The Relation Between Normal and Abnormal Development of the Embryo of the Frog:

X. A Re-examination of the Early Stages of Normal Development from the Point of View of the Results of Abnormal Development.

By

T. H. Morgan.

With 23 figures in text.

Eingegangen am 13. März 1905.

The preceding studies, I—IX, on the influence of external agents on development have shown that the same general types of abnormalities appear over and over again independently of the agent employed. One of the most striking results is the frequent appearance of an abnormal embryo in the upper hemisphere of the egg, although in the normal development the material out of which the embryo develops is supposed by most recent writers to come from the lower hemisphere. This difference in the two cases suggested that some, at least, of the embryo-forming material comes from the upper hemisphere, and this led me to carry out the experiment of destroying the upper four cells with the results described in the Vth paper of this series. It was found that parts of the embryo failed, under these circumstances, to appear.

The development of certain types of abnormal embryos in the upper hemisphere, and the development of the normal embryo below the equator of the egg, might be harmonized in either of two ways; — either the material that produces the abnormal embryo is different from that which goes to form the normal, or else there is a down-wandering of material of the upper hemisphere at a stage earlier than has, heretofore, been suspected. It seemed that this latter possibility might be tested by a re-examination of the normal development during the cleavage, blastula, and gastrulata stages; for,

despite the fact that these stages have been so often described, a renewed study has convinced me that some of the most essential changes occurring during the early development have been overlooked. In a word, a study of the early stages has shown that normally a large part of the material of the upper hemisphere is transferred downwards during the segmentation and early gastrula stages, and that this is the material out of which the dorsal parts of the embryo (the »embryo« in a narrower sense) develops. The failure of this material to be carried downwards in many forms of abnormal development accounts for the appearance of the embryo on the top of the egg. The evidence on which this statement rests will be given in detail in the following pages.

The Cleavage Period and the Enlargement of the Segmentation Cavity.

The segmentation cavity appears as a conspicuous space in the upper hemisphere at the eight-cell stage. As shown in Fig. 1 the cavity is at first confined largely to the upper hemisphere, although its lower end may extend below the equator. A 16-cell stage is shown in Fig. 2. During the following cleavage-stages, 32-, 64-cell

Figs. 1 and 2.

stage, etc., the segmentation cavity may have very different outlines in different eggs, and at different times. Nevertheless it tends to become larger and to broaden out laterally as development proceeds. It is during this period that some of the surface cells of the upper

hemisphere begin to pull into the interior, as REED has recently shown. The following figures show sections of eggs in which these

Figs. 3 and 4.

3

4

changes are taking place. A 32-cell stage is shown in Fig. 3; a 54-cell stage (with 6 cells inside) in Fig. 4; a 64-cell stage (with

Figs. 5 and 6.

5

6

12 cells inside) in Fig. 5, and a 105-cell stage (with 26 cells inside) in Fig. 6. These eggs were all from the same bunch.

The next series begins with segmentation stages much more advanced than the last. The first two eggs, Figs. 7 and 8, are in

the same stage of development. On egg has been flattened, in cutting, in one plane, and the other in the plane at right angles to the former. Unless one is on his guard the different shapes of eggs due to compression in cutting might lead to erroneous conclusions. In fact, the egg itself, at this and at the following stages, is spherical. The roof and sides of the segmentation cavity are rather thick in these two figures. The floor of the segmentation cavity is at about the level of the equator of the egg.

A stage when the blastopore has just appeared is drawn in Fig. 9. The segmentation cavity is still broader from side to side

Figs. 7 and 8.

and not so high (the flattening of the egg exaggerating this effect). The yolk mass rises relatively high up on the floor. The roof of the segmentation cavity is thinner. This thinning of the roof can not be entirely accounted for by an increase in area, for, the surface of the egg is not much greater, if at all greater, than in the stage preceeding. We can, however, account for the thinning out on the assumption that the side-walls have been carried further downwards. The relatively higher position of the yolk on the floor of the segmentation cavity must be compensated for in some way, and the postulated downgrowth of the side-walls would make good the space formerly occupied by the yolk. It is, therefore, significant to find at this time a narrow crevice appearing around the sides of the segmentation cavity between the yolk and the ectodermal wall of the

segmentation cavity. This crevice first appears at the anterior end, and later along the sides and last at the posterior end. The crevice has been supposed by BRACHET and others to represent a split that appears between the yolk and the more superficial cells; but I shall try to show on the contrary that it is due to the pushing upwards of the yolk, rather than to a splitting downwards in the solid wall of the sides of the egg.

The next stage is represented in Fig. 10, in which the gastrulation is a little more advanced. The posterior side of this section

Figs. 9—11.

is drawn to a larger scale in Fig. 10 A. The posterior edge of the roof of the segmentation cavity is seen to be thinner than before, and the smaller and darker cells extend further down on the sides. As yet the crevice has not appeared at the posterior end.

The next stage, Fig. 11 A, shows the dorsal lip of the blastopore further advanced. The yolk on this side of the egg is thrown high up into the segmentation cavity, a condition often figured by previous writers, who have not, I think, fully appreciated the importance of this fact¹). The posterior side of the same egg is shown in Fig. 11.

