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is somehow determined by variations of certain properties along the egg axes. I shall not discuss this problem here but refer to the discussion of the amphibian embryo (1966).

The principal polarities in the sea urchin embryo are the animal-vegetal (AV) and the vegetal-animal (VA) gradients (cf. RUNNSTRÖM 1929; HÖRSTADIUS 1935; LINDAHL 1936). That these polarities are concerned with the cell transformation processes is directly established by the fact that the strongly ciliated apical tuft cells (cl-cells) are formed at the animal cap, whereas mesenchymal sf-cells are formed from the cells around the vegetal pole (cf. Fig. 3 and 4). Between these extremes is found one further region of cl-cells, the presumptive ectoderm, and the endodermal cells, the presumptive intestine. As we shall discuss below there is good reason to presume that the endodermal cells, situated between the two gradients, undergo both transformations and become cf-cells.

III. Energy metabolism

1. Respiration

I shall in the present discussion make certain assumptions which, although maybe obvious, should be specified at the outset. The first one is that the oxygen consumption is a direct measure of the energy consumption, which implies that there exists a respiratory control mechanism. The energy will

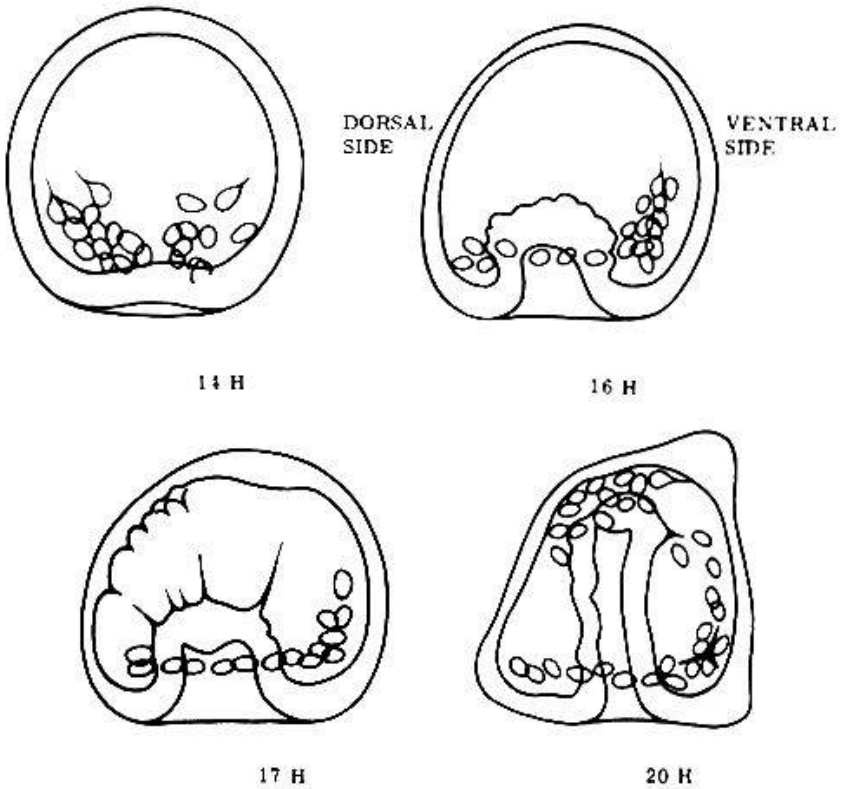


Fig. 4. Gastrulation in the sea urchin embryo. The formation of filiform pseudopodia in primary and secondary mesenchyme mechanocytes is indicated (from GUSTAFSON and WOLPERT: Int. Rev. Cytol. *15* [1963]).

be used partly for sustaining the cortical tension, the movements of the cells and other physical processes, and partly for the synthesis of various chemical compounds. Without trying to estimate the possible distribution of energy consumption between these two kinds of processes I shall make the further assumption that regions of the embryo which are distinguished by a lively chemical activity also consume more energy than the remaining parts.

The oxygen consumption during sea urchin development has often been studied (e.g. GRAY 1927; LINDAHL 1936, 1939b; BOREI 1948; WHITELEY and BALTZER 1958; IMMERS and RUNNSTRÖM 1960). The curves obtained exhibit a number of typical traits (Fig. 5). Shortly after fertilization the rate of respiration commences to rise, following appoximately an exponential course; around hatching this increase is interrupted by a phase of constancy lasting for some hours, but in the mesenchyme blastula the rate of oxygen consumption increases again, at first steeply, later on more slowly, after 30 hours a decline may even be observed. This latter phenomenon is probably reflecting an exhaustion of energy sources (cf. LØVTRUP 1959d). The phase of constancy apparently indicates that the formation of new energy consuming activities suddenly comes to a standstill, a quite astonishing phenomenon which has been the subject of much speculation. It must be stressed, however, that this conclusion is valid only on the assumption that the energy consumption is uniform throughout the embryo.

