On the determination of the dorso-ventral polarity in *Xenopus laevis* embryos

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SUMMARY

1. When embryos, or dorsal or ventral half-embryos, of *Xenopus laevis* are subjected to unilateral restriction of oxygen supply, the posterior end will always appear at the aerobic side, while the development of the anterior end, oriented towards the anaerobic side, will be partly suppressed. The shorter the time treatment lasts, the more normal the development will be.

2. When the restriction of oxygen effects an inversion of the dorso-ventral polarity, development is retarded, otherwise not.

3. Measurements of oxygen consumption show a substantial reduction in the experimental embryos, as compared with normal ones. The change in oxygen consumption in inverted embryos is delayed relative to non-inverted ones, but there is no significant difference in the total consumption of oxygen.

4. Our results support the idea that the dorso-ventral polarity is associated with a gradient in oxygen consumption, and various kinds of evidence suggest that oxygen consumption is, in part, required for the formation of Ruffini's flask-cells, responsible for the initiation of invagination.

5. It is suggested that the basic mechanisms involved in the determination of the normal, and the inverted, dorso-ventral polarity are fundamentally different, the latter being in fact an induction of a new polarity.

INTRODUCTION

The dorsal region of an amphibian embryo is distinguished by being morphogenetically more active than any other region during the early stages of embryonic development. The influence of this region upon the epigenesis of the remaining embryo was demonstrated in a spectacular fashion through the induction of a secondary embryo by a dorsal lip transplant (cf. Spemann, 1938).

This circumstance has raised the question of the factors responsible for the determination of the dorso-ventral polarity – or gradient – thus prevailing in the amphibian embryo. It is known that the fertilizing spermatozoon enters the egg at the ventral side, in the presumptive median plane of the embryo. This implies that the sperm entrance point is located opposite to the grey crescent which outlines the dorsal region of the embryo. It had been surmised, and was

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demonstrated decisively by Ancel & Vintemberger (1948), that the sperm is the causal agent in the determination of the dorso-ventral polarity, i.e. before fertilization has occurred the egg is spherically symmetrical such that any plane through the animal-vegetal axis may become the median plane of the embryo. These authors also showed that the visible cortical changes elicited by fertilization are not a necessary condition for the establishment of the dorso-ventral polarity, for the cytoplasmic derangement which ensues from forced rotation of the egg in a fixed plane may serve the same purpose.

Numerous attempts have been made to discover how such apparently simple dorso-ventral disparities ensure that the dorsal region acquires its demonstrable 'organizing' capacity. At an early stage the notion was adopted that the dorsal side may be distinguished by a higher activity on the chemical and physiological level than any other region of the embryo. The first observation supporting this idea was made by Bellamy (1919) who found that the dorsal region of the amphibian gastrula is particularly sensitive to toxic agents. More conclusive, maybe, was the demonstration by Gilchrist (1928, cf. also Glade, Burrill & Falk, 1967) that, when a temperature gradient is applied over an amphibian embryo, the dorsal side will be formed towards the warmest side. Similarly, it was possible to show that the direction of the dorso-ventral polarity may be re-oriented through unilateral restriction of oxygen consumption, the dorsal side always forming where there is free access to oxygen (Løvtrup & Pigon, 1958). These results concur in supporting the contention that the dorsal region is the metabolically most active one and that, somehow, in this activity proper an element of determination is acquired, since interference with it affects the orientation of the dorso-ventral polarity.

The question of whether this demonstrable activity differential is associated with a measurable difference in energy consumption has attracted much attention. As a measure of the latter, differences in oxygen consumption have usually been used, but it is possible to raise two objections against this method, viz., that glycolytic activity is very high in the early embryo (Cohen, 1954; Gregg, 1962) and also that, apparently, the oxidative metabolism at this phase in some way differs from that prevailing in later development, as indicated by a distinct difference in respiratory control (Gregg, 1960). Yet the fact that development always becomes blocked before or during gastrulation under anaerobic conditions nevertheless suggests that some oxidative process is involved in the determination of the dorso-ventral polarity.

Several published papers deal with the dorso-ventral differential in oxygen consumption in the amphibian embryo. Some of the early contributions describe success, others failure, in revealing a difference between the dorsal and the ventral side in this respect (cf. Needham, 1950). In the present context we shall confine attention to the works in which the Cartesian diver technique has been used to measure the respiration of explants. The first of these, published by Boell & Needham (1939), reported that, on a total nitrogen (TN) basis, no
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measurable difference could be ascertained. Ornstein & Gregg (1953) found that, on a dry weight (DW) basis, dorsal explants respire some 20% faster than ventral explants, while Sze (1953) observed a somewhat larger difference which was preserved, but reduced, when calculated on TN basis.

