Metabolic gradients and morphological polarization in embryonic development of hydroid polypes

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One of the main problems of modern embryology is the problem of relations between morphogenetic and metabolic processes. For several decades this problem was studied under the influence of the theory of physiological gradients and of some of its modifications (Child, 1941; Daleq & Pasteels, 1938). The general principle of these conceptions was that the processes of cell differentiation were strictly determined by previous regional metabolic conditions (metabolic, or physiological gradients). The gradients were supposed in their turn to be determined by the heterogeneity of the embryo's environment. Therefore, a simple and non-reversible chain of relationships was postulated: heterogeneity of environment \( \rightarrow \) graded metabolic differences \( \rightarrow \) regional differences at the cellular or supracellular levels. No possibility of any kind of inverse relations—that is, influence of cellular and supracellular events upon the metabolic processes—was taken into account in these conceptions.

More recently however a number of facts were obtained which demonstrate the possibility of reverse relations. For example, in echinoderm embryos some cases of the 'retardation' of metabolic differences as compared with the morphological ones were reported (Child, 1953). A large group of data, concerning the influence of cell density upon the metabolic activity of cell populations (Lopashov, 1961; Guidice, Mutolo & Moscona, 1967; Pfohl & Guidice, 1967), may also be interpreted in the same sense.

As to hydrozoan embryos, which are the objects of the present study, the corresponding data are scanty and to some extent controversial. On one hand, for some hydromedusan eggs (Child, 1925) the direction of the animal–vegetal (AV) axis is reported to be strictly determined by the redox gradient, which in its turn reflects the aeration gradient, established between the attached and non-attached poles of the oocyte. The first one becomes the lower pole of the redox gradient and corresponds to the vegetal pole. Later on the AV axis coincides

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with the direction of planula elongation, the animal pole becoming the anterior end and the vegetal pole the posterior end of the larva.

In the hydroid *Amphisbetia*, however, a complete inversion of larval polarity with respect to the egg polarity is reported (Teissier, 1931). Namely, a basal (attached) part of the egg, being at the lower level of oxidative gradient, becomes the anterior, and not the posterior pole of the planula. After such a transformation, the inversion of the gradient takes place: the anterior planula pole becomes the higher point of the gradient. Thus, in this case the usual time relations between the metabolic and the morphogenetic processes are partially inverted. However, the general direction of elongation still coincides here with the AV axis.

Taking these facts into account, the necessity of further studies in this field is evident. The aim of the present investigation is to establish the relations between certain metabolic processes and the first steps of morphogenesis in two species of marine Hydrozoa.

**MATERIALS AND METHODS**

Two species of marine Hydrozoa—*Clava multicornis* (Athecata) and *Obelia loveni* (Thecaphora)—were studied. The general outlines of their early development are as follows.

The oocytes of *C. multicornis* are firmly attached to the blastostyle by one side and in many cases adjoin the neighbouring embryos at the other sides (there often occur up to four embryos in one sporosac). Therefore, a natural aeration gradient is established: the ‘closed’ sides, contacting with other rudiments, are poorly supplied with oxygen when compared with the ‘opened’ ones. In *O. loveni* only oocytes and mature eggs are under similar conditions. The advanced *Obelia* embryos can move freely in the sporosac cavity, all their parts being thus under approximately similar conditions of oxygen consumption.

The developmental course of both species is relatively simple. A chaotic cleavage results in the formation of a solid morula, which splits later into ecto- and entoderm. The embryo is elongated and becomes ciliated, thus transforming into a planula. Its anterior end becomes slightly wider than the posterior one. All these processes constitute what we designate ‘morphological polarization’.

To indicate metabolic gradients the following methods were employed.

