part of the vein lying farther forwards. A venous connexion is established between the front end of the inter-renal vein and the tip of the liver, and the channel which so arises, commencing behind in the inter-renal vein, traversing the substance of the liver and ending in the hepatic vein, forms the posterior vena cava (Fig. 194, C, *p.v.c*). In later stages the liver tissue disappears over the greater part of the posterior vena cava so that it passes naked through the splanchnocoele.

The anterior cardinal vein with its intercalated section of lateral cephalic persists as the internal jugular vein of the adult. The Duct of Cuvier also persists in the adult and is now termed the **anterior vena cava**.

The anterior abdominal vein arises as a pair of small veins in the ventral body-wall. These unite in front in the region of the liver and open into the left duct of Cuvier according to Hochstetter. Later the two veins fuse into a single unpaired vessel except at their hinder ends where they become connected with the renal portal vein on each side. Anteriorly the opening into the duct of Cuvier becomes replaced by an opening into the hepatic portal vein.

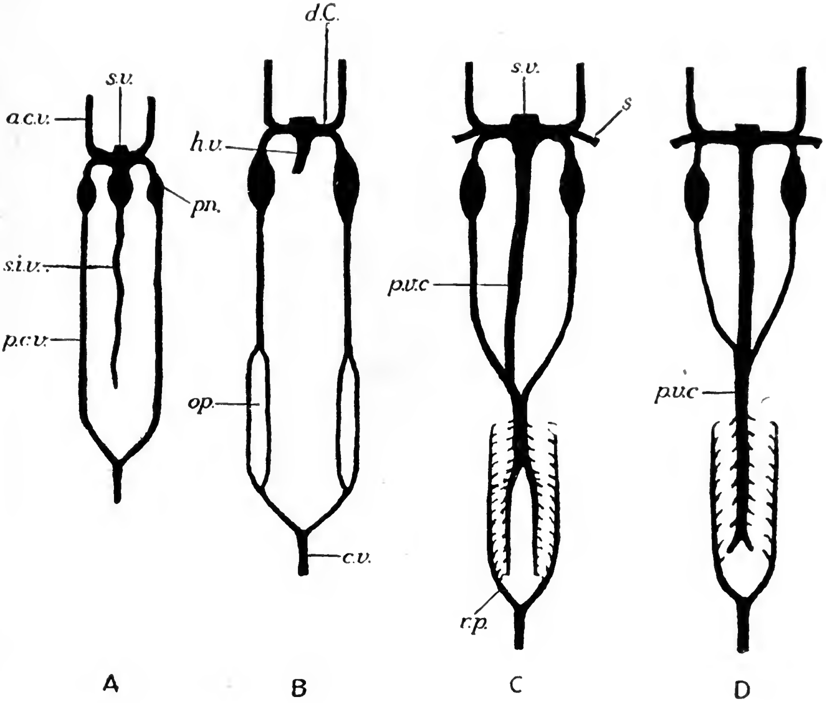


Fig. 194.—Development of main venous trunks in *Salamandra* according to Hochstetter (1888).

a.c.v, anterior cardinal vein ; *c.v*, caudal vein ; *d.C*, duct of Cuvier ; *h.v*, hepatic vein ; *op*, opisthonephros ; *p.c.v*, posterior cardinal vein ; *p.v.c*, posterior vena cava ; *pn*, pronephric sinus ; *r.p*, renal portal ; *s*, subclavian ; *s.i.v*, subintestinal vein ; *s.v*, sinus venosus.

Sauropsida.—Here we find the same main venous trunks as in the lower groups and, in correlation with the large quantity of yolk, the ventral or vitelline system of veins develops precociously as compared with the dorsal or cardinal system.

Lacerta, which has been investigated in detail by Hochstetter (1892), may be taken as an example of the Reptiles. The first veins to make their appearance are the lateral vitelline veins which pass forwards on the dorsal side of the yolk-sac, converging in front to form the hind end of the heart. The two vitelline veins become connected by a transverse anastomosis dorsal to the gut and just behind the dorsal pancreatic rudiment (Fig. 195, B). The parts of the veins in front of this anastomosis break up into a network in the substance of the liver, and the two networks become continuous with one another (Fig. 195, C). The two vitelline veins now form another anastomosis

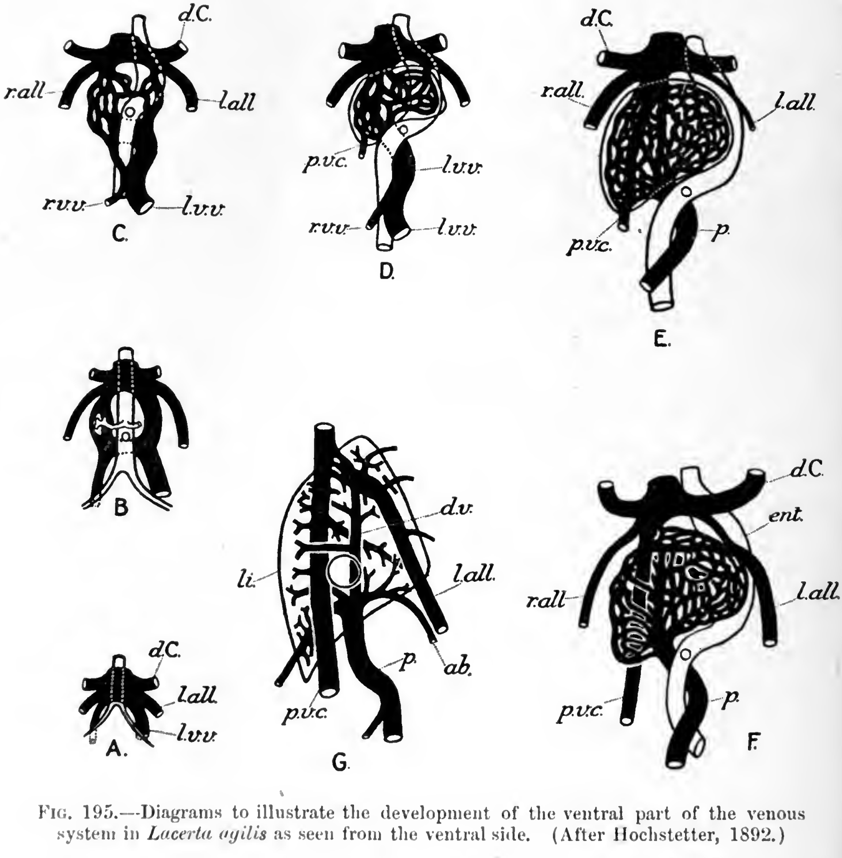


FIG. 195.—Diagrams to illustrate the development of the ventral part of the venous system in *Lacerta agilis* as seen from the ventral side. (After Hochstetter, 1892.)

ab, abdominal vein; *d.C*, duct of Cuvier; *d.v*, ductus venosus; *ent*, alimentary canal; *l.all*, left allantoic vein; *li*, liver; *l.v.v*, left vitelline vein; *p*, portal vein; *p.v.c*, posterior vena cava; *r.all*, right allantoic vein; *r.v.v*, right vitelline vein.

with one another farther back and ventral to the alimentary canal (Fig. 195, C). The right vitelline vein becomes reduced and finally obliterated in the region in front of this ventral anastomosis so that the whole blood-stream passes forwards to the level of the dorsal anastomosis by the persistent left vein (Fig. 195, D, *l.v.v*). In the region anterior to the dorsal anastomosis the left vein now diminishes in size and finally disappears, first in the region behind the hepatic network (Fig. 195, D) and then in the region in front of the network (Fig. 195, E). Results of these changes are (1) that the hepatic network receives its blood supply by a single afferent vessel—the hepatic portal vein—which curves round the gut and is derived in great part from the left vitelline vein—and (2) that its blood drains away to the heart by a single efferent vessel—the hepatic vein—derived from the front end of the original right vitelline vein.

At a comparatively early stage in development a direct channel becomes established, by the widening out of the venous spaces along the middle of the hepatic network, so that a considerable proportion of the blood is able to pass forwards from the portal vein through the liver without actually traversing the network itself. This channel—the **ductus venosus** (Fig. 195, G, *d.v*) persists till nearly the period of hatching but then becomes obliterated so that all the portal blood has to traverse the hepatic network.

The posterior vena cava makes its appearance as a gradually widening channel through the hepatic network towards its right side (Fig. 195, D, E, *p.v.c*). This portion of the liver becomes prolonged backwards as a slender lobe ensheathing a prolongation of the blood channel mentioned. This prolongation fuses at its tip with the tip of the right opisthonephros, continuity becomes established between the venous spaces of the two organs and finally, as in the Amphibian, the liver tissue disappears over a large stretch of the slender lobe already mentioned so that the vena cava is now for a considerable length free from either liver or kidney.

At an early stage a branch of the vitelline vein develops close to its front end. This is the **allantoic** or umbilical vein (Fig. 195, *r.all* and *l.all*). These veins soon become asymmetrical, the left for a time being smaller than the right (Fig. 195, D, E). A little later however the left vein establishes a connexion with the hepatic network (Fig. 195, F); the portion of the vein posterior to this connexion becomes much widened, and the blood-stream from it courses by an enlarged direct channel of the network into the posterior vena cava (Fig. 195, F, G). The blood-stream being diverted through this channel, the portion of the left allantoic vein in front of it shrinks in size and disappears, as does the whole of the right allantoic vein (Fig. 195, F, G). The result is that there persists a single (left) allantoic vein which drains the blood from the allantois into the posterior vena cava near its front end. The allantoic vein increases in size with the allantois but becomes obliterated at the time of hatching when the allantois is cast off. The mesenteric vein develops as a branch of the portal vein (left vitelline vein) a short distance behind its entry into the liver: it increases in size as the vitelline diminishes with the consumption of the yolk and eventually it alone persists as the peripheral portion of the definitive portal vein of the adult.

The subintestinal vein is apparently present only in its post-anal portion which persists as the caudal vein of the adult. In front of the anus, where the ventral wall of the primitive alimentary canal has become intensely modified in connexion with the storage of yolk, the subintestinal vein has disappeared from the course of development.

As regards the dorsal venous system (Fig. 196), the two posterior cardinal veins converge posteriorly and become continuous with the caudal vein. The portions in the region of the opisthonephros become resolved into their external and internal components connected by numerous sinus-like spaces and channels amongst the kidney tubules (Fig. 196, A). With the development of a capillary network in the substance of the opisthonephros the larger blood spaces become divided into an afferent set connected with the external component and an efferent set connected with the internal one. The external channel remains continuous with the caudal vein and forms the renal portal vein. The two internal components fuse together in their anterior portion (Fig. 196, B) and become continuous with the intrahepatic portion of the vena cava. Posteriorly they remain separate and lose their continuity with the caudal vein (Fig. 196, B, C). The blood from the kidneys being now able to pass to the heart by the direct route through the posterior vena cava, the portions of posterior cardinal lying in front of the kidneys are no longer required and soon disappear (Fig. 196, C).

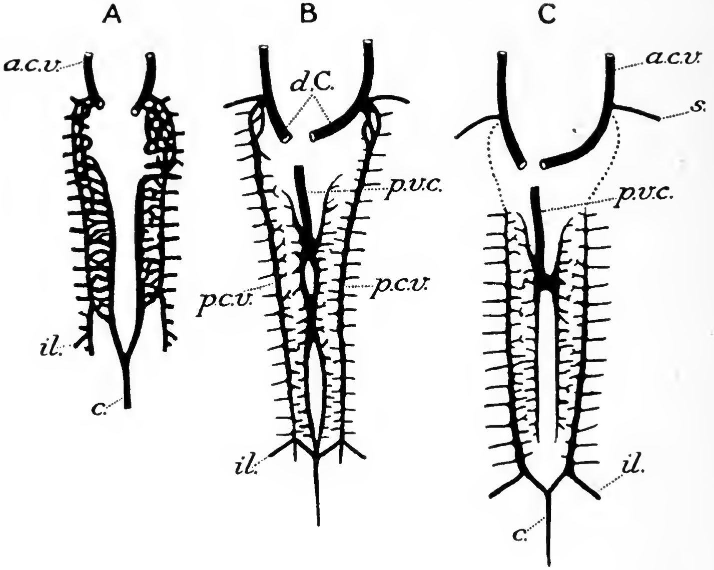


FIG. 196.—Diagram illustrating the development of the dorsal venous system in *Lacerta* according to Hochstetter, as seen from the ventral side.

a.c.v, anterior cardinal vein ; *c*, caudal vein ; *d.C*, duct of Cuvier (=ant. vena cava) ; *il*, iliac vein ; *p.c.v*, posterior cardinal vein ; *p.v.c*, posterior vena cava ; *s*, subclavian vein.

In the head region the anterior cardinal becomes in great part replaced by a lateral cephalic vein in a manner similar to that already described for *Lepidosiren*.

Birds.—In Birds the development of the venous system pursues, as we should expect, a similar course to that already described for Reptiles. Amongst the differences in detail the most striking is that the two vitelline veins become completely fused into a single vessel, the ductus venosus, through the hepatic region, before there are any signs of a hepatic network. This may be regarded as a backward extension of the fusion of the two vitelline veins which gives rise to the heart. The ductus venosus secondarily becomes surrounded by the liver rudiment and a network of channels spreads out from it in the liver substance. The allantoic veins behave as in *Lacerta* except that a small vestige of the left is said to persist throughout life.

The reduction of the tail region in modern birds has brought with it a modification of the caudal vein which is here paired, taking the form of a simple backward prolongation of the posterior cardinal. The main channel of the posterior cardinal runs along the *outer* edge of the opisthonephros but later on a slender channel appears along its inner edge—that on the right side being continuous with the posterior vena cava of which it forms simply a backward prolongation. The two inner channels undergo fusion so that the blood from the kidneys can drain away entirely into the posterior vena cava and this is followed as in other cases by the atrophy of the portion of posterior cardinal lying in front of the opisthonephros. This atrophy extends as far forwards as the subclavian vein which in the Fowl opens into the posterior cardinal vein some distance from its front end. The portion of posterior cardinal lying in front of this point is consequently saved from disappearance and persists as a portion of the definitive subclavian vein of the adult. It will be understood that the blood of the outer channel of the posterior cardinal, which reaches it from the caudal vein, from the posterior limb, and from the body-wall, passes entirely through the opisthonephric network towards the posterior vena cava, in other words that there is at this time a typical renal portal system.

As the metanephros develops, its tubules are also mixed up with the sinuses connecting external and internal channels of the posterior cardinals, so that it too has for a time a functional renal portal system. Later on however one of the channels through the metanephros becomes enlarged and the blood-stream passes directly through it to the posterior vena cava without traversing the meshes of the network. A true renal portal system then no longer exists and the reason for its disappearance is no doubt to be found in the fact that the vascular network of the kidney has become connected with the arterial system. Obviously this will give a much more efficient circulation than the original one, owing to the higher blood pressure in the dorsal aorta and renal arteries than in the renal portal veins which have the systemic network of capillaries interposed between them and the heart. Further the quality of the blood supplied in this way to the kidney is better—being arterial instead of venous—and for both these reasons we can readily understand the tendency in the more highly developed vertebrates for the renal portal system to disappear.

It has already been remarked that the posterior cardinals do not pass back into an unpaired caudal vein as in the Lizard. A vestige however of the unpaired condition may perhaps be recognized in the development of an anastomosis between the two vessels just behind the metanephros. From the transverse bridge so formed a connexion (**coccygeo-mesenteric** vein) is established with the portal vein in the mesentery.

The anterior cardinal vein together with an intercalated portion of lateral cephalic persists as the jugular vein of the adult bird.

The posterior cardinal vein undergoes in the Bird a curious change of position in relation to the root of the iliac artery which it crosses behind the mesonephros. At first it lies on the ventral side of this artery: then it develops an accessory channel round the dorsal side of the vessel and finally the whole blood-stream passes by this dorsal channel while the ventral one disappears. This affords a good example of the way in which a vein may in the course of evolution pass an apparent barrier formed by an artery, nerve or other organ.

INTER-SEGMENTAL VEINS.—In the body wall there develops a series of veins corresponding with the intersegmental arteries and opening into the cardinal veins.

VEINS OF LIMBS.—The vascular network of the limb-bud drains into the posterior cardinal vein. In the Bird (Evans, 1909) the drainage during its earliest stages is into the allantoic vein. Later numerous channels arise connecting the network with the posterior cardinal and presumably one or two of these become enlarged and persist as the definitive veins draining the hind limb.

LYMPHATIC SYSTEM.—The venous system has its obvious roots peripherally in the capillary network of blood-vessels, but it is also provided with a much less conspicuous set of tributary channels which constitute the lymphatic system. This extension of the vascular system retains a lower grade of evolution than the remainder. Its channels are less sharply defined, the lining endothelium over most of its extent having a much feebler development of the backing of connective tissue and muscle which forms the thick wall of the vein or artery. In its peripheral portions the lymphatic spaces may have remained practically in the primitive condition of intercellular chinks of the mesenchyme, while in its central portions, as it approaches the points at which it opens into the ordinary veins, its walls may be well developed and muscular. The lymphatic system serves to drain off the plasma which has oozed out from the capillary blood-vessels and forms the internal medium bathing the surface of the living cells of the body, and to return it to the blood-stream.

The fluid is peopled by amoeboid corpuscles but is without the red corpuscles which have no power to escape through the walls of the blood-vessels.

Our present knowledge of the ontogeny of the lymphatic system is in great part due to the labours of Huntington and McClure whose papers should be consulted as regards details. It seems clear that as a general rule lymphatic channels develop, later than the blood-vessels, as intercellular chinks in the mesenchyme which become continuous and form definite channels, the bounding cells becoming converted into thin endothelium.

SPLEEN.—The spleen arises in *Lepidosiren* and *Protopterus* (Bryce, 1905; Purser, 1917), which may be taken as typical examples, in the form of a condensation of the mesenchyme of the gut-wall. Blood spaces soon make their appearance in the rudiment which later becomes intercalated in the course of the main venous channel leading from intestine to liver. Later on the main blood-stream passes to the liver by a direct channel, the spleen now lying on a lateral loop: later still the afferent part of this loop becomes replaced functionally by a new arterial connexion.

The spleen rudiment frequently arises in close proximity to that of the pancreas and this has led to statements that the spleen is actually derived from the pancreas but the probability seems to be that such statements are based upon erroneous observation.

LITERATURE

Allis. Zool. Jahrb. (Anat.), xiv, **1900**.
Bemmelen, van. Zool. Anzeiger, ix, **1886**.
Bemmelen, van. Meded. tot de dierkunde. Amsterdam, **1887**.
Boas. Morph. Jahrb., vii, **1882**.
Bryce. Trans. Roy. Soc. Edin., xli, **1905**.
Choronshitzky. Anat. Hefte (Arb.), xiii, **1900**.
Dohrn. Mitt. Zool. Stat. Neapel, vii, **1886**.
Dohrn. Mitt. Zool. Stat. Neapel, viii, **1888**.
Evans. Amer. Journ. Anat., ix, **1909**.
Gegenbaur. Jenaischer Zeitschrift, ii, **1866**.
Goette. Abhandlungen zur Entwickelungsgeschichte der Tiere, v. Hamburg and Leipzig, **1890**.
Greil. Morph. Jahrb., xxxi, **1903**.
Hochstetter. Morph. Jahrb., xiii, **1888**.
Hochstetter. Morph. Jahrb., xvi, **1890**.
Hochstetter. Morph. Jahrb., xix, **1892**.
Hochstetter. Hertwigs Handbuch der Entwicklungslehre, iii, **1906**.
Hochstetter. Voeltzkows Wissenschaftliche Ergebnisse, iv. Stuttgart, **1906***.
Hoffmann. Morph. Jahrb., xx, **1893**.
Hoyer. Bull. intern. Acad. Sci. Cracovie. Comptes rendus des Séances, **1900**.
Huntington and others. Anat. Record, ii, **1908**.
Huntington. Anat. Record, iv, **1910**.
Kellicott. Memoirs N.Y. Acad. Sci., ii, **1905**.
Kerr, Graham. The work of J. S. Budgett. Cambridge, **1907**.
Kerr, Graham. Proc. Roy. Phys. Soc. Edin., xvii, **1907***.
Kerr, Graham. Proc. Roy. Phys. Soc. Edin., xviii, **1910**.
Langer. Morph. Jahrb., xxi, **1894**.
Mackay. Phil. Trans. Roy. Soc., B, clxxix, **1889**.

McClure. Anat. Record, ix, **1915**.
Maurer. Morph. Jahrb., xiv, **1888**.
Mollier. Hertwigs Handbuch der Entwicklungslehre, i, **1906**.
Müller. Abhand. Akad. Wiss. Berlin, **1845**.
Müller, F. W. Arch. mikr. Anat., xlix, **1897**.
O'Donoghue. Proc. Zool. Soc. Lond., **1912**.
Purser. Quart. Journ. Micr. Sci., lxii, **1917**.
Rabl, C. Leuckarts Festschrift. Leipzig, **1892**.
Rabl, H. Arch. mikr. Anat., lxix, **1907**.
Ridewood. Proc. Zool. Soc. Lond., **1899**.
Robertson. Quart. Journ. Micr. Sci., lix, **1913**.
Röse. Morph. Jahrb., xvi, **1890**.
Rückert. Biol. Centralblatt, viii, **1888**.
Rückert. Hertwigs Handbuch der Entwicklungslehre, i, **1906**.

CHAPTER VII

THE EXTERNAL FEATURES OF THE BODY

THE preceding chapters have dealt with the ontogenetic evolution of the various organ systems of the vertebrate body. The present chapter will sketch in outline the development of the external characteristics in so far as these have not already been referred to.

(1) DEVELOPMENT OF GENERAL FORM.—The groups of vertebrates in which the egg is typically holoblastic will be considered first.

CROSSOPTERYGII.—Of the two surviving genera *Polypterus* alone has been studied (Graham Kerr, 1907) and the main features in the development of its body-form may be gathered from an inspection of Fig. 197.

It will be seen that the head-end of the embryo is the first to project freely above the general surface of the body (Fig. 197, A). The tail projection soon however makes its appearance (Fig. 197, B) and during subsequent stages grows much more actively in length, the embryo soon assuming a somewhat tadpole-like shape, with a laterally compressed hinder region and a rounded swollen anterior region formed by the main part of the yolk-laden egg. Two organs, the cement-organ (*c.o*) and the external gill (*e.g*), make their appearance as slight bulgings of the surface at a very early stage. During subsequent stages the hinder, laterally compressed region grows rapidly at the expense of the mass of yolk which becomes consequently reduced in volume and at the same time loses its spherical shape so that it projects less prominently. It will be noticed that during the later stages the post-anal region grows particularly actively, the anus thus coming to lie at a relatively greater and greater distance from the hinder end of the body and giving rise to a rapidly growing true "tail" region. During the later stages (Fig. 197, D, E, F) the head undergoes much increase in length, its active forward growth beginning about stage 31. The mouth is at first widely gaping (Fig. 197, E) but by stage 34 the mouth-hinge becomes functional and it can be closed. By stage 36 the anterior swelling due to the yolk has practically disappeared.

ACTINOPTERYGII.—Amongst the Actinopterygian fishes the Ganoids which still retain the holoblastic segmentation show very

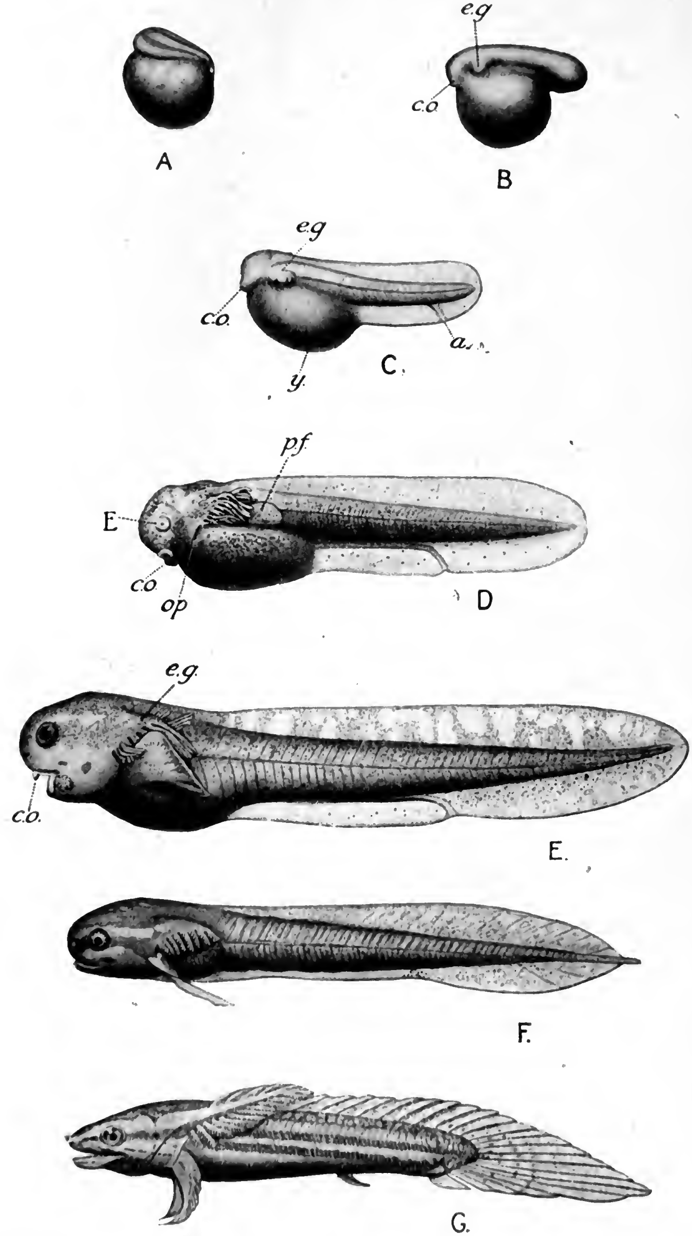


FIG. 197.—Stages in the development of *Polypterus*. (All except A after drawings by Budgett.)

A, stage 12; B, 23; C, 27; D, 31; E, 33; F, 36; G, larva 30 mm. in length. *a*, anus; *c.o*, cement organ; *E*, eye; *e.g*, external gill; *op*, operculum; *p.f*, pectoral fin; *y*, yolk. (A-E × 11; F × 8.)

much the same arrangement in early stages as will be seen in the Lung-fishes (Fig. 200, A), the dorsal part of the embryonic body being curved round the periphery of the egg. As the embryo increases in length the growth of the posterior end is specially active and the general proportions become very much as in *Polypterus*. The somewhat tadpole-like appearance of the larva, caused by the persistent spherical shape of the main mass of yolk, is again apparent—especially in *Amia* and *Lepidosteus* (Fig. 198, A). As in *Polypterus* the intestinal portion of the alimentary canal rudiment is relatively slender in form, arising by a process of actual backgrowth of the posterior trunk region rather than by gradual modelling of the yolk as is the thick intestinal rudiment in the Lung-fish. Conspicuous characteristics of the actinopterygian Ganoid larvae are the presence of well-developed cement-organs and the absence of external gills.

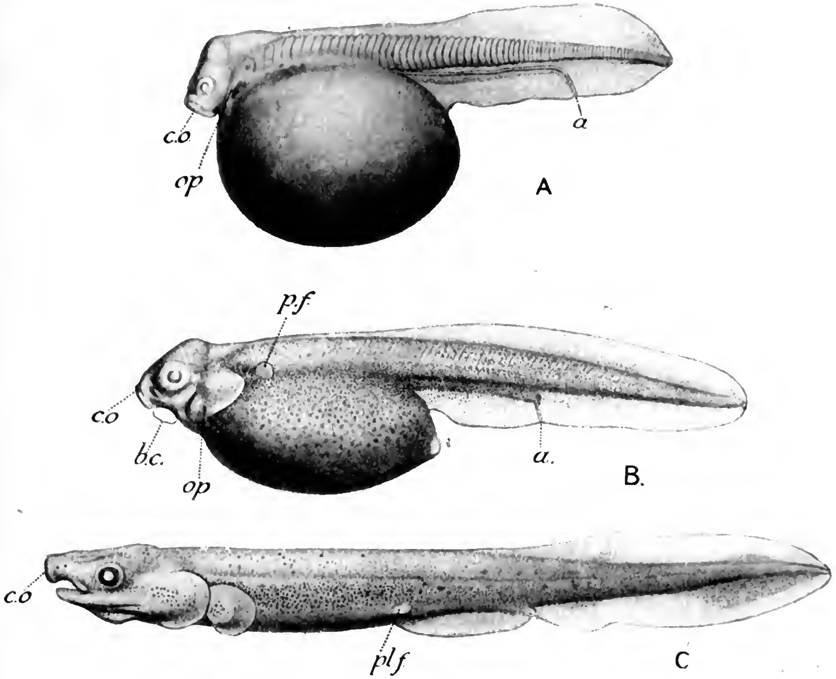


FIG. 198.—Stages in the development of *Lepidosteus*.

a, anus ; *b.c*, widely gaping buccal cavity ; *c.o*, cement organ ; *op*, operculum ; *p.f*, pectoral fin ; *pl.f*, pelvic fin.

In the Teleostean fishes there has come about with the high development of telolecithality a great reduction in the angular extent of the embryonic rudiment during its early stages. Consequently there is very slight ventral curvature round the yolk. In the

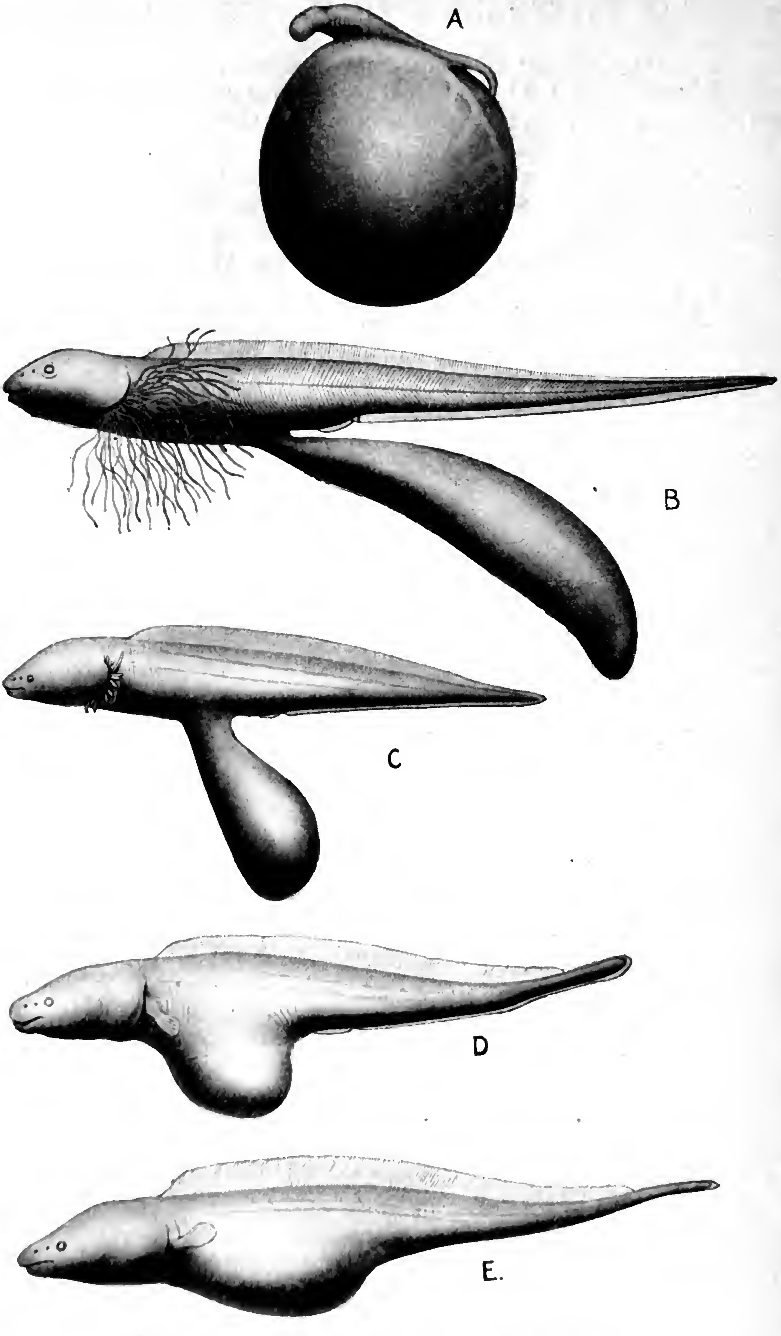


Fig. 199.—*Gymnarchus niloticus.* (A after Assheton.)

A, seventh day ; B, tenth day ; C, fourteenth day ; D, age unknown ; E, forty-third day.

Ganoids *Amia* and *Lepidosteus* the main mass of yolk retains its form for a considerable period, causing a great bulging of the ventral body-wall anteriorly. In the Teleost this is still further accentuated, the bulging forming the yolk-sac which remains prominent even in larvae sufficiently developed to be able to swim actively. An extreme case of the prominence of the yolk-sac is afforded by *Gymnarchus* (Fig. 199) where it shows a peculiarly elongated form for a certain period. Cement organs are as a rule absent in Teleostei: so also are external gills though in rare cases the latter have physiological representatives in filamentous prolongations of the gill lamellae (Fig. 199, B).

Great variety of form exists amongst the larvae of Teleostean fishes, more especially amongst those of pelagic habit. Familiar examples are seen in the pelagic larvae of the Eels—much compressed from side to side, transparent and colourless—even the blood being free from haemoglobin—and much greater in bulk than the immediately succeeding phase in the life-history. The larvae of the Flat-fishes (Pleuronectidae—Flounder, Plaice, Sole, etc.) are again of special interest owing to the extraordinary asymmetry which they develop. They are at first quite symmetrical and in no way abnormal. The larva swims at this time with its laterally compressed body vertical after the manner of a Bream but later develops the habit of swimming on its side. The side of the head-region which is below now grows more actively than the other so that the head becomes strongly asymmetrical and the eye of the lower side becomes gradually transferred to the upper, the right and left eyes being now both on the same side of the head. Correlated with this asymmetry in form there comes about a corresponding asymmetry in colour, the chromatophores being collected together on the upper side and giving it its characteristic obliterative colouring. In some genera it is the right side of the body which is above, in others the left—while in a few species it appears to be indifferently the one or the other.

DIPNOI.—Both of the dipneumonic Lung-fishes—*Lepidosiren* and *Protopterus*—have been investigated (Graham Kerr, 1900 and 1909: Budgett, 1901). They closely resemble one another and *Lepidosiren* will be chosen here for description (Fig. 200).

During the early stages of the modelling of the embryonic body (Fig. 200, A) the latter is curved round the egg, occupying about 290° in angular extent. The head-region becomes demarcated as a slight, somewhat lance-shaped protuberance above the general surface of the egg due to the neural rudiment. The branchial region becomes marked at an early stage by a slight elevation of the surface which soon becomes divided by shallow oblique grooves into the series of branchial arch rudiments. About stage 25 (Fig. 200, B) the tip of the head and the tip of the tail project sharply above the general surface: the external gills (*e.g*) are now in the form of four distinct little knobs on each side, and the cement organ (*c.o*) has made its appearance as a crescentic structure on the ventral side curving round the tip of the

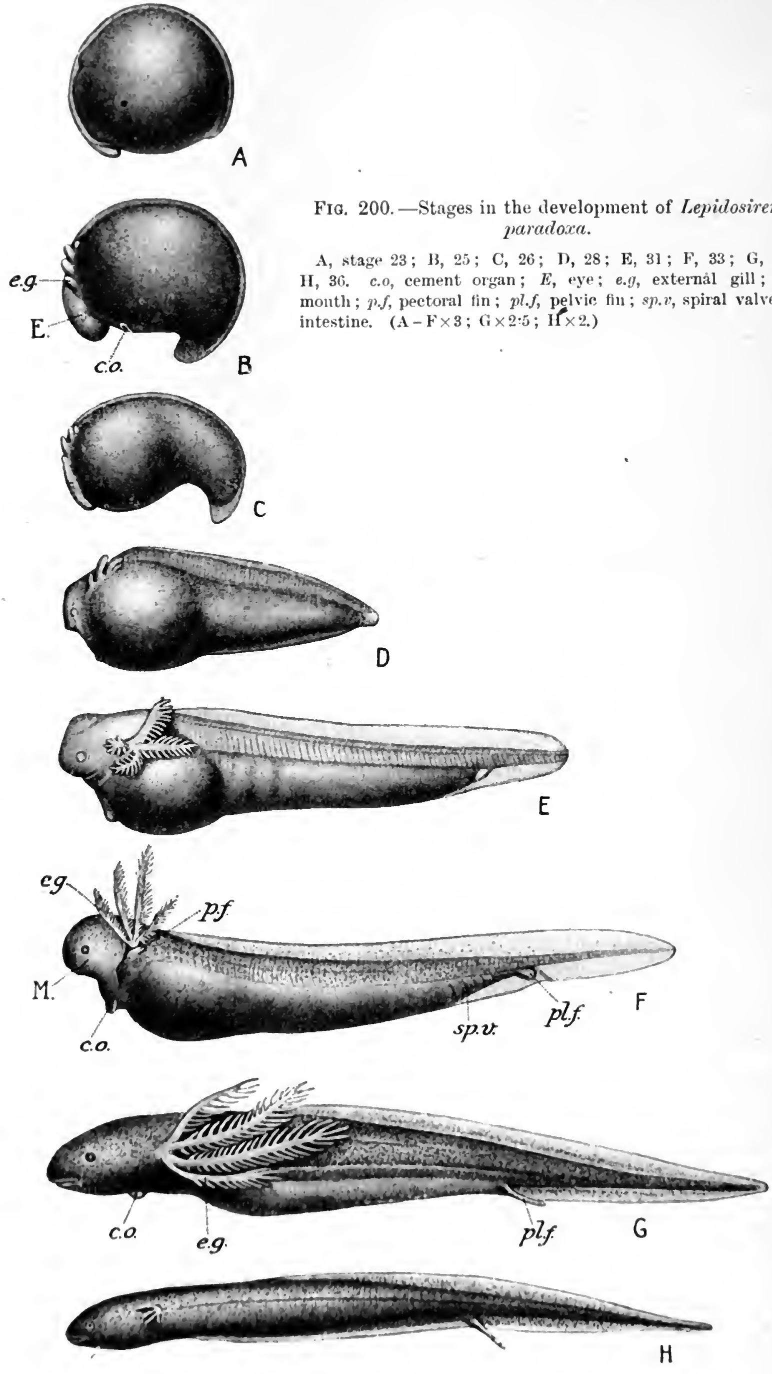


FIG. 200.—Stages in the development of *Lepidosiren paradoxa*.

A, stage 23; B, 25; C, 26; D, 28; E, 31; F, 33; G, 35; H, 36. *c.o*, cement organ; *E*, eye; *e.g*, external gill; *M*, mouth; *p.f*, pectoral fin; *pl.f*, pelvic fin; *sp.v*, spiral valve of intestine. (A – F × 3; G × 2·5; H × 2.)

head. The posterior part of the body now becomes laterally compressed, it grows rapidly in length and the larva assumes a somewhat tadpole-like form—the apparent "tail" being at first bent ventrally (Fig. 200, C). The anus is situated close to the tip of this portion of the body, therefore it is, strictly speaking, not tail but rather posterior trunk-region. About this period hatching takes place. The tail-like hinder region now straightens out (Fig. 200, D) and grows rapidly in length, the growth being at first mainly pre-anal and the true tail-region developing later. As in Crossopterygians the tail is throughout protocercal. As in *Polypterus* again the head-region for a considerable period shows no active growth in length: it is not until about stage 31 (Fig. 200, E) that its growth becomes active and the head-region begins to develop the modelling of its definitive features. The external gills grow actively in length after hatching: each develops a double row of pinnae along its external margin and eventually all four become fused together at their bases. They reach their maximum about stage 35 and thereafter undergo a process of atrophy resulting in their complete disappearance. The limbs make their appearance about stage 31, each as a little knob bearing a striking resemblance to the first stage of an external gill. The cement organ increases in size forming a large cushion-like and very conspicuous organ in the larva of stages 32-34 (Fig. 200, F). Eventually it shrivels up and disappears without leaving a trace behind.

In *Protopterus* as already mentioned the general features of development agree very closely with those of *Lepidosiren.*

Of *Ceratodus* developmental material was obtained by Caldwell in 1884 and by Semon in 1891. Semon's material has formed the basis of a long series of investigations by himself and others which together constitute an important contribution to Vertebrate morphology (Semon, 1893–1913; 1901*).

While the general modelling of the body shows a general resemblance to that of *Lepidosiren* and *Protopterus* there are certain well-marked differences in detail. Perhaps the most striking of these is that the head-region shoots ahead in its development and grows actively in length so as to project freely in front of the yolk at a much earlier period than in the other genera (Fig. 201, A, B). Again the main mass of yolk undergoes a more uniform process of lengthening so that it assumes a somewhat spindle-like form and allows the body as a whole to become slender and "fish"-like, the "tadpole" shape due to the persisting spherical mass of yolk in *Polypterus* or *Lepidosiren* being here absent. During the later larval stages the divergence of *Ceratodus* from the other two Lung-fishes towards the more typical fish condition becomes marked by the paddle-like form of the limbs and the much greater development of the median fin round the hinder end of the body. It will be noticed also that two conspicuous features of the young *Lepidosiren* or *Protopterus*—the Cement organ and the external gills—are completely absent in *Ceratodus.*

In the Urodela the least specialized subdivision of the Amphibia the evolution of external form closely resembles that of the Lung-fishes. An early stage of such a relatively primitive member as *Necturus* might readily be mistaken for the corresponding stage of

FIG. 201.—Development of *Ceratodus forsteri*. (From Semon—*In the Australian Bush*.)
A, stage 32; B, 34; C, 38; D, 41; E, 45; F, 48. (Magnification about 6½ diameters.)

Ceratodus and an almost equally striking resemblance is shown by an Axolotl or Newt about the time of hatching, except that in this case there are the well-developed external gills which were as we have seen absent in *Ceratodus* though present in the other two Lung-fishes.

In the case of the Anura it is perhaps premature to make general statements regarding the differences in form which distinguish them from the more primitive Urodela, for different species differ greatly in the size of the egg and its richness in yolk and the great majority of them have not as yet had their development worked out.

The head-region projects less prominently, sometimes being in its early stages quite flattened out on the yolk (*Alytes, Phyllomedusa*) while in other cases the embryo elongates as a whole there being for a time no marked break in contour between head, trunk and tail. In such cases growth in length may for a time be most active ventrally, so as to cause a curvature of the embryo with its concavity on the dorsal side (*Rana*). In the later stages the tail-region is highly developed, the splanchnocoele being greatly shortened and widened and the head also very broad giving the characteristic tadpole type of larva.

Particular interest attaches to the development of such types of Amphibia as possess heavily yolked eggs. A good example is afforded by the Gymnophiona such as *Hypogeophis* (Fig. 202). A conspicuous difference from the condition seen in the Teleostomes (Figs. 197, 198, 199) lies

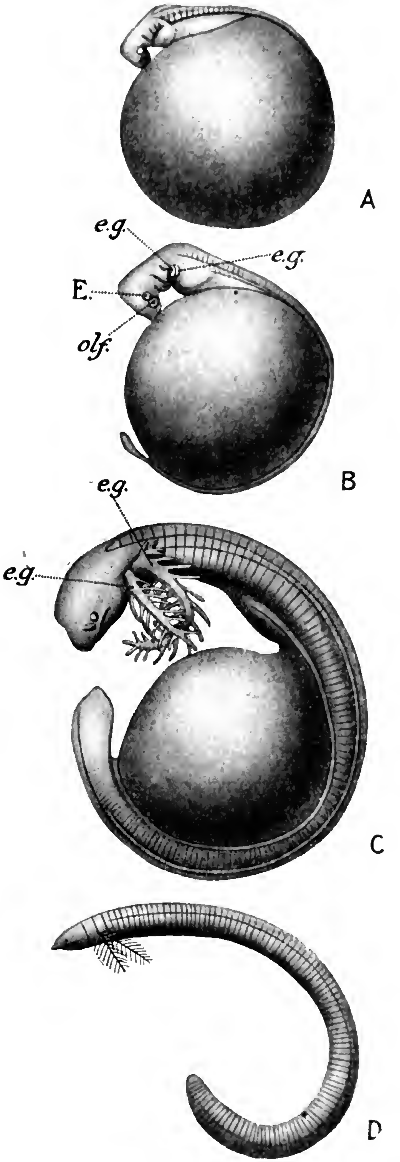


FIG. 202.—Embryos of *Hypogeophis rostratus*. (After Brauer, 1899.)

A, B, C×4; D×1⅓. (For details of B cf. Fig. 87, C). *E*, eye; *e.g*, external gill; *olf*, olfactory organ.

in the fact that here as in the Dipnoi the embryonic body during the early stages of its differentiation has a much greater angular extent, curving round the mass of yolk cells instead of being restricted to a small extent near the apical pole. Another important point to notice is the well-marked downward flexure of the head during early stages—a feature which has already been correlated (p. 93) with the presence of a large supply of yolk. The active forward growth of the head-region leads to the rounded main mass of yolk being situated well back, just in front of the anal region (Fig. 202, C), instead of anteriorly as in the tadpole-shaped larvae of *Lepidosiren* or *Protopterus* or the Ganoid fishes.

ELASMOBRANCHII.—Of the isolated groups of Vertebrates characterized by having meroblastic eggs the most nearly primitive is that of the Elasmobranch fishes. Unfortunately for purposes of comparison we are not, up to the present, acquainted with any member of the group possessing small eggs poor in yolk.

Here as in other groups with typically meroblastic eggs the early rudiment of the body of the embryo—or more correctly of the dorsal portion of the body—extends through a relatively small angular extent, in striking contrast with the 200° or more of a Lung-fish or of one of the Gymnophiona. As the embryo proceeds with its development it grows actively in length, headwards and tailwards, so as to project freely in a tangential direction, remaining in connexion with the main mass of the egg (yolk-sac) by a narrow yolk-stalk. During the forward growth of the head growth-activity is less pronounced on the ventral side so that the gill-clefts are forced into an oblique position and the head undergoes pronounced cerebral flexure.

AMNIOTA.—In the non-mammalian Amniota[1] the first point to notice is that although the size of the egg and the absolute amount of food-yolk contained within it are relatively enormous yet the degree of telolecithality is less extreme than it is in the case of the meroblastic eggs of fishes. Consequently the segmentation process resulting in blastoderm formation spreads in an abapical direction past the level at which the posterior end of the embryonic rudiment will be developed: as a result of this the entire embryonic rudiment lies at its first appearance well within the boundary of the blastoderm, instead of its hinder end being coincident with that boundary as is the case in Elasmobranchs.

Here again the embryonic body grows forwards and backwards free from the surface of the egg, the backward growth being much less pronounced than in the case of the fishes in anticipation of the ultimate lesser degree of development of the tail-region correlated with its diminished locomotor importance in the adult.

The most striking feature however is one which may perhaps be correctly expressed by saying that the clogging influence of the

---

[1] The external features in their development are well illustrated by the developing Bird (see the figures in Chap. X.).

yolk upon the growth in length of the ventral side of the body is much more marked than in other Vertebrates. The result is a strong ventral curvature of the body. The ventral flexure of the head in the mid-brain region already seen in the Elasmobranchs and Gymnophiona is here still more marked, but in addition the whole body is strongly curved ventrally to such an extent as to form more than one complete turn of a spiral. This curvature is in its incipient stages of great morphological interest as providing a possible explanation of the downward indentation of the blastoderm by the head and tail regions, and their consequent ensheathment in blastodermal pockets, which led eventually to the evolution of the amnion.

The formation of the amnion and the separation of true amnion from false amnion involve as will be gathered from Chapter VIII. a solution of continuity of the somatopleure or original body-wall and probably this has initiated what is perhaps the most striking feature of amniote development—the loss, at the time of hatching, of a relatively large proportion of somatopleure together with the allantois.

As a matter of minor detail it should be mentioned that in the case of Birds as compared with the lower Amniota the embryo is distinguished during a long period by the relatively enormous size of the head. It seems reasonable to regard this as in the main an anticipation of the great development of the eyes and optic lobes in the adult.

In the foregoing short sketch the author has confined himself to the main branches of the Vertebrate stem. He has omitted all reference to three different types near the base of that stem, namely *Amphioxus*, the Lamprey, and the Myxinoid, which are of great interest in themselves but which are of less importance for enforcing general principles of Vertebrate development. Of the three types the first will be found fully described by MacBride in Vol. I. and it will easily be seen how in the general form of body, as in various other characteristics, the young Amphioxus has diverged widely from the more typical Vertebrates. The Myxinoids, so far as they are known from Bashford Dean's researches on *Bdellostoma* (1899), appear also to be highly specialized. The Lampreys on the other hand have diverged to a much less extent from the normal. The most striking features during the early development of the body are (1) that the head-region, as was the case in *Ceratodus* and *Hypogeophis* and as is the case also with *Bdellostoma*, shows a marked activity in its growth in length, the mass of yolk persisting longest posteriorly and (2) that the outgrowth of the tail is delayed until a comparatively late period. A marked negative feature in all three of the types mentioned is the absence of all trace of paired limbs.

(2) THE MEDIAN FINS.—The primitive Vertebrate, with its segmented musculature arranged along the two sides of the body and its skeletal axis and central nervous system lying at the mesial plane, is clearly a creature constructed for swimming by lateral

flexure after the manner of an Eel. To secure greater efficiency the body, more particularly its purely motor post-anal portion, becomes compressed from side to side, the compression being most marked near the margin of the body where the thin almost membranous median fin is produced.

In development the median fin arises as a projecting fold of slightly thickened ectoderm into which later on mesenchyme penetrates. In what the evidence points to as being the primitive condition this fin rudiment is continuous and extends round the hind end of the body. In such relatively primitive Vertebrates as Crossopterygians, Lung-fishes, and some Elasmobranchs, it extends forwards on the dorsal side practically to the head-region, while on the ventral side it reaches the anus and may even be continued onwards as a pre-anal median fin, though possibly this has originated in phylogeny independently of the main fin-fold.

