A Physiological Interpretation of the Mechanism Involved in the Determination of Bilateral Symmetry in Amphibian embryos

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INTRODUCTION

Very great interest has been taken in the question of the nature of those factors which determine the bilateral symmetry in amphibian embryos. Numerous experimental observations have been published, and various hypotheses and theories have been advanced to account for the results. The problem has been thoroughly discussed in several reviews (of which many of the more recent will be quoted below) and it would for this reason alone be out of place to review the literature in the present context. For orientation, the present views upon the problem will be presented in brief outline.

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It seems that factors responsible for bilateral symmetry are located partly in the fluid cytoplasm in the interior of the egg, and partly in the more solid cortex. It has previously been thought that the organization of the cytoplasm was determined by the influence of gravity, the constituents being distributed simply according to a density gradient. This idea has had to be abandoned, however, since it has been shown that the cytoplasmic inclusions (especially the yolk granules) are arranged in a definite pattern (Ancel & Vintemberger, 1948; Pasteels, 1951; see also Lehmann, 1945). This organization is labile to some extent, and may be changed experimentally. The influence of gravity on this distribution may be shown by inversion of the egg. Thus, when the egg is kept upside down, the large heavy yolk granules normally occupying the vegetal hemisphere, and the lighter cytoplasm in the animal hemisphere, will partly change places. The cortex or pellicle of the egg comprises a layer of pigmented cytoplasm, a very thin hyaline layer, and the cell-membrane proper (Ancel & Vintemberger, 1948; Dalcq & Dollander, 1948; Holtfreter, 1948). There is yet

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another structure which may be considered to belong to the cortex, viz. the coat (Holtfreter, 1943; Ancel & Vintemberger, 1948; Dollander, 1951). This coat is a thin extracellular pigmented protein layer covering the entire egg surface.

Among the cytoplasmic factors the distribution of yolk is of importance; the invagination generally seems to occur near the margin between a yolk-covered and a yolk-free area (cf. Weigmann, 1926, 1927; Ancel & Vintemberger, 1948). Anomalous yolk distribution obtained by inversion may thus lead to abnormal invagination. It has been suggested by Holtfreter & Hamburger (1955) that 'yolk plays a passive role in this respect, serving merely as a support to direct cytoplasmic shifting'. The material in the yolk-free region may also be of importance. Rather little is known about the composition of the cytoplasm of amphibian eggs. Using cytochemical methods Brachet has shown that both RNA and proteins containing reactive sulphydryl groups were located almost exclusively in the animal hemisphere, maybe partly associated with mitochondria-like inclusions (cf. Brachet, 1945). The possible morphogenetic influence of this part of the egg's cytoplasm has been stressed by some authors (see the discussion by Lehmann, 1945).

The ideas about the relative influence of the cortex vary widely. Lehmann (1945) seems to maintain the view that the cortex has next to no influence, and certainly the fact that invagination under experimental conditions may occur almost anywhere on the egg surface, may seem to support this view. The other extreme is represented by the theory of Dalcq & Pasteels (1937, 1938), which postulates a cortical gradient field of specific morphogenetic substances with centre in the grey crescent.

The truth may lie somewhere between these extreme views. It cannot be questioned, however, that the formation of the grey crescent in the egg cortex is an event of great importance for the determination of the dorso-ventral axis. The respective functions of the cytoplasmic and the cortical factors have been shown in inversion experiments with eggs after the appearance of the grey crescent. Under these conditions the invagination is found to occur at the margin of yolk closest to the original grey crescent (Weigmann, 1926, 1927; Penners & Schleip, 1928 a, b; Pasteels, 1938, 1939; see also the discussion by Ancel & Vintemberger, 1948). This observation shows that the cortical factors, localized in the grey crescent, are stabilized before the cytoplasmic factors. It is thus possible to distinguish between the function of the two sets of factors. The cortical 'dorsalization' factors seem to be responsible for determining in which region of the egg invagination will occur. The cytoplasmic 'symmetrization' factors determine, on the other hand, the direction of invagination and thus the plane of bilateral symmetry.

