Glucose Phosphoenolpyruvate Phosphotransferase Activity and Glucose Uptake Rate of *Klebsiella aerogenes* Growing in Chemostat Culture

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Glucose-limited cultures of *Klebsiella aerogenes* NCTC 418 (and the supposedly identical strain NCIB 418) possessed a glucose phosphoenolpyruvate (PEP) phosphotransferase activity that varied markedly and progressively with growth rate, from more than 250 nmol min$^{-1}$ (mg dry wt cells)$^{-1}$ at $D = 0.1$ h$^{-1}$ to less than 100 nmol min$^{-1}$ (mg dry wt cells)$^{-1}$ at $D = 0.8$ h$^{-1}$. When relieved of the glucose limitation, substrate was used at a rate that bore no precise relationship to the cells’ phosphotransferase activity. Similarly, glucose-sufficient (phosphate- or potassium-limited) cultures metabolized glucose at high rates, whereas the cells possessed only moderate glucose PEP phosphotransferase activities. These results are compared with those reported for glucose-limited cultures of *Escherichia coli* and for variously limited cultures of *K. aerogenes*. Glucose-sufficient cultures, as well as glucose-limited cultures that had been temporarily relieved of glucose limitation, excreted partially oxidized products of glucose catabolism in considerable amounts. The relevance of this ‘overflow’ metabolism to studies of glucose transport using [U-$^{14}$C]glucose is emphasized.

INTRODUCTION

In a growing culture, heterotrophic organisms must possess the capacity to take up carbon-substrate at a rate sufficient to meet the cells’ biosynthetic and bioenergetic demands. But the relationship which this uptake rate bears to the cells’ uptake potential [i.e. the maximum activity of the uptake system(s)] is not well documented; nor is it known whether the enzymes of the uptake system are subject to ‘feedback’ control. In other words, it is not clear by what means, and to what extent, say, glucose uptake is regulated in organisms growing (either in batch or continuous culture) on this substrate. In this connection, it was reported recently (Herbert & Kornberg, 1976) that ‘over a wide range of growth rates, two strains of *Escherichia coli* growing aerobically in continuous culture under glucose limitation utilized glucose at rates identical with those at which cells harvested from the chemostats transported [$^{14}$C]glucose’. However, this finding contrasts sharply with the observations of Neijssel *et al.* (1977) that similarly limited chemostat cultures of *Klebsiella aerogenes*, as well as of two strains of *E. coli*, expressed an immediate and substantial increase in respiration rate when temporarily relieved of the glucose limitation. This clearly indicated that the cells’ capacity to transport glucose was markedly greater than could be expressed in the glucose-limited steady state culture. Nevertheless, the precise relationship between glucose transport capacity, growth rate and the maximum activity of the enzymes of the uptake system(s), measured *in vitro*, remained to be established.

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In enteric bacteria, uptake of glucose, and of several other hexoses, is mediated principally by a multicomponent phosphoenolpyruvate (PEP)-dependent phosphotransferase system (Kundig et al., 1964). Mutant strains of such bacteria that were affected in their ability to form components of this system (HPr and Enzyme I) were simultaneously affected in their ability to take up a number of hexoses (Lin, 1970). Nevertheless, the existence of alternative glucose uptake system(s) cannot be excluded and, indeed, is indicated by the fact that mutants of *E. coli* that lacked specifically glucose PEP phosphotransferase activity but which were able to synthesize glucokinase could still grow on glucose, albeit at a diminished rate. In contrast, other mutants that lacked both glucokinase and glucose PEP phosphotransferase activity could no longer utilize glucose as growth substrate (Curtis & Epstein, 1975). Thus, although there can be little doubt that under many conditions the glucose PEP phosphotransferase system provides the principal mechanism by which glucose enters the growing cell (Kornberg & Reeves, 1972a, b), the contribution which other uptake systems may make to the overall utilization of glucose is unclear.

