Insulin-Induced Accumulation of D-Xylose Against an Apparent Concentration Gradient in Diaphragm Muscle, *in Vivo*

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ABSTRACT The effect of insulin administration upon D-xylose-1-C^{14} penetration into the diaphragm and gastrocnemius muscles of functionally nephrectomized normal, hypophysectomized, and adrenalectomized rats has been examined. It was found in all groups that after the administration of tracer amounts of D-xylose, this sugar enters the cell water of diaphragm to a greater extent than in gastrocnemius muscle, both in the presence and absence of exogenous insulin. Insulin increases the apparent intracellular distribution of D-xylose in both muscles in all three types of rats. After insulin administration, the intracellular concentration of D-xylose in diaphragm muscle was estimated to be about two times greater than D-xylose concentration in plasma; D-xylose accumulation was not observed in gastrocnemius muscle of insulin-treated rats. Intracellular accumulation of D-xylose occurs in diaphragm of insulin-treated rats at plasma concentrations of D-xylose ranging from 4 to 2200 μg/ml; however, a "saturation" phenomenon appears to be operative, since intracellular distribution declines as plasma D-xylose concentration is increased within this range. A decline in intracellular D-xylose distribution also occurs in gastrocnemius as plasma D-xylose is increased, suggesting that entry into this muscle as well does not exhibit the characteristics of a simple diffusion process. The significance of these *in vivo* observations is briefly discussed in relation to widely accepted assumptions concerning sugar permeability in muscle.

INTRODUCTION

This study was initially undertaken to clarify certain divergent results concerning the extent to which D-xylose penetrates into diaphragm muscle *in vivo*, before and after the administration of exogenous insulin. Whereas Kipnis and Cori (1) reported that D-xylose distributes in a volume equivalent to about 50 to 55 per cent of the cell water of diaphragm, and insulin treatment increased the apparent intracellular volume of D-xylose distribution, Park *et al.* (2) using different experimental conditions, reported that D-xylose failed to penetrate into the fiber water of diaphragm or skeletal muscle unless insulin was admin-

istered. Park *et al.*, using eviscerated nephrectomized rats to remove the possibility of endogenous insulin release and to reduce possible sugar transformation, administered pentose intravenously at a slow continuous rate. Kipnis and Cori administered D-xylose in a single intravenous injection to nephrectomized rats, with the result that the plasma concentration of pentose was initially high and then fell rapidly during the early period of their experiments; the intracellular distribution of D-xylose reached a plateau between about 90 and 120 min. at which time it was considered that an equilibrium state had been achieved.

In the present study the apparent distribution of D-xylose in rat diaphragm muscle was measured under *in vivo* conditions, using functionally nephrectomized rats injected subcutaneously with D-xylose, so that blood levels of pentose were not subject to marked fluctuations during the course of the experiment. To evaluate the possibility that hormones released in consequence of the stress of the operative procedure involved in nephrectomy might influence the results, the distribution of C^{14}-labeled D-xylose and inulin was measured in diaphragm and gastrocnemius muscle in normal animals and in hypophysectomized and adrenalectomized rats as well. During the course of these studies, it was unexpectedly observed following the administration of insulin to normal, hypophysectomized, or adrenalectomized rats, that D-xylose is accumulated against an apparent concentration gradient in diaphragm but not in gastrocnemius muscle.

METHODS

Intact, hypophysectomized (2 day), or adrenalectomized (6 day) rats of a Sprague-Dawley strain, weighing 120 to 190 gm, were used. Adrenalectomized rats were maintained with 0.9 per cent saline as drinking water. On the day of the experiment, animals were functionally nephrectomized under avertin anesthesia, and then immediately injected with insulin or glucose solution. In each experiment a portion of each group of rats received insulin (1 u/100 gm. body weight) intraperitoneally accompanied by 100 mg glucose/100 gm to prevent hypoglycemia; the remainder of each group received 25 mg glucose/100 gm as control injections. Immediately thereafter D-xylose-1-C^{14} (National Bureau of Standards, S.A. 2.26 μc/mg) or inulin-carboxyl-C^{14} (New England Nuclear, S.A. 3 μc/mg) was injected subcutaneously. The usual dosage of D-xylose employed (1.8 to 2.5 μc/rat) resulted in plasma D-xylose concentrations ranging between 3 to 10 μg/ml. In one experiment, the total amount of D-xylose injected was increased by addition of non-radioactive D-xylose to give plasma concentrations as high as 2200 μg/ml. At 90 to 100 min. after tracer injection the rats were reanesthetized (nembutal), plasma was immediately obtained from aortic blood, and tissues (diaphragm and gastrocnemius muscle) were excised without delay, avoiding contamination by excess blood. Tissues were blotted in a uniform manner on dry filter paper and extracted with water near 100° without a prior wash. Tissue extracts and diluted plasma (1:50) were deionized with a mixed exchange

resin (amberlite MB3), then plated and counted in a gas flow chamber. A previous report describes the technics involved (3).