One objection may be raised against the explant experiments, namely that the rate of oxygen uptake in explants appears to exceed that of the whole embryos (cf. Needham, 1950). However, if this source of error is not too great, then it seems necessary to conclude that there is a dorso-ventral difference in oxygen consumption which is small enough to explain the negative findings reported, yet large enough to give significant results. This conclusion seems to be supported by the determinations of oxygen consumption in dorsal and ventral half-embryos reported by us (Landström & Løvtrup, 1974). We did not find any significant difference between the two groups, but DW determinations (unpublished experiments) show that the ventral blastomeres account for about 60% of the total DW. The ventral embryos are thus substantially larger than the dorsal ones, and hence our result corroborates those discussed above.

The present paper is a report of some experiments we have performed to find possible solutions to some questions concerning physiological aspects of the determination of the dorso-ventral polarity in the embryo of Xenopus laevis.

MATERIAL AND METHODS

The present experiments were carried out on embryos of X. laevis. For further details concerning the biological material, see Landström & Løvtrup (1974).

Manometric determinations were made by means of the automatic electromagnetic diver respirometer, as described by Løvtrup (1973) and Landström & Løvtrup (1974). This method is inspired by the Cartesian diver respirometer (Holter & Linderstrom-Lang, 1943). The respiration chamber - or diver - is a glass capillary, closed at one end and painted at the open end with black epoxy varnish containing about 35% carbonyl iron (w/w) (cf. Fig. 1). The biological object is placed at the closed end in a drop of medium and the lower end is filled with flotation medium (0.5 N-NaOH, 0.5% Triton X-100). These liquids are separated by an air bubble which serves partly for oxygen supply and partly for buoyancy adjustment. The loaded diver, which must rise in the flotation medium, is transferred to the flotation vessel, beneath which is an electromagnetic coil. The current in the latter, controlled by a beam of light (IR diode) passing through the flotation vessel, will pull the respiration chamber down through the vessel, and it will come to rest when it cuts off so much light that the remaining current balances its buoyancy. As the oxygen in the air bubble is consumed, the diver becomes heavier, and its position is continuously adjusted so as to keep it floating. The current is registered on a recorder. The sensitivity is adjusted to the needs, but the most sensitive respiration chambers
Fig. 1. Diver respiration chamber used in the present experiments. A, top part with embryo; B, air bubble; C, flotation medium; D, black varnish containing carbonyl iron (about 35% w/w).

used so far have a sensitivity of 0.1 nl per mm ( recorder range 100 mV). In the plots represented below each point represents the slope of the recorded graph for a 20 min period in each successive hour.

In order to carry out experiments with unilateral restriction of the oxygen supply, the shape of the respiration chamber has to be as shown in Fig. 1. This model was made in the following way. A Pyrex tube (17 mm outer diameter, 1 mm wall thickness) is heated over a flame and pulled out to capillaries (1.75–2 mm outer diameter). By means of a diamond point, pieces of about 40 mm are cut off. The middle part of one such capillary is heated in a micro-flame and pulled out to an external diameter of about 1.5 mm and a length of about 10 mm. Heating again, the middle is pulled out to a very thin capillary and the latter broken off to give two units of about 20 mm length. The divers are finished by fusing the tapering end and painting the open end with epoxy varnish. (Before being painted, the diver is cleaned in alcohol, bichromate and distilled water.)
The upper, narrower part of the diver has to accommodate an embryo tightly, to prevent diffusion of oxygen between the wall and the embryo, and rotation of the latter. On the other hand, the embryo should not be deformed excessively, since this may unduly interfere with development. The internal diameter of this upper part is therefore very critical, and the divers must consequently be tested carefully before they are adopted for use.

To ensure stability of the floating diver the upper part must be as short as possible; for our purpose a length of 3 mm was found to be appropriate. This size implies the presence of about 1.5 µl of medium in contact with the embryo. Half of this volume, supposed to be present above the embryo, will contain about 50 nl dissolved oxygen at the beginning of the experiment. At the estimated rate of oxygen consumption and on the assumption that no oxygen diffusion takes place, anaerobic conditions must prevail above the embryo within 2 h.

