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Hans L. KORNBERG

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THE NATURE AND CONTROL OF CARBOHYDRATE UPTAKE BY *ESCHERICHIA COLI*

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THE NATURE AND CONTROL OF CARBOHYDRATE UPTAKE BY *ESCHERICHIA COLI**

H. L. KORNBERG

*Department of Biochemistry, University of Leicester UK***

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The uptake of carbohydrates from their growth media by *E. coli* is brought about by two main processes. Both require the expenditure of metabolic energy; since the hydrophilic carbon compounds that serve as nutrients cannot traverse the hydrophobic barrier presented by the cell membrane unaided, both processes also necessitate the presence of specific proteins in that membrane.

In the first of these processes, metabolic energy is transduced, in a manner not yet fully established, to effect the vectorial translocation of the carbohydrate but without changing its chemical identity. If the metabolism of that carbohydrate is prevented by the presence of some mutation that blocks an early step, the carbohydrate thus taken up 'actively' can be shown to accumulate, in concentration many times that present in the external milieu. For example, the 'active' transport of labelled galactose by an *E. coli* mutant devoid of galactokinase activity was shown by Horecker, Thomas and Monod already some 15 years ago [1] to lead to a massive accumulation of the sugar in the cell; if unlabelled galactose was now added, the labelled material readily exchanged with it and the radioactivity was thus displaced from the cell. Similar experiments have been done with mutants impaired in the catabolism (but not the uptake) of arabinose. In the presence of an energy source, such mutants take up labelled arabinose and also concentrate it many-fold; again, the addition of unlabelled arabinose virtually totally 'washes out' the labelled sugar from the cell [2]. This shows that indeed nothing happens chemically to the sugar, and that it is taken up, unchanged, by the mutant. When the experiment is performed with starved bacteria, in the absence of an energy source, it is found that there is an uptake of H^+ ion concomitant with the uptake of the sugar; both accumulation of sugar and H^+ uptake are arrested by the addition of agents that uncouple oxidative phosphorylation, such as ClCCP [3]. Findings such as these very strongly support the view, first formulated by Dr Peter Mitchell [4], that the inward movement of the sugar is energised by a transmembrane gradient of protons and/or of electrical charge.

Although there are a number of carbohydrates that are thus taken up by 'active transport', a second, and different, mechanism is also found in *E.coli* and in other bacteria capable of anaerobic growth. This also requires the input of metabolic energy, but the source of that energy is not an electro-chemical gradient but the metabolite phosphoenolpyruvate (PEP), which donates its phosphate stoichometrically to the sugar. In consequence, the sugar taken up appears inside the cell not in the form in which it is present in the medium but as its phosphate ester; pyruvate is also formed as the other product of the reaction. The main features of this process, which was discovered by Dr Saul Roseman and his colleagues [5] and was designated the 'PEP-dependent phosphotransferase system' (abbreviated PT-system from now on) are illustrated in fig.1.

The system includes at least five components although other constituents of the overall process are still being brought to light in the course of purifica-

* To avoid duplication, only 4 out of the 26 slides that accompanied the oral presentation are reproduced here: these 4 have not been published previously. References to the data contained in the remainder are given in the text.

** Present address: Department of Biochemistry, University of Cambridge, UK.

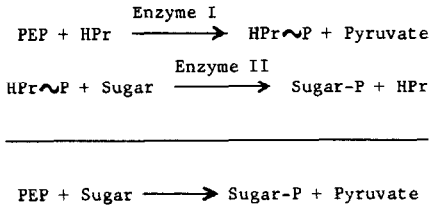

Fig.1. Overall mechanism of the PT-system (after [5]).

tion. An enzyme (Enzyme I) catalyses the transfer of phosphate from PEP to a small histidine-containing protein (HPr), thus forming a phosphohistidyl protein that is still capable of donating the phosphate group to suitable recipients. These recipients are, of course, ultimately the sugars that are the substrates of the overall transport process. However, for any one sugar to be thus taken up into the cell and phosphorylated, an appropriate and often inducible Enzyme II must be present; in contrast to the other components of the PT system, the Enzyme II activities appear to be firmly bound to the matrix of the membrane.

I propose in this lecture to confine myself wholly to discussion of this latter, *group translocation*, process and not to discuss 'active transport' any further. In particular, I shall present some work from my laboratory that bears on the following questions:

1. How many Enzymes II act on any one sugar?
2. How many sugars are acted upon by any one Enzyme II?
3. What factors regulate the rate at which any one sugar is taken up?
4. What factors regulate the uptake of any one sugar in the presence of another?