¹) H. V. WILSON, however, has drawn particular attention to this throwing

Compared with Fig. 10, through the same region, it will be seen that the roof of the segmentation cavity is continuing to thin out. The crevice between the ectoderm and the yolk at the posterior corner of the segmentation cavity has appeared, and in this case, especially, looks as though it were formed by the rolling upwards of the yolk cells.

In the next stage, Fig. 12, the changes noted for the last stage have been carried still further.

Finally in Fig. 13 the posterior lip of the blastopore is well marked (the section is turned in an opposite direction from the last). The crevice at the posterior end between the ectoderm and the yolk

Figs. 12 and 13.

12

13

is now relatively long; and the yolk at the sides is thrown high up into the segmentation cavity, while the floor of the segmentation cavity has even sunken down¹).

In Fig. 14 is represented a late segmentation stage from a different bunch of eggs. The roof of the segmentation cavity is very thin, and the material pressed more outwards and downwards at the sides. A later stage from the same series is drawn in Fig. 15. The segmentation cavity is much broader, and its floor is now pressing somewhat upwards. The side walls of the segmentation cavity are still quite thick, but less so than in the preceeding stages. In this

upward of the yolk at the anterior end of the archenteron, and has discussed its meaning.

¹) The top of this egg has bulged inwards as a result of the action of some of the hardening fluids.

particular bunch of eggs, from which these figures were taken, the contrast between the thickness of the top and sides of the segmentation cavity is greater than usual. In other series the top is relatively thicker. For exact results, therefore, it is important to compare directly only stages from the same batch of eggs, which are much more like each other at every stage than are eggs from different bunches.

In order to study the later stages of gastrulation I have been obliged to resort to another set of eggs. Fig. 16 *A* shows a stage in which the yolk plug is present and much reduced in size. The

Figs. 14 and 15.

14

15

most striking feature in this section is the position of the yolk mass that has been carried upwards along the inner wall of the segmentation cavity, while the central portion is, it appears, sinking downwards a little. While the yolk plug is being drawn in, the yolk continues to extend upwards around the inner wall of the segmentation cavity, and finally meets near the top. The segmentation cavity becomes reduced in size, and finally sinks more into the interior of the yolk mass, where it finally disappears.

A somewhat older stage is represented in Fig. 16 *B*, from another bunch of somewhat smaller eggs. The yolk in front of the archenteron is not carried so high up towards the top of the egg as in the last figure. There is a group of elongated yolk-cells just above

the ventral lip of the blastopore that is very conspicuous in the section, and probably indicates an active process of withdrawal of cells in this region.

A stage, when the yolk plug is just disappearing within the blastopore, is shown in longitudinal section in Fig. 17 (turned in the opposite direction from Figs. 16). The archenteron has opened out into a large cavity. The segmentation cavity has become surrounded by yolk, and has sunken into the interior. In fact there are two cavities in the yolk in this egg, both of which appear to be parts of the original segmentation cavity. Such a result might follow from a condition like that shown in Fig. 16 *A*. The ectoderm now covers the whole surface of the egg, and is correspondingly reduced in

Figs. 16 and 17.

thickness as a comparison of the figures will show. Much of the ectoderm that formed at first a part of the roof of the segmentation cavity has now been carried far down over the sides of the egg.

All of the preceding sections have been longitudinal ones, i. e., sections through the top of the egg and the middle of the dorsal lip. I shall call cross-sections those that are at right angles to the last, and cut across the middle of the yolk hemisphere. As the lateral lips of the blastopore extend around the egg they will be cut across in these sections. Since the lips appear from before backwards a single series of sections of this sort often shows a series of stages in the process of blastopore-formation with the associated changes in the downward movement of the superficial material.

Fig. 18 is through the middle of an embryo in which the blastopore has just appeared. It is the 45th section from the anterior end in a series of 85. In this region of the egg, the crevice between

the ectoderm and the yolk has not yet appeared, although the floor of the archenteron has begun to lift upwards. A little older stage is shown in the Fig. 19 (the 32 section of a series of 70). In this embryo the blastopore had formed a very short crescent. The egg

Figs. 18—20.

was cut slightly obliquely, so that the right side of the section shows a more advanced condition than the left. The smaller cells at the side appear to have reached a lower level than in the preceding stage.

A cross-section of a slightly older embryo is shown in Fig. 20. This is the 20th section (in a series of 54), and, therefore, anterior

to the middle of the egg. The inturning at the lateral lips of the blastopore can be seen on each side of the lower part of the section. Comparing this section with the preceding, it will be seen that the small cells at the sides now extend much further down, while the yolk floor rises higher up, and that the crevice between the ectoderm and the yolk is longer. As one passes through this set of

Fig. 21.

sections towards the anterior end, the lateral lips of the blastopore are better developed, and are drawn nearer together, Fig. 20 A; the yolk rises higher in the segmentation cavity, and the small cells at the sides appear to cover relatively more of the surface. Passing towards the posterior end the reverse conditions are met with; Fig. 20 B. Cross-section through older stages, when the dorsal lip of the blastopore has begun to advance over the yolk, show the following important changes.

