CHAPTER VIII

PFLÜGER'S EXPERIMENTS ON THE FROG'S EGG

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

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

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

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

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

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

THE EFFECT OF GRAVITY ON THE DIRECTION OF THE CLEAVAGE

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

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

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

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

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

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

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

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

What has just been said with regard to the second planes of cleavage holds equally well for the third cleavage-planes. If the egg be rotated through 180 degrees *after* it has divided twice (into four parts), then the third furrows come in as though no change had taken place, *i.e.* nearer the former upper pole. But if the egg had been rotated one hour after fertilization (or even after the first cleavage), the third furrows would appear on the *new* upper hemisphere, *i.e.* nearer the present upper pole. In this last case the four upper cells resulting from the third division are smaller than the lower four. This shows that the four upper cells of the normal eight-cell stage are smaller, not because they are black, but, according to Pflüger, on account of their position in relation to the force of gravity. Embryos develop from these eggs, but they show many abnormal structures.

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

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

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

The direction of the plane of first cleavage was noted and marked by a line scratched on each glass. The beginning of the nervous system appeared in about forty-eight hours. In twelve eggs the median plane of the body coincided with the first cleavage-plane, or at most the two planes did not differ more than 10 degrees. In four eggs there was an angle of 30 to 60 degrees between the two planes, and in one egg one of 90 degrees. Pflüger concludes that it is highly probable from this result that the plane of the first cleavage and the median plane of the body coincide. The exceptions may be due to the rough treatment of the eggs.[1] Newport ('51) had previously made a similar experiment on normal eggs, *i.e.* eggs not fixed artificially, and had reached the same conclusion as Pflüger, but Newport's results were unknown to Pflüger when he made his experiments.

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

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

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

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

CONCLUSIONS FROM THE EXPERIMENTS

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

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

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

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

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

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

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

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