From the literature quoted above it appears that the cytoplasmic and cortical factors taken together establish the axial polarity, and thus the bilateral symmetry. The localization of the grey crescent determines the future dorsal side. The next question obviously concerns which factors determine the site of the
grey crescent. This problem has been thoroughly discussed by Ancel & Vintemberger (1948), who themselves have carried out a series of extremely careful studies on the mechanism involved in the determination of the localization of the grey crescent. It is necessary here to deal with this question in some detail, because the nature of this mechanism is of importance for the physiology of the determination process. The following description of the processes going on in the egg up to the end of grey crescent formation is, when not otherwise stated, based upon the work of Ancel & Vintemberger. It should be mentioned that most of this work has been done on eggs of *Rana fusca*. For reasons mentioned below the interpretation given here deviates on several points from that given by Ancel & Vintemberger, to whose work the reader may refer for further details.

A few minutes after activation or fertilization, a contraction of the cortex occurs, having its centre at the animal pole. As a consequence of this contraction the edge of the pigmented cortex is pulled away from the visible yolk margin, and a grey ring or belt is formed below the egg equator. On sections this process is revealed by thickening of the cortical pigment layer and wrinkles on the surface round the animal pole. It seems reasonable to believe that this contraction occurs in the very elastic surface coat (cf. Holtfreter, 1943). The contraction is reversible; after about 10 minutes the pigment border has more or less returned to the original location. If the egg is in water so that the normal swelling of the jelly may occur, the egg will begin the rotation of orientation as soon as the cortical contraction is over. During this rotation the egg orientates itself, under the influence of gravity, so that the animal-vegetal axis is vertical. The whole egg rotates inside the vitelline membrane, and it may be that the cortical contraction prepares the rotation by disrupting the immediate contact between vitelline membrane and egg surface. It should be mentioned in this context that according to Holtfreter (1943) there seems to be a more firm contact between vitelline membrane and egg surface at the animal than at the vegetal end of the egg. The rotation takes about 20 minutes, and subsequently the perivitelline space begins to form. It has been clearly shown by Ancel & Vintemberger that the primary mechanism involved in this process is the osmotic absorption of water from the outside. A precondition may be the already mentioned disconnexion of the contact with the vitelline membrane at the animal pole. In the shaping of the perivitelline space other factors may be involved, e.g. gravity and the elastic tension of the coat (cf. the observation that calcium influences the size of this space). The increase in size of the perivitelline space goes on for about 90 minutes.

The next stage, the process leading to the formation of the grey crescent, goes on between the first and second hour after fertilization, and is called by Ancel & Vintemberger the rotation of symmetrization. According to these authors the entire cortex rotates about 30° around an axis perpendicular to the plane of symmetry. In this way the pigmented cortical layer is lifted at the dorsal side,
thus forming the grey crescent. At the ventral side only the outermost layers of the cortex cross the yolk margin, as indicated by a slight accumulation of pigment at the ventral side of the vegetal hemisphere. The resistance of the yolk against the cortical rotation is thought to explain why only this small fraction of the cortex can cross the border. Ancel & Vintemberger can only suggest gravity as the force driving this rotation of the cortex while the interior of the egg remains static. It is hardly possible, however, to imagine that any great differences could arise between the accumulation of material at the dorsal and ventral sides. Especially when the above-mentioned resistance at the vegetal border is considered, it seems necessary to conclude that such rotation is not possible. There are several experimental observations which do not conform with this rotation, but before we discuss them, the experiments of Ancel & Vintemberger must be described in further detail. In their studies these authors used three different methods for determining the plane of bilateral symmetry: forced inclination (135°) of the animal-vegetal axis (in a humid chamber), directed rotation of orientation, and fertilization. In the first method the middle of the grey crescent will be at that point of the vegetal border which was highest in the inclined egg. In the second method the plane of symmetry is determined by the plane of rotation, again the highest point on the border will be in the middle of the grey crescent. Finally, in the third method the point of sperm entrance determines the plane of symmetry, and the grey crescent will form on the side opposite that of the sperm entrance. The common feature in the effects of these three procedures seems to be that at the dorsal side the contact with the yolk is disrupted in a narrow strip of the very thin vegetal cortex. The mechanism involved in the two first methods is gravity: yolk sliding down when the egg is kept inverted; and when the egg is rotated yolk moves faster than cortex, thus coming to lie slightly ahead of the latter. In the fertilized egg an asymmetrical contraction seems to occur, with centre at the point of sperm entrance. On sections this contraction is indicated by the accumulation of cortical pigment around the point of sperm entrance; a similar phenomenon is not observed in activated eggs. Whereas the effect of this contraction is very slight on the ventral side, due to the short distance to the pigment border, the contraction is quite considerable at the dorsal side, so that a distinct asymmetry is observable in the pigment distribution. For the following discussion it is necessary to make one assumption, viz. that the contact between yolk and cortex is so firm that only slight movements of the vegetal cortex are possible. If we imagine that a strip of this thin cortex is deprived of the yolk lining, we must expect that the tension in the cortex of the animal part of the egg is no longer in equilibrium. The elastic forces of the cortex (coat) will therefore slowly and gradually stretch this strip until a new equilibrium is reached. The area comprising this region of stretched material would be the grey crescent. It is reasonable to assume that the whole animal cortex slides ventrally, as indicated by experiments with electrolytic marking made by Ancel & Vintemberger, and likewise, as mentioned above,
that the deeper lying cortical material is accumulated at the ventral side. The excess coat seems to be incorporated or absorbed by the immobile coat at the ventral side of the vegetal end, as indicated by pigment accumulation. Such a coat reabsorption process has been described by Holtfreter (1943). The explanation given here is in agreement with the results of Ancel & Vintemberger except for the fact that their marking experiments also indicated movement in the direction from the vegetal pole towards the lower edge of the grey crescent. This observation may find an explanation in the fact that a coagulum fixed at the vitelline membrane may make a trace on the surface when the egg is moving inside the membrane. If the vitelline membrane egg-surface connexion is firmer at the animal side, it may be that the vitelline membrane with the fixed coagulum has been moved along the vegetal surface during the grey crescent formation, thus making the observed trace. There are some observations made by Ancel & Vintemberger which conform better with the mechanism suggested here than with the proposed rotation. Thus, the marking experiments show that dorso-lateral points do not move in planes parallel with the plane of symmetry, but unexpectedly they converge towards this plane. Also, the vital staining experiments of Banki (1929) show that a stretching occurs at the dorsal, but not at the ventral side (cf. Banki's pictures with those of Holtfreter (1943) showing stretching of the coat during wound-healing).

