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Autor: Løvtrup, Søren

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conclude that there is a correlation between the rate of oxygen consumption (energy supply) and the rate of nucleic acid (including DNA) and protein synthesis (energy requiring processes), an altogether plausible contention.

VI. Protein synthesis

All or most proteins synthesized during embryogenesis may be classed as either structural or catalytic proteins. The former are generally more difficult to determine quantitatively, and consequently the overwhelming part of the available data are concerned with enzymes. It should be mentioned that much work has been devoted to studies of changes in proteins characterized by their solubility or immunological properties (cf. RANZI 1962; PERLMAN 1959). In spite of their importance for the understanding of embryogenesis I have not included this work in the present survey.

It was emphasized by GUSTAFSON and HASSELBERG (1951) in their study of enzyme differentiation in the sea urchin embryo that the results should be interpreted with regard to their cytoplasmic localization. This suggestion can be followed rigorously only with respect to those ubiquitous enzymes, which follow a more or less similar pattern in all cells, in which case the correlation between localization and enzyme pattern is quite satisfactory. One might say that these changes pertain to the growth processes in the embryo.

However, during development extensive differentiation processes occur, leading to profound changes in the enzyme pattern. The most important differentiation processes occurring during the early development of the sea urchin are the cell transformations. Enzymes which are typical for either of the various cell types have therefore been treated in a separate section whereas the ubiquitous enzymes are to be found under the heading corresponding to their cytoplasmic localization. Since no enzymes have been studied which are exclusively located in either the nuclei or the microsomes, these two fractions are not included in the following discussion. All questions about localization have been referred to the paper by DE DUVE et al. (1962).

1. Ubiquitous enzymes

a) Mitochondria

Changes in morphology, number, and distribution. – The question about the changes occurring within the mitochondrial population has attracted the interest of several authors. Variations have been observed in three different parameters, viz., morphology, number, and distribution among the different prospective embryonic regions.

Various methods have been used, thus microscopical observation after vital staining by GUSTAFSON and LENIQUE (1952; 1955); this approach as well as counting in homogenates was employed by SHAVER (1956, 1957), and electron microscopy by BERG et al. (1962) and BERG and LONG (1964). Each of these methods are subject to limitations in some respect. The first one may reveal differences in stainability rather than in number, in the second

one losses may occur, and in the third one distortions of form may take place during the preparation of the material (cf. SHAVER 1957; BERG and LONG 1964; GUSTAFSON 1966).

As far as the appearance of the mitochondria is concerned it seems that a differentiation gradually occurs, this change comprises both the size, the cristal density and the shape. The arrangement of the cristae seems to be very irregular in embryonic mitochondria. Distinct differences seem to obtain between animal and vegetal cells (cf. Table 6).

The number of mitochondria also increases, already before the 32-cell stage there is a distinct rise in mitochondrial density. This phase is of short duration, during several hours there is no further change, but in the advanced blastula the mitochondrial number increases again. This rising phase lasts until about the 25th hour, from then on a decline in relative mitochondrial density (RMD) takes place (Fig. 18; GUSTAFSON and LENIQUE 1952, 1955).

Since, as we have seen, there is a change in the shape of the mitochondria during development we may presume that the mitochondria of the earlier type gradually disintegrate, and are replaced by particles of the more advanced types. This phenomenon of mitochondrial disintegration was described by GUSTAFSON and LENIQUE (1952). It therefore seems possible that the results obtained by these authors actually represent the resultant of two separate curves as suggested in Fig. 18.

The early changes could not be confirmed by SHAVER (1956), but the rapid increase in the blastula, followed in the late gastrula by a distinct decline in mitochondrial number was observed, in confirmation of the results obtained by the former authors (cf. Fig. 18 and 19). As shown in the latter figure there is a further rise in the mitochondrial population in the pluteus larva.

The lack of agreement between the results obtained by the two methods might reflect an increased stainability of the early mitochondria, but this explanation does not agree with the observation that incorporation of amino

Table 6

Differentiation of mitochondria in the sea urchin embryo

		Relative[4] size	Relative[4] cristal density	Shape[1–4]	RMD[1,2]
Cleavage		1.00	1.00	spherical or oval	low
Gastrula	animal	1.24	1.15	spherical, rod-shaped?	high
	vegetal[5]	~1.7–2.5	1.25	rod-shaped	medium

[1] GUSTAFSON and LENIQUE (1952); [2] GUSTAFSON and LENIQUE (1955); [3] SHAVER (1956); [4] BERG and LONG (1964); [5] Includes primary mesenchyme and invaginated cells.

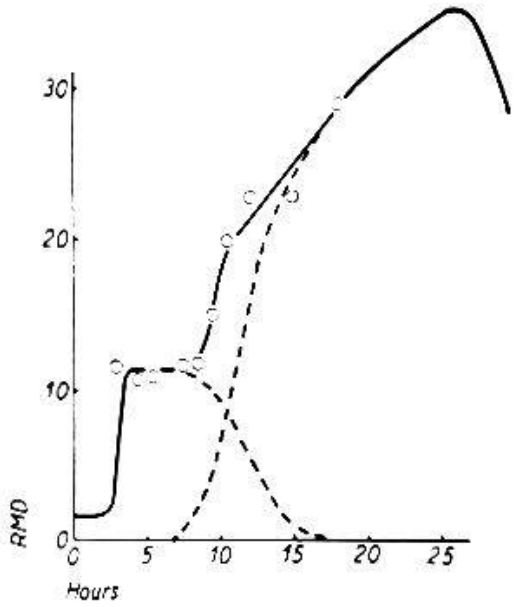

Fig. 18. Changes in relative mitochondrial density (RMD) during embryonic development of the sea urchin. The solid curve is composed from results published by GUSTAFSON and LENIQUE: Exp. Cell Res. *3* (1952); *8* (1955). The stippled curves represent a suggested resolution of the observed curve.

acids into the mitochondrial fraction occurs to a considerable extent even during the very early stages of development (HULTIN 1953e; NAKANO and MONROY 1958; cf. Table 7). The results of the latter (Fig. 20) show close agreement with the results of GUSTAFSON and LENIQUE (1955), especially if correlation is made for the decline in the activity in the acid-soluble fraction, which represents the most likely source of the ^{35}S-methionine incorporated in the mitochondria.

These incorporation experiments, as well as the agreement with the results of SHAVER concerning the later stages, suggest that the approach used

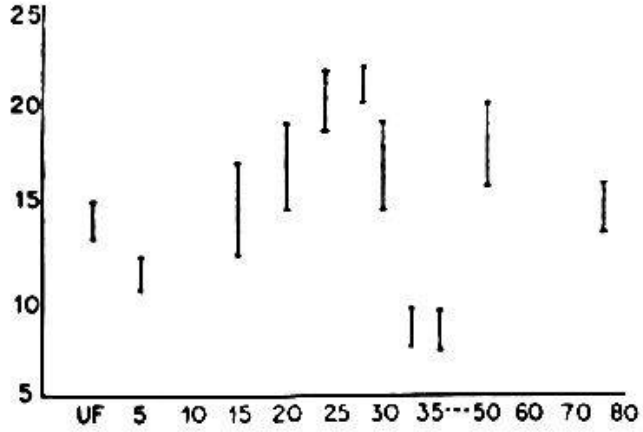

Fig. 19. Changes in the total number of mitochondria during the development of Strongylocentrotus purpuratus at 17° C. The vertical lines indicate the confidence limits (from SHAVER: Exp. Cell Res. *11* [1956]).

by GUSTAFSON and LENIQUE gives quite consistent results. This conclusion is important when we approach the problem of mitochondrial distribution. GUSTAFSON and LENIQUE (1952) reported a gradient in relative mitochondrial density (RMD) from a maximum at the animal to a minimum at the vegetal pole in the blastula gastrula stages (cf. also CZIHAK 1962). Thus the apical tuft cells are very rich in mitochondria, the mesenchyme cells correspondingly poor. These observations were substantiated by results on animalized and vegetalized embryos. For later stages two other regions of high RMD were described, the cells forming the ventral ciliated bands, and the intestinal cells. This observation was confirmed by SHAVER, and it is therfore extremely difficult to understand that he was unable to confirm the first AV-gradient (cf. 1956, 1957). BERG et al. (1962) could not either detect any AV-gradient; in a more recent paper it seems that some mesenchyme cells poor in mitochondria have been observed (BERG and LONG 1964).

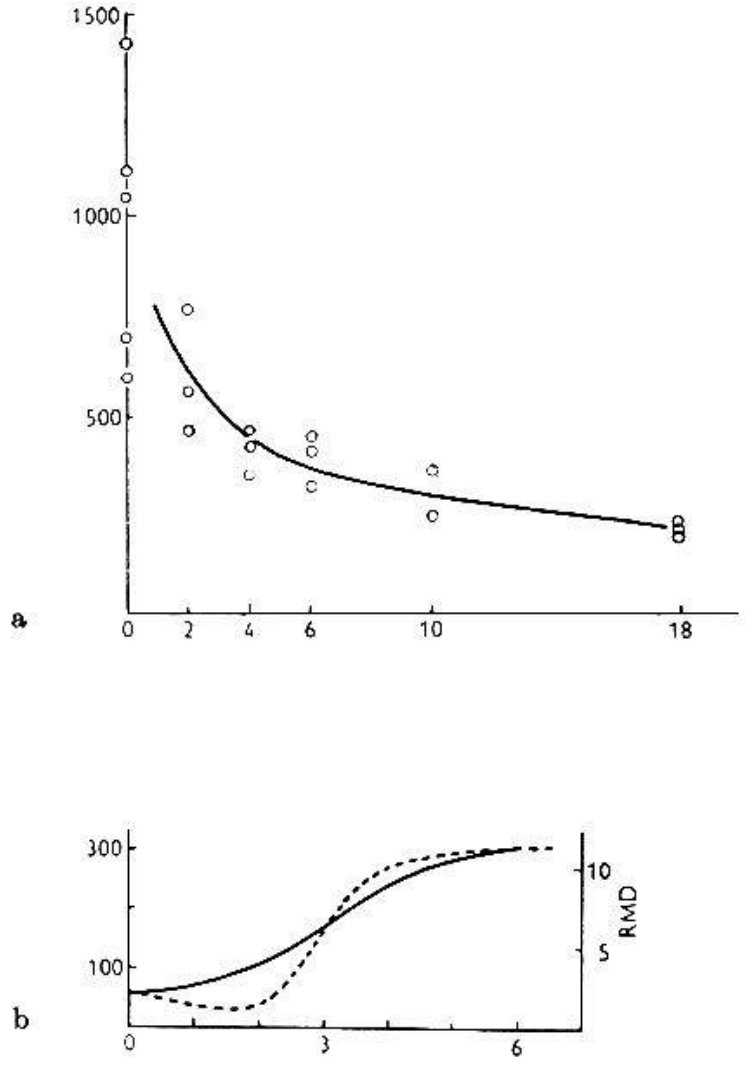

Fig. 20.—a) Isotope content (^{35}S-methionine) of the acid-soluble fraction during the development of Paracentrotus lividus.—b) Comparison of the curves for relative mitochondrial density (RMD) and the isotope incorporation by the particulate fraction during the first 6 h of development (from NAKANO and MONROY: Exp. Cell Res. *14* [1958]).

