PRELIMINARY NOTES

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Oscillations of nucleotides and glycolytic intermediates in aerobic suspensions of Ehrlich ascites tumor cells

Several studies of transient metabolic changes associated with the addition of glucose to aerobic suspensions of ascites tumor cells have been reported[1–4]. Metabolic intermediates have not been reported to oscillate in such systems. However, glucose has been found capable of inducing oscillations of intermediates in intact and broken-yeast systems, muscle extracts and a reconstructed glycolytic system[5–10]. In these systems transition from aerobic to anaerobic conditions appeared to be a necessary prerequisite for sustained oscillations. The study of oscillating systems promises to yield further insight into metabolic control phenomena since the oscillations provide a continuum of transient-state changes which can be analyzed by use of phase diagrams[5]. Under the conditions described in this communication, oscillations of NAD^+, fructose 1,6-diphosphate and ATP occurred in aerobic suspensions of intact ascites tumor cells.

Ehrlich ascites tumor cells were maintained in Swiss white mice by weekly transplantation. After 10–11 days of growth, the cell suspensions were removed from the peritoneal cavity by aspiration and diluted with 5 vol. of ice-cold isotonic, cation-fortified, 55 mM phosphate (pH 7.35) buffer[11], containing a little heparin. These cells were then centrifuged at $54 \times g$ for 5 min. The non-sedimented cells (red cells *plus* a large number of colorless cells) were removed by aspiration. This process was repeated. After this second centrifugation the supernatant solution was colorless and nearly cell free. The cells remaining after aspiration were again diluted, and then sedimented at $900 \times g$ for 5 min. The supernatant was decanted. The packed cells were diluted to an approximate concentration of 4 vol. %. The exact cell concentration was determined in capillary tubes as described by McKee, Lonberg-Holm and Jehl[12]. The 4 % cell suspensions were stirred aerobically at 37°. Glucose (1 M) was added after prescribed incubation times, to give a final concentration of 5.5 mM. Aliquots were taken at convenient times before and after glucose addition. The exact time of sampling was recorded by a second person. The aliquots taken were added to 7 % $HClO_4$ and filtrates were prepared by a protocol similar to that described by Estabrook and Maitra[13]. Concentrations of glycolytic intermediates and adenine mono- and dinucleotides were measured by coupling with specific enzymes, and following changes in concentration of NADH fluorimetrically using a Turner 110 fluorimeter equipped with a Corning 7-60 primary filter and a secondary filter system utilizing a combination of Wratten 2A and 48 filters.

Fig. 1 shows the changes in NAD^+ levels which occur immediately before and after glucose addition in washed cells which had been incubated for 10, 30 and 60 min prior to glucose addition. In all systems, glucose induced transient changes in the NAD^+ level. However, only in the system preincubated for 30 min do these changes

persist as fairly regular oscillations. Similar, but not identical oscillations were found in 3 other incubations performed under similar conditions. The ability of glucose to induce regular oscillations might be correlated to the metabolic state of NAD^+ prior