The dorso-ventral axis of the embryo must be known before it is placed in the diver. Unfortunately, the grey crescent is often difficult to observe in X. laevis, and the direction of the axis therefore may have to be estimated on the basis of the direction of the cleavage divisions and the size of the blastomeres. According to Nieuwkoop & Faber (1956), the first cleavage plane coincides
approximately with the plane of bilateral symmetry. The second cleavage, perpendicular to the latter, divides the embryo into two smaller dorsal, and two larger ventral, blastomeres, as readily visualized when the embryo is observed from the animal pole (Fig. 2).

A diver, rinsed and filled with medium, is placed in a Petri dish together with a healthy-looking embryo in an early four-cell stage, mechanically stripped of its jelly. By means of a hair loop, the embryo is pushed into the chamber. Inside the latter, the dorso-ventral axis of the embryo is established and oriented in a direction parallel to the long axis of the diver, with the dorsal or ventral side towards the open end. As shown in Fig. 3, the embryo is subsequently gently sucked into the upper part by means of a very fine capillary pipette, according to the method described by Lovtrup & Pigon (1958).

Concerning the measurements proper, reference is again made to the previous publications (Lovtrup, 1973; Landström & Lovtrup, 1974).

The morphogenetic development of the embryo was followed during the experiment through a microscope, placed outside the thermostat. At the end of the experiment, the embryo was taken out and examined with respect to various morphological characters. The subsequent development of the experimental embryos was also recorded.

In some experiments the development, but not the respiration, was studied of normal embryos, and of dorsal and ventral half-embryos, confined in closed capillary tubes, thus simulating the condition in a diver. Ventral or dorsal blastomeres were isolated from an advanced four-cell-stage embryo as previously described (Landström & Lovtrup, 1974). Before being placed in a capillary, the half-embryos were allowed to recover in 20% Ringer, in which the calcium concentration was raised to 0.015 M to encourage wound healing (Holtfreter, 1943). Since this tonicity enhances the chance of exogastrulation, the embryos were placed in 7.5% Ringer before sucked into the closed capillary tubes, the wound side turned towards the closed end.

Whereas few normal embryos suffer from being placed in a capillary, the half-embryos, dorsal as well as ventral ones, frequently succumb under our experimental condition, in spite of all precautions.
RESULTS

The inversion of dorso-ventral polarity through unilateral restriction of the oxygen supply described by Lovtrup & Pigon (1958) has, so far, been studied only in *Ambystoma mexicanum*. In some initial experiments, to be reported below, it was ascertained that the same phenomenon can be observed in *X. laevis*. The morphological development of the experimental embryos, even after they have been taken out of the respiration chambers, is not quite normal, a fact which will be briefly described before the results on oxygen consumption are reported.

Table 1. *Orientation of the dorso-ventral polarity under conditions of unilateral restriction of the oxygen supply*.

<table>
<thead>
<tr>
<th>Initial orientation of embryo</th>
<th>Number of experiments</th>
<th>Blastopore and tail towards aerobic side</th>
<th>Abnormal development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Random</td>
<td>14</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>Dorsal side out</td>
<td>39</td>
<td>34</td>
<td>5</td>
</tr>
<tr>
<td>Ventral side out</td>
<td>37</td>
<td>30</td>
<td>7</td>
</tr>
</tbody>
</table>

*Polarity inversion*

Fertilized eggs were sucked into plain capillary tubes, fused at one end, and allowed to develop for 50 h at 18 °C. As seen from Table 1, in all cases where the development was quasi-normal, the blastopore and the tail were located towards the aerobic side. Most of the instances reported as 'abnormal development' represent cytolysis at the anaerobic side; in some few cases, the gastrulation at the aerobic side was distorted to appear like exogastrulation.

In a series of control experiments, fertilized eggs were sucked into capillaries open at both ends. Here it was found that the blastopore appeared close to or at the dorsal side of the embryo.

The floating divers are oriented vertically, which means that the side of the embryo exposed to air is turned downwards, implying that our results may be thought to be an effect of gravity. This interpretation may, however, be excluded, for in the early experiments (Lovtrup & Pigon, 1958) and in those reported here, the capillaries were oriented horizontally in Petri dishes filled with medium.

