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**Contributions to the Embryology of Amphibia:—
The Mode of Blastopore Closure and the
Position of the Embryonic Body.**

By

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With Plates I—IV.

Although the earlier phases of the amphibian development have been studied within recent years by many able investigators such as PFLÜGER ('83), ROUX ('88*a*), O. HERTWIG ('92, '95*b*), O. SCHULZE ('88*b.c.*), MORGAN and TSUDA ('93*b*), KOPSCH ('90), ASSHETON ('94*a*) and EYCLESYMER ('98), it is a remarkable fact that some of the most fundamental points are still far from being settled. Above all, the questions: (1) in what manner the blastopore is closed, and (2) on what portion of the egg-surface, the fundamental parts of the embryonic body are formed, have been answered by many different writers in as many different ways. The opinions held in regard to them can, however, be classified into three categories:—

(1). The blastopore is closed by the coalescence of the lateral lips (Concrescence theory), and the fundamental parts of the embryonic body are formed upon the lower hemisphere of the egg. (PFLÜGER, ROUX, HERTWIG, MORGAN, *et al.*).

(2). The blastopore is closed, mostly by the overgrowth of the ventral lip, and the fundamental parts of the embryonic body are formed upon the upper hemisphere of the egg. (O. SCHULZE; and others who accept the old views).

(3). The blastopore is closed by the overgrowth of the dorsal, ventral, and lateral lips, and the fundamental parts of the embryonic body are formed, partly upon the upper, and partly upon the lower hemisphere of the egg. (ASSHETON, KOPSCH, EYCLESHYMER, *et al.*).

As is well known, the facts on which the views of many of these writers are based, were obtained either from experimental study or from abnormally developing eggs (*spina bifida*, etc.). It has always seemed to me that one ought to exercise extreme caution in making use of this class of facts and that after all, the best way of studying normal processes of development would be to study normal eggs by some method which would not interfere with the normal course of events. For some years past I had thought that the eggs of our *Rhacophorus schlegelii* would furnish peculiarly favourable material for the investigation of the questions under discussion. As I have already stated in my former paper,¹⁾ the eggs of this animal are absolutely without pigment, and among other favorable peculiarities, allow the invagination cavity to be seen through faintly. The "Wachsthumsrand," or the "Equatorial Zone" comparable to the "Germinal Ring" of the fish-egg, may be recognized

1) *Annotationes Zoologicae Japonenses*, Vol. I. Part III, (1897).

from the end of the blastula stage, spreading gradually upwards and downwards, until finally it covers the entire surface of the egg. And the fundamental parts of the embryonic body appear certainly to be formed within this spreading zone. My desire to subject these eggs to a thorough study by some new and suitable method was realized in the spring of 1899 and of 1900 by using, at the suggestion of Prof. K. MITSUKURI, the "Prismen-Rotator"¹⁾ of ZEISS, obtained by him and kindly placed at my disposal. This instrument, as is probably well known, and as is fully described in the place cited, enables one to study the upper and lower views of an egg as well as the lateral view of it from any point in 360° of its circumference, without once touching the egg and thus without the fear of calling forth abnormalities by handling. The results of this study, together with some additional matter, are recorded in the following pages; and although I am not so sanguine as to think that I have found answers to the questions propounded satisfactory to all, yet I hope that the facts set forth will be received as some contributions toward the final settlement of the problems.

For the sake of convenience, I divide the present article into four parts:—

I. General Account of External Developmental Changes in *Rhacophorus* Eggs.

II. Observations upon Eggs of *Rhacophorus*, *Rana*, and *Bufo* fixed on the Prismen Rotator of ZEISS.

III. Explanation of the Facts observed in the Second Part by Changes in the Interior of the Eggs brought out in Sections.

IV. Experiments by Puncture of Eggs.

1) Zeit. f. wiss. Mikrosk. Bd. XIV. p. 304.

Before going further, I wish to express my heartfelt thanks to Prof. K. MITSUKURI for much invaluable advice and for his kindness in working over the manuscripts of the present article.

I.

GENERAL ACCOUNT OF EXTERNAL DEVELOPMENTAL CHANGES IN *RHACOPHORUS* EGGS.

During several years past, I have frequently gone over the external changes which take place in the eggs of *Rhacophorus*. Some features which are peculiar to this species are certainly remarkable, and seem to me to deserve special notice from the point of view of comparative embryology. Some of these, as well as the general habits and the mode of the egg-deposition of this animal, I have already described briefly in my former paper (*loc. cit.*). I may, however, add a few more facts here.

As already stated, the eggs of this animal are absolutely without pigment and are placed in a frothy mass, concealed in subterranean cavities in the muddy banks of paddy-fields, ponds, and other shallow bodies of water. Deposition takes place mostly at night, during the breeding season which extends from April to May. It is an interesting fact that in the earlier part of the season, the animal deposits its eggs mostly on slopes which face east, south, or south-east, while toward the end of the season, when the atmospheric temperature has become mild, any spot favorable for deposition, even if it faces north or north-east, is indifferently chosen.

The eggs just deposited are extremely delicate and flabby,

and the frothy envelope is strongly viscous. The eggs, however, become gradually firmer in texture and are ready to begin segmentation in 4-5 hours after deposition.

During the breeding season of 1899, I kept a pair of the animals in a glass-flask on my table, in the manner already described in my former paper, and obtained a number of eggs from them. Another pair kept in a glass-cup and carried about for three days in my pocket deposited only a limited number of eggs. These eggs as well as those produced by the first-mentioned pair showed more or less abnormality in their development—due, it seems to me, to the forced arresting of oviposition and to the unnatural treatment to which the eggs had been subjected. Even the eggs deposited in a natural position are apt to show some abnormalities if they are subjected to unfavorable treatment. This tendency to abnormalities is not, however, peculiar to the eggs of this animal. So far as my experiences go, those of other Amphibians living in Japan (such as *Rana japonica* or *Bufo japonica*) show themselves similarly sensitive even to slightly altered conditions in their environment. I am now inclined to think that the process of segmentation in *Rhacophorus* eggs as described in my former paper is in some respects abnormal, as the eggs then observed were deposited by a pair of animals in captivity.

The first and second cleavage-lines make the figure of a cross, as is usual in amphibian eggs (Figs. 1-3). The third cleavage plane appears, in most eggs, symmetrically upon the second cleavage-line at nearly equal distances from the crossing point of the first two cleavage-lines (Fig. 4). Thus, the third cleavage must be considered as in a vertical, rather than in the horizontal plane, (to which I have previously referred it), and shows itself in four separate lines starting from the second cleavage-line

toward the egg periphery. In some cases, however, the third cleavage-line appears to encircle horizontally a small area around the upper pole of the egg as recorded in my former paper. There are many other individual differences with respect to the third cleavage. The fourth cleavage appears, in general horizontally encircling an area which takes in about two-thirds of the upper hemisphere (Figs. 5 and 6). It does not appear as a continuous line, but as seven or eight separate lines or pits upon the earlier (first, second, and third) cleavage-lines, and at nearly equal distances from the upper pole. The segmentation-lines which appear later than this are very irregular and are confined mostly within the circle formed by the fourth cleavage-line. Most of the later cleavage-lines including even the second and third can not be traced clearly to the lower pole of the egg. Indeed it is the first only which can be distinctly seen to reach the lower pole. In living eggs of later stages, all the cleavage-lines become superficially indistinct in the lower hemisphere while they are distinct in the upper hemisphere (Fig. 7), so that the eggs of these stages present the appearances of meroblastic forms, though when examined in sections they are seen to be truly holoblastic.

During the earlier part of the season, segmentation is finished at the end of the second day after deposition. Later, with the rise in the atmospheric temperature, the process is gone through faster, so that it is finished at the end of the first day after deposition or even in less time. Toward the end of the segmentation process, a part of the the upper hemisphere of the egg becomes gradually translucent (Fig. 8). This area which is, as a rule, circumscribed by a circular depression (Fig. 9) occupies from the first nearly two-thirds of the upper half, and corresponds approximately to the circle enclosed by the fourth

cleavage-line. As I have assured myself by a study of sections, it is the superficial expression of the segmentation-cavity within, which is seen through its very thin roof. The size of this area, therefore, indicates, in every stage, the extension of the cavity within, and naturally diminishes gradually with the progress of development, until it vanishes entirely, with the advancing closure of the blastopore (Figs. 9-14). For the sake of convenience, I shall refer to this translucent area hereafter as the "area of the segmentation cavity," and to the area encircled by the blastopore lips as the "blastoporic area."

In 24-48 hours after deposition, the dorsal lip of the blastopore makes its first appearance close below the egg-equator. Although there are many individual differences, this is seen, in most cases, 10° - 20° below the equator. In some rare cases, it may approach to within 4° or 5° of the equator, while in other cases, it rarely is as far as 25° below. These cases were found mostly among eggs which had been taken out of their natural frothy envelope and reared in water. According to my experiences, however, these slight individual variations seem to make no serious differences in the development of the future animal, and indeed it seems to me that the eggs of Amphibia are endowed with a strong power of adaptation and resistance to unnatural and injurious influences.

The encircling of the egg by the blastopore lips is generally accomplished in 3-10 hours, and the complete closure of the same in 15-24 hours after the first appearance of the dorsal lip, varying according to the condition of the atmospheric temperature. The gradual diminution in the size of the translucent area of the segmentation cavity proceeds in the normally growing eggs *pari passu* with the gradual closure of the blastopore lips, so that by

the time the lips have entirely encircled the subequatorial region, the former is reduced to about two-thirds of its former dimensions (Fig. 10).

Between the translucent area above, and the blastopore lips below, a broad opaque band may be recognized encircling the equatorial zone of the egg. It gradually spreads upwards and downwards concurrently with the gradual diminution of the blastoporic area and of the area of the segmentation-cavity (Figs. 9-13). This I shall call the "Equatorial Zone." I am inclined to think that it corresponds to the embryonic zone found in the eggs of Selachii, Teleostei and other groups of fishes. It is not, however, so prominent, in normal eggs, as it is said to be by some investigators in other amphibian eggs under the name of the "Embryonic Ring," the "Medullar Ring," or the "Unwachsungs-rände." It is true that in some abnormal eggs, in which the blastoporic area and the area of the segmentation cavity have been disturbed in any way, it may become as prominent as it has been figured by these writers. In such cases, the neural plate may also often become more conspicuous than usual, and the correlation which is ordinarily found between the reduction in size of the area of the segmentation cavity and of the blastoporic area may be lost. By an examination of sectioned eggs, I have assured myself that the equatorial zone is, in the first stage of gastrulation, nothing but a simple accumulation of blastoderm-cells around the egg-equator.

When the equatorial zone has come to occupy about the middle two-thirds of the egg-surface, the neural plate may be faintly detected by two shady lines within the zone, with a slightly projected anterior margin (the projecting tongue of EYCLESHYMER ['98] found in *Amblystema*) (Figs. 10 and 11). In some eggs,

a slight notch may be noticed at the middle point of the dorsal blastopore-lip. But such eggs can not be perfectly normal, I think, for they show sooner or later some abnormalities. In eggs which develop normally, the dorsal lip is always entire and the blastopore is a perfect circle in shape, until it is completely closed.

Up to this stage, the egg always rests on about the middle point of the blastoporic area with the translucent area of the segmentation cavity on the exact top. As the closure of the blastopore progresses, the resting-point of the egg is gradually shifted toward the ventral face of the future embryo. Thus, when we look at an egg at about the end of the gastrulation process, the now much reduced blastopore may be seen on the dorsal side of the embryo, nearer the equator of the egg than before, (Fig. 12-14). At the same time the greatly reduced translucent area of the segmentation cavity will be seen to have shifted anteriorly, in front of the future embryo.

Such a change in the position of the blastopore may be considered as due to the overgrowth of the ventral blastopore lip being greater than that of the dorsal lip, and to the eccentric closure of the blastopore thus brought about. But careful observations have convinced me that here there is a real rotation of the egg as a whole on its horizontal axis. In this, I find myself in agreement with many of my predecessors. But I am unable to follow PFLÜGER ('83), ROUX ('88*a*), MORGAN (97), *et al.*, when they state that the dorsal lip of the blastopore grows over the lower yolk hemisphere about 170° or 180° from the spot of its first appearance before the rotation of the egg takes place. Observations which I shall record in the sequel incline me strongly to the view that in normal eggs, the dorsal lip does not grow over the yolk hemisphere beyond its middle point, (the yolk pole in the

strict sense), and soon begins to move back again, to what is apparently its first starting point, *by the gradual rotation of the egg as a whole.*

At a certain stage of this rotation, the barely perceptible neural plate may be seen in a vertical position with the future head-end upward (Figs. 11 and 12), for the axis of the plate is shorter than the diameter of the egg. Even in later stages, the embryonic body does not lie in an exactly horizontal position but always at certain angles of inclination to the horizontal axis of the egg.

The neural groove is, in most eggs, to be perceived in 40–50 hours after the egg-deposition, while the neural folds appear somewhat later, becoming distinct only 10–20 hours later according to temperature. These folds are closely approximated one to the other from their first appearance.

As I have already stated in my former paper, the great peculiarity in the development of this species is that the head as well as the remaining parts of the body are up to certain late stages flattened out on the spherical mass of the yolk. Accordingly, the hyomandibular arches on both sides are strongly depressed and situated along the latero-frontal sides of the head, while the heart which usually appears below the head in anuran eggs, is here situated *in front of* the same. The body of the curved embryo is raised less than in other species and is so speak wedged into the yolk mass, along the posterior and dorsal surface of the egg. All this reminds one strongly of what is seen in the eggs of the Ganoid fishes.

During the fifth day or the following night, the neural groove is completely closed by the coming together of the neural folds. At this stage (Fig. 15), there is around the closed blastopore a

circumjacent translucent zone which denotes the extent of the peristomal mesoblast. Now follows the gradual rising up of the embryonic body over the general surface of the spherical egg. First, the posterior portion is raised, and then the head-portion, carrying with it both the hyoid and mandibular arches which thus come to occupy the lateral and ventral portion as is usual with other forms (Fig. 16). At such stages, some of the mesoblastic somites have already been formed, these appearing first at the neck-region and gradually increasing in number in the posterior direction. The general outline of the pronephric duct may also be recognized externally, on both sides of the foremost two or three somites.

In this species, the embryo may grow to the perfect larval form within the gradually expanded vitelline membrane. The gradually growing tail may elongate and lie coiled mostly on the left side of the egg, while the head is being gradually raised. Fig. 17 represents a larva, on the eighth day after deposition, forced out of the vitelline membrane by needles. Before being taken out, it could be seen moving about in the slightly distended envelope. The yolk which is now covered by the skin is still quite spherical, and on its dorsal surface the main part of the trunk is wedged in, so that the general aspect of the larva in this stage resembles that of some teleostean embryo.

The larva, growing further, acquires gradually the usual tadpole shape. Fig. 18 represents a tadpole which, on the tenth day after deposition, had but lately hatched out of the vitelline membrane and was moving about within the partly melted frothy substance. The dendritic gills are already formed, and though small are larger than those of *Rana* or *Bufo* at the corresponding stage. Blood-circulation in the gill-filaments and the pulsation of the heart may be clearly seen from without under a dissecting

microscope. Also a small amount of blue-black pigment makes its first appearance in the pectoral region at this stage.

Fig. 19 represents a greatly advanced tadpole on the eleventh day after deposition. At this stage, the larvæ which measure $1\frac{1}{2}$ –2 cm. in length are already escaped into the water together with the melted frothy substance. They are now sprinkled with a large amount of pigment not only at the pectoral region but also along the dorsal surface of the head and of the thoracic region. The blood-vessels on the surface of the yolk-mass and along the mid-lateral line of the larval body are conspicuous. In the fully grown tadpole, however, the blood-vessels as well as the heart become entirely invisible, on account of the dense pigment accumulated as usual within the skin. The suctorial discs are rather rudimentary and appear late as a pair of small papilla-like spots in front of the mandibular arches. Such incomplete formation and retardation in appearance of the suctorial discs are probably due to the fact that the eggs are deposited underground and out of water. Only when the tadpoles escape into the water, is the necessity of some attaching organ felt.

Recently BUDGETT ('99) has called attention to the probable similarity in development of *Rhacophorus* as given by myself in my former paper and of *Phyllomedusa hypobranchialis* as described by him. He says: “* * * from what he (Ikeda) mentions of the appearance of the embryo which develops in a froth much as is the case with *Palidiocola*, I think the development of this form will be found quite like that of *Phyllomedusa*.” Basing my judgment on the description and figures of *Phyllomedusa* given by BUDGETT, I may perhaps point out the points of similarity and difference between that genus and *Rhacophorus*, some of which are by no means insignificant.

In *Phyllomedusa*, segmentation seems to approach the typical holoblastic mode much more closely than in other anurans, the blastomeres of the upper and lower hemispheres showing less difference in size. In *Rhacophorus*, quite the contrary is the case, segmentation approximating more to the meroblastic type as already stated. Again, the head of the *Rhacophorus* embryo when raised over the general surface of the egg is not proportionally as large as that of *Phyllomedusa* as figured by BUDGETT. Also, the gills are not so enormously large in the Japanese genus as in the South American. The embryo of *Rhacophorus* as a whole is rather small in comparison with the yolk-mass which remains almost always spherical in shape. Moreover, the main part of the embryonic body when seen in sections is wedged into the yolk along its dorsal surface. On the other hand, the mode of the tail formation and the shape and the formation of the mesoblastic somites, as well as the rudimentary condition of the suckers, are strictly alike in the two genera.

II.

OBSERVATIONS UPON EGGS OF *RHACOPHORUS*, *RANA*, AND *BUFO* FIXED ON THE PRISMEN-ROTATOR OF ZEISS.

The Prismen-Rotator of ZEISS, as I have before stated, enables one to study the upper and lower views of an egg as well as the lateral view of it from any point of its circumference, without once touching the egg and thus without the risk of calling forth abnormalities by handling. A full description of it

is given in the place already cited, and therefore it suffices here to say that it is a system of mirrors placed in the center of a small dish which can be rotated around its vertical axis at will, and has a graduated scale around its rim to enable one to locate any point exactly on its circumference. The upper view of an egg is obtained by looking at it from above, as in any ordinary case of examination by microscope. The view of its lower hemisphere is reflected on a mirror, and is observed by slightly sliding the dish and thus bringing the objective of the microscope above this mirror. In a similar way, another mirror gives the reflection of the lateral view of the egg, and by rotating the dish, and with it the egg, a view from any point of its circumference can be obtained.¹⁾

Method:—My first care was of course to find a suitable method for fixing an egg on the mirror-surface. I proceeded at first in the manner of PFLÜGER. That is, an egg was taken out of the frothy substance, with the thin layer of the innermost egg-envelope, placed on the dry surface of the mirror and exposed to the air for two or three minutes. When the egg seemed safely fixed, water was poured into the revolving-dish by means of a pipette, until the egg was submerged. This method was not entirely satisfactory, for an egg fixed in this way was apt to detach itself from the mirror surface, thus vitiating the experiment.

