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Utilization and phosphorylation of sugars by *Escherichia coli* *

by
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In the last few years, it has been firmly established that the selectivity in the uptake of sugars by certain microorganisms is controlled by exigencies of a structural order.

In the *E. coli*, MONOD and coworkers have shown that certain non metabolizable glycosides can be accumulated intracellularly under the control of membrane agents that Monod calls permeases. In addition, the existence of a glucose permease has been suggested.

In yeasts, SOLS has demonstrated the selective uptake of sugars by means of transport agents, which he calls transportases, that do not imply phosphorylation.

We have initiated the study of the transport of monosaccharides in the *E. coli*, type A.

As first information, we have studied the development of *E. coli* on a series of sugars followed nephelometrically (fig. 1). This study completes the exploratory work carried out by KAPLAN.

Figure 2 shows the effect of various sugars on the respiration of *E. coli* grown in glucose.

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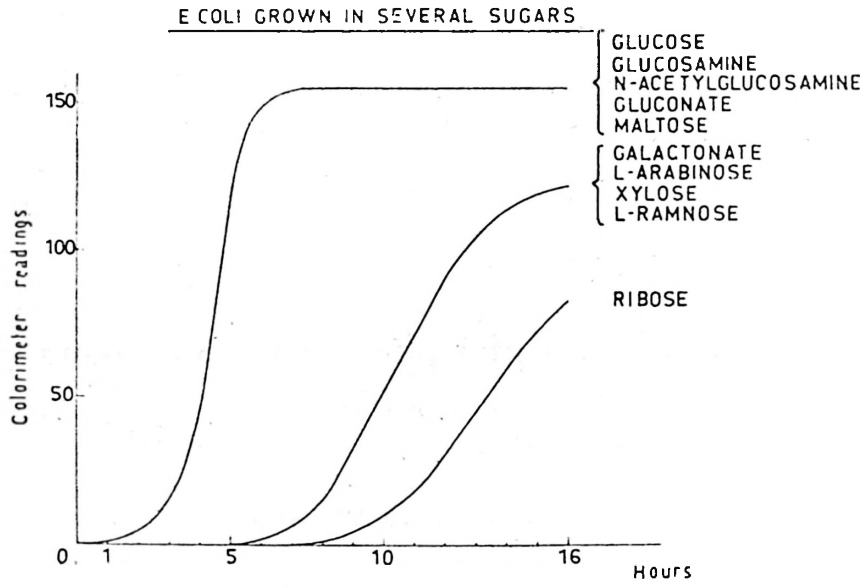


Fig. 1

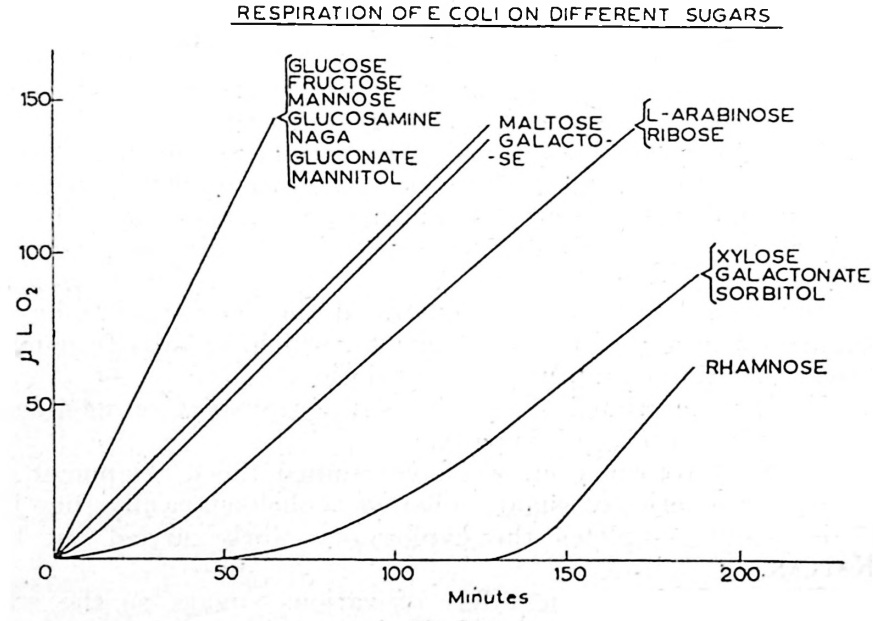


Fig. 2

Uptake of hexoses by suspensions of *E. coli*.

We have investigated the comparative uptake of a group of hexoses which have in common their structural relation-

ship and the fact that they can be utilized by the *E. coli* without previous adaptation. This study includes 2-deoxy-glucose, a non metabolizable derivative of great potential value as a tool in the study of glucose metabolism. The general proceeding consisted in incubating the microorganism in the presence of neutral phosphate buffer with a metabolizable hexose, alone or associated with a structurally related compound, as a possible inhibitor. At the end of the incubation the disapearance of substrate was estimated by differential methods.