1. Vital staining with Nile blue sulphate, Neutral red and Janus green. These dyes have been used for indicating redox gradients by a number of authors (e.g. Child, 1953; Hörstadius, 1952). According to these authors, the dyes may be used in initially oxidized (coloured) as well as in the initially reduced (colourless, leucobasic) form. In this study the oxidized form was always used. The dyes were dissolved in sea water in concentrations 1–2 mg/100 ml. The embryos were incubated in this solution for 10–20 min. The dyes are maintained in embryonic tissues for several days at least.
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2. Autoradiography was used to study the following metabolic aspects:

(A) The distribution of the 'free-radical processes': that is, the metabolic processes, which are accompanied by the emanation of the free radicals. For this purpose the so-called 'inoculated copolymerisation' method, proposed by Tarusow & Koslow (1961), was used. This original method is based on the ability of the soluble monomere acryl-amide to form a non-soluble polymere in the presence of free radicals. When a tissue is incubated in the solution of labelled monomere acryl-amide $^{14}$C, one may judge by the number of grains the relative concentration of free radicals in a given area. The method demonstrates mainly short-lived radicals. Its reliability is approved by a number of authors (e.g. Pyrusian & Aristarchov, 1969). So far as free radicals are by-products of quite different metabolic reactions, the method seems to be adequate for detecting metabolic gradients of the most unspecific character.

(B) The distribution of RNA synthesis. $[^3]$H]Uridine was used as a labelled precursor (specific activity 6 mCi per mm).

(C) The distribution of the protein synthesis: in this case $[^3]$H]lysine was used (specific activity 125 mCi per mm).

(D) The protein transfer from blastostyle to oocyte was also studied with the use of labelled lysine. In these experiments the embryos were fixed at different times after incubation in precursor solution. The changes in precursor concentration in blastostyle and in the oocyte were measured at different times after incubation.

In all autoradiographic experiments the embryos or larvae were incubated in sea-water solutions of labelled precursors (5 μCi/ml) for 40–50 min. Then they were fixed in Bouin solution and embedded in paraffin wax or paraffin-celloidin. The 6 μ sections were treated with 5 % cold perchloric acid and were afterwards coated with photoemulsion 'R' (produced by Photochemical Institute, Moscow). After a 3-week exposure the slides were developed and stained with Ehrlich's haematoxylin.

A similar technique was used for histological purposes.

RESULTS

1. Redox gradients and their relations to morphological polarization

(A) Clava multicorhins. In oocytes and embryos (up to the planula stage) there is always a definite correlation between the distribution of stained regions and their orientation towards the external environment. Namely, just after staining only the 'opened' parts of the embryos become coloured, while the 'closed' ones (adjacent either to blastostyle or to other embryos) remain uncoloured. The result was the same for all the dyes employed.

Therefore in the oocytes and early embryos the 'classical' vital staining gradients may be detected, these gradients being closely correlated with the asymmetry of the external environment. These gradients can be designated
Fig. 1. Different kinds of orientation of the redox gradients and morphological axes in the embryos of *Clava multicornis* (A, B) and *Obelia loveni* (C). The stippled area indicates the higher end of the gradient; the broad end corresponds to the presumptive anterior pole. *a-b* = longitudinal blastostyle axis.

A = solitary *Clava* embryo; B = two *Clava* embryos in one sporosac. $B_1$ = elongation perpendicular to the blastostyle axis. $B'_1$ = the homologous poles of both the larvae are oriented to the same side. $B''_1$ = to the opposite sides. $B_2$ = elongation parallel to blastostyle axis. $B''_1, B''_2$ = homologous poles in both neighbouring embryos are oriented similarly, but in $B'_2$ the anterior pole while in $B''_2$ the posterior one coincides with the opened side of the egg. $B''_3$ = the orientation of the homologous poles of both embryos is reversed. $B_3$ = completed planula having an identical structure in spite of the mode of the previous embryo orientation. $C_1$ = oocytes, $C_2$ = homogeneously stained embryo lying freely in the sporosac cavity, $C_3$ = bi- or unipolar gradients in advanced embryos. $C_4$ = planula with a unipolar antero-posterior gradient.
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'exogenic' or 'primary' gradients. It was found, however, that the direction of the morphological polarization of an embryo can be oriented in quite different ways in respect to the direction of its primary gradient. The following cases demonstrate this point (Fig. 1, A, B):

1. In solitary C. multicornis embryos the direction of elongation is as a rule perpendicular to the longitudinal axis of the blastostyle and to the direction of the primary gradient (Fig. 1, A). In rare cases both directions coincide, but then the anterior larval pole can arise from the closed as well as from the opened egg pole (that is, from either end of the primary gradient).