I next tried collodion which, I believe, has already been used by some investigators. This was far from satisfactory, for although it is a good fixative, it becomes opaque on the application of water, and often kills the egg, probably by the injurious effect

1) While this instrument has been designed for special use in investigations such as I have undertaken, it has not, so far as I know, been used in the study of frog's eggs.

of the ether used in dissolving it. I now tried gelatine. An egg was taken out of the forthy substance with forceps, and its orientation was noted. Gelatine which had previously been warmed on a sand-bath was dropped from the point of a needle on the mirror-surface and on its cooling, the egg was placed in the desired position on the gelatine drop which was then further cooled by pouring water into the mirror-dish. This gave some very satisfactory results (see Egg C). But if the drop of gelatine be too large or too thick in its consistence, the egg will be strongly compressed and may become deformed by the swelling up of the gelatine caused by absorbing water. Moreover, in the later part of the breeding season when the weather gets warm, the gelatine seems to become too soft to keep the egg in its place and it may thus become detached.

The method I have found most satisfactory so far, is the following:—instead of fixing the egg directly on the mirror, a small square piece of cover-glass, about the size of the mirror is cut, and this being gently heated for about 20–25 seconds, on the warm edge of a sand-bath which should never be hotter than one's finger can bear, the egg is quickly placed on it. The coagulation of the egg-envelope attaches the egg firmly on the glass which is then to be fixed on the mirror by gelatine in the manner described above. The least possible quantity of gelatine, of very weak consistence is enough to fix a small piece of glass on the mirror, the two surfaces coming into close contact with each other and not detaching to the end of the experiment. A very necessary precaution is that the water should be poured into the mirror-dish with the greatest possible gentleness. Eggs prepared in this way can be observed continuously for five or more days without the least inconvenience.

Owing partly to the fact that I spent the first three weeks or more of the breeding season (in 1899) in making tentative experiments in methods, and partly to the fact that I was determined to observe only absolutely normal eggs, removing from the mirror-surface eggs that showed the least sign of abnormality, the number of cases I have observed is not at all proportionate to the large number of eggs which I have examined. Only thirteen eggs (Eggs A-M), have been subjected to continuous observation, and of these only three (Eggs C, H, and I) gave entirely satisfactory results, while the remaining ten showed more or less imperfection during the observation.

During the spring of 1900, I subjected the eggs of two other anurans found in Tokyo, (*Rana japonica* and *Bufo japonica*) to the same observations. The number of the eggs employed by me three were of the former, and seven of the latter species. Of these, those that gave sufficient results were respectively one of the first, and two, of the second species. This small number was owing partly to the brevity of the breeding season in these species, as compared with *Rhacophorus*, and partly to the clouding of the mirror, the amalgam appearing unable to stand prolonged submergence in water.

I may here add a few words of explanation in regard to Figs. 20-50 which have been selected out of innumerable drawings to illustrate this section. The heavy horizontal line under every lateral, and some posterior views of the egg is the level of the mirror surface and is to be referred to as the standard line. The second thick horizontal line through the centre of the egg is drawn parallel to the standard line in order to denote approximately the equator of the egg. The third line, vertical to the standard line, passing through the egg-centre gives the vertical

axis of the egg. In a similar manner, the position and size of the blastopore in every stage is indicated by the vertical and horizontal dotted lines, respectively vertical or parallel to the standard line. I may also explain here that when a "side-view" of an egg is mentioned, that view of the egg is meant which has the middle point of the dorsal lip of the blastopore just on the edge of the egg-outline. When this edge is on the right hand of the figure, the view is the "left" view; and if on the left hand, it is the "right" view. The "posterior view" of an egg is the one which has the middle point of the dorsal lip of the blastopore on the middle line of the microscopic field. When once the positions of these views are accurately determined on the graduated scale on the rim of the rotating dish, they are very useful in orienting the egg and in finding these same views again in the further course of development. I generally made it my practice to prepare two sets of drawings at every stage. The first set was drawn from what I judged by my eye to be the side and posterior views of the egg without reference to the graduated scales of the rotating dish. The second set was drawn by turning the dish to points on the graduated scale which ought to give these views as determined in the earliest observed stage of the egg. When discrepancies appeared between these two sets, I generally determined what ought to be considered as the true side and posterior views, by a comparison of the drawings and a careful study of the actual object.

I had hoped to give the exact measurements of the changes in the position and size of the gradually closing blastopore at every stage. I found, however, that basing such measurements on sketches or drawings which necessarily have to be made rapidly in order to mark the conditions at any given moment, would lead

to errors. One or more accessory apparatuses specially designed for the purpose may be necessary to accomplish this object.

I should like to add here that my observations thus far have determined the fact that all the eggs within the vitelline membrane of *Rhacophorus* as well as of other Amphibians studied by me are in their earliest stages incapable of executing any horizontal movements around the vertical axis, *if they are carefully placed in their natural position* :

Having premised these remarks, I may now proceed to describe the eggs observed. I begin with *Rhacophorus*.

An egg-mass of that animal was obtained at 10 a.m., April 27th. It had probably been deposited during the preceding night. From it an egg (Egg C, Figs. 20-38) was taken out and fixed on the mirror by the gelatine method at 2:25 p.m., on the same day. It had already gone through the earlier phases of segmentation: cell-outlines were visible on its upper hemisphere, while obscure on its lower half (Fig. 20). At 2 p.m., on the next day (April 28th), the first trace of the dorsal blastopore lip had appeared at about 6° below the equator (Fig. 21). The posterior view of the egg (Fig. 21) was seen at 217° of the rotating mirror-dish. At 130° and 310° ($217-90=127$; $217+90=307$), were respectively the right, and left side views. Fig. 23 gives the left side view sketched at 3:20 p.m., on the same day: the middle point of the dorsal lip has moved slightly downward and its limbs have grown longer. Fig. 22 gives a view of the upper hemisphere of the egg seen from above at 2:30 p.m. The large translucent area of the segmentation-cavity is found occupying the centre of the egg-outline. On that day, the blastopore lips did not quite encircle the lower hemisphere but on the next morning (April

29th), they had already united in a circle. The area enclosed by them is circular in shape as may be seen in Fig. 24 which gives a view of the lower half of the egg seen from below at 7 a.m. The egg is resting upon the exact middle point of this area (Figs. 26 and 27). The upper view of the egg is given in Fig. 25 which shows that the somewhat diminished but still large area of the segmentation cavity is in the center and stands in exact opposition to the blastoporic area of the lower view (Fig. 24). Figs. 26 and 27 represent respectively the left, and right side views of the egg at 8:10 a.m., on the same day: the dorsal and ventral lips of the blastopore are exactly equidistant from the standard line (the mirror-surface), showing that the rims of the blastopore are growing over the yolk mass at the same rate of progress all around its circumference.

When seen at 9:43 a.m., on the same day, the egg was seen to have rotated about 20° backward (Fig. 28, left side view) so that instead of resting on the middle point of the blastoporic area as heretofore, it was now resting nearly on the ventral lip of the blastopore, and the dorsal lip had again moved slightly upward. The area of the segmentation cavity at the upper pole had also rotated slightly forward and kept its position exactly opposite the blastoporic area of the lower pole. Both these areas had now dwindled to about two-thirds of their original size. Note on the right edge of the egg-outline the faintly marked embryonic body stretching between these two areas. Figs. 29 and 30 give respectively the lower, and upper views of the egg sketched fifteen minutes later, and in them the eccentric position of the blastoporic area, and of the area of the segmentation cavity is well brought out.

At 11:27 a.m., on the same day, the dorsal lip of the blastopore (Fig. 31, left side view) had moved upward greatly, and

the closure of the blastopore had further progressed and the ventral lip had been brought further backward so that the entire blastoporic area could now be seen in the posterior view of the egg. The area of the segmentation cavity still kept its position exactly opposite the blastoporic area.

Further progress in these changes may be seen in Figs. 32-35 which give the left side view of the same egg at successive stages of development sketched respectively at 12:50, 2:04, 3:30 and 4:50 p.m., on the same day. These figures show that while the closure of the blastopore is going on, both the dorsal and ventral lips of it are gradually taken upward toward the equator by rotation. There is, however, an apparent difference between the behavior of the dorsal, and of the ventral lips beyond a certain point. When the dorsal lip in its upward return has reached about the point where it made its first appearance, it apparently ceases to rise any further, while the ventral lip continues to do so (Figs. 33, 34 and 35). This is undoubtedly due to the fact that the dorsal lip, partaking of the general growth of the whole periphery of the blastoporic area toward its center, grows downward toward the center of the area, and the rate of this downward growth must be exactly the same as that of its upward progress by the rotation of the whole egg, so that it remains apparently stationary. On the ventral lip, these two motions are combined, and there is naturally a steady upward progress. In earlier stages, the rate of the egg-rotation must be faster than that of the growth of the blastopore lip, so that there is an upward movement of the whole blastoporic area toward the equator. Conditions like those just described might lead one to suppose that the blastopore in certain later stages is closed only by the rapid overgrowth of the ventral lip.

At 10:30 a.m., next morning (April 30th., the fourth day after deposition), the blastopore was nearly closed, leaving only a small yolk-plug, and the general outline of the neural plate could be well recognized. Fig. 36 gives the left side view, and Fig. 37 the posterior view of the egg at this stage, sketched respectively at 10:37 and 10:50 a.m. These figures show that the dorsal lip of the blastopore has again grown a little downward while the position of the blastopore remains nearly unaltered. This is probably due to the fact that the rotation of the egg had ceased before the closure of the blastopore was finished, and thus bereft of the counterbalancing movement the downward growth of the dorsal lip toward the center of the blastopore is able once more to make itself felt. It seems to me that this is a strong argument in favour of the view that the blastopore is closed by the overgrowth of its lip all around its circumference.

Fig. 38 shows the left side view of the egg on the morning of May 1st., (the fifth day). The neural groove is already closed, and the first traces of the mandibular arches, the optic vesicles etc. are visible.

The facts brought out by the study of this egg (Egg C) are as follows:—

1). The entire lip of the blastopore, the dorsal, ventral and lateral parts, grow equally from all around toward the center of the blastoporic area, from the first until the blastopore is completely closed, although at certain late stages, this is obscured by the rotation of the egg. ホニヤリヌル

2). The rotation of the egg as a whole by which the dorsal blastoporic lip is again brought back to its first starting point begins when the dorsal lip has grown downwards over the yolk mass about 70°, proving that the dorsal lip never travels beyond the yolk-pole.

3). The arc passed by the egg in its rotation as a whole around its horizontal axis is about 80° , because the final position of the closed blastopore or the former yolk-pole never reaches the equator and is about 10° below it.

4). The rate of the egg-rotation in earlier stages is obviously faster than the rate of the downward growth of the dorsal blastoporic lip. Gradually slowing, it becomes equal to the latter, at about the time when the lip has returned to its first starting point. Finally it becomes slower, before it entirely stops.

5). As to the location of the embryonic body in the egg, the main parts of it appear between the upper and lower poles of the egg, along the meridian of the middle point in the dorsal lip of the blastopore (the blastoporic meridian). The lower yolk-pole corresponds to the tail end, while the foremost point of the head end is about 30° – 35° from the upper pole.

6). The initial vertical axis of the egg is at the end brought nearly to coincide with the antero-posterior axis of the embryonic body, while the lateral axis of the egg remains strictly unaltered.

The egg-mass from which Egg H was taken, was deposited early on the morning of May 11th. As the eggs were in the first cleavage stage, I tried to fix an egg in haste in order to determine, if possible, the relations of the plane of the first cleavage to the future axis of the embryo. But the three eggs I mounted one after another all showed abnormalities and in the mean time the first cleavage processes were passed. I therefore on the next morning fixed a new egg (Egg H) on the mirror surface and made the following observations.

The first appearance of the dorsal blastopore lip was noticed

at 2:15 p.m., on the same (2nd) day, (May 12th). The right, and left side views were respectively at 97° and 277° ($=97+180$), and the posterior view at 187° ($=97+90$) of the rotating dish. In this case, the spot where the dorsal lip of the blastopore first appeared was far below the egg-equator, being about 20° below that line. By 5:25 p.m., on that day, the lip had travelled about 15° further downward and the edges of the blastopore hardly extended to the lateral meridian of the egg (*i.e.* the meridian 90° from that of the dorsal lip at its first appearance). During the night which was quite warm, a great progress was, however, made, and when observed next morning (the 3rd day or May 13th) the blastopore was completely encircled and the rotation of the egg had begun. At 6:50 a.m., the egg was resting, just on the ventral lip of the blastopore, which was very much smaller in diameter than (only about $\frac{1}{3}$) that of Egg C at the corresponding stage.

At 8:40 a.m., the resting point of the egg was found at a certain distance ventrad of the ventral lip of the blastopore so that the whole of it could be seen in the posterior view of the egg. The position of the dorsal lip at the time was about 30° below the equator.

During this day, I was able to look at the egg every hour up to 5:30 p.m., and had thus the opportunity of observing the actual closure of the blastopore under microscope, which event took place at 4 p.m. The shape of the gradually diminishing blastopore was always circular, while the yolk-plug ready to be withdrawn into the interior was elongated a little dorso-ventrally. Also, no doubt owing to the small size of the blastopore to start with, its closure in this egg was finished sooner than the rotation of the egg, which was continued after that event over an arc of

about 10° . When seen at 7:30 next morning (the 4th day), the general outline of the embryonic body with the well defined neural groove and folds was already established. The posterior end of the neural groove at the time was about 25° below the equator showing that the spot which was the dorsal blastoporic lip must have returned, as in the first egg (Egg C), nearly to its starting point, after the blastopore was completely closed.

Egg H was therefore like Egg C in the mode of the blastopore closure, and in the location of the embryo. But the spot at which the dorsal blastoporic lip first appeared was much lower down than in the egg first observed, and was closed much earlier, while the arc travelled by the whole egg in its rotation was less, being about 60° .

Egg J was taken out from a mass obtained on the morning of May 20th. The eggs were all in the first cleavage, but I again failed in my attempt to observe the relation of that plane to the future embryo, as an egg of that stage on being fixed on the mirror showed signs of abnormalities. So, Egg J was fixed on the mirror the next morning. At 9:50 a.m., of the same day (May 21th.), the dorsal blastopore lip made its first appearance at a spot about 12° below the equator. The left, and right side views of the egg were to be seen respectively at 160° and 340° ($=160+180$) of the rotating dish, while the posterior view was at 250° ($=160+90$). When seen about ten minutes later (10 a.m.), the dorsal lip had grown about 6° downwards, and at 2 p.m., about 9° further down. Thirty minutes later (2:30 p.m.), in the left side view the dorsal lip had grown down another 9° , so that the lip had moved downwards over the yolk altogether about 24° in 4 hours and 40 minutes. The rate of growth was not con-

stant in this case, being rapid at the first and last observations and quite slow in the interval. The acceleration in the first instance was without doubt to be accounted for by the artificial application of heat as the means of fixing the egg, while the speed in the last period mentioned was perhaps due to the rise of the atmospheric temperature. Such irregularities are, however, of common occurrence and may be due to both artificial and natural causes.

By 3 p.m., of the same day, the dorsal lip had moved to about 60° below the equator, and now the blastopore lip was closed in a complete circle. At this time, the ventral lip was found at a level 5° or 6° higher than the dorsal lip. In the posterior view, also, there could be noticed some differences (2° or 3°) in the level of the right and left lateral lips. The rim of the blastopore was, therefore, in this egg somewhat wavy. The highest part was at a point about 25° – 30° to the right of the middle line of the dorsal lip (*i.e.* of the blastoporic meridian), showing that some trouble in the overgrowth of the lip had unfortunately occurred in this region. When, however, I looked at the egg at 6:30 p.m., all such unevennesses of the blastopore lip had disappeared and it had now grown downward evenly all around to about 66° below the equator.

When seen at 7:30 on the next morning (the third day), the blastopore had completely closed and the yolk-mass had been withdrawn into the interior. The closure had taken place during the night which was unusually warm. The point at which the blastopore was closed was to be found about 30° to the right of the former middle line of the posterior view, and moreover was far below the equator (about 20°). Such an irregularity was no doubt due to the same cause as that which produced the uneven

downward growth of the blastopore rim, although there is a possibility that I may have mistaken the exact position of the posterior view at the start. The dorsal lip of the blastopore in this egg did not return to its first starting point, though the egg rotated 5° or 6° further after the complete closure of the blastopore. The embryonic body formed of this egg was entirely normal.

Although observations on the later features of the blastopore closure and the beginning of the egg-rotation are wanting, the earlier phases observed in this egg clearly show that the blastopore was closed, as in the former cases, by the equal downward growth of the lips all around over the yolk-mass, until the dorsal lip arrives at about 66° below the equator. The position of the embryo also coincides in the main with that in the former eggs, although somewhat swung out of the blastoporic meridian in its posterior portion. The only difference is that the dorsal blastoporic lip did not return to its starting point, which is to be accounted for by the smaller amount of the egg-rotation.

I shall add a few more details obtained from the study of Egg G which unfortunately became unstable in the later stages through the insufficient fixation of the cover-glass upon the mirror-surface. In this egg, the dorsal lip of the blastopore appeared first at 5 p.m., on the second day at about 10° or 12° below the equator, and had moved down to about 50° by 9:50 a.m., on the third day by which time also the blastoporic-circle was completed. Afterwards, the whole edge of the blastopore moved further down, until at 3:10 p.m., on the third day the circle arrived at about 66° below the equator. When seen at 5:30 p.m., from the left side, the egg had rotated about 35° . At this time, the dorsal lip of the blastopore was found at 32° or 33° below the equator, and

the ventral lip at about 80° , so that the diameter of the blastopore occupied about 50° of the egg circumference, whereas it had occupied 70° or more when it first assumed the circular shape.

At 6 p.m., the blastopore has diminished to about 35° in diameter, but the egg had become somewhat unstable, rotating also when the mirror-dish was rotated, and by the next morning had become utterly useless from the same reason.

In the spring of 1900, I studied the subject under consideration in the eggs of two other anurans (*Rana japonica* and *Bufo japonica*). The spawning season of the former species about Tokyo is from February to March, and of the latter species from the end of March to the middle of April.

On the morning of February 2nd, several masses of the *Rana* eggs were obtained. From among them I fixed on the Prisms Rotator an egg in the early gastrulation stage in which the dorsal lip of the blastopore had already appeared and encircled about one-third of the circumference. This year, I carried on my researches in a basement room in order to have a perfectly steady floor, but, although this end was attained, the temperature of the room was comparatively low, and the development of the egg progressed very slowly. This proved very unfortunate, as owing probably to prolonged submergence the mirror became clouded, until on the sixth day nothing more could be done with it.

As the gastrulation process had been going on for sometime, I could not ascertain on this egg the actual starting point of the dorsal blastopore lip. Fig. 39 represents the view of the egg from the lower pole at 4:30 p.m., on Feb. 2nd (the first day). The right and left side-views were obtained respectively at 20°

and 200° , and the posterior view at 290° , of the rotating dish. The complete encircling of the blastopore-lips was observed at 2:17 p.m., on the next day, when the lips had grown down to about 64° or 65° below the equator. At this time and later, up to the morning of the fourth day, the gradually diminishing blastoporic area was always found at the exact center of the lower hemisphere (Fig. 40). At 4:05 p.m., on the fourth day, the blastopore lips had grown down to 72° or 73° below the equator, and the egg was just beginning its rotation as a whole. On the next morning (the fifth day), the egg was found to have rotated about 13° or 14° and the closure of the blastopore had also advanced somewhat. At 3:32 p.m., on the same day, the right side view of the egg was as given in Fig. 41: the egg had rotated so that it was now resting, nearly though not yet quite, on the ventral lip of the blastopore which was now reduced in diameter to $\frac{4}{5}$ of the size in Fig. 40. Further progress is seen in Fig. 42 sketched at 3 p.m., on the sixth day: the egg is now resting exactly on the ventral lip of the blastopore which is still further reduced in size. I could unfortunately make no observation beyond this point, owing as before mentioned to the clouding of the mirror, but the facts brought out prove that in the main, the mode of the blastopore-closure and of the egg rotation in *Rana* are exactly like the same processes in *Rhacophorus*.