There are many lines of evidence suggesting that this is not the case. The

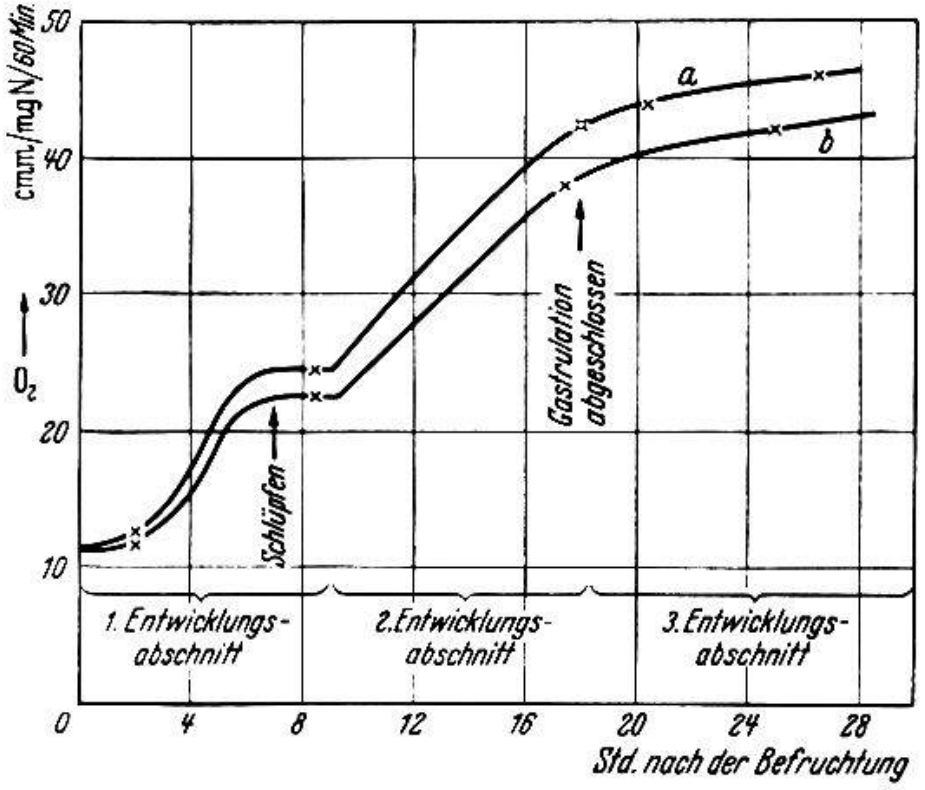


Fig. 5. Oxygen uptake during the embryonic development of Paracentrotus lividus. The curves represent two different batches.—Abscissa: Hours after fertilization. Ordinate: Oxygen consumption per mg N and hour (from LINDAHL: Z. vergl. Physiol. 27 [1939b]).

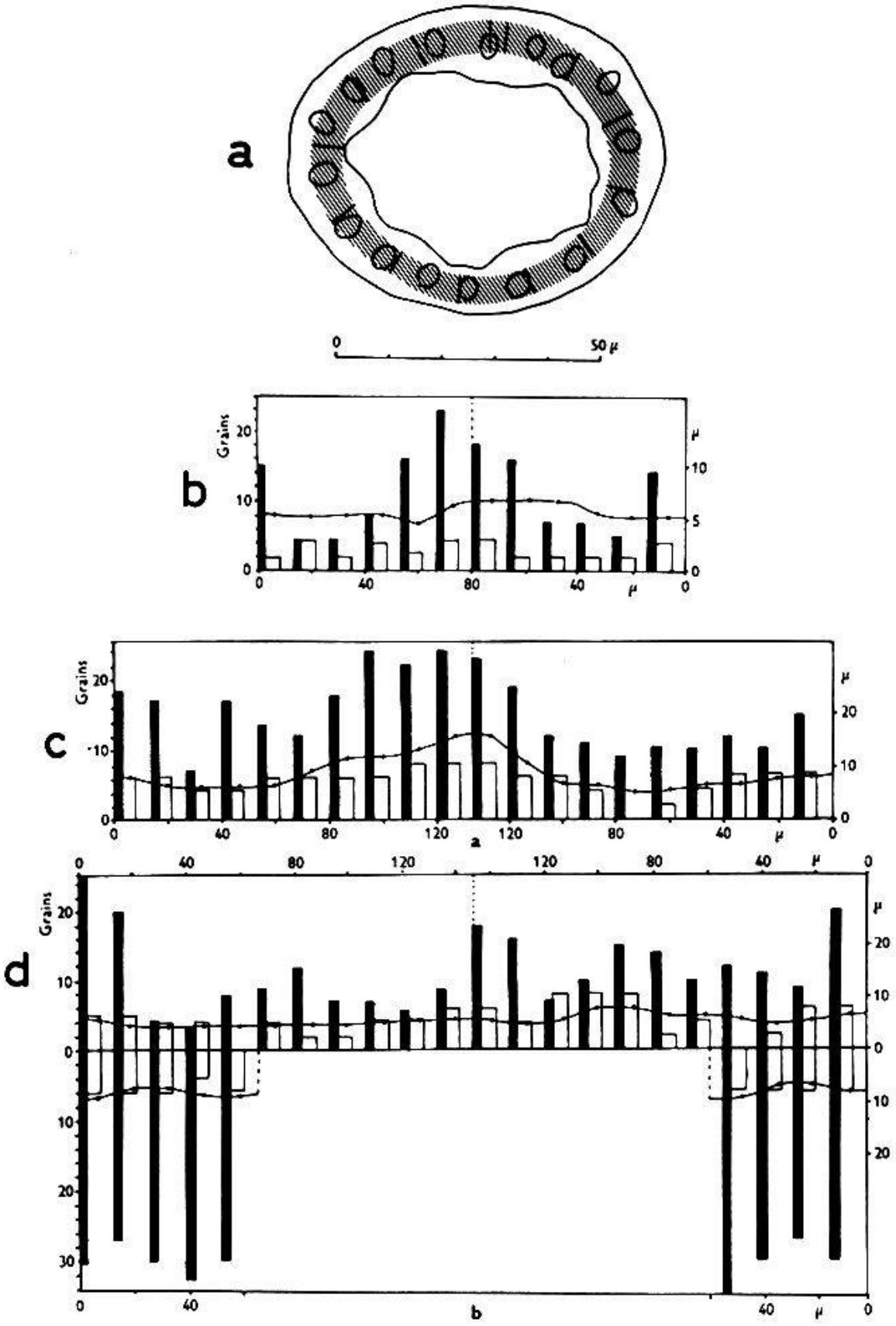


Fig. 6. Incorporation of L-leucine-^{14}C. In the histograms are shown the regional differences in an annular section (a). The ends of the histograms represent the vegetal pole, the centre the animal pole, colums turned downwards the invaginated part of the gastrula. The filled columns represent the number of grains, the open ones the number of nuclei, and the curve the thickness of the body wall.—b = Early blastula 8 h after fertilization (Paracentrotus lividus). c = Mesenchyme blastula 14 h after fertilization (Psammechinus microtuberculatus). d = Prism 24 h after fertilization, same species as c (from MARKMAN, Exp. Cell Res. 23 [1961a]).

