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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.

Recent comparisons made between SCHNEIDER's colorimetric and HOFF-JØRGENSEN's microbiological methods have shown that, unless special precautions are taken, the latter easily is the more reliable assay for DNA (LØVTRUP and ROOS 1961, 1963 a, b), and it therefore seems justified to scrutinize the methods employed in the third group. There can be no doubt, according to the very careful investigations of HUTCHISON and MUNRO (1961), that methods based upon the original SCHMIDT-THANNHAUSER method can give satisfactory results for both nucleic acids. However, this method and other methods involving separation of DNA and RNA, have been worked out on tissues where the contents of the two nucleic acids are of the same order of magnitude. In the sea urchin egg the RNA content is 6–20 000 pg (e.g. SCHMIDT et al. 1948; ELSON et al. 1954; AGRELL and PERSSON, 1956); if thus the results of groups 1 and 2 are correct, the ratio DNA/RNA will lie in the range $^1/_{150}$–$^1/_{1000}$. If this is true, only the greatest optimist could expect to accomplish complete separation of the two compounds by chemical or physical means (cf. ABRAMS 1951).

It therefore seems that the results of BRACHET (1933), ELSON et al. (1952) and of WHITELEY and BALTZER (1958) give the most correct picture of the course of DNA changes in the sea urchin embryo, but it should be emphasized that the disagreement between these and the remaining results pertain mainly to the first part of the curve. The colorimetric methods seem to give slightly

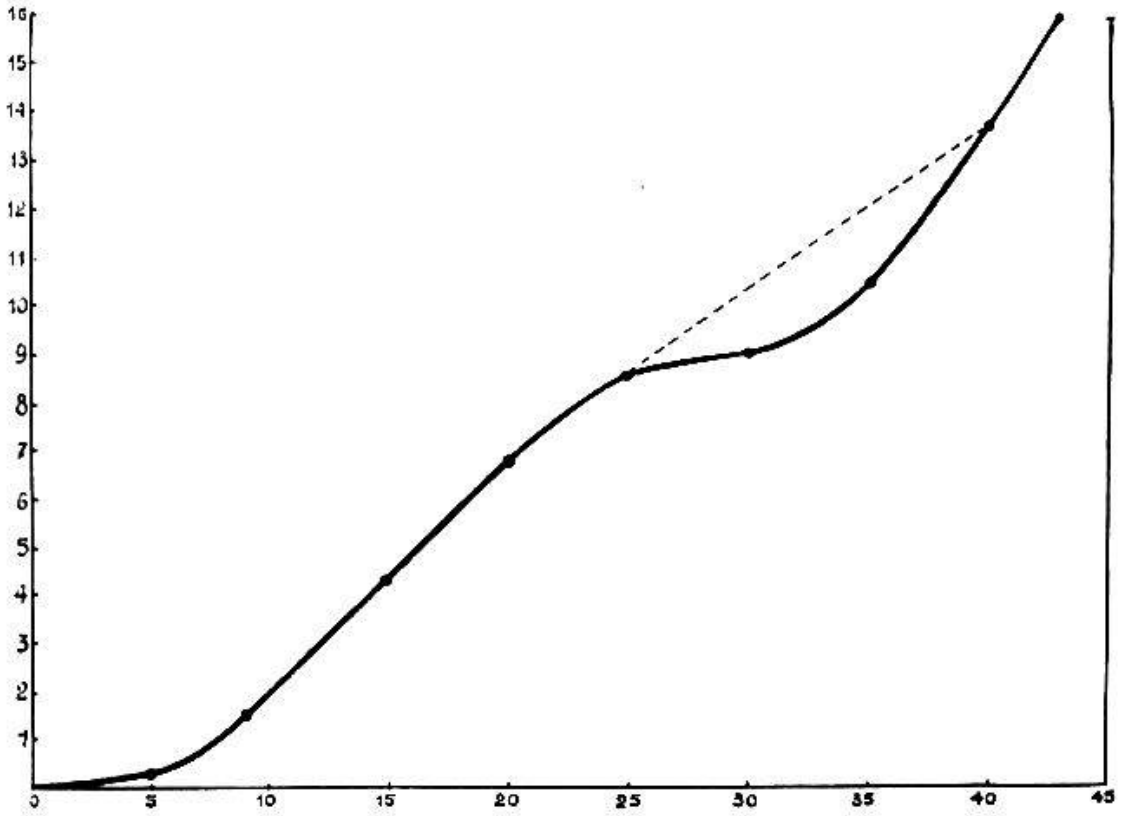

Fig. 12. Colorimetric determination of DNA in Paracentrotus lividus.—Abscissa: Hours of development. Ordinate: DNA, mg per g dry weight (from BRACHET: Arch. Biol. [Liège] *44* [1933]).

lower values than the other methods employed, but it should be noticed that the last value recorded by BRACHET represents a somewhat earlier stage than the ones listed for the other authors.