Table 7

Incorporation of ^{15}N-adenine into various cellular fractions in embryos of Psammechinus miliaris. Per cent ^{15}N excess in purified proteins (from HULTIN [1953e]).

	Cleavage stages	Early mesenchyme blastula
Mitochondria	0.11	0.44
Microsomes	0.23	0.42
Supernatant 1	0.14	0.41
Supernatant 2	0.11	0.25

The results reported may apparently be generalized into the statement the acquisition of the c-property (ciliated cl-cells, intestinal cf-cells) entails a large increase in RMD; a corresponding, but smaller increase seems to be associated with the f-property.

Some of the observations made by GUSTAFSON and LENIQUE (1952) suggest that the characteristically large mitochondria of vegetal cells (presumably part of the f-properties) are present in the intestine.

Enzymes. – Several mitochondrial enzymes have been studied with regard to their changes during sea urchin development, viz., malate and succinate dehydrogenases (GUSTAFSON and HASSELBERG 1951), cytochrome oxidase (DEUTSCH and GUSTAFSON 1952), aspartate and alanine transaminases BLACK 1964), cathepsin B, glutaminase I and ATPase, the latter three by GUSTAFSON and HASSELBERG (1951). The transaminase and the ATPase activities are presumed to represent two or more enzymes, out of which one is bound to the mitochondria (DE DUVE et al. 1962).

It was shown by GUSTAFSON and HASSELBERG (1951) that the activity of most mitochondrial enzymes show a similar pattern of change, involving a large increase in activity during the mesenchyme blastula stage. These changes could neatly be correlated with the increase in mitochondrial number subsequently established. However, as we shall presently discuss, certain irregularities obtain, suggesting that the mitochondrial population is subjected not only to growth, but also to differentiation, a conclusion in excellent agreement with the morphological observations discussed in the preceding section.

It was inferred that during embryogenesis different mitochondrial populations successively replace each other. It might be presumed that this occurrence would be reflected in the enzyme activity curves, at least in the form of periods with constant activity. The determinations on mitochondrial enzymes do not include points enough to reveal finer details in the curves, but it is interesting to note that one curve obtained by GUSTAFSON and

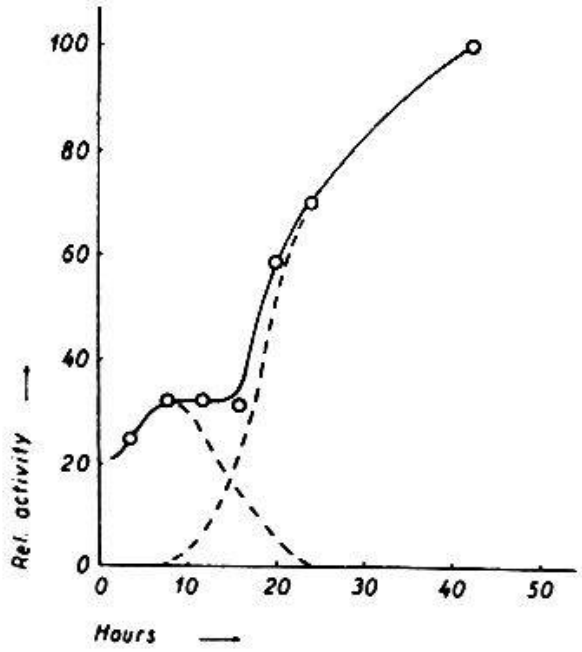

Fig. 21. Changes in succinate dehydrogenase activity during the development of Psammechinus miliaris (redrawn from GUSTAFSON and HASSELBERG: Exp. Cell Res. 2 [1951]). The stippled lines represent a suggested resolution of the observed curve.

HASSELBERG for SDH (Fig. 21) actually shows constant activity for several hours. As I have indicated in the figure, this part of the curve may indeed be composed of a declining and an increasing component, representing the two first mitochondrial populations, respectively.

Considering the large increase in RMD occurring after hatching it appears that the early mitochondria have a large SDH content, as compared with those formed later.

The changes in cytochrome c oxidase (DEUTSCH and GUSTAFSON 1952) present a very complicated picture (Fig. 22). If we resolve curve 1 into two components, we get two curves which, with respect to time, correspond reasonably well to the curves established for the changes in mitochondrial number and SDH activity (Fig. 18 and 21). A very conspicuous difference obtains with respect to activity, however, the cytochrome oxidase being very high compared to that of SDH during the early stages.

From the work of MAGGIO (1959) it is known that in isolated mitochondria there is an activation of the cytochrome oxidase activity—amounting to 20–35%—immediately after fertilization, but no further changes occur in the specific activity during the following 3 h. This implies that no mitochondrial differentiation takes place during this period. It seems that the very steep increase in enzyme activity (cf. Fig. 22) at a time when there is no increase in mitochondrial number according to Fig. 18 might be accounted for by the findings of MAGGIO. However, if the observed enzyme activity were bound to the mitochondria and if, as suggested by MAGGIO's results, no change in specific activity occurs, then the increase in mitochondrial number and in cytochrome oxidase activity should be proportional. This

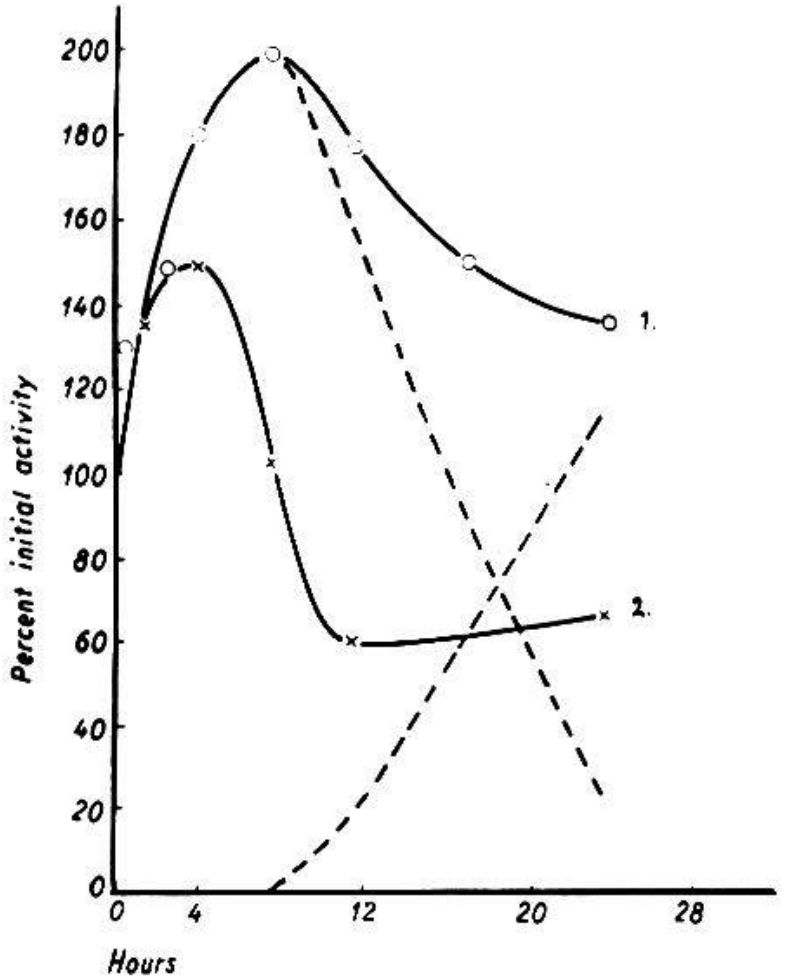

Fig. 22. Changes in cytochrome c oxidase activity during the development of Psammechinus miliaris (redrawn from DEUTSCH and GUSTAFSON: Ark. Kemi *4* [1952]).—The stippled lines represent a suggested resolution of the observed curve.—It should be emphasized that all the curve resolutions suggested in the present paper (cf. Fig. 7, 18, 21, and 22) at best are very crude approximations. If it be possible to make similar resolutions on the basis of experiments, a number of interesting details in the curves may be anticipated.

expectation is not fulfilled; thus, although the mitochondrial density increases at least five-fold, the rise in enzyme activity is only about 50%, after correction for the fertilization activation. This disagreement suggests that a considerable part of the activity represents an extramitochondrial cytochrome oxidase. This question was investigated by HUTCHENS et al. (1942), who found that 67% of the total activity in an egg homogenate is present in a supernatant fraction, and only 1% in the mitochondria. However, as they could account only for 80% of the total activity, it cannot be excluded that the mitochondrial enzyme is more active in the unfractionated homogenate. These authors employed a manometric method, whereas DEUTSCH and GUSTAFSON (1952) as well as MAGGIO (1959) used spectrophotometry. It is not impossible that different methods may give somewhat divergent results, but all the same it seems warranted to conclude that there are two different cytochrome oxidase fractions in the early embryo, both of which decline rapidly during early development.

It might be possible by calculations to get an approximate estimation of the contents in each of these fractions, but a renewed experimental attack ought to give more satisfactory information. The differences between curves 1 and 2 in Fig. 22 suggest that in the latter case the enzyme activity in the second generation of mitochondria is relatively lower than in the first batch.

None of the curve resolutions performed above (Fig. 7, 18, 21, and 22) have been suggested directly by the observed results, the justification for this expedient is mainly to be found in indirect evidence, first of all through the agreement between different lines of approach thus established. However, the results of IMMERS and RUNNSTRÖM (1960) on the DNP stimulation of the respiration (Fig. 23) show two clearly separated curves; the minimum uniting them is located at the end of the phase of constant respiration. If, as these authors suggest, the curve of DNP-stimulated oxygen uptake represents the maximum oxidative capacity in the embryo, then it seems absolutely unavoidable to conclude that two different mitochondrial (or extramitochondrial) oxidative systems are present in the sea urchin embryo, the first one being gradually replaced by the second one during gastrulation.

