A Comparison of the Respiratory Inhibitions Induced by D-Glucose and 2-Deoxy-D-Glucose in Ehrlich Ascites Carcinoma Cells*

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SUMMARY

Several aspects of the effects of D-glucose and 2-deoxy-D-glucose on respiratory metabolism of the Ehrlich ascites carcinoma cells are compared. After a brief stimulatory period which was longer for deoxyglucose than glucose, both compounds inhibited the oxygen consumption rate to a similar extent. Glucose-induced inhibition was released after all the glucose was utilized, but deoxyglucose-induced inhibition was unaltered at a time corresponding to the marked slowing of phosphorylation. Final release from inhibition occurred only after an extended period of respiration. Dinitrophenol initially released the respiratory inhibitions of both glucose and deoxyglucose but then inhibited the rate of oxygen consumption, particularly in the presence of deoxyglucose. A much higher concentration of deoxyglucose than of glucose was needed to induce a respiratory inhibition. Both compounds initially reduced the ATP level. This reduction was transitory in the presence of glucose and more prolonged in the presence of deoxyglucose.

The rate of respiration in Ehrlich ascites cells is markedly inhibited by glucose, mannose, and fructose (Crabtree effect) (10). Since this inhibition is released by uncouplers of oxidative phosphorylation, it has been suggested that the inhibition may be caused by a limiting level of orthophosphate (Pi) or adenosinediphosphate (ADP). This limitation would be expected to be due to utilization of these compounds in glycolytic phosphorylations (1). However, a similar inhibition is caused by 2-deoxy-D-glucose and by glucose in the presence of iodoacetic acid (8). It follows, therefore, either that an intact glycolytic system is not needed for induction of the Crabtree effect or that inhibition caused by the nonglycolyzed sugars has a basis different from that caused by glycolyzable carbohydrates.

It has been suggested that the Crabtree effect is triggered by enhanced hexokinase utilization of ATP, which has been postulated to induce indirectly a mitochondrial ADP deficiency and thus a lowered rate of oxygen consumption (2, 8). If the basic mechanism causing glucose-induced inhibition were the same as the mechanism causing deoxyglucose inhibition, it would be relatively clear that the Crabtree effect was linked to the hexokinase reaction, since deoxyglucose is not glycolyzed beyond initial phosphorylation (8). To date there is little information which could serve as a basis for comparing respiratory inhibitions produced by glucose and deoxyglucose.

In the work reported here several aspects of respiratory inhibition produced by deoxyglucose and glucose are compared. There are some significant similarities in the early phases of inhibition but certain important differences in later periods.

MATERIALS AND METHODS

Tumor preparation.—The Ehrlich ascites carcinoma cells were grown in C57BL mice bred in

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this laboratory. The tumor was inoculated, harvested, and prepared as previously described (9). Fifty-five mM phosphate Locke's buffer solution was used throughout all manipulations and incubations.

Oxygen consumption studies.—Conventional Warburg manometry was used, with 7-ml. vessels containing 0.1 ml. 15 per cent KOH and a 15 × 15-mm. accordioned strip of filter paper in the center well, 0.8 ml. of cell suspension in the main chamber, and in the side-arm 0.1 ml. substrate solution or Locke's solution for the controls. Substrate was tipped in after 15–20 minutes' preincubation. When 2,4-dinitrophenol (DNP) was employed, it was tipped in simultaneously with the substrate.

A Beckman OM-2-oxygen electrode was used to measure oxygen consumption (9). All incubations were made at 38.0° C.

Chemical determinations.—Adenosine triphosphate (ATP) was measured by the glucose-6-phosphate dehydrogenase method of Kornberg, as described by Mommaerts (11).

Chemicals.—All chemicals employed were of C.P. quality unless otherwise stated. The 2-deoxyglucose was either a Nutritional Biochemical product repurified as previously described (9) or a Sigma product which needed no further purification. The hexokinase and glucose-6-phosphate dehydrogenase employed were Sigma products. TPN was obtained from Pabst.

RESULTS

Comparative effect upon respiration.—The effects of deoxyglucose and glucose on respiration are summarized in Table 1. In these experiments respiration was measured manometrically at 2-minute intervals. For purposes of comparison it is convenient to divide duration of the experiment into four phases. The first phase, which was considered to start 2 minutes after substrate addition (i.e., after the initial stimulatory phase, mentioned below), was a period of equal inhibition for both glucose and deoxyglucose. The end of this phase was marked by full utilization of glucose. Interestingly, about half-way through this period there is a marked slowing in the rate of deoxyglucose phosphorylation (8). However, there was no manometric reflection of this, since the respiratory rate remained constant throughout this phase, either endogenously or with added substrate.

