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Enzymes of Carbohydrate Metabolism in *Thiobacillus* species

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A study was made of enzymes of carbohydrate metabolism in representative thiobacilli grown with and without glucose. The data show that *Thiobacillus perometabolis* possesses an inducible Entner-Doudoroff pathway and is thus similar to *T. intermedius* and *T. ferrooxidans*. *T. novellus* lacks this pathway. Instead, a noncyclic pentose phosphate pathway along with the Krebs cycle is apparently the major route of glucose dissimilation in this organism. Glucose does not support or stimulate the growth of strains of *T. neapolitanus*, *T. thioparus*, and *T. thiooxidans* examined, nor does its presence in the growth medium greatly influence their enzymatic constitution. These obligately chemolithotrophic thiobacilli do not possess the Entner-Doudoroff pathway. Their nicotinamide adenine dinucleotide (NAD)-linked isocitrate dehydrogenase activity predominates over their nicotinamide adenine dinucleotide phosphate (NADP)-linked activity; the converse is true for the other thiobacilli. The data suggest that NAD-linked isocitrate dehydrogenase activity in thiobacilli is involved in biosynthetic reactions.

It has been reported that *Thiobacillus intermedius* dissimilates glucose (19) and gluconate (A. Matin, Ph.D. Thesis, University of California, Los Angeles, 1969) mainly via an inducible Entner-Doudoroff pathway. This pathway has also been reported in *Thiobacillus* (*Ferrobacillus*) *ferrooxidans* when grown in glucose-ferrous sulfate medium (F. R. Tabita and D. G. Lundgren, Bacteriol. Proc., p. 125, 1970). Since the same pathway functions during heterotrophic growth of hydrogenomonads on sugars (8), it appeared of taxonomic interest to examine the enzymatic constitution of other mixotrophic, heterotrophic, and obligately chemolithotrophic members of the genus *Thiobacillus* in this respect. Accordingly, representative thiobacilli, grown in media with and without glucose, were analyzed for key enzymes of the various pathways of carbohydrate metabolism. Included were *T. perometabolis* [chemolithotrophic heterotroph (17)], *T. novellus* (chemolithotrophic mixotroph), and *T. neapolitanus*, *T. thiooxidans*, and *T. thioparus* (obligate chemolithotrophs).

MATERIALS AND METHODS

Organisms. Our laboratory strains of *T. thioparus* (16) and *T. perometabolis* (17) were used. *T. novellus* was obtained from M. I. H. Aleem, University of Kentucky, Lexington, Ky.; *T. neapolitanus* from E. J. Johnson, Tulane University School of Medicine, New Orleans, La.; and the thiosulfate-adapted strain of *T. thiooxidans* from M. J. Shively, University of Nebraska, Lincoln, Neb.

Culture conditions. The mineral salts (MS) base described previously (18) was used in the preparation of media for the cultivation of *T. perometabolis* and *T. novellus*. It contained, per 100 ml of medium: NH_4Cl, 0.1 g; $MgSO_4$, 0.05 g; KH_2PO_4, 0.04 g; $K_2HPO_4 \cdot 3H_2O$, 0.06 g; $FeCl_3 \cdot 6H_2O$, 0.002 g; Pfennig's (23) trace salts solution, 3 ml; and bromothymol blue (internal *p*H indicator), 0.003 g. This base was slightly modified for *T. neapolitanus* in that higher phosphate concentrations were used (KH_2PO_4, 0.5%; $K_2HPO_4 \cdot 3H_2O$, 0.5%), and for *T. thiooxidans* in that $K_2HPO_4 \cdot 3H_2O$ was omitted, and the concentration of KH_2PO_4 was raised to 0.2%. Bromophenol blue (0.003%) replaced bromothymol blue as the internal *p*H indicator for *T. thiooxidans*. A small amount of yeast extract (0.03%) was added to the glucose and glutamate MS media used for culturing *T. novellus*. This addition increased the rate of culture development. Supplements made to MS media are indicated in the text. Except for cultures of *T. thiooxidans*, which were maintained between *p*H 4.5 and 5, the *p*H levels of all of the other cultures were kept between 6.8 and 7.0 by periodic additions of sterile Na_2CO_3 or dilute H_2SO_4 solutions. Generation times of *T. novellus* and *T. perometabolis* in various media were calculated from turbidimetric measurements of growth (18). Continuous measurements of growth of the obligate chemolithotrophs could not be made turbidimetrically because of the transient appearance of sulfur during growth; for these organisms only final growth yields were measured.