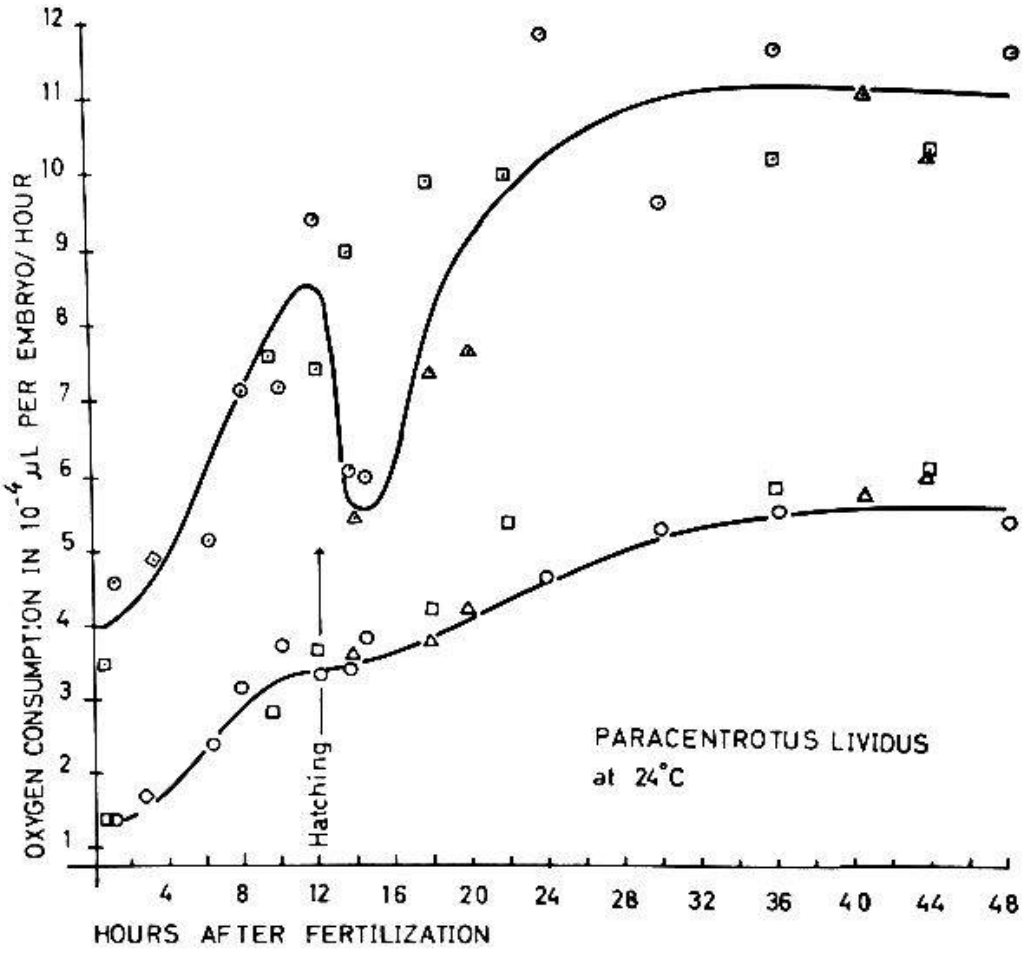

Fig. 23. Lower curve: Normal respiratory rates at different stages of development. Upper curve: Oxygen consumption after addition of 2,4-dinitrophenol (5×10^{-5}M) (from IMMERS and RUNNSTRÖM: Develop. Biol. 2 [1960]).

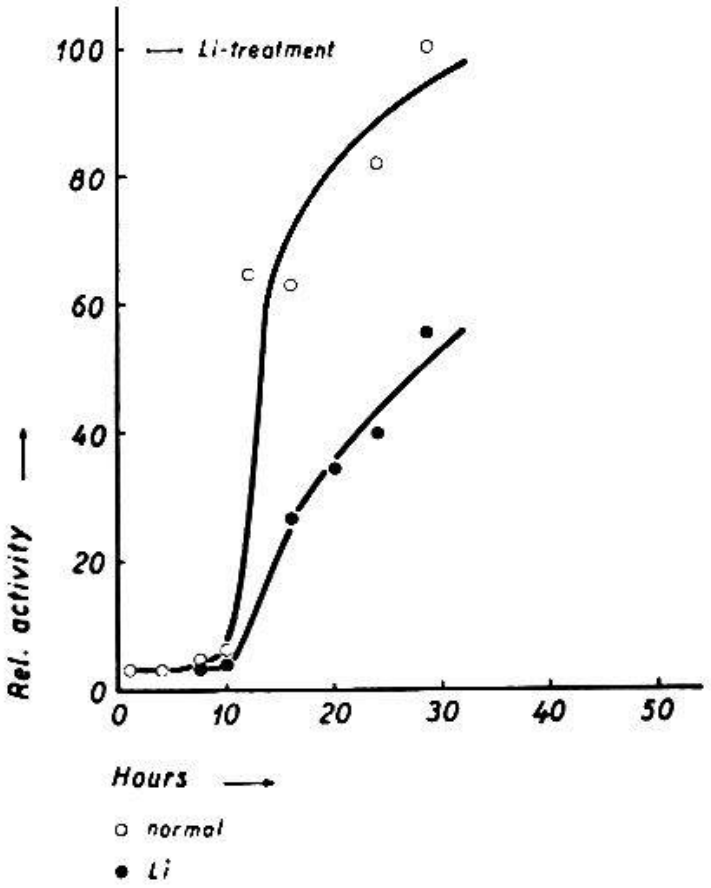

Fig. 24. Changes in cathepsin B activity during the development of Psammechinus miliaris (from GUSTAFSON and HASSELBERG: Exp. Cell Res. 2 [1951]).

Among the remaining enzymes the increase in activity for cathepsin B, glutaminase I and ATPase begins at a time corresponding to the second phase of mitochondrial formation. For the two former enzymes the relative increase seems to be much higher than for the dehydrogenases, suggesting that the early mitochondria are very poor in these enzymes (Fig. 24). Conversely, the relative increase in ATPase activity is quite low (Fig. 25), a phenomenon which of course also might be related to the enzyme content of the first mitochondrial population. It is more likely, however, that this phenomenon should be referred to the fact that, as mentioned above, this enzyme is not located exclusively in the mitochondria. The observed activity may be the sum of a constant, extramitochondrial component, and a mitochondrial enzyme which increases together with the other mitochondrial enzymes.

Similar results were obtained with respect to the transaminases studied by BLACK (1964), two other enzymes known to be localized both in and outside the mitochondria (Fig. 26). Because of the few points, and the low relative increase, it is difficult to draw any definite conclusions, but it seems that the alanine transaminase begins to increase in the late blastula stage, suggesting that it is synthesized together with the other mitochondrial enzymes during the second phase of mitochondria formation. The aspartate transaminase activity, on the contrary, decreases until the pluteus stage. This

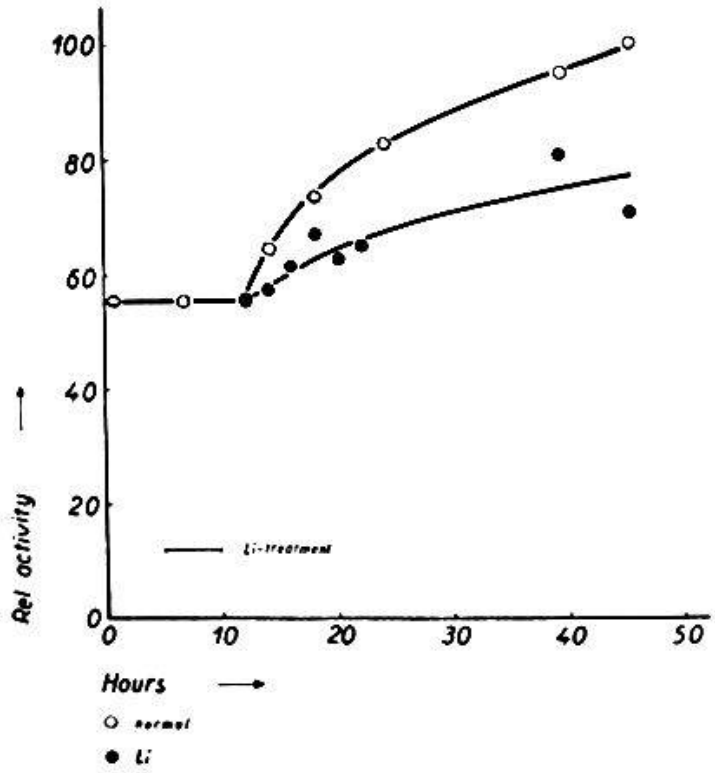

Fig. 25. Changes in ATPase activity during the development of Paracentrotus lividus (from GUSTAFSON and HASSELBERG: Exp. Cell Res. *2* [1951]).

might indicate that this enzyme is synthesized only in association with the third phase of mitochondria formation, the one described by SHAVER (1956). If this interpretation is correct, it is obviously possible to conclude that the particles synthesized in each phase are chemically distinct.

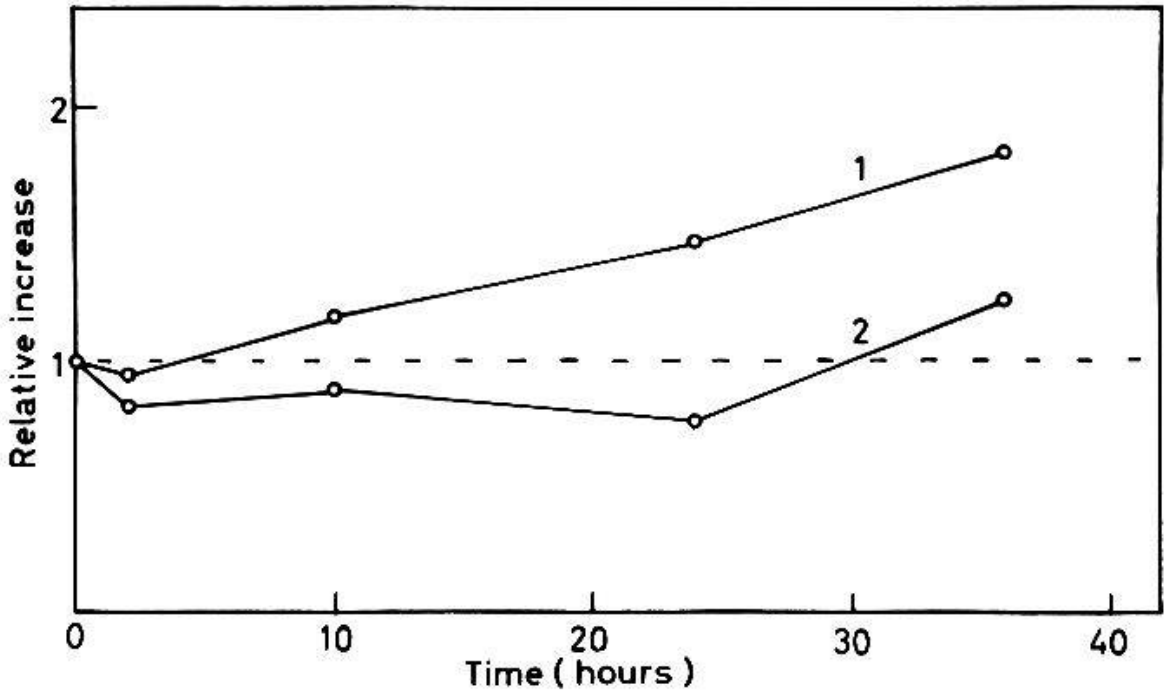

Fig. 26. Changes in transaminase activity during the development of Lytechinus variegatus.—1 = alanine transaminase, 2 = aspartic transaminase (the figure has been drawn on the basis of results published by BLACK [1964]).