Results with D-xylose or inulin are expressed as the per cent volume of distribution between muscle tissue and plasma (100 × CPM per gm wet tissue/CPM per ml plasma). For D-xylose, the values are also given in terms of percentage distribution in intracellular water (100 × CPM per ml of intracellular water/CPM per ml plasma. The estimation of intracellular water was based on the assumption that inulin distributes exclusively in the extracellular compartment, and that the difference between total

TABLE I

DISTRIBUTION OF D-XYLOSE-1-C^{14}
AND INULIN-CARBOXYL-C^{14} IN DIAPHRAGM AND GASTROCNEMIUS
MUSCLE OF NORMAL, HYPOPHYSECTOMIZED,
AND ADRENALECTOMIZED RATS

	Insulin	Distribution of tracers in wet tissues*				Apparent distribution of D-xylose in intracellular water	
		Diaphragm		Gastrocnemius			
		D-xylose‡	Inulin‡	D-xylose	Inulin	Diaphragm	Gastrocnemius
Normal	−	40.0±1.0 (7)	15.6±0.9(9)	23.3±1.0(7)	13.3±1.9(3)	41	16
	+	139.3±8.8(6)	14.7±1.8(6)	78.2±4.3(6)	11.9±1.0(6)	207	103
Hypophysectomized	−	33.2±3.0(5)	14.7±0.4(7)	17.8±1.1(5)	12.2±1.3(4)	31	9
	+	115.0±4.6(6)	13.4±1.6(4)	49.5±4.5(6)	—	166	58
Adrenalectomized	−	55.1±5.8(4)	17.6±0.8(10)	24.8±2.3(4)	9.5±1.0(8)	64	23
	+	113.5±10.4(4)	—	63.5±11.4(4)	—	164	81

* Mean, standard error, and number of observations. Values obtained 90 to 100 min. after tracer administration at plasma concentration of D-xylose between 3 and 10 μg/ml.
‡ D-Xylose-1-C^{14} (S.A. 2.26 μc/mg) *0.8 to 1.1* mg/rat; inulin-carboxyl-C^{14} (S.A. 3.0 μc/mg) *0.8* mg/rat.

water content and inulin space is intracellular water. Total tissue water was determined by drying diaphragm and gastrocnemius muscle samples to constant weight at 100°.

RESULTS

Table I shows the distribution of D-xylose and inulin in diaphragm and gastrocnemius muscle of normal, hypophysectomized, and adrenalectomized rats, before and after the administration of insulin; the calculated apparent intracellular distributions of D-xylose in these muscles are also shown. It will be seen that the calculated intracellular volume of distribution of D-xylose is much greater in diaphragm than in gastrocnemius muscle in all types of animals. The intracellular distribution of D-xylose in the diaphragm and gastrocnemius muscles of normal animals is lowered following hypophysectomy

and increased following adrenalectomy. After insulin administration, the distribution of D-xylose is increased in both muscles in all three types of rats. D-Xylose, excluded from a major fraction of the cell water in gastrocnemius muscle in the absence of exogenous insulin, appears to distribute in most of the cell water after insulin. In all groups studied, insulin increases the apparent intracellular distribution of D-xylose in diaphragm muscle, but in this tissue, in contrast to gastrocnemius, D-xylose appears to accumulate in excess of the external concentration.

