JOURNAL OF BACTERIOLOGY, Feb. 1975, p. 577-582

Vol. 121, No. 2
Printed in U.S.A.

Regulation of the *Thiobacillus intermedius* Glucose Uptake System by Thiosulfate

A. H. ROMANO,* N. J. VAN VRANKEN, P. PREISAND, AND M. BRUSTOLON
Microbiology Section, Biological Sciences Group, University of Connecticut, Storrs, Connecticut 06268

Received for publication 18 November 1974

Cells of the mixotrophic chemolithotroph (facultative autotroph) *Thiobacillus intermedius* which have been grown on a glucose-yeast extract medium, a condition in which glucose is used as a source of energy, accumulate the non-metabolizable analogue 2-deoxy-D-glucose against a concentration gradient in a predominantly unchanged state. On the other hand, cells grown mixotrophically on a thiosulfate-glucose medium, a condition in which glucose provides cell carbon but is not used extensively for energy, and in which enzymes of the Entner-Doudoroff pathway are repressed, do not accumulate 2-deoxy-D-glucose significantly. Similarly, cells grown chemolithotrophically on thiosulfate-carbonate do not take up this sugar. Transfer of thiosulfate-yeast extract-grown cells, which lack the capacity to accumulate 2-deoxy-D-glucose, to a glucose-yeast extract medium results in the induction of the concentrative sugar uptake system. The capacity of induced cells to take up 2-deoxy-D-glucose is inhibited by thiosulfate. Thus, the transport system for glucose appears to be regulated in this organism so that the sugar is accumulated only under conditions where it is utilized as a source of energy, and the presence of the preferred energy source leads to both repression and inhibition of the uptake system.

Pseudomonas aeruginosa utilizes a number of tricarboxylic acid cycle intermediates in preference to glucose (14, 19). This preferential utilization of organic acids is brought about both by the regulation of glucose catabolism (14, 19) and by the regulation of glucose uptake; a number of organic acids both repress the formation of the inducible transport system and inhibit its activity (12, 13). The succinate-glucose diauxie exhibited by *Arthrobacter crystallopoietes* also has been shown to be related to regulation of glucose uptake (7).

A parallel that is interesting from the standpoint of comparative physiology is presented by *Thiobacillus intermedius*, an organism which utilizes an inorganic substance, thiosulfate, as an energy source in preference to glucose. This organism can grow as an autotroph, utilizing thiosulfate as energy source and carbonate as carbon source, as a mixotroph, utilizing thiosulfate as energy source and glucose as carbon source, or as a heterotroph, utilizing glucose in the presence of yeast extract as both energy and carbon source (8–10). In the presence of thiosulfate and glucose, glucose is not used as an energy source, and under such conditions the enzymes of the Entner-Doudoroff pathway are repressed (11). Thus, this organism offers the opportunity of studying the characteristics of the glucose transport system under conditions where glucose is utilized only as a source of carbon, or when it is utilized as a source of energy as well.

We present evidence here, by using the non-metabolizable analogue 2-deoxy-D-glucose, that *T. intermedius* has an inducible, non-phosphorylative, active transport system for glucose that is formed only under conditions where this sugar is used as a source of energy, and that thiosulfate regulates this transport system both by repression and by inhibition of its activity.

MATERIALS AND METHODS

Organism and growth media. *T. intermedius* ATCC 15466 was cultured essentially as described by Matin and Rittenberg (10). All additions were made to a mineral salts solution which contained in grams percent (wt/vol): NH_4Cl, 0.1; $MgCl_2 \cdot 6H_2O$, 0.05; $MgSO_4$, 0.03; KH_2PO_4, 0.04; $K_2HPO_4 \cdot 3H_2O$, 0.06; $FeCl_3 \cdot 6H_2O$, 0.002. The maintenance medium (thiosulfate–yeast extract agar) contained in addition to the above (grams percent): $Na_2S_2O_3 \cdot 5H_2O$, 1.0; yeast extract, 0.1; chlorophenol red, 0.008; agar, 1.5. In preparing experimental media with organic constituents, sodium thiosulfate, yeast extract, or glucose was added separately to the mineral salts after sterilization, at the concentrations specified in Results. In media not containing yeast extract, a trace metals solution was added to give final concentrations (μg per

100 ml) of: $CaCl_2 \cdot 2H_2O$, 100; $ZnSO_4 \cdot 7H_2O$, 88; $CuSO_4 \cdot 5H_2O$, 40; $MnSO_4$, 15; $Na_2B_4O_7$, 10; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 5. For autotrophic growth, bromothymol blue (0.003%) was used as a pH indicator, and sterile Na_2CO_3 was added as carbon source in sufficient quantity to maintain the pH near 6.8 during the course of incubation.