It is remarkable that the transition between the second and third phase has not been reflected in any of the enzyme activity curves. This may in some cases depend on the fact that the determinations were discontinued too early, but in other cases this explanation cannot account for the observations. One possibility which might be invoked is that the enzymes are preserved when the mitochondria disintegrate, but this explanation seems quite unlikely. Another possibility, which can partly explain the findings, is that the enzyme activity in the third generation of mitochondria is higher than that of the second one.

The results discussed in this section show that both growth and differentiation occur with respect to the mitochondrial population. It is remarkable to note that these changes occur at a time when no enzyme changes can be recorded in other cytoplasmic fractions (cf. below). This autonomy certainly suggests that mitochondria form by self reproduction (cf. the discussion by LEHNINGER 1964).

The differentiation of the mitochondria is not a gradual process, rather one population of particles is, in a relatively short period of time, replaced by a new one, which may be different with respect to both morphological and chemical traits. I believe that this phenomenon may be best understood from a phylogenetic point of view (cf. below).

It seems possible to correlate the transfer from the first to the second generation of mitochondria with the transformation of the amoebocytes and mechanocytes, respectively. One might even anticipate two different populations of mitochondria, specific for each of the cell types. There are some indications in support of this contention, thus it was observed by BERG and LONG (1964) that the increase in cytochrome oxidase is higher in vegetal than in animal halves. As the latter contain more mitochondria (GUSTAFSON and LENIQUE 1952) one may presume that they have a low relative content of this enzyme. Likewise, histochemical determination of NAD-cytochrome c reductase shows that this enzyme is mainly confined to the mesenchyme cells (CZIHAK 1962). In contrast the indophenol oxidase activity, up to the mesenchyme blastula at least, seems to be localized in animal-ventral cells (CZIHAK 1963). Whether this activity represents a mitochondrial or an extramitochondrial enzyme may be difficult to decide.

The transition from the second to the third generation apparently cannot be correlated with any morphological event, as mentioned it is not even reflected in the enzyme curves.

I shall not begin a discussion about the possible mechanisms involved in the differentiation of the mitochondria, but only mention that a mechanism like the nuclear-cytoplasmic interaction believed to be responsible for cellular differentiation might be at work in this case. This is particularly suggestive if, as proposed, mitochondria contain DNA (CHÈVREMONT et al. 1959; NASS and NASS 1962).

More mitochondrial enzymes than those treated here are known to be present in the sea urchin embryo, but none of these have been studied with

regard to the changes in activity during development. Some of them have been dealt with in the previous chapter.

Cytochromes and oxidation in the early embryo. – As we have seen, the sea urchin egg contains a very powerful cytochrome c oxidase. It would as a consequence be expected to contain cytochrome c, but in spite of numerous attempts (BALL and MEYERHOF 1940; KRAHL et al. 1941; ROTHSCHILD 1949; BOREI 1951; YCAS 1954), only two cytochromes have been detected spectrophotometrically, belonging to the groups a and b, respectively. MAGGIO and GHIRETTI-MAGALDI (1958) reported the presence of cytochromes a, a_3, b, and c in sea urchin mitochondria; unfortunately the latter was lost during the preparation.

Cytochrome a_3 (= cytochrome oxidase or at least part of this enzyme) cannot be demonstrated spectrophotometrically, according to ROTHSCHILD (1949) because certain pigments interfere with the determination. The approach used by MAGGIO and GHIRETTI-MAGALDI should circumvent this difficulty. In support of its presence ROTHSCHILD (1956) points out that cytochrome c oxidation occurs in homogenates (KRAHL et al. 1941), and the oxidation of dimethyl-p-phenylendiamine and similar substrates has also been used as evidence (cf. RUNNSTRÖM 1956). As to substances of this type it was observed by PAPPENHEIMER and WILLIAMS (1954) that they may be oxidized through a pathway not involving cytochrome c, and that this method therefore cannot provide an unambiguous measure of cytochrome oxidase. The results with cytochrome c oxidation are of course more clearcut, but they do only prove the presence of cytochrome a_3 on the presumption that this is the only terminal oxidase that can react with cytochrome c.

The chemistry of cytochrome oxidase seems to be in a state of ambiguity. It was suggested by KEILIN and HARTREE (1938) that it may consist of two cytochromes, a and a_3, and that it is a copper-containing protein. At present opinions seem to diverge, it may be a+a_3, or a+c, or it may be only one enzyme, possibly then a_3 (cf. WAINIO 1961; HORIO et al. 1961, and the discussion following these papers). KRAHL et al. (1941) tested the effect of two inhibitors of copper containing enzymes, 8-hydroxychinoline and diethyldithiocarbamate on the Arbacia cytochrome c oxidase. None of these produced any inhibition, on the contrary a considerable stimulation was observed with the latter compound. This finding does not support the contention that the terminal oxidase is the same as that found in other animal tissues, and the absence of the a_3 spectrum can therefore be a simple consequence of the fact that the enzyme is not there. This may not hold for the mitochondria, but they are anyhow responsible only for a small fraction of the total cytochrome oxidase activity.

It is of interest to know the activity of the various enzyme preparations in relation to the oxygen uptake of the egg. In the unfertilized Arbacia egg there is—assuming 0.1 mg N per mg dry weight (HUTCHENS et al. 1942)—an oxygen consumption of 4–5 μl per hour and mg N, the corresponding value

for the fertilized egg is 20, whereas a dialyzed enzyme preparation gave a value of 100, referring to the dry weight of the eggs (KRAHL et al. 1941). The homogenates used by HUTCHENS et al. (1942) showed an oxygen consumption of 2400 μl per hour and mg N. The enzyme activities estimated in vitro are thus in general very high compared to those in vivo.

The cytochrome b found in the sea urchin has by YČAS (1954) been suggested to be b_5, the microsomal cytochrome (cf. STRITTMATTER 1961). This, and $a+a_3$ are the main cytochromes present in the midgut of larvae of the Cecropia silkworm. The enzyme activities in preparations from this source have been studied by PAPPENHEIMER and WILLIAMS (1954). These authors found that b_5 is an electron donor in the reduction of cytochrome c by NADH, but b_5 also reacts with NADPH (STRITTMATTER 1951), so that if indeed the cytochrome present is b_5, we may establish the following reaction sequence by addition of cytochrome c to homogenates of sea urchin eggs:

$$\left.\begin{matrix}\text{NADH}\\ \text{NADPH}\end{matrix}\right\rangle \rightarrow \text{flavoprotein} \rightarrow \text{cyt.}b_5 \rightarrow \text{cyt.c} \rightarrow \text{cyt.a} \rightarrow O_2$$

In this scheme cyt. a represents the cytochrome oxidase. This pathway is not inhibited by antimycin A, showing that the Slater factor is not involved (PAPPENHEIMER and WILLIAMS 1954). These authors also showed that cytochrome b_5 is autoxidizable, in a reaction which is uninhibited by cyanide and carbon monoxide, and suggested that this compound represents the principal terminal oxidase in tissues deficient in cytochrome c in general, and in the insect larva during diapause in particular. In this way it becomes possible to account for the CN^-- and CO-insensitive respiration observed in the latter case. If oxidations with b_5 as the terminal oxidase can sustain the energy supply to larvae in diapause then it may be anticipated that the electron transfers in the suggested chain are associated with formation of ATP. It may be presumed that the oxidative phosphorylation observed by LINDBERG and ERNSTER (1948) in homogenates with glucose and other substrates have followed this pathway, and not the normal one present in the mitochondria. It may be relevant, in this connection, to mention the CO-insensitive oxidative phosphorylation observed by MCEWEN et al. (1964) in isolated nuclei.

The terminal oxidase, cytochrome a, in the sea urchin egg, has been studied repeatedly by means of cytochrome oxidase inhibitors, both in vivo and in vitro. With enzyme preparations it has been found that the activity can be suppressed completely with cyanide and azide, and that the inhibition by CO is relieved by illumination (KRAHL et al. 1941). It thus shares a number of properties with the usual cytochrome oxidase. In studies on eggs it has been found that the inhibition by CO does not follow the rules established by WARBURG (1926) for the effect of this substance, and irregularities obtain also with respect to the effect of CN^- (cf. RUNNSTRÖM 1956; LINDAHL 1939a; ROBBIE 1946; ROTHSCHILD 1949, 1956). Apparent insensitivity was observed

in the unfertilized egg, but in the developing embryo sensitivity was gradually acquired. According to LINDAHL the paradoxical situation may be explained by the fact that CO has an activating influence upon the oxygen uptake; when this is corrected for, normal inhibition obtains; a somewhat similar mechanism can account for the results with cyanide. Other opinions have been voiced by ROBBIE (1946) and ROTHSCHILD (1949).

A final solution of this problem does not seem established, but it should be mentioned that if the cytochrome in the sea urchin egg is b_5, or at least has properties similar to those of this compound, then it may be possible to interpret the inhibition experiments along the lines suggested by PAPPENHEIMER and WILLIAMS.

It seems quite interesting, also from a phylogenetic point of view, that the microsomal cytochrome may be the only cytochrome b present in the sea urchin egg. As already mentioned, HUTCHENS et al. (1942) observed that very little cytochrome c oxidase is bound to the mitochondria, most of the activity is found in the supernatant. Whether or not the enzyme in this fraction is bound to the microsomes remains to be seen, but it is certain from the inhibition studies of KRAHL et al. (1941) that the terminal oxidase cannot be cytochrome b_5, both this and cytochrome a must therefore be present in the supernatant.

In the midgut preparations used by PAPPENHEIMER and WILLIAMS (1954) the oxidation of succinate—in the presence of cytochrome c—was very low compared to that observed with NADH, suggesting that the Slater factor limits the reaction rate. Also in this respect there is a great similarity between the silkworm larva and the sea urchin egg, because it has been observed repeatedly that succinate is not oxidized by enzyme preparations from eggs (e.g. KRAHL et al. 1941; GOLDINGER and BARRON 1946; CRANE and KELTCH 1949). In isolated mitochondria a slight succinoxidase was reported (MAGGIO and GHIRETTI-MAGALDI 1958). Most other Krebs cycle substrates, except oxalacetate which probably may form pyruvate, cannot be oxidized either (CRANE and KELTCH 1949).

These results demonstrate the inefficiency of the mitochondria already discussed in a previous section, and also that the extramitochondrial pathway has no means of dealing with intermediates in the tricarboxylic cycle.