Uptake of glucose by *E. coli*

Information relevant to the first two and the fourth of these questions stems from a simple experiment, in which non-catabolisable analogues of glucose were added to a culture of *E.coli* growing on fructose as sole carbon source. It was observed that growth on fructose rapidly ceased [6]. When cultures of the organism were plated on solid media containing fructose and the non-metabolisable analogue, growth was again strongly inhibited but, after a day or so, mutant colonies, which were now resistant to the hitherto

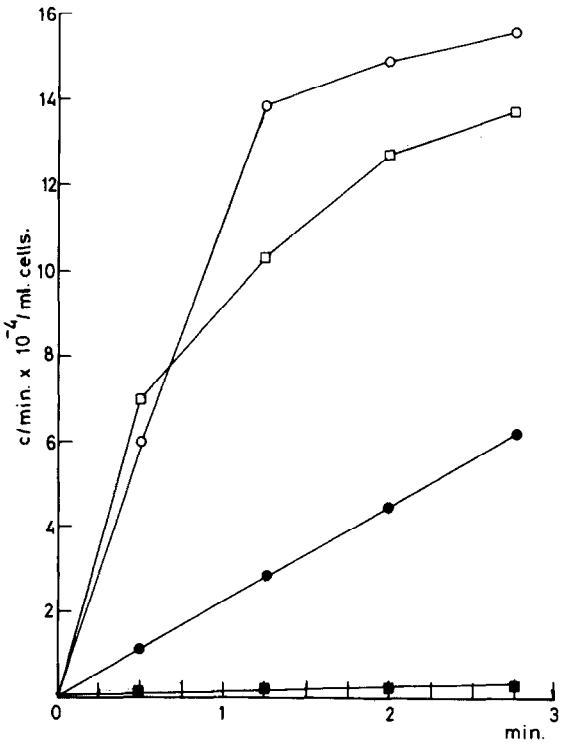

Fig.2. Uptake of [^{14}C]glucose (circles) and of [^{14}C]αMG (squares) by washed suspensions of *E.coli* strain K2.1t (open symbols) and its *umg*-mutant K2.1.22a (closed symbols).

toxic glucose analogue, appeared on these plates. When such mutants were permitted to grow on nutrient agar which had been supplemented with 10 μM ^{14}C-labelled methyl-α-glucoside (αMG), some of the organisms that now grew (and there was not enough αMG in the plates to inhibit the growth even of colonies sensitive to the analogue) were found to be unable to take up the radioactive material. As shown in fig.2 such αMG-resistant organisms differed from their αMG-sensitive parents in being unable to take up labelled αMG when washed suspensions of the cells were exposed to this substance; moreover, the uptake of glucose was also much less than that noted with the parent organism.

A second observation (fig.3) concerns the growth of mutants thus affected. Unlike the parent organism, the mutants are not impaired in their growth on fructose by the addition of glucose analogues such as αMG and 2-deoxyglucose. Furthermore, growth on

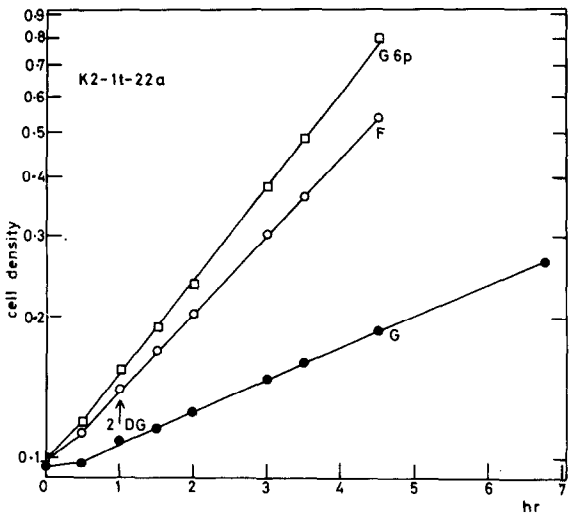

Fig.3. Growth of the *umg*-mutant K2.1.22a on 10 mM glucose (•), glucose 6-phosphate (□) and fructose (○). At the point indicated by the arrow, 5 mM-2DG was added to the flask containing fructose: no inhibition of growth was observed.

glucose occurs at a rate much slower than does growth on glucose 6-phosphate (which can be taken up by *E.coli* without prior hydrolysis [7]). This shows that the step affected in the mutant is between glucose and glucose 6-phosphate, but that the metabolism of glucose 6-phosphate remains normal. The mutation that led to a total inability to take up αMG has therefore also resulted in a severe impairment of the uptake and intracellular utilization of external glucose.

