THE EFFECT OF 2-DEOXYGLUCOSE ON THE METABOLISM OF GLUCOSE, FRUCTOSE, AND GALACTOSE BY RAT DIAPHRAGM*

By HENRY I. NAKADA and ARNE N. WICK

(From the Scripps Metabolic Clinic and Research Foundation, La Jolla, California)

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One of the principal observations on the action of insulin is its ability to increase the rate of disappearance of glucose from the extracellular fluids. One of the many theories of insulin action ascribes this phenomenon to the increased utilization of glucose for glycogen synthesis and oxidation by activating the hexokinase reaction (1). Another proposal is that insulin acts by mediating the transport of glucose across the cell barrier (2, 3). In our studies, glucose analogues being used with isolated rat diaphragms, both of these theories are supported to some extent. The effect of glucose in drastically reducing the uptake and oxidation of fructose by the rat diaphragm can be explained logically by comparing the Michaelis constants of hexokinase for glucose and fructose (4). Glucosamine, which has been shown to be insulin-sensitive, appears to inhibit glucose oxidation by blocking the transfer of glucose into muscle cells (5). On the basis of hexokinase (brain (4)) Michaelis constants for glucose and glucosamine, this amino sugar should have little or no effect on glucose oxidation. Another insulin-sensitive sugar, galactose, appears to follow a metabolic pathway entirely independent of glucose (6). Galactose apparently enters the muscle cells as the free sugar, thus refuting the hexokinase theory in so far as galactose is concerned. The lack of any appreciable competition between glucose and galactose for cell entry indicates the possibility of separate entry pathways for these two sugars. Thus, the theory of sugar transport across the cell membranes appears to be true for galactose, but whether or not it is true for glucose remains to be discovered.

Another glucose analogue that appears to be a useful tool in studying the action of insulin is 2-deoxyglucose. Cramer and Woodward reported that 2-deoxyglucose inhibited yeast fermentation (7) and tumor glycolysis (8). Further studies by these workers have shown that the site of inhibition of yeast fermentation by 2-deoxyglucose was not hexokinase, since the Michaelis constant of yeast hexokinase for glucose was lower than for 2-deoxyglucose (9). Sols and Crane (4) have shown that 2-deoxyglucose was a substrate for brain hexokinase. By using the eviscerated, nephrectomized

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rabbit, Wick, Drury, and Morita (10) have recently shown that 2-deoxyglucose inhibits glucose transfer and glucose oxidation. Their work also indicated that 2-deoxyglucose uptake may have been accelerated by insulin.

The present communication gives the results of further studies on the effect of 2-deoxyglucose on the metabolism of several sugars by the isolated rat diaphragm.

Methods and Materials

The 2-deoxyglucose was prepared as described by Bergmann *et al.* (11) and modified by Cramer (12). Glucose-U-C^{14} was photosynthesized from $C^{14}O_2$ by cantaloupe leaves; galactose-1-C^{14} was obtained from Dr. Isbell of the National Bureau of Standards, fructose-U-C^{14} from the Isotopes Specialties Company, Inc., Glendale, and crystalline zinc insulin was kindly supplied by the Lilly Research Laboratories. The 2-deoxyglucose was determined by the method of Wick *et al.* (10). The procedures for preparing the tissues and conducting the experiments are essentially as described previously (5).

TABLE I

2-Deoxyglucose Uptake with and without Insulin

Experiment No.	2-Deoxyglucose uptake, mg. per gm. tissue	
	No insulin	Plus insulin
1	0.86	1.78
2	1.93	2.31
3	0.50	1.96
4	0.66	1.32
5	0.82	1.17
6	1.61	3.60

Two flasks were used in each experiment, containing paired hemidiaphragms from two rats (trimmed to approximately 350 mg. of wet weight). The final 2-deoxyglucose concentration was 0.01 M, and insulin, when used, was 1.0 unit per flask. The flasks were incubated at 37° for 2 hours with O_2 in the gas phase.

RESULTS AND DISCUSSION

The results presented by Wick, Drury, and Morita (10) suggest that insulin may accelerate the disappearance of 2-deoxyglucose from the extracellular space. The data presented in Table I indicate that insulin does increase the rate of 2-deoxyglucose uptake by isolated rat diaphragms.

The diminution of glucose uptake by the eviscerated, nephrectomized rabbit (10) in the presence of 2-deoxyglucose is confirmed in Table II. It can be seen not only that 2-deoxyglucose does reduce glucose uptake, but

also that glucose reduces the uptake of 2-deoxyglucose. It appears as though 2-deoxyglucose and glucose are competing for the same system which governs the disappearance of glucose from the incubating medium.