The four cross-sections shown in Figs. 21 are through an egg

in which the blastopore has begun its advance. The first figure *A* is the 5th section posterior to the point where the lateral lips fuse in the dorsal lips. The lateral lips are here near together, and the crevice between the ectoderm and the yolk is long. The next section *B* is the 14th from the blastopore, and shows the lateral lips

Fig. 22.

much further apart. The third section is the 35th of the series, and, near the middle of the egg. It will be observed how much further out the lips of the blastopore are at this level. The last section is the 60th, and in it the lateral lips are not developed. Their positions can be approximately located by means of the mesoderm, and are indicated by the two crosses. These points lie relatively still further out at the sides. Thus as the lateral lips advance to become in-

incorporated into the dorsal lip, the material advances toward the middle line. A continuous advance of all the material at the sides must take place, including the invaginating region itself.

The next series, Fig. 22, is through a later stage. The first section, *A*, is the second in front of the one where the dorsal lip opens out at the surface. The archenteron appears as a narrow slit below the surface (on the lower side of the figure). The second section is the fifth one posterior to the dorsal lip, and the 28th of

Fig. 23.

the series. The lateral lips have approached. If we imagine them as meeting in this section, and the yolk plug withdrawn, the width of the archenteron, as shown by section *A*, would now correspond to the distance between the outer ends of the archenteric invagination at the sides. The third figure, *C*, is the 37th of the series. The lateral lips are much further apart and less developed. The distance between them is much greater than the future width of the archenteron at this level. The fourth section, *D*, is the 45th of the series, and much like the last. It lies at the level where the lateral lips

begin to round in towards the posterior end. There are 20 more sections in the series.

The last series Fig. 23 is through an egg in which the blastopore is nearly circular, and much reduced in size. The first section, *A*, is in front of the external opening of the dorsal lip, and shows the archenteron. The next section, *B*, is the second behind the opening of the dorsal lip, and shows the lateral lips near together. The third section, *C*, is through the middle of the blastopore, and shows that here too the lateral lips are now near together, and that compared with Figs. 20 and 21, the approach is greater than can be accounted for by the depth of the archenteron at the sides, so that the whole region must be imagined to have pushed downwards. The fourth section, *D*, is through the posterior end, where the lateral lips are even nearer together than in the preceding.

If these three sets of sections are compared with the earlier stages shown in Figs. 18, 19, it will be seen, that the lateral invaginations, that appear along the lateral lips are much further out at the sides when they first appear than in the later stages, and that the surface between them is greater than the width of the archenteron when it first appears. There must be postulated, therefore, an advance of the sides, including the lateral lips and their invagination, during the closure of the blastopore. This movement appears to be only a continuation of that which has been taking place during the earlier stages.

Provisional Interpretation of the Early Stages.

In the preceding account I have confined myself largely to a description of the changes that take place during successive stages, only intimating at times the character of the processes that bring about the changes. I shall now attempt to give a continuous account of the way in which I suppose the downgrowth of the material to take place. It will be convenient to classify the process under three headings, although in the actual development the process is a continuous one.

First period: The transportation of the material during the cleavage process.

Second period: The changes just prior to and during the appearance of the blastoporic rim.

Third period: The drawing together of the lips of the blastopore.

First Period.

The egg increases a good deal during the segmentation period as would be expected since the large segmentation cavity develops at this time. If the fluid in this space was merely squeezed out of the blastomeres, the egg might remain the same size, although a cavity formed in its interior. That something is contributed to the segmentation cavity by the surrounding blastomeres is probable, since the fluid coagulates in the hardening reagents, although it is probable that the greater part of the fluid is due to absorption of water from the exterior; but whether this water first enters the blastomeres, and is secreted by them into the interior, or whether it enters more directly is quite unknown.

The third meridional cleavage separates largely the material that becomes the roof of the segmentation cavity from that of the floor. It is also probable that some of the material that forms the side walls of the segmentation cavity may come from the upper ends of the four lower cells of the eight-cell stage. From the 32-cell stage onwards, some of the cells of the roof of the segmentation cavity pull into the interior, and apply themselves to the inner wall, especially to the sides. As the segmentation proceeds the material of the roof spreads out, and is carried downwards towards the equator of the egg, so that some of the material that originated higher up on the sides, and, especially, the cells which drew into the interior, become carried further downwards. As this change goes on throughout all the later cleavage periods, the lateral material is carried to, or even below the equator of the egg. At this time the segmentation cavity changes its shape, spreading out more laterally, as the floor rises up higher. As a result of the latter change, room is made for the slipping downwards of cells of the walls of the segmentation cavity. The roof also becomes gradually thinner. In part this thinning out is due to the somewhat increased area of the roof, but also to the extension downwards of the sides.

There is another possible interpretation of the changes that take place during this period, which is the one usually adopted by most writers who have paid any attention to this period of the development. It is usually stated that the superficial material of the egg in the region of the equator continues to divide, and that the smaller cells formed in this way add themselves, as it were, to those of the top, so that the latter appear to extend further downwards. It is

stated, also, that the crevice that appears between the ectoderm and the yolk is due to a split that separates the outer from the inner cells. I have examined these possibilities with some care, and the evidence seems to me to point unmistakeable to the conclusion that the crevice is not a split, but is formed as a result of the ectoderm slipping down as the yolk pushes upwards. Thus one hypothesis will account for the shifting downwards of the material of the surface, for the formation of a crevice where the two regions are moving in opposite directions, and for the rising up of the floor of the segmentation cavity.