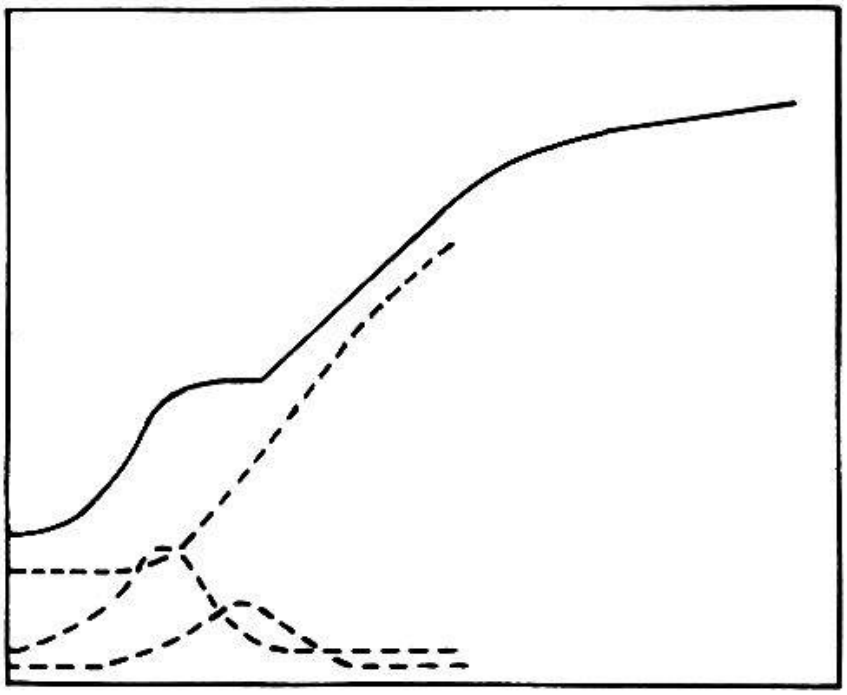


Fig. 7. Suggested resolution of curve b in Fig. 5.

work on reduction gradients (CHILD 1936; HÖRSTADIUS 1952; 1955; LALLIER 1958; BÄCKSTRÖM 1959c; CZIHAK 1962, 1963) suggests that there are two centers of high activity during early development, localized at the animal and the vegetal pole, respectively. The former begins somewhat before the latter. The mechanism underlying the appearance of the reduction gradients has recently been reviewed by GUSTAFSON (1966). I shall not here discuss this question but only, in agreement with this author, conclude that the coincidence in time between the reduction gradients and the waves of synthesis of enzymes of the pentose phosphate (PP) cycle observed by BÄCKSTRÖM (1959b, 1963) suggests that these phenomena are causally correlated. Since, as we shall discuss further in the sequel, these enzymes are part of the main source of energy supply in the early embryo, it is an obvious conclusion that two phases of energy consuming processes, spatially and temporally separated, exist in the early embryo. This point is also born out by the incorporation experiments of MARKMAN (1961a, 1961c). The results obtained show clearly that both RNA and protein synthesis (incorporation of adenine-8-^{14}C and L-leucine-^{14}C, respectively) during early development is particularly high in the most animal region. Gradually the rate of incorporation increases in the vegetal region; in the gastrula the highest rate is observed in the invaginated endodermal cells, whereas that obtaining around the animal pole has decreased considerably (cf. Fig. 6).

If the assumptions stated at the head of this chapter are correct it follows that the rate of respiration is not uniform in the sea urchin embryo; during the phase of constancy we may presume that two maxima of oxygen uptake occur in the animal and vegetal cells, coincident with the maxima of PP cycle enzymes observed by BÄCKSTRÖM (1959b, 1963; cf. Fig. 30). In Fig. 7

I have tried to indicate a resolution of the respiratory curves according to this suggestion; I have assumed that the volume of the animal cells involved in the first maximum comprise $^1/_5$, that of the vegetal cells in the second $^1/_{10}$ of the total volume, and that the rate of oxygen uptake is uniform before the specific activities begin. These assumptions may surely be questioned, but I think that the general argument can be upheld. It will be realized that this suggested resolution of the curves in no way is contradicted by the failure of LINDAHL and HOLTER (1940) to find an animal-vegetal difference in oxygen uptake, since respiration is supposed to increase at both apical ends.

2. Energy sources

We may now turn to the problem about which substances are utilized for covering the energy needs. It seems possible to concentrate the attention on three kinds of substances, viz., carbohydrates, lipids, and proteins, since these constitute the major part of the dry material in the embryo. We shall begin with a survey of some of the analyses made on the contents of the egg and of embryos at various stages.

a) Carbohydrates

The data compiled in Table 1 show that a considerable variation obtains with respect to the observed contents of carbohydrate. It is beyond the scope of the present paper to evaluate the methods employed, but it should be mentioned that HUTCHENS, KELTCH, KRAHL and CLOWES found the various steps involved in the glycogen determination to lead to a gradual loss of total acid-hydrolyzable carbohydrate, suggesting that not all of the polysaccharide present in the egg is insoluble in alcohol-water mixtures, or else that the part of the reducing substances is not polysaccharide. The

Table 1

The carbohydrate content of the sea urchin egg (pM per embryo)*

Authors	Total carbohydrate	Glycogen	Glucose
Paracentrotus lividus:			
EPHRUSSI and RAPKINE (1928)	47.5	45	–
ZIELINSKI (1939)	–	56.5	5.5
ÖRSTRÖM and LINDBERG (1940)	–	29	6.5
Arbacia punctulata:			
PERLZWEIG and BARRON (1928)	10.5	–	-
HUTCHENS et al. (1942)	22.5	10.5	–

* Conversion factors: Paracentrotus lividus: 36 mg N per ml eggs, 0.245 g dry weight per ml eggs, 0.58 ml per 10^6 eggs (ÖRSTRÖM and LINDBERG 1940).—Arbacia punctulata: 25 mg protein per mg N, 5.9 mg N per 10^5 eggs (HUTCHENS et al. 1942). pM: picomole.

difference between the results of ZIELINSKI (1938) and ÖRSTRÖM and LINDBERG (1940) may probably be accounted for if the first possibility prevails.

