THE BEHAVIOR OF THE NUCLEIC ACIDS DURING THE EARLY DEVELOPMENT OF THE SEA URCHIN EGG (ARBACIA)*

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It was found in earlier investigations by Masing (1, 2), by Brachet (3), and by Schmidt (4) that the eggs of Arbacia and other sea urchins contain large amounts of nucleic acids. During the early stages of development (up to the gastrula stage) the increase of the total nucleic acid content of the embryo is small in comparison to the initial nucleic acid content despite the formation of a large number of cell nuclei. In these respects the sea urchin eggs differ fundamentally from avian eggs which contain only extremely small amounts of nucleic acids before fertilization but form large amounts of these compounds during their development.

Brachet observed that unfertilized sea urchin eggs give only very weak Dische tests (5) but very strong furfural tests after being heated with strong hydrochloric acid. He concluded from these facts that the nucleic acid of the unfertilized sea urchin eggs (Paracentrotus) is mainly ribonucleic acid. This interpretation is confirmed in the present paper by the application of improved analytical methods. (An earlier statement to the contrary by Schmidt (4) who inferred the presence of desoxyribonucleic acid from a positive Feulgen test of the nucleoprotein fraction of unfertilized Arbacia eggs was erroneous.)

On the basis of ribose and desoxyribose determinations at various stages of the early embryonic development, Brachet suggested the assumption that ribonucleic acid was gradually transformed into desoxyribonucleic acid during the early development of the sea urchin eggs (3, 7).

Since the analytical methods available for the determination of nucleic acids have been greatly improved in the meantime, we undertook a reinvestigation of this problem with more recent analytical procedures. The results of this investigation are reported in the present paper.

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EXPERIMENTAL

Suspensions of Arbacia eggs were divided into several batches each containing an equal number of eggs per ml. In addition, an aliquot of the original suspension was set aside for the determination of the number of eggs per ml. The counts were carried out in a cell count chamber with the undiluted egg suspensions. All batches except one were then fertilized under comparable conditions. At the desired stage of development each batch was homogenized in a Waring blender after the addition of an equal volume of 10 per cent trichloroacetic acid. By means of this treatment it is possible to achieve the complete mechanical disintegration of the eggs which is an important prerequisite for the quantitative extraction of the non-nucleic acid P fractions.

The analytical procedure used for the determination of the nucleic acids and phosphoproteins was essentially that of Schmidt and Thannhauser (6). One important modification, however, was introduced into the original technique: Instead of calculating the amount of desoxyribonucleic acid P as the difference between the total P and the ribonucleic acid P, we determined the desoxyribonucleic acid P directly in the precipitate obtained on acidification of the alkaline hydrolysate. The precipitate was washed three times with a mixture of 0.5 N hydrochloric acid and 5 per cent trichloroacetic acid and then ashed for the determination of the total P.

The precipitation of desoxyribonucleic acid and the handling of the precipitate was facilitated by adding 1 ml. of a 1 per cent solution of egg albumen to each 5 ml. of the alkaline digest. It is advisable to do this whenever the DNA-containing fraction fails to precipitate in flocculent form.

The modification just described has the advantage of replacing the differential determination of the desoxyribonucleic acid P by its direct determination. Since the amounts of desoxyribonucleic acid in Arbacia eggs as well as in many other animal cells are much smaller than those of the ribonucleic acid the accuracy of the direct determination is considerably higher than that of the differential determination.

It was demonstrated in control experiments with sperm that the amounts of the desoxyribonucleic acid and of the ribonucleic acid introduced with the sperm were small in comparison to those present in the unfertilized eggs. In one experiment the amount of the sperm ribonucleic acid was 0.9 per cent of that of the eggs, the amount of desoxyribonucleic acid 3 per cent of that of the eggs.

RESULTS

Table I demonstrates the result of a representative experiment. The data obtained in seven other experiments on different batches of Arbacia eggs are in essential agreement with those shown in Table I.

Table II shows the amounts of the nucleic acid and other P fractions in the unfertilized Arbacia eggs.