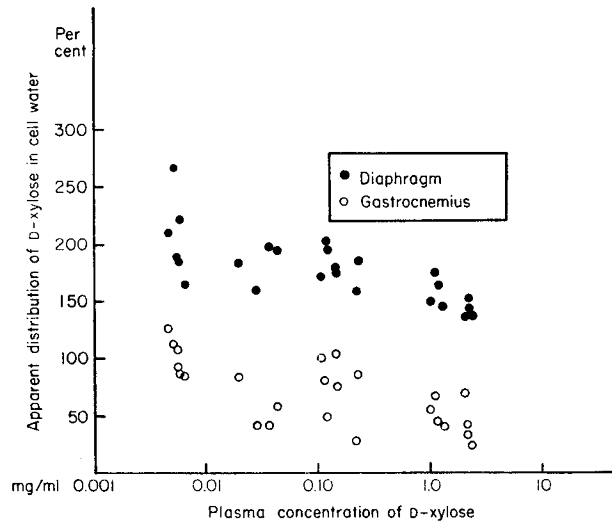

FIGURE 1. Relationship of apparent distribution of D-xylose in cell water of diaphragm and gastrocnemius muscles to plasma concentration of D-xylose. Data are from tissues of functionally nephrectomized normal rats given D-xylose subcutaneously and insulin (1 u/100 gm body weight) plus glucose (100 mg/100 gm) intraperitoneally 90 to 100 min. before sacrifice.

Since apparent accumulation of D-xylose was observed in diaphragm muscle at low plasma concentrations of xylose it became of interest to study the relationship of D-xylose distribution as D-xylose concentration in plasma was increased. Fig. 1 presents such data on the intracellular distribution of D-xylose in gastrocnemius and diaphragm muscle of intact rats given insulin when the D-xylose plasma concentration was varied by injecting variable amounts of D-xylose.

It will be seen that the D-xylose distribution values in both gastrocnemius and diaphragm muscle of insulin-treated rats progressively decline as the plasma concentration of D-xylose is increased from 4 to 2200 μg per ml; however, at all plasma D-xylose concentrations studied, D-xylose appears to accumulate intracellularly against a concentration gradient in diaphragm but not in gastrocnemius muscle.

Although D-xylose is widely believed to be a non-utilizable sugar, there is some evidence that this pentose is metabolized to a slight extent (4); therefore the validity of the conclusion that D-xylose is actually accumulated against an apparent concentration gradient depends on whether the radioactivity in diaphragm muscle and plasma is unmodified D-xylose. We therefore examined the homogeneity of the radioactivity obtained from diaphragm muscle and plasma of insulin-treated animals which had previously received 7.5 μc of D-xylose subcutaneously. The diaphragm muscle and plasma samples of two animals were extracted with hot water, and the extracts obtained were deionized and then lyophilized. The radioactivity in the resulting residue was dissolved in 95 per cent ethanol and chromatographed in a descending system (*n*-butanol:acetic acid:H_2O, 5:1:5) for 19 hrs., during which time authenic D-xylose moves about 10 to 11 cm from the starting line. The chromatogram, examined for radioactivity using a windowless paper strip scanner (Atomic Accessories RSC-5A), showed a single radioactive peak in all cases, which accounted for 93 to 97 per cent of the counts applied. Upon rechromatography, in this system, the radioactivity extracted from diaphragm muscle and plasma was found to be identical in mobility with authentic D-xylose. Radioautography of these paper chromatograms for 5 days, revealed that only a single component was present. There is no evidence from these studies that significant amounts of D-xylose transformation products are present in the aqueous extracts obtained from diaphragm or plasma by our routine procedure and which were employed for the determinations reported here.

DISCUSSION

This study has demonstrated important *in vivo* differences between gastrocnemius and diaphragm muscle with respect to D-xylose permeability. In the absence of exogenous insulin, D-xylose penetrates to a greater extent into the cell water of diaphragm than into gastrocnemius muscle in normal, hypophysectomized, and adrenalectomized animals. Following insulin the apparent intracellular distribution is increased in both muscles, but in diaphragm muscle D-xylose appears to be accumulated against an apparent gradient. The finding that the radioactivity accumulated by insulin-treated diaphragm is liberated from the tissue by boiling and behaves identically with D-xylose in regard to ion exchangers and paper chromatography is strong evidence that D-xylose, and not some radioactive transformation product, is accumulated by the fibers of diaphragm muscle against an apparent concentration gradient.