For Paracentrotus lividus BRACHET (1933; cf. Fig. 12), ELSON et. al. (1952), BALTZER and CHEN (1960) find a plateau or at least a hump on the curve around 20–30 h (the last mentioned authors have chosen to neglect this in drawing their curves). The phenomenon was not observed in this species by WHITELEY and BALTZER (1958), but in Arbacia lixula a very distinct phase of constancy was observed. This curve form indicates that the DNA synthesis in the sea urchin embryo occurs in two distinct phases; a similar phenomenon is well known from the amphibian embryo (cf. 1959c).

2. Reserve DNA

The haploid amount of DNA being around 0.7 pg it appears from the results discussed above that the quantity of DNA in the oocyte (18–40 pg) suffices to supply 13–28 nuclei.

Discussing the question whether this DNA is transformed into chromosomal DNA (in amphibia), BRACHET (1964) advances various arguments against this possibility. Among these are that no genetic meaning can be attached to it, that simple precursors (nucleotides) may be used for DNA-synthesis, and that certain forms of reserve DNA has a base composition deviating from chromosomal DNA in the same species (DURAND 1961). As far as I can see, all these arguments are equally valid for the transformation of RNA to DNA suggested by BRACHET (1933, 1964). This process is supposed to occur at the nucleoside (tide) level, but the same may happen with a DNA reserve.

If the reserve DNA is utilized for synthesis of chromosomal DNA it should become exhausted some time between the third and the fifth cleavage, according to the calculations made above. However, it is exactly at this time that DNA synthesis begins according to HOFF-JØRGENSEN (1954) and AGRELL and PERSSON (1956) (cf. Fig. 13). The latter authors observed that at the same time the synchronous cell divisions come to an end. The mentioned observations were made on Paracentrotus lividus, in two other species, Psammechinus miliaris and Echinus esculentus, the synchronicity lasts until the cell number is 100 and 400, respectively (AGRELL and PERSSON 1956). It would be very interesting to carry out microbiological DNA determinations on these species. It may be mentioned that the transition from synchronicity to asynchronicity in the animal region of the amphibian embryo occurs at an early blastula stage (SCHÖNMANN 1938), thus at a time which corresponds pretty well to the size of the DNA reserve (cf. the discussion in LØVTRUP 1959c).

The transition between the two types of cell division is characterized by an extension of the interphase and the formation of nucleoli (cf. DETLAFF 1964), the latter suggesting increased synthetic activity in the cell, and the

hr. after fertilization		-1/2	3/4	1 1/2	3	4 1/2	6	7 1/2
number of cells per embryo	1	100	100	60	31	15	15	15
	2	–	–	40	10	2	1	–
	4	–	–	–	46	22	–	–
	8	–	–	–	13	41	–	–
	16	–	–	–	–	20	61	27
	>16	–	–	–	–	–	23	58
10^{-6} µg DNA per embryo		17.5	18.3	17.9	18.0	57.5	175	435