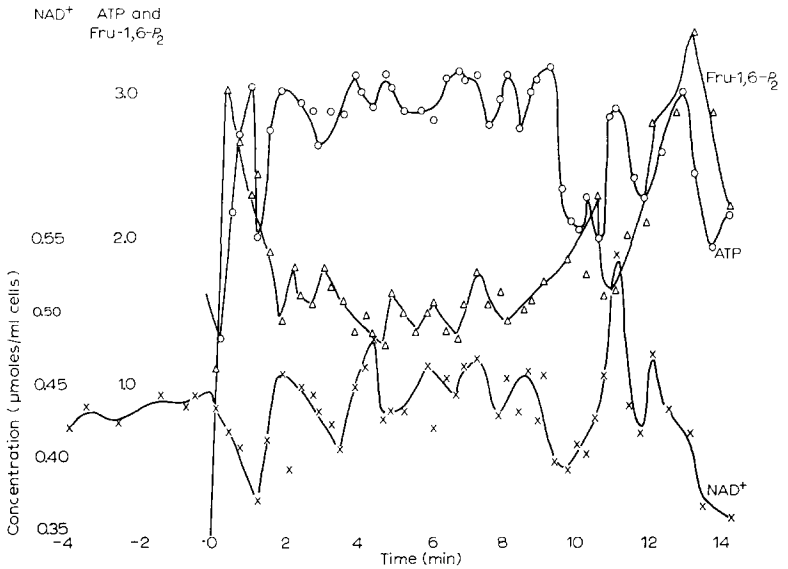

Fig. 1. Changes in NAD concentration upon glucose addition. Samples of washed tumor were preincubated in the absences of exogenous substrate for 10 (□), 30 (△), or 60 min (○) prior to glucose addition, under the conditions described in the text. After 10 min preincubation the NAD level holds constant, after 30 min it tends to rise and after 60 min it is declining. Only in the sample incubated for 30 min were sustained and fairly regular oscillations obtained. The amplitude of these oscillations is much greater than the apparent analytical error, of $\pm$ 0.0125 μmoles/ml cells.

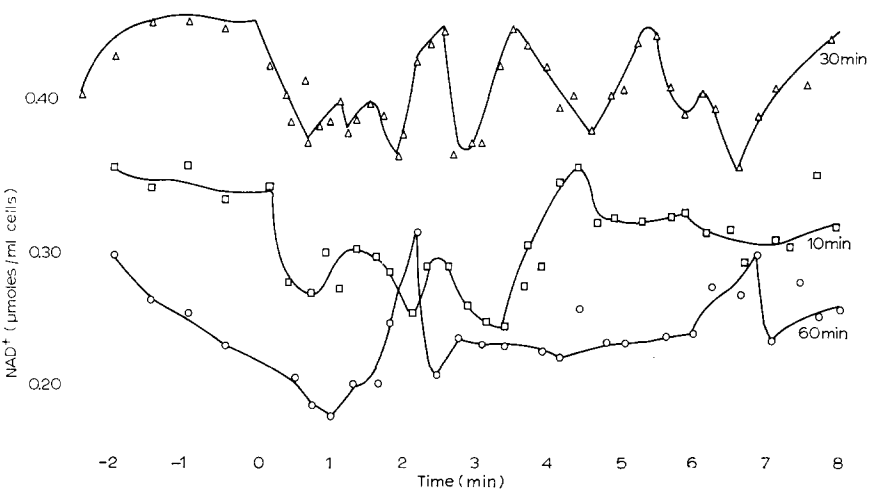

Fig. 2. Glucose-induced oscillations of NAD (×), Fru-1,6-P_2 (△) and ATP (○). The cells were preincubated at 37° for 30 min prior to glucose addition as described in the text. NAD again rises prior to glucose addition. Glucose addition induced a decline of NAD followed by sustained oscillations, which appeared to be dampened during the first 6–8 min, but then to become regenerated. The pattern of ATP and Fru-1,6-P_2 oscillation appears related to the NAD oscillations. The ATP level prior to glucose addition was approx. 3 μmoles/ml cells.

to glucose addition; *i.e.*, those systems in which NAD^+ is accumulating appear to be more prone to oscillate. It is suggested that this increase in the NAD^+ level is an early reflection of substrate limitation.

Fig. 2 shows another experiment in which NAD^+, ATP, and fructose 1,6-diphosphate were measured. These data show that the oscillations are not limited to the pyridine nucleotide. We were not able to show oscillations in O_2 consumption using the Gilson vibrating platinum electrode and more dilute cell suspensions.

When phase-plane plots[5] of fructose 1,6-diphosphate *vs.* ATP concentration are made from the data of Fig. 2, it is found that ATP is out of phase with fructose 1,6-diphosphate throughout the experiment. This is consistent with the concept that fructose 1,6-diphosphate levels change in response to changes in the activity of phosphofructokinase which in turn is controlled by the level of the various adenine nucleotides[14]. Furthermore, these changes appear to be readily and continuously reversed.

We are presently investigating the responses of additional intermediates and are trying to elucidate more definitively the conditions necessary for oscillations.

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Department of Biochemistry,
California College of Medicine,
University of California,
Los Angeles, Calif. (U.S.A.)

K. H. IBSEN
K. W. SCHILLER

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The inhibitory effect of uncouplers of oxidative phosphorylation on mitochondrial respiration

It has been known for some time that excessive concentrations of uncouplers of oxidative phosphorylation will inhibit mitochondrial respiration. For the case of

Abbreviations: FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; TTFB, tetrachlorotrifluoromethylbenzimidazole; TMPD, tetramethyl-*p*-phenylenediamine; ETP, electron-transport particle.