Like *E. coli*, *K. aerogenes* possesses an active PEP-dependent phosphotransferase system for glucose whose activity was found to vary linearly with the growth rate and to extrapolate to a low value at zero growth rate (Carter & Dean, 1977). These changes were contrasted with those found for hexokinase and, like Herbert & Kornberg (1976), the authors concluded that ‘it is the glucose PEP phosphotransferase activity (which could be equated with the rate of glucose uptake) that sets the pace of overall glucose utilization in these growing organisms’. However, Neijssel & Tempest (1976) and Neijssel et al. (1977) have shown not only that glucose-limited cultures of this organism must possess the capacity to take up added glucose at a high rate, irrespective of the rate at which the cultures were growing, but that glucose-sufficient chemostat cultures (phosphate- or K⁺-limited) metabolized glucose at a substantially faster rate than glucose-limited cultures growing at a comparable rate (Neijssel & Tempest, 1975a, b). Clearly, if cultures possessed only the minimum glucose PEP phosphotransferase activity needed to meet the expressed steady state glucose uptake rate (of a glucose-limited culture) then either ancillary glucose uptake mechanisms must operate in the glucose-sufficient cells (and in glucose-limited cultures when relieved of the growth limitation), or else the glucose PEP phosphotransferase activity expressed *in vitro* must not reflect accurately that activity expressed *in vivo*. Consequently we decided to study in more detail the influence of growth conditions both on the actual rate of glucose consumption in the growing cultures and on the apparent activities of the glucose PEP phosphotransferase system.

**METHODS**

*Organisms.* *Klebsiella aerogenes* NCTC 418 was obtained from the Microbiological Research Establishment, Porton, Wilts., and has been used in this laboratory for several years. *Klebsiella aerogenes* NCIB 418 was obtained from the National Collection of Industrial Bacteria, Torry Research Station, Aberdeen. Strains were maintained by monthly subculture on tryptic meat-digest agar containing 0·1% (w/v) glucose.

**Media and growth conditions.** Organisms were grown in 500 ml ‘Porton-type’ chemostats (Herbert et al., 1965) in the media prescribed by Evans et al. (1970) with glucose or lactose added as the carbon source. The glucose in the bulk medium was adjusted to about 10 g l⁻¹ to provide a carbon limitation, and was raised to about 40 g l⁻¹ when growth was to be limited by the supply of phosphate (2 mM) or potassium (1 mM). Under these latter conditions, residual glucose could be readily detected in the culture extracellular fluids at all dilution rates studied. Lactose limitation was established by replacing glucose with 10 g lactose l⁻¹; no free glucose could be detected in the sterilized medium.

Chemostats were operated at 35 °C and a pH of 6·7 to 6·9. The dilution rate was varied as required by the experiment. Fully aerobic conditions were ensured by injecting air at a rate of 0·5 l min⁻¹ into the region of the impeller which was rotating at about 2000 rev. min⁻¹. Under these conditions the oxygen solution rate was in excess of 250 mmol l⁻¹ h⁻¹, as measured by the sulphite oxidation method of Cooper et al. (1944). To provide anaerobic conditions, the air was replaced by pure nitrogen.

**Measurement of glucose uptake rate.** (i) The steady state rate of glucose uptake could be determined from
the steady state bacterial concentration and the difference between the influent and effluent glucose concentrations according to the formula:

\[ q = \frac{f(S - \tilde{S})}{Vx} = \frac{D(S - \tilde{S})}{x} \]

where \( S \) is the glucose concentration (\( \mu \text{mol} \text{ ml}^{-1} \)) in the feed medium, \( \tilde{S} \) the steady state extracellular concentration, \( x \) the steady state bacterial concentration (\( \text{mg dry wt organisms ml}^{-1} \)) and \( D \) the dilution rate (\( h^{-1} \)) which is equal to the medium flow rate (\( f \)) divided by the culture volume (\( V \)).