It should be mentioned that in eggs activated with the animal pole upwards, no determination of the grey crescent site takes place; it may appear at any side of the egg. No asymmetry is to be seen in sections. According to the mechanism outlined above, a very slight asymmetry would suffice to establish that distortion of the equilibrium in the cortical tension needed for the formation of the grey crescent. Such asymmetry might arise during the first cortical contraction.

In perfect agreement with the ideas outlined above are the observations on the permeability to Nile blue of the amphibian egg (Dalcq & Dollander, 1948; Dollander & Melnotte, 1952). It was found that before fertilization the dye penetrates to the same extent at all points of the surface; after fertilization and symmetrization the permeability is reduced at the animal and ventral sides, increased at the dorsal and vegetal regions. This result may be the combined effects of the symmetrical and the asymmetrical contraction. Unfortunately no experiments were made on fertilized eggs before formation of the grey crescent. It would be interesting to see if in these eggs the permeability of the vegetal region was increased, in which case it would be necessary to ascribe this phenomenon to the first symmetrical cortical contraction.

As a conclusion to this discussion on the mechanism involved in the determination of the grey crescent location it may be stated that the process called by Ancel & Vintemberger the rotation of symmetrization in all probability is a stretching or a contraction of symmetrization.

This summary presents the current ideas, in so far as they have been developed upon the basis of morphological observations. The problem has also been
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Before we begin to discuss the physiological aspects of the mechanism involved in the determination of the bilateral symmetry, we may briefly consider the problem of axial determination in biological systems. It seems necessary to assume that the organization of the unfertilized egg is radially symmetrical. This is indicated partly by the rotation and fertilization experiments mentioned above, and partly by the existence of the so-called exogastrulation, an abnormal pattern of gastrulation which has been observed to occur spontaneously, but also may be provoked experimentally (see Holtfreter, 1933). The mechanism involved in exogastrulation seems to be that invagination, instead of beginning only at one side of the egg, occurs along the entire equatorial zone simultaneously. This phenomenon tends to emphasize that the basis of the dorso-ventral polarity which is necessary for a normal gastrulation is the establishment of asymmetric conditions in the egg. This asymmetry at first only involves a quantitative difference; even in normal development invagination occurs at all sides of the egg, but the process is delayed at the ventral side. The possibility that axial determination has its origin in quantitative differences has particularly been emphasized by Child (1941, 1946). Child has also stated the view that external factors must be involved in the establishment of axial polarity. When the idea of Child is applied to the present problem the importance of the formation of the grey crescent is that it allows an axial gradient to be established under the influence of external factors. Child postulates furthermore that the gradients involved in axial determination are metabolic ones. It will be seen that the hypothesis advanced here is in agreement with Child's theory.