In the case of *Bufo*, the eggs whose development I was able to follow during the breeding season of last year were only three in number. Of these the third did not produce satisfactory results, owing to the clouding of the mirror again. Even in the case of the first two eggs which gave tolerably good results, the lower view was rather imperfect from the same cause. I tried therefore to supplement my observations by following three or

four eggs during the spawning season of the present year, but again all were failures. I ought to state also that I tried at first to study the *Bufo* eggs in the basement-room before referred to, but I found the constant low temperature of the room acted injuriously on the *Bufo* eggs and I was therefore obliged to return to the up-stairs laboratory which is kept warmed by hot-water pipes. I will now proceed to describe the two eggs whose development I followed, and in which I was able to make out the relations between the first two cleavage-planes and the future embryonic axis.

The first egg was taken from a mass obtained from the pond in our University grounds on the morning of March 16th. The egg had not yet begun segmentation, when it was fixed on the mirror at 10 a.m. The first cleavage line appeared at 2:50 p.m. on the same day, and had nearly reached the lower pole by 5:30 p.m. When the first cleavage line was placed approximately in the middle vertical line of a side view of the egg the rotating dish was at 175° (Fig. 44) or at 355° . At 6 p.m., the second cleavage line appeared making right angles with the first (Fig. 43). When this line was placed in the middle vertical line of the side view, the dish stood at 85° ($=175^\circ - 90$) or at 265° ($=175 + 90 = 355 - 90$). Having made out the positions of these two cleavage lines on the scale, I was now able to identify the same positions by simply turning the rotating dish to these several readings.

At 8 a.m., on the next morning, the egg had advanced to a stage with 64 or more cells. The first dorsal blastoporic lip appeared at 8:15 a.m., on the third day, about $30^\circ - 34^\circ$ below the equator. When the middle point of the dorsal lip was brought on the middle vertical line of a side view, the rotating dish stood at 85° , corresponding to the similar position of the second

cleavage line. In the same way, in the left and right side views of the egg, when the dorsal lip came to be in the profile, the rotating dish stood at 175° (Fig. 45) and 355° respectively. This showed that the meridian of the blastopore coincided with the plane of the second cleavage and stood at 90° to the plane of the first cleavage.

The blastopore did not close in a circle during this day, but when seen next morning, (the 4th day), the encircling had been completed and the blastopore rim had grown downward all around to about 70° below the equator. Fig. 46 represents the left side view at 8:20 a.m., and corresponds to Figs. 26 and 27 of the *Rhacophorus* egg, and to Fig. 40 of the *Rana* egg. As in all these figures, the dorsal, ventral, and lateral lips of the blastopore are all at the same level and the egg rests on the approximate middle point of the blastoporic area.

At 1:30 p.m., on the same day, the egg (Fig. 47) had rotated about 32° or 33° and the blastopore had diminished to about $\frac{2}{3}$ in its diameter as compared with that in Fig. 46. One hour later at 2:30 p.m., the dorsal lip had returned to its first starting point and the ventral lip was about 20° dorsad of the resting point of the egg, for in addition to the egg rotation the blastopore was now still more diminished in diameter. Toward the evening of that day, the closure of the blastopore was far advanced but the general outline of the embryonic body could not yet be recognized (Fig. 48). On the morning of the fifth day, the blastopore was nearly closed, leaving only a small yolk-plug exposed, and the first trace of the neural groove and folds could be detected in front of the small blastopore. Fig. 49 gives the posterior view of the egg at 8:30 on that morning (the fifth day). Although the embryonic body is not sketched in, it will

not be difficult, I think, to orientate the egg. The final point of closure coincided nearly with the starting point of the dorsal lip, showing that the egg did not rotate more than 55° - 60° . After this, the egg unfortunately became somewhat unstable by the swelling up of the egg envelope. The egg seemed also to show at times certain independent movements which I attributed at the time to some accidental causes.

The second *Bufo* egg that was studied was taken from a mass that was obtained from the same pond on the morning of March 21st. All the eggs had not yet begun segmentation. The particular egg to be studied was soon fixed on the mirror at 10 a.m. The first cleavage line appeared about one hour later (11 a.m.) and reached the lower pole at 12 m. when the second cleavage line began to appear nearly at right angles to the first. In this egg, the frontal view of the first cleavage plane was seen at 115° and 295° of the rotating-dish, and the same view of the second cleavage-plane at 20° ($=115-95$) and 200° ($=295-95$). The dorsal blastoporic lip appeared first at 8 a.m., on the third day, at about 28° or 29° below the equator. In this egg also, the frontal view of the blastopore (the posterior view of the egg) coincided with the frontal view of the second cleavage plane, while the profile or side view of the dorsal lip was the same as the frontal view of the first cleavage plane.

The blastopore rim completed its circle when it had grown downward to about 60° below the equator (2:30 p.m.). At this stage, all the circumference of the rim was at the same level. The same state of things still continued at 3:10 p.m., when the lip had grown down to about 70° below the equator. At 3:30 p.m., the egg had just begun its rotation. At that time, I noticed that the egg moved in an unexpected direction. Thus

at 4:45 p.m. it had rotated about 20° in the opposite direction *i.e.* obliquely ventrad. By careful examination, I was convinced that such unexpected movements of the egg were due to accidental causes, such as the too rapid rotation of the mirror dish, since the vitelline membrane and the outer envelope had become very loose by absorbing a large amount of water. By allowing the egg to stand quietly without any disturbance for ten or fifteen minutes, it gradually returned to its normal position and was rotating in the usual way. Thus, at 5:10 p.m., the dorsal lip had returned to about 50° below the equator, and the ventral lip was at the resting point of the egg, the blastopore being thus 40° in its diameter.

At 8:30 on the next morning (the fourth day), the ventral lip of the blastopore was 6° or 7° posteriorly from the resting point of the egg, while the dorsal lip had again grown *downwards* 4° or 5° , showing that very slight, if any, egg rotation had occurred during the night, although the blastopore had lessened 3° or 4° in its diameter. This was probably due to the fact that the temperature was low during the night. During the day, I was not able to observe the egg up to 2 p.m., when the blastopore had already nearly closed and the yolk-plug had mostly withdrawn into the interior. The neural groove and fold were also recognizable. The arc of rotation in this egg was evidently less than in the first, and the final position of the closed blastopore did not coincide with the first starting point of the dorsal lip, being at about 55° below the equator. The difference between the former and the latter was thus about 25° , and the arc passed over by the egg rotation was about 35° . I thought it quite probable that in this case the rotation would be continued after the complete closure of the blastopore, as in one of the *Rhacophorus* eggs, but

unfortunately I could not observe this, for the egg began at this stage, to show some independent movements within the vitelline membrane, which seemed to increase gradually. As the first *Bufo* egg had showed similar movements at the corresponding stage, I gave close attention to the matter. After examining the conditions carefully, I came to the conclusion that such movements were not natural but, were caused in some way by the strong sun-light which was falling at the time directly on the mirror and the egg—a conclusion which was soon proved to be false, as will be seen further on.

The third egg of *Bufo* was taken from a mass deposited in the same pond early on the morning of March 27th. The eggs had not yet begun segmentation, and one of them was fixed on the mirror at 8:30. The first cleavage appeared at 9:45 a.m., and the second cleavage at 11 a.m. I watched this egg at intervals of time up to the fourth day, but unfortunately owing to the clouding of the mirror, and to the unusual amount of pigment in the egg, I am unable to give details about the exact spot at which the dorsal blastopore lip appeared, or about the mode of the blastopore closure. While, however, looking at the egg at 12 m., on the fourth day when the blastopore had just closed and the neural groove and folds had become clearly recognizable, I noticed a curious behavior of the egg. As in the last two cases, the egg began to show movements within the vitelline membrane. The outer envelope was not swollen out as in the first egg nor was the sun-light at the time falling on it, as in the second egg. The movements were at first very slow and seemed somewhat irregular so that they seemed somewhat accidental, and due to some external stimulus. After a while, however, the motion became more rapid and seemed to show

a considerable regularity. By continuous observation for one hour or more, I finally made out that the egg was rotating regularly with the axis of the embryo for its rotation-axis. Fig. 50 is the anterior view of the egg. The direction of rotation as indicated by the arrows, was from right to left *i.e.* contrary to the motion of the clock-hands. The thick lines on the figure show the different positions of the neural groove during rotation. The time occupied by one turn of the egg was not quite constant, but the differences were small, as the following measurements show : 2'.17", 2'.21", 2'.20", 2'.30", 2'.20", 2'.15" *etc.* Thus the mean time required by one turn was 2'.20". The position of the anterior and posterior ends of the neural groove also varied slightly at every turn. Such regular rotating motion of course could not have been accidental, and the somewhat irregular movements which I had noticed in the first two eggs must have been the beginning of this regular motion. I can not understand the purpose of such motion. Nor have I been able to ascertain the means by which it is performed. If minute cilia are present, I could not bring a sufficiently high power to bear on the egg to make them out. So far as I know, such motion has not been noticed by any previous observer.

Although my observations on the eggs of both *Rana* and *Bufo* are somewhat meagre, the results as regards the most important points, such as the mode of the blastopore closure and the location of the embryo body, are exactly similar to what have been obtained from the study of the *Rhacophorus* eggs. The principal points in these results in the three genera may be summed up as follows:—

1). The dorsal lip of the blastopore always makes its first

appearance some degrees below the equator, although there are variations in this distance from the equator according to different species and to different individuals of the same species. In *Rhacophorus*, it is generally at 10° – 20° below the equator, although in rare cases it may go up to 5° or down to 25° . In *Bufo*, it is at about 28° – 30° . The same fact may be made out from the papers of MORGAN and TSUDA ('95*b*), ASSHETON ('94*a*), KOPSCH ('00), EYCLESHYMER ('95*a*, '98), and other authors.

2). The completion of the blastopore lip in a circle, or in other words the first appearance of the ventral lip may also vary; it takes place generally at about the time when the dorsal lip has grown down to about 50° below the equator in *Rhacophorus*, and to about 60° in *Rana* and *Bufo*.

3). The blastopore remains circular throughout its existence, since the dorsal and the ventral, as well as the lateral lips close in towards the center of the blastopore area equally from all around. Owing to the rotation of the egg as a whole, it looks, however, in later stages as if there were a difference of growth in different parts. That the entire rim of the blastopore takes part in its closure has already been recognized directly or indirectly by SCHULZE ('88*b.c.*), ASSHETON ('94*a*), EYCLESHYMER ('95*a*, '98), and KOPSCH ('00), in opposition to the views of PFLÜGER ('83), ROUX ('88*a*), MORGAN ('93, '97) *et al.* The former authors, however, seem to me to be of the opinion that there is a certain difference between the growing ratios of the dorsal and the ventral lips of a blastopore. And moreover, the fact that the actual final closing point of the blastopore is the former yolk pole (the center of the blastopore area), of the egg, has not yet been maintained by any previous writer. This is probably due to the circumstance that the methods hitherto employed are not

calculated to bring out clearly the equal growth of the whole periphery of the blastopore lips towards the yolk pole.

4). The point at which the blastopore finally closes is in reality always what is the lowest point of the yolk hemisphere (or the yolk pole *in s. str.*) of the unsegmented egg. The distance traversed by the dorsal lip from its first appearance to the time of closure is therefore about 70° – 80° in *Rhacophorus*, and 68° – 70° or less in *Bufo* and *Rana*; that by the ventral lip is about 20° – 40° in all the species; and that by the lateral lip ranges between these two extremes. This estimation agrees well with those given by ASSHETON (60° – 70°), and KOPSCH (*circa* 75° – 80°).

5). When the dorsal, ventral, and lateral lips of the blastopore have grown down to a level which is 60° – 75° below the equator, the whole egg begins to rotate slowly on its transverse horizontal axis, the resting point of the egg gradually shifting toward what becomes the ventral face of the future embryo, and the dorsal lip apparently returning toward its starting point. This greatly obscures the actual mode of the blastopore closure (or the equal growth of the lips over the yolk hemisphere).

6). This rotation of the egg within the vitelline membrane is very slow and may cease, sometimes before, and sometimes after the process of the blastopore closure is completed.

7). The dorsal lip of the blastopore in normally growing eggs, therefore, never goes ventrad beyond the middle point of the blastoporic area (the yolk-pole).

8). In *Rhacophorus*, the correlation in size between the upper translucent area of the segmentation cavity and the lower blastoporic area becomes specially obvious in eggs fixed on the mirror. They not only diminish in size together, but also maintain their position opposite each other, even after the rotation

of the egg has begun. If pigment were absent in the eggs of *Rana* and *Bufo*, similar relations could no doubt be made out.

9). As to the location of the embryonic body, the results of the present investigation are in entire accord with the views of ASSHETON ('94a), and EYCLESYMER ('98). The anterior half of the embryonic body is formed on the upper hemisphere of the egg, and the posterior half on the lower hemisphere. I can not, however, in any way make out that the two halves of the embryonic body are in reality formed from the two separate centers of the formative area, as was supposed by these two authors. And as to the orientation of the embryonic axis, I agree in the main with KORSCH'S statements:—"Und dass die von Pol zu Pol gezogene Axe der Furchungsstadien nicht die dorsoventrale Axe des Embryos ist, sondern dass sie beim jungen Embryo schräg von caudal oben nach cranial unten verläuft," (p. 21, '00). I have only to point out that according to the present observations the head portion of the young embryo is turned always *upwards* (not "unten") and at the same time the caudal portion is turned *downwards* (not "oben").

I have described above how I found in two *Bufo* eggs that the axis of the embryonic body coincided with the plane of the second cleavage line. According to many previous investigators, the coincidence ought to be with the first cleavage plane rather than with the second, while some recent writers, as JORDAN and EYCLESYMER ('94b), entertain the view expressed in the following quotation:—"It seems to us a more reasonable supposition that the direction of the early cleavage planes and the embryonic axes have not vital connection, and

that the coincidence, where it exists, is in itself of no fundamental significance" (p. 413).

In order to test this point further, I made the following observations on *Rhacophorus* eggs. I took eggs either before, or just after, the first appearance of the first cleavage line and fixed them, each on a cover-glass, in the manner already described at the beginning of the article, by heating it slightly on the edge of a sand-bath for about 30 seconds. The eggs were put as quickly as possible in a vessel full of water, and reared in the usual way. As soon as the first cleavage appeared, its direction was plainly marked on the cover-glass by two points. In this way, I prepared four groups of eggs.

In the first group, only one egg out of five was found to have been well fixed on the cover-glass: the rest unfortunately fell off. In the single egg, the axis of the embryo, when it appeared, was exactly at right angles with the plane of the first cleavage.

In the second group, three eggs out of five were well fixed. In the first of these the embryonic axis (the head end) was inclined 50° to the right of the first cleavage plane. In the second egg, the embryonic axis was at right angles to the first cleavage plane. In the third, the anterior half of the embryo was at right angles to the first cleavage plane, while the posterior half was somewhat oblique and deflected about 30° to the left from the embryonic plane. The main axis of the embryo must, however, be considered to be at right angles to the first cleavage plane.

In the third group, only three out of ten eggs were found to have been well attached to the cover-glasses. In one of the three, the inclination of the embryonic axis to the first cleavage plane was about 50° , and in another 20° to the right. In the

third egg, the embryonic axis was exactly at right angles to the same plane.

In the fourth group, only three eggs out of five were good. In one of these, the embryonic axis nearly coincided with the first cleavage; in the second, it was inclined about 60° , and in the third, 40° to the first cleavage plane.

When tabulated, the results may be expressed as follows:—

	The no. of eggs fixed well on the cover glass.	Eggs with the inclined embryonic axis —	Eggs in which the embryonic axis was at right angles to the first cleavage plane.	Eggs in which the embryonic axis coincided with the first cleavage plane.
Group I.	1 (among 5)		1	
Group II.	3 (among 5)	1 (50°)	2	
Group III.	3 (among 10)	2 ($\begin{matrix} 1-50^\circ \\ 1-20^\circ \end{matrix}$)	1	
Group IV.	3 (among 5)	2 ($\begin{matrix} 1-60^\circ \\ 1-40^\circ \end{matrix}$)		1
Total	10.	=5	+ 4	+ 1

When the two *Bufo* eggs in which the embryonic axis was at right angles to the first cleavage plane are added to the list, the total of such eggs becomes 6 out of 12. I admit that the number is altogether too small to allow us to draw any general conclusion, but the fact remains that the percentage of amphibian eggs in which there is a definite relation between the embryonic axis and the earlier cleavage planes is quite large, and that in such cases, the first named axis coincides with the second

cleavage plane and is at right angles to the first. I am inclined to think that the first segmentation cleavage divides the substance of the egg into two parts corresponding to the dorsal, and ventral halves of the future embryonic body, supposing that there is on the whole no interchange of substance during the course of development.

III.

EXPLANATION OF THE FACTS OBSERVED IN THE SECOND PART BY CHANGES IN THE INTERIOR OF THE EGG BROUGHT OUT IN SECTIONS.

Method :—The *Rhacophorus* egg presents several peculiar difficulties for sectioning: the closely applied chorion and the innermost envelope of the frothy substance become exceedingly tough, when hardened, so that it is very difficult for paraffin to penetrate into the egg. Moreover, the egg being absolutely without pigment, becomes so translucent, when clarified in turpentine or xylol, that orientation becomes impossible. Again, owing to the extreme tenuity of the roof of the segmentation cavity, it is apt to cave in when the egg is transferred from one liquid into another of a different specific gravity.

After some experiments, the mode of procedure I adopted was as follows :—The orientation of the egg was secured by first fixing it in any desired position on a thin triangular sheet of frog's liver by a drop of albumen. This was then passed through the ascending grades of alcohol, being kept 13 or 14 hours in absolute alcohol at the end. If a shortening of the time was desired, absolute alcohol was changed two or three times in one

hour. This was then imbedded in the usual celloidin-paraffin method, care being taken that the egg should be thoroughly permeated by celloidin by being kept two or three days in a weak solution of it in a mixture of absolute alcohol and ether in equal parts.

After cutting and mounting, sections were stained on slides. Most satisfactory results were obtained by subjecting them to double-staining with the water solution of the acid fuchsin G (ca 2%) and methyl-blue (ca 3%). This is a slight modification of Auerbach's method for the double-staining of certain sexual elements. A slide is placed in a solution of the first dye for 15-20 minutes, washed, and then put in a solution of the second dye for 20-30 minutes. After a second washing, it is put into absolute alcohol and the excess of blue colour is washed out by stirring it with forceps. At a certain stage of discoloration, it becomes very beautiful. The sections stained in this way show the nucleus in blue, while the cytoplasm as well as the yolk-spherules are red or reddish purple in colour.

Fig. 51 is a cross-section passed through the approximate center of a *Rhacophorus* egg which is at about the stage represented in Fig. 8 and is intermediate between Figs. 20 and 21 of Egg C. The segmentation cavity has not yet become enlarged to its full extent, and its roof is still thick being composed of three or four layers of cells.