The darkening of the egg that takes place during the later cleavage period, which I called attention to in 1891, is due to the movement of darker cells downward, and is not due, primarily, as I supposed, to the development of the pigment in the cells around and below the equator of the egg.

Second Period.

The preceding account has anticipated to some extent the changes that take place during this second period. The changes begin when the yolk floor rises up in the segmentation cavity, causing the appearance of the crevice around the sides. The period culminates with the appearance of the blastoporic rim. The changes begin at the anterior end, where the dorsal lip of the blastopore appears, and slowly extend around the egg. Just after the appearance of the rim, the movement downwards seems to be more rapid, although I can not bring forward a conclusive proof that this is the case. The change in shape of the yolk cells near the region at which the rim is to appear is significant.

Third Period.

As soon as the blastoporic rim appears the subsequent transfer downwards of the material can not be mistaken. The edges of the lip advance, rolling in somewhat as they advance, so that a few of the cells situated just outside and on the lip may be seen to roll under. At the same time the whole yolk field begins to move upwards under the advancing lips.

The dorsal lip appears first and advances rapidly backwards, while the lateral lips are forming. In fact, the advance of the dorsal lip is largely due to the incorporation into it of the material that is being constantly brought up to the middle line by the lateral lips.

As the lateral lips develop faster nearer the anterior end, the result is that the dorsal lip appears to advance more rapidly backwards than the lateral lips advance from the sides, but there can be little doubt that this backward advance is not due, to any extent, to the material that first appears at the dorsal lip moving backwards, but to the lateral lips coming up to the middle line. When the blastopore has been reduced to a small circular or elongated opening the advance is more nearly equal from all sides. The ventral lip undergoes an exactly similar series of changes, so that the yolk surface is not only slipping forward under the dorsal lip, but under the sides and under the posterior lip also, as KORSCH's photographs have shown.

Measurements of the egg at the time when the dorsal lip first appears, and after the blastopore has just closed, show that the egg does not increase in size during this period. This fact has an important bearing on the interpretation of the changes that are taking place. It shows, in the first place, that the thick blastoporic rim does not rise up over the lower surface of the egg, but that the latter slips in under the blastoporic rim. Equally important is the bearing of this fact on the length of the embryo that is formed. If the point at which the dorsal lip appears on the surface be indicated, and the point at which the posterior lip first appears be also marked, it will be found that the region covered by the blastopore is somewhat longer than the length of the dorsal side of the embryo. If to this length we add that of the head region of the embryo, which experiments indicate develops in front of the first position of the dorsal lip, then the embryo-forming material appears to extend over a much longer region of the egg than that actually covered by the embryo. This condition has led to endless confusion. The explanation is found in the fact, that, as the yolk draws into the interior of the egg the surface material out of which the embryo is to develop is actually as a whole drawing somewhat together, i. e., the downward movement involves a real drawing together of the material at both the anterior and posterior ends as well as at the sides. This point will be clearer when some other changes in the development have been considered.

During the period of overgrowth the ectoderm gradually becomes thinner, owing to the greater area it has to cover. The mesoderm is carried towards the middle line and forms a thin sheet over the dorsal surface. In the dorsal lip itself there is present at all

stages a relatively large amount of material, not because the cells there are multiplying and growing more rapidly than elsewhere, but because the material from the sides is being continually brought up to the dorsal lip.

KOPSCH has given some diagrams to illustrate his view of the location of the material that goes to form the embryo, and to show how this material is carried into the embryo. He also tries to show that ROUX's theory, namely, that the second plane of cleavage usually lies approximately between the material that goes to form the anterior and the posterior halves of the embryo, is erroneous or impossible. KOPSCH's diagrams illustrate very well, it seems to me, the location of the material at the time when the posterior lip of the blastopore is about to develop, and the later stages that follow, but in this respect the diagrams give little that is new. They fail to show, however, the earlier location of the material, and for this reason fail to show that ROUX's theory is incorrect. On the contrary, I think, ROUX's view gives a much more approximate idea of the relation of the embryo-forming substance to the position of the second plane of cleavage than does that of KOPSCH.

The Origin of Ectoderm, Mesoderm, and Endoderm.

The primary object of this paper is not to consider the question of the origin of the germ-layers of the embryo, but to trace the movements of the materials. Since, however, the two questions are closely related, a short account may be given of the origin of the layers.

Ectoderm. The roof and the walls of the segmentation cavity become the outer covering of the embryo. The transfer downwards of this material has already been sufficiently described. The surface cells, that lie just below the walls of the segmentation cavity, also form part of the ectoderm, but, as I have tried to show, most of these cells have been relatively higher up on the egg during the early segmentation and blastula stages. Experiments with the eggs indicate that the ectoderm arises from the upper four cells of the eight-cell stage and also from the upper part of the lower four cells, but the exact limits with reference to the third plane of cleavage is difficult to determine.

