CHAPTER X

MODIFICATION OF CLEAVAGE BY COMPRESSION OF THE EGG

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

EFFECT OF COMPRESSING THE SEGMENTING EGG BETWEEN PARALLEL PLATES

The eggs of Rana fusca are on an average 1.5 mm. in diameter. The distance between the two glass plates in the experiment was 1.4 mm., for if less the eggs were burst by the pressure. Since all of the jelly around the egg was not removed, the actual diameter of the egg, as subsequent measurements showed, was less than the distance between the two parallel plates (1.4 mm.). For instance, a compressed egg (after it had been killed and hardened)[1] measured in its longer diameter, parallel to the two plates, 1.83 mm., while the shorter diameter, at right angles to the plates, was 1.2 mm. The two axes, therefore, stand in the relation of 2 : 3. These figures apply to eggs compressed in the direction of the egg-axis, from above downward. When the egg is compressed from side to side it will withstand more pressure. With a distance of 1.37 mm. between the two parallel plates, an egg compressed laterally measured in its longer diameter 1.96 mm., while its shorter diameter, from plate to plate, measured 0.91 mm. The two axes therefore bear to each other the ratio of 1 : 2. The experiments may be described in detail under the following categories.

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

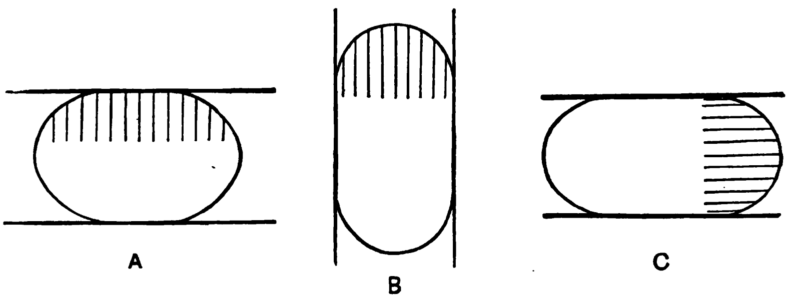


FIG. 30.—Diagram showing three positions of eggs under compression.

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

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

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

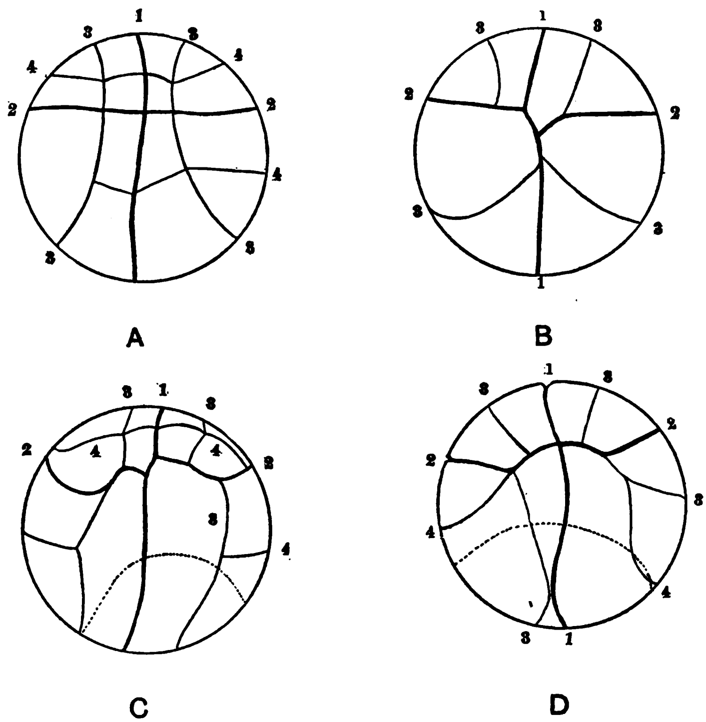


FIG. 31.—A, B. Egg compressed axially (Diagram A, Fig. 30). A. Above; B. below. C, D. Egg compressed laterally (Diagram B, Fig. 30). C. One side; D. other side.

ceding furrows) are also *vertical* and at right angles to the plates, and are generally *parallel* to the *first* furrow (Fig. 31, A, B). The egg is now divided into eight cells, all lying in one horizontal plane. In the black hemisphere the third furrows abut against the second furrows (Fig. 31, A), but below they as often run into the first furrows, as shown in the figure (Fig. 31, B). The fourth furrows are also vertical (*i.e.* at right angles to the plates) and generally run parallel to the *second* planes of cleavage, as seen in the figure (Fig. 31, A, B). There is no segmentation-cavity as yet present in these compressed eggs.

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

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

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

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

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

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

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

Hertwig ('83, b) has described the first cleavage of one of these eggs compressed by plates inclined 45 degrees to each other. The first cleavage divides a smaller protoplasmic portion from a larger yolk-portion. It does not therefore divide the egg, as in the preceding cases, symmetrically.

