THE NUCLEIC ACID OF THE EGGS OF ARBACIA PUNCTULATA

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(Received for publication, October 25, 1934)

Quantitative determinations of the phosphorus distribution in the eggs of a variety of species by Tschernorutzki (1), Massing (2), and Needham and Needham (3) have indicated the probable presence of nucleoproteins in unfertilized ova. Unfortunately, however, the presence of vitellins in such cells renders the exact interpretation of such analyses somewhat difficult since, with current methods of analysis, it is possible that phosphoproteins of this type may be mistaken for nucleoproteins.

In view of these facts it is of interest to determine the presence of nucleic acid in unfertilized ova by means of an actual isolation, which permits an examination of the characteristics of the substance isolated. For this purpose we have selected the eggs of the sea urchin, Arbacia punctulata, whose alecithal nature facilitates the ease of the necessary chemical manipulations. In this connection it should be noted that Mathews (4) states: "The author got a substance with some of the properties of nucleic acid in some quantity from the unfertilized eggs of the sea urchin. It could not be positively identified, however, as the quantity was too small."

EXPERIMENTAL

The isolation of the crude nucleic acid described below was made upon material obtained at Woods Hole at the height of the breeding season. Various fractions of the crude product obtained during a number of years were subsequently combined and purified.
Isolation of Crude Nucleic Acid—The ovaries distended with ripe ova were removed from the animals immediately after collection, suspended in approximately 50 volumes of sea water, and strained through bolting-cloth. The eggs thus obtained were washed with sea water four times by decantation and subsequently centrifuged to remove as much water as possible. Exactly 50 gm. of the resulting egg mass were vigorously shaken with 75 cc. of distilled water and the suspension thus obtained was alternately frozen and thawed twice to insure complete cytolysis.

Nucleic acid was isolated from the resulting mixture by the classical method of Levene (5) as follows: Sodium chloride (3.7 gm.) was dissolved in the mixture, which was then faintly acidified with acetic acid. Finely powdered sodium hydroxide (3.7 gm.) and 3.7 gm. of crystalline sodium acetate were then added and the mixture heated in a boiling water bath until quite fluid. This required about 20 minutes. After being cooled to room temperature, the solution was neutralized with acetic acid, precipitated by the addition of a slight excess of a saturated solution of picric acid, made distinctly acid to litmus with acetic acid, and filtered. The filtrate, which was definitely opalescent and could not be clarified by prolonged centrifugation, was poured into an equal volume of alcohol. After standing overnight, the resulting precipitate was washed in the centrifuge with 50 per cent alcohol until free of picric acid, dehydrated in absolute alcohol, and dried in vacuo. The quantity of the crude product so obtained varied with different lots of eggs, the yield being from 0.09 gm. to 0.30 gm. per 50 gm. of eggs. During the course of several seasons a total of 12.75 gm. of this crude nucleic acid was obtained from 4820 gm. of eggs.

Purification of Nucleic Acid—The composite material was red in color, due to the presence of echinochrome, the characteristic pigment of the Arbacia egg. This was readily removed by trituration with acetone, whereby a pure white product was obtained. Upon analysis this was found to contain 1.92 per cent of nitrogen and 0.94 per cent of phosphorus, about one-tenth the quantities expected for a nucleic acid. Several years ago the writer was able to isolate glycogen from the Arbacia egg (see Perlzweig and Barron (6)) and in view of the analytical figures given above it seemed likely that this substance was the impurity in the crude
nucleic acid. Consequently, the crude product was purified by the copper salt method which Levene (7) has found to be satisfactory for the separation of liver nucleic acid from glycogen.

The crude product (12.4 gm.) was suspended in 600 cc. of water and brought into solution by the dropwise addition of 6 N sodium hydroxide. The opalescent solution so obtained was treated with 20 per cent cupric chloride, as directed by Levene (7), and the copper nucleinate separated. The latter was decomposed with 3 per cent alcoholic hydrogen chloride and the liberated nucleic acid again converted to the copper salt. This process had to be repeated three times before the nucleic acid obtained would yield a clear solution with sodium hydroxide. By this means 1.08 gm. of a pure white product were obtained. This was equivalent to 0.028 per cent of the original weight of the eggs.

The purified product yielded a negative biuret reaction, negative tests for pentose, and a positive Feulgen (8) reaction. Upon analysis it was found to contain 16.35 per cent of nitrogen and 10.13 per cent of phosphorus. Upon hydrolysis in the usual manner with 5 per cent sulfuric acid, 0.6212 gm. of the nucleic acid yielded 0.0708 gm. (11.4 per cent) of crude guanine and 0.1653 gm. of adenine picrate, equivalent to 0.0631 gm. (9.87 per cent) of adenine. The calculated values for a desoxyribosenucleic acid, C_{35}H_{55}N_{16}P_{4}O_{28}, are respectively N 16.76, P 9.89, guanine 12.05, and adenine 10.77.

Identification of Glycogen—The various mother liquors from the purification of the crude nucleic acid were combined and concentrated in vacuo to a volume of 400 cc. A small quantity of insoluble material was centrifuged off and the glycogen precipitated by the addition of an equal volume of alcohol. The precipitate was dissolved in a minimum of water and reprecipitated with alcohol. This process was repeated four times; then the product was washed with 50 per cent alcohol until free of chlorides, dehydrated with absolute alcohol, and dried in vacuo over sulfuric acid. The resulting product (7.2 gm.) yielded typical reactions with iodine and orseillin-BB. Upon hydrolysis with N hydrochloric acid, it yielded 97 per cent glucose, which was identified by the formation of the typical methyl phenylhydrazone.

