A CRABTREE-LIKE EFFECT WITH ISOLATED ASCITES TUMOR MITOCHONDRIA

Leonard A. Sauer

The Rockefeller Institute, New York, N. Y.

Received August 4, 1964

The ease with which the Crabtree effect can be studied in ascites tumor cells has led to many theories for explaining the mechanism of the effect, but no one of these has enjoyed universal acceptance (cf. review by Ibsen, 1961). Most, however, have stressed the importance of the hexokinase reaction and its relation to oxidative phosphorylation. Studies on hexokinase in ascites tumor cells have shown that a large fraction of the enzyme is mitochondrial bound (Wu and Racker, 1959; McComb and Yushok, 1959; Blecher, 1963; and Sauer (unpublished)). This could mean that hexokinase is in an unique structural relationship with the enzymes involved in energy production in these cells. Therefore, experiments with isolated mitochondria from these cells might throw light on the mechanism. The experiments reported here demonstrate, for the first time we believe, that addition of glucose to mitochondrial suspensions from ascites tumor cells results in a response that resembles in many ways the response observed with intact ascites cells. This mitochondrial response is caused by the formation of low concentrations of glucose-6-phosphate, which under these experimental conditions, acts to inhibit the bound hexokinase.

The Ehrlich tetraploid (obtained from Dr. Ray Wu) and ELD or Ehrlich hyperdiploid (obtained from Dr. Theodore S. Hauschka) ascites tumors used in this study were grown, transferred, and harvested as previously described (Sauer, et al., 1960). Bloody samples were not

used. Mitochondrial preparations were prepared from homogenates made in 0.25 M sucrose + 1 mM EDTA, and dry weights were determined by methods previously described (Sauer, et al., 1962). Oxygen consumption was measured using a Clark oxygen electrode. Glucose-6-phosphate (Gl-6-P) was quantitatively determined spectrophotometrically by the Gl-6-P dehydrogenase-linked reduction of TPN. Samples taken for these analyses were heat denatured in boiling water for 2 minutes and the flocculent precipitate removed by centrifugation. This procedure did not cause detectable hydrolysis of a known Gl-6-P solution. Calculations of oxygen consumption and ADP/O ratios were made by the method of Chance and Williams (1955), while respiratory control ratios (RC) were computed as defined by Chance (1959).

Figure 1 indicates the response of mitochondria isolated from the ELD and Ehrlich tetraploid ascites cells to repeated small additions

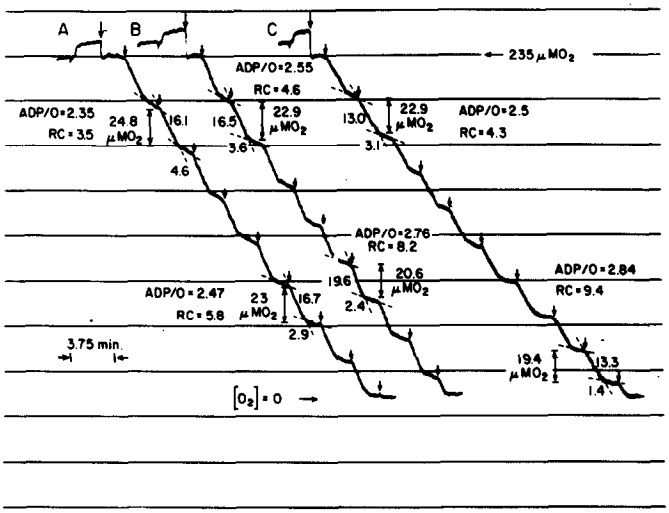

Figure 1. Respiratory control in ascites tumor mitochondria. Suspending medium contains: tris (pH 7.4), 30 mM; KCl, 20 mM; EDTA, 1 mM; sucrose, 50 mM; bovine plasma albumin, 1%; $MgCl_2$, 9 mM; alpha-ketoglutarate, 5 mM; and unless otherwise stated Pi (pH 7.4), 5 mM. Temp.: 25°C. Chamber volume is 6.5 ml. Trace A - 8.2 mg. ELD mitochondria. Trace B - same as trace A plus 4.8 mM fluoride. Trace C - 6.4 mg. Ehrlich tetraploid mitochondria otherwise same as trace B. Respiratory rates, recorded on the traces, are in μM O_2/minute. The first arrow at the top indicates mitochondria addition, while subsequent arrows indicate repeated ADP additions (0.04 ml. of 0.018 M ADP).