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

EFFECT OF COMPRESSING THE EGG IN A GLASS TUBE

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

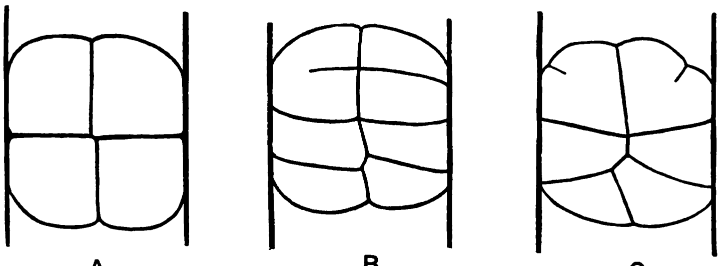


FIG. 32. — Segmentation of egg enclosed in a tube. (After Hertwig.) A. Four-cell stage. B, C. Eight-cell stage, above and below.

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

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

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

CONCLUSIONS FROM THE EXPERIMENTS

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

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

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

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

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

Sachs's law for the direction of new cleavage-planes seems to apply to the compressed eggs. According to Sachs, the form of the whole mass determines the position of the cleavage-planes. Hertwig refers the processes of cleavage directly to the changes that take place in the nucleus. He thinks that the nucleus tends to assume the centre of its sphere of activity, which is the centre of the protoplasmic mass. This is not necessarily the centre of an egg in which the yolk is unequally distributed. Hertwig thinks that the nuclear spindle will then elongate in the direction of the greatest protoplasmic mass. If we apply Hertwig's hypothesis to the segmenting frog's egg, we see that it appears to explain in part the various phenomena. In the egg compressed in the direction of its primary axis and with the primary axis vertical (category 1), the greatest protoplasmic mass will be, for the first spindle, in a horizontal plane; similarly for the second spindle. Hence the cleavage-planes that come in at right angles to the cleavage-spindle must be vertical and at right angles to the plates. The third cleavage-planes will be for the same reason vertical, and even the fourth planes may be so. The number of consecutive divisions at right angles to the compressing plates must, however, soon reach a limit, because the mass of protoplasm in each cell will soon be thicker vertically than horizontally. When this happens, the next cleavage comes in horizontally or parallel to the plates.

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

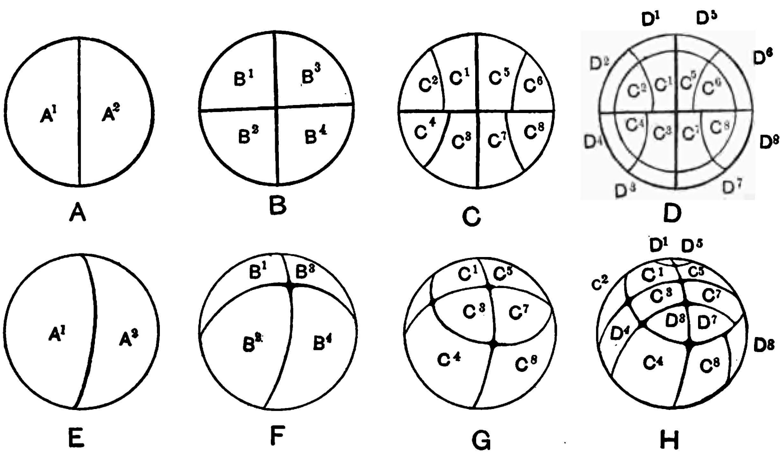
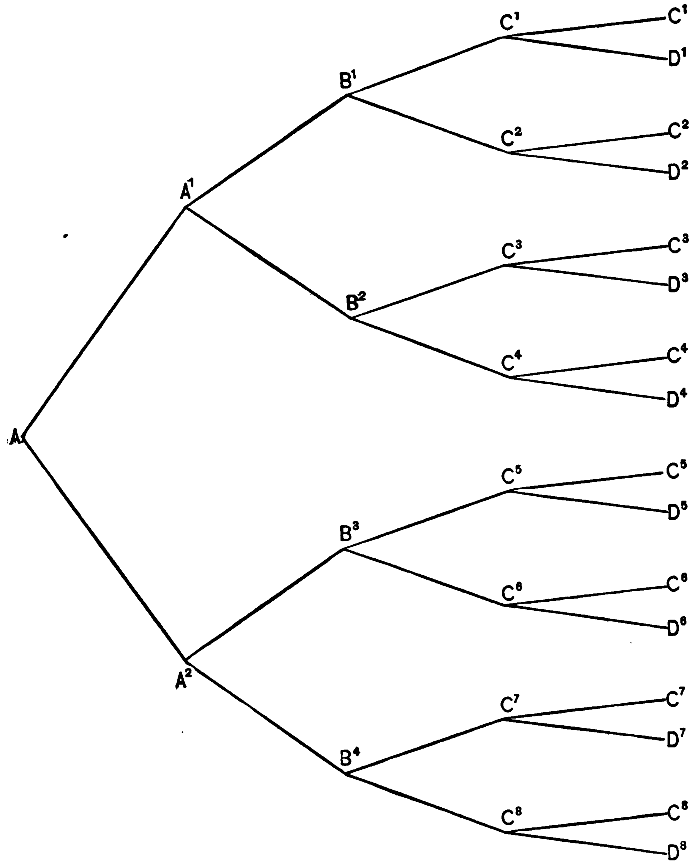


FIG. 33.—Diagrams to show the distribution of nuclei in compressed (A–D) and normal egg (E–H). In the upper series (A–D) the black hemisphere is turned toward the observer; in the lower series (E–H) the egg is seen from the side and in part from above the black hemisphere.

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



THE DISTRIBUTION OF THE NUCLEI IN THE COMPRESSED EGG

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

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

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

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

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