Preparation of cell-free extracts. Large batches of cells were obtained as described (18) and disrupted by passage through a chilled French pressure cell. *T. novellus* and *T. perometabolis* suspensions were passed through the cell once; all the other suspensions were processed twice. After treatment, the mixtures were centrifuged at 12,000 × *g* for 20 min at 4 C to obtain the crude extracts. These were separated into soluble and particulate fractions by centrifugation at 105,000 × *g* for 90 min at 4 C.

Enzyme assays. Enzyme assays were carried out by using standard procedures with minor modifications by measuring reduction or oxidation of pyridine nucleotides at 340 nm in a Cary 15 recording spectrophotometer. The assay mixtures (0.25 ml total volume) contained 0.01 to 0.7 mg of cell extract protein, 5×10^{-2} M tris(hydroxymethyl)aminomethane (Tris)-maleate buffer (*p*H 7), and the following compounds (quantities given in micromoles). Glucokinase (EC 2.7.1.2): $MgCl_2$, 2; glutathione, 2.5; nicotinamide adenine dinucleotide phosphate (NADP), 0.25; adenosine triphosphate (ATP), 1.25; glucose-6-phosphate dehydrogenase, 0.5 unit; glucose, 5 (1). Glucose-6-phosphate dehydrogenase (EC 1.1.1.49): $MgCl_2$, 2; glutathione, 2.5; NADP or nicotinamide adenine dinucleotide (NAD), 0.25; glucose-6-phosphate (disodium salt), 2.5 (14). Phosphogluconate dehydrogenase (EC 1.1.1.44): $MgCl_2$, 2; glutathione, 2.5; NAD or NADP, 0.25; phosphogluconate (trisodium salt), 1.25 (24). Phosphogluconate dehydrase (EC 4.2.1.12): $MnCl_2$, 0.3; glutathione, 2.5; lactic dehydrogenase, 0.5 unit; reduced nicotinamide adenine dinucleotide (NADH), 0.05; phosphogluconate (trisodium salt), 1.25 (20). 2-Keto-3-deoxy-phosphogluconate (KDPG) aldolase (EC 4.1.2.14): glutathione, 2.5; lactic dehydrogenase, 0.5 unit; NADH, 0.05; KDPG, 0.25 (21). Fructose diphosphate aldolase (EC 4.1.2.13): glutathione, 2.5; sodium arsenate, 2.5; NAD, 0.25; phosphoglyceraldehyde dehydrogenase, 0.5 unit; fructose diphosphate (sodium salt), 5 (29). Phosphoglyceraldehyde dehydrogenase (EC 1.2.1.12): glutathione, 2.5; sodium arsenate, 2.5; NAD, 0.25, fructose diphosphate aldolase, 0.5 unit; fructose diphosphate (sodium salt), 5 (13). Isocitrate dehydrogenase, NADP-linked (EC 1.1.1.42), and NAD-linked (EC 1.1.1.4): $MnSO_4$, 1; NADP or NAD, 0.25; DL-isocitric acid (neutralized by NaOH), 2.5 (9, 22). NADH oxidase (EC 1.6.99.3): NADH, 0.05. Reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (EC 1.6.99.1): NADPH, 0.05.

NADP- and NAD-linked isocitrate dehydrogenases, and NADH and NADPH oxidases were assayed only in the crude extracts. All of the other enzymes were assayed in both soluble and particulate fractions. Data are presented only for soluble fractions which contained 90% or more of these activities. Enzyme activities are expressed as enzyme units per milligram of protein contained in the crude extracts from which the soluble and particulate fractions were prepared. An enzyme unit is defined as that amount of an enzyme which converts 1 μmole of its substrate per minute under the conditions specified.