In the second case the ribose in the soluble RNA fraction may be a possible source of error; if we assume the reserve RNA be 10 000 pg, and the average molecular weight of a nucleotide 300, then there is about 30 pM ribose in this fraction. This value corresponds pretty well to the difference between the values of ZIELINSKI and ÖRSTRÖM and LINDBERG. The glycogen isolated from the sea urchin egg is similar to usual glycogen in most respects (HUTCHENS et al. 1942), but many results would be easier to explain on the assumption that another carbohydrate reserve is present with properties different from those found in other animal tissues.

ÖRSTRÖM and LINDBERG observed that 18% of the total carbohydrate—mainly glycogen—disappears in the 10 min following fertilization. ZIELINSKI found a decline of 4% during the first 2 h of development. EPHRUSSI found a 7% decrease in the glycogen content after 12 h, and no traces left after 40 h. In contrast to that no changes were observed in total carbohydrate after 12 h, and after 40 h there was still left about 63% (20 pM) of the original content. These results show that the carbohydrate reserves are used mainly for synthetic purposes, only about one third is claimed for supply of energy. In the pluteus there is 6–7000 pg DNA, with an average molecular weight of a nucleotide of about 300 this amount corresponds to about 20 pM deoxyribose, showing that a large part of the glucose is used for DNA-synthesis.

b) Lipids

EPHRUSSI and RAPKINE (1928) have determined the lipids in eggs and embryos of Paracentrotus lividus. Using the same conversion factors as in Table 1 their results show that the total lipid fraction amounts to about 30 000 pg in the egg, 27 600 pg in the 12 h embryo and 24 600 after 40 h of development. According to HAYES (1938) the egg of Arbacia punctulata contains 5650 pg. of this about 30% is lost during the 1st h; after 43 h of development 37% of the original content still remains. The lipids disappearing seem to be confined almost exclusively to the saponifiable fraction.

c) Proteins

Innumerable Kjeldahl determinations have been made on sea urchin eggs, but most often the results have been used for reference to other parameters, whereas data referring to the nitrogen content per egg during development are rare. EPHRUSSI and RAPKINE (1928) found that the nitrogen content in the egg, calculated as protein, corresponds to about 67% of the dry weight. A decrease was found during development, but when corrected for the increased ash content, the percentage of protein rather went up. Allowing for the reductions in the contents of lipids and carbohydrate the protein content seems to be quite constant. The determinations of total N by GUSTAFSON and HASSELBERG (1951) confirm this concluison.

It has been pointed out by BOELL (1955) that the ammonia formation demonstrated by HUTCHENS et al. (1942) implies that protein is combusted for energy supply. In contrast to ÖRSTRÖM (1941) who observed a transient ammonia formation after fertilization, these authors registered a continuous production of NH_3. According to LINDBERG (1945) this finding seems to result from a methodological error.

3. Oxidative metabolism in the early embryo

In spite of the relatively large variation in the various data presented above it seems certain that only carbohydrate and lipids are used to any significant extent for supply of energy during sea urchin development, whereas the protein reserves are used almost exclusively for synthetic purposes. This result is in complete agreement with my own observations on amphibian embryos (cf. e.g. 1953a, 1959a).

Normally the final oxidation of both carbohydrate and lipids (= fatty acids) passes via the Krebs cycle in the mitochondria. As we shall presently discuss the mitochondria in the sea urchin embryo during the early stages of development are quite normal as regards the activity of various enzymes, and a rather extensive production of new mitochondria occurs. Yet, in spite of this they seem to play an insignificant role in the energy metabolism. Several lines of evidence support this point. Thus, on the basis of incorporation studies involving labelled NH_3, alanine, CO_2 and acetate, HULTIN (1953c, d) was led to the conclusion that mitochondrial activity is very low before the mesenchyme blastula stage, but rises very rapidly with the beginning of this developmental phase (cf. Fig. 8). This suggests that there is a very distinct difference between the first and second generation mitochondria with respect to activity.

The slight inhibition by fluoracetate of the oxygen uptake in sea urchin eggs also suggests that acetate metabolism plays a minor role in the overall oxidative metabolism (CLELAND and ROTHSCHILD 1952b), a finding which completely confirms HULTIN'S observations.

Studies of the formation of CO_2 from labelled glucose has given results which suggest that during early development this substance is oxidized in the PP cycle, whereas the Krebs cycle seems not to be involved in glucose turnover (KRAHL 1956; BÄCKSTRÖM et al. 1960). This must imply that intermediates accumulate without being metabolized by the mitochondria.

As regards the apparent deficiency in the mitochondrial function it seems possible that the activity of one or more enzymes is so low as to limit mitochondrial function. It is, in this connection, interesting to note that KRAHL et al. (1941) found the succinate dehydrogenase (SDH) activity too low to be of significance for the respiration of the egg; similarly CRANE and KELTCH (1949) found that in a cell-free system the oxygen uptake in the presence of oxalacetate is only about half the respiration of intact fertilized eggs.