The next two figures are the median sagittal (Fig. 52), and the middle transverse (Fig. 53), section of eggs corresponding to the stage represented in Fig. 23 of Egg C. The segmentation cavity has now become enormously enlarged, and its roof very thin, being composed of only two layers of cells (the epi-

dermal, and the neural layers of the epiblast). The shaded portions on both sides of the segmentation cavity are the sections of the equatorial zone. Fig. 62 gives a magnified view of the roof drawn from another section of the same series: the cells of the outer layer are somewhat flattened and slightly smaller than those of the inner layer. As before mentioned, this extreme tenuity of the roof accounts for the translucency of the area of the segmentation cavity in an external view.

Fig. 54 is the median sagittal section of an egg corresponding to Figs. 10 and 11 or to Figs. 26 and 27 of Egg C. The blastopore lip has already closed in a circle and is at the same level all around.

The next two figures (Figs. 55 and 56) are respectively a median sagittal, and a middle transverse section of the eggs corresponding to that represented in Fig. 12 or in Figs. 28-30 of Egg C in which the rotation of the egg has commenced. The archenteron has acquired a distinct lumen along the posterior and dorsal surface of the yolk-mass, while the segmentation cavity has become pushed forward and diminished in size in an inverse ratio to the enlargement of the first named cavity.

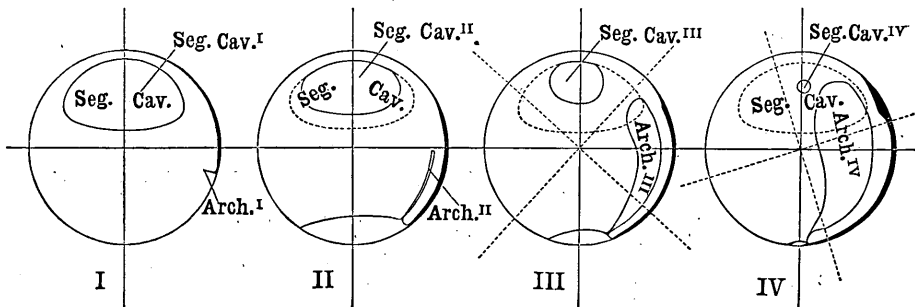
Fig. 57 is a median sagittal section of an egg corresponding to Fig. 13 or to Figs. 32 and 33, in which the blastopore has become moderately closed and the rotation has progressed considerably. The segmentation cavity has now vanished, leaving only small irregular cavities at the anterior end of the large archenteric cavity. This accounts for the fact that the translucent area can no longer be recognized in a superficial view.

Figs. 58 and 59 give respectively a median sagittal, and a middle transverse section of the stage represented in Fig. 14 or in Figs. 36 and 37. Here the blastopore is on the point of being

closed, the yolk-plug having already been withdrawn into the interior. The first trace of the neural groove may be recognized along the median line of the neural plate which is now raised somewhat over the general surface of the egg.

Figs. 60 and 61 give the corresponding sections of the stage represented in Fig. 15 or in Fig. 38. The blastopore is completely closed, and the neural groove is nearly so, so that the general outline of the embryo-body can now be distinctly made out.

In the above series of figures, the horizontal line drawn under each gives the surface on which the egg was resting at a given stage, and the lines drawn parallel and vertical to this show the horizontal and vertical axes of the egg as it then stood. The dotted line in Figs. 55, 57, 58, and 60 shows, on the contrary, the ideal vertical axis or the line which passed through the upper and lower poles of the egg at the blastula stage, and which shifts its position through the gradual rotation of the egg around its transverse horizontal axis. If the egg were supposed to be held to its initial vertical axis without any rotation, the series would become as in Woodcuts I-IV. The two dotted lines at right



The thick line in front of the blastopore indicates the extension of the neural plate. Seg. cav. I, Seg. cav. II, Seg. cav. III, Seg. cav. IV, the gradually diminishing seg. cavity. Arch. I, Arch. II, Arch. III, Arch. IV, the gradually enlarging archenteron.

angles to each other in Woodcuts III., and IV., indicate the *actual* vertical and horizontal axes drawn in full lines in the plate figures. The relative position and size of the segmentation cavity and the archenteron are easily understood from this series.

If now these two series (Figs. 51–61 and Woodcuts I–IV.) are carefully studied, it will become clear that the position of an egg at a given stage is entirely due to the position of its center of gravity. Up to the stage of Fig. 54 or of Woodcut II., the mass and the segmentation cavity are uniformly disposed with regard to the initial vertical axis of the egg, hence the center of gravity lies in this axis, and on its lower pole the egg rests. With the formation and gradual enlargement of the archenteric cavity which goes on hand in hand with the reduction in size of the segmentation cavity, (Figs. 66–61 and Woodcuts III–IV.), the dorsal and posterior part of the egg gradually diminishes in weight while, on the contrary, the anterior and ventral part becomes heavier by degrees. The center of gravity is, naturally, shifted forward, but as the archenteron keeps its symmetry in regard to the sagittal plane, this shifting takes place along that same plane. Hence such rotation of the egg as we have noticed in the study of the external features. In fact, it would be impossible for an egg constituted as given in Woodcut IV., to keep the unnatural position given in that figure: if left to itself, it must fall forward and assume the position given in Fig. 60.

That the rotation is sometimes continued after the complete closure of the blastopore, is due without doubt to the fact that at the time of the latter event, the archenteric cavity has not yet reached its maximum size. The archenteron, however, at its highest development never extends below the original vertical axis and is confined within the dorsal half.

As above stated the egg-rotation is a fact which has already been noticed by all investigators in the growing eggs of Amphibia. But the degrees passed by the rotation as well as the period at which the rotation takes place, are different according to different writers. Thus according to PFLÜGER ('83), ROUX ('88*a*), MORGAN ('93*b*, '97) *et al.* the egg has to rotate, about 170° or more, after the complete closure of blastopore. O. SCHULZE ('88*b*) maintains that the egg rotation begins from the beginning of the gastrulation process, so that it counterbalances, from the first, the downward growth of the dorsal blastopore lip over the yolk hemisphere.

Meanwhile the fact that the egg-rotation takes place by the change in position of the center of gravity of the egg during the gastrulation, has already been noticed by all my predecessors, and is now generally accepted.

I will now go over categorically some of the other points brought out in the study of the sections:—

1). The growth in size of the segmentation cavity from the condition seen in Fig. 51 to that of Fig. 53 is no doubt due to the thinning out of the roof which, as mentioned before, is three or four cells thick at first but becomes later strictly two-cell layered. This thinning appears to me to be brought about by the downward shifting or migration toward the equator of the cells composing the roof which no doubt also increase by division.

2). It seems probable to me that the equatorial zone is produced by the accumulation of these downward migrating cells rather than by the multiplication of the cells which were here from the first. This view is strengthened, it seems to me, by what is seen in Figs. 63 and 64 which are respectively the magnified sagittal sections of the zonal mass at the blastoporic,

and the anti-blastoporic regions of an egg in the stage of Fig. 52. In each of these figures, although the lower part of the zonal mass passes over gradually to the yolk mass, it is in its upper part sharply marked off by a line from the yolk cells, as if the cells had moved down from the roof of the segmentation cavity with which they form one continuous sheet, and accumulated in a mass here.

In Fig. 63, the first trace of the blastoporic slit interrupts the region of transition from the equatorial zone to the yolk-mass. In other words the cells directly above the first blastoporic slit are not the true zonal cells, but forms transitional between them and the yolk cells. Such a state of things leads me to accept the statement of MORGAN and TSUDA that "the blastopore makes its first appearance on the less pigmented and further developed side of the egg, and, moreover, at a short distance only from the group of large cells around the lower pole," (p. 381), although there is no pigment in my materials. These transitional cells are however doubtless soon transformed into the zonal cells which are in their turn differentiated into the permanent tissue cells roofing over the Archenteron. This process of transformation no doubt goes on all around the lower edge of the equatorial zone, while the cells themselves multiply also by division *in situ*. As the result of these formative changes, the equatorial zone spreads downward toward the yolk pole. I am therefore able to agree with ASSHETON ('99), when he says: "In this way there is a gradual apparent creeping of small (black) cells over the surface of the egg—though in reality it is conversion of large cells into smaller *in situ* as, I believe, is now generally accepted," (p. 227).

3). The equatorial zone is, however, only a temporary structure. The blastopore lip extends gradually on each side from the

point of its first appearance in the dorsal median line, along the lower edge of the equatorial zone, until the two limbs of it meet each other and complete the circle in the ventral median line. Before the lip becomes established, the component zonal cells are no doubt differentiated into the permanent epiblast cells, and by this means the epiblast grows down toward the lower pole. As the downward growth of the epiblast is thus taking place before the lip is definitely established, it seems to me that the individual or specific variations in the exact spot at which the dorsal blastoporic lip first appears, are not of so much importance as some writers believe.

The establishment of the lip itself, we must regard as due to invagination. While this goes in deep at the dorsal portion, it becomes at all other portions of its circumference evaginated again to add to its downward growth, as explained below.

The growth downward of the blastopore lip, after once it is established, is, I believe, nothing but the continuation of the cell multiplication and the differentiation of these cells *in situ*. Although I have no direct proof of it, yet I have no doubt that the most active seat of cell-multiplication is at the edge or just inside the edge of the lip. Cells multiplied accumulate inside the lip as a sort of invagination and become soon evaginated to add to the downward growth of the lip. This takes place all around the circumference of the lip except at the dorsal median line where the underlying archenteric cavity has to be deepened and enlarged. The formation of the blastopore lips and their growth are therefore not produced by either invagination or cell-multiplication alone, but conjointly by these two processes.

4). As to the manner in which *the segmentation-cavity diminishes in size*, most previous writers have stated that this

is caused by the gradual growth of the archenteron which pushes the yolk-mass ventrad and thus causes the segmentation cavity to become obliterated in the *ventral half* of the egg. But according to my own observations, the segmentation cavity becomes smaller not only by the gradual growth of the archenteric cavity but also by the rising upward of the yolk-mass which takes place along the inner face of the epiblast, roofing over the segmentation-cavity as the blastopore lips grow downward. In other words, the cavity becomes reduced centripetally, *in situ*. Thus as shown in Figs. 57, 58, and Woodcut IV., the segmentation cavity and the blastoporic area both of which have now become very small are found, as before, at, or close to the former vertical axis of the egg, without changing their original positions relative to each other. So far as my experience goes, I have never yet met with a case in which the final point of the disappearance of the segmentation cavity was *below* the original vertical axis of the egg (the dotted line in Figs. 57, 58, and 60), although I am not in a position to assert that such cases may not occur as individual variations. Also, according to my views, the atrophy of the segmentation cavity has not so much to do with the rotation of the egg as the growth of the archenteric cavity, since in the former process the yolk cells rise *en masse* so to speak, and do not greatly change the position of the centre of gravity.

As a case of individual variation, there is that of Fig. 57 in which the segmentation cavity communicates freely with the archenteric cavity, the barrier separating the two cavities having broken down. Such a case is often met with. In fact, seeing that the yolk mass in the living egg is not solid but must be very mobile, being composed of loosely associated yolk cells, it would be strange if such and other slight differences were not found in various individuals.

5). As to the *first formation and growth of the archenteric cavity*, I agree mostly with ASSHETON ('94a p. 225-226, p. 227, p. 229). As represented in Fig. 63, the first trace of the blastopore appears as a horizontal linear slit below the cell mass of the equatorial zone, and is bounded by a kind of cells which is not the true zonal but transitional between the yolk-cells and the zonal. With ASSHETON we may consider this as formed by a splitting between cells, or as a linear accumulation of the intercellular space; but we ought to remember that it is produced in direct continuation of the *intra-vitelline* space, and must be regarded morphologically as a modified form of invagination, the only difference from the typical form being that cells situated on the outer surface of the egg do not actually migrate inward.

The further growth of the archenteron seems to take place very much as ASSHETON describes:—posteriorly the cavity elongates by the downward growth of the dorsal blastopore lip, in the manner already described, while anteriorly it grows upward by a splitting between cells or by an accumulation of intercellular space accompanied by the multiplication and differentiation of cells *in situ*. This splitting process for the formation of the archenteron cavity and the growth of the blastopore lips seems to me also to be the direct continuation of the segmentation process. For my part, however, I do not quite see the necessity for distinguishing primary and secondary centres of growth, as ASSHETON has done.

The enlarging of the archenteric cavity as well as the reduction of the segmentation-cavity may be regarded from another point of view, simply as a process of rearrangement of the yolk cells in the egg interior. This has already been expressed by KOPSCH ('00) when he says "Da unsere Urteil hinsichtlich der

Verwertung des durch die Furchung geschaffenen Zellenmaterials beim Aufbau des Embryos in erster Linie abhängt von der Zellumlagerungen, welche bei der Gastrulation von sich gehen, so müssen wir die Vorgänge bei der Gastrulation zum Mittelpunkte der Betrachtung machen," (p. 19).

6). In *Rhacophorus* eggs, the gradual *growth of the neural plate* is indicated by a very conspicuous structural change. It is that the lower or neural layer of the epiblast becomes two-cell layered. This has been already noticed by ASSHETON ('94a) who says: "In the frog the nervous layer soon becomes thickened" (the number of cell-layers not specified) "along the future dorsal surface of the embryo, and over the rest of the embryo the nervous layer becomes reduced to a layer of one cell only in thickness, like the epidermic layer" (p. 169). This process takes place not only on the dorsal blastopore lip (Fig. 65 a) but also all around the rim of the blastopore (Fig. 65 b). However, no further progress is made except at the dorsal median portion where it spreads gradually forward and laterally over the dorsal surface of the future embryo (Fig. 66¹), until it reaches a point 30°-40° distant from the original upper pole when it stops. The region over which this change takes place I have indicated in Figs. 54-60 by crossed lines and in Woodcuts I-IV., by heavy black lines. By a careful study, I have ascertained that the upper limit of the two-cell layered portion of the mucous layer coincides approximately with the forward extension of the archenteric cavity at every stage, and therefore at the final stage marks the head end of the future embryo. This again shows clearly that the location of the embryo in the egg is along the meridian of the blastopore with its head end situated 30°-40° from the original upper pole of the egg.

1) Dorsal portion of the egg in Fig. 57 under a higher power.

7). The growth in length of the embryo in *Rhacophorus* and other anuran eggs takes place after the complete closure of the blastopore or the finishing of the gastrulation process.

IV.

EXPERIMENTS BY PUNCTURE OF EGGS.

After making out the normal course of development in the anuran eggs, it occurred to me that experiments by puncturing eggs would be interesting, if for no other reason than to determine what effects these injuries would have on the normal course. Accordingly during the breeding season of 1900, some experiments were made on the *Rhacophorus* eggs.

The puncturing of the eggs was done with the sharpest possible needles from the outside through the vitelline membrane and the thin innermost compact layer of the frothy substance. The injuries which I inflicted on the eggs proved on the whole rather severe, but this was an advantage than otherwise, for slight punctures heal rapidly and often leave no trace as has been observed by many previous investigators. Moreover as remarked already by ASSHETON, punctures however slight have the effect of causing eggs to deviate from the normal course, and if such is the case, it is preferable to have the deviations stand out unmistakably by making the injuries somewhat severe.

I will anticipate the main result of these experiments by the statement that the *normal course of development can not be made out from the results obtained by the experiments.*

The number of eggs experimented upon was in all forty,

divided into four groups, each of which consisted of ten eggs from the same egg-mass. As I was most successful with the fourth group, I shall begin with that:

Fourth Group. (Figs. 67-84).

This series of eggs was obtained from a mass probably deposited on the morning of May 4th. When taken on the morning of May 5th, the eggs were near the end of the segmentation process. At 2:20 p.m., of the same day, the first trace of the dorsal blastopore lip appeared. Puncturing operations were commenced at 2:30 p.m., (*i.e.* 10 minutes after the first appearance of the dorsal lip), and finished at 4:30 p.m. I will give my remarks on each egg observed in the form of notes¹⁾ :—

Egg No. 1. (Figs. 67 *a-e*, Fig. 76).

- May 5, 2:30 p.m. :—(Fig. *a*). Punctured just a little to the right of the yolk-pole (as seen in the posterior view).
 4:04 p.m. :—(Fig. *b*). Left side of the blastopore lip more inclined downwards than the right, and the ex-ovate moved also slightly to the right. Otherwise normal. (Fig. *b*).
 May 6, 7:55 a.m. :—(Fig. *c*). Ex-ovate attached to the right lip of the now circular blastopore. Otherwise normal.
 4:11 p.m. :—(Fig. *d*). Medullary folds and groove faintly recognizable. Ex-ovate attached to the right margin of the now very much smaller circular blastopore.
 May 7, 4:00 p.m. :—(Fig. *e*). Blastopore already closed. Medullary groove ready to be closed by the closely approximated folds. Embryo normal, except that the ex-ovate is at the right margin of the posteriormost part of the medullary groove.

This egg shows that an injury near or at the yolk pole causes perhaps the least amount of deviation from the normal course.

1) The time given in the notes is in many cases only approximate within few minutes.

In fact the development of the embryo in this egg is entirely natural and proves again the correctness of the observations before recorded. Fig. 76 shows diagrammatically the location of the embryo and the closing point of the blastopore.

Egg No. 2. (Figs. 68 *a-f*, Fig. 77).

May 5, 12:37 p.m.:—(Fig. *a*). Punctured a little to the left of the median line, closely under the left limb of the dorsal blastopore lip.

4:06 p.m.:—(Fig. *b*). Previously distinctly visible dorsal lip utterly unrecognizable. Ex-ovate visible. Area of the segmentation-cavity diminishing in the normal manner.

May 6, 8:02 a.m.:—(Fig. *c*). Blastopore with distinct lips, again visible, closed in circle, but larger as compared with the same stage of Egg No. 1 (Fig. 67 *c*). Ex-ovate attached inside the blastopore near its left margin. In front of, and parallel to the dorsal lip, another *accessory* lip present, formed perhaps by the reappearance of the vanished original lip. Area of the segmentation-cavity irregular in shape and found eccentrically a little to the left of the median line, showing that the rearranging process of the yolk-cells inside was disturbed.

4:14 p.m.:—(Fig. *d*). Area of the segmentation-cavity entirely vanished. Shape of the greatly diminished blastoporic area triangular, being a little elongated dorso-ventrally. Ex-ovate still attached within the blastopore near the left margin. Accessory dorsal lip still visible and in its front the neural plate and the shallow medullary groove faintly recognized.

May 7, 9:40 a.m.:—(Fig. *e*). Blastopore completely closed; ex-ovate still attached to the part where the blastopore closed. *Accessory lip has now grown around and enclosed a new circular patch in front of the actual closed blastopore.* Embryonic body raised a little over the egg-surface in front of the accessory lip; comparatively short and somewhat incomplete.

4:03 p.m.:—(Fig. *f*). Embryonic body, medullary groove and folds distinctly recognizable. Distal end of the medullary groove has not yet reached the final closing point of the actual blastopore, there being interposed the circular patch formed by

the accessory dorsal lip. Ex-ovate attached to the closing point of the actual blastopore. Embryo short, although otherwise not greatly abnormal, its axis turned about 14° – 15° to the right.

It seems to me that in this egg, the blastoporic area has somehow become divided into two entirely separate parts: (1) the upper smaller part which was represented by the accessory lip and which completed itself in a circle separate and smaller; and (2) the lower, larger, main part which closed itself finally at the point of puncture, the overgrowth of the yolk having been effected mostly by the ventral and lateral lips. Moreover the embryonic body must all have been formed in front of the injured spot, *i.e.* mostly in the original upper hemisphere of the egg. Fig. 77 gives the diagram of this egg.