All incubations were carried out at 30 C. Liquid cultures were grown in 250-ml flasks containing 30 ml of medium and were shaken on a rotary shaker at 250 rpm or a reciprocating shaker at 100 strokes per min. Liquid cultures were checked routinely for contamination by streaking on the thiosulfate-yeast extract agar, where *T. intermedius* gives rise to very small, compact yellow colonies with an extensive zone of acid formation (9), and subculturing in thioglycolate broth; no growth is detected after overnight incubation at 30 and 37 C.

Uptake studies. Cells were harvested by centrifugation at 20,000 × *g* for 10 min, washed twice with 0.9% NaCl, and then suspended in mineral salts solution to the desired cell density (0.25 to 0.4 mg [dry weight] per ml). The cell suspensions (5 or 10 ml final volume) were incubated at 30 C in a water bath for 30 min to allow temperature equilibration; [U-^{14}C]2-deoxy-D-glucose (International Chemical and Nuclear Co., Irvine, Calif.) was added at the concentration specified and specific activity of 0.2 μCi/μmol. Samples (1 ml) were removed at suitable time intervals, filtered immediately through membrane filters (0.8 μm pore size; Millipore Corp., New Bedford, Mass.) and washed with 15 ml of mineral salts solution at ambient temperature. The filter with the adherent cells was transferred to a vial containing 10 ml of Brays (3) scintillation fluid for counting in a Packard liquid scintillation spectrometer.

Extraction and chromatography of accumulated material. Cells grown in mineral salts plus 0.5% glucose and 0.5% sodium thiosulfate for 72 h were harvested, washed, and resuspended as described to a density of 0.26 mg (dry weight) /ml. Cell suspension (5 ml) was incubated at 30 C with 0.25 mM [^{14}C]2-deoxy-D-glucose (0.2 μCi/μmol) for 5 min, and then filtered and washed as described above. The filter with the cells was placed in 5 ml of water at 90 C and extracted with stirring for 15 min. The suspension was filtered. The residue on the filter, representing nonextracted substances, was counted directly; the filtrate, representing extracted substances, was analyzed for sugar phosphates by a modification of the chromatographic method of Winkler (21); a sample of 0.5 ml was applied to a column (4 by 0.6 cm) containing Bio-Rad AG1-x2 anion exchange resin (100 to 200 mesh), which had been converted to the formate form. The free sugar fraction was eluted with five 0.5-ml portions of water; sugar phosphates and other anionic substances were subsequently eluted with seven 0.5-ml portions of 0.5 M ammonium formate in 0.2 M formic acid. Eluted materials were collected directly in scintillation vials for radioassay.

RESULTS

Effect of energy and carbon source during growth on the capacity to take up 2-deoxy-D-glucose.

The time courses of 2-deoxy-D-glucose uptake by cells grown autotrophically on thiosulfate-carbonate, mixotrophically on thiosulfate-glucose, and heterotrophically on glucose-yeast extract are shown in Fig. 1. It is clear that only those cells that were grown on glucose-yeast extract were capable of accumulating 2-deoxy-D-glucose to a significant degree. An estimate of intracellular concentration of this sugar in glucose-yeast extract-grown cells from these data, based on a cellular water content of 80% (4 μliters of H_2O per mg of cell [dry weight]) and on an assumption that all the water was accessible to solute, yields a figure of 4.5 nmol/μliter of H_2O, or 4.5 mM 2-deoxy-D-glucose. This represents a 22-fold intracellular concentration of substrate over that of the external medium. Thus, an active transport system is indicated, which apparently was induced under conditions where glucose was used as the energy source; the system was not present in cells grown under conditions where thiosulfate was used as energy source, although glucose was present.

