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Phosphorylation of sugars by the intestinal mucosa*

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The absorption of sugars from the intestine is an old problem perhaps slightly out of fashion. It has a new interest if considered as a special case of the general phenomenon of active transfer of sugars across cell membranes.

For the past twenty years it has prevailed the hypothesis that the mechanism of active absorption of sugars from the intestine involved phosphorylation of these sugars. When this hypothesis was formulated by Verzar in the early thirties the information on enzymatic phosphorylation of sugars was scanty indeed: about all that was known about hexokinase was just this name.

Recently, work of Priscilla Hele (1) in England, of Csaky (2) in the U. S. A., and of Bissegger and Laszt (3) in Switzerland has given results suggesting that the rates of phosphorylation of sugars by intestinal mucosa homogenates or extracts are parallel to their rates of absorption *in vivo*. Such a parallelism, if substantiated, would be as close to a conclusive proof of the phosphorylation theory as it appears presently possible. I have carried out a critical study of the phosphorylation of sugars by the intestinal mucosa. When starting it I had the advantage of the knowledge of a number of properties of several purified animal hexokinases, specially the substrate

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specificity of brain hexokinase, recently obtained by work in collaboration with Crane (4).

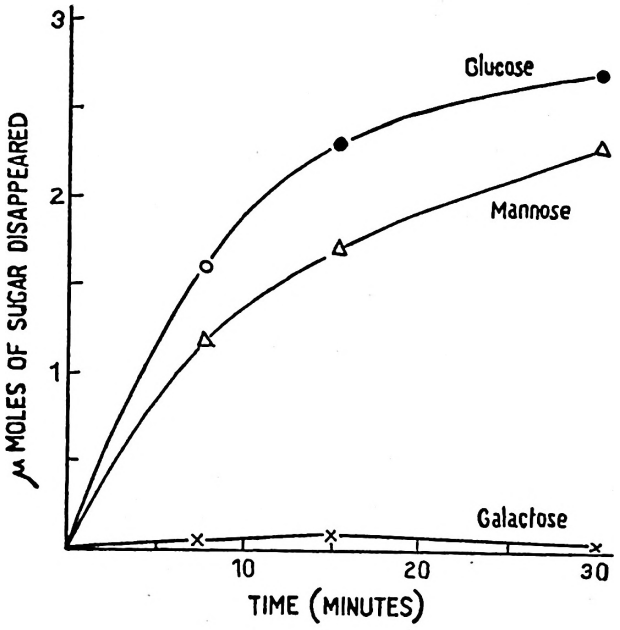

Fig. 1

Preliminary exploratory studies with homogenates of rat intestinal mucosa indicated the existence of such an amount of interfering factors (endogenous sugar, enzyme unstability, phosphomonoesterase, ATPase) that it became obvious that any simple approach like just sugar disappearance upon incubation with added ATP might give highly misleading results.

Interference by endogenous sugar was minimized by fasting the animals twenty four hours and by not depending on small disappearances of sugar, which means relatively long incubations. Apparent enzyme unstability was overcame by preparing the homogenates in ice-cold isotonic mannitol containing ethylenediaminetetraacetate, testing them without delay, and avoiding long incubations. A compromise of the order of 10-20 minutes appeared optimal. Phosphomonoesterase activity was practically eliminated, mainly by competitive inhibition with phosphate in addition to the classical fluoride. The still high rate of ATP disappearance — by direct hydrolysis or otherwise — in these conditions was indirectly overcame by adding

a large enough excess of ATP : some 10 μM of ATP per μM of sugar.

PHOSPHORYLATION BY INTESTINAL MUCOSA

(Observed velocities at 6×10^{-3} M)

Fructose	120	Galactose	< 5
Glucose	100	3-Methylglucose	»
2-Deoxyglucose	90	L-Sorbose...	»
Glucosamine	75	Xylose...	»
Mannose	75	L-Arabinose	»
Allose	10	N-Acetylglucosamine ...	»

Fig. 2

The *first slide* illustrates one experiment in standard conditions. An homogenate of the pooled mucosas of two rats was tested in parallel with glucose, mannose, an galactose. The

