Zusammenfassung

Der histochemische Nachweis der alkalischen Phosphatase bei *Acanthochites fascicularis* L. (Polyplacophora, Mollusca) erbringt den Beweis eines ausgedehnten Vorhandenseins des Ferments. Das Integument, das Bindegewebe und das Muskelgewebe enthalten keine oder nur Spuren von Desmophosphatase. Der Verdauungsapparat, insbesondere die Zuckerdrüse und der Mitteldarm sind dagegen reich an Phosphatase, desgleichen die Niere, die Spermatogonien und Spermatozyten, die Nukleolen der Ovozyten und das Epithel des Ovariums.

Apyrase Distribution in Sea-Urchin Eggs

One of the earliest steps following the fertilization of a sea-urchin egg is a very considerable amount of molecular rearrangement[1] which is necessary for the initiation of development. There is no satisfactory explanation of where the extra energy for initiating these reactions comes from since O_2 consumption in some species is increased[2] while in others it is decreased[3]. By analogy with muscle it might seem reasonable that rapid demands for energy could be met by phosphate bond energy. If this be so, the unfertilized egg would either have to have ATP-ase systems already present, or, the activation of such enzyme systems would have to be one of the early steps following fertilization.

Apyrase activity in frog eggs has been identified after 50 hours of development and has been found to exist in at least 3 protein fractions or complexes[4]. In *Strongylocentrotus*, whole egg extracts, and Weber solution extracts yield apyrase activity and there is some evidence that fertilized eggs contain more apyrase activity than unfertilized eggs[5].

Chemical. ATP was obtained as the Ba salt. This was dissolved, the metal precipitated and the p_H adjusted to 7. Stock solutions of 10 mg free ATP per ml were prepared and kept at –20°C until used. Calcium activator 0·01 mg/ml final concentration was used in most cases and buffer for protein extraction was 0·1 M glycine-NaOH, p_H 9. Proteins were precipitated with 80% trichloracetic acid and phosphate determinations were made with ammonium molybdate-β-naphtholsulfonic acid using a photoelectric colorimeter.

Eggs. Living material was obtained fresh daily and eggs were collected, strained through gauze and washed several times with filtered sea-water. In some cases eggs were subjected to lyophile processes from a preliminary freezing at –20°C. Fertilized eggs were obtained by treating eggs with sperm for such a time as to give 90% membrane elevation (c. 10 min. at 25°C) and were then lyophiled as above. All extracts derived from eggs were handled at 1°C throughout chemical treatment.

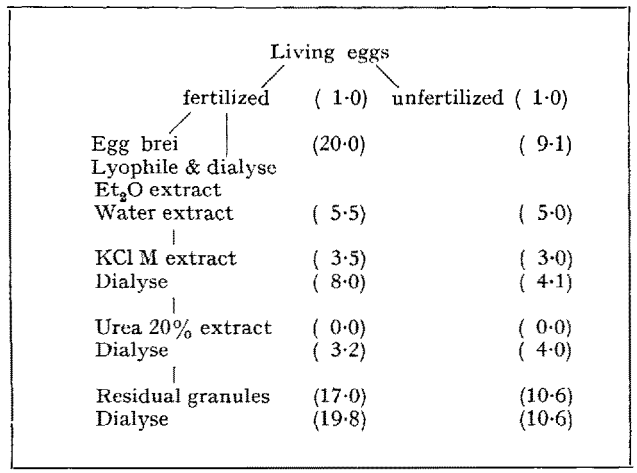
Apyrase Activity. In the case of whole eggs tested for surface apyrase activity, 100 mg wet weight of eggs was added per ml of ATP (1 mg/ml) solution in sea-water, and held at p_H 9 with glycine buffer. For whole egg homogenates and for lyophiled fractions, 50 mg dry weight of substance was used per 10 mg of ATP solution (p_H 7, with $CaCl_2$ activator). Incubation was for 1 or 2 hours at 25°C and 1 ml of 80% CCl_3COOH was added and the tubes iced at the end of the run.

Results. The data fall under the following general classifications: (1) examination of the apyrase activity of the surface of living eggs; (2) the apyrase activity of a brei of fresh eggs; and (3) activity in lyophiled, dialysed and protein-fractionated eggs.

Living *Arbacia* eggs, both fertilized and unfertilized and with jelly coats removed, were analysed for surface apyrase activity in sea-water at p_H 7 and 9. There was no appreciable hydrolysis of ATP after 2 hours at 25°C. Recent results[1] with yeast have shown that this cell has several types of phosphatases at the cell surface including apyrase. These enzymes, however, have an optimal activity at p_H 3·5 and almost no activity at p_H 7.