Purine N: Total P Ratio in the Ribonucleic Acid Fraction of Arbacia Eggs.—Unfertilized Arbacia eggs were homogenized in a Waring blender and freed from acid-soluble and lipid P compounds as described (6). 400 mg. of the dry powder were refluxed with 5 ml. of a 2 per cent solution of sulfuric acid for 2 hours. The hydroly-
sate was made up to 10 cc. and filtered. From 5 cc. of the clear filtrate the purines were precipitated by the addition of a hot saturated solution of silver sulfate in 2 per cent sulfuric acid. After standing for 24 hours in the refrigerators, the silver precipitate was centrifuged, washed 3 times with a saturated solution of silver sulfate,

<p>| TABLE I |
| Amounts of Desoxyribonucleic Acid and Ribonucleic Acid in Arbacia in Early Embryonic Stages |</p>
<table>
<thead>
<tr>
<th>Date of experiment</th>
<th>Embryonic stage</th>
<th>DNA $\times 10^{-4}$ P per embryo</th>
<th>RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 17, 1947</td>
<td>Unfertilized</td>
<td>0.6</td>
<td>22.0</td>
</tr>
<tr>
<td></td>
<td>Morula (8 hrs.)</td>
<td>3.4</td>
<td>22.8</td>
</tr>
<tr>
<td></td>
<td>Pluteus (24 hrs.)</td>
<td>10.7</td>
<td>21.5</td>
</tr>
</tbody>
</table>

and decomposed with 5 cc. N hydrochloric acid in a boiling water bath. In the supernatant from the silver chloride, the total N was determined according to Kjeldahl.

Total nucleic acid P: 0.44 per cent
Total purine N: 0.46 per cent

Found: \( \frac{\text{Purine N}}{\text{Nucleic acid P}} \) : 1.05

Calculated: \( \frac{\text{Purine N}}{\text{P}} \) in yeast nucleic acid: 1.13

DISCUSSION

It was found that during the first 24 hours of the development of the Arbacia egg (pluteus stage), the amount of ribonucleic acid remains practically unchanged, whereas the amount of desoxyribonucleic acid steadily increases.

It follows from our analyses that the early development of the Arbacia egg involves a striking change of the mutual proportion of the two components of the nucleic acid fraction in favor of the desoxyribonucleic acid. The average ratio $\frac{\text{DNA}}{\text{RNA}}$ is 0.05 in the unfertilized egg, 0.17 in the morula (8 hours), and 0.46 in the pluteus (24 hours).
In this respect our observations are in essential agreement with those of Brachet. In our experiments, however, the change of the proportions between both nucleic acids is entirely caused by the increase of desoxyribonucleic acid per embryo, while Brachet found not only an increase of the amount of desoxy-pentose, but also a very considerable decrease of the total pentose per gram of dried embryos. Brachet interpreted this decrease of the total pentose per gram of embryos as being caused by the decrease of ribonucleic acid. This interpretation is not compatible with the results of our determinations of ribonucleic acid.

It should also be noted that Brachet relates his figures to the unit of weight while our values represent those per embryo. It appears that only the latter method of calculation permits an answer to the question as to whether the amount of a substance in a developing egg actually changes.

The observations presented in this paper demonstrate that the nucleic acids of the developing sea urchin egg cannot be considered as a closed metabolic system in the sense that the intense synthesis of desoxyribonucleic acid would proceed at the expense of the ribonucleic acid accumulated in the mature egg. They show that this synthesis is accompanied by a corresponding rise of the total nucleic acid P for which the new formation of desoxyribonucleic acid is exclusively responsible, whereas the amount of ribonucleic acid per embryo remains practically unchanged. It is obvious that the rise of the total nucleic acid fraction could not be detected in some of the earlier investigations (1, 3) since its extent during the early phases does not exceed the range of analytical errors due to the fact that the desoxyribonucleic acid represents only a very small fraction of the total nucleic acid P in the mature egg.

Thus, the formation of new nuclei during the cleavages of the sea urchin egg involves the formation of nucleic acid from other cell constituents. Since the total nucleic acid P in the mature Arbacia egg amounts merely to approximately 25 per cent of the total P there is an abundance of possible P sources available for the synthesis of nucleic acids. Whether or not the metabolic pathway of the biological synthesis of desoxyribonucleic acid goes through an intermediary stage of ribose compounds is still an open question the answer to which is beyond the scope of the present investigation.

From the biological point of view it should be emphasized that the presence of a large excess of RNA in the nucleic acid fraction of the unfertilized sea urchin egg can no longer be considered as an exceptional occurrence. It has recently been shown by Davidson and Waymouth (8), by Schmidt and Thannhäuser (6), and by Schneider (9) that ribonucleic acid is quantitatively predominant in the nucleic acid fraction of many adult animal tissues.

**SUMMARY**

1. The unfertilized Arbacia egg contains an average of \(20 \times 10^{-6}\) ribonucleic acid and \(0.7 \to 1 \times 10^{-6}\) desoxyribonucleic acid.
During the first 24 hours of development, the amount of ribonucleic acid per embryo remains practically unchanged whereas that of desoxyribonucleic acid steadily increases. At the end of this period, the amount of desoxyribonucleic acid per embryo is 10 to 15 times larger than that of the unfertilized egg.

BIBLIOGRAPHY