metrically in whole cells decryptified with toluene (Gachelin, 1969), by linking the phosphoenolpyruvate-dependent phosphorylation of glucose to the reduction of $NADP^+$ in the presence of glucose 6-phosphate dehydrogenase, or by linking the release of pyruvate, consequent on the phosphoenolpyruvate-dependent phosphorylation of glucose or of glucose analogues, to the oxidation of NADH in the presence of lactate dehydrogenase. Both procedures gave similar rates of reaction and, when added glucose was limiting, both assay systems reflected equivalent nucleotide changes on a molar basis.

It was found that mutant K2.1t contained a highly active phosphotransferase system for glucose with K_m below 25μM, the activity of which was not significantly affected by changes in glucose concentration between 0.1 and 50mM. This system was present but in greatly decreased amounts in both mutant K2.1.22a and mutant R5s. However, only mutant R5s contained a second, inducible, phosphotransferase system for glucose, with K_m in the region of 10mM. Since this value is close to the glucose concentration at which mutant R5s grows at half-maximal rate, it is probable that this mutant, which still lacks the system that effects the uptake of α-methyl glucoside, contains a component of fraction II of a phosphotransferase system (Kundig & Roseman, 1971) sufficiently altered in its sugar specificity to permit the uptake of glucose when this hexose is present at high concentrations.

Brice, C. B. & Kornberg, H. L. (1967) *Proc. Roy. Soc. Ser. B* **168**, 281
Gachelin, G. (1969) *Biochem. Biophys. Res. Commun.* **34**, 382
Kornberg, H. L. (1972) in *The Molecular Basis of Biological Transport, Miami Winter Symp.* (Woessner, J. F. & Huijing, F., eds.), vol. 4, North-Holland, Amsterdam, in the press
Kundig, W. & Roseman, S. (1971) *J. Biol. Chem.* **246**, 1407
Kundig, W., Ghosh, S. & Roseman, S. (1964) *Proc. Nat. Acad. Sci. U.S.* **52**, 1067

The Control of Glycerol Utilization by Glucose Metabolism

By W. EDGAR, I. S. FORREST, W. H. HOLMS and B. JASANI (*Department of Biochemistry, University of Glasgow, Glasgow W.2, U.K.*)

Escherichia coli strain ML308 shows diauxic growth on a mixture of glucose and glycerol in simple defined media under aerobic conditions. When cells have been trained to glycerol and are growing on an excess of this substrate, the addition of even a small amount of glucose inhibits almost immediately synthesis of glycerokinase. Glycerol utilization falls by about 67% at the time of glucose challenge and continues at this low rate for about 50min, when it ceases altogether, and it does not start again until glucose is exhausted. If the amount of glucose added is sufficiently large, a diauxic lag can be seen before growth on glycerol recommences. After glucose challenge the glycerol carbon continues to enter protein, nucleic acid and lipid with a slight increase in the proportion entering the last. The delay before glycerol utilization is completely shut off suggests that this process requires protein synthesis. This is supported by the observation that addition of glucose to washed-cell suspensions metabolizing glycerol depresses the rate of glycerol metabolism but never abolishes it.

These phenomena are a clear example of catabolite inhibition (McGinnis & Paigen, 1969). In this case glycerol metabolism could be depressed by fructose 1,6-diphosphate inhibition of glycerokinase (Lin & Zwaig, 1968). However, fructose 1,6-diphosphate at 10mM and above inhibits glycerokinase of this strain to a maximum of 93%. In cultures growing on glucose, glycerol or both, direct measurement shows the intracellular concentration to be about 3mM in all cases. It would seem that the total inhibition of glycerol metabolism cannot stem from glycerokinase inhibition by fructose 1,6-diphosphate, but possibly involves antagonism of glycerol permeation across the bacterial membrane.

Lin, E. C. C. & Zwaig, N. (1968) *Science* **153**, 755
McGinnis, J. F. & Paigen, K. (1969) *J. Bacteriol.* **100**, 902

Effect of *n*-Alkanes on the Transport of Glucose in *Candida* sp. Strain 107

By C. O. GILL and C. RATLEDGE (*Department of Biochemistry, University of Hull, Hull HU6 7RX, U.K.*)

Candida sp. strain 107 is a hydrocarbon-utilizing yeast that has a propensity for producing large amounts of intracellular lipid (Ratledge, 1970). Addition of *n*-alkanes (C_{13} to C_{15} in chain length) to this yeast growing exponentially on glucose resulted in the immediate formation of oxidation products of the alkanes and a decrease in glucose consumption (Ratledge, 1968). The constitutive nature of the *n*-alkane-oxidizing system has been confirmed by use of an oxygen electrode: washed suspensions of the yeast grown on either glucose or *n*-alkanes both immediately oxidized all individual alkanes from C_{10} to C_{18}.

