Summary. DNA derived from black and white rats, on injection into newborn albino rats, failed to produce pigmentary changes in the hair and the eyes. Similarly, DNA derived from normal rats failed to correct a genetically determined absence of glucuronyl transferase in newborn jaundiced rats.

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Influence of 6-Deoxy-6-Fluoroglucose on Glucose Utilization in Rat Epididymal Adipose Tissue and Rat Diaphragm.* (24477)

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The compound 2-deoxyglucose (2-DOG) has been shown to inhibit glucose metabolism as early as the hexokinase step(1). However, one difficulty which has been experienced in studies with this compound is a result of a lack of specificity in its blocking action. Thus it is known that 2-DOG blocks glucose metabolism at the isomerase step(2) as well as the hexokinase step and other sites of action of this drug remain as distinct possibilities (2,3). 6-Deoxy-6-fluoroglucose (6-FG) is a glucose analogue which may circumvent the difficulty of multiplicity of action. Because of the absence of a hydroxyl group in the 6 position, the sugar obviously cannot be phosphorylated by hexokinase. Thus it would appear that if 6-FG does competitively inhibit glucose metabolism in mammalian tissues it should do so prior to or at the hexokinase step. Studies of 6-FG action in rat kidney tissues have indicated that this compound is indeed a competitive inhibitor of glucose utilization (4). Moreover, it appears that 6-FG does inhibit at or prior to the hexokinase step in rat kidney slices. A surprising feature of the study with rat kidney tissue has been the anomalous effect of an increased concentration of 6-FG on glucose oxidation. At low 6-FG concentrations a true competitive response to increasing concentrations of the fluoro derivative is observed. At high 6-FG concentrations, however, a plateau is observed in the curve of inhibition of glucose oxidation over a wide range of 6-FG values. These data have been interpreted to suggest the presence of an alternate pathway of glucose metabolism before the hexokinase step. The object of the studies reported here is to determine whether the inhibitory pattern observed with rat kidney tissues is applicable to other mammalian tissues.

Material and methods. The procedures employed in the preparation and incubation of rat adipose tissues and isolated rat diaphragms have been previously described(5,6). When used as substrates concentrations of glucose and acetate were maintained at 0.01M in the incubation medium. 2-DOG was obtained from Nutritional Biochemicals Corp., Cleveland, O. 6-FG was prepared according to the procedure of Helferich *et al.*(7,8) as revised by Blakley(9).

Results. The data of Table I show that fluoride ion is an effective inhibitor of oxidation of glucose and acetate in rat epididymal

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TABLE I. Comparison of Effects of 6-Deoxy-6-Fluoroglucose and Fluoride Ion on Oxidation of C14-Labeled Acetate and Glucose in Rat Adipose Tissue.

Inhibitor	Substrate	Inhibition, %
6-FG (.03 M)	U. L. glucose	51
	Acetate-1-C^{14}	−6
F^- (")	U. L. glucose	90
	Acetate-1-C^{14}	96
F^- (.003 M)	U. L. glucose	64
	Acetate-1-C^{14}	42

adipose tissue. On the other hand, 0.03M 6-FG is effective only as an inhibitor of glucose oxidation.

The influence of 6-FG and 2-DOG concentrations on oxidation of glucose to CO_2 in the isolated rat diaphragm is shown in Fig. 1. At low concentrations, 6-FG is as effective an inhibitor of glucose oxidation as 2-DOG. However, with increasing concentrations of these glucose analogues, 2-DOG is seen to exhibit a more potent inhibition of $C^{14}O_2$ formation.

A similar pattern is seen to exist in rat adipose tissue (Fig. 2) with the exception that the differential between the two curves is not as extensive at high inhibitor concentrations.

Comparison of the relative effectiveness of 6-FG as an inhibitor of glucose utilization in rat kidney tissue(4), rat adipose tissue, and isolated rat diaphragm is given in Fig. 3. The effectiveness of 6-FG as an inhibitor is seen to increase in the series: kidney, diaphragm, adipose.

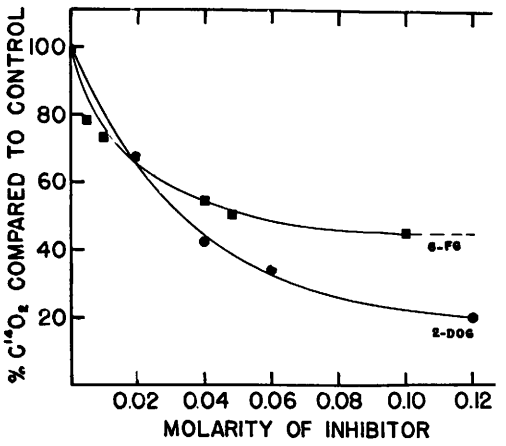

FIG. 1. Effect of 6-FG and 2-DOG on glucose oxidation to CO_2 in isolated rat diaphragm. Each point represents avg of 3 separate determinations.