In the Lung-fishes the median fin-fold during the course of development never loses its continuous and practically symmetrical arrangement round the tip of the tail. It retains throughout life the primitive symmetrical **(protocercal)** form. In the Crossopterygians (apart from the anterior portion of the dorsal fin which becomes divided up into a series of finlets) the same holds until a very late stage in development, the tail of the adult becoming very slightly asymmetrical though the term protocercal is usually and justifiably still applied to it. A similar protocercal tail occurs in the Amphibian larva while the tail of the adult Newt or Crocodile is simply a protocercal tail in which there is no longer a membranous fin-fold present.

It is however characteristic of the fishes in general that, in accordance with their high specialization as expert swimmers, the median fin during ontogeny loses its homogeneous character—certain portions of it, probably those portions which are in mechanically the most favourable positions, becoming enlarged while the intervening portions become reduced to the point of complete disappearance. The result is that the place of the originally continuous fin-fold is taken by a series of separate fins—one or two dorsal, a caudal or tail fin, and on the ventral side, an anal fin. Of these the caudal fin—most favourably situated of all the series to serve as a propelling organ—becomes specially enlarged. It is also characteristic of the more efficient swimmers that the part of the caudal fin lying on the ventral side of the axis becomes particularly developed. We may probably associate this with the function of rotating the body about its long axis as may be seen in a shark when it seizes its prey. The unsymmetrical condition of the tail so produced is termed **heterocercal**. When carried to its extreme the tip of the vertebral axis becomes tilted upwards and, so far as external appearance goes, a (secondarily) symmetrical condition is arrived at, as is seen in the **homocercal** tail of the Teleostean fishes. It will be understood that the protocercal, heterocercal and homocercal conditions merge into one another and cannot be distinguished by any rigid definition.

They clearly represent successive grades in the evolution of the tail as a more and more perfect organ of propulsion, a process of evolution which has come about independently in the various groups of fishes. Thus even the Lung-fishes—the surviving members of which group possess the primitive protocercal type of tail—during the geological periods when they most flourished showed numerous forms in which there was a highly developed heterocercal tail.

Again the assumption of a sluggish mode of life, or the simplification of the swimming movements, is frequently correlated with reversion of the tail towards the protocercal condition. This is clearly seen in many teleostean fishes, such as the Eels and many deep-sea bottom-frequenting fishes. In such cases all trace of the unsymmetrical condition may have disappeared from ontogeny but there is no room for doubt regarding an ancestral heterocercal or homocercal condition—for in their general structure these fishes are highly evolved Teleosts and the group as a whole is characterized by the tail being homocercal.

In the case of the surviving Lung-fishes the general archaicism in structure, and more especially the extremely archaic character of the paired fins, are in favour of the protocercal character of the tail being persistent rather than revertive—apart from the evidence of embryology which fails to disclose any trace of a pre-existing heterocercal phase.

(3) THE LIMBS.—One of the characteristic structural features of the Vertebrata is the presence of the two pairs of limbs, pectoral and pelvic. Two main types of such limbs can be recognized—the fin type for swimming and the pentadactyle or leg type for moving on a solid substratum. As the former is on the whole characteristic of fishes, and as fishes are on the whole more nearly primitive than are terrestrial Vertebrates, the idea has naturally arisen and has now attained perilously near to the position of a dogma that the leg type of limb has been evolved out of the fin; and elaborate attempts have been made to define the manner in which this has come about. It is necessary at the outset to emphasize the importance of keeping an open mind upon this question: there exists the possibility—which as will be seen later is not lightly to be brushed aside—that pentadactyle limb and fin are not in the relation of lineal descent at all but that they have been derived from a common ancestral type of limb differing from either.

No limbs exist in *Amphioxus* or in the Cyclostomata. There is however a general tendency in Vertebrates which have assumed an eel-like form of body for the limbs to degenerate and disappear, and it is well to bear in mind the possibility that this has happened in the case of both of the types mentioned.

The limb at its first appearance in embryonic development forms a little projection from the body surface—a core of mesenchyme enclosed in an ectodermal sheath. In *Lepidosiren* or *Protopterus* or a Urodele it is in the form of a rounded knob identical in appearance with the rudiment of an external gill, but in a large proportion of the Vertebrates it is narrower in a dorsi-ventral than in an antero-posterior direction so as to have the form of a short longitudinally-running ridge.

As is usual where active increase of surface is about to take place the projecting limb rudiment is foreshadowed by a thickening of the ectoderm and by a condensation of the underlying mesenchyme.

In *Torpedo*, one of the Rays—fishes characterized by the great antero-posterior extension of the large pectoral fin—the two limb rudiments which are at first distinct (Rabl, 1893) become for a time joined together by a transitory ectodermal thickening. This phase, in which the two fin rudiments are as it were parts of a continuous ridge, was the earliest stage observed by Balfour (1878) and it afforded him an embryological basis for the lateral-fold view of the phylogenetic origin of the Vertebrate limbs (see p. 445).

As the limb rudiment develops it shows in many cases characteristic changes in its position. First it shows a movement of rotation. This is well illustrated by the case of *Ceratodus* as described by Semon (1898). Here the pectoral limb rudiment becomes rotated in such a way that its originally pre-axial or headward edge becomes dorsal and its originally lower or ventral surface comes to face in a headward direction. In other words, if one could observe the developing left pectoral limb from a point away to the animal's left side the limb would be seen to undergo a clockwise rotation. It results from this that when the fully developed limb is folded back alongside the body its outer surface is that which was originally ventral. A rotation similar in direction though varying in angular extent in different forms occurs also during the development of the pectoral fin in Crossopterygians and Actinopterygians. In Tetrapoda, on the other hand, a rotation of the limb rudiment in the opposite direction takes place—the pre-axial edge becoming ventral. Not improbably this may be regarded as a secondary modification foreshadowing the pronate position of the fore-limb characteristic of terrestrial progression.

The pelvic fin in *Ceratodus* undergoes a similar rotation but in the opposite direction to that of the pectoral: the left pelvic fin regarded from a point away on the animal's left would be seen to undergo a counter-clockwise rotation. The result is that the originally dorsal surface comes to face headwards or, when the fin is folded back alongside the body, outwards.

The corresponding rotation of the pelvic limb in other fishes and in the lower Tetrapods appears to stand in need of further investigation.

It is clear from the facts of Comparative Anatomy that the paired limbs have undergone extensive shiftings along the surface of the vertebrate body in adaptation to its general form and its method of movement (see below, p. 448). It is of interest—though not necessary for establishing the fact of such phylogenetic shifting—to enquire whether any record of it occurs in ontogeny. Obvious evidence which at once suggests itself in this connexion is the presence of abortive muscle-buds in front of or behind those which become incorporated in the definitive limb. These seem clearly to indicate that the part of the body surface superficial to these buds was at one time part of the actual limb. But unfortunately such abortive buds occur both anterior and posterior to the definitive limb and there is no means of fixing definitely the time in phylogenetic evolution from which the two sets of abortive buds date. They may date from the same period, in which case they might merely afford evidence of the process of narrowing of the limb base which has undoubtedly taken place during the later evolution of fins; or they may date from different periods, in which case the anterior set might be taken as evidence of a backward movement of the limb, and the hinder set as evidence of a forward movement occurring at a different period—movements which again have undoubtedly taken place. In view of the impossibility of determining to what extent the evidence in any particular case is to be interpreted in these two different directions it seems on the whole advisable to leave this muscle-bud evidence on one side.

Other evidence has been adduced from cases where the actual limb rudiment as a whole (*i.e.* the projection from the surface of the body) seems to be displaced during development. For example in the figures illustrating the development of the pelvic limb in *Spinax* (p. 207) it will be seen that the anterior limit of the fin is in successive stages of development opposite myotomes 21, 26, 28 and 31; the hinder limit at the same stages opposite myotomes 30, 38, 38 and 39, and the middle of the fin base opposite myotomes 25, 32/33, 33/34 and 35/36. It seems quite allowable in such a case to speak of the limb as having undergone a backward displacement. In other cases, as that of *Scyllium* according to Goodrich (1906), ontogenetic development discloses no evidence of such backward migration.

The limb rudiment gradually increases in size and assumes its definitive form and as it does so it becomes equipped with its characteristic skeletal and neuromuscular arrangements in the manner already described.

PHYLOGENETIC ORIGIN OF THE LIMBS.—The limbs are organs highly characteristic of the Vertebrata. While they exist typically as two pairs, pectoral and pelvic, one or both pairs readily disappear in groups where they are no longer needed. They are particularly prone to disappear in those Vertebrates which assume an elongated form of body and revert to the archaic method of moving by lateral flexure. Thus in Eels the pelvic limb has disappeared, in *Symbranchus* both pairs. In *Lepidosiren,* the most elongated Lung-fish, we see both pairs in process of reduction. We see the same in elongated Urodeles such as *Amphiuma,* while in the Gymnophiona both pairs have vanished. In Reptiles we see beautifully how the limbs undergo reduction (*Chalcides*) and complete disappearance (Amphisbaenidae, *Anguis*) in those various groups of Lizards which have developed an elongated snake-like form. So also in Snakes.

In cases where the limbs are completely gone in the adult it may be possible to observe them during early stages of development. The young Symbranchus (Fig. 203) has for a time huge pectoral fins which it uses as organs of respiration (Taylor, 1914). In Gymnophiona and Blind worms (*Anguis*)[1] minute limb rudiments have also been observed in the embryo. In other cases no trace of the missing limbs has been found during early development. In view of this general tendency of the limbs to disappear in Vertebrates which have assumed an eel-like or snake-like form of body it is well, as

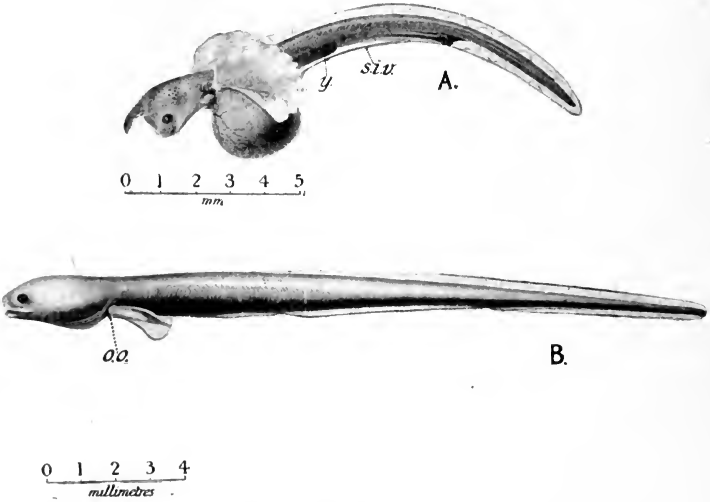


FIG. 203.—*Symbranchus marmoratus.* Larvae showing pectoral fins. (After Taylor, 1914.)

o.o, opercular opening; *s.i.v*, subintestinal vein; *y*, yolk.

already indicated, not to assume a dogmatic attitude in regard to such Vertebrates as Lampreys or Hag-fishes. The possibility is not excluded that even these Cyclostomes are descended from ancestors in which limbs were present.

The interesting question now emerges—How did the limbs of the Vertebrate originate in evolution? Few morphological speculations have excited more interest and more controversy than this. Two main hypotheses have been propounded and each has found supporters amongst the most eminent morphologists. Although in the opinion of the present writer it is no longer necessary to fall back upon either of these views, a simpler possibility having presented itself, a

---

[1] The elementary student may be warned not to mistake the rudiments of the paired penes in Snake embryos for limbs!

short sketch of each and of the arguments for and against it may now be given. The two hypotheses indicated are the "Lateral Fold" hypothesis of Balfour, Mivart, Thacher and others and the "Gill-septum" hypothesis of Gegenbaur and his school. Each hypothesis concerns itself with the origin of the paired limbs of fishes—the fin being regarded as the primitive type of limb from which the pentadactyle limb has been evolved later on.

THE LATERAL-FOLD HYPOTHESIS.—It will be remembered that the mode of development of the median unpaired fins indicates clearly that these fins are simply persisting and exaggerated portions of a once continuous median fin-fold. According to the lateral-fold hypothesis of the origin of the limbs the paired fins of fishes are similarly to be looked on as persisting and enlarged portions of a continuous fin-fold which once extended along each side of the body. The hypothesis rests upon a tripod basis of Embryological, Anatomical and Palaeontological fact.

Balfour in his Development of Elasmobranch fishes (1878) wrote: "Along each side of the body there appears during this stage (G-I) a thickened line of epiblast, which from the first exhibits two special developments: one of these just in front of the anus, and a second and better marked one opposite the front end of the segmental[1] duct. These two special thickenings are the rudiments of the paired fins, which thus arise as special developments of a continuous ridge on each side, precisely like the ridges of epiblast which form the rudiments of the unpaired fins." "If the account just given of the development of the limbs is an accurate record of what really takes place, it is not possible to deny that some light is thrown by it on the first origin of the vertebrate limbs. The facts can only bear one interpretation, *viz. that the limbs are the remnants of continuous lateral fins.*"

Further embryological support to this hypothesis has been provided (1) by the fact that the muscles of the limb are of segmental origin, derived from a number, often a considerable number, of myotomes (see p. 207) and that apparently vestigial muscle-buds have been found both headward and tailward of the series which take part in the muscularization of the definitive fin, and (2) by the fact that the fin type of limb commonly shows a marked narrowing of its base of attachment during the process of development, the rudiment having during early stages the form of a longitudinal ridge attached throughout its length to the body.

Regarding the evidence upon which the fin-fold hypothesis rests the following criticisms may be expressed. (1) The ectodermal ridge described as connecting the two limb rudiments in Elasmobranchs turns out to be a characteristic not of Elasmobranchs in general but only of Rays (*Torpedo*) *i.e.* of forms in which there is an enormous, admittedly secondary, extension of the pectoral fin along the side of the body. The Elasmobranchs less specialized in this respect—the

---

[1] = Archinephric.

Sharks and Dog-fishes—do not, so far as is known, develop this ridge.

(2) The fact that the myotomes—from which the limb rudiment, like other portions of the body, has to derive its equipment of voluntary muscles—are themselves metameric and that the skeletal elements necessarily correspond in position with the muscles seems to render it unnecessary to seek any further evolutionary explanation of the tendency on the part of the musculature and skeleton of the limb to exhibit a markedly metameric appearance during early stages in its development.

The occurrence of abortive muscle-buds in front of the definitive limb is taken—quite reasonably—as evidence pointing to a tailward shifting of the anterior margin of the limb having taken place, and similarly the presence of abortive buds behind the definitive limb is taken as evidence of a headward shifting of the hinder margin of the limb. But this shifting of the anterior and posterior margins of the limb may have in evolution taken place either synchronously (*i.e.* together with a narrowing of the base of attachment of the fin) or at different periods as the limb shifted backwards and forwards as a whole in accordance with variations in adaptational requirements. The present writer sees no convincing reason for rejecting either of these possibilities—and if either be possible then the evidence loses its value as support of one view rather than the other.

(3) The narrowing of the limb proximally and its expansion distally is a process which would naturally take place as the fin became more efficient as a propelling organ—just as in the evolution of a racing oar or paddle with its broad blade and slender shaft—and, accordingly, too great weight should not be attached to the occurrence of such a process during ontogeny in arguing as to the evolutionary *origin* of the limbs.

As regards anatomical evidence stress is laid on the exceedingly close structural resemblance in skeleton and musculature between the paired and the unpaired fins. On the other hand it is suggested that, seeing that lateral and paired fins are organs similar in function and built up out of similar muscular elements, a close similarity in their anatomical arrangements may quite probably be merely a case of that secondary convergence of which so many striking examples are known in the animal kingdom.

An ancient fossil fish, *Cladoselache,* is brought in to corroborate the view, its paired fins having each a broad longitudinally-running base of attachment and being apparently supported by separate rays without any continuous basal skeleton. But it is pointed out (1) that what signs there are of basal skeleton may be readily interpreted as representing the axis of a fin of the Ceratodus type laid back against the side of the body (see Fig. 169, E, p. 353), and (2) that the structure of the tail is of a very highly developed and powerful type and that it is most unlikely that a powerful swimmer, such as the highly evolved tail demonstrates *Cladoselache* to have been, should have retained its paired fins in a relatively primitive and inefficient condition.

Finally there are great physiological difficulties in the way of accepting the lateral-fold hypothesis. There are no more fundamental characteristics of the Vertebrate body than the arrangement of its longitudinal muscles in segmental masses along each side of the body, and the position of its skeletal axis, its central nervous system and its main arterial trunk in the region of the mesial plane. It is quite clear that such a creature is built for swimming by waves of lateral flexure after the manner of an Amphioxus, a Lamprey or a Lung-fish. Any new swimming organ that became evolved in primitive Vertebrates must have had some advantage over, or at least not interfered with, this primitive method of swimming. It is difficult to see how the supposedly ancestral lateral fold could possibly have complied with these conditions. The suggestion that the lateral fold may have functioned at first as a balancing organ or as a "bilge keel", will not bear examination from the point of view of elementary physics. Rabl suggests that the two lateral folds may have acted primitively as a kind of parachute and that they became muscularized at their anterior and posterior ends, the intermediate portion undergoing atrophy (thus originating the two pairs of limbs). The skeletal elements on this view would also develop at the ends of the ridge first, and spread backwards (pectoral fin) or forwards (pelvic fin). Thus would be explained the reversal of the position of the anterior and posterior edges of the two fins in *e.g. Ceratodus*. Such an explanation however fails entirely to meet the difficulty that there exists not merely an antero-posterior reversal in the structure of the two fins but also a dorsi-ventral one.

THE GILL-SEPTUM HYPOTHESIS.—This hypothesis was based by Gegenbaur (1872) on facts of adult anatomy. In some of the Elasmobranchs (*Pristis*) the central gill ray attached to the branchial arch is enlarged and the rays next to it have come to have their bases of attachment shifted secondarily from the arch on to this enlarged ray, so as to produce an arrangement recalling the biserial archipterygium of *Ceratodus* with its central axis and lateral rays; Gegenbaur suggests that the archipterygium with its limb girdle has in fact been evolved out of such an arrangement of rays attached to a branchial arch and that the limb itself is serially homologous with the gill septum.

In support of this view it is pointed out that branchial arch and limb girdle are each in early stages of development in the form of a continuous curved rod of cartilage; that this becomes usually segmented in the case of the branchial arch but that even in the girdle it also shows traces of segmentation in some ancient fossil forms (Pleuracanthids, Acanthodians); that in some cases the perichondrium of the pectoral girdle is known to be innervated by that typical branchial nerve the Vagus; that in the lower forms the *trapezius*, one of the muscles associated with the fore-limb, is innervated by the same nerve; and that connected with the ordinary branchial arches there are myotomic muscles as well as splanchnic, so that the basis already exists for a muscularization purely myotomic.

On the other hand the objection is urged against the Gegenbaur hypothesis that it involves a very great shifting of the pelvic fin backwards from its assumedly original position at the hinder end of the branchial region. This objection need not be taken seriously in view of the extensive shiftings of the limbs which are definitely known to have taken place. Thus in Rays we commonly find that the pectoral girdle has moved back to a position in relation to the segmentation of the body far posterior to the position which it occupies in Sharks: in Urodele Amphibians the hind-limb has taken up positions, as indicated by the position of the sacrum, varying between the 14th (*Triton palmatus*) and 63rd vertebra (*Amphiuma means*) while in the Anura—where in accordance with the leaping habits it is advantageous to have the attachment of the hind-limb far forward—the sacrum has come to be as far forward as the 9th or even (*Hymenochirus*) the 6th vertebra[1]: in Plesiosaurs and Birds a still more striking backward migration of the pectoral girdle with its attached limb has taken place (*e.g.* in the Swan as compared with *Archaeopteryx* through 14 or 15 segments): and finally in many Teleostean fishes the pelvic fins have become so shifted forwards along the sides of the body as to attain to an actually jugular position.

The fact that the limb girdles are embedded in the somatopleure while the branchial arches lie in the splanchnopleure has again been raised as a difficulty in the way of accepting the Gegenbaur theory. The difficulty is not so serious as it seems at first sight. The chief obstacle in the way of a splanchnopleural organ becoming shifted outwards into the somatopleure is clearly the coelomic cavity—but in the branchial region this tends to be in great part obliterated. As regards blood-vessels, nerves, etc.—these form by no means insuperable barriers to the change in position of skeletal elements. Such skeletal tissue may, as has already been indicated in Chapter V., spread past a blood-vessel or nerve and if it then becomes absorbed behind the obstacle there is brought about a complete transposition of the two structures.

The criticism that the musculature of the limbs is myotomic in origin while that of the branchial arches is splanchnic is provided against by the mixed character of the muscularization of the branchial arches, taken in conjunction with the demonstration that in such a case replacement of splanchnic muscle by myotomic may take place (p. 217).

Rabl considers the metameric origin of the muscles etc. of the limb to be enough by itself to undermine the Gegenbaur hypothesis, but it is difficult to see how the musculature could be otherwise than metameric in origin seeing that it has to be derived from the segmentally arranged myotomes.

---

[1] Gadow, in *Cambridge Natural History*.

The muscularization of the jugular pelvic fin of Teleostean fishes is admittedly secondary: the limb rudiment becomes muscularized by the myotomes to which it happens to be opposite at the time muscularization begins: but if this fact be admitted it is not open to us to deny the possibility of a similar process having taken place in the successive positions taken up by the pelvic limb in the course of the movements which it has undergone during phylogenetic evolution.

A further objection urged against the Gegenbaur hypothesis is that there have not been discovered, up to the present, any examples of the intermediate stages between gill-septum and limb which must have existed if this hypothesis be a true theory. This objection appears to be a valid one.

Again it is urged that in those Vertebrates which would appear, in this respect, to have retained most nearly the primitive condition (Cyclostomata, Elasmobranchii) the gill-septa are fixed firmly in position and are therefore not likely to become converted into motor organs, which must necessarily project beyond the surface and be freely movable. This objection like the last appears to be a valid one.

It will be apparent from the short sketch which has been given of the two rival views of the evolutionary origin of the limbs of Vertebrates that neither can be regarded as wholly satisfactory. However these hypotheses are old, as the science of Embryology goes. They were designed to fit the data available at the time they were formulated and the great bulk of subsequent work upon this particular problem has consisted in the adducing of new facts which appear conveniently to fit on to those already accumulated by the supporters of one view or the other. In a rapidly advancing science like Embryology however it is advisable to have from time to time a stocktaking of the facts of contemporary knowledge with the object of seeing whether the more extensive body of available facts suggests the same working hypotheses as were suggested by the facts known at earlier periods or, as is always possible, something quite different. The putting this principle into practice is more conducive to progress and more stimulating to research than the mere accumulation of further facts to support or to confute the working hypotheses of earlier times.

THE EXTERNAL GILL HYPOTHESIS.—Applying this principle to the problem of the evolutionary origin of the limbs one finds an important set of data which were not available to Gegenbaur or Balfour. In their day there was no proper appreciation of the importance of the fact that there existed in three of the less specialized groups of Vertebrates—Urodele Amphibians, Lung-fishes and Crossopterygians—those organs which have been described in Chapter III. under the name External Gills. The mode of development of these organs is now known in all three of the groups mentioned and the evidence appears to be conclusive that they are truly homologous throughout.

It has been shown that there is a tendency for the External Gills to become eliminated—as *e.g.* in various Anurous Amphibians: it has been shown further that in some of the main groups of Vertebrates in which they do not occur their disappearance may be accounted for by the evolution of a new physiological substitute—the vascular surface of the yolk-sac.

Having regard to these facts and to the relatively archaic character of the groups in which they actually occur the conclusion is considered justifiable that such external gills are organs of high antiquity in the Vertebrate stem. Further, from their distribution upon the various arches it is inferred that in all probability an external gill was once present upon each visceral arch. But it has also been shown to be probable that the series of visceral clefts—and therefore of visceral arches—was formerly more extensive, extending farther back along the body than it does in existing Vertebrates. It is therefore concluded that in an earlier phase of its evolution the phylum whose modern representatives we call Vertebrates was characterized by the possession of a series of external gills extending tailwards beyond the limit reached by the branchial region of existing Vertebrates.

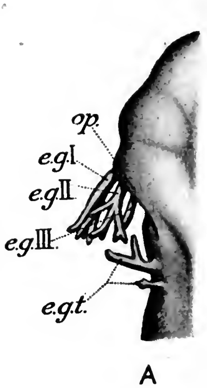


Fig. 204. — Left side of head region of a larva of *Rana temporaria* upon which, four days previously, a piece of skin from the branchial region of another embryo had been grafted. (After Ekman, 1913.)

*e.g.*I, II, III, external gills (autositic); *e.g.t*, external gills (parasitic) which have developed from the implanted piece of skin; *op*, operculum.

But such external gills are potential organs of support—as shown by the "balancers" of Urodeles (see Fig. 88, p. 157)—and also potential organs of movement—as shown by the well-developed musculature by which they can be flicked backwards. In other words these organs—and these alone among the organs of the Vertebrata—possess the qualifications which have to be postulated for the evolutionary forerunner of the Vertebrate limb.

In view of such considerations as those just set forth the present writer believes the most plausible working hypothesis of the evolutionary origin of the limbs—having regard to our present-day knowledge—to be that which interprets them as modified external gills, belonging to visceral arches farther back in the series than those forming the branchial arches of existing Vertebrates. The limb girdle would on this hypothesis, as on that of Gegenbaur, be interpreted as representing a branchial arch skeleton, the difference from the Gegenbaur view having to do rather with the nature of the projecting limb itself.

Fig. 200 illustrates how close may be the general resemblance between the earliest stages of development of limb and external gill. The same is brought out also by Fig. 204, representing part of a frog larva on which had been grafted a piece of skin from the gill-producing region of another larva. The external gills of the graft have gone on developing and are remarkably limb-like in appearance.[1]

The External Gill Hypothesis as to the evolutionary origin of the limbs fits in well with other facts which are now known. In the breeding male Lepidosiren the hind limb regularly and the pectoral limb occasionally (Agar, 1908) take on temporarily the characters of an external gill both in structure and in function (Fig. 205). This remarkable fact—otherwise a morphological mystery—becomes at once understandable on the hypothesis outlined above, as a simple "reversion" towards an ancestral condition. Fig. 205 brings out clearly a further peculiarity of these gill-like limbs of the male Lepidosiren, namely that the respiratory outgrowths of the limb are in the case of the pectoral limb attached to its ventral side, in the case of the pelvic to its dorsal side. But it has already been shown that the definitively ventral side of the pectoral limb is homologous with the definitively dorsal side of the pelvic limb—the difference in position being due to the rotation in different directions undergone by the limb rudiments in the course of their development. This reversed position of the respiratory filaments in the two sets of limbs clearly then fits in exactly with the view that they are ancient morphological characteristics of the limb which have reappeared in the male Lepidosiren.

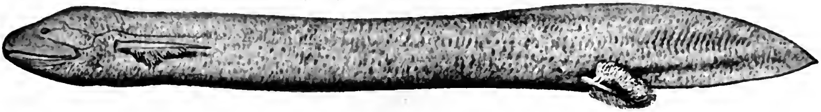


Fig. 205.—*Lepidosiren*, breeding male showing apparent reversion of both pectoral and pelvic limbs to the branchial condition. (From a specimen in the Zoological Museum of the University of Glasgow.)

The striking resemblance between the pectoral girdle and the branchial arches in some of the more ancient Fishes again finds its explanation in the morphological identity of the two structures. It is now established that the swim-bladder of Fishes is morphologically a lung, and that the lung is to be regarded as at the least an extremely ancient organ in the Vertebrate phylum. This points to the probability that the early Vertebrates were creatures which clambered

---

[1] Reference should also be made to Fig. 88 (p. 157) which brings out clearly the remarkably limb-like character of the Urodele "balancers."

Budgett (1901) mentions the case of an abnormal Protopterus larva which had failed to develop the pinnae upon one of its external gills. "This bare shaft so much resembled the pectoral limb that the larva appeared to have two pectoral limbs on one side."

about amongst the vegetation of shallow water and we may suppose that in this early stage the limb was of a crude styliform shape such as we see exemplified in the metamorphosed external gill of the Urodele balancers, or in the actual limb of the larva of *Lepidosiren.*

On this hypothesis the ancestral styliform limb has pursued two divergent lines of evolution. The one of these is found in those Vertebrates which have developed along the lines of becoming specialized for efficient swimming. Here it has become a fin, an early stage of this evolution being represented by the crude paddle of *Ceratodus.* That this biserial archipterygial type does actually represent an extremely early type in the evolution of fins seems to be demonstrated by two facts taken in conjunction with one another—

1. That this thick and clumsy organ represents functionally a relatively inefficient type of swimming organ as compared with the thin flat fin of most existing Fishes, and

2. That palaeontology shows it to have been a widely distributed type of fin in the early days of the evolution of the main groups of Fishes. It was in fact the predominant type of limb amongst ancient Elasmobranchs, Ganoids and Lung-fishes.

Evidence is not entirely wanting to show how the Crossopterygian type of fin on the one hand (as seen in the existing Polypterus) and the Actinopterygian type on the other (as seen in *Amia* and other Ganoids and Teleosts) may have been evolved out of the biserial archipterygial type. This evidence cannot be gone into here but so far as Crossopterygians are concerned the student should note the close resemblance of the pectoral fin of the young Polypterus (Fig. 197, E) and of its supporting skeleton (Fig. 169, F) to the modified archipterygial fin of the ancient Shark *Pleuracanthus* (Fig. 169, B).

Along the other line of evolution the styliform limb has given rise to the pentadactyle leg with its expanded foot and its characteristic jointing. It is of great interest in this connexion to watch the clumsy movements of a Lepidosiren larva and to note that the hind limb by which the creature pushes itself along becomes bent twice upon itself precisely in the way which would. give rise to the ankle and knee-joints of one of the lower Tetrapoda. Occasionally the appearance is rendered still more suggestive by the tip flattening out slightly into a foot-like expansion. The observer watching a Lepidosiren larva performing such movements finds it difficult to avoid the suspicion that he is witnessing something very like what took place in the early stages of the evolution of the pentadactyle limb. Should this be the true history of the origin of that type of limb it would explain the unsatisfactory and wholly unconvincing results of the efforts of comparative anatomists to derive the skeletal elements of the pentadactyle limb from those of one or other type of fin.

Embryology offers no explanation of the number of digits being so generally five. The physiological advantage of the expanded foot being divided up into separate radiating digits is obvious, as is that of the double nature of the adjoining portion of the limb skeleton to facilitate rotation round the axis of the limb. There are also mechanical advantages in there being a central digit with one on each side of it. Possibly the presence of an additional digit outside of these is to be looked on as of the nature of simple reinforcement.

The modification of the pectoral limb in the case of Birds for purposes of flight is of great interest, but nothing is known as to the phylogenetic transition from Reptile to Bird in this connexion. To the present writer it seems most probable that the Birds were evolved out of aquatic Reptiles in which the fore-limb was specialized for use in swimming under water, after the manner of existing Penguins, and that the function of aerial flight was evolved directly from such movement under water. On this hypothesis the more or less terrestrial habits of modern Birds would be regarded as a secondary acquirement.

(4) EVOLUTIONARY ORIGIN OF THE TAIL REGION.—It is characteristic of Vertebrates that the anus loses its practically terminal position and comes to be situated some distance forwards on the ventral side, the overhanging hinder end of the body forming the tail. This opens up a question of much morphological interest—though one to which we are not yet in a position to give any certain answer—as to the phylogenetic origin of the tail.

It seems clear that the tail arose in ancient aquatic Vertebrates as an adaptation to swimming and on the whole it seems most probable that it came into existence through the gradual migration forwards of the anus upon the ventral side. Such a shifting forwards of the anal opening from the hinder end of the body is a familiar feature in many groups of invertebrates where it is associated as a rule with a tubicolous habit and has doubtless for its object the getting rid of excretory products which would otherwise be discharged into the depths of the tube, or burrow, or shell. In the Vertebrate the forward shifting of the anal opening has probably its physiological significance in the increasing efficiency of the tail as the main motive organ—the disappearance from it of the alimentary canal, and its surrounding splanchnocoele, being correlated with the conversion of the tissues on each side of the skeletal axis into a solid mass of muscle. Probability is added to this conjecture by the fact that we see what appears to be a continuation of the same process in the most efficient group of modern swimming Vertebrates (Teleostei) where in the most highly developed forms the alimentary canal and splanchnocoele come to be restricted to a relatively small region immediately behind the head, the remaining and main part of the body being entirely "tail."

In actual ontogeny the tail region is developed not by the withdrawal from it of gut and splanchnocoele but as an actual outgrowth, the hind end of the body continuing to sprout out past and dorsal to the anal opening. It is of course conceivable that in phylogeny the tail arose similarly as an outgrowth of the body dorsal to the anus but this seems in every way less probable than the method of evolution sketched above.

LITERATURE

Agar. Anat. Anzeiger, xxxiii, **1908**.
Balfour. Monograph on the Development of Elasmobranch Fishes. London, **1878**.
Budgett. Trans. Zool. Soc. Lond., xvi, **1901**.
Caldwell. Journ. Proc. Roy. Soc. New South Wales, xviii, **1884**.
Dean, Bashford. Kupffers Festschrift. Jena, **1899**.
Ekman. Morph. Jahrb., xlvii, **1913**.
Gegenbaur. Untersuchungen zur vergl. Anat. der Wirbelthiere, iii. Leipzig, **1872**.
Goodrich. Quart. Journ. Micr. Sci., l, **1906**.
Kerr, Graham. Phil. Trans. Roy. Soc. London, B, cxcii, **1900**.
Kerr, Graham. The Work of J. S. Budgett. Cambridge, **1907**.
Kerr, Graham. Keibels Normentafeln, iii. Jena, **1909**.
Rabl. Morph. Jahrb., xix, **1893**.
Semon. Zoologische Forschungsreisen, i. Jena, **1893–1913**.
Semon. Keibels Normentafeln, iii. Jena, **1901***.
Taylor. Quart. Journ. Micr. Sci., lix, **1914**.

CHAPTER VIII

ADAPTATION TO ENVIRONMENTAL CONDITIONS DURING EARLY STAGES OF DEVELOPMENT

I. PROTECTIVE ENVELOPES OF THE ZYGOTE.—The Zygote or fertilized egg is in the Vertebrata as in other groups provided with protective envelopes. Of such we may recognize three fundamentally distinct types which are conveniently designated as primary, secondary and tertiary envelopes respectively. A **primary envelope** is a cuticular covering of the surface of the zygote: it is therefore produced by the living activity of the protoplasm of the macrogamete or zygote itself. A typical example of a primary envelope is the vitelline membrane which is formed on the surface of the Echinoderm egg in response to the act of fertilization. The "vitelline membrane" which covers the surface of the egg of a Bird is commonly looked on as a primary envelope.

A **secondary envelope** is one which is formed by the activity of the surrounding cells while the egg is still contained in its ovarian follicle. It may be cuticle-like in its nature or it may be composed of cells.

Finally **tertiary envelopes** are formed by the excretory activity of the oviducal lining, being deposited upon the surface of the egg as it travels down the oviduct. Of such a nature are the complicated protective envelopes which surround the egg of a Bird or Reptile, or the simpler jelly-like investment found in the case of most Amphibians.

Apart from tertiary envelopes the most conspicuous envelope of the Vertebrate egg is usually what is known as the **zona radiata** or zona pellucida—the former name being given to it from the fact that it is pierced by numerous very fine canals which give it a characteristic radiate appearance when seen in section. These fine canals apparently contain protoplasmic bridges connecting the protoplasm of the egg with that of the follicle-cells which surround it while still in the ovary, and doubtless having for their function the passing in of food-material from the follicle-cells into the egg-cell.

The zona radiata is, as a rule, most conspicuous during early intra-ovarian stages while the egg is undergoing active growth during the storing up of yolk. Later it thins out and becomes less conspicuous. The zona radiata is usually looked upon as primary in its nature but this is by no means settled and some competent authorities regard it as secondary.

Outside the zona radiata there may often be found a second envelope which does not show the perforations characteristic of the zona radiata: this also in the case of the large heavily yolked eggs becomes thinned out during the process of growth. Envelopes of this type are specially conspicuous in those Vertebrates in which there is no great development of tertiary envelopes secreted by the oviducal wall, *e.g.* Teleostean fishes. In such cases the outer layer of the envelope, lying immediately external to the typical radiate layer, frequently shows characteristic modifications, consisting of closely packed villi or columns composed of cells which swell up and become strongly adhesive, serving to attach the eggs to one another or to a solid object (Roach—*Leuciscus rutilus*, Bleak—*Alburnus*, Herring—*Clupea harengus*). In the Actinopterygian Ganoids a similar condition is found. In *Ceratodus* this special outer layer is not found, there being here a tertiary envelope of jelly. In *Lepidosiren* and *Protopterus* the tertiary envelope is as a rule no longer formed, the eggs lying loose in the bottom of the burrow, though it is of interest to notice that in *Lepidosiren* the secretion of a jelly-like tertiary envelope round the eggs is still occasionally found as an individual variation. In *Petromyzon*, as in the Teleosts alluded to above, a radiate envelope is found inside a villous one which becomes swollen up and sticky on the absorption

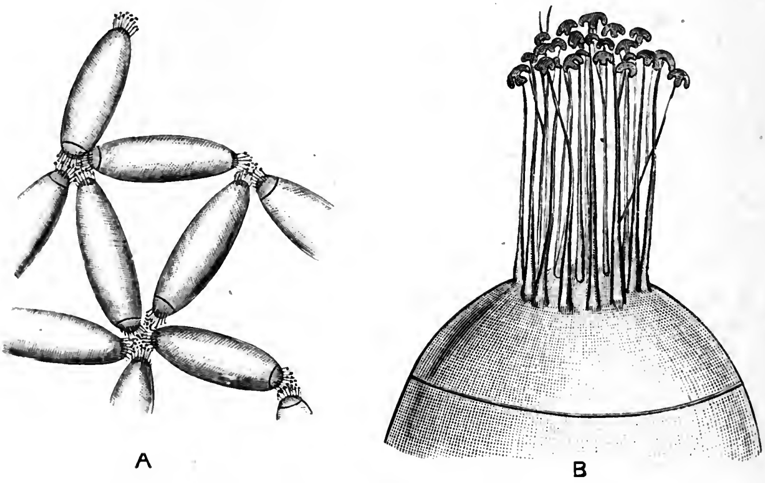


FIG. 206.—A, cluster of eggs of *Bdellostoma*, attached together by the interlocking of their anchoring filaments; B, apical portion of the egg-shell, showing the anchoring filaments projecting from the middle of the separable "lid." (Figure by Bashford Dean, from *The Cambridge Natural History*.)

of water. In the Myxinoids the egg is contained in a characteristic elongated shell provided at each pole with a group of stiff anchoring filaments ending each in a lobed umbrella-shaped expansion (Fig. 206). The piece of shell covering the germinal pole is marked off by a deep incision from the rest so as to form a lid which is forced off at the time of hatching.

Whatever the true nature of the envelopes under discussion, whether primary or secondary, they already exist round the egg before fertilization takes place, and as the substance of the envelope is, as a rule, impenetrable by spermatozoa there necessarily exist one or more openings or **micropyles** through which the fertilizing spermatozoon makes its way into the egg. In the Myxinoids one such micropyle is found in the middle of the lid, surrounded by the concentric circles of anchoring filaments. The presence of a micropyle in Lampreys and in Lung-fishes is not definitely established. In *Lepidosiren* it has been observed that the envelope enclosing the coelomic, and therefore unfertilized, egg is thick and gelatinous while after fertilization it becomes dense and horny. Possibly therefore during the first-mentioned condition it is penetrable by the spermatozoa. In Teleostean fishes a micropyle occurs at the apical pole, and so also with Actinopterygian Ganoids except that in the Sturgeons there exist a group of openings (5-13 in the Sterlet, according to Salensky) instead of a single one.

Of the more complicated arrangements of tertiary envelopes found in Vertebrates no better example could be taken than those found in the case of the Fowl's egg. These will be found described in Chap. X. In Birds in general the envelopes resemble those of the Fowl, differences occurring in details of relative size, shape, and colour of the shell. The "egg" (*i.e.* the zygote with its envelopes) appears to be largest relatively in *Apteryx* where it reaches about a quarter of the weight of the parent.

The shape of the shell is impressed upon it by the pressure of the oviducal wall and differences in shape are no doubt due to differences in the peristaltic contraction. Thus the strong contraction of the oviducal muscles which, acting on the headward side of the egg, propels it onward, if combined with comparatively slight contraction on the tailward side of the egg will naturally cause the egg to assume a more or less markedly conical shape—the end of the egg directed towards the cloaca being broader than the other end. In some cases, as that of eggs laid on bare ledges of rock, this conical shape has probably had a definite natural selection value, in causing any rolling movement of the egg to follow a strongly curved path. In other cases where there is less marked inequality of pressure on the two poles of the egg the shape is more regularly ellipsoidal.

The eggs of Birds being commonly exposed to light and to view they very often show a characteristic colouring, either throughout the thickness or merely in the outer layer of the shell. In very numerous cases the natural selection value of the colouring as a means of making the egg less conspicuous is obvious.

In Reptiles the tertiary envelopes resemble those of Birds though in many cases, as in various Lizards and Turtles, there is no definite rigid shell. On the other hand there may be a certain amount of lime deposited in the outer layers of the shell-membrane. The albumen varies in amount: in *Sphenodon* it forms only a very thin layer (Dendy, 1899).

In Elasmobranch fishes the egg is again enclosed in a layer of albumen and this in turn surrounded by a shell. The shell is of a horny consistency and is frequently rectangular and pillow-shaped. Characteristic differences are found in different genera and species. Thus in the Skates (*Raia*) each angle is prolonged so that the egg has an outline like that of a hand-barrow. In *Scyllium* (Fig. 207) the prolongations become long spirally coiled anchoring filaments: in *Pristiurus* two short prolongations occur at one end while the other end is simply rounded.

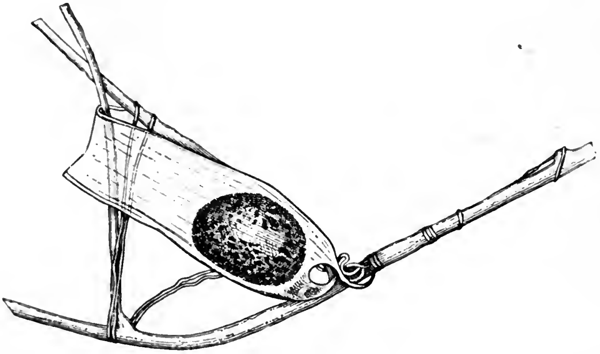


FIG. 207.—Egg of *Scyllium*, held in position by its four elastic filaments being wound round a plant. (Figure by Kopsch, from *The Cambridge Natural History.*)

II. MODIFICATIONS OF THE ENVELOPES AND OTHER ADAPTIVE MODIFICATIONS OCCURRING DURING THE EARLY DEVELOPMENT OF THE AMPHIBIA.—The Amphibians form a group of Vertebrates which have taken less or more completely to a terrestrial existence in their adult condition. They have not been able to emancipate themselves entirely from the ancestral aquatic habitat, possibly on account of the feeble development of the horny outer layer of the epidermis. They are still as a rule entirely aquatic during the early stages of their development, the eggs being laid in water and the young animal passing its larval existence in the water.

In a number of cases, particularly in Anura inhabiting tropical regions with a well-marked dry season, very interesting adaptations are found whereby the young animal is enabled to pass a more or less prolonged period out of the water. In the first type of these adaptations we find special modifications of the tertiary envelope which is normally a simple mass of jelly deposited round the egg.

The first type of such adaptation is exemplified by various species of *Hylodes* and by *Rana opisthodon* in which the eggs are simply deposited in free air in damp spots, each surrounded by a transparent spherical protective shell. In *R. opisthodon* (Boulenger, 1890) the young Frog before hatching develops on the tip of its snout a small conical protuberance apparently used like the egg-tooth of Reptiles and the similar organ in Birds to tear open the egg-envelope. A further interesting adaptive feature is that the young unhatched Frog possesses on each side of its body a series of vascular flaps of skin somewhat resembling the gill-flaps of an Elasmobranch fish and apparently functioning as respiratory organs.

In a considerable number of tropical Anura the oviducal secretion which surrounds the eggs is, at the time of laying, beaten up by rapid movements of the hind feet of the parents into a fine foam or froth with numerous entangled air-bubbles. This may be deposited on the surface of a pool where it floats about like a fleck of ordinary foam with the developing eggs scattered through it (*Paludicola fuscomaculata*). At a particular stage in development a digestive ferment apparently is secreted, probably by ectodermal gland cells, which liquefies the jelly and allows the larvae to drop through into the underlying water.[1] In other cases the mass of foam is deposited in an excavation in the ground, so situated that rain-water readily trickles into it (*Engystoma ovale*), or merely in a damp spot. In the case of the Japanese *Rhacophorus* (*Polypedates*) *schlegeli* (Ikeda, 1897) the burrow is made in a bank by the margin of standing water and after the mass of egg-foam has been deposited the pair of Frogs make their way out by excavating a tunnel which slopes downwards and opens near the water's surface. Here again at the appropriate stage of development the jelly liquefies and the young larvae are carried down by it into the water.

In the case of *Phyllomedusa hypochondrialis* the process of oviposition was observed by Budgett (1899) in the Gran Chaco. The eggs are deposited during the night, the female clambering up amongst the leaves of a suitable plant by the margin of a pool, with the male on her back (Fig. 208). With their hind legs the two Frogs bend the margins of a leaf together so as to form a funnel into which the eggs are poured together with the fertilizing sperm. The eggs are enclosed in a mass of firm adhesive jelly which causes the leaf to

---

[1] It is probable that such ferments play an important part in softening the egg-envelopes preparatory to hatching in various animals. Thus in *Lepidosiren* the process of hatching is rendered possible by the softening of the egg-shell brought about apparently by digestive ferment secreted by the ectoderm covering the body (Graham Kerr, 1900). The same appears to be the case in Teleosts (Wintrebert, 1912). In *Xenopus* amongst Amphibians a similar process apparently takes place and in this case Bles (1905) attributes the formation of the ferment not simply to the diffuse activity of the ectoderm cells but to the action of a special "frontal gland." It seems not improbable that the formation of such hatching ferments will be found to occur very generally in aquatic Vertebrates.

retain its funnel shape. The eggs develop within the jelly up till the stage of a tadpole of 9-10 mm. in length. During this process the jelly apparently liquefies, until only a thin membranous bag containing watery fluid surrounds each embryo. Eventually the remains of the jelly with its contained tadpoles trickles downwards into the water. If, as sometimes happens, the margin of the water has retreated from immediately below the leaf the tadpoles may still make their way for a distance of several inches to the pool by active jumping movements, helped it may be by a shower of rain.

Fig. 208. — *Phyllomedusa hypochondrialis*, female carrying male on her back during oviposition. (After Budgett, 1899.)

In the allied *Phyllomedusa sauvagii*, from the same neighbourhood, a similar mode of oviposition occurs, though here the nest is composed of several leaves (Fig. 209). Agar (1909) finds in this case that both at the commencement and end of oviposition there are laid a large number of spheres of jelly which contain no egg in their interior.[1] The eggs are thus protected both above and below by a thick mass of eggless spheres. During the later stages of development the layer of envelope next the surface of each egg becomes greatly distended by the accumulation of fluid within it, the jelly between the eggs meanwhile diminishing in volume. The larvae with their huge external gills have thus considerable room in which to move freely. Eventually the envelope ruptures and the larva hatches. The nest thus comes to be occupied by a seething mass of tadpoles, floored and roofed in by a thick mass of jelly formed by the empty spheres. Eventually — in from 12-24 hours after the bulk of the larvae have hatched — the jelly begins to deliquesce and the larvae drop down with it into the water.

Fig. 209. — *Phyllomedusa sauvagii*, mass of spawn. (After Agar, 1909.)

Similar nesting habits occur in other tropical Hylids, *e.g. Phyllo-*

---

[1] In the common Frog (*Rana temporaria*) apparently empty capsules may be formed in quantity in the oviduct before eggs begin to enter it (Wezel, 1908). Interspersed with the normal eggs Agar found 2-3 per cent of such eggless capsules. These appear to be deposited round small solid particles such as fragments of shed epithelium (Lebrun, 1891).

In *Centrophorus*, where the left ovary is no longer functional, empty tertiary envelopes are frequently still formed in the left oviduct (Braus, 1906).

medusa iheringii (von Ihering, 1886), *Hyla nebulosa* (Goeldi, 1895), *Rhacophorus reinwardtii* (Siedlecki, 1909). In the last mentioned the eggs are deposited in a mass of foam enclosed in one or several leaves (Fig. 210). At the appropriate time the central portion of the mass liquefies and the colourless tadpoles make their way into this central fluid—the superficial layer of the mass being hard and dry. Eventually the lower part of the mass softens and the liquid containing the tadpoles trickles out on to the ground where the larvae are able to continue their development in the smallest puddles.

In the second type of such adaptations the eggs or young are carried about, away from the water, by one of the parents. In the simplest of such cases no structural modification of the parent's body is involved. Thus in *Alytes obstetricans* the male draws the strings of eggs out of the cloacal aperture of the female and loops them round his thighs—the portion of oviducal secretion lying between successive eggs becoming highly elastic and gripping the thighs tightly. Oviposition takes place on land and the male pays only occasional visits to the water. When one of these happens at the appropriate period the young hatch in the form of tadpoles while the male parent resumes his terrestrial habits.

In a number of cases the transport of the young by the parent takes place at a later period, when the tadpole stage has been reached, the larvae adhering to the back of the male parent and so being transported from one pool to another (Fig. 211, A). This habit occurs in various species of *Dendrobates* and *Phyllobates* (Brandes u. Schoenichen, 1901).

FIG. 210.—*Rhacophorus reinwardtii*, mass of spawn with hatched tadpoles. (After Siedlecki, 1909.)