2. If there are two or more embryos in the sporosac (Fig. 1, B), they can elongate either in parallel (Fig. 1, B2) or in perpendicular (Fig. 1, B3) direction with respect to the blastostyle axis. At the same time the direction of elongation is usually oblique to the direction of the primary gradient, for this latter is oriented in distolateral-basimedial direction (in relation to the sporosac symmetry). The most important point here is that the positions of homologous poles of neighbouring embryos can be opposite with respect to each other (Fig. 1, B1): in some cases anterior (Fig. 1, B2), in other cases posterior (Fig. 1, B211) larval poles can coincide with, for example, the higher pole of the primary gradient.

It can be deduced from these data that the exogeneous primary redox gradient does not determine the direction of embryonic polarization. These two events seem to be determined by independent factors.

In completely formed larvae new gradients of colour distribution are established, these gradients being in perfect correspondence with the direction of morphological polarization. The highest points of these gradients coincide as a rule with the anterior planulae poles (Fig. 1, B3).

The final pattern of the colour distribution is established after the morphological polarization has been completed. The corresponding redox gradients may be designated 'secondary' ones.

(B) Obelia loventi (Fig. 1, C). The oocytes and mature eggs reveal a distinct and obviously exogenous 'primary' gradient, diminishing from the external (opened) to the internal (closed) part (Fig. 1, C1). Later on, when the embryos are detached from the blastostyle and lie freely in the sporosac cavity, these gradients become vague and often completely disappear. Non-elongated embryos stain homogeneously (Fig. 1, C2). After the elongation becomes evident, uni- or, more rarely, bipolar gradients arise, coinciding with the direction of elongation (Fig. 1, C3). A completed planula always reveals a distinct antero-posterior gradient which may be designated a 'secondary' one (Fig. 1, C4). The disappearance of the primary gradient in Obelia is obviously connected with the detachment of an embryo from the blastostyle and, consequently, with the equalization of oxygen supply for all its parts. A similar phenomenon of the disappearance of a gradient can be observed in early C. multicornis embryos artificially removed from sporosacs. Approximately 10 h after removal the
embryos tend to be stained homogeneously. (A relatively long duration of the period of disappearance of any regional differences in staining demonstrates that some considerable reconstructions of oxidative mechanisms are taking place here, the aeration changes being only the triggers for these reconstructions.)

'Secondary' gradients are revealed in Obelia embryos relatively earlier than in Clava, but in Obelia also they show no particular relation to the primary ones. The phenomenon of the redistribution of the staining pattern, which is connected with the replacement of the primary gradient by the secondary one, is quite visible in Obelia. Thus, if the presumptive anterior planula pole does not correspond with the higher pole of the primary gradient and is therefore primarily uncoloured, later on the stain, being previously absorbed there in leucobasic state, undergoes oxidation and becomes coloured; and vice versa,

2. The reflection of the primary gradients in the structure of cell nuclei

In the embryos of both species studied a distinct correlation between the direction of the primary gradient and the structure of cell nuclei can be observed (Fig. 2).

Starting from the early cleavage the nuclei of the 'closed' and 'opened' sides of the embryo are distinctly different. The latter possess clear nucleoplasm, distinct nucleolus and granulated chromatin ('clear nuclei'—Fig. 2, a), while the former are irregularly compressed, homogeneously stained, strongly basophilic

Fig. 2. Different kinds of cell nuclei in C. multicornis embryo of early parenchymula stage. a = 'clear nuclei', situated at the 'opened' side. b = 'dark nuclei', situated at the 'closed' side (adjacent to blastostyle (bl) or to another embryo = em). In the ectoderm both kinds of nuclei are represented while in the endoderm there are only dark nuclei.
and possess no visible nucleoli (‘dark nuclei’ Fig. 2, b). In the ectoderm nuclei of both kinds can be found, while in the endoderm mainly dark nuclei are present.

As development proceeds, the number of dark nuclei decreases: they are replaced by clear ones. The replacement seems to be due not to the atrophy of dark nuclei, but to their direct transformation into clear ones. It was possible to follow a number of intermediate steps of the transformation, whereas no signs of nuclear atrophy were ever observed. By the stage of free-swimming planula all the dark nuclei become transformed into clear ones.