Another possibility has been suggested by CLELAND and ROTHSCHILD

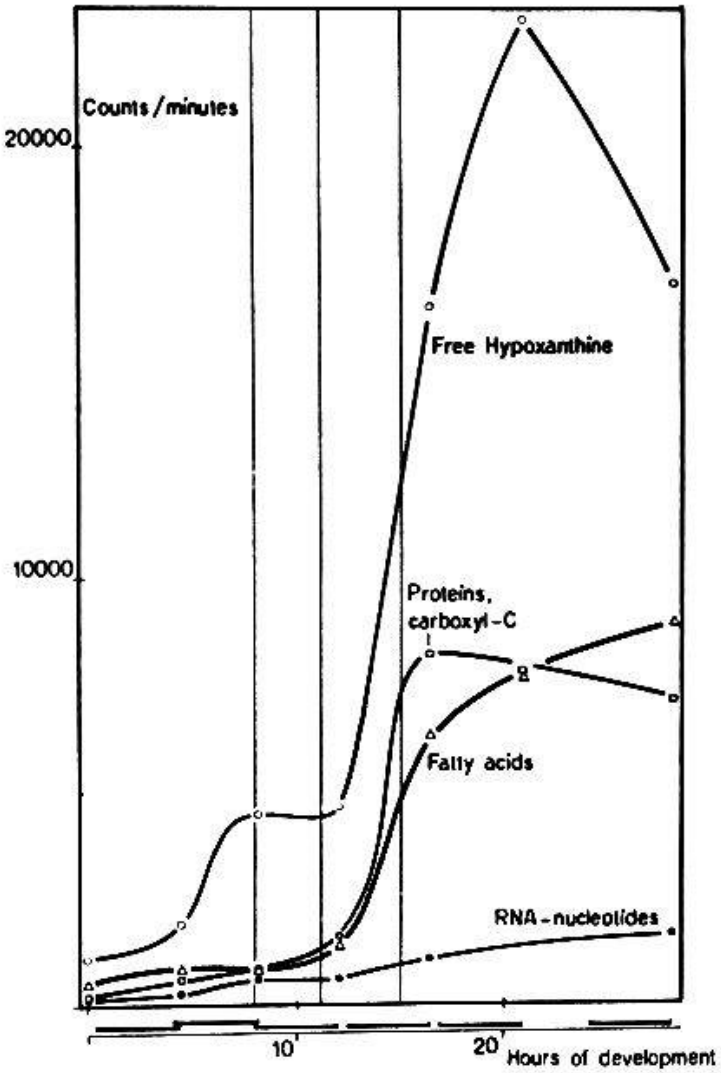


Fig. 8. Incorporation of 1-^{14}C-acetate in embryo of Psammechinus miliaris at different stages of development. Vertical lines indicate hatching, appearance of mesenchyme cells and start of invagination, respectively. The periods of isotope treatment are indicated by horizontal lines at the bottom of the figure (from HULTIN: Ark. Kemi *6* [1953d]).

(1952b). On the basis of studies of the oxidation of pyruvate these authors came to the conclusion that in many enzyme preparations the activity of the condensing enzyme, catalyzing the reaction: "acetate" + oxalacetate → citrate, is a limiting factor. If this is correct the block is to be found at the very entrance to the Krebs cycle, and thus to the mitochondria. An accumulation of acetate could possibly be anticipated as a consequence of this enzymatic deficiency.

The low mitochondrial activity seems to preclude that either of the potential energy sources to any substantial extent is completely oxidized during early development.

4. Utilization of energy sources

Determinations of RQ constitute the classical approach to the analysis of the contribution of various energy sources to the total consumption of energy, and also in studies on sea urchin development has this method been

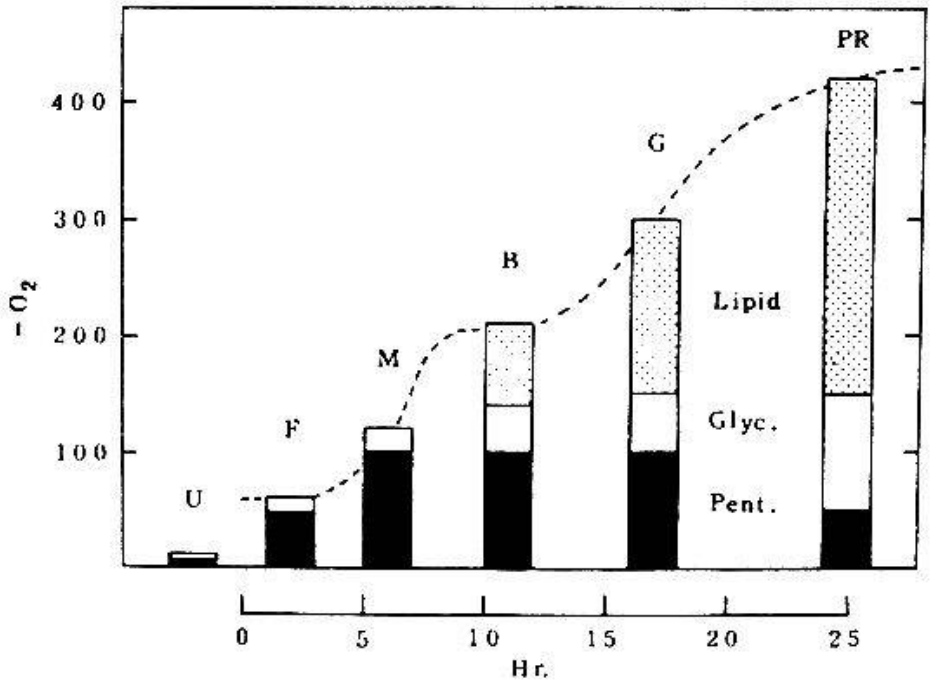


Fig. 9. Utilization of energy sources during the development of Anthocidaris crassipina. —Abscissa: Hours after fertilization at 20° C. Ordinate: μl O_2 per 10^6 embryos and hour.—U = unfertilized eggs, F = fertilized eggs, M = morula, B = swimming blastula, G = gastrula, PR = prism.—Black zone (Pent.): Carbohydrate oxidation involving pentose phosphate cycle enzymes. White zone (Glyc.): Carbohydrate oxidation involving glycolysis and mitochondrial enzymes. Dotted zone: Oxidation of lipids. Dashed line: Changes in the rate of oxygen uptake (from ISONO, quoted by GUSTAFSON in: WEBER: Biochemistry of animal development [1966]).

applied (e.g. LASER and ROTHSCHILD 1939; ÖHMAN 1940; HUTCHENS et al. 1942). However, RQ determinations presuppose that the energy sources are completely oxidized, and since that precondition is not fulfilled in the early sea urchin embryo, this procedure may lead to erroneous conclusions.

The accuracy of the chemical determinations do not suffice to correlate oxygen supply and consumption of energy sources, especially in view of the incomplete oxidation of occurring glucose. Fortunately, ISONO (quoted by GUSTAFSON 1966) has investigated the relative contribution of the energy sources, also with respect to the metabolic pathways, during the first 25 h of development (Fig. 9).

