bolic effects and the tissue distribution of antimony when administered alone or with simultaneous injections of thyroxin. It was found that: 1) there appeared to be little effect on the body weight or oxygen uptake following ingestion of antimony trioxide in the amounts employed in these studies; 2) thyroxin activity was intensified by antimony trioxide resulting in a greater degree of hypermetabolism than was observed with thyroxin alone; 3) the relative distribution of antimony in the tissues was not significantly altered by injections of thyroxin; 4) the glandular hyperplasia observed in the thyroids of the antimony group was not present in the thyroxin-antimony group which suggests a difference in the mechanism of action.

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Influence of Insulin on Uptake of Monosaccharides by the Isolated Rat Diaphragm. (20023)

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The mechanism whereby insulin produces an increase in the phosphorylation of glucose is unknown. The effect of insulin may be due to some direct action on the hexokinase system or to some action on the permeability of the cell which makes more substrate available for phosphorylation. The latter hypothesis was developed by Levine *et al.*(1) who demonstrated that the volume of body fluid throughout which d-galactose is distributed in the eviscerated, nephrectomized dog is increased after the administration of insulin. Since galactose is neither utilized nor excreted by the eviscerated dog, Levine postulated that insulin exerts its effect by facilitating the rate at which the hexose enters the cell. Subsequently Levine and his colleagues(2) reported that insulin exerts a similar effect on other sugars having the same configuration as d-glucose in the first 3 C atoms.

In order to test further the hypothesis that insulin exerts an action on the transfer of sugars across the cell membrane, the effect of insulin on the *in vitro* uptake of several hexoses and pentoses by the isolated rat diaphragm was studied. The sugars used were highly purified preparations of d-glucose,

d-galactose, d-fructose, d-xylose, d-arabinose, and l-arabinose.

Method. Male rats of the Wistar strain, weighing between 150 and 200 g were fasted overnight and killed by stunning and decapitation. In each experiment the 4 hemidiaphragms from 2 rats were placed in 5 ml of a chilled solution of phosphate-saline(3) containing 0.2% of the particular sugar being studied. The tissues were shaken in the cold solutions for 5-7 minutes, blotted gently on filter paper, and transferred to Warburg vessels in such a manner that each flask contained a left hemidiaphragm from one rat and a right hemidiaphragm from the other rat. The incubation mixture consisted of 2 ml of the same solution of sugar in buffer as was used in the pre-equilibration period, but one flask of each pair contained amorphous insulin in addition. The concentration of insulin in the incubation mixture was 0.02 unit per ml when glucose was present, while 0.1 unit per ml was used with the other sugars. The vessels were maintained at 37°C and shaken at a rate of 116 cycles per minute in an atmosphere of oxygen. Generally an experiment at each of 4 different incubation periods, 30, 60, 120 and 240 minutes, was performed each day. At the end of the incubation period the tissues were blotted and weighed on a torsion balance. The average wet weight of tissue in each flask was 250 mg. The *uptake of each sugar* was calculated by subtracting its final concentration, after removal of the tissue, from that of a portion of the medium incubated simultaneously without tissue. For determination of glucose, fructose, and xylose the Nelson method was used(4); with galactose and the 2 isomers of arabinose the method was modified slightly in that the time of heating with the copper reagent was increased to 40 minutes. The standard solutions always consisted of the sugar being studied. In about half of the experiments on the uptake of fructose the results obtained with the Nelson method were confirmed by parallel determinations with the more specific method of Roe(5).

Results. Sugars that are utilized, that is, transformed to other substances and removed as such from the metabolic pool will be removed from the extracellular medium so long

TABLE I. Effect of Insulin on the Uptake of Monosaccharides by Isolated Rat Diaphragm.