It will facilitate the following discussion if we begin by presenting the hypothetical interpretation, but before this is done we may briefly state the following demands to be made of this hypothesis: (1) The interpretation must, as far as possible, account for all morphological, physiological, and biochemical results obtained in studies of this phase of embryonic development. (2) It must be epigenetic rather than preformationist, i.e. the assumptions as to the organization of the egg should be as few as possible. (3) It should be formulated in such terms that experimental verification is possible.

It will now be attempted to show that in the literature strong support may be found for the following postulate: The determination of the dorsal area consists in creating such conditions that the rate of oxygen consumption can be much higher in this area than in any other part of the egg. On this basis we will discuss the possible nature of the cytoplasmic and cortical factors which are involved.

It is well known that the normal segmentation process up to the beginning of gastrulation may occur anaerobically, indicating that the energy requirements
for this process are quite low. This is corroborated by the observations that consumption of energy reserves (carbohydrate) is low (Brachet & Needham, 1935; Gregg, 1948), that no RNA or protein synthesis is demonstrable (Brachet, 1945; Løvtrup, 1955), and that the DNA synthesis which is associated with segmentation goes on at the cost of preformed reserves (Zeuthen, 1951; Hoff-Jørgensen & Zeuthen, 1952; Hoff-Jørgensen, 1954; Løvtrup, 1955; Gregg & Løvtrup, 1955).

In contrast to this, gastrulation cannot occur under anaerobic conditions (see Brachet, 1945). In the normal amphibian embryo the respiratory rate shows a slight increase during early development (segmentation), and this increase continues up to the end of gastrulation, following an exponential curve (Atlas, 1938). This respiratory increase must primarily represent the energy consumption of all those processes involved in the normal gastrulation process (cell-divisions and cell-movements, synthesis of various chemical compounds, &c.). This is also suggested by the observation that in lethal hybrids, in which development is blocked before gastrulation, hardly any respiratory increase is found (Barth, 1946; Chen, 1953; but cf. Brachet (1954) who observed respiratory increases in some hybrid species). Likewise the glycogen consumption is very low in hybrids compared with normal embryos (Gregg, 1948). We may thus conclude that among the processes involved in gastrulation some are dependent upon the simultaneous occurrence of oxidative reactions. The main task of these reactions is presumably to supply energy. We must infer from this that the dorsal region, in which invagination occurs, and in which high metabolic activity can be demonstrated (cf. below), has a higher rate of oxygen consumption than other parts of the embryo.

For the supply of energy by oxidation it is necessary to have an energy source, mitochondria, and oxygen. We may consider first the question of the oxygen supply. It will be realized that this may be influenced by the cortex, in so far as the passage of oxygen into the egg may be controlled by a membrane of low permeability. The protein coat which surrounds the mature amphibian egg has extremely low permeability to water (Krogh, Schmidt-Nielsen, & Zeuthen, 1938; Holtfreter, 1943; Prescott & Zeuthen, 1953). There is no reason to believe that a membrane having a low permeability to water is not also relatively impermeable to oxygen (cf. Krogh, 1937), and it therefore seems rather certain that the supply of oxygen is limited by the coat. The stretching of the protein coat which in the previous section was shown to be associated with the formation of the grey crescent leads to increased permeability to dyes (Dalcq & Dollander, 1948; Dollander & Melnotte, 1952). We may expect that the permeability is increased to oxygen also, which is the condition necessary for establishing the postulated axial metabolic gradient. In this way those energy-requiring processes may be initiated which eventually lead to gastrulation. We do not know much about the chemical nature of these processes, except that they include synthesis of DNA, RNA, and proteins (see e.g. Hoff-Jørgensen & Zeuthen, 1952;
Brachet, 1945; Løvtrup, 1955). The energy source and the mitochondria must be localized in the cytoplasm. As far as the former is concerned, we know that the principal energy source during early development is glycogen (see e.g. Brachet & Needham, 1935; Gregg, 1948; Løvtrup, 1953a; Løvtrup & Werdinius, 1957). This substance is mainly confined to the animal hemisphere in the early amphibian gastrula (Gregg & Løvtrup, 1950). During gastrulation considerable amounts of glycogen disappear in the dorsal region (Heatley & Lindahl, 1937; Jaeger, 1945). It may be argued that glycogen may disappear without being oxidized; it might equally well be used for synthetic purposes. This argument does not hold, however, for except at high temperatures the oxygen consumption corresponds closely to the carbohydrate utilization (see Løvtrup, 1953a, b; Løvtrup & Werdinius, 1957). Mitochondria are present in the amphibian egg (Boell & Weber, 1955), but nothing is known about their distribution.