Egg No. 3. (Figs. 69 *a-f*, Fig. 78).

May 5, 12:40 p.m.:—(Fig. *a*). Punctured at the periphery of the translucent area of the segmentation cavity, in front of, and in the line of the first appearing dorsal blastopore lip.

4:40 p.m.:—(Fig. *b*). Dorsal blastopore lip has grown somewhat downward and in length, and shows a more than usually crescentic curve at the highest point of which the ex-ovate is now attached.

May 6, 8:12 a.m.:—(Fig. *c*=posterior, Fig. *d*=left side view). Very peculiar aspect. Blastopore circle finished, but unusual in appearance. Ventral part round and smooth, but a gradually narrowing arm sent dorsad, and the ex-ovate attached at the tip. Segmentation cavity likewise peculiar, and elongated antero-posteriorly.

4:19 p.m.:—(Fig. *e*). Area of the segmentation cavity entirely vanished. Blastopore grown much smaller and almost circular, but still connected by a narrow slit with the ex-ovate. General outline of the embryonic shield faintly recognized.

May 7, 9:10 a.m.:—(Fig. *f*). Medullary groove and folds well re-

cognizable. Blastopore already closed leaving a small yolk-plug. Ex-ovate detached and adhering to the inner face of the vitelline membrane, but its scar still visible as a plug-like protuberance of the yolk substance in front of the normal yolk-plug. Embryonic body shorter than normal.

The first effect of puncture in this egg seems to have been a sending upward of the dorsal blastopore lip toward the point of injury. This was probably due to the exudation of more or less yolk substance in the intervening space. The dorsal lip does not seem to have grown downward at all after this, thus causing a peculiar pointed bay in the blastoporic area. The growth over the yolk seems to have been undertaken entirely by the ventral and lateral lips. The whole of the embryo was without doubt formed entirely on the upper hemisphere, as in No. 2. Fig. 78 represents diagrammatically the location of the embryo body. The oblique dotted line indicates the part over which the dorsal blastopore lip was pulled upward.

Egg No. 4. (Figs. 70 *a-f*, Fig. 79).

May 5, 2:43 p.m.:—(Fig. *a*). Punctured on both sides of the dorsal blastopore lip slightly below its level, and at about equal distances from it (the right puncture just a little nearer and heavier, hence its ex-ovate larger).

4:14 p.m.:—(Fig. *b*). No noteworthy peculiarity. Translucent area grown smaller. Dorsal lip grown slightly downwards.

May 6, 8:19 a.m.:—(Fig. *c*). Blastopore circle completed but very peculiar in appearance. Two horns sent outward from its upper lateral corners to each ex-ovate. Dorsal lip curved contrary to the usual way and has a hanging festoon-like outline, showing that the middle point is growing fastest downward. Segmentation cavity of an unusual shape, being elongated antero-posteriorly and somewhat out of the median line on the left side.

4:22 p.m.:—(Fig. *d*). Segmentation-cavity entirely vanished.

Blastopore circle grown much smaller and become oval in shape; detached from the smaller left ex-ovate, although the basal part of the horn by which it reached the latter still remains; approximated much closer to the larger right ex-ovate; dorsal lip again in the normal crescentic curve.

May 7, 9:14 a.m.:—(Fig. *e*). Blastopore just closed leaving a comparatively large yolk-plug to which the right ex-ovate is attached. Left ex-ovate found near the left margin of the neural plate, though its distance from the right ex-ovate is nearly the same. Neural plate clearly recognizable.

4:15 p.m.:—(Fig. *f*). Yolk-plug withdrawn into the interior. Right ex-ovate attached to the posterior end of the right medullary fold. Left ex-ovate in the same position as before. Embryo itself normal, but its position greatly revolved leftward.

In this egg, the blastopore was closed at the heavier right puncture, and the growth over the yolk was undertaken mostly by the ventral, and left lateral lips. As the axis of the embryobody has veered greatly leftward with the right ex-ovate as the center, the main part of the body has been formed within the early equatorial zone, as shown in Fig. 79.

Egg No. 5. (Figs. 71 *a-f*, Fig. 80).

May 5, 3:57 p.m.:—(Fig. *a*). Punctured at the approximate middle point of the region antipodal to the first appearing dorsal blastopore lip, at about the level of the equator.

4:14 p.m.:—(Fig. *b*). Dorsal lip grown somewhat downward seems to be moving leftward.

May 6, 8:25 a.m.:—(Fig. *c*). Blastopore circle completed, and grown moderately small; ex-ovate attached within it near the ventral lip. Area of the segmentation cavity diminished in size, and has shifted its position opposite as usual to the blastopore circle.

4:26 p.m.:—(Fig. *d*). Broad neural plate faintly recognized. Blastopore greatly reduced in size; ex-ovate attached to its left margin. Area of the segmentation-cavity entirely vanished.

May 7, 9:16 a.m.:—(Fig. *e*). Blastopore completely closed; yolk plug no longer visible. Ex-ovate attached to the left side of the closing point of the blastopore. Head and neural folds faintly recognizable although the posterior part of the neural groove is not yet distinct.

4:18 p.m.:—(Fig. *f*). Embryo formed and quite normal. Ex-ovate attached to the left side of the tail end.

Here we must conclude that the blastopore was closed at the punctured point (*i.e.* at the point opposite the first dorsal lip) by an excessive growth of the dorsal and lateral lips: as the embryo was situated in front of this, it must have been formed entirely on the lower hemisphere as given diagrammatically in Fig. 80.

Egg No. 6. (Figs. 72 *a-g*, Fig. 81).

May 5, 1:35 p.m.:—(Fig. *a*). Punctured closely below the dorsal blastopore lip, slightly to the right of the median line.

4:17 p.m.:—(Fig. *b*). Nothing noteworthy.

May 6, 8:32 a.m.:—(Fig. *c*=posterior, Fig. *d*=left side view). Segmentation-cavity and blastoporic area similarly acuminate toward the punctured point.

4:30 p.m.:—(Fig. *e*). Blastopore circle much smaller and now circular in shape. Ex-ovate attached to its upper margin. Area of the segmentation cavity entirely vanished.

May 7, 9:20 a.m.:—(Fig. *f*). Embryonic area defined. Ex-ovate attached to its posterior end. Blastopore already closed.

4:21 p.m.:—(Fig. *g*). Embryonic body well formed, normal in every way except that it is short. Ex-ovate attached to its posterior end.

Fig. 81 gives a diagrammatic representation of this egg. The embryo except for its shortness is entirely normal as is also its location. This is probably due to the circumstance that the puncture was slight, compared with Egg No. 2. Although the point of injury was different, the results in this egg are very much like those seen in Egg No. 3.

Egg No. 7. (Figs. 73 *a-f*, Fig. 82).

(Cf. Egg No. 3).

May 5, 12:50 p.m.:—(Fig. *a*). Punctured closely above the approximate middle point of the dorsal lip.

4:19 p.m.:—(Fig. *b*). Dorsal lip slightly grown downward; its middle point bent and drawn upward toward the ex-ovate with which it is in direct contact. (Cf. Fig. 69 *b*).

May 6, 8:35 a.m.:—(Fig. *c*). Area of the segmentation-cavity unusually small. Blastopore circle still large and acuminate toward the ex-ovate. (Cf. Figs. 69 *c* and *d*).

4:32 p.m.:—(Fig. *d*). Segmentation-cavity no longer visible. Blastopore circle small, and somewhat triangular in shape, with the apex touching the ex-ovate. Neural plate faintly recognizable.

May 7, 9:24 a.m.:—(Fig. *e*). Blastopore closed; ex-ovate attached to the point of its closure. Yolk-plug withdrawn. Neural plate distinct.

4:25 p.m.:—(Fig. *f*). Medullary groove ready to be closed. Ex-ovate attached to the posterior end of the right medullary fold.

The results as represented in Fig. 82 are very much as in Egg No. 3, as well as in Egg No. 6.

Egg No. 8.

Failure.

Egg No. 9. (Figs. 74 *a-f*, Fig. 83).

May 5, 1:15 p.m.:—(Fig. *a*). Punctured at the approximate animal pole, somewhat heavily, as the fluid contents of the segmentation cavity were forcibly ejected at the time.

4:23 p.m.:—(Fig. *b*). Segmentation cavity collapsed; even a small quantity of yolk substance had been protruded. Dorsal blastopore lip growing downward normally.

May 6, 8:44 a.m.:—(Fig. *c*). Shrinkage of the upper hemisphere recovered; area of the segmentation-cavity disproportionately

small compared with the blastoporic area. First ex-ovate had been lost without leaving any trace; in its place a large globular mass protruded anew. Blastopore lip grown considerably downward.

4:41 p.m.:—(Fig. *d*). Segmentation cavity no longer visible. Blastopore considerably reduced in size. Large ex-ovate in front, and to the right of the now faintly recognizable medullary plate.

May 7, 9:30 a.m.:—(Fig. *e*). Medullary groove and folds evident. Ex-ovate in front.

4:27 p.m.:—(Fig. *f*). Embryonic body distinct, shorter than normal. Medullary groove ready to be closed. Ex-ovate a short distance in front of the head end.

The shortness of the embryonic body is probably due to the exudation of the second large ex-ovate. The development is otherwise normal. Compare Diagram Fig. 83.

Egg No. 10. (Figs. 75 *a-f*, Fig. 84).

(*Cf.* Egg No. 5).

May 5, 2:43 p.m.:—(Fig. *a*). Punctured on the ventral median line, approximately 45° below the equator.

4:26 p.m.:—(Fig. *b*). Normal.

May 6, 8:46 a.m.:—(Fig. *c*). Blastopore circle completé. Ex-ovate at its ventral lip.

4:45 p.m.:—(Fig. *d*). Area of the segmentation cavity vanished. Blastopore much smaller and the ex-ovate attached to its left dorsal margin.

May 7, 9:30 a.m.:—(Fig. *e*). Blastopore nearly closed, leaving a small yolk-plug. Ex-ovate out of the blastopore, but still closely attached to the dorsal lip. Neural plate faintly visible.

4:30 p.m.:—(Fig. *f*). General outline of the embryo formed. Ex-ovate attached to the posterior end of the left neural fold.

We must conclude that the blastopore was finally closed at the point of injury, the ventral lip ceasing to grow when it reached the ex-ovate about 45° below the equator, the task of overgrowth

being thereafter undertaken by the dorsal and lateral lips. The location of the embryo must therefore be, as in Fig. 84, on the lower hemisphere of the egg. The results are exactly as in Egg No. 5, the only difference being that as the puncture was further down in this egg, the position of the embryo was different in the same degree.

First Group. (Figs. 85-86).

The eggs of this group were picked out from a mass obtained on the morning of April 17th., (deposited in all probability on the morning of the 16th.) and were at the time at the end of the segmentation process. The first trace of the dorsal blastopore lip appeared at 2 p.m., of the same day. Puncturing was begun at 2:30 and finished at 4:40 p.m.

Egg No. 1.

April 17, 2:30 p.m.:—Punctured at the approximate center of the lower yolk hemisphere as in Group IV., Egg No. 1. (*Cf.* Figs. 67 *a-e*). Watched until April 19, 2:12 p.m. when the embryo had been completely formed in the normal way, with only the ex-ovate attached to the distal end of the embryonic body. Results are exactly as in the egg referred to: hence details may be omitted.

Egg No. 2.

April 17, 2:30 + p.m.:—Punctured below the dorsal lip slightly to the left of the median line, at a point which was two-thirds of the distance from the yolk pole to that lip (as in Group IV., Egg No. 2, Figs. 68 *a-f*). Puncture unfortunately rather severe and an excessive amount of egg-contents exuded.

April 18, 2:00 p.m.:—Upper hemisphere strongly depressed. Area of the segmentation cavity vanished. Still more matter exuded

and covered the posterior portion of the egg so that the blastopore could not be seen.

April 19, 2:20 p.m.:—Head portion of the embryo seen beneath, and in front of, the exuded matter.

Although exact observation could not be made, there is no reason to suppose that the course of development was, in the main, otherwise than was seen in Group IV., Egg No. 2, or Nos. 6 and 7.

Egg No. 3.

April 17, 2:30 + p.m.:—Punctured as in Group IV., Egg No. 3, (Figs. 69 *a-f*), but somewhat higher. Liquid-contents forced out of the segmentation cavity whose roof became shrunken. Exudation, however, soon ceased, and the roof recovered its usual appearance. Then the distance between the punctured point and the dorsal lip became reduced to about $\frac{1}{2}$. This is in all probability due to the upward movement of the blastopore toward the punctured point as in Group IV., Egg No. 3.

April 18, 2:15 p.m.:—Area of the segmentation cavity and blastoporic area reduced in size in equal proportions. But the distance of the ex-ovate from the dorsal lip remains the same.

April 19, 2:30 p.m.:—General outline of the embryonic body evident. Blastopore completely closed, and at the same distance from it as before, the ex-ovate.

How the ex-ovate keeps its distance from the dorsal lip unaltered, after the first rather sudden approximation of the two may probably be explained in this way. After the first pulling upward of the dorsal lip toward the injured point, it ceases to grow any further downward, due somehow to the injury inflicted above it and the consequent exudation of the contents within, and the overgrowth of the blastopore lips over the yolk is performed mostly by the ventral and lateral lips. The final point of the blastopore closure is therefore near the point of the

first appearance of the dorsal lip, and the embryo is formed almost entirely on the upper hemisphere of the egg (Fig. 78). The results are therefore identical with those of the egg of the corresponding number in Group IV.

Egg No. 4. (Figs. 85 *a-c*, Fig. 85').

April 17, 2:30 + p.m. :—(Fig. *a*). Punctured on both sides of the dorsal blastopore lip, slightly below its level and at equal distances from it (as in Group IV., Egg No. 4, Fig. 70 *a*). Right puncture heavier than left in this egg also, but the two punctures further from the dorsal lip and from each other than in that egg. Ex-ovate produced from the punctures in profile in the posterior view and about 20° below the equator. Another unexpected small and transverse ex-ovate formed directly beneath the dorsal lip which, however, disappeared next day.

April 18, 2:30 p.m. :—(Fig. *b*). Right and left ex-ovates somewhat nearer each other. Blastopore oval and closer to the right (larger) ex-ovate. Main axis of the future embryo-body somewhat curved toward the right side. This state of things can probably be explained by unequal growth of blastopore lips. Punctures and ex-ovates must check the growth of the blastopore lips, when they are reached in the downward growth. In this case, the growth has not entirely ceased but been only retarded, longer on the right side than on the left, because the injury was severer on the former side. Dorsal lip was also somehow checked in its further downward growth, when it reached the level of the ex-ovates, and overgrowth must have been done mostly by the ventral lip. Hence the position of the blastopore at the level of the ex-ovates; its shape is transversely oval, because retarded at the ex-ovates; it is nearer the right ex-ovate because the left lip was, so to speak, released sooner at the left ex-ovate and had grown more than the right.

April 19, 2:30 p.m. :—(Fig. *c*). Blastopore nearly closed, leaving only a small yolk-plug. Right ex-ovate detached from the punctured

point and carried to the right of the middle of the embryo. Left ex-ovate in the former position. Embryo entirely recovered from its lateral curvature and quite normal.

As in Group IV., Egg No. 4, the blastopore closed in this egg near the right point of puncture, the growing of its rim over the yolk being performed mostly by the ventral and left lateral lips. But unlike that egg (Fig. 79), the position of the embryo was more normal, although the main portion of it was formed on the upper hemisphere (Fig. 85').

Egg No. 5. (Figs. 86 *a-c*, Fig. 86').

April 17, 2:30 + p.m.:—(Fig. *a*). Punctured at the approximate middle point of the region opposite the first appearing dorsal lip, as in Group IV., Egg No. 5, Fig. 71 *a*. The point of injury was a little below the level of the dorsal lip, and the exudation of the contents somewhat serious so that the upper hemisphere shrunk near the puncture. It soon recovered, however.

April 18, 2:40 p.m.:—(Fig. *b*=view from the original lower pole). Area of the segmentation cavity disappeared. Blastopore small, and about 30° dorsad from the ex-ovate.

April 19, 3:00 p.m.:—(Fig. *c*). Blastopore closed, about 35° dorsad of the ex-ovate, the increased distance (5°) being equal to about half the diameter of the blastoporic circle in Fig. *b*. Embryonic body distinct.

Unlike the egg of the corresponding number in Group IV., the blastopore did not close in this egg at the punctured point, but 35° below it (Fig. 86'). We must suppose that the ventral lip was able to overcome the obstacle presented by the puncture and to grow further downward. The final closing point of the blastopore becomes as usual the tail end of the embryo which is not therefore formed entirely on the lower hemisphere as in Fig. 80.

Egg No. 6.

April 17, 2:30 + p.m.:—Punctured in the middle line, close below the dorsal blastopore lip (*Cf.* Group IV., Egg No. 6, Figs. 72 *a-g*).

Blastopore finally closed at the point of puncture, and the embryo stretching forward from this point was distinctly shorter than normal, though not as short as in Group IV., Egg No. 6.

The results are therefore exactly like those in the egg of the corresponding number in Group IV.

Egg No. 7.

Not punctured, as there were 1 large, and 3 or 4 small natural ex-ovates in the anti-blastoporic region. Twenty four hours later, they had, however, all become detached from the egg and attached to the inner surface of the vitelline membrane. Hence quite useless for the present investigation.

Egg No. 8.

Punctured closely above the first dorsal blastopore lip as in Group IV., Egg No. 7 (Figs. 73 *a-f*, Fig. 82). In this egg the injury was slightly to the right of the middle line and lighter. Twenty-four hours later, blastopore circular and already small; ex-ovate attached to its right margin. Hence stage like Fig. 73 *c* missed, if present. Another twenty-four hours, ex-ovate attached to the right margin of the closed blastopore. Embryo short but otherwise normal.

The results are therefore absolutely the same as those in Group IV., Egg No. 7.

Egg No. 9.

Chiefly instructive in regard to the formation of ex-ovates. Punctured at the approximate lowest point of the yolk

hemisphere as in Egg No. 1 of this and Group IV. Exudation rather less than usual.

Twenty-four hours later, ex-ovate enormously elongated, within the vitelline membrane, into a long streak the base of which was attached to the left margin of the moderately closed blastopore. The point in the vitelline membrane through which puncturing was effected shifted considerably upward from the dorsal lip. Careful examination showed that as after puncture I had placed the egg with the yolk hemisphere to one side, the egg rotated within the vitelline membrane to its natural position, and in so doing the ex-ovate was gradually elongated into a long streak by additional exudation. I tried to restore the egg to the proper position, but the exudation outside the outer envelope had become firmly attached to cotton-fibres in a bed of which substance the egg had been placed. So the egg was left as it was.

Another twenty-four hours, some more exudation which seemed, however, completely detached from the egg-surface. Embryo seems normal in its formation beneath the large, but detached ex-ovate.

The final closing point of the blastopore and the location of the embryonic body were somewhat obscured by the exudations, but as the ex-ovate was seen distinctly attached to the dorsal lip up to the evening of the second day after puncture, we may suppose that the final point of the blastopore closure was, as usual, at the punctured point (in this case, the yolk-pole), and that the embryo must have been formed as in other cases of this kind.

