HIGH PRESSURE EFFECTS ON ENDOGENOUS ADENINE NUCLEOTIDE LEVELS IN SEA URCHIN EGGS PRIOR TO FIRST CLEAVAGE

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In an earlier report (5), it was shown that under specified conditions the application of high hydrostatic pressure to FL amnion cells resulted in an increase of measurable endogenous adenine nucleotides, although the more slowly metabolizing primary amnion cells showed no such increase

The data seemed to indicate that in FL cells this increase was not due to an enhanced synthesis during experimental manipulation, but rather the "unmasking" of a previously unidentifiable fraction and its resultant availability for measurements.

It was considered that utilization of the sea urchin egg in comparable experiments could provide a means for (1) testing the biological extent of this phenonemon, (2) comparing the relatively metabolically inert unfertilized egg with the rapidly metabolizing fertilized egg, and (3) relating the phenomenon to some specific morphological state in the sequence of events preceding first cleavage.

METHODS

The initial series of experiments was performed on the eggs of *Arbacia punctulata* at the Woods Hole Marine Biological Station. Eggs and sperm were harvested via the KCl injection method. The eggs were washed twice with filtered sea water, fertilized, and kept in finger bowls at 20°C for approximately 25 min. They were then collected, placed in a fixation-type pressure chamber (6), and a pressure of 10,000 psi at 20°C was applied at 35 min postinsemination for a 15-min period. After this 15-min period, and while still under pressure, the egg suspension was mixed with an equal amount of 10% perchloric acid (PCA). The pressure was then released and a small sample was removed for cytological analysis. The remainder of the fixed suspension was transferred to a homogenizer and disrupted in the cold. Following total disruption of the eggs, as noted microscopically, a large sample of the material was centrifuged in the cold and the subsequent residue was stored for protein determinations. The supernatant was neutralized with KOH and analyzed for individual adenine nucleotides by a combination of enzymatic and spectrophotometric methods identical with that previously described for similar experiments on amnion cells (5). Protein determinations were made by the method of Lowry et al. (7).

Each batch of eggs was pretreated for viability and rejected if less than 95% underwent first cleavage. Each pressure experiment involved two nonpressurized, but otherwise identically treated, control specimens. One control sample was fixed at the time of pressure application and another at the time of release.

The second, and more extensive series of experiments was performed on the eggs of *Paracentrotus lividus* at the Naples Zoological Station. The eggs were collected by the ovary excision method and washed twice with filtered sea water.

The pressure technique was altered somewhat in these experiments. The results with the *Arbacia punctulata* eggs indicated an extensive breakdown of ATP in both the control and pressure series upon fixation in PCA at a temperature of 20°C (Table I). In order to prevent this breakdown, it was decided to conduct the entire experiment at a temperature of 0°–2°C.

TABLE I

Pressure Effects on Eggs of Arbacia Punctulata

20°C	mμM/mg Protein			
	ATP	ADP	AMP	Total SD
	Fixation under pressure			
Unfertilized egg				
Atmospheric pressure	—	13.9	18.8	32.7 ± 3.2
15-min 10,000 psi	—	13.4	17.5	30.9 ± 3.1
Fertilized egg				
35-min postinsemination	—	13.4	18.2	31.6 ± 3.0
50-min postinsemination	—	13.1	18.7	31.8 ± 3.3
35-min postinsemination + 15-min 10,000 psi	—	19.2	28.9	48.1 ± 4.9
	Fixation after release of pressure			
35-min postinsemination + 15 min 10,000 psi + 2 min atmos. pressure	—	13.6	17.6	31.2 ± 3.2

Previous experiments on amnion cells (5) had shown that this lower temperature did not interfere with this pressure effect, which is immediately reversible upon release of pressure at 20°C, and is maintained indefinitely at 0°–2°C, thus obviating the need for fixation while under pressure.

The eggs were inseminated, kept at 18°C, collected at specific time intervals, and rapidly chilled by addition of iced sea water. They were then centrifuged gently in the cold, and a concentrated suspension was transferred to the pressure chamber at 0°–2°C. A pressure of 10,000 psi was then applied for 15 min, released, and the eggs were transferred to a tube for rapid centrifugation in the cold. The sea water supernatant was then removed and the egg residue suspended in 4 cc of cold 6% PCA. Following this, the procedure duplicated that used with *Arbacia puntulata.*

Each experiment had its individual controls, and samples for microscopic observation were taken throughout the procedure.

RESULTS

The results of the experiments performed on *Arbacia punctulata* are shown in Table I. Each figure is the average of six determinations. The lack of measurable ATP probably results from loss due to hydrolysis which can occur at 20° in the presence of PCA. The interesting figure lies in the total of the adenine nucleotides measured following pressure treatment. The data show an increase of about 50% in measurable adenine nucleotide. The experiments on the unfertilized egg show no such increase, with both control and pressurized samples yielding the same results.

In several experiments performed on fertilized eggs at 35 min postinsemination, a pressure of 10,000 psi was applied for 15 min at 20°C and then released. At 2 min following the release of pressure, the eggs were mixed with the PCA. The results of these experiments show identical values for adenine nucleotides in controls and pressurized samples, indicating a rapid return to normal levels following pressure release.