Mesoderm. The cells of the mesoderm can first be recognized as such at the time of the appearance of the dorsal lip of the blasto-

pore. As the lateral lips develop the mesoderm also appears around the egg in the same relative position in regard to the blastoporic rim that it occupies in the dorsal lip. When first recognizable the mesoderm is made up a group of cells just below the »crevice«, and between the ectoderm and the yolk. It is from the beginning directly continuous with the inner layers of the wall of the segmentation cavity, and often presents the appearance of developing directly out of these cells as they push downwards. Even after the blastopore lips have formed and are closing in, there is always a close connection between the inner layers of the ectoderm and the mesoderm at the edge of the rim as though cells from the former were pushing in to become mesoderm as the lateral lips become incorporated in the dorsal lip. On the other hand a part of the group of mesoderm cells resembles so closely the yolk cells in the region in which the mesoderm first appears, that it seems possible by a single division taking place to convert the yolk cells into mesodermal cells. It is not improbable that some of the mesoderm arises also in this way. The three possible sources that I have given are not mutually exclusive, for, the cells of the inner wall of the segmentation cavity are directly continuous with the yolk cells in the region where the mesoderm is formed, so that the distinction between them may be an arbitrary one.

The group of mesodermal cells is carried downwards in the dorsal lip of the blastopore, or rather, the lateral lips bring up their mesoderm to the mid-dorsal line to add it to the mesoderm already there. In this way the mesoderm comes to lie over the dorsal surface of the embryo, beneath the medullary plate.

I have already referred to the tongue of cells that grows upwards around the inside of the dome-shaped roof of the segmentation cavity. This is the material out of which the mesoderm of the sides, and ventral surface of the embryo is subsequently formed. The material arises in close proximity to the cells that produce the first formed mesoderm, although the divisions that transforms them into mesoderm do not occur until a relatively late stage.

The »tongue« of cells appears to be formed by the yolk cells pulling upwards at and near the region of the top of the archenteric invagination, which takes place entirely around the egg. The part of the mesoderm formed in this way represents, therefore, invandering endoderm cells.

Endoderm. The yolk makes up, of course, the main bulk of the endoderm. The point of chief interest at present is the origin of the cells of the roof of the archenteron. This is a difficult question, for a discussion of which we still lack, perhaps, sufficient data, although some points, at least, can be made out with a high degree of probability.

The origin of the slit-like archenteron first demands attention. What can actually be observed may be stated in a few words. A depression, or pit, appears in the yolk cells just below the region where the mesoderm can be identified; Fig. 9. In surface view the pit is deeply pigmented, and sections show that this is due to the pigment that accumulates in the outer, pointed ends of the cells, that appear, from their shape, to be drawing in from the surface.

There is present in sections a line of superficial cells just above the pit that more nearly resemble the yolk cells than the ectoderm higher up, although they grade off insensibly into the latter. These cells roll around into the pit, as the lip of the blastopore develops, and form, therefore, a part of the dorsal wall of the archenteron. This same process occurs not only at the dorsal lip, but around the whole rim of the blastopore. When the lateral lips are brought to the middle line these cells, that have rolled under its edge, will form the cells of the mid-dorsal wall of the archenteron.

As the blastopore extends downwards the pit becomes a narrow slit, Fig. 12. At the top of the slit there can always be found a group of wedge shaped cells with their pointed ends turned outwards and downwards. These cells are exactly like those that first appeared when the dorsal lip developed. A crucial point is whether these cells are the same ones that first appeared, or whether new ones have continually assumed the shape and the position of those that preceded them. There is, in fact, some indirect evidence from the form of the different cells at the top of the archenteron to lead one to the conclusion that some of the pointed cells become added to the dorsal wall of the archenteron, while others that lie on the anterior end of the floor become successively pointed, move to the anterior end, and in turn may be added to the dorsal wall. Most of them, however, in all probability move further upwards into the yolk. The determination of just what changes are taking place at the top of the archenteron is extremely difficult, but the inturning of the yolk during the period of gastrulation, combined with the observed shape of the yolk cells at the top of the archenteron, make it pro-

bable, that some of the superficial yolk cells that disappear under the advancing rim of the blastopore must become incorporated in the yolk mass at or near the top (and at the sides of the lateral rim, and at the top of the posterior rim) of the archenteron. The actual number of cells across the lower hemisphere between the points where the lateral lips appear is actually greater than the number forming the cross-section of the floor of the archenteron. The rolling around of cells at the anterior end of the archenteron makes the archenteron longer in this direction than the actual space overgrown by the dorsal lip, so that the ultimate number of cells forming the floor in this direction may be greater than the number over the corresponding surface area of the yolk field.

There is another point in favour of the view that the cells of the floor of the archenteron change shape at the anterior end, one after another. In this way we can account for the movement of the yolk surface under the lips of the blastopore. As each cell changes its shape in this region, so that its end next to the archenteron becomes smaller, it occupies less room, and the line of cells lower down are drawn further up towards the upper end. As each approaches the turning point, it also changes shape, and exerts a similar drag on the cells lower down. I suggest this view with some hesitation, for it seems undesirable to add another account to the many that are already in existence to explain the process of gastrulation of the frog's egg; but as a working hypothesis, and one that is accord with the most recent results I trust it may at least be given a serious hearing.