Under normal conditions the grey crescent forms at the yolk margin, and invagination also occurs in this region. The present hypothesis assumes that the distribution of glycogen and mitochondria is radially symmetrical, but otherwise it only demands that they should be present at the yolk margin. Whether they are localized in a ring (marginal plasm, see Lehmann, 1945), in a layer on top of the yolk or are evenly distributed in the animal hemisphere is of no consequence for the hypothesis. It was mentioned in the preceding section that the cytoplasmic factors may determine the direction of invagination. It is very difficult to imagine that factors freely dispersed in the cytoplasm can exert such influence. If, therefore, glycogen and mitochondria are not fixed to certain structures, the question may be raised whether it is simply the direction of the yolk margin which determines the direction of invagination. Even if passively, the yolk would thus play an important role. It is not possible at the moment to decide this question, but it should be emphasized that there may be two types of cytoplasmic factors, viz. glycogen and mitochondria, which are involved in the establishment of the metabolic gradient, and yolk, which may determine the direction of invagination.

It is important to test the consequences of the hypothesis outlined above. The main point is that in the intact egg the oxygen supply limits the rate of oxygen uptake. This is supported by the fact that the oxygen consumption in homogenates is considerably higher than in whole eggs (Brachet, 1934; Spiegelman & Steinbach, 1945). Likewise, Needham (1942) has calculated from results on the oxygen uptake of explants that they respire at a rate which is about three times higher than in the intact egg.

It is possible to interpret these results differently. Thus the homogenate experiments were thought to represent a change in the substrate-enzyme orientation, and the explant results were considered a result of the inertness of yolk. The results obtained by comparing the oxygen uptake in dorsal and ventral explants may speak more convincingly in favour of the present hypothesis. As mentioned above, it has been possible from the disappearance of glycogen to infer that the
metabolic rate is much higher in the dorsal than in the ventral side, but it has never been possible to show very striking differences between the respiration of ventral and dorsal explants (see, for example, the detailed discussions by Needham, 1942, and Brachet, 1945, and the recent papers by Ornstein & Gregg, 1952, and Sze, 1953). This lack of agreement between expected and observed results may find a natural explanation if, as suggested here, the difference in metabolic rate is caused not by differences in the content of cytoplasmic factors involved in respiration, but by the rate of oxygen supply. In the explants, oxygen may diffuse through the exposed surfaces of the cells inside the coat, and the differences in oxygen uptake between the explants may therefore be quite low. The possibility exists that a coat may be formed at the exposed surfaces (see Holtfreter, 1943). If this happens, the oxygen consumption should be reduced for both explants, but it is not possible to foretell the consequences with respect to the difference in respiration between the explants.

With oxygen diffusion as a limiting factor, the results obtained on consumption of energy sources at different temperatures (Løvtrup, 1953b) may find a natural explanation. It was observed that during early development there was a discrepancy between loss of energy reserves (carbohydrate) and oxygen consumption, which led to the conclusion that considerable quantities of carbohydrate were lost without being oxidized. As the increase of diffusion rate with temperature is much lower than that of chemical reactions, the inefficient carbohydrate utilization may reflect that the oxidation of lactic acid formed by glycolysis is lagging behind due to lack of oxygen. In this connexion it should be mentioned that measurable amounts of lactic acid are formed in the embryos under aerobic conditions, but a considerable increase is observed during anaerobiosis (Lennerstrand, 1933; Cohen, 1955). The very low temperature coefficient observed for respiration during the early developmental stages also corroborates the explanation given above (Løvtrup, 1953b).

The strongest support in favour of the suggestion that the supply of oxygen is a limiting factor may perhaps be found in the results of Cohen (1955) showing that the amphibian embryo, up to the end of gastrulation, is partially anaerobic. This was indicated by a burst of oxygen uptake which follows transfer of embryos from nitrogen or air to an oxygen atmosphere. Cohen has discussed the possibility that the oxygen supply into the interior of the egg may be limited by the rate of diffusion. Some calculations which he carried out to test this point did not give an unambiguous answer. As Cohen did not take into consideration the possible existence of a membrane acting as a diffusion barrier, the results of his calculations do not invalidate the present hypothesis. They are important, however, because they indicate that the rate of diffusion of oxygen in the cytoplasm is slow enough to permit a gradient of oxygen supply when the permeation is limited to a certain region of the egg surface.

