CHAPTER IX

EXPERIMENTS OF BORN AND OF ROUX

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

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

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

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

Examination of sections of an inverted egg shows that forty-

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

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

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

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

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

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

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

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

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

THE CLEAVAGE OF THE EGG IN A CENTRIFUGAL MACHINE

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

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

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

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

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

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

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

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

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