A PRIMARY METABOLIC CHANGE OF FERTILIZATION: INTERCONVERSION OF PYRIDINE NUCLEOTIDES

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Addition of sperm to unfertilized eggs initiates new metabolic reactions necessary for cell division and differentiation. The mechanism of activation of metabolism has been extensively studied. Several changes, which are possibly involved in this activation, have been found to occur in the first few minutes after sperm addition (reviews by Rothschild, 1956; Runnström, 1959). In the sea urchin egg, the best documented early changes are: (1) an increase in respiratory rate (Warburg, 1908; others summarized by Rothschild, 1956); (2) a change in calcium binding (Mazia, 1937); (3) an increased rate of K+ transport (Monroy-Oddo and Esposito, 1951); and (4) an excretion of acid into the medium (Runnström, 1933). It is not known whether any of the above changes are the causal factors of activation, or whether they are consequences of it. Indeed, there are undoubtedly numerous unknown reactions of importance in initiating post-fertilization metabolism.

In the hope of learning more about the metabolic activation caused by fertilization, this problem was studied with the fluorometric techniques developed by Chance and his colleagues (1962) for *in vivo* measurement of reduced pyridine nucleotide. Information about the pyridine nucleotides is important, since these coenzymes indicate the oxidation-reduction activities of the cell. The initial experiments showed that shortly after sperm addition there occurred a dramatic increase in fluorescence of the cell suspension. This report describes the kinetics of this phenomenon, the results of chemical analyses to identify the fluorescent compounds, and the metabolic significance of the observed changes.

Materials and Methods: Gametes of the sea urchin, Strongylocentrotus purpuratus, were obtained by injection of 0.5 M KCl.* Cell counts were by the method of Shapiro (1935). For fluorescence measurements, 6 ml of an egg suspension (1 to 1.5 x 10^5 cells/ml) were placed in a cylindrical cuvette and stirred with a magnetic stirrer. Temperature was 17.5° C. Measurements were with a fluorometer similar to that described by Chance and Legallais (1963). Enzymatic analyses of pyridine nucleotides were by the method of Estabrook and Maitra (1962). Reduced pyridine nucleotides were extracted at room temperature with homogenization in 1M KOH-0.05M versene. Oxidized pyridine nucleotides were extracted at 0° C with homogenization in five per cent perchloric acid. In each experiment, recoveries were determined before and after fertilization. The values given below are corrected for these recoveries, which ranged from 49 to 100 per cent.

Results and Discussion: A typical experiment showing the fluorescence increase following sperm addition is shown in Figure 1. In this experiment, the increase began 40 seconds after sperm were added, with a half-reaction time of 40 seconds. This fluorescence change resulted solely from fertilization of the egg by the sperm. It was not due to sperm alone, as shown by the absence of fluorescence changes when sperm were added to sea water containing no eggs, to already fertilized eggs, and to infertile eggs.

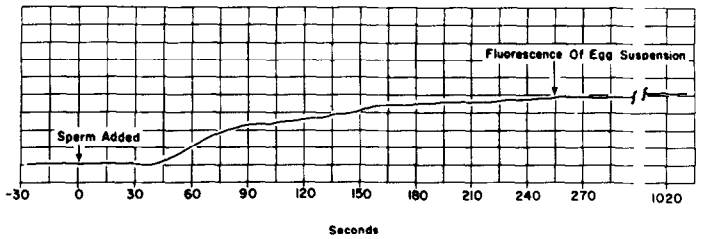

Figure 1. 366mμ excited fluorescence of sea urchin eggs following sperm addition.

*The sea urchins were the gift of Dr. Daniel Mazia, to whom I express my gratitude.

To determine whether the fluorescence change resulted from the reduction of pyridine nucleotide, cells were sampled at rapid intervals following fertilization, coincident with *in vivo* measurement of fluorescence. Subsequent enzymatic analysis, as seen in Figure 2, showed that the fluorescence increase parallels the large increase in TPNH. The sum of the reduced pyridine nucleotides was linearly related to the fluorescence, which demonstrates that the *in vivo* fluorescence change can be ascribed solely to the reduction of pyridine nucleotide. Other enzymatic analyses of reduced pyridine nucleotides, done with a total of four different batches of eggs, gave similar results. In these experiments, TPNH was found to increase three to five-fold after fertilization. DPNH, which was measured near the lower limit of sensitivity, initially decreased after fertilization, followed by a subsequent increase over the pre-fertilization level.

