THE DEVELOPMENT OF THE FROG'S EGG


The MMCo.

THE DEVELOPMENT OF THE FROG'S EGG

AN INTRODUCTION TO

EXPERIMENTAL EMBRYOLOGY

BY

THOMAS HUNT MORGAN, PH.D.

PROFESSOR OF BIOLOGY, BRYN MAWR COLLEGE

New York

THE MACMILLAN COMPANY

LONDON: MACMILLAN & CO., LTD.

1897

Norwood Press
J. S. Cushing & Co. — Berwick & Smith
Norwood Mass. U.S.A.

PREFACE

THE development of the frog's egg was first made known through the studies of Swammerdam, Spallanzani, Rusconi, and von Baer. Their work laid the basis for all later research. More recently the experiments of Pflüger and of Roux on this egg have turned the attention of embryologists to the study of development from an experimental standpoint. Owing to the ease with which the frog's egg can be obtained, and its tenacity of life in a confined space, as well as its suitability for experimental work, it is an admirable subject with which to begin the study of vertebrate development.

In the following pages an attempt is made to bring together the most important results of studies of the development of the frog's egg. I have attempted to give a continuous account of the development, as far as that is possible, from the time when the egg is forming to the moment when the young tadpole issues from the jelly-membranes. Especial weight has been laid on the results of experimental work, in the belief that the evidence from this source is the most instructive for an interpretation of the development. The evidence from the study of the normal development has, however, not been neglected, and wherever it has been possible I have attempted to combine the results of experiment and of observation, with the hope of more fully elucidating the changes that take place. Occasionally departures have been made from the immediate subject in hand in order to consider the results of other work having a close bearing on the problem under discussion. I have done this in the hope of pointing out more definite conclusions than could be drawn from the evidence of the frog's egg alone.

In treating the general problems of development, I have tried to keep as near to the evidence as possible. I have intention-

ally avoided at times the discussion of the more theoretical problems arising from the experiment, for it seems to me that such discussions are out of place in a volume of this sort. Only the early stages of the development have been considered, because almost all of the experimental work on the frog's egg has been done on the early stages, and also because I am more familiar with the development and with the experiments of this period. Moreover, the later stages have been recently most admirably described by Marshall in his *Vertebrate Embryology*.

A few words of personal explanation may be added. For several years I have been collecting the material for the present volume, but as the literature is so extensive and as I have had other work to do first, I made but slow progress. In the summer of 1893 I set seriously to work, and owe much to the admirable facilities offered by the University of Berlin. I take pleasure in acknowledging my indebtedness to Geheimrath Professor Fr. E. Schulze for many privileges and kindnesses extended to me in Berlin. The work was continued irregularly during the winter of 1893–1894 while enjoying the opportunities of the Stazione Zoologica in Naples. During the winter of 1894–1895 the material was brought together and in the summer of 1896 at Zürich the manuscript was almost completed. I gladly take this opportunity to thank Professor Arnold Lang for many courtesies extended to me during two visits to Zürich. Dr. Driesch has most kindly looked over some of the chapters, and has made many valuable suggestions. Dr. H. H. Field has also examined a part of the manuscript and helped me in several directions. To Professor E. B. Wilson I am under heavy obligations, and owe much to his valuable suggestions and corrections. To Dr. H. Randolph I owe a debt of gratitude for kindly advice and criticism. I am also greatly indebted to Professor Joseph W. Warren and to Professor E. A. Andrews for advice in connection with the revision of the proof.

CONTENTS

CHAPTER V

CHAPTER VI

CHAPTER VII

CHAPTER VIII

CHAPTER IX

CHAPTER X

CHAPTER XI

CHAPTER XII

CHAPTER XIII

CHAPTER XIV

CHAPTER XV

CHAPTER XVI

INTRODUCTION

THE eggs of most of our species of frogs are laid in the spring. In some cases they are set free almost immediately on the emergence of the frogs from their winter sleep; in other cases the eggs are not laid until some weeks or even months after the frogs have awakened. In almost every instance the eggs are deposited in water and usually in quiet pools or ponds, or in protected bays along streams where the water has backed up and has come to rest. Sometimes the bunches of eggs are stuck to sticks, grass, submerged sedge, or even to stones; in other cases the bunches are not fastened.

The copulation precedes and lasts through the laying-period; a single male fertilizing all the eggs laid by one female. The sperm pours out of the cloaca of the male at the moment when the eggs pass out of the female.

Both the male and the female sexual products, the eggs and spermatozoa, are ripened during the summer and autumn of the year preceding the deposition of the eggs, — at least this is the more usual process. The origin of these sexual cells must first be studied in order to more fully understand their *relation to each other, and the part they play in the subsequent development.*

DEVELOPMENT OF THE FROG'S EGG

CHAPTER I

THE FORMATION OF THE SEX-CELLS

THE development of the sex-cells is generally divided into three periods: 1) a multiplication-period, during which the primitive germ-cells pass through a large number of divisions; 2) a growth-period, in which the primitive germ-cells, that have become reduced in size through repeated division, grow larger; 3) a maturation-period, when only two divisions take place, between which the nucleus does not pass into a resting-stage. At the end of this last division the male germ-cells undergo histological changes by which they become transformed into spermatozoa.[1]

SPERMATOGENESIS

The changes that take place in the testes of the frog have not been so fully worked out as in some other animals; we may therefore follow, first, the method of development of the

[1] This is a modification of the terminology of v. la Valette St. George, whose nomenclature of spermatogenesis is still often used. La Valette's classification is as follows: —

The primordial germ-cells give rise to spermatogonia, which cease to divide after a time and increase in size. Each spermatogonium is thus converted into a primary spermatocyte. Each primary spermatocyte divides into two cells, the spermatocytes of the second order, and each of these divides once more, without a resting-period, to form two spermatids. In this way four spermatids are formed from each primary spermatocyte. Each spermatid is then changed directly into a spermatozoön.

spermatozoön in two forms in which the process is better known, and then consider the special case of the frog.

The development of the spermatozoa of Gryllotalpa, the mole-cricket, has been described by vom Rath ('92, '95). As the process of spermatogenesis is relatively simple in this form, and as it is, according to vom Rath, much like the process that takes place in the frog, we may therefore first briefly consider the changes in Gryllotalpa.

First Period. A cell in the resting-stage at this time shows a large nucleus with a distinct membrane enclosing a network of fine chromatin (Fig. 1, A). The beginning of the cleavage is indicated by the withdrawal of the chromatin from the nuclear membrane and the thickening of the fibres of the

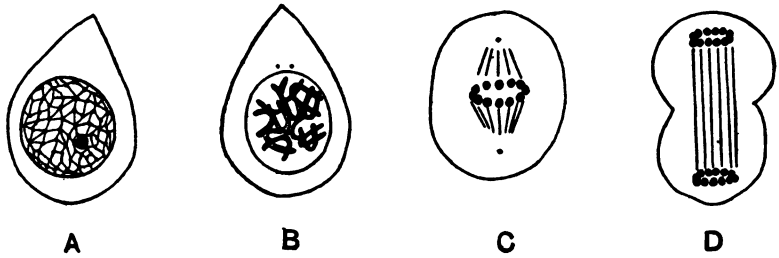


FIG. 1. — Division of sperm-mother-cells in Gryllotalpa. (After vom Rath.)

chromatic network. The tangled mass of threads, or network, then takes a somewhat excentric position. This thread seems to consist of linin, on which chromatin-granules are arranged. Sometimes the thread can be seen to be split along its length into two parts. The halves of the thread remain, however, closely sticking to each other. The double thread then breaks up by *cross-division* into twelve equal segments, or chromosomes (Fig. 1, B). The chromosomes next become shorter, and finally spherical, and come to lie in an equatorial plate (Fig. 1, C). When the chromatin is still in the skein-stage, two minute bodies are seen in the protoplasm just outside of the nuclear membrane (Fig. 1, B). These are the two centrosomes, which separate more and more from each other, and finally come to lie on opposite sides of the nucleus. A protoplasmic spindle develops between the two centrosomes (Fig. 1, C) and the fibres of the spindle become fixed to the

chromatin-granules of the equatorial plate. Each of the twelve chromatin-granules divides into two equal parts and the halves migrate toward one or the other of the centrosomes (Fig. 1, D). The cell-protoplasm next divides into two parts, so that two new cells are formed. Each cell contains twelve chromosomes. In this way the primitive sperm-cells continue to increase in number by a series of cell-divisions, all like that just described.

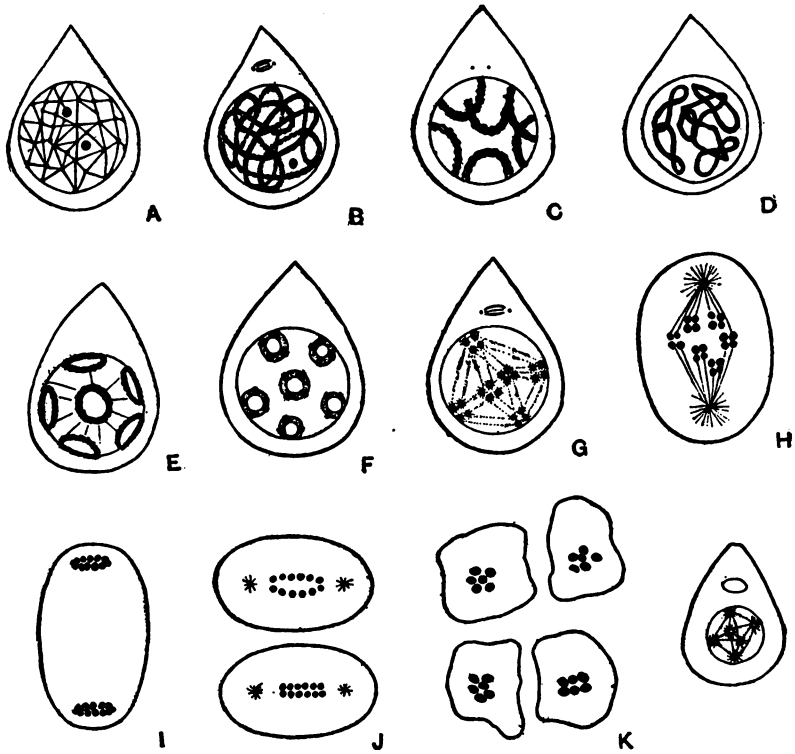


FIG. 2.—The formation of spermatozoa in Gryllotalpa. The two maturation-divisions. (After vom Rath.)

Second Period. A period of rest then follows, during which the cells grow larger. During this time the chromatin is again arranged in a fine network.

Third Period. Two successive and most peculiar cell-divisions now take place. The chromatin-network becomes thicker, and forms a tangled skein of threads (Fig. 2, A, B).

Each thread is split longitudinally into two parts. Two centrosomes again appear. The chromatin-thread next breaks up into *six* bent rods or chromosomes (Fig. 2, C). There is some doubt as to the way in which the next change is brought about. The account of vom Rath, which we follow here, seems to be in harmony with the process that is known to take place in some other forms during this period of development of the germ-cells. It appears that the halves of each of the six bent rods begin to separate from each other except at the ends of the rods, where the halves remain united. Each rod is in this way converted into a ring (Fig. 2, D). These rings are often so bent on themselves that they form a loop. The six chromatin-rings lie close to the periphery of the nucleus. The rings contract and become smaller and thicker (Fig. 2, E). This stage lasts but a short time and is succeeded by a stage shown in Fig. 2, F, G. Out of each ring four star-like granules are formed, the tetrad or "Vierer-gruppe." The four granules of each set are closely held together by clear linin threads. If each granule be counted as a distinct chromosome, then there are present at this time six groups of four chromosomes each, or twenty-four chromosomes. These twenty-four chromosomes become attached to the fibres of the achromatic spindle (Fig. 2, H) and arrange themselves into an equatorial double plate. Then twelve of these granules united in pairs wander toward one pole of the cell and twelve toward the other pole, and the division of the cell takes place (Fig. 2, I). This process is spoken of as the *first maturation-division*. Without passing into a *resting-stage*, a second division of each cell follows (Fig. 2, J). A new karyokinetic spindle is formed and the twelve chromosomes are separated into two plates of six chromosomes each, which go to their respective poles. Each of the two new cells contains therefore only six chromosomes (Fig. 2, K). The number of chromosomes is now *reduced to half the normal number present in the other cells of the body of the animal*. Each of the four cells formed by these two consecutive divisions of the sperm-mother-cell then differentiates into a spermatozoön (Fig. 2, L). Each spermatozoön consists of three parts, — a head, a middle piece, and a tail. The head is formed almost entirely out of the nucleus of the parent-cell of the spermatozoön, as

seen in Fig. 7, A, B, C. It is probable that a very thin layer of cytoplasm covers the outer surface of the head. The chromatin is densely packed into the head-piece, and cannot be resolved into its component chromosomes. The middle piece lies just back of the head.[1] In some animals this middle piece is known to enter the egg with the spermatozoön and a part of it becomes the centrosome, which then divides into two centrosomes and around these arise the achromatic rays of the dividing egg. The tail of the spermatozoön is generally described as coming from the cytoplasm of the cell.

The development of the spermatozoön in the salamander has been carefully studied by Flemming ('87), vom Rath ('93), Meves ('96), and others. There are certain remarkable processes that take place in the spermatogenesis of these Amphibia that seem to occur also in the frog, but as they have not been as carefully worked out in the latter form we may examine first the changes that take place in the salamander. Each year after the male has lost its supply of sperm, new spermatozoa begin to develop. The epithelial cells lining the cavities of the testes divide at first after a type of cleavage called, by Flemming, homœotypic. This first period of activity produces the first generation of spermatocytes, which divide according to another type, the heterotypic.

The cells of the second generation of spermatocytes also divide in the same way, but with an occasional homœotypic cleavage. Finally, in the third generation of spermatocytes, both types of cleavage occur. The products of the third generation transform directly into spermatozoa. In the *heterotypic division* the process is as follows. The chromatin is at first arranged in a thick thread, having a definite arrangement. The skein-stage follows, and a longitudinal splitting of the chromatin-thread is apparent. A thickening of the thread then takes place, and it breaks up into twelve chromosomes (only *half* the number present in other cells of the body) (Fig. 3, A, B). At the free ends of the bent chromosomes, each of which is split longitudinally, the halves fuse together (Fig. 3, B), but elsewhere the

[1] Its origin in the frog has not been definitely made out. It is probably cytoplasmic in origin (Fig. 7, A, B, C).

halves of the chromosomes separate from each other along the longitudinal line of division. The process is similar to the ring-formation of Gryllotalpa. In this way twelve loops are formed from the twelve chromosomes. The bent ends of the new loops or rings correspond to the middle portions of the earlier rods or chromosomes (see the + and − signs in Fig. 3, A, C). Meanwhile the achromatic spindle between the centrosomes has developed, and the loops of chromatin are arranged on the threads of the spindle, as seen in Fig. 3, B. At the next stage each loop

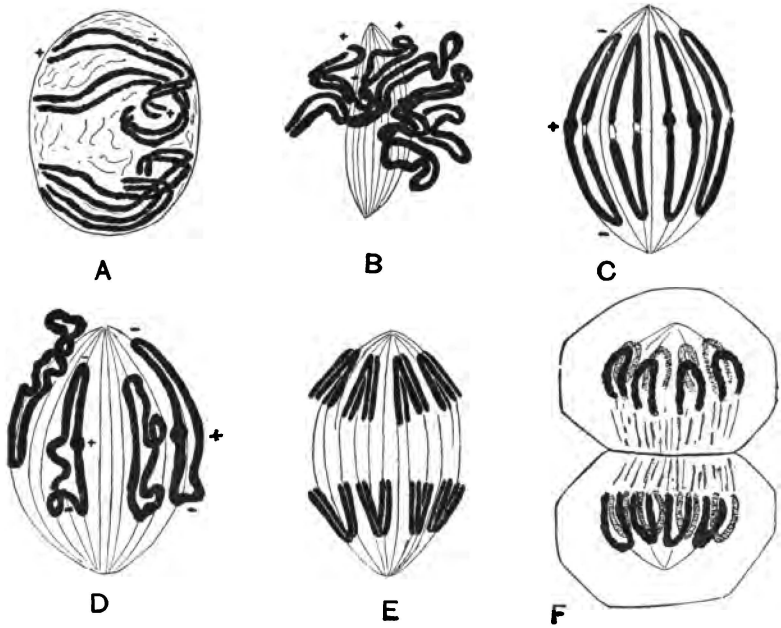


FIG. 3.—Heterotypic type of nuclear division in Salamandra. (After Flemming.)

breaks at the equator, *i.e.* at the point where the ends of the rods fused at an earlier period, and begins to migrate toward its centrosome (Fig 3, D). While this migration of the twelve bent chromosomes is taking place, each chromosome may be seen again to split longitudinally, although the two halves remain in contact (Fig. 3, E). The cell then passes into a resting-stage.

In the *homœotypic division* the first phase, the spireme, is simi-

lar to the last, *i.e.* it is a skein (Fig. 4, A) with longitudinally split thread. Twelve bent rods appear and become shorter than the bent rods of the heterotypic type. These rods then arrange themselves about the middle of the achromatic spindle (Fig. 4, B). The twelve bent rods divide each into two by separation along a longitudinal line, and twenty-four rods are present. Immediately twelve of these migrate toward one pole, and twelve toward the other, and the cell-division follows (Fig. 4, C, D, E, F). The cells then come to rest.

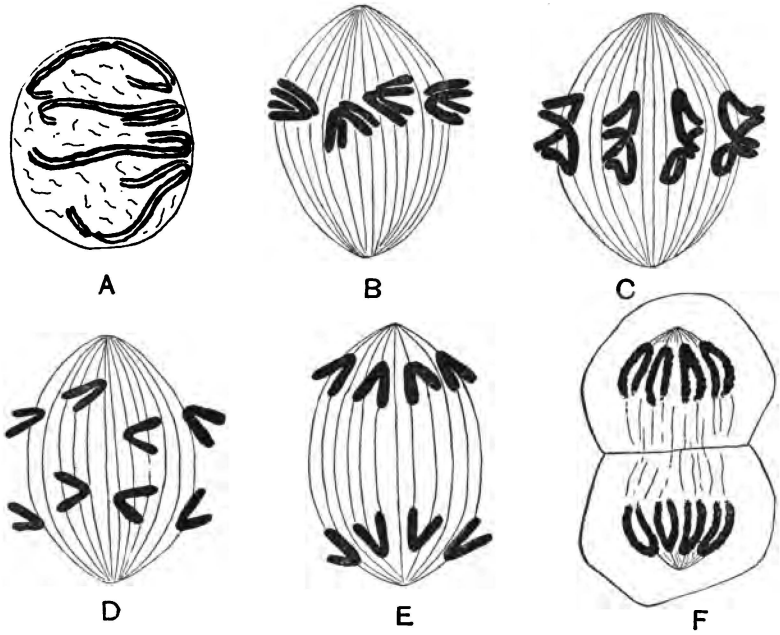


FIG. 4. — Homœotypic type of nuclear division in Salamandra. (After Flemming.)

The end result in the two types of cleavage is the same, but the details are, as described, different. It is important to note that the number of chromosomes is half that of the number of chromosomes in the other cells of the body.

Vom Rath maintains that a fourth generation of cells appears in the development of the spermatozoa of the salamander. Flemming supposed that at the end of the third generation of cells, described above, the differentiation into spermatozoa began, but

vom Rath has found that at the end of the third generation large cells appear with huge nuclei (Fig. 5, B), in which there are twelve groups of chromosomes. Each group or tetrad is composed of four granules. There are, therefore, present forty-eight spherical chromosomes united in groups of four. These tetrads arose from a heterotypic spindle, and in the following way. As the twelve loops (which are now double, making twenty-four loops) passed toward one pole they became much thicker (Fig. 5, A). The middle point of union of each of the twenty-four loops broke (Fig. 5, B), and the portions rounded up, so that there were present forty-eight chromosomes arranged in twelve groups of four chromosomes each (Fig. 5, B). Immediately after the formation of the tetrads the groups of chromosomes arranged themselves along the rays of the achromatic

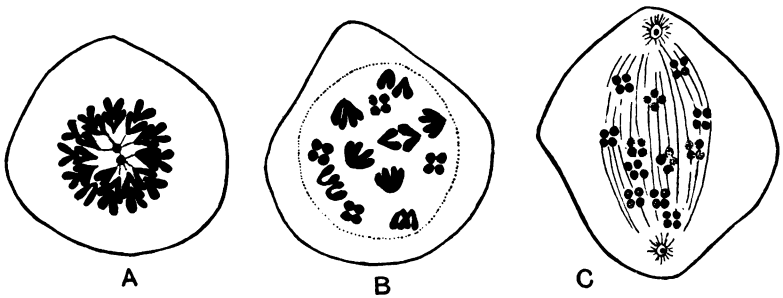


FIG. 5. — Formation of tetrads in testis of Salamandra. (After vom Rath.)

spindle (Fig. 5, C). The tetrads next passed toward the equator of the spindle, and there they divided, so that two of each of the four chromosomes passed toward one pole of the cell (as in Gryllotalpa). In this way two new cells are formed with twenty-four chromosomes each. A second division succeeds without an intervening resting-stage, and the number of chromosomes is reduced, so that each cell has twelve chromosomes. The cells resulting from the last division, having each twelve chromosomes, differentiate each into a spermatozoön.

The second division, according to some workers (Boveri, Hertwig, and Brauer), is the result of a second *longitudinal division*. But vom Rath holds that this second division in the Amphibia and in Gryllotalpa is the result of a *cross-division* of

the threads. According to Boveri, the meaning of the formation of the tetrad is only the precocious separation of the chromatin-threads for two rapidly succeeding divisions (without an intermediate resting-stage). The doubling of the chromosomes previous to division has, he thinks, no further significance than the preparation for two quickly succeeding divisions. It is not obvious, however, in the *development of the spermatozoön* why this rapid division should take place at this time and at no other in the life of the cell.

Meves ('96) has most recently reëxamined the development of the spermatozoön in the salamander. His results differ in several respects from the earlier results of Flemming, and in one essential respect from the work of vom Rath. According to Meves, the germ-cells undergo many divisions in the upper part of the testis. The chromatic figure is that of the usual type of division; and *twenty-four chromosomes* are present. As a result of the division, the cells become smaller, and each cell becomes surrounded by a layer of connective tissue. Each of these cells then divides many times according to the usual type of division, so that clusters of cells are produced surrounded by a common wall of connective tissue. Then follows the resting-period, in which the cells enlarge. After this the maturation-divisions take place. Meves thinks that most probably each cell divides only twice during this period, as in other forms. The first division is heterotypic, and now for the first time the number of *chromosomes is reduced to twelve.* Without a resting-period each cell again divides, the twelve chromosomes splitting longitudinally. This second division is homœotypic. Each cell, containing twelve chromosomes, then transforms directly into a spermatozoön.

Meves shows therefore that Flemming was mistaken in regard to the number of cell-generations that are present in the spermatogenesis of the salamander, and further that Flemming failed to make out the real sequence of the generations and the number of chromosomes present in each. More important is Meves' statement that, *normally*, there is not a formation of tetrads as vom Rath had affirmed. At present it is impossible to decide between the divergent accounts of Meves and vom

Rath, and we must suspend judgment until further work throws more light upon the question.

The spermatogenesis of the frog has not been worked out in the same detail as that of the salamander, yet vom Rath ('95) has made certain important statements in regard to it. The prophase of the mitoses, before the ripening period, has in the frog a close resemblance to the skein-stage of the heterotypic and homœotypic variations in the salamander. But, on the other hand, in the metakinetic stage the peculiarities of the homœotypic and heterotypic forms, as described by Flemming,

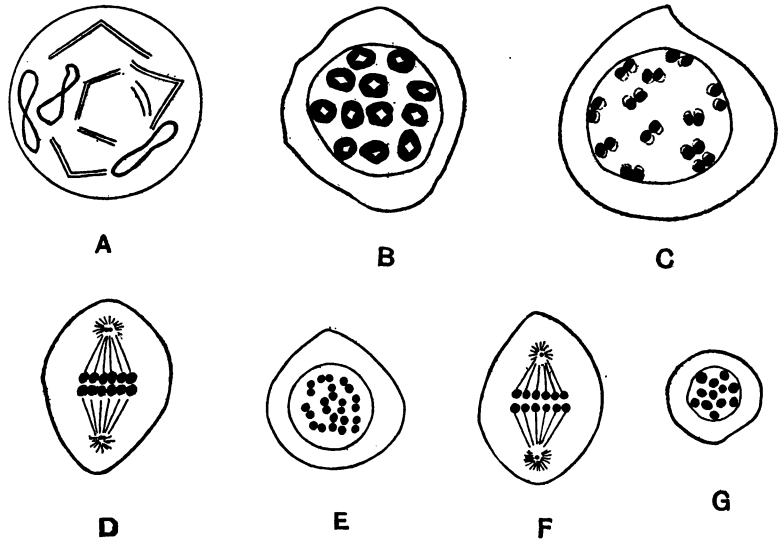


FIG. 6. — Stages in the last maturation-division of sperm-cells of Frog. (After vom Rath.) (Figs. C, D, F slightly modified.)

are absent. The formation of the chromatin-rings and tetrad-groups in the frog (Fig. 6, A, B, C) differs from that of the salamander and is much more like that of Gryllotalpa. The rings, owing to the strong contraction of the segments, are relatively small in the frog, but proportionately thick (Fig. 6, B). From each ring arise the four spherical chromosomes of each tetrad-group (Fig. 6, C). The ring-stage lasts quite long in Rana, judging from the frequency of its presence. The rings lie at the periphery of the nucleus.

The shape of the spermatozoön is very different in different species of frogs. In some species the head of the spermatozoön is drawn out into a fine point (Fig. 7, A, E); in other forms it ends bluntly (Fig. 7, D). The middle piece is easily found in some spermatozoa, but in others only by the application of special reagents. In the European toad (Fig. 7, A) the tail of

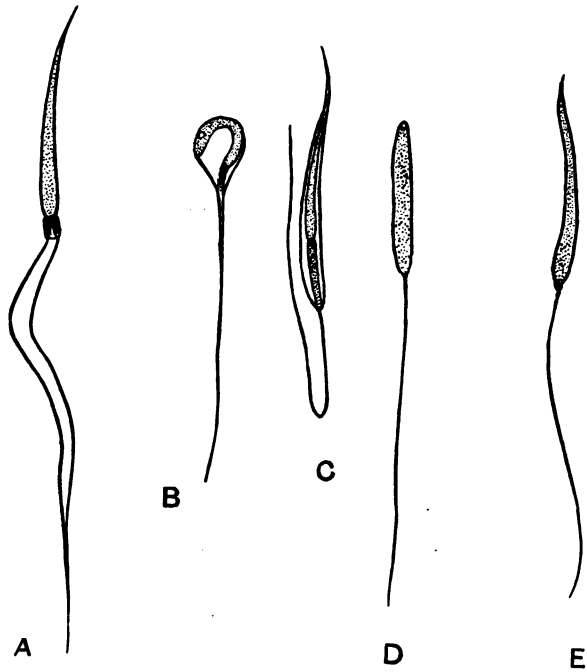


FIG. 7. — Spermatozoa. A. Bufo cinereus. B–C. Two stages in development of spermatozoön; D. Fully formed spermatozoön of Hyla arborea. E. Spermatozoön of Rana esculenta (the tail is too short). (After v. la Valette St. George.)

the spermatozoön is formed by a flat membrane with a thickened border. In Hyla arborea and Rana esculenta (Fig. 7, D, E) the tail of the spermatozoön is like a long lash or thread. In Rana esculenta the head measures .015–.021 mm. in length and the tail .04 mm. in length.

"DIRECT" DIVISION OF THE GERM-CELLS

In the testes of the frog and of other Amphibia are often found germ-cells whose nuclei have very irregular outlines.

Centrosomes can generally be demonstrated in these resting-cells. Other cells have the nuclei broken up into a number of smaller spheres. Still other nuclei may have a deep depression on one side, as though the nucleus were dividing into two by constriction. These nuclei have often been described as dividing by amitotic or direct division, *i.e.* without the characteristic mitotic division. Meves ('91) has stated that in the testes of the salamander amitotic division occurs regularly, and he believes that such cells will later form spermatozoa. Other authors (Bellonci and vom Rath), admitting that such a division may take place, affirm that such cells are in process of degeneration, and never subsequently form spermatozoa. Vom Rath declares that cells that have once divided by amitosis can never again divide by karyokinesis (mitosis), and that such cells degenerate later, and do not ever develop into sex-cells.

OÖGENESIS

The origin of the egg in the ovary of the frog has been studied by Schultze ('87, e), but many important details are still unknown. The egg derived from one of the cells of the outer layer of the ovary is surrounded by a large number of follicle-cells. The nucleus of the egg consists of fine chromatic threads, of a nuclear sap, and of scattered nucleoli. As the egg enlarges the nucleus also enlarges, and the chromatin stains more faintly and appears in the form of scattered threads. The nucleoli stain well and become larger and more numerous as the egg enlarges, and are found generally around the periphery of the nucleus. Certain portions of the protoplasm now begin to stain dif-

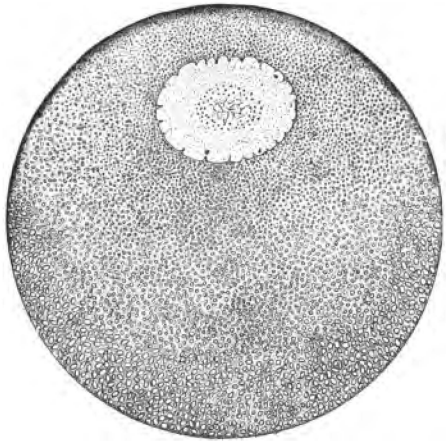


FIG. 8. — Ovarian egg of Rana.

ferently from the rest, and are spoken of as the yolk-nuclei. They seem, in some way not fully understood, to be connected with the development of the yolk-granules.[1] The yolk-granules, at first small and scattered, grow larger and become more numerous (Fig. 8). Before the egg leaves the ovary, the nucleus wanders toward the periphery and places itself under the black pole of the egg (Fig. 9). When the surface of the egg is examined, it shows a lighter area, owing to the displacement of the pigment-granules in the region occupied by the nucleus. The nucleoli at this time migrate toward the centre of the nucleus,[2] there disintegrate, and finally disappear. The chromatin-material draws together at this time into threads which stain more deeply, the nuclear membrane disappears, an achromatic spindle develops, and the egg is ready to extrude the first polar body.

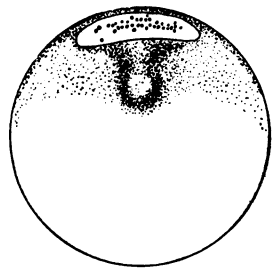


FIG. 9. — Section of ripe ovarian egg. (After Hertwig.)

COMPARISON OF SPERMATOGENESIS WITH OÖGENESIS

The method of extrusion of the polar bodies is described in the next chapter, but we may anticipate this account in order to consider here a remarkable parallel that has been discovered between the formation of the polar bodies and the formation of the spermatozoa. In the latter, as we have seen, two successive divisions follow each other during the maturation-period *without an intervening resting-stage.* The tetrad-groups are present at the beginning of the process. After the two maturation-divisions the number of chromosomes is reduced to half the number characteristic for the species.[3] The same phenomena appear when the polar bodies are extruded from the egg. After the extrusion of the first polar body, the spindle for the second

[1] Will ('84) describes the yolk-nuclei as arising from constrictions of the nucleus set free with their nucleoli into the protoplasm.

[2] Schultze affirms that the later chromatin comes from these nucleoli, but Born has corrected this statement.

[3] The reduction in number of the chromosomes seems in some forms to take place before the tetrad-period.

polar body forms immediately *without a resting-period.* Further, it is found, after the extrusion of the second polar body, that the number of chromosomes in the egg is reduced to half the number characteristic for the somatic cells. Tetrads have also been described as occurring just before the extrusion of the polar bodies. In many cases the first polar body divides into two, so that three polar bodies are present. These three polar bodies and the egg seem to correspond to the four spermatozoa from each spermatocyte. All four spermatozoa are functional, but only the egg (and not its three polar bodies) is capable of development. Weismann has utilized the discovery of the reduction of the number of chromosomes to build up an elaborate and highly speculative theory of heredity. The reduction division is, according to Weismann, not simply a quantitative division of the chromatic thread, but is at one stage at least a qualitative division. The reduction of the chromosomes to half the number present in the other cells of the body seems, according to Weismann and others, to be a preparation for fertilization. Since the spermatozoön brings into the egg only half the number of chromosomes found in the somatic cells of the animal, and since the egg-nucleus supplies the other half, the number of chromosomes will thus remain constant for the species from generation to generation.

CHAPTER II

POLAR BODIES AND FERTILIZATION

WHETHER the egg leaves the ovary by means of its own activity, or by some other mechanism, we do not know. That the egg itself takes some part in the process seems possible from the fact that it is set free only at a particular moment of its maturation, *i.e.* at a time when certain processes have taken place in its interior. This same process takes place simultaneously in all the eggs in the ovary. The separation of the egg from the ovary is not dependent upon the act of copulation, for several cases are on record in which isolated females were found to have eggs in the body-cavity and oviducts.

The egg set free in the cœlomic cavity is covered by an extremely thin membrane, the egg-membrane or vitelline membrane. The egg itself is very soft and easily broken if handled. Later, when in the oviduct, the protoplasm seems to become more firm.

The egg shows a white and a dark hemisphere. The relative distribution of superficial pigment in the egg determines the extent of the white and dark surfaces. The outer layer of pigment in the black hemisphere seems to be in close contact with, or fixed to, the vitelline membrane, but the pigment lying in the protoplasm beneath the outer layer is free to move with any movement of the protoplasm (Figs. 8, 9). The relative extent of surface of the egg that is black or white is variable in different species, and even in different females of the same species; but all the eggs from one female show approximately the same distribution of pigment.

EXTRUSION OF THE FIRST POLAR BODY AND EGG-LAYING

Just prior to the extrusion of the egg into the body-cavity of the frog, the nucleus undergoes a remarkable change, so that in

place of a large watery nucleus only a small mass of chromatic substance, lying in the protoplasm, is present. An achromatic spindle appears, and the chromatin in the form of granules is arranged at the equator of the spindle. The spindle lies at the surface of the egg near the centre of the black hemisphere (Fig. 11, A). It lies also in the centre of the fovea, which is found on the surface of the egg. The fovea marks the former position of the large ovarian nucleus, and although the nuclear membrane of the original nucleus has disappeared, and its watery cavity has been encroached upon by the surrounding protoplasm, yet the pigment has not penetrated very deeply into this region. The eggs pass in this condition from the body-cavity into the oviducts. Newport ('51) believed that, owing to the close attachment of the oviducts at their inner openings to the walls of the pericardium, at each contraction of the heart the slit-like openings of the oviducts would gape open, and any eggs in the vicinity might be forced into the mouths of the tubes. Also, he thought that owing to the muscular movements of the body, and the resulting shifting of the internal organs, the eggs sooner or later pass near the openings of the oviduct, and are then carried into the tube. At any rate, there seems to be not much ground for the older statement that the mouths of the oviducts actually grasp the eggs by a muscular movement like that of swallowing. According to Nussbaum ('95), the eggs, when set free from the ovary into the body-cavity of the frog, are carried into the open mouths of the oviducts by the motion of the cilia of the cœlomic epithelium. These cilia drive anteriorly any bodies lying free in the body-cavity. If, for instance, eggs taken from one frog be placed in the vicinity of the openings of the oviducts in the body-cavity of another frog, they will be carried into the open mouths of the oviducts by the action of the cilia in that region.

The cilia do not cover the entire surface of the cœlomic epithelium, and there are certain recesses in the body-cavity destitute of cilia. The eggs that accumulate in these recesses will be sooner or later forced out into the general cavity as a result of the alternate contractions and expansions of the ventral musculature of the body-wall, as well as by the changes

produced by the filling and emptying of the lungs, and by the movements of the heart.

Swammerdam's account in 1737 describes the passage of the egg from the ovaries to the oviducts by way of the cœlomic space. Spallanzani in 1785 observed that the females of Bufo igneus, isolated before union with the male, could still lay their eggs. One of the tree-frogs has its eggs in the uterus before it unites with the male. On the other hand, Spallanzani stated that females of the stinking toad if isolated while the eggs are still in the ovaries will retain their eggs, but if separated after having paired will then deposit their eggs. According to the evidence of several authors, Rana temporaria when isolated will, in certain cases at least, set free its eggs.

It has been suggested that the embrace of the male is mechanically necessary in order that the eggs may pass from the ovary into the oviducts, but this is certainly not always the case, and if not necessary in one form is probably not necessary in others. The sexual excitement set up by the tight embrace of the male may however be necessary in some species for the successful performance of egg-laying. The eggs pass one by one down the length of the oviducts, ultimately to reach the lower portion of the tube, the so-called uterus, where the eggs accumulate. If a frog is killed at the height of the breeding season, free eggs are often found in the body-cavity, and a series of eggs passing individually down the ovarian tubes, as well as an accumulation of eggs in the uteri. In their passage through the oviducts the eggs undergo certain internal changes and receive also their egg-coats. In the tubes of the oviducts the nuclear spindle divides, so that half of the original chromatin goes

FIG. 10. — Egg in jelly. (After Schultze.)

to one pole of the spindle, and half to the other. The spindle has assumed, during this time, a radial position with respect to the egg, so that we may speak of a distal and of a proximal or central end (Fig. 11, A). The distal end pushes out into a protrusion of protoplasm that has simultaneously formed at this

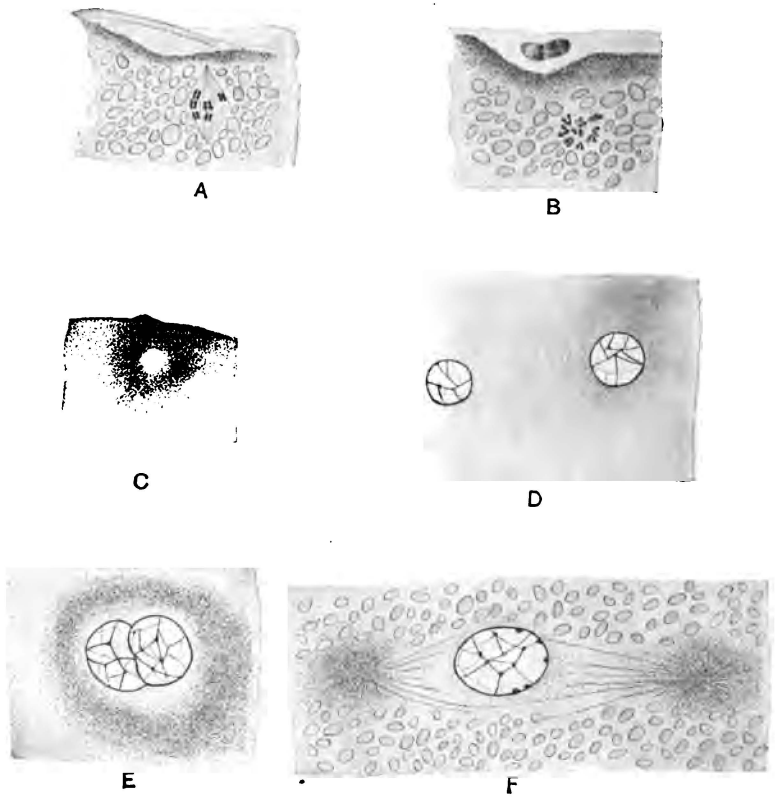


FIG. 11. — Extrusion of first polar body and fertilization of egg of Toad. (From preparations made by Helen D. King.) A. First polar spindle. B. First polar body extruded; second polar spindle present. C. Entrance of spermatozoön. D. Male and female pronuclei. E. Apposition of two pronuclei. F. First segmentation-spindle.

point of the surface of the egg. This protrusion of protoplasm with its enclosed half of the nucleus gradually pinches off from the surface of the egg, and there is thus formed the first polar body (Fig. 11, B). The egg gets a thin layer of gelatinous

substance around it soon after entering the oviduct, *i.e.* before it has reached the first part of the convoluted portion. This is the so-called chorion, — a thin investing membrane which adheres closely to the vitelline layer around the egg. During the remainder of the passage through the oviducal tube the egg gets two other distinct gelatinous layers (Fig. 10). The middle layer of the three is, according to Newport, a watery layer of considerable thickness. The outer gelatinous covering is also thick and serves to stick the eggs together in a bunch, and even to stick the bunches of eggs, when laid, to surrounding objects.

The spawning of certain species of frogs takes place very rapidly, and by a single effort. Newport says that the process takes place in a few seconds or less than a minute, and that all the eggs that have accumulated in the uteri are laid at once. When laid, the egg-cluster forms a rounded mass which is, at first, scarcely as large as a walnut. The eggs then seem to consist almost entirely of dark-colored "yelks" with thin gelatinous envelopes. "Up to about this period the ova remain undisturbed in the water in a mass as they are expelled, and lie indiscriminately, some with the dark and some with the white portion of the yelk uppermost or horizontal. But during the time that has passed since the ova have been in contact with the water, the envelopes have imbibed fluid and expanded until these investments of the yelk have a thickness equal to about two-thirds of the diameter of the yelk itself."

"The yelks, that have remained up to this time with their white surface uppermost, now change their position spontaneously by a partial rotation of the whole mass of each on its axis, within the vitelline membrane, until the dark surface of the whole is placed uppermost. Whether this change of position is merely the result of expansion of the vitelline membrane at this period, or whether it be also connected, as we may fairly believe, with changes going on in the interior of the yelk, I am not prepared to decide."

THE JELLY OF THE EGG, AND THE SECOND POLAR BODY

The jelly around the frog's egg serves, no doubt, as a protection to the egg. The soft eggs are kept in spherical shape

and protected from injury from without. The slime protects them from water-snails that will eat the eggs if they are shelled out from the jelly. The jelly may also protect them against water-birds. The eggs and young tadpoles seem, however, in themselves to be distasteful to certain crustacea (Bernard and Bratuschek, '91).

This jelly has the physical peculiarity of allowing the sun's rays to pass through, but hinders reflection of the rays from the interior to the outside. The result is that in the sunlight the mass of eggs is at a higher temperature than the surrounding water, and as the eggs of many frogs are laid in the early spring, when the water is quite cool, this property of the jelly helps to hasten their development.

Hertwig ('77) thought that a change takes place in the interior of the egg after fertilization, so that a difference in the specific gravity of different parts of the egg is brought about. Schultze ('87), however, pointed out that at this period the egg contracts slightly from its vitelline membrane, and between the egg and its membrane a fluid collects, that is probably squeezed out of the egg itself. The egg, freed from its innermost coat which held it in place, then rapidly orients itself with respect to gravity. Unfertilized eggs will also, after a time, slowly rotate, and in these it can be seen that the separation of the egg from its membrane is less perfect than in fertilized eggs. "At the *moment* when the ovum is expelled from the body, the envelope is merely a thin gelatinous layer, its entire diameter being equal only to about one-sixth of the diameter of the yelk. After it has been *one minute* in water, and begun to imbibe and expand, it is then equal to about one-fourth of the diameter of the yelk. At the end of *two minutes* it is enlarged to one-third, and in *three minutes*, to one-half the diameter of this body. In four minutes, it exceeds three-fifths, and in six minutes, two-thirds, and it continues to imbibe fluid and expand at the same rate, until, at from ten to fifteen minutes, it very nearly equals in thickness the whole diameter of the yelk; and at half an hour it is one-fourth greater than this. At the end of three hours the membranes have acquired nearly their full size."