Discussion. The inhibitory action of fluoride ion on glycolysis scheme and the Krebs' cycle is a well established phenomenon. Thus the possibility arises that the inhibition observed with 6-FG is not an effect of 6-FG *per se* but rather an effect of fluoride ion hydrolyzed from this glucose analogue during tissue incubation. This possibility appears to be refuted by the data of Table I. A further observation consistent with the conclusion that fluoride ion is not responsible for the 6-FG effect is that 6-FG has been shown to behave kinetically as a competitive inhibitor of glucose utilization(4). Fluoride ion on the other hand is known to be a non-competitive inhibitor of glycolysis.

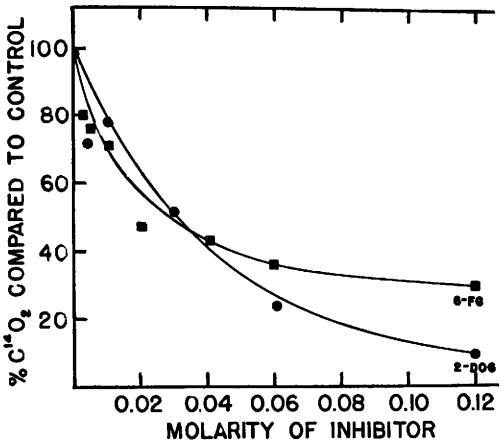

FIG. 2. Effect of 6-FG and 2-DOG on glucose oxidation to CO_2 in rat epididymal adipose tissue. Each point represents avg of 3 separate determinations.

Earlier studies with rat kidney slices and sugars variously labeled with C^{14} have indicated that the site of 6-FG action occurs prior to formation of glucose-6-phosphate(4). Moreover, it has been suggested that an unknown pathway leading to glucose oxidation exists in kidney tissues prior to the hexokinase step. This conclusion was reached on the basis of the observation that at high 6-FG concentrations the curve of inhibition of glucose oxidation to CO_2 approaches a maximum at approximately 35 to 45% inhibition, indicating that some non-6-FG sensitive pathway is permitting glucose oxidation while the 6-FG sensitive pathway is almost completely inhibited.

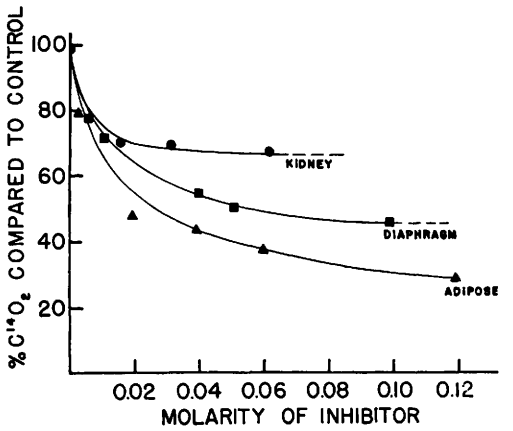

FIG. 3. Comparison of effect of 6-FG on rat kidney, rat diaphragm, and rat epididymal adipose tissue.

The pattern observed in kidney tissue appears to be qualitatively repeated in rat diaphragm (Fig. 1) and rat epididymal adipose tissue (Fig. 2).

Comparison of the response of all 3 tissues to 6-FG concentration (Fig. 3) indicates that the inhibited pathway of glucose oxidation contributes to an increasingly greater extent to total formation of CO_2 in the series: kidney<diaphragm<adipose.

The demonstration that 6-FG inhibits glucose oxidation in such diverse specialized tissues as kidney, diaphragm, and adipose as well as in lower organisms such as yeast preparations(10) suggests that the process interfered with by 6-FG is a universal metabolic pathway. Work to determine the exact site of action of this analogue is currently underway.

Summary. The effect of 6-deoxy-6-fluoroglucose (6-FG) on oxidation of glucose has been examined *in vitro* in rat epididymal adipose and rat diaphragm tissue. It was found that 6-FG at all concentrations studied was an effective inhibitor of the oxidation of glucose. However, at high 6-FG concentrations, inhibition curve of glucose oxidation approaches a maximum at approximately 75% and 50% for adipose and diaphragm tissue respectively. The inhibition curve obtained with 2-deoxyglucose, which was used for comparison purposes, appeared to asymptotically approach 100% inhibition of glucose oxidation in these tissues. These results have been interpreted to mean that 2 or more pathways previous to the hexokinase reaction appear to exist for oxidation of glucose in these tissues, and that only one of these pathways is inhibited by 6-FG.

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