In the most interesting cases however the transport of the eggs or young by the parent is associated with the making use of some particular structural feature of the latter—either permanent or specially developed for this purpose. In *Rhacophorus reticulatus* (Günther, 1876) the eggs are carried about by the female, adherent to its ventral surface. In *Hyla goeldii* (Boulenger, 1895) the eggs adhere to the dorsal surface of the female, only in this case the skin of the parent responds to the stimulus afforded by the presence of the eggs and grows up into a slight ledge surrounding them (Fig. 211, B). In *Pipa americana* (Bartlett, 1896) the cloaca of the female is protruded at the time of oviposition as a large spout-like structure which projects forwards between the dorsal surface of the female and the ventral surface of the male. The eggs pass out one by one through this and are distributed at fairly equal intervals over the dorsal surface of the trunk of the female. The skin now proliferates actively, growing up so as to form highly vascular partitions between the eggs, each of the latter coming to be enclosed in a deep pit. The mouth of this becomes closed in by a dark-coloured operculum, possibly formed of hardened epidermal secretion. Each egg is thus enclosed in a little chamber in which it passes through the early stages of its development, including a modified tadpole stage, and issues forth eventually (after about 82 days) as a young Toad.

In another set of Anurous Amphibians the eggs undergo their development in a spacious single cavity within the parental body. In *Rhinoderma darwini* (Jimenez de la Espada, 1872; Plate, 1897) this cavity is the enlarged unpaired croaking sac of the male, into which the eggs, to the number of from 5 to 15, are swallowed and from which the young issue after completing the tadpole stage. In the genus *Nototrema* the brood cavity is a special large pouch lying beneath the skin of the back, lined by involuted epidermis and opening to the exterior just in front of the cloacal aperture. In different species of the genus there is much difference in the length of time during which the developing embryo is retained within the pouch, the length of this period being apparently correlated with the size of the egg and the amount of food-yolk stored within it. Thus in *N. marsupiatum* there may be as many as 200 eggs in the pouch, each measuring about 5 mm. in diameter (Brandes u. Schoenichen, 1901), and the young make their way out as typical tadpoles which doubtless lead for a time a free aquatic existence before metamorphosis takes place.

FIG. 211.—A, male of *Phyllobates trinitatis* carrying tadpoles; B, female of *Hyla goeldii* carrying eggs. (After Boulenger, 1895.)

In *N. oviferum* (Weinland, 1854) the eggs are much larger (10 mm.) and fewer in number (about 15) and in this case as in the allied *N. testudineum* and *N. fissipes*, which also possess large eggs, the young go on developing within the pouch until after the period of metamorphosis.

In *Nototrema* an interesting adaptive feature characterizes the external gills. These organs are present upon branchial arches I and II, each consisting of a long slender stalk, which passes at its outer end into a thin highly vascular membrane formed by the fused and expanded outer ends of the two external gills. The two membranes so formed, one on each side of the body, are closely applied to the inner surface of the thin egg-envelope. The outer surface of the envelope is in turn in intimate contact with the highly vascular lining of the pouch which sends projecting folds in between the eggs. We have here clearly an adaptive arrangement to minister to the respiratory needs of the developing young, analogous with that provided by the allantois of a Reptile or Bird.

It appears somewhat puzzling that the eggs of *Nototrema* should come to be contained in a pouch the opening of which is much smaller than the cross-section of the egg. The probability appears to be (Boulenger, 1895) that the pouch is formed in response to the presence of the eggs upon the animal's back, a ridge growing up round the eggs as in the case of *Hyla goeldii* but in this case continuing its growth towards the mesial plane until the corresponding upgrowths from the two sides meet and completely roof in the pouch-like cavity. Support is given to this explanation by the condition in *N. pygmaeum* where the opening of the pouch is in the form of a median longitudinal slit, prolonged forwards as a kind of seam or raphe along which the roof of the pouch readily tears and which presents all the appearance of having been formed by the coming together of two originally separate lips.

It must never be forgotten that such peculiarities of development as have been alluded to in the above-mentioned Anura involve adaptive modifications on the part of the young individual itself. The most frequent of such modifications is physiological adaptation, as shown for example by the fact that the transference of the young individual to water before the normal time is commonly fatal. In other cases structural adaptations of a more conspicuous kind are apparent. Thus in *Pipa* the late tadpole stage, although enclosed within its cell, develops a broad and highly vascular tail which doubtless serves for respiratory and possibly nutritive interchange with the maternal tissues: again in *Nototrema* the external gills show the peculiar modification already alluded to. The true external gills are in several cases absent, their function being taken over by the vascular surface of the yolk, while in such a case as *Rana opisthodon* special new respiratory organs have been developed.

In the various modifications of development dealt with in the preceding section we have to do with attempts, so to speak, on the part of isolated members of a particular group of Vertebrates (Anura) to lessen the degree of their dependence upon the ancestral aquatic habitat. Such attempts amongst the existing Amphibia are not altogether successful: the group as a whole remains chained to a watery, or at least humid, environment.

The lower Vertebrates which made a real success of terrestrial existence, emancipating themselves entirely from the aquatic environment, are represented to-day by the Amniota, and it remains now to study their special modifications of development.

III. ADAPTIVE MODIFICATIONS IN THE DEVELOPMENT OF THE AMNIOTA.—It is characteristic of many Vertebrates that, associated with the provision of special arrangements for nourishing the young individual, the time of commencing an independent life on its own account is greatly delayed. In such cases where a considerable proportion of the whole development takes place within the shelter of the egg-shell (or of the parental body) we have to do with what is known as **embryonic** in contradistinction to **larval** development. During embryonic development the young individual is free from the necessity of fighting and fending for itself; it is to a great extent sheltered from the struggle for existence, and in correlation with this we find remarkable hypertrophies and modifications of various parts of the body taking place which in a free state would render life impossible.

The first of these modifications makes its appearance in the lower, aquatic, Vertebrates in the form of a pronounced bulging of the ventral side of the body. In the more primitive holoblastic Vertebrates this is caused by the great thickening of the ventral endoderm (Fig. 80, E, p. 146), its cells being much enlarged and packed with granules of yolk. Where this distension of the endoderm cells is most marked anteriorly there is brought about the tadpole shape of body as seen in the Ganoids and *Lepidosiren*: or, on the other hand, the distended region may be situated towards the hinder end as in *Petromyzon*, *Ceratodus* or the Gymnophiona. In such cases as development proceeds the large yolk-cells go on segmenting, the yolk within them is gradually used up, and the mass of endoderm, becoming more and more attenuated, ceases to project beyond the general outline of the body.

In the meroblastic egg, as has already been shown, the proportion of living protoplasm amongst the yolk has been reduced to vanishing point so that except superficially the yolk never segments. Typically it becomes gradually enclosed in the endoderm which spreads over its surface. There is thus formed what is known as the **yolk-sac**, a structure usually of enormous size as compared with the rest of the embryo. It will readily be understood how impossible a free active existence would be while there is a large yolk-sac present. The assimilation of yolk and its transport to the actively growing parts of the embryo are brought about mainly by the rich development of superficial blood-vessels forming the vitelline network. In typical Teleosts, *e.g.* Salmonids, the yolk-sac becomes at an early period completely separated from the dorsal part of the endoderm which becomes the functional gut, the yolk absorption taking place entirely by the vitelline vessels.

An important point to be remembered is that the vitelline network though primarily nutritive in function is necessarily also respiratory, gaseous interchange taking place between the blood circulating in its vessels and the medium which bathes its surface. The vitelline network is the primary breathing organ in the great majority of Vertebrates during early stages of development. In cases where the embryo lies in contact with maternal tissues the respiratory exchange takes place ultimately, through the thin intervening layers of fluid or envelope, between the blood circulating in the vitelline network and that circulating in the oviducal lining of the mother. In this way all the necessary preliminary conditions are provided for the evolution of a placenta, and as will be shown later these conditions are actually taken advantage of in some cases and a simple yolk-sac placenta is formed.

In the more highly developed types of yolk-sac the splanchnic mesoderm which surrounds the vitelline vessels sprouts inwards, forming irregular vascular septa which project into the yolk-sac. This modification, which brings about a great increase in the assimilatory surface, reaches such a development in Birds that towards the end of incubation these ingrowths form an irregular meshwork of vascular trabeculae traversing the whole of the yolk right to its centre.

Eventually the yolk, whether in the form of a yolk-sac or a mass of heavily yolked cells, is enclosed within the ventral wall of the body. In the holoblastic Vertebrates this comes about as already indicated by the simple spreading of the blastoderm over the surface of the yolk so as completely to enclose it. In the Fowl the spreading of the blastoderm, and its derivatives the endoderm and mesoderm, round the yolk is never quite completed, there remaining a small circular patch at which the yolk is separated from the albumen only by the remains of the vitelline membrane (cf. Fig. 215, *v.m*).

Further in the Amniota the region of somatopleure bounding the coelomic space in which the yolk-sac lies becomes converted into amnion and serous membrane (cf. Fig. 215, A), and is eventually cast off, playing no part in the formation of the definitive body-wall. The yolk thus lies outside the limits of the definitive body-wall, projecting through the umbilical funnel which is bounded all round by the stalk of the amnion. Eventually, shortly before hatching, the edges of the umbilical opening are drawn over the yolk-sac in a manner which will be described later (see p. 475). In *Lacerta vivipara* in which the yolk-sac is reduced the remains of it are simply cast off according to Strahl.

The most remarkable of the excrescences adaptive to an embryonic existence are the organs known as **Amnion** and **Allantois**—portions of the embryonic body which become greatly hypertrophied and perform important functions during embryonic life but which are eventually, for the most part, shed about the time of birth or hatching and play no part in the formation of the body of the adult.

AMNION.—The most nearly primitive subdivision of the Amniota is the group Reptilia and we accordingly turn to it and more especially to the Chelonia, which have been worked out by Mitsukuri (1891), to provide a foundation for our description.

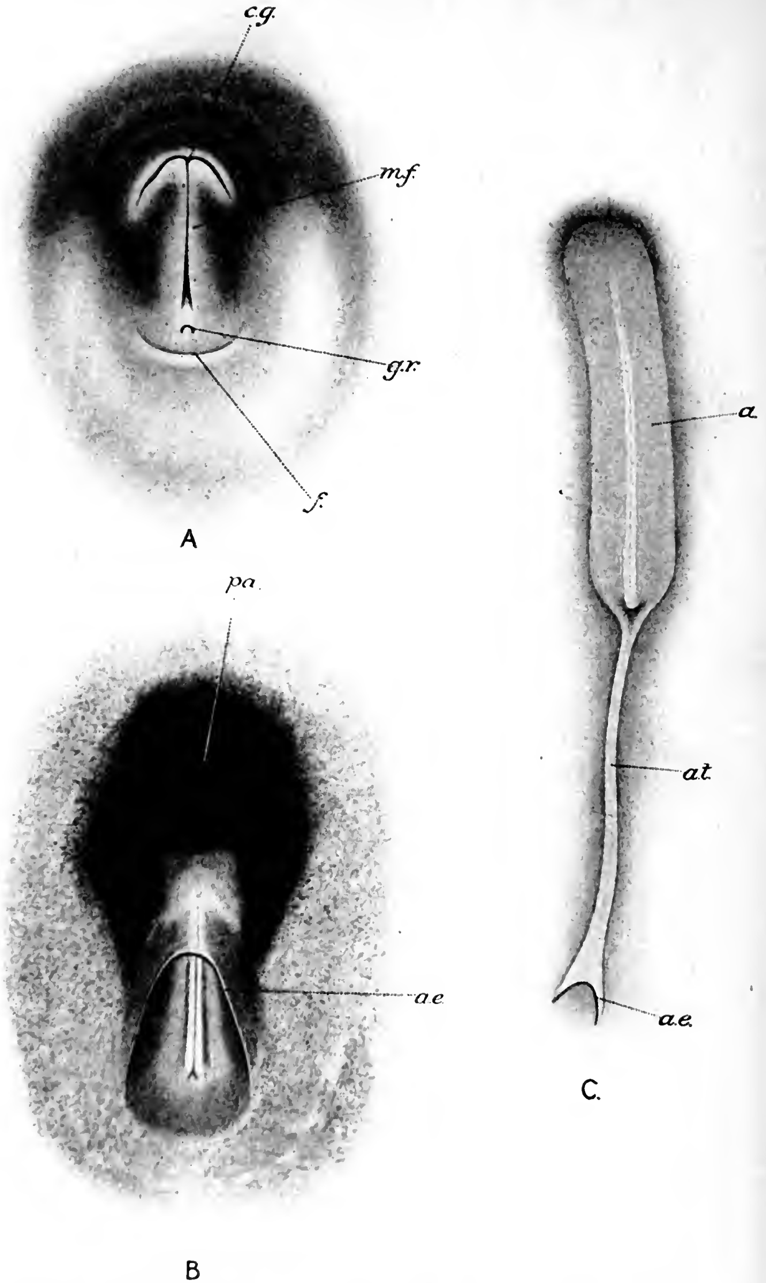


Fig. 212.—Chelonian blastoderms illustrating the development of the amnion. (A and C after Mitsukuri, 1891.)

A, *Clemmys*; B, *Chelydra*; C, *Clemmys*. *a*, amnion with neural rudiment seen indistinctly through it; *a.e*, edge of amniotic flap; *a.t*, amniotic tunnel; *c.g*, cephalic groove; *f*, inconstant fold which is sometimes present; *g.r*, gastrular rim; *m.f*, medullary fold; *p.a*, proamnion with head of embryo showing through it.

In Chelonia the first indication of amnion formation appears at a stage like that represented in Fig. 212, A. The future body of the embryo, indicated by the medullary folds, lies flat on the surface of the egg, extending out all round into the blastoderm. The first sign of the amnion is produced by the front end of the medullary plate coming to dip downwards so as to form a deep slit or groove (Figs. 212 and 213, *c.g*) curving tailwards on each side as seen from above. The posterior wall of this slit forms the anterior limit of the head of the embryo while its anterior wall forms the rudiment of the amnion (Fig. 213, *a.e*). The portion of blastoderm in front of and to the side of the head of the embryo is as yet two-layered, the mesoderm not yet having spread into it, and it follows that the amniotic rudiment is also two-layered. This region of the blastoderm, which is still without mesoderm and which in this case forms the amniotic rudiment, is termed the **proamnion**. As development proceeds the head end of the embryo increases in size and as it does so it dips more and more downwards so as to deepen the cephalic groove or slit in front of it. While this is going on there takes place active growth of the ectoderm along the sharp edge of the amniotic rudiment (Fig. 213, *a.e*) in such a way that this edge becomes prolonged backwards as a solid flap, covering over the body of the embryo from before backwards. This amniotic flap continues to grow tailwards, its growing edge concave and prolonged backwards on each side (Fig. 212, B, *a.e*), until it reaches the tail end of the embryo, so that the whole of the latter is covered in by an amniotic roof. Nor does the process stop now: it goes on with the result that there is formed a long tunnel (Fig. 212, C, *a.t*) continuous in front with the amniotic cavity, *i.e.* the cavity between

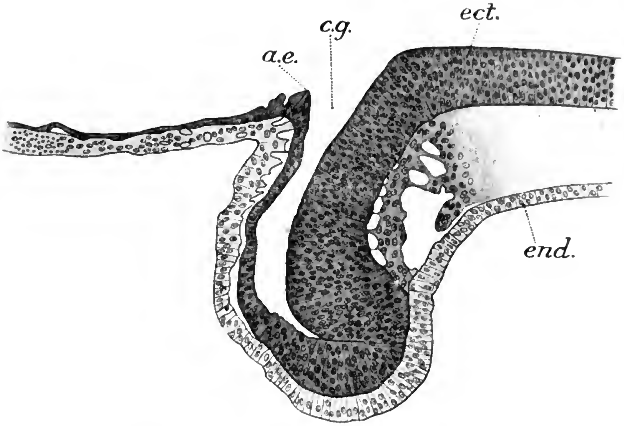


FIG. 213.—Sagittal section through the head end of a Chelonian embryo. (After Mitsukuri, 1891.)

a.e, amniotic edge ; *c.g*, cephalic groove ; *ect*, ectoderm of medullary plate ; *end*, endoderm.

the body of the embryo and the amnion, and terminating behind in an opening bounded above by a concave free edge (*a.e*).

An important point to realize is the relation of the amnion to the cell layers. The first rudiment, as has been indicated, is composed of the two primary layers ectoderm and endoderm, and this applies also to the lateral prolongations backwards of the free edge. The whole of the amniotic roof however except these marginal parts is formed at first of solid ectoderm and of ectoderm alone (Fig. 214, A, *a.f*). As development goes on the mesoderm extends between ectoderm and endoderm and then splits into somatic and splanchnic layers. The result of this is that the endoderm, with its covering of splanchnic mesoderm, sinks down and no longer projects upwards on each side into the base of the amnion (Fig. 214, B). The somatic mesoderm on the other hand does continue to project into the base of the amnion just as did the endoderm previously (Fig. 214, B). The originally simple ectodermal roof of the amniotic cavity undergoes a process of splitting from its lateral margin inwards and as this split extends towards the mesial plane the amniotic fold of

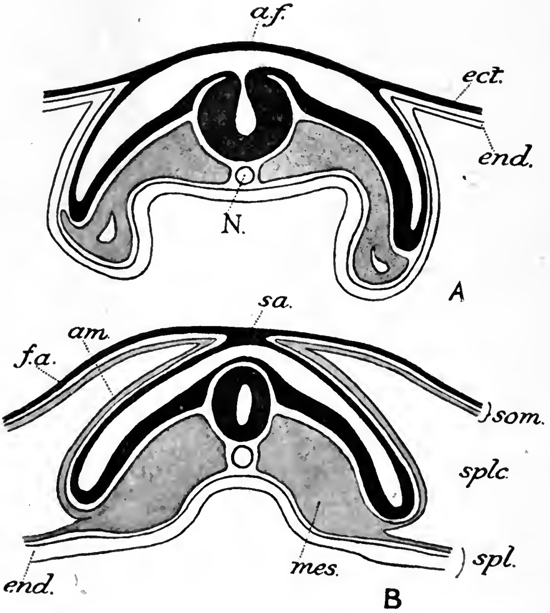


FIG. 214.—Diagrammatic transverse sections through Chelonian embryos (*Clemmys*. A, stage with 2-3 mesoderm segments; B, 6-7 segments) illustrating the relations of the amnion. (Based on figures by Mitsukuri, 1891.)

a.f, amniotic flap; *am*, amnion; *ect*, ectoderm; *end*, endoderm; *f.a*, false amnion; *mes*, mesoderm segment; *N*, notochord; *sa*, sero-amniotic junction; *som*, somatopleure; *spl*, splanchnopleure; *splc*, splanchnocoele.

somatic mesoderm spreads with it. Except along the middle line the amniotic roof thus becomes double—the inner roof being formed of ectoderm internally and somatic mesoderm externally, the outer roof of somatic mesoderm internally and ectoderm externally. Of these two roofs the inner is the amnion (Fig. 214, B, *am*), the outer is the **false amnion** or **serous membrane** (*f.a*). The portion which retains its original condition of being formed of unsplit ectoderm (*sa*) may be called the **amniotic isthmus** or the sero-amniotic connexion (Mitsukuri). During later stages of development this becomes reduced to a thin vertical partition in which form it persists throughout, except in the region of the head where it disappears entirely so that there is here a continuous coelomic space stretching from side to side between amnion and serous membrane.

The posterior tubular prolongation of the amniotic cavity becomes obliterated through part of its extent and in this way the amniotic cavity becomes completely closed.

The first-formed part of the amnion, lying in front of the head of the embryo, remains for a time proamniotic in character, *i.e.* composed of ectoderm and endoderm, but eventually the mesoderm and coelomic space spread in between the two primary layers and the portion of the amnion in question comes to resemble the rest.

As the body of the embryo becomes constricted off from the yolk-sac the basal edge of the amnion continuous with that of the embryonic somatopleure becomes tucked inwards so that the amnion, which formed in earlier stages a mere roof, comes to form a complete envelope. The amniotic cavity is filled with secreted fluid in which the body of the embryo floats.

BIRDS.—The process of amnion formation in the Birds shows conspicuous differences from that which has been described for the more primitive Reptiles. Two of the chief of these differences seem to be associated with the fact that the amnion develops relatively later in the Bird, at a period when the head and anterior body region of the embryo project prominently above the general level of the blastoderm and when the mesoderm has already split into splanchnic and somatic layers. Correlated with this fact we find (1) that in the Bird the amniotic rudiment has to grow upwards so as to surround the projecting head and trunk, and (2) that the upgrowth is composed of somatopleure only.

The amnion may be said to originate as a kind of wall, formed of an upwardly projecting fold of somatopleure, which comes to surround the actual body of the developing embryo. This wall is not absolutely vertical: it is tilted, or inclined inwards, towards the middle of the embryonic body. With increasing growth it projects more and more over the body of the embryo, its free edge bounding a gradually diminishing opening, through which the body of the embryo is visible when looked down upon from above. Eventually this opening is reduced to vanishing point and the body of the embryo is completely covered in by a double roof formed by the amnion and the serous membrane.

The amniotic fold does not develop with equal activity throughout its extent. Its growth is much more active anteriorly than elsewhere, with the result that the headward portion of the fold becomes extended rapidly backwards as an **amniotic hood** over the head and anterior end of the body of the embryo (cf. Figs. 233, 235, 236). The last remnant of the amniotic opening is consequently situated quite near the hind end of the body.

Correlated with the later appearance of the amniotic hood—at a time when the coelomic cavities are extensively developed—it is at no period composed throughout, from side to side, of a simple layer of unsplit ectoderm as was the case with the Chelonian. It is of interest to notice however that the sero-amniotic isthmus has not altogether disappeared, although it never has the breadth that it has in early stages in the Chelonian.

The details of amnion formation are readily observable in the Fowl and have been fully described by Hirota (1894). The process takes place as follows: The first step consists in the appearance of a crescentic upgrowth of blastoderm just in front of the head of the embryo at about the stage of 14 segments. At this period the mesoderm has spread forwards on each side but has not yet extended into the space immediately in front of the embryonic head (proamnion). Where the mesoderm is present it has split to form the coelome and owing to this being filled with secreted fluid the somatopleure bulges up somewhat so as to be conspicuously marked off from the flat proamniotic area. The amniotic fold makes its appearance just about the anterior boundary of the proamnion. As it increases in height it overlaps the head of the embryo and grows backwards over it as the amniotic hood (Fig. 233). Into the fold the mesoderm and coelomic cavities have already penetrated. Where the mesoderm from the two sides meet in the mesial plane of the hood the two portions of coelome do not open freely into one another but remain separated by a septum of mesoderm—the mesodermal sero-amniotic isthmus. At an early period of the backgrowth of the amniotic hood the ectoderm in the middle of its free posterior edge is seen to project headwards as a small wedge, the base of which is formed by the growing edge. As this wedge is carried backwards by the continued progress of the amniotic edge it leaves behind it a kind of trail in the form of a continuous line, or rather partition, of ectoderm connecting the ectoderm on the outer surface of the amniotic fold with that on its inner surface. This is clearly the ectodermal sero-amniotic isthmus of the Reptile persisting in a much attenuated form; the attenuation being due to the fact that the coelomic spaces have extended much nearer to the mesial plane than in the corresponding stage of amnion-formation in the Reptile.

Up till about the time when the amniotic hood has completed its backgrowth its cavity—the amniotic coelome—remains divided into two separate halves by a septum, which in front is purely mesodermal but throughout the rest of its extent is traversed by the ectodermal sero-amniotic isthmus. The anterior, purely mesodermal, part of the septum disappears early in the fourth day so as to make the amniotic coelome continuous from side to side, but the rest of the septum persists throughout the whole period of development although its central ectodermal portion becomes gradually reduced and by the tenth day has completely disappeared.

Towards the end of the second or early in the third day the tail of the embryo begins to project, bending ventrally and dipping downwards as it does so. As it does this the tail comes to be hidden under a projecting amniotic fold precisely as happened at the head end except that here the coelomic cavity is already completely continuous across the mesial plane there being no trace of a septum or sero-amniotic isthmus. The free edge of this "tail fold" of the amnion is, as was that of the "head fold," concave only here the concavity is directed headwards. Early in the fourth day the concave edges of the head and tail folds become continued into one another at about the level of the hind limb rudiment, so that the body of the embryo is now surrounded by a continuous amniotic fold—most highly developed anteriorly where it forms the amniotic hood, less so in the caudal portion and least of all laterally. The more or less elliptical opening bounded by this fold, through which the dorsal surface of the embryo is exposed, gradually shrinks as the fold grows and eventually, during the first half of the fourth day as a rule, it becomes obliterated and the amniotic cavity closed.

The true amnion at first closely ensheaths the head and trunk of the embryo but from about the fifth day onwards watery amniotic fluid is secreted into its interior so as to form an extensive water jacket in which the embryo is suspended (Fig. 215). For a considerable period the embryo is gently rocked to and fro in the fluid by the slow rhythmic contractions of muscle fibres which develop in the somatic mesoderm covering the amnion on its outer surface.

The development of the amnion in the Sauropsida in general is adequately illustrated by the two types which have been described. There occur variations in detail. Thus the inequality in the activity of growth between the anterior and posterior portions of the amniotic fold so marked as a rule may be practically absent (Chameleons), or it may reach an extreme limit, the posterior portion of the fold being obsolete and the anterior portion continuing its backgrowth past the tail end of the embryonic body to form an amniotic tunnel, as in the Chelonians above described (*Sphenodon*, Gannet—*Sula*, Puffin—*Fratercula*).[1]

ALLANTOIS.—The allantois may also be conveniently studied in the Bird. In the Fowl it makes its first appearance as a little clear

---

[1] Schauinsland, 1906.

vesicle, projecting from the ventral side of the trunk near its hind

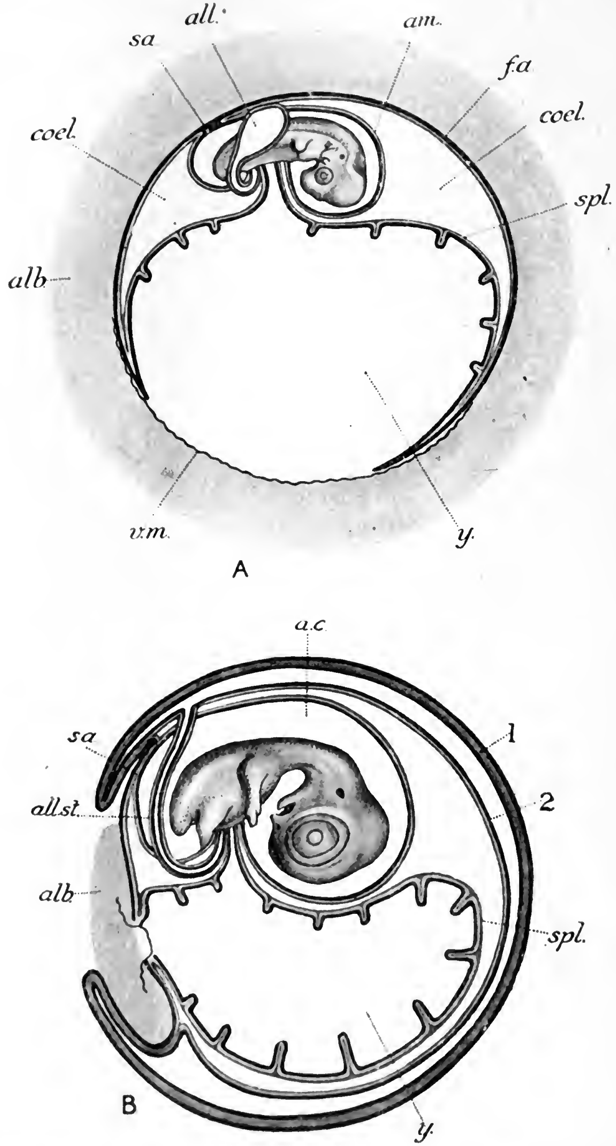


Fig. 215.—Diagrams illustrating the arrangement of amnion, allantois, etc., in the Fowl. (After Lillie, 1908.)

A, fourth day; B, ninth day. *a.c*, amniotic cavity; *alb*, albumen; *all*, allantoic cavity; *all.st*, allantoic stalk; *am*, amnion; *coel*, coelome; *f.a*, false amnion, or serous membrane; *sa*, seroamniotic isthmus; *spl*, splanchnopleure; *v.m*, vitelline membrane; *y*, yolk; 1, outer wall of allantois fused with serous membrane; 2, inner wall of allantois.

end (Figs. 239, 240), and serving for the reception of the renal secretion. The study of sections shows that the allantois is simply a pocket of the ventral wall of the gut towards its hind end—corresponding exactly with the bladder of an Amphibian. It is thus lined with endoderm and covered externally with splanchnic mesoderm. The allantois like the bladder of the Amphibian bulges into the splanchnocoele. As development goes on the allantois, distended with fluid, increases in size, projecting on the right or upper side of the embryo till it comes in contact with the inner surface of the somatopleure (Fig. 215, A, *all*), and with still further growth flattens out against the somatopleure taking a somewhat mushroom-like shape. In the case of an independently living animal such as an Amphibian the allantoic outgrowth of the gut can only increase in size within the restricted space of the splanchnocoele which is already occupied by the viscera. In the Bird embryo on the other hand there are available for the growth of the allantois the wide-spreading extensions of the coelome, on the one hand between amnion and serous membrane and on the other over the surface of the yolk. The allantois accordingly spreads out all round towards the limits of this space (Fig. 215, B). As it does so it loses its rounded vesicular form, its proximal (Fig. 215, B, 2) and distal walls (Fig. 215, B, 1) approaching one another. The mesoderm covering its outer surface tends to undergo secondary fusion with that of neighbouring structures. Thus about the end of the sixth day it fuses with the adjacent surface of the amnion. Again towards the time of hatching a similar fusion takes place with part of the yolk-sac. The most important of these fusions

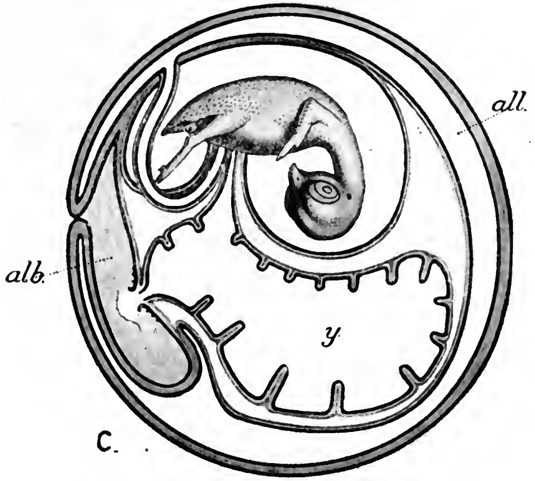


FIG. 215A.—Diagram illustrating the arrangement of amnion, allantois, etc., in the Fowl. (After Lillie, 1908.)

C, twelfth day. *alb*, albumen ; *all*, allantoic cavity ; *y*, yolk.

however is that, which commences early in the fifth day, with the inner surface of the serous membrane.

At a comparatively early period (during the fifth day) the mesoderm covering the allantois becomes vascular and as the organ becomes flattened its proximal or inner and its distal or outer walls become strikingly different as regards their vascularity, the outer wall developing an extremely rich network of capillary blood-vessels with very small meshes, while the inner wall possesses merely a sparse network together with the large vessels of supply. This difference between the two walls of the allantois becomes conspicuous about the end of the sixth day of incubation in the common Fowl. The difference is associated with the fact that the distal wall of the allantois is destined to become the great respiratory organ, taking over this function from the vascular area of the yolk-sac by which it is performed during the early stages of development. In correlation with the more efficient performance of this function the albumen, or white, as it gradually shrinks in volume and acquires greater density gravitates down to the lower side of the egg thus bringing the mushroom-shaped allantois close up to the shell membrane on the upper side. The process is still further facilitated by the ectoderm of the serous membrane becoming reduced to a very thin—hardly distinguishable—layer in the region where it is underlain by, and fused with, the allantois. The capillary network thus comes into very close relation with the shell membrane and the overlying porous shell, and gaseous exchange can readily take place between the blood circulating in the network and the external atmosphere.

As development goes on the respiratory needs of the embryo become greater and greater and these are met by the allantois spreading outwards all round its periphery, so as to provide a greater and greater respiratory area. During this spreading outwards of the allantois the three main allantoic vessels are somewhat retarded in their growth with the result that each one causes an indentation of the growing edge of the allantois beyond which the allantois bulges on each side.

When the growing edge of allantois comes, after about nine days' incubation, into the neighbourhood of the remaining mass of albumen, a new phenomenon appears inasmuch as the allantoic margin with its covering of serous membrane proceeds to grow onwards close under the shell membrane as a circular fold recalling the amniotic fold and enclosing the mass of albumen (Figs. 215, B, 215A, C). The ectodermal lining of the cavity so formed sprouts out into the albumen in the form of irregular projections which become vascularized from the allantoic mesoderm and no doubt play a part in absorbing the last remains of the albumen.

By about the end of the second week of incubation the shell membrane is lined throughout the whole of its extent by the highly vascular outer wall of the allantois. This remains the breathing organ until—a day or two before hatching—the young chick's beak penetrates the air-space and pulmonary breathing begins. The allantoic circulation then gradually becomes sluggish and stops, and eventually by a process of autotomy the allantois is separated from the body of the embryo and is left behind as the vascular membrane seen lining the fragments of shell from which a young bird has hatched.

ENCLOSURE OF YOLK-SAC WITHIN THE EMBRYONIC BODY.—As already indicated the yolk-sac becomes eventually (about a couple of days before hatching in the case of the common Fowl) enclosed within the body-wall. The process by which this is brought about appears to be as follows (H. Virchow). With the growth of the embryo a great increase takes place in the area over which the amnion is fused with the proximal wall of the allantois (cf. Fig. 215A, C), the compound and highly muscular membrane so formed extending eventually almost completely round the yolk-sac. At its edge it is continued onwards by the somatopleure, this latter terminating round the circular area where the yolk remains exposed. The yolk-sac is thus contained in a space the wall of which is formed of the following components in sequence starting from the body of the embryo: (1) amnion, (2) amnion fused with proximal wall of allantois, (3) proximal wall of allantois and (4) somatopleure in the region of the distal pole of the yolk-sac. The proximal portion of this wall, being formed of amnion, is necessarily continuous with the body-wall of the embryo at the umbilical opening and further those parts of it formed from amnion and allantois are highly muscular and contractile. During the later stages of development this wall slowly contracts and as it does so the yolk-sac is pushed into the umbilical opening which closes after it.

EVOLUTIONARY ORIGIN OF THE AMNION.—As regards this question, which has excited much controversy, the following appears to the present writer to be the working hypothesis which fits most easily the facts so far as they are known.

(1) The amnion originated as a *fold of blastoderm* round the body of the embryo (Fig. 216, A, B).

As has already been shown the amnion arises in this way in ontogeny in the Reptilia which are generally recognized as being the most primitive Amniotes. The same holds for the Birds and for some of the Mammals.

The Mammalia as a group are admittedly descended from ancestors in which the egg was large and meroblastic as it is in the Reptilia. This is indicated, apart from other convincing evidence, by the fact that they still exhibit in ontogeny a well-developed though yolkless "yolk-sac." It follows then that it is inadmissible to regard facts derived from the study of certain mammals in which the mode of amnion formation during ontogeny is of a different, even though apparently simpler, type as constituting important evidence in regard to the phylogenetic origin of the amnion, as has been done in particular by Hubrecht (1895).

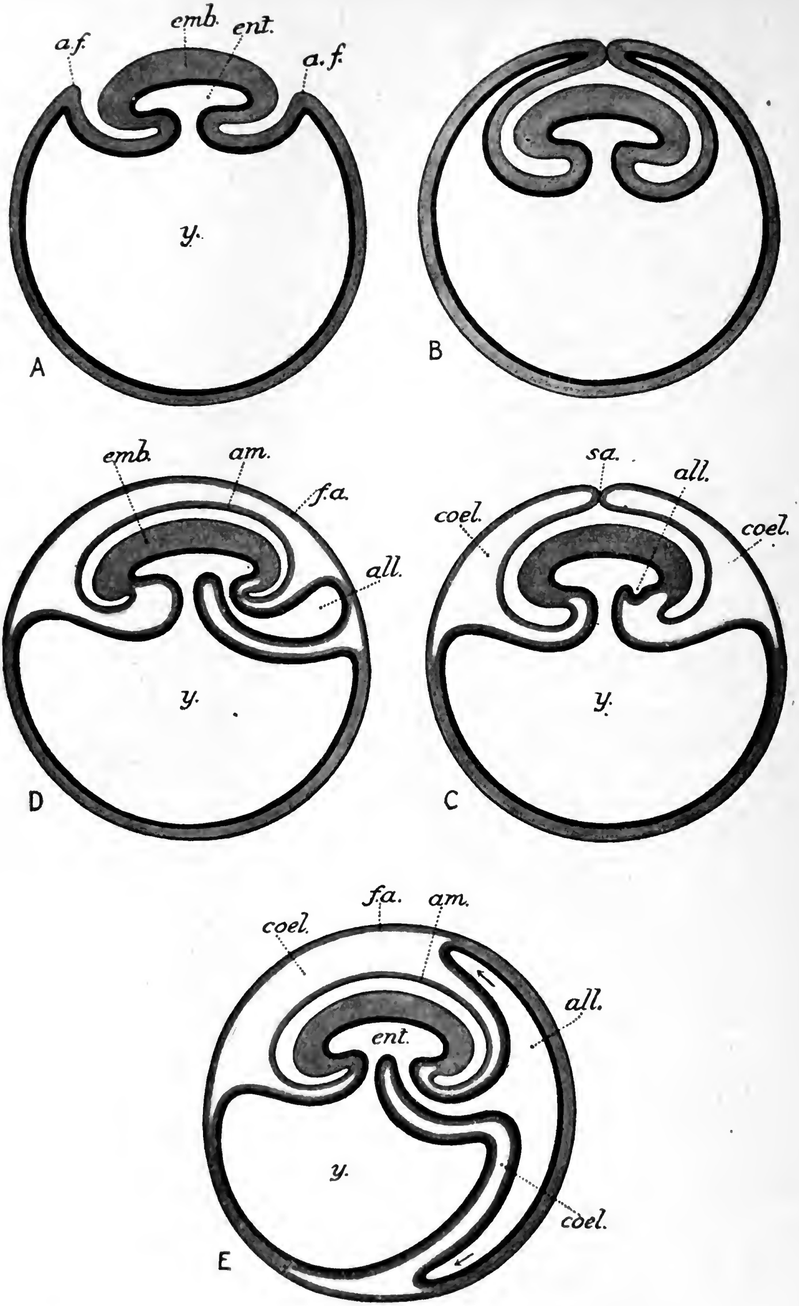


FIG. 216.—Diagram illustrating the evolution of amnion, etc.

a.f, amniotic fold ; *all*, cavity of allantois ; *am*, amnion ; *coel*, coelome ; *emb*, body of embryo ; *ent*, cavity of enteron ; *f.a*, serous membrane ; *sa*, sero-amniotic isthmus ; *y*, cavity of yolk-sac.

(2) The amniotic fold consisted at first of yolk-sac wall, the body of the embryo being forced down into the yolk-sac as it increased in size, possibly by the resistance of the rigid protective shell associated with the assumption of a terrestrial habit.

The tendency towards predominant development of the anterior portion of the amniotic fold may probably be correlated with the predominant growth and ventral flexure of the head end of the body which would cause it to dip down into the yolk-sac particularly markedly.

The delay in the appearance of mesoderm in the region of the proamnion may similarly have been originally due to the pressure of the downwardly flexed head.

(3) The yolk-sac with its richly developed superficial network of blood-vessels was the respiratory organ of the embryo at this early phase in the evolution of the Amniota. It follows that the portion of it nearest the shell, and therefore in the most favourable position for carrying on the breathing function, would tend to increase in area and would therefore bulge more and more over the body of the embryo (amniotic fold, Fig. 216, A, B, *a.f*) so as eventually to utilize the whole of the inner surface of the shell.

(4) The egg being now terrestrial the excretory poisons produced by the activity of the already functional renal organs could no longer pass away by diffusion into the surrounding water. It would obviously be disastrous were they to accumulate in the space round the embryo and they therefore had to be retained within the body. This led to the great and precocious enlargement of the receptacle for these excretory poisons, already present in the pre-amniote ancestor, the allantoic bladder.

(5) This precocious enlargement of the allantois in turn necessitated the early increase in size of the coelomic cavity to accommodate it.

(6) The allantoic wall—a part of the gut-wall—was naturally vascular, like the rest of the gut-wall, and with its great increase in size it would come in contact with the inner surface of the somatopleure. But as soon as it did this respiratory exchange would take place between its blood—through the substance of the somatopleure—and the medium outside. The allantois would thus constitute a new, though at first small, breathing organ.

(7) As the embryo grew its respiratory needs would grow also. Meanwhile of its two respiratory organs the one—the yolk-sac—would be shrinking in size and therefore diminishing in efficiency while the other—the allantois—would be increasing in size as it became more and more distended. This would lead to the supplanting of the yolk-sac by the allantois as the main respiratory organ. As the allantois increased in size it would tend to extend in the position of greatest respiratory efficiency, *i.e.* close under the somatopleure.

(8) With the development of the allantois and coelome the splanchnopleure would be freed from the somatopleure and the

upgrowth round the body of the embryo—the amniotic fold—would now become purely somatopleural (Fig. 216, C).

(9) As soon as the amniotic fold extended so far over the body of the embryo as to roof it in completely it would at once assume a new importance in protecting the delicate body of the embryo, enclosed within it as in a water jacket, from the dangerous jars and shocks incidental to a terrestrial existence. In correlation with the importance of this function of the closed amnion we might expect to find a tendency for its closure to be accelerated. As a matter of fact it will be found that in various mammals, including man, the amniotic cavity is closed from the beginning.

Fig. 217.—Egg-shell of *Acanthias* enclosing two eggs. (The divisions of the scale represent millimetres.)

IV. Viviparity in the Lower Vertebrates.—In many different groups of animals the embryonic phase of development is passed within the oviduct (uterus) of the mother. The advantages of this are obvious, for not only is the young individual sheltered to a great extent from the struggle for existence, as it is even within an egg-shell, but it forms for the time being as it were part of the body of a complete adult individual with its full equipment for holding its own in the struggle. It is in the group Mammalia amongst Vertebrates that viviparity reaches its highest development, as the final touch in their adaptation to a terrestrial existence, but it is of interest to notice that the phenomenon occurs, in a less highly elaborated form, here and there amongst the lower Vertebrates—Fishes, Amphibians, and Reptiles.

Thus among the Elasmobranch fishes[1] there are numerous genera in which the early stages in development are passed through in the uterus. In such cases we find in the first place a well-marked tendency towards the reduction of the protective egg-envelopes which are no longer necessary. Thus there is found, as a rule, during early stages a typical set of egg-envelopes, but the horny shell is very thin and weak as compared with that of oviparous Elasmobranchs and as development goes on (embryo of 7-8 cm. in *Acanthias*) it becomes still thinner, breaks up, and disappears.

A curious feature in such cases is the tendency for a group of eggs to be enclosed in a common set of envelopes instead of each egg having its own set. Thus in *Acanthias* (Fig. 217) there are commonly from two to six eggs enclosed in a common shell; in *Trygonorhina* two or three, in *Rhinobatus* seven or eight.

The embryo within the uterus is still nourished primarily by the yolk in its yolk-sac. This primitive mode of nourishment has not

---

[1] See Gudger, 1912.

yet been replaced by a process of absorption from the uterine wall as is the case in the Mammalia. But the uterine wall already plays a part though a minor one in providing food material for the young individual by its glandular activity. The beginnings of this are seen in the albuminous fluid enclosed within the egg-shell, and it is possible that the elongated gill-filaments of the embryo play a part in absorbing nourishment from this. A further development consists in the secretion of an abundant "uterine milk" which is drawn into the pharynx through the spiracles by precociously occurring movements like those of respiration and passed on into the digestive tract.

In accordance with its glandular activity the lining of the uterus frequently undergoes an increase of area by growing out into villi or **trophonemata** (Wood-Mason and Alcock, 1891). In the Sting-Rays specially enlarged trophonemata may be drawn into the pharyngeal cavity of the embryo through its greatly dilated spiracles so that their secretion reaches the alimentary canal of the young fish directly (Fig. 218).

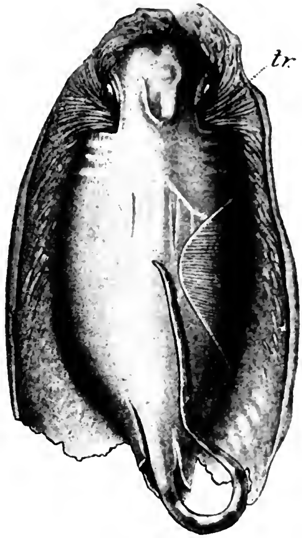


FIG. 218. — Portion of uterus of *Pteroplataea micrura* slit open to show an embryo with the trophonemata (*tr*) projecting into its spiracles. (After Wood-Mason and Alcock, 1891.)

During the later stages of intra-uterine development there usually comes about an intimate relationship between the surface of the yolk-sac and that of the uterine lining and in association with this there is found a varying degree of specialization of the uterine lining (Ercolani, 1879; Widakowich, 1907). This latter may be smooth (*Squatina angelus, Notidanus cinereus*), or project into longitudinal folds so as to give increase of surface (*Acanthias vulgaris, Scymnus lichia*), or grow out into papillae or trophonemata (*Torpedo, Pteroplataea*). Or finally it may develop folds which interlock with grooves on the surface of the yolk-sac, the uterine and yolk-sac surfaces being in the most intimate contact so as to constitute physiologically a definite yolk-sac placenta (*Carcharias glaucus, Mustelus laevis*, etc.).

Amongst the Teleostean fishes viviparity occurs occasionally, in at least half-a-dozen different families; the Cyprinodontidae, Scorpaenidae and Embiotocidae furnishing the greatest number of cases. They are particularly numerous amongst the Embiotocidae and Scorpaenidae of the western coast of North America (Eigenmann, 1894).

The eggs are retained in the ovary, either in the follicle, or in the cavity of the ovary; more rarely in the dilated oviduct or uterus. The developing embryo may depend for its nourishment upon the yolk (Scorpaenidae); it may absorb nourishment by the surface of the yolk-sac which grows out into villi (*Anableps*); or the nutritive secretion of the ovarian wall may be taken into the alimentary canal and there digested (Embiotocidae).

Among the Amphibia true viviparity is rare. A well-marked case occurs in *Salamandra atra* (Wiedersheim, 1890). Here a large number (40–60) of eggs pass into the oviduct when breeding is about to take place but of these all except the one (in rare cases as many as four) next the cloacal opening simply break down forming a kind of broth which fills the oviducal cavity. The embryo nourishes itself, after it has used up its own yolk supply, by gulping down and digesting this fluid, which contains not merely the yolky debris of disintegrated eggs, but also large quantities of red blood corpuscles derived from extensive haemorrhages of the uterine wall.

Perhaps the most striking feature of the Mammalia is the extreme degree of adaptation which they typically show to an intra-uterine mode of development in which the embryo leads a parasitic existence attached to the uterine lining of the mother. In accordance with this the external ectoderm of the blastocyst becomes modified to form organs of attachment which eventually, in the region of the yolk-sac and more especially in the region of the allantois, become vascularized and elaborated into the complex nutritive and respiratory organs named **placentae**. This being so, it becomes of much interest to enquire whether amongst those Amniota which are lowest in the scale of evolution—the Reptiles—there are any foreshadowings of the type of adaptation to intra-uterine development found in the Mammalia. Probably numerous such cases exist but at the present time, with our extremely imperfect knowledge of Reptilian development, we are acquainted with only a few. The most interesting of these is that of the Italian Lizard *Chalcides tridactylus* (*Seps chalcides*). Giacomini's description of this (1891) may be said to form the foundation of what will one day probably form an important chapter in Vertebrate embryology.

The eggs, which measure about 3 mm. in diameter, are first found in the oviducts early in May, while the first young are born towards the end of July, the period of gestation thus being between one and two months.[1] The eggs become spaced out along the oviduct or uterus, so as to give it a moniliform appearance, each egg being arranged with its apical pole towards the mesometrium. At about the middle of gestation the "egg" presents the appearance shown in Fig. 219, A, the whole forming a kind of blastocyst about 7 mm. in diameter. The outer surface is formed by the ectoderm of the serous membrane. Within the serous membrane there can be seen the allantois with transparent, richly vascular, wall and the yolk-

---

[1] About sixty-five days according to Mingazzini.

sac, more opaque than the allantois and already much smaller than the latter as seen in surface view. The edges of the allantois and the mushroom-shaped yolk-sac fit closely together and between them is the body of the embryo contained in the amnion. As in other Sauropsidans or Prototherian Mammals the yolk-sac lies on the embryo's left, the allantois upon its right—upon the side, in this case, next the mesometrium. As development proceeds the exposed area of yolk-sac becomes gradually reduced by the encroachment of the allantois. The latter however remains merely in contact with the edge of the yolk-sac and never comes to surround it. Over the yolk-sac area there remain visible for a long time the remains of the vitelline membrane (cf. Bird). Both allantoic and yolk-sac regions of the surface develop placental arrangements, the former being physiologically the more important of the two.

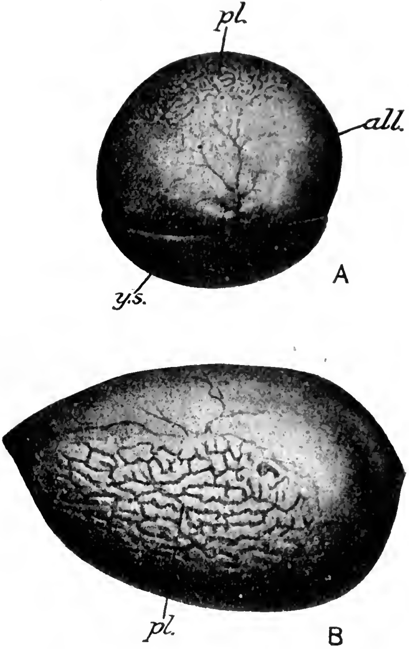


FIG. 219.—" Egg " of *Chalcides tridactylus*. (After Giacomini, 1891.)