Pentose Nucleic Acid—In connection with some other experiments it was observed that when Arbacia eggs are heated with N
hydrochloric acid in a water bath the resulting fluid after de-
proteinization with tungstic acid yields distinct tests for pentoses.
Since recent investigations (Jones and Perkins (9), Jorpes (10))
have shown definitely that pentose nucleic acids occur in various
mammalian tissues, and Calvery (11) has found such substances
in chick embryos, it seemed possible that in the Arbacia egg such a
substance might be responsible for the pentose reactions noted
above. While no definite evidence can be offered to substantiate
this, the following preliminary experiment is of interest in this
connection.

100 gm. of Arbacia eggs were ground in a mortar with 10 gm. of
sand and 150 cc. of water. The resulting mixture was held over-
night in an ice box, subsequently boiled for 15 minutes, and
filtered. The residue was extracted with two 25 cc. portions of
boiling water and these extracts added to the filtrate. The latter
was cooled to room temperature and treated with 6 N acetic acid
until no further turbidity developed. An equal volume of alcohol
was added and the resulting precipitate, which settled rapidly, was
centrifuged off, washed with 50 per cent alcohol, then with acetone
to remove echinochrome, finally with absolute alcohol, and air-
dried, 0.8 gm. of a pale yellow horny mass being so obtained.
This was ground in a mortar with 50 cc. of 1.5 N sodium hydroxide
previously cooled to 0°. An equal volume of ice water was added
and the mixture placed in an ice box for 2 hours, with occasional
shaking. At the expiration of this time a small quantity of a
gummy material remained undissolved. Without separation of
this, the mixture was acidified to litmus with acetic acid and shaken
for a few minutes with 0.5 cc. of Merck’s 5 per cent “dialyzed iron.”
The filtrate, which was slightly opalescent, was made acid to Congo
red with hydrochloric acid, precipitated with an equal volume of
alcohol, and allowed to settle overnight.
The snow-white rubbery precipitate so obtained was washed
free of chlorides with 50 per cent alcohol, dehydrated with ab-
solute alcohol, and dried in vacuo, whereby 0.17 gm. was obtained.
This product yielded a strong biuret reaction; hence an attempt
was made to purify it by the method of Osborne and Harris (12),
which is stated to be an excellent method for the purification of the
pentose nucleic acid obtained from yeast. The entire yield of the
crude product was stirred with 2.5 cc. of warm 10 per cent sodium
acetate solution in which about one-third of the product dissolved to form an exceedingly viscous solution which could not be clarified by centrifugation. It should be noted that such behavior is quite different from that exhibited by even highly impure yeast nucleic acid under the same conditions. The solution was filtered and the filtrate diluted with 0.5 cc. of alcohol and made acid to Congo red by the addition of a few drops of 6 N hydrochloric acid. At this point a slight turbidity developed—not a distinct precipitate, as occurs with yeast nucleic acid under the same conditions. Upon standing overnight, a definite precipitate appeared. This was centrifuged off, was freed of chlorides, and dried in the same manner as were the earlier precipitates. The yield of the vacuum-dried product was only 0.02 gm.

As at the time facilities for microanalysis were not available, only a qualitative examination of the product was feasible. It contained nitrogen, but yielded negative biuret and Feulgen reactions. 10 mg. of the substance were hydrolyzed by heating with 2 cc. of 5 per cent sulfuric acid on a water bath for 0.5 hour. The resulting solution was found to contain phosphorus, to yield a minute precipitate with ammonia (guanine?), a brown precipitate with ammoniacal silver nitrate, and a positive pentose test with phloroglucinol. The quantity of material available did not suffice for further examination, but it is hoped to extend these observations in the near future.

DISCUSSION

From the foregoing it is evident that the unfertilized eggs of the sea urchin, *Arbacia punctulata*, contain a nucleic acid of the deoxyribose type which appears to be identical with that which has been obtained from a variety of mammalian tissues, from chick embryos, and, more recently, from *Mycobacterium tuberculosis* (Coghill (13)). So far as the writer has been able to determine, this is the first time that such a nucleic acid has been isolated from an unfertilized egg or from an invertebrate source.

With regard to the pentose derivative isolated from unfertilized *Arbacia* eggs, it should be noted that while its properties resemble those of a ribose nucleic acid, it has not been definitely characterized as such. Nevertheless the presence of a pentose derivative of this nature in the eggs is of interest in connection with the
recent conclusions of Brachet (14), who as a result of his investigations of the ova of another species of sea urchin, *Paracentrotus lividus*, Lamarck, has concluded that subsequent to fertilization desoxyribose nucleic acid is formed at the expense of a pentose nucleic acid initially present in the unfertilized egg. This interesting hypothesis is based upon this author's findings that the quantity of desoxyribose nucleic acid in the unfertilized eggs is so small as to be analytically indeterminable, and that following fertilization the pentose content of the eggs decreases, while a corresponding increase in desoxyribose nucleic acid occurs.

In the case of the *Arbacia* egg, Brachet's hypothesis appears to be untenable, for the quantity of the pentose derivative which we have been able to isolate is of essentially the same order of magnitude as the quantity of desoxyribose nucleic acid obtainable from the eggs.

**SUMMARY**

A nucleic acid of the desoxyribose type and a pentose derivative which may be a nucleic acid have been isolated in approximately equal amounts from the unfertilized eggs of the sea urchin, *Arbacia punctulata*.

**BIBLIOGRAPHY**

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