INHIBITION OF THE CRABTREE EFFECT IN ASCITES CARCINOMA CELLS BY 2-DEOXY-D-GLUCOSE[1,2]

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It has been repeatedly confirmed since the original observation of Crabtree (1929) that the oxygen consumption by ascites carcinoma cells is markedly depressed below the endogenous level when a glucose substrate is present above a certain minimum concentration. The substitution of fructose, mannose, or 2-deoxy-D-glucose (2-DOG) for glucose as the substrate produces a similar reduction in oxygen consumption[3] (Ibsen, 1961). That fructose and mannose will produce the Crabtree effect is not surprising in view of the general utilizability of these hexoses. On the other hand, the similar depression of cellular respiration by 2-DOG should be of more than passing interest since this compound, as far as known, cannot be metabolized beyond the 2-DOG-6-phosphate stage by the ascites carcinoma cells (Ibsen *et al.*, 1958).

In this report we present data showing that 2-DOG simultaneously inhibits the rate of glycolysis of glucose and increases its rate of oxidation. Since this dual effect of 2-DOG has not been previously reported, and

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with further work may aid in elucidating the control mechanisms involved in the Crabtree effect, we are reporting these preliminary studies at this time.

Methods: Ehrlich ascites carcinoma cells grown for 7-10 days in white Swiss mice were employed. The cells were withdrawn and washed 4 to 5 times by gentle differential centrifugation to remove red blood cells and ascites fluid. Calcium free Krebs-Ringer phosphate medium (KR) adjusted to pH 7.4 was the washing and incubating fluid. The packed cells were suspended in 7 volumes of KR in all experiments except 8 and 9 where the dilution was 1:64. Cells were incubated in a total volume of 2 ml of KR. All incubations were carried out at 37° C for 2 hours in 50 ml Erlenmeyer flasks in a Dubnoff metabolic shaking incubator. The $C^{14}O_2$ was collected by trapping the CO_2 in an alkaline center well and precipitating the CO_2 (with carrier) as $BaCO_3$. In the presence of 2-DOG the glucose was determined by the radioactivity of the osazone; otherwise, the glucose was determined by the glucose oxidase method. Lactic acid was determined by the method of Barker and Summerson (1941).

Results and Discussion: Figure 1 shows the effect of concentration of 2-DOG on the oxidation of glucose-U-C^{14} to $C^{14}O_2$, residual glucose, and residual (net) lactate with the ascites carcinoma cells. 2-DOG inhibits glucose disappearance from the incubating medium and lactate appearance, in accordance with its known sites of blocking action (Wick et al., 1957); the extent of inhibition increases with increase in molar concentration of 2-DOG. In contrast, 2-DOG at concentrations up to 0.06M increases the rate of $C^{14}O_2$ production with an apparent peak of stimulation near molar ratios of 2-DOG to glucose of 3:1. The decrease in glucose oxidation rates with concentrations of 2-DOG greater than 0.03M cannot be readily explained.

Since we are measuring glucose oxidation by the expired $C^{14}O_2$, it was essential to determine if the Crabtree effect could be demonstrated by this technique. A series of experiments were conducted in which ascites carcinoma