A, 7 mm. in diameter, showing yolk-sac (*y.s*), allantois (*all*), and foetal portion of allantoic placenta (*pl*); B, 15-16 mm. in longest diameter, seen from apical pole, showing foetal portion of allantoic placenta (*pl*).

The **allantoic placenta** is already becoming apparent at the stage shown in Fig. 219, A, in the form of an elliptical area at the mesometrial pole which adheres to the uterine lining by means of numerous little projections which interlock with similar projections on a corresponding uterine area. As development goes on the egg assumes an elongated shape (Fig. 219, B). The whole of the uterine lining in contact with the outer surface of the "egg" is provided with a rich capillary network lying close beneath the uterine epithelium and here and there insinuating itself between the epithelial cells. Over the allantoic placental area the maternal projections now form undulating ribbons attached along one edge and free along the other. On the surface of these ribbons the uterine epithelium instead of being flattened as it is elsewhere is columnar and has a glandular appearance. With the ribbon-like projections just mentioned there interlock the somewhat similar projections of the foetus. These are also covered with columnar epithelium close under which lies a rich capillary network. The latter is not confined to the actual placental projections for even the smooth parts of the surface over the allantoic area are provided with an extraordinarily rich network of capillaries which show an even more marked tendency than those of the uterus to penetrate into the epithelium. Over the smooth area the foetal and maternal surfaces are in intimate contact, so that the two capillary networks lie parallel and close to one another, separated only by two very thin epithelial membranes. In the region where foetal and maternal projections interlock chinks are apparent between the two in which there appear to be traces of a fluid material—probably nutritive and secreted by the maternal epithelium which as already mentioned has in this region a glandular appearance.

The **yolk-sac placenta** is less highly developed. In the region of the centre of the yolk-sac flattened ridge-like projections also appear which interlock with corresponding uterine projections and become vascularized as the mesoderm spreads beneath them. Between the two surfaces is the remnant of vitelline membrane but this gradually disappears so that foetal and maternal surfaces come into intimate contact.

Chalcides (*Gongylus*) *ocellatus*, another Italian lizard, is also viviparous and in it occur similar though less marked adaptations to viviparity (Giacomini, 1906). Here in the later stages of gestation the general arrangement of the foetal envelopes resembles that in *C. tridactylus*. The allantoic region of the foetal surface is smooth and possesses a rich capillary network. It lies in immediate contact with the uterine lining, which in this region is covered with very thin flattened epithelium overlying an extremely rich network of maternal capillaries.

The portion of uterine lining in relation with the vitelline region of the foetal surface is less richly vascular, is covered with thicker epithelium of vacuolated cells with large nuclei, and is thrown into low folds which interlock with corresponding folds of the foetal surface so as to form an incipient yolk-sac placenta. The foetal epithelium of this region is thickened and in places columnar and appears to have an absorbent function. As in *C. tridactylus* remains of membrane are to be seen for a time between the foetal and maternal surfaces in this region.

To sum up, we find in *Chalcides ocellatus* a less advanced stage of adaptation to intra-uterine development than in *C. tridactylus*. Probably similar conditions will be found in various other viviparous Lizards as *e.g.* in the Australian *Trachysaurus* and *Tiliqua scincoides* (*Cyclodus boddaerti*) (Haacke, 1885).

In the Blind-worm (*Anguis fragilis*) and in the Viper (*Vipera*) and Smooth snake (*Coronella austriaca*) viviparity also occurs but here in a still more definitely incipient form—a thin shell persisting throughout development and the foetal envelopes and uterine lining remaining practically unmodified.

Thus in the three sets of Reptiles above mentioned we see three steps in the evolution of viviparity:

(1) the mere retention of the egg within the uterus, the shell still remaining and no intimate relations being developed between foetal and maternal tissues (*Anguis, Vipera, Coronella*),

(2) the rupture at an early stage and eventual disappearance of the shell, and the coming into intimate relations of foetal and maternal tissues, both becoming highly vascular and there being an attempt at the formation of a yolk-sac placenta (*Chalcides ocellatus*),

and (3) the development of an allantoic placenta (*C. tridactylus*).

LITERATURE

Agar. Proc. Zool. Soc. Lond., **1909**.
Bartlett. Proc. Zool. Soc. Lond., **1896**.
Bles. Trans. Roy. Soc. Edin., xli, **1905**.
Boulenger. Trans. Zool. Soc. Lond., xii, **1890**.
Boulenger. Proc. Zool. Soc. Lond., **1895**.
Brandes und Schoenichen. Abhandlungen Naturforsch. Gesell. Halle, xxii, **1901**.
Brauer. Zool. Jahrb. (Anat.), xii, **1899**.
Braus. SB. kgl. preuss. Akad. Wiss., **1906**.
Budgett. Quart. Journ. Micr. Sci., xlii, **1899**.
Dendy. Quart. Journ. Micr. Sci., xlii, **1899**.
Eigenmann. Bull. U.S. Fish Commission, xii (1892), **1894**.
Ercolani. Mem. Accad. Bologna (Sez. Sci. Nat.), x, **1879**.
Espada, Jimenez de la. An. Soc. Española de Historia Natural, i, **1872**.
Giacomini. Monitore zoologico italiano, ii ; and Arch. ital. Biologie, xvi, **1891**.
Giacomini. Mem. Accad. Bologna (Sez. Sci. Nat.), Ser. 6, iii, **1906**.
Goeldi. Proc. Zool. Soc. Lond., **1895**.
Gudger. Proc. Biol. Soc. Washington, xxv, **1912**.
Günther. Ann. Mag. Nat. Hist. (4), xvii, **1876**.
Haacke. Zool. Anz., viii, **1885**.
Hirota. Journ. Coll. Sci. Tokyo, vi, **1894**.
Hubrecht. Verh. Akad. Amsterdam, 2de Sect., **1895**.
von Ihering. Ann. Mag. Nat. Hist. (5), xvii, **1886**.
Ikeda. Annot. Zool. Japonenses, i, **1897**.
Kerr, Graham. Phil. Trans. Roy. Soc., B, cxcii, **1900**.
Lebrun. La Cellule, vii, **1891**.
Lillie. The Development of the Chick. New York, **1908**.
Mitsukuri. Journ. Coll. Sci. Tokyo, iv, **1891**.
Plate. Verh. Deutsch. Zool. Ges., Kiel, **1897**.
Schauinsland. Hertwigs Handbuch der Entwicklungslehre, I. Jena, **1906**.
Siedlecki. Biol. Centralblatt, xxix, **1909**.
Virchow, H. Internat. Beiträge zur wiss. Medizin, I, **1891**.
Weinland. Arch. f. Anat. u. Phys., **1854**.
Wetzel. Arch. Entw. Mech., xxvi, **1908**.
Widakowich. Zeitschr. wiss. Zool., lxxxviii, **1907**.
Wiedersheim. Arch. mikr. Anat., xxxvi, **1890**.
Wintrebert. C. R. Soc. Biol. Paris, lxxii, lxxiii, **1912**.
Wood-Mason and Alcock. Proc. Roy. Soc. Lond., xlix, **1891**.

CHAPTER IX

SOME OF THE GENERAL CONSIDERATIONS RELATING TO THE EMBRYOLOGY OF THE VERTEBRATA

In the course of the preceding chapters many of the general principles of vertebrate embryology will have made themselves apparent: the present chapter will deal shortly with some others of these principles which seem to require special notice.

(1) The Ontogenetic Evolution of the Zygote into the completely formed Individual.—The Vertebrate commences its individual existence as a zygote — a single cell — in which the specific characteristics, derived from the paternal and maternal ancestors, are already present though not recognizable. That this latter statement is accurate is demonstrated by such a fact as the following. The pelagic fertilized eggs of different species of Teleostean fishes show no trace of the specific features which characterize the adults. Such distinguishing features as are present and enable a specialist to identify them are mere differences in size, amount of yolk, colour of oil globule and so on, and have nothing to do with adult characteristics. Yet if a selection of such eggs are allowed to develop together under a homogeneous set of environmental conditions each is found gradually to unfold the complete array of characteristics which distinguish its own kind. As the various zygotes have developed under the same identical set of environmental conditions it follows that the differences which gradually become apparent cannot be due to the moulding influence of external conditions: they must have been already present though in invisible form in the zygote.

It follows further that the evolution of the zygote into the adult is in the main not a process of acquiring greater and greater complexity, in the sense of acquiring new properties, but rather of the localization—the segregation—of special peculiarities in particular portions of the individual, so that these portions assume a specific character and become recognizable as definite tissues or organs. The peculiarities were there to begin with, but they were diffuse and therefore unrecognizable—somewhat in the same way as the various colours of the spectrum are present in ordinary "white" light but are invisible until they are sifted out from one another by the action of a prism.

The lesson learned from the developing pelagic zygote—that in its case the full equipment of the complete individual is provided from internal sources—is one which should ever be borne in mind. It makes it easier to realize that in other cases, where the developing organism exists in a less homogeneous environment and where it has to fend for itself, characters impressed upon it directly by the environment, however conspicuous, are still superficial as compared with the really fundamental characters already present in the zygote.

The course of ontogenetic development from the zygote stage involves two main processes, (1) increase in bulk accompanied by the assumption of a multicellular condition, and (2) differentiation of parts *i.e.* the segregation, into localized portions of the living substance, of peculiarities which were in the zygote distributed without definite arrangement. The topographical differentiation of the developing embryo does not necessarily keep exact pace with the subdivision into cells. Thus in *Amphioxus* the egg appears to be still homogeneous throughout up to the time when it has segmented into 8 or even 16 blastomeres for even at this stage a blastomere isolated from its neighbours experimentally may go on for a time pursuing the same course of development as it would have done were it a complete zygote. In other cases, as appears to be the rule in the Frog, the first step in segregation—the segregation of characters belonging to the right and left halves of the body into corresponding hemispheres of the egg—would appear to take place in the zygote stage *i.e.* before the appearance of the first cleavage furrow.

The progressive segregation of specific characters in the various parts of the developing individual is beautifully brought out in the case of various invertebrates by the elaborate studies on "Cell lineage," some of which have been fully described in Vol. I.

The animal individual lives its life under a particular set of environmental conditions, constituted by the external medium—water or air—with its other living inhabitants: the latter play an important part, it may be by such comparatively simple and direct methods as by affecting the composition of the external medium, it may be by far more complex and obscure influences due to biological inter-relationships. The individual is able to go on living because of its organization and its living activities being fitted, in the most intimate manner, into the particular set of conditions which constitute its environment.

So also with the various parts of the body—organs, tissues, individual cells—of the young developing individual. Each lives amidst an environment of extreme complexity and of perfectly definite type of complexity, conditioned by the nature of the body of which it forms a portion and by the character of the parts of the body which are in proximity to it. Its living substance is everywhere bathed by—and no doubt intimately adapted to life in—an internal medium, watery fluid laden with the products of metabolism of the living substance as a whole. The differences in function of the various organs and tissues necessarily involve differences in their metabolic activity and therefore differences in the chemical nature of the contributions which they make to the complexity of the internal medium as well as differences in the substances which they withdraw from the internal medium for their own needs.

Physiologists recognize that changes in the constitution of the internal medium play an important part in exciting and controlling vital activity.[1] In the ordinary life of the animal important examples of such influence are afforded by changes in the activity of the normal function of an organ, as for instance when the pancreas secretes actively in response to the presence in the internal medium of a special substance secreted by the intestinal wall when stimulated by food material. Other examples are afforded by changes in growth-activity—as of the skin in response to a change in the amount of substance secreted by the thyroid, or of the mammary gland in response to the presence of substances produced by the metabolic activity of the foetus.

There is no reason to doubt that the living cells and tissues and organs of the embryo are similarly adapted to and influenced by the constitution of the internal medium, and if this be so the influence in question must play an important part in development. A possible example is afforded by the experimental result that the grafting of the developing optic cup of an Amphibian embryo into near proximity to ectodermal tissue (such as the pigment-layer of the retina, the wall of the brain, the olfactory epithelium, the external ectoderm of the head or trunk) is apt to induce that ectoderm to develop into a lens (Bell, 1907). Such influence upon one portion of embryonic substance by another portion in its neighbourhood may well be exercised through chemical or other changes produced by the specific metabolism of the latter in the internal medium in its neighbourhood.

A corollary to the consideration outlined above, which has an important bearing upon much work in experimental embryology, is that it is unwise to place reliance upon the mode of development of an organ-rudiment being normal, unless its environment is normal.

(2) CELLULARIZATION OF THE ZYGOTE: CELLULAR CONTINUITY AND DISCONTINUITY.—Protoplasm being a soft semi-fluid substance a particle of it, as it increases in volume during the process of growth which is associated with normal metabolism, would soon reach a mechanically unstable condition, in which retention of its characteristic form, or even cohesion, would be impossible. In nature a corrective to this is provided by the protoplasm undergoing fission. In the Protozoa the products of this fission normally break apart and

---

[1] For their bearing upon evolutionary change see Parker (1909).

lead an independent existence, while in the Metazoa the subdivision is less complete and the growing mass of living substance continues to exist as a coherent individual. The physiological advantages of subdivision of the individual body into cellular units is apparent. It renders possible the intercellular deposit of rigid skeletal materials which act as a support to the organism as a whole: it facilitates localization of function and enables blocks of units specialized for particular functions to be transferred during ontogeny to the positions in which they will be most useful: it enables other units to move hither and thither, either by their own activity or by being swept along in a circulating stream of fluid, to wherever they are specially needed in the course of the ordinary vital processes: and it is of enormous importance in relation to attacks upon the organism from without, whether by limiting the area of injury to comparatively small tracts of living substance or by enabling portions of the living substance specialized for defence to be mobilized and ready to concentrate at the point of attack.

Modern science impresses upon us the importance of regarding the individual not merely as an aggregation of cells and organs, but rather as a mass of living substance imperfectly subdivided up into cells and organs: imperfectly because each cell and each organ is inextricably linked up in the living activity of the whole individual. It brings to our notice numerous tissues in which the actual living substance of the constituent cells is linked up by intercellular bridges of protoplasm: it tells us of particular cases of developing embryos where similar intercellular continuity is apparent. The question is thus raised: are we correct in our belief that actual complete separation of cells takes place as a general rule when they undergo fission during ontogeny? More especially is it really the case that the individual blastomeres of the segmenting egg become completely separated from one another: is it not rather the case that the apparently complete separation is only apparent, that the individual blastomeres remain continuous through fine protoplasmic bridges: and that cases of intercellular continuity observed in the adult are merely expressions of the fact that such bridges persist throughout the whole period of development?

That the latter is really the case has been held by various workers and supported particularly strongly by Sedgwick (1895, 1896). It will however have been gathered by the reader from Chapter I. that such a view is in the opinion of the present writer not tenable. The fact that the blastomeres of a segmenting egg tend to take a spherical form, or at least to be bounded by convex surfaces, seems by the ordinary laws of surface tension to indicate that these blastomeres are not continuous with one another. Continuity of substance between the cells of the embryo or adult is therefore when it occurs a secondary and not a primary phenomenon. At the same time the present writer's observations lead him to agree with Sedgwick that such intercellular continuity of protoplasm is much more widely spread than is generally recognized.

(3) YOLK.—Theoretically the most primitive type of zygote should from the beginning be able to absorb food for itself. As an actual fact however the zygote is provisioned for a shorter or longer period by the highly nutritious fat and proteid, in the form of yolk which is stored up in its cytoplasm.[1] With increasing specialization the amount of this store becomes greater and greater so as to lengthen the period during which the young individual is provisioned and freed from the necessity of working for its own living. A good example of a high degree of such specialization amongst Vertebrates is afforded by the relatively huge egg of the Ostrich.

It has of course to be borne in mind that the degree of specialization in this direction is to be estimated not merely by the absolute amount of yolk present but still more by the relative amount of yolk in proportion to protoplasm. Thus two eggs may be described as equally richly yolked although very different in size provided that the proportion of yolk to protoplasm is similar in the two cases. In correlation with this we find that a group characterized by heavily yolked eggs may evolve in the direction of producing more and more numerous, and therefore necessarily smaller, eggs. Good examples of this are seen in the Teleostean fishes where the eggs may be produced in enormous numbers and of very minute size although still retaining a proportionately large supply of yolk.

In C. Rabl's discussions of his "Theory of the Mesoderm" (1889) an important place is taken by repeated losses and re-acquisitions of yolk during the phylogeny of the Vertebrata. Rabl arranges Cyclostomes, Elasmobranchs, Ganoids, Amphibians, meroblastic "Protamniota" and Mammals, in a linear series, and concludes that Ganoids and Amphibians have undergone a diminution of yolk and have therefore reverted to the holoblastic condition; that meroblastic Protamniota have re-acquired a large amount of yolk and have therefore reverted to the meroblastic condition; and that finally Mammals have lost their yolk and again become holoblastic. In the opinion of the writer there is no sufficient justification for any one of these assumptions except the last. There is, as is well known, definite evidence to show that Mammals are descended from ancestors with large and heavily yolked eggs and that the small size and practically yolkless character of their holoblastic eggs are secondary acquirements. In this case the loss of yolk has brought in its train profound changes in the early processes of development but of such there are no signs in those other cases in which Rabl supposes loss of yolk to have taken place. It must also be remembered that in the Mammal there is an obvious physiological reason for the loss of yolk—namely that the food material needed during the development of the embryo is provided from the tissues of the mother.

---

[1] For a detailed account of the development of the yolk in the egg of one of the lower Vertebrates (*Proteus*) see Jörgensen (1910).

On the recapitulation hypothesis the segmentation and other early stages of ontogeny represent ancestral evolutionary stages common to all Vertebrates. The differences to be observed between such stages in different members of the group are consequently not to be looked on as ancestral but rather as due to the influence of disturbing secondary factors. Of these by far the most important is the presence of the particles of yolk, this dead substance clogging and retarding the living activity of the egg protoplasm. The extent to which it does this in any particular region of the egg is roughly proportional to its relative amount as compared with the living protoplasm in that portion of the egg. The yolk is as a rule of higher specific gravity than the protoplasm. Correlated with this it tends to be in proportionally greater amount in the lower parts of the egg than in the apical part, with the result that the processes of cell division and of development generally are relatively more slowed down in these lower portions—in extreme cases brought to a full stop—by its retarding influence. Typical examples of this are seen in the holoblastic but unequally segmenting eggs of the ordinary Amphibia. In this case it is possible by replacing experimentally the action of gravity by a more potent force (by centrifugalizing the eggs) to concentrate the yolk still more than is natural in the lower hemisphere with the result that the egg is now converted into a meroblastic one (O. Hertwig) the lower hemisphere being unable to segment. On the other hand by inverting the egg and so allowing the yolk granules to settle down towards the apical pole under the influence of gravity it is possible to cause the segmentation furrows to start from the abapical pole and spread towards the apical.

The influence of yolk upon the gastrulation process will have been realized from the perusal of Chapter II.: it is well illustrated by the series *Amphioxus* (Fig. 18), *Petromyzon* (Fig. 23), *Rana* (Fig. 25), *Lepidosiren* (Fig. 21), *Hypogeophis* (Fig. 27) and *Torpedo* (Fig. 28). Put in a single sentence it may be said to consist above all in the gradual subordination of the process of invagination to those of overgrowth and delamination. In the succeeding stage it makes itself apparent more particularly in the modification of the mode of origin of the mesoderm, the outgrowth of hollow enterocoelic pouches being replaced by the delamination of a solid mass or sheet.

The storage of yolk carries with it not merely the modifications just indicated in the processes of segmentation and gastrulation. Its influence becomes retrospective and affects even preceding stages during the growth of the intra-ovarian egg. This is shown more especially by the precocious concentration of yolk in that portion of the egg which will later become endodermal. Thus is the telolecithal condition brought about and telolecithality itself is seen to be really a foreshadowing of a particular adaptive feature of later stages of development (p. 183).

In examining sections of later stages of Vertebrate embryos in which the eggs are rich in yolk it is readily seen that there are conspicuous differences between different parts of the embryo's tissues in regard to the yolk contained in their cells, for example endodermal structures are frequently marked out by larger yolk granules which cause them to stain more deeply with yolk-staining dyes. The condition of the yolk in a tissue may indeed give a useful hint as to the cell layer to which it belongs and as a matter of fact evidence of this kind has played a conspicuous part in many embryological discussions.

It is important to bear in mind however the physiological significance of the character in question. It appears to be closely related to the metabolic processes in the tissue concerned. As a given tissue in a yolky embryo goes on with its growth and development its yolk is gradually used up, a necessary preliminary being its breaking down into fine particles easily assimilable. Tissues or cells undergoing active growth and multiplication have their yolk in this fine-grained condition: those which are for the time being comparatively inert retain their yolk in a coarse-grained form. Thus a disturbing factor is introduced which has to be carefully borne in mind when using the character of the yolk as a criterion of the morphological nature of a given cell or tissue.

A still further disturbing factor lies in the fact that while yolk is being used up and disappearing from view in one part of the body it may be deposited in cells elsewhere—as for example takes place in eggs during their period of growth within the ovary. Such increase in the amount of yolk however, accompanied commonly by increase in the size of the individual granules, is naturally relatively rare in comparison with the breaking down of yolk which is occurring through the general tissues of the embryo. It follows that on the whole *coarsely* granular yolk in a cell or tissue affords more reliable evidence as to its nature than does fine-grained yolk—which may be and usually is merely a symptom of active metabolism.

(4) RECAPITULATION.—The fascination as well as the philosophical interest of the study of Vertebrate embryology rests in great part upon the recapitulation of phylogenetic evolution during the development of the individual. In the early days of evolutionary embryology this idea was accepted in an unquestioning and uncritical spirit and it was supposed that all that had to be done to obtain an accurate and fairly complete picture of the phylogenetic history of any particular animal was simply to work out its ontogenetic development. The more extensive knowledge which we have regarding embryological phenomena to-day serves on the one hand to confirm fully the truth of the general principle and on the other hand to indicate how its working is interfered with by various disturbing factors.

The *main* controlling factor in ontogeny is the character of the adult. This is the motive power throughout the developmental period. Just as according to Newton's First Law a moving body tends to continue in a state of uniform motion in a straight line, so in ontogeny the developing individual tends to progress constantly towards the goal of adult structure. Not in this case however necessarily by the straightest and shortest path. The structure of the adult is the expression of the action of Heredity. The earlier stages are not exempt from the same influence. Each step in the development of the ancestor tends to be repeated in the development of the descendant. The descendant then during its ontogeny tends to pursue the same, it may be devious, path as the ancestor. If in the course of generations the adult structure becomes shifted onwards in a process of evolution, this merely means the adding on of a new portion at the latter end of the ontogenetic path. The earlier portions of this path, built up of similar increments representing previous steps in evolutionary progress, are repeated as before, and so the complete process of individual development forms a record or recapitulation of phylogenetic history.

It cannot be too constantly borne in mind that the factor just indicated is the supreme factor in ontogenetic development. Other factors may be superficially conspicuous, may have far-reaching influence upon details, but this factor—the tendency to repeat ancestral steps in development up to and including the final characters of the adult—is and must always be paramount.

Modern advances in knowledge of the facts of embryology, together with the assumption of a properly critical frame of mind, have shown, however, that the picture of past evolution afforded by the phenomena of individual development is at the best but a blurred and imperfect one, and that this must necessarily be so is readily realized when we remember that a large proportion of the characters of any organism are adaptive to its special mode of life. The circumstances under which a developing organism exists are, as a rule, widely different from those under which its ancestors proceeded along the evolutionary path, and in correlation with this its adaptive features are equally distinct. As we study the development of any species of animal we do not then see before us a complete and perfect picture of its evolutionary history, but merely gain fleeting, and it may be misleading, glimpses through the obscuring clouds of adaptive features.

A further disturbing factor is indicated by the consideration that in past evolutionary history each stage in evolution was represented by a complete functional organism, all the parts of which were necessarily at correlated stages of development so as to form a functional whole. Many modern animals however develop under conditions in which the different systems of organs are no longer forced to keep accurate step with one another, and the result is that some lag behind while others, particularly organs of great histological complexity in the adult—such as the brain or the eye—are accelerated in their early development, so as to give time for the complicated histogenetic processes that have to be completed before the organ can become functional. It will be realized that this latter type of disturbance affects the development of the individual as a whole much more than it does its component organs, the result being that embryology frequently affords a much more perfect picture of the evolution of single organs than it does of the organism as a whole.

In reference to the ontogenetic record of phylogeny an interesting question presents itself regarding the reliability or otherwise of the information derived from the study of larval forms. To what extent may a particular type of larva be taken as probably representing a corresponding phase in the evolutionary history of the group: to what extent are its features to be regarded as ancestral, to what extent as mere modern adaptations to the environmental conditions among which the particular creature now pursues its individual development? In connexion with various groups among the Invertebrata larval forms have played a conspicuous part in phylogenetic speculation—in some cases without due discrimination in interpreting their features as ancestral—the climax perhaps being reached by the view which regards such pelagic larvae as trochospheres or nauplii—precociously developed and free-swimming heads without any trunk—as representing ancestral forms (cf. Graham Kerr, 1911).

In considering whether a particular stage of development is to be taken as probably repeating an ancestral stage of the adult special attention should be directed towards its mode of life, with the object of estimating the degree to which it diverges from the probable mode of life of the ancestral stage. If its mode of life is strikingly aberrant, *e.g.* parasitic where the normal habit of the group is free-living, or pelagic where the normal habit is not pelagic, then we must always keep in mind the possibility or probability that its most conspicuous features are mere modern adaptations and are therefore worthless as evidence of ancestral conditions.

Again it should be considered whether in the main features of its organization it agrees with animals which are admittedly allied to it.

Larvae occur in the following Vertebrates—*Amphioxus, Petromyzon,* Crossopterygians, Ganoids, many Teleosts, Lung-fishes and the majority of Amphibians. Applying such criteria as are indicated above we should rule out as probably devoid of phylogenetic significance the larva of *Amphioxus* on account of its quite aberrant "pleuronectid" asymmetry (see Vol. I. Chap. XVII.). We should again rule out the Teleostean larvae on account of their extreme diversity. In Urodele Amphibians and Dipneumonic Lung-fishes on the other hand we see larvae which appear to be distinctly of a common type. And in Crossopterygian and Actinopterygian Ganoids we again find larvae which differ from these in detail rather than in fundamental characteristics. Consequently we should incline towards the view that the type of larva in question does not depart very widely from the common ancestral type out of which existing Vertebrates have evolved.

Again in considering whether a particular feature of structure is to be regarded as ancestral or as a modern adaptation the following questions should be asked: (1) Is the feature peculiar to one group of Vertebrates or does it occur in several groups, and (2) if it occurs in several groups do the various animals possessing the peculiarity in question undergo their larval stages in similar sets of environmental conditions?

If the particular feature occurs in several groups derived from a common ancestral form this obviously increases the probability of the feature itself being ancestral. If however the several groups show similar sets of environmental conditions during their larval stages this introduces the element of doubt whether the similar features may not after all be merely adaptations to these similar sets of conditions.

Again it is important to make out whether the particular similarity has to do merely with parts of structure in direct functional relationship to external conditions. If there be deep-seated correspondences in structure with no such direct functional relationship to external factors then this gives greatly increased probability to these correspondences being truly ancestral in their nature.

The morphologist in trying to decipher the record of evolutionary history from the data of comparative anatomy or embryology is constantly impressed by the potency of nature's economy of living substance. An organ no longer required may be eliminated within a very short period of evolutionary time. Thus in some species of Mackerel (*Scomber*) so important an organ as the air-bladder has been eliminated: in various Frogs and Toads the external gills have been eliminated from development. Thus negative embryological evidence is of peculiarly little weight in relation to phylogenetic problems.

(5) THE PROTOSTOMA HYPOTHESIS.—This is a working hypothesis which links together and in a sense explains a number of features in the early development of Vertebrates which are otherwise extremely puzzling. The more important of these features may be summarized as follows:

In *Amphioxus* as has already been shown the dorsal side is at first occupied by the widely open gastrular mouth. Later this becomes roofed in by a backgrowth of the gastrular rim anteriorly. A similar process of backgrowth appears to take place in the gastrulation of lower Vertebrates in general. The roof of the gastrular cavity formed by this process gives rise later not merely to the dorsal wall of the alimentary canal but also to notochord and central nervous system.

I. Now occasionally there are appearances which suggest that this archenteric roof consists really of two lateral halves which have become fused together along the sagittal plane. Thus in *Protopterus* the down-growing dorsal lip of the blastopore is frequently indented by a median incision. Again in Urodeles the medullary plate is frequently traversed by a fine superficial groove which passes forwards along the median line.

II. Then there occur curious cases of abnormality in which the dorsal region of the body is actually divided into two halves by a longitudinal split in the mesial plane. Thus Oscar Hertwig found (1892) that by fertilizing frog's eggs which had become over-ripe, either by retention within the oviduct or by being kept for from one to four days in a moist chamber, he obtained a certain number of abnormal embryos of the type shown in Fig. 220, A, where a large expanse of yolk-cells is visible in dorsal view instead of being completely covered in as would be the case normally. In transverse section (Fig. 220, B) such an embryo was found to have two half

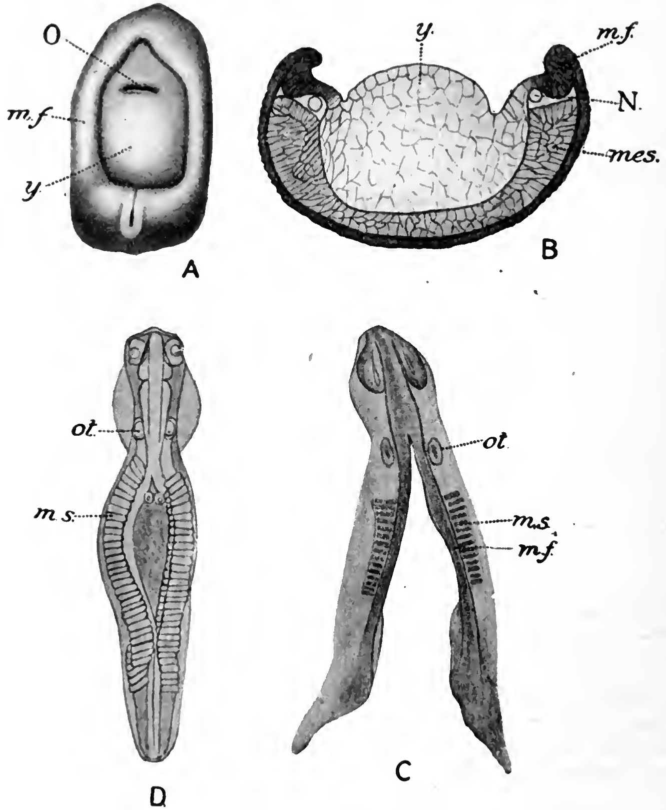


Fig. 220.—Abnormal embryos illustrating the Protostoma theory.

A, abnormal Frog embryo seen from the dorsal side; B, transverse section through hinder third of ditto (after O. Hertwig, 1892); C, abnormal Trout embryo (*S. fario*) in dorsal view (after Kopsch, 1899); D, abnormal embryo of Pike (*Esox lucius*) (after Lereboullet, 1863). *m.f*, medullary fold; *m.s*, mesoderm segment; *mes*, mesoderm; *N*, notochord; *O*, opening leading down into enteron; *ot*, otocyst; *y*, mass of yolk-cells.

neural rudiments and two notochords of half the usual size, widely separated by the mass of yolk or endoderm.

Similar abnormalities have been observed in Teleostean fishes. *E.g.* Fig. 220, D shows a Pike-embryo which is normal towards its anterior and posterior ends but interrupted for some distance in the mid-dorsal line by a wide cleft in which the yolk is visible. Again in Fig. 220, C a similar cleft is seen to traverse the whole length of a Trout-embryo from the hind-brain region tailwards.

An important feature of such abnormal embryos of fish and amphibians is that they frequently proceed with their development, the lips of the fissure closing up and the two sets of half-organs being brought together in the mesial plane, undergoing complete fusion and the individual becoming in fact entirely normal. The importance of this return to the normal on the part of such split embryos is that it indicates that the departure from the normal during the split condition is far less fundamental than would appear at first sight.

Here then we have to do with two very remarkable phenomena. Firstly there is the abnormality itself—the fact that the dorsal region of the body is for a time in the form of two distinct halves. Abnormalities of such a definite type as this usually have a definite evolutionary or other meaning and it is necessary to search for such a meaning in this particular case. Secondly, there is the fact that an embryo almost completely bisected in this way is frequently able to right itself and become perfectly normal. This again suggests the question whether this power of righting itself has not some special evolutionary meaning.

III. In the higher meroblastic Vertebrates we have seen that there exists along the middle line of what corresponds with the archenteric roof of *Amphioxus* (*i.e.* the region which becomes converted into the dorsal part of the body, including notochord and medullary plate) the structure known as the primitive streak. We have also seen that in the lowest Vertebrates possessing it, this primitive streak represents the line of fusion of the gastrular lips, and that we are therefore justified in attaching the same significance to the primitive streak in those higher forms in which the actual process of fusion can no longer be observed. That this interpretation is correct is indicated by the occasional occurrence of openings in the line of the primitive streak communicating ventrally with the enteron and dorsally with the outer surface of the medullary plate, or its derivative the floor of the neural tube (pp. 51, 53). Such neurenteric communications are readily explicable by the view that they represent simply parts of the line of fusion of the gastrular lips where the actual fusion has not been completed. Here again we have a phenomenon which demands explanation—the occurrence of what seems to be the vestige of a slit-like gastrular mouth along the mid-dorsal line.

IV. We have another remarkable body of facts associated with the fate of the blastopore or remnant of the gastrular mouth in various groups of the animal kingdom. Thus within the limits of the groups Annelida or Mollusca the blastopore in some forms becomes the mouth, in others the anus. No one would doubt for a moment that the mouth opening is homologous throughout these groups yet in one member of the group it can be traced back to the blastopore while in another member it is the anus which can be so traced. In other forms the gastrular mouth simply vanishes away during development and in some of these cases it assumes a curious elongated slit-like form along the mid-neural line before it disappears.

It is the merit of the Protostoma theory that it—and it alone—affords an explanation of these four very different but equally puzzling bodies of facts. It falls therefore to be accepted by the Vertebrate embryologist as one of his working hypotheses.

The Protostoma theory is simply a special development of the theory of the evolution of the coelomate Metazoa which is generally accepted by morphologists, namely that the animals in question have passed, during the remoter parts of their evolutionary history, through a Protozoan and later a Coelenterate stage. The peculiarity of the Protostoma theory is that it includes within the coelenterate period a stage corresponding in its main structural features with the Actinians of the present time, characterized by the presence of an elongated slit-like mouth, dilated somewhat at each end and surrounded by a specially concentrated portion of the ectodermal nerve plexus. The portion of the surface on which the slit-like mouth was situated was thus the neural surface.

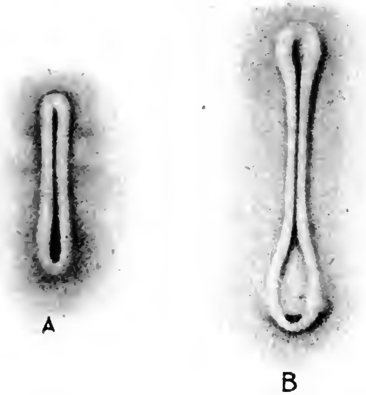


Fig. 221.—View of neural rudiment in embryo of (A) *Peripatus* (after Sedgwick, 1884); and (B) *Lepidosiren*. In the case of *Lepidosiren* the embryo is shown as it appears when straightened out.

Sedgwick (1884) was led to the idea by his studies on the development of *Peripatus*. He found in the species investigated by him a stage (see Fig. 221, A) in which the gastrula-mouth formed a long slit traversing the neural surface and surrounded by the ectodermal neural rudiment. As development went on the gastrular mouth or protostoma became obliterated, except in its dilated terminal portions, by fusion of its lips. The terminal parts remained open as mouth and anus respectively. The portions of nerve rudiment between the two openings became the ventral nerve cords while the portions in front of the mouth and behind the anus gave rise respectively to the supra-oesophageal ganglia and the suprarectal commissure. According to Sedgwick this stage in the development of *Peripatus* repeats the features of an Actinozoon-like Coelenterate ancestor, not merely of *Peripatus,* and therefore of Arthropods in general, but of such other groups as Annelids, Molluscs and Vertebrates.

It will be noted that on this protostoma hypothesis an important physiological distinction has at an early period of evolution marked off the Vertebrates from the other groups mentioned. This distinction came about with the acquisition of different habits of movement. In the stem which gave rise to Annelids, Arthropods, Molluscs, movement took place with the neural surface next the substratum (as in those modern Medusae which are able to creep on a solid surface—*e.g. Cladonema*), while in the Vertebrate stem on the other hand the neural surface was directed away from the solid substratum (as in the modern Actinian when it creeps). This difference in the position of the body in relation to the substratum would naturally lead in time to the different types of dorsiventrality so apparent in the fundamental organization of the two diverging stems. It is frequently stated by critics of the protostoma hypothesis that it involves a reversal of dorsal and ventral sides during the evolution of Vertebrates from their invertebrate ancestors but it will be gathered from what has been said that this criticism rests on a misunderstanding.

It will be readily seen that the protostoma hypothesis successfully explains the four categories of puzzling facts already enumerated. The paired appearance of the gastrular roof would be a reminiscence of the fact that originally it was actually paired: the split along the back of the abnormal embryos would mean the temporary re-appearance of the ancestral split or mouth: the primitive streak would be the scar along which the lips of this ancestral mouth or protostoma underwent fusion: and the converting of blastopore now into mouth now into anus would be an imperfect reminiscence of the fact that in phylogeny it gave rise to both.

On this hypothesis the various signs of a split along the neural surface of the vertebrate embryo, whether in the form of a dorsal furrow or a primitive streak or an actual opening, are interpretable as reminiscences of the protostoma slit which traversed the neural surface of the Actinozoon-like ancestor.[1] It is of interest to notice that in two Vertebrates at least there exist what seem to be obvious traces of neural rudiment extending round behind the anal part of the protostoma precisely as in *Peripatus.* In Fig. 221, B, is shown an embryo of *Lepidosiren* spread out in one plane, with the neural rudiment in the form of a ridge which is continuous behind the blastopore or anus. If it be reflected that this opening may be

---

[1] That the primitive streak and primitive groove are closely related to the gastrula mouth was perceived by Rauber (1877) but a clear evolutionary explanation of this relationship was first given by the protostoma theory of Sedgwick (1884) and Hertwig (1892).

regarded as being continued forwards by a potential slit, represented *e.g.* by the primitive streak of other forms, it will be realized how close is the resemblance to the conditions in *Peripatus.* The pre-anal portions of the neural rudiment in *Lepidosiren* come together in the mesial plane to form the spinal cord, while the postanal portion flattens out and disappears so that the anal opening comes to lie entirely behind the posterior limit of the central nervous system. It is clear that if the development of the anal opening were delayed until the neural folds had already come together it would make its appearance completely behind the central nervous rudiment and with no obvious connexion with it. This is very possibly the case in Vertebrates other than those mentioned.

Although the anal opening of Vertebrates is thus brought into the relations with the nervous system that we should expect on the protostoma hypothesis there is no such definite evidence in the case of the mouth. It is true that in some cases the dorsal furrow has been traced to the neighbourhood of the mouth and that the mouth opening has in some cases at first the form of a sagittally placed slit, but in no case, up to the present, has the neural rudiment been traced round in front of the mouth. This difficulty however is greatly lessened when we correlate the facts just mentioned in regard to the anal opening in *Lepidosiren* with the relatively late appearance of the mouth opening of Vertebrates as discussed on p. 193. It may well be that the non-inclusion of the mouth opening within the obvious neural rudiment is due simply to the pre-oral parts of the medullary folds having already flattened out and disappeared before the oral opening makes its appearance. If this is the case it carries with it the interesting consequence that the supra-oesophageal or pre-oral ganglia of *Peripatus* have disappeared in the Vertebrate and it is therefore waste of energy to discuss what parts of the brain of a Vertebrate are homologous with the supra-oesophageal ganglia of Invertebrates.

This Protostoma idea, dealing as it does with extremely remote phases of the Vertebrate phylogeny, must not be looked on as a definitely proved theory, nor can it be expected ever to reach that dignity, but it is a fascinating working hypothesis which serves, and which alone serves, to link together and in a sense explain a considerable body of otherwise mysterious and apparently inexplicable facts of Vertebrate embryology.[1]

(6) THE VERTEBRATE HEAD.—The two phyla of the animal kingdom which have reached the highest stage of evolutionary development—the Arthropoda and the Vertebrata—are alike characterized by the possession of a well-developed head. In the

---

[1] In considering the difficulties in the way of the theory afforded by cases where the gastrula becomes roofed in by a process of simple backgrowth without any trace of protostoma (e.g. *Amphioxus*), it is well to bear in mind the parallel case of the amnion—of which a large portion may be formed by simple backgrowth, although the sero-amniotic isthmus and the ingrowth of mesoderm from the two sides seem to point clearly to a former formation by the meeting of two lateral folds.

evolution of a head we may take it that the principal factors involved are probably the following:

(1) The habit of active movement in a direction corresponding with the prolongation of the axis of the body,

(2) The concentration of organs of special sense towards the end of the body which is in front during movement,

(3) The concentration of nerve centres to form a brain in proximity to these organs of sense.

In the case of the Vertebrate the brain has reached a comparatively large size and in correlation with this the protecting skeleton has become highly developed and has lost the flexibility which is characteristic of it in the trunk. Further in the Vertebrate the walls of the buccal cavity and pharynx have become highly specialized, particularly in the matter of their skeleton, in relation to the functions of ingestion and mastication of the food on the one hand, and of respiration on the other.

Each of these various factors involves structural change, not affecting merely one organ but causing modification of the whole complex arrangements of the head-region. Thus associated with the loss of flexibility we find (1) loss of segmentation of the skeleton, (2) disappearance or great modification of the myotomes, (3) corresponding changes in the nerves supplying these myotomes and (4) disappearance of the coelomic cavities.

The full appreciation of the importance of this feature of the Vertebrata makes it, in the present writer's opinion, impossible to doubt that the possession of a definite head is a feature that has come down from the unknown ancestral form from which the Vertebrate stock has evolved. If this be correct it follows that the relatively feeble differentiation of the head end of the body seen in *Amphioxus* is to be regarded as a secondary condition, correlated with the peculiar mode of life of this animal, and devoid of phylogenetic significance.

It has already been pointed out that organs of great complexity in the adult tend to be laid down at an early stage of individual development, time being thus obtained for the development of their complex detail. It is perhaps in direct relation to this principle that the highly complex head-region of the Vertebrate, which comes to assume control over most of the activities of the individual, develops particularly early in ontogeny — the various developmental processes making themselves as a rule first apparent in the head region and spreading thence tailwards along the trunk. This fact is of practical importance to the embryologist for in the case of segmentally repeated organs it enables him to find a series of developmental stages within the body of a single embryo.

Though this precocious cephalization is a marked feature of Vertebrate ontogeny it never goes within this phylum to the length it does amongst certain Invertebrates where the larval stage

(Nauplius, Trochosphere) is practically a precociously developed and free-living head which has not yet developed a trunk.

As will have been gathered, one of the most conspicuous features of the head-region is the loss of segmentation in organs in which it was once present.

Metameric segmentation, which first makes its appearance in typical form in the Annelida, is probably to be associated primarily with the coelome and its lining the mesoderm. The coelome is distended with coelomic fluid and the turgidity so caused gives firmness to the body. The physiological advantage of the coelomic cavity being subdivided into successive compartments is obvious. The segmentation of other organs is to be looked on as secondary to that of the mesoderm, and more especially to that of the muscles. Thus the segmented character of the nervous system of an Annelid or Arthropod is due to the ganglion-cells tending to become concentrated at the level of the masses of muscle which work the parapodia or limbs. So also the segmentation of the skeleton which permits flexure of the body is correlated directly with the segmentation of the musculature which causes that flexure.

So, conversely, with the disappearance of segmentation in the head of the Vertebrate. Correlated with the loss of flexibility in the brain region the myotomes which produce the flexure have disappeared, and correlated with this in turn the ensheathing skeleton has lost its segmentation and the segmentally arranged motor nerves have also gone. The process has taken place from before backwards. It has been carried to the greatest extent in front, to the least at the hinder limit of the head.

It is definitely established that the head of the Vertebrate has at least in part come into being by the modification of what was once the anterior portion of the trunk. With the gradual evolution and increase in size of the brain—so characteristic of the phylum Vertebrata—this organ has gradually encroached upon the spinal cord, and its protective skeleton the chondrocranium has *pari passu* encroached upon the vertebral column. This is clearly indicated by the fact that included within the limits of the skull are nerves which are serially homologous with those of the trunk. Putting on one side the probability—as many would regard it—that cranial nerves III, IV, V, VI, VII, IX and X are really homologous with the spinal nerves, we find behind the Vagus a series of **spino-occipital** nerves (Fürbringer, 1897), which although included within the limits of the skull are yet undoubtedly members of the same series as the spinal nerves. The number of those is very different in the different subdivisions of the Vertebrata as may be gathered from an inspection of Fig. 222. In all probability they will be found also to show considerable variation in different individuals of the same species.

During the evolution of the head there is some reason to believe that its extension backwards has taken place by successive steps. In the most ancient recognizable stage the cranium (Palaeocranium —Fürbringer) extended no farther back than the vagus nerve. This phase is represented—either persistent or revertive—in the adult stage of modern Cyclostomes: it is also seen in the young Lepidosiren of stage 34 (see Fig. 154, B, p. 309).

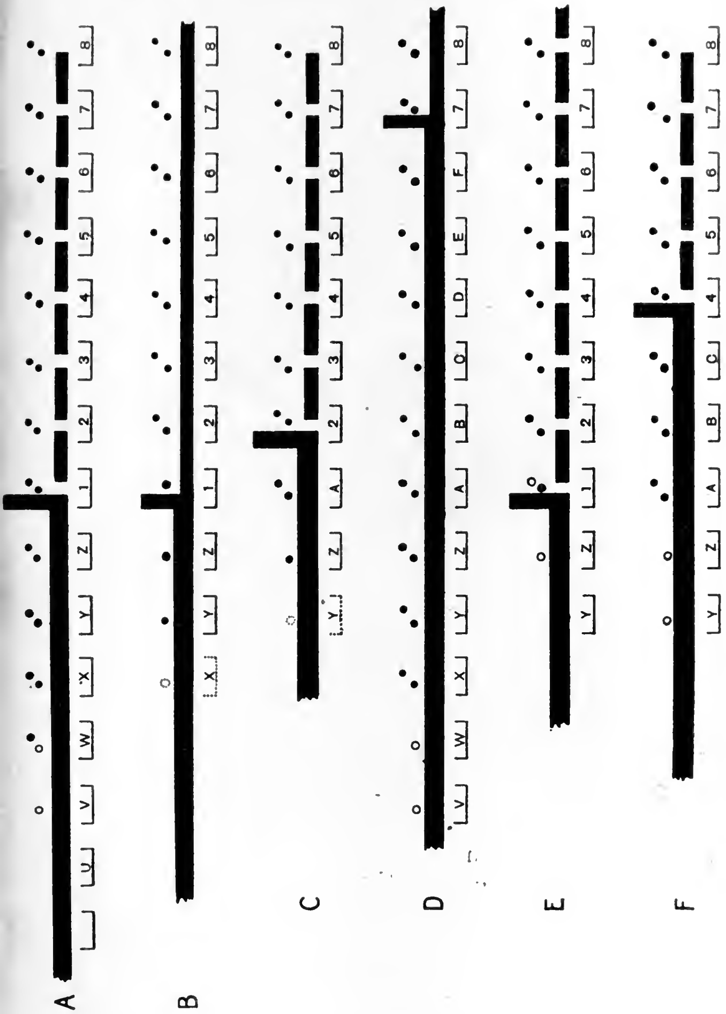


Fig. 222.—Diagram illustrating the relations of the hinder limit of the cranium in an Elasmobranch (A), *Lepidosiren* (B), *Polypterus* (C), *Acipenser* (D), an Amphibian (E), and a Reptile (F), as seen from the left side.

The cranial floor is indicated by the broad horizontal black band: it is demarcated from the vertebral column by the vertical band which represents the occipital limit of the cranium. Dorsal and ventral nerve roots are shown as black dots, when transitory as rings, when occurring only occasionally as dotted rings. The myotomes are indicated by rectangular outlines. The spino-occipital myotomes are lettered according to Fürbringer's system—the anterior batch (occipital) with the concluding letters of the alphabet, the posterior batch (occipito-spinal) with the commencing letters (*A*, *B*, *C*). Trunk myotomes not yet incorporated in the head are designated by numbers.

The next phase is seen in the adults of such relatively primitive groups as the Elasmobranchs, the dipneumonic Lungfishes and the Amphibians, in which an occipital region has been added on to the palaeocranium.

Finally the hinder limit in the other Vertebrates has been shifted still farther back—one segment (*Polypterus*), three segments (Amniota) or as many as five segments (*Acipenser*).

As far forwards as the hinder limit of the palaeocranium there is, as already indicated (p. 317), clear evidence that the cranial wall represents a series of neural arches which have undergone fusion. As indicated on the same page it is difficult to avoid extending this homology to the mesotic portion of cranial wall lying still farther forwards. As regards the prechordal portion of the cranium there is no definite evidence, but if we regard the trabeculae as primitively in continuity with the parachordals we have to grant the possibility of even this part of the cranium being in series with the portions farther back and therefore also originally vertebral in constitution.

In conclusion it must be remembered that the series of myotomes is also continued into the head-region, and the occurrence of typical myotomes as far forwards as the premandibular or oculomotor segment (p. 210) may be taken as strong evidence that the segmentation of the mesoderm originally extended throughout the head-region including its pre-chordal portion.