Fig. 1 presents the results of the effect of increasing concentrations of 2-deoxyglucose on the uptake of C^{14}-labeled glucose, fructose, and galactose. The uptake of glucose is reduced considerably at relatively low concentrations of 2-deoxyglucose, but the inhibitory effect is not so marked at higher concentrations. The effect on fructose appears to be similar to that on glucose, except that the inhibition at low concentrations is more pronounced. The uptake of 2-deoxyglucose in these experiments appears

TABLE II

Effect of 2-Deoxyglucose (2-DG) on Glucose (Glu) Uptake and Effect of Glucose on 2-Deoxyglucose Uptake

Substrates	Sugar uptake, mg. per gm. tissue									
	Experiment 1		Experiment 2		Experiment 3		Experiment 4		Experiment 5	
	Glu	2-DG	Glu	2-DG	Glu	2-DG	Glu	2-DG	Glu	2-DG
Glucose (0.01 M).....	3.56		3.92		3.65		3.26		3.65	
" (0.01 ") + 2-deoxyglucose (0.01 M).....	2.20	1.18	2.13	1.42	2.79	1.43	2.09	0.82	2.11	1.10
2-Deoxyglucose (0.01 M)..		2.38		1.68		2.20		2.09		1.33

For the three flasks in each experiment, the diaphragms from three rats were divided nearly equally among the flasks. The weights of diaphragm in each vessel were adjusted to approximately 350 mg. The final volume was 3 ml., and 1.0 unit of insulin was added to each vessel. Incubations were carried out at 37° for 2 hours with O_2 in the gas phase. Initial and final sugar concentrations were obtained by the Miller and Van Slyke titration method.

to be a function of its concentration; the uptake of galactose was not at all inhibited.

The radioactive respired CO_2 from these sugars was also measured (Fig. 2). In contrast to the decreased uptake of glucose, the oxidation of glucose was impaired relatively slightly by 2-deoxyglucose. Fructose, on the other hand, had its oxidation considerably inhibited and galactose oxidation was unaffected.

The same pattern of events found in previous competition studies is reflected in these experiments. Galactose, which is acted on by hexokinase only under special conditions (4), had neither its uptake nor oxidation inhibited by 2-deoxyglucose. Since the K_m values of hexokinase for both glucose and 2-deoxyglucose (9) are considerably smaller than that of fructose, one can again interpret the effect of 2-deoxyglucose on fructose uptake

and oxidation on competition for the hexokinase reaction. But this line of reasoning fails when applied to the effect of 2-deoxyglucose on glucose, since the K_m value of hexokinase for glucose is lower than that of 2-deoxyglucose. The fact that glucose uptake was reduced much more than was glucose oxidation suggests that 2-deoxyglucose can block at two or more sites; *viz.*,

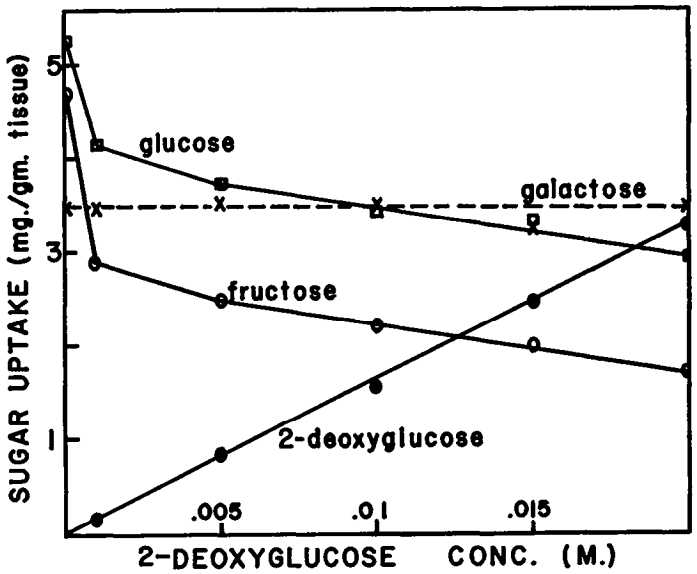

FIG. 1. The effect of 2-deoxyglucose on the uptake of glucose, fructose, and galactose. For each experiment, the diaphragms from six rats were divided nearly equally among the six flasks. The weights of diaphragms in each vessel were adjusted to 350 ± 10 mg. Each flask contained the designated radioactive carbon-labeled sugar (0.01 M), 1.0 unit of insulin, as well as the designated amounts of 2-deoxyglucose. Incubations were carried out at 37° for 2 hours with oxygen in the gas phase. Sugar uptakes were calculated from the radioactivity that remained in the diaphragms after six rinses in cold isotonic NaCl. Radioactivity of the diaphragms was determined by wet-oxidizing the tissue and counting the carbon as $BaCO_3$. The concentration of 2-deoxyglucose was determined by titration.

first at the site of cell entry, as shown by blocking the uptake of glucose and fructose, and second at the hexokinase reaction as shown by blocking of fructose oxidation but not the oxidation of glucose.

