SIMULTANEOUS MEASUREMENT OF TPNH FORMATION AND RESPIRATION FOLLOWING FERTILIZATION OF THE SEA URCHIN EGG

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The preceding paper showed that an early metabolic change following fertilization of the sea urchin egg was the conversion of part of the cell's DPN into TPN and TPNH (Epel, 1964). The earliness and rapidity of this conversion suggested that this reaction might be involved in the activation of the post-fertilization metabolism.

Another metabolic change which occurs after fertilization is a large increase in respiratory rate (reviewed by Rothschild, 1957). This increase undoubtedly reflects the post-fertilization activation of DNA synthesis (e.g., Nemer, 1962), protein synthesis (Hultin, 1961; Wilt and Hultin, 1962; Nemer, 1963), ion transport (Monroy-Oddo and Esposito, 1951), and other reactions associated with cell division and early differentiation.

This paper reports on the simultaneous measurements of the post-fertilization TPNH formation and increase in respiratory rate. Besides the importance of knowing the temporal sequence of these two changes, such measurements should also indicate whether TPN-TPNH formation is directly involved with metabolic activation, or is a secondary consequence of it.

Materials and Methods: Methods for obtaining and handling of sea urchin eggs were as previously described (Epel, 1964). TPNH formation was determined by measuring the post-fertilization fluorescence increase, since enzymatic analyses had shown that the bulk of this increase could be attributed to TPNH (Epel, 1964). The in vivo fluorescence measurements were as previously described (Epel, 1964). Respiration was measured with a Clark-type oxygen electrode (Yellow Springs Instrument Co.) immersed in the cell suspension. The

electrode response time was 3 to 7 seconds, as measured by adding an extremely dense sperm suspension to the eggs and determining the lag period before attainment of the new respiratory rate. The electrode output amplification was adjusted so that 10 per cent of the oxygen in air-saturated sea water corresponde to the width of the recorder chart paper. The recordings of fluorescence and respiration were synchronized by connecting the chart motors of both recorders to a single on-off power switch.

Results: In eight experiments, done with eggs from four different females, the increase in fluorescence began at 41.7 ± 3.8 seconds after sperm addition. The increase in respiratory rate began at 58.5 ± 5.1 seconds after sperm addition. The lag period between fluorescence and respiration was 16.8 ± 6.2 seconds. As the electrode response time was 3 to 7 seconds, the actual lag period was between 13.8 and 9.8 seconds. The test of the null hypothesis that the 9.8 second difference was not meaningful showed that p was less than 0.01. The above results thus demonstrate that the increase of fluorescence precedes the increase of respiratory rate.

A typical experiment illustrating the simultaneous measurement of fluorescence and respiration is shown in Figure 1. A slight decrease in fluorescenc is seen to begin at 30 seconds, followed by the usual increase beginning at 45

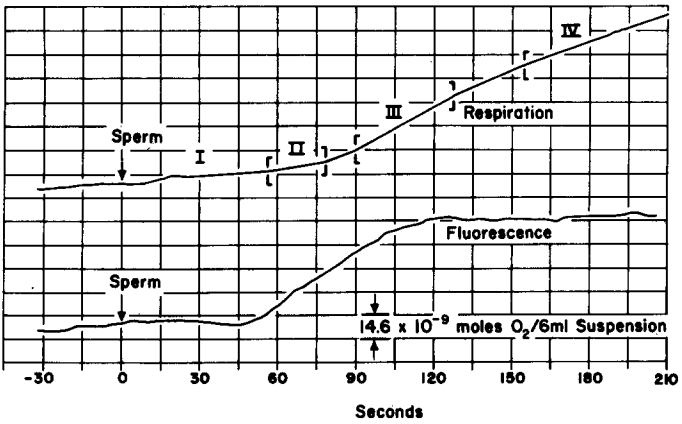

Figure 1. Synchronized recorder tracings of fluorescence and respiration. Egg concentration was 121,000 eggs/ml in a 6 ml volume.

seconds. The first increase in respiratory rate, evidenced as a change of slope, is seen to begin at 58 seconds (labelled II). This is followed by a much sharper increase at 78 seconds (labelled III). At about 130 seconds the respiratory rate gradually decreases to a lower rate (labelled IV).