Therefore, the temporary existence of the dark nuclei can be interpreted as a sign of fully reversible physiological depression in the tissues of the ‘closed’ side due to an insufficient oxygen supply.

Fig. 3. The diagram of changes in the concentrations of free-radicals at the opposite poles of C. multicornis embryos. Solid line = data for the ‘opened’ sides (up to parenchymula stage) and for the anterior poles (later stages). Broken line = data for the opposite sides and poles.

3. The distribution of the free-radical processes in oocytes and embryos of C. multicornis

At the earlier stages (from oocyte up to parenchymula) the difference between the free-radical concentrations of the ‘opened’ and ‘closed’ sides was measured (Fig. 3, lines Ia, Ib; Fig. 4, A); at the later stages (from parenchymula up to
free-swimming planula) the corresponding differences between the anterior and posterior ends were measured (Fig. 3, lines IIa, IIb; Fig. 4, B).

As to the primary (exogeneous) gradients, there is a greater concentration of free-radical at the 'closed' (adjacent to blastostyle) side than at the 'opened'

Fig. 4. Incorporation of [14C]acryl-amide into the morula (A) and planula (B) of Clava multicornis. O. = ‘opened’, Cl. = ‘closed’ side; bl = blastostyle; Ant. = anterior; Post. = posterior pole (×100).
side in the oocytes. Still higher free-radical concentration is observed in the cells of the blastostyle itself. Such a mode of distribution of the free-radical processes may be related to the synthetic activity of the blastostyle and the adjacent oocyte regions (see below for the data concerning lysine incorporation).

During early cleavage the free-radical concentration is diminished to some extent in all parts of the egg, remaining however higher at the 'closed' side. It results in the almost complete disappearance of the initial gradient.

Starting from the morula up to the 2-layered embryo, along with the general increase of the free-radical concentration, there is a more rapid rise at the 'opened' side as compared with the 'closed' one. As a result of this, a typical exogeneous gradient of the free-radical processes is established, which completely coincides with the redox gradient described above.

By the parenchymula stage, however, this gradient practically disappears (the difference between the opposite parts becoming insignificant); this disappearance is the result of both decrease of free-radical processes at the 'opened' side and increase at the 'closed' one. Just about this time the embryos can move freely in the sporosac, so that the regional differences in oxygen availability become insignificant.

While comparing the lines Ia and Ib (Fig. 3) a purely 'aerational' and temporal character of the primary gradients becomes obvious: these gradients are caused by some transitional regional differences in the rate of metabolic increase (which is slower at the 'closed' side), rather than by any stable regional metabolic distinctions.

Approximately at the same time as the reduction of the primary gradients, very slight morphological differences between the presumptive anterior and posterior larval poles appear, thus permitting measurement of 'secondary gradients' and study of their intermingling with the remaining primary gradients.

Three main cases may be distinguished:

1. The direction of planula elongation is perpendicular to the blastostyle axis (Fig. 1, A). In this case the primary and secondary gradients are clearly situated in perpendicular directions and are not therefore superimposed. In such embryos the regular differences in the intensity of the free-radical processes along the presumptive antero-posterior axis can already be seen at the parenchymula stage (Fig. 3, line II, points a₁, b₁). The expanded end of the body coincides with the lower pole of the gradient (point a₁). Assuming that the proportions of the body are not altered during the subsequent embryo development (which seems to be highly probable), the presumptive posterior pole appears to demonstrate a little higher intensity of the free-radical processes than the anterior one. Later on these relations are sharply reversed (points a₂ and b₂). This transformation is due solely to the decrease of metabolism at the posterior pole. A typical 'secondary' gradient of the free-radical processes is thus established, remaining without any principal changes up to the free-swimming planula stage (points A and B).

2. The direction of the elongation is parallel to the blastostyle axis. Now the
presumptive anterior pole corresponds to the ‘opened’ egg side, and the posterior one to the ‘closed’ egg side (Fig. 3, lines II, points a3, b3). In this case the primary gradient simply turns into the secondary one without any reorientation. Here the secondary gradient differs from the primary one only in its stability (the differences between the points a3 and A, b3 and B are insignificant).