Egg No. 10.

Punctured on the ventral median line about 68° below the equator, *i.e.* somewhat lower than in Group IV., Egg No. 10 (Figs. 75 *a-f*).

Twenty-four hours later, blastopore diminished to a very small circular spot to the ventral margin of which the ex-ovate was attached.

After another twenty-four hours, the ex-ovate had un-

fortunately become detached and was found a short distance forward from the tail end of the embryo. Embryo quite normal.

Although the detaching of the ex-ovate obscured the final stage, there can be no reasonable doubt that the closing point of the blastopore was at, or near, the punctured point, and so the location of the embryonic body nearly the same as Egg No. 5 of this group or Egg No. 10 of Group IV.

Second Group. (Figs. 87-92).

The eggs of this group were obtained from a mass deposited late on the morning of April 21. The dorsal blastopore lip appeared at 8:10 a.m., April 23. Puncturings were carried out between 8:20 and 9:30 of the same morning.

Egg No. 1. (Figs. 87 *a-d*, Fig. 88).

- April 23, 8:20 a.m.:—(Fig. *a*). Punctured at the approximate middle point of the yolk-pole (*Cf.* Group I., Eggs Nos. 1 and 9, and Group IV., Egg No. 1). Needle-point somewhat blunt and did not penetrate well. Only a small ex-ovate.
- April 24, 7:20 a.m.:—(Fig. *b*). Blastopore much reduced in size, and somewhat oval in shape. Ex-ovate near the dorsal lip showing that this lip has grown faster than the ventral. Another unexpected ex-ovate discovered near the ventral lip, accounting for the slow growth of that lip, and the shape of the blastopore.
- 3:30 p.m.:—(Fig. *c*). Blastopore much smaller. Dorsal ex-ovate now excluded out of it and found to the left of the median line of the neural plate consisting of a larger anterior, and a smaller posterior globule arranged close together longitudinally. Former no doubt the original ex-ovate, and the smaller formed at the time of exclusion. Ventral ex-ovate at the ventral blastopore lip.
- April 25, 5:40 p.m.:—(Fig. *d*). General outline of the embryonic body distinct. Embryo, however, somewhat incomplete and its axis

more or less curved to right and left in a wavy manner. Dorsal ex-ovate as before. Ventral ex-ovate unfortunately detached and lost without trace. But a mass of yolk has exuded anew from the closing point of the blastopore, probably at the time the ventral ex-ovate was detached.

In the normal course of development, the dorsal lip ought to have stopped when it reached the first ex-ovate formed by puncturing. But in this egg there was formed unexpectedly a second ex-ovate which stood in the way of the ventral lip growing over the yolk. Accordingly the dorsal lip went on growing toward the ventral lip, until the blastopore finally closed at or near the second ex-ovate. The embryo was thus formed as in Fig. 88. Seeing that all these disturbances took place, it is no wonder that the embryo body is somewhat abnormal.

Egg No. 2.

Punctured at the point intermediate between the dorsal lip and the yolk-pole, on the blastopore meridian. (Therefore lower and more in the middle line than in the eggs of the corresponding number in Groups I., and IV.). Although exudation at the moment was slight, the pressure of the somewhat blunt needle must have been rather strong. At any rate a number of larger and smaller unexpected ex-ovates appeared below the dorsal lip and near the puncture-point. Afterwards there were further exudations at this point as well as from the left limb of the dorsal blastopore lip when it had grown down some distance, showing that the arrangement of the internal contents must have been considerably disturbed. The results of all these were that the dorsal, ventral and lateral, lips were all hindered in their growth and the embryo was abnormal, being shorter, and the neural groove and folds being incomplete, especially near the head-end. The closure of the blastopore was also incomplete, a large yolk-plug being present at the end of the third day. But in the main,

the closing point of the blastopore was at or near the punctured point, and the anterior $\frac{2}{3}$ or $\frac{3}{4}$ of the embryonic body were formed in front of the point at which the dorsal lip first appeared.

Egg No. 3.

April 23, 4:15 p.m.:—Taken from another egg-mass and punctured at the upper pole, as in Group IV., No. 9. Fluid contents of the segmentation cavity forcibly ejected, and the upper hemisphere shrunken. After a few minutes ejection stopped.

April 24, 7:25 a.m.:—Shrinkage of the upper hemisphere recovered, although the area of the segmentation cavity unusually small. Blastopore circle completed and grown down to about 50° below the equator. Neural plate faintly visible.

2:20 p.m.:—Small ex-ovate found in front of the neural plate and to the right of the median line.

April 25, 5:00 p.m.:—Blastopore completely closed. General outline of the embryonic body evident, with well-defined neural groove and folds. Ex-ovate found 20° – 50° forward from the head-end of the embryo, about opposite the final closing point of the blastopore. Embryo quite normal.

This egg was therefore like Group IV., Egg No. 9 with this difference, that in the latter the embryo was shorter, owing to the exudation of the large secondary ex-ovate after the first was lost.

Egg No. 4. (Figs. 89 *a-e*, Fig. 90).

April 23, 8:20 + a.m.:—(Fig. *a*). Punctured on both sides of the dorsal blastopore lip, some distance below its level (as in the eggs of the corresponding number in Groups I., and IV.). In this egg, left puncture at the periphery of the egg when seen in the posterior view; right puncture higher and nearer the dorsal lip.

2:40 p.m.:—(Fig. *b*). Dorsal lip grown downwards and elongated, left limb reaching the left ex-ovate; right limb slightly above the right ex-ovate.

April 24, 7:00 a.m.:—(Fig. *c*). Blastopore lips grown further down-

ward, and the circle complete; ovate in shape, the pointed extremity extending toward and reaching the right ex-ovate. Place of the small left ex-ovate already grown over and now outside the blastopore.

2:15 p.m.:—(Fig. *d*). Blastopore circle grown much smaller. But quite unexpectedly a new short transverse ex-ovate was found attached close to the ventral lip. Right ex-ovate now also outside the blastopore circle, but a string-like prominence with rough surface connecting it with the new transverse ex-ovate (its nature not clear). We may suppose that a large new obstacle appearing to hinder the progress of the ventral lip, the right lip had time allowed it to overcome the right ex-ovate and to grow down toward the ventral lip producing somehow the string-like prominence in its track. If this new obstacle had not appeared, the blastopore might have closed much nearer the right ex-ovate. Neural plate not yet recognizable, although usually seen at this stage.

April 25, 5:07 p.m.:—(Fig. *e*). General outline of the embryonic body with partly closed neural groove and closely approximated folds distinct. Embryo short and curved to the right. Right ex-ovate attached to the right side of the embryonic head; left ex-ovate in the former position. Transverse ex-ovate at the posterior end of the embryo.

The actual point of the blastopore closure and the location of the embryonic body were somewhat obscured in this egg. But the former, we may suppose, was a little to the left of the yolk-pole, and the embryonic body was directed from this point obliquely toward the point which corresponded to the right edge of the dorsal blastopore at its first appearance, as shown in Fig. 90. The inclination of the embryonic axis to the former blastopore meridian is about 45°.

Egg No. 5.

April 23, 3:40 p.m.:—Taken out of another egg-mass. It was intended to puncture the egg at the anti-blastoporic region as in the

eggs of the corresponding number in Groups I., and IV. But when punctured, the point was found to be above the level of the dorsal lip, and to the right of the anti-blastoporic meridian (*i.e.* to the left when seen from the blastoporic meridian). Then another puncture was made: This time, it was at the level of the dorsal lip but slightly to the left of the anti-blastoporic meridian (*i.e.* to the right, when seen from the blastoporic meridian). From the first puncture, a small quantity of the egg-contents exuded through the egg envelope; from the second, a small protuberance was produced as an ex-ovate inside the envelope.

April 23, 7:15 a.m.:—Blastopore circle already finished. Ventral lip has overcome the first ex-ovate and passed 10° beyond it, but not yet detached from the second ex-ovate which is found close to this lip. Dorsal and lateral lips grown much further, 55° – 60° below the equator.

3:07 p.m.:—Blastopore reduced to a small circular spot; second ex-ovate close to the right lateral lip; first ex-ovate about double the same distance from the ventral lip.

April 24, 5:15 p.m.:—Blastopore closed. Embryonic body formed normally. First ex-ovate at the distal end of the embryonic body; second ex-ovate, on the right side of the right neural fold; distance between the two unaltered. Growth therefore must be by the dorsal and lateral lips.

As the blastopore was closed about 10° below the first ex-ovate, and the embryo extended dorsad from this, nearly the whole of the embryonic body must have been formed on the yolk hemisphere, below the first dorsal blastopore lip. (*Cf.* Fig. 80).

Egg No. 6.

Punctured close below the dorsal blastopore lip in the median line, (*Cf.* the eggs of the corresponding number in Groups I., and IV.). Twenty-four hours later, the blastopore circle completed and all going well. Eight hours later, a large new tubercle-like ex-ovate was discovered close below the ventral lip. This was probably due in some way to the

pressure of the left hand needle during the operation which was done with the right. After this, the course of development was checked, and the egg was set aside.

Egg No. 7.

- April 23, 8:20 + a.m. :—Punctured at the yolk-pole (as in Group I., Eggs Nos. 1 and 9, and Group IV., Egg No. 1). The point of puncture was however not at the actual pole, but slightly to the left when seen in the posterior view. Exudation somewhat forcible.
- April 24, 7:22 a.m. :—Egg entirely recovered. Blastoporic area and area of the segmentation cavity diminishing in the normal way. Ex-ovate near the middle point of the blastoporic area.
- 2:50 p.m. :—Blastopore now reduced to a small spot. General outline of the neural plate faintly recognizable. Ex-ovate close to the left blastopore lip.
- April 25, 5:20 p.m. :—Blastopore completely closed. General outline of the embryonic body well established, entirely normal. Ex-ovate attached to the left margin of the closed blastopore.

Except that the axis of the embryo did not coincide with the blastopore meridian, but was bent slightly to the left, because the punctured point which became the tail end was slightly to the left, the results agree entirely with those in the above mentioned eggs in Groups I., and IV.

Egg No. 8.

- April 23, 9:00 a.m. :—Punctured near the right limb of the dorsal blastopore lip. Injury not severe, and a small quantity of the egg-contents exuded outside the egg-envelope.
- 2:50 p.m. :—Dorsal blastopore lip increased in length and grown a little downward, so that the right limb nearly reached the ex-ovate.
- April 24, 7:30 a.m. :—Moderately reduced blastoporic area peculiar in shape: ovate with the pointed end turned toward the ex-ovate with which it was directly connected. Lateral and ventral lips not distinct.

2:50 p.m.:—Blastopore greatly reduced in size, but a very large mass of the egg-contents had been exuded from the ventral margin of the small blastopore, concealing from view that region. First ex-ovate still attached to the right lateral lip. Neural groove faintly recognized in front of the dorsal lip.

April 25, 5:25 p.m.:—Large secondary ex-ovate larger in size by additional exudation covering the posterior part of the egg but apparently entirely detached from the exuding part. Blastopore barely closed in front of the large ex-ovate. Original ex-ovate attached to the right margin of the tail fold, showing that the blastopore must have closed near the region when the dorsal lip first appeared. Embryo very imperfect: head could not be detected in the external view; tail well formed.

Although the formation of the embryonic body in this egg was very incomplete, the results in regard to the final point of the blastopore closure and the location of the embryonic body agree in the main with those obtained from Group IV., Nos. 2, 6, and 7; Group I., Nos. 6 and 8, and No. 6 of this group.

Egg No. 9. (Figs. 91 *a-d*, Fig. 92).

April 23, 9:00 + a.m.:—(Fig. *a*). Puncturing was intended to be at the exact yolk-pole, but was in fact slightly to the right in the posterior view.

April 24, 7:55 a.m.:—(Fig. *b*). Blastoporic area greatly reduced, circular in shape but connected with the ex-ovate by an arm on the right lip.

2:45 p.m.:—(Fig. *c*). Blastopore now reduced to a very small circle. Ex-ovate now outside of it to the right. Neural plate faintly visible.

April 25, 5:30 p.m.:—(Fig. *d*). Blastopore completely closed. General outline of the embryonic body well made out: *ex-ovate is at the exact axial end of the embryo, and the final closing point of the blastopore a little to the left of it did not become the tail end.*

The diagram of this egg is given in Fig. 92. Although there

is the last-noted peculiarity, the main results are the same as in other eggs which received similar injury: the blastopore closed at the punctured point and the embryo was situated as in the normal course of development.

Egg No. 10.

Intended to be punctured as in the eggs of the corresponding number in Groups I., and IV., but owing to failures in manipulation the results were meagre and not worth recording; there was nothing contrary to what had already been made out in other eggs.

Third Group. (Figs. 93-96).

The eggs of this group were taken from the mass deposited probably on the early morning of April 27. The dorsal blastopore lip appeared at 12:30 p.m., April 28. Puncturing operations were performed on them between 12:40 and 1:35 p.m. of the same day.

Egg No. 1.

Punctured at the approximate yolk-pole, as in the eggs of the same number in the three other groups. Ex-ovate not large. All went well during the first two days, excepting that at one time the surface of the yolk-hemisphere near the ventral lip had a rough appearance. But when examined 8:55 a.m., third day (April 30) a large and oval mass of yolk substance had been protruded outward as a yolk-plug along the dorsal blastopore margin. Original ex-ovate a little outside of the much reduced blastopore area near its ventral lip. At 5:06 p.m., (the same day) the peculiar yolk-plug had been largely withdrawn within the egg-interior. Blastopore become a small circular spot. Ex-ovate further removed from the

blastopore on the ventral side. Neural groove, though not neural folds, recognizable. At 7:00 a.m., fourth day (May 1), embryo formation not advanced. Blastopore completely closed at about the place of the exuded yolk-plug.

This egg must somehow have been damaged by the puncturing operation more than at first appeared. The final closing point of the blastopore was not at the point of puncture, which was the yolk-pole in this case, but at a point about 20° dorsad of that pole on the blastopore meridian, and the incomplete embryo stretched forward from that point. This is undoubtedly to be accounted for in this way: the ex-ovate stood for a time in the way of the growth of the ventral lip, but a greater obstacle in the shape of a peculiar yolk-plug effectually stopped the downward progress of the dorsal lip. Thus the ventral lip had time to overcome its obstacle and to grow up toward the dorsal lip, where the blastopore closed.

Egg No. 2.

Punctured about 20° below the dorsal blastopore lip in the middle line (as in the corresponding number of the other groups). Exudation little and injury seemed not very great. Four hours later, dorsal lip grown down close to the ex-ovate. Blastoporic region somewhat bulged outward. At 8:23 a.m., next day (April 29), a large new tubercle-like ex-ovate had been produced hanging down from the dorsal region, in front of the dorsal blastopore lip. Original ex-ovate now attached to the middle basal part of the new tubercle, anteriorly from the dorsal lip. At 5:35 p.m., blastoporic circle completed but acuminate toward the tubercle, as also the area of the segmentation-cavity from above. At 9 a.m., third day (April 30), area of the segmentation cavity disappeared. Blastopore area still large. At 5 p.m., blastopore greatly reduced in size. Tubercle-like ex-ovate smaller and grown in length, whereby the original ex-ovate was pushed further

forward from the dorsal lip. At 7 a.m., fourth day (May 1), blastopore closed just behind the tubercle. Embryonic body not recognizable.

Although the definite location of the embryonic body was not made out, it is evident that the blastopore was closed at the place of the greatest obstacle to the growth of the blastopore lips.

Egg No. 3. (Figs. 93 *a-h*, Fig. 94).

April 28, 12:50 p.m.:—(Fig. *a*). Punctured about midway from the upper pole to the dorsal blastopore lip on the blastopore meridian. Not only liquid contents but also some solid yolk-substance exuded.

4:34 p.m.:—(Fig. *b*). Dorsal lip grown downward and increased in length; its right limb and ex-ovate nearer each other, as has been often noticed before in similar cases.

April 29, 8:30 a.m.:—(Fig. *c*=left side view, Fig. *d*=posterior view). Blastopore circle completed and grown small; shape ovate, acuminate toward the ex-ovate, which is found in the same relative position to the dorsal lip.

5:38 p.m.:—(Fig. *e*). Blastopore greatly reduced in size. Area of the segmentation cavity completely disappeared. Ex-ovate in the same relative position.

April 30, 9:04 a.m.:—(Fig. *f*). Blastopore nearly closed, leaving a small oval yolk-plug exposed. Ex-ovate about 30° above it in the same relative position. Medullary groove and folds not distinct.

5:12 p.m.:—(Fig. *g*). Blastopore completely closed. General outline of the embryonic body well made out: at its tail end is attached the ex-ovate.

May 1, 7:04 a.m.:—(Fig. *h*). Embryo formation more advanced. Ex-ovate in the same position as before.

The blastopore was probably closed in this egg at about the point where the dorsal lip first appeared, if we may judge from the position of the ex-ovate. But curiously enough, the tail end of the embryo did not coincide with the closing point of the blastopore,

but with the punctured point, as happened in Group II., Egg No. 9. The main parts of the embryonic body were, therefore, formed entirely upon the upper hemisphere (Fig. 94).

Egg No. 4. (Figs. 95 *a-g*, Fig. 96).

April 28, 1:35 p.m.:—(Fig. *a*). Punctured on both sides below the level of the dorsal lip as in the eggs of the same number in other groups. Punctures just seen in profile in the posterior view; about 20° below the level of the dorsal lip which was 8° or 9° below the equator. Right puncture heavier and exudation outside the envelope. Left puncture much lighter, slightly protruded.

4:38 p.m.:—(Fig. *b*). Dorsal lip grown downward and increased in length: its left limb longer and nearly reaching the left ex-ovate which has grown a little larger; its right limb still some distance from the right ex-ovate.

April 29, 8:45 a.m.:—(Fig. *c*=left side view, Fig. *d*=ventral view). Blastopore circle completed and ovate in shape, touching with the acuminate end the right ex-ovate. Left ex-ovate grown a little larger but firmly attached to the punctured point is far outside the blastopore circle (about 70° from the left lip), and curiously near the area of the segmentation cavity.

5:42 p.m.:—(Fig. *e*). Area of the segmentation cavity nearly disappeared. Left ex-ovate in the same relative position. Right ex-ovate still attached to the right lip of the further reduced blastopore.

April 30, 9:07 a.m.:—(Fig. *f*). Blastopore become still smaller and circular in shape, with the right ex-ovate yet closely attached to the right lip. Left ex-ovate more remote from the left lip. In front of the blastopore neural plate recognizable.

5:14 p.m.:—(Fig. *g*). Left ex-ovate approximately in the neck region of the future embryo. Right ex-ovate still attached to the right lip of the now greatly reduced blastopore. The egg unfortunately died after this.

As the blastopore closed at the right point of puncture where the injury was greater, the growth of the blastopore rim must

have been performed mostly by the dorsal, ventral, and left lips. And to judge from the relative position of the two ex-ovates, in regard to each other and to the embryo, the location of its body must have been as in Fig. 96. This is similar to Group IV., Egg No. 4 (Fig. 79), but the inclination of the embryo body to the blastopore is greater in this case than in that egg.

Egg No. 5.

Punctured at a point approximately antipodal to the place of the first appearance of the dorsal lip, as in the eggs of the corresponding number in the other groups. Development proceeded as in those eggs. Blastopore gradually closing presented an ovate shape, with the ex-ovate attached to its acuminate ventral end. When it was nearly closed, there was found a small yolk-plug on the top of which the ex-ovate somewhat reduced in size was attached. It finally closed here. Embryonic body extended from here forward.