Characteristics of the uptake system. To determine whether the 2-deoxy-D-glucose that was taken up by glucose-yeast extract grown

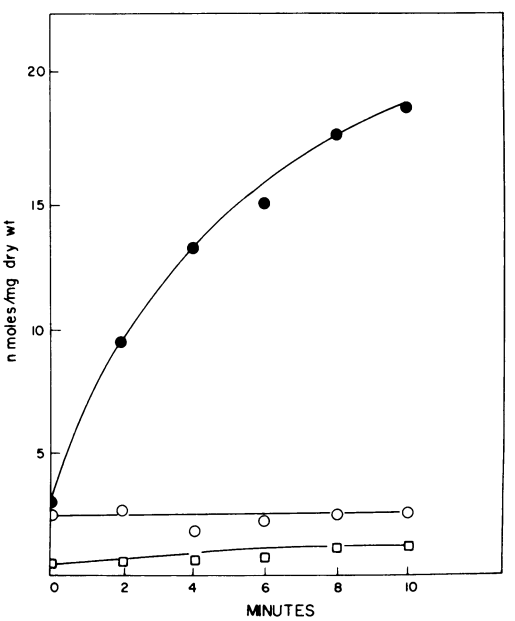

FIG. 1. *Time course of 2-deoxy-D-glucose uptake. Cells grown on mineral salts plus 0.5% glucose and 0.5% yeast extract for 72 h (●), mineral salts plus 0.5% thiosulfate and 0.5% glucose for 72 h (○), or mineral salts plus 0.5% thiosulfate and sodium carbonate for 2 weeks (□), incubated with 0.2 mM [^{14}C]2-deoxy-D-glucose (0.2 μCi/μmol).*

cells was phosphorylated, cells were allowed to take up labeled 2-deoxy-D-glucose for 5 min and extracted with hot water, and the extract was chromatographed on anion-exchange resin (see above). Hot water extraction removed 97% of the radioactivity from the cells, indicating that there was negligible incorporation of 2-deoxy-D-glucose into cellular polymers. Anion-exchange chromatography yielded one major fraction, accounting for 96% of the total radioactivity applied to the column, which appeared in the neutral fraction eluted with water (fractions 1 to 6; Fig. 2). Only small amounts appeared in subsequent fractions, eluted with 0.5 M ammonium formate in 0.2 M formic acid, which would contain anionic products such as sugar phosphates or sugar acids. Paper chromatography of the hot water extract with a *n*-butanol-acetic acid-H_2O (4:1:1 by volume) system yielded a single radioactive spot, corresponding to 2-deoxy-D-glucose (not shown). Thus, 2-deoxy-D-glucose is taken up by an active transport system, whereby the sugar is unchanged, rather than by a group translocation system, such as the phosphoenolpyruvate-glucose phosphotransferase system (16).

The uptake of 2-deoxy-D-glucose shows saturation kinetics with respect to substrate concentration with an apparent K_m of 1.6×10^{-5}. A Lineweaver-Burk plot of 2-deoxy-D-glucose uptake in the absence and presence of nonradioactive glucose (Fig. 3) shows that glucose competitively inhibits 2-deoxy-D-glucose uptake with an apparent K_i of 3.3×10^{-7}. This indicates that 2-deoxy-D-glucose is transported via the glucose transport system. Further evidence that these sugars share the same stereospecific carrier, and that 2-deoxy-D-glucose is taken up into a free intracellular pool that is readily exchangeable with external substrate, is provided by the observation that addition of nonradioactive glucose to a cell suspension that has been allowed to take up radioactive 2-deoxy-D-glucose leads to a rapid efflux of the radioactive sugar from the cell (Fig. 4).