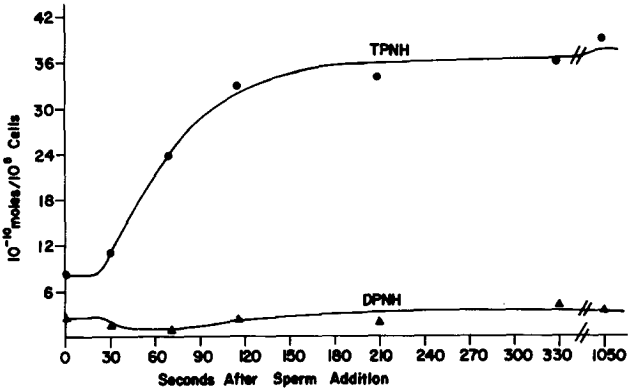

Figure 2. Results of enzymatic analysis of reduced pyridine nucleotides. This can be compared to Figure 1, since the eggs used for these two experiments were from the same female.

In a different experiment, oxidized pyridine nucleotides were extracted at rapid intervals following sperm addition. Reduced pyridine nucleotides were extracted before fertilization and ten minutes after fertilization, which was at a time when the *in vivo* fluorescence measurements indicated no further change.

The results of this experiment are shown in Figure 3 and Table I. Figure 3 shows that within 30-40 seconds after sperm addition, a large decrease in DPN is initiated, which is paralleled by an increase in TPN. Reference to Table I shows that this decrease in DPN actually represents a 28 per cent loss in diphosphopyridine nucleotide, which can be completely accounted for by the 2.6 fold increase in triphosphopyridine nucleotide. This means that the rapid increase in TPN and TPNH upon fertilization occurs at the expense of DPN.

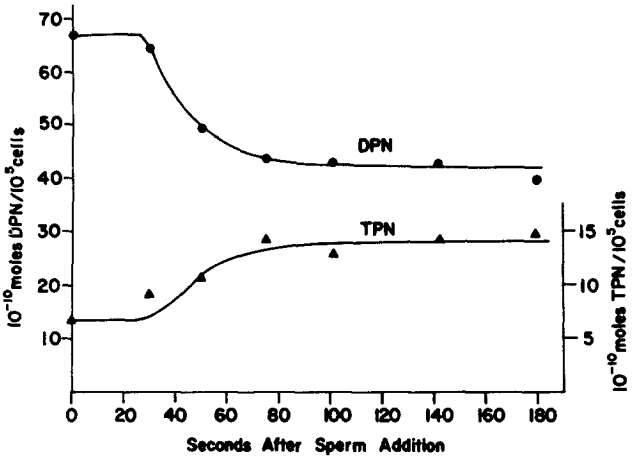

Figure 3. Results of enzymatic analysis of oxidized pyridine nucleotides. Figures 1 and 2 are not strictly comparable to Figure 3, as the eggs were from different females.

TABLE I. Pyridine Nucleotide Content of Sea Urchin Eggs

	UNFERTILIZED	FERTILIZED
DPN*	66.8 ± 0.9**	41.6 ± 1.8
DPNH	5.6 ± 2.6	10.6 ± 2.1
Σ DPN + DPNH	72.4	52.2
TPN	6.6 ± 0.5	13.9 ± 0.7
TPNH	6.6 ± 2.2	20.5 ± 0.1
Σ TPN + TPNH	13.2	34.4
Total pyridine nucleotides:	85.6	86.6

*10^{-10} moles per 100,000 cells.