(ii) The glucose uptake potential of glucose-limited cultures was measured by adding a cell-saturating pulse of glucose (about 10 mM final concentration) to the culture, turning off the medium pump and measuring the residual glucose concentration at set time intervals (normally 3 min). Over the short period of the experiment (15 min) the bacterial concentration, as measured by the absorbance of the culture at 540 nm, did not increase substantially and thus the rate of glucose uptake could be calculated from the relationship:

\[ q = \frac{S_0 - S_t}{x \times 60} \cdot (\mu \text{mol mg}^{-1} \text{ h}^{-1}) \]

where \( S_0 \) and \( S_t \) are the glucose concentrations (\( \mu \text{mol} \text{ ml}^{-1} \)) immediately following the pulse and at some time interval ('t' (min) thereafter. Again \( x \) is the steady state cell concentration at the start of the experiment.

Glucose was assayed with glucose oxidase, using the Galox reagent and galactose by the Galax reagent of AB Kabi (Stockholm, Sweden) and bacterial dry weight was determined by the procedure of Herbert et al. (1971). Total oxidizable carbon present in the culture fluids was measured with a Beckman TOC Analyser model 915A.

**Enzyme assays.** Glucose PEP phosphotransferase activity was measured in suspensions that had been decrpytified according to Kornberg & Reeves (1972b). About 10 ml culture (containing up to 5 mg dry wt organisms ml \(^{-1} \)) were centrifuged for 10 min at 3000 \( g \) (4 \( ^\circ \)C) and the organisms were washed once with 10 ml 0.2 M-NaH \(_2\)PO \(_4\)/NaOH buffer (pH 7.2) containing 2 mM-MgSO \(_4\). They were then resuspended in the same buffer to a concentration of about 2.2 mg dry wt organisms ml \(^{-1} \) and cooled to 4 \( ^\circ\)C in ice/water. Exactly 2 ml of this suspension was blown into a test tube containing 10% (v/v) toluene in ethanol and was mixed at maximum speed on a Vortex-Genie mixer (Scientific Instruments, U.S.A.) for 2 min. The quantity of toluene/ethanol mixture used varied from 35 \( \mu \)l for cells grown at low dilution rate to 25 \( \mu \)l for the cells grown at high dilution rate; optimal decrpytification was established for each culture at every growth rate. The glucose PEP phosphotransferase activity of the toluene-treated cells was measured as follows. The reaction mixture (final volume 1 ml) contained: 0.2 M-NaH \(_2\)PO \(_4\)/NaOH buffer (pH 7.2) containing 2 mM-MgSO \(_4\); 1.27 cmol; NADH, 0.25 \( \mu \)mol; lactate dehydrogenase (EC 1.1.1.27), 2 units; and cell suspension, 0.1 ml. The reaction was started, after equilibration at 35 \( ^\circ\)C, by adding 5 \( \mu \)mol glucose, and the decrease in absorption at 340 nm was followed. Activities are expressed as nmol glucose phosphorylated min \(^{-1} \) (mg dry wt cells) \(^{-1} \) and have been corrected for NADH oxidase activity, which was low.

Hexokinase activity was determined using cell-free extracts. Organisms from about 70 ml culture (4 to 5 mg dry wt organisms ml \(^{-1} \)) were harvested by centrifuging at 3000 \( g \) for 10 min, washed with 20 ml 20 mM-Tris/HCl buffer (pH 7.5), centrifuged as above and resuspended in 10 ml of the same buffer. They were then ruptured by sonic oscillation, using a Branson Sonifier model B-12 (Branson Sonic Power Co., Connecticut, U.S.A.; 60 W; 6 \( \times \) 30 s, with 30 s periods of cooling). Cell debris was removed by centrifuging at 27000 \( g \) for 10 min, and the supernatant was used for the enzyme assay. All operations were performed at 4 \( ^\circ\)C. For the enzyme assay, the reaction mixture (final volume 1 ml) contained: Tris/HCl buffer (pH 8.2), 100 \( \mu \)mol; NADP, 0.75 \( \mu \)mol; ATP, 5 \( \mu \)mol; MgCl \(_2\), 5 \( \mu \)mol; glucose-6-phosphate dehydrogenase (EC 1.1.1.49), 1.2 units; and cell extract, about 0.3 mg protein. The reaction was started by adding 5 \( \mu \)mol glucose, and the increase in absorption at 340 nm was followed. Activities are expressed as nmol glucose phosphorylated min \(^{-1} \) (mg protein) \(^{-1} \). All assays were performed at 35 \( ^\circ\)C.