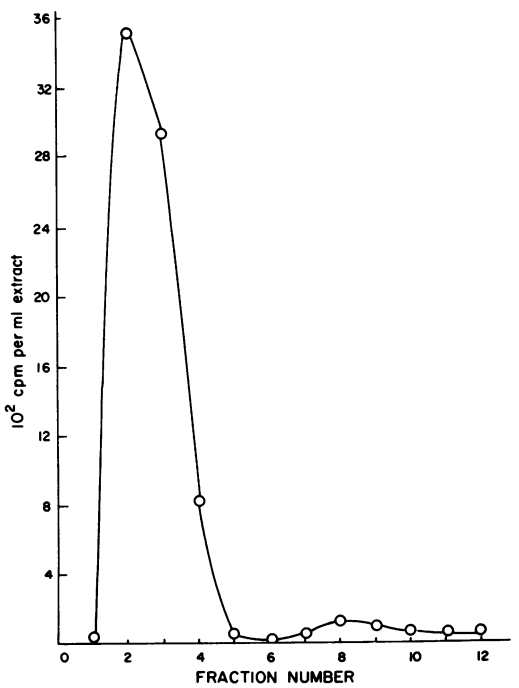

FIG. 2. *Ion exchange chromatography of a hot-water extract of cells grown on mineral salts plus 0.5% glucose and 0.5% yeast extract for 72 h, that were allowed to take up* [^{14}C]*2-deoxy-D-glucose.*

Regulation of the transport system. Cells grown on thiosulfate-yeast extract have a low capacity to transport 2-deoxy-D-glucose. When such cells are washed and suspended in a glucose-yeast extract medium, the capacity to transport 2-deoxy-D-glucose is induced (Fig. 5). That this induction involves the synthesis of a new protein is indicated by its inhibition by chloramphenicol. There was considerably less induction of the transport system in the thiosulfate-glucose medium (Fig. 5). These data, taken together with those of Fig. 1, indicate that the glucose transport system is inducible, and its synthesis is repressed by thiosulfate.

Thiosulfate also inhibits 2-deoxy-D-glucose transport by cells that have been induced by glucose. Both the rate of uptake and final steady-state concentration of 2-deoxy-D-glucose was decreased by the addition of sodium thiosulfate at the same concentration used in growth media (Fig. 6). A Lineweaver-Burk plot

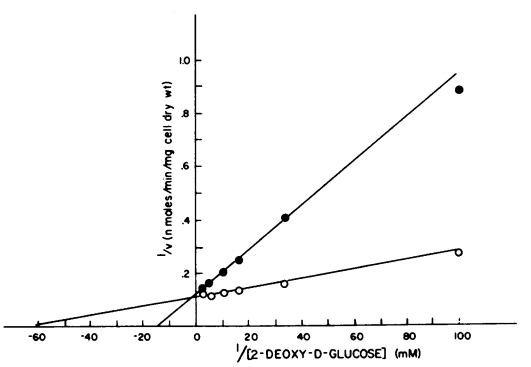

FIG. 3. *Lineweaver-Burk plot of the inhibition of 2-deoxy-D-glucose uptake by D-glucose. Cells grown on mineral salts plus 0.5% glucose and 0.5% yeast extract for 72 h were incubated with* [^{14}C]*2-deoxy-D-glucose (0.2 μCi/μmol) with no further addition* (○), *and with* 10^{-6} *M D-glucose* (●).

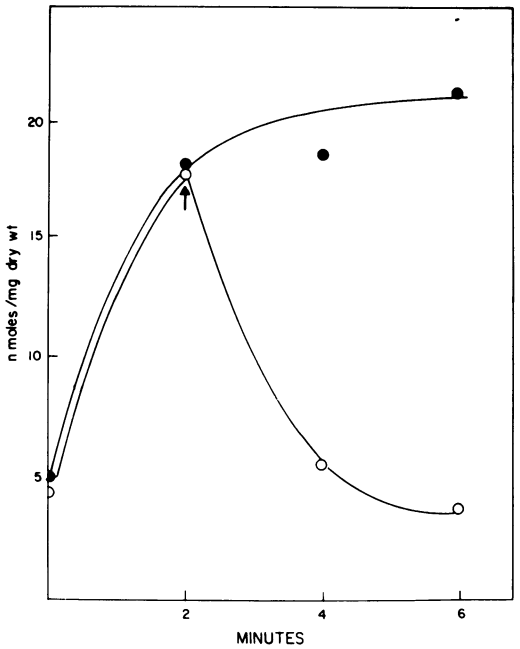

FIG. 4. *Exchange of intracellular 2-deoxy-D-glucose with external D-glucose. Cells grown on mineral salts plus 0.5% glucose and 0.5% yeast extract for 72 h were incubated with 0.5 mM [^{14}C]2-deoxy-D-glucose (0.2 μCi/μmol) with no further addition (●), or with 1 mM D-glucose added at 2 min (○).*

of the uptake of 2-deoxy-D-glucose by glucose-induced cells in the absence and presence of sodium thiosulfate (Fig. 7) shows the characteristics of uncompetitive inhibition by thiosulfate, with respect to 2-deoxy-D-glucose concentration.

