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DIFFUSION ACROSS RAT DIAPHRAGM

II. MOVEMENT OF SUGARS; EFFECT OF INSULIN AND OTHER AGENTS

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SUMMARY

Diffusion of sugars was measured across surviving rat diaphragm. Selective properties of this living barrier were shown by the fact that galactose moved across it much faster than glucose. Insulin slowed down the diffusion of those sugars whose uptake is known to be accelerated by the hormone. Anti-insulin serum and glucagon had an opposite effect. Diffusion of glucose was also slowed down by anoxia and electrical stimulation and accelerated by adrenaline and 2,4-dinitrophenol. Diffusion of xylose was enhanced by the presence of glucose. These results are easier to interpret in terms of the specific sugar-binding properties of the cellular matrix than according to the membrane-permeability theory.

INTRODUCTION

Penetration of sugars into living structures has been studied by many groups of investigators mostly on erythrocytes and muscle cells. The latter have acquired a particular significance when it became known that sugar uptake by muscle is accelerated by insulin. It is generally assumed that the transport of sugars, as that of most other small molecules and ions is controlled by the permeability properties of the plasma membrane. Specific penetration characteristics are explained on the assumption of carriers forming reversible combinations with the sugar to transport it across the membrane.

Rat diaphragm has been a favorite preparation for sugar transport studies. The present paper describes experiments in which diaphragm was used as a diffusion barrier in the way described in the preceding paper. The results obtained suggest that sugar transport can perhaps be explained in terms of the binding properties of a cellular matrix rather than by the membrane hypothesis.

METHODS AND MATERIALS

Diffusion of sugars was measured by the technique described in the first paper[1]. A fragment of rat diaphragm was interposed as a barrier between two compartments A and B. Compartment A contained 2 ml of bicarbonate–Krebs–Ringer solution in

which the sugar was dissolved at the concentration of 0.015 M. Compartment B contained 20 ml of the same salt solution without the sugar.

Diffusion rates were measured by collecting 1-ml samples from compartment B at 20-min intervals for 100 min and estimating the amount of sugar passing through the barrier. In all other respects, the technique as well as the calculation of the results and their expression by means of the two parameters, a and b, was the same as in the ion-transport experiments.

Estimation of various sugars was done by the following procedures: D-glucose was estimated by the glucose oxidase method[2], D-galactose, fructose and sucrose by the anthrone technique[3], D-xylose by the orcinol method[4], 2-deoxyglucose by the cysteine–sulfuric acid method[4] and ethanol according to NEISH[5].

Anti-insulin serum was prepared by the method of ARMIN *et al.*[6]. It was administered to rats 1 h before removal of the diaphragm. At this time the blood sugar was between 300 and 400 mg per cent.

All the other agents were added *in vitro* to compartment A at the concentrations indicated. Crystalline glucagon was obtained from Eli Lilly and Co.

RESULTS

Fig. 1. shows the rates of diffusion of different sugars across the rat diaphragm, compared with their rate of passage across a sheet of cellophane utilized under the same conditions. It is seen that all sugars pass more slowly through muscle tissue

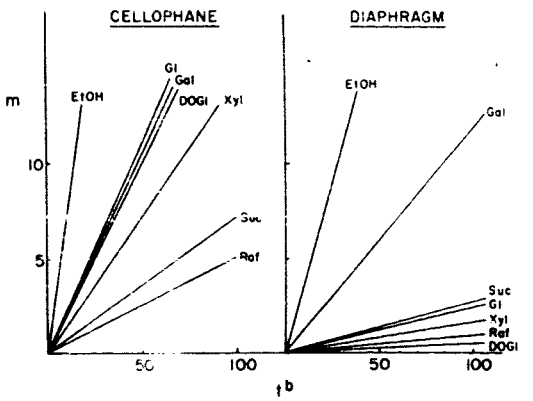

Fig. 1. Diffusion of sugars across cellophane and rat diaphragm. Abscissa: t^b (min); ordinate: m, fraction of solute diffusing (per cent). Left, diffusion across cellophane; right, across diaphragm. EtOH, ethanol; Gl, glucose; Gal, galactose; DOGl, 2-deoxyglucose; Xyl, D-xylose; Suc, sucrose; Raf, raffinose.

probably because of the greater thickness of the barrier. It is to be noted, however, that the order of diffusibility of the sugars has changed, indicating that the living system possesses a selectivity while the cellophane lets the sugars go through approximately in the order of their diffusion coefficients.