Protein was determined by the biuret method using bovine serum albumin as a standard (see Herbert et al., 1971).

**Chemicals and enzymes.** Substrates, cofactors and enzymes were obtained from Boehringer Mannheim, Amsterdam. All other chemicals were of reagent grade.

**RESULTS**

With strains of *E. coli* and *K. aerogenes* the primary uptake process for glucose seemingly involves a glucose PEP phosphotransferase system whose activity can be conveniently assessed using the ‘decrpytification’ procedure of Kornberg & Reeves (1972b). When
Fig. 1. Glucose PEP phosphotransferase (PT) activities and glucose uptake rates (qglucose) of Klebsiella aerogenes strains NCTC 418 (a) and NCIB 418 (putatively identical to strain NCTC 418) (b) growing in glucose-limited chemostat culture at different dilution rates: ○, phosphotransferase activity of decryptified cells; △, actual rate of glucose utilization in the growing culture; ■, rate of glucose consumption following the addition of a pulse of glucose (10 mM final concentration).

applied to samples from glucose-limited cultures of K. aerogenes NCTC 418, this procedure revealed activities that varied significantly with growth rate and were maximal in cells taken from the slowest growing cultures (Fig. 1a). Thus, at D = 0.1 h⁻¹, cells possessed the potential to transport glucose at a rate that was some 12- to 13-fold higher than that actually expressed in the growing (glucose-limited) culture. Surprisingly, the apparent activity of the glucose PEP phosphotransferase decreased markedly with increasing growth rate such that at D > 0.7 h⁻¹ the cells consumed glucose at a rate in excess of that which could be fully accounted for by the apparent phosphotransferase activity.

To determine the relationship between the apparent uptake potential, as derived from the glucose PEP phosphotransferase activity, and that which could be realized by the growing cells, glucose-limited cultures growing at selected dilution rates were relieved of substrate limitation by the sudden addition of a cell-saturating concentration of glucose (about 10 mM) and the rate of consumption of glucose was measured. The results obtained (Fig. 1a) showed that irrespective of the growth rate, cells were able to consume glucose at a fast rate. At the higher growth rates, the rate of consumption of the added glucose was only marginally greater than that of the growing (glucose-limited) culture, but at the low growth rates it was markedly greater.

The fact that fast-growing cultures of K. aerogenes consumed glucose at a significantly greater rate than could be accounted for by the activity of the glucose PEP phosphotransferase suggested that either the decryptification procedure did not completely unmask (or partially inhibited) the component enzymes, or glucose could enter the cell by auxiliary mechanism(s). Sensitivity to toluene treatment was found to vary with the growth conditions, but the values reported in Fig. 1(a) represent the maximal activities found at each growth rate and were highly reproducible. Nevertheless, they still could be underestimates.

To test whether other mechanisms for glucose uptake operate in K. aerogenes, it was necessary to grow the organism under conditions where the synthesis of the glucose PEP phosphotransferase was repressed. Carter & Dean (1977) reported that K. aerogenes
Glucose uptake in K. aerogenes

Fig. 2. Changes in the concentration of galactose (○) and glucose (●) following the addition of an equimolar solution of glucose plus galactose (each 10 mM final concentration) to a lactose-limited culture of Klebsiella aerogenes NCTC 418. The changes in glucose concentration following the addition of glucose alone (△) to a second lactose-limited culture are compared with the combined glucose plus galactose uptake rates (▲) normalized to the same starting concentration (i.e. 10 mM). Dilution rate was 0·13 h⁻¹.