DISCUSSION

T. intermedius possesses an inducible active transport system for glucose that does not involve group translocation by the phosphoenolypyruvate-glucose phosphotransferase system. In this regard, the glucose transport system is the same as that described for *P. aeruginosa* (5, 12, 13) and other strictly aerobic bacteria (15). Further metabolic similarities to the pseudomonads include the presence of the Entner-Doudoroff pathway as the chief means of glucose dissimilation (11) and the susceptibility of both glucose uptake and glucose dissimilation to regulation by preferentially utilized sources of energy. The only difference is that, in the case of *P. aeruginosa*, organic metabolites such as succinate or citrate are the preferred substrates which regulate glucose dissimilation (14, 19) and transport (12, 13), whereas in *T. intermedius* the regulatory metabolite is the inorganic energy source, thiosulfate. Matin and Rittenberg (11) have shown that the thiosulfate represses synthesis of enzymes of the Entner-Doudoroff pathway, and that whereas glucose is incorporated into cellular material in the presence of thiosulfate it is not utilized as a source of energy. We now show that the glucose uptake system is both repressed and inhibited by thiosulfate (or a metabolite derived from it); thus, a system for the active accumulation of glucose against a concentration gradient is present only under conditions where glucose is utilized as a source of energy. Active accumulation of glucose is apparently not required for the utilization as a source of carbon.

It is of interest to note that, in other facultative autotrophic or mixotrophic organisms, the utilization of sugars is also modulated by the presence of the appropriate inorganic energy source. For example, in *Hydrogenomonas* sp., the utilization of fructose is both repressed (4, 17) and inhibited (2) by hydrogen. Also, in *T. ferrooxidans*, glucose is utilized only after the iron in the medium is depleted (18). It would be of interest to determine whether the regulation

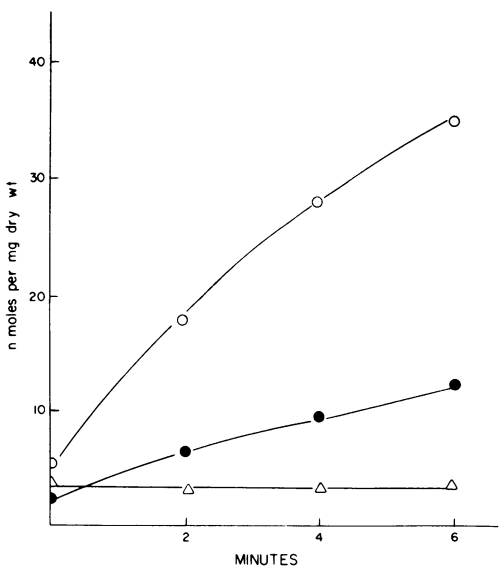

FIG. 5. *Induction of the glucose transport system. Cells grown for 72 h in mineral salts plus 1% sodium thiosulfate and 0.1% yeast extract were harvested, washed, and suspended in mineral salts plus 0.5% glucose and 0.5% yeast extract (○), mineral salts plus 0.5% glucose, 0.5% yeast extract, and chloramphenicol (50 μg/ml; △), or mineral salts plus 0.5% glucose and 0.5% sodium thiosulfate (●). After 24 h incubation at 30 C, cells were harvested, washed, and incubated in presence of 0.25 mM [^{14}C]2-deoxy-D-glucose (0.2 μCi/μmol).*

of sugar utilization in these organisms is manifest at the level of transport.

Little can be said regarding the mechanism of the inhibition of 2-deoxy-D-glucose transport by thiosulfate, or the characteristics of the transport system itself, on the basis of data reported here. However, the kinetics of uncompetitive inhibition that is exhibited by thiosulfate with respect to 2-deoxy-D-glucose concentration indicates that the thiosulfate does not decrease the affinity of the transport carrier for the sugar, since the apparent K_m of the system is decreased. Rather, it may be reasonable to suppose that thiosulfate may interfere with the functioning of an additional component of the system, which may be the energy-coupling system. In any case, the characteristics of uncompetitive inhibition points up an important consequence in physiological terms that is apparent on inspection of Fig. 7A; inhibition of transport is minimal at low sugar concentrations and becomes greater at higher sugar concentrations. Thus, when the organism is growing in the presence of thiosulfate, it still has a high affinity system for taking in glucose, present in low concentration, that it can incorporate into cellular carbon. In environments where sugar concentration is high, a mechanism is present to exclude excessive amounts of sugar when the preferred energy source is present.