The repeated efforts to demonstrate cytochromes c and a_3 in the sea urchin egg seem to stem from the conviction that negative results must be due to experimental errors, since these compounds are found in almost all aerobic cells. Yet, there are exceptions to this rule, thus it was shown by MØLLER and PRESCOTT (1955) that in the protozoa Amoeba proteus, Chaos chaos, and Tetrahymena geleii only cytochrome a (or cytochrome e oxidase), cytochrome b, and cytochrome e can be found. It appears that cytochrome e = cytochrome c_1 and cytochrome b_5 are very closely related (PAPPENHEIMER and WILLIAMS 1954; STRITTMATTER 1961). Without entering into a discussion of cytochrome nomenclature I think it can be concluded that the content of cytochromes in the sea urchin egg is very similar to that of the investigated

protozoa. It should be mentioned that the terminal oxidase in these organisms does not react with cytochrome c (ANDRESEN et al. 1951), and that the oxygen uptake is inhibited by cyanide.

It has been argued above that the egg cell and the first blastomeres may represent a phylogenetically very primitive cell, an amoebocyte, exhibiting various archaeic biochemical traits. To the list of those already established it seems that we may add lack of cytochrome c. That certain living protozoa are similar in this respect is very interesting from a comparative biochemical point of view, but it is necessary to point out that most protozoa apparently possess cytochrome systems more similar to those found in metazoa. Maybe this phenomenon reflects a series of independent mutations along a metazoan and one or several protozoan lines, originating from a cell possessing a primitive cytochrome system of the type found partly in Chaos chaos, partly in the sea urchin egg.

There are two points which should be emphasized. The first one is that the discussion here does not imply the absence of cytochrome c in all eggs, the formation of this substance may have become possible at various stages of the phylogenetic development, by independent mutations. A study of the pattern of distribution of cytochrome c among various eggs might indeed furnish very interesting information of importance for the problem of phylogenesis. The other point is that the presence of cytochrome c in sea urchin sperm has been demonstrated beyound doubt (ROTHSCHILD 1948; KEILIN and HARTREE 1949). This finding does not invalidate the reasoning advanced above, since the sperm cell is no amoebocyte, but belongs to the class of epitheliocytes; it may thus represent a higher phylogenetic stage. If mitochondria are self reproducing, then the mitochondrial cytochrome c containing material in the sperm (cf. AFZELIUS 1955) may be necessary for the formation of later mitochondrial generations with more developed chemical properties.

The extramitochondrial cytochrome c oxidase system as well as the inefficient mitochondria present in the egg seem to be typical for the class of amoebocytes, since they are preponderant as long as most cells belong to this cell type. As we have seen, when the amoebocytes are transformed into other cell types new kinds of mitochondria are produced, and the very peculiar extramitochondrial cytochrome c oxidase activity disappears, being replaced by the usual mitochondrial enzyme system.

b) Lysosomes

Thanks mainly to the work of DE DUVE and his collaborators (cf. review 1963) it is today possible to distinguish several fractions of large granules, previously isolated together in the mitochondrial fraction. The most important of these accessory granules is the lysosome. Among the enzymes studied in the sea urchin embryo by GUSTAFSON and HASSELBERG (1951) it is possible to distinguish a separate group of enzymes, acid phosphatase, cathepsin D and aryl- or phenylsulfatase, purported to be localized in the lysosomes.

With respect to the two first enzymes the localization seems relatively certain, but the situation is somewhat unclear concerning arylsulfatase. From rat liver three different enzymes are known, designated by the letters A, B, and C. A and B are lysosomal enzymes with a pH-optimum around 6; they have little or no activity with p-nitrophenylsulphate as substrate. C, on the contrary is a microsomal enzyme with a slightly alkaline pH-optimum, and it is active towards the mentioned sulfate ester (DODGSON et al. 1955; ROY 1958). Judging from the substrate affinity the enzyme investigated by GUSTAFSON and HASSELBERG is arylsulfatase C, but it has a pH-optimum around 6, suggesting that it is of the lysosomal type (A or B). In spite of the uncertainty concerning the classification of this particular enzyme, the experimental results establish the fact that no increase in lysosomal enzymes occurs during the investigated phase of sea urchin development. Although it cannot be excluded that lysosomal enzymes are synthesized, it seems likely that, in contrast to the mitchondria, no increase occurs in the number of these particles. The results presented in Fig. 27 are typical for the enzymes in this and the following group.

c) Supernatant

The following enzymes may be referred to the supernatant fraction: glutathione reductase (BÄCKSTRÖM 1959a), dipeptidase (HOLTER and LINDAHL 1940), adenosine deaminase, pyrophosphate (and hexametaphosphate?) hydrolase, and aldolase (GUSTAFSON and HASSELBERG 1951). No increase occurs in the activity of any of these enzymes suggesting that the supernatant fraction is subjected to no increase (growth) during the investigated period of development.

As already discussed in a previous chapter the presence of aldolase in the sea urchin embryo is questionable.

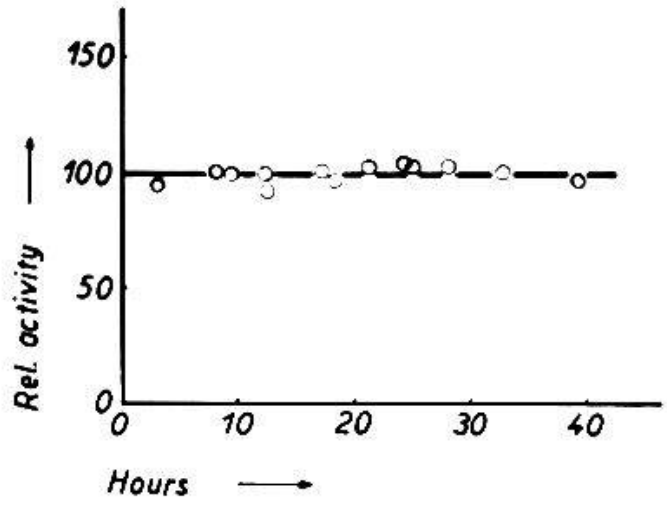

Fig. 27. Activity of cathepsin D during the development of Psammechinus miliaris (from GUSTAFSON and HASSELBERG: Exp. Cell Res. *2* [1951]).

2. Cell class specific enzymes

Under this heading will be treated partly certain enzymes which with some assurance can be referred to the new cell types arising during embryogenesis. There are other enzymes, for which such a correlation is not possible, but having in common that their activity decreases during development it may be suspected that they are enzymes typical for amoebocytes. I have, without further justification, classed these as amoebocyte enzymes.

a) Amoebocyte enzymes

Catalase. – During development the catalase activity decreases in two phases (DEUTSCH and GUSTAFSON 1952). The first, slight decrease lasts until the mesenchyme blastula stage, the second during the remaining part of the investigated developmental period (Fig. 28). The decreasing activity may, as suggested by the authors mentioned, reflect a gradual formation of inhibitors. I may suggest that compared to the other cell types, a high content of catalase may be a typical trait of amoebocytes, and that the gradual decrease in enzyme activity represents the continuous reduction in the number of sl-cells.

According to DE DUVE et al. (1962) catalase is found in a special type of granules, containing besides uricase and D-amino acid oxidase. It would be interesting to know about the changes in the activity of the latter enzyme, if present.

2'-deoxyribosyl 4-aminopyrimidine 5'-phosphate deaminase. – This enzyme, dAPDase, has been discovered by SCARANO (cf. 1961). It catalyzes the conversion of deoxycytidylic acid to deoxyuridylic acid and of deoxymethylcytidylic acid to thymidylic acid. It is found in large amounts in the sea

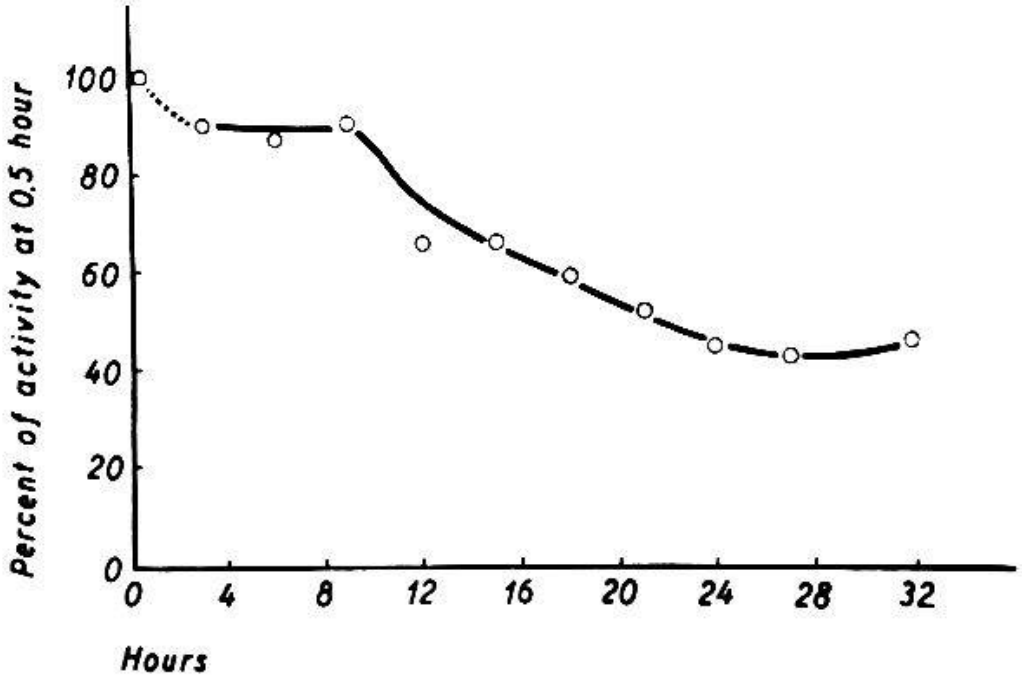

Fig. 28. Changes in catalase activity during the development of Psammechinus miliaris (from DEUTSCH and GUSTAFSON: Ark. Kemi *4* [1952]).