"The expansion of the envelope is greatly retarded at the

end of the third or fourth hour, until after cleavage of the yelk has taken place, when it again proceeds, but much more slowly than at first."[1]

In Rana fusca the extrusion of the *second* polar body takes place one half-hour after fertilization, and the process can be seen under a low magnifying glass or even with the naked eye. A whitish speck appears in the black hemisphere near the point at which the first polar body was extruded. It is necessary, however, to make sections of the egg to discover the further changes that are taking place. Schultze ('87) has given a careful description of the process. The nucleus that remains in the egg after the extrusion of the first polar body assumes once more a horizontal position, but *does not go into a resting-stage* (Fig. 11, B), *i.e.* the chromatic loops or threads do not re-fuse into a network nor does a nuclear membrane form. The chromatin arranges itself on a new spindle. The latter then assumes a more or less radial position, and the second polar body is extruded half an hour after the egg is laid. It is probable that the second polar body is not extruded under normal conditions until after a spermatozoön has entered the egg.

One and a half hours after the egg is laid, another change may be seen taking place. Near to or at the apex of the black pole the egg is seen to flatten, and an accumulation of fluid is found here between the egg and its vitelline membrane (Fig. 10). At or near the centre of this flattened portion one may see the fovea, and near or in it the polar bodies appear on the flattened disc. This chamber formed between the flattened egg and the inner membrane was seen by Newport and called the "respiratory chamber." It may ultimately be as large as one-sixth the diameter of the whole egg. Schultze points out that it lies somewhat excentrically with respect to the egg-axis (Fig. 10). The clear fluid in this chamber has been supposed to be the watery contents of the original large nucleus of the egg, which has been squeezed out of the egg. Very little evidence has as yet been given to support this view. Some of the older embryologists thought that this fluid represented

[1] Newport ('51), p. 193.

the original egg-nucleus itself, which was squeezed out of the egg at this time. Now, however, since we know the complete history of the nucleus during this period, the suggestion of its entire loss by the egg does not call for serious criticism.

Entrance of Spermatozoön and Copulation of Pronuclei

The sperm of the male is poured out into the water, and probably over the eggs themselves at the moment when they are laid, and the spermatozoa begin at once to bore into the jelly of the egg-mass (Fig. 10).

Kupffer has described the entrance of the spermatozoön into the eggs of Bufo variabilis. When the head of a spermatozoön touches the egg-membrane, the protoplasm of the egg draws back slightly at the point of contact, but quickly returns again to its first position. The period of penetration of the spermatozoön from the moment of contact of the sperm-head until the spermatozoön disappears into the egg, lasts in some cases from one to one and a half minutes, in other cases only three-fourths of a minute. Several spermatozoa were observed by Kupffer to enter each egg.

Other spermatozoa reach the egg-membrane, but do not seem to be able to enter the egg. In the regions where these spermatozoa lie, the surface of the egg rises up in small protuberances. This process occurs about fifteen minutes after the first spermatozoa have entered, and lasts about one or two minutes, after which the protuberances sink back into the egg. The spermatozoa in the regions of the protuberances are left outside the egg-membrane. This peculiar phenomenon is described by Kupffer as a counter demonstration of the egg against those spermatozoa that have not been able to enter. Eggs that have been artificially fertilized show, when cut into sections, that one hour after fertilization a dark pigmented streak is formed, reaching from the pigmented coating of the egg into the yolk-mass. The process takes place in the upper or dark hemisphere, and regularly at one side of the centre of the dark field near to the edge of the white border. The streak takes a somewhat oblique course toward the centre of the egg. At

the central end the dark streak is rounded, and encloses a clear spot.

In this clear region one sees a distinct pronucleus about nine microns (μ) in diameter. Eggs one and a half hours after fertilization show that the pigmented streak has penetrated deeper into the egg, and in the frog the male pronucleus has enlarged to 32 by 22 μ (Fig. 11, D, for the toad).

At this stage another nucleus is present in the frog's egg, and this lies not far from the end of the pigmented streak (Fig. 11, D). This measures 22 μ, and has the same structure as the male nucleus. These two nuclei are undoubtedly the male and female pronuclei. We now know that the female pronucleus has come directly from the original egg-nucleus, which has, after extruding its two polar bodies, penetrated once more deeper into the egg. The complete history has not been traced in the frog, but there can be no reasonable doubt as to what takes place. In the newt (and in the toad) the history has been followed, and it is found that the female pronucleus arises from the egg-nucleus after the extrusion of the polar bodies.

In the next half-hour Hertwig has found that the nuclei approach more nearly to each other, and the pigment-streak penetrates deeper into the egg, the swollen end enlarges, and the two large oval male and female pronuclei are then found together in the swollen end of the streak (Fig. 11, E). In a preparation of an older stage both nuclei have increased in volume to 35 μ, and have flattened against each other. *They then fuse into one nucleus* which measures 44 μ (Fig. 11, F, toad). The resulting nucleus, the segmentation-nucleus, is surrounded by clear protoplasm and then by a pigment-coat. From the segmentation-nucleus a streak of pigment extends to the dark surface of the egg and marks the path of entrance of the spermatozoön. All preparations after two and a half hours showed the union of the two pronuclei.

If the jelly be examined after the eggs have been laid, several or many spermatozoa can be seen boring their way through the jelly toward the egg. Some will have reached the inner layers, and still others lie in the outer coats (Fig. 10). It is probable that after one spermatozoön has succeeded

in forcing its way through the inner coat and *into* the egg, changes then take place in the egg that prevent or make difficult the further entrance of other spermatozoa. The contraction of the egg, noted above, may possibly have something to do with the process. If, however, two or more spermatozoa should reach the surface of the egg at about the same moment, it is not improbable that more than one might enter.[1] Both may then pass toward the female pronucleus, but in the frog it is probable that after one male pronucleus has fused with the female pronucleus, the further progress of other male pronuclei that happen to get into the egg is stopped.

It is sometimes said that the female pronucleus *attracts* the male pronucleus, but the approach of the two may be due to changes in the protoplasm; for the migration of the pronuclei through the egg is probably in most cases brought about by the protoplasm of the egg under the influence of the pronuclei, and the pronuclei themselves are merely passively carried along.

In the newt (Jordan, '93) it seems to be usual for more than one spermatozoön to enter, but only one of these fuses with the female pronucleus. The others subsequently degenerate and go to pieces. In the eggs of other animals, as the starfish, polyspermy, or the entrance of more than one spermatozoön into the egg, brings about disastrous results, causing irregular division of the nucleus and subsequent irregularities in the segmentation of the egg. In these eggs the field of action is small, and the male pronuclei or their centrosomes mutually influence one another and the female pronucleus. In large eggs with much yolk, such as those of the Amphibia and of the Sauropsida, the spermatozoa may be too far apart to affect one another or the segmentation-nucleus, and after the fusion of one male pronucleus with the female the movement of the other male pronuclei towards the female pronucleus seems to stop.

The head of the spermatozoön enters the egg to become the male pronucleus. The tail of the spermatozoön is left at the

[1] It is probable that Kupffer's ('82) account does not apply to eggs under normal conditions.

surface of the egg, or if a part enter the egg it takes no share in the subsequent changes. The middle piece of the spermatozoön is now known to contain a body that plays a most conspicuous part in many animals in the division or cleavage of the egg. The middle piece enters with the head of the spermatozoön. It contains the centrosome, which divides, and around each centre an elaborate system of rays develops. The two centrosomes migrate to opposite sides of the segmentation-nucleus, and between the two appears the spindle of the first cleavage. In the frog the history of the middle piece and centrosome, and the origin of the segmentation-spindle have not yet been worked out.

CHAPTER III

EXPERIMENTS IN CROSS-FERTILIZATION

A NUMBER of attempts have been made to fertilize the eggs of one species of frog with the spermatozoa of another species. Rusconi experimented in 1840 with the toad (♂) and the green water-frog, Rana esculenta (♀). Lataste in 1878 attempted to cross-fertilize the eggs of different species of urodeles with Pelobates fuscus and P. cultripes.

EXPERIMENTS OF PFLÜGER AND OF BORN

The most extensive and important work is that of Pflüger ('82) and of Born ('83). These investigators have made a large number of experiments in crossing different races and species of Anura. When the sperm of Rana fusca was placed with the eggs of Bufo vulgaris, the eggs segmented and developed as far as the "morula" stage, and then without exception died.[1] Conversely, when the sperm from B. vulgaris was used with the eggs of R. fusca, no result followed, not even the segmentation of the egg (except in one experiment where two eggs out of one hundred divided irregularly). Eggs of R. fusca placed in a water-extract of the testes of R. esculenta remained unfertilized. But eggs of R. esculenta placed with the sperm of R. fusca developed regularly, with few exceptions, as far as the blastula stage, and then died. Crossing various species of Tritons gave no results. But eggs of Rana fusca were acted upon by the sperm of Triton alpestris and Triton tæniatus, inasmuch as they began to show irregular cleavage-lines. Later they died. The reverse cross gave no result.

Rana fusca and Rana arvalis are very similar in appearance, but are apparently separate species. Cross-fertilization was

[1] Pflüger ('82).

here possible (R. fusca, ♂, R. arvalis, ♀). Tadpoles developed from the crossed eggs, and some of these ultimately transformed into frogs. Pflüger got similar results with the same species, and also found that the reverse cross (R. fusca, ♀, and R. arvalis, ♂) gave no result. Born found that the eggs of Bufo cinereus could readily be fertilized with the sperm of Bufo variabilis. All the eggs segmented regularly, the larvæ left the jelly, and developed into frogs.

In respect to the closeness of the relation between the species, Born says that we can be quite certain that the two species of Rana arvalis and R. fusca are much more nearly related than the two species of Bufo. The success of cross-fertilizing depends apparently less on the degree of relationship, as shown by the similarity of color and habits, than on the similarity of the male sexual products (Pflüger). Although R. fusca and R. arvalis seem to be very closely allied species, they have very different spermatozoa; in fact, the spermatozoa are as different as the spermatozoa of R. fusca and R. esculenta.[1] The two species of toads (Bufo) have very similar spermatozoa, which differ only in size, but this difference is so slight that, were the two kinds mixed together, one could scarcely distinguish between them. It is *apparently* owing to the difference in form of the spermatozoa of the R. fusca and R. arvalis, and to the similarity of the spermatozoa of B. cinereus and B. variabilis that the results are due.

Pflüger has made a large number of reciprocal crosses between different *races* of R. fusca. "The different races are as fertile *inter se* as are individuals of the same race." Pflüger concluded, after comparing the results of all of his experiments on cross-fertilization, that in general *those spermatozoa are most successful* for purposes of cross-fertilization *that have the thinnest and most pointed heads*. That in general those eggs are most easily fertilized that belong to species having spermatozoa with thick heads. The results, then, he thought, depend largely upon mechanical conditions; for where the head is small and pointed, the spermatozoön can bore its way more successfully into the eggs

[1] R. arvalis and R. esculenta have similar sperm. Born and Pflüger found that the crossed eggs segmented irregularly, and that later the embryos all died.

of its own and of other species. If the head is large, the spermatozoön can force its way only into those eggs that are adapted to spermatozoa with large heads. For instance, the spermatozoa of R. fusca have thinner heads than any others, and the head is, moreover, very pointed. These spermatozoa can fertilize eggs of nearly all other species (R. arvalis, R. esculenta, B. communis). Conversely, the thick-headed spermatozoa of R. arvalis and the blunt-headed spermatozoa of R. esculenta cannot get into the eggs of R. fusca.

The spermatozoön of B. communis, which has a *very pointed* but *somewhat larger* head than that of R. fusca, appears nevertheless to be able at times to penetrate the eggs of R. fusca and to fertilize them. That the spermatozoön of Triton can enter the eggs of R. fusca is explained very easily when we remember that the sharp thin head of the Triton spermatozoön is best adapted of all species to penetrate any egg. We see, too, that the thick-headed spermatozoön with a blunt anterior end, such as those of R. arvalis and R. esculenta, cannot fertilize the eggs of any other species. And finally, to confirm the conclusion, we find that these two species, R. arvalis and R. esculenta, which have large-headed spermatozoa, are alone capable of *reciprocal* crossing. Pflüger believed that the eggs have the greatest capacity for cross-fertilization at the height of the breeding season, and the same statement holds, but in a much less degree, for the spermatozoa.

Experiments on Other Forms

Hertwig has objected to Pflüger's conclusions on the ground that the eggs of the sea-urchin are much more capable of cross-fertilization after they have begun to suffer change either from being kept some time in sea-water, or from the application of drugs. He thought that the frogs kept by Pflüger had been also under artificial conditions. Further, Hertwig concluded, from his results on sea-urchins, that the possibility of crossing does not depend entirely upon the external conditions, but to a large extent upon some unknown property of the egg. Eggs in good condition are able to prevent the entrance of foreign spermatozoa, but as soon as they begin to lose their irritability, they can no longer resist the entrance.

Born obtained some interesting results as to the relations existing between the number of spermatozoa in a fluid-extract of the testis and the power of the fluid-extract to fertilize eggs. He insists that in some cases there is a necessary connection between the two. It is far from clear how this is possible, and the result may depend on other causes which are introduced along with the solutions employed. Moreover, the further question of polyspermy of such eggs complicates the results. Born believes that many cases of irregular segmentation of crossed eggs are due to the entrance of several or many spermatozoa into the egg, which act as centres for protoplasmic accumulations. Such a segmentation he calls "barock" segmentation. On the other hand, Pflüger suggests that the *irregular* cleavage of certain of the crossed eggs is the result of the disintegration of the male pronuclei, so that the chromatin is scattered, and then acts on the protoplasm, producing an irregular division.

Recent results have shown that polyspermy is a normal occurrence in some amphibian eggs, and, despite the presence of several spermatozoa, normal cleavage and normal embryos result. The changes that take place within the cross-fertilized eggs must be more carefully studied before a final decision can be reached in regard to the meaning of some of the experiments described above.

We must not confuse two factors that enter into the problem of cross-fertilization. On the one hand, the spermatozoön may not be able to push through the gelatinous coatings of the egg, or it may not be able to bore through the outer surface of the egg itself, or it might be unable to enter the protoplasm if the latter were entirely free from its coats.[1] On the other hand, even if the spermatozoön could successfully enter and combine with the female pronucleus, it does not follow that the egg would develop. We now know that so many factors enter into the problem of fertilization of the egg that it is not surprising when we find that two pronuclei that have ever so slight differences are not able to carry out the complicated machinery of cell-division and development.

[1] As in the case of naked pieces of protoplasm of the egg of species of sea-urchins.

The eggs of the starfish can be fertilized by the spermatozoa of the sea-urchin, — forms much more different than any two species, genera, or even families of frogs, and the early stages of segmentation, and the formation of a swimming blastula and gastrula may be passed through; but the later embryonic development is not carried out, and after a time the gastrulas die.[1]

Hertwig's experiments ('77) on polyspermy in the eggs of echinoderms show that when several spermatozoa enter the same egg a karyokinetic spindle is formed around each of the resulting male pronuclei and many or all of the pronuclei divide. Often the spindles are so near together that they mutually influence one another and most complicated karyokinetic figures result. Subsequently the protoplasm breaks up around the pronuclei in a most irregular way, and generally such eggs do not give rise to even the earliest stages of development. The phenomenon is so similar to the "barock" segmentation of the frog's egg that it seems possible that in the latter the result is brought about in the same way as in the echinoderms.

EXPERIMENTS OF RAUBER AND OF BOVERI

Rauber, in 1886, tried to carry out the following interesting experiment. The segmentation-nucleus of a frog's egg, one hour after fertilization, was removed by means of a fine pipette. The same process was carried out with a toad's egg. The nucleus of the toad's egg was then placed in the frog's egg that had had its nucleus removed, and the nucleus of the frog's egg was placed in the toad's egg. Unfortunately, neither egg developed. The results of such an experiment would be of the greatest importance if the experiment could be successfully carried out; for in this way we should hope to discover whether the characters of the embryo come from the nucleus or from the protoplasm of the egg.

Boveri, in 1889, made somewhat similar experiments with the egg of the sea-urchin. When the eggs are shaken in a small tube, they are broken into fragments, some with nuclei and others without. When a sufficiently large non-nucleated

[1] Morgan, '93, *Anat. Anzeiger.*

fragment is penetrated by one spermatozoön, the fragment develops. Such a fragment contains only half the number of chromosomes of the normal fertilized egg.[1] Boveri isolated some of these fragments, and said that they give rise to small embryos normal in structure. Boveri stated, further, that if a non-nucleated fragment of the egg of one species of sea-urchin is entered by one spermatozoön of another species, the resulting larva is like the larva of the father (*i.e.* it is like the larva of the individual from which the spermatozoön comes). If this result should prove true,[2] it would show that the nucleus and not the protoplasm determines the character of the larva.

---

[1] Morgan, '95, *Anat. Anzeiger.*

[2] Seeliger ('95) and myself ('95) have repeated Boveri's experiment and have tried to show that the evidence on which Boveri based his conclusion in regard to the paternal character of the crossed larva is insufficient.

CHAPTER IV

CLEAVAGE OF THE EGG

WHEN the egg comes to rest in its membranes after fertilization has taken place, it will be found that the egg-axis assumes an oblique position with respect to the vertical. The degree of obliquity may be different for the eggs of different species of frogs, but in some species it is carried so far that, when the egg is looked at from above, a crescent of the white hemisphere can be seen on one side of the egg. Roux has stated that the declination of the egg-axis takes place only *after* the entrance of the spermatozoön, and *toward* that side into which the spermatozoön has penetrated.[1] He was able to determine this by artificially fertilizing the egg at definite points. By means of a small pipette, water containing spermatozoa was brought in contact with the jelly somewhere near the upper hemisphere of an egg. Presumably the spermatozoön will then take the shortest path to the egg. Roux found that the egg after a time *generally* rotated on its axis toward the point at which the artificial fertilization was supposed to have taken place.

NORMAL CLEAVAGE

The first furrow appears on the egg about two and one half to three hours after fertilization, the time depending in part on the temperature of the water. A rather wide furrow appears in the flattened area near the black pole, and rapidly extends over the upper surface of the egg, and then moves more slowly over the lower or white surface. The sides of the furrow are often wrinkled, probably a mechanical result of the

[1] Roux believes the obliquity to be a usual phenomenon *after* fertilization for some species; in others the obliquity is only occasionally seen. Schultze finds it to be as much as forty-five degrees in Rana fusca.

infolding of the outer harder crust of the egg. These wrinkles are best seen in the upper hemisphere; subsequently they disappear. It will be found on cutting in two an egg in the process of cleavage that the furrow is also extending *through*

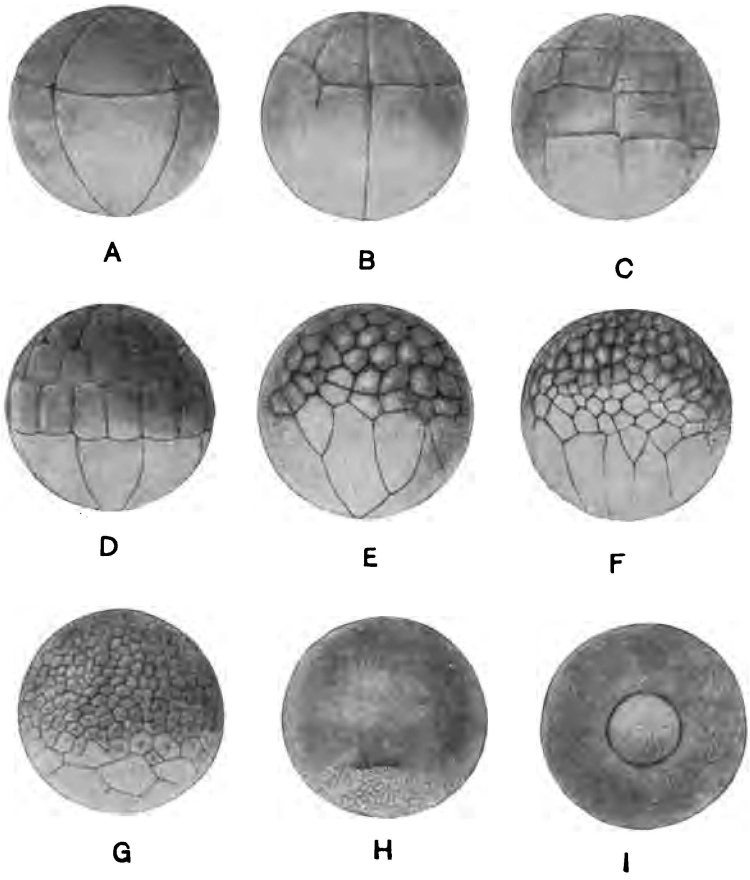


FIG. 12. — Segmentation of egg and formation of blastopore (H, I). A. Eight-cell stage. B. Beginning of sixteen-cell stage. C. Thirty-two-cell stage. D. Forty-eight-cell stage (unusually regular). E, F. Two sides of same egg in later cleavage. G. Still later cleavage. H. Dorsal lip of blastopore. I. Circular blastopore (with lower pole toward observer).

the protoplasm of the egg, *i.e.* dividing the contents into two parts. When the superficial furrow has encircled the egg, the substance also has been divided.

If a series of sections be made through the egg at different stages in the process of cleavage, we should see that prior to the division of each blastomere the nucleus had divided into two parts. This takes place by the ordinary process of indirect

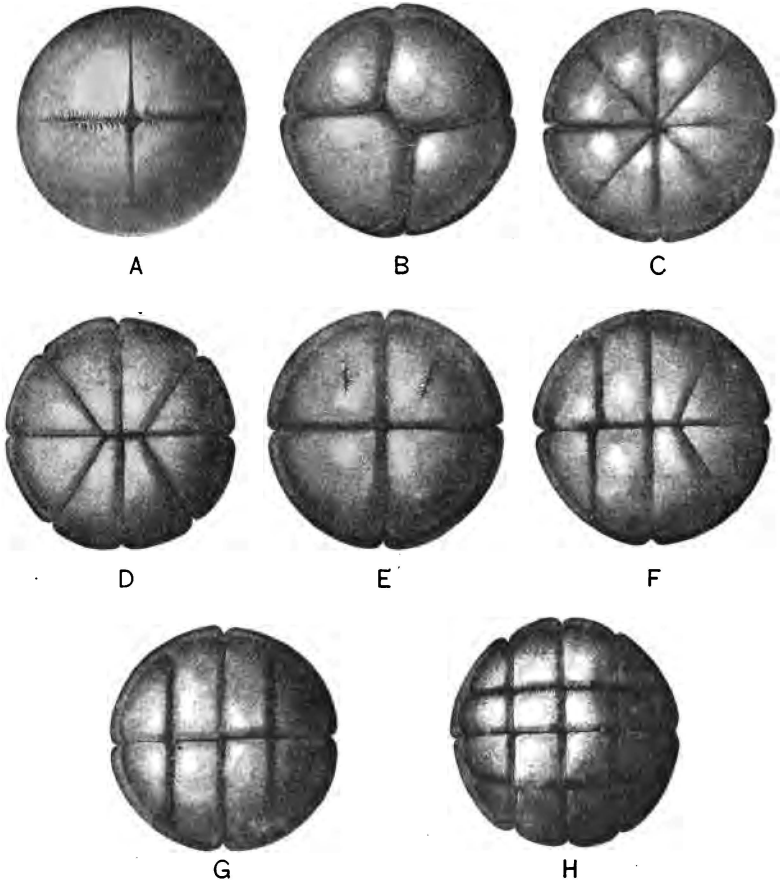


FIG. 13. — Segmentation of egg (two, eight, sixteen, and thirty-two cell stages, after M. Schultze), as seen from above. A. Two-cell stage; beginning of second furrows. B. Eight-cell stage, with cross-furrow. C, D, F, G. Sixteen-cell stages. E. Eight-cell stage (regular type). H. Thirty-two-cell stage.

or karyokinetic division. Half of the chromatin passes to one pole of the nuclear spindle, and the other half to the other pole. As the spindle elongates, it carries with it the surrounding pig-

ment. The first cleavage-plane always passes directly between the separating halves of the segmentation-nucleus.

There is an infinite number of possible planes through which the first cleavage might divide the egg into equal portions. What, then, determines the particular plane taken? We can think of this plane as determined by external conditions, or by the internal structure of the egg, or by a combination of the two. In the first place, it seems probable that at the first division of the segmentation-nucleus each resulting half will get half of the chromatin of the male and half of the chromatin of the female pronucleus. The first plane of division *must therefore pass at right angles to the plane of apposition* of the two pronuclei. That is to say, it will also pass through the path of penetration of the spermatozoön (the male pronucleus), and therefore approximately through the point at which the spermatozoön has entered. This, according to Roux, is what actually takes place. Moreover, since the egg has rotated as a whole in the direction of the point of entrance of the spermatozoön, the first cleavage will pass exactly through the highest point of the white crescent, as seen from above.

On the other hand, there is no direct evidence to show that the two apposed pronuclei retain throughout subsequent changes the position of *first apposition*, and there is much to show that in the frog's egg, as well as in other eggs, the dividing nucleus, or the direction of its spindle, is very susceptible to modifications in the surrounding conditions.

There is also some evidence to show that the declination of the axis of the frog's egg is not necessarily determined by the entrance of the spermatozoön, but by the arrangement of the internal constituents of the egg itself. If, therefore, it could be shown that the declination is present in unfertilized eggs, and that in fertilized eggs the plane of first cleavage passes more or less through the highest point of the white crescent, then we should conclude that the plane of first cleavage is prearranged in the egg. It would follow as a corollary that the nuclear spindle orients itself with *respect to the egg*.

There is direct evidence to show that in the newt some such process as this does take place. Jordan ('93) has shown that the spermatozoön may enter at any point of the surface of the

upper hemisphere, yet the plane of first division is always *across* the long axis of the egg. Hence, it is fair to assume that the segmentation-spindle does so orient itself after the fusion of the male and female pronuclei that half of the male and half of the female chromatin are carried apart in the direction of the long axis of the egg, whatever may have been at first the position of apposition of the two pronuclei. I have dwelt on this point at some length because it is one of great importance for our understanding of the relation between egg and embryo; and because it is much to be desired that the present state of doubt should be cleared away.

After the protoplasm has divided into two equal parts, the egg "rests" for a time. During the division-period the hemispheres or blastomeres round up to some extent; but as soon as the division is completed they flatten against each other, so that the cleavage-plane is not so distinctly seen on the surface of the egg. The same process of flattening generally takes place also when the dividing egg is brought into preserving fluids.

During the time of division we may speak of each blastomere as tending to become itself a sphere, but, owing to the lack of room, the rounding of the two parts is very imperfect. In other eggs (*e.g.* the eggs of the sea-urchin), where it is possible to remove the egg-membranes, it has been found that then each of the blastomeres approaches more nearly the spherical form, or even becomes a complete sphere. We see from this that the external conditions may at least modify the form of cleavage of the egg.

It is sometimes said that during the division the two new parts or blastomeres tend to repel each other until after the division is completed, and to attract each other after the division is finished. Such a statement is, however, of little value, and may convey an entirely wrong impression of the changes taking place. One thing seems to be certain, that during the division of the egg the *spheres* or cells have an influence on one another. Whether unseen protoplasmic connections weld them together, or whether it is merely a question of *contact action*, has not yet been fully determined.[1]

[1] See Roux's experiments on cytotaxis ('96).

The nucleus of each blastomere during the resting-period undergoes a series of changes, the so-called reconstructive process taking place. The chromatin-granules or chromosomes are again surrounded by a nuclear membrane, and the granules fuse into a thread or network. At the next division of the egg the nuclear chromatin is again set free in the protoplasm by the absorption of the nuclear membrane. A spindle is formed and the chromatin in each cell is again exactly halved.

The second cleavage-furrow appears about three-quarters of an hour after the appearance of the first. Each of the two blastomeres divides in a plane at right angles to the preceding division. The furrows begin, generally simultaneously, in the upper hemisphere of each of the first two blastomeres, and push toward the lower pole (Fig. 13, A). The upper and lower ends of these new cleavage-planes are sometimes exactly opposite to each other, so that the effect is as though the whole egg had been divided by a single furrow in a plane at right angles to the first. In many cases, however, the new planes of division are not quite opposite, but reach the upper and lower poles of the egg at different points along the first plane of division. A "cross-line" is thus formed. The same result may be brought about even subsequent to division by a shifting or readjustment of the blastomeres on one another. As a rule, when a cross-line occurs in the upper pole, another one is formed in the lower pole, and the two stand in space at right angles to each other, as is shown in the diagrammatic reconstruction in Fig. 14, A. The same result can be obtained by compressing four clay spheres together until a single sphere results. It will be found in such a model that the cross-lines above and below are generally at right angles to each other.

The third furrows come in at right angles to the preceding planes of division, and are therefore horizontal (Fig. 12, A). The third planes of division do not lie at the equator of the egg, but, taken together, form a small circle in the black hemisphere or on the border-line between the black and white areas. Above there are four smaller dark blastomeres and below four larger white blastomeres. The four upper blastomeres are of approximately the same size, but in some species of frogs it seems that one is a little smaller than the rest, one is somewhat

larger, and two are intermediate in size. The *smallest blastomeres of the upper four always lie nearest the summit of the white crescent;* the largest is its *vis-à-vis*. If we think of the third planes of cleavage as lying in a single plane not quite at right angles to the first and second, but tilted a little, we get a clearer conception of the conditions present. The fourth cleavage-period comes in from a half to three-quarters of an hour later. As an idealized form, we may think of the new planes as forming two great circles at right angles to each other, and lying vertically and between the planes of the first and second cleav-

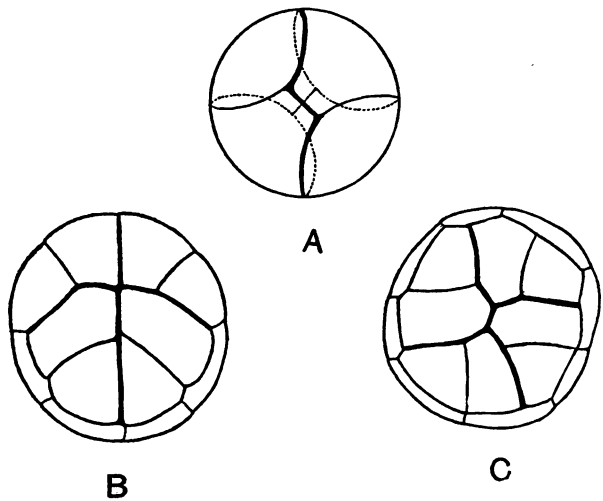


FIG. 14.—A. Diagram of four-cell stage to show cross-line. B, C. Sixteen-cell stage of two eggs. (After Rauber.)

ages (Fig. 13, C). This regular form is rarely if ever attained, and the greatest amount of variation is found to exist.

Remak said that frog's eggs divide much more *regularly* when carried in from places where they were normally laid. If allowed to stand quietly after being laid, they soon begin to divide irregularly. Vogt has also observed that those eggs of the salmon develop most regularly that have been kept in motion. It does not seem probable, however, that the motion itself could have anything to do directly with the matter; but if the egg be not supplied with a sufficient amount of fresh water,

etc., it might no doubt segment irregularly, or it may be that the motion equalizes the external conditions so that the eggs keep a more nearly spherical shape and hence divide more regularly.

Max Schultze ('63) and Rauber ('82) have made the most careful study of the variations in the planes of division of the fourth cleavage. In Figs. 13, D, F, G, and 14, B, C, are shown the upper hemispheres of several eggs. If we examine the position of the planes of the last (fourth) divisions, we see that in the upper hemisphere each new cleavage-plane fails generally to reach the black pole of the egg, but passes to one or to the other side. In the lower hemisphere the new planes fall far short of the white pole.

Occasionally we find eggs in the upper hemisphere of which one or more of the fourth planes reach the black pole itself, and, therefore, lie more nearly radial in position (Fig. 13, C). In other cases, however, one or more of the new fourth cleavage-lines may even be nearly in the horizontal plane. In the lower pole also there is much variation, and occasionally a blastomere is divided into a very small and a very large part, owing to the sudden turning aside of the new cleavage-line, so that it meets one of the first two cleavage-planes before it has extended far into the lower hemisphere. Other eggs at this same stage show a strictly bilateral arrangement of the cells in the upper hemisphere. In Fig. 13, D, F, G, we see that the fourth cleavage-planes have met the same furrow (first or second). Also in Fig. 14, B, a bilateral symmetry is present, formed in a somewhat different way, as the figure shows; and in this egg the lower hemisphere also is symmetrically divided.

During the fifth cleavage-period the irregularities in the division of the cells is generally so great that we cannot speak definitely of any special direction of the new planes. Nevertheless there is a tendency for some of the new furrows to come in at right angles to the last planes of division. Therefore, many and occasionally all of the new fifth cleavage-planes are horizontal (Fig. 12, C). The eight cells in the upper hemisphere divide into equal or nearly equal parts, but the eight blastomeres of the lower hemisphere divide unequally into eight upper smaller blastomeres containing pigment, and eight lower blastomeres which are the white blastomeres around the lower

pole. The division of the lower eight blastomeres is sometimes so regular that a circle of eight dark cells is formed around the equator of the egg (Fig. 12, C). After this division thirty-two cells are present. At this period the distribution of the cells over the dark and light regions of the egg is such that the cells on the side of the egg showing the light crescent above are smaller than the corresponding cells on the opposite side.

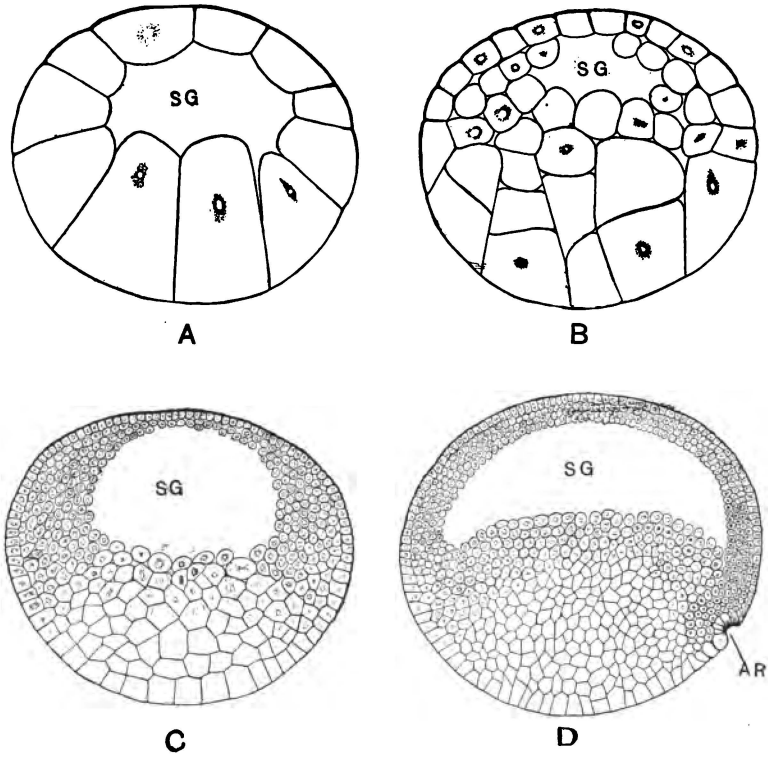


FIG. 15. — Stages in the segmentation (A, B, C) of the egg, and the beginning of gastrulation (D). AR. Beginning of archenteron. SG. Segmentation-cavity.

Up to the present time all the divisions of the cells started on the outside of the egg or blastomeres and progressed inward. As early as the eight-cell stage, a cavity appeared between the cells in the upper hemisphere of the egg. This is the "segmentation-cavity." It is filled with an albuminous fluid which

probably comes entirely, or in part, from the surrounding cells. This cavity gets larger and larger as development proceeds (Fig. 15, A). If an egg be cut open after the thirty-two-cell stage, it will be found that many, perhaps all, of the cells or blastomeres are undergoing division into outer and inner cells (Fig. 15, B). We may speak of this process as a *delamination*. Before the egg has divided into sixty-four cells, as seen on the surface, this delamination into inner and outer cells has in most cells taken place.

The cell-divisions now proceed more rapidly and with great irregularity. The rhythm also soon becomes lost, so that while some cells are dividing others are resting. Not only have the outer blastomeres continued to divide at the surface, but also below the surface of the egg new blastomeres are being cut off from the outer cells; the inner blastomeres also continue to divide. In the upper part of the egg a large segmentation-cavity forms. Its roof is covered by several layers of small deeply pigmented cells, its sides by larger cells, and its floor is formed by the large whitish yolk-bearing cells (Fig. 15, C).

If the surface of the egg be carefully examined during these later stages, it will be found that the cells over one side are distinctly smaller than those over the opposite side. We see that the side of the egg containing the most pigment is made up of larger cells. In Fig. 12, G, H, the opposite sides of an egg are shown, and here the less pigmented cells are seen to be smaller than the cells in the same position on the other side of the egg. Sections show, moreover, that this difference in size is not only found on the surface of the egg, but also in the interior as well. During the early periods of cleavage the egg has become neither more nor less pigmented on its surface, and has retained the same distribution of pigment as in the unsegmented egg.

Besides the variations in the cleavage noted above, others are more rarely found that depart much further from the usual typical forms. The first furrow, for instance, may divide the egg into very unequal parts. The second furrow may appear before the first has reached the lower pole. The third furrows may stand *vertically*, passing from near the upper pole into the lower hemisphere, *i.e.* the third furrows occupy the position of the fourth furrows of the usual type of cleavage.

CORRESPONDENCE OF THE FIRST CLEAVAGE-PLANE AND THE MEDIAN PLANE OF THE EMBRYO

If the egg when in the two-cell stage be fixed[1] so that it cannot rotate in a horizontal plane, and if such an egg be carefully watched until the moment when the medullary folds have just appeared, it will be found that the position of the plane of first cleavage corresponds approximately, or even exactly, to the median plane of the body of the embryo. This experiment was first made by Newport in 1851, subsequently by Pflüger ('83), and Roux ('85), and later by other workers.[2] If, however, during the subsequent cleavage-periods, *i.e.* during the eight and sixteen cell stages, etc., the position of this plane be kept in mind, it will be found that the later blastomeres from one or the other side often pass over the imaginary plane that corresponds to the plane of first division. Striking, therefore, as is the coincidence of the first plane of cleavage and the middle plane of the embryo, it remains to be proved, I think, that there is any direct causal connection between the first cleavage-plane and the median line of the body. It may be that the two phenomena are coincident because the internal arrangement of the egg that determines one may also, but independently, determine the other. In the newt Jordan ('93) has shown that the first plane of cleavage corresponds approximately to the cross-plane of the body. That is, the first two blastomeres correspond to the anterior and posterior parts of the body respectively. He suggests that the shape of the egg-capsule of the newt may be the cause determining the plane of first division. Some other factor than that of the position of the first plane of cleavage seems to determine the position of the embryo on the egg, for in the teleost's egg, where the symmetry and bilaterality of the cleavage is even more sharply marked than in the frog or newt, there seems to be no relation at all between the first cleavage-planes and the planes of the adult body.[3]

---

[1] For method, see Pflüger ('83), Roux ('85), and Morgan ('91).

[2] Rauber ('86) has later contradicted these results, but it is probable that there is an error in his experiment.

[3] Clapp ('91), Morgan ('93).

ROUX'S EXPERIMENTS WITH OIL-DROPS

The arrangement assumed by the blastomeres after each cleavage has attracted much attention. A system of soap-bubbles, or of balls of clay compressed into a sphere, gives somewhat similar figures. In this connection Roux has made a most instructive series of experiments. A small wine-glass is half filled with dilute alcohol and then sufficient oil is poured in to form a large drop. A stronger (lighter) alcohol is now poured on top of the oil, which assumes a spherical or nearly spherical shape. The drop lies suspended between the two

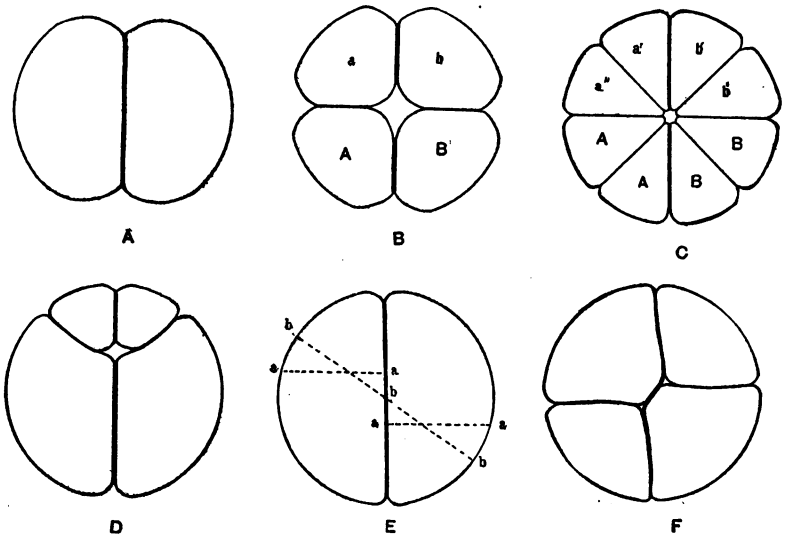


FIG. 16.—Systems of oil-drops. (After Roux.) In C, the lowest drops should be marked A′, B′; and those next them A″, B″.

alcohols and its periphery just touches the walls of the glass.[1] It is possible to divide this sphere of oil into equal or unequal parts by means of a glass rod and, if precautions are taken, the drops will not for a time flow together. The drops tend each

[1] Roux recommends olive or paraffine oil. I find that thick cotton-seed oil gives as good or better results when suspended between fifty and seventy per cent. alcohols. A smaller drop is to be used when more than two divisions are to be made.

to become spherical, but the wine-glass holds them together and their surface-tensions cause them to assume definite relations to one another. When the drop is divided into two equal parts, the halves, if little compressed, arrange themselves as shown in Fig. 16, A, and if much compressed, as shown in Fig. 16, E.

If each drop is again divided,[1] the resulting four drops arrange themselves as shown in Fig. 16, B. A large central cavity, similar to the segmentation-cavity of many segmenting eggs, is present in the centre between the four drops.

If the drops had been first divided unequally, we should find that the smaller drop, having a stronger tendency to become round, caused the region of contact of the two drops to bend in toward the larger drop. If each of these two drops is again divided, the four parts arrange themselves as shown in Fig. 16, D. The same result is brought about if we divide the drop at first equally and then each of the products unequally (Fig. 16, D).

If we first divide a drop equally and then each of the two unequally, but at different ends of each drop, as shown by the dotted lines in Fig. 16, E, the resulting four drops arrange themselves as shown in Fig. 16, F. Moreover, and this is a point of much importance, it is a matter of indifference in what direction the smaller drops are cut off. For instance, if each of the first two drops is divided along the dotted lines, *a*–*a*, *a*–*a* (Fig. 16, E), the result is the same as when the division takes place along the line *b*–*b*, *b*–*b*. In either case the drops arrange themselves as shown in Fig. 16, F. The two larger drops come together at the centre of the system and flatten somewhat against each other, producing a cross-line. The two smaller drops are pushed out more toward the periphery of the system.

If we adopt the method of lettering shown in Fig. 16, B, we can follow more readily the further divisions. Dividing equally two of the four drops of Fig. 16, B, we find the

[1] In dividing the drops it is better to move the rod always from the centre toward the periphery. The plane of the first division is indicated in the figures by the heavier line.

arrangement of the six resulting drops to be that shown in Fig. 17, A. We can write out the arrangement in the form of equations: thus (in Fig. 16, B) $a = A = B = b$; and (in Fig. 17, A) $b' = b''$, $a' = a''$.

Dividing *unequally* a and b so that a' is less than a'' and b' is less than b'', the drops arrange themselves as shown in Fig. 17, B. A large central cavity is present in the centre of the system.