This egg, therefore, substantiates the results obtained in the eggs of the same number in Groups II., and IV.

Egg No. 6.

Punctured at the approximate middle point close under the dorsal lip (see the corresponding number in other groups). $3\frac{1}{2}$ hours afterward, appeared somewhat peculiar: two dorsal lips, one above the other, as in Group IV., Egg No. 2 (Fig. 68.d). The higher was probably the original one. A large, rectangular ex-ovate exuded afresh behind the lower and actual dorsal lip. Original ex-ovate attached to the middle point of this. At 9:05 a.m., next day (April 29), the area of the segmentation-cavity strongly acuminate toward the ex-ovates (as in Group IV., Nos. 3 and 6 and No. 2, of this group), and much more than the blastopore.

Blastopore grew smaller and in the end would probably have closed somewhat behind the large secondary ex-ovate

which was further enlarged by fresh exudation. Embryonic body stretched forward from this and was short compared with the normal. Original ex-ovate on the middle point of the secondary ex-ovate as before.

Although the results are imperfect, the manner of the blastopore closure and the location of the embryonic body are as in Egg No. 6 of Groups I., and IV.

Egg No. 7.

Punctured close above the middle point of the dorsal blastopore lip. Injury very slight. Ex-ovate very small. Dorsal lip seems to have been able to overcome the injury easily, for at 9:09 a.m., next day (April 29), it had already cleared from the ex-ovate and grown downward to about 35° below the equator. Segmentation cavity grown moderately small as also the blastopore circle which was not entirely circular but somewhat oval, the dorsal lip being drawn up a little toward the ex-ovate. Soon after this, ex-ovate became detached, and therefore development proceeded entirely normally and the embryo formed was normal.

This incidentally proves that, as remarked by ASSHETON ('94a), an egg is able to recover sooner or later from a slight injury, and is able to develop in an entirely normal manner.

Egg No. 8.

April 28, 1:24 p.m.:—Punctured at both the upper and lower poles. Lower injury much more severe, so much so that one hour after the lower hemisphere was somewhat diminished in size and there was a distinct space between the egg and the vitelline membrane.

April 29, 9:20 a.m.:—Blastopore area and area of the segmentation cavity equally diminished in a moderate degree. Unfortunately a new ex-ovate produced in front *i.e.* dorsad of the lower puncture. Dorsal lip had, however, already grown over the new obstacle and was nearer the original ex-ovate than the ventral lip.

5:55 p.m.:—Blastopore area and area of the segmentation cavity much diminished in size in equal ratio. Dorsal lip, however, not grown any further, so that the reduction of the blastopore area at this stage is due to the growth of the ventral and lateral lips. Secondary ex-ovate beneath the dorsal lip still more increased in size.

April 30, 9:23 a.m.:—Blastopore area diminishing faster than the area of the segmentation cavity, though both are now much reduced. Two ex-ovates still at their antipodal positions.

5:25 p.m.:—Area of the segmentation cavity disappeared. Blastopore closed. Rudiment of medullary groove (primitive groove?) seen in front of the lower ex-ovate. Other embryonic parts not made out.

May 1, 7:14 a.m.:—No further progress.

The final point of the blastopore closure in this egg was no doubt about the lower punctured point (yolk-pole), and the location of the imperfect embryo was as usual between the two poles.

Egg No. 9.

Punctured at the approximate upper pole (see Group IV., Egg No. 9; Group II., Egg No. 3). Puncture light. Development proceeded normally and the embryo formed normal. At the end the ex-ovate found about 20° forward from the embryonic head-end, and about 25° to the right of the produced embryonic axis.

Egg No. 10.

April 28, 1:30 p.m.:—Punctured at a point approximately midway between the yolk-pole and the anti-blastoporic point, as in the eggs of the corresponding number in other groups. Piercing rather forcible, but not as much as in Group II., Egg No. 10. Soon afterwards a second small ex-ovate was produced unexpectedly close under the dorsal lip.

5:10 p.m.:—Dorsal lip grown somewhat downwards and increased in length, has changed its position very peculiarly to the left side of the egg between the first and second ex-ovate.

April 29, 9:36 a.m.:—Blastopore circle already completed, and somewhat reduced in size; not circular in shape, but elongated antero-posteriorly between the two ex-ovates, showing that growth downward has been accomplished mostly by the lateral lips. Secondary ex-ovate nearly excluded out of the area, although still connected with the dorsal lip by a narrow channel. Original ex-ovate still attached to the ventral lip. Left lip entire but right lip somewhat zigzag, showing that growth was more or less disturbed.

6:02 p.m.:—Area of the segmentation cavity already disappeared. Blastopore a very small but elongated spot in front of the original ex-ovate which is attached to the ventral lip. Secondary ex-ovate in the same position as before. Neural plate not recognizable.

April 30, 9:30 a.m.:—Blastopore closed. Location of the embryonic body still doubtful.

5:30 p.m.:—Embryonic body with the neural plate and the primitive groove faintly recognizable in front of the original ex-ovate. Secondary ex-ovate entirely disappeared.

May 1, 7:24 a.m.:—Embryonic body distinct. Original ex-ovate attached to the tail end. Embryo has its axis somewhat curved to the left; otherwise normal.

The results in this egg are in the end strictly like the eggs of the corresponding number in the other groups.

For the sake of convenience I append here a table showing where the punctures were made on each egg:—

The results of the above experiments by the puncture of eggs may be summed up as follows:—

1). When the puncture is single and is at or below the level of the dorsal blastopore lip at its first appearance, the blastopore always closes at the point of puncture. Group I., No. 5, Group II., No. 1, Group III., Nos. 1, 2, and 3 appear to be exceptions to this general statement, but in these cases there were disturbing conditions such as the detachment of the ex-ovate formed; or the formation of unexpected accidental ex-ovates on other parts of the egg-surface.

2). When two or more punctures are made at the same time on different parts of an egg, the blastopore closes at the one which causes the greatest injury. In Eggs No. 4 of Groups I., III., and IV., the right puncture was always the severer of the two: hence the blastopore closed at that point. The egg of the same number in Group II., is only an apparent exception, for an accidental ex-ovate had been produced near the ventral lip which hindered its further growth.

3). The extent to which various portions of the blastopore lip grows is not constant in punctured eggs but depends entirely on the position of the pierced point. When the blastopore arrives in its downward growth at any injury, the portion of its lip which touches the injury is hindered in its further course, and while this portion is, so to speak, trying to get over the obstacle, the other parts of the lip have time to grow over a larger extent than is normal. If the obstacle is not serious (Group I., No. 5), or is in some way detached (Group I., Nos. 4, 9, and 10, Group III., No. 7), the hindered portion may get over it and grow beyond, showing only more or less retardation. If the obstacle is serious, the downward growth may be entirely stopped and

other portions of the blastopore lip have to compensate by covering a larger extent.

4). When the puncture is made on any part of the upper hemisphere it has no direct effect on the closure of the blastopore but may affect more or less the process of the reduction of the segmentation cavity or in other words the internal re-arrangement of the egg materials, and may thus indirectly cause disturbance in the growth of the blastopore lip as well as in the formation of the embryonic body.

5). In most cases, the final closing point of the blastopore coincides with the tail end of the future embryo. But occasionally there are exceptions to this general rule. In Group II., No. 9, and Group III., No. 3, the ex-ovates were at the tail end of the embryo.

6). In accordance with the statement under the last heading, the location of the embryonic body in punctured eggs is very variable. Thus, in Group I., Nos. 3, 6, and 8; Group II., No. 8; Group III., Nos. 3 and 6, and Group IV., Nos. 2, 3, 6, and 7, the embryonic body was formed almost entirely upon the upper hemisphere. But in No. 5 of all the groups, it was on the lower yolk hemisphere. And several intermediate stages between these two extremes may be seen in other eggs. The most peculiar cases are the eggs No. 4 of Groups II., III., and IV., in which the embryos appeared obliquely along the former equatorial zone.

7). There is, however, a general tendency in every egg for the blastopore to close at the yolk-pole (*s. str.*), and for the embryonic body to have its axis coinciding with the plane of the blastoporic meridian. The former fact is evident in No. 5 of Groups I., and II., and Group III., Nos. 7, and 9, while the latter appears in Group I., No. 4 in which the curved axis of

the embryo has entirely recovered its normal condition by the detachment of the right ex-ovate.

Such a tendency we must regard as the result or sum of the long course of selection, and is the reason of the great resisting power which amphibian eggs offer to various disturbing circumstances. And there must be many such disturbances, sometimes no doubt very slight, to which the frog's egg-masses must be subjected in their natural environment in spite of their gelatinous envelope. This probably accounts for the fact that individual variations in the frog's egg in regard to the segmentation process and the blastopore closure are so innumerable, that one is at a loss to find out exactly which is the normal course.

8). The greatest injury is done to the process of the blastopore closure and to the formation of the embryonic body when an egg is punctured at the region of the first appearance of the dorsal blastopore lip, and the resulting embryo shows more or less imperfections. This has already been remarked by ASSHETON and others.

9). The least injury is done, when an egg is punctured at either pole or on the future ventral side along the anti-blastoporic meridian.

10). Not only a puncture, but various other causes such as misplacement of eggs, insufficient supply of fresh water, unconscious pressure of a blunt needle during an experiment *etc.*, may disturb the internal arrangement of the egg-contents and cause abnormalities in development, often greater than those produced by a puncture.

11). From what has been detailed, it seems evident to me that the results obtained by puncturing eggs or by other similar methods, can never be depended on to reveal the normal course

of development in the naturally growing eggs. Thus, in the above experiments the facts observed in Group I., Nos. 5 and 10; Group II., Nos. 1 and 10; and Groups III., and IV., No. 10 appear to speak strongly for MORGAN'S views, while the eggs, No. 5 of Groups II., III., and IV., would become data favorable to the opinions of PFLÜGER, ROUX and others. Again a third group (Group I., Nos. 3, 6, and 8; Group II., Nos. 6 and 8; Group III., Nos. 3 and 6; Group IV., Nos. 2, 3, 6, and 7) would go to support SCHULZE and others who maintain the older views. The truth is: none of these shows the normal course, and we must look deeper for a general law that would include and explain all these cases.

12). For my own part, I am inclined to accept the *isotropism* of the frog's egg. According to this view there is no fixed law that the embryonic body in Amphibia must be formed in one particular region and in no other part of the egg-surface. Whenever there is sufficient reason, the embryo can be formed at any part of the egg-surface. It is true that in the ordinary course of the normal development the embryonic body, as already mentioned, appears along the meridian of the blastopore within the equatorial zone. This general rule has been produced by a long course of inheritance and is no doubt most beneficial to the growth of the egg. But whenever there occurs any great obstacle so that the egg can not grow in the usual way, it does not stop its growth at all, but seems always trying to overcome the obstacle in one way or another, and to continue its development. In this manner there result various kinds of abnormalities, natural or artificial. If it were true that the embryonic body could be formed only at one particular region and in one particular manner, there ought not to be normal embryos produced in

abnormal ways. That such are actually produced, as has been brought out by the foregoing investigation, is solely due to the isotropic nature of the Amphibian eggs so far as regards the formation of the embryonic body.

Consequently, I feel justified in saying that the embryonic body in Amphibia may be formed indifferently at any part of the egg-surface according to the nature of the disturbing circumstances.

It is needless for me to state that the isotropic nature of Amphibian eggs has already been insisted on by many eminent writers beginning with PFLÜGER, and including BORN, O. SCHULZE, etc. But it seems to me that most of these writers have been inclined to place too much weight on the influence of gravity, and have overlooked or underestimated a factor much more efficient in the production of abnormalities, namely, the varying ratio in which different parts of the blastoporic lip accomplish their task in enclosing the yolk-mass—the ratio which determines, in reality, the final closing point of the blastopore, and consequently the position of the embryo.



Postscript.

While I was engaged in writing the present paper, an interesting article by H. V. WILSON on the "Formation of the Blastopore in the Frog Egg" appeared in *Anat. Anzeiger Bd. XVIII.*, No. 9-10, 1900, pp. 209-239. It appears that WILSON and I have been studying independently the same subject at the same time. Moreover the main results of his observations and experiments by pricking the eggs, are largely in agreement with mine, though there are some differences in details.

The most striking points of agreement in our observations are as follows:—1. The blastopore is closed by the equal overgrowth of the blastopore lip all around. 2. The first appearance and later growth of the archenteric cavity is brought about, by the gradual accumulation of the intercellular spaces. 3. The vertical rotation of the Amphibian egg is caused by the change in the specific gravity of the different parts of the egg during the gastrulation. 4. The change in the specific gravity of the different parts is brought about by the enlargement of the archenteric cavity, with the concomitant suppression of the segmentation cavity, etc.

The points of more or less difference between the results of WILSON and of myself are as follows:—1. WILSON observes that the "Ectoderm cells close to the dorsal lip, and close to the ventral lip, gradually disappear round their respective lips. Such cells, it would seem, must become a part of the archenteric lining." This I can not corroborate from my materials. 2. WILSON states that the ex-ovate produced by pricking the eggs cannot be regarded as necessarily a fixed point, for the ex-ovate

in some instances shifts its position. As already mentioned, the ex-ovates produced from heavy punctures in my materials, may undoubtedly be regarded as fixed point of reckoning.

This view of WILSON on the second point is, probably, based upon a notion which I believe to be untenable, *viz.* that every part of the blastopore lip in pricked eggs always grows over the yolk in equal proportion just as in the normally growing egg. For this reason, WILSON'S explanation of the result of his experiments 9 and 10 seems rather hard to understand.

There is no difficulty in reality, I think, in explaining these complex phenomena when we once admit, that inequality in growth in various parts of the blastopore lip may, and does usually, occur in eggs punctured or otherwise experimented upon.



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POSTSCRIPT.

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PLATE I.

Explanation of Letters in the Figures.

- A. C. = Archenteron cavity.
- E. = Equatorial line.
- S. C. = Segmentation cavity.
- V. = Vertical axis of the rotating eggs.
- V' = Original vertical axis of eggs.

Plate I.

Figs. 1-19. Developmental stages of the *Rhacophorus* egg, magnified about 11 or 12 times.

Figs. 1-6.—Segmentation up to the fourth cleavage stage.

In Fig. 2, the second cleavage-plane should have been marked II instead of IV, which is an error.

Fig. 7.—Side view of an egg, 7-10 hours after deposition.

Fig. 8.—Side view of an egg, 10-15 hours after deposition, when the first dorsal lip of the blastopore has not yet appeared.

Fig. 9.—Posterior view of an egg, 20-25 hours after deposition, with the dorsal lip just appeared, and with the fully grown upper area of the segmentation cavity which had already begun to appear in the last stage.

Fig. 10.—Posterior view of an egg, 35-45 hours after deposition, with the broadly spread equatorial zone interposed between the blastopore lip, which has just completed its encircling, and the tolerably reduced area of the segmentation cavity.

Fig. 11.—Posterior view of another egg, 40-50 hours after deposition, still standing vertically upon the middle point of the blastopore area.

Fig. 12.—Posterior view of another egg, 45-55 hours after deposition, with the faintly visible outline of the neural plate.

Fig. 13.—Left side view of a much advanced egg, 50-60 hours after deposition, when the egg has rotated considerably and the blastopore and the area of the segmentation cavity placed opposite each other have been greatly reduced in nearly equal ratio.

Fig. 14.—Left side view of an egg, 55-65 hours after deposition, when the blastopore was completely closed and the axial region of the embryonic body may be well recognised by the presence of the deep neural groove as well as the slightly raised neural folds.

Fig. 15.—Left side view of another egg, 60-70 hours after deposition, when the neural groove has just closed. The embryonic body is flattened out over the surface of the yolk mass.

Fig. 16.—Left side view of a much advanced egg, 70-90 hours after

deposition, when the body of the embryo with several mesoblastic somites and the rudiments of the Wolffian duct etc. are hardly raised over the general surface of the egg.

Fig. 17.—Fish-like stage of an embryo on the 8th day after deposition. Sketched after being taken out of the vitelline membrane.

Fig. 18.—(a) Tadpole on the 10th day after deposition when it has just hatched out but is still within the frothy substance. Number of pigment spots beginning to appear at the thoracic region of the tadpole over the upper surface of the spherical yolk mass. (b) Blood-vessels in the external gills.

Fig. 19.—Tadpole on the 11th day after deposition.

Figs. 20–38. Successive stages of the *Rhacophorus* Egg C, fixed on ZEISS'S "Prismen Rotator" and observed with ZEISS'S Oc. I × Obj. a^2 . The horizontal basal lines under many of the figures in this Plate indicate the surface of the mirror on which the egg is resting. The lines drawn vertical to these show the vertical axis of the rotating egg in successive stages. The horizontal lines parallel to the basal lines through the central point of these figures denote the equatorial plane of the rotating egg in successive stages. The dotted and other lines in several figures all denote the approximate position of the blastopore in the successive stages of the rotating egg.

Fig. 20.—Side view of Egg C sketched at 2:30 p.m. of the first day after deposition. Stage intermediate between Figs. 7 and 8.

Fig. 21.—Posterior view of Egg C sketched at 2:25 p.m. of the second day after deposition and at the scale 217° of the mirror dish.

Fig. 22.—Upper view of Egg C showing the centrally situated large area of the segmentation cavity, sketched at 2:30 p.m. of the second day.

Fig. 23.—Left side view of Egg C, sketched at 3:20 p.m. of the second day.

Fig. 24.—Lower view of Egg C, sketched at 7:00 a.m. of the third day after deposition. The blastopore is diminishing by equal growth of every part of its lip.

Fig. 25.—Upper view of Egg C, sketched about 5 minutes later than Fig. 24. It shows that the diminution of the area of the segmentation cavity is also taking place centripetally.

Figs. 26, 27.—Left, and right, side views of Egg C just before its vertical rotation begins. Sketched at 8:10 and 8:15 a.m. of the third day.

Fig. 28.—Left side view sketched at 9:43 a.m. of the third day when the egg has just begun to rotate, resting now on the ventral lip of the gradually reducing blastopore.

Figs. 29, 30.—Lower, and upper, views of Egg C, sketched respectively at 9:57 and 10:00 a.m. of the third day. Both the areas of the

segmentation cavity and the blastopore are equally diminished in size and similarly acquiring an eccentric position.

- Fig. 31.—Left side view of Egg C, sketched at 11:27 a.m. of the third day.
Fig. 32.—Left side view of Egg C, sketched at 0:50 p.m. of the third day.
Fig. 33.—Left side view of Egg C, sketched at 5:14 p.m. of the third day.
Fig. 34.—Left side view of Egg C, sketched at 3:30 p.m. of the third day.
Fig. 35.—Left side view of Egg C, sketched at 4:50 p.m. of the third day.
Fig. 36.—Left side view of Egg C, sketched at 10:37 a.m. of the fourth day, when the blastopore has nearly closed, and the general outline as well as the location of the embryonic body become equally well recognisable.
Fig. 37.—Posterior view of Egg C, sketched at 10:50 a.m. of the fourth day.
Fig. 38.—Left side view of Egg C, sketched at 8:30 a.m. of the fifth day.

Figs. 39–42. Selected to show successive stages of a *Rana* egg (*R. japonica*) observed in the same way as Egg C, ZEISS. Oc. 1 × Obj. a^2 .