The work of ISONO has not been available to me, and I have therefore had no opportunity to evaluate the results. Since furthermore the species studied by him (Anthocidaris crassipina) is different from the one on which the reported chemical analyses have been obtained, no direct comparison between the results is possible. All the same it seems possible to use ISONO's findings for a comparison between oxygen consumption and substrate utilization in Paracentrotus lividus. The area under the respiratory curve in Fig. 9 may be divided into three components, supposed to represent oxidation of carbohydrate through the pentose phosphate (PP) cycle or through the Krebs cycle, and of lipid through the latter pathway. It can be estimated that they correspond to 35, 20, and 45%, respectively, of the total oxygen consumed.

Using these values, and various data on oxygen and substrate consumption, it is possible to make a balance sheet (Table 2). It is seen that if the glucose supposed to pass through the PP cycle is not completely oxidized then the carbohydrate consumption must be much higher than anything which can be accounted for by chemical analyses. Even if an intermediate is accumulated and oxidized when the mitochondrial activity becomes more efficient, it is still impossible to account for the oxygen consumption during the development of Paracentrotus lividus. It seems unavoidable to conclude that there are present carbohydrate or other reserves that are not determined by the methods employed, or else that the oxygen consumption values are too high. In the American species the discrepancy is not very large, especially considering that a major part of the carbohydrate left in the embryo will represent glucose transformed into deoxyribose, a process which consumes 0.5 mole O_2 per mole. It should be mentioned, however, that during the early phases of development HUTCHENS et al. (1942) could not register a loss in the carbohydrate content corresponding to the oxygen consumption.

Altogether it seems that, unless the paper of ISONO has cleared up this problem conclusively, there are still several questions concerning the energy supply in sea urchin development that await their final solution.

5. Metabolic pathways in the glucose metabolism during early development

The breakdown of glucose preceding the final oxidation in the mitochondria may occur along two main metabolic pathways, either through glycolysis or through the PP cycle. Until the presence of the latter was suggested by LINDBERG (1943) glycolysis was presumed to be the major pathway for glucose breakdown, even if lack of inhibition by iodoacetate (RUNNSTRÖM 1933) suggested certain peculiarities.

Since then much study has been centered around the question whether both pathways exist in the embryo, or whether only the PP cycle is present. Various experimental approaches have been employed in this connection, mainly studies of isolated enzyme reactions and of the effect of inhibitors. Since the latter method may give the most ambiguous results, I shall deal first with those in the former group.

KRAHL et al. (1954) have studied the hexokinase activity in Arbacia punctulata. As appears from Table 3, glucose, 2-deoxyglucose, mannose and fructose are phosphorylated at appreciable rates, but glucose-6-phosphate (G-6-P) and fructose-6-phosphate (F-6-P) are hardly attacked. This very low phosphofructokinase activity does not support the contention that there is an active glycolytic pathway.

Studying the NADP reduction with various substrates KRAHL et al. (1955) could establish the presence of glucose-6-phosphate dehydrogenase (G-6-PDH), 6-phosphogluconate dehydrogenase (6-PGDH), phosphoglucomutase, hexose isomerase, and fructose-1,6-diphosphatase. The two first are

Table 2

Energy metabolism during the first 25 hours of sea urchin development*

	Oxygen consumption per embryo (pM)				Substrate consumption per embryo						
					Lipids (pg)		Glucose (pM)			Total	
	Total	Lipids (45%)[1]	Glucose in Krebs cycle (20%)[1]	Glucose in PP (35%)[1]	calculated[2]	found	Krebs cycle[3]	PP cycle[4]	PP cycle with complete oxidation[3]	calculated	found
Paracentrotus lividus	670[5]	300	135	235	3350	5400[6] (40 h)	22	115	39	61	18[6]
Arbacia punctulata	130[7]	59	26	45	655	0?[8] 2000?[8]	4.3	22.5	8.3	12.6	8.5[8]

* Conversion factors: P. lividus: 36 mg N per ml eggs; 0.58 ml per 10^6 eggs (Örström and Lindberg 1940). A. punctulata: 25 mg N per g wet weight; 5.9 mg N per 10^6 eggs (Hutchens et al. 1942)

[1] Isono (quoted by Gustafson 1966)

[2] 1 g fat requires 2.02 l or 0.09 mole oxygen for complete oxidation

[3] 1 mole glucose requires 6 moles oxygen for complete oxidation

[4] 1 mole glucose requires 2 moles oxygen for conversion to acetate

[5] Lindahl (1939b)

[6] Ephrussi and Rapkine (1928). The values are not corrected for the increase in ash content. If that is done, the lipid consumption becomes negligible, and that of glucose 12.5 pg

[7] Hutchens et al. (1942)

[8] Hayes (1938)

Table 3

Relative rates of phosphorylation of various hexose compounds by hexokinase preparations from Arbacia punctulata (from KRAHL et al. 1954).

	Manometric method	Photometric indicator method
Glucose	1.0	1.0
2-Deoxyglucose	2.0	2.0
Glucosamine	0.6	–
Mannose	1.2	1.2
Fructose	1.8	1.7
Galactose.....	0.0	–
Glucose-6-phosphate	0.0	–
Fructose-6-phosphate	0.2	

normal components of the PP cycle, the third and the fourth of the glycolytic pathway. When fructose-1,6-diphosphate (HDP) was incubated with NAD, the coenzyme reduction proceeded at a rate of 1–8% of that observed with NADP in the presence of G-6-P. The HDP-NAD reaction is supposed to involve two steps, splitting of the substrate by aldolase into two triosephosphates and oxidation of the latter by triosephosphate dehydrogenase (TPDH). In this procedure the observed rate must be that of the slowest reaction; in spite of the slight activity the result must be considered quite remarkable, since JANDORF and KRAHL (1942) could observe no TPDH activity in egg extracts. These results show at best that glycolysis may occur in the sea urchin egg, but that it is of extremely low quantitative importance. However, the findings are not conclusive as to the presence of the glycolytic pathway; it cannot be excluded that the observed reduction of NAD depends upon the activity of other enzymes than TPDH.

