BBA 41132

Stimulation of ascites tumor cell respiration by low concentrations of 2-deoxyglucose

Various evidence has suggested that the intracellular P:O ratio in Ehrlich ascites carcinoma cells is near 3. From comparisons of steady-state rates of anaerobic glycolysis and endogenous respiration[1] or from the depression of respiratory rate in the Crabtree effect and the rate of aerobic glycolysis[2], P:O ratios between 2.5 and 3.0 may be obtained, assuming a constant rate of ATP hydrolysis under the different conditions. Phosphate and oxygen consumption during the period of sharp respiratory stimulation immediately after glucose addition also indicate a ratio of 3 (ref. 3). The transitory Crabtree effect after addition of small amounts of glucose is also consistent with a P:O of 3 (ref. 4), and the transient stimulation of respiration after addition of glucose to iodoacetate-poisoned cells implies a ratio of 2 or more[5]. Recently, however, MORTON AND LARDY[6-8], described a new method for estimating intracellular P:O ratios by measuring oxygen and inorganic phosphate uptake after addition of 2-deoxyglucose to serve as a phosphate trap. They observed ratios of 3 for immature bull spermatozoa under certain conditions, but found ratios less than one for ascites tumor cells. In view of this conflicting piece of evidence, the phosphorylation of deoxyglucose and its effect on respiration is being re-examined. Previous work[9,10] indicated that the effect of deoxyglucose on respiration is complex, since higher levels of deoxyglucose cause first a stimulation and then a depression of respiration. The ability to produce a prolonged depression of respiration is also exhibited by several other sugars which are also phosphorylated but not glycolyzed[11]. In comparison, low concentrations of deoxyglucose elicited a less complicated response, causing a simple, transient stimulation[10]. The magnitude and duration of the stimulation was uncertain because a steady-state oxygen electrode method was used[12]; hence, the stimulation of respiration was re-estimated by a kinetic method which allowed exact calculation of oxygen consumption[4,5,12]. The stoichiometry between the stimulated oxygen consumption and the deoxyglucose added was compared over a range of low sugar concentrations (0.04–0.77 mM) to obtain the effect of a brief burst of phosphorylation without the more profound changes in metabolism engendered by the extensive phosphorylation at high levels of deoxyglucose[10,13,14].

The Gilson Medical Electronics oxygraph, Model K, a polarographic device employing a bare, vibrating platinum cathode, was used for measurement of respiratory rates as described previously[4,5,12]. 1 ml of phosphate–Locke buffer (isotonic 54 mM phosphate, pH 7.35) was placed in the 2-ml oxygraph chamber and 0.20 ml of Ehrlich ascites tumor cell preparation, washed and suspended in the buffer to a concentration of 28–55 % (v/v), was added and mixed in with a brief stream of bubbles. The fall in oxygen tension was recorded for 2 min and then 0.10 ml buffer of 0.5–10.0 mM 2-deoxyglucose was also blown in and recording was continued for 4 or 5 min. Rates of respiration were calculated from the slopes of the tracings. Results from a representative experiment, shown in Fig. 1, illustrate the effects of increasing deoxyglucose concentration. Addition of the buffer without sugar causes a transient slowing of respiration which varies in magnitude from one tumor preparation to another but

which is consistent within any one preparation; this artifact must be taken into account in any estimation of respiratory stimulation[4]. The two low deoxyglucose concentrations (0.04 and 0.08 mM) cause an initial spike in respiration lasting 30–45 sec and then approach the control curve. The higher concentration (0.77 mM) elicits a more prolonged stimulation but eventually produces a depression of respiration, evident after 3 min. Such a later depression of respiration develops when the deoxyglucose concentration is in excess of 0.2 mM.

Total stimulation of respiration was estimated by graphically integrating between the control curve (buffer only added) and the curve obtained after addition of the sugar. Only the periods of stimulation were included, the later periods of depressed respiration being neglected. The average results from 4 different experiments are shown in Fig. 2. Conditions used are summarized in Table I. Despite variations in tumor strain, mouse strain, and tumor age, the results proved consistent, and a linearity is evident between the respiratory stimulation and the amount of deoxyglucose added up to 0.20 μmole of the sugar (equivalent to 0.15 mM). The failure of linearity above this level is correlated with the appearance of the later respiratory inhibition, which may indicate that the inhibitory effects are beginning before the stimulated phase ends. The slope of the proportional region indicated by the line in Fig. 2 gives 0.142 μmole excess O_2 consumed during the stimulation per μmole deoxyglucose (dGlc) added; the P:O ratio may then be calculated as $\frac{1}{2}$ (Δ dGlc/ΔO_2) or $\frac{1}{2}$ (1.00/0.142) = 3.5. When the possible error in the points is taken into account, the ratio could be as low as 3 or as high as 5; hence, the results can be reconciled with a P:O ratio of 3 but are inconsistent with ratios less than one[6]. A later communication will describe the reasons behind the low P:O ratios obtained by MORTON AND LARDY[6] in some detail, but in general they are a consequence of the long time interval (15 min) used by these authors. Within 5 min after addition of a high concentration

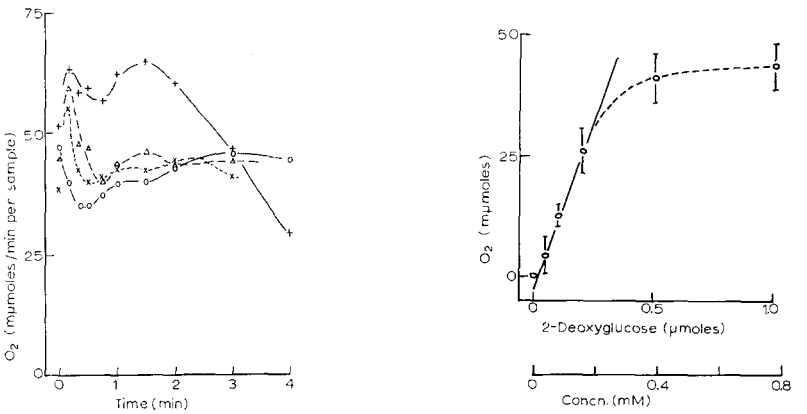

Fig. 1. Rates of oxygen consumption after addition of low concentrations of deoxyglucose. Expt. C (Table I). O—O, buffer without deoxyglucose added at zero time; ×---×, deoxyglucose added to a final concentration of 0.04 mM; △---△, 0.08 mM; +—+, 0.77 mM.