Eggs disrupted by grinding with sand, as well as eggs fractionated by chemical means were assayed for apyrase activity with the results (for *Arbacia*) as indicated in Figure 1. It is to be noted that the egg granule fraction gave activity results practically equal to those obtained with unfractionated egg brei. The other various fractions, with the possible exception of the KCl extract of fertilized eggs (dialysed), were practically inactive. In this respect similar results have been obtained for the apyrase activity of granules in the cells of chicken embryos[2].

Fig. 1



Arbacia egg fractionation scheme. Figures in parentheses represent per cent hydrolysis of ATP per hour for 50 mg dry weight of egg material.

Further experiments on another form (*Paracentrotus*) were carried out to test the general validity of the idea that the protoplasmic granules were the site of apyrase activity. These data are included in Table 1. Here the lyophiled but unfractionated eggs showed considerably

Table I

Apyrase activity of fractions of *Paracentrotus* eggs

Fraction	Per cent hydrolysis of ATP/hr/50 mg eggs	
	fertilized	unfertilized
Lyophiled, ether extracted and dialysed eggs . . .	5·0	5·1
Lyophiled, ether extracted and dialysed egg granules	25·8	13·7
Lyophiled and dialysed egg granules	18·0	13·8

[1] Cf. J. RUNNSTRÖM, L. MONNÉ, and E. WICKLUND, Nature *153*, 313 (1944).

[2] D. M. WHITAKER, J. Gen. Physiol. *15*, 183 (1931).

[3] D. M. WHITAKER, J. Gen. Physiol. *16*, 475 (1933).

[4] L. G. BARTH and L. JAEGER, J. Cell. Comp. Physiol. *30*, 111 (1947).

[5] W. M. CONNORS and B. T. SCHEER, J. Cell. Comp. Physiol. *30*, 271 (1947).

[1] A. ROTHSTEIN and R. MEIER, J. Cell. Comp. Physiol. *32*, 77 (1948).

[2] H. B. STEINBACH and F. MOOG, J. Cell. Comp. Physiol. *26*, 175 (1945).

less activity than the granules isolated from them. The pretreatment of the lyophiled powder with ether-petroleum ether extraction before it was suspended in water seemed to yield higher activities than when the powder is used untreated. This extraction removed a considerable portion of the egg pigment which is contained almost entirely in some of the granules. Ether extraction is thought to allow better wetting of the granular material and hence a higher apparent apyrase activity.

Discussion. Since apyrase appears, at least to some extent in many different fractions of the egg, one has the impression that present methods of separation are not satisfactory for removing preferentially this enzyme, or else that it is a widely distributed substance adsorbed to many proteins. The fact that in the frog[1] there are 3 protein fractions giving reasonable apyrase activities, and that in other experiments[2] apyrase activity is not separated into a single fraction are in general accord with the present experiments. In this connection, experiments by RUNNSTRÖM[3] indicate that the unfertilized egg has an appreciable amount of ATP normally present. There is no reason to suppose that this substance is not uniformly distributed throughout the egg, hence it is necessary to suppose that such apyrase as is present is in some way "inactive". The experiments of CENNAMO and MONTELLO[4] show that upon cytolysis sea-urchin eggs give an acid reaction which can quantitatively be equated to ATP breakdown. The question then arises as to whether some of the observed apyrase activity can be ascribed to the "activation" of the enzyme during the chemical treatment of the egg materials. At any rate, since chemical treatments of fertilized and unfertilized eggs were the same the observed differences in apyrase activity can be considered real.

It is difficult to evaluate the possibility of utilization of ATP by the fertilized egg as far too little is known about the chemical processes taking place. Evidence has been presented that in the developing frog egg[5] ATP is broken down under anaerobic conditions, and is resynthesized under aerobic conditions. Both of these reactions take place while there is considerable apyrase activity in the cell. Since the ATP concentration in the egg after fertilization tends to remain constant, if we grant an increased apyrase activity it must follow that the synthetic activities responsible for the production of ATP are increased proportionally with the increase in apyrase[6].

I am indebted to Prof. R. DOHRN for the use of the facilities of the Zoological Station, to Dr. A. MONROY for much valuable advice, and to Prof. J. RUNNSTRÖM for reading the manuscript.

LORIN J. MULLINS[7]

Zoological Station, Naples[8], August, 22 1949.