However, a severe impairment is still not a total abolition, and it is clear that at least one other route must operate to effect the uptake of glucose. It is known [8] that some systems involved in the active transport of galactose can also take up glucose; fortunately, these systems are not present in the strain of *E.coli* used in our experiments, nor are other systems that might conceivably bypass the uptake of glucose by some Enzyme II of the PT system [9].

A clue to the identity of that other route came from studies of the uptake of fructose by *E.coli*. It was known that the major route for the entry of fructose from the external milieu into metabolic pathways involved, as initial step, the activity of an inducible, high-affinity PT-system that catalyses the formation of fructose 1-phosphate from fructose and PEP [10,11]. The fructose 1-phosphate that thus appears in the cell is then further phosphorylated to fructose 1,6-bisphosphate by an ATP-linked kinase [12], and this provides the starting material for cellular syntheses as well as for the provision of energy. Mutants that lacked the initial uptake step had been isolated by my colleague, Dr Tom Ferenci. However, such mutants were of two types. In one type, labelled fructose was not taken up whether supplied at 0.1 mM or at 5 mM; in contrast, the other type that also did not take up 0.1 mM fructose did take it up when 5 mM fructose was supplied [6]. Subsequent genetical analysis revealed the presence of two loci, situated respectively at about minutes 41 and 36 on the *E.coli* genome [13]. The first of these specifies the high-affinity uptake system already mentioned but the second specifies an Enzyme II of the PT system that had only low affinity for fructose, but was also inducible by fructose and depended on the provision of PEP to form fructose 6-phosphate. Since it seemed inherently unlikely that the Good Lord had equipped *E.coli* with a PT-system specific for the uptake of fructose in case the organism encountered that sugar in concentrations greater than 2 mM, Dr Maurice Jones-Mortimer and I signalled our ignorance of its physiological role by diffidently describing this latter gene as *ptsX*.

Since the product of the PtsX system is a sugar phosphorylated in the 6-position, it seemed reasonable that this system might also act on glucose. This expectation was borne out. Mutants that lacked not only Umg but also PtsX activity were virtually totally unable to grow on glucose or, when labelled glucose was added to cultures growing on a carbon source that did not cause catabolite repression, were unable to take up that labelled sugar [14]. Similar findings, which shed light on questions 1 and 2 that I posed earlier, were independently and simultaneously reported by Dr Wolfgang Epstein and his colleagues working in Chicago [15]. It is evident that there are at least two Enzymes II that are normally concerned in the uptake of glucose; moreover, the second of these can also handle fructose.

Control of glucose uptake

There are good reasons for believing that the rates

at which micro-organisms utilize carbohydrates are determined by the rates at which these carbohydrates enter the cells. Evidence to this effect stems mainly from two types of observation. In one, the rate at which suspensions of lactose-grown *E.coli* hydrolyse *ortho*-nitrophenylgalactoside (ONPG) was found to be only about one-tenth of the rate at which the same cells, when disrupted with toluene, hydrolyse this lactose-analogue: clearly, the transport of the galactoside into the cells limited the rate of its hydrolysis by internal β-galactosidase [16]. A second type of observation stems from the work of Horecker, Thomas and Monod [1] with an *E.coli* mutant devoid of galactokinase activity, to which reference has already been made. It will be recalled that this mutant could still take up galactose but could no longer convert it to galactose 1-phosphate: consequently, galactose accumulated in high concentrations inside the cell. However, the rate at which the mutant took up galactose was identical to the rate at which the parent organism took up this sugar and, of course, no galactose accumulated in those latter cells. It follows that the rate of metabolism of galactose 1-phosphate cannot be less than the rate at which galactose was taken up. Similar conclusions have been reached with mutants impaired in the metabolism of gluconate [17].

However, the carbohydrates used for those studies all enter *E.coli* by 'active transport'. It may thus be asked whether phosphotransferase activity likewise limits the rate at which glucose can be utilized by *E.coli*. And, if the activity of the PT system indeed imposes a rate-limiting step to metabolism, it would further be proper to ask whether it is the maximal activity of an insufficiency of some component of that system, or the sub-maximal activity of an abundance of these components, or the availability of PEP, or all three of these factors, that impose the limitation.