(7) EMBRYOLOGY AND THE EVOLUTION OF THE VERTEBRATE.—The special charm and the chief importance of the study of embryology reside in the fact that it is one of the main branches of evolutionary science. The greater part of what is ordinarily called evolutionary research deals with the possible methods and causes of evolutionary change. The data of Embryology on the other hand form a branch of synthetic evolutionary science which deals not with possible causes or methods but with the actual facts of evolutionary change, striving to map out the course along which this has proceeded. In compiling the record of evolutionary progress we are dependent upon Comparative Anatomy and Palaeontology as well as Embryology, and in formulating conclusions care has to be taken that whenever possible they are based on the data of all three sciences. In cases where these data are not in agreement care must be taken to bear in mind the main disturbing factors which are liable to invalidate the conclusions in each case. In reasoning from Embryology and Comparative Anatomy the possibility that particular features are modern adaptations to existence say within a uterus or egg-shell or under any other set of conditions different from those of the ancestor has to be borne in mind. In the case of Palaeontology and Comparative Anatomy there exists the same danger of error as besets the protozoologist when he endeavours to construct a continuous life-history out of a number of isolated observations on the dead animal—the error of arranging observations in a series which is not natural, or on the other hand, if the seriation be done correctly, of reversing its direction. In Palaeontology errors of this type are peculiarly apt to arise on account of the extraordinary imperfection of our knowledge. If a series of organisms *a, b, c, d,*

become known from a series of geological deposits *A, B, C, D,* this affords convincing evidence in most cases that the particular organisms lived at the time the particular deposits were laid down: the conclusion may also be fairly justifiable that not only did they exist but that they were abundant at the period in question. The conclusion however which is so apt to be drawn that *a, b, c, d,* actually made their first appearance in the same order as the deposits *A, B, C, D,* is quite unreliable. They may have existed in smaller numbers for immense periods of time before the periods corresponding to *A, B, C, D,* when they were really abundant, and the order of their first appearance may have been *d, c, b, a,* or any other. Such a geological series is in fact in itself of little value as an index to the order of evolution. In Embryology on the other hand where the evolutionary stages occur as part of a continuous process, each dependent upon its predecessor, we appear to be safe in assuming that the record, however incomplete, is at least arranged in proper sequence.

Another principle to be borne in mind, when the attempt is being made to work out the evolutionary history of a particular group, is that conclusions must be based upon broad knowledge of structure as a whole. No implicit reliance must be placed upon evidence relating to one system of organs unless it is corroborated by the evidence of other organs. Failing this precaution the investigator is liable to the pitfall afforded by convergent evolution of organs of similar function. Here again the palaeontologist finds himself much hampered as compared with the embryologist, for as a rule all evidence except that of the skeletal system has passed completely beyond his ken.

EVOLUTIONARY ORIGIN OF THE VERTEBRATA.—In the preceding portions of this book it has been shown that Embryology provides us with a record—in at least its main outlines—of the evolutionary changes which the various organ-systems have undergone within the group Vertebrata. For, amongst others, the reasons stated at the foot of p. 491 the record is less clear regarding the evolutionary history of the complete individual. Even however if we had this record complete for the various types—Fish, Amphibian, Reptile, Bird—we should find ourselves still confronted with the interesting problem of the first origin of the primitive Vertebrate type:—from whence came these lowly original Vertebrates out of which the various existing types of Vertebrate have been evolved?

This problem of the ancestry of the Vertebrata is naturally a fascinating one and it has attracted much attention and been the theme of voluminous writing. Enthusiasts have at different times endeavoured to demonstrate that the Vertebrates are descended from this phylum or from that. It is perhaps best not to take such attempts very seriously. They have served a useful purpose in arousing interest and stimulating research but they have little claim to a place in the permanent literature of Zoology.

We are naturally unable to get any evidence bearing upon the problem from Palaeontology. The most ancient Vertebrates of which fossil remains are known had probably already evolved to a far greater distance from the original type of Vertebrate than that which separates them from the existing Vertebrates of to-day. And the probability is that the earliest Vertebrates went on existing and evolving through long ages before they developed those complex skeletal structures which are alone adapted for preservation as fossils in the geological record. Comparative Anatomy fails us too—for up to the present no existing type of animal has been discovered which can justifiably be interpreted as an unmodified survivor of the original Vertebrates.

It is Embryology alone which yields us examples of Vertebrates in the earliest stages of evolution but the data afforded by that science do not carry us beyond the formulation of a few very broad and general conclusions regarding the prevertebrate phases in the evolutionary history of the phylum.

(1) The fact that Vertebrates, like other Metazoa, commence their existence as a unicellular zygote appears to justify us in postulating a unicellular *i.e.* a Protozoan ancestral stage.

(2) The fact that there occurs in the admittedly more primitive Vertebrates a gastrula stage appears to justify us in postulating a diploblastic or Coelenterate ancestral stage.

(3) The facts which are united together in the Protostoma hypothesis *suggest* that the coelenterate ancestor evolved along lines somewhat similar to those of the modern Sea-anemones with their elongated slit-like protostoma dilated at each end and surrounded by a concentration of the ectodermal nerve-plexus.

(4) The facts that the coelome was probably originally segmented (as indicated by *Amphioxus*), that the excretory organs are in the form of nephridial tubes, that the vascular system consists fundamentally of longitudinal vessels on opposite sides of the alimentary canal connected together by vascular arches, the blood passing tailwards in the vessel on the neural side of the alimentary canal—suggest that there intervened between the coelenterate phase and the vertebrate phase a stage which possessed many features in common with those animals which are grouped together to-day in the phylum Annelida. We may suppose that this annelid-like creature became evolved from an Anemone in which the body had become drawn out, as in the genus *Herpolitha* or one of the brain corals, and which had become actively motile. In the two diverging stems which gave rise to Annelids and to Vertebrates respectively we may take it that a difference existed in the normal position of the body—the former progressing with their neural, the latter with their abneural, surface underneath. It is conceivable that this difference may have been associated with the difference between a creeping mode of life in which the chief sensory impressions were related to the solid substratum and a swimming mode of life in which they rather came from above.

Addendum to Chapter IX.—More than once in the course of this volume reference has been made to the "Theory of Germinal Layers" or the "Germ Layer Theory." This theory, which has played a great part in the development of embryological science in the past and still dominates to a great extent embryological research, had its foundations in observations made by these pioneers of embryological science — Wolff, Pander, von Baer and Remak. Wolff (1768) observed that the alimentary canal in the Bird embryo is developed out of a thin membrane or leaf ("Blatt") and inferred that the other organs go through a similar stage. Pander (1817) gave the name "blastoderm" to the first membrane-like stage of the embryo as a whole, saw how this became differentiated into the three layers—outer, middle and inner—and traced out the development from these of the main organ-systems. Von Baer (1828) carried on and elaborated Pander's work, recognized that the middle layer was double, and that it was secondary to the two primary layers: the outer and the inner. He also extended his observations to forms other than the Fowl and laid the foundations of Comparative Embryology. Remak (1855) finally worked out the germ-layers in terms of the Cell-theory, traced the origin of the coelome to a split in the middle layer, and worked out more precisely the relations of the layers to the definitive organ-systems.

One of the most important steps in the development of the Germ Layer Theory was made by Huxley (1859) who as a result of his researches upon the Medusae recognized the two primary cell-layers in these animals (named by Allman "ectoderm" and "endoderm") and suggested the comparison of them with the two primary layers of the Vertebrate embryo.

Embryology, like Morphology in general, first became a real living science as a result of Darwin's demonstration of the fact of evolution. In the *Origin of Species* (1859) the principle of recapitulation is already admitted. "Embryology rises greatly in interest, when we thus look at the embryo as a picture, more or less obscured, of the common parent-form of each great class of animals." The idea was further elaborated by Fritz Müller (1864).

Kowalevsky (1871, etc.) and other embryologists had demonstrated the wide-spread occurrence among the Invertebrates of an early stage of development more or less cup-shaped in form and consisting only of the two primary cell-layers, and the important advance was made synchronously by Lankester and Haeckel of perceiving in this two-layered stage a repetition of a common ancestral form.

Lankester (1873) recognized amongst the Metazoa two distinct grades of complexity of structure so far as their cell-layers were concerned—the diploblastic grade (represented by the Coelenterate) consisting of the two primary layers, and the triploblastic grade with an interposed middle layer. Further he recognized that each Metazoon—whatever its definitive condition—passes in the course of development through a diploblastic stage which he termed the **planula**. Such a planula stage he regarded as a repetition of a common ancestral stage of evolution.

Haeckel (1872) about the same time as Lankester also developed the idea that the diploblastic stage of ontogeny was to be interpreted as the repetition of an ancestral form: Haeckel called this ancestral form *Gastraea*. The main difference between Haeckel's view and Lankester's was that the former regarded the endoderm as having arisen by a process of invagination—as it actually does arise in ontogeny in the great majority of cases—while Lankester regarded it as having arisen by a process of delamination from the outer layer.

As regards the middle germ-layer ideas remained somewhat vague until Agassiz (1864) showed that in the Starfish the mesoderm arose in the form of an outgrowth of the archenteric wall. The same was found to be the case in various other Invertebrates, and in 1877 Kowalevsky showed how in *Amphioxus* the mesoderm was during an early stage in the form of archenteric pockets. In the same year Lankester developed the generalization that the coelome is to be regarded as uniformly enterocoelic in origin and comparable with the diverticula of the archenteric lining seen in Coelenterata.

The separation of such mesodermal cells as are in their early stages free and amoeboid under the common name mesenchyme was first made by O. and R. Hertwig (1882).

The later developments of the theory of the mesoderm involved in the Protostoma theory have already been alluded to earlier in this volume and the same applies to what the author regards as the chief qualification of the germ-layer theory indicated by modern work, namely that the boundary between two layers where they are continued into one another must be regarded not as a sharply marked line but as a more or less broad debatable zone.

LITERATURE

Agassiz. Contributions to the Natural History of the United States of America, v. Boston, **1864**. [Vol. v printed as vol. v, pt. 1, of Mem. Mus. Comp. Zoology Harvard.]
Baer. Über die Entwickelungsgeschichte der Thiere. Beobachtung und Reflexion, i. Königsberg, **1828**.
Bell. Arch. Entwick. Mechanik, xxiii, **1907**.
Fürbringer. Gegenbaurs Festschrift. Leipzig, **1897**.
Haeckel. Die Kalkschwämme. Berlin, **1872**.
Hertwig, O. Arch. mikr. Anat., xxxix, **1892**.
Hertwig, O. and R. Jenaische Zeitschrift, xv, **1882**.
Jörgensen. R. Hertwigs Festschrift. Jena, **1910**.
Kerr, Graham. Proc. Roy. Phys. Soc. Edin., xviii, **1912**.
Kopsch. Internat. Monatsschr. Anat. u. Phys., xvi, **1899**.
Kowalevsky. Mém. Acad. Sci. St-Pétersbourg, (7), xvi, **1871**.
Kowalevsky. Arch. mikr. Anat., xiii, **1877**.
Lankester. Ann. Mag. Nat. Hist., (4), xi, **1873**.
Lankester. Quart. Journ. Micr. Sci., xvii, **1877**.
Lereboullet. Ann. Sci. Nat., 4, Zool., xx, **1863**.

Müller. Für Darwin. Leipzig, 1864.

Pander. Beiträge zur Entwickelungsgeschichte des Hühnchens im Eye. Würzburg, 1817.

Parker. American Naturalist, xliii, 1909.

Rabl. Morph. Jahrb., xv, 1889.

Rauber. Primitivstreifen und Neurula der Wirbelthiere. Leipzig, 1877.

Remak. Untersuchungen über die Entwickelung der Wirbelthiere. Berlin, 1855.

Sedgwick. Quart. Journ. Micr. Sci., xxiv, 1884.

Sedgwick. Quart. Journ. Micr. Sci., xxxvii and xxxviii, 1895 and 1896.

Wolff. Nov. Comment. Acad. Sci. St-Pétersbourg, xii, 1768.

CHAPTER X

THE PRACTICAL STUDY OF THE EMBRYOLOGY OF THE COMMON FOWL

For gaining practical experience in the study of embryology there is no type of material so convenient as that of the early stages in the development of the Common Fowl. Freshly laid eggs can be obtained practically anywhere and to obtain the various stages of development all that is necessary[1] is to keep the eggs at a suitable temperature (about 38° C.) either under a sitting hen, or in one of the incubators which can be purchased, or even in a simple water-jacketed oven such as can be made by any tinsmith. If an incubator be purchased it will be provided with a proper heat regulator for use with electricity, gas or oil, while with the most primitive water-bath it is possible to arrange a lamp so as to give a temperature sufficiently constant as to carry the eggs through at least the first few days of incubation—the most important period for purposes of study. Bird embryos—apart from their use in learning practical embryology—provide admirable material for giving practice in the ordinary methods of section-cutting which are in such constant use in Zoology, Anatomy, Physiology, and Pathology. This chapter will then be devoted to giving an account of the development of the Fowl with directions as to the technique involved in its practical study.

In the description which follows the developmental phenomena will be described in their natural sequence but on account of the practical difficulties involved in the extraction and preservation of blastoderms of the first day of incubation it will be found best, in actual laboratory work, after studying the new-laid egg and its envelopes, to proceed to the stage of about 42 hours' incubation and gain some practice in the manipulation of it before attempting the earlier stages. In the following technical instructions the sequence is followed which has been found to be in practice most convenient for beginners.

TECHNICAL DIRECTIONS[2]

I. New-Laid Egg.—Fill a glass vessel about 4½ inches in diameter and 2 inches in depth with normal salt solution [water

---

[1] Provided the eggs have been fertilized.

[2] The reader is assumed to have an elementary knowledge of the ordinary methods of cutting sections. See, however, the Appendix.

100 c.c., common salt ·75 gramme] heated to a temperature of about 40° C. Submerge the egg upon its side in the salt solution and remove the side of the shell which is uppermost by cutting with a pair of strong scissors and then lifting off the isolated piece of shell with blunt forceps. Take care to keep the point of the scissors or forceps close to the inner surface of the shell so as to avoid risk of injury to the true egg or "yolk."

II. EGG AFTER 42 HOURS' INCUBATION.—Open the egg as before. On removing the piece of shell the blastoderm will be seen as a circular whitish area on the upper side of the yolk. Excise the blastoderm by making a series of rapid cuts with the large scissors through the vitelline membrane a short distance external to the boundary of the blastoderm. Should the yolk happen to be tilted round so that the blastoderm is not uppermost but rather at one side make the first cut *below* the blastoderm so that the elasticity of the vitelline membrane will tend to pull it upwards when the cut is made. Otherwise the blastoderm may be lost by its being pulled downwards.

Having isolated the circle of vitelline membrane, with its adherent blastoderm, slide it off the yolk by pulling gently on one side with the forceps. Remove the remains of the egg from the dish so as to keep the salt solution clean. Take hold of the circle of vitelline membrane at one edge with the forceps and wave it backwards and forwards beneath the surface of the salt solution. The blastoderm will gradually become detached. Should it not do so at once the separation should be started by freeing it from the vitelline membrane with a scalpel at one edge. Notice the difference in appearance between the vitelline membrane and the blastoderm which has been detached from it. If the blastoderm is yellow from adherent yolk this should be washed off either by waving the blastoderm backwards and forwards in the salt solution or by gently directing jets of salt solution on the yolky surface of the submerged blastoderm by a wide-mouthed pipette.

The blastoderm should now be brought near the surface of the salt solution and a watch-glass slipped under it by which it may be lifted from the larger vessel. The blastoderm is so delicate that it must be kept submerged in the fluid: no attempt must be made to lift it above the surface by forceps.

A microscope coverslip slightly larger than the blastoderm should now be submerged in the watch-glass and the blastoderm floated over it *dorsal side above.* The dorsal or upper side of the blastoderm can easily be identified from the fact that the edges of the blastoderm tend to curl *upwards.* Having floated the blastoderm over the coverslip the latter should be gently raised to the surface of the fluid with a pair of large forceps. Take care to keep the coverslip absolutely horizontal and lift it out of the fluid very carefully so that the blastoderm is stranded on its upper surface, the lower surface of the blastoderm being in contact with the coverslip. The superfluous salt solution should be drawn away with blotting-paper so as to bring the blastoderm into close contact with the glass; take great care that the blotting-paper does not actually touch the blastoderm as in that event it will be apt to stick to it. Now take the coverslip between the finger and thumb and with the aid of a pipette place a very small drop of corrosive sublimate solution (or other fixing fluid) upon the centre of the blastoderm. This will cause the blastoderm to adhere to the coverslip. Now invert the coverslip and drop it on to the surface of some fixing fluid in a watch-glass.

The blastoderm is then passed through the various operations of staining, dehydrating and clearing, preparatory to mounting whole or conversion into a series of sections as the case may be. The advantage of having the blastoderm adherent to a coverslip is that it makes it easier to handle and above all it keeps it from becoming wrinkled or folded. The blastoderm if fixed in corrosive sublimate can usually easily be detached from the coverslip at the stage of clearing if it has not already become free at some preceding stage. Should it adhere obstinately it should be placed in acidulated alcohol for an hour or more.

The examination of the blastoderm should be carried out as follows:

1. Study the blastoderm and embryo as a whole under a, preferably binocular, dissecting microscope while it is submerged in the fixing fluid. As the fixing fluid penetrates the embryo the various details in its structure come into view. Continue the examination of the surface relief in the alcohol which is used for getting rid of the excess of the fixing agent. After examining from the dorsal side invert the blastoderm and examine from below.

2. Repeat the examination of the embryo as a whole as a transparent object after staining and clearing. If the individual embryo is to be cut into sections a careful drawing should be made at this stage, the outline being preferably drawn by means of the camera lucida.

3. Study serial sections cut transversely to the axis of the embryonic body.

[Sagittal and horizontal sections will also be useful for study after the transverse ones.]

III. EARLY SECOND-DAY BLASTODERM.—The same method is used as for the 42-hour stage but special care must be taken on account of the more fragile character of the blastoderm. In all probability the blastoderm will remain adherent to the vitelline membrane in spite of repeated shaking and the process of detachment will have to be started by gently easing up the edge of the blastoderm on the side next the forceps in which the edge of the circle of vitelline membrane is held.

To get rid of adherent yolk the circle of vitelline membrane should be laid on the bottom of the dish of salt solution, blastoderm uppermost. A pipette with a wide mouth should be held vertically a few millimetres above the blastoderm and the india-rubber bulb squeezed rhythmically so as to wash away the particles of yolk by *very gentle* currents of salt solution. When the blastoderm is lifted out of the solution stranded upon the coverslip it is very apt to become folded. When this happens, on account of the fragility of the blastoderm, no attempt should be made to stretch it out by the use of needles or forceps. The folds should rather be straightened out by a current of salt solution allowed to flow out from the orifice of a pipette held vertically just over the centre of the blastoderm.

IV. Early Blastoderms.—Open the egg as before. Let the albumen run off until the vitelline membrane over the blastoderm is exposed. Raise the egg until the blastoderm touches the surface of the salt solution and then bring a wide-mouthed pipette of Flemming's solution, held vertically, into such a position that its tip just touches the film of fluid over the blastoderm. Let the solution flow down slowly on to the vitelline membrane covering the blastoderm. If there is any albumen overlying the blastoderm this should be carefully stripped off as it coagulates. A small piece of black bristle should be stuck into the vitelline membrane on each side to mark the line joining the chalazae so as to facilitate the orientation of the blastoderm for section-cutting. The fixing fluid should be allowed to act for several minutes and then a circle of vitelline membrane may be excised with the blastoderm adhering to it. Float out the circle of vitelline membrane on a coverslip with the blastoderm above and submerge in a watch-glass of fixing fluid. If the circle of blastoderm adheres to the coverslip so much the better: it may be separated in the clearing agent.

Instead of a pipette as above indicated being used for the fixing fluid a small rim of cardboard, *e.g.* the rim of a small pill-box lid, may be placed on the surface of the yolk, raised up slightly out of the salt solution, so as to enclose the blastoderm and then the little tank so formed may be filled with Flemming's solution which will gradually diffuse downwards. Minchin recommends a triangular instead of a circular rim for facilitating subsequent orientation.

For fine work it is preferable to embed the whole yolk in celloidin and then after the celloidin has been hardened to cut out the portion in the region of the upper pole for sectioning. This method consumes however much more time than does the paraffin method.

V. Third-Day Egg.—A. Open the egg as before.

B. Study the embryo and blastoderm while still alive and in situ. A large outline drawing should be made. The details of the body of the embryo will be seen better later but the arrangement of the blood-vessels can best be studied now while the circulation is still active. As a rule they can be seen distinctly through the vitelline membrane but if not the latter should be carefully stripped off. A Greenough binocular microscope with its lowest power objectives is a useful accessory for examining the blood-vessels.

C. Excise the embryo with the surrounding portion of blastoderm, float it on a slide, cover with coverslip supported by wax feet at the corners and examine as a transparent object, comparing the various features with those shown in Figs. 235 and 236.

D. Excise a second embryo with its surrounding blastoderm. Float it on to a coverslip, embryo above, and submerge it in a watch-glass of fixing fluid. Watch it carefully under the lens or preferably low-power binocular as the tissues gradually become opaque. The amnion will be seen particularly clearly during this process. A drawing should be made of the embryo enclosed in its amnion as an opaque object.

E. Carefully strip off the amnion with a pair of needles[1] and study the configuration of the head end of the embryo.

F. Stain and mount the embryo.

G. Prepare series of sections (*a*) transverse to trunk region, (*b*) horizontal through trunk region and therefore approximately sagittal in the region of the head which is lying over on its left side.

VI. THE FOURTH DAY.—On placing the egg in the salt solution the broad end will tilt up more decidedly than before owing to the increase in size of the air space. Care should therefore be taken to make the first perforation of the shell close to the broad end so as to allow the air to escape. Care must also be taken not to injure the vascular area as the whole blastoderm is now much closer to the shell than it was in earlier stages. As soon as the egg has been opened a careful drawing should be made while the embryo is still alive and in situ. The main features of the vascular system in particular should be carefully worked out at this stage. If the circulation becomes sluggish through cooling a little warm salt solution should be added but care must be taken not to bring about a great and sudden rise of temperature as in this case the greatly accelerated heart-beat is apt to cause rupture of a vessel.

The body of the embryo, allantois, etc., are covered over by the thin transparent serous membrane or false amnion as becomes apparent if the attempt is made to push a blunt needle down into the space round the allantois. This membrane should either be cut through with a pair of fine scissors, just outside the boundary of the allantois, or carefully stripped off with fine forceps. When this has been done it is possible to shift the body of the embryo into such a position that it with its blood-vessels can be observed in side view. Until this has been done it is impossible to get a proper view of the body of a well-developed embryo of this age owing to its dipping down out of sight into the yolk-sac.

The embryo should now be excised by cutting round outside the boundary of the vascular area and floated into a watch-glass of clean warm salt solution. The embryo may now be studied as a transparent object on the stage of the dissecting microscope. It is better

---

[1] Bearing in mind that steel needles must not be allowed to touch corrosive sublimate solutions. Picric acid solutions are convenient fixing agents to use for D and E.

however in the first attempt to proceed at once to fix the embryo. An essential preliminary is to remove the true amnion which closely ensheaths the body of the embryo. In doing this it is best to commence at the region between the heart and the tip of the head where a couple of fine needles may be used to tear the amnion. Its anterior portion may then be seized with fine forceps and pulled backwards over the embryo's head. The operation is simplified by carrying it out immediately after submerging the embryo in fixing fluid as the action of the fluid makes the amnion slightly opaque and therefore more easily visible. If however corrosive sublimate be the fixing fluid fine splinters of coverslip should be used for dissecting off the amnion unless this is done prior to immersing in the fixing fluid. The embryo should again be carefully studied during the process of fixation, many details becoming particularly distinct before the creature becomes completely opaque. Finally the embryo should be studied, preferably with the binocular, as an opaque object, and then prepared either for section cutting or for mounting whole.

VII. Six Days.—Open freely into the air-space. Carefully tear away part of its inner wall so as to expose part of the vascular area, great care being taken not to injure the latter. Notice the direction in which the vessels of the vascular area converge: this will indicate the direction in which the embryo is to be found. Work towards the embryo, picking off the shell piece by piece, using *blunt* forceps. Frequently the escape of the air from the air-space allows the vascular area to sink down and leave a wide space between it and the shell membrane. In other cases however it remains in close contact with the shell membrane and in this event the greatest care must be taken not to injure the vascular area as by doing so the very fluid yolk is allowed to escape and the salt solution rendered so opaque that observation of the embryo in situ is made almost impossible.

Notice that the allantois has increased much in size, that it has become richly vascular and that it is spreading outwards in a mushroom-like manner underneath the serous membrane. It has already spread so far as to cover the embryo nearly completely.

It is best now to remove the shell entirely and to examine its contents as they lie submerged in the warm salt solution (as shown in Fig. 242).

With fine sharp scissors cut through the serous membrane just outside the limit of the allantois, commencing on the dorsal side of the embryo where the allantois is not yet closely applied to the yolk-sac. It is easy to do this owing to the coelomic cavity having spread outwards well beyond the limits of the allantois. The allantois being now no longer flattened out, by its continuity with the serous membrane all round, its vesicular character becomes apparent, as well as the difference in character of the vascular network on its proximal and distal walls. The relations of the vascular allantoic stalk to the vascular yolk-stalk should be noted: also the fact that the amnion is now widely separated from the embryonic body by secreted amniotic fluid. If the embryo is a well-advanced one towards the end of the sixth day the amnion, which is now muscular, may exhibit periods of muscular contraction during which the embryo is rocked to and fro in the amniotic fluid. These movements must be distinguished from the occasional contractions of the muscles of the embryonic body which also occur about this time though they are much less conspicuous.

After a careful study of the living embryo with the allantois and yolk-sac hanging from its ventral side it may be excised along with a circle of vascular area, floated into a watch-glass and examined alive with a lens or binocular, and then treated with fixing fluid such as Bouin's solution. The latter brings out the surface modelling which should be carefully studied especially in the region of the gill clefts.

Dissect off the amnion, add more fixing fluid and after superficial fixation renew the Bouin's solution. It is a good plan to suspend the embryo by the yolk-sac so that the weight of the head causes the neck to become somewhat straightened. After the embryo is sufficiently fixed the neck may be cut through and the lower surface of the head studied for the relations of the olfactory rudiments and mouth.

Sagittal sections through the head are particularly instructive at this stage.

VIII. SEGMENTATION.—To obtain segmentation stages hens which are regular layers should be chosen. In such cases the egg is laid at a slightly later time on consecutive days. As a rule egg-laying is confined to the forenoon and early afternoon and when an egg is due after the end of this period it is retained within the oviduct and not laid until next day. The retention of an egg in this way inhibits the process of the ovulation so that a new egg is not shed from the ovary until the preceding one has been laid.

HISTORY OF THE EGG UP TO THE TIME OF LAYING.—The egg arises as a single cell of the left ovary[1] which grows to a relatively enormous size as yolk is deposited in its cytoplasm. The yolk is of a characteristic yellow colour but in particular tracts the disintegration of its granules into finer particles gives it a white colour. Of this white yolk a mass occupying the centre of the egg is continuous through a narrow isthmus with a tract lying immediately beneath the germinal disc ("Nucleus of Pander") and this latter is prolonged as a thin superficial layer over the surface of the egg. Between the superficial layer and the central mass are a number of thin concentric layers of white yolk.

---

[1] The right ovary and oviduct which are present in early stages undergo atrophy, never becoming functional. This is probably to be regarded as an adaptive arrangement which has been developed in Vertebrates with large eggs to avoid the dangers which would be involved in the synchronous passage of a pair of eggs of great size, more especially if contained in a rigid shell, into the narrow terminal portion of the passage to the exterior.

As the egg increases in size it bulges out beyond the surface of the ovary, becoming eventually dependent from the ovary by a thin stalk at the end of which it is enclosed within the distended follicle. The wall of this is richly vascular except on the side away from the stalk where an elongated patch—the "stigma"—marks the position in which the follicle-wall will rupture to set the egg free.

When this process (**ovulation**) is about to take place the thin membranous lips of the oviducal funnel become active, apply themselves to the follicle containing the ripe egg and grip it tightly. The follicle then ruptures and the egg is as it were swallowed by the oviducal funnel. Within the funnel fertilization takes place provided that spermatozoa are present.[1]

The egg proceeds now to travel slowly down the oviduct, propelled onwards by the peristaltic contraction of the oviducal wall, the entire passage occupying about 22 hours. As it does so the albumen is deposited on its surface by the secretory activity of the oviducal epithelium. The first to be deposited is rather denser than that formed subsequently. It forms a sheath immediately outside the vitelline membrane and extending in tapering spindle-like fashion for some distance up and down the oviducal cavity: the two prolongations are the **chalazae** (Fig. 223, *ch*).

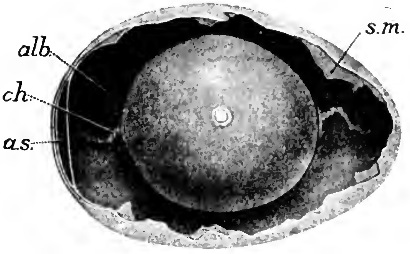


FIG. 223.—Unincubated egg of the Fowl.

a.s, air-space; *alb*, albumen; *ch*, chalaza; *s.m*, shell membrane. In the centre—at the apical pole—is seen the germinal disc with the white "Nucleus of Pander" showing through it.

The envelope of dense albumen enclosing the egg is not merely propelled onwards; it also undergoes a clockwise rotation about the axis along which it is travelling, caused probably by the cilia present on the oviducal epithelium. Owing to the prolongations of the albumen in front and in rear of the egg not undergoing this rotation the chalazae become twisted upon themselves in opposite directions.

Layer after layer of albumen (Fig. 223, *alb*) is deposited round the egg and chalazae until the full size is reached. The character of the secretion then changes and the shell membrane (Fig. 223, *s.m*) is formed. Finally in the dilated hinder part of the oviduct ("uterus") the secretion is in the form of a thick white fluid which, deposited on the surface of the shell membrane, gradually takes the form of the hard and rigid shell perpetuating the characteristically "oval" form impressed upon the egg envelopes during the passage down the oviduct. In composition the egg-shell consists of calcium salts infiltrating a slight organic basis of keratin-like material. Structur-

---

[1] The spermatozoa remain alive and active within the oviduct for a period of about three weeks.

ally the greater part of its thickness consists of calcareous trabeculae forming a fine sponge work. The inner surface of the shell is rough, projecting into minute conical papillae, while the outer surface is covered by a smooth apparently structureless layer perforated by numerous fine pores.

SEGMENTATION.—If the egg has been fertilized it proceeds with its development as it slowly travels down the oviduct. The process of segmentation is accomplished during this period and consequently the obtaining segmentation stages involves the sacrifice of the parent hens. Owing to the difficulties in the way of obtaining a complete series our knowledge remained for long fragmentary but recently (1910) a number of stages have been described and figured by Patterson which give a fairly complete picture of the process (Fig. 224). From these data we may take it that the early phases of segmentation are based on the normal plan where a meridional furrow appears traversing, or passing close to, the centre of the germinal disc *i.e.* the apical pole of the egg, and is followed by a second meridional furrow perpendicular to the first. In the third phase there is occasionally a regular set of four vertical furrows but more usually the process now becomes irregular (Fig. 224, C). In the next phase also there may be a fairly regular development of latitudinal furrows demarcating a group of about eight cells round the apical pole but typically there is no such regularity.

The initial furrows, which make their appearance as above indicated, gradually extend. They eat their way downwards into the thickness of the germinal disc, never however cutting completely through it. They also extend outwards towards the edge of the disc which however again they never quite reach. The apparent segments into which the germinal disc is mapped out by the early furrows are therefore not really isolated from one another —there being still continuity between the segments on the one hand peripherally and on the other on the lower side of the disc next the yolk.

Complete blastomeres are first marked off when, about the time the latitudinal furrows appear, division planes make their appearance parallel to the surface, cutting off the small segments in the centre from the underlying deep layer of the germinal disc.

The later stages of segmentation are quite irregular. Division planes make their appearance in all directions by which the germinal disc becomes completely divided up into small segments except on its lower surface and round its edge where there remains a syncytial mass in which the nuclei divide without their division being followed by any protoplasmic segmentation. It is to be noted that the process of segmentation throughout goes on more actively towards the centre of the disc, more slowly towards its margin, so that the blastoderm comes to be composed of smaller cells towards the centre and larger towards the periphery.

The result of the segmentation process is that the original

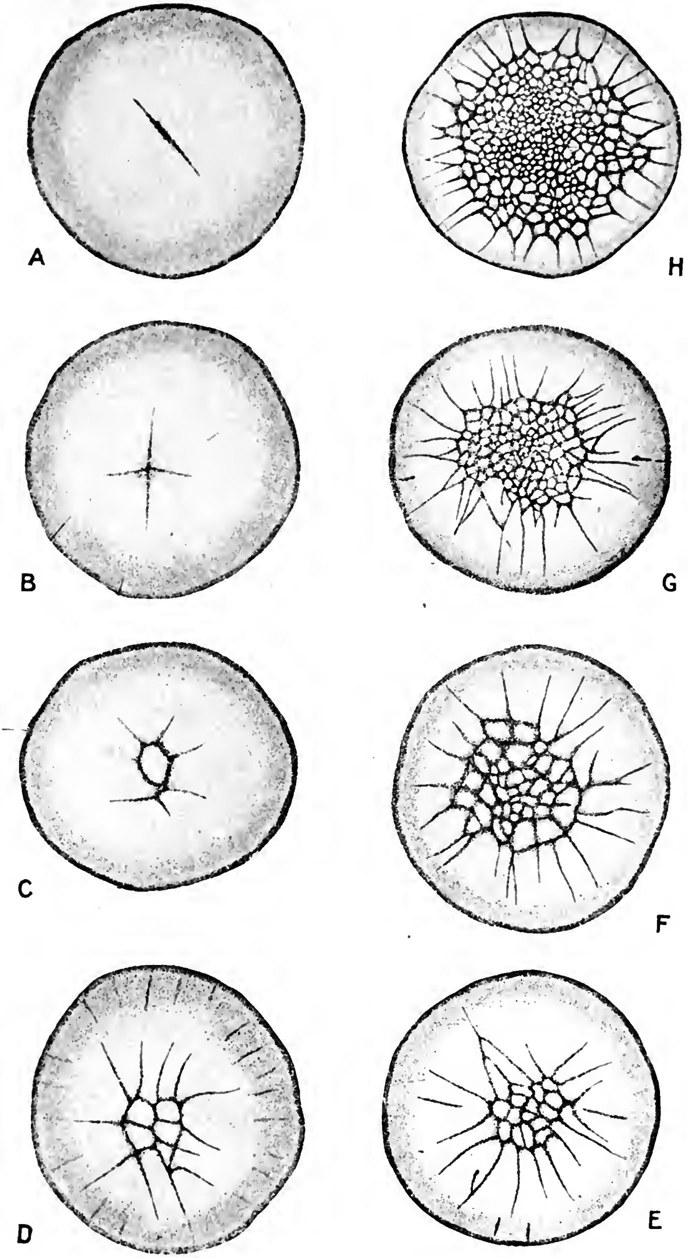


FIG. 224.—Views of the blastoderm of the Fowl's egg during segmentation. (After Patterson, 1910.)

A, 3 hours after fertilization ; B, 3¼ hrs. ; C, 4 hrs. ; D, 4-5 hrs. ; E, about 5 hrs. ; F, 5½ hrs. ; G, 7 hrs. ; H, 8 hrs.

germinal disc comes to be represented by a lenticular blastoderm lying at the apical pole of the egg and corresponding to the mass of micromeres of such a holoblastic egg as that of *Lepidosiren*. The superficial layer of cells become fitted closely together and form a definite epithelium—which is destined to become the ectoderm. The cells of the lower layers on the other hand are rounded with chinks between them representing the segmentation cavity. The lowest of all have the appearance of being incompletely cut off from what is ordinarily termed the white yolk lying below them but which is really a syncytial layer full of fine granules of yolk and with scattered nuclei.

Apparently a few accessory sperm nuclei are usually present in the fertilized eggs and faint traces of abortive segmentation may be visible round them (*cf.* Elasmobranch, Fig. 8, B*, p. 14).

At the time of laying the blastoderm forms a small whitish disc covering the apical pole of the egg. Sections show it to consist of an upper layer of ectoderm and of a lower layer consisting of numerous rounded micromeres lying about in the fluid of the segmentation cavity. These micromeres become larger towards the lower face of the blastoderm and they are more crowded together round the periphery.

It must not be supposed that all newly-laid eggs show exactly the same degree of development. As a matter of fact great variation occurs, one of the chief variable factors probably being the length of time occupied in the passage down the oviduct. Where this time is longer, as *e.g.* towards the end of the laying season, the stage of development of the egg when laid is more advanced.

THE FIRST DAY OF INCUBATION.—After the egg has been laid the lowering of the temperature leads to such a slowing of its vital processes that development appears to come to a standstill. If kept at a low temperature it retains its vitality for a considerable period but makes no appreciable advance in development. If the temperature be raised by incubation the developmental processes are at once accelerated and comparatively rapid changes come about. The blastoderm increases in size, its margin spreading outwards, and at the same time there comes about a distinct difference in appearance between its central and marginal parts—the central portion assuming a dark transparent appearance (**pellucid area**) which contrasts strongly with the whiter "**opaque area**" surrounding it. The examination of sections at once explains this difference in appearance: the more opaque appearance peripherally is seen to be due to the lower layer cells being there closely crowded together.

An important change soon comes over the lower layer cells, in as much as those next to the yolk, in the region underlying the pellucid area, lose their rounded shape, become somewhat flattened and adhere together edge to edge to form a continuous membrane—the (secondary) endoderm. This appears first beneath the posterior portion of the pellucid area; it gradually extends

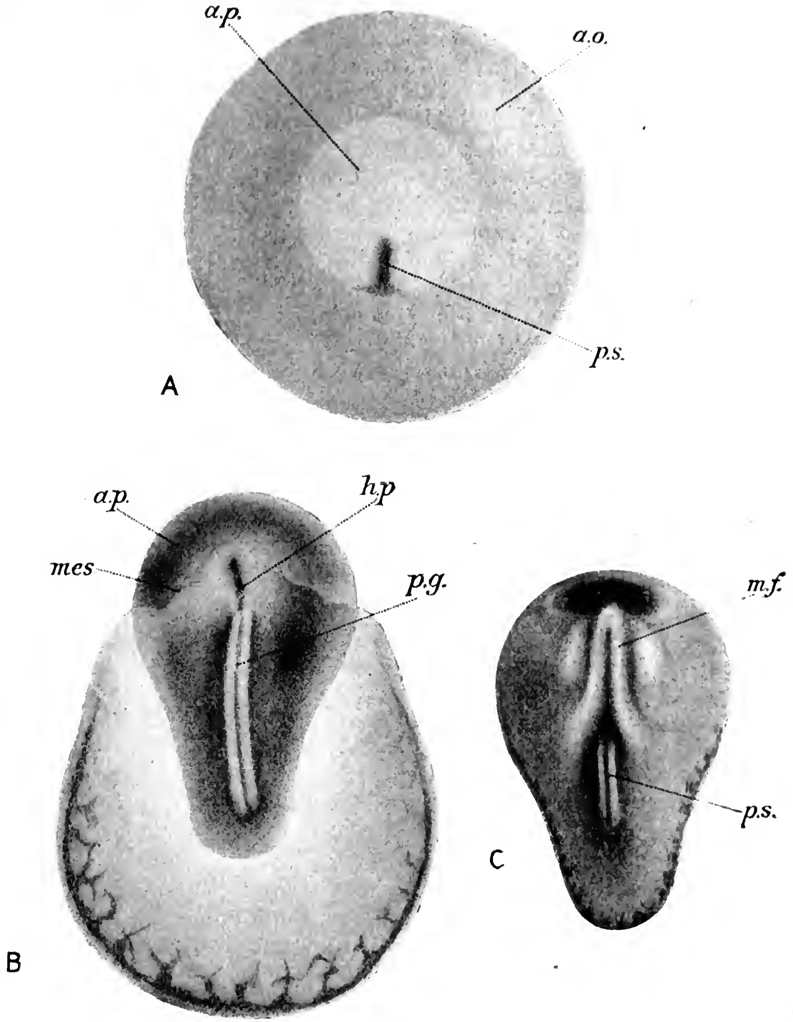


FIG. 225.—Illustrating three stages of the blastoderm of the Fowl during the second half of the first day of incubation.

a.o, opaque area ; *a.p*, pellucid area ; *h.p*, head process ; *mes*, boundary of sheet of mesoderm ; *m.f*, medullary fold ; *p.g*, primitive groove ; *p.s*, primitive streak.

forwards and outwards, and eventually is continuous all round with the thickened marginal part of the blastoderm.[1]

---

[1] This thickening of the posterior edge of the blastoderm presents in sagittal section a striking resemblance to a gastrular lip growing back over the yolk and Patterson (1907) believes that an actual process of involution — a reminiscence of gastrulation by invagination — takes place. It must not be forgotten that any explanation of such obscure developmental phenomena in Birds must, to be reliable,

A gradual change takes place in the shape of the pellucid area which, up till now circular, assumes an oval or pear shape (Fig. 225, B)—the long axis perpendicular to the long axis of the egg-shell, and the narrow end being next the observer when the broad end of the egg-shell is to the left. This narrow end may be called posterior from its relations to the rudiment of the embryo which appears later. Together with the gradual change in the shape of the pellucid area there takes place the development of the primitive streak. This makes its appearance usually during the first half of the first day of incubation, as a linear opacity stretching forwards along the long axis of the pellucid area in its posterior third. As the first day of incubation goes on the primitive streak becomes more and more distinct. A longitudinal groove develops along its middle—the primitive groove—while on each side of this it forms a ridge, the primitive fold.

If a number of eggs be examined during the first day of incubation it will be seen that the primitive streak, as is commonly the case with vestigial organs, shows extreme variability. More especially its hinder end is commonly bent to one side or the other, or even bifurcates into two branches. At its front end one or both halves of the primitive streak swell up into a slight knob while the primitive groove becomes somewhat deeper and wider.

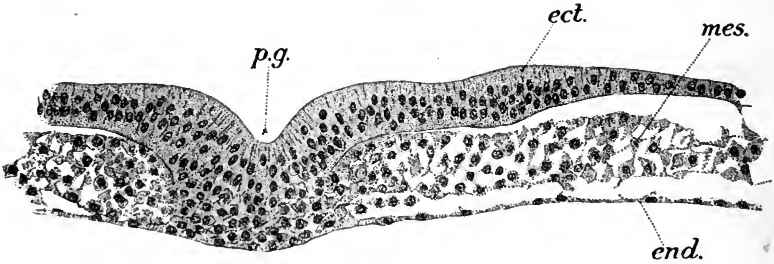


FIG. 226.—Transverse section through primitive streak of the Fowl.

ect, ectoderm ; *end*, endoderm ; *mes*, mesoderm ; *p.g*, primitive groove.

The primitive streak is shown by transverse section to originate from a linear tract of ectoderm along which the cells are undergoing rapid proliferation, as is indicated by the relatively numerous mitotic nuclei. The cells budded off by the ectoderm are aggregated together in a compact mass along the course of the primitive streak while on each side they become loosened out and wander away into the space between ectoderm and endoderm to take part in forming the sheet of mesoderm.

---

rest on a firm basis of knowledge of Reptilian development: At the present time however our knowledge of the exact relationship of these developmental stages of Birds to the corresponding stages of Reptiles is not in the present writer's opinion adequate to form a trustworthy basis for their interpretation.

For a short distance in the region of its front end the mass of cells forming the primitive streak is continuous not only with the ectoderm but with the endoderm as well: the primitive streak of this region may be defined as a tract along which there is cellular continuity between the ectoderm and the endoderm.

During the latter half of the first day what is known as the "Head process" makes its appearance (Fig. 225, B, *h.p*). In a view of the whole blastoderm this has the appearance of being a somewhat less distinct prolongation forwards of the primitive streak—in front of the knob which marks its apparent front end. The study of transverse sections shows that the so-called head process is exactly similar in structure to the primitive streak immediately behind it, except that it is separated from the overlying ectoderm by a distinct split and that there are no primitive folds or primitive groove over it. On its lower side there is perfect continuity with the endoderm—as is the case with the anterior part of the obvious primitive streak into which it is continued.

During the same period of incubation there appears the first sign of the surface relief of the body of the embryo in the form of what is known as the **head fold** (Fig. 227, A, *h.f*). This is formed by the blastoderm bulging upwards and forwards, forming a projection bounded in front by a steep face crescentic in shape as seen from above, the two horns of the crescent directed backwards. The projection increases in prominence: its front edge soon comes to overhang, the blastoderm becoming tucked underneath it both in front and at the sides, the two horns of the crescent which the fold formed at its first appearance gradually extending farther and farther backwards. The projection is destined to give rise to the head end of the embryo and there are certain important details to be noticed about its structure which can be made out best by the study of sagittal sections.

The region of the blastoderm where the head fold develops is composed of the two primary layers, ectoderm and endoderm, the mesoderm not yet having spread into it. It follows that the head rudiment has a double wall, its outer sheath of ectoderm enclosing an inner wall, quite similar in shape, composed of endoderm. It will be understood that this inner wall of endoderm is continued at its hind end into the flattened layer of endoderm which lies on the surface of the yolk. In other words the endoderm within the head rudiment may be described as forming a very short wide tube, blind anteriorly but opening behind into the yolk. This endodermal tube is the rudiment of the front part of the endodermal lining of the alimentary canal of the adult and is termed the foregut.

Soon after the commencement of the formation of the head fold the ectoderm of the medullary plate becomes raised up into a longitudinal ridge (Fig. 227, A, *m.f*) upon each side of the median line. Between the two ridges is a groove—the medullary groove: the ridges themselves are the medullary folds: the two medullary folds

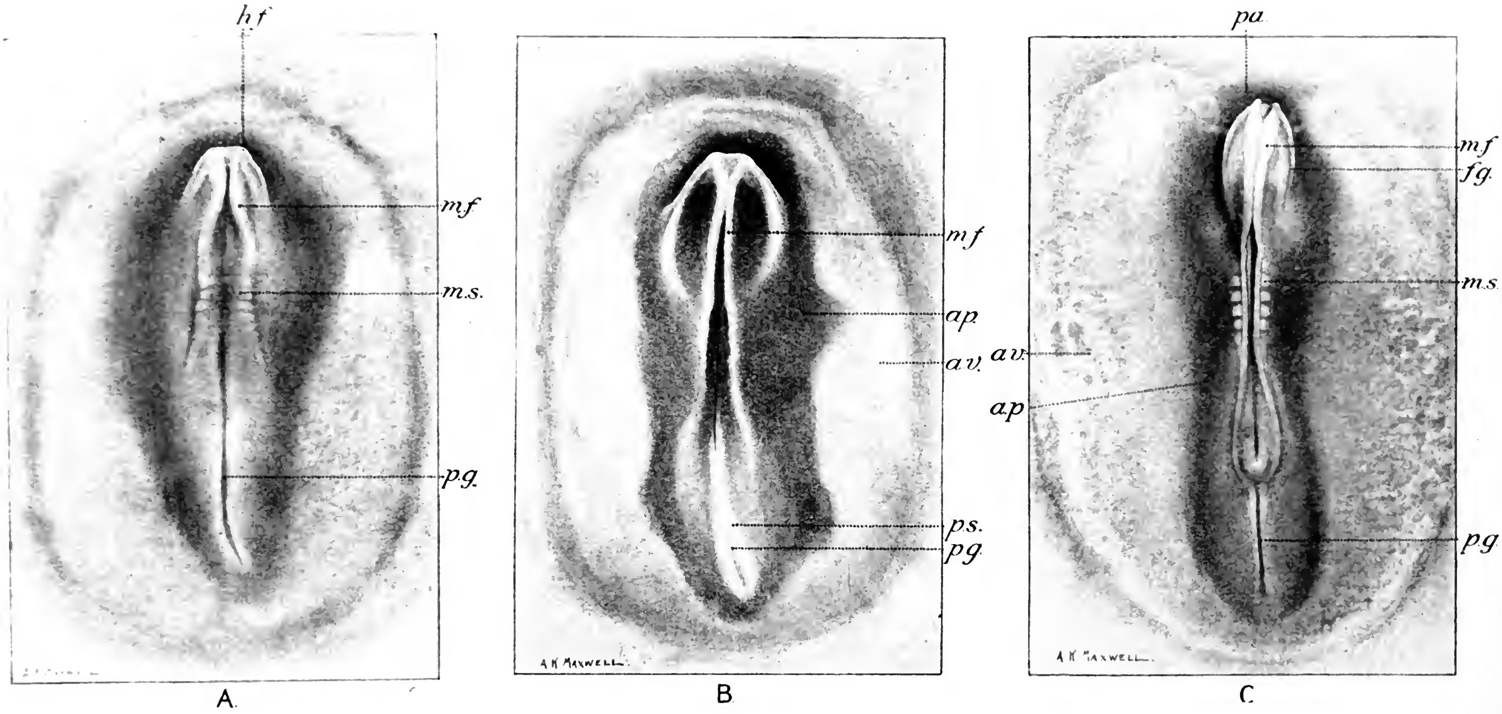


FIG. 227.—Fowl embryos at about the end of the first day of incubation seen by reflected light.

A, 3 mesoderm segments; B, no segments yet demarcated; C, 5-6 segments. *a.p*, pellucid area; *a.v*, vascular area; *f.g*, foregut; *h.f*, head; *m.f*, medullary fold; *m.s*, mesoderm segments; *p.a*, proamnion; *p.g*, primitive groove; *p.s*, primitive streak.

are continuous anteriorly. The two medullary folds gradually extend backwards and at the same time they become more prominent and arch over towards one another until at about the end of the first day they meet. It is to be noticed (Fig. 227, B) that the first meeting of the medullary folds is some little distance back from their anterior end, in about the position in which the division between mesencephalon and rhombencephalon will develop later. Towards their anterior end the folds remain less prominent than they are farther back with the result that the meeting of the two folds is here considerably delayed.