Although recent work has demonstrated that sugars are accumulated against a gradient in microorganisms (5, 6), and in kidney slices (7), heretofore this phenomenon has not been reported in muscle. In this tissue, it has been considered that sugars enter by a diffusion process (which may or may not have

an energy requirement), to equilibrate in fractions of the cell water, the size of the fraction depending upon the presence of insulin and/or muscle work. This view, in large part, is based upon the fact that in the intact rat diaphragm *in vitro*, incubated with varying concentrations of D-xylose (or D-galactose) ranging from 0.05 to 5.0 mg/ml, the uptake of sugar is directly related to the sugar concentration of the medium, so that the apparent intracellular distribution is independent of the external sugar concentration over a considerable range (1, 8). It should be emphasized, however, that *in vitro* studies involve a diaphragm muscle preparation in a resting state in contrast to the *in vivo* situation, in which diaphragm is a working muscle as will be discussed later. In most of the *in vivo* experiments (4, 9, 10), in which it has been reported that sugars like D-xylose are not accumulated against a gradient, the studies have involved analysis of skeletal muscle which our studies demonstrate does not exhibit the accumulation phenomenon.

We have no definite explanation for the fact that neither Kipnis and Cori nor Park *et al.* observed accumulation of D-xylose against an apparent gradient in diaphragm muscle in their experiments with insulin-treated rats. However, our results in other respects are similar to those of Kipnis and Cori, but differ from those of Park *et al.* Park *et al.* report values which indicate that mannitol distributes in a larger space than D-xylose in both diaphragm and skeletal muscle, suggesting that D-xylose fails to penetrate into muscle fibers in the absence of insulin, whereas our findings as well as those of Kipnis and Cori indicate the D-xylose space to be considerably higher in diaphragm muscle than the space of inulin or raffinose, resulting in an apparent intracellular distribution of D-xylose of 41 per cent and 53 per cent, respectively for these two studies in intact rats. Second, our studies show marked differences in the distribution of D-xylose between gastrocnemius and diaphragm muscle, both before and after insulin administration (consistently observed in normal, hypophysectomized, and adrenalectomized rats); whereas Park *et al.* state that D-xylose distribution in gastrocnemius is essentially the same as in diaphragm, both before and after insulin administration. The basis for this difference between our findings and those of Park *et al.* is not known. However, in considering the results on gastrocnemius and diaphragm muscle, it should be emphasized that in our animals, which were first anesthetized and then relatively inactive for 90 min., diaphragm is a working muscle, whereas gastrocnemius is essentially a resting muscle. Since it has been established that muscular work, induced by electrical stimulation, facilitates the penetration of sugars such as D-xylose into skeletal muscle (4, 9, 10), the lower uptake of D-xylose by gastrocnemius relative to diaphragm, might be explained on the basis of differences in work activity between these muscles *in vivo*. It has been reported (10), that the effects of muscle work and insulin are additive in facilitating sugar entry into muscle, and it is possible that the differences observed between

diaphragm and gastrocnemius muscle following administration of exogenous insulin may likewise be an expression of the differences in insulin action upon resting and working muscles.

There remains to be considered the nature of the mechanism whereby D-xylose is accumulated against an apparent gradient in insulin-stimulated diaphragm muscle *in vivo*. It seems reasonable, in view of the present findings and the *in vitro* studies previously cited, to consider that the apparent concentration of D-xylose achieved in cell water results from two processes: one similar to diffusion (or "facilitated" diffusion, *cf.* reference 11) whereby in the presence of insulin sugar equilibrates in most of the cell water, and a second process, which may be designated as "active," whereby the apparent D-xylose concentration in the cell is elevated over the external concentration in the plasma. As to the nature of the active component, one might postulate a sugar pump in the cell membrane as has been suggested with wide acceptance in the case of monovalent cations and amino acids; alternatively, it is possible to postulate cytoplasmic "binding" sites for D-xylose, involving weak forces, wherein the hydroxyls of sugars are hydrogen-bonded to surfaces of polymeric intracellular structures. Our data provide no basis for differentiating between these alternative possibilities. The fact that the intracellular D-xylose distribution in insulin-treated diaphragm falls progressively as the plasma D-xylose concentration is increased, might be explained as a "saturation" phenomenon on either basis. In gastrocnemius muscle of insulin-treated rats, in which the apparent intracellular concentration of D-xylose does not exceed the outside concentration, a sugar pump or binding sites need not be postulated. However, the present data indicate that in this muscle, as well as in diaphragm, the apparent intracellular distribution of D-xylose decreases as plasma D-xylose concentration increases.

The present *in vivo* findings are not in agreement with the widely held concept that the entry of sugars like D-xylose into muscle has the characteristics of a simple diffusion process. The finding that D-xylose is accumulated in diaphragm muscle against an apparent gradient suggests that it may be profitable to reexamine the basic assumptions of sugar permeability theory.

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