

Synthetic activities

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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

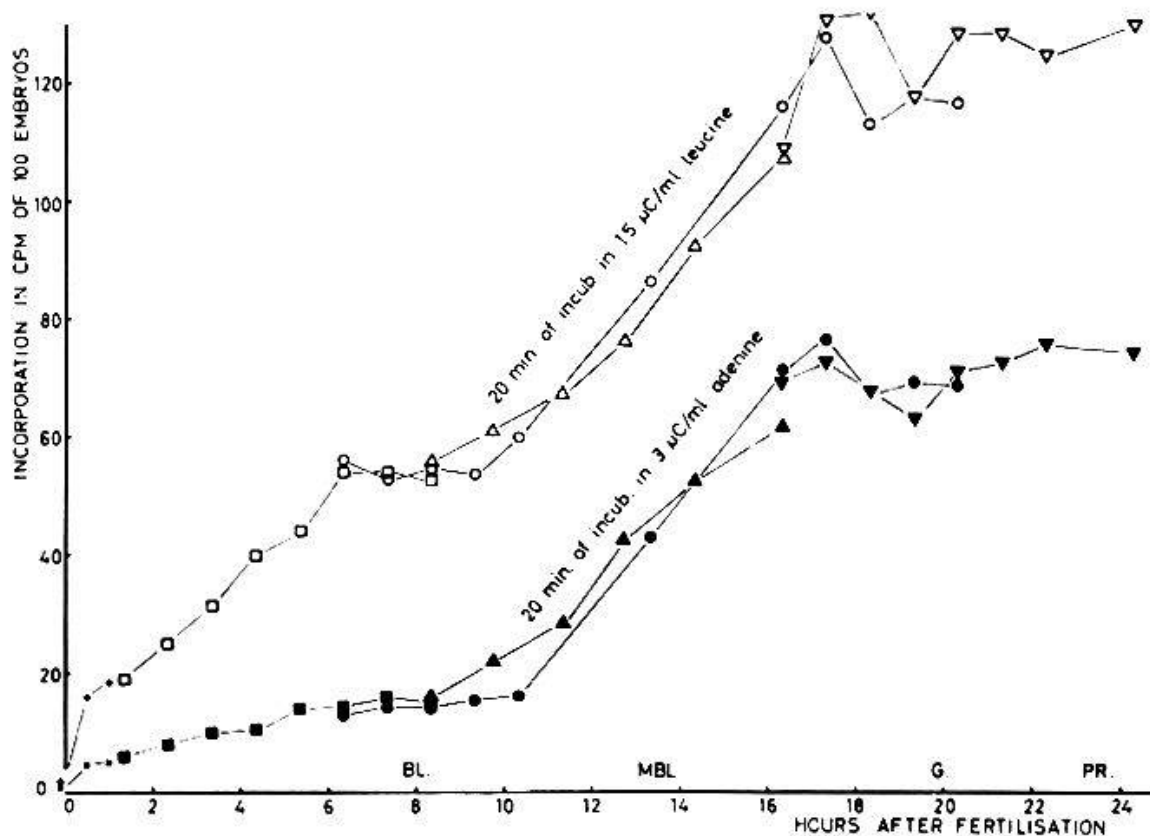


Fig. 10. Incorporation of L-leucine- ^{14}C (open symbols) and adenine-8- ^{14}C (filled symbols) into embryos of *Paracentrotus lividus* at various developmental stages.—BL = blastula, MBL = mesenchyme blastula, G = gastrula, PR = prism (from MARKMAN: Exp. Cell Res. 23 [1961 b]).

purpose. The carbohydrates and lipids present in the pluteus larva are presumably incorporated in the larval body. More than half of this carbohydrate was shown to be used for deoxyribose synthesis, most of the remaining may be used for synthesis of mucopolysaccharides (cf. IMMERS 1955, 1956). The major part of the lipid reserves is probably used for elaboration of various membrane structures. However, very little is known about the synthetic activities involving these two groups of substances, and we shall therefore concentrate our attention upon the reserves of nucleic acids and proteins, and their utilization for various synthetic purposes.