The fact that the diaphragm does not behave as an inert membrane is further shown in Table I which lists the diffusion parameters a (rate constant) and b ("binding" index) of a few sugars with and without insulin (0.1 unit/ml) added to the upper

TABLE I

EFFECT OF INSULIN ON THE DIFFUSION OF SUGARS ACROSS THE DIAPHRAGM
a, rate constant; *b*, "binding" index.

	Control		*Insulin*	
	$a \pm S.D.$	$b \pm S.D.$	$a \pm S.D.$	$b \pm S.D.$
Glucose	0.023 ± 0.014	1.217 ± 0.14	0.004 ± 0.004	1.515 ± 0.07
Galactose	0.122 ± 0.016	0.790 ± 0.10	0.046 ± 0.018	1.032 ± 0.07
D-Xylose	0.018 ± 0.007	1.178 ± 0.11	0.010 ± 0.006	1.295 ± 0.12
2-Deoxyglucose	0.005 ± 0.003	1.400 ± 0.16	0.001 ± 0.001	1.570 ± 0.18
Fructose	0.025 ± 0.008	1.127 ± 0.10	0.015 ± 0.006	1.198 ± 0.09
Sucrose	0.026 ± 0.010	1.090 ± 0.09	0.022 ± 0.009	1.125 ± 0.13

TABLE II

EFFECT OF ANTI-INSULIN SERUM

	$a \pm S.D.$	$b \pm S.D.$
Glucose	0.070 ± 0.022	0.993 ± 0.12
2-Deoxyglucose	0.023 ± 0.016	0.965 ± 0.11

TABLE III

EFFECT OF GLUCAGON AND INSULIN ON THE DIFFUSION OF GLUCOSE

	$a \pm S.D.$	$b \pm S.D.$
Control	0.023 ± 0.014	1.217 ± 0.14
Glucagon	0.039 ± 0.016	0.830 ± 0.11
Glucagon + insulin	0.020 ± 0.015	0.952 ± 0.10

bathing solution. It is seen that the diffusion rate of glucose, galactose, xylose, 2-deoxyglucose and fructose decreases under the influence of insulin. The binding index increases under the same conditions. The difference is highly significant for the first two sugars. Sucrose diffusion is not influenced by insulin.

Diaphragms taken from animals injected with anti-insulin serum show an opposite effect. As shown in Table II, they allowed glucose and deoxyglucose to diffuse faster, presumably because the antibody had neutralized the endogenous insulin present in the muscle.

Glucagon at 100 μg/ml, added *in vitro* to the bathing solution, also increased the passage of glucose across the diaphragm and its effect was reversed by insulin (Table III).

The striking discrimination shown by diaphragm between glucose and galactose was studied by submitting the preparation to various experimental conditions. Table IV summarizes the results of these experiments. It is seen that adrenaline at 5 μg/ml produces a considerable increase in the diffusion of glucose while galactose transport is decreased. This reverses the ratio of diffusion rates for glucose and galactose from 0.189 in the control preparation to 4.6 in the adrenaline-treated diaphragms.