- Fig. 39.—Lower view of the *Rana* egg sketched at 4:30 p.m. of the first day, after being fixed on the "Prismen-Rotator."
Fig. 40.—Lower view of the same egg sketched at 7:40 a.m. of the fourth day, after being fixed on the "Prismen-Rotator."
Fig. 41.—Right side view of the same egg sketched at 3:32 p.m. of the fifth day, after being fixed on the "Prismen-Rotator."
Fig. 42.—Right side view sketched at 3:00 p.m. of the sixth day.

Figs. 43–49. selected to represent successive stages of a *Bufo* egg (*B. japonica*) No. 1. Observed in the same way as Egg C.

- Fig. 43.—Upper view of the egg at the first appearance of the second cleavage line.
Fig. 44.—Frontal view of the first cleavage line, corresponding to the later left side view of the egg.
Fig. 45.—Left side view of the egg sketched at 8:30 a.m. of the third day after deposition.
Fig. 46.—Left side view of the egg sketched at 8:20 a.m. of the fourth day.
Fig. 47.—Left side view sketched at 1:30 p.m. of the fourth day, when the egg has rotated around its transverse horizontal axis about 30°–35°.
Fig. 48.—Posterior view of the egg, sketched at 4:00 p.m. of the fourth day.
Fig. 49.—Posterior view of the egg, sketched at 8:30 a.m. of the fifth day.
Fig. 50.—Anterior view of a *Bufo* egg No. 3 in its peculiar rotatory motion. The arrows show the direction of the rotation, and the meridional lines represent successive positions of the neural groove in the moving egg. The dots made between the thick lines indicate different positions which the anterior extremity of the neural groove assumes by oscillation in every turn of the egg.

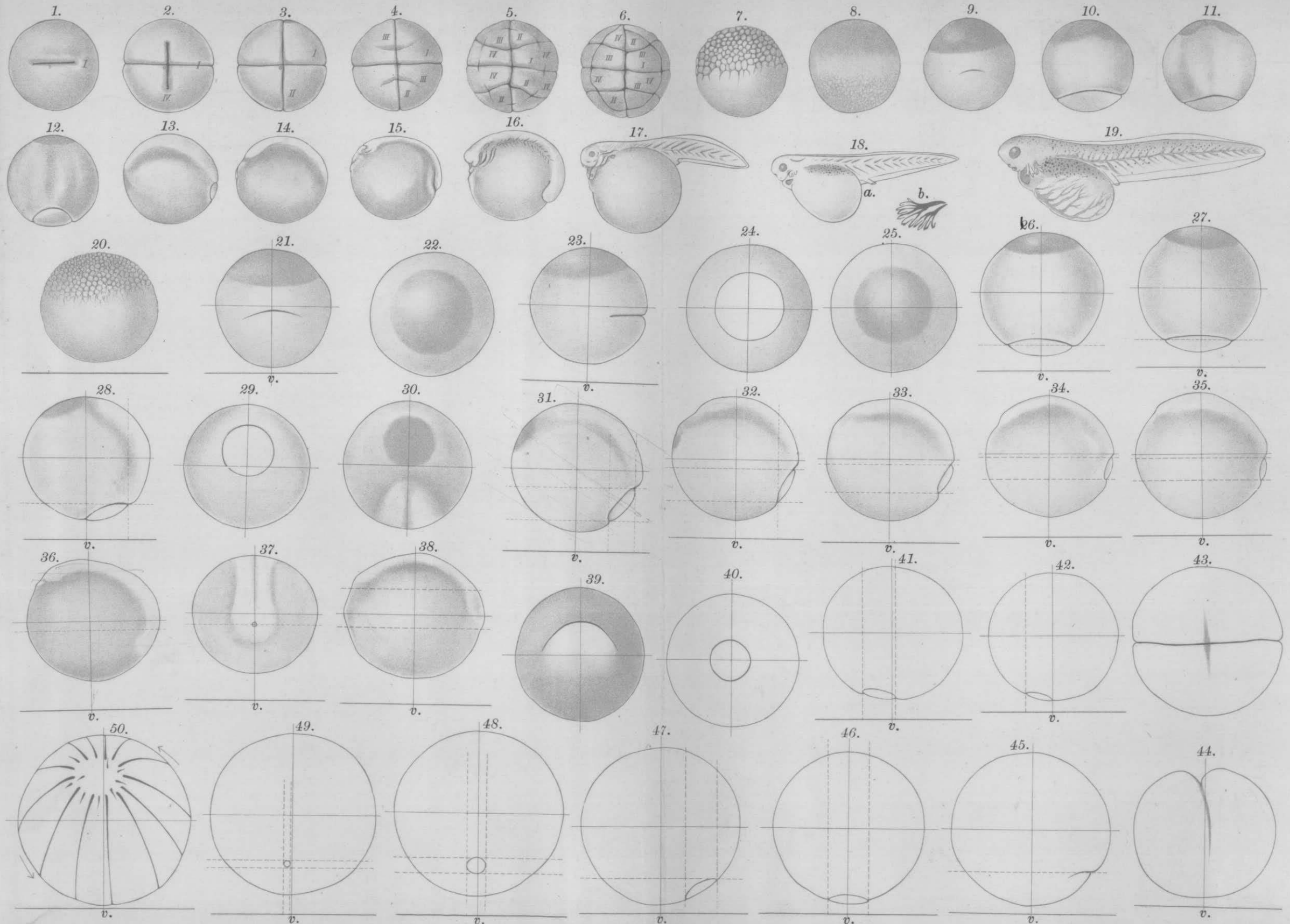


PLATE II.

Plate II.

In the figures contained in this plate, the sparingly dotted spaces indicate the yolk-mass. The closely dotted parts in Figs. 52 and 53 are sections of the equatorial zone overlaid by the recently differentiated epiblast, while in all other figures they represent both the hypoblast and epiblast without discrimination. In Figs. 54-61, the parts of the epiblast, which are marked with a double row of lines, show the thickening of that layer around the blastopore and in the neural plate.

All the figures drawn from the middle sagittal or the middle transverse sections of the *Rhacophorus* eggs in their successive stages by the drawing prism. WINKEL Oc. 1 × Obj. 1.

- Fig. 51.—Cross-section through the approximate center of an egg which corresponds to about the stage represented in Fig. 20.
- Fig. 52.—Middle sagittal section of an egg which corresponds to the stage represented in Figs. 9 and 23.
- Fig. 53.—Cross-section of another egg in the same developmental stage as the above, through the approximate center but in a somewhat oblique direction. The parts densely dotted in Figs. 52 and 53, are the sections of the equatorial zone overlaid by the recently differentiated epiblast.
- Fig. 54.—Middle sagittal section of an egg which corresponds to the stage represented in Figs. 10 and 24-27, when the egg has not yet begun its rotation.
- Figs. 55, 56.—Median sagittal and middle transverse sections of two different eggs in a stage corresponding to that represented in Fig. 12 and Figs. 28-30.
- Fig. 57, *a*, *b*.—Two median sagittal sections of two different eggs which correspond to the stage represented in Figs. 13, 32 and 33.
- Fig. 58.—Sagittal section of another egg corresponding to the stage represented in Figs. 14, 36 and 37.
- Fig. 59.—Middle transverse section of another egg in the same stage.
- Figs. 60, 61.—Middle sagittal, and middle transverse, sections of the eggs in a stage which is represented in Figs. 15 and 38.

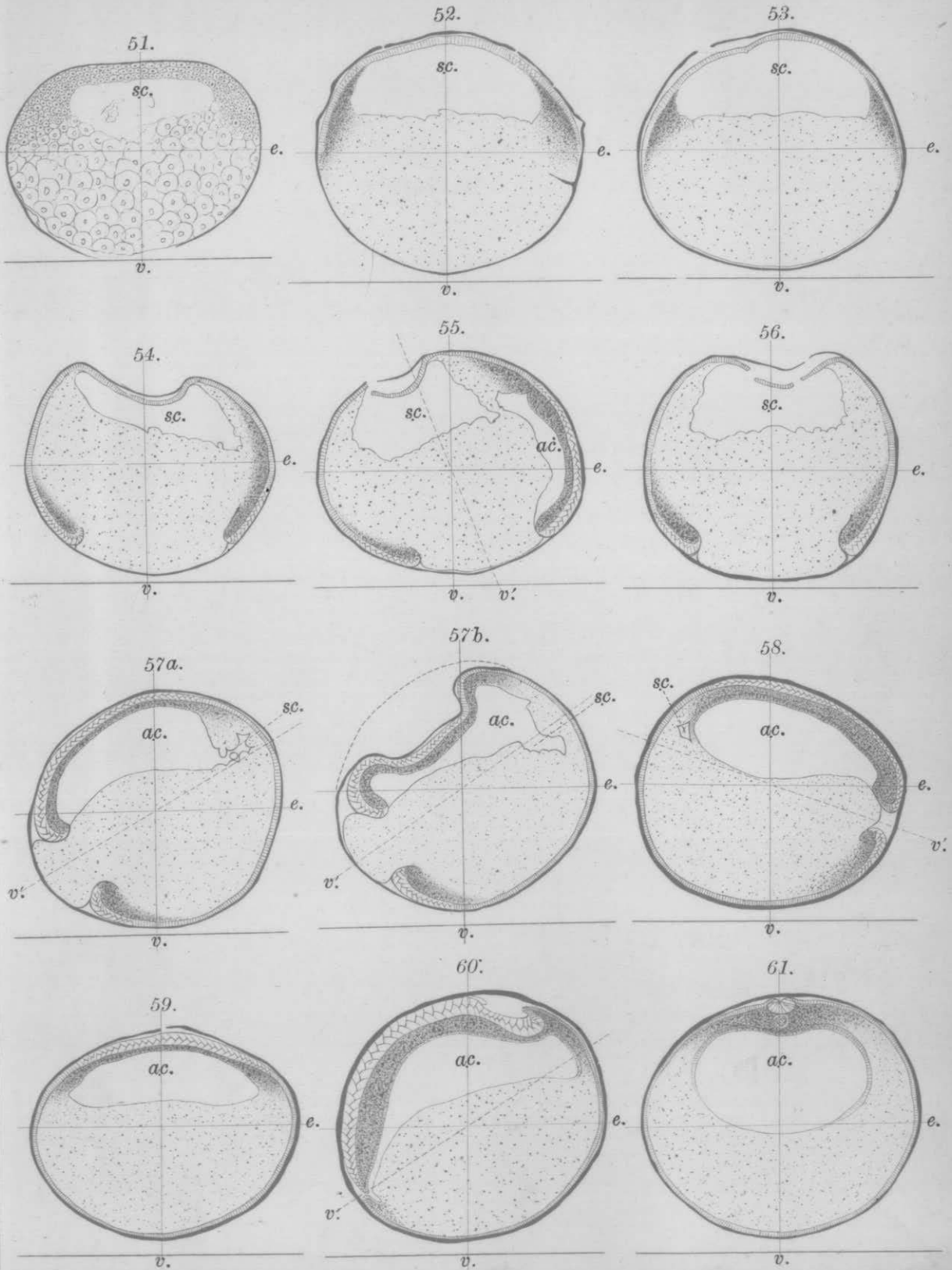


PLATE III.

Plate III.

- Fig. 62.—Roof of the segmentation cavity, under strong magnification, drawn from a section of the same series as that from which Fig. 53 was drawn. WINKEL, Oc. 3 × Obj. 5.
- Fig. 63.—Blastoporic region of the same section as Fig. 52, under a higher magnification. WINKEL, Oc. 3 × Obj. 5.
- Fig. 64.—Antiblastoporic region of the same section as the last. WINKEL, Oc. 3 × Obj. 5.
- Fig. 65.—Blastoporic region drawn from the same sagittal section as Fig. 54, under a higher magnification. WINKEL, Oc. 3 × Obj. 5.
- Fig. 65'.—Antiblastoporic region of the same section as above. WINKEL, Oc. 3 × Obj. 5.
- Fig. 66.—Roof of the archenteron cavity in the section represented in 58. WINKEL, Oc. 3 × Obj. 5.
- Fig. 67 *a-e*.—Successive stages of Egg No. 1, Group IV., which was punctured at the approximate yolk pole.
- Fig. 68 *a-f*.—Successive stages of Egg No. 2, Group IV., which was punctured closely under the left side rim of the first dorsal lip of the blastopore.
- Fig. 69 *a-f*.—Successive stages of Egg No. 3, Group IV., which was punctured directly above the first dorsal lip of the blastopore on the median line.
- Fig. 70. *a-f*.—Successive stages of Egg No. 4, Group IV., which was punctured on both sides below the first dorsal lip of the blastopore.
- Fig. 71 *a-f*.—Successive stages of Egg No. 5, Group IV., which was punctured at the approximate middle point of the antiblastoporic region.
- Fig. 72 *a-g*.—Successive stages of Egg No. 6, Group IV., which was punctured closely under the first dorsal lip of the blastopore.
- Fig. 73 *a-f*.—Successive stages of Egg No. 7, Group IV., which was punctured directly above the middle point of the first dorsal lip of the blastopore.

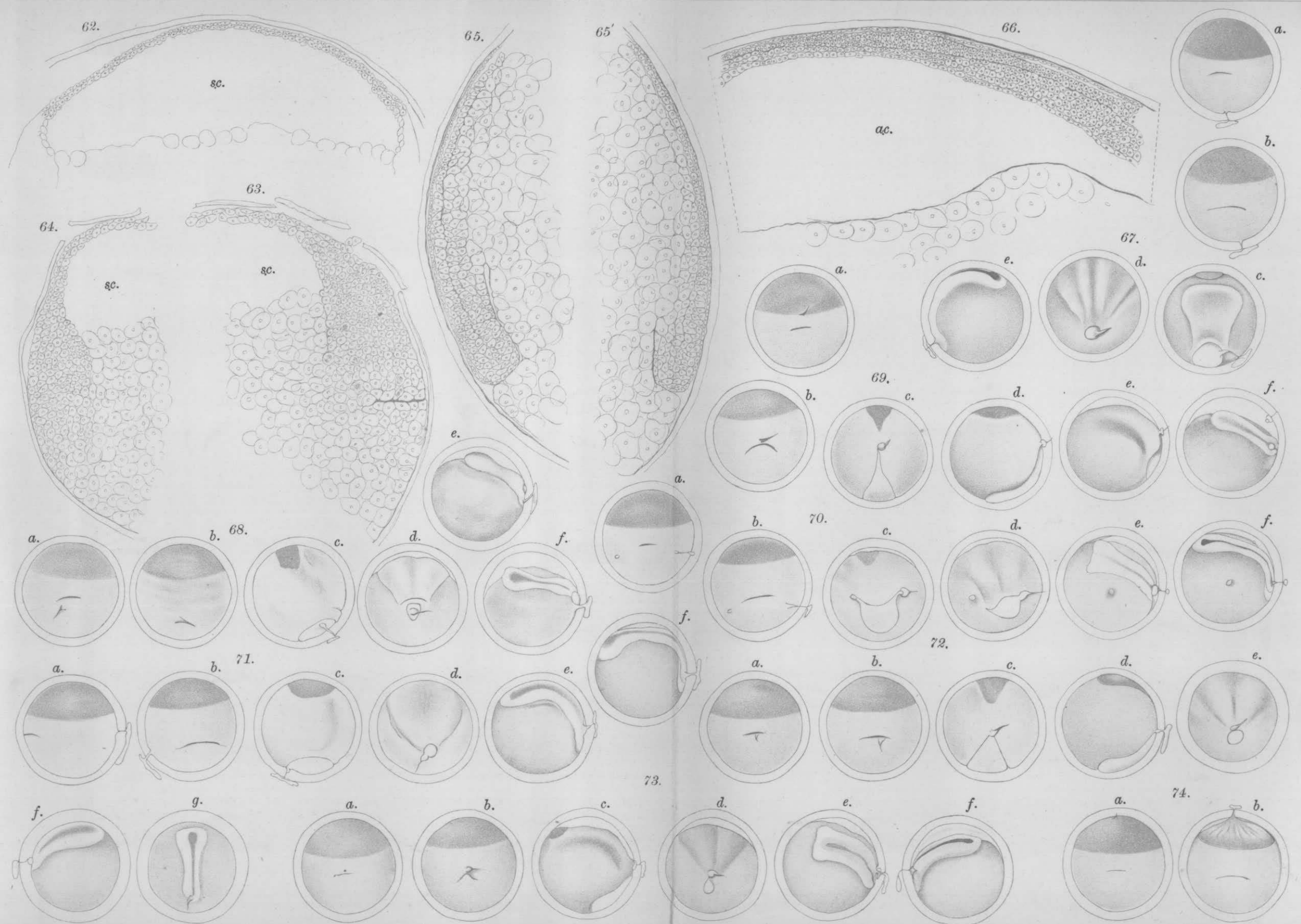


PLATE IV.

Plate IV.

Fig. 74 *a-f*.—Successive stages of Egg No. 9, Group IV., which was punctured at the upper pole.

Fig. 75 *a-f*.—Successive stages of Egg No. 10, Group IV., which was punctured at the sub-antiblastoporic region.

Figs. 76–84. Diagrammatic representations of the results obtained from Eggs Nos. 1, 2, 3, 4, 5, 6, 7, 9, 10, Group IV., in regard to the location of the embryonic body and the punctured region of every egg. The curved line above the egg-equator, in every figure, is the lower limit of the early area of the segmentation cavity, while the dotted line denotes the future lip of the blastopore.

Fig. 76.—Diagram of Egg No. 1, Group IV., Figs. 67 *a-e*.

Fig. 77.—Diagram of Egg No. 2, Group IV., Figs. 68 *a-f*.

Fig. 78.—Diagram of Egg No. 3, Group IV., Figs. 69 *a-f*.

Fig. 79.—Diagram of Egg No. 4, Group IV., Figs. 70 *a-f*.

Fig. 80.—Diagram of Egg No. 5, Group IV., Figs. 71 *a-f*.

Fig. 81.—Diagram of Egg No. 6, Group IV., Figs. 72 *a-g*.

Fig. 82.—Diagram of Egg No. 7, Group IV., Figs. 73 *a-f*.

Fig. 83.—Diagram of Egg No. 9, Group IV., Figs. 74 *a-f*.

Fig. 84.—Diagram of Egg No. 10, Group IV., Figs. 75 *a-f*.

Fig. 85 *a-c*.—Successive stages of Egg No. 4, Group I.

Fig. 85'.—Diagrammatic representation of the results obtained from the above egg.

Fig. 86 *a-c*.—Successive stages of Egg No. 5, Group I.

Fig. 86'.—Diagrammatic representation of the results obtained from the above egg.

Fig. 87 *a-d*.—Successive stages of Egg No. 1, Group II.

Fig. 88.—Diagrammatic representation of the results obtained from the above egg.

Fig. 89 *a-e*.—Successive stages of Egg No. 4, Group II.

Fig. 90.—Diagrammatic representation of the results obtained from the above egg.

Fig. 91 *a-d*.—Successive stages of Egg No. 9, Group II.

Fig. 92.—Diagrammatic representation of the results obtained from the above egg.

Fig. 93 *a-h*.—Successive stages of Egg No. 3, Group III.

Fig. 94.—Diagrammatic representation of the results obtained from the above egg.

Fig. 95 *a-g*.—Successive stages of Egg No. 4, Group III.

Fig. 96.—Diagrammatic representation of the results obtained from the above egg.