The results of the experiments with *Paracentrotus lividus* are presented in Table II. In these experiments the eggs were pressurized before fertiliza-

TABLE II

Pressure Effects on Eggs of Paracentrotus Lividus

Pressure and subsequent treatment at 0°–2°C		mμM/mg Protein			
		ATP	ADP	AMP	Total SD
Unfertilized egg					
	P	26.8	5.0	3.7	35.5 ± 3.8
	C	27.5	4.2	3.9	35.7 ± 3.7
Fertilized egg					
No. min postinsemination					
5	P	24.5	4.2	3.1	31.8 ± 3.2
	C	25.2	4.3	3.5	33.0 ± 3.0
15	P	22.3	7.5	3.8	33.6 ± 3.2
	C	24.3	7.2	3.5	35.0 ± 3.7
30	P	23.6	5.2	3.1	31.7 ± 3.2
	C	25.3	5.9	2.4	33.6 ± 3.5
45	P	24.8	15.9	7.3	47.1 ± 4.5
	C	24.2	6.8	3.0	34.0 ± 3.2
	P1	24.3	6.4	3.2	33.9 ± 3.5
	P2	3.2	12.3	17.7	33.1 ± 3.4
	C2	5.2	14.2	15.2	33.6 ± 3.5
60	P	23.2	6.0	3.3	32.5 ± 3.3
		24.3	6.8	3.1	34.2 ± 3.5

P = 15 min at 10,000 psi.
C = Atmos. pressure control.
P1 = 15 min at 8,000 psi.
P2 = 15 min at 10,000 psi at 18°C + 2 min at atmos. pressure.
C2 = Atmos. pressure control at 18°C.

tion, at 5 min after fertilization, and at 15-min intervals after fertilization until 60 min had elapsed. Each figure represents a minimum of six experimental determinations. The data show a large increase of measurable adenine nucleotide (ca. 40%) as a result of exposure to high pressure at 45 min postfertilization. In these experiments it is readily seen that the increase appears in the ADP and AMP values while the ATP level remains constant.

Microscopic observation placed this 45-min sample at exactly the same morphologic stage as had been noted in *Arbacia*, i.e., early prophase, the total time for the first division cycle being somewhat longer for *Paracentrotus* than *Arbacia*.

Five experiments were performed at the 45-min interval at 18–19°C rather than 0°–2°C, and in each case the control and pressure samples yielded identical results; *i.e.*, the pressure apparently did not bring about any increase in the adenine nucleotide level. However, it is readily seen that a shift between the individual nucleotides is rather marked, again indicating that ATP breakdown occurs at this temperature. The failure to note a net increase in adenine nucleotide under these conditions coincides with the previous data on *Arbacia* and FL amnion cells, since about 2 min at atmospheric pressure elapsed between release of pressure and addition of PCA. As shown before, this is sufficient time for return to normal levels at 18°C.

A series of experiments was also performed at the 45-min interval at 2°C, but with pressures ranging up to a maximum of 8000 psi. In no instance was there any increase in the adenine nucleotide level, indicating that the critical pressure for appearance of this fraction is at or near 10,000 psi.

DISCUSSION

These experiments show that there is no significant fluctuation of adenine nucleotide levels during the time interval from fertilization to first cleavage when these eggs are kept at atmospheric pressure; and these results are in agreement with the ATP data of Chambers and Mende (2) and Swann (9). The cycle fluctuations of high energy phosphate during the division cycle of *Lytechinus* reported by Barnett and Downey (1) are not expressed in the adenine nucleotide fractions when measured at atmospheric pressure, but may be reflected in the high pressure changes. Application of 10,000 psi during early prophase makes available for measurement an adenine nucleotide fraction hitherto undetected. The appearance of this fraction specifically at the time of early prophase suggests the possibility of a role in either the assembly of the mitotic apparatus or the increase in cortical gel strength prior to division (10). The disruptive effect of high pressure on the formation and maintenance of the mitotic apparatus has been clearly shown by Zimmerman and Marsland (11), and more recently Zimmerman and Silberman (12) have shown the acute sensitivity of *A. punctulata* eggs to pressure at early prophase. It should also be noted that the increase in TCA-soluble sulfhydryl–containing protein associated with mitotic apparatus formation (4) occurs at about the same time period at which the adenine nucleotide appears. Also, Miki (8) has recently reported an increase of ATPase activity in the cortex of the egg at this time.

The source of the pressure-evoked adenine nucleotide fraction is as yet unknown. It would seem highly improbable that a metabolic synthesis could occur during the period of specifically imposed conditions of the low temperature (0–2°C) and high pressure (10,000 psi). The more probable interpretation would appear to be that high pressure treatment causes changes in molecular configurations, allowing the reversible disruption of enzyme-substrate complexes which could conceivably play an important role in either the assembly of the mitotic apparatus (3) or the enhancement of cortical gelation which occurs at this time. Further speculation as to the specific source or function of the adenine nucleotide would not seem justified until more definitive experiments are carried out.

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