Judging from incorporation studies involving purines and amino acids, the synthesis of both types of macromolecules begins at an early stage of development. As shown by MARKMAN (1961b) the rate of incorporation changes with time according to a curve with several phases (cf. Fig. 10).

It has been shown by KAVANAU (1954) that the protein synthesis occurs in distinctly separate stages (Fig. 11). From the time correlation these are seen to correspond to the phases of protein and RNA synthesis observed by MARKMAN, and especially to the changes in G-6-PDH and 6-PGDH activity (BÄCKSTRÖM 1959b, 1963; cf. Fig. 30).

On the basis of his studies on the incorporation of $^{15}\text{NH}_3$ and ^{15}N -alanine HULTIN (1953b, c) was able to demonstrate that a fundamental change

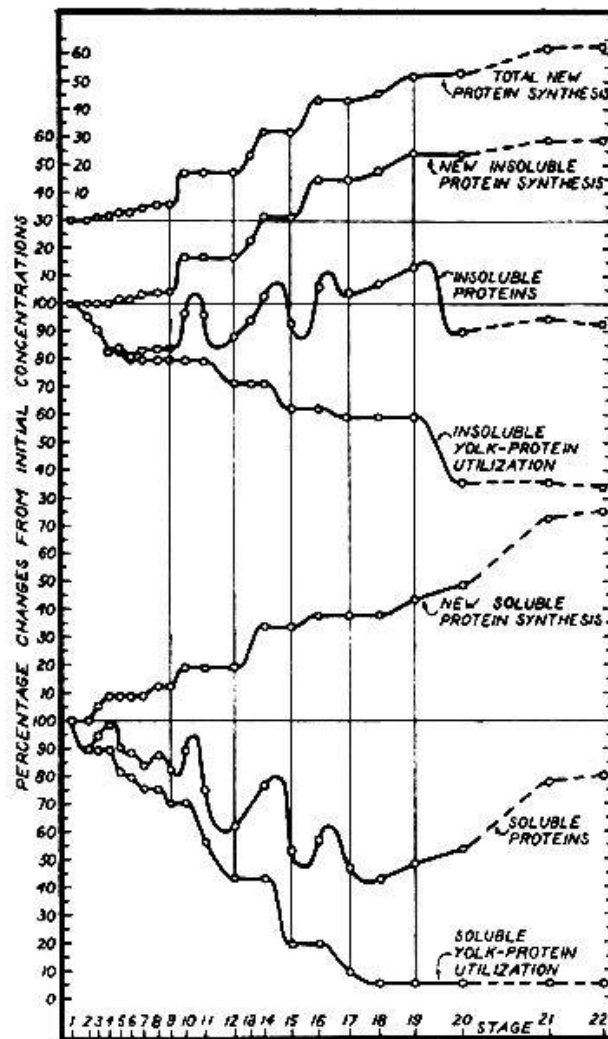


Fig. 11. Average percentage changes in various protein fractions during the development of *Paracentrotus lividus*. The curves illustrate quite clearly that protein synthesis occurs in several distinct phases (from KAVANAU: Exp. Cell Res. 7 [1954]).

occurs in the amino-acid-protein metabolism at the mesenchyme blastula stage. Before this stage "the nitrogen metabolism of amino acids is substantially restricted to exchange reactions at the amino acid level", but subsequently "the metabolic accessibility of the amino acids is considerably increased ..., the exchange reactions between amino acid nitrogen and ammonia being successively intensified" (1953c, p. 550). HULTIN considers this change to be correlated with increased mitochondrial activity.