Table 1. Glucose PEP phosphotransferase (PT) activities, hexokinase activities and glucose uptake rates of Klebsiella aerogenes NCTC 418 growing on glucose in phosphate- and potassium-limited chemostat cultures at different dilution rates

<table>
<thead>
<tr>
<th>Dilution rate (h⁻¹)</th>
<th>PT activity*</th>
<th>Hexokinase activity†</th>
<th>q_{glucose}⁺ (in situ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate-limited</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0·18</td>
<td>65</td>
<td>78</td>
<td>113</td>
</tr>
<tr>
<td>0·40</td>
<td>81</td>
<td>ND</td>
<td>125</td>
</tr>
<tr>
<td>0·63</td>
<td>78</td>
<td>77</td>
<td>150</td>
</tr>
<tr>
<td>Potassium-limited</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0·17</td>
<td>38</td>
<td>ND</td>
<td>177</td>
</tr>
<tr>
<td>0·34</td>
<td>45</td>
<td>ND</td>
<td>234</td>
</tr>
<tr>
<td>0·42</td>
<td>82</td>
<td>ND</td>
<td>250</td>
</tr>
</tbody>
</table>

ND, Not determined.

* Specific activities are expressed as nmol glucose phosphorylated min⁻¹ (mg dry wt cells)⁻¹.
† Specific activities are expressed as nmol glucose phosphorylated min⁻¹ (mg protein)⁻¹.
⁺ Expressed as nmol glucose utilized min⁻¹ (mg dry wt cells)⁻¹.

NCIB 418 grown in a lactose-limited chemostat culture was devoid of the phosphotransferase. However, when strain NCTC 418 was grown under lactose limitation we found that it possessed a substantial glucose PEP phosphotransferase activity that varied from 90 to 38 nmol min⁻¹ (mg dry wt cells)⁻¹ as the dilution rate was increased from 0·13 to 0·74 h⁻¹. Nevertheless, at D = 0·13 h⁻¹ the cells took up glucose at a rate of 128 nmol min⁻¹ (mg dry wt cells)⁻¹, again suggesting the possible presence of a second uptake system.

The galactose permease is capable of transporting glucose into E. coli (see Kornberg, 1976) and may be present, and function similarly, in lactose-limited K. aerogenes. We investigated this possibility by pulsing a lactose-limited culture (D = 0·13 h⁻¹) with an equimolar mixture of glucose and galactose (each 10 mM final concentration). The rate of glucose consumption under these conditions was significantly diminished to 81 nmol min⁻¹ (mg dry wt cells)⁻¹ (Fig. 2), which was close to the activity of the glucose PEP phosphotransferase activity of the decryptified cells. Galactose was taken up simultaneously at 45 nmol min⁻¹ (mg dry wt cells)⁻¹, which was about equal to the difference in glucose uptake rates.
observed between lactose-limited cultures pulsed with glucose alone and with glucose plus galactose (Fig. 2). Though by no means definitive, this experiment lends support to the conclusion that the glucose PEP phosphotransferase activity in the decryptified cells was not an underestimate of that extant in the untreated (actively growing) cell.

Neijssel & Tempest (1975b) reported that glucose-sufficient cultures of *Klebsiella aerogenes* NCTC 418 consumed glucose at a substantially higher rate than did glucose-limited cultures growing at the same dilution rate (0.17 h⁻¹). It was of interest, therefore, to determine the glucose PEP phosphotransferase activity of such cells and to correlate this with the glucose uptake rate expressed by the growing culture. The results obtained with phosphate-limited cultures (Table 1) show that the glucose PEP phosphotransferase activity was markedly decreased to a value that was clearly insufficient to account for the expressed rate of glucose uptake. Increasing the dilution rate from 0.18 to 0.63 h⁻¹ caused the glucose consumption rate in situ to increase by about 30%, in agreement with the previous findings of Neijssel & Tempest (1975a), and there was a similar increase in glucose PEP phosphotransferase activity. But at all growth rates the glucose consumption rate was substantially in excess of the maximum phosphotransferase activity that could be measured in the decryptified cells. Extracts of these phosphate-limited organisms possessed a moderate hexokinase activity. Assuming that these organisms contained about 65% protein (Herbert, 1976) then it could be calculated that the combined glucose phosphorylating potential of the two enzymes, glucose PEP phosphotransferase and hexokinase, was sufficient to account for the overall rates of glucose consumption at $D = 0.18$ and 0.40 h⁻¹, but was insufficient at $D = 0.63$ h⁻¹. In the latter case, the difference, though possibly significant, was small [128 versus 150 nmol min⁻¹ (mg dry wt cells)⁻¹].