During these later hours of the first day important advances are taking place in the development of the mesoderm. In the first place it is to be noted that the anterior limit of this layer is gradually extending forwards, encroaching more and more upon the proamnion —the part of the blastoderm in front of the head fold which is still two layered. In the second place the mesoderm becomes considerably thickened and more compact in the region near the median line—adjacent to the head process or notochord. This thickened portion of the mesoderm becomes divided by transverse splits into a series of blocks—the mesoderm segments—lying one behind the other (Fig. 227, A and C, *m.s*). The first pair of splits to make their appearance are placed obliquely, sloping outwards and backwards: they mark the hind boundary of the first or most anterior segment. A little later a pair of similar splits develop a little farther back forming the hinder limit of the second segment, and so on, segment after segment becoming separated off from the still continuous mesoderm lying farther back.

While this portion of the mesoderm is becoming segmented it is at the same time becoming sharply marked off by its greater thickness from the lateral mesoderm lying farther out from the axis. Towards the end of the first day a further important development takes place in the mesoderm in as much as isolated splits appear in it parallel to its surface and these gradually spread and finally become continuous so as to divide the mesoderm into the outer somatic layer next the ectoderm and the inner splanchnic next the endoderm. The cavity which has made its appearance between somatic and splanchnic layers of mesoderm is the coelome. The portion lying within the myotome, which soon becomes filled up by immigrant cells derived from its wall, is the myocoele (Fig. 228, *mc*). The portion lying farther out, in the lateral mesoderm, is the splanchnocoele (*splc*). The two layers lying external to this cavity—the somatic mesoderm and the ectoderm —constitute the somatopleure or body-wall: the corresponding layers lying internal to the cavity—the splanchnic mesoderm and the endoderm—constitute the splanchnopleure or gut-wall.

While the changes above described have been taking place the blastoderm has constantly been increasing in area and by the end of the first day it forms a cap covering an extent of about 90° at the upper pole of the egg. In the opaque area—the part of the blastoderm lying outside the boundary of the pellucid area—there are present the same layers of cells as in the pellucid area—the ectoderm, which extends farthest peripherally, the endoderm which passes into a thick yolk syncytial layer peripherally (germinal wall), and the mesoderm the outer part of which is still unpenetrated by the coelomic split. The part of the opaque area where mesoderm is present assumes a very characteristic mottled appearance (Fig. 227, C, *a.v*) caused by the rudiments of blood-vessels and blood: hence the name vascular area which is given to this part of the blastoderm. When the embryo has reached the stage with about seven mesoderm segments the secretion of fluid (plasma) commences within the blood islands.

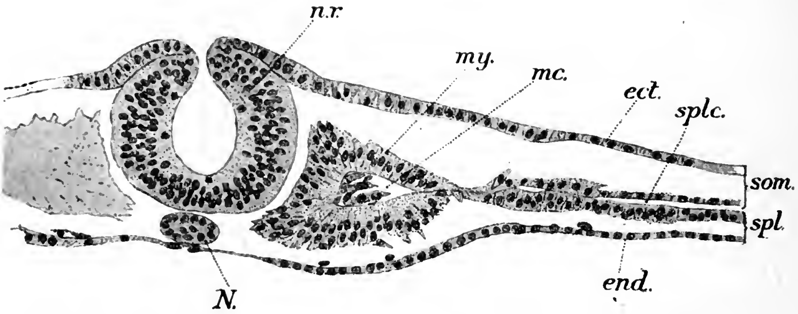


FIG. 228.—Transverse section through the body of a Fowl embryo about the end of the first day of incubation.

ect, ectoderm; *end*, endoderm; *mc*, myocoele; *my*, myotome (mesoderm segment); *N*, notochord; *n.r*, neural rudiment; *som*, somatopleure; *spl*, splanchnopleure; *splc*, splanchnocoele.

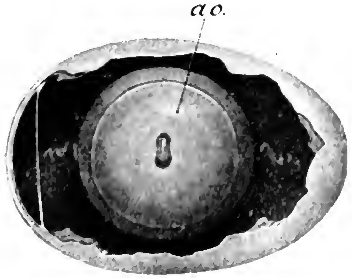


FIG. 229.—Egg of the Fowl about the middle of the second day of incubation.

a.o, circular opaque area. In the centre is the dark pellucid area, with the rudiment of the embryonic body lying along its axis.

THE SECOND DAY OF INCUBATION.—The general appearance of an egg opened during the second day of incubation is seen in Fig. 229. The blastoderm has increased considerably in size and now covers about 110°. The pellucid area has assumed a somewhat fiddle-like shape.

On examining the excised blastoderm about the commencement of this day it is seen that the formation of the head fold has progressed considerably and the head rudiment projects more conspicuously above the general level of the blastoderm. Within the head rudiment the foregut can be seen and it is noticeable that it stretches farther back than does the outer wall of the head rudiment. In other words the head fold of the endoderm has spread farther back than that of the ectoderm. This is brought out clearly by a sagittal section such as that shown in Fig. 230. Such a section also brings out the fact that while the greater part of the portion of blastoderm tucked in beneath the head of the embryo is two-layered (proamnion), there being no mesoderm present, this does not apply to the farthest back part of the fold. Here, in the wide space between ectoderm and endoderm, mesoderm has penetrated which will give rise to the pericardiac wall and the heart. The medullary folds have met over a considerable extent but still remain separate at their extreme front ends as well as over the whole extent which will later form the spinal cord. Here they bound a deep neural groove. Towards their posterior ends the two medullary folds diverge to pass on either side of a lance-shaped area (**rhomboidal sinus**) which they enclose by converging towards one another behind it. Along the centre of the floor of the rhomboidal sinus the primitive streak is still visible separated by a knob-like elevation from the part of the primitive streak which lies farther back.

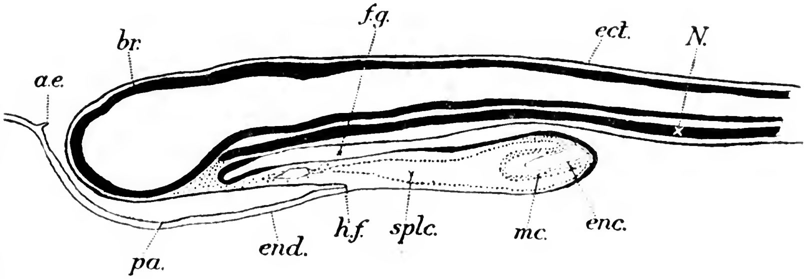


FIG. 230.—Diagrammatic sagittal section through anterior end of Fowl embryo with 15 segments.

a.e, rudiment of amnion ; *br*, brain ; *ect*, ectoderm ; *enc*, endocardium ; *end*, endoderm of yolk-sac ; *f.g*, foregut ; *h.f*, posterior limit of head fold of ectoderm ; *mc*, myocardium ; *N*, notochord ; *pa*, ectoderm of proamnion ; *splc*, splanchnocoele.

The mottled appearance characteristic of the vascular area is now seen to be continued inwards, though much more faint, across the pellucid area to the body of the embryo.

An embryo with about ten segments is shown in Fig. 231. The pellucid area is still somewhat fiddle-shaped with the body of the embryo lying along its axis. Apart from the increase in number of the mesoderm segments the most conspicuous advances in development are in the central nervous system. The medullary folds have met and fused together to enclose the neural tube except towards their hind ends where they still bound the rhomboidal sinus on each side. The forebrain region is greatly dilated, its projection on each side being the optic rudiment (*o.r*). It will be noticed that a slight notch in its wall in the mesial plane anteriorly indicates that at this point the two neural folds have even yet not completely fused. Posteriorly the neural folds seem to be continuous with the lips of the primitive groove. A faint continuation forwards of the primitive groove may be seen in the floor of the rhomboidal sinus.

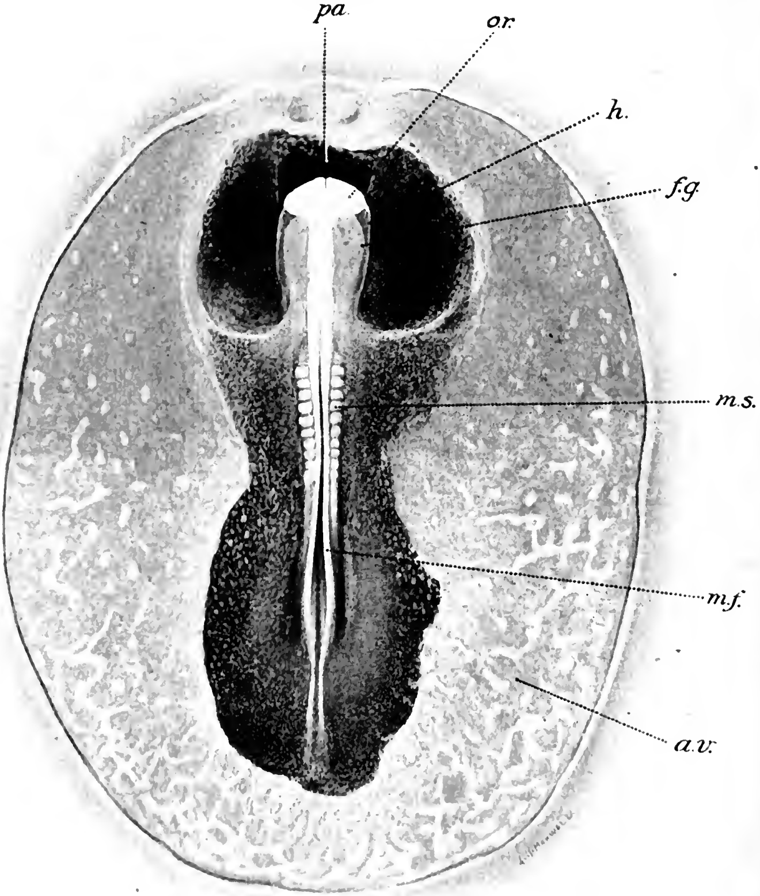


FIG. 231.—Blastoderm with Fowl embryo with about 10 or 11 mesoderm segments.

a.v, vascular area ; *f.g*, foregut ; *h*, head ; *m.f*, medullary fold ; *m.s.* mesoderm segment ; *o.r*, optic rudiment ; *pa*, proamnion.

Important mesoderm features are to be noticed. The mottled appearance of the vascular area produced by the rudiments of blood-vessels developing in the splanchnic mesoderm is conspicuous. The formerly isolated vascular rudiments (white in the figure) are now becoming joined up to form a network and the network can be traced —less distinct and on a smaller scale—across the pellucid area. At its anterior and inner corner the network is continuous with a short and wide vessel which slopes obliquely forwards and inwards and disappears beneath the hind end of the foregut (shown more clearly in Fig. 232, *v.v*). This vessel is the rudiment of the vitelline vein, which drains the blood from the vascular area towards the heart. Another conspicuous vessel rudiment is the **terminal sinus** — a marginal vessel which bounds the vascular area externally. In front of the head of the embryo is a somewhat rectangular area of the blastoderm distinguished by its being very transparent (Fig. 232, *pa*). This is the proamnion—its transparency being due to the fact that the mesoderm has not yet spread into this region of the blastoderm. On each side of the head of the embryo the surface of the blastoderm bulges upwards into a dome-like swelling (Fig. 232, *splc*). This is due to a precocious splitting of the mesoderm in this region to form a large coelomic space. The bulging appearance is produced by the coelomic space being tensely filled with fluid. The raising up of this region of somatopleure is preliminary to the formation of the head fold of the amnion.

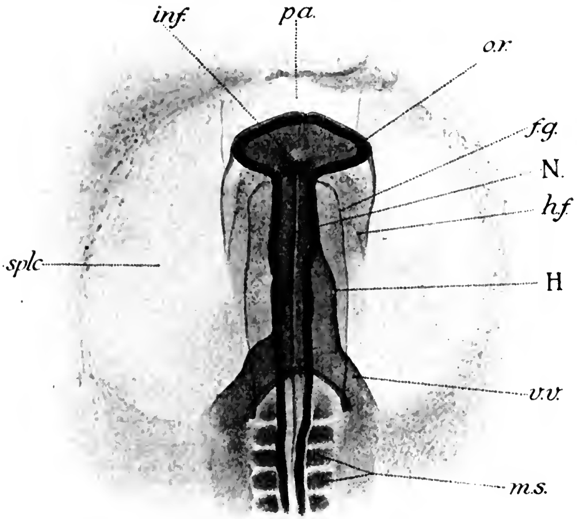


Fig. 232.—Head of Fowl embryo of same stage as that shown in Fig. 231, more highly magnified and seen by transmitted light.

f.g, foregut; *H*, heart; *h.f*, hinder limit of head fold of ectoderm; *inf*, infundibulum; *m.s*, mesoderm segments; *N*, notochord; *o.r*, optic rudiment; *pa*, proamnion; *splc*, patent portion of splanchnocoele containing coelomic fluid; *v.v.* vitelline vein.

By turning over the excised blastoderm and examining it from below or by staining and then examining it in dorsal view by transmitted light (Fig. 232) it will be seen that between the two coelomic spaces there lies a ٨-shaped structure. The two diverging limbs of the ʌ posteriorly are the vitelline veins already alluded to (*v.v*), while the median portion (*H*)—a straight tube passing forwards beneath the foregut—is the rudiment of the heart and ventral aorta. It will be noticed that the two vitelline veins when traced backwards from the heart are seen to fit round the tunnel-like opening of the foregut. In the forebrain region is seen the downwardly projecting pocket of its floor—the infundibulum (Fig. 232, *inf*)—and extending back from this in the middle line the notochord (*N*). On each side of this posteriorly are seen the mesoderm segments (*m.s*).

In a slightly more advanced embryo with about fifteen mesoderm segments the tucking in of the blastoderm under the head has proceeded considerably further. The neural tube has become closed in entirely except for the slit-like remnant of the rhomboidal sinus posteriorly. The optic rudiments projecting prominently from the forebrain on each side and beginning to be narrowed slightly at their base give the brain a conspicuous T-shape. The wall of the brain in its posterior region shows a series of puckerings one behind the other marking it off into a series of what used to be called brain "vesicles." Of these the anterior one, the largest and most distinct, is destined to become the mesencephalon while those behind it enter into the formation of the rhombencephalon. The latter are often interpreted as vestiges of a once present segmentation of the brain, but are regarded by the author of this volume as being adequately accounted for by the active growth of the brain within its confined space, aided possibly by the varying consistency of the mesenchyme outside it (see p. 101).

On each side of the head region posteriorly, just in front of the first obvious mesoderm segment, the rudiment of the otocyst has made its appearance as a cup-like depression of the ectoderm.

The heart, growing in length more rapidly than the neighbouring parts of the body, has been forced into its characteristic bulging outward on the right side. The first traces of hæmoglobin are making their appearance in the posterior portion of the vitelline network.

An important new feature becomes visible about this stage in the form of a whitish line on the bulging roof of the splanchnocoele on each side. The lines in front curve in towards one another, meeting in front of the proamnion and sweeping back in a wide curve on each side. This line is the first rudiment of the amniotic fold. As the fold becomes more and more prominent it bends backwards and inwards, arching over the head region, and towards the end of the second day (Fig. 233) forming the amniotic hood which ensheaths the head portion of the embryo.

Many of the important details in the structure of the second day blastoderm can only be made out by the study of series of transverse sections. In studying the stage now under consideration it is advisable to begin with a section taken from about the middle of the total length of the embryo such as that represented in Fig. 234A. The blastoderm some little distance away from the median line of the embryo is seen to consist of the usual two double layers—the somatopleure (*som*) composed of ectoderm and somatic mesoderm and the splanchnopleure (*spl*) composed of splanchnic mesoderm and endoderm. In immediate contact with the lower surface of the endoderm in the complete egg there would be the yolk. In the splanchnic mesoderm overlying the endoderm are seen the blood-vessels of the vascular area. When traced inwards towards the mesial plane the two layers of mesoderm are seen to come together to form the narrow protovertebral stalk or nephrotome which joins up the lateral mesoderm to

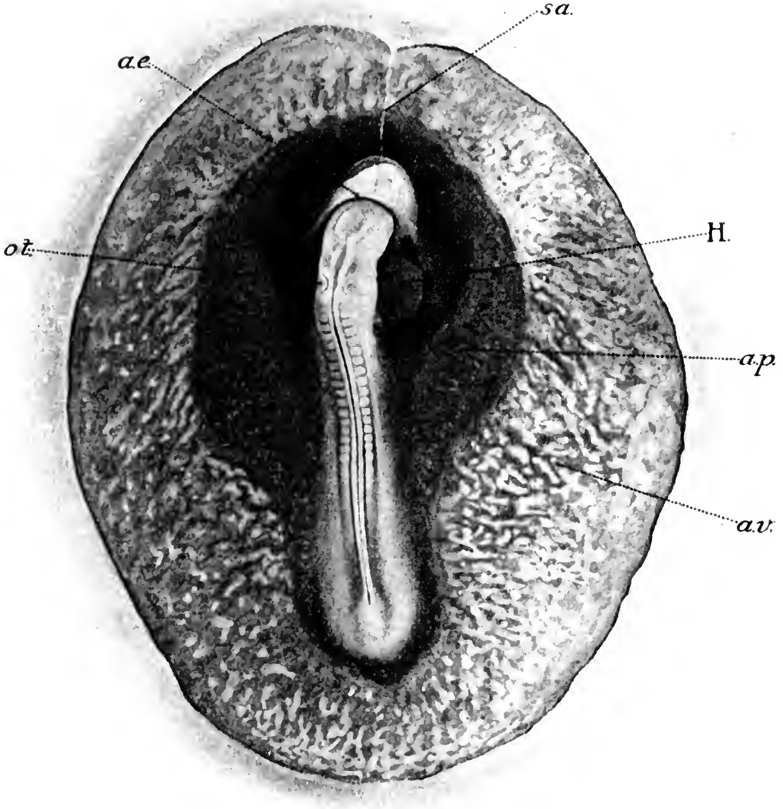


FIG. 233.—Blastoderm and embryo Fowl with 18 mesoderm segments.

a.e, backgrowing edge of amniotic hood ; *a.p*, pellucid area ; *a.v*, vascular area ; *H*, heart ; *ot*, otocyst ; *sa*, sero-amniotic connexion.

the mesoderm segment. Immediately above the nephrotome, between it and the ectoderm, is seen the rudiment of the archinephric duct—a rod of cells which is gradually extending tailwards.

In the centre of the section is the neural tube (*s.c*) with its thick walls and the solid notochordal rudiment (*N*) lying immediately below it. The blood-vessel (*A*) on each side between nephrotome and endoderm is the dorsal aorta which is at this stage double.

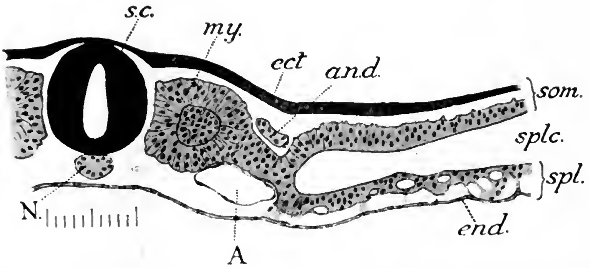


Fig. 234a.—Transverse section through the middle of a second-day Fowl embryo (15 segments).

A, paired dorsal aorta; *a.n.d*, archinephric duct; *ect*, ectoderm; *end*, endoderm; *my*, myotome; *N*, notochord; *s.c*, spinal cord; *som*, somatopleure; *spl*, splanchnopleure; *splc*, splanchnocoele.

Working back towards the tail end of the embryo it is seen that subsequent sections show less and less advanced stages of development in concordance with the fact that development proceeds from the head end tailwards. Thus the neural tube opens out by the slit-like rhomboidal sinus; the archinephric duct disappears; the notochord passes back into the undifferentiated tissue of the primitive streak.

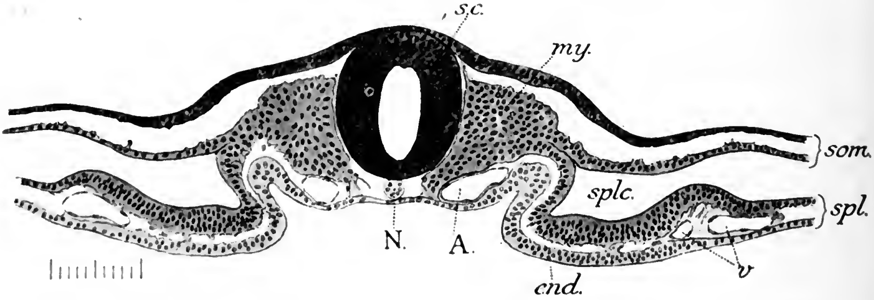


Fig. 234b.—Transverse section through a second-day Fowl embryo just behind the hinder limit of the foregut.

A, dorsal aorta; *end*, endoderm; *my*, myotome; *N*, notochord; *s.c*, spinal cord; *som*, somatopleure; *spl*, splanchnopleure; *splc*, splanchnocoele; *v*, vessels of vascular area.

On the other hand the examination of sections farther forward towards the head region brings into view various important further developments. Such a section as that shown in Fig. 234b illustrates clearly an early stage in the folding off of the foregut from the cavity of the yolk-sac—a fold of splanchnopleure growing inwards on each side below what will become the foregut. The large vessels seen in the splanchnopleure external to the fold just mentioned are tributaries of the vitelline veins, and a few sections farther forwards they would be found to be united together to form the main vitelline vein on each side.

As the series of sections is traced forwards the two folds of the splanchnopleure are seen to approach one another and finally to meet and undergo fusion, so that there now exists a foregut cavity shut off (as seen in transverse section) from the yolk-sac, the walls of the two structures being still connected by a median vertical partition formed by the fusion of the endoderm from the two sides. A little farther forward this partition disappears from the section and the foregut as seen in section (Fig. 234C) is quite isolated from the endoderm of the yolk-sac wall. The vitelline veins have also fused to form the tubular heart. It is seen that the splanchnic mesoderm ensheaths the endothelial wall of the heart (*enc*) on each side and that where it does so it is somewhat thickened (*mc*) as compared with the same layer in the region overlying the yolk-sac. This localized thickening of the splanchnic mesoderm is destined to give rise to the entire thickness of the heart wall except the lining endothelium. It is seen to be continuous with the extra-cardiac portions of the splanchnic mesoderm by the dorsal (*d.mc*) and ventral mesocardium (*v.mc*).

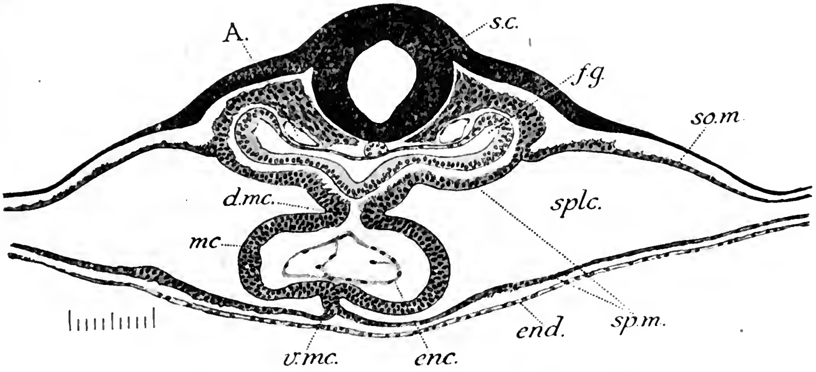


FIG. 234C.—Transverse section of a second-day Fowl embryo passing through the rudiment of the heart.

A, dorsal aorta; *d.mc*, dorsal mesocardium; *enc*, endocardium; *end*, endoderm; *f.g*, foregut; *mc*, myocardium; *s.c*, spinal cord; *so.m*, somatic mesoderm; *sp.m*, splanchnic mesoderm; *splc*, splanchnocoele; *v.mc*, ventral mesocardium.

Traced forwards through the series of sections the heart is seen to narrow in calibre as it tapers off into the ventral aorta. Towards its front end the latter gives off a large branch on each side which passes outwards and upwards round the foregut to become continuous with the dorsal aorta. These two hoop-like vessels which connect up ventral and dorsal aortae are the first pair of aortic arches.

Still further forward the region of the forebrain and optic rudiments is reached (Fig. 234D).

Owing to the folding off of the head rudiment the section of the head itself appears completely detached from the blastoderm and the latter is beginning to form a depression which will later become more marked and in which the head will lie. In the blastoderm it will be noticed how away on each side it shows the normal four layers of cells—ectoderm, somatic mesoderm, splanchnic mesoderm, endoderm—while on the other hand in the region underlying the head of the embryo it is only two layered the mesoderm being here absent.

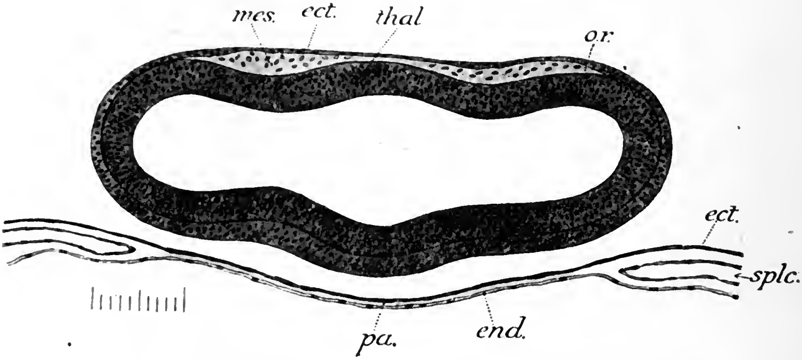


FIG. 234D.—Transverse section of a second-day Fowl embryo passing through the optic rudiments.

ect, ectoderm ; *end*, endoderm ; *mes*, mesenchyme ; *o.r*, optic rudiment ; *pa*, proamnion ; *splc*, splanchnocoele ; *thal*, roof of thalamencephalon.

This two-layered region of blastoderm is the proamnion before alluded to.

The head itself is occupied almost entirely by the brain rudiment—the thalamencephalon in the centre (*thal*) continued outwards on each side as the optic rudiment (*o.r*). For the most part the external ectoderm is closely apposed to the surface of the brain but dorsally the former is commencing to recede from the latter, the space between the two being occupied by mesenchyme (*mes*).

THE THIRD DAY OF INCUBATION.—During the later hours of the second and earlier hours of the third day of incubation there take place a number of important changes which render this period perhaps the most interesting of all to the morphologist. For the student who is training himself practically in the technique of embryological observation there is no finer material than that afforded by Bird embryos of about this age for learning one of the most important parts of that technique namely the interpretation of serial sections.

It is advisable to make a careful study of the anatomy of an embryo of about the stage shown in Fig. 235 or 236.[1]

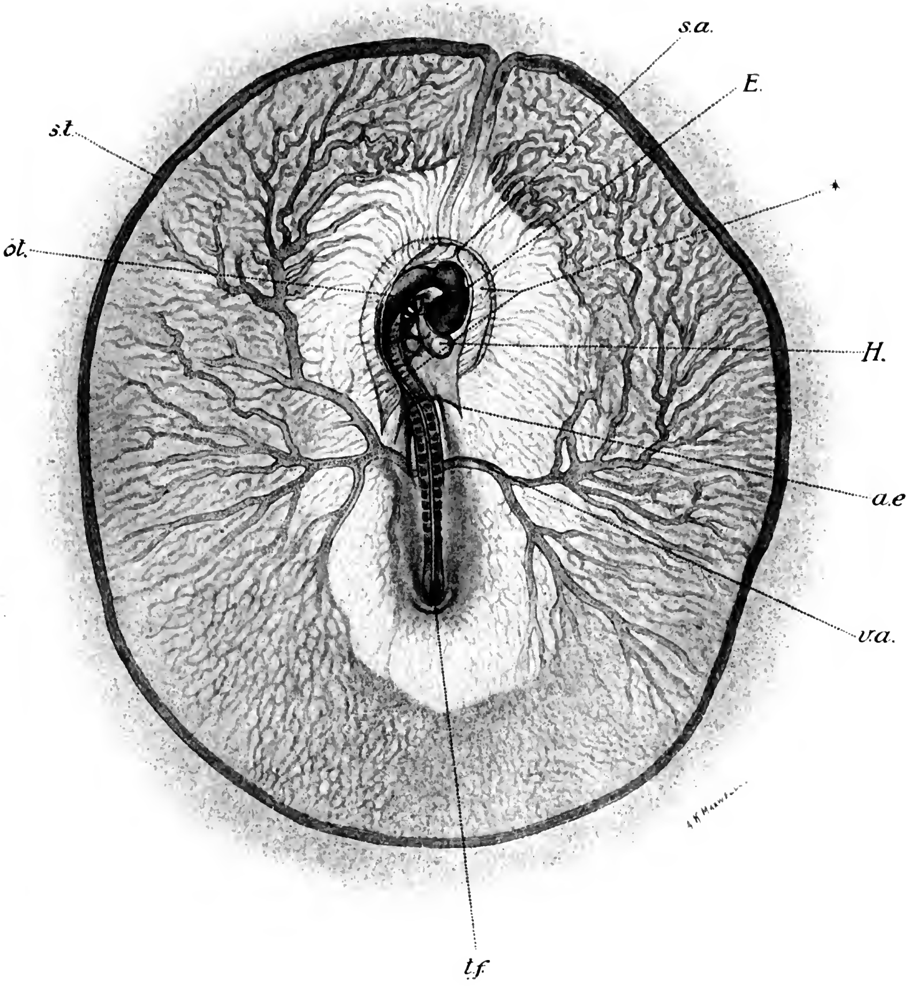


Fig. 235.—Third-day Fowl embryo with the vascular area.

a.e, edge of amnion; *E*, eye; *H*, heart; *ot*, otocyst; *s.a*, sero-amniotic connexion; *s.t*, sinus terminalis; *t.f*, tail-fold; *v.a*, vitelline artery; *, portion of splanchnopleure involuted to form a recess round the head of the embryo.

---

[1] It is customary to mount transverse sections with the posterior or tailward surface of the section next the slide: consequently the figures represent the sections as seen from in front and the side of each figure towards the right-hand side of the page corresponds to the left-hand side of the embryo.

On opening the egg it is at once seen that the blastoderm has increased considerably in size, the outer limit of the opaque area having spread downwards as far as about the equator of the egg. The vascular area has also increased considerably and is still bounded by the conspicuous terminal sinus which anteriorly turns inwards and passes back parallel to the corresponding part of the sinus of the other side to open into the vitelline vein close to its inner end. Of these two veins which run parallel to the long axis of the embryo the right is reduced in size and eventually disappears.

The yolk has assumed a more fluid consistency; the proportion of white yolk has increased; the albumen has shrunk considerably in volume, and the air space has increased correspondingly.

The free edge of the amniotic hood (Fig. 235, *a.e*) has grown back so as to ensheath all the head and anterior trunk region of the embryo. It follows that when examined *in situ* the front part of the body is seen through two layers of somatopleure. Of these the outer—the serous membrane—forms a kind of roof which passes outwards all round into the general blastoderm. The inner—the true amnion—closely invests the head end of the embryo and is visible in profile as a sharp line immediately outside the outline of the head itself. Anteriorly the amnion very often seems to be prolonged into a sharp peak (Fig. 235, *s.a*): this is the sero-amniotic connexion.

The free edge of the amniotic fold, somewhat arch-like in outline, may die away posteriorly (Fig. 235) or it may be already continued into the lateral and caudal parts of the fold (Fig. 236)—but even if present these are still low and inconspicuous as compared with the headward part of the fold.

As regards the body of the embryo it is seen that the folding off of this from the yolk is proceeding rapidly. The head and anterior part of the trunk project freely and, correlated with this and with the ventral flexure of the head region, the latter has come to lie over on one side, usually the left, so that it is seen in profile when the blastoderm is looked down upon from above. At the extreme hind end the tail region is also seen to be in process of becoming marked off from the blastoderm by a tail fold (Fig. 235, *t.f*) of the same nature as the head fold. Similarly the trunk region between the regions of head and tail fold is becoming demarcated from the blastoderm outside it by a lateral fold (Fig. 236).

The body of the embryo has increased considerably in length and this growth in length is particularly active towards the dorsal side of the embryo where there is greater freedom from the clogging effect of the yolk. The result of this difference in rate of growth between dorsal and ventral sides is that those parts of the embryo which are detached from the general blastoderm assume a strong flexure towards the ventral side. This is particularly pronounced in the head region, the head being completely bent upon itself so that the front end of the brain is reversed in position, what was its ventral side having come to be dorsal.

The mesoderm segments have increased in number there being

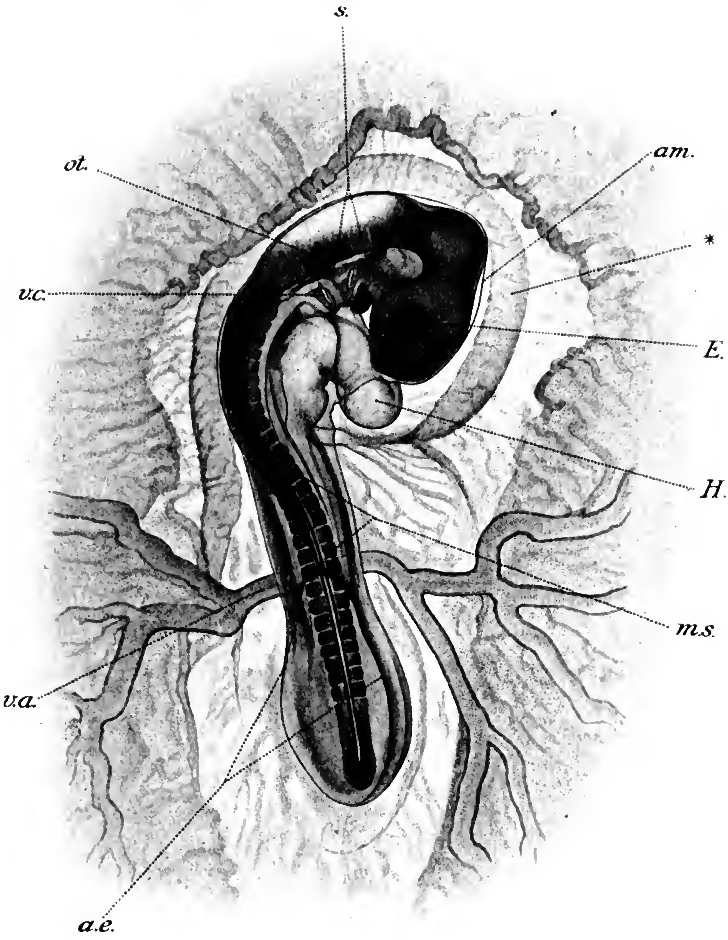


FIG. 236.—Third-day Fowl embryo (No. 47) viewed as a transparent object.

a.e, edge of amnion; *am*, amnion; *E*, eye; *H*, heart; *m.s*, mesoderm segments; *ot*, otocyst; *s*, indications of preotic mesoderm segments (?); *v.a*, vitelline artery; *v.c*, visceral cleft II; *, portion of splanchnopleure bulging downwards into the yolk, forming a recess in which lies the head of the embryo.

now about 25-30 metotic segments and those towards the anterior end are showing a considerable amount of dorsiventral growth. In some embryos (Fig. 236) the series of definitive mesoderm segments is continued far into the head region by what appear to be the ghostly vestiges of formerly existing segments (see pp. 210, 211).

The central nervous system has made important advances in development. The brain shows a relatively large increase in size as compared with the spinal cord: thalamencephalon, mesencephalon and rhombencephalon are marked off by definite constrictions—the mesencephalon being particularly prominent at the bend of the head. The greater part of the roof of the rhombencephalon is assuming its definitive thin membranous character. The three great organs of special sense have made their appearance. The eye (*E*) forms a large conspicuous cup-like structure lying at the side of the fore-brain. Its rim is cleft ventrally by the choroid fissure (Fig. 236). Its mouth is partially blocked by the round lens rudiment. The otocyst (*ot*) is also conspicuous—a pear-shaped sac, its narrow end dorsal, lying at the side of the hind brain. The olfactory organ is represented by a slight dimple of thickened ectoderm near the tip of the head.

The side walls of the foregut are perforated by visceral clefts. The series of these develop from before backwards and by this stage three have commonly appeared—clefts I, II, and III of the series.

It is perhaps the vascular system which shows the most interesting features during the third day. The heart is still in the form of a simple tube, but its active growth in length has caused a great increase in the curvature which was already pronounced about the middle of the second day. Its γ-like curvature is shown in Fig. 236. At its morphologically front end the heart is continued into the ventral aorta and this at its end gives off a series of vessels, the aortic arches, which pass up round the sides of the foregut between adjacent gill-clefts and open dorsally into the aortic root which lies just dorsal to the clefts. Like the clefts themselves the aortic arches develop in sequence from before backwards and by this stage arches I, II, and III have made their appearance (Fig. 241, A).

At its front end the aortic root can be traced for some distance into the head as the dorsal carotid artery (Fig. 241, A, *d.c*). Posteriorly the two aortic roots become hidden from view by the myotomes but the study of sections shows that they have here united to form the unpaired dorsal aorta. Still farther back this vessel again becomes paired and a little behind the point of bifurcation each of the branches gives off a large vitelline artery (*v.a*) which passes outwards at right angles to the axis of the body to supply the vascular area.

Of the venous system the most conspicuous components are the great vitelline veins (Fig. 241, A, *v.v*) which, receiving numerous branches from the vascular area, pass forwards converging towards one another to form by their fusion the hind end of the heart. Examination of the vascular area shows that the branches of the vitelline arteries and of the veins accompany one another in their ramifications. In the living condition, in which all these arrangements of the vascular system should be studied, the arteries are seen to be more deeply coloured and more conspicuous than the veins. The two vitelline veins by their fusion form the hind end of the tubular heart and on tracing this forwards a somewhat Y-shaped vessel is seen opening into it laterally. The stalk of the Y which is very short, though showing considerable variability within its limits, is the right duct of Cuvier (Fig. 241, A, *d.*C). The branches of the Y are the cardinal veins. Of these the posterior (*p.c.v*), coming from the region of the kidneys, is only visible for a short distance, being soon hidden as it is traced backwards beneath the myotomes. The anterior cardinal vein (*a.c.v*) on the other hand can be traced forwards for a long distance into the head from which it drains the blood back towards the heart. It will be noted that here in the embryonic Bird we find exactly the same arrangement of main veins—duct of Cuvier, anterior cardinal and posterior cardinal—as is characteristic of the adult condition of lowly organized fish-like Vertebrates.

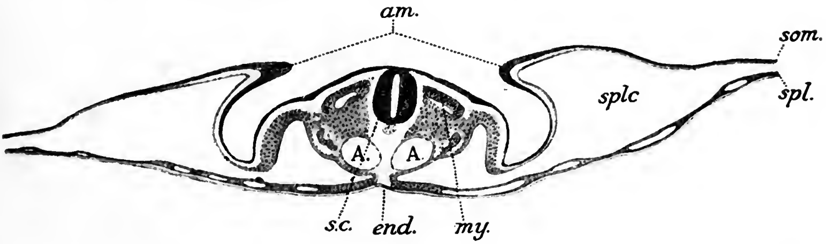


Fig. 237a.—Transverse sections through third-day Fowl embryo. (Partly based on figures by Duval.) A, Through the hinder part of the trunk region.

A, dorsal aortae; *am*, amniotic folds; *end*, endoderm; *my*, myotome; *s.c*, spinal cord; *som*, somatopleure; *spl*, splanchnopleure; *splc*, splanchnocoele.

For the study of such details of structure as cannot be made out in the whole embryo the most useful sections are series cut transversely to the long axis of the trunk region. These should be supplemented by series parallel to the sagittal plane in the head region.

It is well to commence the study of the transverse sections with one through the hinder trunk region, about the level of the vitelline arteries. Such a section is depicted in Fig. 237a.

In comparing this section with a corresponding section through the second-day chick (Fig. 234a) the same general features will be recognized—the differences being mainly differences in detail. The most conspicuous of these is caused by the development of the amniotic fold of the somatopleure which rises up on each side, arching towards the median plane over the dorsal side of the embryo (*am*). Traced forwards through the series the amniotic folds of the two sides are seen to meet and undergo fusion so as to give rise to the inner true amnion and the outer false amnion or serous membrane: the former continuous at its inner edge with the somatopleure of the embryo's body, the latter at its outer edge with that of the blastoderm. It will be readily seen that the space between true and false amnion is morphologically part of the splanchnocoele. It will also be realized that both true and false amnion being somatopleural in nature are composed of ectoderm and somatic mesoderm but that the relative position of these two layers is reversed in the amnion as compared with the false amnion.

Important changes have taken place in the mesoderm. The mesoderm segment is no longer connected with the lateral mesoderm the nephrotome having become converted into renal structures—the archinephric duct and mesonephric tubules. The relations of these will be understood by referring back to the general description of renal organs in Chapter IV. (p. 254). The inner wall of the segment has lost its epithelial character and broken up into a mass of actively proliferating mesenchyme cells. Many of these cells will wander away in amoeboid fashion and settle down round notochord and spinal cord to form the protective sheath in which eventually develops the vertebral column. Collectively these amoeboid cells constitute the sclerotome which is therefore much more diffuse in its origin than in the lower vertebrates illustrated on p. 285.

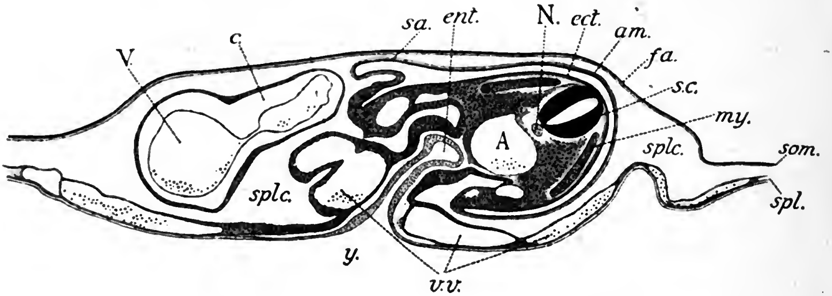


FIG. 237B.—Transverse section just behind the point of union of the two vitelline veins.

A, dorsal aorta; *am*, amnion; *c*, conus arteriosus; *ect*, ectoderm; *ent*, enteron; *f.a*, false amnion or serous membrane; *my*, myotome; *N*, notochord; *s.c*, spinal cord; *sa*, sero-amniotic isthmus; *som*, somatopleure; *spl*, splanchnopleure; *splc*, splanchnocoele; *V*, ventricle; *v.v*, vitelline veins; *y*, yolk.

Certain blood-vessels are visible in the section. In the splanchnic mesoderm of the yolk-sac numerous vessels of the vitelline network are visible: over the mesonephros may usually be seen the posterior cardinal vein, while on each side of the mesial plane ventral to the notochord are the two dorsal aortae.

As the series of sections is traced towards the head the most conspicuous change is the increasing asymmetry due to the body of the embryo coming to lie over more and more upon its left side. Fig. 237B represents a section just behind the posterior limit of the foregut.

The body of the embryo lying over on its left side is closely invested by the amnion (*am*) while over this lies the thin roof (*f.am*) constituting the serous membrane. At *sa* the two membranes are united by the sero-amniotic connexion. In the mesoderm of the two folds of splanchnopleure which are approaching one another to floor in the alimentary canal (*ent*) are seen the two large vitelline veins (*v.v*). The ventricle and the conus are seen cut longitudinally in the wide coelomic space lying to the right of the body of the embryo.

A section a little farther forward in the series has the appearance shown in Fig. 237C. The definitive gut (*ent*) is completely separated at this level from the yolk-sac, and corresponding with this the two vitelline veins, which in sections farther back lay one on each side of the yolk-stalk, are now completely fused into a large median vessel, the *ductus venosus* (*d.v*), which is simply the backward prolongation of the heart. The posterior liver rudiment, a blindly ending pocket of the gut-wall projecting forwards ventral to the ductus venosus, is seen in the section figured (*li.2*), although its communication with the gut-wall is no longer visible, lying as it does several sections farther back. At this level however a second pocket-like outgrowth of the gut-wall has made its appearance (*li.1*). This is the anterior liver rudiment. It will be noticed that it lies dorsal to the ductus venosus. In the coelomic space ventral to the ductus venosus and liver rudiments, and quite isolated, is the rounded section through the ventricular region of the heart (*V*).

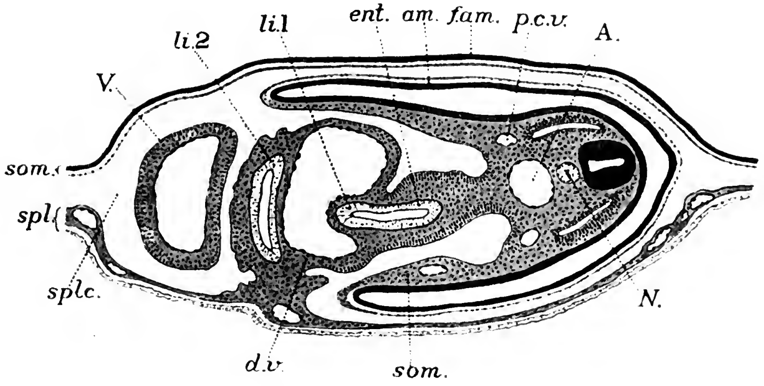


FIG. 237C.—Transverse section a little in front of the hind end of the heart.

am, amnion; *A*, dorsal aorta; *d.v*, ductus venosus; *ent*, alimentary canal; *f.am*, false amnion; *li.1*, anterior liver rudiment; *li.2*, posterior ditto; *N*, notochord; *p.c.v*, posterior cardinal vein; *som*, somatopleure; *spl*, splanchnopleure; *splc*, splanchnocoele; *V*, ventricle.

In the sections studied so far the body-wall of the embryo is widely open on its ventral side—the opening being bounded by the recurved edge along which the somatopleure of the body is continuous with the non-embryonic region of the somatopleure forming the amnion. As however the folding off of the embryo progresses the edge alluded to grows inwards and the opening bounded by it becomes reduced in size. It will be gathered readily from Fig. 237D that through the opening in question the splanchnocoele, included within the definitive body of the embryo, is continuous with that part of the coelome which lies outside (extra-embryonic coelome). In the section figured the heart is seen to be cut through in two places. Reference to the figure of the whole embryo (p. 535) will show that the piece of heart which lies towards the left side of the embryo (*at*) is the atrium, while that on the embryo's right (*C*) is the ventricle or conus. In the section figured a large blood-vessel (*d.C*) is seen cut longitudinally in the somatopleure. By tracing this vessel through neighbouring sections it will be found to open at its ventral end into the atrial part of the heart while dorsally it splits into the two cardinal veins—anterior and posterior. These relations show the vessel in question to be the duct of Cuvier. The only other point calling for special mention in the section figured is that the ventral part of the pharyngeal cavity projects outwards upon either side: this dilated ventral part of the pharynx forms the rudiment of the lung.

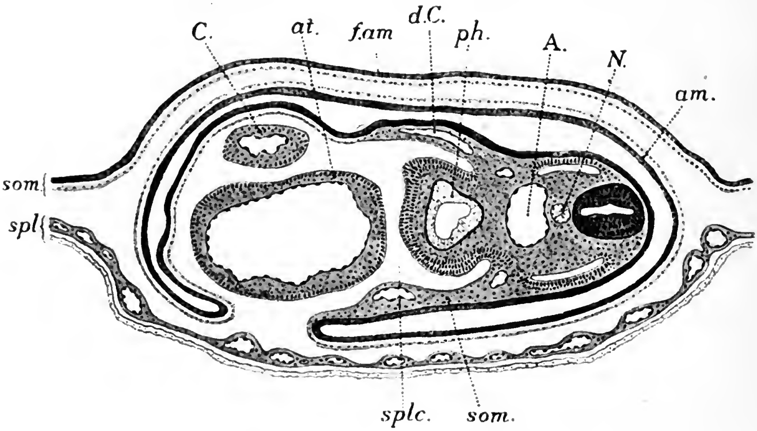


FIG. 237D.—Transverse section a short distance behind the front end of the heart.

A, dorsal aorta; *am*, amnion; *at*, atrium; *C*, conus; *d.C*, duct of Cuvier; *f.am*, false amnion; *N*, notochord; *ph*, pharynx; *som*, somatopleure; *spl*, splanchnopleure; *splc*, splanchnocoele.

In the region in front of the heart the dorsiventral depth of the body of the embryo becomes comparatively suddenly reduced and in the vacant space within the amnion so provided there appears a new structure quite detached from the rest of the section. The structure in question is a section through the recurved tip of the head (see figure of whole embryo). In Fig. 237E this shows the thick-walled forebrain (*f.b*) with its wide ventriocular cavity while upon each side and ventrally[1] there is seen a localized thickening (*olf*) of the ectoderm: this is the dimple-like rudiment of the olfactory organ. To return to the main part of the section—there is seen in its centre the wide pharyngeal space and on the embryo's left side the pharyngeal wall projects out to the ectoderm as an endodermal pocket—the rudiment of the second visceral cleft (*v.c.II*). Immediately ventral to the pharynx is the ventral aorta (*v.A*). On the left side of the embryo the aortic root (*a.r*) is seen immediately dorsal to the pharynx, while on the right side—the section not being accurately transverse—a hoop-like aortic arch (*a.a.III*) is seen passing dorsalwards round the side of the pharynx from ventral aorta to aortic root. The large

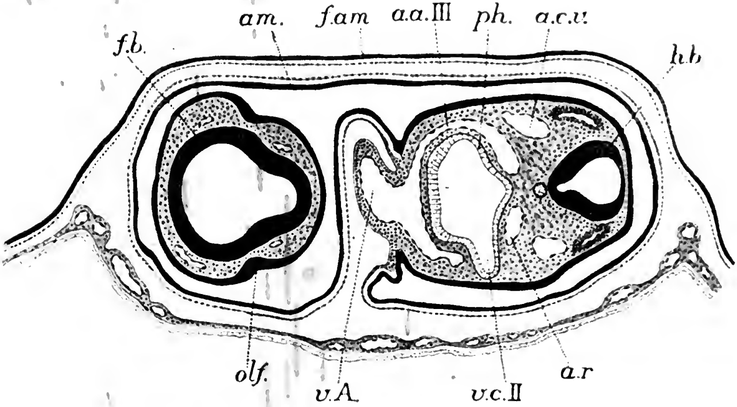


FIG. 237E.—Transverse section passing through the second visceral cleft and the olfactory rudiment.

a.a.III, third aortic arch: *a.c.v*, anterior cardinal vein; *am*, amnion; *a.r*, aortic root; *f.am*, false amnion; *f.b*, forebrain; *h.b*, hind brain; *olf*, olfactory rudiment; *ph*, pharynx; *v.A*, ventral aorta; *v.c.II*, second visceral cleft.

vessel lying dorsal and slightly external to the aortic root (*a.c.v*) is the anterior cardinal vein. Traced tailwards it is found to open into the dorsal end of the duct of Cuvier. The neural tube (*h.b*) is seen to have a thin roof and widely expanded lumen indicating that it is now passing into the region of the hind brain.