To establish the latest stage of development at which inversion is still possible, we placed embryos at different stages in tubes and kept them there until the outline of the blastopore was a closed circle, i.e. stage 11. As appears from Table 2, it is possible to invert the polarity as long as gastrulation has not yet started. Thus, whether the embryo is confined in a capillary tube at stage 3 or at stage 8/9, blastopore formation begins at the side turned towards the open end, with predictable consequences for morphological development. When the embryos had reached stage 10 before the experiment began, their development was
Determination of polarity in Xenopus severely impaired (exogastrulation) if left in the tube, but if they were taken out at stage 11 the brief period of oxygen restriction, about 2 h, had little effect on their subsequent development. This is shown in Fig. 4, where it will also be observed that for the other embryos the morphogenetic derangement is proportional to the time they have been enclosed in the capillary tube. It is of interest to note that the formation of melanocytes in the anterior part of the body evidently is influenced by the oxygen consumed by the cells located at the ‘ventral’ side of the embryo.

Table 2. Polarity inversion as a function of the developmental stage

For each stage, 10 embryos were confined in glass capillaries. It may be noticed that whereas the abnormalities observed in stage 3 and stage 6 embryos involved cytolysis and developmental arrest, those confined between stages 10 and 11 continued to develop, but in a highly abnormal fashion.

<table>
<thead>
<tr>
<th>Duration of confinement (stages)</th>
<th>Polarity inversion</th>
<th>Abnormal development</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-11</td>
<td>—</td>
<td>2</td>
</tr>
<tr>
<td>8/9-11</td>
<td>10</td>
<td>—</td>
</tr>
<tr>
<td>7-11</td>
<td>10</td>
<td>—</td>
</tr>
<tr>
<td>6-11</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>3-11</td>
<td>8</td>
<td>2</td>
</tr>
</tbody>
</table>

The morphological development of the experimental embryos, which were kept under conditions of restricted oxygen supply for 50 h at 18 °C, was followed during this period and, subsequently, after transfer to completely aerobic conditions. Since, as will be discussed presently, the development of the anterior end of the experimental embryos is impaired, the characters used for the staging of the embryos were such as are confined to the posterior end of the embryo.

As shown in Fig. 5, it is not possible to register any difference in the rate of development between the controls and the embryos which had their dorsal side turned outwards initially, but those oriented in the opposite direction exhibit a distinct retardation. Fig. 6 shows the external appearance of the various embryos after 20 h; it is readily seen that the gastrulation in Fig. 6B is delayed compared to that prevailing in the other embryos. As shown by previous observations (Landström & Lovtrup, 1974) our strain of X. laevis is distinguished by a remarkably constant rate of development, thus the onset of gastrulation may be predicted with an accuracy of ± 20 min. In this case the delay demonstrated

**Figure 4**

The effect of confinement on morphological development. The embryos shown are all of the same age (96 h at room temperature). From above: control, stages 44-45; non-inverted embryo, confined between stages 10 and 11; inverted embryo, confined between stages 8/9 and 11; inverted embryo, confined between stages 7 and 11; inverted embryo, confined between stages 6 and 11; inverted embryo, confined between stages 3 and 11.
Fig. 5. Rate of development of 18 °C. ○, Dorsal side initially turned outwards; ▲, ventral side initially turned outwards; ○, controls. Staging according to Nieuwkoop & Faber (1956).

Fig. 6. Appearance of embryos 20 h after fertilization. A, Dorsal side initially turned outwards, stage 12; B, ventral side initially turned outwards, stage 10½; C, control, stage 12.

in Figs. 5 and 6 clearly is significant. Fig. 7 shows embryo 40 h old; the same difference as in Fig. 6 is observed, but furthermore a retardation of the anterior end of the enclosed embryos is readily visualized. Finally, Fig. 8 shows three embryos after 96 h of development. It appears that the tail is
Fig. 7. Appearance of embryos 40 h after fertilization. A, Dorsal side initially turned outwards, stage 22; B, ventral side initially turned outwards, stages 17–18; C, control, stage 22.

Fig. 8. Appearance of embryos 96 h after fertilization. A, Dorsal side initially turned outwards, stage of head region 32–34, stage of tail region 41–42; B, ventral side initially turned outwards, stages approximately as in A; C, control, stages 41–42. The experimental embryos have had free access to oxygen for 46 h.
Table 3. Blastopore formation in half-embryos as a function of the oxygen supply

The numbers in parentheses represent the percentage values when the cytolysed embryos are included.

