Inversion of the Dorso-ventral Axis in Amphibian Embryos by Unilateral Restriction of Oxygen Supply

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INTRODUCTION

According to the hypothesis advanced by Løvtrup (1958) the supply of oxygen is one of the factors responsible for the determination of bilateral symmetry in amphibian embryos. The protein coat covering the outside of the egg is known to have a very low permeability (Holtfreter, 1943), and it was suggested in the hypothesis that the formation of the grey crescent consists in a stretching of this coat by which the permeability is increased (cf. the work of Dalcq & Dollander (1948) and of Dollander & Melnotte (1952) on permeability of Nile blue). In this way the radial symmetry of the egg is changed to a bilateral symmetry from a metabolic point of view. As a consequence of the increase in permeability those oxidative, energy-supplying processes which are associated with gastrulation are enabled to proceed at a higher rate at one side of the egg. This hypothesis can of course only be valid for eggs which require oxygen for gastrulation. In some amphibian species, particularly in toads, gastrulation may occur under anaerobic conditions (Waddington, 1956). It seems necessary to assume that the bilateral symmetry is somehow built into the structure of these eggs.

In order to test the hypothesis mentioned one might cut off the supply of oxygen from the dorsal side of the egg, where the grey crescent is located, but not from the ventral side. This may be accomplished by placing the eggs in closed tubes with the grey crescent towards the closed end of the tube. Failure to develop would, of course, only permit the already-known conclusion that oxygen is necessary for gastrulation. Support for the suggested hypothesis might be found, however, if inversions of the dorso-ventral axis occurred, i.e. if gastrulation could be forced to take place at the ventral, aerobic side.

In the axolotl egg with which we have carried out our experiments it may be difficult to observe the grey crescent, and a different approach was therefore necessary. The first division plane does not invariably coincide with the future plane of bilateral symmetry (cf. the discussion by Ancel & Vintemberger, 1948).

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However, it is unquestionable that there will be an equal number of blastopores on each side of a plane going through the animal-vegetal axis perpendicular to the division plane, if the position of the grey crescent is either unknown or disregarded. In experiments with tubes as mentioned above, the possibilities are as follows. If oxygen were not essential for gastrulation, all eggs would gastrulate, about half of them towards the aerobic and the other half towards the anaerobic side. If oxygen is necessary two possibilities exist. If inversion is impossible, half of the eggs should gastrulate with blastopores towards the aerobic side, while the development of the other half should be blocked. Finally, if inversion were possible, significantly more than half of the eggs should gastrulate towards the aerobic side. This latter possibility, if it occurred, might be considered a support for the hypothesis advanced.

**METHOD**

Eggs of the axolotl (*Siredon mexicanum*) were used in our experiments. They are easily freed of the jelly coat and can be obtained relatively easily at different times of the year. The present experiments were carried out in May 1957 and January 1958. Glass tubes sealed at one end and about 3 mm. in length were prepared from Pyrex tubing. The internal diameter of the tube must be selected rather strictly, so that the eggs devoid of their jelly coats fit tightly into the tubes, without being appreciably deformed. The glass tubes were washed in hot chromic-sulphuric acid, and rinsed for 48 hours in running tap-water. They were then placed for a few days in daily changed distilled water. Eventually they were stored in distilled water. About 30–60 minutes before an experiment the distilled water was sucked out of the glass tubes, and they were subsequently transferred into Holtfreter solution.

A few hours after oviposition the eggs were transferred into dishes with Holtfreter solution. Eggs in the two-blastomere stage were picked out and freed of the jelly coat by means of watch-maker's forceps. These eggs were placed in the tubes with the division plane in the direction of the axis of the tube. As mentioned above, no attention was paid to the possible localization of the grey crescent. The eggs were sucked into the tubes by means of a very fine capillary pipette (see Text-fig.).
When the egg was properly placed and oriented within the tube, the pipette was withdrawn. The tubes with eggs were transferred to fresh Holtfreter solution, which was subsequently changed every day, and were kept at a temperature of 16°–17° C. After 24 hours the tubes were inspected. Eggs showing any signs of damage were discarded, and the same was done if the tubes were so wide that rotation of the egg was possible.

There were always some eggs which slipped out of the tubes during the first 24 hours of the experiment. These were used as controls, to determine the percentage of eggs which died before gastrulation. In the first experiments we tried to use as controls eggs placed in tubes open at both ends. However, it is difficult to place the eggs inside the tubes, as the very gentle 'sucking' procedure cannot be used, and when forced into the tubes the eggs slip out very easily.

When gastrulation had begun, after approximately 60 hours, the eggs were inspected every 12 hours.

RESULTS

The results of the experiments are summarized in the Table. The experimental eggs were divided into three groups: (1) gastrulation at the aerobic side; (2) gastrulation at the anaerobic side; (3) no clear gastrulation. These latter eggs died sooner or later. Control eggs were divided into two groups: (1) normal gastrulation; (2) no development.

The two preliminary experiments in May are not conclusive because of the lack of controls. The large number of eggs in the group with no clear gastrulation may be because development of the eggs is less satisfactory during the warm period of the year. A few observations were made on the gastrulation of the eggs in tubes, which deserve mentioning. First of all, whereas the beginning of gastrulation occurs within a rather narrow time interval in the controls, it was spread over about 2 days in the experimental eggs. In some of the 111 cases reported as showing gastrulation at the aerobic side, abnormalities in the gastrulation pattern were observed, indicating possibly the effect of inversion of the dorso-ventral axis. Finally, we found that some of the eggs in the 'no clear gastrulation' group showed finger-like outgrowths, which means that whereas some processes were going on, gastrulation was completely inhibited.

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DISCUSSION

The present experiments were performed in order to test the hypothesis of the mechanism of establishment of bilateral symmetry advanced previously. The significance of the results is unquestionable, and they give good support to the hypothesis. They do not, of course, give any definite proof; many more experiments will have to be performed before the validity of the hypothesis is established.

It may be argued that the inhibition of gastrulation at the anaerobic side is a result of an accumulation of metabolic waste products. This possibility certainly exists, but it does not explain the observed inversion of the dorso-ventral axis.

Another objection that might be raised is that the eggs, lying in the tubes, could perhaps rotate within their membranes. Against this it may be argued that the tubes in most cases were arranged so that the animal poles were turned upward; thus the driving force of rotation was absent. However, even if rotation occurred in a plane parallel to the axis of the tube, there should be the same probability that a dorsal side was changed from the anaerobic to the aerobic side, as that of the opposite event. Rotation in a plane perpendicular to the axis of the tube would be of no consequence. It should be mentioned though that the eggs fit very tightly in the tubes, so that rotation seems very unlikely to have occurred. However, in order to be completely sure on this point, some form of marking might be employed.

It should be mentioned that Lindahl a long time ago made some experiments with sea-urchin eggs which may indicate that similar conditions prevail in these embryos (Lindahl, 1936). Lindahl sucked eggs through fine capillaries, and the side of the egg which went first through the capillary almost invariably became the ventral side, i.e. the side at which invagination occurs. It is reasonable to assume that the membrane on the side of the egg going first through the pipette has been stretched most. If this is true, the increased permeability of the stretched membrane would explain the axial determination observed.

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REFERENCES


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