Radiorespirometry. Radiorespirometric measurements were made by using the ionization chamber method described by Davidson and Schwabe (6), which employs a small ionization chamber, a vibrating reed electrometer, and a potentiometric recorder. The ionization chamber was calibrated with $Ba^{14}CO_3$ of known specific activity by using a Cary-Tolbert CO_2 gas generator. At the air flow rate used in the experiment (50 ml/min), its sensitivity was found to be 3.7×10^{-3} μCi per hr per mv. The electrometer readings during the experiment were up to 1,000 times the background, which was between 0.2 to 0.5 mv. After the experiment, a smooth curve was drawn through the recorder tracings, and the rates of $^{14}CO_2$ evolution were calculated for points at 5-min intervals along the curves using the following formula:

μmoles of $^{14}CO_2$ evolved per hr per ml of culture = 3.7×10^{-3} μCi per hr per mv × [(observed mv × μmoles of unlabeled glucose)/(μCi of labeled glucose × ml of culture)].

Cells of *T. novellus*, grown in glucose broth, were washed in potassium phosphate buffer (0.006 M; *p*H 6.8) and resuspended in the same medium but lacking glucose. Each incubation chamber contained 2 ml of cell suspension (ca. 33 mg of cell protein) and 1 mg of labeled glucose with a total activity of 0.5 μCi. Incubation temperature was 30 C. Respiration of glucose-*1*-^{14}C, glucose-*2*-^{14}C, glucose-*3*-^{14}C, glucose-*3,4*-^{14}C, and glucose-*6*-^{14}C was studied. The respiration of glucose-*4*-^{14}C was calculated from measurements with glucose-*3,4*-^{14}C and glucose-*3*-^{14}C (32).

Attempted culture of T. neapolitanus on glucose. Cultivation of *T. neapolitanus* in glucose-MS medium (MS plus 0.1% filter-sterilized glucose) was attempted in a continuous-flow dialysis system consisting of flanged Bellco spinner flasks separated by a semipermeable membrane (P. C. Pan, Bacteriol. Proc., p. 125, 1970). The membrane used was derived from ordinary dialysis tubing, which had been boiled in 10^{-4} M Tris-10^{-4} M ethylenediaminetetraacetic acid tetrasodium salt, cooled, and washed in deionized distilled water. The apparatus was sterilized by autoclaving, after which 130 ml of sterile medium was introduced aseptically into each of the two flasks. The medium in one flask was inoculated with washed cells of *T. neapolitanus* harvested from an exponential-phase thiosulfate (1%)-glucose (0.5%) culture. The uninoculated medium in the second flask was replaced continuously with sterile medium at a rate of 60 to 70 ml/hr.

Glucose-*1*-^{14}C and glucose-*6*-^{14}C were obtained from Calbiochem, Los Angeles, Calif. Glucose-*2*-^{14}C, glucose-*3*-^{14}C, and glucose-*3,4*-^{14}C were purchased from New England Nuclear Corp., Boston, Mass. Sources of other special chemicals and analytical procedures have been specified (18, 19).

RESULTS

Enzymes of glucose metabolism. Among the thiobacilli examined in this study, only *T. perometabolis* responds to the presence of glucose in the growth medium by a marked increase (ca. threefold) in the specific activity of glucokinase (Table 1). Glucose also causes a fivefold increase in the activity of glucose-6-phosphate dehydrogenase in this organism.

Glucose-6-phosphate dehydrogenase activities

TABLE 1. *Enzymes of glucose metabolism in Thiobacillus species*[a]

Enzyme	*T. novellus*			*T. perometabolis*		*T. neapolitanus*		*T. thiooxidans*	
	YE (4 hr)	GL (18 hr)	G (8 hr)	C (8 hr)	GC (7.5 hr)	S (ND)	SG (ND)	S (ND)	SG (ND)
Glucokinase[b]	126	108	148	27	85	490	590	40	40
Glucose-6-phosphate dehydrogenase	147	200	234[c]	25	129	110	140	68	162
Phosphogluconate dehydrase	<2	<2	<2[d]	7	50	<2	<2	<2	<2
NAD-linked phosphogluconate dehydrogenase	334	374	585	7	6	11	16	32	33
NADP-linked phosphogluconate dehydrogenase	21	13	15	5	5	63	81	10	11
Fructose diphosphate aldolase	52	32	21	7	11	16	20	13	14
Phosphoglyceraldehyde dehydrogenase	178	424	500	46	45	1,320	1,380		

[a] Values are specific activities (10^{-4} enzyme units per milligram of protein) after growth in indicated medium. YE, 0.5% yeast extract; G, 0.4% glucose-0.03% yeast extract; GL, 0.35% glutamate-0.03% yeast extract; C, 0.3% casein hydrolysate; GC, 0.5% glucose-0.3% casein hydrolysate; S, 1% thiosulfate; SG, 1% thiosulfate-0.5% glucose. Generation times are given in parentheses; ND, not determined.