Measurements of the rates at which different labelled sugars are taken up by washed *E.coli* reveal that the sugar specific components of the PT-system are formed inducibly [18]. An apparent exception to this is that glucose can be taken up by many strains of *E.coli* even though they have not previously been exposed to that sugar; in contrast, other strains take up glucose and its analogue, αMG, at only low rates unless the cells have previously encountered glucose [19]. Direct assay of phosphotransferase activity in cells rendered permeable with toluene showed that these activities correspond remarkably closely to the rates at which intact cells take up labelled αMG [19]. However, the rates of αMG uptake or phosphorylation thus measured were barely sufficient to account for the rate at which the 'constitutive' strain grew on glucose in batch culture, and were quite inadequate to account for the growth of the 'inducible' strain under these conditions.

This discrepancy disappeared when the two strains were grown in continuous culture with glucose as the limiting nutrient [20]. Samples of either strain taken from the chemostat took up glucose at rates that were not only closely similar but were much higher than those at which batch-grown cells took up this sugar. Furthermore, the specific activities of the PT-system thus measured varied with the growth rate of the cells and accounted quantitatively for the rate at which the glucose nutrient was utilized by the growing culture (fig.4). It thus appears that the PT-system

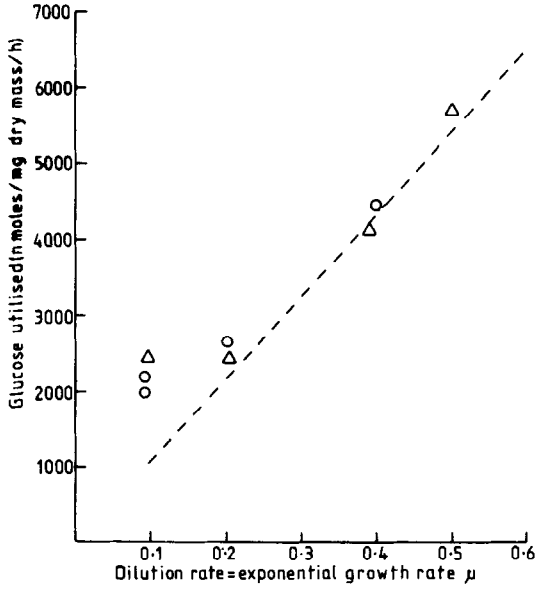

Fig.4. Relationship of the overall utilization of glucose by two strains of *E.coli*, – the 'inducible' B11 (△) and the 'constitutive' K2.1t (○) – at various exponential growth rates in continuous culture with glucose as limiting nutrient, to the rate of glucose uptake of cells harvested from the chemostat at various μ. The dotted line indicates the rate of glucose utilization calculated from the known cell densities and μ.

indeed represents a rate-limiting step in carbohydrate utilization and that, in the case of glucose, its physiological activity may be subject to 'catabolite repression' [21]. There is thus good reason to believe that both the amounts of component proteins of the PT-system and their physiological activities are subject to metabolic control.

Since PEP is a stoicheiometric reactant in the uptake of glucose, it would be expected that any condition that limits the availability of PEP would limit also the rate at which glucose is utilized. This expectation is borne out by the behaviour of a number of mutants that are impaired in enzymes of carbohydrate metabolism involved in the generation of PEP from the catabolism of glucose. Such mutants may grow poorly or not at all on glucose, yet may grow well on glucose 6-phosphate. For example, mutants devoid of fructose 6-phosphate kinase (Pfk^-) exhibit this behaviour [22]. The uptake of glucose involves the investment of 1 mole of PEP per mole of glucose taken up. Pfk^--mutants must catabolise the glucose 6-phosphate thus formed via the oxidative pentose phosphate pathway, in which, ultimately 1 mole of fructose 6-phosphate and 1 mole of glyceraldehyde 3-phosphate are formed. In the absence of Pfk activity, the fructose 6-phosphate is presumably utilized for cell wall biosynthesis or for partially regenerating glucose 6-phosphate; the glyceraldehyde 3-phosphate is the only precursor of PEP. Since the carboxylation of PEP is the only anaplerotic reaction operative in *E.coli* growing on carbohydrates [23], there is thus competition for this lone molecule of PEP and growth on glucose is consequently negligibly slow. Experiments with washed cells confirm that such cells take up [^{14}C]glucose to only a negligible extent but that the simultaneous provision of any (unlabelled) precursor of PEP greatly stimulates the uptake of labelled glucose [22]. A similar explanation has been advanced for the failure of mutants devoid of phosphoglucoseisomerase and also 6-phosphogluconate dehydrogenase to grow on glucose [24].