All this synthetic activity is sustained by the conversion of inert reserves present in the egg. Since the content of amino acids decreases (e.g. GUSTAFSON and HJELTE 1951; KAVANAU 1954), and the total content of nucleic acid increases (e.g., ELSON et al., 1954) it may be inferred that there is a synthesis of nucleotides from amino acids. Determinations of the nucleic acids show that there is a substantial increase in the DNA content, whereas the RNA content remains constant, or declines slightly. However, as there is a quite extensive incorporation of isotopes into the RNA fraction throughout development, it follows that the RNA present at the outset must re-

Table 5

DNA content in sea urchin gametes and embryos (pg per gamete or embryo)*

CRANE (1947)	SCHMIDT et al. (1948)	VILLEE et al. (1949)	VENDRELY and VENDRELY (1949)
<i>Arbacia punctulata</i>			
sperm			0.7**
egg	700-1000	120 (3 h)	220**
pluteus	10 700		
Separation, P determination	Separation, P determination	Separation, P determination	Colorimetric deoxyribose determination
<i>Paracentrotus lividus</i>			
BRACHET (1933)	ELSON and CHARGAFF (1952); ELSON et al. (1952)	HOFF-JØRGENSEN (1954)	WHITELEY and BALTZER (1958) BALTZER (1960)
sperm		0.7	
egg	20-30	18	40 ©
pluteus	8000	700	4-5000
Colorimetric deoxyribose determination	Separation, microbiol. thymine determination	Microbiol. deoxyriboside determination	Colorimetric deoxyribose determination
		Separation, UV absorb.	Separation, UV absorbation

* Conversion factors: *Arbacia punctulata*: 5.9 mg N per 10⁶ eggs, 0.025 mg N per mg wet weight (HUTCHENS et al. 1942). *Paracentrotus lividus*: 4.07 ml eggs per g dry weight, 1.7 × 10⁶ eggs per ml (ÖRSTRÖM and LINDBERG, 1940).

** *Abacia equituberculata*.

*** No correction for change in dry weight.

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present reserves which are gradually transformed during development. The question about the utilization of these for synthesis of either nucleic acid, as well as the possible presence of a DNA reserve, will be discussed on the following pages.

V. Nucleic acid synthesis

1. DNA

Many reports have been published dealing with the DNA content of sea urchin gametes and embryos. Data from several of these have been compiled in Table 5. It is seen that with respect to the DNA content of the sperm there is good agreement, showing that the haploid amount of DNA is somewhat below 1 pg. The values obtained at the end of development, in the pluteus stage, are also essentially in accord, showing that *Paracentrotus* larva at this time contains around 0.005 mg DNA corresponding to about 3600 cells. The *Arbacia* pluteus seems to have a somewhat higher DNA content. With respect to the unfertilized egg the agreement is less striking, the recorded values ranging between 12 and 1000 pg. In order to clear up this point it is necessary to consider the analytical approach.

In principle the various methods employed can be divided into three groups. In the first one no separation between RNA and DNA is made, the latter is determined directly by a method specific for DNA (BRACHET 1933; VENDRELY and VENDRELY 1949; HOFF-JØRGENSEN 1954; WHITELEY and BALTZER 1958). In the second and third groups the nucleic acids are separated, in the former DNA is determined by a specific method (ELSON and CHARGAFF 1952), in the latter by an unspecific method (CRANE 1947; SCHMIDT et al. 1948; VILLEE et al. 1949; AGRELL and PERSSON 1956; BALTZER and CHEN 1960). The results in the third group are substantially higher (3-50 times) than those in the two first groups, with the exception of the values published by VENDRELY and VENDRELY. It is obvious that the difference cannot be blamed on the separation step (cf. groups 1-2 and 2-3); consequently either the specific methods give too low, or the unspecific too high values.

CHEN et al. (1961) propose that microbiological methods give too low results, but those obtained at the pluteus stage by ELSON et al. (1954) and by BALTZER and CHEN (1960) are almost identical, and the early values obtained by WHITELEY and BALTZER (1958) by a colorimetric method are pretty close to those obtained with the microbiological methods. GREGG (1957) has objected to the microbiological method of HOFF-JØRGENSEN that since no separation of the nucleic acids is involved, even free deoxynucleotides (or -sides) may be determined. However, these substances dissolve easily in 90-95% acetone, so if acetone-dried material is employed, any free nucleotides will be removed. Furthermore, HOFF-JØRGENSEN got very closely the same result as ELSON and CHARGAFF who separated the nucleic acids.