3. The direction of the elongation is the same as that of the previous case, but the presumptive anterior pole corresponds to the ‘closed’ embryo side. In these cases the primary gradients prevail up to the hatching of the embryos from sporosacs (Fig. 3, lines II, points a4, b4), and are only later substituted by the secondary (inverted) gradients (see the increase from point a4 to point A and the decrease from point b4 to point B).

Special attention was paid to the question of whether the primary gradient is reinforced by the newly arising secondary one in case (2) and whether it is weakened in case (3). If this were the case, the gradient a3 b3 would be significantly steeper than the gradient a4 b4. However, no significant differences between these two gradients were observed. Therefore, it is to be concluded that in cases (2) and (3) the secondary gradients are completely inhibited by the primary ones and can be revealed only in case (I).

The above data give some support for the idea that at least in the most pure case (I) the establishment of the secondary gradient is due to the inhibitory activity of the anterior pole, this being the cause of the drop of the free radical concentration at the posterior pole. It is difficult to say, however, whether this fact can be regarded as a general rule and, even if it is so, whether one can compare it with the inhibitory interactions of the parts observed in adult hydroids (Tardent, 1955; Rose, 1957; Burnett, 1966).

4. The distribution of [3H]lysine-incorporation in the oocytes and the embryos of C. multicornis

As one can see from Figs. 5 and 6, [3H]lysine is intensely incorporated during the whole course of embryonic development as well as the whole of oogenesis except the phase of the dissolution of the germinal vesicle. The rate of incorporation into the ‘closed’ oocyte side is significantly higher than that into the ‘opened’ one. The rate of incorporation into the blastostyle cells is six times greater than that into the ‘closed’ oocyte side. When fixing the oocytes at different times after incubation in the precursor solution, a gradual increase of label concentration at the ‘closed’ oocyte side and a proportional decrease in the blastostyle cells is observed (Fig. 5): at 3 h after incubation the label concentration in the oocyte becomes higher than that in the blastostyle, while at 24 h the relations of the label concentrations in the oocyte and blastostyle become reversed with respect to the initial ones. Both the total label concentration in the oocyte and the blastostyle and the label concentration in the ‘opened’ oocyte side remain approximately constant during the whole period. These data
Fig. 5. Changes in the label concentration in the oocyte and blastostyle of C. multicornis at different times after incubation in [3H]lysine solution. 1 = 'opened' oocyte side, 2 = 'closed' side; bl = blastostyle; T = total concentration.

Fig. 6. Diagram of changes in the intensity of [3H]lysine incorporation. Details as in Fig. 3.
demonstrate that proteins synthesized in the blastostyle cells are transferred to
the closed part of the oocyte.

During early embryonic development no significant regional differences
concerning the lysine incorporation into the cytoplasm are observed (lines I,
Fig. 6). Therefore, no essential cytoplasmic primary gradients of lysine incor-
poration are revealed. However, some slight differences are registered in cell
nuclei: the percentage of the completely inactive nuclei at the ‘closed’ side is
markedly greater than that at the ‘opened’ one (Table 1). At the same time, the
average number of grains per active nucleus is constant everywhere.

Table 1. Differences in the incorporation of $[^{3}H]$lysine and $[^{3}H]$uridine
in cell nuclei of the ‘opened’ and ‘closed’ side of Clava multicornis embryos

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<tr>
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<th>$[^{3}H]$Lysine</th>
<th>$[^{3}H]$Uridine</th>
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<tbody>
<tr>
<td></td>
<td>Average grain</td>
<td>Average grain</td>
</tr>
<tr>
<td></td>
<td>number per nucleus</td>
<td>number per nucleus</td>
</tr>
<tr>
<td>Per cent of nuclei</td>
<td>labelled</td>
<td>labelled</td>
</tr>
<tr>
<td>Opened side</td>
<td>6.8</td>
<td>70</td>
</tr>
<tr>
<td>Closed side</td>
<td>6.2</td>
<td>48</td>
</tr>
<tr>
<td>Entoderm</td>
<td>6.7</td>
<td>56</td>
</tr>
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Considering the lack of primary gradients of lysine incorporation (except dur-
ing oogenesis) the early establishment of secondary gradients is of special
interest (Fig. 6, II). By the parenchymula stage the incorporation at the pre-
sumptive anterior end (Fig. 6, II, point $a_1$) is 25% greater than that at the
posterior one (point $b_1$). The significantly larger amount of synthetic activity
at the anterior end compared with the posterior one occurs during all subsequent
development; it is observed even in those embryos in which the orientation of
the morphological axis is completely reversed in relation to the direction of vital
staining and free-radical gradients (Fig. 6, II, points $a_4$ and $b_4$). In 13 embryos
out of 20 studied the incorporation was significantly greater at the anterior end
(corresponding in these cases to the lower end of the primary gradient), in five
embryos it was approximately the same at both opposite poles and only in
two cases was there a slightly greater rate of incorporation at the posterior pole
(corresponding here to the higher end of the primary gradient). In free-
swimming larvae the incorporation rate was always significantly higher at the
 anterior end.