Anoxia decreases both glucose and galactose diffusion and electrical stimulation

TABLE IV

DIFFUSION OF GLUCOSE AND GALACTOSE UNDER VARIOUS CONDITIONS

	Glucose		*Galactose*	
	a ± S.D.	*b* ± S.D.	*a* ± S.D.	*b* ± S.D.
Control	0.023 ± 0.014	1.217 ± 0.14	0.122 ± 0.015	0.790 ± 0.10
Adrenaline	0.212 ± 0.028	0.750 ± 0.09	0.046 ± 0.015	0.968 ± 0.12
Anoxia	0.010 ± 0.012	1.162 ± 0.14	0.057 ± 0.018	0.928 ± 0.11
DNP	0.074 ± 0.025	0.752 ± 0.08	0.062 ± 0.018	0.815 ± 0.09
Stimulation	0.002 ± 0.004	1.570 ± 0.28	0.089 ± 0.021	0.828 ± 0.10

produces the same effect, more markedly on glucose transport. High doses of 2,4-dinitrophenol (5 mM) have a tendency to equalize the rate of passage of the two sugars.

The effect of one sugar on the diffusion of the other is shown in Fig. 2. It is seen that the transport of D-xylose is accelerated in the presence of increasing concentrations of glucose.

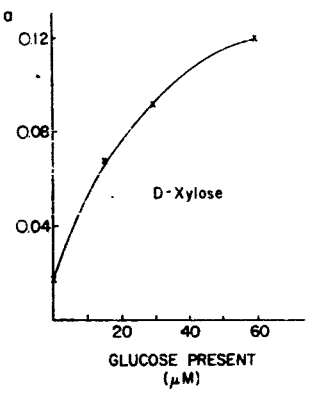

Fig. 2. Action of glucose on the diffusion of D-xylose. Abscissa: initial concentration of glucose in compartment A; ordinate: *a*, diffusion rate. The initial concentration of xylose was 15 μmoles/ml in all experiments.

DISCUSSION

The complexity of the problem connected with the diffusion of ions and small molecules was mentioned in the preceding paper. All the evidence points to the probability that diffusion takes place, in part at least, across the cells themselves. The data presented in this paper further support this assumption. It is difficult to imagine how a purely extracellular transport could be modified by insulin and the other agents studied. In a few experiments, diffusion of glucose and galactose was measured across the membranous part of the diaphragm which contains few or no muscle fibers. The rate of passage of the two sugars was not significantly different (0.053 ± 0.018 for glucose and 0.045 ± 0.015 for galactose).

A further complicating factor for the diffusion of sugars is that some of them are actively metabolized during their passage across the muscle. This explains

probably why glucose has a lower diffusion rate than galactose, but does not account for the slow passage of xylose and 2-deoxyglucose.

The uptake of sugars by muscle has been the object of many investigations[7]. On the whole, the diffusion of sugars across the diaphragm was found decreased under the same conditions which are known to increase sugar uptake; *e.g.*, insulin, anoxia and excitation. The increased diffusion of xylose in the presence of glucose is the counterpart of the inhibition of xylose uptake by glucose[8]. An exception is the action of DNP which increases glucose uptake but, in our experiments, increased the diffusion of this sugar (it decreased, however, the passage of galactose). The difference may be due to the high concentration of DNP used in the present work.

In all these experiments, permeability phenomena are closely associated with metabolic processes. This is true also for the non-metabolizable sugars whose transport is also dependent on metabolic energy. Our data support the hypothesis of RANDLE[7] according to which energy is required not for the transport of sugars but for their exclusion from the cell and which implies that insulin acts by cutting down the supply of energy.

There is fairly general agreement on the necessity for the sugars to combine with some cell constituent in order to be transported. The nature of this combination, however, is controversial: WILBRANDT[9] favors combination with mobile carriers which transport the sugar across the membrane, others[10, 11] prefer fixed binding sites which would bind certain sugars and keep others out. Insulin and other agents could act by changing the affinity of these binding sites.

The results reported above tend to support the fixed binding site assumption by showing that the conditions which favor the uptake of sugars slow down their diffusion across the muscle cell. The observations showing accelerated diffusion of xylose in the presence of glucose suggest competition for binding sites rather than for transport channels.

The nature and location of the binding sites is just as uncertain as that of the carriers. They may involve protein side groups but we could not find a correlation between sugar transport and protein structure, similar to the one described in the preceding paper for ion transport. It is also impossible to decide whether the sites are located on the surface of the cell or in the lattice structure of the intracellular matrix, as suggested by HECHTER AND LESTER[12].

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