of ADP. In all experiments the ADP/O and RC ratios increased to a steady value as the trace proceeded.[1] RC ratios of 8 to 10 are commonly seen with alpha-ketoglutarate as substrate, with values occasionally as high as 22.

Figure 2 shows the effect of added glucose (1 mM) on the mitochondrial preparations in the presence of endogenously generated ATP. In both preparations glucose addition resulted in a sudden burst of respiratory activity followed by a 2.5 to 3.5 fold inhibition of respiration. To obtain the value of this inhibition the activated respiratory rate was divided by the inhibited rate. Additions of ATP, more glucose, or another sugar during the inhibited period had no effect. The

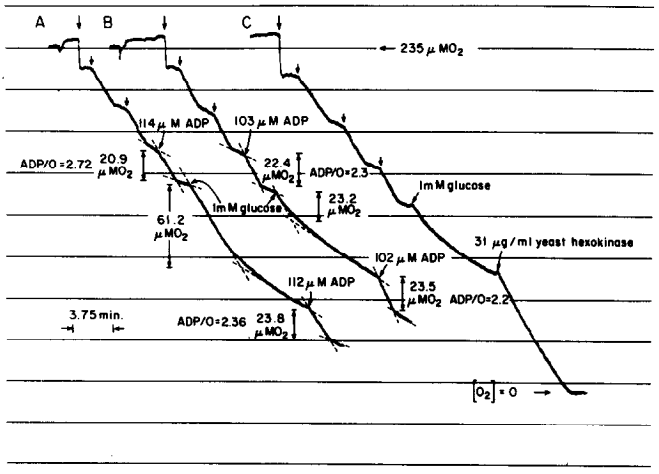

Figure 2. Effect of glucose in mitochondrial respiration. Suspending medium is the same as in Figure 1. Additions of ADP, glucose and yeast hexokinase are noted on traces. Trace A - 6.4 mg. Ehrlich tetraploid mitochondria. The change in oxygen concentration following glucose addition is 61.2 μM O_2. An average calculated ADP/O ratio of 2.54 leads to 312 μM G1-6-P generated. Analysis at end of experiment found 310 μM G1-6-P. Trace B - 8.2 mg. ELD mitochondria. Calculated G1-6-P concentration is 105 μM; found, 183 μM. Trace C 8.2 mg. ELD mitochondria. Same conditions as trace B except Pi is 1 mM. 590 μM G1-6-P were found when O_2 was exhausted.

1. In previous manometric experiments with ELD mitochondria (Sauer, et al., 1962) the usefulness of added fluoride was demonstrated. In these experiments its usefulness was confirmed, as shown in trace B.

inhibition, however, could be relieved by another ADP addition, as indicated, or by adding dinitrophenol (not shown), demonstrating that the respiratory inhibition was due to depression of the hexokinase reaction and therefore to a lack of phosphate acceptor.

From the increased respiratory activity following glucose addition it was possible to calculate the G1-6-P generated. In making these calculations, we have taken as the ADP/O ratio the average of the values obtained before and after glucose addition, and have assumed that all the ADP generated from the hexokinase reaction is rephosphorylated. These calculated values agree reasonably well with the values found on analysis. Some deviation is to be expected since the inhibition is not total. Under these conditions, it is interesting that for the same degree of respiratory inhibition, different amounts of G1-6-P were generated by the two preparations. Thus, ELD mitochondrial preparations were inhibited at calculated values ranging from 80 to 130 μM G1-6-P. Mitochondrial preparations from the Ehrlich tetraploid, however, were not inhibited until G1-6-P values of 250 to 300 μM were generated.