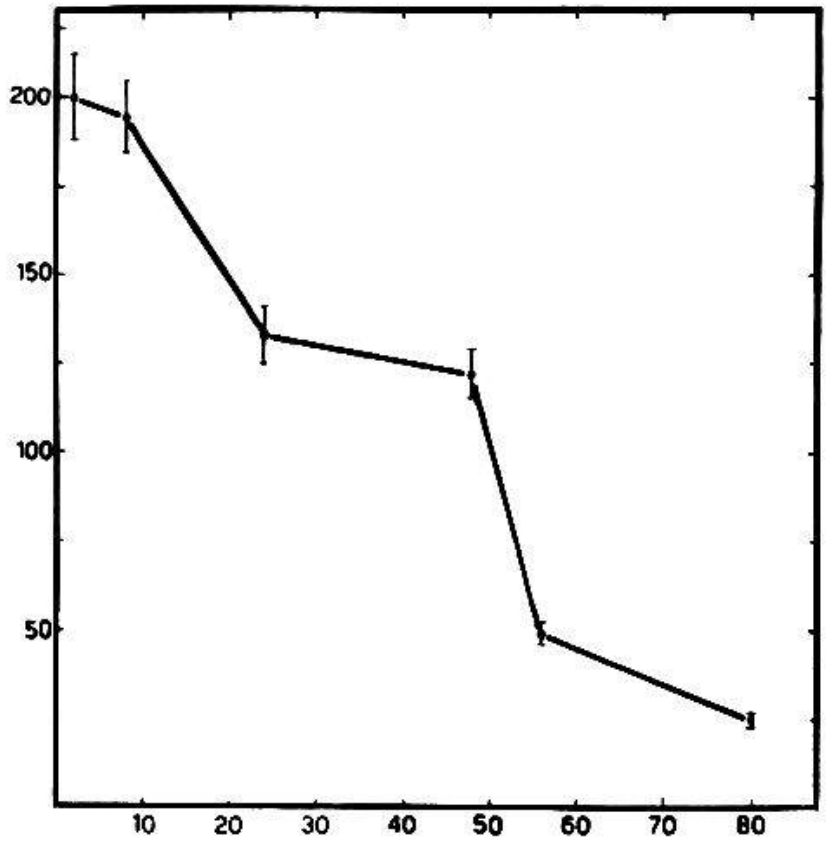

Fig. 29. Changes in 2'-deoxyribosyl 4-aminopyrimidine 5'-phosphate deaminase activity during the development of Sphaerechinus granularis (from SCARANO: Symp. on germ cells and development [1961]).

urchin egg, but the activity gradually diminishes during development. This decrease occurs in two phases, just as the DNA-synthesis, suggesting a correlation between these two phenomena (Fig. 29). The timing does not correspond to that shown for DNA-synthesis in Paracentrotus lividus, but this discrepancy may be correlated with the fact that the species in which the dAPDase has been investigated, Sphaerechinus granularis, apparently develops more slowly.

The pattern of change suggests that this enzyme is characteristic for the amoebocytes, disappearing gradually as these are transformed into other cell types.

Deoxyribonuclease. – MAZIA (1949) has followed the activity and the subcellular localization of deoxyribonuclease during the development of Arbacia punctulata. The activity remained constant throughout, but from being completely soluble at the outset the enzyme could be sedimented to 90% at the end of development. MAZIA concludes from these results that the enzyme becomes localized in the nucleus.

It seems possible to distinguish two different DNAases, one active at alkaline or neutral pH, and activated by Mg^{++}, the other with an acid pH optimum, and Mg^{++}-independent. The former is a mitochondrial enzyme, the latter is found in the lysosomal fraction (DE DUVE et al. 1962). The en-

zyme investigated by MAZIA (1949) was active at neutrality, Mg^{++} was added but it was not stated whether it was required or not.

According to these characteristics the enzyme investigated by MAZIA may be the mitochondrial enzyme, and since the centrifugal force applied (20 000 g for 15 min) should be strong enough to sediment the mitochondria it does not seem excluded that the enzyme was actually bound to these particles.

The question about the localization remains open, but I would suggest that the primitive mitochondria in the amoebocyte may be distinguished by not containing any DNAse, in contrast to the more evolved mitochondria contained in the other cell types. The constant curve observed by MAZIA might thus be composed of a gradually decreasing and a gradually increasing component. It could be argued that chances for the resultant be a horizontal line would be minimal, but the number of points, and the precision of the determinations would hardly warrant the detection of smaller changes.

Glucose-6-phosphate and 6-phosphogluconate dehydrogenase. – BÄCKSTRÖM (1959b) has followed the changes in the G-6-PDH activity during development. This enzyme is localized in the supernatant, but the activity is not constant like that of the other enzymes in this fraction, on the contrary, there is a substantial increase in activity up to the stage of hatching, after which the enzyme activity gradually diminishes. Several peaks were observed on the curve, both during the rising and the declining phase (Fig. 30).

The absence of activity in the unfertilized egg shown in the figure can

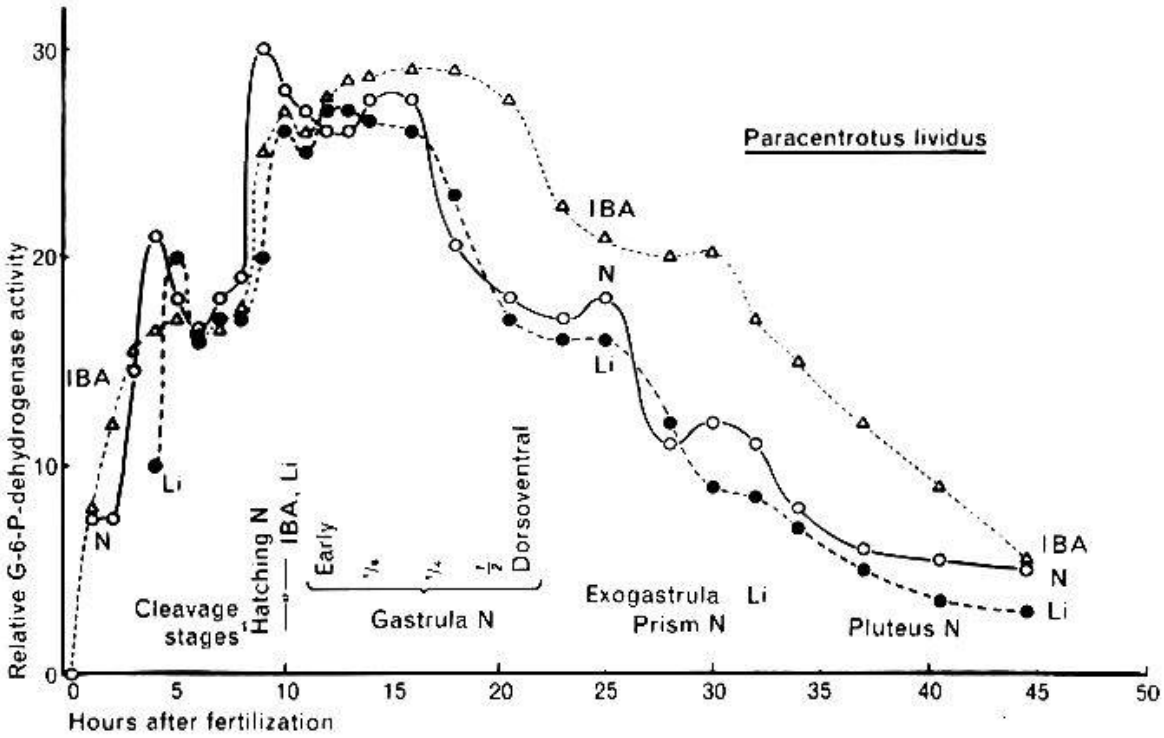

Fig. 30. Changes in glucose-6-phosphate dehydrogenase activity during the development of Paracentrotus lividus (N = normal embryos, Li = vegetalized embryos, IBA (o-iodosobenzoic acid) = animalized embryos (from BÄCKSTRÖM: Exp. Cell Res. *18* [1959b]).

hardly be a general phenomenon, in other batches of Paracentrotus lividus, and in Psammechinus miliaris there was measurable activity at this stage. This confirms the findings of KRAHL et al. (1955); these authors furthermore did not observe any change immediately after fertilization in Arbacia punctulata. G-6-PDH is the first of the enzymes in the PP cycle. This metabolic pathway has been suggested to be of general occurrence in embryonic cells, but as we have discussed above the presence of the whole cycle does not seem to have been proven.

However this may be, it seems that the metabolic pathway involving G-6-PDH represents the main source of energy supply in the early embryo. Changes in the energy consumption must of course entail changes in the oxygen consumption and in the activity of the particular enzymes, but not necessarily the enzyme synthesis which apparently takes place. The curves obtained by BÄCKSTRÖM (1959b) need not, indeed, represent enzyme synthesis, they might as well represent enzyme activation. However, as appears from the discussion elsewhere in this paper, I do not think one ought to take recourse to this explanation unless definitely required, and there seems to be no reason to do it in the present case. The fact that the content of G-6-PDH is high during early development and gradually declines suggests that this enzyme is a distinguishing, but of course not a unique feature of the amoebocyte, as well as mitochondria of a morphologically distinct and chemically inefficient type.

As we have already discussed there are indications that mitochondria of this type are produced during pre-hatching development, and other enzyme systems, including G-6-PDH might be synthesized as well. There is no reason to doubt that the egg may possess the necessary apparatus for synthesis of amoebocyte proteins, and as we know from the isotope studies of HULTIN (1953c) and MARKMAN (1961b), the rate of incorporation in the protein fraction is relatively high during early development, as compared with for instance the incorporation into nucleic acids (cf. Fig. 10).

The two phases of enzyme increase observed before hatching suggest energy requiring activity occurring in two separate regions of the egg and I believe that BÄCKSTRÖM (1959b) is correct in proposing the first phase to be associated with development of the animal, the second with the development of vegetal cells. Each of these phases of enzyme increase may be associated with increased oxygen consumption, as suggested above (cf. Fig. 7). In my opinion this correlation definitely excludes that the enzyme synthesis, as well as the mechanism of glucose oxidation, can be of specific importance for either animal or vegetal determination proper. Their only role seem to be the supply of energy to both processes. The first peak occurs slightly before or simultaneously with the onset of the synthesis of acetylcholine esterase typical for epitheliocytes, and a similar correlation obtains between the second enzyme and alkaline phosphatase, the enzyme characterizing the mechanocyte (cf. the following sections). It may be envisaged that in association with their own transformation the amoebocytes respond

to the increased energy demands by synthesizing the enzymes which form part of their main source of energy supply. The decline would reflect that the transformation has occurred, that the rate at which G-6-PDH is synthesized by the new cell types is too low to keep up with the breakdown.

I would like to insist that these two peaks correspond only to the cells near the poles, the cells at the apical tuft, and the presumptive primary mesenchyme cells. Gradually even the remaining cells transform; the third peak, occuring during early gastrulation might represent the secondary mesenchyme cells, the fourth and fifth might represent phases of transformation of either endodermal or ectodermal cells (cf. BÄCKSTRÖM 1959b).

The peaks during the later part of development are quite modest, although the number of cells involved, according to the present interpretation, is much larger. This lack of correlation may be seen on the background that the cells at this stage of development are well supplied with mitochondria, direct oxidation may no longer be the main pathway for energy supply. This is revealed among other things by the plain fact that the rate of oxygen consumption after hatching is considerably higher than before, although the G-6-PDH activity is substantially reduced. The fluctuations in energy consumption purportedly demonstrated by the enzyme curves are not reflected by the oxygen consumption curves (cf. Fig. 5). This could hardly be expected, since the differential activity at any moment is concentrated in a small part of the embryo.

Studies on 6-PGDH has shown that its activity changes according to a pattern very similar to that observed for G-6-PDH, with the exception that the decrease is less distinct in the pluteus (BÄCKSTRÖM 1963).

b) Epitheliocyte enzyme

Acetylcholine esterase. – Among the enzymes investigated in the sea urchin embryo, only two remain, acetylcholine esterase (AChE) and alkaline phosphatase (APh).