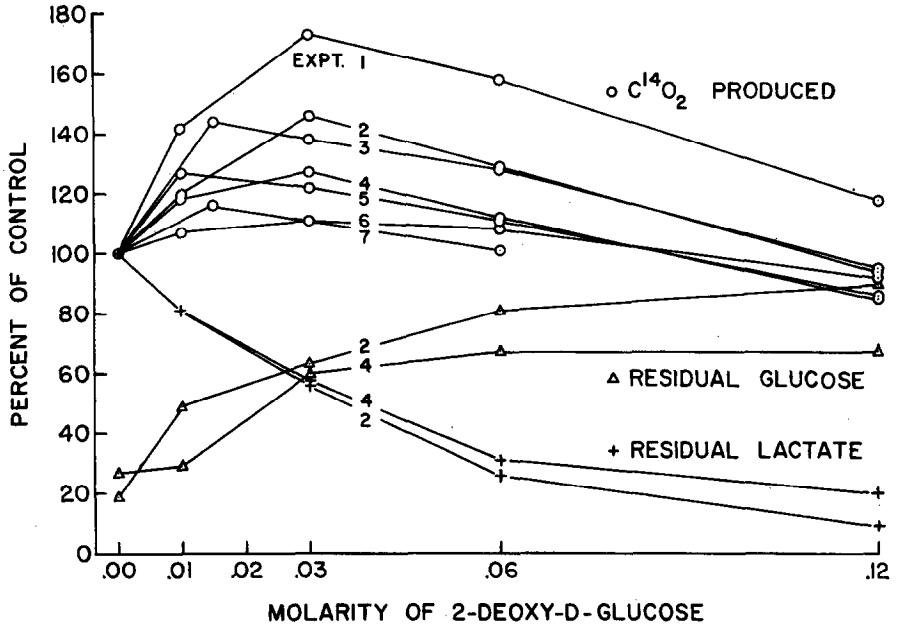

Figure 1. Effect of concentration of 2-deoxy-D-glucose on oxidation of glucose-U-C^{14} (0.01 M) to $C^{14}O_2$, residual glucose, and residual lactate with Ehrlich ascites carcinoma cells. Experimental flasks incubated for 2 hours in absence of 2-DOG taken as control for $C^{14}O_2$ and residual lactate; non-incubated (zero time) flasks without 2-DOG taken as control for residual glucose.

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cells were incubated with a range of glucose-U-C^{14} concentrations. Figure 2 shows that the highest rate of glucose oxidation occurs with the lowest concentration of glucose tested (0.0006M), and with higher glucose concentrations the rate of glucose oxidation decreases. The rate of oxidation is particularly sensitive to glucose concentrations up to 0.0025M. These data are interpreted to be an expression of the Crabtree effect, and comparable to the customary oxygen uptake studies employed by other workers. It is of interest that with these cells the uptake of glucose and the inhibition of glucose oxidation to $C^{14}O_2$ both reach maximal values at 0.0025M glucose, and remain constant for all higher glucose concentrations.

The exact metabolic alteration produced by 2-DOG cannot be determined from these experimental observations. However, any proposed mechanism must

be compatible with these three conditions: 1) a stimulation of $C^{14}O_2$, 2) a decrease of residual (net) lactate production, 3) a decrease in amount of glucose taken up by the cells.

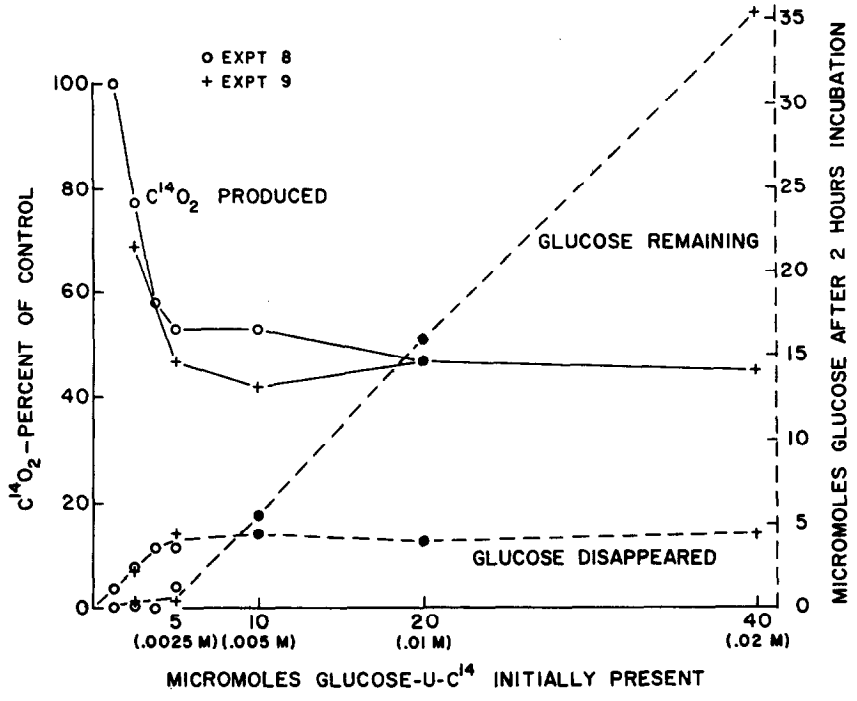

Figure 2. Effect of glucose-U-C^{14} concentration on its oxidation to $C^{14}O_2$, and on its disappearance with Ehrlich ascites carcinoma cells. Oxidation values at 0.0006M glucose taken as 100%.

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