Concomitant with complete disappearance of glucose was an increased rate of oxygen consumption indicating the beginning of phase 2. The respiratory rate of cells respiring endogenously or in the presence of deoxyglucose remained as in phase 1, except for a slight tendency for the rate to increase in the presence of deoxyglucose. This increase in oxygen consumption rate in the presence of glucose can be considered a release from respiratory inhibition. The inability of glucose-treated cells to achieve full endogenous rate may be due to a pH change accompanying conversion of glucose to lactate. From the beginning of this phase to the end of the experiment respiration in the presence of glucose remained constant.

TABLE 1

A COMPARISON OF THE EFFECTS OF DEOXYGLUCOSE AND GLUCOSE ON RESPIRATION

Phase	Exp.	Duration (min.)	Respiratory rate (cu mm O_2 utilized/min)		
			Glucose	Deoxy-glucose	Endoge-nous
I	1	16	0.84	0.85	1.48
	2	18	0.83	0.80	1.42
II	1	12	1.17	0.90	1.53
	2	14	1.24	0.92	1.39
III	1	26	1.11	0.94	1.35
	2	26	1.24	1.03	1.27
IV_A	1	40	1.23	1.25	1.37
	2	30	1.09	1.06	1.92
IV_B*	1	31	1.24	1.28	1.28
	2	30	1.13	0.99	0.84

The final cell concentrations in these studies were 13.9% for Exp. 1 and 13.8% for Exp. 2, or 0.11 ml. of cells. The final concentrations of glucose and deoxyglucose were 6.2 and 6.8 mM, respectively. Glucose utilization and deoxyglucose phosphorylation were measured as described previously (8). Within experimental error all the deoxyglucose utilized could be accounted for as the phosphorylated compound. Determinations were done in duplicate. Meaning of the phases are described in the text.

* Phase IV_B is merely an extension of Phase IV_A by the amounts of time indicated.

Commencement of phase 3 in the presence of deoxyglucose was marked by a period of irregular respiration and the first obvious signs of a gradual and progressive decline of endogenous respiration. The fourth phase can be considered to begin when oxygen consumption in the presence of deoxyglucose stabilized at a rate similar to or higher than the somewhat decreased endogenous rate. That the rate was the same with glucose may be a coincidence. Cells which were exposed to glucose had a high lactate content, whereas the other cells were substrate-deficient. This last period can be considered as marking the time of full release from deoxyglucose-induced inhibition.

It appears, then, that similar levels of glucose

and deoxyglucose initially reduced respiration of aliquots of the same tumor preparation to comparable levels. However, deoxyglucose kept the cells in inhibition almost 3 times longer than glucose.

Charts 1 and 2 compare the initial effects of glucose and deoxyglucose on respiration. Both com-

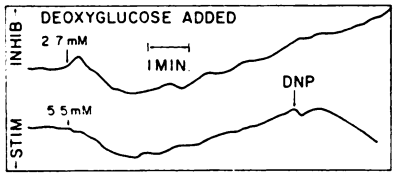

CHART 1.—Initial phases of the respiratory inhibition induced by deoxyglucose and the effect of dinitrophenol on deoxyglucose inhibition as measured with the oxygen electrode. There were 0.30 ml. of cells in 22 ml. of buffer. DNP was added as indicated to give a final concentration of 6.4×10^{-5} M.

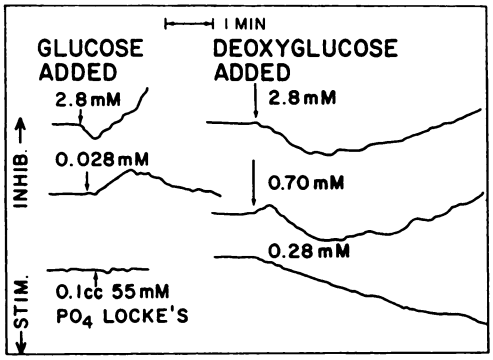

CHART 2.—A comparison of the initial stimulations and closely following inhibitions produced by glucose and deoxyglucose as measured with the oxygen electrode. There were 0.20 ml. of cells in 20 ml. of buffer.

pounds could induce an initial short stimulation, but the stimulation observed in the presence of deoxyglucose was of longer duration. Packer and Golder (12) have also observed that deoxyglucose stimulated respiration to a greater extent than did glucose. We have not consistently observed a respiratory stimulation in the presence of glucose even at high glucose levels and found it much more difficult to induce at still lower levels. Chance and Hess (2–4) appear to more consistently obtain such an initial stimulation. However, they too have reported cases of no initial stimulation with glucose (3).