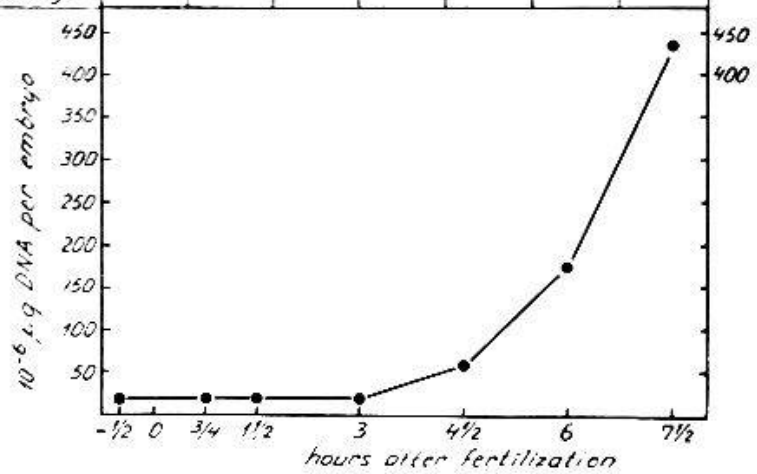

Fig. 13. Microbiological determination of DNA in Paracentrotus lividus. In the table is shown the percentage of embryos at different stages of development (from HOFF-JØRGENSEN: Colston Papers 7 [1954]).

former that the synthesis of some product has become the limiting factor for the rate of cell division.

The agreement between DNA analyses and morphological observations as to the time transition suggests that the DNA present in the unfertilized egg is a reserve which can be and is utilized for the first cell divisions, and that only when these reserves are used up does the synthesis of new DNA begin. The reserves may of course comprise even proteins, as suggested by MAZIA (1961). Interpreted in this way the results of the DNA analyses get a biological meaning and importance.

GRANT (1958) has, in the amphibian egg, tried to study the nature of the DNA reserve by extraction with cold PCA (perchloric acid) before microbiological determination. He found that about 50% of the DNA disappears, suggesting that half of the reserve is of relatively low molecular weight. We have repeated GRANT's experiments; in a number of cases we could confirm his results, in other cases PCA extraction was found to be without any effect. The analysis of the data is not yet completed.

3. RNA

Total RNA remains constant or decreases slightly during development (e.g. ELSON et al. 1954; BÄCKSTRÖM 1959 a; TOCCO et al. 1963). In the two

former papers one or more oscillations were observed in the curves. As we shall discuss below isotope experiments have shown a lively incorporation into RNA during all phases of development, indicating a changeover between different RNA fractions, one of which may constitute a reserve. Because of this circumstance, determinations of total RNA cannot give any information about the RNA-synthesis in the embryo.

This problem has been attacked by Tocco et al. (1963) by fractionation of egg homogenates. As shown in Fig. 14 these authors found a decrease in the fraction containing microsomes + non-sedimentable RNA, whereas that contained in nuclei, mitochondria, and heavy microsomes increased during development. These three fractions amount to 5% of total RNA at the beginning, and to 25% at the end of development. The obvious source of this RNA is the former fraction, but as mentioned by Tocco et al. (1963), the various incorporation experiments show that de novo synthesis of RNA nucleotides also occurs, there are thus two potential sources of RNA.

Brachet and Jeener (1944) observed a conspicuous difference between the RNA in amphibian eggs and various adult tissues; whereas in the latter only traces of RNA could be found in the supernatant after high speed centrifugation, 60–80% of the total RNA was recovered in this fraction in the former case. It has been surmised that this non-sedimentable type of RNA may represent a cytoplasmic reserve. It would have been very interesting to know the changes in this fraction during development, but in the result presented in Fig. 14 this fraction is combined with a microsomal fraction. In some ^{32}P incorporation experiments a further separation was accomplished; the results in Fig. 15b show clearly that the rate of incorporation in the microsomal fraction increases—two phases are indicated—whereas that of the supernatant fraction remains almost constant, a finding compatible with the role ascribed to the non-sedimentable RNA, considering that it does not contain only reserve RNA.