Figure 2 depicts the respiratory rate derived from Figure 1 by slope determination at the indicated intervals. It is seen that the first increase in respiratory rate, phase II, results in a doubling of the initial rate. After 20 seconds, the rate increases precipitously to one 6.2 times the initial rate. This rate, phase III, lasts about 25 seconds, and then gradually decreases to one 4.6 times the initial rate.

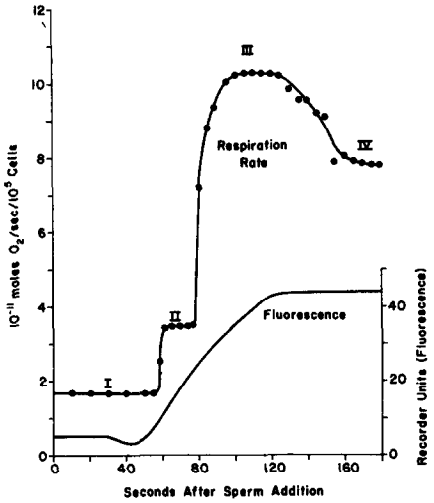

Figure 2. Comparison of respiratory rate with the fluorescence increase.

Discussion: The finding that there is a large burst of oxygen consumption following fertilization agrees with the similar polarographic analysis done by Ohnishi and Sugiyama (1963). These workers, however, did not observe the initial slight burst labelled phase II in Figures 1 and 2. Our observation of this phase was probably made possible by the high amplification and rapid chart paper speed used in our experiments.

The finding that TPNH formation begins prior to the increase in respiratory rate is surprising. A priori, one would expect that, simultaneous with TPN-TPNH formation, there would occur a slight increase in respiratory rate.

This would be expected since the formation of TPN-TPNH from DPN requires ATP (Kornberg, 1950), and since an increased rate of ATP utilization should result in an increased respiratory rate (Chance, 1959). Calculation of the energy required for pyridine nucleotide conversion, however, shows that the ATP used would be only 3 to 6 per cent of the steady-state level. It is thus conceivable that a slight lag might occur before the ADP level increased sufficiently to influence the respiratory rate.

The analysis of the first increase in respiratory rate (phase II) shows that this extra respiration would produce ATP of the magnitude required for the observed TPN-TPNH formation. Referring to Figure 2, the increment in respirato rate during phase II is 1.8×10^{-11} moles O_2/sec/10^5 eggs. As the duration of phase II is 20 seconds, the extra O_2 consumed is 36×10^{-11} moles/10^5 eggs. Assuming a P:O ratio of 3, the ATP made above the basal rate during phase II is 21.6×10^{-10} moles ATP/10^5 eggs. Similar calculations from seven other experiments showed that the increment in ATP formation during phase II was $39.7 \pm 17.4 \times 10^{-10}$ moles ATP/10^5 cells. These values are in excellent agreement with the 20 to 40×10^{-10} moles DPN per 10^5 cells converted to TPN-TPNH.

Summarizing the results and the preceding analysis of the results, the experimental data showed that TPNH formation preceded the increase in respirato rate. It was further shown, by calculation from the data, that the initial increase in respiratory rate could be a consequence of the increased utilization of ATP in the synthesis of TPN-TPNH from DPN. The above results, therefore, establish pyridine nucleotide conversion as a primary energy-utilizing reaction of fertilization.

There are two plausible interpretations for the large and rapid post-fertilization increase in TPN-TPNH. The first is that this increase is involved in activation of the hexose monophosphate shunt. This interpretation is supported by qualitative evidence of an increase in shunt activity, as determined by measuring $C^{14}O_2$ from C_1, C_2, C_6 labelled glucose (Krahl, 1956; Backström et al., 1961). It is also supported by a reported post-fertiliza-

tion increase in activity of glucose-6-phosphate dehydrogenase and 6-phosphogluconic acid dehydrogenase (Backström 1959; 1963).

A second interpretation, which is not mutually exclusive of the first, is that the increase in both shunt activity and TPNH level is a causal factor in the increased rate of synthesis in the fertilized egg. This explanation is suggested by the biosynthetic role of TPNH in reductive synthesis (Klingenberg and Bücher, 1960; Lowenstein, 1961). The low level of TPNH in the unfertilized egg could result in a low rate of synthesis. This would occur since many syntheses require both oxidations and reductions of the substrate. This need for reducing power would be met by the large increase in TPNH after fertilization.

The resultant increase in rate of synthesis would require more ATP, which could explain the finding that the enormous increase in respiration rate occurred only after some TPNH had been formed.

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