It was observed by Brachet (1934) that the oxygen consumption is almost independent of the oxygen tension. This finding is neither compatible with
Cohen's results nor with the ideas advanced in this paper. New experiments are obviously needed to elucidate this particular point.

If stretching of the surface coat gives rise to an increase in permeability to oxygen, one might expect that the stretchings of the coat occurring during the preparatory phases of mitosis might cause a temporary increase in the oxygen uptake. Such rhythmic oxygen uptake, synchronized with the early mitotic divisions have been demonstrated (Brachet, 1945; Zeuthen, 1946). Of the two possible interpretations of the results mentioned by the authors, variations in oxidation intensity or increase of permeability to oxygen, the present hypothesis thus decides in favour of the latter.

Any conditions which influence the properties of the coat may lead to changes in the pattern of gastrulation. In Ca-free or hypertonic solutions the permeability of the membrane is increased, and in such media a high frequency of exogastrulation is observed (Holtfreter, 1933). The mechanism involved seems to be that, because of the increased permeability, oxygen may be supplied at an increased rate all around the egg, leading to simultaneous invagination along the entire marginal zone, thus giving rise to an exogastrula. The appearance of accessory organizers and other developmental anomalies in overripe eggs (Witschi, 1934; Briggs, 1941), which according to Holtfreter (1948) is a result of deterioration of the surface coat, is also easy to understand in the light of the present hypothesis.

It seems important to see whether the morphogenetic effects of lithium and thiocyanate may be incorporated in the present hypothesis. These ions may exert what may appear quantitative effects, in that treatment with lithium may lead to microcephaly, and treatment with thiocyanate to macrocephaly (see the review by Ranzi, 1953). Lithium is known to increase, and thiocyanate to decrease the viscosity of protein solutions, and it would be tempting to explain the above-mentioned morphogenetic effect by assuming that the permeability of the coat is decreased and increased, respectively, by these ions. The action of thiocyanate on the coat does not seem to have been investigated, but certain observations may be found concerning the action of lithium. Thiosulfate counteracts the formation of the grey crescent (Dollander & Laurent, 1952), apparently in support of the above explanation. Sodium citrate has the same effect, although it has none of the morphogenetic effects of lithium. This need not invalidate the suggested mechanism, because lithium and citrate have quite different actions on the mechanical properties of the coat (Dollander & Labadie, 1952). By exposure to high concentrations of lithium the coat is easily destroyed (Dollander & Laurent, 1951). It may well be that the quantitative morphogenetic effects of lithium and thiocyanate are exerted through their action on the mechanical properties of the coat, although more experiments are needed to prove this. It seems certain, however, that lithium at least has more specific, maybe qualitative effects. These may be exerted by suppression of the synthesis of proteins as suggested by Gustafson (1950).
CONCLUSION AND SUMMARY

A hypothetical interpretation has been advanced to explain the physiological mechanism operating in the determination of the bilateral symmetry in amphibian embryos. The basic assumption of the hypothesis is that the establishment of the dorso-ventral polarity consists in creating such conditions that oxidative processes may go on at a higher rate in one side of the egg. Of the three factors necessary, glycogen, mitochondria, and oxygen, the two former are found in the cytoplasm of the animal hemisphere. Very little is known about the localization of these components, but presumably they are distributed with radial symmetry. The third factor, oxygen, must enter through the cortex. It is known that the protein coat of the amphibian egg has a very low permeability, and it is suggested that the rate of oxygen supply is limited by the diffusion resistance of this coat. The grey crescent is assumed to represent a region of higher permeability, formed by stretching of the coat. A considerable number of observations may find a simple explanation in the light of this interpretation. It is not possible to enter upon a discussion of the applicability of the hypothesis to other species. It should only be mentioned that the importance of mitochondria in morphogenetic processes, and the question of the surface as a diffusion barrier for oxygen have been investigated in relation to the early development in sea-urchin embryos (see Gustafson & Lenicque, 1952; Lindahl & Öhman, 1938; Lindahl, 1940). Elements of the hypothesis have thus for some time been commonplace in sea-urchin studies. To apply the hypothesis directly to echinoderms meets with some difficulties, but it remains to be tried whether it is possible to overcome them.

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REFERENCES


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