In tracing the series of sections further forwards it will be realized that the front part of the head region is, owing to its reflexed position, actually being traced in a morphologically tailward direction. In the section figured (Fig. 237F) the reflexed portion of the head is cut at the level of the eye rudiments (*opt*) which are seen to be in the optic cup stage with the inner or retinal layer

---

[1] It will be realized from an inspection of the figure of the entire embryo that the recurved part of the head is reversed in position. Its ventral side lies therefore in the figure towards the right.

distinctly thickened as compared with the outer or pigment layer, and with a narrow optic stalk passing to the thalamencephalon near its floor. In the mouth of the optic cup is the lens but this is seen better a few sections farther on in the series.

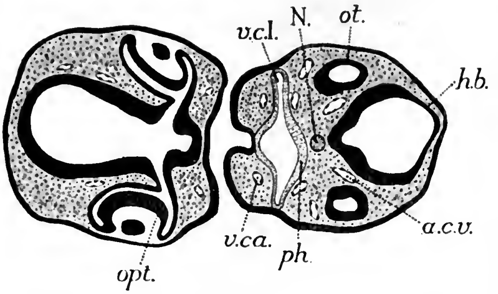


FIG. 237F.—Transverse section passing through the rudiments of the eye and otocyst.

a.c.v, anterior cardinal vein ; *h.b*, hind brain ; *N*, notochord ; *opt*, optic cup ; *ot*, otocyst ; *ph*, pharynx ; *v.c.1*, first visceral cleft ; *v.ca*, ventral carotid.

Turning to the other half of the section it is seen that it is no longer connected with the extra-embryonic somatopleure: in other words the series of sections has now passed the hinder limit of the headfold of the somatopleure. The pharynx passes out as a pocket on each side towards the ectoderm—the rudiments of the first pair of visceral clefts (*v.c.I*). The neural tube has become greatly increased in size forming the hind brain with its widely expanded cavity—the fourth ventricle. On each side is a large thick-walled sac—the otocyst. Examination of neighbouring sections shows that it is still connected with the outer skin by a narrow neck. In the spongy connective tissue which forms packing between the various organs are seen a

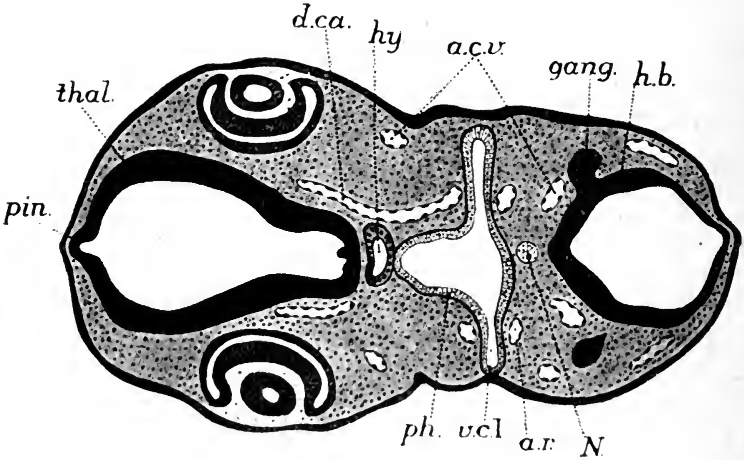


FIG. 237G.—Transverse section passing through the eye and just in front of the otocyst.

a.c.v, anterior cardinal vein ; *a.r*, aortic root ; *d.ca*, dorsal carotid artery ; *gang*, ganglion of eighth cranial nerve ; *h.b*, hind brain ; *hy*, pituitary body ; *N*, notochord ; *ph*, pharynx ; *pin*, pineal organ ; *thal*, thalamencephalon ; *v.c.1*, first visceral cleft.

number of blood-vessels such as ventral and dorsal carotids and anterior cardinal veins.

As will be gathered by sliding a straight-edge forward over the figure of the whole embryo, its edge parallel to the plane of the sections, there comes a point in the series where the sections through the reflexed part of the head and the rest become continuous. This happens as soon as the deep niche in the bend of the head is passed. Such a section is represented in Fig. 237G. Comparison of this figure with the preceding one will make clear the fact that the extreme ends of the section are both of them morphologically dorsal. The brain is cut through twice—on the right of the figure is the hind brain while on the left is the thalamencephalon distinguished by the pocket-like rudiment of the pineal organ (*pin*). The thin optic stalk lies outside the section, but the structure of the optic cup otherwise is well seen. The lens is in the form of a closed vesicle which has by this stage become completely nipped off from the external ectoderm. Immediately ventral to the thalamencephalon is the pituitary involution cut transversely. The section passes through the ganglia of the auditory nerve (*gang*) and on the embryo's right through the nerve root connecting the ganglion with the medulla oblongata. Various blood-vessels are cut through: their names and relations with one another are most easily determined by sliding a straight-edge along the drawing of the embryo as a whole.

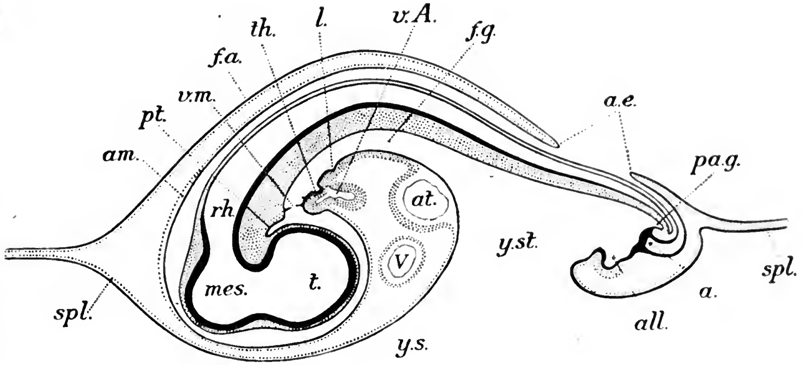


FIG. 238.—Diagrammatic sagittal section through third-day Fowl embryo. The notochord and dorsal aorta are omitted Ectoderm and endoderm are indicated by continuous lines, mesoderm (except endocardium) by dots.

a, position of anus, not yet perforate; *all*, allantois; *am*, amnion; *at*, atrium; *a.e*, amniotic edge; *f.a*, serous membrane; *f.g*, foregut; *l*, lung rudiment; *mes*, mesencephalon; *pa.g*, postanal gut; *pt*, pituitary involution; *rh*, rhombencephalon; *spl*, splanchnopleure of yolk-sac; *t*, thalamencephalon; *th*, thyroid; *V*, ventricle; *v.A*, ventral aorta; *v.m*, remains of velar membrane; *y.s.* cavity of yolk-sac; *y.st*, cavity of yolk-stalk.

The study of this stage should be completed by examining series of sections parallel to the sagittal plane in the head region and interpreting them by what has been made out from the whole embryo and the series of transverse sections. The most instructive sections are those in or close to the sagittal plane. Fig. 238 shows diagrammatically a sagittal section through the whole length of the embryo, but it will of course be understood that, owing to the head of the embryo having come to lie over on its left side while the trunk region retains its original position, a section which is sagittal in the head region will, in actual fact, be practically horizontal in the trunk.

The feature that dominates the section is the cerebral flexure—the strongly marked curvature of the head region towards the ventral side. The brain is of relatively enormous size: a distinct dip in its roof marks the boundary between the thin-roofed rhombencephalon which lies behind it and the region in front of it—the cerebrum—which will give rise to mesencephalon, thalamencephalon and hemispheres.

The next instructive feature brought out by such a section is the general relation of gut to yolk-sac. The rounded head-fold of the splanchnopleure has extended far back so as to floor in the foregut (*f.g*). The velar membrane (*v.m*) has just ruptured so that the foregut communicates in front with what will become the stomodaeum into which also opens the pituitary involution of the ectoderm (*pt*). The floor of the foregut dips downwards to form the rudiments of the thyroid (*th*) and lung (*l*). In a slightly more advanced embryo the two liver rudiments would be seen also as pocket-like outgrowths of the enteric floor in the neighbourhood of the atrial end of the cardiac tube.

The posterior end of the definitive alimentary canal is also becoming folded off from the yolk-sac though the cavity of the yolk-stalk—the communication between the definitive alimentary canal and the cavity of the yolk-sac—is still very wide. The position of the future anal opening is indicated by a thick septum (*a*) composed of fused ectoderm and endoderm. Dorsal and posterior to this the enteron extends back as a blindly ending pocket—the remains of the postanal gut (*pa.g*), while anterior to the anus the enteric floor dips downwards as the rudiment of the allantois (*all*). The latter is covered with a thick layer of mesoderm and bulges into a dilated portion of the splanchnocoele. Towards the front end of the embryo a still more widely dilated portion of the splanchnocoele accommodates the cardiac tube. At its anterior (*v.A*) and posterior ends (*at*) this is ensheathed in the thick mesoderm on the ventral side of the foregut, while its middle portion (*V*) hangs free in the cavity.

Finally the amniotic fold of the somatopleure is seen to extend almost completely over the body of the embryo, the amniotic edge (*a.e*) bounding a comparatively small opening near the tail end.

Having studied in some detail the features characteristic of an individual third-day embryo it will be convenient now to give a general sketch of the chief advances in development which take place during this day.

At the commencement of the day the body of the embryo lay flat along the surface of the yolk: only at its head end was it clearly demarcated from the surrounding blastoderm and this head region owing to the commencing ventral curvature was beginning to lean over on to its left side. During the course of the third day the tucking in of the blastoderm under the definitive body proceeds apace so that the body becomes more and more completely demarcated from the part of the blastoderm forming the yolk-sac wall, and the yolk-stalk becomes correspondingly narrowed. The preponderance of growth activity on the dorsal side which leads to the ventral curvature is during the early hours of the day especially marked in the region of the mesencephalon but as the day goes on becomes very pronounced about the level of the heart and still later in the tail region. Thus the axis of the body develops strong ventral curvature especially marked at three different levels—mesencephalic, cardiac and caudal. Along with this increasing curvature the whole body of the embryo comes to lie over on its left side so that the observer looking down upon the egg from above sees the body of the embryo in profile from its right side.

During the day the embryo becomes ensheathed in the amnion in the manner already described. The vitelline network of blood-vessels attains to its highest development, forming as it does the organ for respiration as well as for absorption of the food and its transport into the body of the embryo. Correlated with the lying of the embryonic body over on its left side the paired venous channels which convey the blood from the vitelline network into the heart gradually lose their symmetry, those of the right side dwindling in size while their fellows show a corresponding increase.

In the brain the main regions become established: the roof of the thalamencephalon and medulla oblongata assume their thin membranous character while the hemispheres bulge out in front of the thalamencephalon. The central canal of the spinal cord becomes reduced to a vertical slit by the thickening of the side walls. The olfactory rudiment makes its appearance: the auditory rudiment becomes converted into the closed pear-shaped otocyst, still however connected with the ectoderm by a solid strand of cells. In the eye the lens thickening has become involuted and converted into a closed vesicle with its inner wall markedly thickened. The optic cup has been completely formed and the retinal layer differentiated from the thin and degenerate pigment layer. In the latter the first deposition of pigment takes place during the later hours of the day.

The definitive alimentary canal is still open towards the yolk-sac over about half its extent but in addition to the foregut there becomes folded off during the course of the third day a considerable extent of hind-gut, the ventral wall of which commences to bulge out to form the rudiment of the allantois towards the close of the day. The hind-gut is still closed posteriorly but the foregut late in the third or during the fourth day becomes thrown into communication with the stomodaeum by rupture of the velar membrane. The pituitary rudiment makes its appearance. The four gill-pouches are formed and reach the ectoderm, the fourth in the closing hours of the day, and the first or it may be the first two become perforate. The thyroid rudiment makes its appearance and during the latter half of the day becomes closed. The pulmonary rudiment develops and becomes constricted off from the pharynx except at its front end. About the beginning of the day the two liver rudiments appear and during its course the process of anastomosis begins between the branches which sprout out from them. During the latter half of the day the pancreatic rudiments make their appearance—first the dorsal, then the left ventral, then the right ventral.

During the course of the day the mesoderm segments increase from about 20 to 25 up to about 40. Early in the day the Wolffian duct becomes tubular and in the latter half of the day it completes its backward growth and reaches the cloaca. The germinal epithelium becomes recognizable.

The skeleton remains throughout the day purely notochordal.

The heart retains its S-shape and during the latter half of the day the atrial septum begins to develop. The two dorsal aortae begin about the commencement of the third day to undergo their fusion to form the definitive unpaired aorta. In addition to the first one or two aortic arches which are already present the third makes its appearance (Fig. 241, A, III, p. 550), then the fourth, and during the latter half of the day the sixth, while the first becomes obliterated. As regards the venous system the most important feature is the assumption of the same general plan of the main trunks as is characteristic of Fishes.

Finally it should be noted that during this day the body of the embryo becomes enclosed within the amnion.

It will be realized even from the bare summary that has been given that the third day of incubation of the Fowl's egg is morphologically the most important of all and the student will be well advised to devote a good deal of time to making a detailed study of embryos of this period.

THE FOURTH DAY OF INCUBATION.—By the end of the fourth day of incubation the blastoderm has spread about half-way round the yolk. The vessels of the vascular area are conspicuous, though it is to be noticed that the terminal sinus is becoming relatively less so than it was during the third day. The folding off of the body of the embryo has progressed greatly. By the extension backwards of the head fold the region of the heart has become floored in on its ventral side. Posteriorly the tail fold is deepening in a similar fashion. Between head fold and tail fold the somatopleure of the embryonic body is prolonged ventralwards into a very short and wide tube—the somatic stalk the wall of which is reflected dorsalwards as the true amnion. The latter is now complete and closely invests the body of the embryo. Lying loosely within the somatic stalk and of much smaller diameter is the splanchnic or yolk stalk—the continuation of the splanchnopleure in a ventral direction as it passes out into the wall of the yolk-sac. The body of the embryo has undergone a great increase in size. The growth of its tissues has been particularly active in its dorsal region and this has led to a continuation of the flexure towards the ventral side which was already well marked in the third day embryo.

An important new feature in the fourth day embryo is provided by the two pairs of limb rudiments each in the form of a dorsiventrally flattened ridge with rounded edge and broad base of attachment to the body. The head of the embryo at once attracts attention by its relatively enormous size. This is due to the relatively immense size of the brain and eyes. We have here to do apparently with a case of the precocious growth in size of organs which in the fully developed condition possess extreme complexity of minute structure. The main regions of the brain can be seen very distinctly: the relatively large mesencephalon with its bulging dome-like roof, the thalamencephalon with the pineal rudiment, the rapidly growing rudiments of the hemispheres, and the hind-brain with its relatively thin and membranous roof. The three main special sense organs are all conspicuous—the olfactory organ, the eye with its choroid fissure and lens, the pyriform otocyst. Arranged in a row ventral to the otocysts are the pharyngeal clefts—three or four in number. In the case of cleft I the ventral part of the cleft is becoming much narrowed by the approach of its anterior and posterior walls. The dorsal end of the cleft on the other hand remains dilated: it corresponds to the spiracle of fish-like forms.

The heart, which forms a large structure lying between the tip of the head and the region of the fore limbs, is still in the form of a coiled tube but the appearance of localized bulgings of its wall foreshadows its division into the various chambers characteristic of the adult. Thus the curve of the tube lying posteriorly and on the right is becoming dilated to form the ventricle: the part morphologically in front of this leading towards the ventral aorta is slightly dilated to form the conus arteriosus, while the curve lying anteriorly and on the left side shows a slight bulging on each side foreshadowing the two auricles. Slight constrictions separate these various bulgings—an atrio-ventricular constriction narrowing the cavity to form the auricular canal, and a less conspicuous one between ventricle and conus.

The general arrangement of the peripheral vessels is intermediate between that of the third day (Fig. 241, A) and that of the fifth day (Fig. 241, B) and need not be described in detail. Aortic arches I and II undergo in turn a gradual process of obliteration while arches IV and VI make their appearance farther back if they have not already done so. It is also during this day that arch V makes its brief appearance.

The allantoic veins, which at first are merely veins of the body-wall, during the fourth day establish their connexion with the allantois, and in the course of the day the right vein disappears.

The allantois itself forms a conspicuous new feature for towards the end of the day it begins to project distinctly from the ventral side of the embryo about the level of the hind limb.

Owing to the increasing size and complexity of the embryo the elementary student will not as a rule prepare complete series of sections later than the third day. He will however find it profitable to have transverse sections through the developing sense organs, sagittal sections through the head, and transverse sections through the posterior trunk region.

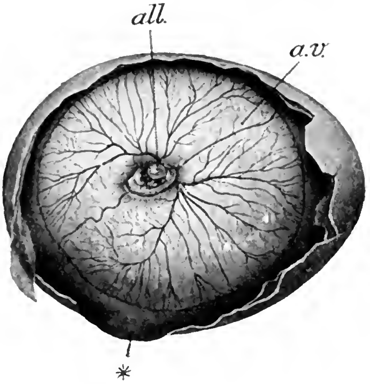


FIG. 239.—Fowl's egg opened at the end of the fifth day. The embryo enclosed in its amnion is sunk down in the centre of the vascular area, the allantois projecting upwards towards the serous membrane—a transparent membrane through which the embryo and allantois are seen. The increasing fluidity of the yolk is shown by the outward bulging of the yolk-sac wall over the broken edge of the shell at the lower side of the figure. The albumen now lies completely underneath the yolk so as to be invisible in a view from above.

a.v, vascular area; *all*, allantois; *, bulging of yolk over broken edge of shell.

From the study of sections the following advances in development during the fourth day may be made out.

In the brain the rudiment of the paraphysis makes its appearance and the pineal outgrowth begins to sprout out into diverticula about the end of the day. The olfactory rudiment becomes connected with the buccal cavity by a slight groove. The rudiments of lagena and recess make their appearance as slight bulgings of the otocyst wall. The cavity of the lens becomes obliterated by the growth of its inner wall: pigment becomes conspicuous in the outer wall of the optic cup: the layer of nerve fibres in the retina becomes recognizable: mesenchyme begins to invade the cavity of the optic cup and about the end of the day also intrudes between the lens and the ectoderm.

The post-anal gut becomes reduced to a solid strand of cells and finally disintegrates. The yolk-stalk becomes narrowed to a fine tubular channel. The gall-bladder begins to dilate towards the close of the day: the dorsal pancreas begins to develop outgrowths: and the rudiments of the caeca make their appearance.

The mesoderm segments increase in number to about 50. Early in the day, if it has not done so already, the Wolffian duct opens into the cloaca. The mesonephric glomeruli begin to appear and the tubules become elongated and coiled. In the posterior region of the mesonephros secondary tubules make their appearance while in the anterior region a process of degeneration becomes apparent. During the second half of the day the ureter begins to sprout out from the Wolffian duct and about the end of the day the rudiments of Müllerian ducts and of the metanephric units may become recognizable.

In the heart the atrial septum becomes completed about the end of the fourth day and the endothelial cushions begin to develop.

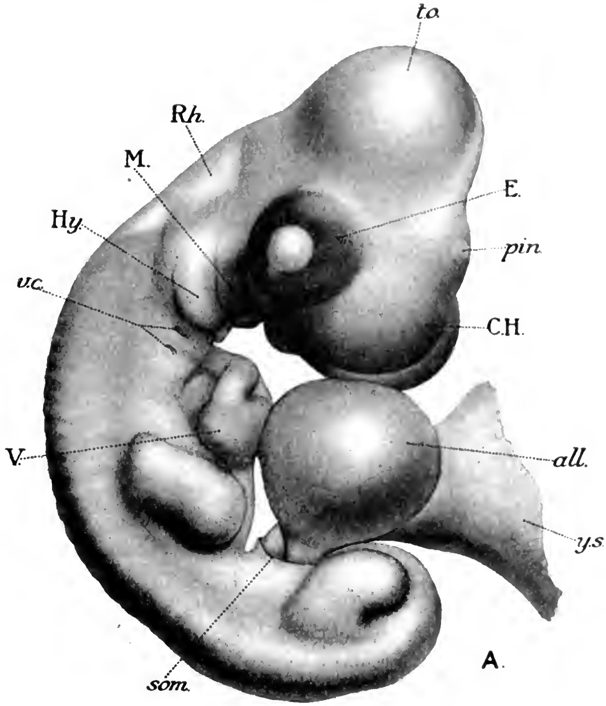


Fig. 240.—Chick extracted from the egg at about the middle of the fifth day of incubation.

all, allantois; *C.H*, cerebral hemisphere; *E*, eye; *Hy*, operculum; *M*, mandibular arch; *pin*, pineal rudiment faintly visible as slight elevation on roof of thalamencephalon; *Rh*, thin roof of rhombencephalon; *som*, edge of somatopleure cut through where it becomes reflected back over the body of the embryo to form the amnion; *t.o*, roof of mesencephalon (optic lobe); *V*, ventricle; *v.c*, visceral clefts III and IV; *y.s*, yolk-sac.

Fifth Day.—The progress in development during the course of the fifth day is illustrated by Figs. 239-241. The albumen has so shrunk in volume as to be no longer visible in a view of the opened egg from above: the yolk has become extremely fluid: the vascular area has increased considerably in size. The allantois is now a conspicuous object and the mesoderm covering its surface is beginning to develop blood-vessels. The head of the embryo is, as before, of relatively very large size: the flexure in the region of the mesencephalon is still more pronounced. The operculum (Fig. 240, *Hy*) is conspicuous, growing back from the hyoid arch over the posterior visceral clefts. The limb rudiments now project freely though their form is that of simple flippers without any of the peculiarities of the leg or wing of the Bird. The body of the embryo is floored in on its ventral side completely but for the rounded opening (*som*) along whose lips the somatopleure is continued into the amnion and through which emerge the narrowing yolk-stalk and the stalk of the allantois.

The study of the living embryo *in situ* shows the general plan of the blood system to be as is shown in Fig. 241, B. The heart still betrays its tubular origin though the chambers are clearly recognizable as dilatations. Three aortic arches (III, IV and VI) are distinctly visible and occasionally the fleeting vestige of the penultimate arch as in the specimen represented in the diagram. In front of the aortic arches the ventral aorta is seen extending forwards as the ventral carotid (*v.c*): the pulmonary artery (*p.a*) passes back from the sixth arch. Dorsally the aortic root extends forwards into the head as the dorsal carotid artery (*d.c*). A little distance behind the liver the vitelline artery (*v.a*) leaves the dorsal aorta and farther back the allantoic artery (*a.a*) a branch of which, the iliac artery, passes to the hind limb.

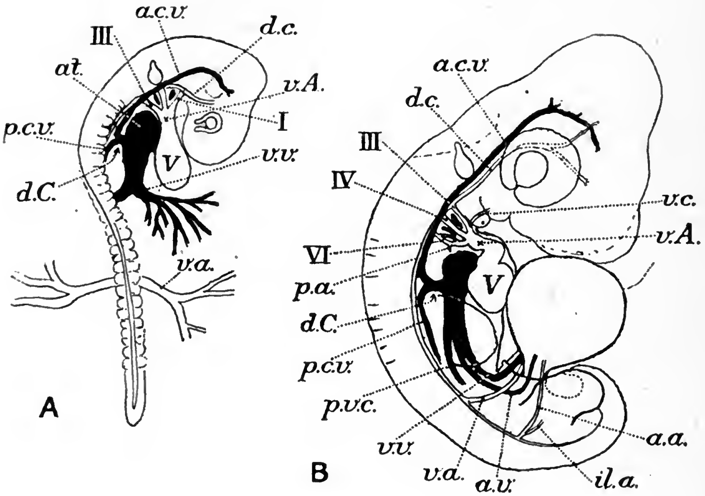


FIG. 241.—Diagram showing the main parts of the vascular system as seen in a Fowl embryo during the third day (A) and the fifth day (B).

a.a, allantoic artery ; *a.c.v*, anterior cardinal vein ; *at*, atrium ; *a.v*, allantoic vein ; *d.C*, duct of Cuvier ; *d.c*, dorsal carotid ; *il.a*, iliac artery ; *p.a*, pulmonary artery ; *p.c.v*, posterior cardinal vein ; *p.v.c*, posterior vena cava ; *v.A*, ventral aorta ; *v.a*, vitelline artery ; *v.c*, ventral carotid ; *v.v*, vitelline vein ; I-VI, aortic arches.

In the venous system the duct of Cuvier is seen, continuous at its dorsal end with the anterior and posterior cardinal veins. The former (*a.c.v*) branches through the head: the latter (*p.c.v*) can be traced dimly back into the region of the kidney. The main blood-stream to the heart comes from the vitelline vein (*v.v*) and is joined within the substance of the liver by the blood from the left allantoic vein (*a.v*) and the posterior vena cava (*p.v.c*).

Ignoring the vitelline and allantoic vessels which are clearly adaptations to the peculiar conditions of the developing embryo the main plan of the blood system is seen to be clearly the same as is characteristic of Fishes.

By cutting off the head after fixing and viewing it from below (Fig. 245, A) the modelling of the face can be studied. The fronto-nasal process (*f.n*) is bounded on each side by the shallow oro-nasal groove connecting it with the buccal cavity. The ridge forming the outer boundary of the olfactory organ is demarcated from the maxillary process by a faint transverse groove passing outwards towards the eye—the **lachrymal groove**. Posteriorly the stomodaeal opening is bounded by the mandibular ridge with a distinct break in the middle line between the two mandibular arches.

Of other developmental features of the fifth day we may note the following. The first indications of turbinals appear on the mesial wall of the olfactory organ, and of semicircular canals in the otocyst. The optic stalk becomes solid: the rudiments of the ocular muscles become recognizable. The pituitary body begins to form outgrowths. The rudiments of thymus and bursa fabricii make their appearance: the bronchi begin to develop branches. The formation of new mesonephric tubule rudiments comes to an end and the mesonephros begins to show signs of functional activity. The atrial septum develops secondary perforations. The fourth aortic arch on the left side, and the portions of aortic root immediately behind the third arch undergo reduction. The horizontal septum of the ventral aorta begins to extend back into the conus and the anterior portions of the posterior cardinal veins begin to undergo atrophy.

Sixth Day.—During the sixth day of incubation the body of the embryo increases rapidly in size and in correlation with this it dips down into the very fluid yolk, pushing the splanchnopleure of the yolk-sac wall in front of it, so that it is almost hidden from view when the egg is first opened. The amnion is now raised up from the body of the embryo by a marked accumulation of amniotic fluid (Fig. 242). The allantois has increased greatly in size and in the natural condition is flattened mushroomwise against the inner surface of the serous membrane. In the embryo excised as directed on p. 513 it will be seen that the somatopleure of the embryonic body is completely closed in ventrally except for a small circular space round which it is reflected outwards in a funnel-like fashion and continued into the thin membranous amnion. Through the funnel-like opening a slender probe can be passed from the extra-embryonic coelomic space beneath the serous membrane into the portion of coelome enclosed within the body of the embryo which will become the definitive splanchnopleure or body-cavity. Through the opening there pass out the stalks of the yolk-sac and the allantois (Fig. 246, B) each conspicuous owing to its large blood-vessels. The peripheral distribution of the vitelline and allantoic vessels shows a characteristic difference (Fig. 242)—the vitelline network (vascular area) terminating, in the now greatly reduced terminal sinus at a considerable distance from the distal pole of the yolk-sac while on the other hand the allantoic network is most richly developed on the distal side of the allantois (p. 474).

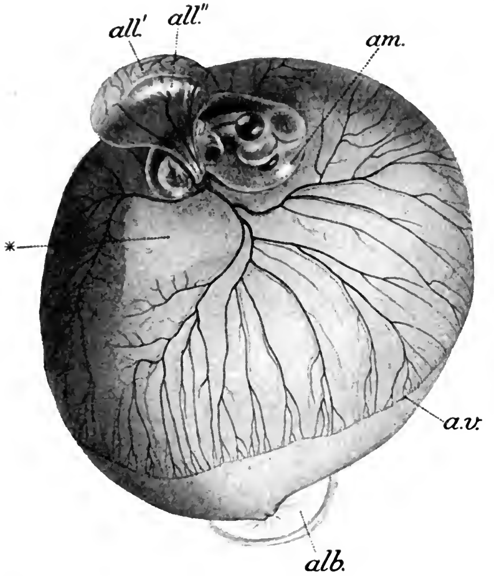


FIG. 242.—Common Fowl. View of contents of the egg-shell extracted at the end of the sixth day of incubation. The serous membrane has been removed so as to allow the allantois to be displaced slightly in order to give a clearer view of the body of the embryo contained within its amnion.

a.v, edge of vascular area; *alb*, remains of albumen; *all'*, outer wall of allantois; *all''*, inner wall of allantois; *am*, amnion; *, portion of vascular area lying, in the natural position, beneath the head of the embryo and free from blood-vessels.

The body of the embryo now for the first time begins to show indications of bird-like form, and faint traces of digits and of feather-rudiments may become apparent about the end of the day.

In the eye the rudiment of the pecten, which first became recognizable during the fourth day, is now conspicuous as an ingrowth of mesenchyme through the choroidal fissure, bounded on each face by the inflected lips of the fissure.

The tongue begins to project and the thyroid becomes constricted off from the pharynx. The oesophagus towards the end of the day loses its cavity; the dilatation of the gizzard becomes evident; the intestine begins to grow actively in length (Fig. 246, B). The three pancreatic rudiments become continuous with one another.

The muscles of the body begin to exhibit contractility, the trunk occasionally showing twitches of ventral flexure. The ureter develops outgrowths to form the primary collecting tubes of the metanephros about the beginning of the sixth or the end of the fifth day and the terminal part of the duct of the opisthonephros may become incorporated in the cloaca so as to give the ureter its independent opening. About this time the first indications of sexual differentiation become recognizable, the genital strands beginning to show signs of degeneration in the female.

The main portions of the skeleton become laid down in prochondral tissue and, towards the end of the day, in cartilage.

The heart begins to assume its definitive external form; the ventricular septum develops and the conus septum begins to do so. The fourth aortic arch becomes obliterated on the left side.

Seventh Day (Figs. 243 and 244).—The mushroom-shaped allantois is spreading actively all round beneath the serous membrane. The amnion is beginning to show waves of contraction passing along its wall. The brain and eyes and consequently the head as a whole are of relatively enormous size. In sections the roof of the fourth ventricle is found to be developing irregular folds in which the vessels of the choroid plexus will appear. All three turbinal rudiments are present in the nose. The crop is beginning to expand. The visceral clefts are all closed. The glands of the stomach are beginning to make their appearance as rudiments. The cavity of the enteron disappears for some distance forwards from the point of origin of the allantois. The Müllerian ducts may show incipient asymmetry. The notochord is beginning to be constricted by the vertebrae. The first traces of ossification are making their appearance, especially in the skeleton of the limbs.

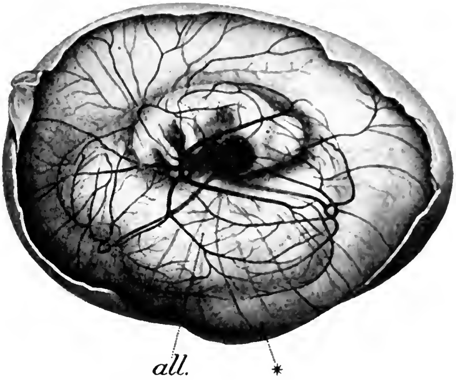


Fig. 243.—Fowl's egg opened during the seventh day. The body of the chick is seen dimly through the highly vascular allantois. The vessels of the allantois can be distinguished from those of the vascular area by their turning back at the edge of the allantois while those of the vascular area pass onwards uninterruptedly. The highly fluid character of the yolk is shown by the yolk-sac wall bulging outwards over the broken shell at the point marked *.

all, allantois.

The septum of the conus arteriosus is complete and the muscular coat extends into it from each side: the pocket-valves are becoming excavated. The fourth aortic arch on the left side has disappeared while the portion of aortic root between arches III and IV on the right side, and behind arch III on the left side, are becoming obliterated.

Eighth Day.—The movements of the amnion now reach their highest degree of activity. The fronto-nasal process (Fig. 245, C) is growing out to form the pointed beak while the lower jaw is taking a similar pointed form, the two mandibular arches being now continued into one another ventrally without a break. The rudiments of feathers are beginning to make themselves apparent.

In the brain the cerebellum is becoming folded on itself so as to bulge outwards. The oro-nasal grooves are covered in to form the tubular communication between nose and mouth. The lachrymal groove is no longer visible: the lachrymal glands are developing as solid ingrowths of ectoderm. The pituitary body now forms a rounded mass of branched glandular tubes lying between the trabeculae and communicating with the buccal cavity by a narrow tubular duct opening immediately over the glottis. The air-sac rudiments make their appearance on the surface of the lung (Fig. 246, C, *a.s*).

The mesonephric tubules have been growing actively up till now: the metanephric units are making their appearance: the Müllerian duct reaches the cloaca if it has not already done so although no actual communication is established until about six months after hatching.

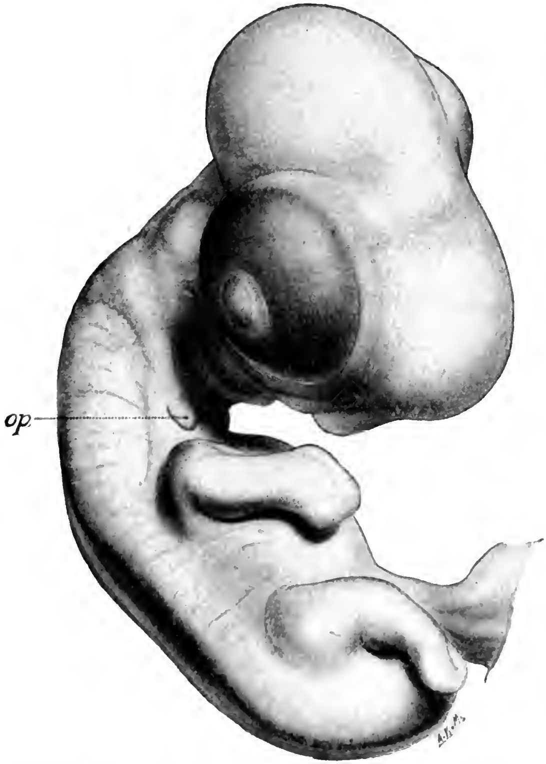


FIG. 244.—Chick extracted from egg during seventh day showing operculum (*op*).

Ossification becomes conspicuous in the limb-bones and the investing bones of the head. The keel of the sternum forms an ossification distinct from the two lateral rudiments of the body of the sternum.

The terminal sinus of the vascular area has disappeared. The septum of the conus is now completely traversed by muscle so that both aortic and pulmonary cavities are completely ensheathed by muscle. The splitting apart of the two vessels is inaugurated by the appearance of a longitudinal incision along the line of attachment of the septum.

As regards the further progress of development the following approximate times may be mentioned.

About the ninth day the oesophagus gradually becomes patent again. On the tenth day the arterial arches have practically assumed the definitive condition and the metapodial skeleton is ossified.

Up to about the eleventh day the contractions of the amnion remain very active, but thereafter they gradually become more gentle until during the closing days of incubation they stop. The mesonephros also attains to its maximum activity and there commences the process of degeneration which will continue till the time of hatching: tubules have developed throughout the length of the metanephros.

By the twelfth day the duct of the pituitary body has become reduced to a solid cellular strand: the exact time at which this happens is very variable; it may be as early as the sixth or seventh day. The lachrymal duct, which originated as a solid ingrowth of ectoderm along the line of the lachrymal groove, now becomes tubular. About the twelfth or thirteenth day the cavity reappears over the greater part of the rectum except just at the hinder limit of the occluded portion immediately in front of the allantois. Here the cavity remains blocked till nearly the time of hatching.

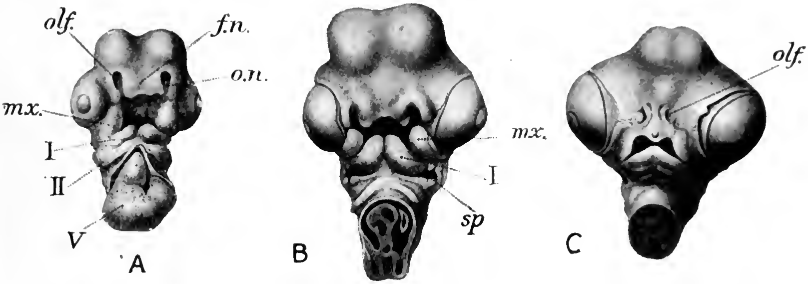


FIG. 245.—View of head of Fowl embryo as seen from below. (After Duval, 1889.)

A, five days; B, six days; C, eight days. *f.n*, fronto-nasal process; *mx*, maxillary process; *olf*, olfactory opening; *o.n*, oro-nasal groove; *sp*, hyomandibular cleft; *V*, ventricle; I, II, visceral arches.

About the thirteenth day the cartilaginous skeleton is complete and the rudiments of claws begin to develop.

About the fifteenth day the Eustachian valve develops in the heart.

By the sixteenth day the albumen has all gone and the yolk-sac wall becomes completed ventrally.

About the nineteenth day the yolk-sac becomes enclosed within the body-wall and the partition between mesenteron and proctodaeum breaks down so that the alimentary canal communicates with the exterior.

About the twentieth day the umbilicus closes. The violent struggles of the young bird cause its beak to penetrate the air-space: its lungs are filled with air: its further struggles cause its beak to break the shell and it emerges, leaving behind the broken shell lined with the cast-off allantois and serous membrane.

Correlated with the process of hatching important changes take place in the circulation: the gap in the atrial septum (foramen ovale) becomes closed so that the blood arriving in the right auricle can only reach the left auricle by the circuitous route through the right ventricle and pulmonary circulation, and the allantoic vein, duct of Botallus, and ductus venosus in the liver become obliterated.

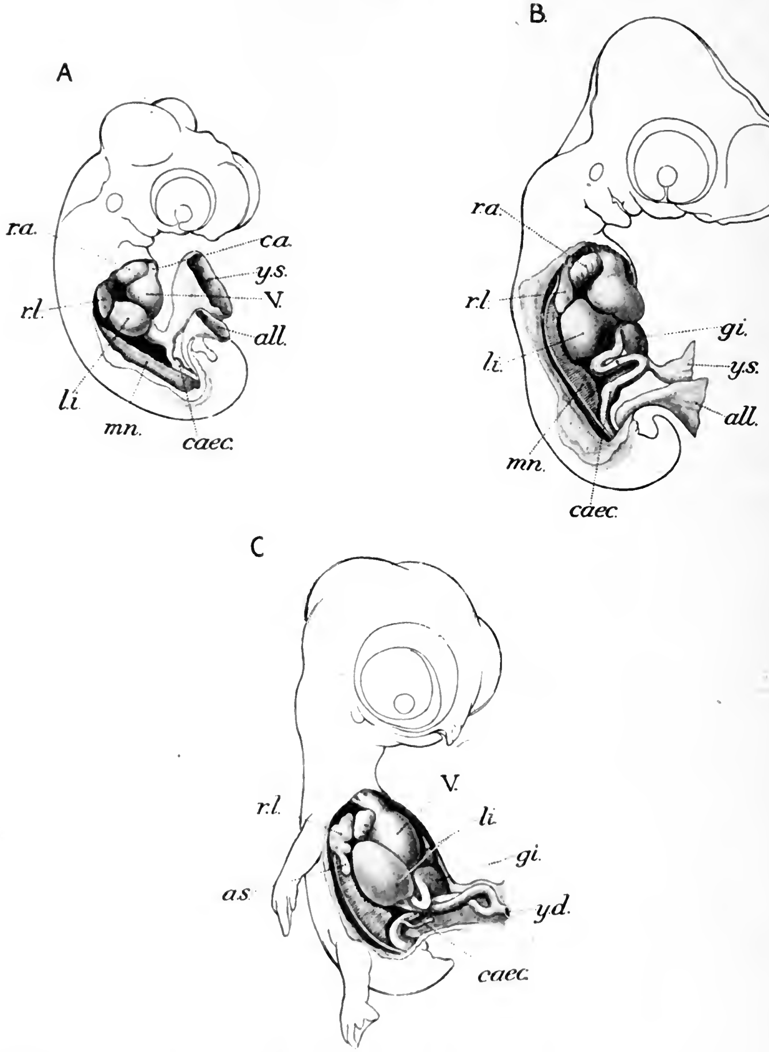


FIG. 246.—Dissections from the right side showing the general arrangement of the viscera of a Fowl embryo at the end of the fifth (A), sixth (B), and eighth (C) days of incubation. (After Duval, 1889.)

a.s, abdominal air-sac; *all*, allantois; *c.a*, conus arteriosus; *caec*, caecum; *gi*, gizzard; *li*, liver; *mn*, mesonephros; *r.a*, right auricle; *r.l*, right lung; *V*, ventricle; *y.d*, yolk-stalk; *y.s*, yolk-sac.

LITERATURE

Duval. Atlas d'Embryologie. Paris, 1889.

Foster and Balfour. The Elements of Embryology. Second Edition, edited by A. Sedgwick and W. Heape. London, 1883.

Keibel und Abraham. Keibels Normentafeln zur Entwicklungsgeschichte der Wirbeltiere, II. Jena, 1900.

Lillie. The Development of the Chick. New York, 1908.

Marshall. Vertebrate Embryology. London, 1893.

Patterson. Biol. Bulletin, xiii, 1907.

Patterson. Journ. Morph., xxi, 1910.

The most complete account of the development of the Fowl is that by Lillie. It, and Duval's Atlas if a copy can be obtained, for it is unfortunately out of print, should form part of the equipment of every embryological laboratory.

CHAPTER XI

HINTS REGARDING THE PRACTICAL STUDY OF THE EMBRYOLOGY OF THE VARIOUS TYPES OF LOWER VERTEBRATES

AMPHIOXUS.—The interest and importance of *Amphioxus* to the student of Vertebrate morphology are due to the fact of its position near the base of the Vertebrate phylum. It is true that in its adult structure *Amphioxus* is intensely specialized in correlation with its burrowing habit. Further, it is necessary to recognize that a burrowing like a pelagic mode of life, in which the environmental conditions are comparatively uniform, is likely to lead to a kind of fixing of the organization which will be fatal to its adaptability to new sets of conditions and consequently to its capacity for evolving along new lines. We must therefore regard it as improbable that the Vertebrata passed through an ancestral condition of specialization for a burrowing habit and the specialized features of the later stages of the life history of *Amphioxus* cease on that account to have a phylogenetic interest. The main interest to the Vertebrate morphologist lies therefore in the earlier stages before the specialization of the adult has developed—in such features as segmentation, gastrulation and the origin of the main systems of organs. And the interest of these stages is heightened by the fact that food yolk—that potent disturbing factor—is present to a far smaller extent in the egg of *Amphioxus* than in that of any other of the lower Vertebrates.

Unfortunately the known localities in which fresh embryological material of *Amphioxus* can be obtained in abundance are still few, and in most laboratories recourse must be had to preserved material purchased from supply stations such as the Naples aquarium.

The best locality so far known for obtaining developmental stages of *Amphioxus* is the pantano or shallow lagoon at Faro near Messina. Here the spawning takes place each evening, when conditions are favourable, during the summer months from April to July. The eggs pass to the exterior through the atriopore. If in a dish on board a boat the eggs are liable by its movements to become distributed through the water and they are then apt to become drawn by the inspiratory current in amongst the buccal cirri. When the

Amphioxus becomes inconvenienced by such entangled eggs amongst the cirri it is able suddenly to reverse the respiratory current so as to clear them away, and in this way there is produced a misleading appearance as if the eggs were being laid through the mouth. The first meiotic division has been completed before oviposition while the second is in the spindle stage at this period. Fertilization probably takes place immediately, spermatozoa being disseminated through the water.

It is best (Cerfontaine, 1906–7) to bring the adults into the laboratory and wait until they spawn which operation may be considerably delayed. To a dish of pure sea-water is added a little sea-water containing sperm then the eggs, collected with a pipette as soon as extruded, are added.

Batches of eggs are fixed periodically, preferably in strong Flemming's solution or Hermann's solution. After dehydration they are placed in a mixture of 2 parts clove oil and 1 part collodion in which they may be kept indefinitely. For examination whole the egg or embryo is placed on a slide or coverslip in a drop of the clove-oil-collodion. After the specimen has been arranged in the desired position by means of needles a drop of chloroform is applied in order to cause the collodion to solidify. The whole is then cleared with cedar oil and mounted in canada balsam. For the preparation of sections the procedure is similar, only in this case the slide or coverslip should be coated with paraffin as a preliminary to allow the collodion block to become detached, and the latter should be embedded in paraffin.

PETROMYZON.—The various species of Lamprey make their way up streams to suitable gravelly spots for spawning in the spring or early summer (April, May, in the northern hemisphere). Material for embryological study is best got by "stripping" the ripe males and females *i.e.* by passing the hand back along the body with gentle pressure so as to force out the eggs or sperm. The gametes from the male and female are collected separately in two small dishes: they are then mixed together, stirred gently with a feather, and water added. This "dry" method gives a smaller proportion of unfertilized eggs than when the eggs are received from the fish directly into water (Herfort, 1901). As fixing agent the ordinary corrosive sublimate and acetic acid is quite satisfactory.

MYXINOIDS.—The only Myxinoid eggs that have been obtained in any numbers are those of *Bdellostoma* which are dredged near Monterey, California, on shelly and gravelly bottom at a mean depth of about 12 fathoms (Bashford Dean, 1899). Much still remains to be done in working out the details of their development but it is clear that this is of a highly peculiar and specialized type.

ELASMOBRANCHII.—The eggs are fertilized in the upper part of the oviduct. They may traverse the oviduct comparatively rapidly and be laid as in Birds at an early stage of development [*Chimaera*. Scylliidae, *Cestracion*, *Raia*] or they may remain in the oviduct for a prolonged period and the young born in an advanced stage [*Notidanus*, *Mustelus*, *Galeus*, *Carcharias*, *Zygaena*, *Lamna*, *Alopias*, *Cetorhinus*, *Acanthias*, *Scymnus*, *Squatina*, *Torpedo*, Trygonidae, Myliobatidae]. Amongst the viviparous Elasmobranchs preserved developmental stages of *Torpedo* (Fig. 247) may be obtained from Naples, and of *Acanthias* from various marine laboratories.

Amongst the oviparous forms certain species of Skate (*Raia*) are used as food-fishes and their eggs can frequently be obtained in quantity at trawling centres. In such cases arrangements can be made with local fish-dealers to send on by post the " skate-purses " taken from the oviducts when the fish are cut up.[1] The eggs of the different species differ in size and in the characters of the shell—shape, colour, degree of translucency (Williamson, 1913). Of the European species *R. batis* is the most convenient species to use; the normal period of spawning is from December to April but the retarding effect of the low temperature is so great that December eggs are practically overtaken in their development by the April eggs. The complete period of development is roughly 20 months, most of the eggs hatching about August.

The eggs should be posted in damp seaweed. On arrival the soft sticky marginal zone of the shell, which separates off except at one end and serves to anchor the egg to the sea-bottom, is removed, and the date is marked in ink with a wooden style upon the flat portion of shell between the two horns.

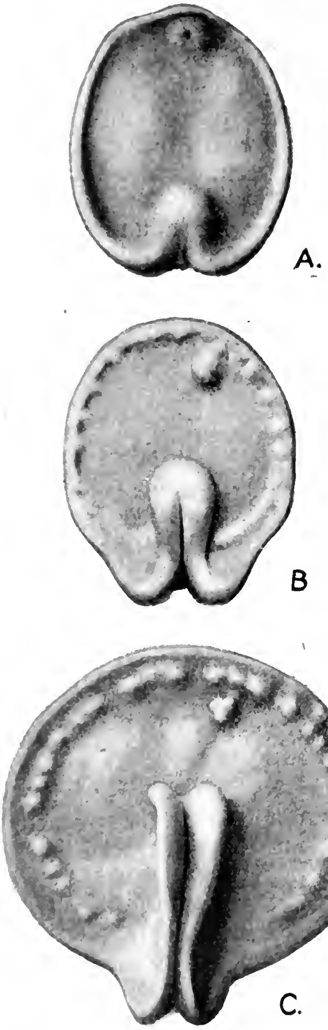


FIG. 247.—Blastoderm of *Torpedo* with medullary folds (× 18). (After Ziegler, 1892.)

A, stage four (Scammon, 1911); B. stage six; C, stage ten. The rounded projection near the anterior edge of the blastoderm is the bulging roof of the segmentation cavity. In C the blood-islands form a row of conspicuous elevations of the surface of the blastoderm parallel to its edge.

---

[1] Jamieson observed out of many thousands of eggs only one case of the inclusion of two eggs within a common shell.

For hatching boxes it is convenient to take ordinary fish boxes freely perforated with auger holes, provided with a cross partition in the centre, and pitched inside and out to discourage the growth of seaweeds. The hatching boxes are moored afloat in pure sea-water within a breakwater or other shelter. About 20 eggs are placed in each compartment.

On alternate days the boxes are drawn a few times backwards and forwards through the water to dislodge any sediment that may have accumulated. Once a week they are hauled out of the water and each egg-shell tested by rubbing the finger over its surface. If a slippery mucus-like layer has developed on its surface the egg is useless and should be got rid of.