<table>
<thead>
<tr>
<th></th>
<th>Cytolysis</th>
<th>No blastopore formation</th>
<th>Blastopore formation</th>
<th>Blastopore %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventral half-embryos</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambient oxygen supply</td>
<td>27</td>
<td>20</td>
<td>—</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Restricted oxygen supply</td>
<td>30</td>
<td>5</td>
<td>11</td>
<td>69 (24)</td>
</tr>
<tr>
<td>Dorsal half-embryos</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambient oxygen supply</td>
<td>10</td>
<td>—</td>
<td>16</td>
<td>100 (62)</td>
</tr>
<tr>
<td>Restricted oxygen supply</td>
<td>13</td>
<td>—</td>
<td>8</td>
<td>100 (38)</td>
</tr>
</tbody>
</table>

developed to approximately the same extent in all cases, while the anterior end is retarded in the experimental ones. A slight difference seems to exist between the two previously enclosed embryos.

These results thus demonstrate that the inversion of the dorso-ventral polarity is associated with a distinct check on morphological development which, at the onset of gastrulation, amounts to about 2 h. This difference is gradually accentuated during gastrulation, but subsequently it remains relatively constant at a value around 7 to 8 h. This observation suggests that the recorded retardation is primarily associated with the polarity inversion proper. Once this hurdle has been overcome, development proceeds at the same rate as in the non-inverted embryos.

The results of the experiments on half-embryos are summarized in Table 3. It can be seen that, when the cytolysed embryos are omitted, blastopore formation occurs in 100% of the dorsal half-embryos, irrespective of the conditions of oxygen supply. In the ventral ones, the often-recorded complete suppression of blastopore formation in ambient oxygen was corroborated. However, when subjected to unilateral restriction of the supply of oxygen, a significant number of ventral half-embryos form a blastopore and undergo gastrulation. When they are taken out of the tubes, whether at the gastrula or the tail-bud stage, further differentiation of tail structures takes place, but the development of the anterior region is completely suppressed (Fig. 9).

Oxygen consumption

The results obtained from the respiration measurements are summarized in Fig. 10. The oxygen consumption of the embryos with the original ventral side exposed to the air bubble is seen to be significantly lower than that observed in those oriented the opposite way. The oxygen consumption of the latter is approximately half of that of the controls, except for the first 10 h, where the rate is seen to be somewhat higher, in relative terms. In Fig. 11 the oxygen
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Fig. 9. Appearance of embryos 65 h after fertilization. A, Control embryo, stages 33–34; B, dorsal half-embryo, stages 33–34; C, ventral half-embryo developed inside capillary tube; D, ventral half-embryo in ambient oxygen. The experimental embryos have had free access to oxygen for 15 h.

Fig. 10. Oxygen consumption under normal and experimental conditions. Each point represents the mean of three individual values; the standard error of the mean is indicated in some instances. ●, Dorsal side initially turned outwards; ▲, ventral side initially turned outwards; ○, normal respiration.

consumption is plotted against the morphological development. As would be anticipated from the results in our previous article (Landström & Løvtrup, 1974), the respiration is substantially lower in the experimental embryos than
Fig. 11. Oxygen consumption as a function of morphological development under normal and experimental conditions. ●, Dorsal side initially turned outwards; ▲, ventral side initially turned outwards; ○, normal respiration.

Table 4. Total oxygen consumption during gastrulation in the two sets of experimental embryos

The time indications are the age of the embryos and, in parentheses, the periods of time to which the oxygen consumption value refers.

<table>
<thead>
<tr>
<th>Onset of gastrulation (stage 9+)</th>
<th>Dorsal side initially turned outwards</th>
<th>Ventral side initially turned outwards</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (h)</td>
<td>Total oxygen consumption (nl)</td>
<td>Time (h)</td>
</tr>
<tr>
<td>12 (9)</td>
<td>355</td>
<td>15 (12)</td>
</tr>
<tr>
<td>25 (22)</td>
<td>1000</td>
<td>30 (27)</td>
</tr>
<tr>
<td>Oxygen consumption during gastrulation</td>
<td>—</td>
<td>645</td>
</tr>
</tbody>
</table>

in the controls. As concerns the former, the oxygen uptake is slightly lower in the inverted embryos, but, although this finding concurs with the morphological observations reported above, the difference is hardly significant.