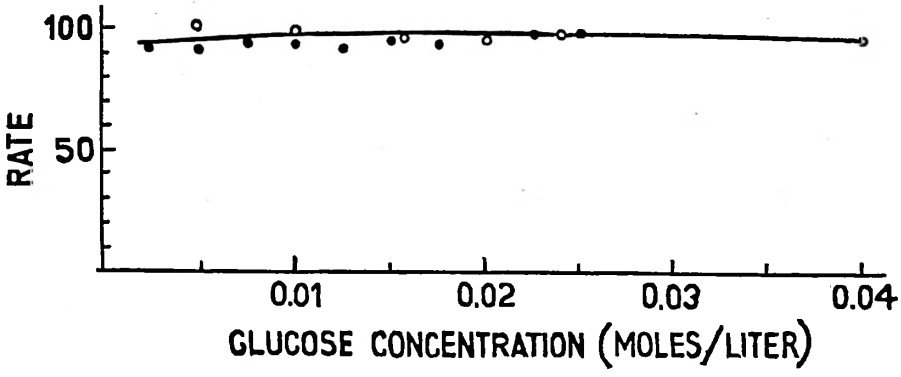

Fig. 3

3 μM of added glucose approached complete disappearance within thirty minutes. Mannose utilization was somewhat slower. With galactose there were no significant changes.

The results with all the sugars tested in these conditions are presented in the *second slide*. The figures can be taken only as an orientation, since they were obtained at a single,

arbitrary substrate concentration. But I wish to emphasize here the absence of phosphorylation of 3-methylglucose.

The effect of the concentration of glucose on its phosphorylation rate was studied by estimation of total ketose formed. This method was developed after observing a high activity of phosphoglucose isomerase and phosphofructokinase together with negligible utilization of fructose diphosphate in our conditions. The results shown in the *third slide* indicate a conside-

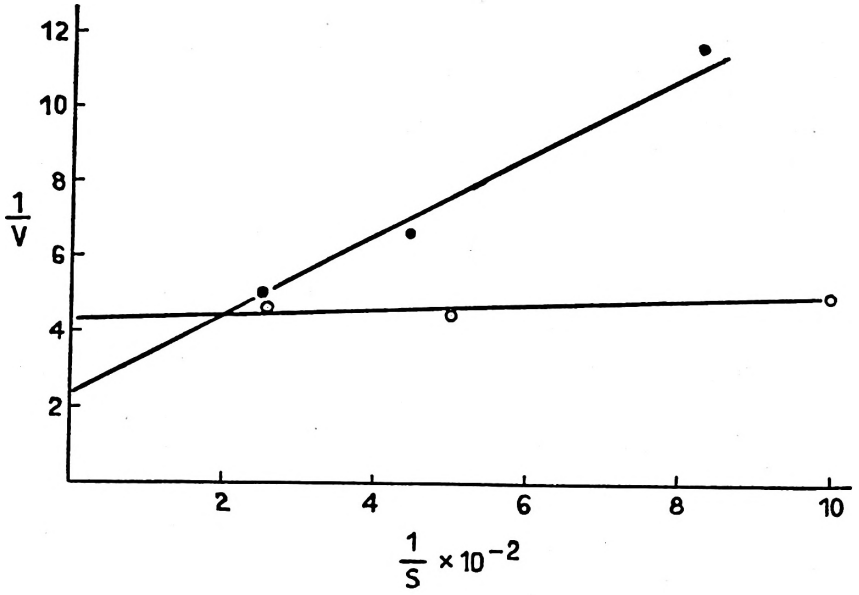

Fig. 4.

rable affinity for glucose : the Michaelis constant cannot be greater than about 2×10^{-1} M ; it might be much smaller.

The rate of phosphorylation of fructose is, on the contrary, markedly affected by its concentration in the vicinity of our standard conditions. The experiment presented in the *fourth slide* indicates a Michaelis constant for fructose of about 4×10^{-3} M and a maximal rate 1.8 times that of glucose. Experimental confirmation of the high maximal rate of fructose phosphorylation was obtained at 5×10^{-2} M substrate concentration. This was accomplished with a new method : estimation of esterified sugar. By this procedure it was observed that the rate of allose phophorylation also increased with the concentration. Among the sugars not appreciably phosphorylated at 6×10^{-3} M

concentration, L-sorbose alone was appreciably phosphorylated at the higher concentration, the observed rate being ca. 20 per cent of the glucose rate. When the sugar added was either galactose or 3-methylglucose the precipitate isolated with barium and alcohol from nucleotide-free filtrates was negative to the anthrone test for carbohydrates.