If each of the four equal drops (Fig. 16, B) be equally divided, the resulting eight drops arrange themselves as shown

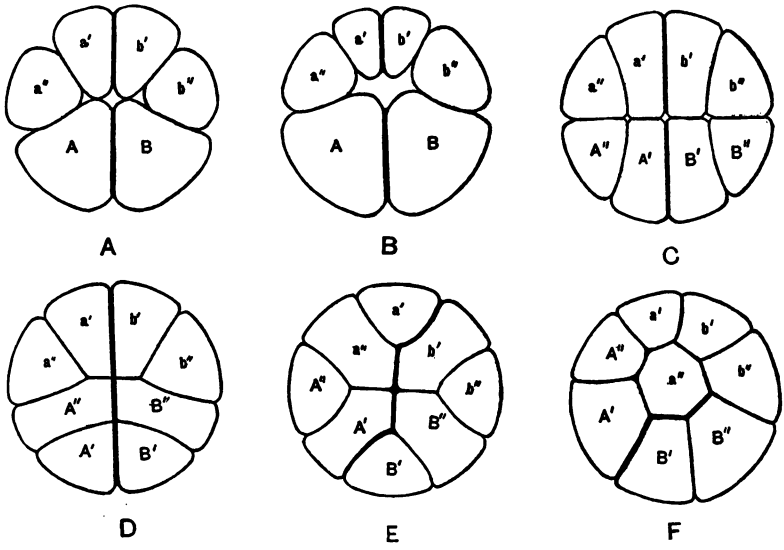


FIG. 17.—Systems of oil-drops. (After Roux.)

in Fig. 16, C. A central cavity is present, but *smaller* than when only four equal drops formed the system.

If we divide each of four equal drops (Fig. 16, B) *unequally* so that a'' is less than a' and b'' is less than b', also A'' is less than A' and B'' is less than B', the resulting eight drops arrange themselves as shown in Fig. 17, C. The four larger drops come together in the centre, pushing the smaller drops more toward the periphery of the system.

If we divide four equal drops (Fig. 16, B) so that a'' is less

than a', b'' is less than b', but A' is less than A'' and B' is less than B'', then the drops assume the form shown in Fig. 17, D.

If we divide four equal drops (Fig. 16, B) so that a' is less than a'', b'' is less than b', A'' is less than A', and B' is less than B'', the resulting eight drops arrange themselves as shown in Fig. 17, E. In this system it is instructive to note how far the first division-plane is drawn out of its straight course as a result of the shifting of the drops on one another. It is not unusual for one of the drops to glide into the centre of the system, as shown in Fig. 17, F. This produces a more stable arrangement than when a large central cavity is present.

Most of these systems are found also in the segmenting frog's egg, as can be seen by comparing these figures of the oil-drops with the figures of the segmenting frog's egg (Fig. 13) by Max Schultze made in 1863. Rauber has also given figures showing arrangements of the upper eight blastomeres (Fig. 14), like the systems of oil-drops shown in Fig. 17, D and E.

A careful comparison between the systems of oil-drops and the arrangement of the blastomeres of the frog's egg shows, as Roux points out, that while in many cases the agreement is perfect, yet occasionally the blastomeres assume an arrangement that oil-drops of the same size would not assume. For instance, Roux figures an arrangement of the blastomeres like that of Fig. 17, C, but here the blastomere corresponding to a' is less than a'' and A' is less than A''. In this egg the *smaller* blastomeres meet in the centre, but this never occurs in the system of oil-drops.

Roux removed a part of a blastomere so that it became suddenly smaller. A new arrangement ought now to have taken place among the blastomeres if they conformed entirely to the laws regulating the oil-drops. In one case where four blastomeres were present, the blastomere that had been reduced in size did move out more toward the periphery of the system, and the two neighboring blastomeres pushed in more toward the centre to form a cross-line. In other experiments, however, the blastomeres did not rearrange themselves in conformity with the systems of oil-drops. For instance, in one experiment in which material was drawn out of one of the first

four blastomeres, the inner end of the reduced blastomere retained its central position. In another instance material was taken out of that one of the four blastomeres that had already made a broad cross-line with its *vis-à-vis*. Although this blastomere was much reduced in size and made smaller than any other blastomere of the system, yet it retained the same cross-line as before; *i.e.* it was not pushed out to the periphery. Even when the experiment was made *at the time of appearance* of the second cleavage, the newly forming blastomeres did not in all cases adjust themselves in agreement with the laws regulating the oil-drops.

These results show that the conditions present in the frog's egg do not allow the blastomeres to assume always the arrangement shown by the same number of oil-drops having the same relative size. Roux points out several differences in the two cases. The walls of neighboring blastomeres seem to stick together, and this would prevent the blastomeres from gliding freely over one another should any change take place to disturb the equilibrium. Moreover, the blastomeres are living contractile bodies, and through their own internal activity may interfere with the mechanical tendencies of the system. The nature of the surface of each blastomere and the sort of changes taking place in the surface may also affect the arrangement.

It will be seen then, as has been said, that there may be factors present in the frog's egg that so influence the arrangement of the blastomeres that the systems do not always conform to those of the oil-drops. Nevertheless, the results from the latter give us an ideal scheme showing the effect produced by one set of factors, — that of surface-tension. It seems highly probable that surface-tension is also an important factor in the segmenting egg, but other conditions present prevent its free play.

HISTORICAL ACCOUNT OF THE CLEAVAGE OF THE FROG'S EGG

The earliest observations on the segmentation of the animal-ovum were made upon the frog's egg. Swammerdam ('37) saw, but did not understand, the first cleavage-furrow of the egg. Spallanzani, in 1785, observed the first two furrows cross-

ing each other at right angles. Prévost and Dumas, in 1824, gave for the first time a definite description of the cleavage of the frog's egg. They described the first furrow beginning in the black hemisphere and stretching out into the white hemisphere. They saw, moreover, the small lateral creases or folds along the edges of the first cleavage-furrow. The second furrow, they said, cuts the first at right angles. When the dark hemisphere is divided into four segments, they saw that then a third equatorial furrow forms near the boundary of the two hemispheres. The next furrows, they said, appear parallel to the first.

Rusconi ('26) observed that the furrows were not simply surface-lines, but cut up the yolk into separate parts, producing finally a large number of small pieces, which he believed were the elements from which the different parts of the body developed. Von Baer's description of the process, in 1834, is much more exact than the accounts of his predecessors. His interpretation, too, is much clearer and nearer to the truth. He said that the advance of the first furrow into the lower hemisphere goes on as though it were overcoming great difficulty. The tearing apart of the yolk into halves is brought about as a result of a living activity, and the power to divide the ovum does not reside only in the surface of the ovum, but extends throughout the whole mass. Von Baer noticed that after the division the cross-diameter of the egg is greater than the vertical diameter in the proportion of six to five, and he said that the difference would be greater were it not for the egg-membrane. The tendency, he said further, is to form two spheres which are, however, compressed against each other by the membrane. Since the division of the white hemisphere progresses more slowly, and since the third division is nearer to the upper hemisphere, we can understand why the dark portions are always smaller than the white portions. When the surface appears again smooth (owing to the smallness of the portions into which the ovum has divided), the egg is very distinctly larger than at first. Von Baer concluded that material is taken up from the outside to form the albumen, and hence to enlarge the ovum. He interpreted the process of cleavage as the self-division of the individual to form innumerable smaller units. In the later

stages these smaller bodies fuse by a vital process into a new whole, and a new individual is thus produced from the fragments of the first.

Schwann and Schleiden promulgated the cell-theory in 1838–1839. This produced an effect on all subsequent interpretations of the segmentation of the frog's egg. The main points to settle were: first, whether the process of cleavage is a process of cell-division, *i.e.* whether the egg is a cell that divides; second, whether the bodies that result from the segmentation of the egg pass over into the cells of the embryo. The search for the nucleus, before and after the process, also occupied the attention of workers on the subject. Bergmann ('41) was the first to treat the process of cleavage from the cell standpoint. The first divisions of the egg did not produce true cells, he said; yet as the results of these divisions went over directly into the cells of the embryo, therefore the division of the batrachian egg is the introduction of cell-formation into the yolk. Later, he said that the yolk may be thought of as strongly disposed to form cells, but that nuclei are wanting. Reichert's interpretation ('46) was a step backwards. Kölliker, in 1843, described the segmentation-spheres as without a membrane and containing spore-like bodies which multiplied endogenously. When these bodies are set free, he thought, they become the cells from which the tadpole is built up. Cramer ('48) thought that the early cleavage-spheres formed membranes (cell-walls) and were the progenitors of the true cells of the body. Remak ('50–'55) argued that the cleavage-process was the beginning of cell-division, and that the products resulting from division formed the cells of the embryo. This statement marked a distinct advance and is the standpoint taken at the present time. Moreover, Remak thought it highly probable that there was a continuity of the original egg-nucleus with the cleavage-nuclei. Max Schultze, in 1863, described admirably the process of cleavage of the frog's egg. He spoke of the egg as a cell with protoplasm and nucleus, and of the process of cleavage as cell-division. Ordinary cell-division depends, he said, on the contractility of the protoplasm. The same property belongs to the egg-yolk, since it divides like a true cell.

CHAPTER V

EARLY DEVELOPMENT OF THE EMBRYO

In the preceding chapter the cleavage of the egg has been described to the period when the blastopore is about to appear on the surface. During the subsequent development the cells continue to divide, so that at no time can the cleavage or the cell-division be said to cease. At each successive stage the number of cells is greater than in the preceding stage. This statement does not imply, however, that the formation of each new structure is introduced by new cell-divisions in the region where the change is about to begin, because many changes take place in regions where cell-division is not more rapid than elsewhere.

The spherical form of the "egg" or young embryo is soon lost. In the present chapter we shall follow the changes that can be seen taking place on the exterior of the living embryo; and in the following chapter we shall attempt to make out the movements of cells and groups of cells that take place in the interior of the embryo during this period.

The Blastopore

On that side of the egg where the smaller cells are found, a short horizontal line of pigment[1] appears amongst the white cells below the equator of the egg (Fig. 12, I). This line marks the beginning of the archenteron, and the cells bounding the upper or darker side of the pigment-line form the dorsal lip of the blastopore. The dorsal lip becomes crescentic in outline, with the concavity of the crescent turned toward the white hemisphere (Fig. 19, I, II). If the living egg be watched, it

[1] There is a great deal of variation at first in the shape of the blastopore.

will be found that changes take place at this time in the blastoporic region with great rapidity.

Pflüger has described ('83) these changes, and we may follow his admirable account, subsequently adding other facts that have since been discovered. The eggs which Pflüger studied[1] were taken from the uterus at twelve o'clock midday, and placed in a row on a thick glass mirror. The mirror was then put into a dish, and water added to the depth of 2 mm. In this way, owing to the reflection of the lower pole by the mirror, both hemispheres of the egg could be watched. During the night, when the temperature was low, the eggs developed more slowly. At six o'clock in the morning the thermometer stood at 16° C. At this time the eggs showed on the lower hemisphere, and in the upper fourth of that region and therefore just beneath the equator, the first trace of the dorsal lip of the blastopore. By ten A.M. the long horizontal split (dorsal lip of the blastopore) had become distinctly marked as an indentation of the surface of the egg. At eleven A.M., the dorsal lip had moved somewhat further below the equator of the egg, *i.e.* toward the lower pole. The "split" is now broader, and its corners turned down so that it forms a crescent, with the lower pole of the egg-axis as its middle point. The diameter of the crescent is to the egg-diameter as 2 : 3. From the corners of the crescent a furrow continues to extend on each side around the white hemisphere. The progress of the dorsal lip toward the lower pole is not due to a rotation of the egg *as a whole*, but to the *migration of the dorsal lip over the white hemisphere*. At half-past twelve o'clock (twenty-four hours after fertilization), the dorsal lip has progressed further toward the lower pole. The crescent has at the same time extended so as to form a half-circle whose diameter is somewhat less than in the preceding stage. It stands now in relation to the diameter of the egg as 1 : 2.

By one o'clock P.M., the semicircle forming the dorsal and lateral lips of the blastopore has extended so as to form a complete circle (Fig. 19, A, IV). The white yolk-cells protrude from the centre of this circle and form the so-called

[1] Bufo cinereus.

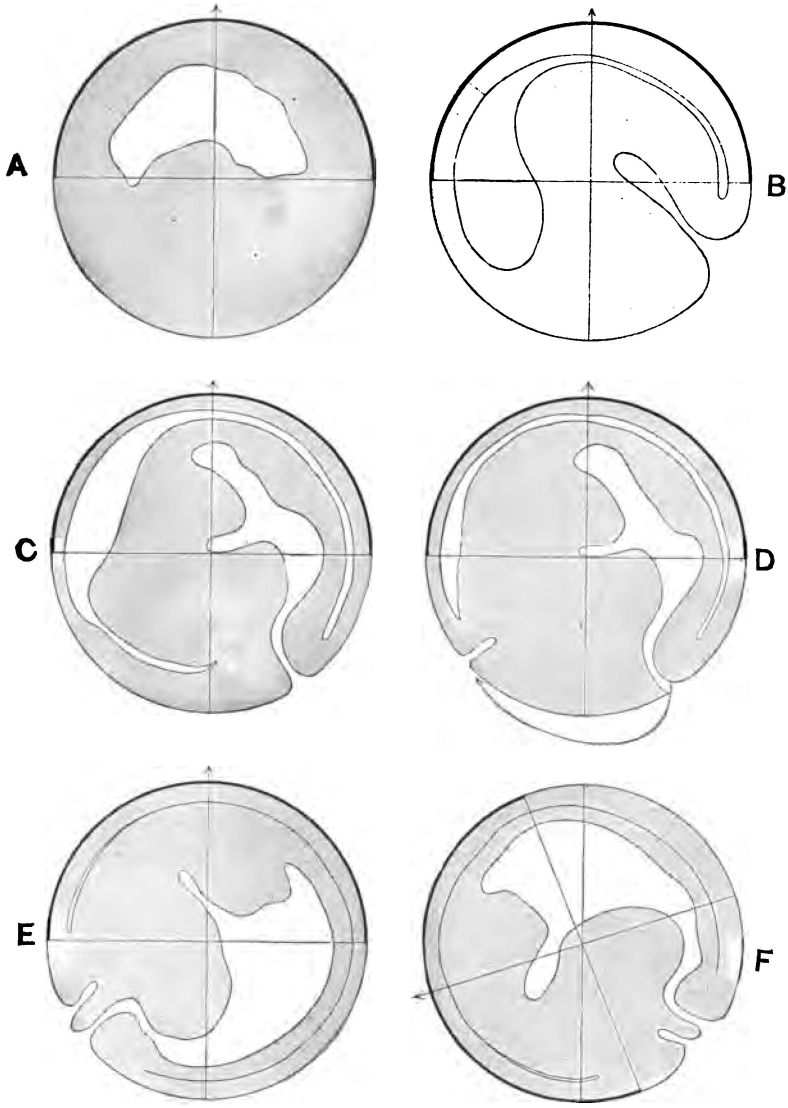


FIG. 18.—Diagrams to show extent of movement of dorsal lip of blastopore and rotation (F) of embryo. (After Pflüger.)

yolk-plug. The diameter of the circle around the yolk-plug is still smaller than before. At 2.15 P.M., the opening containing

the yolk-plug — the so-called opening of Rusconi, or blastopore — is still smaller. The periphery of this circular blastopore is deeply pigmented. At 4.15, the opening is further reduced and measures no more than one-eighth of the diameter of the egg. The blastopore will now be found to have progressed so far that it again lies just beneath the equator of the egg, but on the side of the egg opposite to that at which the dorsal lip first appeared. We can summarize by saying that the dorsal lip of the blastopore has moved over a meridian of the egg from a point near the equator across nearly to the opposite point of the equator. The movement takes place over the lower white hemisphere, and during the process the position of the egg remains unchanged. The arc traversed by the dorsal lip of the blastopore is, however, not as much as 180 degrees, because it started below the equator and does not quite reach the equator at the opposite side. But the arc is certainly more than 90 degrees, and varies in different eggs.

So far we have traced the history of the blastopore from six o'clock in the morning to 4.15 in the afternoon of the same day. Then a remarkable process begins. The blastopore moves back as a whole in exactly the opposite direction until, at 7.45 in the evening, it has come back to the point from which it started in the morning. This reverse movement of the whole blastopore is brought about by quite a different process from the first movement of the dorsal lip. *The whole egg rotates around a horizontal axis.*[1]

The overgrowth of the lower pole by the dorsal and lateral lips of the blastopore has covered the lower hemisphere with cells that do not contain as much pigment as do the cells that lie around the upper pole, *i.e.* the original black hemisphere. Hence when the egg rotates as a whole in the way just recorded, a somewhat lighter area will be carried into the new upper hemisphere, while the original upper hemisphere will now come to lie nearly on the lower side of the egg. In the lighter upper region, as we shall soon see, the central nervous system develops. The rotation of the whole egg appears to take place through 180 degrees, although it is possible that the

[1] An axis at right angles to the median plane of the later embryo.

central nervous system may also grow forward somewhat so that the actual rotation is not great.

Perhaps the whole process may be made clearer by reference to a series of sections through the egg. These are taken through the meridian that corresponds to the middle plane of the body; it therefore passes through the upper and lower poles (Fig. 18). The arrows indicate the primary axis. The dorsal lip of the blastopore has formed in Fig. 18, B, and in Fig. 18, C, D, the migration of the lip has gone further over the lower hemisphere. The ventral lip of the blastopore has also formed. Figure 18, D, corresponds to a stage in which the blastoporic circle is completed. In Fig. 18, E, we see that the dorsal lip has travelled further over the lower pole toward the ventral lip. Finally,

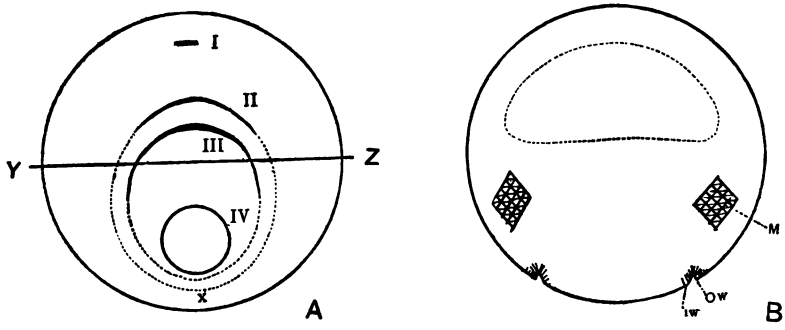


FIG. 19. — A. Diagram to illustrate overgrowth of dorsal lip of blastopore. I-IV represent different stages. B. Diagram of cross-section through Z-Y of A, to show lateral lips of blastopore, and mesoderm (M), and (IW) inner wall of archenteric pit. OW. Outer wall.

in Fig. 18, F, the egg is represented as having rotated as a whole to bring the embryonic portion above.

The changes that take place during the closure of the blastopore are perhaps more clearly shown by the following experiment. By means of a fine pointed needle it is easy to puncture the egg slightly at any given point. When the outer surface of the egg is pierced, there follows a protrusion of material as soon as the needle is withdrawn. At other times when the surface is only *indented* (*not* pierced) by the needle, there follows a blunt protrusion of material and the surface remains unbroken. In the latter case the marks do not always last as

long as do those produced by the first method, but as less harm is done to the egg, one can often get more satisfactory results.[1]

If when the first trace of the blastopore appears on the surface of the egg (Fig. 19, A), a slight injury is made to the surface of the white hemisphere at the side opposite the blastoporic lip, *i.e.* at a point 150 degrees from the dorsal lip of the blastopore (Fig. 19, A, at *x*), we shall find that in the course of four hours the blastopore will form a crescent, and that the distance from the dorsal lip to the point of injury is much less than at first (Fig. 19, III). A circular line of pigment in the white hemisphere shows the line along which the lateral and posterior lips of the blastopore will appear. It will be seen in this case, that the point of injury lies therefore outside of the yolk-plug, *i.e.* posterior to the ventral blastoporic lip.

In the course of four hours more it will be found that the circular blastopore is much smaller than before, and that the dorsal lip now lies much nearer to the point of injury (Fig. 19, IV). The dorsal lip has travelled over more than two-thirds of the original distance from its starting-point to the point of injury. By making a new experiment with an egg that has reached this stage of development, it will be found that when the outlines of the blastopore have become sharply defined, the later closure takes place at nearly an equal rate from all points of the circumference, perhaps, however, still somewhat more rapidly from the dorsal lip backwards.

Where the diameter of the circle representing the outline of the egg equals 27 mm.,[2] the distance between the blastopore and the injury measures 24 mm. In the first four hours the blastopore moves through 8 mm. In the next four hours it travels through 7 mm., and is therefore now only 9 mm. from the point of injury. By this time the blastopore is circular in outline, and the injury lies just outside (2 mm.) of the circle. The blastopore now measures 7 mm. in diameter. Assuming that from this time forward the blastopore grows together at an equal rate toward its centre, then the *dorsal lip* will pass over about one-half of the diameter of the blastopore, or 4 mm.

[1] This method can be used only with great caution.

[2] The numbers refer to the measurement of the figure, and not to the egg itself.

The dorsal lip has passed then, in all, through 19 mm. of the white area; the ventral lip (from behind, forward) through 3 mm.

If the region overgrown by the dorsal lip be compared with the length of the medullary folds which soon appear in the same region of the embryo, it will be found that the latter, when they first appear, are somewhat longer than the region overgrown. If, however, we deduct from the length of the medullary folds the thickness of the anterior connective that joins the right and left sides of the nerve-plate, we shall find that the remaining length of the medullary folds corresponds very closely with the length of the region overgrown. We must, therefore, conclude that the anterior connective lies just in front of the point at which the first trace of the dorsal lip of the blastopore appeared.

We have assumed the point of injury to be a fixed point and the overgrowth to be due to the progress of the dorsal lip. It might equally well have been assumed that the overgrowth was only apparent and was produced by the sliding forward of the whole of the white area beneath the dorsal lip of the blastopore. The end-result would be the same, but the process different. There can be no question, however, that the movement is really due to the progress of the dorsal lip. Other experiments where two or more points of the surface are injured show very conclusively that the movement is a backward growth of the rim of the blastopore.

Comparing the statements made above with those of Pflüger, it will be found that they differ in three unimportant respects. The rapidity of the overgrowth of the very early stages, before the complete establishment of the crescent, was not noted by Pflüger. The distance travelled by the dorsal lip, as just described, is somewhat less than that given by Pflüger. Pflüger thought that the dorsal lip moved over about 180 degrees, but added that the amount of the movement differed in different individuals, and was probably between 90 and 180 degrees. My own results make the region of overgrowth about 120 degrees. From Pflüger's figures we are led to believe that the whole blastopore after the establishment of its ventral lip continues to move somewhat nearer to the equator of the side nearest to the ventral lip. If this really

does take place, in the way shown by Pflüger's figures,[1] it can only be due to a slight rotation of the egg as a whole in this direction, for experiments show that the entire movement of the ventral lip is forward, *i.e.* toward the dorsal lip.

The yolk-plug is finally withdrawn into the interior of the egg and the blastopore remains as a round or often somewhat elongated opening. Its subsequent changes we shall follow later.

EXTERNAL CHANGES AFTER THE CLOSURE OF THE BLASTOPORE

Let us next examine the changes that appear at this time in the region that now lies anterior to the blastopore and on the upper surface of the egg. There is much variation in the early stages of development of the embryos of a given species, and in different species the variations are even greater. The differences in level of different regions are the result of movements of the ectoderm. To see these to best advantage, the *living* egg must be placed in the direct sunlight, and the surface studied with low powers of the microscope.

An embryo in which the yolk is still exposed is shown in Fig. 20, A. Passing forward from the yolk-plug over the upper surface of the egg is a broad groove, the so-called "primitive groove." At the anterior end of the primitive groove is a circular elevation. On each side of the primitive groove, at IM, the inner medullary folds are seen. Outside of these we find a depression, and farther on each side, at EM, the outer medullary folds. A sickle-shaped depression lies just in front of the blastopore.

A later stage of the same embryo is shown in Fig. 20, B. The primitive groove is narrower, the medullary folds are more distinct, and anteriorly a continuation of the lateral folds has formed. This will later be called the head-fold. Anteriorly and laterally, there is formed on each side a lateral extension of the medullary plate, the so-called "sense-plate."

The medullary plates now begin to roll in, producing a deep furrow, the medullary furrow with the primitive groove at its

[1] Pflüger ('83), Pl. II., Figs. 4 and 5 (see Fig. 18, F).

bottom (Fig. 20, C). The lateral sense-plate is split into an anterior and posterior part on each side. The more anterior part may still be called the sense-plate, SP, and the posterior, the gill-plate, GP.

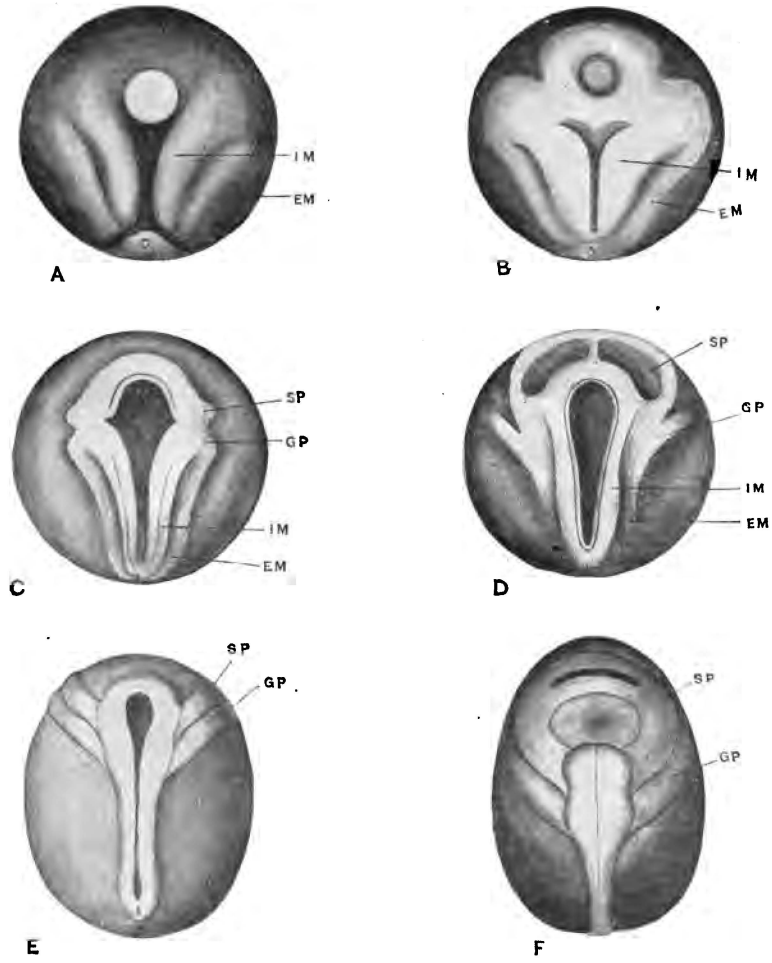


FIG. 20.—Surface views of young embryo of Rana. (After Schultze.) IM. Inner medullary plate. EM. Outer medullary plate. GP. Gill-plate. SP. Sense-plate. BP. Blastopore.

A later stage is shown in Fig. 20, D. Here the sense-plate is found to have extended laterally and forward, and the two

sides have met in front of the medullary folds. The gill-plate is also seen, but the outer medullary folds are no longer conspicuous. The inner medullary folds are closing in to form a tube. The blastopore is reduced to an elongated slit-like opening.

A still later stage is drawn in Fig. 20, E. The outline of the whole egg is now elliptical, with the long axis in the direction of the long axis of the embryo. The medullary folds are also much longer, and have approached each other in the middle line. A deep furrow lies between the two halves. The

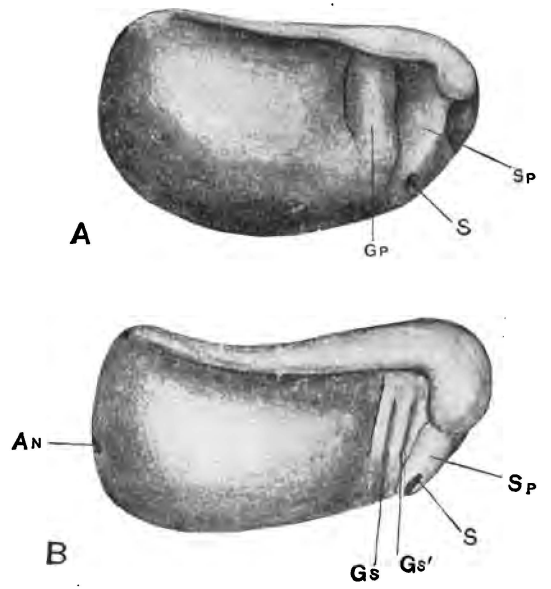


FIG. 21.—Embryos of Rana. (After Schultze.) Gs, Gs'. Two gill-slits. GP. Gill-plate. SP. Sense-plate. S. Suckers. AN. Anus.

folds have more nearly approached at the middle of their length, and are more widely separated at the anterior and posterior ends. At the posterior end the medullary folds are overarching the small elongated blastopore. The sense-plates and the gill-plates are distinctly visible.

The medullary folds now fuse along their whole length, leaving, as we shall see, a central canal, which is the overarched medullary furrow. The elongation of the embryo continues as

seen in Fig. 21, A. The anterior end of the medullary tube shows on each side a lateral protrusion, the eye-bulb. At the anterior end of the body we can see the sense-plate and on each side the broad gill-plate. Lying in the sense-plate on each side is a deeply pigmented area which is the forecast of the "suckers," S. There is a depression in the middle line, in the centre of the sense-plate. This depression marks the mouth-depression, and indicates the point at which, later, the stomodæal invagination will take place. (See Fig. 20, F.)

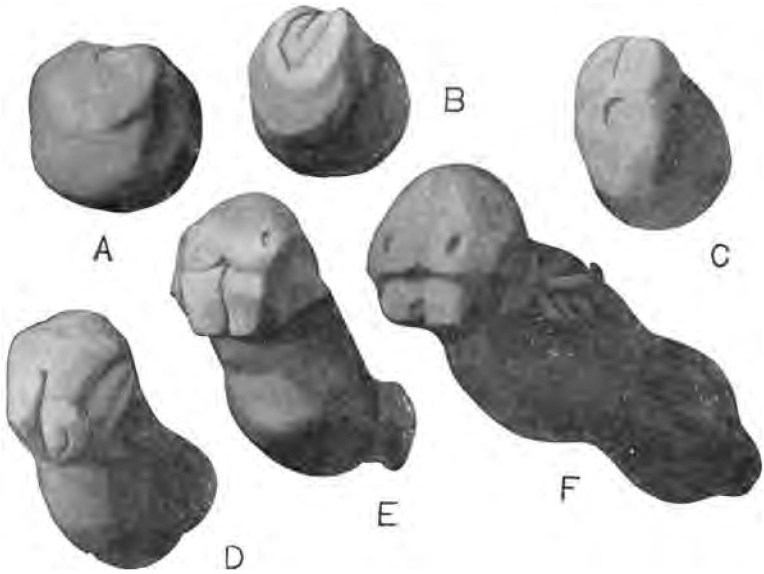


FIG. 22.—Development of embryo. Anterior view, showing sense-plate, nasal pits, stomodæum, and gills. (After Ziegler.)

A later stage is shown in Fig. 21, B. The relation of the parts is much as in the last figure. The anterior end of the medullary tube is larger than before, and the protuberances of the eye-evaginations are more apparent. In the gill-plate on each side appears a vertical depression and later another depression behind it, GS, GS′. These depressions mark the external gill-slits. The anus has shifted to a more ventral position. The suckers have each elongated ventrally, and have fused into a pigmented V-shaped figure. The outer medullary

plates take no part in the rolling in to form the medullary tube, but flatten out and seem to disappear.

Ziegler ('92) has made several excellent figures of living embryos of Rana temporaria (Figs. 22, 23). The first of these shows young embryos as seen from in front, so that the sense-plate is turned toward the observer (Fig. 22). A longitudinal groove appears in the middle of the sense-plate, and subsequently a transverse groove develops across the sense-plate (Fig. 22, D, E). The depression that later forms the mouth lies at the crossing-point of the longitudinal and trans-

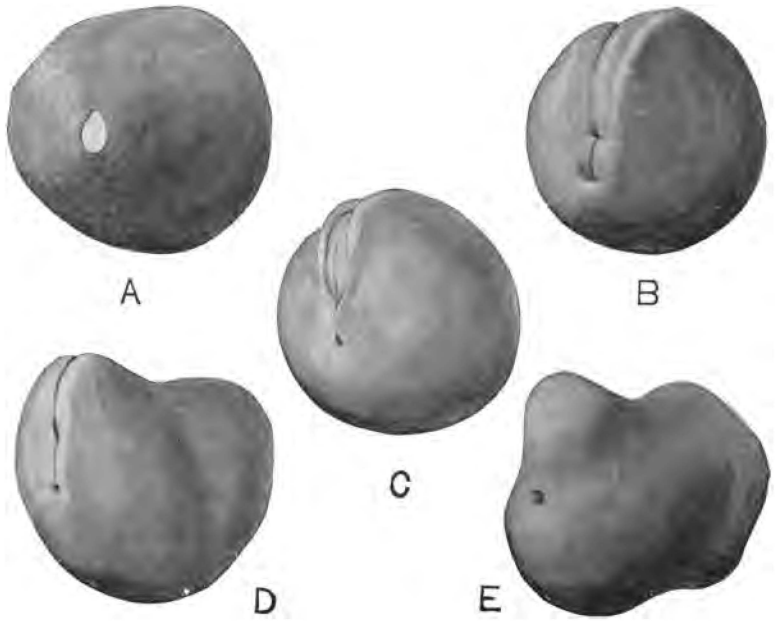


FIG. 23. — Development of embryo, showing closure of blastopore and formation of anus. (After Ziegler.)

verse grooves. There is present on each side above the mouth a thickened ridge that forms the superior maxillary process. Below and behind the mouth a pair of ridges appear that meet in the middle line. These are the sub-maxillary processes which later form the lower jaw. A pair of depressions of the surface ectoderm *below* the mouth-area mark very early the

beginning of the "suckers," or adhesive glands (Fig. 22, D). The nasal pits appear above the mouth (Fig. 22, F).

The outlines of the three brain-vesicles can also be faintly seen in surface view. A pair of swellings on each side of the fore-brain shows the position of the eye-evaginations (Fig. 22, D). In the pharyngeal region there first appears on each side a vertical ridge, and later another ridge parallel to and behind the first (Fig. 22, D, E). On these ridges gills appear as protrusions of the surface, and later a third ridge and gill are formed behind and somewhat beneath the others. Some time after hatching, the *gill-slits* break through to the exterior between the ridges or gill-arches, and at about the same time, the mouth breaks through into the cavity of the pharynx.

In the head-region the beginnings of some of the spinal ganglia may be seen, and a series of mesodermal blocks also appear and may be dimly seen from the outer surface. These structures do not, however, appear as distinctly in surface views of the embryo of Rana temporaria as they do in the embryos of some other species.

Soon after the nerve-tube has closed, the dorso-posterior end of the body begins to extend backwards to form the tail (Fig. 23, D, E). The anal opening lies just behind and ventral to this region of posterior growth. The anus seems to shift to a more ventral position during the elongation of the tail. At first the tail is a thick outgrowth of the posterior end of the body, but as it grows longer it flattens from side to side, and in later stages a thin fan-like border or fin develops on its upper and lower margin (Fig. 38).

CHAPTER VI

FORMATION OF THE GERM-LAYERS

THE period that we are now about to examine is marked by extensive movements of parts of the segmented egg as a result of which the organs are formed. During the segmentation-period the cells retain, as we have seen, the position in which they arise, but with the appearance of the blastopore a new period is initiated in which extensive movements of cells and groups of cells take place.

HIS'S EXPERIMENTS WITH ELASTIC PLATES

His ('94), from his studies of the behavior of elastic plates, has concluded that many of the phenomena of the developing embryo are the mechanical result of the tensions set up in the different layers. In the embryo the shoving, compression, or extension is supposed to result from the unequal growth of different parts. When a cell-plate lifts itself up into a fold, as a result of more rapid growth in that region than elsewhere, there is present on the concave side a positive tension ("Druckspannung") and on the convex side a negative tension. Under these conditions the cells become conical, *i.e.* they are small on the concave side and broad on the convex side of the fold. Each embryonic cell tends of itself to become spherical and only the surrounding conditions, resulting from the growth of surrounding parts, determine the shape of each cell at any period of development. His has tried to explain many of the changes taking place in the early embryo as the result of this simple folding principle. The inrolling of the medullary plate, the formation of the eye-outgrowth from this plate, the formation of the mouth-cavity and the gill-slit-folds, etc., are examples of some of these changes. His pointed out how closely the forms

taken by many of these structures in the embryo resemble the folds that can be produced mechanically by pulling out or pushing in a thin elastic plate of rubber. If this interpretation is true, it means that at different periods in the development, regions of more rapid growth appear, now here, now there, and as a mechanical result of the conditions present, such structures as the medullary folds, the eye-outgrowths, etc., are produced. The cells change their shape in response to surrounding conditions, *i.e.* they do not by their individual activity or movement change their shape to produce the successive changes of the embryo, but the shape of many cells is changed as the result of growth or increase in mass of certain regions. For instance, a cell becomes conical not through its own initiative, but because the surrounding pressure forces it into a conical shape.

THE FORMATION OF THE EMBRYO BY CONCRESCENCE

The period of overgrowth of the blastopore when the so called process of gastrulation is going on has been described in Chapter V. We may now follow the changes that take place in the interior of the egg during that time.

When the dorsal lip of the blastopore appears, the cells have shown little tendency to arrange themselves into sheets or layers. However, even when the segmentation-cavity is covered by a roof of small cells, the cells of the outer layer have begun to flatten against one another and to form a thin layer of cells over the outer surface of the black hemisphere. In the lower hemisphere the larger white cells do not show such an arrangement. In the equatorial region, where the black and white cells meet, a careful examination of sections will show that there exists a more or less defined ring of cells stretching around the embryo, forming a broad zone (Fig. 15, D). The *inner* cells of this ring contain a good deal of pigment around the nuclei. The yolk-granules of these inner cells are smaller than the yolk-granules in the large white cells of the lower hemisphere, and the cells of the ring seem to contain also a larger amount of clear protoplasm. This inner zone of cells passes, on the one hand, by insensible gradations into the cells of the outer surface of the ring and internally it is continuous

with the inner region of large yolk-cells. *This ring of cells, as subsequent development shows, is the beginning of the embryo, and the ring itself is composed of the material which subsequently forms the central nervous system, the mesoderm, the notochord, and a part of the endoderm.* An understanding of the subsequent development depends on a knowledge of the changes that take place in this ring.

The material of the ring is intimately involved in the movements that take place during the overgrowth of the lower hemisphere by the lips of the blastopore. During this period, we must picture to ourselves the ring as rising up and drawing together over the lower white hemisphere, so that ultimately it leaves its equatorial position and its halves come together to form the embryo. (Fig. 24, A, B, C.)

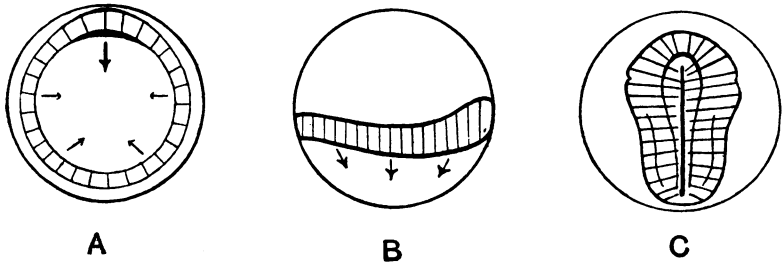


FIG. 24.—Diagrams illustrating germ-ring and concrescence of lips of blastopore.

As the dorsal lip of the blastopore progresses over the white hemisphere, its progress is due to the movement and fusion along a meridian of the material of the equatorial ring. We are to think of the material of the ring as moving toward the middle line from the *right* and *left* sides (for with the establishment of the dorsal lip the *ring* becomes bilateral) and fusing continuously in the dorsal lip (Fig. 24). The advance of the blastopore is merely the expression of the absorption into its dorsal lip of the material of the two sides of the ring. As soon as the material from the sides reaches the median line in the dorsal lip of the blastopore, it remains stationary and new material is added behind that just laid down. The material of the equatorial ring is thus carried into a meridian of the egg. With the disappearance of the yolk-plug below the

surface, the final stages of overgrowth are completed. The ventral lip of the blastopore has moved somewhat forward, as previously explained, and this slight forward movement probably takes place by the growth toward the median line of the material at the sides of the ventral lip.

There are other changes closely bound up with the preceding phenomena and, although these changes take place simultaneously, it will be necessary first to consider them separately, and then to try to combine them into a single statement. The changes involve, 1) the formation of the archenteron, 2) the progression of the blastoporic rim over the lower hemisphere, 3) the origin of the middle layer or mesoderm.

THE FORMATION OF THE ARCHENTERON

1) When the dorsal lip appears, certain cells pull away from the surface, leaving their outer pigmented ends exposed for a time (Fig. 15, D, Fig. 12, H). These cells are near the border-line between the black and white regions, but lie distinctly amongst the white cells. The next change involves the sinking in beneath the surface of the region in which these cells are present. The dorsal lip of the blastopore now begins its movement over the lower hemisphere. From the surface we can see that the crescent becomes longer and longer, the horns extending outwards along the black-white border but well within the white. The same changes that took place where the dorsal lip first appeared, now take place also wherever the crescent extends. First certain superficial cells pull into the interior of the egg leaving only their pigmented ends at the surface, and then this area of pigment sinks below the general surface. Simultaneously the edges of the blastopore roll over the inturned (invaginated) cells. The same changes also take place at the posterior or ventral lip of the blastopore, when the two horns of the lateral lips have met there. It is necessary to examine sections that have been cut in several planes in order to follow the changes that take place during the further overgrowth of the blastopore. If we examine a median longitudinal (sagittal) section at the time when the dorsal lip has just begun to roll over, we find (Fig. 25, A) that a narrow space is left between the dorsal lip and the surface of the lower hemisphere over

which the dorsal lip has begun to roll. We find, at the upper end of this crevice, the pigmented ends of those cells that were previously at the surface. During later stages the space, which we may at once speak of as the archenteron, becomes longer, due to a further progression of the dorsal lip over the white hemisphere. If the section were taken somewhat to one side of the median line, the length of the archenteron would be found to be less than in the median line, because the rolling in has been relatively less. If we make a section at right angles to the last in the plane Y–Z, in Fig. 19, A, we cut the two horns or ends of the crescent. The cavity on each side is just beginning, owing to the smaller amount of closing in from the sides of the lateral lips of the blastopore. (Fig. 19, B.)

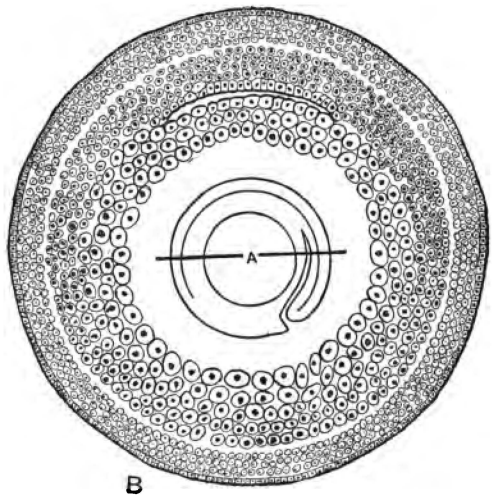


FIG. 25.—A (small figure inside B). Longitudinal section through young embryo. B. Cross-section of last. (After Schultze.)