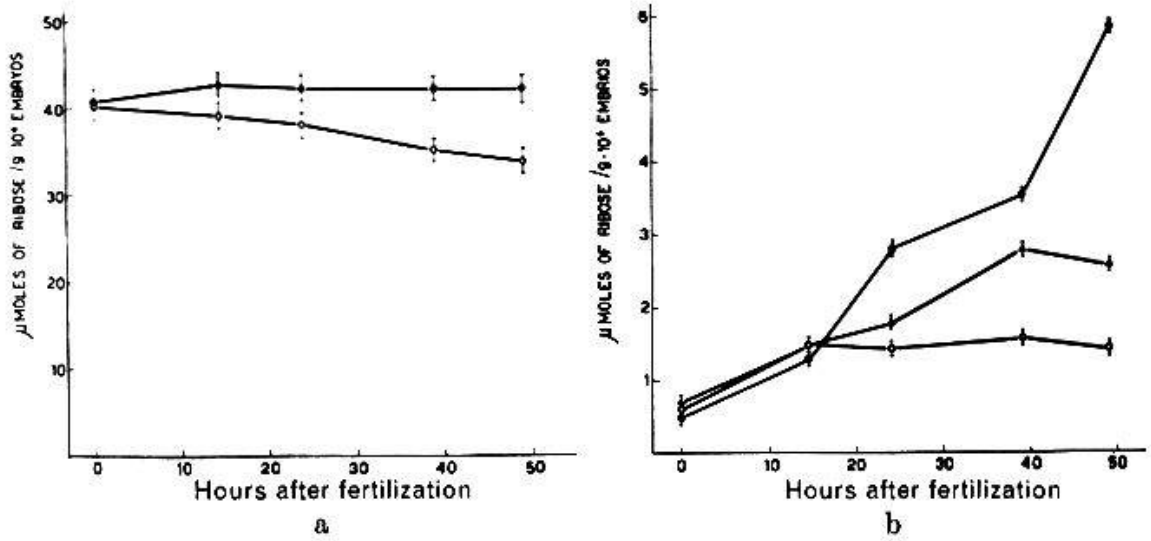

Fig. 14. RNA of subcellular fractions during the development of Paracentrotus lividus.— a: • = total content, o = microsomes + nonsedimentable fraction. b: • = nuclei, o = mitochondria, ▲ = heavy microsomes (from Tocco et al.: Exp. Cell Res. *31* [1963]).

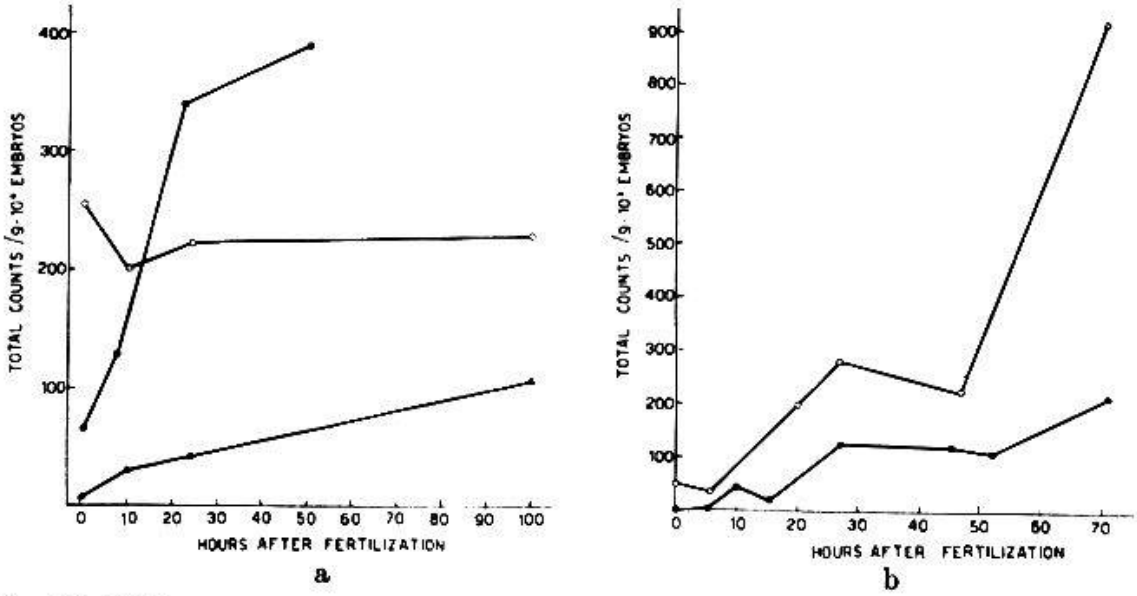

Fig. 15. ^{32}P-incorporation into RNA of subcellular fractions during the development of Sphaerechinus granularis.—a: • = nuclei, o = mitochondria; ▲ = heavy microsomes. b: • = non-sedimentable fraction, o = microsomes (from Tocco et al.: Exp. Cell Res. *31* [1963]).