INHIBITION OF THE FRUCTOSE'S PHOSPHORYLATION

Glucose	+ + + +
Mannose	+ + + +
2-Deoxyglucose	+ + +
Glucosamine	+ + +
N-Acetylglucosamine	+ + +
Mannoheptulose	+ + +
Xylose	+ +
1,5-Sorbitan	+
1,4-Sorbitan	—
Galactose	—
3-Methylglucose	—

Fig. 5

The *fifth slide* summarizes the results of semiquantitative experiments of competitive inhibition of the phosporylation of fructose by the other sugars studied. They give an order of affinities roughly similar to those of purified brain and heart hexokinases. Here again galactose and 3-methylglucose appear as inert. Some other sugars, including N-acetylglucosamine and xylose, behave as inhibitors. On the contrary, N-acetylglucosamine did not affect the phosphorylation of L-sorbose that can be observed at high concentrations of the latter.

These results indicate the existence in the intestinal mucosa of the rat of a common hexokinase, with a pattern of substrate specificity at least broadly similar to that of brain, and a small independent ketokinase activity, perhaps similar to that found by the Coris in skeletal muscle.

There is fairly good evidence that the hexokinase of the intestinal mucosa phosphorylates at C6 the pyranose form of the aldohexoses and the furanose form of fructose. Glucose-6-phosphate can inhibit the enzyme, although the apparent

affinity is ten times smaller than that for purified particulate brain hexokinase.

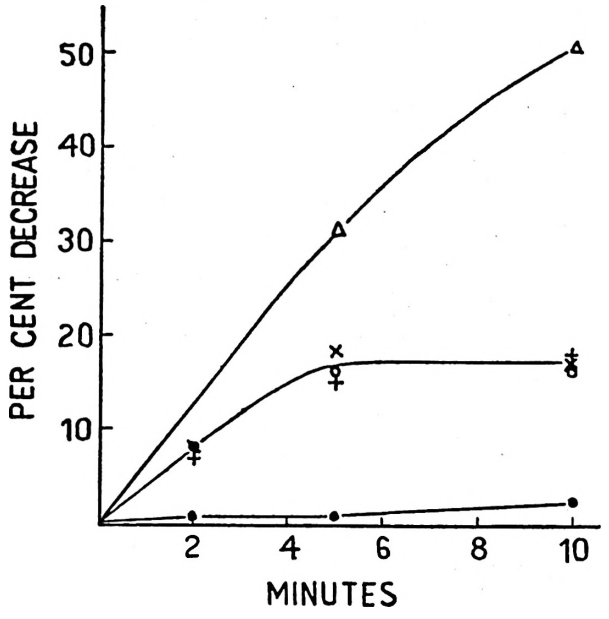

Fig. 6

Before concluding I wish to describe an experiment that illustrates the danger of uncritically looking for possible very labile enzymes. The experiment presented in the *sixth slide* was carried out with an homogenate of intestinal mucosa of a *non-fasted* rat, and with incubation times shorter than usual, as customary in attemps to detect presumptive labile enzymes. As usually, glucose phosphorylation was observed as a reference. The triangles correspond to sugar disappearance from glucose. The crosses correspond to sugar disappearance with galactose, but in one of the two series (that represented by inclined crosses) the galactose was added *after* the incubation! The circles correspond to L-sorbose: the open ones by sugar disappearance and the full ones by the more specific ketose disappearance, both analyses having been carried out in aliquots of the same filtrates. These results clearly indicate that the carly flattening off given by certain sugars is not due to any labile enzyme but to the ending of an artifact.

The last *slide (7th)* compares the rates of absorption from the intestine with the rates of phosphorylation. The order of

magnitude of the absorption rates are taken from the literature. Only 2-deoxyglucose had not previously been tested for absorption. And it was of primary importance because of the readiness with wich it is phosphorylated. I have found that it is not selectivelyy absorbed. The drastic lack of correlation between absorption and phosphorylation by hexokinase rules out the latter as primarily related to the mechanism of active absorption and strongly suggests that phosphorylation is not a part of the process.

A detailed report on the hexokinase activity of the intestinal mucosa will appear in the Biochimica et Biophysica Acta.

	Relative rates	
	Absorption	Phosphorylation
GALACTOSE	110	<5
GLUCOSE	100	100
3-METHYLGLUCOSE	90	<5
FRUCTOSE	50	180
MANNOSE	ca. 25	75
XYLOSE	»	< 5
L-SORBOSE	»	»
GLUCOSAMINE	»	75
2-DEOXYGLUCOSE	»	90

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