It holds for both that more than one enzyme is known to attack their typical substrates, and various expedients (substrate specificity, activation, etc.) are required to distinguish between the isozymes (cf. AUGUSTINSSON 1950; STADTMAN 1961). Both enzymes are relatively diffusely distributed among the cellular fractions (DE DUVE et al. 1962).

AUGUSTINSSON and GUSTAFSON (1949) have studied the content of AChE during the development of Paracentrotus lividus. No demonstrable enzyme activity could be recorded in the unfertilized egg, but during development a gradually increasing curve was observed (Fig. 31). Tracing this curve back to zero activity it can be estimated that the synthesis begins 4–5 h after fertilization. On the basis of their results and on various evidence from the literature the authors concluded that there is a close correlation between the development of ciliary or contractile activity and synthesis of AChE.

It was possible by treatment with Li^+ to distinguish three different phases of synthetic activity (Fig. 31). The first phase begins, as mentioned, a few

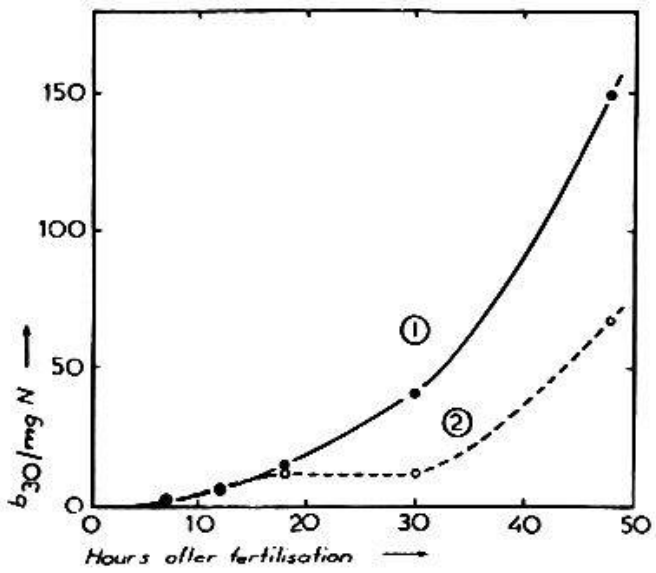

Fig. 31. Changes in acetylcholine esterase activity during the development of Paracentrotus lividus.—1 = normal embryos, 2 = vegetalized Li-treated embryos (from AUGUSTINSSON and GUSTAFSON: J. cell. comp. Physiol. *34* [1949]).

hours after fertilization and lasts to about 15–18 h, corresponding to late gastrulation. This synthesis must reflect partly the development of the apical tuft and partly the formation of ciliated cells in the remaining part of the embryo.

The second phase lasts from 15–18 h to 30–35 h and reflects the development of the ventral ciliary bands and other ciliated ectodermal structures.

The third phase beginning after 30–35 h corresponds to the development of the intestine which at this time begins to show contractility.

WILLMER (1960) has also pointed out the correlation between ciliation and AChE, and if we adopt his cell classification scheme, we might say that the appearance of AChE activity demonstrates the occurrence transformation sl→cl. If this formulation is correct it follows that no activity should be found in the early embryo, containing only amoebocytes, and this expectation is confirmed by experimental evidence. It appears that the cell transformation with respect to the c-property occurs at a quite late stage in the endoderm and certain parts of the ectoderm. This timing was actually suggested by the results of the G-6-PDH discussed in the previous section. It should be emphasized that the time of transformation need not be correlated with the time of determination in any simple fashion.

c) Mechanocyte enzyme

Alkaline phosphatase. – As suggested AChE may be typical of epitheliocytes, and the synthesis of this enzyme accordingly an index of the formation of cells with the c-property. A similar correlation seems to obtain between APh and the f-property. The absence of either enzyme from the amoebocytes in the early embryo would appear a necessary requirement for the acceptance of this contention, and for AChE this expectation was ful-

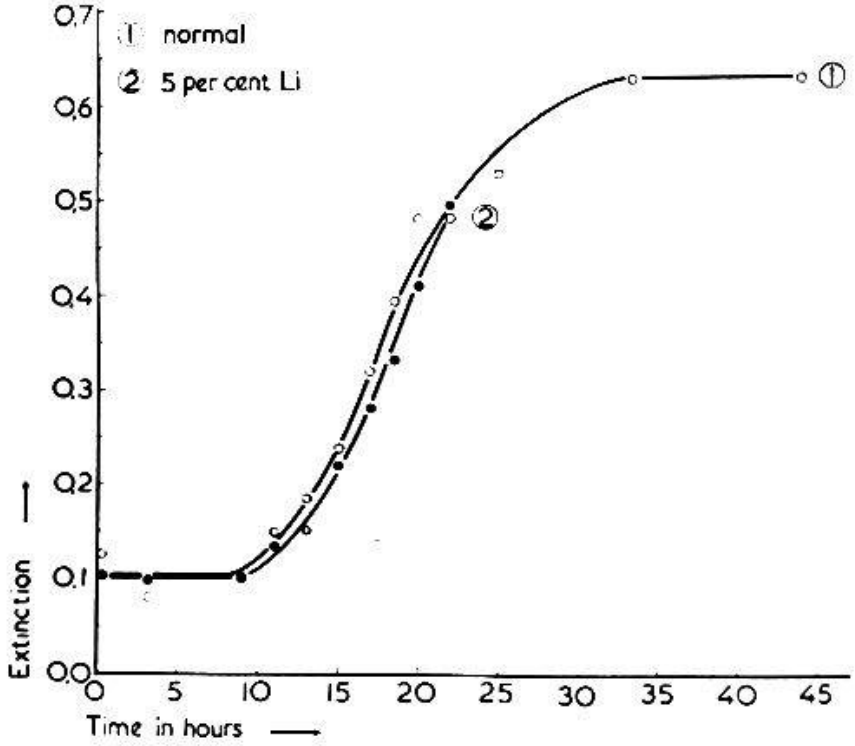

Fig. 32. Changes in alkaline phosphatase activity during the development of Psammechinus miliaris.—1 = normal embryos, 2 = vegetalized Li-treated embryos (from GUSTAFSON ans HASSELBERG: Exp. Cell Res. *1* [1950]).

filled. Matters are somewhat more obscure with respect to APh, for as shown in Fig. 32 there is substantial APh activity in the unfertilized egg (GUSTAFSON and HASSELBERG 1950). The moderate increase in relative enzyme activity during development shows that the amount of enzyme present at the beginning is quite substantial. In amphibian embryos matters are simpler, for here the enzyme activity before gastrulation is hardly measurable, the presence of APh at this stage can be questioned. The very low activity at the early stages is reflected in the large relative increase (cf. KRUGELIS 1950; KRUGELIS et al. 1952; DE CESARIS COROMALDI 1955; LØVTRUP 1955, 1958, 1959b).

There seems to be two possibilities to account for the experimental findings without violating the principles advocated above. The first one is that the activity at alkaline pH represents residual activity of the acid phosphatase. As we have seen above, the activity of this enzyme remains constant during development, and since its activity in the gastrula stage is not much lower than that of APh (Fig. 33), it follows that in the early stages it must have a much higher activity than the latter enzyme (cf. Fig. 32). The second possibility is that, as already mentioned, several alkaline phosphatases exist, one of which is specific for sf- and cf-cells, and that the activity present in the sea urchin egg represents an enzyme different from this one. The available evidence cannot decide the question but I would like to men-

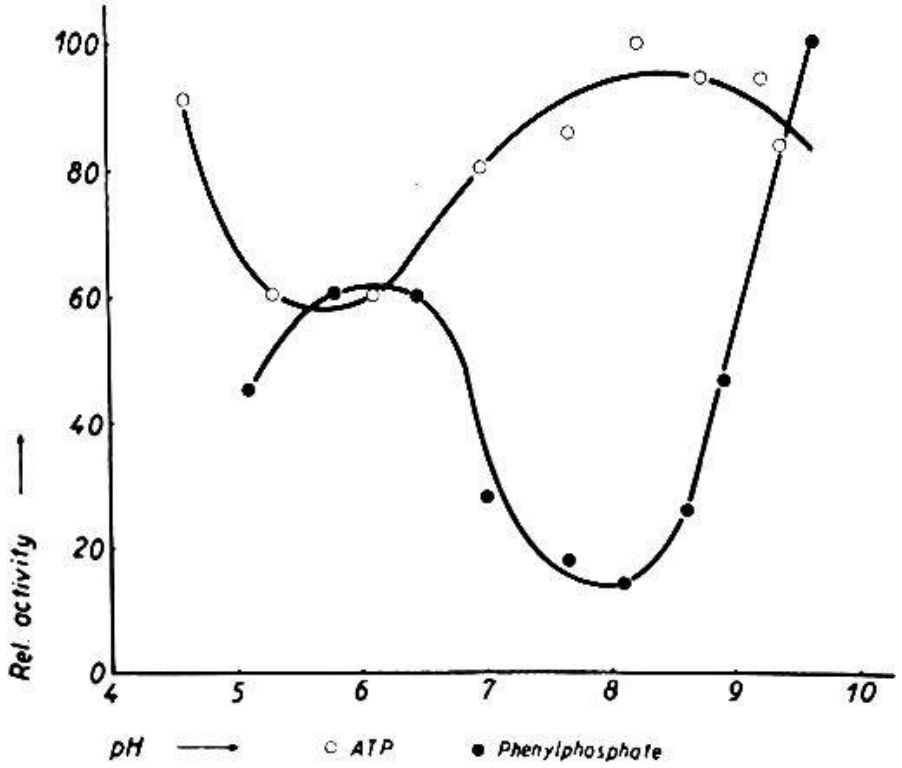

Fig. 33. Activity-pH curves for the splitting of ATP and phenylphosphate by extracts of Paracentrotus lividus gastrulae (from GUSTAFSON and HASSELBERG: Exp. Cell Res. 2 [1951]).

tion a few more facts in support of the view that APh is typical for cells possessing the f-property. Firstly, it is well known that several kinds of mechanocytes, for instance those concerned with bone formation, are rich in APh. Secondly, it has been possible to demonstrate acid, but not alkaline phosphatase activity in various protozoa (HOLTER and LOWY 1959; PIGON 1962). Since these—except probably the trypanosomas, which contain APh (HARVEY, 1949)—are either amoebocytes or epitheliocytes, this observation supports the suggestion that synthesis of APh constitutes part of the complex of properties characteristic for the f-property.

However, the strongest evidence in favour of my thesis is maybe the localization of the APh activity appearing after the mesenchyme blastula stage. By histochemical methods (Fig. 34) it has been found that all this activity is localized in the mesenchymal sf-cells and the endodermal cf-cells (EVOLA-MALTESE 1957; HSIAO and FUJII 1963). The latter authors published values suggesting that the relative increase in activity is much lower with glycerol-2-phosphate as substrate than is that found with phenylphosphate (GUSTAFSON and HASSELBERG 1950). This observation indicates that we are dealing with at least two separate enzymes with different substrate affinities, the one synthesized during development hydrolyzing phenylphosphate at a greater relative rate than the one present in the egg. Similar observations have been made with amphibian material (LØVTRUP 1955; URBANI 1962).