UTILIZATION OF THE HEXOSES.

Resting suspensions of *E. coli* could utilize without detectable lag : glucose, fructose, mannose and N-acetylglucosamine. The table I summarizes the relative rates of the utilization of these hexoses, which were fairly constant in different lots, although the absolute rates showed considerable variation.

TABLE I

Hexose uptake by E. coli *resting cells*

SUGAR	Utilization
Glucose	100
Mannose	59
Fructose	41
N-acetylglucosamine	23
2-deoxyglucose	0

UTILIZATION OF HEXOSES IN THE PRESENCE OF RELATED COMPOUNDS.

Figure 3 represents schematically the inhibitions observed in the captation of glucose, fructose and N-acetylglucosamine, in the presence of tested related compounds, which were at a molar concentration ten times greater than that of the substrate. The consumptions were studied at two intervals during incubation and the percentages of inhibitions were in agreement. Average values have been represented in the figure.

The great affinity for glucose, as compared with the other sugars tested, is apparent. On the other hand, the fact that 2-deoxyglucose inhibits the utilization of all these hexoses, although it is not metabolizable by the *E. coli*, nor even is a

MUTUAL INTERFERENCE IN HEXOSE UPTAKE

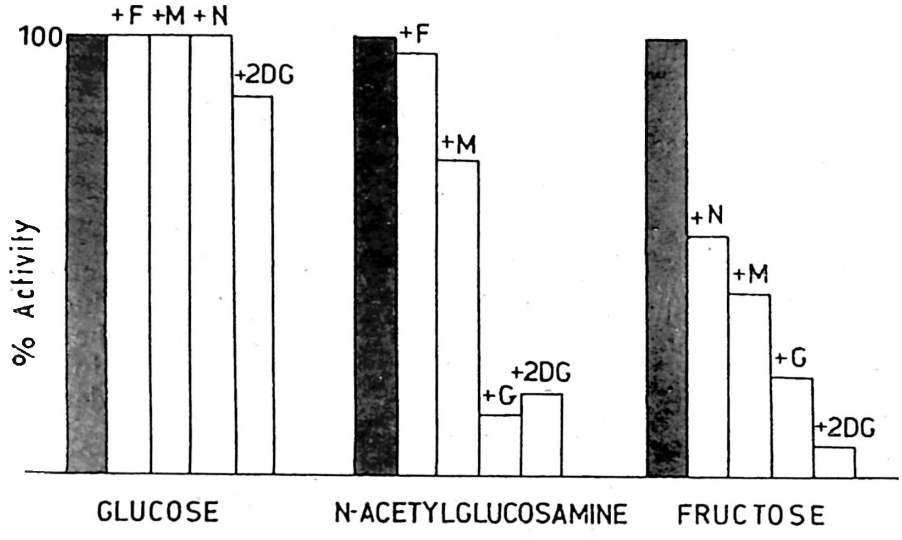


Fig. 3

INHIBITION OF NAGA UPTAKE BY 2DG

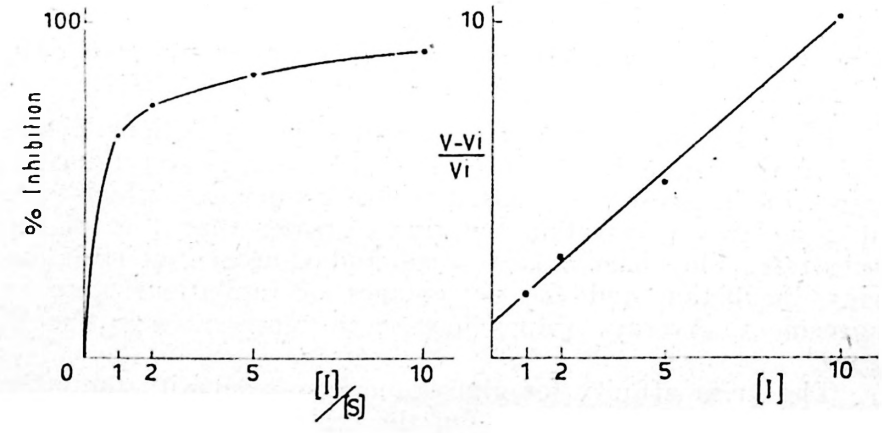
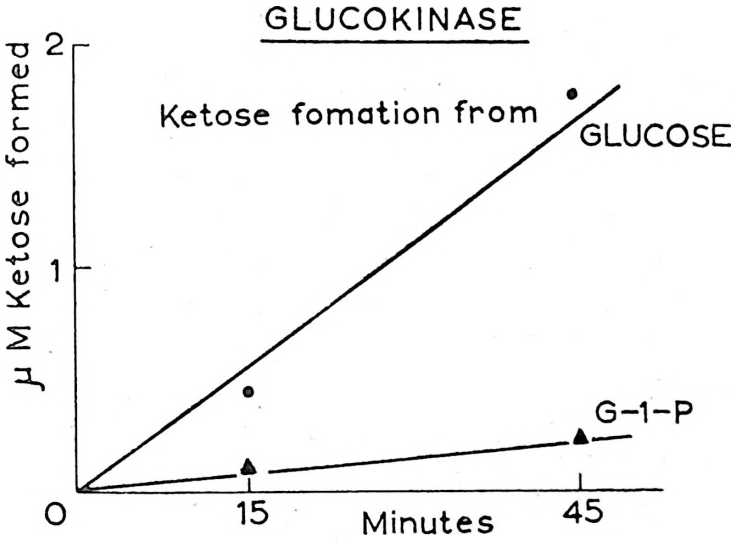