As suggested originally by BRACHET (1933), and recently reiterated by Tocco et al. (1963) there is no evidence speaking against the assumption that during embryogenesis nuclear DNA is formed partly from the RNA reserve present. This nucleic acid may thus be formed from stores of DNA and of RNA, and by de novo synthesis, whereas for RNA synthesis only the two latter possibilities are involved. Various isotope experiments discussed above show that amino acids are precursors for the bases. As discussed in the previous chapter both ribose-5-phosphate and deoxyribose-5-phosphate may be formed from glucose. It would be interesting to know whether the enzymes catalyzing the conversion of ribonucleotides to deoxyribonucleotides (cf. REICHARD 1961) are present in the embryo, and if so, at which stage they make their appearance. The possible formation of deoxyribose-5-phosphate suggests that enzymes of this types may not be necessary for the conversion between the two types of nucleotides.

4. Isotope incorporation studies

The question whether the RNA reserves may be used indiscriminately for synthesis of either nucleic acid seems to be of great theoretical interest, and several authors have tried to study this problem. The tracer experiments of VILLEE et al. (1949) with ^{32}P, of ABRAMS (1951), and of SCARANO and KALCKAR (1953) with glycine-1-^{14}C and adenine-8-^{14}C all show that the specific activity is much higher for DNA than for RNA. This has been used as an argument against RNA as a DNA precursor, but this reasoning is only valid if all the RNA present is actively participating in the turnover. If a major part is an RNA reserve, then the determination of specific activities may lead to erroneous conclusions; both VILLEE et al. and ABRAMS admit

that their results do not contradict the possibility that a small fraction of the total RNA can be a DNA precursor.

From the isotope dilutions found in his experiments ABRAMS (1951) calculated that some of the purines in both nucleic acids are derived from de novo synthesis with glycine as precursor but that the major part stems from an endogenous source. This source might be RNA if it were not for the fact that the value of the ratio of the specific ratio of guanine/adenine was different for DNA and RNA, being about two in the former, and one in the latter case. This finding led to the conclusion that "any simple conversion mechanism of RNA or its hydrolysis products to DNA is ruled out" (lc. p. 241).

However, if this is the only hindrance for the acceptance of a both chemically and biologically plausible mechanism the situation seems hopeful, for it appears from the results of HULTIN's $^{15}NH_3$-incorporation experiments (1953a) that during an essential part of the developmental period the incorporation in RNA guanine is about twice as high as in RNA adenine (Fig. 16).

As to the high ratio of the specific activities for guanine/adenine a few comments may be warranted. This has been observed, directly or indirectly, with both glycine-1-^{14}C (ABRAMS 1951), $^{15}NH_3$ (HULTIN 1953a) and adenine-8-^{14}C (SCARANO and KALCKAR 1953). In the two latter cases a decrease in the ratio was observed towards the end of development. As stated by ABRAMS, this result seems to exclude that adenine is a precursor for guanine. If not, the quantities of guanine and adenine precursors in the pool must be of decisive consequence for the incorporation of labelled compounds, thus if