A section at right angles to the last section in the plane of the line in Fig. 25, A, is shown in Fig. 25, B. The archenteron is seen in the upper part of the section. Its upper or dorsal wall is made up of small cells, while its floor is formed of large cells filled with yolk. The segmentation-cavity fills the centre of the section.

During the time when the yolk-plug is withdrawing from the surface, the segmentation-cavity becomes smaller, owing, without doubt, to the intrusion of the large yolk-mass into its interior, and finally, when the archenteron begins to open, the segmentation-cavity is almost entirely obliterated. The segmentation-cavity is thus utilized by the embryo, for into this cavity is pushed the yolk-mass as the latter is overgrown by

the blastopore-lips. This statement does not necessarily imply, however, that the segmentation-cavity was prepared especially in view of the subsequent changes.

It will be seen from the foregoing account that the walls of the archenteron are formed as the blastopore closes in. The floor of the archenteron (Fig. 25, B) is nothing more than the surface of the lower white hemisphere that is overgrown. The origin of the roof and sides of the archenteron is somewhat difficult to understand. We have seen that around the crescent of the blastopore certain cells have pulled in, leaving a depression on the surface. It is impossible to say just how far the cells that pull in continue to be drawn inward, because simultaneously the lips of the blastopore roll over. This brings us to a discussion of the second topic.

THE OVERGROWTH OF THE BLASTOPORIC RIM

2) There are at least two ways in which we may think of the closing in of the lips of the blastopore, *i.e.* there are two ways, either of which might explain the covering of the white by the black cells. We may think of the *free edge* of the blastopore as growing toward a middle point. Or we may imagine that the lateral and dorsal edges actually roll in toward the middle line. The latter process seems to be that which probably takes place, for Jordan ('93) has seen the outer dark cells actually rolling over and into the archenteron in the living egg.

The dorsal and lateral walls of the archenteron will then be formed in part, or entirely, from those cells of the surface that have rolled in and have come to lie beneath the surface. These are the cells, therefore, that have been at one time situated at the surface of the embryonic ring, and inasmuch as the advance of the dorsal lip takes place very largely by the fusion of the lateral lips, it follows that the material for the greater part of the dorsal wall of the archenteron comes from cells at one time on the outer surface of the egg. I am inclined to think that at first there is also an actual in-pulling of cells along the blastoporic rim so that cells at one time below the outer surface come also to stand, later, at the sides of the archenteron, *i.e.* where the dorsal and ventral walls meet.

THE ORIGIN OF THE MESODERM

3) It is difficult to give an account of the method of development of the mesoderm, because there are almost as many different descriptions of the process as authors who have described it. I have without hesitation set aside those accounts where the author has transparently sought to find his preconceived theories demonstrated in his drawings of the sections of the embryo. In the second place, several of the more recent accounts have started out, I think, with a false conception of the position of the embryo on the egg and its method of formation, hence in these accounts the *method* of the formation of the mesoderm is likely to be erroneously described, although in several cases the actual drawings of the sections have been, I believe, accurately made. I have followed as far as possible those interpretations that are in conformity with the experimental results relating to the growth of the embryo. Certain abnormal embryos, to be described later (Chapter VII), that first appear as a ring around the egg throw, I think, also much light on the subject.

The cells that are to form the mesodermal layer are present at the time when the dorsal lip of the blastopore has first appeared, and even just prior to that time. The innermost of those cells forming the ring around the egg are the cells that become the mesoderm (Fig. 19, B). These cells are carried up to the median dorsal line of the embryo by the closure of the blastopore (Fig. 24, A, B, C). They will then be found forming a layer or sheet of cells (Fig. 25, B) that separates itself on the outer side from the thick layer of small ectodermal cells (that has been simultaneously lifted up) and that is separated on the inner surface, but not very sharply if at all, from the dorsal and dorso-lateral walls of the archenteron. A continuous sheet of tissue is formed in this way over the dorsal surface stretching across the middle line. According to some accounts, the fusion of this mesoblastic sheet with the endoderm is much closer in the mid-dorsal line than on each side. We may, however, think of the mesodermal layer and endodermal layer as coming up *together* to the median line from the sides, so that we are to think of the

mesodermal and endodermal cells as being together from the beginning.

DIFFERENT ACCOUNTS OF THE ORIGIN OF THE ARCHENTERON AND MESODERM

Before following further the fate of these concentric coats or layers of cells, the so-called "germ-layers," we may for a moment examine some other descriptions that have been given as to the method of formation of the archenteron in the frog. The most common view of the method of gastrulation of the frog has been that a process of *invagination* takes place at the dorsal lip of the blastopore. This process is supposed to be brought about by the drawing inwards and upwards of a fold of the outer wall, so that a blind sac forms. As this presses forward into the yolk, the latter pushes before it and fills up the segmentation-cavity. At the same time the mesoderm is described as growing forward from the region of the blastopore over the dorsal surface of the embryo.

Other authors represent, however, the dorso-lateral edges of the archenteron proliferating cells along the two sides to form the mesoderm, while in the mid-dorsal line a solid block of *endoderm* cuts off to form the notochord. Hertwig has gone so far as to affirm that at the dorso-lateral edges of the archenteron there are traces of a pair of lateral pouches along each side, and that these give rise to the cells that push in between the ectoderm and endoderm to form the middle layer.

Robinson and Assheton ('91) assert that the old account of the formation of the archenteron by invagination is entirely erroneous, and that the cavity of the archenteron owes its existence to a process of progressive splitting or separation of the large yolk-cells of the lower hemisphere, and that this splitting extends up into the yolk beneath the upper hemisphere. The dorsal lip of the blastopore remains approximately stationary where it first formed, and the anus develops around this point.[1]

[1] In a later account Assheton ('94) has much altered his former view. He describes only the anterior end of the archenteron as formed as a split amongst the endoderm-cells, while the posterior third of the archenteron is, he thinks, the result of the overgrowth of the dorsal and lateral lips of the blastopore.

Both assumptions are, I think, erroneous, as a study of the changes that take place in the dorsal lip will convince any one who will take the trouble to follow in the living egg the method by which the closure of the blastopore takes place.

LATER DEVELOPMENT OF THE MESODERM AND ORIGIN OF THE NOTOCHORD

Schultze ('88), who has studied the formation of the middle germ-layer of the frog, has given an accurate account of the condition of the mesoblast in the embryo during the period of overgrowth of the blastopore. He has done this, too, despite the fact that he believes the embryo of the frog to be formed over the upper or black hemisphere of the egg. This belief has not, however, in my opinion, vitiated in any degree his description of the position of the mesoblast after its formation. I have, therefore, reproduced his figures in Fig. 26, A–E.

If a cross-section be made through an embryo (in the plane of the dark line of Fig. 25, A) at the time when the blastopore has assumed a crescentic shape, we find over the surface of the section a thick envelope of ectoderm. The ectoderm is at this time composed of about four layers of cells (Fig. 25, B). In the outermost layer the cells are columnar in shape. In the centre of the section there is a large segmentation-cavity surrounded by large yolk-bearing cells. The archenteron, as seen in cross-section, is a large, arched cavity, its lower wall formed by yolk-cells and its dorsal wall covered by a layer of small cells showing a tendency to become flattened against one another. Above the upper wall of the archenteron, and between it and the ectoderm, is a thick layer of cells. This layer stretches out on each side of the embryo as a lateral sheet, but the edges of the sheet merge insensibly into the yolk-bearing cells at the sides. Where this middle layer (mesoderm) is sharply defined, we can easily distinguish its cells from those of the endoderm, for the mesodermal cells are smaller and pigmented. At the free edge of the sheet it becomes, however, impossible to distinguish between the cells of the mesoderm and of the endoderm.

If we examine a complete series of sections through this

embryo, we find that the layer of mesoderm is inserted between ectoderm and yolk-cells over all the posterior half of the embryo. There is a small antero-ventral region into which the mesoderm does not extend. At a point posterior to the section described above, we find the mesoderm extending much farther *ventrally*, so as to nearly encircle this region of the embryo. The blastopore is completely encircled by the sheet of mesoderm.

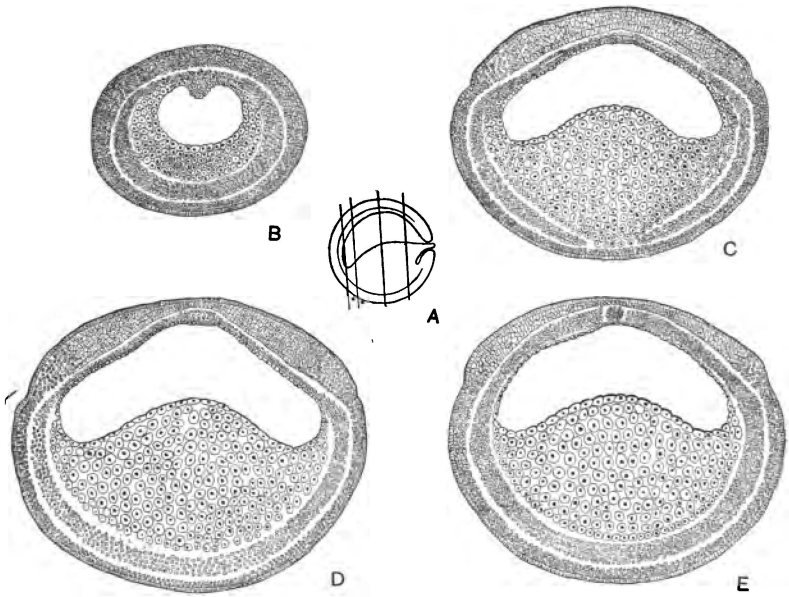


FIG. 26. — A. Longitudinal section through a young embryo of Rana. B, C, D, E. Cross-sections of last in planes of lines in A.

Cross-sections through an older embryo are drawn in Fig. 26, B, C, D, E. The embryo has flattened along the mid-dorsal line. The ectoderm has become thinner along this line, where a faint groove can be seen on the surface of the living egg, — the primitive groove. On each side of the mid-dorsal line, the ectoderm is somewhat thicker than before, and the cells are more closely packed together. The ectoderm over the surface of the embryo consists of an outer layer and of several inner layers of cells. The cavity of the archenteron has opened out and is very large.

As before, its ventral wall is composed of larger and yolk-bearing cells. Above and laterally the walls are formed of smaller cells. The latter have now arranged themselves in a definite layer, and have become somewhat flattened (Fig. 26, B, C, D). This layer is also sharply separated from the mesoderm. The mesoderm, as compared with its previous condition, has undergone important changes. It has extended further ventrally, and has met from the right and left sides in the mid-ventral line along most of the ventral surface. Over the dorsal and dorso-lateral walls of the archenteron it forms a thinner layer of cells than in the earlier embryo (Fig. 25, B).

There is still a ventral region of the embryo where the ectoderm and the yolk-cells are in contact, *i.e.* a region into which the mesoderm has not extended (Fig. 26, C). The medullary plate is seen in cross-section. It will be noticed that the plate is much thinner in the mid-dorsal line than at the sides. On each side the medullary plates show a differentiation into two parts. The most lateral and ventral edge of the plate is formed of cells less closely held together than those nearer the mid-dorsal line. This mass of rounded cells is the beginning of the neural crest.

The mesoderm in the mid-dorsal line is thickened in the posterior sections. According to some writers, this median mesoderm has *always up to this time remained closely fused with the layer of endoderm beneath it.* It marks the beginning of the notochord.

The formation of the notochord takes place from behind forwards, so that in the same embryo different stages of its development may be found (Fig. 26, D, E).

The account given above of the formation of the notochord is not generally accepted, particularly since the formation of the notochord from the endoderm is the method followed by many, perhaps by all other vertebrates. That a median mass of tissue stretches at first across the dorsal median wall of the archenteron in the frog cannot be denied, but many embryologists have preferred an interpretation different from that which I have followed. It is affirmed that there is always a *closer connection between the endoderm* and the tissue lying above it in the dorsal median line than between the endoderm on each side of

the mid-dorsal line and the mesoderm. Further, it is said, that the cord of cells in the median dorsal line remains for a longer time connected with the mid-dorsal endoderm than does the mesoderm at each side with the lateral endoderm, and that the notochord separates from its lateral connections (right and left) with the mesoderm, while it still remains for a time closely fused in the mid-line with the endoderm.

In the newt and in other urodeles the endoderm in the mid-dorsal line thickens and bends upward to form a longitudinal fold. The fold pinches off from the endoderm and forms a cord of cells, — the notochord. In the posterior end of the toad's notochord the same method of development may be seen sometimes to take place.[1]

With such clear evidence of the method of formation of the notochord from endoderm in the newt, it is not surprising that embryologists have attempted to interpret the changes that take place in the frog in the same way. The main difficulty arises from an unwillingness on their part to derive the notochord from the so-called middle germ-layer, or mesoderm. The question therefore turns, *for them*, on what they will call the middle layer in the frog, and what not the middle layer.

Since, however, all the cells in this region have had a common origin, the question is perhaps a trivial one; for we cannot doubt, I think, that had some of the cells in the middle line passed a little to one side or the other of the median line, they would have been capable of becoming mesoderm, and, *vice versâ*, had some of the lateral cells come to lie nearer to the middle line, then they would have taken part in the formation of the notochord.

The notochord separates entirely from the mesoderm and endoderm, and becomes rounded in cross-section. On each side of the notochord the mesoderm becomes thicker, as is shown in Fig. 42. The final stage in the closure of the medullary folds and the changes that take place in the mesoderm will be described in a later chapter.

---

[1] Field ('95).

CHAPTER VII

THE PRODUCTION OF ABNORMAL EMBRYOS WITH SPINA BIFIDA

EMBRYOS of the frog are occasionally found that differ greatly from normal embryos. Roux, in 1888, first described one of these embryos and showed that a knowledge of its structure and method of development helped very much toward an understanding of the processes that take place in the

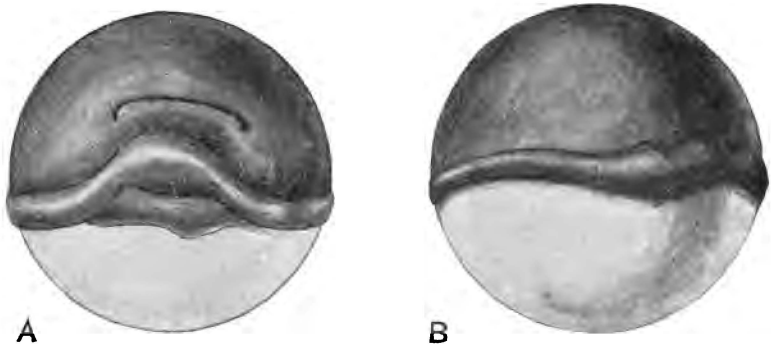


FIG. 27.—Two embryos formed as rings around equator of egg. A. Seen from in front (produced in salt solution). (Morgan.) B. Seen from side. (After Roux.)

normal development. An embryo described by Roux is shown in Fig. 27, B. Around the equator of the egg along the zone between the white and black hemispheres is a thickened ridge. A careful examination shows that this ridge is not uniform in thickness, but is bilateral in form. Each half is somewhat thickened at one end, and resembles half of the medullary plate of the normal embryo. Cross-sections (Fig. 29, B) show that these ridges around the equator of the egg are the two halves of the medullary plate. Instead, however, of being in close

contact, the two half-plates are separated in the middle by the diameter of the egg, but at the anterior and posterior ends the half-plates unite to form the ring. In section, a cord of cells, the notochord, is found beneath each half of the medullary fold; and between the yolk-cells and the ectoderm there is also found a sheet of tissue representing the mesoderm. Hertwig, in 1892, described a large number of these embryos. One is shown in surface view as seen from the white pole, in Fig. 28, A. The embryo is at a later stage of development than that described above. The exposed white yolk, turned toward the observer,

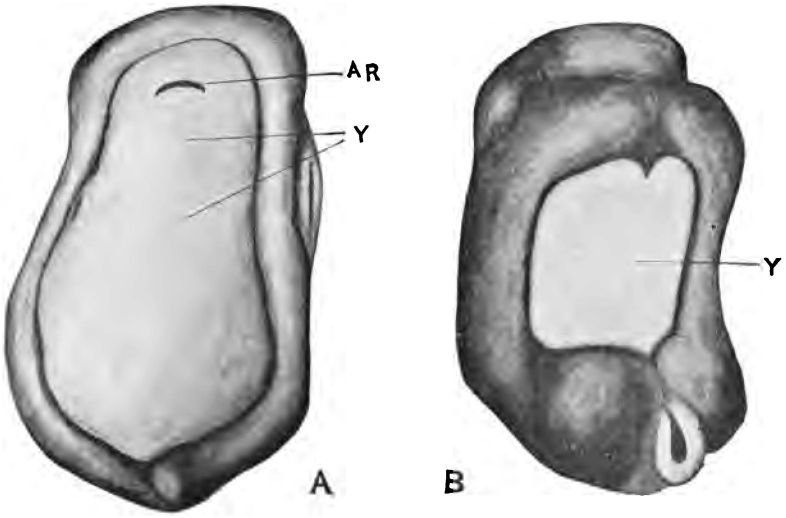


FIG. 28.—Two "spina-bifida" embryos. (After Hertwig.) A. Earlier, B. older stage (different embryos).

is surrounded by a groove, and outside of the groove there is a bounding darker ridge. In the anterior portion of the white is seen a crescent-shaped depression. A cross-section through the middle of the body of an embryo similar to the last is shown in Fig. 29, A. The exposed yolk is seen at Y. On each side of this there is a depression, and beyond the depression a thickened ridge composed of ectoderm cells. Each ridge passes over on its outer side into the ectoderm that covers all the lower part of the embryo. Even in their present stage

of development the ridges are clearly seen to be the widely separated halves of the medullary plate. Beneath each half of the medullary plate there is a cross-section of the notochord, and between the yolk-cells, in the centre of the section, and the ectoderm covering the lower surface, there is a thick sheet of cells representing the mesoderm.

A longitudinal (sagittal) section of the embryo drawn in Fig. 28, A, is shown in Fig. 29, C. The large exposure of yolk-cells (Y) in the upper part of the figure is very conspicuous. A deep and narrow depression, bounded for the most part by a distinct layer of yolk-cells, is found near the anterior end. This depression corresponds to the crescent-shaped opening seen in surface view, and is supposed to correspond to a part of the archenteron of the normal embryo.[1] Ectoderm covers the lower (ventral) surface of this section, and at one point the cells are thickened to form the adhesive glands of the larva. At the posterior end of the embryo a small depression is present, and, as later development shows, this corresponds to the posterior portion of the archenteron of a normal embryo.

Hertwig found that if male and female frogs of certain species be separated and kept apart for several weeks, and the eggs then be artificially fertilized, an abnormal segmentation follows, and, although many of the eggs die, among those that live a large number show this condition of spina bifida.

In 1893 I made a series of experiments attempting to produce artificially embryos showing spina bifida, and found that they could be made by two entirely different methods. If the segmented egg, before the blastopore-lips appear, be placed in water to which .6 per cent. of salt (NaCl) has been added, the later development is modified. The dorsal lip of the blastopore appears in its normal position but does not continue to extend over the white hemisphere. The corners of the lips gradually extend around the equator of the egg. A sharp line or depression separates the black and white hemispheres, and on the black side of the depression a circular ridge appears, which marks the beginning of the medullary ring (Fig. 27, A).

Similar embryos may also be produced if the dorsal lip of

[1] Possibly it represents in part the liver-diverticulum.

the blastopore is injured with a needle at the moment of its appearance, or if the yolk-mass in front of the dorsal lip is injured so that the yolk protrudes from the general rounded surface of the egg. The blastopore is thus prevented from extending backward, and its material differentiates, *in situ*, along the equatorial line. The lateral lips tend to approach the middle line and to fuse, but the medullary folds may appear before the fusion has taken place. There is thus pro-

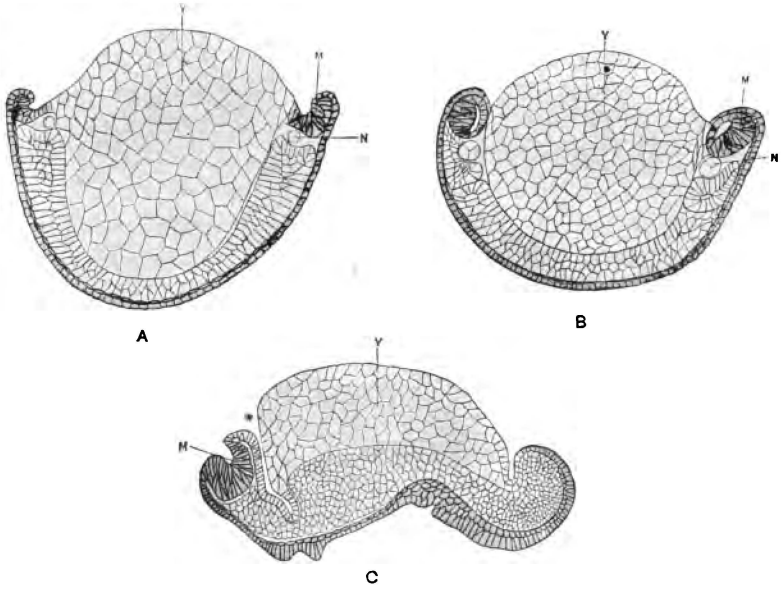


FIG. 29.—Cross (A, B) and longitudinal (C) sections through an embryo with spina bifida. (After Hertwig.) M. Half medullary plate. N. Half notochord. Y. Yolk.

duced an embryo with an exposure of yolk in the mid-dorsal line. The exposure is more or less extensive, according to the extent of fusion anteriorly of the blastopore, and to the extent of fusion forwards of the lateral and ventral lips.

These embryos with spina bifida show that the material for the mid-dorsal surface of the embryos appears first as a ring around the equator of the egg or a little below the equator. If this material is prevented from reaching the mid-dorsal surface, *it differentiates in situ.* Hence the production of a ring-like medullary plate and a double notochord.

It is important to know definitely the origin of the material that forms the equatorial ring. We have seen that the ring appears at the same time that the blastopore-lips extend around the equator of the egg. Does this material also extend out laterally from the dorsal lip of the blastopore along the sides, or is the material already present as a circular ring of tissue, from which the lips of the blastopore differentiate? A study of the normal embryo combined with experiments gives, I believe, a conclusive answer to these questions. In the first place, if the *dorsal lip* be entirely destroyed, so that it cannot advance, nevertheless the lateral lips still appear and extend backward. If a point of the surface be injured just in front of one (or both) of the advancing *corners of the dorso-lateral lips*, the advance of the latter would be stopped if an actual transfer of material were taking place; nevertheless, on the posterior side of the point of injury, a depression of the surface, marking the blastoporic rim, appears, and continues to extend backward. The same thing happens if injuries be made at two consecutive points in the direction of extension of the lateral lip. Now if material were actually transferred backward from the dorsal lip and around the equator of the egg, its movement would be stopped when the dorsal lip was seriously injured, so that the lateral lips of the blastopore, and, later, the medullary folds, would not appear, or else their appearance would be delayed. Further, if there were, in reality, any such transfer backward of material around the equator, its progress would be stopped when the material reached the points of injury made along the line of the lateral lip. On the contrary, the appearance of the lateral lips, after the destruction of the dorsal lip, takes place as though no hindrance were present.

The experiments point clearly to the conclusion that there is no backward transfer of building material, but that the material for the dorsal surface is already present as a ring around or near the equator of the egg.

If the normal embryo be studied by means of sections at the period of the extension of the lateral lips of the blastopore, the material of the ring is found to be already present in the region into which the lateral lips extend. The evidence from these various sources proves *that the production of the embryos*

showing spina bifida is owing to the differentiation in situ of cells that in the normal embryo are first carried to the dorsal surface before they differentiate into their definitive organs.

Roux first pointed out that the embryo described by him showed that the material for the two sides of the embryo is laid down in a ring, and that by the growing together (concrescence) of this ring along the mid-dorsal line of the embryo, the two halves of the body are brought together. The same method of formation of the embryo by concrescence has been described as taking place in other vertebrate embryos, and certain writers have even affirmed that this is the method by which all embryos of vertebrates are formed. In the main, Roux's conclusion for the frog seems to be correct,[1] but in one respect not an unimportant exception must be taken to his statement. If the material be laid down as a ring of tissue around the equator, and if, by its coming together (apposition), the two halves of the embryo result, it follows that the embryo should be at least as long as one semicircle of the surface of the egg. Further, we have seen that the anterior end of the medullary plate lies somewhat above the point of appearance of the dorsal lip of the blastopore, so that the embryo would be, on Roux's supposition, even longer than a semicircle. But if we measure the medullary plate of the embryo at the time of its *first appearance*, we find that in length it is only about one-third of the length of the circumference of the egg. It follows, then, that as the material comes to the mid-dorsal line in the normal embryo, it must also become more concentrated, so that the length of the medullary plate is less than the length of the material of its halves. *There is an accrescence or concentration of material combined with a concrescence or union of material from the two sides.*

[1] Although Roux did not foresee the possibility that material might grow around the equator from the dorsal lip of the blastopore, my own experiments show, I think, that such a transfer does not take place.

CHAPTER VIII

PFLÜGER'S EXPERIMENTS ON THE FROG'S EGG

IN order to discover how far the development depends on the surrounding conditions to which the egg is subjected, we must change those conditions and observe the result. In this way we may hope to find out to what extent the phenomena of development are dependent on conditions outside of the egg, and how far they result from the egg itself.

Pflüger made, in 1883, a brilliant series of experiments that have been the point of departure for much of the later work on the frog's egg; therefore, in this chapter, I shall give a somewhat detailed account of Pflüger's work. The results are arranged in an order different from that followed by Pflüger, with the hope of making clearer a necessarily brief abstract.

The following orientation of the egg will facilitate the description of the experiments. If the middle[1] point of the black hemisphere of the frog's egg (the "black pole") is imagined to be connected with the analogous point of the white hemisphere (*i.e.* with the "white pole") by a straight line passing through the centre of the egg, this line forms the *primary diameter* or *primary axis* of the egg. An imaginary primary equator and a system of parallels and meridians belong to such a diameter. When the frog's egg segments, the first two cleavage-planes are found to be vertical in whatever position the egg may lie. The line of intersection of these first two planes passes through the centre of the egg, forming what we may

[1] Pflüger does not notice that in the normal egg at rest this "middle part" is not necessarily the *highest* part of the egg. Correspondingly, the lower pole need not be the lowest point of the egg. For the present, however, we must disregard this distinction.

call the *cleavage-axis* or *secondary axis*. To this axis there also belong an imaginary secondary equator, parallels, and meridians. If the egg should be turned, *after cleavage*, so that neither the primary nor the secondary axis is vertical, then the diameter that stands *at the time* vertical may be spoken of as the *tertiary axis*.

It will be seen, from what has been said, that the imaginary primary and secondary axes (with their systems) turn with the egg, *i.e.* may be thought of as constituent parts of the egg; while the tertiary axis only corresponds to any diameter of the egg that is for the moment vertical.

THE EFFECT OF GRAVITY ON THE DIRECTION OF THE CLEAVAGE

In normal eggs the first and second cleavages are vertical, the third horizontal. The question arises, "Does there exist a causal relation between the cleavage-planes and the egg-axis, as has always been assumed without question, or do the first two cleavages go through the primary axis, only because the latter coincides with the force of gravity?" This can be tested by preventing the normal rotation of the egg, and Pflüger found a simple method by which this is possible.

When the frog's egg is removed from the uterus, it is covered by a thin coat of gelatinous substance which quickly absorbs water and, if sufficient water is present, a space appears after fertilization between the egg and its innermost membrane. If an egg is taken from the uterus and placed in a dry watch-glass, and only a drop of water containing sperm is added, then the membrane swells somewhat, and sticks firmly to the glass; if now the right amount of water is added, the surface of the egg remains in contact with the egg-membranes and the egg cannot rotate as it does under normal conditions. The watch-glass containing the egg may be turned in any position, and the egg turns with it, so that any desired point of the egg's surface may be placed uppermost. Let us imagine an egg to be so turned that the black pole lies on one side. In the course of three hours the first division comes in, but now the plane of the first cleavage may not correspond to the primary

axis. It follows always the direction of the force of gravity, *i.e.* it passes through the *vertical diameter* of the egg.

The second cleavage also is vertical, and its position is also determined by the position of the egg, and by the position of the plane of the first cleavage. The third cleavage-planes often show irregularities. Generally they are at right angles to the first two, and lie nearer the upper pole of the egg, or, in other words, their position is also influenced by the force of gravity, for they lie nearer to the pole that stands uppermost at the time. It is a remarkable fact that the subsequent cleavages are more rapid in the upper than in the lower hemisphere, no matter what region of the egg has been placed uppermost. Embryos develop from these eggs that have been turned into abnormal positions, and the embryos differ from normal embryos only in the relative distribution of pigment over the surface of the body. Many have the upper surface of the body a light brown color with dark spots; others have the head, the back, and upper surface of the tail almost free from pigment, and of a whitish-yellow color. The belly in these embryos is more or less deeply pigmented. In a few days, however, new pigment develops over the dorsal surface of the embryo. It should be noted that these paler embryos often show abnormalities, such as bizarre excrescences, irregular movements, slower development, and that after a few days they begin to die.

Pflüger concluded from his experiments that an egg may be divided in all possible directions by the early cleavage-planes according to the position in which the experimenter places the egg, and from such an egg a normal tadpole may develop.

It is not, however, entirely a matter of indifference what angle is made between the cleavage-planes and the primary axes. It is certain that if the upturned hemisphere contains more white than black, a normal embryo may develop; but if the upturned hemisphere be entirely white, *i.e.* if the egg has been rotated through 180 degrees, embryos may occasionally develop, but they are nearly always abnormal and soon die. It is difficult, in fact almost impossible, to keep the white hemisphere upward; for in nearly every case Pflüger found that later a partial rotation of the egg took place, so that a crescent of black appeared above the horizon. One exceptional case is worth recording.

An egg was observed that had its white hemisphere turned *exactly* upwards until the first cleavage came in. More water was then added, and the egg retained its reversed position and continued to segment energetically and with wonderful regularity. The upturned white hemisphere was soon divided into many small cells, while the cells in the lower black hemisphere were larger. A later examination of the egg showed that during the cleavage the egg had rotated through about 45 degrees, bringing a portion of the black hemisphere above the horizon. Still later the egg seemed to rotate back again into its first reversed position. After a time the development stopped and the egg died.

If, as the preceding experiments seem to show, there exists a relation between the force of gravity and the position of the first three cleavage-planes, it is important to know whether gravity acts only at the moment of cleavage or whether the action is a slow and continuous one. Pflüger found that if the egg at the two-cell stage be rotated a few seconds before the appearance of the *second* furrow, so that a new angle is made by the primary axis with the direction of the force of gravity, then the second furrow comes in as though the egg had not been changed, and may therefore make any possible angle with the direction of the force of gravity. The same experiment can be made if more water is added to an egg that has already segmented once in an abnormal position. The egg may then rotate so that the first cleavage-plane is no longer vertical; nevertheless, the second furrow always comes in at right angles to the plane of the first furrow, and, therefore, may make any possible angle with the direction of the force of gravity.

A different result follows if the egg has been rotated one hour after fertilization and therefore some time before the time of the first cleavage. The plane of cleavage of the *second division* is then affected, and coincides with the direction of the force of gravity. We must conclude that an interval of one hour at least is necessary to produce any change in the egg.

What has just been said with regard to the second planes of cleavage holds equally well for the third cleavage-planes. If the egg be rotated through 180 degrees *after* it has divided twice (into four parts), then the third furrows come in as

though no change had taken place, *i.e.* nearer the former upper pole. But if the egg had been rotated one hour after fertilization (or even after the first cleavage), the third furrows would appear on the *new* upper hemisphere, *i.e.* nearer the present upper pole. In this last case the four upper cells resulting from the third division are smaller than the lower four. This shows that the four upper cells of the normal eight-cell stage are smaller, not because they are black, but, according to Pflüger, on account of their position in relation to the force of gravity. Embryos develop from these eggs, but they show many abnormal structures.

Pflüger also rotated eggs through 180 degrees after the third cleavage had come in. In four hours and a half the cells of the new upper hemisphere were of the same size as those of the new lower hemisphere. A normal egg at this time would have shown a great difference in the size of the cells of the upper and lower hemispheres. It follows from the last experiments that gravity may affect not only the first, second, and third cleavage-planes, but the later stages as well. "Gravity," Pflüger said, "according to some unknown law regulates the cleavage-planes. A simple explanation of the phenomenon does not seem possible in the light of the facts."

THE RELATION OF THE PLANES OF CLEAVAGE TO THE AXES OF THE EMBRYO

Pflüger made other experiments to determine whether, under normal conditions, there exists any relation between the planes of cleavage and the axes of the embryo. He placed seventeen eggs in as many watch-glasses and added water containing sperm. The axes of the eggs were vertical.

The direction of the plane of first cleavage was noted and marked by a line scratched on each glass. The beginning of the nervous system appeared in about forty-eight hours. In twelve eggs the median plane of the body coincided with the first cleavage-plane, or at most the two planes did not differ more than 10 degrees. In four eggs there was an angle of 30 to 60 degrees between the two planes, and in one egg one of 90 degrees. Pflüger concludes that it is highly probable from this result that the plane of the first cleavage and the

median plane of the body coincide. The exceptions may be due to the rough treatment of the eggs.[1] Newport ('51) had previously made a similar experiment on normal eggs, *i.e.* eggs not fixed artificially, and had reached the same conclusion as Pflüger, but Newport's results were unknown to Pflüger when he made his experiments.

Pflüger was led from certain results of his experiments to observe carefully the position of the egg at the time when the normal embryo was developing. He found, as has been already described, that the dorsal lip of the blastopore appeared in the white below the equator of the egg. He noted in the living egg that the blastopore slowly migrated over the white hemisphere, and that it finally closed nearly 180 degrees from the point of its first appearance. Subsequently the whole egg slowly rotated, so that the small blastopore traced the same path (but in a reversed direction) over which the dorsal lip of the blastopore had passed. The results show that the nervous system develops over the lower white hemisphere of the egg. The material for the nervous system comes from the substance of the lips of the blastopore as they move over and cover the lower hemisphere. This material, from which the nervous system is formed, is at first somewhat lighter in color than the pigmented hemisphere of the egg. It is darker, however, than the white material of the lower hemisphere.

If in normal eggs the first cleavage-plane corresponds to the median plane of the body of the embryo, does the same relation hold for eggs that have segmented in abnormal positions? In other words, does the median plane of the body in eggs that have been turned so that the primary axis is no longer vertical still correspond to one of the primary meridians of the egg, or to one of the secondary (*i.e.* segmentation) meridians? Pflüger's observations showed that in eggs with oblique primary axes the plane of the first cleavage is not identical with the median plane of the embryo, but forms different angles with it. In forty-eight eggs there were thirty-three in which the median plane of the embryo coincided with the *primary axis*. In the

[1] Or else to a very early rotation of the egg, either as it shifts around its centre of gravity during gastrulation, or from the action of surface-cilia. — T. H. M.

remaining eggs there were eight in which the median plane of the embryo made angles between 10 and 25 degrees with the primary axis. In five cases the angle was between 25 and 45 degrees, and in two the angle was as great as 45 to 90 degrees. Pflüger concluded that in abnormally turned eggs the median plane of the embryo belongs to the system of meridians of the primary axis of the egg, — as in normal eggs; and that the cleavage of the egg only breaks up the building material into small building blocks, and it is of no importance in the subsequent stages of development how the splitting up has taken place.

CONCLUSIONS FROM THE EXPERIMENTS

From the orientation of the embryo with respect to the primary axis in whatever position the egg may be, it might seem that the material of the egg is not *isotropic*. That is to say, the position of the embryo is fixed in the egg, and the embryo assumes its predetermined position regardless of the method of segmentation. A more careful examination will show however, Pflüger believes, that the egg is isotropic.

It is obvious that although in most cases when the egg lies with an oblique primary axis, the median plane of the body belongs to the system of primary meridians, yet there are theoretically an infinite number of these meridians any one of which might happen to be uppermost and to coincide with the median plane of the body; and Pflüger's tables show that there must be a great many possible primary meridians any one of which may become the median plane of the embryo.

In the second place, the dorsal lip of the blastopore never develops on the *upper* hemisphere, however the egg may be turned. Pflüger says that, in all, he has probably examined a thousand eggs, and never once found the blastopore above. It appears always in the *white below the equator*. Again, in an egg abnormally placed the head always develops *above* and the body *below*. These relations could not exist for all positions of the egg, if the position of the embryo were prefixed in its relation to the primary meridians.

Pflüger considered one other possibility; namely, that the semi-fluid contents of the egg may rearrange themselves in eggs

abnormally turned, so that the predetermined material takes a definite position, and the blastopore always appears in its proper hemisphere. A rearrangement, Pflüger believed, does not take place, because the egg, if set free, even after it has been turned for two hours, will tend to rotate into its normal position. Such an egg, set free in its membrane, places the primary axis vertical, and this rotation will take place even after the first and second furrows have appeared; and this would not be the case had there been a rearrangement of the contents.

Pflüger noticed, however, in eggs that had been turned into abnormal positions, that the *upper*, white hemisphere is often darker in the later stages than it was at first, and conversely, the black hemisphere may appear lighter owing to the loss of a part of its pigment. This is brought about, Pflüger believed, by a streaming of the pigment-granules of the egg, and is not a result of the rotation of the contents as a whole.

The position of the dorsal lip of the blastopore is determined, then, in part by the position of the primary axis, and in part by the tertiary axis, since the blastopore is *always* in the lower hemisphere, however the egg be turned. "*The primary axis determines the meridian, and the tertiary axis the parallel in which the dorsal lip of the blastopore shall appear.*"

Since these statements are true for all possible positions of the primary axis, it follows that all primary meridians are of equal value. If we think of an egg with inclined primary axis and imagine this egg rotated around such an axis, then all the primary meridians of the egg will in turn come uppermost. Whichever one is brought to rest in the vertical plane, that one will symmetrically halve the opening of the blastopore when the latter develops, and on that one the embryo will lie with its head turned upwards. It is this vertical meridian that coincides with the direction of the force of gravity. In this meridian, every part is not of equal value, because the blastopore appears only in a certain region, and the position of the embryo is thus fixed. The appearance of the blastopore on the vertical meridian below the equator marks the crystallization-point of the whole organization. In other words, the egg-substance has at this time one meridian polarized. Pflüger says: "I think of each half of the egg after this as polarized, for both halves are

then of equal value and are composed of equivalent molecular rows. Gravity alone has determined which of all possible meridians shall be the controlling one."

He adds: "I imagine that the fertilized egg bears no more relation to the later organization of the animal than the snowflakes bear to the size and structure of the glacier that develops from them. From a germ there always arises the same structure because the external circumstances remain the same. The glacier that develops out of the snowflakes has always the same form, so long as the external conditions are unchanged."

CHAPTER IX

EXPERIMENTS OF BORN AND OF ROUX

PFLÜGER, as we have seen, believes that when the frog's egg is rotated so that the white hemisphere is turned uppermost, no rotation of the contents of the egg takes place. Born ('84, b) repeated this experiment of Pflüger and sought, by making actual sections of the eggs, to find out whether any changes do take place in the interior of the reversed egg.[1]

Sections through normal, fertilized or unfertilized frogs' eggs show that there is a peripheral, darkly pigmented rind in the form of a shell thickest at the black pole (30 to 40 microns) and fading away at the white pole (Fig. 8). Beneath the black rind in the upper hemisphere lies a brownish pigmented protoplasm. In the centre of this and just under the black pole is found in cross-section a clearer spot containing the nucleus. The yolk lies within the white hemisphere. The yolk appears coarsely granular, while the protoplasm in the dark hemisphere is finely granular.

CHANGES THAT TAKE PLACE IN THE INTERIOR OF THE EGG AFTER ROTATION

Born observed in the living egg that when the white hemisphere is kept upward, it gradually becomes darker in color, owing to the appearance of a grayish-white area. Pflüger had noticed the same phenomenon. This area grows larger in proportion to the length of time that the egg has been turned.

Examination of sections of an inverted egg shows that forty-

[1] After the first account of Born's had appeared, other papers dealing with the same subject by Roux, Rauber, and O. Hertwig were also published. These authors all agree with Born.

five minutes after inversion a rearrangement of the contents has begun. The heavier white yolk has begun to sink down on one side, taking the shortest path toward the bottom of the inverted egg. As the heavier yolk sinks down in response to the action of the force of gravity, the granular protoplasm rises up on the opposite side. The two sorts of substances do not mix during the interchange of position, but keep sharply separated from each other. The pigment-rind remains fixed, but loses something of its thickness. After an interval of forty-five minutes to two hours, the finely granular protoplasm has reached the highest point of the egg, and has spread out under the surface of the white hemisphere. The yolk has passed to the lower hemisphere of the inverted egg, and now lies inside of the black rind.

This description of the movement of the contents of the egg applies to all those cases in which the white pole does not stand exactly upward, or, in other words, where the egg is turned less than 180 degrees. When the egg is completely inverted the force of gravity causes the contents of the egg to rearrange themselves in a somewhat different way. The yolk sinks down on all sides, while the lighter protoplasm rises up through the centre of the egg, carrying with it the nucleus.

Pflüger believed that eggs which had been rotated through 180 degrees, and kept in that position, did not segment because of the covering up of a micropyle, where the black pole came in contact with the lower surface of support. Born thinks that such eggs do not segment, owing to the inability of the spermatozoa to pierce the white rind which is uppermost.[1]

In the normal egg the path of the spermatozoön can be followed by the trail of pigment passing in from the surface of the egg, which marks the direction taken by the spermatozoön. This pigment-line can also be followed in the partially inverted egg, and it is seen that the male pronucleus is also carried along in the streaming protoplasm.

Born found in eggs that have been partially inverted, that the first cleavage-plane is generally vertical, passing through

[1] The problem of the extrusion of the second polar body in these eggs should be examined.

the highest point of the egg. Sometimes, however, the plane of cleavage is oblique to the vertical, *i.e.* occasionally it does not pass through the highest point of the egg. The position of this vertical or nearly vertical plane of cleavage bears generally some relation to the path, or meridian, of streaming of the contents of the egg. The first plane of cleavage corresponded with the streaming meridian in about one-third of one hundred recorded cases. In nearly all of the remaining two-thirds, the first cleavage-plane stood nearly at right angles to the streaming meridian.[1]

Born's results throw a new and important light on Pflüger's experiments. The force of gravity acts on the rotated egg only to bring about a rearrangement of the contents of the egg in accordance with the specific gravity of the substances present. This is the only connection between the direction of the force of gravity and the direction of the planes of cleavage. We also see why a certain amount of time is necessary after the reversal of the egg for the rearrangement to take place.

THE CLEAVAGE OF THE EGG IN A CENTRIFUGAL MACHINE

Roux ('84) tested in another way the effect of gravity on the segmentation of the frog's egg. "What would happen," he asked, "if an egg were so placed that at every moment a new point was turned uppermost? Further, if gravity acts only so as to rearrange the contents of the egg, what would take place if a centrifugal force were applied to the eggs before cleavage?" Such a centrifugal force ought to cause the egg to orient itself in respect to the direction of that force, in the same way that gravity causes the egg to turn.