Sugar	30 min.			60 min.			120 min.			240 min.		
	Uptake, mg/g	Insulin effect, mg/g	P	Uptake, mg/g	Insulin effect, mg/g	P	Uptake, mg/g	Insulin effect, mg/g	P	Uptake, mg/g	Insulin effect, mg/g	P
d-glucose	1.09 ± .26* (6)	+.82 ± .18	<.01	1.84 ± .09 (12)	+1.55 ± .14	<.001	3.01 ± .15 (6)	+1.54 ± .29	<.01	5.35 ± .35 (5)	+1.99 ± .44	<.02
d-fructose	—	—		1 ± .28 (6)	+ .55 ± .25	>.05	1.74 ± .14 (10)	+ .75 ± .15	<.001	2.58 ± .37 (10)	+1.84 ± .40	<.01
d-galactose	.67 ± .14 (8)	+.33 ± .09	<.01	.74 ± .11 (8)	+ .44 ± .10	<.01	1.26 ± .21 (8)	+ .11 ± .09	>.2	1.30 ± .12 (8)	+ .23 ± .16	>.1
d-xylose	.64 ± .04 (6)	+.11 ± .12	>.4	.89 ± .31 (8)	+ .22 ± .26	>.4	1.02 ± .20 (6)	+ .10 ± .13	>.4	1.44 ± .24 (6)	+ .31 ± .14	>.05
d-arabinose	.29 ± .12 (6)	+.14 ± .08	>.1	.68 ± .29 (6)	— .10 ± .17	>.5	.76 ± .20 (6)	+ .21 ± .21	>.3	.72 ± .27 (6)	— .24 ± .27	>.4
l-arabinose	.83 ± .26 (6)	+.08 ± .34	>.8	1.10 ± .19 (6)	— .10 ± .34	>.7	.99 ± .17 (6)	+ .63 ± .26	<.05	1.71 ± .32 (6)	+ .23 ± .31	>.4

* Mean ± S.D.
Numerals in parentheses represent No. of experiments.

as the enzymatic systems involved in such transformations remain intact. Sugars which are merely transported across the membrane into the cell will be removed from the medium until they can no longer be stored as such.

If it be assumed that the extracellular compartment of muscle is about 10% of the wet weight(6) and that this compartment reaches equilibrium with the incubation medium during the 5 minutes of pre-equilibration, then the maximum uptake that can be attributed to diffusion in 2.0 ml of a medium with a sugar concentration of 200 mg % is given by the expression: $(2)\ (.9)\left(\frac{2}{2 + .9\ (\text{wet wt})}\right)$. Accordingly, with a wet weight of 0.25 g, the maximal uptake by the muscle as a result of diffusion from the medium will be 1.6 mg per g.

The data summarized in Table I reveal that the isolated rat diaphragm removes appreciable quantities of all sugars studied. The uptake of d-galactose, d-xylose, and l-arabinose approaches, as an asymptote by 4 hours, the value attributable to maximal diffusion of the sugars into the intracellular compartment. The uptake of d-arabinose, however, is maximal at 2 hours and is about half that of the other pentoses; the difference between the mean total uptakes of the 2 isomers of arabinose by 4 hours is highly significant (P 0.01).

The effect of insulin on the uptake of glucose by the muscle is apparent at all incubation periods. A statistically significant effect of insulin on the uptake of d-fructose is apparent only after one hour of incubation. With d-galactose, a significant increase in uptake is produced by insulin after 30 and 60 minutes of incubation but not thereafter. With l-arabinose, the effect of insulin is significant after 2 hours incubation but not thereafter. With the other sugars, no statistically significant effect of insulin could be demonstrated at any period of incubation. It is pertinent to note that some of the positive effects of insulin would have been missed if only one or another incubation period were utilized.

Discussion. The data reported herein reveal that d-galactose, d-xylose, and l-arabinose are taken up by the diaphragm at a rate which can be attributed to diffusion of the sugars into the cell. Whereas the uptake of d-arabinose is about half that of the other pentoses, the uptake of d-glucose and d-fructose exceeds that attributable to maximal diffusion. The contrast between the uptake of d-arabinose and that of the other non-utilizable sugars suggests that the terminal molecular configuration may determine whether or not the sugar can continue to enter the cell until its intracellular concentration reaches that of the extracellular medium.

In accord with the postulate of Levine and his colleagues, insulin enhances the uptake of d-galactose and l-arabinose. In disagreement, however, is the observation that insulin does not influence appreciably the uptake of d-xylose and increases markedly the uptake of d-fructose.

Consequently, the effect of insulin cannot be attributed solely to the proximal molecular configuration of the sugar. The data, however, support the hypothesis that insulin acts on some process, other than the hexokinase reaction, which facilitates the rate of which some sugars enter the cell.

Summary. The uptake of d-galactose, d-xylose, and l-arabinose by the isolated rat diaphragm is such as can be attributed to equilibration with extracellular fluid. The uptake of d-glucose and d-fructose exceeds that attributable to diffusion while the uptake of d-arabinose does not approach that of the other pentoses. Insulin enhances the uptake of d-glucose, d-fructose, d-galactose, and l-arabinose. The data support the hypothesis that insulin acts on some mechanism other than the hexokinase reaction.

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