When the egg has reached the desired period of development it is removed from the water, placed in a horizontal position with the more strongly convex side below and opened by carefully removing the greater part of the less convex side of the shell. The isolated piece of shell must be lifted off very carefully as the albumen is very adhesive and the vitelline membrane extremely delicate.

In the early stages the embryo is almost invisible in the fresh state so the egg, still held carefully in a horizontal position, is gently submerged in fixing fluid. The blastoderm then comes into view and after a short time may be excised and floated into a watch-glass to complete fixation and the subsequent processes.

In later stages (Fig. 248) where the body of the embryo is constricted off from the yolk-sac, it is narcotized by submersion in sea-water containing 3% alcohol and then the yolk-stalk is ligatured with thread and the embryo excised for further treatment.

Embryological material of the Sharks is to be preferred to that of the Skates or Rays on account of their less specialized character but unfortunately it is more difficult to obtain in quantity. Small sharks of the genus *Scyllium* and allied genera occur commonly round the shores of the various continents and their eggs may be found attached to seaweed at extreme low tides.

On the British coasts a well-known spawning ground for *Scyllium canicula* exists at Careg Dion about 2½ miles from Beaumaris on the Anglesea side of the Menai Straits in between 3 and 4 fathoms of water and in spots not exposed to strong tidal currents.[1] The eggs are deposited usually in the morning, the shorter stouter pair of filaments which issue first from the cloacal opening being trailed about amongst tufts of the seaweed *Halidrys siliquosa* until they become entangled when the fish swims round so as to wind the elastic filaments firmly amongst the seaweed. The eggs can only be obtained at very low and specially favourable spring tides and as White finds at one time embryos of all stages of development it would appear that oviposition is not limited to any definite season.

Scyllium not infrequently deposits its eggs in aquaria and at the

---

[1] For the details in regard to this locality I have to thank Professor Philip J. White of Bangor.

Berlin Aquarium it has been observed that pairs of eggs were deposited at intervals of about ten days. The methods of technique mentioned in connexion with the Skate are also applicable to the eggs of *Scyllium*.

It should not be forgotten that, as mentioned earlier in this volume, one of the greatest desiderata in Vertebrate embryology is an oviparous shark with eggs of small size.

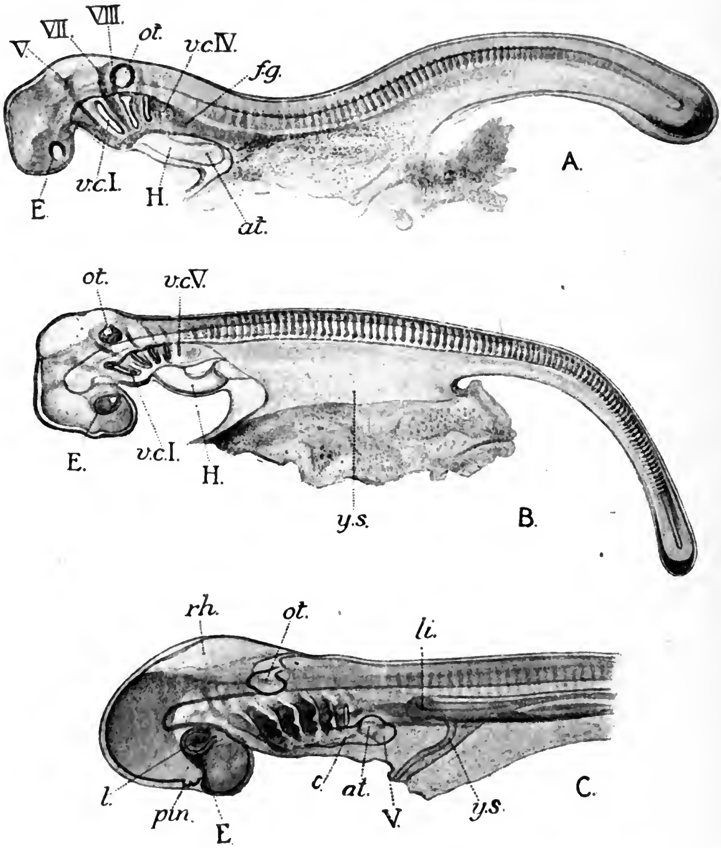


FIG. 248.—*Raia batis*, embryos.

at, atrial portion of heart; *E*, eye; *c*, conus; *f.g*, foregut; *H*, heart; *l*, lens; *li*, liver; *ot*, otocyst; *pin*, pineal organ; *rh*, thin roof of fourth ventricle; *v.c.*I, etc., visceral clefts; *y.s*, yolk-stalk; V, VII, VIII, cranial nerves.

TELEOSTOMI.—The most archaic and therefore the morphologically most important surviving member of this group is *Polypterus* and strenuous efforts have been made to obtain developmental material. Harrington lost his life on an expedition to the Nile with this object. Budgett made two expeditions to the Gambia, one to Nigeria and the Nile, and a fourth to the Niger Delta with the same object in view. The three first expeditions were fruitless but on the fourth he was fortunate enough to obtain ripe males and females and to accomplish fertilization of a number of eggs. Unhappily Budgett did not live to work out this precious material, falling a victim to blackwater fever soon after his return to England. The Budgett material has been investigated (Graham Kerr, 1907) but further material is urgently needed to work out much of the detail.

On the Gambia and on the Upper Nile Budgett found females with eggs in the oviducts during July and August; in the Niger Delta during August and September. During these periods he found that at any one time only a small proportion of males had active motile spermatozoa in their urinogenital sinuses so that it looks as if the actual breeding season of each individual male were very short. The fertilizations which were successful were effected with teased-up testis, the tubules being much distended and the sperm clear instead of opaque as it frequently is. In some cases Budgett found that eggs from the splanchnocoele gave a larger percentage of successes than those from the oviduct.

The fertilized eggs adhered strongly to the bottom of the dish and this supports the statements made by the natives that in nature the eggs are attached to sticks and stems of plants under the water.

Nothing is known regarding the development of the other surviving Crossopterygian—*Calamichthys*.

Of the Actinopterygian ganoids, whose haunts are more accessible and less unhealthy than those of *Polypterus*, the development has been worked out more or less completely in the case of each of the main types—the Sturgeon (*Acipenser*), the Garpike (*Lepidosteus*), and the Bowfin or Dogfish (*Amia*).

At the large fishery stations such as those on the Elbe or Delaware Rivers ripe Sturgeons are caught during a brief season on their way into the river to spawn. The eggs and spermatozoa may be obtained by "stripping" the fish *i.e.* by firm pressure passed backwards along the sides of the body, or by opening the fish. The eggs are immediately placed in a dish and a little of the sperm mixed with a small volume of water is poured over the eggs, the whole being stirred gently for about ten minutes. They are then distributed in a single layer over the bottom of a submerged shallow tray made with coarse mosquito netting to which the eggs adhere firmly within twenty minutes. The trays are then placed in wooden hatching boxes with gauze ends and moored in the river so that they are traversed by a constant current. The dark-coloured somewhat tadpole-like larvae hatch out in from three to six days.

Lepidosteus (Dean, 1895) breeds at Black Lake, N.Y., normally between the middle of May and the middle of June, the eggs being fertilized at the moment of spawning and being distributed over the bottom in shallow water, adhering firmly to stones and other solid objects. For laboratory purposes it is best to employ artificial fertilization as in the case of the Sturgeon.

Amia (Dean, 1896) spawns at Black Lake during the latter half of April or May. The eggs are deposited on a compact site over which the vegetation is pressed aside so as to form a clear space with about a foot of water over it. The eggs, fertilized at the moment of laying, adhere to roots or other portions of the water-plants. The rate of development as in other cases varies greatly with the temperature and from four days to fourteen have been observed to elapse between the deposition of the eggs and their hatching.[1]

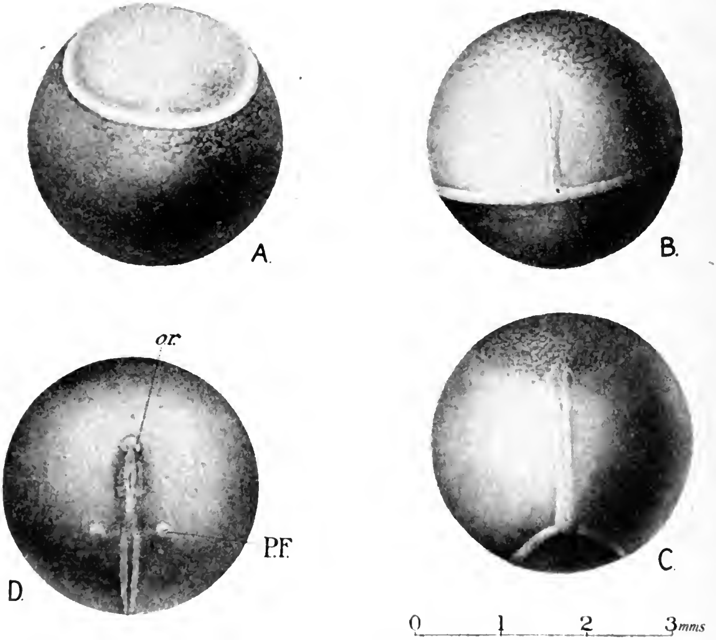


FIG. 249.—Stages in the development of *Symbranchus*. (After Taylor, 1914.) *o.r*, optic rudiment; *P.F*, pectoral fin rudiment.

Of Teleostei (Figs. 249 and 250) by far the most convenient for systematic laboratory work are the Salmon (*Salmo salar*) and the Trout (*S. fario*), eggs of which can be obtained in quantity from the various hatcheries. The eggs obtained by "stripping" are fertilized artificially and may then be sent by post packed in damp moss. Small hatching boxes suitable for laboratory use can also be purchased.[2]

The eggs and larvae of marine Teleosts are often obtained in great

---

[1] Excellent developmental material of *Lepidosteus* and *Amia* may be obtained from the Woods Hole Laboratory or from Mr. J. C. Stephenson, Washington University, St. Louis.

[2] *E.g.* from the Solway Fisheries Co., Dumfries, Scotland.

numbers in the tow-net but these are not so convenient for investigation on account of their reduced size. As there is little doubt that the Teleostei have been evolved out of ancestral forms with large eggs investigations are particularly desirable on those teleosts, mostly freshwater forms inhabiting warm climates, in which the large size of the egg has been retained. There is an important field for investigation in the embryology of tropical freshwater fishes. Of individual families the Siluridae, Characinidae and Gymnotidae call especially for investigation.

Dipnoi.—The Lungfishes form a group of much importance to the Vertebrate morphologist on account of, on the one hand, their great antiquity and the retention of many archaic features in their organization and, on the other hand, of the fact that they present to us foreshadowings of various features which become prominent characteristics in the tetrapoda or terrestrial animals. A knowledge of their embryology consequently became one of the great desiderata of Vertebrate Embryology. The first discovered of the three surviving representatives of the group—*Lepido-*

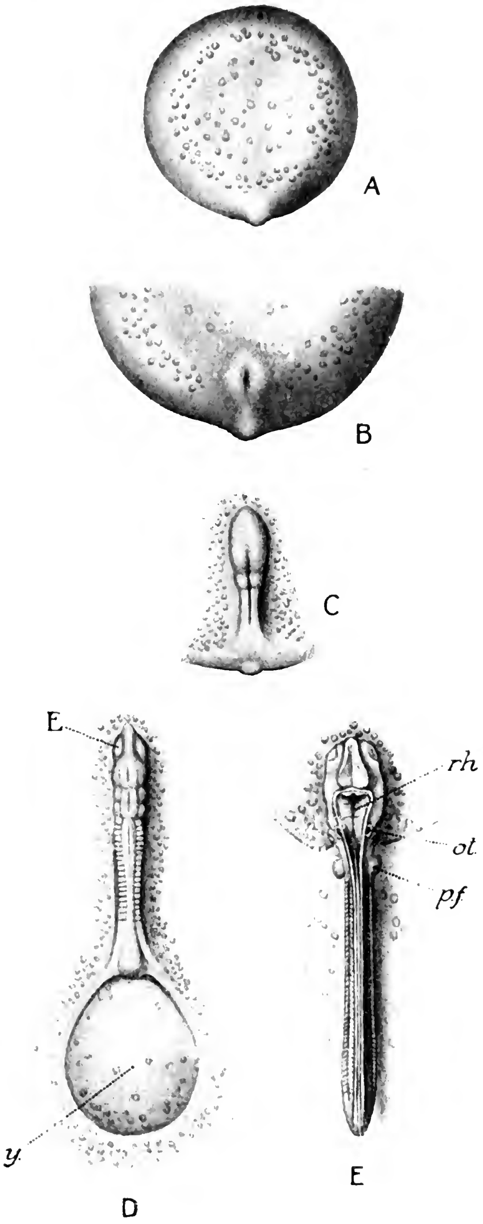


Fig. 250.—Blastoderms and embryos of Trout (*Salmo fario*). (After Kopsch, 1898.)

E, eye; *ot*, otocyst; *p.f*, pectoral fin; *rh*, rhombencephalon; *y*, exposed surface of yolk.

siren—remained unknown so far as its development was concerned until 1896 when Graham Kerr succeeded in obtaining abundant embryological material in the Gran Chaco of South America.

The developmental stages of *Protopterus*, the next representative of the group to become known to science, were first obtained on the Gambia River by Budgett who had taken part in the Lepidosiren expedition a few years earlier. *Ceratodus*, the last of the surviving genera to become known in the adult condition, was the first to be made known embryologically by Caldwell and Semon as already mentioned (p. 435).

The Lung-fishes like other animals living under similar conditions breed at the commencement of the rainy season (*Protopterus*, Gambia, August; *Lepidosiren*, Chaco, November but incidence of rainy season irregular and may be delayed—till *e.g.* June—or omitted altogether; *Ceratodus*, September to December). In the case of *Ceratodus* the eggs are scattered loosely about amongst the water plants, while in *Protopterus* and *Lepidosiren* they are deposited in a special burrow at the bottom of the swamp where they are guarded by the male parent.

Dipnoans live well in captivity and there is little doubt that it will be found easy to induce them to breed by using similar methods to those described under the heading Amphibia. It is particularly desirable that this should be done in the case of *Lepidosiren* on account of the large size of its histological elements which make it a peculiarly suitable type for the investigation of various problems of histogenesis.

The eggs of Dipnoi, especially of *Lepidosiren*, are of large size and this makes it especially advisable to use celloidin in addition to paraffin methods of embedding. When paraffin is used it is necessary to remove the egg envelope by slitting it up with fine scissors, care being taken to keep the point of the scissors close to the envelope so as to avoid injury to the surface of the egg.

Corrosive sublimate and acetic acid is a good stock fixing agent. For stages before hatching 10% formalin is convenient.

AMPHIBIA.—The most easily obtained embryological material is that of the common Frogs of the genus *Rana* the masses of spawn of which are familiar objects in pools during the early weeks of spring in temperate climates. The exact time differs with climate and also with species, some species such as *R. esculenta* in Europe and *R. catesbiana* in North America lagging several weeks behind the others. The spawn, fertilized as deposited in the early morning, may conveniently be kept during its development in earthenware pans. The water should be left stagnant and unchanged during the period prior to hatching as under these circumstances the spawn is less liable to be attacked by fungus but the hatched larvae should be at once transferred to clean water.

Investigations are greatly needed on the embryology of Anura outside the genus *Rana* (cf. Figs. 251, 252, 253 and 254). The different genera and species differ greatly in the size of the egg and its richness in yolk and there is no group of Vertebrates which offers anything like the same facilities for studying the influence of yolk upon the course of development. Further it will be only after greatly extended studies on different species that we shall be in a position to have a really comprehensive idea of typical Anuran development.

FIG. 251.—String of eggs of unknown Frog from the Gambia. Individual variations in the rate of development are indicated by the varying size of the yolk-plug.

Many tropical species of Frogs and Toads are to be obtained alive from animal dealers and in these it may be taken as a general rule that breeding takes place at the commencement of the rainy season, or in other words when environmental conditions become favourable after a prolonged period during which they have been unfavourable. By bearing this principle in mind such tropical amphibians may usually be induced to breed in captivity. Bles in his excellent account of the life-history of *Xenopus* (1905) describes a method which will be found to be of general use. The pair of animals were kept in a Budgett tropical aquarium consisting of a glass bell-jar 20 inches in diameter dipping into a galvanized iron water-tank heated by a small Bunsen burner and oxygenated by plants of *Vallisneria*. During summer the temperature of the water in the bell-jar was kept at about 25° C. The water was not changed. The frogs were fed daily with small earthworms or thin strips of raw calf's liver until they would eat no more. In December the temperature was allowed to fall to 15°-16° during the day and as low as 5°-8° during the night. As the temperature rose with the onset of spring the frogs became more active, waking up out of the lethargic condition induced by the winter's cold. Breeding was induced by simulating the natural conditions of the rainy season. The temperature was raised to about 22° C. Each morning and evening about two gallons of the water was drawn off, allowed to cool for twelve hours and then returned to the aquarium in the form of a fountain of spray from the upturned

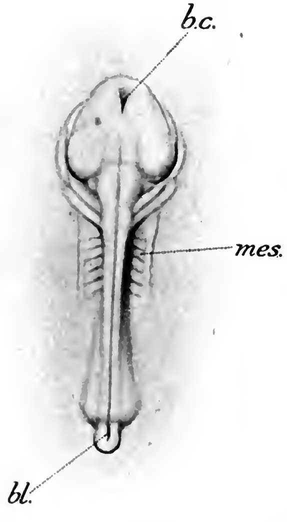


FIG. 252.—Embryo of *Phyllomedusa hypochondrialis* flattened out in one plane.

b.c, buccal cavity; *bl*, blastopore; *mes*, mesoderm segments.

end of a glass siphon drawn out to a fine point so as to produce the effect of a shower of rain. Within a week or two breeding took place.

The chief difficulty in the way of cutting sections of Frog's eggs is due to the presence of the jelly-like envelope. This may be got rid of by prolonged soaking, six months or more, in ·5% formalin (Ogushi, 1908), or by fixing in Zenker's fluid and leaving the eggs in this fluid renewing it after 2 to 3 days and continuing the treatment for 8 to 14 days or longer, shaking gently so as to remove the envelopes (Kallius, 1908).

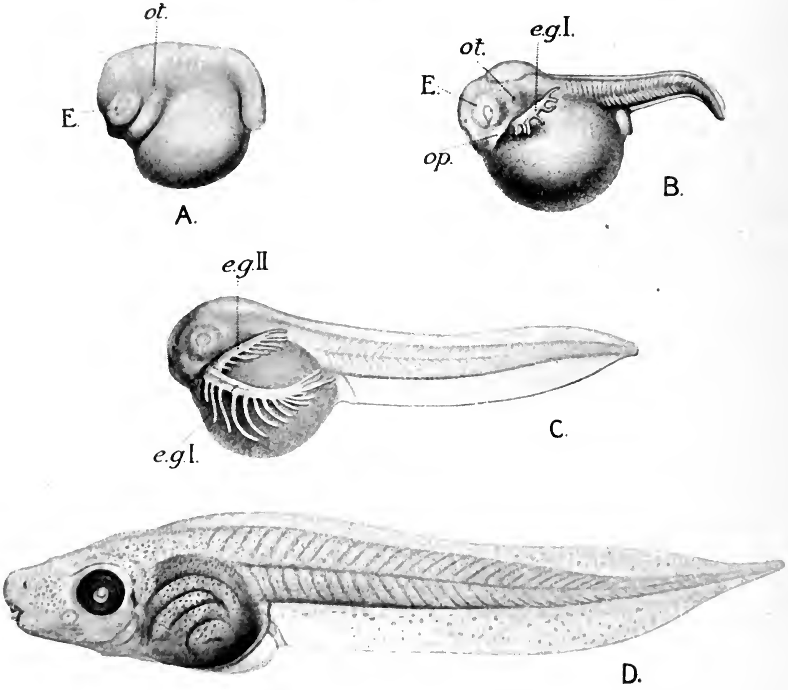


Fig. 253.—Stages in the development of *Phyllomedusa hypochondrialis*. *E*, eye; *e.g*, external gill; *op*, operculum; *ot*, otocyst.

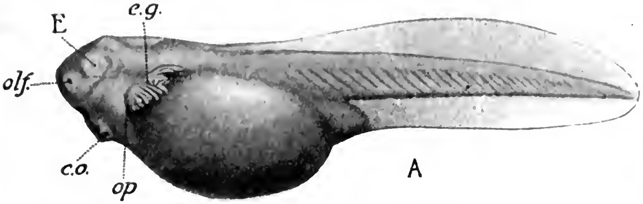
For cutting sections paraffin is commonly used but it should be supplemented by celloidin *e.g.* the clove-oil method mentioned under *Amphioxus*.

In the Urodeles the eggs are commonly laid singly in water and attached to water plants (*Triton*) or other solid objects such as logs or stones (*Proteus*, *Necturus*). In *Cryptobranchus* and *Amphiuma* they form a beaded string, adjacent envelopes being connected together by a narrow isthmus.

Fertilization is rarely external (*Cryptobranchus*—Smith, 1912). In the Newts the female takes up a spermatophore into the cloaca.

Such internal fertilization leads up to the condition in the Salamanders where fertilization takes place in the upper part of the oviduct and the developing embryo is retained for a less or more prolonged period within the body of the parent. In *Salamandra maculosa* larvae about an inch in length are born in May resulting from fertilization during the preceding summer.

As in the Anura wide differences exist in the richness of yolk and consequent size of the egg—the latter varying from under 2 mm. in the Newts to 6 mm. (*Necturus*) or 7 mm. in diameter (*Cryptobranchus japonicus*): so that here again though not to the same extent as in the Anura there is an excellent field for investigation into the influence of yolk upon developmental processes.



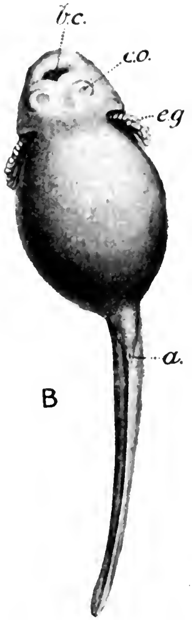


FIG. 254.—Tadpole of unknown Frog from Tropical Africa.

A, side view; B, ventral view. *b.c*, buccal cavity; *c.o*, cement organ; *a*, anus; *E*, eye; *e.g*, external gill; *olf*, olfactory organ; *op*, operculum.

The eggs of Urodeles are commonly collected under natural conditions and kept in earthenware dishes. Or the adults just about to breed may be brought into the laboratory and allowed to deposit their eggs in a suitable aquarium.

The Urodela form one of the relatively primitive groups of Vertebrates and their embryology deserves much greater attention than it has hitherto received. Most of the older literature deals with special details in the development of the Newts but comprehensive monographs, including "normal plates" on the development of such genera as *Proteus, Siren* and *Amphiuma* are much wanted. A general account of the development of the American species of *Cryptobranchus* has been given by Smith (1912), while the Japanese species has been dealt with by Ishikawa (1918), De Bussy (1915) and Dan. de Lange, Jr. (1916). Of *Necturus* normal plates with accompanying tables have been worked out by Eycleshymer and Wilson (1910).

The Gymnophiona—though an aberrant group of Amphibians highly specialized for a burrowing existence—are of much embryological interest and have provided the material for work of great morphological importance, such as that of Brauer upon the excretory organs. A general account of the development of *Ichthyophis* will be found in Sarasin (1887–90) and of *Hypogeophis* in Brauer (1897).

The eggs, fertilized internally, are normally deposited in the soil and the embryologist has, as a rule, to depend upon such scanty material as can be obtained by digging in the damp soil of localities where Gymnophiona are abundant. *Typhlonectes* in South America and *Dermophis* in West Africa are viviparous.

Of the group in general it may be said that a comprehensive monograph on the development of each genus beyond *Ichthyophis* and *Hypogeophis* is a great desideratum.

As standard fixing agents for Amphibia corrosive sublimate and acetic acid, and for the later larval stages strong Flemming's solution, may be used. For the early stages (segmentation and gastrulation) quite good results are obtainable from eggs that have been preserved alive in 10% formalin: in this case it is well to treat the egg before dehydration for an hour or two with corrosive sublimate solution as without this precaution the formalin-preserved eggs are difficult to stain well. When any other fixing agent than formalin is used it is necessary, as a preliminary, to remove the egg envelopes. In the case of the larger eggs of the Urodela and Gymnophiona this can be accomplished with the aid of fine scissors and forceps.

REPTILIA. — For gaining practical knowledge of Reptilian development the student will find the group Chelonia most convenient as it is possible to obtain[1] excellently preserved series of developmental stages of Terrapins (*Chrysemys*) and Snapping Turtles (*Chelydra*). In particular localities especially in warm climates he may have opportunities of obtaining the eggs of Lizards, Snakes or Crocodilians. In all cases the same technique may be used as in the case of the Fowl.

AVES.—The Birds, although showing conspicuous differences in external appearance and in minute details of structure, form a very compact evolutionary group and there is little likelihood of important differences in principle existing in their development. Interesting differences in detail however are to be found—such as the presence or absence of neurenteric canals. Groups which there is any reason to suspect of being particularly archaic—such as Divers, Grebes, Penguins—are worthy of careful scrutiny for possible persistence of Reptilian features.

LITERATURE

Bles. Trans. Roy. Soc. Edin., xli, **1905**.

Brauer. Zool. Jahrbücher (Anat.), x, **1897**.

de Bussy (de Lange), L. P. Eerste ontwikkelingsstadiën van *Megalobatrachus Maximus*, Schlegel. Amsterdam, **1905**.

Cerfontaine. Arch. de Biologie, xxii, **1906**.

Dean, Bashford. Journ. Morph., xi, **1895**.

---

[1] *E.g.* from Mr. J. C. Stephenson, Washington University, St. Louis, or The Marine Biological Laboratory, Wood's Hole.

Dean, Bashford. Quart. Journ. Micr. Sci., xxxviii, **1896.**

Dean, Bashford. Kupffers Festschrift. Jena, **1899.**

Herfort. Arch. mikr. Anat., lvii, **1901.**

Ishikawa. Mitt. Deutsch. Gesell. Natur- und Völkerkunde Ostasiens, xi, 2, **1908.**

Kallius. Anat. Anz., xxxiii, **1908.**

Kerr, Graham. The Work of John Samuel Budgett. Cambridge, **1907.**

Kopsch. Arch. mikr. Anat., li, **1898.**

de Lange, Dan., Jr. Onderzoek. Zoöl. Lab. Groningen, iv, **1916.**

Ogushi. Anat. Anz., xxxiii, **1908.**

Sarasin, P. and H. Ergebnisse naturwissenschaftlicher Forschungen auf Ceylon, ii. Wiesbaden, **1887–90.**

Scammon. Keibels Normentafeln, xii. Jena, **1911.**

Smith. Journ. Morph., xxiii, **1912.**

Taylor. Quart. Journ. Micr. Sci., lix, **1914.**

Williamson. Fisheries, Scotland, Sci. Invest., 1912, i. **1913.**

Ziegler, H. E. and F. Arch. mikr. Anat., xxxix, **1892.**

APPENDIX

THE GENERAL METHODS OF EMBRYOLOGICAL RESEARCH

Embryology is one of the youngest of the sciences and it offers a wide field for fascinating and important research. Regarded as a branch of morphology its main object is to gain information concerning the lines along which the structure of existing groups of animals has evolved. In the phylum Vertebrata there is an immense amount of work still to be done and it is important that the would-be researcher should be guided by certain general principles as to the technique of the subject, otherwise he is apt to achieve no more than the addition of relatively unimportant details to the vast accumulation of details which during the past few decades has tended to hide away general principles and incidentally to smother interest in the subject.

The incompetent or inexperienced investigator frequently betrays himself by his choice of subject: he chooses a problem of relatively minor interest when there lie ready at his hand others which are of real importance, or he chooses a subject really important but of such difficulty that the probabilities are heavily against the feasibility of its solution under existing conditions. The beginner then should see that he has the aid of some competent adviser before he decides upon his line of research.

Having chosen his particular problem he has next to decide regarding the particular animals upon which his research is to be carried out. The earlier workers were guided mainly by the accessibility of the material. Fowls and Rabbits — of which embryos were easily obtained and easily investigated—provided the material for the great pioneers of vertebrate embryology and the embryology of to-day suffers much from the difficulty of getting rid of general ideas founded on such narrow bases. Now that embryology has taken its place as a branch of evolutionary science we recognize the importance of basing our general ideas upon the phenomena of development as displayed by the more primitive existing groups. In attempting any important problem of vertebrate morphology evidence must be got from Elasmobranchs, Crossopterygians, Lung-fishes, Urodeles, before we can feel completely confident as to general principles: in other words we must go to groups which are admittedly archaic. Apart from directly adaptive features an animal which is archaic in its adult structure may be expected to show primitive features in its development. Naturally we should not look for this in cases where development takes place under peculiar conditions, for these necessarily involve adaptive modification. A pitfall into which investigators frequently stumble is that, starting from some particular group—say *Amphioxus*, or the Mammalia—with whose structure they happen to be thoroughly familiar, they assume its general organization to be primitive. As a matter of fact it may be assumed with considerable probability that every existing vertebrate is to a certain extent a mixture of primitive features and specialized. It is only by careful comparative study that it can be decided which features are probably primitive and it is quite certain that these will not be found all within one group. Consequently speculations based upon the intensive study of one particular group are to be distrusted, though there is always less ground for distrust if the group is one which is recognized for reasons other than embryological, as being on the whole archaic.

When minute histological details are concerned another qualification which should be possessed by the animal chosen for investigation is large size of its cell units.

The material should be abundant. Not only should there be a continuous series of stages but there should be numerous specimens of each stage. There is no such thing as an absolutely normal individual: the conception "normal" is an abstraction based upon the observation of numerous individuals. Only by observing numerous individuals can we therefore arrive at a knowledge of normal development. Work carried out on a few specimens may of course provide isolated observations of much interest and value but it is inadequate to serve as a basis for general conclusions.

In all descriptive embryology it is necessary to have some method of specifying the stage of development of individual embryos. Unfortunately there has been a great lack of uniformity as to the particular method of doing this. One of the most frequently used is that of specifying the period of time during which development has been going on as for example a "chick embryo of 40 hours' incubation." This method is quite unsatisfactory, owing to the fact that the actual stage of development of any individual embryo is a function of other factors in addition to mere time, such as temperature and individual idiosyncrasy. Thus in many tropical freshwater animals a statement of the age of the embryo is practically worthless unless accompanied by a record of the temperature, and even then there remains the unknown element of individual peculiarity such as is for example illustrated by Fig. 251 where a number of sister eggs of a Frog are seen to have "lost step" with one another to a marked extent even at a comparatively early stage of development. In other words eggs or embryos of the same age are liable to vary greatly in their degree of development, and a statement of their age is not adequate as a precise indication of the stage of development. The want of precision varies in different cases: it is less for example in a Eutherian mammal where development takes place at a fairly definite temperature than it is in a Fish or Amphibian inhabiting a tropical pool or swamp where the temperature is liable to great variation.

It is necessary then in referring to particular stages of development to define them by structural features. Here however a new difficulty presents itself in the fact that the relative rate of development of different organ-systems is not the same in different individuals. It follows that if a number of individuals be grouped together as being at the same stage of development as judged by a particular organ A it will be found that other organs B, C, etc. are not exactly at the same stage of development—some are less developed some more in the various individuals. Still for practical purposes this is a useful way of indicating roughly the stage of development. For example early stages in the development of Vertebrates may be defined by giving the number of mesoderm segments which have developed—these being fairly conspicuous structures and definable by a number. A much better system, however, is to use numbered stages defined by the general external form—the first structural feature met with in the examination of an embryo. Keibel has published "normal plates" of the development of various Vertebrate types in which standard stages in development are defined by accurate figures. Unfortunately some of the normal plates are incomplete as regards the earlier stages during segmentation and gastrulation, but wherever the plates extend over the whole period of development they should be made use of by the working embryologist as his standard stages. Where no normal plates exist the embryologist should make it his first business to construct one by carefully working over the external features of development and defining by careful drawing and description a series of stages which he judges to be roughly equidistant.

The embryology of any animal is an account of the observable changes which take place in its structure from the zygote stage up to the adult. Logically the investigation of its embryology should proceed similarly from zygote to adult but in actual practice it is better to work in the opposite direction—to commence by getting a clear idea of the adult organization and then to work back from the known to the unknown of earlier stages.

An embryological investigation should commence with a careful study of the entire embryos or larvae at the various stages. Each stage should be examined first alive by transmitted and reflected light, careful note being taken of any movements due to muscular contraction, ciliary action etc. Particular attention should be paid to the arrangement of the blood-vessels, the time of commencement of heart movements, of circulation of the blood and of the appearance of haemoglobin in the corpuscles. The appearance of chromatophores should be noted: the seat of their first appearance and their reactions—whether by changes of form, movement of pigment granules in their protoplasm, or by actual migration—in response to changes in direction or intensity of light. During this phase of the work constant use should be made of the binocular microscope and rough sketches should be made.

Embryos of each stage should be submitted to the action of various fixing agents and it is important to watch the embryo *during* the process of fixing, for the fluid as it gradually penetrates the tissues often makes special structures stand out distinctly for a short space of time—to disappear again with further penetration. The fully fixed embryo should be subjected to further careful scrutiny by reflected light under the Greenough binocular. To detect small inequalities of the surface it will be found necessary to arrange the lighting carefully. The light from an incandescent gas-mantle may be concentrated by a large condenser and caused to illuminate the embryonic surface in a tangential direction. It is often well to cover the specimen with a little house of opaque cardboard or metal resting on the stage of the microscope and possessing two apertures one in its roof through which the observation is made and one at the side through which light is admitted. The embryo must of course be completely submerged in fluid and is preferably contained in a round glass dish with a layer of pitch or black wax on the bottom in which, if necessary, small excavations can be made in which the embryo can rest securely in the desired position. The glass vessel should be rotated slowly during the observations so as to allow of the incidence of the light from different directions. It is important to observe a number, preferably a considerable number, of embryos of the same stage, as owing to individual variation particular features may be much more distinct in some than in others.

A number of thoroughly typical specimens of each stage should be picked out for further investigation and these should be carefully drawn under the camera lucida, a piece of millimeter scale being placed by the side of the embryo and drawn at the same time so as to form a reliable record as to dimensions.

At this stage the normal plates should be constructed if not already in existence and the embryos classified in accordance with them.

For the study of internal structure the great method is that of cutting the embryo into serial sections[1] but a much older method, that of dissection, should by no means be ignored. Careful dissections made under the Greenough binocular are often extraordinarily instructive. It is advisable to experiment with embryos fixed according to various methods as different methods give different degrees of consistency, opacity etc. Van Beneden and Neyt's fluid will be found in many cases to give very good results.

In section-cutting a fetish to beware of is excessive thinness of sections. The expert section cutter is liable to become so interested in his feats in accomplishing the preparation of sections of an extraordinary degree of thinness that he is apt to forget that the criterion of good sections is not simply their degree of tenuity but the relation which their thickness bears to the size of the cell-elements of the particular embryo. Thus while in some cases it is of advantage to have sections so thin as 1 μ[2] or even ·5 μ, in other cases, such as segmentation and gastrulation stages of some of the large heavily-yolked holoblastic eggs, the sections should reach as much as 80 μ or 100 μ in thickness.

Before an embryo is cut into sections its soft protoplasm has to be supported by infiltration with some suitable embedding mass. For this purpose the two substances used at the present time are paraffin of high melting-point and celloidin. Of these the first is used frequently alone but the student should realize from the beginning that if he is to obtain reliable results, especially where yolk is present in the embryonic tissues, he must use both methods and control and check the results obtained from one by those obtained from the other.

The process of infiltrating the embryo with paraffin is usually carried out in a hot-water oven heated by oil, gas or electricity and kept at a temperature just above the melting-point of the paraffin by a thermostat. The melted paraffin may be contained in small copper pans preferably plated inside with silver or nickel. An essential preliminary is a very thorough dehydration followed by a very thorough soaking in the clearing agent. To get the best results it is well to take the embryo through

---

[1] A useful guide for beginners is *Section-Cutting* by P. Jamieson in preparation. For those who already possess an elementary knowledge of the subject an excellent work of reference is Bolles Lee's *Microtomist's Vade-mecum.*

[2] $1 \mu = \frac{1}{1000}$ millimeter.

three changes each of 90% alcohol, absolute alcohol, and xylol or other clearing fluid. The actual process of infiltration with paraffin should last for the minimum time (which will have to be determined by experiment[1]) and be carried out at the minimum temperature.

It may be remembered that the complicated and bulky water-bath with its thermostat is in no way necessary for the embedding process. A very simple apparatus which is perfectly efficient consists of a small metal trough (copper, or tinplate) resting upon a metal table kept heated at one end by a small flame. By sliding the trough lengthwise along the table a position can be found such that the entire thickness of paraffin is fluid at the end next the flame and solid towards the other end. Between these two points stretches an inclined plane of solid paraffin upon the surface of which the embryo rests without any risk of the temperature rising appreciably above melting-point. A simple embedding trough of the kind indicated is of great use in the field as there is no method of storing and transporting embryos so free from danger of accident or of histological deterioration as having them embedded in solid paraffin.

To get a block of paraffin in good condition for section-cutting the embryo should be transferred to a bath of fresh paraffin as soon as it is infiltrated. With certain clearing agents, *e.g.* cedar oil, it is well to give two or three changes of paraffin. The vessel containing the embryo in a considerable volume of paraffin should now be floated on cold water so as to give a homogeneous translucent block of solid paraffin. On no account should the vessel be actually submerged in the cold water for in this event the contraction of the inner paraffin as it cools within the already rigid outer layers will lead to the formation of cavities into which the water penetrates.

For the actual process of section-cutting it is necessary to use a mechanical microtome. The Cambridge Rocking microtome is one of the most convenient for ordinary embryological work while the Reinhold-Giltay microtome is a most excellent instrument both as regards accuracy and rapidity of working.

The paraffin block containing the embryo is trimmed down so as to be rectangular in section and is then fixed by the interposition of a hot spatula to the paraffined surface of the microtome carrier in such a position as may be necessary to give the required direction of sections.

Where the object is a "difficult" one, *e.g.* containing much yolk, it is advisable to have it surrounded by a paraffin block of considerable size. A considerable mass of paraffin above the specimen makes it cut better, while a considerable mass to the side causes successive sections, with their long edges, to adhere better together and form a continuous ribbon. The embryo should be near one of the lower corners of the block to facilitate exact orientation.

For thorough investigation of the structure of embryos it is advisable to have specimens cut into sections in the three sets of planes—transverse, sagittal or longitudinal vertical, and coronal or longitudinal horizontal. To obtain these it is necessary to have the embryo orientated exactly on the microtome. In most cases this can be accomplished with a sufficiently close approximation to accuracy when fixing the paraffin block on to the

---

[1] *E.g.* for a Chick at about the middle of the second day about 20 minutes will be found to be sufficient.

carrier, especially if care has been taken to trim the surfaces of the block parallel to the three chief planes of the embryo.

Where greater accuracy is needed, as in the case of very small embryos, they should be arranged in position in the melted paraffin with warm needles under the prism binocular microscope. This may be done by placing the watch-glass or other vessel on the top of a small flat copper cistern full of water, provided with inlet and outflow tubes, and heated up by contact with the top of the water-bath or hot stage. In the bottom of the embedding vessel is placed a small plate of glass on the upper surface of which are engraved parallel lines intersecting one another at right angles. When the embryos have been accurately orientated with regard to the engraved lines a stream of cold water is allowed to run through the cistern and this causes the paraffin rapidly to solidify. When the block is quite hard the glass plate is picked off and the ridges formed by its engraved lines serve as accurate guides to the position of the embryo.

Still greater accuracy is obtainable by arranging that the melted paraffin in which the embryo is being orientated is already in its definitive position on the holder of the microtome, the paraffin being kept melted as long as necessary by an electric current passing through a loop of high resistance wire.[1]

For the actual cutting care must be taken that the razor (solid ground) or other knife has a very fine edge which does not show irregularities when examined under the low power of the microscope. The blade should be thoroughly cleaned with pure spirit before commencing work. If very thin sections, *e.g.* of 1 μ in thickness, are required it is well to commence with sections of 5 μ, then without stopping to change to 4 μ, then to 3 μ, then to 2 μ, then to 1 μ—cutting a continuous ribbon throughout and going ahead rapidly when the 1 μ sections are cutting properly.

The celloidin method should be constantly used as a check on the paraffin method. Where yolky eggs or embryos are being cut the celloidin method gives the only trustworthy sections as by it the yolk granules are held in position and prevented from sticking on the edge of the knife, ploughing through the tissues and destroying much of the fine detail, as is always liable to happen if paraffin alone is used under such circumstances.

In cases where there is no need for specially thin sections (say under 25 μ) a convenient method is that in which the celloidin block is hardened by exposure to chloroform vapour and then cleared by immersion in cedar-wood oil.

The block of celloidin is usually fixed to a block of wood which is gripped by the holder of the microtome. Care should be taken that such wooden blocks are baked for several days so as to ensure their being absolutely dry. Otherwise moisture will diffuse out and produce a milky opacity in the celloidin which ought to be absolutely clear and transparent.

Sometimes it will be found that the block becomes too hard and will not cut properly, its edges frilling or breaking. This is sometimes due to the presence of a trace of chloroform in the cedar oil used for clearing. When this is the case the cut surface of the block should have perfectly pure cedar oil applied to it with a brush just before each section is cut.

---

[1] A special apparatus for this purpose is made by the Cambridge Scientific Instrument Company.

To obtain thinner sections it is necessary to embed the celloidin block containing the object in paraffin. This may be done simply by transferring the block saturated with cedar oil to melted paraffin. A better method is to use a solution of celloidin in clove oil of about the consistency of treacle. The object, thoroughly permeated by this and surrounded by a small quantity of the celloidin, is hardened and cleared in chloroform. The block is then carefully trimmed with one face accurately parallel to the plane of the required sections. It is now immersed in melted paraffin for a minimum time (ten minutes suffices for a small object). After cutting and mounting the sections the slide is immersed in xylol until the paraffin is dissolved out, then in absolute alcohol, then in a mixture of equal parts of absolute alcohol and ether until the celloidin is removed. The slide is now taken down through the series of alcohols and the sections stained and mounted in the ordinary way.

The arriving at a clear idea of the structure of an embryo from the study of a series of sections involves fitting the successive sections together into a continuous whole. To a great extent this reconstruction of the whole from the successive sections can be done mentally but where complicated structures are being investigated, some aid is either absolutely necessary or at least desirable for the sake of accuracy. The present writer finds the most reliable as well as the most convenient of such aids in the method of reconstruction by means of glass plates.[1] Successive sections are drawn with a hard (9 H) lead pencil by means of a camera lucida upon finely ground sheets of glass such as is used for photographic focusing screens and then the successive drawings are fitted together, a fluid of as nearly as possible the refractive index of the glass being interposed between them so that the ground surfaces disappear and the heap of plates appears as a clear block with the structures drawn running through it and appearing as a kind of solid model.

The following details may be noted. Sections are cut to a standard thickness of 10 μ (*i.e.* $\frac{1}{100}$ mm.): the glass plates are 1 mm. thick: the drawings are made at a magnification of 100 diameters. But it will be found in practice that much use can be made of the method even if these three dimensions are not so exactly correlated. The outlines made with pencil of the particular organ that is being studied are filled in with water colour. Vermilion is the most generally useful colour for it retains its opacity and light-reflecting properties to an unusually high degree when submerged in fluid of high refractive index. When the plates are dry No. 1 is laid, ground side up, on a flat surface—preferably a glass stage with a mirror beneath so that light may be reflected up through it—a few drops of the fluid used, *e.g.* clove oil or cedar oil or a mixture of fennel oil (two parts) and cedar oil (one part) as recommended by Budgett[2] are placed by a pipette on the centre of the ground surface and then plate No. 2 is lowered gently into position and fitted into its place over plate No. 1. The outlines of the drawings should be made to coincide exactly, and the two plates should be pressed firmly into contact care being taken to avoid interposed air bubbles which act as elastic cushions and prevent the upper plate from settling down into contact with the other. Successive

---

[1] *Quart. Journ. Micr. Sci.*, xlv, 1902.

[2] *Trans. Zool. Soc. London*, xvi, Pt. 7, 1902.

plates are fitted on in a similar manner until the particular organ stands out like a solid model in the mass of plates.

The same set of drawings may be used for different organs: the clove oil is removed by treating with strong spirit, and the water colour by holding under the tap, and then, after drying, a new organ can be coloured in. By colouring merely the cavity of an organ the relations of the cavity can be displayed as by an injection. When finally done with the drawings are removed by scrubbing with "Monkey brand" soap.

By this method, after a little practice, reconstructions can be made with great rapidity and accuracy.

Though less accurate and much more tedious the older method of reconstructing with plates of wax is useful for building up a permanent model. Its use is also indicated where only a single specimen is available. Instead of wax plasticine may be used [1] which allows of a kind of dissection being made, in as much as particular parts of the model may be bent out of the way to display structures which would otherwise be hidden.

In investigating the development of the skeleton the cartilage is often found to pass by imperceptible gradations into unmodified mesenchyme. The absence of sharply defined surfaces in such cases makes the reconstruction method unreliable and it is advisable to supplement it by subjecting the embryo to treatment with a specific stain which picks out the cartilage while leaving the other tissues uncoloured so that the cleared and transparent specimen may be studied as a whole under the binocular microscope.

An excellent stain for this purpose is v. Wijhe's Methylene Blue.[2] The embryo is fixed preferably in ·5% watery solution of corrosive sublimate, with 10% formalin added just before use, and preserved in alcohol. When about to be stained it should be treated for a day or two with alcohol containing $\frac{1}{4}$% hydrochloric acid—care being taken to renew this so long as it develops any yellowness due to traces of iodine. The stain consists of a solution of $\frac{1}{4}$% methylene blue in 70% alcohol to which 1% hydrochloric acid has been added some time before use. The embryo is stained for a week and is then treated with 70% alcohol containing $\frac{1}{4}$% hydrochloric acid and renewed several times the first day and thereafter once daily until no more colour comes away. The embryo is now dehydrated, cleared gradually in xylol, passed through stronger and stronger solutions of canada balsam in xylol, and preserved eventually in balsam so thick as to be solid at ordinary temperatures though liquid at 60° C.

An excellent method of cleaning small cartilaginous skeletons is to place them amongst Frog tadpoles which remove the muscle etc. from the surface of the cartilage by means of their oral combs.

In regard to the general principles of embryological research it need hardly be said that, as in other branches of science, accuracy of observation occupies the first place. And yet, curiously, accuracy may become a fault. In those branches of science which are more effectively under the control of mathematics it is well recognized that in any type of investigation there is a limit of probable error of observation—due to instrumental or sensory imperfections or to disturbing factors of one kind or another—

---

[1] Harmer, *Pterobranchia of Siboga Expedition*, 1905.

[2] *Proceedings* Akad. Wetensch. Amsterdam, June 1902.

beyond which it is mere waste of time to push observation. In all biological observation the limit of probable error is particularly high yet this fact is peculiarly apt to be ignored and it is no unusual thing to find dimensions or other numerical data stated to three or four places of decimals when anything beyond the first place is worthless for the reason indicated.

To secure accuracy of observation not merely training and experience in the art of observing is needed but also a proper psychological outlook: the observer must be able to take a completely detached point of view and must ever be on the watch to guard against some particular hypothesis or preconceived idea causing actual error instead of fulfilling its proper function of keeping the powers of observation tuned up to the highest pitch of alertness.

The whole spirit and aim of scientific investigation is directed towards the seriation of facts and the devising of general expressions or formulae which unite them together. In this it contrasts with the more primitive state of mental development which observes isolated phenomena, noting the differences between them but blind to the common features which link them together. In embryology as in other departments of knowledge the able investigator sees the general principles which run through and organize the masses of detail: he interests himself in discovering the likeness which is hidden under superficial difference; he is constructive not destructive.

In this volume embryology is treated as a branch of morphology but it must be borne in mind that morphology and physiology are inseparably intertwined. The living body whether of an embryo or an adult is above all a piece of exquisite mechanism fitted to live and move and have its being, and to ignore this is to make morphology as sterile and as misleading as would be the study of machinery apart from the movements and functions of its various parts. More particularly in attempting to delineate the evolutionary past of an organ, or set of organs, speculation must always be rigidly controlled by the reflexion that at each phase in evolution it must have been able to function.

When at length the stage is reached of putting results into form for publication the first thing to aim at is absolute clearness of expression. It must be remembered that clearness of language and clearness of thought are closely interdependent. Sloppy obscure language means sloppy obscure thought. The greatest care should be taken in the correct and precise use of technical terms. Argumentation in regard to scientific and other matters is, when the disputants are equally well informed, due as a rule to some word or expression being used in slightly different senses. Elegant literary style, however desirable, must always be subordinate to clarity and precision of language. Indeed actual harm is sometimes done to scientific progress by the writer whose literary skill carries away not merely himself but others of uncritical and impressionable mind. Scientific problems are eventually settled not by skill in dialectic but by increase of knowledge.

As a rule the proper presentment of an embryological thesis involves pictorial illustration. In this the elaborate coloured lithographs of former days may conveniently be replaced to a great extent by simple line or half-tone drawings in India ink or process black which can be reproduced photographically and inserted in the text in contiguity with the passage which they illustrate. Their function is to render more clear the statements of the author: they represent as accurately as possible phenomena as observed by the skilled and trained eye with a brain behind it. Actual photographs, which represent merely details lying in one particular plane and as seen by the untrained photographic lens, should be avoided. Apart from the imperfections indicated they are so blurred by the ordinary processes of reproduction as to be liable to misinterpretation and in these days of skilful manipulation they are of course useless as guarantees of truth.

INDEX

(References to **figures in Clarendon** type)

END OF VOL. II

Printed by R. & R. Clark, Limited, *Edinburgh.*

[illegible] 1947 ~~[illegible], Alice~~
Nov 2/48 Dickinson, Thos. [illegible]
Lawrence, Thos. Jones.
~~Nov 17/49~~ ~~J. [illegible] St.~~