[b] We have not attempted to distinguish between glucokinase and hexokinase activities.

[c] Activity of NAD-linked glucose-6-phosphate dehydrogenase, 115×10^{-4} units.

[d] Activity of this enzyme in gluconate-grown *T. novellus* was also less than 2.

in *T. novellus* and *T. thiooxidans* are also significantly increased by growth in glucose-containing media. The constitutive levels of glucokinase and glucose-6-phosphate dehydrogenase are considerably higher in *T. novellus* and *T. neapolitanus* compared to the other two organisms. A high constitutive level of glucokinase has also been reported in *Nitrosocystis oceanus* (31).

Phosphogluconate dehydrase activity was not detected in *T. novellus*, *T. neapolitanus*, *T. thiooxidans*, or *T. thioparus* cells grown with or without glucose. *T. novellus* extracts also gave negative results when $MgCl_2$ (2 μmoles), $FeSO_4$ (1.5 μmoles), or a mixture of inorganic trace salts [Pfennig (22); 0.05 ml, v/v] was used in the assay mixture instead of $MnCl_2$. Thus, these organisms lack the Entner-Doudoroff pathway. Direct assays for KDPG aldolase showed its presence in *T. novellus* (specific activity of glucose and gluconate grown cells: 300×10^{-4} enzyme units/mg of protein) and its absence in *T. thioparus*. In *T. thiooxidans* or *T. neapolitanus*, the failure to detect phosphogluconate dehydrase activity could be due either to the absence of the dehydrase, or the aldolase, or both, since the two enzymes were assayed jointly. In contrast, both enzymes are present in *T. perometabolis*, and their combined activity increases by sevenfold when this organism is grown in the presence of glucose.

Mixed extracts of *T. perometabolis* cells grown with and without glucose had activities predictable from the per cent of the extracts in the mixture and their individual specific activities. It is therefore likely that the differences in activities reflect differences in the amount of enzymes synthesized under the two growth conditions.

T. novellus grown in various media possesses a high specific activity of NAD-linked phosphogluconate dehydrogenase. In this organism and in *T. thiooxidans*, the NAD-linked activity is higher than NADP-linked phosphogluconate dehydrogenase activity. The converse is true for *T. neapolitanus*. In *T. perometabolis*, these activities are low and almost equal. Growth in the presence of glucose causes a significant increase in the specific activity of the NAD-linked enzyme in *T. novellus*, but not in the other species.

The specific activity of fructose diphosphate aldolase fluctuates significantly with growth conditions only in *T. novellus*. This enzyme is least active in glucose-grown cells, intermediate in glutamate-grown cells, and most active in those grown in yeast extract broth. In the other thiobacilli, the specific activity of this enzyme is low and shows relatively little variation in cells grown with and without glucose.

Significant fluctuations in the specific activities of phosphoglyceraldehyde dehydrogenase in response to growth conditions occur only in *T. novellus*. It may be noted that the pattern of variation of this enzyme is similar to that of glucose-6-phosphate dehydrogenase and NAD-linked phosphogluconate dehydrogenase, and opposite to that of aldolase.

Isocitrate dehydrogenases. NAD- and NADP-linked isocitrate dehydrogenases are present in most of the thiobacilli examined and with interesting differences in their relative abundance (Table 2).

In the obligate chemolithotrophs, NAD-linked isocitrate dehydrogenase is more active than the NADP-linked enzyme; indeed, the latter enzyme could not be detected in *T. thiooxidans*, as has also been reported by Hampton and Hanson (10).