This lack of effect of 2-deoxyglucose on glucose oxidation is at variance with the results reported by Wick, Drury, and Morita (10), who found that in eviscerated, nephrectomized rabbits 2-deoxyglucose inhibited both the transfer and oxidation of glucose. Since, in their experiments, the 2-deoxyglucose concentration within the cell could build up over a number of hours, it was believed possible that results similar to those found in the rabbits might be obtained if 2-deoxyglucose were incubated with the diaphragms

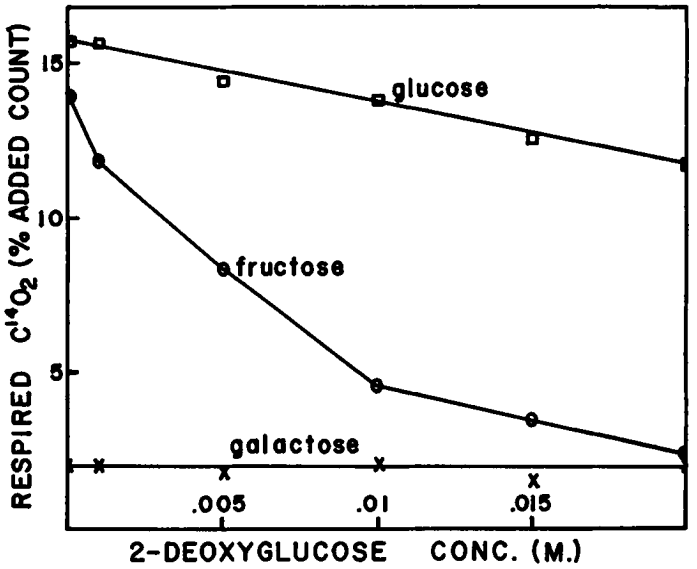

FIG. 2. The effect of 2-deoxyglucose on the oxidation of glucose, fructose, and galactose. The respired $C^{14}O_2$ values were obtained from the experiments listed on Fig. 1.

TABLE III

Effect of Preincubating Rat Diaphragms with 2-Deoxyglucose on C^{14}-Glucose Uptake and Oxidation

Substrates	Not preincubated		Preincubated	
	C^{14}-Glucose uptake, mg. per gm. tissue	C^{14}-Glucose oxidation, μmoles per gm. tissue	C^{14}-Glucose uptake, mg. per gm. tissue	C^{14}-Glucose oxidation, μmoles per gm. tissue
Glucose-U-C^{14}	6.43	11.8	7.0	12.0
" + 2-deoxyglucose	5.1	8.7	3.4	3.8

For the four flasks in this experiment, diaphragms were cut into four approximately equal pieces and divided among the flasks. Each flask contained approximately 350 mg. of wet tissue suspended in a final volume of 3 ml. of Krebs-Ringer phosphate buffer (pH 7.4). The substrate concentrations were glucose-U-C^{14} (0.01 M), 2-deoxyglucose (0.02 M), and insulin (0.7 units per flask). Incubations were carried out at 37° for 2 hours with oxygen in the gas phase.

prior to the addition of radioactive glucose. The results of such an experiment (Table III) show that preincubation of the diaphragms with 2-deoxyglucose for 15 minutes does result in a substantial reduction in glucose oxidation as well as further reduction in the uptake of glucose.

It has been reported that 2-deoxyglucose-6-phosphate is not an inhibitor of hexokinase and is not further metabolized (4, 13). If this is the case, then one explanation for the inhibiting effect of 2-deoxyglucose on glucose oxidation could be that the intracellular concentration of the deoxysugar is sufficiently higher than that of glucose so that a competition for hexokinase occurs. Since the oxidation of glucose is being measured as CO_2, the actual site of inhibition could occur at some enzyme other than hexokinase. The metabolism of 2-deoxyglucose and the mechanism of its inhibition of glucose are under investigation.

SUMMARY

1. The isolated rat diaphragm was used to study the action of 2-deoxyglucose and its effect on other sugars. The uptake of 2-deoxyglucose and the acceleration of this uptake under the influence of insulin have been demonstrated.

2. 2-Deoxyglucose retarded the uptake of glucose and glucose depressed the uptake of 2-deoxyglucose.

3. 2-Deoxyglucose inhibited the uptake of glucose and fructose, but had no effect on galactose uptake.

4. Fructose oxidation by the rat diaphragm was drastically reduced by 2-deoxyglucose, glucose oxidation was depressed slightly, and galactose oxidation was not affected.

5. Glucose oxidation and uptake by rat diaphragms can be inhibited by preincubation of the diaphragms with 2-deoxyglucose.

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