5. The distribution of $[^{3}H]$uridine-incorporation in oocytes and embryos
 of C. multicornis (Fig. 7, A, B)

During early oogenesis when the oocyte diameter does not exceed 280 $\mu$, the
labelled uridine is actively incorporated into its nucleolus. Later on the rate of
incorporation falls. Oocyte cytoplasm is labelled during the whole of oogenesis. A significantly (19%) greater amount is incorporated at the ‘closed’ side compared with the ‘opened’ one (Fig. 8). The incorporation into the blastostyle cells is more intense than that into the ‘closed’ oocyte side.

In the embryos and free-swimming larvae neither primary nor secondary regular regional differences in uridine incorporation are observed (Fig. 8). In 17 out of 30 free-swimming larvae the rate of incorporation is higher at the anterior pole while in 13 larvae at the posterior pole. Generally, no definite tendency is observed. No significant regional differences in nuclear RNA metabolism are observed either (Table 1): the differences in percentage of active nuclei between the opposite sides are not significant. Only in the entoderm is the percentage of actively incorporating nuclei higher than that in the ectoderm.

Fig. 7. Incorporation of [3H]uridine into the oocyte (A) and planula (B) of *Clava multicornis*. *O.* = ‘opened’, *Cl.* = ‘closed’ side; *bl* = blastostyle; *Ant.* = anterior, *Post.* = posterior pole (× 100).
While summarizing all the above data three correlated findings should be especially noticed:

1. It was found that in the hydrozoan embryos studied here the regional and temporal changes in the rates of the different metabolic processes are not correlated. For example, the local increase of the free-radical concentration is not necessarily connected with the increase of RNA or protein synthesis. On the contrary, the rate of protein synthesis can increase even in the regions with minimal free-radical concentration. The lack of correlation between the rate of protein and RNA synthesis (so far as it can be found by autoradiography) can also be established: there are no significant gradients of RNA synthesis at any stage while the secondary gradients of lysine incorporation are clearly seen. Finally, some signs of the lack of correlation between the rates of metabolic processes in nuclei and cytoplasm have been revealed at the earlier stages (compare the absence of regional differences in lysine incorporation into the cytoplasm, and the presence of some differences in nuclear activity).

These data may be compared with the corresponding ones obtained for other species (see Markman, 1957; Brachet, 1960; Child, 1953, for gradients in the sea-urchin embryo; Brachet, 1960, 1966, for gradients in the frog embryo): one can see that in Hydroza the degree of independence in regional rates of
the different metabolic processes is much more expressed than in the embryos of higher animals. Therefore hydrozoan embryos may provide a suitable new model for studying metabolic regulation in early development.

2. The lack of any definite relation between the direction of primary gradients and that of morphological polarization was demonstrated. In fact, the primary gradients (evidently exogeneous) have no definite morphogenetic significance in the species studied. As is particularly clear for Clava, the larval axes can be oriented in quite different ways with respect to those external factors which may be the cause of its polarization.

Thus, in Hydrozoa the morphological polarization is realized in quite a different manner from that in the majority of other species, where the special polarizing factors, the ovarian structures or any other environmental factors can easily be identified (Raven, 1961). In Hydrozoa the direction of the morphological axis initially seems to be an 'occasional' one. Later on it is stabilized. In relation to this it is of interest to remember that the 'stabilization of occasionality' (Quastler, 1964) is considered as an initial evolutionary pathway for establishing organization.

The intrinsic mechanisms of morphological polarization in Hydrozoa are worth special study. These investigations are now in progress. The aim of the present study is only to underline the lack of strict dependence of the internal polarization on the heterogeneity of the external environment.