To sum up: The anterior cavity of the archenteron is lined both above and below by the yolk cells. This part of the archenteron has been formed by the drawing upward of the yolk cells some of which roll around the anterior end of the archenteron. The mid-roof of the rest of the archenteron is formed by the cells that have rolled under the rounded lips of the blastopore, and have been brought to the middle line by the advance and fusion there of the lateral lips of the blastopore. The side walls of the archenteron, except for its anterior end, are formed by the yolk cells that draw in along the lateral lips of the blastopore, and also possibly to a limited extent by cells that have moved around the upper end of the archenteric cavity of the lateral lips. The side walls, as a whole, also are brought nearer to the middle line by the advance of the lateral lips.

The »Mechanics« of the Downward Movement of Material.

The postulation of an extensive movement of material, such as that which I have attempted to trace, invites, almost demands in fact, an attempt to account for the mechanics of the process. I shall attempt, therefore, to give a provisional account of how the process takes place, admitting freely that the difficulties involved in an attempt of this sort with so little data at hand make the hypothesis far from satisfactory.

The first beginning of the downward movement of the material at the top of the egg is associated with the decrease in thickness of the walls, and with the enlargement of the segmentation cavity. That the enlargement of the cavity itself is the mechanical means by which this change is affected seems to me improbable. On the contrary its enlargement appears to be the result of the changes in its roof and floor, and not the cause of them. The results must, therefore, be due to the activity of some region of the egg-substance. The thinning out of the roof suggests that the pressure of its small cells may cause its sides to push downwards against the less resisting yolk. On the other hand, if it were simply a question of increased pressure in the roof it is not clear why the roof might not simply turn into the segmentation cavity, and thus relieve the pressure. If, however, the yolk cells responded actively to the increase in pressure from above, and changed their shape, a re-adjustment might be continually taking place in this way. In fact, the movement upwards of the whole floor of the archenteron suggests that a re-adjustment of some sort is going on. The assumption would be simpler, however, if we postulate only a change of shape in the yolk cells, which pushing upwards allow the sides of the egg to slip downwards. This downpull causes all of the cells of the roof of the segmentation cavity continually to readjust themselves to the new conditions, and the roof becomes thinner. The re-adjustment may not be simply a passive process, but the cells may each respond to the local conditions of tension of the region in which they lie. Whether, then, both the yolk cells and the surface cells of the roof are actively engaged in the downgrowth, or the yolk alone is responsible, while the cells of the roof only actively readjust themselves to the conditions, can not be stated. By removing the roof

of the segmentation cavity in the living egg this point might be determined.

At the time of appearance of the blastopore there can be little doubt that a change in shape of the yolk cells is largely responsible for the changes that take place, and if my account of the inturning of the yolk into the blastopore is correct, this change of shape of the cells may entirely account for the results. It may be that the cells of the blastoporic rim also take an active part in the process of overgrowth of the lower hemisphere, by changing shape and do not simply draw together after the fashion of an elastic band as the yolk withdraws. In fact, there must be very extensive changes in the relative positions of the cells to each other during this time, and these changes also can be best accounted for on the assumption of the contractile power of each cell in response to the surrounding conditions of tension or of pressure of the parts. The process may not be dissimilar in kind from that by which artificially exposed surfaces of later embryos are covered over. I have tried to show that this process also must be due to a process of contraction of the cells.

That the rubber-band idea of the contraction process is insufficient is also made probable by a number of other considerations. The drawing together of the blastoporic rim begins at the anterior end, and goes on there for some time before the rest of the rim is formed. Moreover the movement is for some time towards the anterior end, as well as towards the middle line, so that the movement goes on in a definite direction, and is not equal in all directions. If we consider the process of contraction as due to a response of the individual cells we get quite a different conception of the process than when we compare it to the process as seen in a stretched band. In fact the phenomenon is an internal one, and the conditions are not impressed on the regions from without. If the molecules of a piece of rubber could contract or expand locally the process would be somewhat more like the results caused by a change in shape of the cells. The power of the individual cells to change shape by a process of contraction, comparable to that of a muscle, gives the peculiar character to many of the phenomena of development. Whether the phenomenon of contractility which appears to be one of the fundamental characteristics of living things is in its ultimate analysis a »vital« or a mechanical process does not concern us here, but I do not think it probable that the change in shape of the cells that takes

place during development can be explained, as RHUMBLER has tried to show¹⁾, as due to differences in the osmotic and other physical conditions on different sides of the same cell, but rather to a process of contraction like that shown by a muscle cell, whatever the ultimate character of the process may be.

It would be tempting to extend this view to many of the developmental phenomena, for, an examination will show that a large number of the changes through which the embryo passes may be accounted for on the assumption that the individual cells have the power to change their shape by certain parts of the cell contracting in response to local conditions, internal and external. I need scarcely do more than name the different forms under which this phenomena appears to occur: The cleavage of the egg is probably a contractile process; the inwandering and immigration of amoeboid cells belong to this category, if we may extend to this process the same explanation that JENNINGS has recently discovered for amoeba. The invagination and the formation of pouches of many kinds can also be explained as the result of the change in shape of the cells of a given region. The rounding up of solid organs and the delimitation of groups of cells, in general, may be accounted for in the same way. In fact, may we not ask ourselves whether the so called formative forces, or factors, or principles (whichever term we prefer) may not be an expression of the phenomenon of response and contraction?