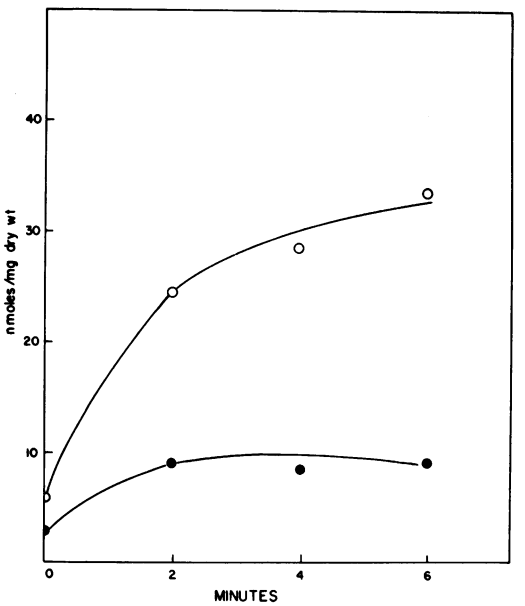

FIG. 6. *Inhibition of 2-deoxy-D-glucose uptake by sodium thiosulfate. Cells were grown on mineral salts plus 1% thiosulfate and 0.1% yeast extract, washed, and induced by transfer to mineral salts plus 0.5% glucose, and 0.5% yeast extract for 24 h. They were then harvested and incubated in the presence of 0.25 mM [^{14}C]2-deoxy-D-glucose (0.2 μCi/μmol) with no further addition* (○), *or with 31 mM sodium thiosulfate* (●).

We are continuing to study the mechanism of glucose transport and its regulation in this organism. It is possible that a metabolite derived from thiosulfate oxidation, such as adenosine 5'-triphosphate may act as a negative effector on the permease. Alternatively, if glucose transport is dependent upon electron flow catalyzed by a specific dehydrogenase (as yet unknown in this case) according to a model similar to that proposed by Kaback (6), it is

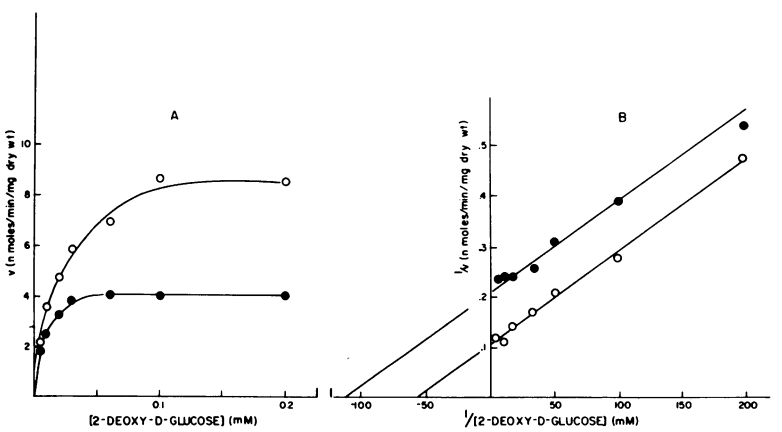

FIG. 7. *Kinetics of the inhibition of 2-deoxy-D-glucose uptake by sodium thiosulfate. Velocity versus concentration plot (A) and Lineweaver-Burk plot (B). Cells were grown for 72 h on mineral salts plus 1% sodium thiosulfate and 0.1% yeast extract, washed, and induced by transfer to mineral salts plus 0.5% glucose and 0.5% yeast extract for 24 h. They were then harvested and incubated with* [^{14}C]*2-deoxy-D-glucose at concentrations indicated (0.2 μCi/μmol), in the absence* (○) *and presence* (●) *of 31 mM sodium thiosulfate.*

possible that the introduction of electrons from thiosulfate oxidation into the electron transport chain at the level of cytochrome *c* (1, 20) might cause a slowing of electron flow higher up the chain. These and other possibilities are being investigated.