In the heterotrophic and mixotrophic thiobacilli examined, as in *T. intermedius* (19) and *Hydrogenomonas* species (30), the NADP-linked enzyme predominates under all growth conditions tested. In *T. novellus*, the specific activity of the NADP-linked but not of the NAD-linked isocitrate dehydrogenase is proportional to growth rates in the three media tested (Tables 1 and 2). In *T. perometabolis*, relatively little difference is observed with respect to these activities in cells grown with and without glucose. Growth rates of this organism are comparable in the two media (Table 1).

NADH and NADPH oxidases. All of the thiobacilli examined possess NADH and NADPH oxidase activities (Table 2). Again, NADH oxidase activities are proportional to growth rates in *T. novellus*. In the obligate chemolithotrophs, the activities of this enzyme are relatively low and constant.

NADPH oxidase activities are low in all of the species tested and do not vary significantly in response to different growth conditions. That these activities could be due to a transhydrogenase was not ruled out.

Effect of ATP on glucose-6-phosphate dehydrogenase activities. Glucose-6-phosphate dehydrogenase activities in extracts of the three thiobacilli examined show a concentration dependent ATP inhibition (Fig. 1), which varies with the species. The data suggest differential sensitivity of glucose-6-phosphate dehydrogenase from the three species to ATP. However, the measurements were made with crude extracts and quantitative interpretation of the data is unwarranted. Inhibition of glucose-6-phosphate dehydrogenase activity by ATP has been reported in many other organisms (2–4, 15, 19).

Radiorespirometric measurements with T. novellus. The data (Fig. 2A and B) show that CO_2 release from C-1 of glucose is most rapid and most extensive. By 45 min, about 60% of this carbon atom is respired, whereas only 27 to 43% of the other carbon atoms is respired. There is a clear lack of equivalence in the respirations of glucose carbon atoms 1 and 4, and 3 and 4. The kinetics of respiration of C-3 and C-6 also differ from each other but less markedly.

Attempted culturing of T. neapolitanus in glucose-MS medium. *T. neapolitanus*, *T. thioparus*, and *T. thiooxidans* grew in thiosulfate-glucose medium without detectable inhibition or stimulation of final growth yields up to glucose concentrations of 0.5%. Attempts to grow these organisms with glucose as the sole source of carbon and energy were unsuccessful when conventional cultural procedures were employed, i.e., flasks of medium incubated with constant shaking. It has been reported that the organisms will grow in glucose-MS medium if toxic products of glucose metabolism are continuously removed (5; P. C. Pan, Bacteriol. Proc., p. 125, 1970). Because of these reports and because the specific activities of several enzymes of glucose metabolism are relatively high in *T. neapolitanus* (Tables 1 and 2, reference 11), cultivation of this organism was attempted with glucose as the sole source of carbon and energy under conditions of contin-

TABLE 2. *Isocitrate dehydrogenases and NADH and NADPH oxidases in Thiobacillus species*[a]

Enzyme	*T. novellus*			*T. perometabolis*		*T. neapolitanus*		*T. thiooxidans*		*T. thioparus*	
	YE	GL	G	C	GC	S	SG	S	SG	S	SG
NADP-linked isocitrate dehydrogenase	13,300	5,500	7,500	3,900	3,550	206	225	<2	<2	183	650
NAD-linked isocitrate dehydrogenase	99	128	195	295	236	1,466	1,380	1,167	1,330	760	1,000
NADH oxidase	2,033	750	1,000	272	255	59	65	42	40	33	38
NADPH oxidase	25	24	26	59	46	39	47	—[b]	—	—	—

[a] Values are specific activities (10^{-4} enzyme units per milligram of protein) after growth in indicated medium. YE, 0.5% yeast extract; G, 0.4% glucose-0.03% yeast extract; GL, 0.35% glutamate-0.03% yeast extract; C, 0.3% casein hydrolysate; GC, 0.5% glucose-0.3% casein hydrolysate; S, 1% thiosulfate; SG, 1% thiosulfate-0.5% glucose.

[b] Not determined.