The strain of *K. aerogenes* used in these experiments (NCTC 418) has been maintained in this laboratory for six years, and it is conceivable that marked changes in some of its properties may have occurred over this period. Hence we obtained from the National Collection of Industrial Bacteria a fresh culture of strain NCIB 418 and determined its glucose PEP phosphotransferase activity when grown at different dilution rates in a glucose-limited chemostat culture. The results obtained (Fig. 1b) show that a similar decrease in the phosphotransferase activity accompanied a progressive increase in the growth rate, but that at each growth rate the activity expressed by these cells was substantially higher than that found with our original NCTC 418 strain (Fig. 1a). Moreover, the capacity of these
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Glucose-limited cells to take up glucose, when relieved of the growth limitation was correspondingly increased (Fig. 1b). Although the glucose PEP phosphotransferase activity of glucose-limited *K. aerogenes* NCIB 418 decreased markedly with increase in growth rate (as did the activity in *K. aerogenes* NCTC 418), the glucose uptake potential did not decrease correspondingly (Fig. 1b). Thus, whereas with the slowly growing cultures the measured glucose PEP phosphotransferase activity was greatly in excess of the unconstrained glucose uptake rate, it was markedly less than that required to account for the glucose uptake rate expressed by the more rapidly growing cultures when relieved of their glucose limitation. Interestingly, the glucose that was consumed by cultures when subjected to a glucose pulse was only partially assimilated into cell substance and products of glucose catabolism accumulated in the medium in substantial amounts, as shown by the associated changes in the extracellular level of total oxidizable carbon (Fig. 3). The extent of this ‘overflow’ metabolism varied progressively with the dilution rate such as to suggest that, following the glucose pulse, growth continued at the same rate as that extant in the steady state culture and that excess glucose was largely converted to soluble products. That glucose-limited cultures could not immediately accelerate their rate of cell synthesis, when shifted to glucose sufficiency, was apparent from the fact that there was no rapid increase in the absorbance of the culture following the pulse and is consistent with the findings of Harvey (1970) obtained with similarly limited cultures of *E. coli*.

**DISCUSSION**

Once a glucose-limited chemostat culture of micro-organisms is in a steady state, the extracellular glucose concentration generally is low and constant, and the rate of glucose uptake by the cells is proportional to its rate of supply. But the relationship which this expressed rate of glucose uptake bears to the cells' potential, or unconstrained, uptake rate is by no means certain. Implicit in the relationship between growth-limiting substrate concentration and growth rate formulated by Monod (1942) is the suggestion that the cells' uptake potential for the growth-limiting nutrient does not vary with the growth rate and that at \( \mu_{\text{max}} \) it is fully expressed. Clearly, glucose-limited cultures of *K. aerogenes* NCTC 418 (Fig. 1a) and NCIB 418 (Fig. 1b) behaved predictably in that the unconstrained rate at which cells consumed glucose following a sudden addition of a cell-saturating concentration of this substrate was similar at all growth rates tested. Surprisingly, however, this unconstrained uptake rate bore no obvious relationship to the measured *(in vitro)* activity of the cells' glucose PEP phosphotransferase. At low growth rates the phosphotransferase activity (and hence the putative uptake potential) greatly exceeded the unconstrained glucose uptake rate, whereas at high growth rates it was markedly insufficient to account for the measured maximum rate at which glucose could be consumed. The reason for the progressive decrease in glucose PEP phosphotransferase activity with increasing growth rate (Fig. 1a, b) is not immediately obvious; either the activity measured with toluene-treated cells was unrepresentative of that activity *in vivo* or ancillary mechanisms for glucose uptake must operate in organisms growing at the higher dilution rates.