Zusammenfassung

Sowohl befruchtete als auch nicht befruchtete Seeigeleier enthalten allem Anschein nach aktive Apyrase Die Aktivität scheint in der Hauptsache in den körnigen Teilen des Protoplasmas lokalisiert zu sein. Gleich nach der Befruchtung steigt die gesamte Apyraseaktivität der Eier an. Diese gesteigerte Enzymtätigkeit findet sich wiederum hauptsächlich in den Zellkörnchen. Das Verhältnis zwischen Apyraseaktivität und Energiebedarf während der Entwicklung wird untersucht.

[1] L. G. BARTH and L. JAEGER, J. Cell. Comp. Physiol. *30*, 111 (1947).
[2] W. M. CONNORS and B. T. SCHEER, J. Cell. Comp. Physiol. *30*, 271 (1947).
[3] J. RUNNSTRÖM, Biochem. Z. *258*, 257 (1933).
[4] C. CENNAMO and S. MONTELLO, Exper. *3*, 10 (1947).
[5] L. G. BARTH and L. JAEGER, Physiol. Zool. *20*, 133 (1947).
[6] J. BRACHET, *Embryologie chimique* p. 148 (Liège 1945).
[7] Merck Fellow of the National Research Council, Washington.
[8] Present Adress: Department of Biophysics, Johns Hopkins University, Baltimore.

Über die Einwirkung von Ultraschall auf Gelenkflüssigkeit

Die hohe Viskosität der Gelenkflüssigkeit ist hauptsächlich durch ihren Gehalt an Hyaluronsäure, einer hochmolekularen Polysaccharidsäure, bedingt[1]. Enzymatische oder rein chemische Vorgänge, die depolymerisierend wirken, vermindern die Viskosität von Hyaluronsäurelösungen[2]. Wir haben den Einfluß von Ultraschall auf die Viskosität von Kniegelenkpunktaten und von Hyaluronsäurelösungen aus Glaskörper untersucht. Die Gelenkflüssigkeiten wurden außerdem im Elektrophoreseversuch geprüft.

Die Beschallung der Lösungen erfolgte (ohne Ausschluß von Luft) in gewöhnlichen Reagenzgläsern. Die Gläser tauchten in ein mit Wasser gefülltes und von außen gekühltes Gefäß, das dem Schallkopf aufgesetzt wurde. Die vom Quarz (10 cm²) abgegebene Schallenergie betrug 55 W (gemessen in Wasser), die Frequenz 800 kHz. Die Viskositätsmessungen wurden mit einem Viskosimeter nach OSTWALD bei 37° C gemacht. Die (rohen) Hyaluronsäurelösungen wurden aus filtrierten Rinderglaskörpern nach Fällung mit Alkohol erhalten.

	Material	Beschallungsdauer (Minuten)	Relative Viskosität (37° C)
I	Gelenkflüssigkeit	0	12,8
	(H., chronische Synovitis) . .	5	6,6
		10	2,7
		20	2,1
II	Gelenkflüssigkeit	0	4,7
	(M., Meniskusläsion)	5	2,0
		10	1,6
III	Gelenkflüssigkeit	0	2,4
	(Sch., Tibiakopffraktur) . . .	1	2,2
		10	1,6
IV	Hyaluronsäure (Glaskörper) .	0	10,5
		10	5,1
		20	5,0
V	Hyaluronsäure (Glaskörper) .	0	6,8
		20	4,0

Die Einwirkung von Ultraschall führt zu einer beträchtlichen Abnahme der Viskosität der Gelenkflüssigkeit. In der Tabelle sind die Ergebnisse von Versuchen mit drei Gelenkpunktaten verschieden hoher Viskosität aufgeführt. Nach einer Beschallungsdauer von zehn Minuten ist die Viskosität in Versuch I auf 21 %, in Versuch II auf 43 % und in Versuch III (in dem etwas dickere Reagenzgläser verwendet wurden) auf 67 % der Viskosität der unbeschallten Probe gesunken. Die aus Glaskörper gewonnenen Hyaluronsäurelösungen, die nur sehr geringe Proteinmengen enthalten, zeigen nach der Beschallung ebenfalls eine bedeutende Viskositätsabnahme.

Während der Beschallung steigt die Temperatur der Versuchslösung auf maximal 43° C. Fünfzehn Minuten langes Erwärmen von Gelenkflüssigkeit bei 44–46° C bewirkt jedoch keine Erniedrigung (sondern sogar eine Erhöhung) der Viskosität.

[1] Vgl. K. MEYER, Physiol. Rev. *27*, 335 (1947). – CH. RAGAN und K. MEYER, J. Clin. Invest. *28*, 56 (1949).
[2] Literatur siehe: L. HAHN, Fermentforschung *17*, 417 (1944).