Summary.

1) The frequent appearance of abnormal embryos in the upper hemisphere of the egg can be explained either on the grounds that the abnormal and the normal embryo are developed out of different material, or else that the embryo-forming material lies at first higher up in the upper hemisphere, than has heretofore been suspected, and that it is carried downwards below the equator of the egg before the blastopore appears. These alternatives could be tested either by removing two or four of the upper blastomeres in the eight-cell stage (the results have been described in the VI-paper of this series), or by a reexamination of the early stages of normal development. The results of such an examination are given in the present paper.

¹⁾ I wish to state however that I owe much to RHUMBLER's clear analysis of the nature of the factors involved in the gastrulation process.

2) The segmentation cavity grows larger during the early cleavage period and during this time its roof is becoming thinner, Figs. 2—8. Towards the close of this period, the floor of the segmentation cavity rises higher up, and the cavity becomes in consequence broader horizontally, Figs. 9—10. At the same time that the yolk mass moves upwards, the lateral walls of the segmentation cavity push downwards over the sides of the egg. The crevice that appears in the interior between the yolk and the ectoderm, Figs. 9—13, appears when the processes just described have reached a certain stage. The crevice owes its origin to the rolling upwards of the yolk mass as the lateral walls push downwards.

3) The appearance of the blastopore is due to certain yolk cells changing shape, which causes at first an indentation of the surface and leads later to the formation of the slit like archenteron in the yolk. This process continuing at the anterior end, an extension forward of the archenteron into the yolk is brought about; the yolk mass during the same time pushes upwards higher into the segmentation cavity, Fig. 12. The shape of the cells at the top of the blastoporic invagination suggests that in a change of shape may lie the cause of the moving of the surface cells of the lower hemisphere under the advancing lips of the blastopore. As each cells reaches the top of the invagination it becomes pointed at its outer end, and as the cell changes shape the next cell is drawn higher up in the interior. The new cell then undergoes a similar change in shape, and the process repeats itself until all the surface cells have been drawn under the lips of the blastopore. The interior yolk cells of the lower hemisphere may also draw upwards by changing their shape.

4) The ectoderm of the embryo is formed from the cells of the roof and sides of the segmentation cavity that are pushing downwards during the entire cleavage period, and are later carried over the lower hemisphere by the advance of the blastopore lips. The mesoderm also arises high up in the egg. It arises in part from cells that in the early cleavage formed part of the inner wall of the roof of the segmentation cavity. From their beginning these cells are continuous with the yolk cells at the lower edge of the segmentation cavity, and these cells also appear to be added to the mesoderm. Thirdly, the inner layer of the ectoderm at the edge of the blastopore rim is always continuous with the mesoderm, and as the blastopore closes it is not improbable that some of the inner ectoderm cells are added to the mesoderm.

The anterior portion of the walls of the archenteron is lined by yolk cells, and is formed by the rolling around of yolk cells at the top of the archenteron. The floor of the remaining portion is the yolk of the lower hemisphere. The roof of the mid-dorsal part is formed by cells that have rolled around the edge of the blastopore rim and have been brought, for the most part, to the middle line by the advance of the lateral lips. The lateral walls of the archenteron are lined by the cells that first draw in at the rim, especially at the lateral lips; possible also some of these cells come from cells that by changing shape at the top of the invagination have come to pass around the upper end of the archenteric split to be added to the outer walls.

5) The »mechanics« of the downward movement of material finds its explanation in the power of the yolk cells to change shape. The entire movement of the floor of the segmentation cavity can be explained in this way. The change is especially noticeable at the blastoporic invagination. The downward, compensatory movement of the outer ectoderm cells may be due to a passive re-arrangement, or the cells may also take an active part in the process. Which of these processes occurs can not be stated.

The power of individual cells to change their shape is due to their contractile power which is no doubt the result of a stimulus, but the real nature of the phenomenon of contractility is not known at present, although it does not appear to correspond to any one, known physical process. It is suggested as a tentative view that the so-called formative principle may be also a expression of this contractile power.

Zusammenfassung.

1) Das häufige Erscheinen unnormaler Embryonen auf der oberen Eihemisphere kann entweder auf der Grundlage erklärt werden, daß normaler und unnormaler Embryo sich aus verschiedenem Material entwickeln, oder aber daraus, daß das embryobildende Material weiter nach oben an der oberen Hemisphäre gelegen ist, als man bisher annahm, und daß es bis unter den Ei-äquator vor dem Auftreten des Blastoporus hinabgelangt. Diese Alternative läßt sich erhärten: entweder durch Entfernung von zwei oder vier der oberen Blastomeren im Achtzellenstadium (die bezüglichen Ergebnisse sind in der VI. Arbeit dieser Serie niedergelegt), oder durch ein Wiederdurchprüfen der frühen normalen Entwicklungsstadien. Die Ergebnisse einer solchen Nachprüfung sind in gegenwärtiger Arbeit niedergelegt.