The increased respiratory activity that follows glucose addition could be inhibited in both mitochondrial preparations by the prior addition of G1-6-P. For example, with the Ehrlich tetraploid mitochondrial preparation the addition of 300 μM G1-6-P abolished the response to added glucose. When 100 μM G1-6-P was added, however, the response was not abolished, but instead, respiration commenced and remained linear until an oxygen consumption equivalent to the generation of about 200 μM G1-6-P was reached, then the typical inhibition began. The sum of the known added plus the calculated generated G1-6-P concentrations always totaled 250 to 300 μM G1-6-P, the inhibitory concentration for these mitochondria. Qualitatively similar results were observed with ELD mitochondrial preparations.

Also shown in Figure 2 is the effect of yeast hexokinase addition

during the glucose inhibited state (trace C). This resulted in a prompt reversal of the respiratory inhibition, in keeping with the known lack of effect of G1-6-P on yeast hexokinase (Crane and Sols, 1953). When 2-deoxyglucose (DG) or fructose were added to ELD mitochondria instead of glucose, a respiratory depression was not seen; rather, there was a linear rate of respiration until the oxygen was exhausted. This is in keeping with the above results since it is known that the 6-phosphates of these sugars do not inhibit tumor hexokinase (McComb and Yushok, 1959). Ehrlich tetraploid mitochondria, however, showed an inhibition following fructose but not DG addition. Since G1-6-P could be easily detected after adding fructose, the finding by Wu and Racker (1959) that these mitochondria contain G1-6-P isomerase is confirmed.

Figure 3 shows the repeatedly observable effect of varying the in-

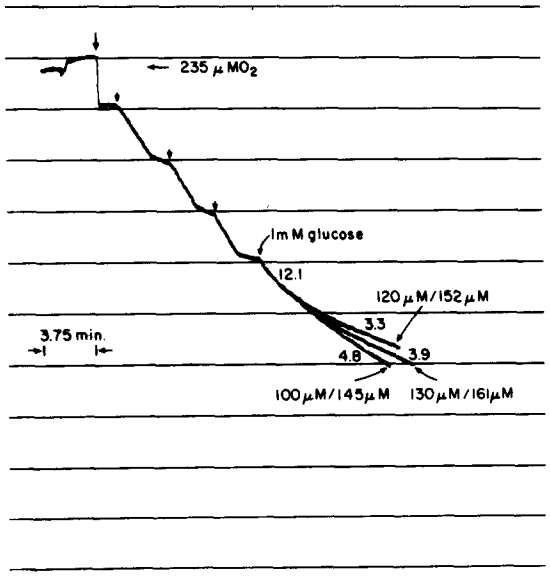

Figure 3. Effect of Pi on glucose inhibition of mitochondrial respiration. 8.2 mg. ELD mitochondria. Suspending medium is the same as trace B, Figure 2. Pi concentrations are: upper curve, 1 mM; middle curve, 2.3 mM; and lower curve, 9.2 mM. Ratios at end of curves represent G1-6-P calculated/G1-6-P found. Respiratory rates written along trace are in μM O_2/minute.

itial amounts of inorganic phosphate (Pi) on the response of the ELD mitochondrial preparation to glucose addition (results with Ehrlich tetraploid mitochondria were similar). The portions of the traces before glucose additions were superimposed to allow direct comparison. It is seen that the respiratory inhibition was outstanding when low concentrations of Pi were added and became progressively less as the initial Pi concentration was increased. Indeed, raising the Pi concentration to 15 mM (not shown) abolished the inhibition due to glucose, but since the upper portion of the trace was also distorted, its meaning is not clear. This abolition is not due to a relief of Pi lack for oxidative phosphorylation since in the presence of low Pi (1 mM) the glucose inhibited state can be reversed by ADP, or yeast hexokinase (Figure 2, trace C). A likely explanation for the Pi effect is the relief of the end product inhibition of hexokinase, as noticed by Tiedemann and Born (1960), and Rose, et al. (1964).