Fig. 2. Total stimulation of oxygen consumption as a function of deoxyglucose added. Average of data from Expts. A–D; vertical bars give mean deviation. Values were calculated by graphically integrating between rate curves obtained after deoxyglucose addition and the curve obtained after buffer addition (Fig. 1). Lower abscissa scale gives final concentration of deoxyglucose in mM; other scales give total amount per sample.

of deoxyglucose, the rate of deoxyglucose phosphorylation has declined to near zero while oxygen consumption is continuing at a low but steady rate; hence, changes calculated over a 15 min interval give much lower ratios than rates estimated during the first 2 or 3 min after deoxyglucose addition.

The tendency of this approach to yield ratios greater than 3 may reside in oversimplifications in the assumptions behind the calculation, which may be summarized as follows:

(a) No ATP is drawn away from the endogenous ATP-utilizing reactions for deoxyglucose phosphorylation. Any such diversion of ATP from the endogenous reactions would decrease the stimulation of oxidative phosphorylation and thereby increase the apparent P:O ratio. The deviation of the curve in Fig. 2 from linearity above 0.2 mM suggests that ATP is diverted at higher sugar levels, and there is no guarantee that this does not occur to a lesser degree at low sugar levels. The magnitude of such an artifact would be impossible to estimate with the existing data, but its effect should be minimized by the extrapolation to zero deoxyglucose concentration.

(b) The 2-deoxyglucose used is pure. This assumption is probably valid. The preparation, obtained from Mann Research Laboratories, Inc. was stated to be chromatographically homogeneous. Incubation with hexokinase and ATP and chemical and enzymatic analysis of the products indicated that the deoxyglucose 6-phosphate formed contained less than a 1 % contamination with glucose 6-phosphate or fructose 6-phosphate. With sufficient periods of incubation with cells, the deoxyglucose was totally consumed, demonstrating that there was no significant nonmetabolizable component.

(c) All the deoxyglucose is phosphorylated during the period of stimulated respiration. Less than complete phosphorylation would increase the apparent P:O ratio calculated from the total sugar added. Estimates of deoxyglucose consumed in separate experiments indicated that a 0.15 mM concentration was at least 90 % utilized by 2 min, the usual duration of stimulated respiration. This supports the general validity of the third assumption but does not rule out the possibility that a small percentage of the deoxyglucose is remaining at the end of the period of stimulated respiration. Assuming a 90 % phosphorylation would lower the calculated P:O ratio from 3.50 to 3.15.

TABLE I

CONDITIONS USED IN EXPERIMENTS ON RESPIRATORY STIMULATION BY 2-DEOXYGLUCOSE

Note: Between Expts. A and B, the original hypotetraploid strain of Ehrlich ascites carcinoma (I) was discontinued and a new hyperdiploid strain (II) was started.

Expt.	*Temp.*	*% Cell concn. (v/v)*	*Tumor age (days)*	*Tumor strain*	*Mouse strain*
A	23°	4.3	9	I	Swiss
B	23°	5.5	10	II	Swiss
C	24°	7.5	13	II	Strong A
D	23°	8.5	7	II	Strong A

This project is supported by a grant (CA-10723) from the National Institutes of Health, the U.S. Department of Health, Education, and Welfare, Bethesda, Md.

ELMON L. COE

Department of Biochemistry,
Northwestern University Medical School,
Chicago, Ill. (U.S.A.)

1 J. H. QUASTEL AND I. J. BICKIS, *Nature*, 183 (1959) 281.
2 K. H. IBSEN, E. L. COE AND R. W. MCKEE, *Nature*, 183 (1959) 1471.
3 B. HESS AND B. CHANCE, *J. Biol. Chem.*, 234 (1959) 3031.
4 I.-Y. LEE AND E. L. COE, *Biochim. Biophys. Acta*, 131 (1967) 441.
5 E. L. COE, *Biochim. Biophys. Acta*, 93 (1964) 209.
6 B. E. MORTON AND H. A. LARDY, *Biochemistry*, 6 (1967) 43.
7 B. E. MORTON AND H. A. LARDY, *Biochemistry*, 6 (1967) 50.
8 B. E. MORTON AND H. A. LARDY, *Biochemistry*, 6 (1967) 57.
9 K. H. IBSEN, E. L. COE AND R. W. MCKEE, *Biochim. Biophys. Acta*, 30 (1958) 384.
10 K. H. IBSEN, E. L. COE AND R. W. MCKEE, *Cancer Res.*, 22 (1962) 182.
11 W. D. YUSHOK, *Cancer Res.*, 24 (1964) 187.
12 E. L. COE AND M. H. COE, *Anal. Biochem.*, 11 (1965) 65.
13 R. B. MCCOMB AND W. D. YUSHOK, *Cancer Res.*, 24 (1964) 193.
14 K. K. OVERGAARD-HANSEN, *Biochim. Biophys. Acta*, 104 (1965) 330.

Received July 16th, 1968