Preliminary Notes

Structural factors involved in the control of metabolism in ascites-tumor cells

It has been shown that light-scattering measurements can be used to observe size changes in suspensions of intact cells[1] and isolated mitochondria[2,3]. It became of interest to determine whether this method could also be employed for the measurement of changes in mitochondrial size within living cells. If successful this might allow correlation of metabolic and structural changes which would be helpful in clarifying mechanisms of metabolic control.

Fig. 1. shows a simultaneous recording of oxygen utilization and light-scattering in a 10% suspension of Ehrlich ascites-tumor cells. The addition of glucose to the suspension causes an initial increase in the rate of respiration and an increased absorbance at 650 mμ. The optical change then levels off and at about the same time respiration slows down to a rate considerably less than the initial endogenous state. The changes in respiration are in general agreement with the results reported by CHANCE AND HESS[7]. The absorbance changes must be due to light scattering since

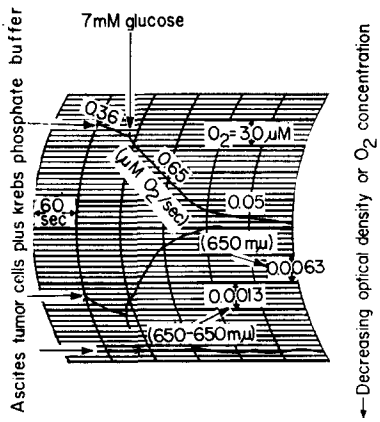

Fig. 1. Respiratory and light-scattering changes accompanying the addition of glucose to Ehrlich ascites-tumor cells. 7-day old ascites cells were withdrawn from the peritoneal cavity of mice, washed, and suspended in Krebs phosphate buffer. Respiration was measured polarographically[4] and the turbidity or light-scattering[5] was recorded simultaneously with a spectrophotometer designed to measure small changes in light transmission in materials of high initial optical opacity[6]. The lower trace at 650–650 mμ was carried out as an instrumental control. As is seen, no change was brought about in the level of this trace throughout the course of the experiment. The rates of respiration calculated in μmoles/sec/l are written above the trace. The calibrations for the optical changes are given in absorbance (optical density). Time moves from left to right. Temperature, 25°.

they have been demonstrated over a wide range of wavelengths between 400 and 700 mμ by the method described in Fig. 1 and by direct determination of light scattered at 90°; moreover the magnitude of these optical changes increased with decreasing wavelength. These findings are in accord with light-scattering theory[5].

From the earlier work[1–3,5] an increase in light scattering is the result of a decrease in particle size. Extensive determinations of packed-cell volume and microscopic examination have shown no change in cell size on addition of glucose. However, an amount of isolated mitochondria equal to that present in a cell suspension yields about the same increase in light scattering on addition of glucose, hexokinase, and

Abbreviations: ADP, ATP, adenosine di- and triphosphate.

ATP, as found with intact cell suspensions. These findings indicate that under certain conditions light-scattering changes of intact cells may result from changes in mitochondrial size within the cells.

This finding may be explained by the following considerations. Glucose addition causes a transient increase in intracellular ADP. CHANCE AND HESS[8-9] showed that the ADP became available to mitochondria, which have a high affinity for this substance. This resulted in the acceleration of respiration seen in Fig. 1, which is similar to the effect of ADP on the respiration of isolated mitochondria. The ability of intramitochondrial ADP and ATP to cause shrinking of mitochondria in suspensions[3,10-12] would explain the light-scattering increase which occurs simultaneously with the respiration increase.

The inhibition of respiration occurs simultaneously with an inhibition of glycolysis[8-9], and in this circumstance no further change in mitochondrial size is observed. To explain these inhibitions LYNEN AND KOENINGSBERGER[13], RACKER[14], and CHANCE AND HESS[7] have proposed that a compartmentalization exists in the cell with respect to availability of metabolites for cytoplasmic and mitochondrial reactions. They postulated specifically that ATP accumulates in mitochondria and is no longer available for extramitochondrial oxidation of glucose. Thus it was envisaged that glycolysis would decline due to the unavailability of ATP, and respiration would be inhibited due to lack of ADP. The decreased mitochondrial size observed here may be identifiable with the "compartmentalization" postulated by the aforementioned investigators[7,13-14]. Since data are available which show that ATP does not escape from contracted as readily as from swollen mitochondria[15-16], the decrease in mitochondrial size may explain the unavailability of extramitochondrial ATP and thereby the inhibitions observed.

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