In Table 2 effects of varied levels of glucose and deoxyglucose on respiration are compared. The average depth of inhibition remained constant in both cases. This constancy was not observed at very low glucose levels when inhibition was studied by oxygen electrode technics (5). There was a great deal of difference in the depth of inhibition found in different tumor preparations with glucose (0.2 to 0.5 cu mm O_2/min), and therefore the degree of deoxyglucose inhibition shown in Table 2 cannot be compared with the glucose inhibition. It can also be seen that the lowest level of deoxyglucose (1.7 mM) did not induce an inhibition, whereas the same amount of glucose caused a distinct inhibition.

Oxygen electrode studies (Chart 2) also indicate that deoxyglucose did not show an effect on respiratory inhibition at a level which was an order of magnitude higher than the minimal quantity of glucose.

Effect of DNP.—Chart 3 shows the effect of various levels of DNP on endogenous respiration and respiration in the presence of deoxyglucose or glucose. It is obvious that DNP stimulated respiration in the presence of glucose. When deoxyglucose was the substrate, DNP also stimulated respiration but for only the first 10 minutes, after which respiration decreased at a rate inversely related to the DNP concentration. Both the stimulatory and inhibitory effects of DNP were less marked endogenously than in the presence of deoxyglucose. The maximum amount of DNP stimulation estimated after 10 minutes' respiration was as follows: endogenous, 17 per cent, with 1.4×10^{-4} M DNP; glucose, 120 per cent, with 2.8×10^{-4} M DNP, and 72.5 per cent with 1.4×10^{-4} M DNP; and deoxyglucose, 58.2 per cent,

TABLE 2

EFFECT OF VARYING DEOXYGLUCOSE OR GLUCOSE LEVELS ON THE RESPIRATORY INHIBITION

Deoxyglucose (mM)	Glucose (mM)	Duration of inhibition (min.)	Total inhibition* (cu. mm. O_2)	Av. depth of inhibition†
	1.6	8	4.3±2.2	0.54±0.22
	3.1	24	10.5±2.3	0.48±0.14
	4.6	38	18.1±0.6	0.48±0.02
	6.2	55	27.8±0.5	0.50±0.02
1.7		zero		
3.4		80	17.4±4.6	0.22±0.05
5.1		80	15.4±1.0	0.20±0.01
6.8		80	19.2±4.4	0.24±0.06

In the deoxyglucose experiment 0.06 ml. of cells was employed, whereas in the glucose experiment 0.05 ml. of a different cell preparation was used. All analyses were done in triplicate. The deviations shown in columns 4 and 5 are standard deviations.

* Difference between endogenous oxygen consumption and consumption in presence of glucose or deoxyglucose for the duration of the inhibition period.

† Average depth of inhibition is the total inhibition (column 4) divided by the duration of inhibition in minutes (column 3).

with 1.4×10^{-4} M DNP. Of particular importance is the fact that during the first 10 minutes respiration in the presence of deoxyglucose and DNP was as high as or higher than endogenous respiration in the presence or absence of DNP. The oxygen electrode pattern shown in Chart 1 confirms the fact that DNP did stimulate respiration after it had been inhibited by deoxyglucose.

Emmelot and Bos (6) have studied the effects of varying DNP concentrations on the respiration of S_3A ascites cells endogenously and in the presence of glucose. Qualitatively they obtained similar data. However, S_3A cells seem to be somewhat more sensitive to lower DNP levels than are Ehrlich cells.

Effect of deoxyglucose and glucose on the ATP level.—In a previous publication (8) ATP was shown to be initially lowered by glucose but then quickly rose to its initial concentration.[1] This cyclic destruction and formation of ATP has since been confirmed (7, 15). Furthermore, deoxyglucose did not raise the ADP level markedly and only slightly elevated the AMP (8). However, no results on the ATP concentration were reported. Chart 4 compares the effects of glucose and deoxyglucose on the ATP content of two aliquots of the same tumor preparation. Both hexoses quickly lowered the ATP content, but deoxyglucose, unlike glucose, was unable to restore ATP utilized in glycolytic phosphorylation.

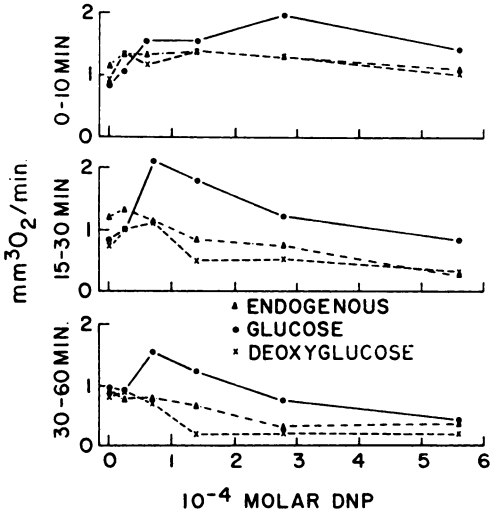

CHART 3.—The effect of varying levels of DNP on respiration in the presence of glucose and deoxyglucose, and endogenously, measured by conventional Warburg procedure. Data are calculated on the basis of respiration per 0.044 ml. of cells.