A wheel rotating around a horizontal axis was used. To this wheel were attached tin boxes into which the eggs were put. A box could be placed at any point along a radius of the wheel. When the machine made eighty-four revolutions a minute, some of the boxes were so placed that the centrifugal

[1] In this case the second cleavage-plane would correspond with the meridian of streaming. Born states that the median plane of the embryos, developing from the rotated eggs, passes through the secondary meridian that cuts the highest edge of the white field in its partially inverted position.

force was twice as great as the force of gravity. When the box was at the lowermost point in a revolution, the centrifugal force and the force of gravity acted together. When the box was at the highest point in its revolution, then the centrifugal force and the force of gravity acted against each other. With the velocity of eighty-four revolutions a minute, the centrifugal force was greater than the force of gravity, even at the highest point of a revolution. At the intermediate points, *i.e.* between the highest and lowest points, the conditions are different for each point, and lie between the two extremes just stated. Under the conditions of the experiment the eggs rotated inside their membranes, so that the black pole turned *inward*, i.e. *toward* the axis of rotation, and the white pole turned outward. In other words, the eggs now oriented themselves with regard to the centrifugal force, and not with regard to the force of gravity. Even when the centrifugal force was only half that given above, the eggs still arranged themselves with reference to that force. In the latter case the force of gravity was barely overcome by the centrifugal force at the highest point of the revolution. If a still shorter radius were used, so that at the highest point of the revolution the force of gravity was three times as strong as the centrifugal force, even then the eggs oriented themselves as before, *i.e.* with the black pole turned toward the axis of rotation. In all of these experiments it will be seen that the centrifugal force is a constant force, while the action of gravity varies in direction at each point in the revolution. If a still shorter radius of the wheel was used, in which case the centrifugal force was still less, then the eggs retained any position that they had when first put into the box.

All of these different possibilities could be realized at the same time by using a series of tin boxes placed at the proper intervals along a radius of the wheel. "The apparatus, laden with ten to eighteen freshly fertilized eggs, was set in motion. I waited with great interest for the appearance of the first cleavage. It appeared at the normal time and the whole cleavage proceeded in exactly the normal way. A normal blastopore appeared, and the formation of the medullary folds, the brain-folds, and closure of the neural tube, and later

the formation of the suckers, gills, and tail, all took place normally. There was no difference in the time of development between the eggs in the machine and the normal eggs outside," used to check the results.

In the eggs acted upon by the centrifugal force, the segmentation-axis corresponded with the egg-axis, and showed no relation to the direction of the force of gravity. The third furrows appeared nearer to the black pole, and the black cells always divided faster than the white cells, regardless of the position of the egg in respect to the force of gravity. The blastopore appeared in its usual position.

Roux concluded that Pflüger's interpretation of his experiments in regard to the action of the force of gravity was incorrect. Roux said that in his own experiment the localized effect of the force of gravity had been done away with, when the eggs were slowly revolved, *i.e.* in those eggs nearest the axis of the machine, and still the cleavage appeared in these eggs irrespective of their position. When the centrifugal force was stronger, it replaced the force of gravity, and the eggs oriented themselves in regard to the new force, and still the cleavage and the subsequent development took place normally.

Roux pointed out that a possible objection might be made as to the sufficiency of his results. Since the eggs were always rotated in a constant plane, it might be affirmed that gravity, acting vertically in the plane of rotation, still acted upon the egg. To meet this objection a new experiment was devised. Single eggs were placed in a glass tube 6 cm. long. This tube, only half filled with water, was closed and fastened to the centrifugal machine. During each rotation of the apparatus, the eggs and the water would fall from one end of the tube to the other, so that the orientation of the eggs would be changed during each revolution. Nevertheless embryos normal in structure were produced. They were, however, small and weak.

CHAPTER X

MODIFICATION OF CLEAVAGE BY COMPRESSION OF THE EGG

In 1884 Pflüger made the important and novel experiment of compressing the unsegmented egg of the frog between parallel plates of glass. In consequence, the cleavage was modified, and there was found to be a direct relation established between the planes of cleavage and the direction of the pressure applied. The first three planes of division were at right angles to the compressing plate. Pflüger explained these results as due to the position which the nuclear spindle would take during its elongation. The long axis of the spindle, he thought, would place itself in the direction of least resistance, *i.e.* in a plane parallel to the glass plates; and since the division of the cell is at right angles to the long axis of the spindle, it will, therefore, be at right angles to the compressing plates. Born ('93) and Hertwig ('93) simultaneously repeated Pflüger's experiment, making also some modifications of the original experiment. Born's account is here followed, as it gives a more detailed report of the results.

Effect of Compressing the Segmenting Egg between Parallel Plates

The eggs of Rana fusca are on an average 1.5 mm. in diameter. The distance between the two glass plates in the experiment was 1.4 mm., for if less the eggs were burst by the pressure. Since all of the jelly around the egg was not removed, the actual diameter of the egg, as subsequent measurements showed, was less than the distance between the two parallel plates (1.4 mm.). For instance, a compressed egg (after it had been killed and

hardened)[1] measured in its longer diameter, parallel to the two plates, 1.83 mm., while the shorter diameter, at right angles to the plates, was 1.2 mm. The two axes, therefore, stand in the relation of 2 : 3. These figures apply to eggs compressed in the direction of the egg-axis, from above downward. When the egg is compressed from side to side it will withstand more pressure. With a distance of 1.37 mm. between the two parallel plates, an egg compressed laterally measured in its longer diameter 1.96 mm., while its shorter diameter, from plate to plate, measured 0.91 mm. The two axes therefore bear to each other the ratio of 1 : 2. The experiments may be described in detail under the following categories.

1) *Eggs compressed in the direction of the primary axis* (Fig. 30, A). The eggs taken from the uterus were placed on a dry plate

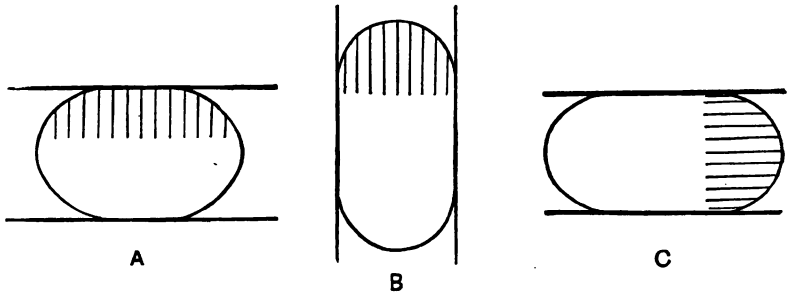


FIG. 30. — Diagram showing three positions of eggs under compression.

of glass, so that the white pole was exactly downward, *i.e.* the egg-axis was vertical. Another glass plate was then placed over the eggs and brought down into contact with two supporting rods, so that the two glass plates were 1.4 mm. apart, and the eggs correspondingly compressed. The eggs were then fertilized and the whole apparatus put into a dish of water. The primary axis of each egg was kept always vertical. When the first furrow comes in, it is *vertical*, *i.e.* at right angles to the glass plates, and passes from the black to the white pole (Fig. 31, A), dividing the egg into two symmetrical halves. The second furrows come in at right angles to the first, and are also

[1] The eggs contracted very little during the process of hardening.

vertical, *i.e.* at right angles to the glass plates. The second furrows may cross the first furrow in the middle of its upper and lower surfaces, so that four cells of equal size result; or the second furrows may sometimes pass to one side of the middle point, so that two cells may be larger than the other two (Fig. 31, A). The last result may be due to a slight obliquity in the position of the axis of the compressed egg. The *third furrows* (which normally are horizontal and at right angles to the pre-

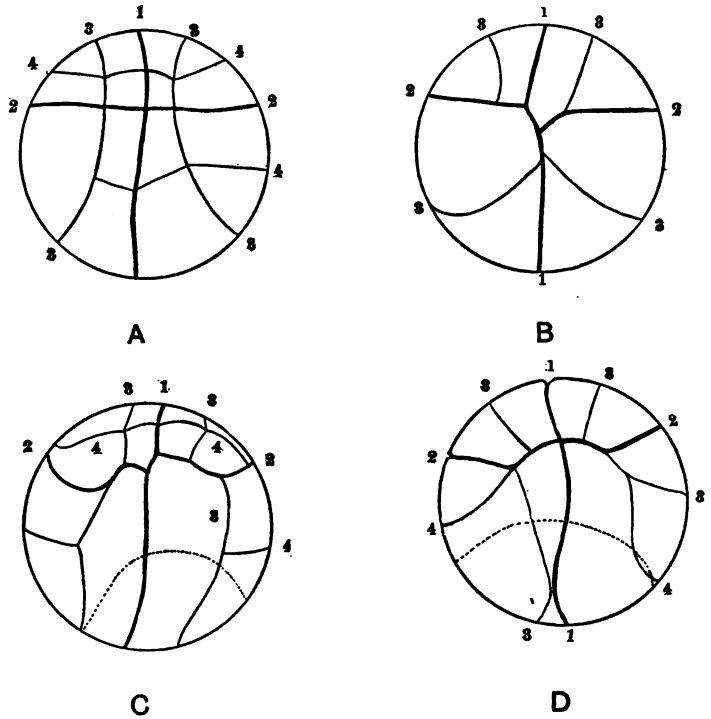


FIG. 31.—A, B. Egg compressed axially (Diagram A, Fig. 30). A. Above; B. below. C, D. Egg compressed laterally (Diagram B, Fig. 30). C. One side; D. other side.

ceding furrows) are also *vertical* and at right angles to the plates, and are generally *parallel* to the *first* furrow (Fig. 31, A, B). The egg is now divided into eight cells, all lying in one horizontal plane. In the black hemisphere the third furrows abut against the second furrows (Fig. 31, A), but below they as often run into the first furrows, as shown in the

figure (Fig. 31, B). The fourth furrows are also vertical (*i.e.* at right angles to the plates) and generally run parallel to the *second* planes of cleavage, as seen in the figure (Fig. 31, A, B). There is no segmentation-cavity as yet present in these compressed eggs.

It is possible to keep these eggs in position until the blastopore appears, and then to follow its movements up to a time when the medullary folds form. The blastopore appears on the under side, *i.e.* on the white hemisphere near *the edge of the egg*. It closes at the opposite edge of the lower surface. The medullary folds also appear on the lower surface of the egg, and remain there until the embryo begins to lengthen. The belly is therefore turned upward.

2) *Eggs compressed laterally*, i.e. *at right angles to the primary axis, with the black pole kept upward* (Fig. 30, B). The eggs were placed between glass plates so that, when the plates were turned vertically, the axis of the eggs also stood vertical, and the compression was from the sides. The first furrow is vertical and at right angles to the two glass plates (Fig. 31, C, D). The furrow passes through the middle of the egg, dividing it into two equal parts. Deviations from this mode of division often occur. The first division sometimes passes obliquely, *i.e.* to one side from above downward, but keeps always at right angles to the glass plates.

The second cleavage comes in also at right angles to the plates, and at right angles to the first furrow, and therefore in a *horizontal* position. It always lies nearer the upper (*i.e.* the black) side of the egg, as shown in the figure (Fig. 31, C, D). Two upper small cells and two lower large cells are formed. The second furrows have come in where, normally, the third furrows lie.

The furrows of the third order appear first in the upper smaller cells. They are at right angles to the glass plates, and parallel to the first furrow, near to which they often lie (Fig. 31, C, D). Occasionally, a furrow of the third order may lie parallel to the second, and not to the first furrow; it may even run along the edge of the compressed egg, and is then parallel to the compressing plates. In the lower cells the furrows of the third order also come in vertically and at right angles to

the plates. They are generally more or less parallel to the first furrow. The furrows of the fourth order come in as a rule at right angles to the last furrows, and therefore vary in position according to the position of the third furrows (Fig. 31, C).

The later development of these eggs is as follows: if the eggs have been much compressed, the blastopore appears always at the periphery of the flattened egg, *i.e.* at the edge, and in the space between the two plates; when the eggs are not so much compressed, the blastopore appears near the edge but more or less upon one or the other surface. Curiously enough, just before the closure of the blastopore, its opening is found to lie at the edge *of the same side at which it first appeared.* Born interprets this result as due to a rotation of the whole egg during the closure of the blastopore. The eggs, he believes, are able to rotate in the space between the glass plates around an axis at right angles to the plates. The medullary fold appears also at the edge of the compressed egg.

3) *Eggs compressed laterally and kept with the black pole to one side* (Fig. 30, C). If the eggs, laterally compressed, are kept after compression in a *horizontal* position, *i.e.* with the primary axis horizontal, other phenomena appear. Under these circumstances, Born says that a streaming of the contents of the egg takes place. The cleavage of these eggs corresponds in general to that of the laterally compressed eggs, with normally directed, *i.e.* vertical, axis.

4) *Eggs compressed between two plates oblique to each other, so that the eggs lie in a wedge-shaped space.* The first two furrows are at right angles to the compressing plates, which are inclined 12 degrees to each other. The furrows of the third order are in the *smaller*, dark and more compressed cells at right angles to the plates, while in the yolk-cells, which are little compressed, the furrows are horizontal. The details of these experiments of cleavage have not been worked out by Born so fully as in the cases where the compressing plates were parallel to each other.

Hertwig ('83, b) has described the first cleavage of one of these eggs compressed by plates inclined 45 degrees to each other. The first cleavage divides a smaller protoplasmic por-

tion from a larger yolk-portion. It does not therefore divide the egg, as in the preceding cases, symmetrically.

Hertwig found that when eggs were compressed from above downward, *i.e.* flattened axially between parallel plates, there was no agreement between the plane of the first cleavage and the median plane of the embryo. Four times the two coincided, approximately, with the first furrow, five times with the second, and six times with neither. The blastopore closes in these eggs, as Born had also shown, at a point of the white hemisphere opposite to that at which it first appeared. In the eggs compressed from the sides and standing with the axis vertical, the blastopore appeared generally at the edge between the two plates, and closed at a point opposite to that at which it had first appeared.[1] Exceptionally in these eggs the blastopore appeared on one of the flattened surfaces, *i.e.* against one of the compressing glass plates.

EFFECT OF COMPRESSING THE EGG IN A GLASS TUBE

Roux has shown that if the frog's egg be sucked up into a glass tube of smaller diameter than the diameter of the egg,

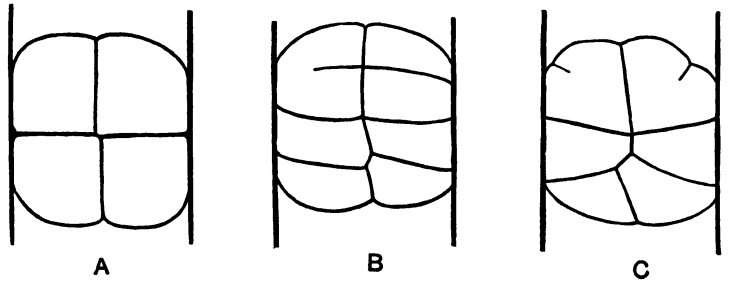


FIG. 32. — Segmentation of egg enclosed in a tube. (After Hertwig.) A. Four-cell stage. B, C. Eight-cell stage, above and below.

the egg will be drawn out into a barrel-shaped body and the cleavage correspondingly modified. The results, however, are not always alike. This is probably due to the presence of a large amount of jelly surrounding the eggs, so that they do

[1] Hertwig found that when *unsegmented eggs* compressed between parallel plates were rotated so that the white pole was turned upward, the egg rotated

not always take the shape of the enclosing tube. In order to avoid this inconstant element, Hertwig ('93) repeated the experiment with eggs from which much of the jelly had been cut away. The fertilized eggs were drawn up into cylindrical tubes in which they assumed a short cylindrical shape (Fig. 32). The eggs lay with the black hemisphere against one side of the tube and this side was turned upward, and the tube kept in a horizontal position. The first cleavage of such an egg is vertical and at right angles to the long axis of the tube (Fig. 32). The second furrows are also vertical and at right angles to the first, therefore in the direction of the long axis of the tube. The third furrows are also vertical and parallel to the first. The result so far is the same as when the eggs are compressed from above downward between parallel glass plates. The fourth furrows are *horizontal* and divide the egg into eight black and eight white cells.

CONCLUSIONS FROM THE EXPERIMENTS

These experiments in which the cleavage has been modified by changing the shape of the egg have an important bearing on the general problem of cleavage of the egg. In the first place, the "induced" form of the cleavage may give us some insight into the causes that determine the direction of the normal cleavage-furrows. In the second place, we see that when an egg is compressed, the sequence of the cell-division is very different from the normal sequence. Since we get normal embryos from eggs modified in this way, it would seem to follow, as Pflüger was the first to point out, that the cleavage simply divides the spherical egg into the building-blocks from which the later embryo forms, and it is a matter of indifference

---

as a whole and tended to turn the white hemisphere downward. If, however, the eggs were compressed after the two, four, or eight cell stage, they then held their position much better when the white pole was turned upward. If the compression was applied when the cleavage of the eggs had gone very far, but before the blastopore appeared, it was again found that the rotation of the egg as a whole takes place (as in the unsegmented egg). An egg that has been turned with its white pole upward at the two or four cell stage and has kept its position during the cleavage-period, no longer tends to rotate as a whole during the later stages of cleavage.

as to the succession of divisions. The value of this statement will be discussed later.

These experiments show clearly that by changing the form of the egg, we change at the same time its method of cleavage. Again, reasoning from these "induced" forms back to normal forms of cleavage, we see also something of the forces at work there. Pflüger did not fail to see the importance of these experiments. He believed, as we have seen, that the direction of the cleavage-planes results from the direction of the pressure, because when the nuclear spindle of the egg or of a blastomere forms, the spindle elongates in the direction of least resistance, that is, at right angles to the direction of the pressure.

The spindle in the egg axially compressed cannot lie at right angles to the plates because of the resistance of the yolk below, but it must elongate in a plane parallel to the plates. Since the cleavage of the protoplasm takes place at right angles to the long axis of the nuclear spindle, the division-planes must appear at right angles to the plates. Born has pointed out that this interpretation of Pflüger cannot be the true one, because the egg is not a solid elastic ball, but a fluid globe with an elastic coat. The pressure, therefore, will be quickly equalized in all directions, and cannot act during the time of cleavage in any given direction.

Sachs's law for the direction of new cleavage-planes seems to apply to the compressed eggs. According to Sachs, the form of the whole mass determines the position of the cleavage-planes. Hertwig refers the processes of cleavage directly to the changes that take place in the nucleus. He thinks that the nucleus tends to assume the centre of its sphere of activity, which is the centre of the protoplasmic mass. This is not necessarily the centre of an egg in which the yolk is unequally distributed. Hertwig thinks that the nuclear spindle will then elongate in the direction of the greatest protoplasmic mass. If we apply Hertwig's hypothesis to the segmenting frog's egg, we see that it appears to explain in part the various phenomena. In the egg compressed in the direction of its primary axis and with the primary axis vertical (category 1), the greatest protoplasmic mass will be, for the first spindle, in a horizontal plane; similarly for the second spindle. Hence

the cleavage-planes that come in at right angles to the cleavage-spindle must be vertical and at right angles to the plates. The third cleavage-planes will be for the same reason vertical, and even the fourth planes may be so. The number of consecutive divisions at right angles to the compressing plates must, however, soon reach a limit, because the mass of protoplasm in each cell will soon be thicker vertically than horizontally. When this happens, the next cleavage comes in horizontally or parallel to the plates.

Hertwig's hypothesis seems, therefore, in harmony with the phenomena of the compressed eggs. Whether it is of general application may be doubted because cases have been recorded

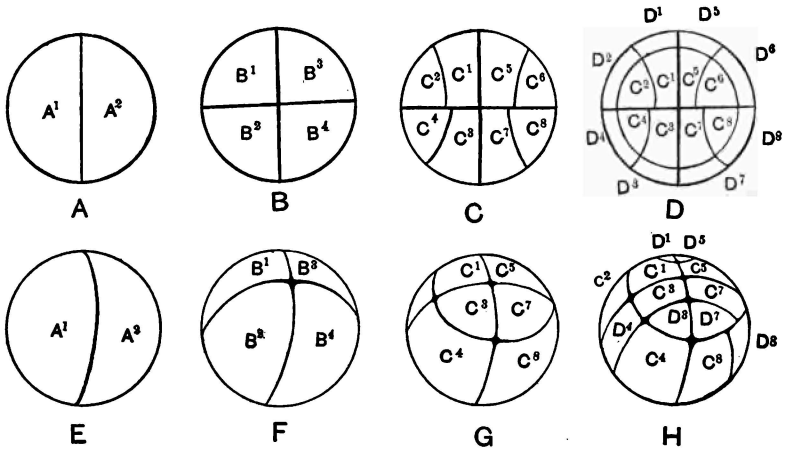
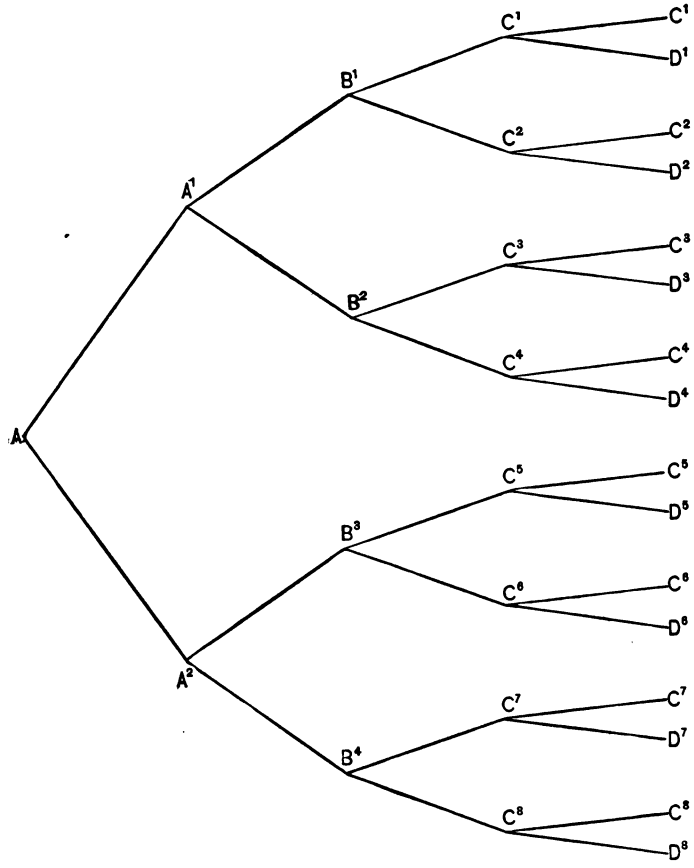


FIG. 33.—Diagrams to show the distribution of nuclei in compressed (A–D) and normal egg (E–H). In the upper series (A–D) the black hemisphere is turned toward the observer; in the lower series (E–H) the egg is seen from the side and in part from above the black hemisphere.

where the elongating spindle does not seem to take the direction of the greatest protoplasmic mass. Further, in certain spherical eggs without yolk, all the axes are equal, and some other cause must be present to determine the direction of the spindle. Even in the compressed egg (category 1) the protoplasm must be radially symmetrical. Finally, it is possible that the phenomena of the greatest protoplasmic mass and the elongating spindle may be only concomitant and not causal phenomena, for the position assumed by the centrosomes, which come to lie at

the apices of the spindle, must also be considered. The centrosomes determine the position of the poles of the nuclear spindle. Moreover, the position of apposition of the two pronuclei of the egg may be a further factor in the first cleavage.



THE DISTRIBUTION OF THE NUCLEI IN THE COMPRESSED EGG

In the experiments recorded above, where the frog's egg is compressed during the cleavage-period, the distribution of nuclei in the protoplasm is different from that in the normal egg. This is illustrated in the accompanying diagrams (Fig. 33). Let us call the segmentation-nucleus A, and its first products

A^1–B^2. The products of these nuclei we may call B^1–B^2, B^3–B^4. The following division will give eight nuclei, C^1–C^8, and at the sixteen-cell stage we may call the nuclei C^1–D^1, C^2–D^2, etc., as shown in the accompanying diagram.

Now let us compare, using this nomenclature, a normal egg (Fig. 33, B) with an axially compressed egg (Fig. 33, A). In the normal egg at the sixteen-cell stage, the nuclei around the upper pole will be C^1–D^1, C^3–D^3, C^5–D^5, C^7–D^7, and those around the lower pole, C^2–D^2, C^4–D^4, C^6–D^6, C^8–D^8. On the other hand, in a compressed egg that has been freed from the compression after the eight-cell stage, so that the fourth furrow has come in horizontally (Fig. 33, A–D), we find that the nuclei in the upper hemisphere are C^1–C^2, C^3–C^4, C^5–C^6, C^7–C^8, and in the lower hemisphere, D^1–D^2, D^3–D^4, D^5–D^6, D^7–D^8. Thus there is an entirely different distribution of the products of the nuclear division in the two cases,[1] yet normal embryos develop from both eggs.

The simplest and most obvious conclusion from this result is, I think, that the sequence of nuclear division during the early cleavage-period has no relation to the subsequent formation of the embryo, and that at this time the nuclei are all equivalent.

---

[1] There are several other possible combinations of these sixteen nuclei, but in no case is the distribution alike in the normal and in the compressed egg.

CHAPTER XI

THE EFFECT OF INJURING ONE OF THE FIRST TWO BLASTOMERES

We have seen that the plane of first cleavage corresponds as a rule with the median plane of the future embryo, so that one of the first two blastomeres gives rise to the cells that form one side of the body of the embryo, and the other blastomere produces the cells of the other side. It would seem then that even at the two-cell stage the axes of the future embryo are definitely laid down. But the most fundamental question remains unanswered; viz., has the egg after its first cleavage divided its material into qualitatively different parts (*i.e.* has the material of the right side of the body been separated qualitatively from that of the left side), or are the first-formed blastomeres still undifferentiated, and their subsequent fate dependent on the relative position they bear to each other as a part of a *whole?*

Roux tried to answer this question by the following ingenious experiment.

Roux's Experiment of "Killing" One of the First Two Blastomeres

As soon as the first furrow had passed through the egg, one of the resulting blastomeres was pierced with a hot needle. In order to carry out the experiment successfully, certain precautions must be taken. The eggs as soon as removed from the uterus are scattered over a glass plate (under water) so that they lie singly. Then water containing spermatozoa is added. After ten minutes this water, clouded by the spermatozoa, is poured off and fresh water is added. When the first furrow in the eggs appears, the water is again poured off. Each egg is held by a pair of forceps and then pierced by a

hot needle. The needle is carefully sharpened, and is re-sharpened after each egg is operated upon. It is best to pierce the blastomere in the black hemisphere near the first cleavage-plane. The needle passes through about a half (or more) of the blastomere. When the needle is withdrawn, a greater or less amount of the contents of the blastomere protrudes where the blastomere has been injured. The egg after operation is returned to the water. It is necessary to keep the eggs under careful observation, because sometimes the blastomere has been only slightly injured and continues to develop more or less irregularly. Such eggs should be removed.

FIG. 34. — A. Hemiembryo lateralis. B. Hemiembryo anterior. (After Roux.)

Roux found that in twenty per cent. of the eggs the uninjured blastomere lived and continued to develop. This blastomere by continued division developed into a form that may be called a "semimorula verticalis," since it is like the vertical half of a normal "morula." "That is to say, it is a hemispherical structure with small deeply pigmented cells above, and with larger non-pigmented cells below." The segmentation-cavity is often absent; sometimes it is represented by a few loosely aggregated cells, and sometimes by a cavity bordered in part by the injured half of the egg (Fig. 35, A). A "semiblastula verticalis" then develops with a well-defined segmentation-cavity. A "semigastrula" stage is next passed through. "Hemiembryones laterales" develop from most of these eggs, as seen in Fig. 34, A. This figure shows that

the right half of an embryo has developed from the uninjured blastomere. Half a medullary plate is present along the line of separation of the injured and uninjured halves. Near the posterior end of the half plate, the yolk of the developed half is exposed over a small region and surrounded by half of a blastopore (?). A cross-section of such an embryo shows (Fig. 35, B) that the half plate has essentially the same form as half of the normal medullary plate; that beneath this half plate a notochord is present forming a rod, round or slightly oval in cross-section; that a small archenteron is present in the developing half, and that a mesodermal sheet is present over the side of the hemiembryo. It is interesting to note that while only half the medullary plate is present, yet the

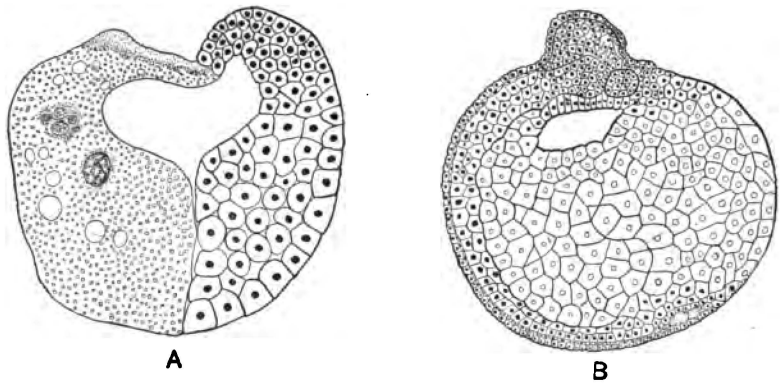


FIG. 35.—Cross-sections through two half-embryos of different stages. (After Roux.)

notochord and archenteron, which are also median structures, form whole structures but of smaller size than the corresponding normal organs. Roux thought that the notochord was very probably composed of only half the number of cells present in the normal notochord, but, owing to a great amount of variation in the latter, it was not possible to determine this relation definitely.

Pflüger, Roux, and Born have shown that sometimes in the normal development the plane of the first cleavage corresponds to the cross-plane of the body of the embryo, *i.e.* the plane of the first cleavage separates the anterior from the posterior end of the body. Under these circumstances, if one of the first two

blastomeres had been killed, we should have anticipated, Roux says, that "hemiembryones anteriores" or "posteriores" would have appeared. Roux claims that such forms do really appear. The same result can be obtained, if, after the second cleavage of the egg, two of the four cells be killed, *i.e.* those two that lie on the same side of the second cleavage-plane. A hemiembryo anterior (?) is shown in Fig. 34, B. It has the anterior end of the medullary folds normally formed, also a normal chorda, mesoderm, and archenteron in this anterior end. In every respect it corresponds to the anterior end of a normal embryo, except that the archenteric cavity is small, resulting, Roux thinks, from the impossibility of pushing the yolk-mass posteriorly, as is done in the normal embryo when the archenteron enlarges. Roux is uncertain whether he has seen any "hemiembryones posteriores," although one embryo that he found, with thick and short blastoporic lips, may represent such a form.[1] Roux made some further experiments in which one of the first four blastomeres was killed, and other experiments in which three of the first four blastomeres were killed. In the first case he obtained three-fourth morulæ and three-fourth blastulæ; in the latter case, one-fourth blastulæ and one-fourth embryos. Roux concluded from his experiments, "that the development of the frog's gastrula and of the embryo immediately following the gastrula-stage is, after the second cleavage-period, a mosaic work of at least four vertical self-developing (or differentiating) parts." "How far this mosaic work is changed by a change in the position of material in the later development, cannot be determined."

In later stages in the development of the hemiembryos a new series of phenomena appear, that result in the "reorganization"

[1] We should expect, *following Roux's argument*, to get as many hemiembryones posteriores as anteriores, yet such does not seem to be the case. Hertwig ('93, A) has maintained that it is absurd to suppose the posterior end of the blastopore could appear when there is no anterior end; but this supposition rests, I think, on an erroneous idea of the way in which the blastopore forms, for I have shown in my experiments ('94) that the posterior lips of the blastopore may appear when the anterior lip has been destroyed. The experiment should be carefully repeated with the four-cell stage, where it is possible to distinguish the two anterior and the two posterior cells.

of the half operated upon, and in the subsequent "postgeneration" of the same.

Sections of eggs that have been successfully operated upon show the kind of change that has taken place in the injured blastomere as a result of the operation. The yolk is found much vacuolated in places, and the protoplasm in the immediate path of the needle has been killed, and much changed. After a time it is found that scattered nuclei or nuclear-like structures are also present in the injured half (Fig. 35, A). These have come from the regular or irregular division of the nucleus of the blastomere that has not in most cases been killed by the hot needle. The developed half is somewhat larger than the injured blastomere, and a sharp line of demarcation is at first present between the two halves. Even in the early stages of some eggs changes are found to take place that precede the "reorganization" of the injured half. Roux describes *three sorts of reorganization-phenomena.* The *first of these changes* involves the formation of cells in the injured half. Nuclei, surrounded by a finely granular protoplasm, appear in the injured blastomere. These nuclei seem to arise from two sources, — from the nucleus of the injured blastomere, and from nuclei (or cells) of the developing half that have transmigrated. Around the nuclei the yolk breaks up into cells. This cellulation of the yolk may take place at very different times. It may be absent in some cases in a semigastrula and be present in other cases in a semimorula or semiblastula. The cellulation of the injured half begins always near the developing half, and extends thence outward. The cells of the injured half are of various sizes, but generally larger than the cells of the uninjured half.

The cellulation of the yolk takes place only in the unchanged non-vacuolated parts. Where the yolk has been much changed, it is worked over by another method, *i.e.* by the *second method of reorganization.* These parts are revived or reorganized by the nuclei or the cells that have now appeared in the injured half. Such parts are either actually devoured by wandering cells or slowly changed under the influence of neighboring cells or nuclei so that they become a part of these cells.

In addition to the two preceding modes, *a third method of reorganization* takes place. When the yolk has been much

injured, the surface may be subsequently covered by ectoderm that grows directly from the developing half over the injured portions. "*Postgeneration*" now begins in the cellulated injured half and ultimately the missing half of the embryo is formed. The surface ectoderm is first postgenerated either by direct overgrowth from the uninjured to the injured side, or by the formation of ectoderm from the cells of the newly cellulated yolk. The missing half of the medullary folds appears very quickly. Half a day or a night is often sufficient to change a hemiembryo lateralis into a whole embryo with a complete medullary plate. The mesoblast grows over to the injured half, but increases in length and breadth by the addition of new cells from the cellulated yolk. The formation of new mesoderm takes place only along the free edge of that already formed. The growth is in a dorso-ventral direction.

The archenteron is postgenerated in a way very different from the way in which the archenteron of the normal embryo is formed. The lacking half of the archenteron arises in connection with the half of the archenteron already present in the hemiembryo. The yolk-cells of the injured half become radially arranged and a slit appears in the postgenerated half extending out from the archenteron of the hemiembryo. The cells surrounding the slit arrange themselves into a lining layer and the slit opens to form the missing half of the archenteron. In general we may say that in the postgeneration of the organs of the injured half, the changes always proceed from the already differentiated germ-layers of the hemiembryo, and the postgeneration takes place where the exposed surfaces of the germ-layers touch the newly cellulated yolk-mass of the injured half.

FURTHER EXPERIMENTS

(By Hertwig, Endres and Walter, Schultze, Wetzel, Morgan)

We may next consider the work of others, who have, after Roux, repeated the same experiment and made further variations of it. Lastly, before a final conclusion can be reached as to the interpretation of the results, we must carefully examine the evidence from similar experiments on other forms. We

shall be then in a position to understand more fully the results of the experiments on the frog's egg.

Hertwig ('93, b) was the first to repeat Roux's experiment, but reached results diametrically opposed to those of Roux. At the two-cell stage, one of the blastomeres was stuck with a hot needle,[1] but unfortunately a detailed description of the method employed is not given by Hertwig. After the operation[2] the egg so turns itself that the uninjured part rotates upward, while the injured half is below. This is owing, Hertwig says, to the development of a blastula and gastrula cavity, within the uninjured and segmented half. The cleavage-stages of the egg are not described! Sections of the blastula stage show that in the cellulated half a segmentation-cavity, having a thin roof, has appeared. This cavity lies, in the present case, in the centre of the developing half. In other embryos, the cavity may lie excentrically, and in some cases *a part of the floor of the cavity may be bounded by the yolk-substance of the undeveloped half.* Hertwig interprets these results to mean that when one of the first blastomeres is injured, the method of development of the other blastomere is very much altered. The injured half lying in contact with the active half plays only a passive rôle in the further development.

The injured blastomere is closely applied to the developing half, and in places passes continuously into the latter. Hertwig thinks that the yolk of the injured blastomere exerts on the developing half an influence similar to that which the food-yolk of meroblastic eggs exerts on the protoplasmic portion that forms the embryo. This injured yolk-material comes to lie in the ventral and posterior portion of the embryo.

Hertwig ventures further to prophesy that if the injured yolk-mass had been taken altogether out of the egg-coat (*i.e.* from its contact with the living half), then there would be formed a normal embryo without defect and like the normal embryo in every respect except its smaller size.

It is of importance to note that Hertwig describes other

---

[1] In a few cases a galvanic stream was used to kill the blastomere.

[2] How soon after is not stated.

embryos that he obtained by Roux's methods, and contrasts these with those described above. Some of the embryos showed the condition of spina bifida, *i.e.* with both sides of the body developed and with a large yolk-exposure in the mid-dorsal line.[1] Others of the embryos were only slightly injured by the operation and developed nearly normally. In these the entire dorsal region was well developed and the blastopore closed to a small ring. Only on the ventral side was a small defect found where the outer and middle germ-layers were absent. In these latter embryos, and in those showing spina bifida, Hertwig believes the injured blastomere was not killed or even sufficiently injured to prevent its partial development. That this is the true explanation cannot be doubted; for it is not at all unusual to find after the operation that the injured blastomere may separate off small portions of itself as cells that develop along with the cells from the uninjured half. Here, it seems to me, is the uncertain part of Hertwig's work. He has not observed, as far as stated, the segmentation of each egg on which he has operated, and consequently his results are open to the objection that in many cases, where he does not suspect it, the *injured cell has also continued to divide* and to form a part of the later embryo.

In nearly all of the embryos described by Hertwig the medullary folds are unequally developed.[2] Hertwig's attempts to meet this fact do not seem to me altogether satisfactory. A large number of the embryos have developed unsymmetrically. The ventral and posterior yolk-mass lies higher up on one side than on the other. In consequence of this, one side of the medullary fold lies nearer to the injured yolk than does the other, and as a result the two sides of the body are unevenly developed. The asymmetrical position of the blastopore on the living part is assumed to be the underlying cause of the later asymmetrical position of the medullary folds; but for the primary reason of the lack of symmetry of the blastopore itself Hertwig gives really no explanation, and to state that it

[1] Among these embryos Hertwig describes one that seems to have been an excellent example of Roux's "hemiembryo lateralis."

[2] There are a few exceptions.

is due to the "yolk lying higher up on one side" is only begging the question. Roux has not failed to notice the incompleteness of Hertwig's explanation, and has interpreted all of Hertwig's results as due to a sudden postgeneration of the injured half of the embryo; *i.e.* Roux believes a half-embryo to have first formed, and then to have been quickly followed by an imperfect formation of the other half. Hence the asymmetry of the embryos.

It is impossible to say how far postgeneration has played a part in the development of the embryos described by Hertwig, but that postgeneration will explain all the difference between the results of Roux and of Hertwig seems highly improbable. Further, as I have said, it seems not unlikely that many of the embryos described by Hertwig have come, not only from the uninjured blastomere, but also from a part of the *injured blastomere*. If this latter supposition be true, we can better understand why the injured yolk forms in many cases an integral part of the developing embryo.

Hertwig has made a most formidable attack on Roux's explanation of postgeneration of the embryo. The subject itself is of secondary importance as compared with the main problem involved in the experiment, and yet of sufficient interest to warrant careful examination. Roux describes the blastomere into which the hot needle has been plunged as dead, and speaks of a later revivification of the dead half of the egg. Hertwig believes that all of that part of the operated blastomere that is later divided up into cells (to be used in the development) is not dead, but only more or less injured. Only a small portion of the injured blastomere is really *dead*, and that is the portion which has become coagulated by the hot needle. This portion cannot later be broken up into cells, but may be either thrown out by the living embryo or assimilated, owing to the power of digestion of neighboring cells. The injured blastomere behaves in the same way that a portion of the body of an animal would if a needle had been stuck into it. The place injured might quickly heal, and the comparatively small region that had been pierced and killed would be reabsorbed again. If the needle had been first heated, the region of injury would only be larger, and the necrotic tissue would be either thrown

off or absorbed. It has been shown by Roux that when a blastomere has been pierced by a *cold* needle, there is a small outflow of yolk, and the injured blastomere continues to divide at the same rate as the uninjured cell. When the needle is heated, the cleavage-process is delayed or prevented, while it continues on the uninjured side; but after a time the injured blastomere may also begin to divide in an irregular way. After two or three days one gets generally from such eggs quite normal gastrulæ and embryos, differing in little or no respect from embryos from uninjured eggs.

The nucleus of the uninjured blastomere may continue to divide, although the protoplasm, owing to its injury, may not be able to do so for some time. The nuclei may scatter themselves through the protoplasm (and yolk), and subsequently take part in the division of this into cells. In extreme cases Hertwig admits that when the needle is very hot, the whole of the protoplasm of the blastomere may be killed, and also the nucleus. Furthermore, it is possible that occasionally the heat may radiate from the one blastomere into the other and *partially* kill this other one also. If the last condition is brought about, the development of the partially injured blastomere may take place only very slowly, if at all. In most cases, therefore, Hertwig believes a "reorganization" of the injured cell takes place, and not a "revivification" of the dead half. In this reorganization, Hertwig thinks that the nucleus of the injured cell itself plays the main part, while Roux believed the process was brought about largely by an immigration of cells (or nuclei) from the uninjured into the injured half. Hertwig's conclusion here seems based rather on *a priori* probability, while Roux's statements rest directly on his own observations. Recently the same ground has been worked over by Endres and Walter, whose results substantiate Roux in every respect.

Endres and Walter ('95) have obtained the typical half-blastulæ and half-gastrulæ and half-embryos which Roux has described. They deny that *whole embryos* develop from one of the first two blastomeres, as Hertwig affirmed. Their figures show in the most conclusive way that *half-embryos* do develop

under the conditions of Roux's experiment. The subsequent postgeneration of the injured half of the egg has also been studied by these authors. They confirm in every detail the method of reorganization and postgeneration of the injured half as described by Roux. *The reorganizing cells have migrated from the uninjured to the injured side*, and there have caused the protoplasm to break up into cells. The injured blastomere is also overgrown *directly* by the ectoderm of the uninjured and developing side. In many of these embryos the right and left side (one side has postgenerated) are separated from each other by a protruding yolk-mass, forming spina-bifida embryos. The reorganization of the *much changed* mass of the injured blastomere is brought about by being assimilated by the cells that have migrated into that region, by the second and third methods of reorganization described by Roux. When the material of the injured half is only incompletely reorganized, there is formed, after postgeneration, a more or less pronounced spina bifida. When the injured material is completely worked over or reorganized and postgenerated, a perfect embryo may be formed.

Schultze ('94, b, d) has made an interesting modification of one of the experiments of Pflüger and obtained most unexpected results. The eggs of Rana fusca removed from the uterus were placed singly upon slides. On each slide had been stuck two thin glass rods from 1.65 to 1.35 mm. in thickness. Between these rods, which are separated from each other by the width of the slide, an egg is placed with the *white pole uppermost*. The egg is then fertilized in this position. After three minutes the spermatozoa may be supposed to have entered, and a glass cover is placed over the egg and brought down into contact with the two glass rods above-mentioned, and there fixed with rubber rings. The egg is by this means slightly compressed and held more or less firmly in position. Each slide is then turned over, *i.e.* through 180 degrees, so that the dark pole of the compressed egg is brought upward. The eggs now in the normal position are put into a dish of water, to remain in this position until the *first furrow* has appeared or even until it has passed through the egg. Then the slide and

its egg *are again rotated through* 180 *degrees*, so that the white pole is once more turned uppermost. Owing to the compression, the eggs retain their inverted position.