**Each value is the average, ± standard deviation, of two samples assayed in duplicate.

The finding that TPNH increases after fertilization agrees with the results of Krane and Crane (1960, 1963). They reported a similar change in eggs of a different sea urchin, Arbacia punctulata, as well as in eggs of a clam, Spisula soliddisma. The results of Table I also prove the hypothesis, advanced by Krane and Crane, that the TPNH increase occurs through phosphorylation of DPN. The major differences from our results are that the above workers measured pyridine nucleotides several hours after fertilization, whereas the measurements reported in this paper show the reaction to occur in the first few minutes after fertilization. Another difference is that the TPN/TPNH ratios found in Arbacia and Spisula are much lower than has been found in S. purpuratus. This may result from species differences, or may indicate that these ratios change in the later stages when the cells are actively engaged in mitosis.

The above results show that an early metabolic transition after fertilization is the synthesis of TPN and TPNH from pre-existing DPN. The enzymes probably involved in the reduction of TPN to TPNH are the dehydrogenases of the hexose monophosphate shunt. In sea urchin eggs, the activity of these enzymes are known to be greater than the activities of the glycolytic dehydrogenases (Krahl et al., 1955). Similarly, the hexose monophosphate shunt is the major pathway of glucose oxidation (Krahl, 1956; Backström et al., 1960).

The enzymatic reaction involved in the synthesis of TPN from DPN is the type mediated by DPN kinase, which catalyzes the reaction

$$DPN + ATP \longrightarrow TPN + ADP$$

This enzyme is present in both fertilized and unfertilized sea urchin eggs (Krane and Crane, 1960). Since its action is only apparent after fertilization, fertilization must result in the activation of this enzyme.

The rapidity of the pyridine nucleotide interconversion suggests that this reaction is of fundamental importance in the activation of the fertilized egg. The metabolic role of TPNH is in reductive biosynthesis (Klingenberg

and Bücher, 1960; Lowenstein, 1961). Examples of TPNH-linked systems which may be of importance to the embryo are lipid synthesis (Wakil, 1962), nucleotide synthesis through TPNH involvement in folic acid metabolism (Friedkin, 1963), and reduction of protein disulphide (Asahi et al., 1961). Reductive synthesis in the unfertilized egg might be limited by the low level of TPNH. The formation of TPNH after fertilization would remove this limitation, resulting in the activation of new synthetic pathways.

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References

Asahi, T., Bandurski, R.S., and Wilson, L.G., J. Biol. Chem. 236: 1830 (1961).

Backstrom, S., Hultin, K., and Hultin, T., Exp. Cell Res. 19: 634 (1960).

Chance, B., Cohen, P., Jobsis, F., and Schoener, B., Science 137: 499 (1962).

Chance, B., and Legallais, V., I.E.E.E. Trans. Bio-Med. Electronics 10: 40 (1963).

Estabrook, R.W., and Maitra, P.K., Anal. Biochem. 3: 369 (1962).

Friedkin, M., Ann. Rev. Biochem. 32: 185 (1963).

Klingenberg, M., and Bücher, T., Ann. Rev. Biochem. 29: 669 (1960).

Krahl, M.E., Keltch, A.K., Walters, C.P., and Clowes, C.H.A., J. Gen. Physiol. 38: 431 (1955).

Krahl, M.E., Biochim. et Biophys. Acta 20: 27 (1956).

Krane, S.M., and Crane, R.K., Biochim. et Biophys. Acta 43: 369 (1960).

Krane, S.M., and Crane, R.K., in Control Mechanisms in Respiration and Fermentation, (B. Wright, ed.), p. 157, Ronald Press, New York (1963).

Lowenstein, J.M., J. Theoret. Biol. 1: 98 (1961).

Mazia, D., J. Cell. Comp. Physiol. 10: 291 (1937).

Monroy-Oddo, A., and Esposito, M., J. Gen. Physiol. 34: 285 (1951).

Rothschild, L., Fertilization, John Wiley and Sons, New York (1956).

Runnström, J., Biochem. Ztschr. 258: 257 (1933).

Runnström, J., Hagström, B.E., and Perlman, P., in The Cell (J. Brachet and A.E. Mirsky, eds.) v.1, p. 327, Academic Press, New York (1959).

Shapiro, H., Biol. Bull, 68: 363 (1935).

Wakil, S.J., Ann. Rev. Biochem. 31: 369 (1962).

Warburg, O., Ztschr. f. Physiol. Chem. 57: 1 (1908).