Fig. 4

substrate for its glucokinase, suggests interference at the uptake level. We shall insist on this point later.

With the object of deciding if the inhibitions are of a competitive type, we have carried out an experiment, illustrated in the figure 4, of competition in the utilizations of N- acetylglucosamine with a gradient of concentrations of 2-deoxyglucose. The inhibitions observed are proportional to the relation between the concentration of inhibitor and that of the substrate, and approach asymptotically to 100 %.



N-Acetyl-GLUCOSAMINEKINASE

Correlation between substrate disappea-
-rance and appearance of acid-stable phosphate

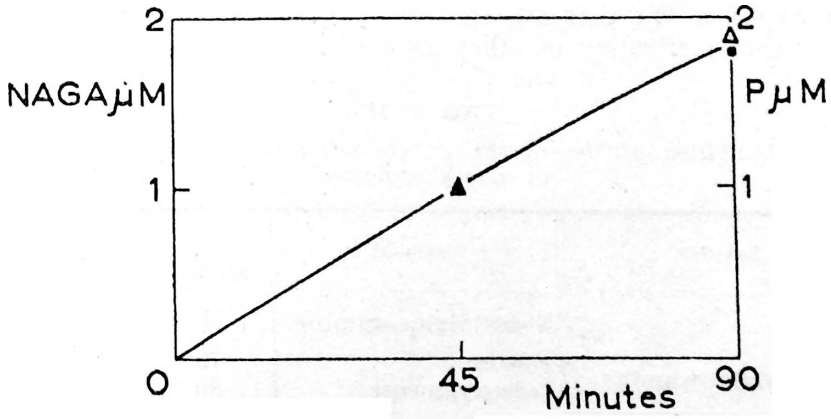


Fig. 5

PHOSPHORYLATIVE ACTIVITIES BY EXTRACTS OF *E. coli*.

The initial metabolic transformation of several of the sugars utilized by the *E. coli* is unknown. And for several of those that are known to be phosphorylable there is uncertainty on the identity of the kinase involved.

Homogenates and extracts of *E. coli*, grown in mineral medium-gloco, contain a glucokinase and a N-acetylglucosaminekinase. Both are stable enzymes. The latter can be precipitated with ammonium sulphate at 35 % saturation, while the glucokinase falls between 35 and 75 % saturation.

Michaelis's constant of glucokinase for glucose, is ca. 3×10^{-4} M. It little inhibited by the presence of N- acetylglucosamine at concentration ten times superior, but it is, indeed, inhibited by glucosamine which is also a substrate for the enzyme with a Michaelis's constant of ca 3×10^{-3} M.

On the other hand, the N-acetylglucosaminekinase is little inhibited by the presence of glucose or glucosamine at a molar concentration ten times greater than that of the substrate.

The product of the reaction of both enzymes is the corresponding 6-phosphate. Figure 5 shows the agreement between substrate disapearance and the apearance of acid stable phosphate. It also presents an experiment which rules out glucose-1-phosphate as primary product of glucose phosphorylation.

* * *

We have seen some results of the competition obtained at the level of uptake of hexoses by resting cells and at the level of their phosphorylation by cell-free extracts. Now we would like to compare some of the results. Table 2 makes it apparent the existence of marked differences. These discrepances raise the question of the possible existence in the *E. coli* of transportases for the uptake of monosaccharides prior to phosphorylation. We plan research along this line as well as further characterization of other kinases in the *E. coli*.

TABLE II

Inhibition of the uptake as compared with inhibition of phosphorylation

Substrate	Inhibitor	% Inhibition	
		uptake	phosphorylation
Glucose	N-acetylglucosamine	0	22
N-acetylglucosamine	glucose	75	33
	2-deoxyglucose	80	0

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