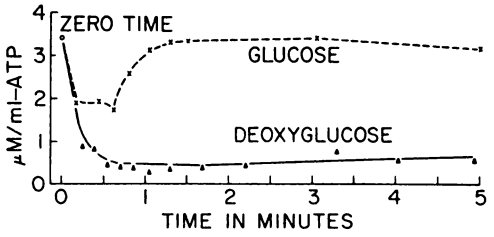

CHART 4.—Effect of glucose and deoxyglucose on ATP concentration. There were 1.5 ml. of cells in 15 ml. of buffer. The final concentrations of glucose and deoxyglucose were 6.2 and 6.8 mM, respectively.

DISCUSSION

Although there is a similarity of the initial stimulation and rapidly following inhibition produced by addition of glucose or deoxyglucose to Ehrlich carcinoma cells, the later phases of inhibition produced by these two compounds appear to be relatively different.

Of primary importance is the observation that DNP reverses both deoxyglucose- and glucose-induced inhibition. This would suggest that, at least during the initial phases, both compounds slow respiration by limiting mitochondrial ADP. Depletion of orthophosphate probably can be ruled out because of high phosphate content of the buffering medium (9). Respiratory inhibition with deoxyglucose cannot be due to utilization of ADP glycolytically, because deoxyglucose undergoes only one metabolic alteration—phosphorylation to 2-deoxyglucose-6-phosphate (8, 14). Therefore, the limitation of mitochondrial ADP must be indirect and related to hexose phosphorylation. Two possible indirect modes of ADP deprivation within the mitochondria have been previously proposed (2, 8).

Another factor suggesting that both glucose and deoxyglucose act by causing a similar limitation is that both compounds initially reduce inhibition to the same level when the same cell preparation is utilized.

Inability of smaller concentrations of deoxyglucose to cause an inhibition may be due to a lower rate of cellular permeability, or to a lower affinity for hexokinase. Furthermore, deoxyglucose is phosphorylated at a slower rate than glucose (8).

It has been shown previously that deoxyglucose

[1] Data previously obtained (8) for the effect of glucose on ATP were described as "probably correct relative to each other, although low by a constant factor." The data presented here, and other unpublished data, show that this is true and that the zero time ATP values should be between 3 and 4 μmoles /ml cells and all the values shown in Fig. 8 (8) of our earlier publication should be about 2 μmoles higher. It should be emphasized that the values for ATP presented here were obtained in different experiments and by a different technic than in our earlier publication (8). The decreases of ATP caused by glucose addition varied with different batches of tumor.

did not markedly increase the ADP or AMP level of the cell (8). Thus, with the relatively larger decrease of ATP there is a significant depletion in the total adenine phosphate content. It is possible that this depletion is achieved by deamination, since deoxyglucose does induce a marked pH rise in poorly buffered media (9). Wu and Racker (15) have reported that, in the presence of glucose and iodoacetic acid, there is a loss of adenine phosphates and an increase of inosine. This depletion of adenine nucleotides may account for the inability of cells to come sharply out of deoxyglucose-induced respiratory inhibition. A decrease of adenine nucleotides may account for the inhibitory effect of DNP which follows the stimulatory period. This concept is supported by the fact that, in the presence of glucose, which can maintain adenine nucleotide as ATP through glycolytic phosphorylation (8), DNP inhibition is delayed. However, it should be emphasized that the initial phases of deoxyglucose inhibition could not be due to this depletion of nucleotides, because DNP would be expected to increase the rate of depletion; and yet DNP does stimulate respiration and release inhibition. Furthermore, enough ATP is available in the cell to allow continued phosphorylation of deoxyglucose at a relatively fast rate for at least 10 minutes.

Thus, it appears probable that, during the first 10 minutes, glucose and deoxyglucose produce inhibition by depleting mitochondrial ADP. Interestingly, Chance and Hess (3, 4) have reached a similar conclusion concerning inhibition in the presence of glucose and iodoacetic acid, in which case glycolysis is also interrupted. On the other hand, Seelich and Letnansky (13) have demonstrated that deoxyglucose decreases the accumulation of α-ketoglutarate and increases the accumulation of citrate when pyruvate is the substrate. This they interpret as an effect on the TPN level possibly caused by a deficiency of ATP. They believe that the deoxyglucose effect is not the same as that produced by glucose. However, analyses for TPN in the presence of deoxyglucose as substrate did not show any consistent change of concentration during the period of inhibition (8).

A complete study of the nucleotide picture employing deoxyglucose and glucose in the presence DNP could be valuable in elucidating the relation between effects of these compounds on respiration.

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