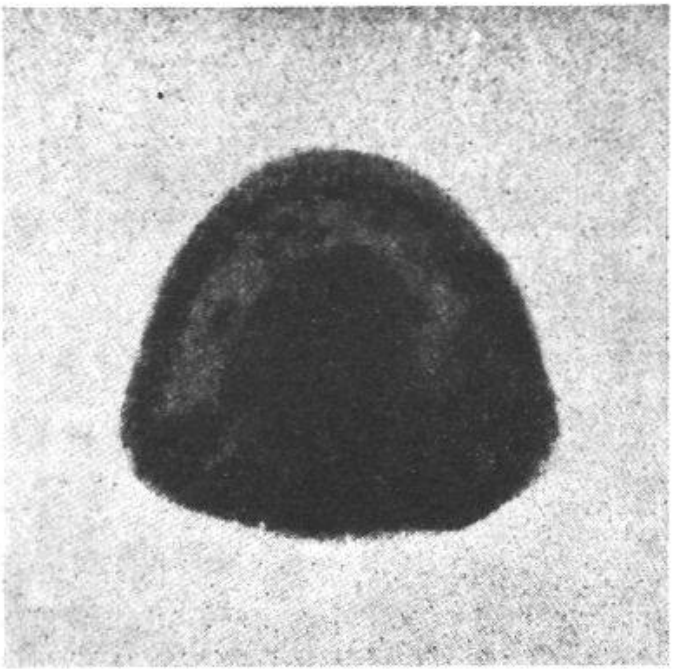

Fig. 34. Localization of alkaline phosphatase activity, as evidenced by the Gomeri method, in a gastrula of Paracentrotus lividus (from EVOLA-MALTESE: Acta Embryol. Morph. exp. (Palermo) *1* [1957]).

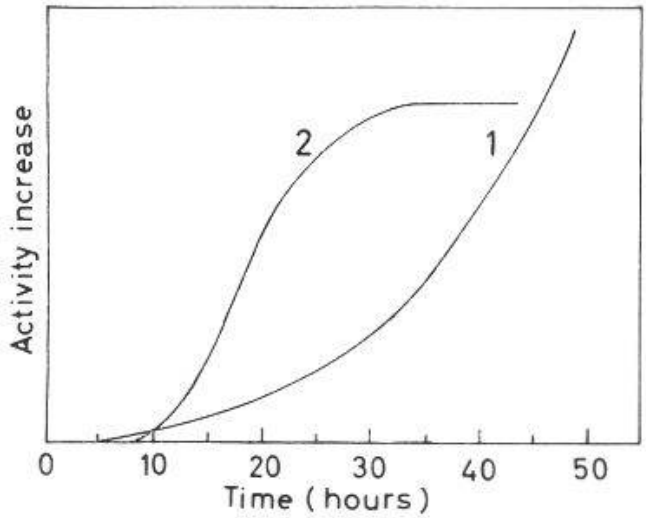

Fig. 35. Superimposition of the curves for acetylcholine esterase (Fig. 31) and alkaline phosphatase (Fig. 32).—1 = AChE, 2 = APh.—The time difference between the onset of synthesis of the two enzymes is clearly shown. In order to facilitate the comparison the early constant APh activity has been subtracted from all APh values.

The difference with respect to the time at which the synthesis of AChE and APh begins is shown in Fig. 35. The correlation with the two maxima in Fig. 30 is quite satisfactory.

d) Cell transformation and cell differentiation

At the conclusion of this section it is necessary to consider briefly the question about the separation of differentiation into two components, one concerned with cell transformation and one with the subsequent cell diffe-

rentiation. In the amphibian embryo it is easy, also with respect to enzyme synthesis, to distinguish between these two phases. Thus, the early, and very slight synthesis of AChE may be correlated with the formation of cells possessing the c-property, whereas the later and much more extensive phase of enzyme synthesis mainly corresponds to the differentiation of the nervous system (e.g. YOUNGSTROM 1938; SAWYER 1943; BOELL and SHEN 1950). A similar distinction can be made for APh activity between the formation of cells with the f-property and the differentiation of the intestine (e.g. LØVTRUP 1955). It might be argued that in the sea urchin embryo the later phases of synthesis of either of these enzymes represent cell differentiation in ectoderm and endoderm, and not cell transformation. It is very difficult to settle this matter as long as the investigations do not comprise enzymes typical for the cell differentiation process—as for instance trypsin in the amphibian embryo (cf. LØVTRUP 1955).

With this one reservation I think it can be accepted that the two enzymes, AChE and APh, can be considered as indices of the primary differentiation, the phase of cell transformations, in sea urchin development.

e) Enzyme patterns in animalized and vegetalized embryos

Animalization consists of a partial or complete suppression of the formation of cells with the f-property. In vegetalization a certain suppression of the acquisition of the c-property may occur, but the main factor seems to be an enhanced spreading of the f-property; the specific feature of vegetalization is the formation of cf-cells from potential cl-cells. We shall analyze the enzyme pattern in animalized and vegetalized embryos on the basis of this description of the processes, without any reference to the possible basic mechanisms involved.

Before we start this discussion be it mentioned that animalizing and vegetalizing agents are suggested to interfere with differentiation processes. As the latter are invariably associated with differential protein synthesis, it follows that we may expect to discover interference when changes in enzyme pattern occur, but in those cases where the activity remains constant there is no reason to presume any effect of the various morphogenetic agents. In all cases where this point has been tested experimentally, the expectation has been borne out (GUSTAFSON and HASSELBERG 1951).

Mitochondrial enzymes. – It was observed that the changes in the mitochondrial enzymes may partly represent a growth process, partly a differentiation process, since different types of mitochondria arise during development.

In embryos treated with Li^+ the synthesis of three mitochondrial enzymes, cathepsin B, glutaminase and ATPase, was found to be reduced by about 50% (GUSTAFSON and HASSELBERG 1951; cf. Fig. 24 and 25). We do not know enough about the enzyme contents of the various types of mitochondria to correlate this finding with the morphological changes.

Glucose-6-phosphate dehydrogenase. – This enzyme was suggested to be typical for amoebocytes, and must consequently disappear as a result of the cell transformations occurring during development. Since neither animalizing nor vegetalizing agents block the transformation processes as such, but only interfere with respect to the cell types arising, one might not expect any interference with the changes in this enzyme. As far as the rise and the decline is concerned this expectation is fulfilled (cf. Fig. 30), but certain displacements of the curves are observable; in animalized embryos the activity decrease is delayed, in vegetalized embryos it is accelerated (Bäckström 1959b). As a matter of fact, the rate of decline is faster in this case than indicated by the figure, for the results were obtained with the «difference method», in which embryos of the same age are compared; as Li^- has a slight retarding influence (cf. Fig. 32) it follows that if the results were compared with reference to morphological development, the curve for the vegetalized embryos should be located further towards the ordinate axis. The relative position of the three curves consequently seems to demonstrate that in Li^+-treated embryos cell transformation is accelerated, in embryos treated with o-iodobenzoic acid it is retarded.

The smoothening of the curves, as compared with that representing normal development, suggests in agreement with morphological observations that the fine pattern in the succession of the cell transformations is partly upset by the morphogenetic agents.

Acetylcholinesterase. – This enzyme, representative of cl- and cf-cells, might expectedly be present in enlarged amounts in animalized, and in reduced quantities in vegetalized embryos. Of these two cases only the latter has been investigated (Augustinsson and Gustafson 1949). It turned out that among the three phases of enzyme synthesis only the second one, representing the ectodermal differentiation, was suppressed. The first one, corresponding to the differentiation of the apical tuft and the ciliation of the superficial cells was not influenced, and the same holds for the third phase, related to the differentiation of the intestine. These results seem to be in conformity with morphological observations. Since the number of endodermal cells presumably is higher in a vegetalized embryo, one might expect the rate of synthesis in the third phase to be greater than in the normal embryo. The curves published by Augustinsson and Gustafson (cf. Fig. 31) do not support this possibility, but the number of determinations do not suffice to decide this question. However, since Li^+ interferes with the oxygen supply and the utilization of the reserve material, it is not unlikely that the synthetic activity may be interfered with. A similar situation obtains in amphibian embryos reared at high temperatures (Løvtrup 1953b).

Alkaline phosphatase. – One may expect that by sufficient strong animalization the synthesis of AlPh becomes completely suppressed. This question has not been investigated. Vegetalization furthers the formation of cells

possessing the f-property, and as we have seen above, it may even accelerate the rate of cell transformation. This acceleration is offset by a slight retardation of development, so no major change in the timing of the onset of synthesis may probably be anticipated, but a distinct enhancement of the rate of synthesis might be expected. Experiments show that the rate and extent of synthesis is unchanged. If one wishes to avoid any specific mechanism to be involved, I think that this result can be explained only by assuming interference with energy supply and utilization of reserve materials, as discussed above.

VII. Conclusion

1. Morphogenesis and phylogenesis

As has been discussed very briefly in the present paper morphogenesis is the outcome of the interplay, active or passive, between a limited number of different cell types. The egg cell, as well as the early blastomeres, belong to one and the same class, cells of the other types derive from the original ones through transformation processes. Of these there seems to be only two, consequently there can be only four different basic cell classes. All other kinds of cells may be regarded as further differentiations of each of these four types. It was shown above that the polarities of the sea urchin embryo determine the cell type distribution, and the same seems to hold for the amphibian embryo (LØVTRUP 1966). The primary morphogenesis can be considered the resultant of the interaction of these four cell types, and a few extracellular structures (cf. LØVTRUP 1965 a–c).

There are a number of interesting conclusions to be derived from the views presented here. BALDWIN (1937) stated: "Biologists have from time to time been impressed by the fact that the members of the animal kingdom fall into a relatively small number of types, in spite of a considerable degree of variation within each type ..." (l.c. p. 104). If the basic morphogenetic events, which of course must influence the pattern of all subsequent development, are determined mainly by the activity of these four cell types, then it seems obvious that the possible number of variations must be quite low. On the other hand, variations within each group of animals presumably is a result of differential protein synthesis, and here the possibilities are almost unlimited.

Another consequence is that phylogenetic evolution must largely be a result of changes in the cell distribution pattern during early embryogenesis. The first animal cell must have been a solitary cell, an amoeba or an amoebocyte. This archaeic cell is, to this very day, represented by each egg cell. The solitary amoeba represents, from an evolutionary point of view a blind alley; only when this cell had acquired the possibility to transform to other cell types were new roads open. The first new cell type which arose apparently was the epitheliocyte, probably in the flagellate form. The reversible transformation amoebocyte $\rightleftarrows$ flagellate can be observed in certain protozoa (cf.