2) Die Furchungshöhle vergrößert sich während der frühen Furchungsperiode, indem sich währenddessen ihr Dach mehr und mehr verdünnt, Fig. 2—8. Gegen Ende dieser Periode erhebt sich der Boden der Furchungshöhle und damit gewinnt die letztere eine größere Horizontalausdehnung, Fig. 9—10. Zur selben Zeit, in der sich die Dottermasse aufwärts bewegt, dringen die lateralen Furchungshöhlenwände nach unten über die Eiseiten vor. Der Spalt, der sich im Innern zwischen dem Dotter und dem Ectoderm zeigt, Fig. 9—13, tritt auf, wenn die eben beschriebenen Prozesse ein gewisses Stadium erreicht haben. Der Spalt leitet seine Entstehung vom Aufwärtsrollen der Dottermasse her, während die Seitenwände nach unten vordringen.

3) Das Auftreten des Blastoporus ist durch die Gestaltveränderung bestimmter Dotterzellen veranlaßt, welche zuerst eine zahnförmige Kerbe der Oberfläche verursacht und später zur Bildung des schlitzförmigen Archenteron im Dotter führt. Durch das Weitergehen dieses Prozesses am Vorderende wird eine Ausdehnung des Archenteron im Dotter nach vorn bewirkt; während derselben Zeit drängt die Dottermasse nach oben höher in die Furchungshöhle hinauf, Fig. 12. Die Gestalt der Zellen am Scheitel der Blastoporeinstülpung legt nahe, daß in dieser Gestaltveränderung die Ursache der Bewegung der der unteren Hemisphäre angehörigen Oberflächenzellen unter die vorrückenden Blastoporuslippen liegt. Beim Erreichen des Einstülpungsscheitels spitzt sich jede Zelle am äußeren Ende zu, und mit der Gestaltveränderung der Zelle rückt die nächste im Innern weiter nach oben. Die neue Zelle unterliegt dann einer ähnlichen Gestaltveränderung und der Prozeß wiederholt sich, bis alle Oberflächenzellen unter die Blastoporuslippen gezogen worden sind. Die inneren Zellen der unteren Hemisphäre können gleichfalls unter Gestaltveränderung nach oben rücken.

4) Das Ectoderm des Embryo bildet sich von den Zellen des Furchungshöhlendaches, welche während der ganzen späteren Furchungsperiode nach unten vorrücken und später durch das Vorrücken der Blastoporuslippen über die untere Hemisphäre transportiert werden. Auch das Mesoderm entsteht weiter oben im Ei. Es entspringt teilweise von Zellen, die in der frühen Furchungsperiode der inneren Wand des Furchungshöhlendaches angehörten. Von ihrer Entstehung an hängen diese Zellen mit den Dotterzellen am unteren Rande der Furchungshöhle zusammen und diese Zellen scheinen gleichfalls zum Mesoderm beizutragen. Drittens ist stets die innere Ectodermlage am Rande der Blastoporuspalte im kontinuierlichen Zusammenhange mit dem Mesoderm, und wahrscheinlich treten beim Blastoporuschluß einige von den inneren Ectodermzellen dem Mesoderm bei.

Der vordere Teil der Archenteronwandung wird von Dotterzellen begrenzt und entsteht durch das Eintreiben von Dotterzellen am Archenteronscheitel. Der Boden des übrigen Teils ist der Dotter der unteren Hemisphäre. Das Dach dieses Teils wird von Zellen gebildet, welche um den Rand des Blastoporuspalts herumgerollt und zum größten Teil durch das Vorrücken der seitlichen Lippen in die Mittellinie gebracht wurden. Die seitlichen Archenteronwände sind von den Zellen begrenzt, die zuerst im Spalt, speziell an den seitlichen Lippen, eingezogen wurden. Wahrscheinlich stammen einige dieser Zellen auch von Zellen ab, die unter Gestaltveränderung am Einstülpungsscheitel um das obere Ende des Archenteron herumgelangt, sich trennen und zu den Außenwänden hinzutreten.

5) Die »Mechanik« der Materialabwärtsbewegung findet ihre Erklärung in der Fähigkeit der Dotterzellen zur Gestaltänderung. Die ganze Bewegung des

Furchungshöhlenbodens läßt sich so erklären. Die Veränderung ist speziell bemerkenswert an der Blastoporseinstülpung. Die kompensatorische Abwärtsbewegung der Außenzellen kann durch ein passives Wiederordnen ihrerseits veranlaßt sein, oder sie können an dem Prozeß aktiv beteiligt sein. Welcher von diesen Prozessen eintritt, läßt sich nicht feststellen.

Die Fähigkeit der Zellindividuen zur Gestaltveränderung beruht auf ihrer Kontraktilität, die ohne Zweifel einem Reiz gehorcht, aber die wahre Natur des Kontraktilitätsphänomens ist zurzeit nicht bekannt. Doch hängt sie anscheinend mit keinem bekannten physikalischen Prozeß zusammen. Als versuchsweise Auffassung sei angedeutet, daß das sogenannte gestaltbildende Prinzip gleichfalls eine Form dieser Kontraktionsfähigkeit sein könnte.