The presence of this enzyme in sea urchin eggs was first questioned by RUNNSTRÖM (1933), when he showed that iodoacetate, an inhibitor of TPDH, did not interfere with the respiration. This observation was confirmed on homogenates by LINDBERG and ERNSTER (1948), and seems to constitute a support of the findings of JANDORF and KRAHL mentioned above. However, the use of inhibitors may be ambiguous, and opposite findings have indeed been reported (cf. below), so these results may not either be entirely conclusive. LINDBERG and ERNSTER also found that the oxygen uptake in homogenates in the presence of HDP and NAD was the same as in the absence of substrate; an increase occurred after addition of iodoacetate. These findings do not lend much support to the contention that either aldolase or TPDH are present in the sea urchin egg.

CLELAND and ROTHSCHILD (1952a) observed the formation of lactate and pyruvate under anaerobic conditions, but as this activity began only after a protracted lag period it was suggested to involve an activation of a glycolytic system through damage to the eggs. Formation of lactate or pyruvate

is not, in itself, a token of glycolytic activity, for triosephosphate may also arise through the PP cycle. The use of inhibitors do not much clarify the issue, for phloridzin, iodoacetate and fluoride all inhibit steps that are common to the two pathways, viz., phosphorylase and hexokinase, TPDH, and enolase, respectively.

Yčas (1954) has attempted to determine the activity of aldolase through the formation of triosephosphate from HDP. But as we have seen above from the work of Krahl et al. (1955) the possibility exists that HDP may enter the PP cycle, so even in this case it is impossible to accept the findings as conclusive evidence.

In a discussion of PP cycle it has been stressed by Pon (1964) how difficult it is, by metabolic experiments, to decide between the presence of either of the two metabolic pathways for glucose, particularly because several of the enzymes are the same for both. It appears from the discussion above that the experimental evidence can neither exclude, nor prove that glycolysis occurs in the sea urchin egg. The tenacious attempts to demonstrate that it is present may stem from the conviction that since glycolysis has been found in next to all animal cells, including, I believe, the adult sea urchin, it should consequently be present also in the egg.

This contention is fallacious, because it does not take into consideration the phenomenon of biochemical evolution. It is well known that during development changes occur not only on the morphological level, but also on the intracellular level, as expressed by the acquisition of new synthetic capacities, etc., and that these ontogenetic changes in many cases may be correlated with phylogenetic development (cf. Wald 1952; Baldwin 1963).

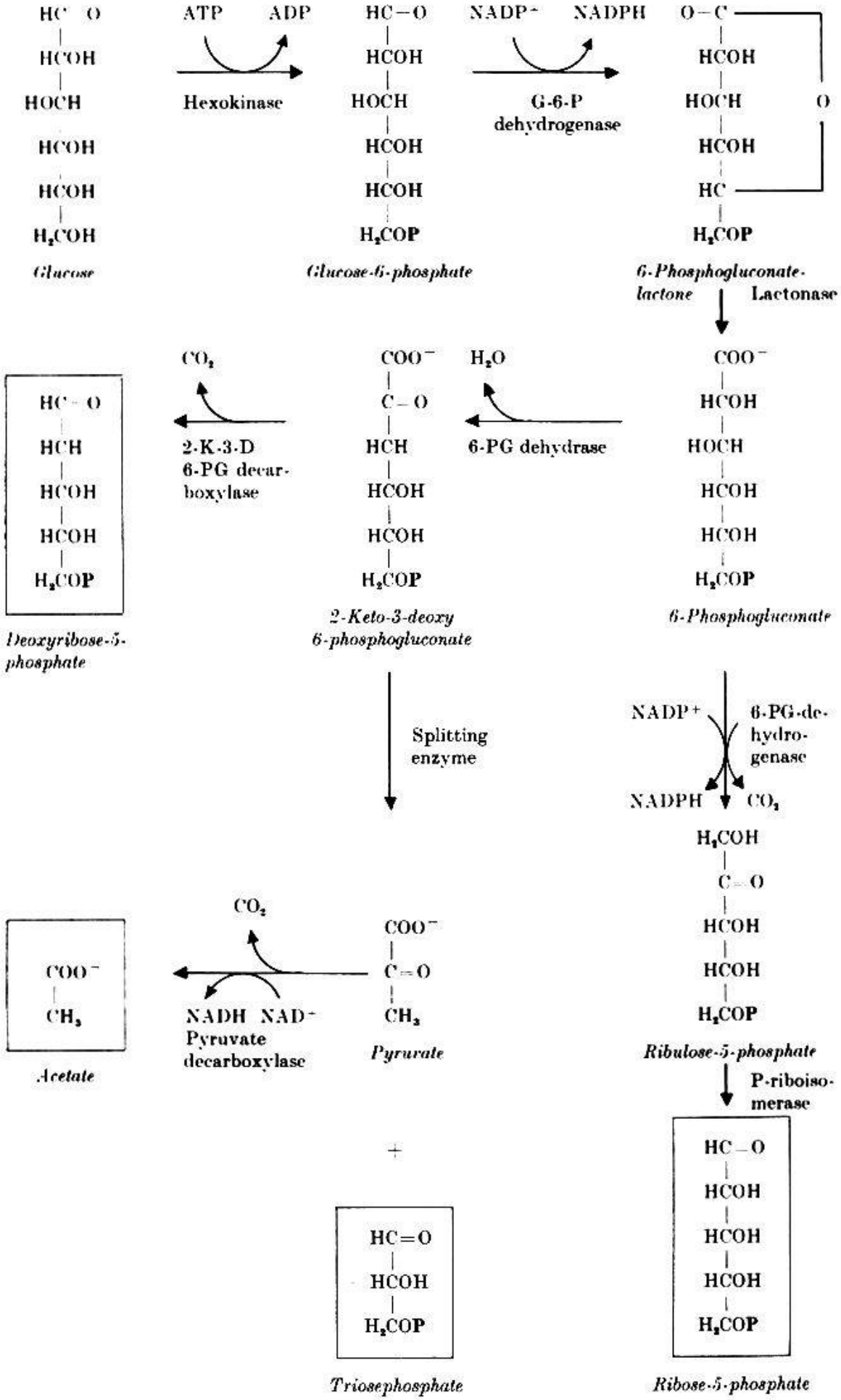
If we are allowed to extrapolate these findings back to the unicellular level, i.e., the egg, then we might expect the latter to exhibit a number of properties which today are found in unicellular organisms, i.e., in protozoa. It seems that many protozoa possess the glycolytic pathway, others have the PP cycle, some even seem to possess none of these. Thus if this phylogenetic view is valid, then there is nothing objectionable in the finding that no glycolysis occurs in the egg. It is obvious that from a practical point of view it is rather immaterial whether or not both pathways are present, because glycolysis under any circumstances is quantitatively unimportant, but theoretically it makes a great difference. It is therefore to be wished that experiments to settle definitely this question may soon be performed.