As shown by Hunter & Kornberg (1979), the sensitivity of *E. coli* to decryptification with toluene varies with growth rate, and the same holds true for *K. aerogenes* (unpublished data). And though the values shown in Fig. 1 were obtained with optimally decryptified cells, it remains a possibility that growth rate-linked changes in membrane architecture rendered components of the glucose PEP phosphotransferase more susceptible to disorganization and/or denaturation. On the other hand, Hunter & Kornberg (1979) provided strong evidence that the growth rate-linked changes in phosphotransferase activity which they observed were not artefactual; they correlated closely with changes in the cells' glucose uptake potential. Hence if, as seems likely, the activities which we measured are real maximum values, then the growth rate-linked changes observed must have some rational
Fig. 4. Hypothetical plots of the relationship between specific growth rate ($\mu$) and substrate concentration ($s$) of glucose-limited chemostat cultures of bacteria. In constructing these plots the following assumptions were made: (1) that glucose uptake is mediated by a primary high-affinity enzyme system that obeys Michaelis–Menten kinetics; (2) that the specific growth rate ($\mu$) is directly proportional to the specific rate of glucose uptake and consumption ($v$) and that the latter increases linearly from 20 nmol min$^{-1}$ (mg dry wt cells)$^{-1}$ at $D(\mu) = 0.1$ h$^{-1}$ to 200 nmol min$^{-1}$ (mg dry wt cells)$^{-1}$ at $D = 1.0$ h$^{-1}$; (3) that the affinity constant ($K_m$) does not vary and has the same value (10 $\mu$m) in all three cases; and (4) that all three cultures possess, and express, the same substrate uptake potential [200 nmol min$^{-1}$ (mg dry wt cells)$^{-1}$] at the maximum growth rate, which is taken to be 1.0 h$^{-1}$. In case (a) the maximum activity of the uptake system ($V_{\text{max}}$) increases linearly as the dilution rate is decreased, attaining a value of 380 nmol min$^{-1}$ (mg dry wt cells)$^{-1}$ at $D = 0.1$ h$^{-1}$; in case (b) the maximum activity does not vary with growth rate; and in case (c) the maximum activity of the uptake system decreases linearly as the dilution rate is decreased, attaining a value of 120 nmol min$^{-1}$ (mg dry wt cells)$^{-1}$ at $D = 0.1$ h$^{-1}$. Thus, from a knowledge of the values $K_m$, $v$ and $V_{\text{max}}$ at each growth rate ($=$ dilution rate) the growth-limiting substrate concentration ($s$) can be calculated from the relationship: $s = (K_m v) / (V_{\text{max}} - v)$. These hypothetical plots do not relate precisely to any known organism, but the trends shown by *Klebsiella aerogenes* and *Escherichia coli* can be equated with cases (a) and (c), respectively.

physiological basis. In this connection, two possibilities might be considered. Firstly, the progressive increase in steady state extracellular glucose concentration (associated with a progressive increase in culture dilution rate) may impose an increasing degree of repression on the synthesis of the enzymes of the glucose PEP phosphotransferase system. Alternatively, one might speculate that the observed changes stem from the fact that the mean cell size of *K. aerogenes* cultures increases threefold as the growth rate increases from 0.1 to 0.9 h$^{-1}$ (Herbert, 1961). Thus if one expresses phosphotransferase activity as units per cell, rather than units per mg dry weight of cells, then the value varies only marginally with growth rate.