After twenty-four hours at 17 degrees C., the gastrulation begins. The rubber bands are then removed from the slide,

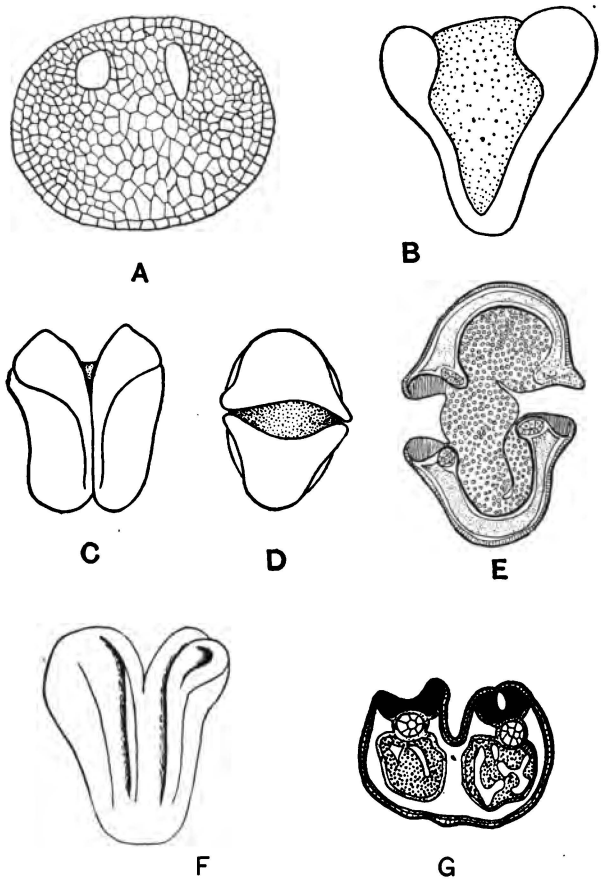


FIG. 36.—Double embryos. A. Section through segmenting egg. (After Wetzel.) B. Double embryos united ventrally. C, D. Double embryos united dorsally. (After Schultze.) E. Cross-section through C. (After Wetzel.) F. Double embryos united laterally, and G, cross-section of same. (After Wetzel.)

the cover-slip carefully cut away from the jelly of the egg, and the slide and egg returned to the water.

If eggs that have been inverted after the two-cell stage are

watched during the later cleavage-period, it will be found that the upper white surface disappears, and often a whitish band is found in the position of the first furrow. Continuous observation also shows that the white hemisphere may slowly sink to one side. At thirty hours the blastopore has appeared in the normal eggs, while on the inverted eggs *two gastrula-invaginations* are found. From each half of the egg a more or less complete embryo may develop (Fig. 36, B, C, D). The two "double monsters" are united to each other in various ways, often with the two ventral surfaces united in one common yolk-mass, as shown in Fig. 36, B. Another of these double forms is shown in Fig. 36, C, D, and a cross-section through the body in Fig. 36, E.

The details of these experiments of Schultze have not yet been published. The method of gastrulation of the halves is not clearly explained, nor does Schultze explain the changes that take place in the interior of the blastomere after the rotation. The results show, however, in the clearest way that each half of the egg, after the first division, has the power to develop all the organs of a single embryo.

Wetzel ('95) has more recently studied the gastrulation-process in some of these embryos and has given a fuller description than Schultze of the origin of the archenteron. A cross-section through the blastula-stage of one of these eggs is shown in Fig. 36, A. Two distinct segmentation-cavities are present in the upper or white hemisphere of the egg. The centre of the double blastula is filled with large yolk-cells. The sides are formed of smaller cells richer in protoplasm and pigment. The structure of this double blastula shows that, in all probability, the contents of each of the first two blastomeres have rotated after the inversion of the egg so that the more protoplasmic portions have come to lie at the outer and upper sides of each blastomere; while the heavier yolk has sunken down to the lower surface along the cell-wall that separated the first two blastomeres from each other.

At a later stage a depression appears on the surface of the egg in the region of the plane that separated the first two blastomeres from each other, *i.e.* approximately in the plane of the

first cleavage. This depression or groove on the surface may divide at either end into two distinct and independent grooves. Cross-sections through such an egg show that the groove on the surface is the result of an invagination to form an archenteron in each half. This means that each half-blastula has begun to invaginate along the common line of contact of the halves. Since the halves are in contact, the overgrowth of each blastopore is impossible. The lips of the blastopore of each half, therefore, have extended around the equator of the egg as in the spina-bifida embryos. A medullary fold appears later along *each* blastoporic rim, and then it becomes apparent that two embryos are present, each a spina bifida, and united by a common central yolk-mass (Fig. 36, C, D, E). The open dorsal surfaces of these two embryos are turned toward each other (Fig. 36, E).

This seems to be the more common type of double monster produced from these eggs. If, however, the blastoporic invaginations begin at different regions of the two hemispheres, many possible variations of the method described will be introduced; Schultze and Wetzel have in fact, as we have seen, described several forms of these double monsters. (Fig. 36, B, F.)

It seemed to me not improbable that Schultze's results explain in part the difference in the results of the experiments of Roux and of Hertwig. If, on the one hand, the uninjured blastomere retain its normal position after the operation, *i.e.* with the black pole turned upward, then there should develop a half-embryo, in Roux's sense. On the other hand, if, after the operation, the position of the egg be reversed so that the white pole of the uninjured blastomere is turned upward, then a whole embryo of half-size might develop. In Roux's experiment it is probable (although not explicitly stated) that the black hemisphere always remained upward after the operation. Hertwig does not say in what position the eggs lay in his experiments. He only says that in the blastula and gastrula stage the heavier injured yolk was down, and the lighter uninjured blastomere was above. If, immediately after the operation, the eggs lay with the injured blastomere below, we should expect some change to take place in the

interior of the uninjured blastomere as a result of its oblique or even inverted position; hence the uninjured blastomere might develop differently than it would have done had it retained its normal position (as in Roux's experiment). In this way we might attempt to reconcile, in part, the different results of Roux and Hertwig. I cannot but think, however, that the main difference is due to the partial development of the injured blastomere in many of Hertwig's experiments, so that cells split off from the injured blastomere took part in the formation of the embryo.

In 1894 I made the following experiments to determine whether one of the first two blastomeres could give rise to a half or to a whole embryo, according to the conditions of the experiment. One of the first two blastomeres was killed with a hot needle in the way described by Roux ('93, c).[1]

In some of the eggs the black pole remained upward after the operation; other eggs were rotated after the operation, so that the white pole was turned upward. The eggs were closely watched for several hours, in order to ascertain with certainty whether the injured half divided or not. In those cases in which this happened, the eggs in question were eliminated from the experiment.

The eggs were placed at first on a moistened glass plate and kept for a time in a moist atmosphere, or else simply thrown into water. The results seemed to be the same. When the black pole of the uninjured blastomere remained up, the blastomere developed, in all the cases observed, into a *half-embryo*. Conversely, those eggs in which the white pole was turned upward, formed, in most cases, *whole embryos of half-size*. In the latter case the cleavage was modified in consequence of the reversed position of the egg. The upturned white hemisphere produced smaller cells than the lower black hemisphere, pointing unmistakably to a rotation of the fluid contents of the blastomere.

[1] The needle was heated each time before piercing an egg. This made a greater injury to the blastomere much more certain. On the other hand, it lowered the percentage of embryos obtained, because in many cases the other blastomere was probably injured also by the heat.

The *half-embryos* and the *whole embryos of half-size* developed independently of the yolk-mass of the injured side. In this respect my results differed very materially from the results of Hertwig. Many of Hertwig's embryos developed in connection with the injured blastomere; mine, on the contrary, developed independently of the injured blastomere. I suspect, as I have said, that this difference may be in part due to this, that Hertwig did not carefully remove from his experiment those eggs in which the injured blastomere continued to segment, and that cells from the injured blastomere took a direct part in the subsequent development.

In one of my experiments, in which the uninjured blastomere had been *reversed* after the operation, it developed into a half-embryo, and not into a whole embryo of half-size. Moreover, in this embryo the medullary folds appeared on the white surface of the egg, showing that a rotation of the contents of the blastomere must have taken place. We must, therefore, conclude that the simple fact of the rotation of the blastomere-contents is not, in itself, the determining factor as to whether a whole or a half-embryo will result, but probably the *kind* of rotation determines this result. The result may also depend in part, I think, upon how far the contents of the uninjured blastomere have retained, after the operation, their organic connection with the other injured blastomere.

In later papers Roux stated that he has often obtained in his experiment other sorts of embryos than those he first described, which he calls "hemiooholoplasten." These are *whole* embryos that have come from the uninjured blastomere without the postgeneration of the other injured blastomere. Roux interprets these as embryos "completely postgenerated," with only a partial use of material from the other side, or even with no material from the injured side. Roux affirms that he has seen all intermediate stages between those embryos that have used all of the yolk-material of the injured side, those that have used only a part of the material of the injured side, and those that have not used any of this material. These embryos differ from one another only in point of size. Roux does not call the embryos that have developed entirely from the material of the

non-injured side, whole embryos of half-size, but he believes that at first there formed a half-gastrula, then a half-embryo. Later this half-embryo completed itself without using material from the injured side! That is to say, by using "wandering cells" the half-embryo has *postgenerated* the other half of its body!

CHAPTER XII

INTERPRETATIONS OF THE EXPERIMENTS; AND CONCLUSIONS

THE results of the experiments of Pflüger, of Roux, and of others have given rise to much discussion in respect to the relation existing between the unsegmented egg and the embryo. The old questions of evolution and epigenesis have been once more brought into the foreground, but divested of their historic meaning.

The results of the experiments on the frog's egg are, however, in the first place, too insufficient in themselves, and in the second place are as yet too uncertain on many points, to warrant general conclusions based on these results alone. The experiments can only be understood if considered in connection with similar experiments on other groups of animals.

ROUX'S MOSAIC THEORY OF DEVELOPMENT

Roux's discussion of the problems of development is deserving of most careful examination, for even in his earliest papers we see foreshadowed many of the possible interpretations that have later been accepted in one or another form. Roux pointed out that the known facts of development showed that a certain *formal self-differentiation* of many parts of the egg takes place during development. This self-differentiation may result from an unequal growth of different substances in the egg which come into activity at different times; and if so, it should be our aim to discover the stimuli that bring these different substances into action, and thus cause the consecutive series of events. The stimuli must come either from without at each stage of development, or the egg may contain within itself the power of progressive development as soon as it is once set into activity. That the egg needs during its development certain things from

its environment is self-evident; a certain amount of warmth and of oxygen, etc., must be present. These, while necessary for the development of the egg, do not necessarily determine the sequence of events; for under the same external conditions, eggs of different animals develop very differently. The results obtained by placing the frog's egg under different conditions also show that the power of progressive development must lie within the egg itself. Roux compared the egg, in this respect, to a complicated piece of machinery which, when once set in motion, would go through a long series of changes depending on its internal structure.

If so much be granted, the next question to be answered is this: do all the parts of the dividing egg work together, *i.e.* interact to form the result, or have the parts of the egg separated from one another by the cleavage the power to develop independently? The first alternative Roux called *the differentiating interaction of the parts*, and the latter alternative, *the self-differentiation of the parts*. With reference to the results of the experiment in which one of the first two blastomeres of the frog's egg was killed or injured, Roux concluded that each of the first two blastomeres shows in this experiment the power of self-development: *i.e.* each half is independent of the other and we may legitimately infer that when both blastomeres are alive, as in the normal development, the same self-differentiation of each blastomere takes place. This independent development goes on till the organs of the body begin to form. Whether the limit of independent development is then reached we do not know, for it is possible that in the complicated series of movements that take place in the formation of some of the organs, the power of independent development may be supplemented or replaced by the action resulting from the *correlation of the parts* to one another, *i.e.* by a mechanical interaction of different parts. Each of the first two blastomeres contains not only the building-material for the corresponding parts of the embryo, but also *the differentiating* and *formative* forces for those parts. The cleavage in the direct, or normal development of the individual, divides *qualitatively* the "germ-plasm," and, in particular, the nuclear material. The development of the frog's gastrula and of the embryo

immediately resulting from the gastrula is, from the second furrow on, a mosaic work of at least four vertical, independent pieces. How far this mosaic work of four pieces is altered by later changes in the position of the material, and by differentiating correlation, is not known.

Roux also stated clearly the relation that exists between the method of self-differentiation, and the method of interaction of the parts on one another, and the bearing of these questions on the older problems of evolution and epigenesis. If many portions of the egg are differentiated owing to their inherent power, and produce in this way the manifold differentiations seen in the embryo, then the egg must have been composed in the beginning of many parts bound up together, and the development is a metamorphosis or an unfolding of its peculiarities; *i.e.* the development is an evolution. Further, the cleavage not only divides the egg into smaller parts, but at the same time *localizes* the differentiated material, so that this material is arranged definitely in relation to later development. This result appears possible only through a qualitative separation of the material during the course of the cleavage. If this is true, we see that the development depends on the molecular structure of the egg, and therefore further analysis is beyond our reach. The *segmented* egg would be then only the *sum of its independent parts*, and during the period of the self-differentiation of these parts, there has been no united action to form a whole. Therefore the whole can have no regulating or formative influence on the parts.

If this view be true, His's principle of germinal localization in the egg has not only a descriptive worth, but also expresses a causal relation, so that organs can be referred to parts of the fertilized egg, and even to the unfertilized egg.[1]

If, on the other hand, development takes place as a result of the interaction of all or many parts on one another, then the fertilized egg may be composed of a very few differentiated parts, which by their interaction produce a greater and greater

[1] We could explain those exceptional cases in which two embryos arise from one egg, if we supposed that after the first cleavage there was a sort of doubling, in each blastomere, of the primary constituents of the body (Roux).

complexity. The development would then be due to the production of many parts out of a few primary ones, *i.e.* the development is a process of epigenesis. There would thus result an ever-changing interaction of the parts to form the whole, by which means there would be also brought into play a regulating influence of the whole back again on the parts, *i.e.* correlation of the parts under the influence of the whole. His's principle of germinal localization would, therefore, have a causal meaning only in so far as it points out the place in the egg where the resulting formation of many-sided changes takes place; and it would be of only secondary value to be able to refer the place of action of these changes to the undifferentiated plasm or to the unfertilized egg.[1]

In conclusion, it should be noted, Roux said, that self-differentiation of the parts and dependent differentiation of the parts, *i.e.* evolution and epigenesis, may be combined in a many-sided activity or *union*, and it would then be our duty, in order to interpret these problems, to use a double foresight and a double care, to make out the part played by each of these factors in the development.

THEORY OF DRIESCH AND OF HERTWIG OF THE EQUIVALENCY OF THE EARLY BLASTOMERES

Studies on other forms show that great care must be taken in interpreting the results of the experiments on the frog's egg. In 1891 Driesch made a series of most important experiments on the eggs of the sea-urchin.[2] The blastomeres were isolated by shaking them apart, and it was found that although each blastomere segmented as a part, *i.e.* as if still in contact with the missing half, yet the open side of the blastula closed over very soon, and a gastrula and embryo having the normal form were produced. Driesch concluded from this and similar experiments that all the blastomeres are equivalent, and that the position of each blastomere in the segmenting egg determines in

[1] The formation of two embryos from one egg would take place, on the theory of interaction of the parts, at the time when the median axis of the body is formed. Two such axes would be laid down instead of one.

[2] Fiedler had made, in 1891, a somewhat similar experiment, but it was not carried sufficiently far to be of great value.

general the fate of the blastomere. If the blastomeres could be interchanged as can the individual marbles of a heap, then the fate of each would be determined by its new position in the whole. This conclusion is directly opposed to Roux's theory of a qualitative division of the blastomeres during the early cleavage.

Hertwig had also stated shortly before Driesch that in his opinion the egg divides quantitatively, and that Roux's experiments did not touch the cardinal point of this problem, because the other injured half of the egg remained in contact with the developing half. Hertwig expressed his belief that if the first two blastomeres of the frog's egg could be separated from each other, each would develop into a whole embryo. Further, he thought that the development of an organism is not a mosaic work, but that the parts develop in relation to one another, *i.e.* the development of a part is dependent on the development of the whole. Wilson ('93) also, from the results of a most careful and important series of experiments on the egg of Amphioxus, concluded that the division of the egg is not qualitative. He found that isolated blastomeres give rise to larvæ smaller in size than the normal, but having the normal form. The differentiation of the blastomeres, Wilson thought, takes place in the later periods of cleavage.

ROUX'S SUBSIDIARY HYPOTHESIS

Roux replied to the criticisms that Driesch, Hertwig, Wilson, and others have made of his theory, and attempted to show that his view is fully compatible with the results that Driesch and others obtained.

Roux ('92, a, '93, b) pointed out that the results of Chabry,[1] Fiedler, and Chun show that in ascidians, sea-urchins, and ctenophors a half-development takes place when one of the first two blastomeres has been removed, and that the experiments of Driesch also showed that an isolated blastomere of the egg of Echinus cleaved as a half, and not as a whole, and that a half-blastula also developed. These results indi-

[1] Later experiments have shown that this statement is not true for ascidians, as Chabry's work might seem, in part, to show.

cate that a certain formal self-differentiation of many parts of the segmenting egg has taken place. On the other hand, the fact of postgeneration shows that in each of the first blastomeres a power sufficient to complete the whole must also be potentially present. In order to awaken this potential power of a blastomere, a disturbance in the development must occur. This latent activity may be only slowly awakened in the development, sometimes sooner, sometimes later.

We have, therefore, to distinguish two sorts of development, —the normal "direct" development, and an "indirect" postgenerative (or regenerative) development. The first or direct is the result of the self-differentiation of the early blastomeres, and of the complexity of their derivatives. The second or indirect is the result of a profound correlation which adds to an imperfect whole the lacking parts. Should the postgeneration set in immediately after the isolation of the blastomere and so convert the blastomere at once into an actual whole, then we should not have found out that each blastomere is really a self-differentiating cell, but we should have erroneously concluded that the first (four) cleavage-cells are qualitatively equivalent. Into this error Roux believed Driesch and Hertwig to have fallen. In the frog, ascidian, and ctenophor each of the first blastomeres is specifically different from the others, but in respect to postgeneration we find that each blastomere has the same potentiality, and each is in reality totipotent. The "idioplasm" in direct (*i.e.* normal) development, called into activity by the process of fertilization, is divided qualitatively and *unequally* during the cleavage, while the material which may later serve for postgeneration and regeneration (which is not active during the normal development) is always equally or quantitatively divided.

According to Roux, the nucleus represents the controlling power of the cell, but the protoplasm acts as a stimulus to the nucleus and hence may indirectly regulate the process of cleavage. "In the telolecithal frog's egg the position of the food-substances and formative substances stands in strict causal relation to the position of the main axes of the embryo." The nuclei of eggs in which the normal arrangement of the contents has been disturbed will be influenced during the first cleavage-

period, so that a qualitative division of the nucleus may result different from the corresponding normal qualitative division. The second cleavage, for instance, may come first (qualitatively) as a result of the position of the nucleus in the protoplasm.

Roux further suggested that the consecutive series of nuclear divisions must be different *in kind* in the normal and in the compressed eggs, and that an "anachronism" has taken place in the latter case. By this "anachronism" Roux has tried to save his theory of qualitative division of the nucleus during the cleavage-period.

To sum up Roux's later position, we may say that in order to vindicate his earlier theory of a *qualitative* division of the nucleus and a resulting self-differentiation of the first-formed blastomeres, he has been obliged in the first place to bring forward his theory of postgeneration, assuming that along with the qualitative division of the nucleus a parallel quantitative division of the germ-material also occurs. Further, Roux assumes that the kind of qualitative *division* of the nucleus is directly influenced by the arrangement of the protoplasm, and, as we have seen above, he is unable to explain satisfactorily the results of the experiment of the compressed egg, except as an "anachronism." These complications into which Roux has been forced are largely the outcome of the primary assumption of a qualitative division of the nucleus. This Roux-Weismann hypothesis of qualitative nuclear division has, however, no known histological facts in its favor. On the contrary, all we know of nuclear divisions speaks clearly in favor of an exact division of the chromatin-material, and a most elaborate mechanism is present to bring about this result.

EXPERIMENTS ON OTHER FORMS

The results obtained from a study of the development of fragments of the unsegmented egg and of isolated blastomeres of ctenophors[1] have a direct bearing on our interpretation of the experiments on the frog's egg. When the first two blastomeres are separated from each other by a sharp needle or cut apart by a pair of small scissors, each continues to cleave as a half, *i.e.*

[1] Chun ('92). Driesch and Morgan ('95).

as though it were still in contact with its fellow-blastomere. When the organs appear in the larva, only half the full number of rows of swimming-paddles appear. Each row, however, has its full complement of paddles. The invagination of ectoderm to form the "stomach" is very excentric in the half-larva, but forms a *closed* tube running from the mouth-opening to the excentric sense-plate. In several respects, therefore, the larvæ were distinctly half-larvæ. But in another respect they were more than half-larvæ. The endodermal cells of the normal larva arrange themselves into four hollow pouches, and the "stomach" invagination passes in the central line of the four pouches. In the half-larva, on the contrary, the endodermal mass forms more than two pouches (*i.e.* more than half the normal number in the whole larva). Two distinct pouches are present and in addition, generally, a third smaller pouch is formed. The latter lies excentrically. In the meeting-point of the three pouches is the excentric "stomach" invagination.

The isolated one-fourth blastomere segments also as a part of a whole, and develops in some cases into a one-fourth larva, having only *two rows* of paddles (*i.e.* one-fourth the normal number), but with *two* endodermal pouches (*i.e.* with one more than one-fourth the normal number). The three-fourth embryos develop six rows of paddles (*i.e.* three-fourths of the normal number) and *four* endodermal pouches. The problem is here a complicated one, for while in one set of organs we find a half-development, in other organs we find more than a half, but yet not the whole development.

The results show, however, beyond question, that, even when isolated from its fellow, the one-half blastomere may give rise to a larva that is in many respects only one-half of the normal larva.

There is yet to be described another series of experiments that have a direct bearing on the interpretation of the preceding results. Roux showed that if a part of the protoplasm be removed from the *unsegmented frog's egg*, the egg may continue in many cases to develop into a normal embryo. The eggs of the sea-urchin lend themselves much more readily to this experiment. They may be broken up into fragments of all sizes

if shaken in a small tube. Those fragments which contain the egg-nucleus may be fertilized and will develop. If the pieces are large enough a gastrula is formed, and still larger pieces develop into normally formed larvæ.

When the *unsegmented egg of the ctenophor* is cut into pieces, there may result either a whole larva or a larva lacking certain parts, and, further, the study of the cleavage of these egg-fragments shows that if the fragment cleaves like the whole egg (but with smaller blastomeres) then a whole larva results, while if the cleavage is irregular the larva is also imperfect. Presumably, in the first case the egg has been cut symmetrically, but in the second case unsymmetrically. Or we might assume that in the one case the egg-fragment rearranged its protoplasm into a new whole, while in the second case it was unable to do so. On either alternative we must conclude that a defect in the protoplasm often brings about a modified cleavage and also a defective embryo, and this takes place even *although the whole of the nuclear material of the unsegmented egg remains present.* There seems, therefore, no escape from the conclusion that *in the protoplasm and not in the nucleus lies the differentiating power of the early stages of development.*

General Conclusions

We have seen that one of the first two blastomeres of the frog's egg may develop into a half-embryo, or into a whole embryo of half-size, according to the conditions of the experiment. So long as the first two blastomeres remain in contact without any disturbance of the cell-contents, each blastomere develops its half of the body. On the other hand, if the protoplasm is disturbed by reversing the position of the egg after the first cleavage, there generally results a whole embryo from each blastomere. Unfortunately, it has not been found possible to separate completely from each other the first two blastomeres of the frog's egg, so that we do not know whether a whole embryo of half-size or a half-embryo would result. In other animals (Echinodermata, Hydromedusæ, Teleostei, Amphioxus, Ascidia, and Salamandra) each of the first two blastomeres, if separated from its fellow, develops into a whole embryo, regardless of the means employed to separate the

blastomeres.[1] On the other hand, the *isolated blastomere* of the ctenophor-egg develops into a half-embryo. These experiments show that the half-development of the frog's egg need not be the result of the presence of the other blastomere, as has been suggested. This is also shown by Schultze's experiment, in which, although both halves are present and in contact, each blastomere develops into a whole embryo.

The results show that in general the first blastomeres are totipotent, *i.e.* each has the power to produce a whole embryo if separated from its fellow, even although it may under certain conditions produce only a half-embryo, as in the frog. Nevertheless, in most forms each isolated blastomere continues to *segment* as though still in contact with the other half. This latter phenomenon shows that the egg-protoplasm has a definite arrangement according to which the cleavage peculiar to each kind of egg is brought about, and there is sufficient evidence to show, I think, that this is a cytoplasmic phenomenon, and is not the result of nuclear interference. We have also seen that some of the isolated blastomeres that cleave as a part may later develop into whole larvæ (echinoderms), while other blastomeres that cleave as a part may give rise to half-larvæ (ctenophors). That these phenomena too are dependent directly on the cytoplasm is shown by the experiment of cutting a piece from the *unsegmented* egg. Under these circumstances, the nucleated fragment of the echinoderm-egg gives rise to a whole embryo, although it segments as a part, while in the ctenophor an imperfect embryo is *generally* formed. The results in these two cases are nearly the same as when the blastomeres of the respective eggs are isolated, although in the latter experiment the entire segmentation-nucleus is present. In the ctenophor the process of self-regulation seems to be

[1] Roux ('95) has stated that the development of a half or a whole embryo may depend upon the method employed to separate the blastomeres. If shaken apart, whole embryos result; if cut apart, half-embryos. Zoja's results ('95) refute such an interpretation. He cut apart echinoderm and hydroid eggs and yet got whole embryos. On the other hand, when the blastomeres of the ctenophor are cut apart, half-embryos result. It must, however, be admitted that disturbance of the contents of an isolated blastomere might be favorable to whole development, as in the frog.

largely absent, either because the blastomeres cannot be brought into a new whole, or because the protoplasm is so fixed, so stiff, that it cannot readily rearrange itself. If from either of these conditions, or from some other, the blastomeres are not capable of rearrangement or reconstruction, an imperfect embryo results.

How far does the totipotence of the blastomeres reach? Does it end with the two-cell, four-cell, or later stages of cleavage? Probably this varies in different eggs. The one-fourth blastomere of Echinus can form a perfect embryo, and even the one-eighth blastomere may develop into a gastrula. The same is true for the egg of Amphioxus. For the frog it is not yet possible to say where the limit lies. In this connection the following facts are of importance. The isolated blastomere of the sea-urchin's egg runs through the same number of divisions that it would have done had it remained in contact with its fellows.[1] Hence the half-embryo has only one-half the number of cells of the normal embryo, and the one-fourth embryo has only about one-fourth the full number. This seems to give, in part, an explanation of the statement made above, viz., that the one-half embryo develops further than the one-fourth, and the latter further than the one-eighth, since the smaller the isolated blastomere the fewer are the cells it produces from which the embryo is formed. The lack of power of development of the small isolated blastomere is not, therefore, dependent on its differentiation. This is also shown by the following experiment. In the blastula-stage of the sea-urchin's egg, pieces may be cut or shaken from the blastula-wall, and, if large enough, they develop into small larvæ. Here also we find that the large pieces can go further in the ontogeny than the smaller pieces, probably owing to the presence of a sufficient number of cells or of sufficient material to form the necessary organs of the embryo.[2]

If the early blastomeres are totipotent, what brings about the later differentiation of these cells? There are sufficient reasons,

---

[1] Morgan ('95).

[2] The same experiment cannot be made on the frog's blastula, because, if cut, the pieces immediately disintegrate.

I think, to conclude that the power of differentiation lies within the egg itself, and does not depend directly on external stimuli. We have seen that Roux and Weismann (particularly the latter) explain this differentiation of the cells as a result of the qualitative division of the nucleus from the very beginning of the cleavage. The nucleus, unravelling its qualities at each division, sends into each cell the proper constituents, and the nuclei, then acting on the cell-protoplasm, cause it to differentiate. On the other hand, Hertwig contends that the early blastomeres are equivalent, and that *differentiation is brought about* by the *interaction of the blastomeres*. In other words, any blastomere that has come to occupy a given position has its fate sealed, because in this position it bears a certain relation to the other blastomeres of the whole ; the whole being simply the sum-total of the blastomeres present. But it is impossible to imagine that the interaction of strictly equivalent blastomeres could bring about a self-differentiation. If it is assumed that the gross-contents (such substances as yolk, etc.) determine the differentiation of each part, still the hypothesis is obviously insufficient for all cases, because, as we have seen, fragments of *any part* of the egg of echinoderms develop into whole embryos, and fragments even of the blastula form new blastulæ, gastrulæ, and embryos. Some of these small blastulæ represent only the "animal" half of the original blastula, and the cells will not, therefore, contain any of the protoplasm or yolk that the cells usually contain that are invaginated, for all this portion of the blastula has been cut off. And since these "animal" pieces gastrulate, we must infer that the gross-contents of the blastomere, or collection of blastomeres, do not *necessarily* cause the differentiation. If, then, neither qualitative division of the nucleus, nor cellular interaction, nor the gross-contents of the blastomeres can be the cause of differentiation of the embryo, what does bring about the differentiation? There are certain facts of inheritance that also have a bearing on this question. The characters of the male are known to be transmitted by means of the spermatozoön. The latter carries into the egg mainly the male nucleus. Therefore, many embryologists have turned to the nucleus as the originator of the differentiation of the cell. Various suggestions have been offered as to the way

in which such an influence could be transmitted from the nucleus to the cytoplasm. Strasburger supposes the nucleus exerts a dynamic influence on the cell-plasm. De Vries and others imagine that organized particles, "pangens," pass out of the nucleus to transform the cytoplasm. Driesch suggests that the nucleus secretes ferments which change the cell-plasm. These hypotheses are purely imaginary, for at present we know almost nothing of the function of the nucleus; and even if we suppose the differentiation comes in some unknown way from the nucleus, still we do not know what could start the process in isolated nuclei that are after the cleavage-period assumed to be equivalent. There is, however, one series of experiments which seems to throw some light on the present problem, although the interpretation is extremely difficult and hazardous. I refer to the experiment on the ctenophor-egg, in which a part of the cytoplasm was cut from the unsegmented egg, and the latter gave rise in most cases to an imperfect embryo. Here, although the entire segmentation-nucleus is present, yet by loss of cytoplasm defects are produced in the embryo. The form, therefore, of the early embryo would seem to result from the structure of the protoplasm, or from the arrangement of the blastomeres after cleavage. In either case the phenomenon is in the first instance cytoplasmic. How can this conclusion be brought into harmony with the facts, stated above, of inheritance of characters through the male pronucleus? Let us assume an imaginary case to show how this union of the two conceptions is possible. If we had used the spermatozoön of one species (or variety) of ctenophor and the egg of another species, and then after fertilization had removed a part of the egg-cytoplasm, we should expect to find the embryo defective, but the organs that were formed we should expect to show a combination of male and female characters. In other words, the imperfect embryo would have resulted from the arrangement of the protoplasm into an imperfect form, but the *kind of organ* would have depended on the structure of the nucleus in each cell. After cleavage, the cytoplasm of each part differentiates into this or that organ, but the kind of differentiation of each part is determined by the nucleus of that part.

If the argument given above should prove true, then the

origin of the differentiation is to be found in the ultimate structure of the cytoplasm of the egg or embryo, although even then we do not know how this mechanism could be started. Whitman ('95) has stated his conviction that it is erroneous to think of the embryo as only the *sum-total* of cells interacting upon one another, but that the embryo itself is to be thought of as a whole, which regulates its parts regardless of cell-boundaries. According to this view, each portion of the embryo has its fate sealed, not because the given portion forms a member of the community of cells, but because the whole directs the fate of each special part. Driesch has pointed out that the egg seems to act like an intelligent being. If so, are the causes of differentiation and of regeneration the same in kind as physico-chemical causes, or do they belong to the category of intelligent acts, and can these latter be accounted for by the *known* principles of chemistry and physics? The plain answer is, we do not know.

CHAPTER XIII

ORGANS FROM THE ENDODERM

WE may now turn again to the history of the development of the normal embryo.

THE CLOSURE OF THE BLASTOPORE, AND THE FORMATION OF THE NEURENTERIC CANAL

During the last stages of the closure of the blastopore its lateral lips rapidly approach each other, and it then becomes an elliptical and later a slit-like opening (Fig. 23). The posterior edge of the blastopore also grows forward for a short distance, and as a result a pocket-like continuation of the archenteron is formed (Fig. 37, A). The depth of this pocket corresponds to the extent of the forward growth of the posterior edge or ventral lip of the blastopore. If the embryo be examined in the region over which the *posterior lip of the blastopore* has advanced, there will be found at first nothing on the surface to mark the region closed over. Some observers have described faint traces of a groove in this region, but such appearances are probably exceptional. Later, however, when the outlines of the medullary folds have appeared, a distinct longitudinal groove appears in this region running posteriorly from the small blastopore (Fig. 23, B). At the ventral end of the groove a distinct depression or pit is soon formed (Fig. 37), which marks the beginning of the anus. It lies at a point opposite to the bottom of the posterior pocket of the archenteron, and corresponds therefore approximately to the region at which the first trace of the ventral lip of the blastopore was found.

As the medullary folds close in to form the nervous system, the blastopore is overarched by their posterior ends. The folds

meet above and posterior to the blastopore, so that the latter can no longer be seen from the surface (Figs. 23, D, E, and 37, A). As a result the central canal of the nervous system

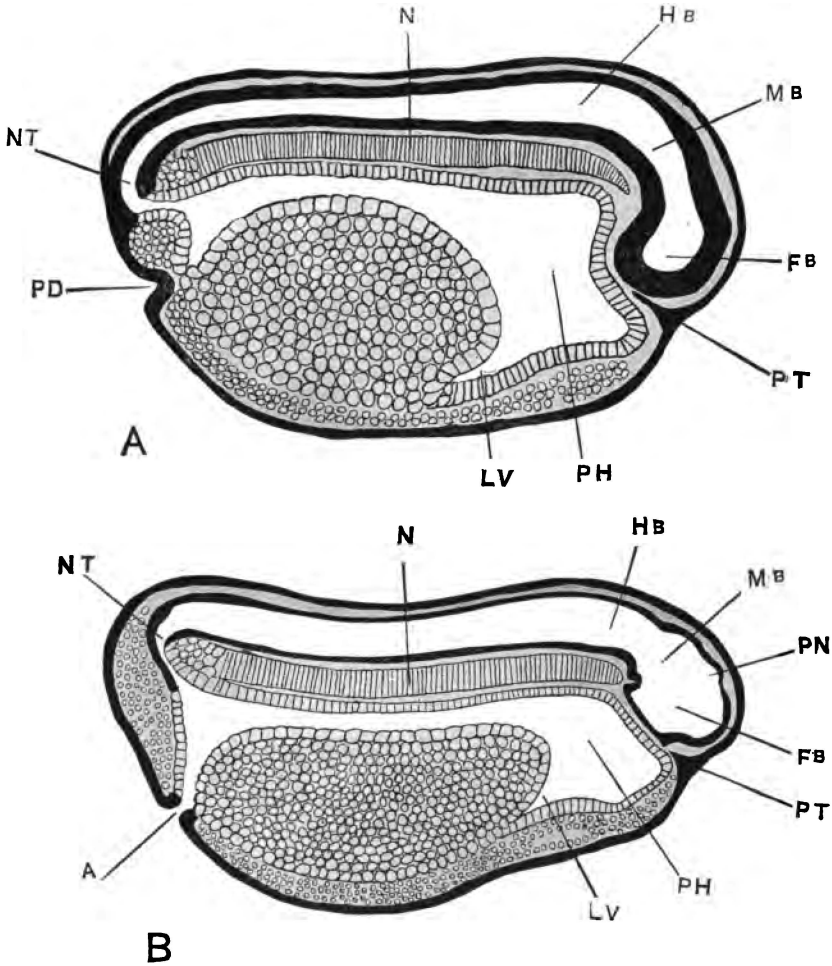


FIG. 37. — Sagittal sections through two stages: A. when blastopore is overarched; B. when anus has formed. (After Marshall, with modifications in A.) A. Anus. FB. Fore-brain. HB. Hind-brain. LV. Liver-diverticulum. MB. Mid-brain. N. Notochord. NT. Neurenteric canal. PD. Proctodæum. PH. Pharynx. PN. Pineal body. PT. Pituitary body.

becomes continuous at its posterior end with the overarched blastopore, and by means of the latter the so-called neurenteric

canal, the central canal of the nerve tube, is directly continued into the archenteron (Fig. 37, A). At this time the archenteron is completely closed in from the exterior, since neither the mouth nor the anus has as yet opened.

The posterior ends of the medullary folds close just behind the blastopore. The groove lying behind the blastopore is not overarched by the folds. During this period the posterior pit of this groove has become much deeper. At first, the pit was separated from the archenteron by a thick layer of cells consisting of ectoderm, mesoderm, and endoderm. The mesodermal cells begin to pull away from this region, and the pit, in consequence, becomes deeper. Then the endodermal cells pull away beneath the pit, and only a single layer of ectodermal cells remains to separate the cavity of the archenteron from the exterior. Finally the latter cells also draw away, and the pit opens into the archenteron. The external opening becomes the anus of the frog. It is at first almost on the dorsal surface of the embryo, but it rapidly shifts[1] to a more ventral position, and at the same time the region above it elongates to form the beginning of the tail. The neurenteric canal is only a temporary structure, and is soon obliterated by the growing together of its walls, although its position may be marked in sections for some time after its actual closure by the irregular line of pigment in the region of the coalescence of its walls.

In the Urodela the changes that take place during the final stages of the blastopore are somewhat simpler. The circular blastopore is reduced to an elongated slit-like opening; but there seems to be some variation in the details of the method of its later reduction. The medullary folds *arch over only the anterior end* of the elongated blastopore, leaving free the posterior end. The anterior end becomes the neurenteric canal. The sides of the middle part of the slit-like blastopore come together and fuse at the time of overgrowth of the medullary folds. The posterior end of the blastopore always remains open to the exterior, and forms the permanent anus.

[1] The method by which the apparent change in position of the anal opening takes place has not been clearly made out.

The main differences that exist between the methods of formation of neurenteric canal and anus in the frog and in urodeles are these: In the frog the ventral lip of the blastopore grows forward during the closure of the blastopore, and only subsequently a new opening forms at the point from which the for-

FIG. 38. — Embryo of Rana temporaria at time of hatching.

ward growth began (Fig. 37, A, B). In the urodeles (newt and Amblystoma) the ventral lip of the blastopore remains stationary, *i.e.* it retains its first position, and the anus forms directly from its posterior end.

THE DIGESTIVE TRACT AND THE GILL-SLITS

The origin of the archenteron has been described in Chapter VI. At the time when the yolk-plug is drawn in from the surface, the archenteron has begun to enlarge (Fig. 26, A). A series of cross-sections (Fig. 26, B–E) of an embryo at this stage show that the dorsal and lateral walls of the archenteron consist of a single layer of endodermal cells, while the floor of the archenteron is formed by the upper surface of the yolk-mass. The uppermost cells of the yolk-mass show, to some extent, a tendency to arrange themselves in a single layer bounding the archenteron.

Shortly after this period the embryo increases in length, and the archenteron is correspondingly drawn out (Fig. 37). The anterior end of the archenteron enlarges, and the yolk-mass is pushed posteriorly. As a result the middle and posterior parts of the archenteric cavity become smaller than they were in the earlier stages (Figs. 39, 40). The walls of the anterior portion of the archenteron are thin, and composed of a single layer of cells. A blind diverticulum extending from this enlarged

anterior portion into the yolk-mass behind (Fig. 37, A, B) forms the beginning of the liver.

The first gill-slits appear at a stage when the medullary folds have rolled over and are about to fuse. At the present stage, the gill-slits are well marked. They appear along the lateral walls of the enlarged anterior end of the archenteron as solid outgrowths of its wall. At the posterior end of the archenteric cavity the position of the blastopore, which has now closed, is marked by a diverticulum, the so-called "post-anal-gut" (Fig. 37). It is in this region that the neurenteric canal of the embryo persists for a short time after the blastopore has been covered over by the medullary folds. The pit-like invagination of ectoderm, the proctodæum, has opened into the postero-ventral portion of the archenteron (Fig. 37, B).

At the time when the tadpole is ready to emerge from the jelly-capsule (Fig. 38), the anterior portion of the archenteron has become larger and longer (Fig. 39), and in the region where the heart forms, ventral to the pharynx, an inward projection of the endodermal wall is present. In the middle region of the embryo the lumen of the archenteron is reduced to a small cavity, as seen in cross-section (Fig. 40), and is now longer from above downward than from side to side. The yolk-mass as a whole is rounded and more compact than in the earlier stages. At the posterior end of the embryo the archenteric cavity bends around the end of the yolk-mass, taking a curved course to open on the ventro-posterior surface of the body by the anus.

During the early stages of development the cells of the embryo have been exceedingly active, but no food has been taken as yet into the digestive tract, for the mouth does not open until some time after the embryo has left the egg-membranes. All the cells of the body contain yolk-granules, which serve in part, beyond doubt, to supply the energy necessary for development. A large amount of yolk is also stored up in the endoderm cells of the ventral yolk-mass, and must also long serve as a source of nourishment for the young tadpole.

The changes in shape that the archenteron passes through seem to be in part a result of the activity of the endodermal cells, and in part the necessary result of the change in shape

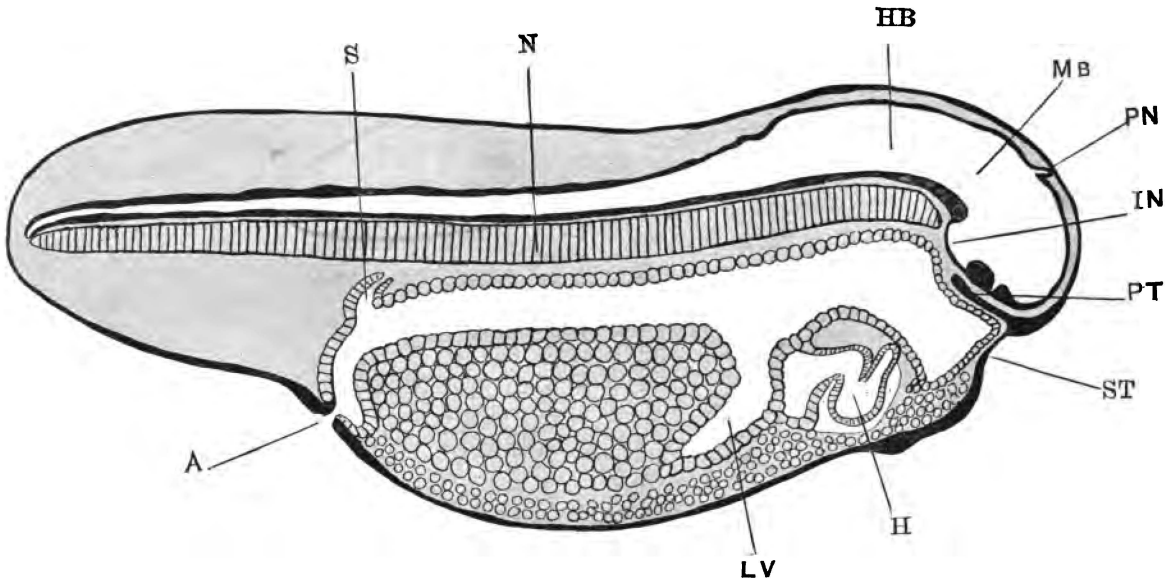


FIG. 39. — Sagittal section through middle plane of body. A. Anus. H. Heart. HB. Hind-brain. LV. Liver-diverticulum. MB. Mid-brain. N. Notochord. PN. Pineal body. PT. Pituitary body. S. Segmental duct. ST. Stomodæum. IN. Infundibulum.

that the whole embryo assumes. The early enlargement of the anterior archenteric cavity and the formation of a single-layered wall at the anterior end, with the subsequent formation of the gill-slits, would seem to be the result of the activity of the endodermal cells of those regions. On the other hand, some of the changes in shape that the lumen undergoes would seem to be due to the change in shape of the whole embryo as it elongates antero-posteriorly, and narrows from side to side. Nevertheless, even in this case the cells do not seem to be

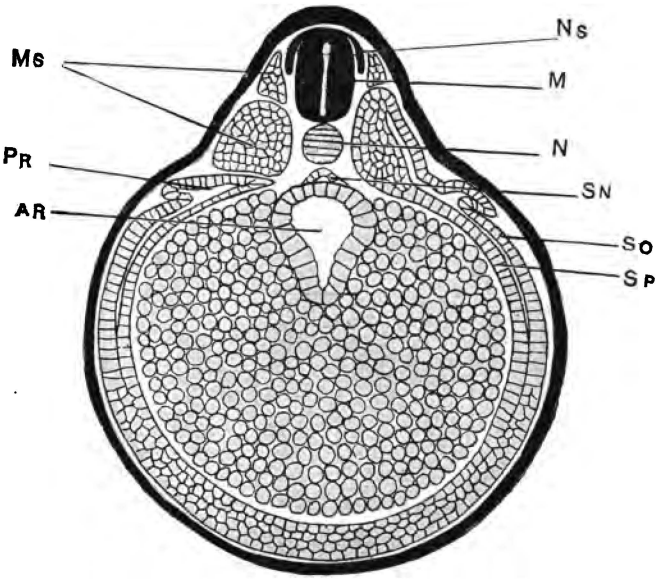


FIG. 40. — Cross-section through the middle of an embryo (3½ mm.). AR. Archenteron. MS. Mesoblastic somites. N. Notochord. Ns. Neural crest. M. Medullary tube. PR. Pronephros. SN. Subnotochordal rod. So, SP. Somatic and splanchnic mesoderm. (After Marshall.)

entirely passive, for the number of cells lining certain parts of the early archenteron is, in cross-section, considerably larger than the number lining the same region at a later stage. Either certain of the cells have pulled away from the surface and have passed into the yolk, or else they have changed their position relative to one another on account of the lengthening of the archenteron. In the latter case the total number of endoderm cells lining the archenteron would still be the

same in the older and younger embryos, or greater in the older embryo as a result of cell-division.