Having disposed of glycolysis for the time being, we shall turn our attention to the PP cycle. This metabolic pathway may lead to formation of ribose-5-phosphate, but it may also accomplish the formation of triosephosphate, which in turn may be oxidized to pyruvate, the first step being catalyzed by TPDH. As we have just discussed this enzyme may not be present, in which case even the PP cycle is excluded as the pathway for energy supply.

We must therefore look for a metabolic pathway leading to pyruvate without TPDH being involved. This possibility is found in the Entner-Doudoroff pathway (Table 4). The primary end products of this metabolic

Table 4

A proposal for the metabolic turnover of glucose in the sea urchin egg



sequence are as indicated triosephosphate and pyruvate, but the possibility obtains that also deoxyribose and ribose may be formed.

Pyruvate may be decarboxylated oxidatively to acetate (RUNNSTRÖM 1933; KRAHL et al. 1942; CLELAND and ROTHSCHILD 1952b). As we have discussed above, it seems that the oxidation of acetate in the mitochondria proceeds at a very slow rate during early development, a certain accumulation thus probably will occur. Could not the much discussed acid, liberated at fertilization, be acetic acid? Under anaerobic conditions it seems that pyruvate may be transformed into lactate, even though the mechanism involved still is veiled by many question marks (cf. CLELAND and ROTHSCHILD 1952a).

It follows from the discussion above that triosephosphate at best can be oxidized very slowly, probably a certain accumulation occurs until more efficient metabolic pathways are established as a result of the cell transformations occurring during development. The accumulation of this intermediate may possibly account for the failure of certain authors in demonstrating a loss in total (reducing) carbohydrate (EPHRUSSI and RAPKINE 1928; HUTCHENS et al. 1942).

It may seem preposterous to suggest a metabolic pathway entirely different from anything otherwise existent in animal cells, even if the available experimental evidence is compatible with this proposal. This statement may not be valid for the inhibition experiments, actually we may not know enough about the enzymes involved in the Entner-Doudoroff pathway to make any certain predictions about the effects of various inhibitors.

However, this kind of pathway is not completely unsubstantiated, if we accept the phylogenetic point of view. The parasitic protozoon, Entamoeba histolytica, contains G-6-PDH but not 6-PGDH. Ribose-5-phosphate is a growth requirement for this organism, and the end products of G-6-P metabolism are pyruvate and triosephosphate (HILKER and WHITE 1959; HILKER 1959 quoted by PON 1964). The presence of 6-PGDH constitutes a difference between the sea urchin egg and this amoeba, suggesting that ribose may be formed by the egg, but before conclusively proven, there seems to be no reason to postulate that other enzymes of the PP cycle are present, and if this is correct it follows that even the PP cycle is absent in the sea urchin egg.

The present discussion has not been intended to establish the mechanism of energy supply in the sea urchin embryo; that can be done only by experiments. I do hope, however, that this survey will show that this question is far from settled, and also that to solve the problem it probably must be approached without any reference to mechanism found in adult tissues.

6. Phosphagen

In studies on the acid soluble phosphorous compounds LINDBERG (1943) found four different fractions in the ovary of Brissopsis sp., viz., inorganic

phosphate (46%), ATP (27%), a very slowly hydrolyzable compound (12%), and arginine phosphate (15%). In the eggs of this species the analyses showed the last compound to be absent, the other three were found in the concentrations 53%, 31%, and 16%, respectively. Later LINDBERG (1945) isolated the slowly hydrolyzable ester from cow brain and found it to be propanediol-1-phosphate (PDP). The compound was found to stimulate oxygen uptake and pentose formation in homogenates of sea urchin eggs. After injection to rats the specific activity of the substance was found, in the liver, to decrease slowly, whereas the activity in other, more easily hydrolyzable phosphate compounds increased, suggesting either that the organic part of the ester is transformed or that the phosphate is transferred from the PDP to other compounds. In the latter case propanediol phosphate would act as a phosphagen. The various observations made by LINDBERG do not contradict this suggestion.

As a matter of fact, the analyses reported above lead to the conclusion that either PDP is the phosphagen of the sea urchin egg, or else this cell has no phosphagen. Whatever is correct, it leads to an extension of the somewhat simplified (cf. BALDWIN, 1963) phylogenetic sequence of phosphagens:

none or PDP	→	arginine phosphate	→	creatine phosphate.
(sea urchin) eggs		invertebrates		vertebrates

The circumstance that slowly hydrolyzable phosphate esters are found in many eggs (cf. NEEDHAM, 1942) is may be a further support for the phylogenetic approach suggested here. It may be mentioned that CRANE and KELTCH (1949) could observe no phosphorylation of arginine in cell free preparation of sea urchin eggs.

In certain protozoa and lower metazoa phosphagens have been found that are slowly hydrolyzable, and distinctly different from arginine phosphate, but as far as can be judged from the rate of hydrolysis they are also different from PDP (cf. SEAMAN 1952).

It seems astonishing that this interesting compound, which apparently is found in, and metabolized by mammalian tissues, has not been subject to further studies. None of the metabolic pathways in the current biochemical repertoire can account for the formation of PDP. It should therefore be pointed out that this substance, together with triose, may be formed by the splitting of fucose, the carbohydrate so typical for eggs (cf. VASSEUR, 1952).

IV. Synthetic activities

The synthetic activity in the embryo involves the formation of new carbohydrates, lipids, nucleic acids and proteins etc. We have seen in the previous chapter that the two former represent the main sources of energy supply, but that only a minor part of the reserves present in the egg is used for this