The patterns of change in glucose PEP phosphotransferase activity, and of glucose uptake potential, shown by glucose-limited cultures of *K. aerogenes* (Fig. 1) are markedly different from those reported for *E. coli* by Hunter & Kornberg (1979) and for *K. aerogenes* NCIB 418 by Carter & Dean (1977). As already mentioned, the data obtained with *E. coli* are consistent; both the phosphotransferase activity and the glucose uptake potential increase with growth rate in parallel to, though not coincident with, the expressed (in vivo) rate of glucose uptake. Although these results were obtained with a mutant of *E. coli* that was devoid of GalP and Mgl activity (i.e., ancillary glucose uptake mechanisms were absent), wild-type strains have been found to behave similarly (unpublished results). This mode of behaviour of *E. coli* is difficult to rationalize since by diminishing its glucose uptake potential at low growth rates it simultaneously decreases its capacity to scavenge glucose from the environment. Thus, in a competitive natural environment *E. coli* would be at a severe disadvantage. In contrast, the behaviour of glucose-limited *K. aerogenes*, reported here, is completely predictable; by derepressing synthesis of its glucose uptake enzymes at low growth rates it enhances its competitiveness. This can be readily appreciated by constructing hypothetical
plots of growth rate ($\mu$) versus growth-limiting substrate concentration ($s$) assuming the Monod (1942) relationship to apply. Three such plots are shown in Fig. 4 which refer to conditions (a) where the uptake system is derepressed at low growth rates, (b) where it is constant at all growth rates, and (c) where it is progressively repressed as the growth rate is decreased. Comparing (a) (representative of $K$. aerogenes) and (c) (representative of $E$. coli) it is clear that, in environments containing only low concentrations of glucose, $E$. coli would not be able to compete effectively with organisms such as $K$. aerogenes.

The results contained in this paper fail to accord with those of Carter & Dean (1977) in three respects. Firstly, we found a high glucose PEP phosphotransferase activity in slowly grown cells that progressively decreased with increasing growth rate; they reported the opposite. Secondly, they observed no significant difference between the glucose PEP phosphotransferase activities of glucose-, phosphate- and potassium-limited cells grown at the same dilution rate; we found marked differences (see Table 1 and Fig. 1). Lastly, they did not detect glucose PEP phosphotransferase activity in lactose-limited $K$. aerogenes, whereas we found moderate activities. Moreover, lactose-limited cultures of $K$. aerogenes NCTC 418 ($D = 0.13$ h$^{-1}$) consumed added glucose immediately and at a substantial rate. Although our laboratory strain of $K$. aerogenes (NCTC 418) appeared to be similar to the NCIB strain used by us and obtained from the same source as the one used by Carter & Dean (1977), the culture conditions employed by the two groups were not identical and this may be the reason for the discrepancy between the two sets of results. In particular, we routinely added trace metals (Ca$^{2+}$, Mn$^{2+}$, Zn$^{2+}$, Cu$^{2+}$, Co$^{2+}$, Fe$^{3+}$ and Mo$^{6+}$) to our culture media since we could not be sure that each of these would be present in adequate amounts as contaminants of the bulk medium constituents.

Finally, we wish to draw attention to the consequences of the ‘overflow’ metabolism expressed by glucose-limited cultures when relieved of their growth limitation. It is clear that slowly growing glucose-limited cultures of $K$. aerogenes are not able to accelerate immediately their rate of growth when supplied with an excess of glucose. Hence glucose enters the cell at a rate vastly in excess of that at which it can be consumed in growth-associated (biosynthetic) processes. Fine control of the uptake of excess glucose seemingly does not occur to a substantial extent, but the intracellular accumulation of intermediary metabolites is circumvented by the cells excreting into the medium partially oxidized products of glucose catabolism (e.g., gluconate, pyruvate and acetate) (Neijssel & Tempest, 1975a, b, 1976). The extent of this overflow metabolism becomes apparent when one compares the rates of excess glucose consumption with the rates of decrease of oxidizable carbon from the culture fluids (Fig. 3); with slowly growing glucose-pulsed cultures this difference is most marked. It follows, therefore, that had we sought to determine glucose uptake, using [U-$^{14}$C]glucose, the values obtained would have been comparable to those of total oxidizable carbon and would have been gross underestimates. It seems reasonable to suggest, therefore, that the rates of glucose uptake reported for $E$. coli in the earlier paper of Herbert & Kornberg (1976) were underestimated as a consequence of overflow metabolism, and, indeed, the later paper of Hunter & Kornberg (1979) lends support to this supposition.

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