Preferential utilization of glucose

When cultures of *E.coli* are placed in growth media containing both glucose and any one of a wide range of carbohydrates, glucose is used preferentially and the proteins required for the uptake and catabolism of the other carbohydrate are formed only after the glucose has been virtually used up. This phenomenon, originally termed the 'glucose effect' [25], is made up of at least two components. By 'catabolite inhibition' [26] glucose inhibits the uptake of other carbohydrates; by 'catabolite repression' [21] glucose (or some product of glucose metabolism) represses the synthesis of enzymes that would normally be induced by those other carbohydrates. As I mentioned earlier in my discussion of the growth in batch culture of 'inducible' strains of *E.coli* [18], this repression may even be exerted by glucose over the uptake of glucose itself.

That 'catabolite inhibition' also plays an important part in the utilization of glucose preferentially to other carbohydrates (and may indeed be the prime cause [27] of that preferential utilization since, if any carbohydrate present cannot penetrate the cell, it also cannot act as inducer of the enzymes required for its catabolism) is shown by a simple experiment in which fructose-grown cells were placed in two flasks, both of which contained growth medium with an equimolar mixture of fructose and glucose as carbon source. The only difference between the flasks was that, in one the fructose was labelled with ^{14}C whereas in the other the glucose was thus labelled. Although the organism had been fully induced for the enzymes of fructose utilization, the subsequent growth of the cells occurred very largely at the expense of glucose: only one molecule of fructose was utilized for about every eight molecules of glucose [6].

That this preferential utilization of glucose to fructose necessitated the function of the PT-system was shown by the abolition of this preference when the experiment was repeated with a mutant into which has been transduced the *umg* marker [6]. Now fructose continued to be utilized although, of course, glucose was present in the medium initially in an equimolar amount. However, this effect was quite non-specific: glucose, which inhibited the uptake of lactose and galactose as well as that of fructose by Umg^+ cells, no longer did so when the organisms were made Umg^- [28]. Clearly, the process of phosphorylating glucose, or the formation of a phosphorylated protein intermediate, or the glucose 6-phosphate product, exerts a pleiotropic inhibition on the uptake of many carbohydrates.

However, the same or similar mechanism also functions to inhibit specifically the uptake of at least one sugar – fructose, and the site at which it acts appears to be the inducible high-affinity system for the uptake of fructose that is specified by the *ptsF*-gene. This was shown by the properties of a mutant DA 1 that was resistant to the toxic effects of 2-deoxyglucose (2DG) when growing on fructose but was still sensitive to this analogue when growing on glycerol [29]. As with Umg^- mutants, glucose was no longer utilized preferentially to fructose by this organism but, although [^{14}C]fructose was incorporated simultaneously with unlabelled glucose when the mutant grew on such a mixture, the proportions of cell carbon derived from these two sugars were unusual: about 35% of the total carbon incorporated was derived from glucose. In contrast, glucose still excluded sugars other than fructose, whether these were taken up by the PT-system or by 'active transport', to about the same extent as it did in wild-type cells. The gene specifying this loss of 'catabolite inhibition', exerted by glucose on the utilization specifically of fructose (and therefore designated *cif*) was found to be highly co-transducible with the fructose uptake system specified by *ptsF*, which suggests that the alteration leading to the Cif^- phenotype has occurred in a glucose-specific component of that fructose uptake system. Since the inhibitory effect of glucose on fructose uptake was largely overcome by mutation at this locus despite the unimpaired activity of the Umg system, it is possible that it is not glucose *per se* but glucose 6-phosphate that interacts with fructose (or with some phosphorylated intermediate of fructose uptake, such as a phosphoprotein or the fructose 1-phosphate product). This conclusion has some experimental support [29]. The incorporation of [^{14}C]fructose by Cif^+ cells growing on this labelled sugar was drastically reduced not only by the presence of unlabelled glucose but also of unlabelled glucose 6-phosphate; in contrast, and as expected, the utilization of labelled fructose by Cif^- cells was relatively little affected by the presence of glucose. But, interestingly, the *cif* mutation appeared to produce relief from inhibition not only by glucose but also by glucose 6-phosphate. It is conceivable that the utilization of glucose preferentially to other sugars may involve a general interference with the energy-transducing mechanisms catalysing the uptake of that second sugar; this may underlie the toxic effects exerted by phosphorylated sugars that have often been noted [30,12].

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