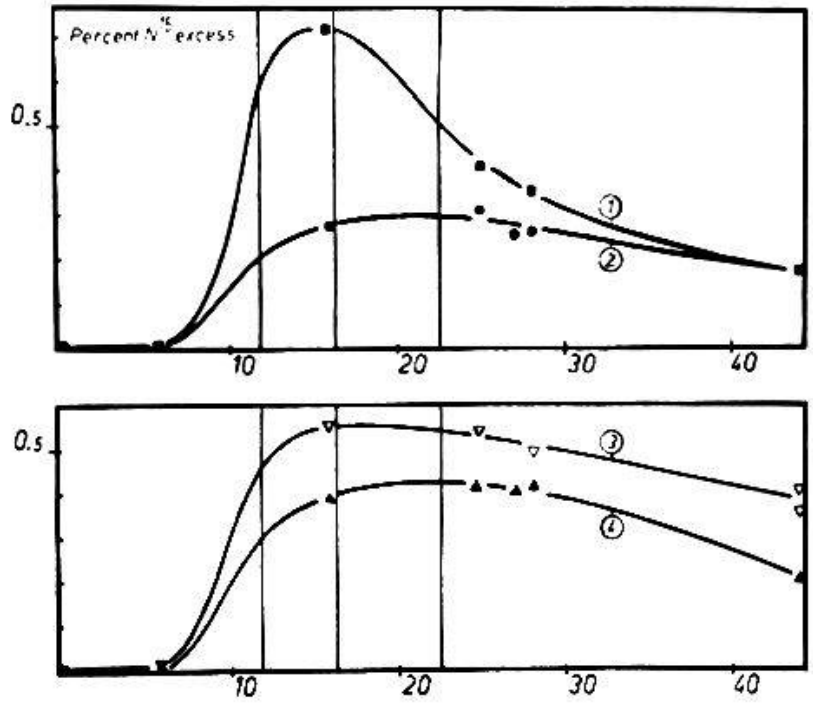

Fig. 16. Incorporation of $^{15}NH_3$ into purines and pyrimidines of RNA in embryos of Paracentrotus lividus at different stages of development.—1 = guanine, 2 = adenine, 3 = uracil, 4 = cytosine (from HULTIN: Arch. néerl. Zool. *10*, 1. Suppl [1953c]).

the guanine is present in limiting amounts, synthesis of this base from simpler precursors may be required to cover the needs. It may or may not be relevant in this connection to mention that the ratios between the various free adenine and guanine nucleotides are very large in the unfertilized eggs and that, for ATP at least, a drastic decrease occurs during development (HULTIN 1957). Here seems to be another possibility for explaining the change in the guanine/adenine ratio. The pool might also be influenced by the composition of the nucleic acid reserves, cf. DURAND (1961) who has demonstrated a DNA store of abnormal composition in the insect Grylla bimaculatus.

However, the adenine/guanine conversion is not completely blocked, for SCARANO and KALCKAR (1953) observed a labelling in guanine after addition of adenine-8-^{14}C. These authors observed an enhanced relative incorporation in guanine with developmental age. The findings of HULTIN (Fig. 16) may thus also be interpreted in the way that the enzymes required for the purine interconversion are absent or of very low activity during the first 10–15 h of development, but not thereafter. The question whether the enzymes are