We have subsequently investigated the effect that *n*-alkanes have on glucose utilization. Both alkane- and glucose-grown cells immediately and rapidly

incorporated [U-^{14}C]glucose, but this could be inhibited by adding *n*-decane or *n*-undecane either simultaneously with the glucose or 30min later. Inhibition, with both types of cells, was directly proportional to the alkane concentration. Glucose, however, had no inhibitory effect on the incorporation of *n*-[1-^{14}C]decane into the cells. The effect of *n*-decane on the rate of glucose assimilation was much greater at a given concentration of alkane with alkane-grown cells than with glucose-grown cells. Moreover, glucose-grown cells showed a maximum inhibition of the rate of ^{14}C incorporation from [U-^{14}C]glucose of about 75% at 3μl of decane/mg dry wt. of cells, but alkane-grown cells showed no such maximum, and at the same concentration of decane incorporation of ^{14}C was 99% inhibited.

This inhibition of glucose assimilation could be due to alkanes or, less likely, because of the rapidity of this effect, to an oxidation product either inhibiting glucose transport directly or interrupting glucose catabolism with subsequent accumulation of glucose or a glucose metabolite that then inhibits glucose transport.

With *n*-alkane-grown cells, in which glucose assimilation was inhibited by *n*-decane to about 1% of the original rate, the rate of uptake of glucose was proportional to the density of cells but independent of glucose concentration. However, the apparent internal glucose concentration, although only reaching a maximum of about 5mM, was still higher than the final external concentration. This low concentration suggests that only residual active transport was being observed under these inhibited conditions. As there are difficulties in interpreting these results because of the metabolism of glucose, we have also used [1-^{14}C]sorbose, which is not metabolized by this yeast but is actively accumulated by it, probably by the same porter system as glucose, reaching an internal concentration in excess of 1M. Uptake of sorbose was most effectively suppressed by *n*-decane, thus leading us to conclude that the process of glucose transport itself is being blocked by the alkane.

The functional differences between glucose- and alkane-grown cells with respect to the effect of alkanes on glucose uptake may possibly be connected with a structural difference between the two types of cells. Considerable differences in structures have already been reported with other species of *Candida* (Ludvik *et al.*, 1968; Bos & de Boer, 1968).

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Bos, P. & de Boer, W. E. (1968) *Antonie van Leeuwenhoek J. Microbiol. Serol.* **34**, 241
Ludvik, J., Munk, V. & Dostalek, M. (1968) *Experientia* **24**, 1066
Ratledge, C. (1968) *Biotechnol. Bioeng.* **10**, 511
Ratledge, C. (1970) *Chem. Ind. (London)* 843

Inhibition of the Utilization of Lactose and other Carbon Sources by 3-Deoxy-3-Fluoro-D-Glucose in *Escherichia coli*

By R. J. MILES and S. J. PIRT (*Department of Microbiology, Queen Elizabeth College, University of London, London W.8, U.K.*)

3-Deoxy-3-fluoro-D-glucose is an unnatural glucose analogue that was first synthesized by Buck *et al.* (1966). It has been shown to be metabolized to 3-deoxy-3-fluoro-D-gluconic acid and perhaps also to 3-deoxy-3-fluoro-2-oxo-D-gluconic acid in *Pseudomonas fluorescens* (White & Taylor, 1970), but it appears not to undergo any significant metabolism in *Saccharomyces cerevisiae* (Miles & Pirt, 1969).

In frozen and thawed cells of *Escherichia coli*, 3-deoxy-3-fluoro-D-glucose was rapidly phosphorylated by the phosphoenolpyruvate-dependent phosphotransferase system, and resting cells took up 3-deoxy-3-fluoro-D-glucose to the extent of 0.03g/g dry wt. of cells. Uptake of 3-deoxy-3-fluoro-D-glucose was not lethal, though at concentrations in the range 0.1–10mM it completely prevented or severely inhibited the utilization of all carbon sources other than glucose. It prevented lactose utilization by inhibition of the synthesis and activity of β-galactoside permease; its effects on the utilization of other carbon sources may also be interpreted as being due to specific inhibition of the synthesis and activity of enzymes and permeases involved in their utilization. 3-Deoxy-3-fluoro-D-glucose-resistant mutants were isolated that were deficient in the Enzyme II of the phosphoenolpyruvate-dependent phosphotransferase system specific for glucose and for 3-deoxy-3-fluoro-D-glucose.

In inhibiting the utilization of carbon sources other than glucose, 3-deoxy-3-fluoro-D-glucose presumably acts as an analogue of glucose, and the physiological significance of the inhibition that it causes may be related to the mechanism(s) whereby glucose brings about its own preferential utilization. 3-Deoxy-3-fluoro-D-glucose also inhibited the utilization of carbon sources other than glucose in *S. cerevisiae*.

The ability of 3-deoxy-3-fluoro-D-glucose to bring about effects analogous to those of glucose, which 2-deoxyglucose and α-methyl glucoside and other glucose analogues are either unable or only partially able to bring about, is presumably an indication of its close stereochemical similarity to glucose.

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Buck, K. W., Foster, A. B., Hems, R. & Webber, J. M. (1966) *Carbohyd. Res.* **3**, 137–138
Miles, R. J. & Pirt, S. J. (1969) *Biochem. J.* **114**, 10P