In the present manometric experiments, we have been able to register a difference in oxygen consumption between the dorsal and the ventral side without suppressing the development completely, as in explant experiments, or in half of the embryo, as in our previous paper (Landström & Løvtrup, 1974).
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Theoretically the possibility exists that, if the rate of oxygen supply is not a limiting factor, the oxygen entering the exposed area may diffuse through the whole embryo to the distal parts, thus ensuring a normal respiration in the whole embryo. The latter explanation is clearly ruled out by our results (Figs. 7, 10 and 11); hence, under the given experimental conditions the oxygen supply is a limiting factor, and the experimental design thus fulfils its intended purpose.

In Table 4 it is shown that the observed retardation in development is associated with a corresponding change in oxygen consumption; when gastrulation begins the amount consumed is the same in inverted and non-inverted embryos. In the table it is further to be seen that in the non-inverted, as in the normal embryos, the onset of gastrulation, as indicated by the first traces of pigment accumulation (stage 9+), occurs after 12 h of development, in the inverted embryos after 15 h. From the tables of Nieuwkoop & Faber (1956) it appears that at their stage 10½ a distinct accumulation of pigment occurs at the ventral side, so the initiation of this process must take place somewhat earlier. And since the embryos of our strain of *X. laevis* reach stage 10½ after 16 h, it seems warranted to conclude that invagination begins at the ventral side approximately at the normal time.

**DISCUSSION**

The incipient blastopore is outlined by a condensation of pigment. This phenomenon is known to be due to the activity of Ruffini’s flask-cells (cf. Holtfreter, 1943), the agents responsible for the initiation of the process of invagination. Since these cells always appear first at the dorsal side of a normal embryo, we may infer that their formation is somehow influenced by the dorso-ventral polarity.

The experiments quoted in the introduction support the inference that the normal dorso-ventral polarity acts by establishing an activity differential in the embryo. We have convinced ourselves that, as in so many other amphibian species, gastrulation is completely suppressed in *X. laevis* under anaerobic conditions. It might be inferred simply that the energy required for the cell transformation involved in the formation of Ruffini’s cells exceeds that which can be supplied by anaerobic metabolic pathways. However, another explanation is afforded by the observations of Wilde & Crawford (1968), according to which, in *Fundulus* at least, the ATP production is not significantly reduced under anaerobiosis, and yet the RNA production is completely suppressed. This mechanism may account for our results, in so far as the synthesis of mRNA unquestionably is required for the mentioned cell transformation.

In either case the observed suppression of gastrulation in the part of an embryo cut off from the supply of oxygen is to be expected, but not necessarily the apparent inversion of the dorso-ventral polarity occurring when the ventral side is turned outwards in the capillary tubes. In order to account for this
phenomenon it must first be observed that the invagination, and thus the formation of Ruffini's cells, gradually spreads around the circumference of the embryo. This fact may be accounted for in two ways: (1) the cell transformation occurs spontaneously, and the function of the dorso-ventral polarity is merely to impose a temporal gradient on this process; or (2) the transformation occurs spontaneously only at the dorsal side, whence it spreads through contact induction around the egg. The former alternative is supported by the fact that, as recorded above, the transformation occurs at approximately the normal time in the inverted embryos, but it is contradicted by the experiment of Curtis (1962) showing that when the grey crescent cortex is excised in a fertilized embryo, development is completely suppressed. According to the latter observation our experiments with 'inverted' embryos cannot represent an inversion of the dorso-ventral polarity, but rather its induction de novo. It was in an attempt to show that this phenomenon is practically realizable that we made the experimental series with ventral half-embryos. As we have shown, this point was in fact borne out although, admittedly, the inversion is less successful in this case than in the whole embryos. We do not know whether the faulty development is imposed by the experimental conditions, or whether it is due to an inherent deficiency in the ventral half-embryos.

Hence we may add another agent to those which can induce a dorso-ventral polarity, namely, unilateral supply of oxygen. And furthermore it is possible to make the generalization that the side of the embryo which has the highest oxygen and energy consumption always becomes the dorsal side, i.e. the side where the gastrulation begins.

Thus it appears that the effect of the dorso-ventral polarity is quantitative rather than qualitative. Its normal function is merely to ensure a higher activity at the dorsal than at the ventral side of those processes in which all the blastomeres are engaged. And if this conclusion is correct then it may be futile to search for cortical or cytoplasmic inductors, evocators or regulators in the embryo, at least not before the direction of the dorso-ventral polarity has become determined.

REFERENCES


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