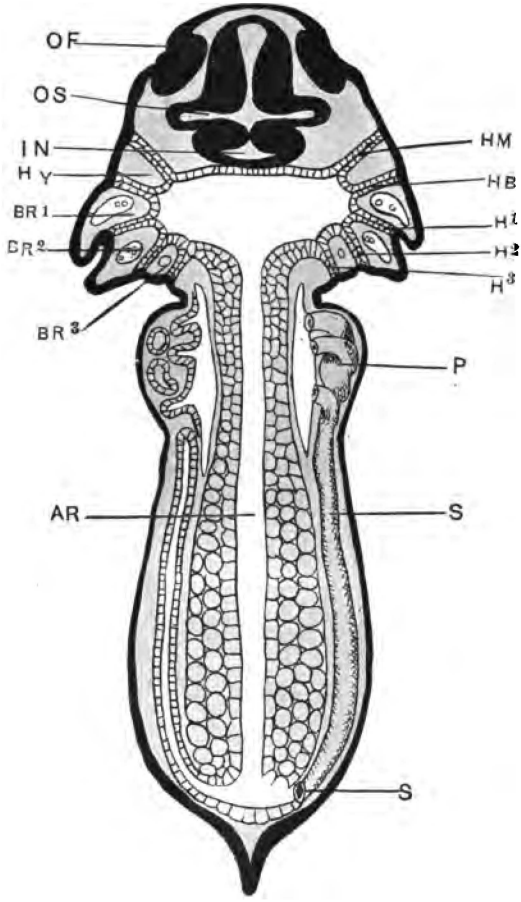


FIG. 41. — AR. Archenteron. BR^1, BR^2, BR^3. Branchial arches. H^1, H^2, H^3. Gill-clefts. HB. Hyoid cleft. HM. Hyomandibular cleft. HY. Hyoid arch. IN. Infundibulum. OF. Olfactory pit. OS. Optic stalk. P. Pronephros. S. Segmental duct. (After Marshall.)

The first three pairs of gill-slits appear almost simultaneously; the first two, however, before the third. When the tadpole leaves its capsule, there are five pairs of gill-slits; the two new pairs have appeared successively behind the third. A horizontal section through the larva (Fig. 41) shows to best advantage the five clefts at this stage. "The gill-pouches form vertical partitions radiating outwards from the pharynx to the surface - ectoderm. Each pouch is formed of a double fold of endoderm, the two layers of which are in close contact with each other. The outer ends of all five pairs of gill-pouches reach the ectoderm and fuse with its inner or nervous layer."[1] The most anterior pouch or cleft

[1] Marshall ('93).

is the hyomandibular cleft, and this is followed successively by the first, second, third, and fourth branchial clefts. The last is the smallest and is often imperfectly developed at this time.

The *visceral* or *gill-arches* lie between the *clefts*. The first arch between the hyomandibular and the first branchial clefts is the hyoid arch (Fig. 41). Then follow the first branchial arch (BR^1), second branchial arch (BR^2), and third branchial arch (BR^3). Behind the fourth branchial pouch there is an imperfectly defined fourth branchial arch.

When the tadpole leaves its jelly-capsule, the pouches are still double-walled, solid partitions; but about the time when the mouth forms, the endodermal lamellæ of some of the pouches separate and place the cavity of the pharynx in communication with the exterior. The second and third branchial clefts open first. Later the first branchial cleft opens, and later still the fourth.

The hyomandibular cleft is at first like the others, but it never opens to the exterior. After its formation it separates from its ectodermal connection, and recedes from the surface. The lamellæ separate, and the cleft appears as a diverticulum of the pharynx.

Two other structures arise from the walls of the pharynx shortly before the hatching of the tadpole. "The lungs arise as a pair of pouch-like diverticula of the walls of the œsophagus. They are at first exceedingly small and have strongly pigmented walls."

The thyroid body appears about the time of hatching as a short median longitudinal groove along the wall of the pharynx. "The groove is shallow anteriorly, but deepens at the hinder end, where it leads into a small conical pit-like depression of the endoderm, forming the pharyngeal floor, just in front of the pericardial cavity. Soon after the mouth opens, the thyroid separates completely from the floor of the pharynx, remaining as a solid rounded mass of pigmented cells, in close contact with the anterior wall of the pericardium."[1]

[1] Marshall ('93).

CHAPTER XIV

ORGANS FROM THE MESODERM

THE mesoderm appears as a distinct layer over the dorsal surface of the embryo at the time when the dorsal lip of the blastopore is moving over the white hemisphere (Fig. 25). At first the mesoderm is in close contact with the endoderm, particularly along the mid-dorsal line. The notochord soon separates from the mesodermal sheets of each side by two vertical furrows, so that from this time forward there are two lateral sheets of mesoderm, separated in the mid-dorsal line by the notochord (Fig. 26, E). Around the anterior and posterior ends of the notochord, the two sheets of mesoderm are continued into each other.

These sheets of mesoderm now rapidly extend ventrally. This down-growth is brought about by additions to the ventral borders of the sheets. The new cells that are added come, probably, from the yolk-cells along the free borders of the mesoderm; the yolk-cells in this region dividing rapidly form smaller cells that are joined to the mesoderm.[1] At the time when the medullary folds appear outlined upon the surface, the lateral sheets of mesoderm have extended ventrally and to a certain extent have fused in the mid-ventral line. The cells of each sheet of mesoderm are arranged over the greater part of their extent into two layers; but on each side of the notochord the mesoderm is somewhat thickened to form the beginning of the segmental plate (Fig. 42); and in this region there is, in the early stages of development, no distinct arrangement of the cells into two layers.

---

[1] According to some authors the ventral extension of mesoderm results from a proliferation of the mesoderm that is first laid down over the dorsal region, but it seems to me there is little ground for such an assumption.

Over the anterior end of the embryo and around the pharynx the mesoderm forms a thin layer of cells, loosely held together (Fig. 26, B). The mesoderm over the dorsal surface of the pharynx and beneath the brain plate is represented by only a single layer of somewhat scattered cells. Around the blastopore there is a thick layer of mesodermal cells which is thickest on the dorsal surface. In general, in the posterior region of the body the mesoderm is thicker than in the middle and anterior regions.

THE MESODERMIC SOMITES

In the following stages of development of the embryo the dorsal ectodermal plate is lifted up and rolled in to form the central nervous system (Fig. 42). The mesoderm lying on

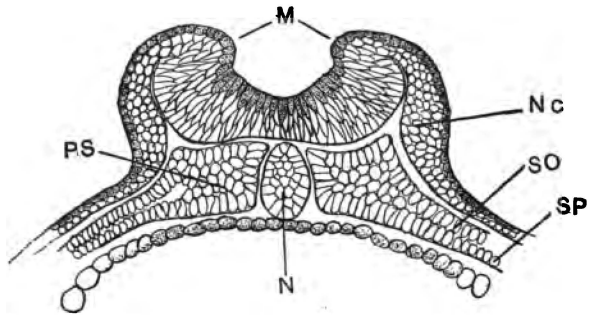


FIG. 42. — Cross-section through middle of embryo. M. Medullary plate. N. Notochord. Nc. Neural crest. PS. Primitive segment-plate. SO, SP. Somatic and splanchnic mesoderm.

each side of the notochord changes shape somewhat during this time. It forms on each side a thick, nearly solid mass of cells, the plate of the primitive segments or segmental plate (Fig. 42). The outermost cells of this mass, *i.e.* those lying nearest to the dorsal surface, now show a tendency to arrange themselves into an epithelial layer. This layer is at first continuous at the sides with the outer or somatic layer of cells of the lateral mesodermal sheets. The two layers of cells of the lateral mesodermal sheets (Fig. 42, SO and SP), the somatic and splanchnic layers, often show a tendency to separate and leave a cavity between them. This cavity filled with fluid

is the cœlom, or body-cavity, and is at first continued into the segmental plate. The cavity in the segmental plate lies between the outer epithelial layer and the inner solid mass of cells.

When the medullary plate of the embryo begins to roll in to form the nerve-tube, each segmental plate begins to break up transversely into a series of blocks or mesodermic somites. The process begins first in the region anterior to the middle of the embryo (Fig. 43). The mesodermic somites are at first somewhat irregular in outline. The first well-marked somite lies at about the level of the ganglion of the vagus nerve. In front of this there are traces of another somite which is partially broken up into loose mesenchymatous tissue. Still further forward, the series of somites is replaced by loose mesenchyme. In the frog the number of head-somites (or structures corresponding to them) is uncertain. At first the primitive segments or somites are not separated from the lateral sheets of mesoderm, but almost immediately after the segmental plate has begun to break up transversely into somites, these begin to separate also from the lateral mesoderm. This separation appears first in the intersegmental borders. At this time the medullary folds have met to form a closed tube. Posterior to the fourth segment, the segmental plate is beginning to break up into blocks, but these have, as yet, no sharply marked outer or ventral boundaries. The body-cavity of the lateral mesodermal sheet is at first, as we have seen, sometimes continued into the cavity of the segmental plate, but when the constriction of the plate from the lateral sheets takes place, this communication (the communicating canal) is lost. Even in the younger stages there is a differentiation of a peripheral epithelial layer surrounding the dense central mass or kernel of the somites. This peripheral part is represented on the

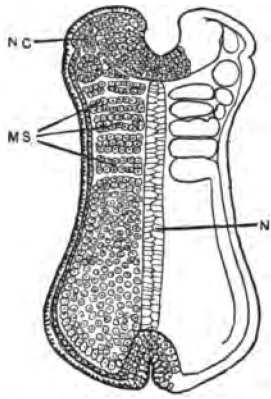


FIG. 43. — Frontal section of Bombinator. (After Götte.) MS. Mesoblastic somites. N. Notochord. NC. Neural crest.

outer side of each somite by the entire somatic layer. Along the ventral and median boundaries of the somites a layer having a loose epithelial character (mesenchyme) is also to be seen. Thus the central mass which is to develop into the myotome lies on the median side of the cœlom, and is wholly surrounded by an epithelial layer. Frontal sections show that this layer can also be traced inward for some distance between successive somites over both their anterior and posterior surfaces (Fig. 44).

"Not merely is mesenchyme produced by the thin peripheral layer of the somites, but in anterior regions considerable portions of the kernels of the somites also undergo a metamorphosis in this direction. Thus, if I be not mistaken, a somite immediately in front of somite 1 has been wholly converted into mesenchymatic tissue. The kernel of the succeeding somite (somite 1) has given rise to a considerable quantity of mesenchyme, and the process has been manifested, though to a less degree, even in succeeding somites."[1]

At the time when fourteen pairs of somites are present[2] the cells of the more anterior somites have begun to differentiate into muscle-fibres. The cells of each somite elongate in the antero-posterior direction and become cylindrical in shape, and each extends the whole length of its somite (Fig. 44, B). Each cylindrical cell has at first but a single nucleus. Around the wall of the cell a layer of fine fibrillæ appears. The original nucleus divides and re-divides into many nuclei, which lie scattered throughout the cell.

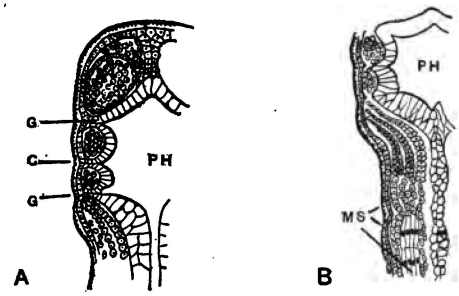


FIG. 44. — Frontal sections through the anterior end of Bombinator. (After Götte.) A. Shows three gill-pouches (G), and mesoderm of arches. B. Shows formation of mesodermic somites (MS). PH. Pharynx.

[1] Field ('91).

[2] Four days after fertilization of the egg, when three pairs of gills have appeared.

The development of the musculature of the head, limbs, and ventral body-wall takes place at a later stage. A description of the origin and development of these structures is beyond the limit of the present account.

THE HEART AND BLOOD-VESSELS

The heart appears at the time when the medullary folds have rolled in, and have met along the mid-dorsal line; it lies below the pharynx, and anterior to the liver (Fig. 37, B). The mesoderm in this region shows a tendency to split into two sheets and, where the heart is about to develop, a cavity, a part of

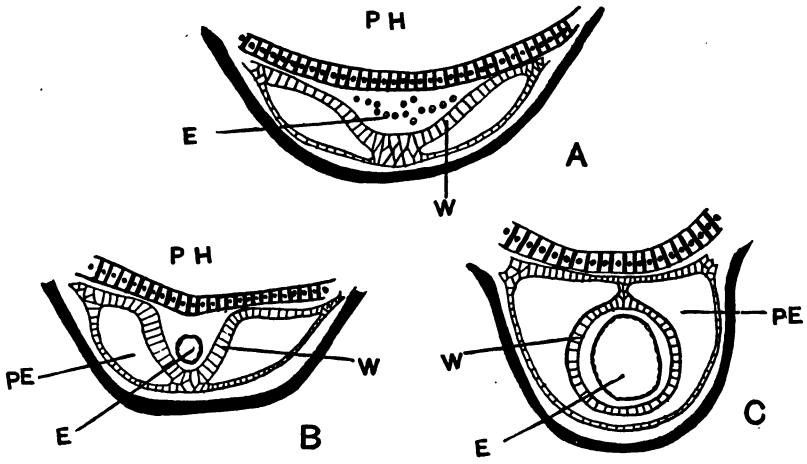


FIG. 45.—Three stages in development of heart. E. Endothelium. PE. Pericardium. PH. Pharynx. W. Wall of heart.

the cœlom, appears between the sheets. A cross-section of the larva (Fig. 45, A) shows on each side of the mid-ventral line in the region of the heart the somatic and splanchnic layers widely separated from each other. The cœlomic cavities of the right and left sides are not continuous across the middle line, but anterior and posterior to this section the cœlomic cavity is found to be continuous before and behind with the general cœlomic space on each side. A few scattered cells lie in the middle line between the splanchnic layer and the ventral wall of the pharynx (Fig. 45, A). These cells have

been described as originating from the ventral wall of the archenteron, and if so, have had a different origin from the other cells of the heart.[1]

At a somewhat later stage of development the walls of the cœlomic cavities of the right and left sides separate further (Fig. 45, B). The splanchnic layer thickens, and begins to surround the proliferation of scattered "endodermal cells." These endodermal cells arrange themselves into a thin-walled tube stretching throughout the heart-region (Fig. 45, B). Subsequent development shows that this tube becomes the endothelial lining of the heart. Around this endothelial tube the thickened splanchnic layers now begin to push in from the sides between the tube and the lower wall of the pharynx. The tube becomes finally entirely surrounded by mesoderm (Fig. 45, C). The mesoderm from the sides that has met beneath the pharynx forms the dorsal mesentery of the heart. The mesoderm around the tube continues to thicken, and forms later the musculature of the heart.

At first the heart has also a ventral mesentery formed by the union of the walls of the cœlomic cavities below it (Fig. 45, B), but later the mesentery is in part absorbed and the cœlomic cavities become continuous below from side to side, forming the pericardial chamber. The outer layer of somatic mesoderm gives rise to the pericardium itself.

The tubular heart is attached at its posterior end to the liver and anteriorly to the wall of the pharynx. It becomes free ventrally and later also dorsally along the middle of its course, and owing to an increase in length is bent on itself into an S-shaped tube (Fig. 39).

When the tadpole is $4\frac{1}{2}$ mm. in length, we find a vessel opening into the posterior end of the heart, the sinus venosus, formed by the union of two large vitelline veins. These veins have appeared on each side of the liver-diverticulum and continue along the yolk-mass in a fold of the splanchnopleure. They are supposed to carry to the heart the food-material absorbed from the yolk. Into the sinus venosus empty also two

[1] At least these cells have arisen from the yolk-cells after the ventral mesoderm has been split off.

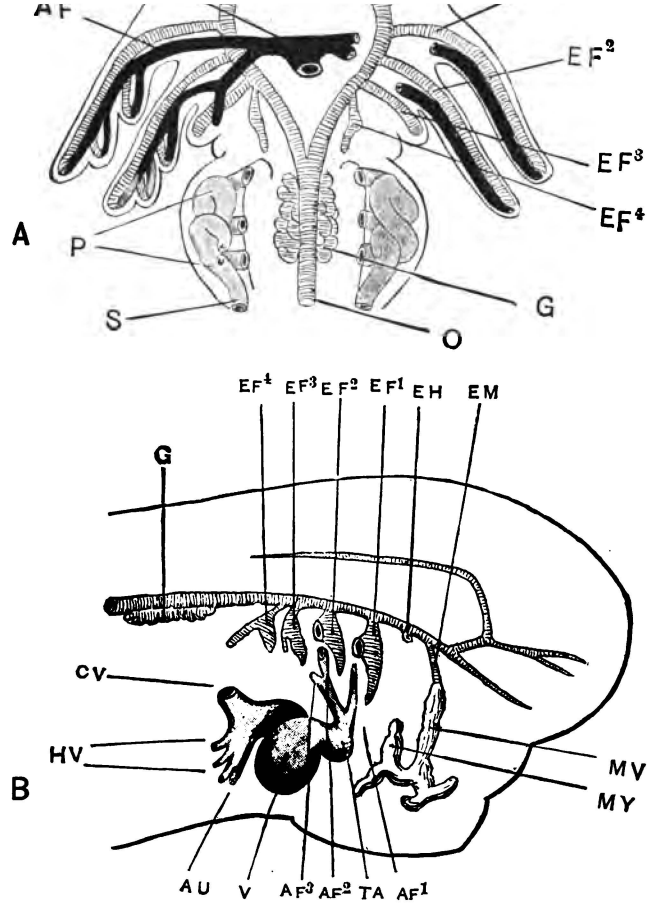


FIG. 46, A. — AF. Afferent branchial vessel. AR. Anterior cerebral artery. CA, CP. Anterior and posterior commissural vessel. EF^1, EF^2, EF^3, EF^4. Efferent branchial vessels of the first, second, third, and fourth branchial arches. EH. Efferent hyoid vessel. EM. Efferent mandibular vessel. G. Glomus. O. Aorta. P. Pronephros. RT. Truncus arteriosus. S. Segmental duct. (After Marshall.)

B. — AF^1, AF^2, AF^3. Afferent branchial vessels. AU. Auricle. CV. Cuvierian vein. EF^1, EF^2, EF^3, EF^4. Efferent branchial vessels. EH. Efferent hyoid vessel. EM. Efferent mandibular vessel. G. Glomus. HV. Hepatic veins. MV. Mandibular vein. MY. Hyoidean vein. TA. Truncus arteriosus. V. Ventricle. (After Marshall.)

veins that have come down from the dorso-lateral region of the embryo. These are the Cuvierian veins formed on each side by the union of the posterior and anterior cardinal veins. The posterior cardinals bring back the blood from the head-kidneys. Around the head-kidneys these veins form sinuses that are enormously large. Each posterior cardinal also receives somatic veins from the posterior part of the body-wall. The anterior cardinal veins bring back blood from the dorsal part of the head-region.

In a larva 4½ mm. in length, the blood-vessels of the branchial region have also appeared. The anterior end of the heart, the truncus arteriosus, divides into a right and left branch, which pass forward and laterally toward the base of the gill-region. In the mandibular arch no vessels are as yet present. In the hyoid arch an irregular space appears in the mesoderm. In the first branchial arches two vessels appear, a large efferent vessel (Fig. 46, for an older embryo) connected with the dorsal aorta, and a smaller afferent vessel. The latter is at present without connection. In the second branchial arch the conditions are like those in the first. In the third branchial arch only a small efferent vessel has as yet appeared. No vessels are present at this time in the fourth branchial arch. The dorsal aorta is represented by a paired vessel in the dorso-pharyngeal region. Opposite the hyoid arch each branch of the dorsal aorta divides into a dorsal and into a ventral branch. The dorsal branches meet each other behind the infundibulum, while the ventral branch passes forward to end blindly (Fig. 46). The two aortæ unite posteriorly into a single vessel at the level of the pronephros (Fig. 46, A).

The condition of the blood-vessels shortly after the tadpole has left its envelopes (it is then 7 mm. in length) is illustrated in Figs. 46 and 47. The heart has enlarged and is further twisted on itself. The aortic bulb-portion and the auricular and ventricular portions are distinctly marked from each other by constrictions of the tube. The right and left branches of the aortic bulb have grown toward the gill-arches, and the afferent vessels of the first and second branchial arches have united with the ventral aortic branches AF^1 and AF^2. The efferent branches, EF^1 and EF^2, of the first and second bran-

chial arches have greatly enlarged, and the efferent and afferent vessels are now also united to each other in each arch by small vessels (Fig. 47) or capillary tubes. The efferent vessels of these two arches are also in communication with the dorsal aorta of their respective sides. There is thus established at this time a circulation of blood from the heart to the dorsal aorta by way of the first and second branchial arches.

In the third and fourth branchial arches the efferent vessels have appeared. In the third arch the beginning of an afferent vessel is seen (Fig. 46). In the hyoid arch blood-vessels appear, as we have seen, at an early stage of development and seem to correspond to those in the branchial arches, but after developing to a certain extent, they begin to degenerate. In the mandibular arch no vessels have appeared at the time when the larva leaves its capsule. Soon after this time a vessel develops in this arch, and a small diverticulum arises from the dorsal aorta (Fig. 46, B, MV), and later the two vessels unite.

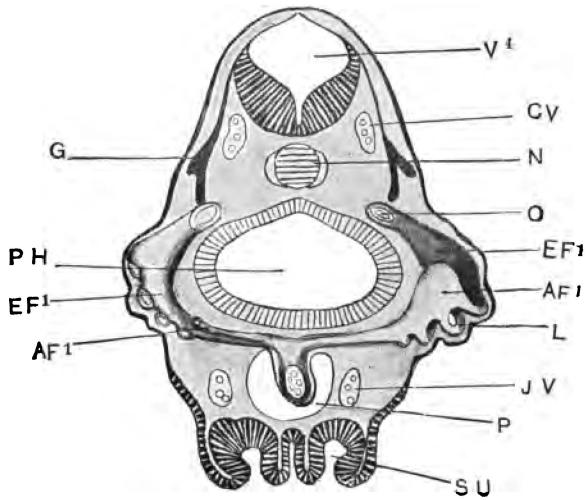


FIG. 47. — AF¹. Afferent branchial vessel. CV. Anterior cardinal vein. EF¹. Efferent branchial vein. G. Pneumogastric nerve. JV. Inferior jugular vein. L. Capillary loop connecting afferent and efferent branchial vessels. N. Notochord. O. Aorta. P. Pericardium. PH. Pharynx. SU. Suckers. V⁴. Fourth ventricle. (After Marshall.)

The origin of the heart has been described, but as yet *the*

method by which the blood-vessels are formed has not been fully considered. The dorsal aorta is the first vessel to arise. A series of isolated lacunæ appear in the mesoderm along the roof of the pharynx, and by opening into one another form a pair of longitudinal vessels. Vessels next appear in the first and second branchial arches. Similar vessels arise later in the third and fourth branchial arches. In the hyoid and mandibular arches the vessels appear, as we have seen, later still. These branchial blood-vessels originate in part as isolated lacunæ in the mesoderm, and in part as outgrowths of already existing vessels. For instance, *lacunar vessels* appear in the mesoderm of the gill-arches, two in each arch. One of these is the efferent lacunar vessel, and later connects with a corresponding diverticulum from the dorsal aorta, and the other lacunar vessel is the afferent vessel of the same arch. This latter vessel grows ventrally toward the diverticulum from the truncus arteriosus and unites with it.

The walls of the blood-vessels are formed directly from the mesodermal cells around the lacunæ. "The blood-corpuscles are free cells that have been left in the lacuna-spaces, or more usually are cells budded off at a later stage from the walls of the vessels into their cavities."[1] At first the blood-corpuscles are simply spherical cells containing yolk-granules. Only after the embryo is hatched do many of the corpuscles begin to acquire the shape and character of red blood-corpuscles.

THE PRONEPHROS

The excretory system of the young embryo is represented on each side by the *pronephros* and the *segmental duct*. Whether the pronephros and duct arise in part from an early ingrowth of ectoderm or whether they develop *in situ* from the somatic mesoderm is perhaps still open to doubt. Field ('91), who has worked out most recently the development of the pronephros and segmental duct in the frog, describes the organ as coming entirely from the mesoderm. We shall follow closely Field's account. The pronephros appears at a stage when the medullary

[1] Marshall ('93).

plate is first formed. It is well marked at the time when the medullary folds have rolled in, but have not yet fused. A thickening of the somatic layer of the lateral mesoderm near the second mesoblastic somite marks the beginning of the pronephros (Fig. 48, A). At a later stage, the mesodermic thickening becomes larger, *and the anterior end arches over toward the cœlomic cavity, to form the first nephrostome.* The ventroposterior part of the nephrostomal thickening is continued backward as a thickening of the somatic wall as far as the seventh somite, to form the segmental duct. A canalization now takes place in the nephrostomal portion and in the segmental duct. Three short tubes or canals appear in the pronephric mass running outward from the cœlom (Fig. 41). Constrictions appear between the first and second, and between the second and third canalized tracts (Fig. 48, B), and short

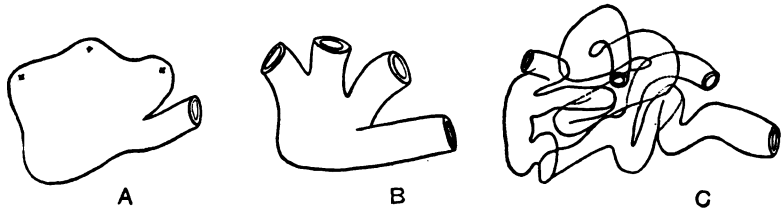


FIG. 48. — Three stages in the formation of the pronephros. (A and C after Field.)

hollow stalks are formed leading ventrally into the longitudinal canal of the segmental duct.

A proliferation of cells from the somatic layer of the mesoblastic somites, dorsal to the pronephros, gives rise to a covering of mesoderm for the pronephros, the *pronephric capsule.* A little later a protrusion of the splanchnic wall opposite to the funnels of the pronephros forms the *glomus* (Fig. 47, B). The glomus becomes filled with blood, and seems to have a direct connection with the dorsal aorta. The bulging portion of the glomus protrudes into the cœlom, and its cavity is separated from the cœlomic cavity by only a single layer of cells.

At the time when the embryo is hatched, the duct of the pronephros, the segmental duct, has fused with the wall of the cloaca, and the lumen of the duct opens into the digestive

tract (Fig. 41). Presumably the pronephros is functionally active at this time. The arrangement of the tubes of the pronephros, and their relation to the common tube or pronephric duct, is shown in Fig. 48, C. The three nephrostomes open into three collecting tubules, and these tubules have elongated independently of one another. The first collecting tubule is short; the second is thrown into several turns and opens into the pronephric duct a short distance from the first. The collecting tubule from the third nephrostome opens some distance behind the point of opening of the second. The segmental duct is thrown into a series of turns between the first and second collecting tubules; and as it leaves the pronephric region it takes at first a tortuous course, and then runs as a straight tube backward to the cloacal opening.

The posterior cardinal veins have appeared at this time, and in the region of the head-kidneys these veins widen into a sinus lying amongst the windings of the collecting tubules of the pronephric duct. The glomus of each side reaching from the region of the first to that of the third nephrostome, and lying exactly opposite the nephrostomes, is well developed (Fig. 46).

So far the description of the development of the excretory system has been that given by Field. The same author adds: "According to the account which at present receives the most general acceptance, the pronephros first appears as an outfolding of the somatopleure in the form of a longitudinal groove. The anterior end of this groove is destined to become the pronephros, the remaining portion is constricted off to form the segmental duct. Since the process of constriction advances from before backward, stages may be found in which a completed tube is continuous posteriorly with a mere groove of the somatopleure. In the anterior region the groove remains in communication with the body-cavity, and grows down toward the ventral surface of the embryo in the form of a broad pocket. The slit-like peritoneal opening of this pouch closes throughout the greater part of its length, leaving, however, two or three regions of incomplete closure, the fundaments of the nephrostomes."

"The nephrostomal tubules are formed by the fusion of the walls of the pouch between two nephrostomes. The regions of

fusion extend in vertical lines from the nephrostomal margin of the pouch nearly to its ventral border, where there is left an unfused and therefore continuous longitudinal tract constituting the canal which I have called the collecting trunk."[1] Field continues, "In opposition to this view, I would maintain: (1) That the first trace of the excretory system consists of a solid proliferation of somatopleure, the pronephric thickening; (2) that the lumen of the system arises secondarily; and (3) that the pronephric tubules do not appear in consequence of the local fusion of the walls of a widely open pouch, but that they are differentiated at an early stage from the hitherto indifferent pronephric thickening."

The pronephric duct of the Amphibia arises, according to one view, as we have seen above, from an *evagination of somatopleure*, its lumen being therefore a detached portion of the body-cavity. A second view of the origin of the duct is, that it arises from a solid proliferation of somatopleure. Field agrees with the latter view. A third view maintains that the duct is ectodermic in origin. Field has shown, however, that in the Amphibia the excretory system develops most probably without any participation of the ectoderm in its formation.

---

[1] "This view of the development of the pronephros, although suggested by Wilh. Müller, was first described in detail by Goette for Bombinator, and was later extended to other Amphibia by the researches of Fürbringer. It has been entirely confirmed by Wichmann, by Hoffmann, and still more recently by Marshall and Bles." (Field, '91, page 281.)

CHAPTER XV

ORGANS FROM THE ECTODERM

THE outer covering-layer of the embryo, the ectoderm, gives rise to the nervous system and organs of special sense (eyes, ears, nose). The adhesive glands or "suckers" are also formed by this layer; and the anterior and posterior divisions of the digestive tract, the stomodæum and proctodæum, have a lining of ectoderm.

In the present chapter we shall follow the development of these organs.

THE CENTRAL NERVOUS SYSTEM

The medullary plate appears on the surface of the young embryo at the time when the yolk-plug is about to be drawn in from the surface. It extends over about one-third of the circumference of the egg and is, at first, quite broad. It is slowly converted into a tube by the drawing together of its material, and by a subsequent over-rolling of its sides to meet in the mid-dorsal line. This change into a furrow, and then into a closed tube, involves extensive *movements* of the material of the plate. Whether the plate moves as a whole, or whether the movement is only the sum-total of changes in shape and position of the individual cells, is not known (compare Figs. 26, 42, 50). While the medullary tube is developing, the embryo as a whole is changing its spherical shape into a more elongated form and the medullary tube is also drawn out.

The medullary plate is formed, for the most part, from a thickening of the *inner layer of the ectoderm* (Figs. 26 and 42). It is continuous on each side with a broad flange or ridge of thickened ectoderm (Fig. 42, Nc). This ridge of cells, the neural crest or ridge, is also lifted up during the formation of

the tube, forming a broad sheet of cells on each side, continuous with the dorsal edges of the closing tube. These lateral sheets are very large and conspicuous at the anterior end of the nerve-tube. The subsequent history of these structures will be followed later.

The first part of the medullary tube to close, is the antero-median portion, and from this point the closure of the tube extends anteriorly and posteriorly. At the anterior end, the tube remains open latest; at the posterior end, the medullary folds arch over the blastopore, as already described.

When the medullary folds have met along the mid-dorsal line, the apposed edges fuse, and the outer layer of ectoderm then becomes continuous over the outer surface of the embryo. A part of the same layer has been cut off and lines the cavity of the neural tube. The nerve-tube soon loses all connection with the overlying ectoderm (Fig. 40).

The anterior end of the nerve tube is larger than the rest, and this end is at first bent down nearly at right angles to the long axis of the more posterior portion (Fig. 37, A). The bending begins at the front end of the notochord. A slight transverse infolding of the wall of the anterior end of the tube takes place soon after its closure, and later another transverse infolding occurs, still further forward. As a result three divisions or vesicles of this region are produced. They correspond to the fore-brain, mid-brain, and hind-brain respectively. The fore-brain (Fig. 37, FB) is the large anterior vesicle. From it develops later the third ventricle, the pineal body, the infundibulum, the optic vesicles, and the cerebral hemispheres. The mid-brain (Fig. 37, B) is the smallest of the three divisions, and gives rise to the optic lobes and to the Sylvian aqueduct. The hind-brain is continued into the more posterior medullary tube. It lies in the same plane with the medullary tube, and represents only a somewhat enlarged part of the tube. The hind-brain becomes the medulla oblongata, and from its roof the cerebellum is formed.

The roof of the fore-brain is very thin. Near the middle of its upper margin an evagination is formed, which is, at first, only a hollow diverticulum (Fig. 37, B), but when the tadpole leaves its capsule, the peripheral end of the outgrowth forms a

small round knob. This knob, the pineal body, lies just below the surface-ectoderm. Later the structure grows forward, and becomes dilated at its distal end. The dilated end or bulb remains connected with the brain by a stalk. White particles develop in the bulb, so that it stands out in strong contrast to the dark surface of the brain.

At the time of closure of the medullary folds, a mid-ventral diverticulum forms from the floor of the fore-brain. This is the infundibulum. It is in close contact with the anterior end of the notochord (Fig. 38). The infundibulum is throughout its subsequent history a wide sac with thin walls. It soon comes into close connection with another structure, the pituitary body (Fig. 39). The pituitary body arises very early, even before the neural tube is closed, as a solid ingrowth, or cord of cells, from the ectoderm, immediately in front of the anterior end of the medullary plate (Fig. 37, A). Later, this small, solid, tongue-like process projects inward from the ectoderm beneath the brain and above the dorsal wall of the pharynx. The inner end of the ingrowth expands into a flattened mass of cells, which lies immediately beneath the anterior end of the notochord. This mass becomes later the pituitary body, while the rest of the process forms a slender stalk connected at one end with the ectoderm.

THE EYES

The eyes develop in part from the walls of the fore-brain. Even before the neural tube is closed, in the embryos of some species of frogs, two pigmented areas may be seen on the antero-lateral walls at the anterior end of the infolding medullary plate. These pigmented areas mark the region from which a pair of evaginations of the fore-brain will develop to form the *optic vesicles*. The hollow vesicles push out laterally toward the sides of the head. Each tubular evagination then becomes constricted, forming a distal hollow bulb and a proximal hollow stalk (Fig. 49). The bulb gives rise to the retina and to the pigment behind the retina, while, according to Marshall ('93), the stalk forms a path along which the fibres of the optic nerve pass from the eye to the brain. The outer hemisphere of the optic bulb flattens and then pushes in so that the former

cavity of the vesicle is nearly obliterated (Fig. 49); and at the same time the inturned wall becomes greatly thickened. There is thus formed an open, cup-shaped structure with the opening of the cup turned outward. The wall of this optic cup lying toward the brain remains thin, and pigment soon appears in it. The inturned wall becomes the retina of the eye.

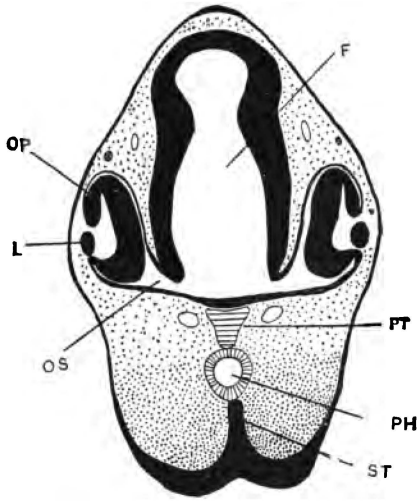


FIG. 49. — Cross-section through head and eyes. F. Fore-brain. L. Lens of eye. OP. Optic cup. OS. Optic stalk. PH. Pharynx. PT. Pituitary body. ST. Stomodæum.

At the time when the optic bulb turns in on itself, a thickening of the inner layer of ectoderm opposite the optic cup takes place. This thickening forms a solid mass of cells projecting into the open mouth of the cup. It becomes hollow and then separates from the ectoderm (Fig. 49), filling up the opening of the optic cup, and forms later the lens of the eye. In the space left between the lens and the retinal layer the vitreous body of the eye forms. The later stages of the development of the eye take place after the embryo leaves its capsule. The nerve-fibres that develop from the retina and pass into the brain along the optic stalks have not yet appeared.

THE EARS

While the neural groove is closing, a pair of thickened circular patches of the *inner layer* of the ectoderm arises, one on each side of the head near the hind-brain. After the closure of the neural tube each area forms a shallow pit with the concavity turned outward, and each is covered by the outer layer of the ectoderm. The pit deepens, the outer edges come together, and a hollow vesicle is formed before the tadpole

leaves the capsule. These auditory vesicles separate from the surface ectoderm. "At the time of the separation the vesicle is a closed sac somewhat pyriform in shape; its lower or ventral portion being spherical and lying opposite the notochord, and its dorsal wall being prolonged upwards into a short blind diverticulum lying at the side of the hind-brain. The wall of the vesicle consists of a single layer of cubical or columnar cells." This ectodermal sac becomes the sensory lining of the inner ear (Fig. 50).

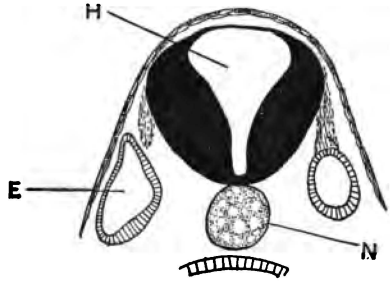


FIG. 50. — Cross-section through hind-brain (H) and inner ear (E). N. Notochord.

THE NERVES

At the time when the medullary plate forms as a thickening of the ectoderm, there also forms, as we have seen, on each side of the plate a *lateral neural ridge or plate of ectoderm.* Each neural ridge appears at first as a continuation of one side of the thickened medullary plate (Fig. 26). A slight constriction on each side marks the line of demarcation between the medullary plate and the neural ridge (Fig. 42). The neural ridges are more conspicuous at the anterior end of the medullary plate; they also develop somewhat earlier in this region. After the medullary plate has rolled up to form the medullary tube, the lateral neural ridges are also carried up, retaining for a time their primitive connection with the outer (now dorsal) part of the medullary tube (Fig. 40).

The neural ridges next become broken up into a series of dorsal nerves, the cells collecting at certain regions, and thinning out and disappearing in the intermediate regions. The dorsal nerves grow down later between the myotomes and the nerve-cord. Accumulations of cells occur at certain regions on each dorsal nerve to form the ganglion of the dorsal root, and nerve-fibres are spun out from the cells of the ganglion. The ventral roots of the spinal nerves appear much later.

Marshall ('93) says the *cranial nerves*, "which are undoubtedly derived from the neural ridges, are the trigeminal, the facial and auditory, and the sensory branches of the glosso-pharyngeal and pneumogastric nerves." These nerves, "although arising from the neural ridges in the same way as the dorsal roots of the spinal nerves, yet differ from these, and agree amongst themselves in certain important features."

"I. The nerves in question, in place of growing downwards like the spinal nerves, alongside the central nervous system, grow outwards close to the surface of the embryo between the epiblast and the mesoblast."

"II. Each of these four nerves acquires a new connection with the surface epiblast some considerable distance beyond the root of origin from the brain, and at about the horizontal level of the notochord; at this place and at any rate in part from the surface epiblast itself, the ganglion of the nerve is formed."

"III. The nerves have special relations to the gill-slits, each nerve dividing into two main branches, which embrace between them one of the gill-slits."

"IV. A special system of cutaneous nerves is developed from the surface epiblast in connection with these four nerves, forming the lateral line system of nerves."

The pneumogastric nerves are "wing-like" expansions of the neural plate, extending more than half-way down the side of the pharynx. At the time when the larva leaves the capsule, a thickening of the ectoderm on each side opposite this nerve and at the level of the notochord develops, and fuses with the nerve. From this double origin arises the ganglion of the pneumogastric. A lateral line thickening has appeared as a solid cord of cells on each side, extending from the pneumogastric backward along the side of the embryo.

It is not possible to enter here into the details of the development of the other cranial nerves enumerated above. The development of the first, third, fourth, and sixth nerves has not as yet been fully worked out. The origin of the optic nerve has been described in connection with the development of the eye.

THE APPEARANCE OF CILIA ON THE SURFACE OF THE EMBRYO

If the living embryo be examined at the time when the neural folds have appeared, it will be seen that the embryo slowly rotates within the jelly-capsule. This rotation is the result of the activity of certain ciliated ectodermal cells. The distribution of these cells over the surface of the body has been recently described by Assheton ('96). Assheton states that at the time when the medullary folds are first visible, and even after they have begun to roll in, there are no traces of cilia on

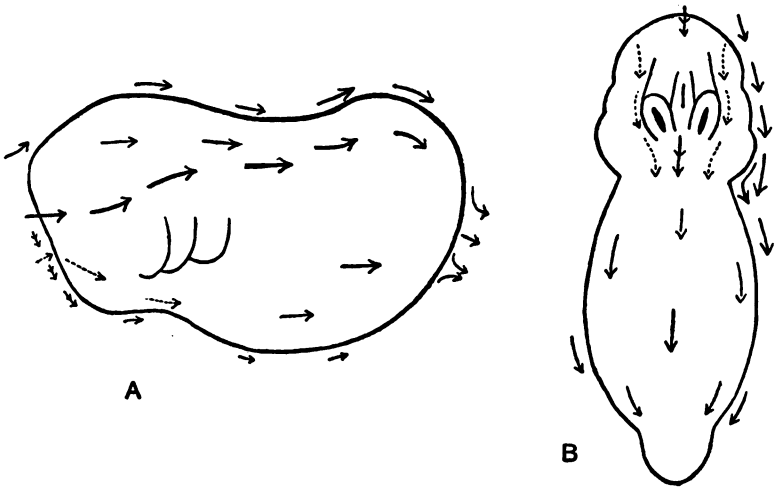


FIG. 51.—Embryo of Rana. The arrows show the direction of currents of water over the surface. A. Side view. B. Ventral view. (After Assheton.)

the surface of the embryo. Before the neural folds have met in the middle line the ectoderm has become ciliated in certain regions, as can be demonstrated by the streaming movement of granules of carmine placed on the surface. The arrows in Fig. 51 show the direction of the flow of granules over the surface. The lateral edges of the anterior end of the medullary folds seem to show the first traces of cilia, and a few hours later (Fig. 51, A) cilia have also appeared along the sides of the folds.