ACKNOWLEDGMENTS

This work was supported by Research grant GB 31543 from the National Science Foundation, and by the University of Connecticut Research Foundation.

We wish to thank Alvah Phillips for valuable discussions on kinetics.

LITERATURE CITED

1. Aleem, M. I. H. 1965. Thiosulfate oxidation and electron transport in *Thiobacillus novellus*. J. Bacteriol. **90:** 95-101.
2. Blackkolb, F., and H. G. Schlegel. 1968. Regulation der Glucose-6-phosphat Dehydrogenase aus *Hydrogenomonas* H16 durch ATP und $NADH_2$. Arch. Mikrobiol. **63:**117-196.
3. Bray, G. A. 1960. A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. Anal. Biochem. **1:**279-285.
4. Gottschalk, G. 1965. Die Verwertung organischer Substrat durch *Hydrogenomonas* in Gegenwart von molekularem Wasserstaff. Biochem. Z. **341:**260-270.
5. Guymon, L. F., and R. G. Eagon. 1974. Transport of glucose, gluconate, and methyl-α-D-glucoside by *Pseudomonas aeruginosa*. J. Bacteriol. **117:**1261-1269.
6. Kaback, H. R. 1972. Transport across isolated bacterial cytoplasmic membranes. Biochim. Biophys. Acta **265:**367-416.
7. Krulwich, T., and J. C. Ensign. 1969. Alteration of glucose metabolism of *Arthrobacter crystallopoietes* by compounds which induce sphere to rod morphogenesis. J. Bacteriol. **97:**526-534.
8. London, J. 1963. *Thiobacillus intermedius* nov. sp. A novel type of facultative autotroph. Arch. Mikrobiol. **46:**329-337.
9. London, J., and S. C. Rittenberg. 1966. Effects of organic matter on the growth of *Thiobacillus intermedius*. J. Bacteriol. **91:**1062-1069.
10. Matin, A., and S. C. Rittenberg. 1970. Utilization of glucose in heterotrophic media by *Thiobacillus intermedius*. J. Bacteriol. **104:**234-238.
11. Matin, A., and S. C. Rittenberg. 1970. Regulation of glucose metabolism in *Thiobacillus intermedius*. J. Bacteriol. **104:**239-246.
12. Midgley, M., and E. A. Dawes. 1973. The regulation of transport of glucose and methyl-α-glucoside by *Pseudomonas aeruginosa*. Biochem. J. **132:**141-154.
13. Mukkada, A. J., G. L. Long, and A. H. Romano. 1973. The uptake of 2-deoxy-D-glucose by *Pseudomonas aeruginosa* and its regulation. Biochem. J. **132:**155-162.
14. Ng, F. M.-W., and E. A. Dawes. 1973. Chemostat studies on the regulation of glucose metabolism in *Pseudomonas aeruginosa*. Biochem. J. **1132:**129-140.
15. Romano, A. H., S. J. Eberhard, S. L. Dingle, and T. D. McDowell. 1970. Distribution of the phosphoenolpyruvate:glucose phosphotransferase system in bacteria. J. Bacteriol. **104:**808-813.
16. Roseman, S. 1969. The transport of carbohydrates by a bacterial phosphotransferase system. J. Gen. Physiol. **54:**138s-184s.
17. Schlegel, H. G., and U. Eberhardt. 1972. Regulatory phenomena in the metabolism of Knallgasbacteria, p. 205-242. *In* A. H. Rose and D. W. Tempest (ed.), Advances in microbial physiology, vol. 7. Academic Press Inc., London.
18. Tabita, R., and D. G. Lundgren. 1971. Utilization of glucose and the effect of organic compounds on the chemolithotroph *Thiobacillus ferrooxidans*. J. Bacteriol. **108:**328-333.
19. Tiwari, N. P., and J. J. R. Campbell. 1969. Enzymatic control of the metabolic activity of *Pseudomonas aeruginosa* grown in glucose or succinate media. Biochim. Biophys. Acta **192:**395-401.
20. Trudinger, P. A. 1961. Thiosulfate oxidation and cytochromes in *Thiobacillus* X. 2. Thiosulfate oxidizing enzyme. Biochem. J. **68:**680-686.
21. Winkler, H. H. 1960. A hexose-phosphate transport system in *Escherichia coli*. Biochim. Biophys. Acta **117:**231-240.