Thus, it is clear that the inhibition of coupled mitochondrial respiration following glucose addition is caused by G1-6-P inhibition of the mitochondrial hexokinase. However, an important problem is whether this mitochondrial reaction represents a part or the whole of what occurs in the intact cell. It could well be the explanation for the early responses seen on glucose addition since the inhibitory G1-6-P concentrations found here are well within the levels found in careful analyses of glucose treated, intact ascites cells. Thus, Wu and Racker (1959) found 0.5 to 0.6 mM hexosemonophosphate/liter intracellular water in the Ehrlich tetraploid. In this study 0.3 mM G1-6-P caused a marked respiratory inhibition of the mitochondria from this tumor. Also, Chance and Hess (1959) found that glucose concentrations as low as 120 μM could cause a respiratory inhibition in intact ELD cells. In this study 80 to 130 μM G1-6-P caused a respiratory inhibition in ELD mitochondria. Since on glucose addition, the intracellular Pi is known to decrease in

these cells (Wu and Racker, 1959; Hess and Chance, 1961) the mitochondrial inhibition would be augmented, as noted above in Figure 3.

The reported Gl-6-P Ki values for particulate bound hexokinases (Crane and Sols, 1953; McComb and Yushok, 1959) are larger than the above data would suggest. The reason for this is not understood, but differences in assay conditions, such as Pi buffer concentrations, must be considered. However, a more specific enzyme difference may be involved since these preparations have different inhibitory Gl-6-P concentrations. The demonstration that DG does not cause an inhibition in these experiments while glucose does may indicate that the early Crabtree effect (polarographic studies) as seen in intact cells on glucose addition has a different mechanism from that seen on DG addition. This has already been suggested for the long term Crabtree effect (manometric studies) (Ibsen, et al., 1962; Hofmann, et al., 1962) seen with these sugars. Experiments are presently under way that, it is hoped, will clarify the above problems.

Acknowledgement

The author would like to thank Dr. Philip Siekevitz for numerous discussions during this work.

References

Blecher, Melvin, J. Biol. Chem., 238: 513 (1963).
Chance, Britton and G. R. Williams, J. Biol. Chem., 217: 383 (1955).
Chance, Britton, in CIBA Foundation Symposium on the Regulation of Cell Metabolism. J. and A. Churchill, Ltd., London, 1959, p. 91.
Chance, Britton and B. Hess, J. Biol. Chem., 234: 2416 (1959).
Crane, Robert K. and Alberto Sols, J. Biol. Chem., 203: 273 (1953).
Hess, Benno and Britton Chance, J. Biol. Chem., 236: 239 (1961)
Hofmann, E., S. König and I. Behne, Naturwissensch., 2: 41 (1962).
Ibsen, K. H., Canc. Res., 21: 829 (1961).
Ibsen, Kenneth H., Elmon L. Coe, and Ralph W. McKee, Canc. Res., 22, 182 (1962).
McComb, R. B. and W. D. Yushok, Biochim. Biophys. Acta, 34: 515 (1959).
Rose, Irwin, J. V. B. Warms and E. L. O'Connell, Biochem. Biophys. Res. Comm., 15: 33 (1964).
Sauer, L. A., A. P. Martin and E. Stotz, Canc. Res., 20: 251 (1960).
Sauer, L. A., A. P. Martin and E. Stotz, Canc. Res., 22: 632 (1962).
Tiedemann, Heinz and Jochen Born, Hoppe-Seyler's Zeit. f. physiol. Chem., 321: 205 (1960).
Wu, Ray and E. Racker, J. Biol. Chem., 234: 1029 (1959).
Wu, Ray and E. Racker, J. Biol. Chem., 234: 1036 (1959).