As the medullary folds grow nearer together, the ciliation extends further back along the sides of the dorsal surface. When the folds have just touched at the anterior end, cilia appear on the antero-ventral surface of the embryo, in the region where the mouth subsequently forms. The direction of the currents set up is from before backward. The whole of the dorsal surface next becomes ciliated. The ciliation spreads rapidly and at the time when seven or eight mesoblastic somites are present (when the embryo is 3 mm. in length) it has extended over the whole surface of the embryo. The currents, however, differ very much in intensity. Figure 51, A, B, shows the direction of the flow, the larger arrows indicating stronger currents. The action of the cilia is strongest at the anterior end of the body. A well-defined stream passes over the bases of the gills, which have begun to appear at this time. Over the ventral surface the currents move slowly and in eddies. At the hinder end of the embryo the action of the cilia directs the currents of water toward the blastopore and anus. When the embryo measures 4 mm., the so-called "suckers" have appeared, and the currents in that region have changed their direction. These "suckers" are in reality mucous glands that secrete a sticky substance by means of which the embryo can fix itself to objects with which it comes in contact. The edges of the glands have well-developed cilia, which direct a stream of water over the stomodæal depression, and thence backward between the glands (Fig. 51, B). In older embryos, when the glands have united to each other in the mid-ventral line, the direction of the currents in this region is altered. The central stream now turns outward around the anterior sides of the glands and passes backward along the sides. In older larvæ (8 mm.) small special currents run over the edges of the adhesive glands and into the depressions within the glands.

The cilia that cause the flow of water over the surface of the embryo are not developed by all the ectodermal cells. Even where the currents are most active, the cilia-bearing cells are slightly less abundant than the non-ciliated cells. Each ciliated cell bears on its outer surface numerous short cilia.

The gill-filaments also carry cilia in the proportion of one ciliated cell to two non-ciliated cells. The effect of the cilia becomes less conspicuous after the larvæ have reached 7 or 8 mm. in length, although even in much later stages the cilia are still found over all parts of the body. Their motion is sufficiently strong to cause the embryo (6 or 7 mm. in length) to move forward, if placed on a glass plate, at the rate of one millimetre in from four to seven seconds.

CHAPTER XVI

EFFECTS OF TEMPERATURE AND OF LIGHT ON DEVELOPMENT

It has been long known that the rate of development within certain limits is dependent on temperature. The development of the frog's egg is very much retarded, or even stopped, in water at the freezing-point. In North America, Rana temporaria often lays its eggs so early in the spring that the water is afterward frozen. The eggs that are caught in the ice are generally killed, but those that lie in the water below often remain alive and will subsequently develop normally.

Hertwig ('94) has shown that the maximum temperature for normal development of the eggs of Rana fusca is about 25 degrees C. Eggs develop very rapidly at this temperature, and in twenty-four hours have reached a stage of advancement corresponding to that at the end of the second day for the average temperature of 16 degrees. A temperature of 25 to 30 degrees C. long continued, or a temperature of 30 to 35 degrees for a short time, injures the eggs; their development is arrested and many die. Eggs that have been partially injured by heat (after two or three hours at 30 degrees C. or after three to eight hours at 26 to 28 degrees C. and then brought into a normal temperature) continue to develop at a slower rate than eggs under normal conditions. The yolk-hemisphere of the egg is first affected, so that the cleavage-furrows do not appear in it. The injured or dead half of the egg lies below, and the segmented portion above.

Hertwig obtained similar results by cooling the eggs. Soon after fertilization the eggs were placed in water at zero C. and kept there for twenty-four hours. During that time they did not segment, but when brought back to a higher (normal) temperature, the egg divided into two, four, etc., blastomeres;

nevertheless, as subsequent development showed, the egg had been injured. Many of these eggs developed in the same way as did those kept at a temperature of 25 degrees C., *i.e.* the segmentation of the yolk-hemisphere was retarded.

Schultze ('95) has also made some experiments on the eggs of Rana fusca in which the eggs were subjected to a temperature of zero C. Embryos in the following stages of development were used: *stage* A, when the dorsal lip of the blastopore had just appeared; *stage* B, at the end of the "gastrula" period; *stage* C, embryos with closed medullary folds. Three days after these had been placed in a chamber at zero C. they were examined and found in the same stage as when put into the cold. Some of the eggs were then removed, and continued to develop normally at a higher temperature. After fourteen days in the cold the remaining eggs were examined. The eggs were still in the same stage as when put into the cold chamber, but those of stage C had died. The others developed normally when brought into a higher temperature.

Thus while Hertwig found that the eggs of Rana fusca were injured by only twenty-four hours at a temperature of zero C., Schultze saw that *certain stages*, at least, were not affected by fourteen days at the same temperature. It is to be noted that Hertwig put the eggs into cold water soon after fertilization, while Schultze used later stages of development.

Not only is the rate of development of the frog-embryo affected by the temperature, but also by *the kind of light* in which it develops. Schnetzler in 1874 compared the development of Rana temporaria in white and in green light. The conditions of the two sets of embryos were nearly the same except as regards the kind of light. The embryos developed much faster in the white light, and the tadpoles underwent sooner their metamorphoses. Yung ('78) made a much more careful and elaborate series of experiments in which the eggs and embryos were subjected to a series of different lights. Instead of colored glass, which is seldom monochromatic, Yung used solutions of different sorts. The eggs were placed in a vessel containing about 5 litres of water; this vessel was then placed in a larger vessel of the same form. A space of 5 to 10 mm. was left between the two vessels. This space was filled with

a fluid that allows only certain parts of the spectrum to pass through. The top of the dish containing the eggs was covered by an opaque lid. An alcoholic solution of "fuchsine cérise" was used to produce a monochromatic red light; a solution of potassium chromate for a yellow light (although this allows a little red and green to pass through); a solution of nitrate of nickel (which is perfectly monochromatic) for a green light; an alcoholic solution of aniline "bleu de Lyon" for a blue light; and an alcoholic solution of aniline violet for a violet light. Parallel experiments took place in the daylight ("white light") and in the dark. The other conditions were the same for all the aquaria; they had the same amount of water, the same extent of surface for aëration, the *same temperature*, and were placed in the same position before a window. Eggs of R. esculenta and of R. temporaria were used.

At the end of seven days it could be seen that the embryos in the violet and in the blue light were more vigorous and in a later stage of development than the others. At the same time the development in the red and in the green was retarded. At the end of a month the tadpoles were in good condition, and the following table shows their mean length in each aquarium.

LARVÆ OF RANA ESCULENTA AT THE END OF ONE MONTH.

Violet.	Blue.	Yellow.	White.	Dark.	Red.	Green.
27	24	22.8	23	19.6	19.1	15.1

The breadth of the embryos shows the same differences. It is interesting to see that in the red and in the green light the tadpoles were even less developed than those in the white light or even in the dark. The result of this series of experiments on R. esculenta agrees with other experiments made by Yung at different times, upon other species of frogs and upon other animals.

APPENDIX

METHODS OF PRESERVATION, ETC.

For general purposes the eggs and embryos may be preserved in a saturated solution of picric acid in seventy per cent. alcohol to which a little sulphuric acid has been added (as in Kleinenberg's picro-sulphuric solution). The segmenting eggs or the early stages of the embryo surrounded by the jelly should be put directly into the fluid. Each egg should have, however, the outer jelly-coats cut off with a pair of scissors, and it is well to use an abundance of the preserving solution. Older embryos may be shelled out in the preserving fluid with *sharp* needles. After from three to five hours the eggs or embryos are transferred to seventy per cent. alcohol, which is changed several times; they should be kept for several days in eighty per cent. alcohol. In this alcohol (eighty per cent.) the inner egg-membrane slowly separates from the egg, and can be easily removed, after which the egg is preserved permanently in eighty-five per cent. to ninety per cent. alcohol. Corrosive-acetic solution gives good results with older embryos. For the early stages of fertilization and of extrusion of the polar bodies the following solution is to be recommended: one per cent. chromic acid, twenty-five parts; water, seventy parts; glacial acetic acid, five parts. Boiling water also gives good results.

Difficulty is often found in cutting the eggs on account of the brittleness of the yolk-portion; but if the following method is carefully followed, there will be no trouble in this regard. The preserved egg or embryo is put into absolute alcohol from two to five hours, turpentine two to three hours, soft paraffine a half-hour (change once), *hard paraffine* a half-hour. The melting-point of the hard paraffine should be from 56

to 58 degrees C. The egg must then be cut at a *temperature of seventy-five to eighty degrees Fahrenheit* (24 to 26 degrees C.); one often succeeds best if the microtome is placed in the sunlight during the cutting.

The segmentation-stages do not need to be stained. The older embryos stain well *in toto* in borax carmine or in hæmatoxylin on the slide. Fresh material cuts and stains better than that long preserved.

Formalin preserves eggs and jelly most admirably for demonstration. The segmentation-stages show particularly well when preserved (permanently) in this solution.

LITERATURE

Assheton, R.

'94, a. On the Phenomenon of the Fusion of the Epiblastic Layers in the Rabbit and in the Frog. Quart. Jour. Micr. Science, XXXVII, '94.

'94, b. On the Growth in Length of the Frog Embryo. Quart. Jour. Micr. Science, XXXVII, '94.

'96. Notes on the Ciliation of the Ectoderm of the Amphibian Embryo. Quart. Jour. Micr. Science, XXXVIII, '96.

Von Baer, K. E.

'28. Ueber Entwickelungsgeschichte der Thiere, '28 and '37.

'28. Geschichte des Froschembryo. Burdach, Die Physiologie als Erfahrungswissenschaft, II, '28.

'34. Die Metamorphose des Eies der Batrachier von der Erscheinung des Embryo und Folgerung aus ihr für die Theorie der Erzeugung. Müller's Archiv, '34.

Van Bambeke, Ch.

'68. Recherches sur le développement du Pélobate brun. Mémoires couronnés de l'Acad. Roy. des Sc. de Belgique, XXXIV, '68.

'70. Sur les trous vitellins que présentent les œufs fécondés des amphibiens. Bull. de l'Acad. Roy. d. Sc. de Belgique, (II), XXX, '70.

'80, a. Fractionnement de l'œuf des Batraciens. Arch. de Biologie, I, '80.

'80, b. Nouvelles recherches sur l'embryologie des Batraciens. Arch. de Biologie, I, '80.

'80, c. Formation des feuillets embryonnaires et de la notochorde chez les Urodèles. Bull. de l'Acad. Roy. des Sc. de Belgique, (II), XLIX, '80.

Barfurth, D.

'93, a. Halbbildung oder Ganzbildung von halber Grösse. Anat. Anz., VIII, '93.

'93, b. Experimentelle Untersuchungen über die Regeneration der Keimblätter bei den Amphibien. Anat. Hefte, III, '93.

'93, c. Ueber organbildende Keimbezirke und künstliche Missbildungen des Amphibieneies. Anat. Hefte, III, '93.

Beddard, F. E.

'94. Notes upon the Tadpole of Xenopus lævis (Dactylethra capensis). Proc. Zoöl. Soc., London, '94.

Bellonci, G.

'86. Sui nuclei palimorfii delle cellule sessuali degli anfibi, '86.

Benecke, B.

'80. Ueber die Entwickelung des Erdsalamanders. Zool. Anz., III, '80.

Bergmann.

'41. Die Zerklüftung und Zellenbildung im Froschdotter. Müller's Archiv, '41.

Bernard und Bratuschek.

'91. Der Nutzen der Schleimhüllen für die Froscheier. Biol. Centralb., XI, '91.

Bertacchini, P.

'89. Sui fenomeni di divisione delle cellule seminali primitive nella Rana temporaria. Rassegna Sc. Med., IV, '89.

Born, G.

'81. Experimentelle Untersuchungen über die Entstehung der Geschlechtsunterschiede. Breslauer ärztl. Zeitschr., No. 3, '81.

'82. Ueber Doppelbildungen beim Frosch und deren Entstehung. Breslauer ärztl. Zeitschr., No. 14, '82.

'83, a. Biologische Untersuchungen, I. Ueber den Einfluss der Schwere auf das Froschei. Pflüger's Archiv, XXXII, '83.

'83, b. Beiträge zur Bastardirung zwischen den einheimischen Anurenarten. Pflüger's Archiv, XXXII, '83.

'84, a. Ueber die inneren Vorgänge bei der Bastardbefruchtung der Froscheier. Breslauer ärztl. Zeitschr., No. 16, '84.

'84, b. Ueber den Einfluss der Schwere auf das Froschei. Verh. d. Med. Section d. Schles. Ges. f. vaterl. Cultur. April 4, '84.

'85. Biologische Untersuchungen über den Einfluss der Schwere auf das Froschei. Archiv f. Mikr. Anat., XXIV, '85.

'86. Weitere Beiträge zur Bastardirung zwischen den einheimischen Anuren. Archiv f. Mikr. Anat., XXVII, '86.

'87. Ueber die Furchung des Eies bei Doppelbildung. Breslauer ärztl. Zeitschr., No. 15, '87.

'92. Die Reifung des Amphibieneies und die Befruchtung unreifer Eier bei Triton tæniatus. Anat. Anz., VII, '92.

'93. Ueber Druckversuche an Froscheiern. Anat. Anz., VIII, '93.

'94, a. Die künstliche Vereinigung lebender Theilstücke von Amphibien-Larven. Jahresb. d. Schles. Ges. f. vaterl. Cultur. Med. Section. Juni 8, '94.

'94, b. Die Structur des Keimbläschens im Ovarialei von Triton tæniatus. Archiv f. Mikr. Anat., XLIII, '94.

'94, c. Neue Compressionsversuche an Froscheiern. Jahresb. d. Schles. Ges. f. vaterl. Cultur. Zool. Bot. Section. 10 Mai, '94.

Cramer, H.

'48. Bemerkungen über das Zellenleben in der Entwickelung des Froscheies. Müller's Archiv, '48.

Cucati, G.

'90. Spermatogenesi nella Rana esculenta. Anat. Anz., V, '90.

Driesch, H.

'95. Zur Analysis der Potenzen embryonaler Organzellen. Archiv f. Entwickelungsmechanik der Organismen, II, '95.

'96, a. Betrachtungen über die Organisation des Eies und ihre Genese. Archiv f. Entwickelungsmechanik der Organismen, IV, '96.

'96, b. Die Maschinentheorie des Lebens. Biol. Centralb., XVI, '96.

Durham.

'86. Note on the Presence of a Neurenteric Canal in Rana. Quart. Jour. Micr. Science, XXVI, '86.

Ecker, A.

'51. Icones physiologicæ, '51–'59.

Endres, H.

'94. Ueber Anstichversuche an Froscheiern. Jahresb. d. Schles. Ges. f. vaterl. Cultur. Zool. Bot. Section. Nov. 15, '94.

'95. Ueber Anstich- und Schnurversuche an Eiern von Triton tæniatus. Jahresb. d. Schles. Ges. f. vaterl. Cultur, '95.

Endres, H., und Walter, H. E.

'95. Anstichversuche an Eiern von Rana fusca. Archiv f. Entwickelungsmechanik d. Organismen, II, '95.

Von Erlanger, R.

'91, a. Zur Blastoporusfrage bei den anuren Amphibien. Anat. Anz., VI, '91.

'91, b. Ueber den Blastoporus der anuren Amphibien, sein Schicksal und seine Beziehungen zum bleibenden After. Zool. Jahrbücher. Abt. f. Anat. und Ontog., IV, '91.

Eycleshymer, A. C.

'92. Paraphysis and Epiphysis in Amblystoma. Anat. Anz., VII, '92.

'93. The Development of the Optic Vesicles in Amphibia. Jour. Morph., VIII, '93.

Fick, R.

'93. Ueber die Reifung und Befruchtung des Axolotleies. Zeitschr. f. wiss. Zool., LVI, '93.

Field, H. H.

'91. The Development of the Pronephros and Segmental Duct in Amphibia. Bull. Museum of Comp. Zoöl., XXI, '91.

'93. Ueber die Gefässversorgung und die allgemeine Morphologie des Glomus. Anat. Anz., VIII, '93.

'94. Die Vornierenkapsel, ventrale Musculatur und Extremitätenanlagen bei den Amphibien. Anat. Anz., IX, '94.

'95. Bemerkungen über die Entwickelung der Wirbelsäule bei den Amphibien; nebst Schilderung eines abnormen Wirbelsegmentes. Morph. Jahrbuch, XXII, '95.

Flemming, W.

'87. Neue Beiträge zur Kenntniss der Zelle. Archiv f. Mikr. Anat., XXIX, '87.

Fürbringer, M.

'77. Zur Entwickelung der Amphibienniere. Dissertation, '77.

'78. Zur vergleichenden Anatomie und Entwickelungsgeschichte der Excretionsorgane der Vertebraten. Morph. Jahrbuch, IV, '78.

Gurwitsch, A.

'95. Ueber die Einwirkung des Lithionchlorids auf die Entwickelung der Frosch- und Kröteneier (R. fusca und Bufo vulg.). Anat. Anz., XI, '95.

'96. Ueber die formative Wirkung des veränderten chemischen Mediums auf die embryonale Entwickelung. Archiv f. Entwickelungsmechanik d. Organismen, III, '96.

Gasser, E.

'82. Zur Entwickelung von Alytes obstetricans. Sitz-Ber d. Naturf. Ges. Marburg, No. 5, '82.

Gebhardt, W.

'94. Ueber die Bastardirung von Rana esculenta mit Rana arvalis. Dissertation, Breslau, '94.

Goette, A.

'75. Die Entwickelungsgeschichte der Unke, '75.

Von Griesheim, A.

'82. Künstliche Befruchtung der Eier von Rana fusca. Dissertation, '82.

Von Griesheim, A., Kochs, W., Pflüger, E.

'81. Beiträge zur Physiologie der Zeugung. Pflüger's Archiv, XXVI, '81.

Herlitzka, A.

'95. Contributo allo studio della capacità evolutiva dei due primi blastomeri nell' uovo di tritone (Triton cristatus). Archiv f. Entwickelungsmechanik d. Organismen, II, '95.

Héron Royer et Ch. van Bambeke.

'81. Sur les Caractères fournis par la bouche des Têtards des Batraciens anoures d'Europe. Bull. Soc. Zool. d. France, VI, '81.

Hertwig, O.

'77. Beiträge zur Kenntniss der Bildung, Befruchtung, und Theilung des thierischen Eies, II Theil. Morph. Jahrbuch, III, '77.

'82. Die Entwickelung des mittleren Keimblattes der Wirbelthiere. Jena. Zeitschr. f. Naturw., XV und XVI, '82–'83.

'85, a. Das Problem der Befruchtung und der Isotropie des Eies, eine Theorie der Vererbung. Jena. Zeitschr., XVIII, '85.

'85, b. Welchen Einfluss übt die Schwerkraft auf die Theilungen der Zellen. Jena. Zeitschr., XVIII, '85.

'85, c. Ueber das Vorkommen Spindeliger Körper im Dotter junger Froscheier. Morph. Jahrbuch, X, '85.

'92. Urmund und Spina bifida. Archiv f. Mikr. Anat., XXXIX, '92.

Hertwig, O. (*continued*).

'93, a. Experimentelle Untersuchungen über die ersten Theilungen des Froscheies und ihre Beziehungen zu der Organbildung des Embryos. Sitzungsb. d. k. Preuss. Akad. d. Wiss. zu Berlin, '93.

'93, b. Ueber den Werth der ersten Furchungszellen für die Organbildung des Embryo. Archiv f. Mikr. Anat., XLII, '93.

'94. Ueber den Einfluss äusserer Bedingungen auf die Entwickelung des Froscheies. Sitzungsb. d. k. Preuss. Akad. d. Wiss. zu Berlin, XVII, '94.

'95. Beiträge zur experimentellen Morphologie und Entwickelungsgeschichte, No. 1. Archiv f. Mikr. Anat., XLIV, '95.

Higgenbotham, J.

'50. Influence of Physical Agents on the Development of the Tadpole of the Triton and the Frog. Phil. Trans. Roy. Soc., London, '50.

'63. Influence des agents physiques sur le développement du tétard de la grenouille. Jour. de la Physiologie de l'homme et des animaux. VI, '63.

Hinckley, Mary H.

'82. Notes on the Development of Rana sylvatica. Proc. Boston Soc. Nat. History, '82.

His, W.

'74. Unsere Körperform, '74.

Hochstetter, F.

'94. Entwickelung des Venensystemes der Wirbelthiere. Ergebnisse der Anatomie und Entwickelungsgeschichte, III, '94.

Houssay, F.

'90. Études d'embryologie sur les vertébrés. L'Axolotl. Archiv de Zool. exper. et gen., (III), VIII, '90.

De l'Isle, A.

'73. De l'hybridation chez les amphibies. Ann. des sc. naturelles, XVII, '73.

Johnson, A.

'84. On the Fate of the Blastopore and the Presence of a Primitive Streak in the Newt (Triton cristatus). Quart. Jour. Micr. Science, XXIV, '84.

Johnson, A., and Sheldon, L.

'86. Notes on the Development of the Newt. Quart. Jour. Micr. Science, XXVI, '86.

Jordan, E. O., and Eycleshymer, A.

'92. The Cleavage of the Amphibian Ovum. Anat. Anz., VII, '92.

Jordan, E. O.

'93. The Habits and Development of the Newt. Jour. of Morph., VIII, '93.

Jordan, E. O., and Eycleshymer, A. C.

'94. On the Cleavage of Amphibian Ova. Jour. Morph., IX, '94.

Kolessnikow, N.

'78. Ueber die Eientwickelung bei Batrachiern und Knochenfischen. Archiv f. Mikr. Anat., XV, '78.

Kupffer, C.

'82. Ueber aktive Betheiligung des Dotters am Befruchtungsakte bei Bufo variabilis und vulgaris. Sitzungsb. d. math.-physik Classe d. k. b. Akad. d. Wiss. zu München, XII, '82.

Lataste, F.

'78. Tentatives d'hybridation chez les Batraciens anoures et urodèles. Bulletin de la Société zoologique de France, III, '78.

Lwoff, B.

'94. Die bildung der primären Keimblätter und die Enstehung der Chorda und des Mesoderms bei den Wirbelthieren. Bull. de la Soc. imper. des Naturalistes de Moscou, VIII, '94.

Marshall, A. M., and Bles, E. J.

'90. The Development of the Kidneys and Fat Bodies in the Frog. Stud. Biolog. Lab. Owens College, II, '90.

Marshall, A. M.

'90. The Development of the Blood Vessels in the Frog. Stud. Biolog. Lab. Owens College, II, '90.

'93. Vertebrate Embryology, '93.

McDonnell, B.

'57. Exposé de quelques expériences concernant l'influence des agents physiques sur le développement du tétard de la grenouille commune. Jour. de la Physiologie de l'homme et des animaux. (I), II, '57.

Massart, J.

'89. Sur la pénétration des spermatoides dans l'œuf de la grenouille. Bull. de l'Acad. Roy. des Sci. de Belgique, (III), XVIII, '89.

Maurer, F.

'88. Die Kiemen und ihre Gefässe bei Anuren und Urodelen Amphibien. Morph. Jahrbuch, XIV, '88.

Meves, F.

'91. Ueber amitotische Kerntheilung in den Spermatogonien des Salamanders. Anat. Anz., VI, '91.

'96. Ueber die Entwickelung der männlichen Geschlechtszellen von Salamandra maculosa. Archiv. f. Mikr. Anat., XLVIII, '96.

Moquin-Tandon, G.

'76. Recherche sur les Première Phases du Développement des Batraciens anoures. Ann. des sciences naturelles, (VI), III, '76.

Morgan, T. H.

'89. On the Amphibian Blastopore. Studies from the Biol. Lab. Johns Hopkins Univ., IV, '89.

'91. Some Notes on the Breeding Habits and Embryology of Frogs. American Naturalist, XXV, '91.

'94. The Formation of the Embryo of the Frog. Anat. Anz., IX, '94.

Morgan, T. H. (*continued*).

'95. Half-Embryos and Whole-Embryos from one of the first two Blastomeres of the Frog's Egg. Anat. Anz., X, '95.

Morgan, T. H., and Tsuda Umé.

'93. The Orientation of the Frog's Egg. Quart. Jour. Micr. Science, XXXV, '93.

Newport, G.

'51. On the Impregnation of the Ovum in the Amphibia. Phil. Trans. Roy. Soc., London, '51.

Nussbaum.

'93. Zur Entwickelungsgeschichte der embryonalen Gefässendothelien und der Blutkörperchen bei den Anuren (Rana temporaria). Abh. Akad. der Wiss. in Krakau, XXII. (Reprinted in Biol. Centralblatt, XIII, '93.)

Nussbaum, M.

'95. Zur Mechanik der Eiablage bei Rana fusca. Archiv f. Mikr. Anat., XLVI, '95.

Orr, H.

'88. Note on the Development of Amphibians. Quart. Jour. Micr. Science, XXIX, '88.

Von Perényi, J.

'89. Die Entwickelung der Keimblätter und der Chorda in neuer Beleuchtung. Anat. Anz., IV, '89. (Page 587.)

Pflüger, E.

'82. I. Hat die Concentration des Samens einen Einfluss auf das Geschlecht? II. Ueber die das Geschlecht bestimmenden Ursachen und die Geschlechtverhältnisse der Frösche. III. Ueber die parthenogenetische Furchung der Eier der Amphibien. IV. Wirkt der Saft nicht brünstiger Männchen befruchtend? V. Die Bastardzeugung bei den Batrachien. VI. Versuche der Befruchtung überreifer Eier. VII. Zur Entwickelungsgeschichte der Geburtshelferkröte (Alytes obstetricans). Pflüger's Archiv, XXIX, '82.

'83. Ueber den Einfluss der Schwerkraft auf die Theilung der Zellen. I, II, III, Theil. Pflüger's Archiv, XXXI, XXXII, '83.

'84. Ueber die Einwirkung der Schwerkraft und anderer Bedingungen auf die Richtung der Zelltheilung. Pflüger's Archiv, XXXIV, '84.

Pflüger, E., und Smith, W. J.

'83. Untersuchungen über Bastardirung der anuren Batrachier und die Principien der Zeugung. Pflüger's Archiv, XXXII, '83.

Ploetz, A. J.

'90. Die Vorgänge in den Froschhoden unter dem Einfluss der Jahreszeit. Archiv f. Anat. u. Phys., (Supplement-Band) '90.

Prévost et Dumas.

'24. Troisième Mémoire. De la génération dans les Mammifers. Ann. des sc. naturelles, II, '24.

Rabl, C.

'86. Ueber die Bildung des Herzens der Amphibien. Morph. Jahrbuch, XII, '86.

Vom Rath, O.

'93. Beiträge zur Kenntniss der Spermatogenese von Salamander maculosa. Zeitschr. f. wiss. Zool., LVII, '93.

Rauber, A.

'82. Neue Grundlegungen zur Kenntnis der Zelle. Morph. Jahrbuch, VIII, '82.

'83. Furchung und Achsenbildung der Wiebelthiere. Zool. Anz., VI, '83.

'86, a. Personaltheil und Germinaltheil des Individuum. Zool. Anz., IX, '86.

'86, b. Furchung und Achsenbildung. II. Zool. Anz., IX, '86.

Reichert, K. B.

'41. Ueber den Furchungsprocess der Batrachier-Eier. Müller's Archiv, '41.

'46. Der Furchungsprocess und die sogenannte Zellenbildung um Inhaltsportionen. Müller's Archiv, '46.

'61. Der Faltenkranz an den beiden ersten Furchungskugeln des Froschdotters und seine Bedeutung für die Lehre von der Zelle. Müller's Archiv, '61.

Remak, R.

'55. Untersuchung über die Entwickelung der Wirbelthiere, '55.

Robinson, A., and Assheton, R.

'91. The Formation and Fate of the Primitive Streak, with Observations on the Archenteron and Germinal Layers of Rana temporaria. Quart. Jour. Micr. Science, XXXII, '91.

Rossi, U.

'90. Contributo alla maturazione delle uova degli Amfibii. Anat. Anz., V, '90.

Roux, W.

'83. Ueber die Zeit der Bestimmung der Hauptrichtungen des Froschembryo, '83.

'84–'91. Beiträge zur Entwickelungsmechanik des Embryo.

No. I. Zur Orientirung über einige Probleme der embryonalen Entwickelung. Zeitschr. f. Biologie, XXI, '85.

No. II. Ueber die Entwickelung der Froscheier bei Aufhebung der richtenden Wirkung der Schwere. Breslauer ärztl. Zeitschr., '84.

No. III. Ueber die Bestimmung der Hauptrichtungen des Froschembryo im Ei und über die erste Theilung des Froscheies. Breslauer ärztl. Zeitschr., '85.

No. IV. Die Bestimmung der Medianebene des Froschembryo durch die Copulationsrichtung des Eikernes und des Spermakernes. Archiv f. Mikr. Anat., XXIX, '87.

Roux, W. (*continued*).

No. V. Ueber die künstliche Hervorbringung halber Embryonen durch Zerstörung einer der beiden ersten Furchungskugeln sowie über die Nachentwickelung (Postgeneration) der fehlenden Körperhälfte. Virchow's Archiv, CXIV, '88.

No. VI. Ueber die morphologische Polarisation von Eiern und Embryonen durch den electrischen Strom. Sitzungsb. d. k. Akad. Wiss. in Wien, CI, '91.

'88, a. Ueber die Lagerung des Materials des Medullarrohres im gefurchten Froschei. Verhandlungen d. Anat. Gesell. zu Würzburg, '88; also Anat. Anz., III, '88.

'88, b. Zur Frage der Axenbestimmung des Embryo im Froschei. Biol. Centralb., VIII, '88.

'89, a. Die Entwickelungsmechanik der Organismen, eine anatomische Wissenschaft der Zukunft. Festrede, '89.

'89, b. Ueber die Entwickelung der Extraovates der Froscheier. Jahresb. d. Schles. Ges. f. vaterl. Cultur, '89.

'92, a. Ziele und Wege der Entwickelungsmechanik. Merkel-Bonnet's Ergebnisse der Anatomie und Entwickelungsgeschichte, II, '92.

'92, b. Ueber das entwickelungsmechanische Vermögen jeder der beiden ersten Furchungszellen des Eies. Verhandl. d. Anat. Gesellschaft Wien, '92.

'93, a. Ueber Mosaikarbeit und neuere Entwickelungshypothesen. Anat. Hefte, Merkel und Bonnet, '93.

'93, b. Ueber die Spezifikation der Furchungszellen und über die bei der Postgeneration und Regeneration anzunehmenden Vorgänge. Biol. Centralb., XIII, '93.

'93, c. Ueber die ersten Theilungen des Froscheies und ihre Beziehungen zu der Organbildung des Embryo. Anat. Anz., VIII, '93.

'93, d. Ueber die Selbstordnung der Furchungszellen. Berichte des naturw.-med. Vereins zu Innsbruck, XXI, '93.

'94, a. Die Methoden zur Hervorbringung halber Froschembryonen und zum Nachweis der Beziehung der ersten Furchungsebenen des Froscheies zur Medianebene des Embryo. Anat. Anz., IX, '94.

94, b. Ueber den Cytotropismus der Furchungszellen des Grasfrosches (Rana fusca). Archiv f. Entwickelungsmechanik der Organismen, I, '94.

'95. Gesammelte Abhandlungen über Entwickelungsmechanik der Organismen, '95.

'96, a. Ueber die Selbstordnung (Cytotaxis) sich "berührender" Furchungszellen des Froscheies durch Zellenzusammenfügung, Zellentrennung und Zellengleiten. Archiv f. Entwickelungsmechanik der Organismen, III, '96.

'96, b. Ueber die Bedeutung "geringer" Verschiedenheiten der relativen Grösse der Furchungszellen für den Charakter des Furchungsschemas. Archiv f. Entwickelungsmechanik der Organismen, IV, '96.

Rückert, J., und Mollier, W.

'89. Resultate über die Entstehung des Vornierensystem bei Triton, Rana und Bufo. Sitz. Ber. d. Ges. für Morph. u. Phys. in München, XIX, '89.

Rusconi, M.

'26. Développement de la grenouille commune, '26.

'36. Zweiter Brief an E. H. Weber. Müller's Archiv, '36.

'40. Ueber künstliche Befruchtung von Fischen und über einige neue Versuche in Betreff künstlicher Befruchtung an Fröschen. Müller's Archiv, '40.

'54. Histoire naturelle, développement et metamorphose de la Salamandre terrestre, '54.

Samassa, P.

'95. Studien über den Einfluss des Dotters auf die Gastrulation und die Bildung der primären Keimblätter der Wirbelthiere. II. Amphibien. Archiv f. Entw.-mechanik d. Organismen, II, '95.

Schanz, F.

'87. Das Schicksal des Blastoporus bei den Amphibien. Jena. Zeitschr. f. Naturwissenschaft, XXI, '87.

Schmidt, V.

'93. Das Schwanzende der Chorda dorsalis bei den Wirbelthieren. Anat. Hefte, II, '93.

Schnetzler, J. B.

'74. De l'influence de la lumière sur le développement des larves de grenouilles. Arch. des sciences physiques et naturelles, LI, '74.

Schultze, M.

'63. Observationes nonnullæ de ovorum ranarum segmentatione, '63.

Schultze, O.

'83. Beiträge zur Entwickelung der Batrachien. Archiv f. Mikr. Anat., XXIII, '83.

'86. Ueber Reifung und Befruchtung des Amphibieneies. Anat. Anz., I, '86.

'87, a. Zur Entwickelung des braunen Grasfrosches. Festschrift f. Kölliker, '87.

'87, b. Die vitale Methylenblaureaction der Zellgranule. Anat. Anz., '87.

'87, c. Zur ersten Entwickelung des braunen Grasfrosches (Ref. Roux). Biol. Centralb., VII, '87.

'87, d. Ueber Axenbestimmung des Froschembryo. Biol. Centralb., VII, '87.

'87, e. Untersuchungen über die Reifung und Befruchtung des Amphibieneies. Zeitschr. f. wiss. Zool., XLV, '87.

'88. Die Entwickelung der Keimblätter und der Chorda dorsalis von Rana fusca. Zeitschr. f. wiss. Zool., XLVII, '88.

'89. Ueber die Entwickelung der Medullarplatte des Froscheies. Verh. d. phys. med. Gesellschaft, Würzburg, XXIII, '89.

Schultze, O. (*continued*).

'94, a. Ueber die unbedingte Abhängigkeit normaler thierischer Gestaltung von der Wirkung der Schwerkraft. Verh. d. Anat. Ges., VIII, '94.

'94, b. Die künstliche Erzeugung von Doppelbildungen bei Froschlarven mit Hilfe abnormer Gravitationswirkung. Archiv f. Entwickelungsmechanik der Organismen, I, '94.

'94, c. Ueber die Einwirkung niederer Temperatur auf die Entwickelung des Frosches. Anat. Anz., X, '94.

'94, d. Ueber die Bedeutung der Schwerkraft für die organische Gestaltung sowie über die mit Hilfe der Schwerkraft mögliche künstliche Erzeugung von Doppelmissbildungen. Verh. phys. med. Ges. zu Würzburg, XXVIII, '94.

Schwink, F.

'88. Ueber die Gastrula bei Amphibieneiern. Biol. Centralb., VIII, '88–'89.

'89. Ueber die Entwickelung des mittleren Keimblattes und der Chorda dorsalis der Amphibien. München, '89.

'90. Ueber die Entwickelung des Herzenendothels der Amphibien. Anat. Anz., V, '90.

'91. Untersuchungen über die Entwickelung des Endothels und der Blutkörperchen bei Amphibien. Morph. Jahrb., XVII, '91.

Sidebotham, H.

'88. Note on the Fate of the Blastopore in Rana temporaria. Quart. Jour. Micr. Science, XXIX, '88.

Spallanzani, L.

1785. Expérience pour servir à l'histoire de la génération, 1785.

Spencer, W. B.

'85. On the Fate of the Blastopore in Rana temporaria. Zool. Anz., VIII, '85.

'85. Some Notes on the Early Development of Rana temporaria. Quart. Jour. Micr. Science, XXV, '85.

Stahl, E.

'88. Pflanzen und Schnecken. (*Jena,*) '88.

Stricker, S.

'60. Entwickelungsgeschichte von Bufo cinereus bis zum Erscheinen der äusseren Kiemen. Sitzungsb. d. k. Akademie der Wiss. zu Wien, XXXIX, '60.

'62. Untersuchungen über die ersten Anlagen in Batrachier-Eiern. Zeitschr. f. wiss. Zool., XI, '62.

Swammerdam, J.

1737. Die Bibel der Natur, 1737.

V. la Valette St. George.

'75. Die Spermatogenese bei den Amphibien. Archiv f. Mikr. Anat., XII, '75.

'86. Spermatologische Beiträge. Dritte Mittheilung. Archiv f. Mikr. Anat., XXVII, '86.

Vogt, K.

'42. Untersuchungen über die Entwickelungsgeschichte der Geburtshelferkröte, '42.

Wetzel, G.

'95. Ueber die Bedeutung der Cirkulären Furche in der Entwickelung der Schultzeschen Doppelbildungen von Rana fusca. Archiv f. Mikr. Anat., XLVI, '95.

'96. Beitrag zum Studium der künstlichen Doppelmissbildungen von Rana fusca. Inaugural Dissertation. Berlin, '96.

Will, L.

'84. Ueber die Entstehung des Dotters und der Epithelzellen bei den Amphibien und Insecten. Zool. Anz., VII, '84.

Wilson, C. B.

'96. The Wrinkling of Frog's Eggs during Segmentation. American Naturalist, XXX, '96.

Yung, E.

'78. Influence des différentes couleurs du spectre sur le développement des animaux. Arch. de zool. experimentale et générale, (I,) VII, '78, and Arch. des sciences physiques et naturelles, '78.

'81. De l'influence des lumières colorées sur le développement des animaux. Mittheil. a. d. zool. Station zu Neapel, II, '81.

'90. Propos scientifiques, '90.

Ziegler, F.

'92. Zur Kenntniss der Oberflächenbilder der Rana-Embryonen. Anat. Anz., VII, 92.

OTHER MEMOIRS REFERRED TO IN TEXT

Boveri, Th.

'89. Ein geschlechtlich erzeugter Organismus ohne mütterliche Eigenschaften. Sitz. d. Ges. f. Morph. u. Physiol. zu München, '89. (Translated in American Naturalist, March, '93.)

Chun, B.

'92. Die Dissogonie der Rippenquallen. Festschrift f. Leuckart, '92.

Clapp, C. M.

'91. Some Points on the Development of the Toad-fish (Batrachus Tau). Jour. Morph., V, '91.

Driesch, H.

'91–'93. Entwickelungsmechanische Studien.

No. I. Der Werth der beiden Furchungszellen der Echinodermentwickelung. Zeitschr. f. wiss. Zool., LIII, '91.

No. II. Ueber die Beziehungen des Lichtes zur ersten Etappe der thierischen Formbildung. Zeitschr. f. wiss. Zool., LIII, '91.

No. III. Die Verminderung des Furchungsmaterials und ihre Folgen. Zeitschr. f. wiss. Zool., LV, '92.

Driesch, H. (*continued*).

No. IV. Experimentelle Veränderungen des Typus der Furchung und ihre Folgen. Zeitschr. f. wiss. Zool., LV, '92.

No. V. Von der Furchung doppeltbefruchteter Eier. Zeitschr. f. wiss. Zool., LV, '92.

No. VI. Ueber einige allgemeine Fragen der theoretischen Morphologie. Zeitschr. f. wiss. Zool., LV, '92.

No. VII. Exogastrula und Anenteria. Mittheil. a. d. zool. Station zu Neapel, XI, '93.

No. VIII. Ueber Variation der Mikromerenbildung. Mittheil. a. d. zool. Station zu Neapel, XI, '93.

No. IX. Ueber die Vertretbarkeit der Anlagen von Ektoderm und Endoderm. Mittheil. a. d. zool. Station zu Neapel, XI, '93.

No. X. Ueber einige allgemeine entwickelungsmechanische Ergebnisse. Mittheil. a. d. zool. Station zu Neapel, XI, '93.

'92. Entwickelungsmechanisches. Anat. Anz., VII, '92.

'93, a. Zur Theorie der thierischen Formbildung. Biol. Centralb., XIII, '93.

'93, b. Zur Verlagerung der Blastomeren des Echinideneies. Anat. Anz., VIII, '93.

'94. Analytische Theorie der Organischen Entwickelung, '94.

Driesch, H., und Morgan, T. H.

'95. Zur Analysis der ersten Entwickelungsstadien des Ctenophoreneies. Archiv f. Entwickelungsmechanik d. Organismen, II, '95.

Hertwig, O.

'90. Vergleich der Ei- und Samenbildung bei Nematoden. Archiv f. Mikr. Anat., XXXVI, '90.

'94. Zeit- und Streitfragen der Biologie, '94.

Hertwig, O., und Hertwig, R.

'87. Ueber den Befruchtungs- und Theilungs-vorgang des Thierischen Eies unter dem Einfluss äusserer Agentien. Jena. Zeitschr. Naturw., XX, '87.

His, W.

'94. Ueber mechanische Grundvorgänge thierischer Formbildung. Archiv f. Anat. u. Phys., '94.

Morgan, T. H.

'93. Experimental Studies on Teleost Eggs. Anat. Anz., VIII, '93.

'95. Studies of the "Partial" Larvæ of Sphærechinus. Archiv f. Entwickelungsmechanik d. Organismen, II, '95.

Rabl, C.

'89. Die Theorie des Mesoderms. Morph. Jahrbuch, XV, '89.

Vom Rath, O.

'92. Zur Kenntniss der Spermatogenese von Gryllotalpa vulgaris. Archiv f. Mikr. Anat., XL, '92.

'95. Neue Beiträge zur Frage der Chromatinreduction in der Samen- und Eireife. Archiv f. Mikr. Anat., XLVI, '95.

Rauber, A.

'80. Formbildung und Formstörung. Morph. Jahrbuch, VI, '80.

Sachs.

'92. Die Anordnung der Zellen in jüngsten Pflanzentheilen. Arbeiten d. botan. Institute in Würzburg, II, '92.

Schwann, Th.

'39. Mikroskopische Untersuchungen über die Uebereinstimmung in der Structur und Wachsthum der Thiere und Pflanzen, '39.

Weismann, A.

'92. Das Keimplasma. Eine Theorie der Vererbung. '92.

Whitman, C. O.

'95. The Inadequacy of the Cell Theory of Development. Jour. Morph., VIII, '93.

Wilson, E. B.

'92. The Cell Lineage of Nereis. Jour. Morph., VI, '92.

'93. Amphioxus and the Mosaic Theory. Jour. Morph., VIII, '93.

Zoja, R.

'95. Sullo svilluppo dei blastomeri isolati dalle uova di alcune meduse (e di altri organismi). Archiv f. Entwickelungsmechanik d. Organismen, I, '95.

INDEX