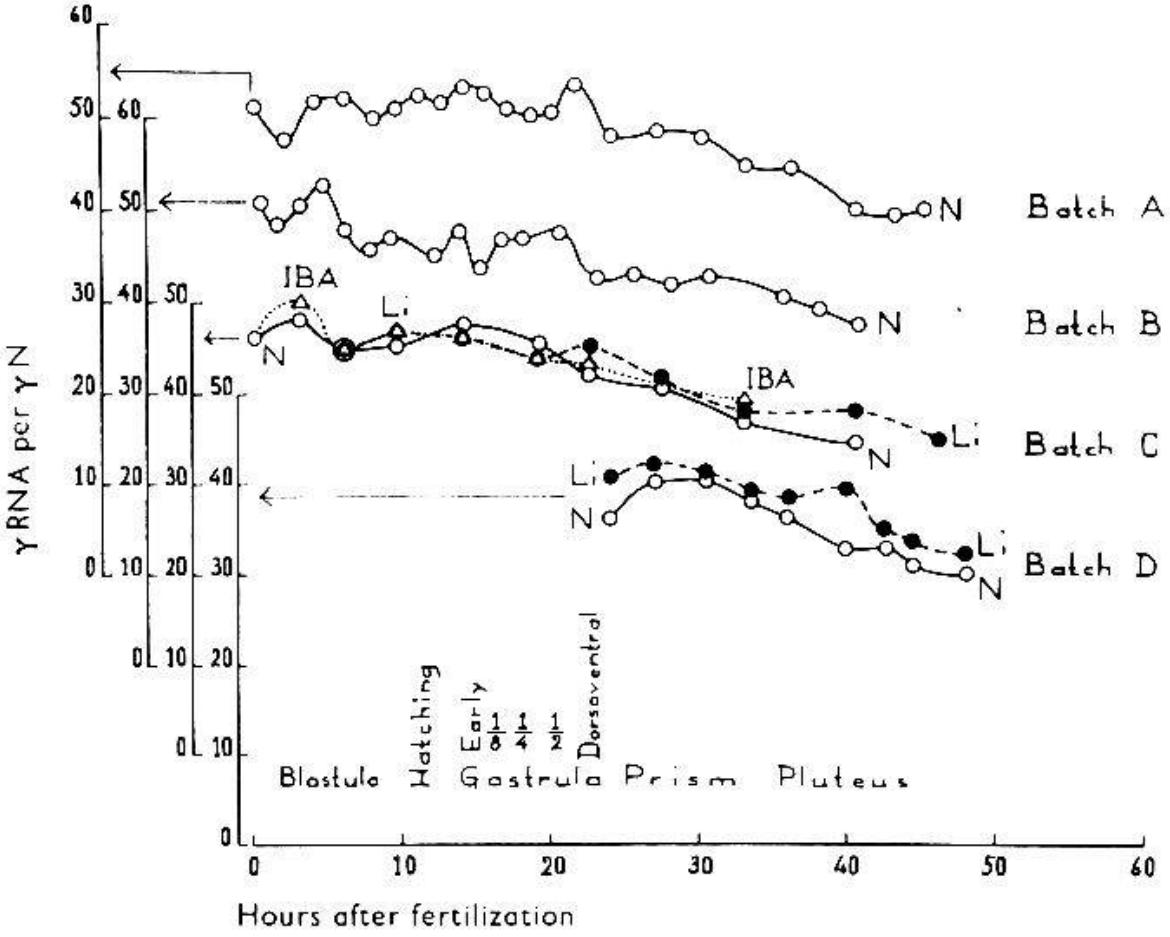

Fig. 17. Changes in the RNA content during the development of Paracentrotus lividus.—N = normal embryos, Li = vegetalized embryos, IBA (o-iodosobenzoic acid) = animalized embryos.—The arrows point to the ordinate for each group of curves (from BÄCKSTRÖM: Ark. Zool. *12* [1959a]).

completely absent might be decided by incorporation experiments with labelled adenine. Unfortunately the incubation periods employed by SCARANO and KALCKAR (0–22, 12–30, and 12–48 h) were too long to settle this problem.

The slight, but significant decrease in RNA during development found by ELSON et al. (1952) and by BÄCKSTRÖM (1959a), and particularly the oscillations found by the latter author (Fig. 17) would seem incompatible with the wisdom of the workings of Nature, were it not for the possibility that the RNA represents a reserve that may be utilized for synthesis of both DNA and RNA. In this perspective BÄCKSTRÖM's findings suggest that in periods of rapid DNA synthesis the RNA reserve is tolled, causing a decrease in total RNA, whereas in periods of extensive RNA formation the content is replaced by de novo synthesis. BÄCKSTRÖM's interesting experiments ought to be repeated with due regard to the possible peculiar physical properties of the embryonic RNA, and under such conditions that statistical evaluation of the data were possible.

5. Correlation between DNA and respiration

COMITA and WHITELEY (1953) and WHITELEY and BALTZER (1958) have tried to correlate DNA content and rate of oxygen consumption (cf. also TYLER 1942 and ZEUTHEN 1952). It is a reasonable contention that respiration increases with the number of cells, even if the size of each cell decreases continuously. However, in order to establish such a correlation it appears insufficient to demonstrate that a mathematical regression can be established, that is generally possible for two gradually increasing phenomena. Agreement between the finer details of the curves should also prevail, and such accord seems to be missing on two points. The first one is that the DNA-curves show no constancy from about 7–10 h, as do the respiratory curves (cf. Fig. 5). This lack of agreement may easily be referred to the few points used to establish the DNA curves. The second point is that after about 20 h there is only a very slight increase in O_2-consumption, after 35–40 h the rate even decreases (LINDAHL 1939b; COMITA and WHITELEY 1953; WHITELEY and BALTZER 1958), but during this phase the DNA-synthesis continues at a constant rate (cf. Fig. 12).

MARKMAN (1961b) has followed very closely the rates of incorporation of adenine-8-^{14}C and ^{14}C-leucine (i.e., the rates of nucleic acid and protein synthesis) during the first 24 h of development. The curves obtained by him (Fig. 10) are almost identical with the respiratory curves except that after 20 h the rate of incorporation seems to be constant. Now, the incorporation of adenine does not represent *only* DNA synthesis, but it represents *also* DNA synthesis (cf. ABRAMS 1951), and the shape of the cumulative "DNA" curve one may establish on the basis of MARKMAN's data actually exhibit the same features as the experimentally observed curves (cf. Fig. 12), except that the hump occurs slight earlier. It therefore seems possible to

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