3. The pattern of metabolic activity has been found to be redistributed during embryo development in accordance with the morphological polarization. This conclusion confirms some recent data (see references in the Introduction) which stress the dependence of metabolism on some morphogenetic processes. In fact, the appearance of the secondary metabolic gradients never precedes morphological polarization and, moreover, in some cases (e.g. free-radical processes, especially in the case B3HII, Fig. 1) the metabolic redistribution occurs significantly later than the visible morphological polarization. In general it is difficult to avoid the conclusion that the morphological reconstructions are the cause of metabolic redistribution, but not vice versa (it is especially clear for the lysine incorporation gradients because of the absence of primary regional metabolic differences). If this is true, the hydrozoan embryos may be a suitable object for studying the simplest forms of 'organismical' (cellular and supracellular) control of metabolism in early development.

SUMMARY

1. The regional differences in the rate of metabolic processes and their relations to morphological polarization were studied in the oocytes, embryos and larvae of Clava multicornis and Obelia loveni (Coelenterata, Hydrozoa). Vital staining with redox dyes and autoradiography of free-radical processes, and of the incorporation of [3H]lysine and of [3H]uridine were employed.
2. During oogenesis the redox gradients decrease from the ‘opened’ to the ‘closed’ (adjacent to blastostyle or to other embryos) side of the egg whereas the gradients of other metabolic processes are oriented in the opposite direction.

3. At the early stages of development redox and free-radical gradients decrease from the ‘opened’ to the ‘closed’ side. Since the orientation of these ‘primary gradients’ has no definite relation to the morphological axis, they do not seem to play any morphogenetic role. No regional differences in the lysine incorporation in the cytoplasm, or the uridine incorporation into nuclei and cytoplasm were observed at these stages.

4. Following the morphological polarization a redistribution of metabolic processes takes place. As a result, the newly established ‘secondary’ redox, free-radical and lysine-incorporation gradients become evident, and they definitely correspond with the direction of the morphological polarization. No regular regional differences in uridine incorporation are noticeable at any stage except oogenesis.

5. The problem of the relation between the metabolic and morphogenetic processes is discussed; a conclusion is drawn that in the studied hydrozoan species the metabolic processes seem to be the consequences of the morphogenetic processes but not vice versa.

МЕТАБОЛИЧЕСКИЕ ГРАДИЕНТЫ И МОРФОЛОГИЧЕСКАЯ ПОЯВЛИВАНИЯ В ЭМБРИОНАЛЬНОМ РАЗВИТИИ ГИДРОИДНЫХ ПОЛИРОВ

Л. В. БЕЛОУСОВ И Т. В. ОСТРОУМОВА

1. Изучались региональные различия интенсивности метаболических процессов и их связь с морфологической поляризацией на эмбрионах и личинках Clava multicornis и Obelia loveni (Coelenterata, Hydrozoa). Использовались методы приживенного окрашивания окислительно-восстановительными индикаторами и авторадиографии свободно-радикальных процессов, включения H3-лизина и H3-уридина.

2. В период овогенеза градиент приживенного окрашивания падает по направлению от открытой к закрытой (прилежащей к бластостилю) стороне яйца, в то время как все прочие метаболические градиенты направлены противоположно.

3. На ранних стадиях развития наблюдаются окислительно-восстановительные и свободно-радикальные градиенты, падающие по направлению от открытой к закрытой стороне. Эти ‘первичные градиенты’ не имеют морфогенетического значения. На этих стадиях не наблюдается региональных различий во включении лизина в цитоплазму и включения уридина в ядро и цитоплазму.

4. После морфологической поляризации наступает перераспределение метаболических процессов. В результате возникают ‘вторичные’ окислительно-восстановительные, свободно-радикальные градиенты и градиенты включения лизина, полностью совпадающие с направлением морфологической поляризации. Ни на одной стадии не наблюдается четких градиентов включения уридина.
5. Обсуждается проблема связей между метаболическими и морфогенетическими процессами. Делается вывод, что в исследованных случаях влияние морфогенетических процессов на метаболические сильнее обратного.

REFERENCES


(Manuscript received 30 December 1968)