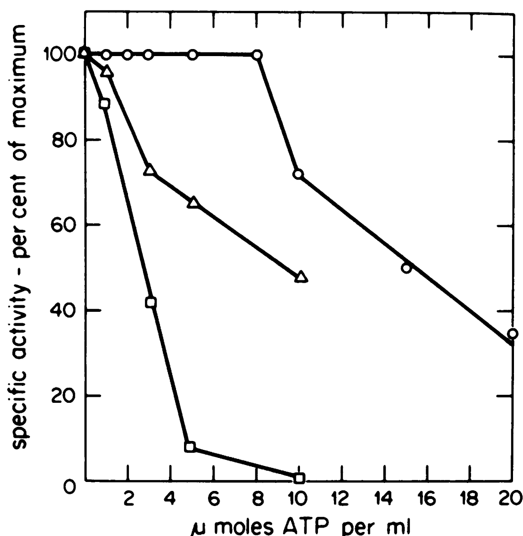

FIG. 1. *Effect of ATP concentration on glucose-6-phosphate dehydrogenase activities in Thiobacillus species. Reaction mixtures contained in 0.25 ml total volume: Tris-maleate buffer (pH 7.0), 12.5 μmoles; glutathione, 2.5 μmoles;* $MgCl_2$, *1.25 μmoles; NADP, 0.25 μmole; glucose-6-phosphate (disodium salt), 0.25 μmole; cell extract protein, 0.1 to 0.2 mg; and ATP as indicated. Symbols:* O, *T. novellus enzyme;* △, *T. neapolitanus enzyme;* □, *T. perometabolis enzyme.*

uous dialysis of the culture. No growth occurred during a 6-day incubation period (Fig. 3), although according to Pan (Bacteriol. Proc., p. 125) growth of *T. neapolitanus* under these conditions is complete within 3 to 5 days. Microscopic examination revealed that cells lost motility within the first 24 hr. No swollen, vacuolated, or branched forms, as reported for undialyzed *T. thiooxidans* cultures by Borichewski and Umbreit (5), were seen at any time during the experiment. The culture was streaked on thiosulfate-MS plates daily. A decrease in viable cells with time was observed, but with some survival on the sixth day. On this day, dialysis was discontinued and $Na_2S_2O_3 \cdot 5H_2O$ (1% final concentration) was added to both flasks of the apparatus. After a lag, growth, accompanied with acid production, occurred (Fig. 3).

DISCUSSION

T. perometabolis is similar to *T. intermedius* (19) in having an inducible Entner-Doudoroff pathway which apparently serves as its major primary route of glucose dissimilation. This conclusion follows from the marked variation in the levels of phosphogluconate dehydrase and glucose-6-phosphate dehydrogenase when *T. perometabolis* is cultured in the presence and absence

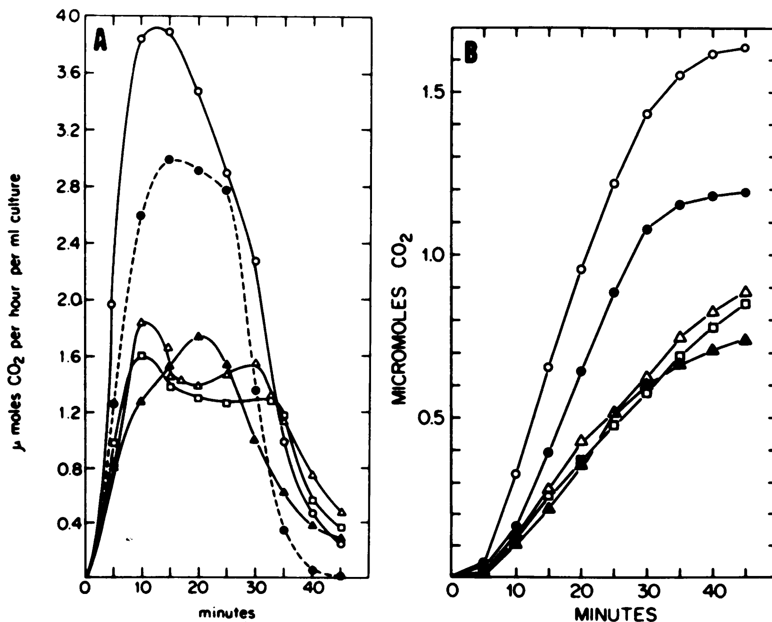

FIG. 2. *Respiration of differentially labeled glucose substrates by T. novellus growing in glucose (0.4%)-yeast extract (0.03%) broth. A, Rates; B, cumulative* CO_2 *yield. Rate of* $^{14}CO_2$ *evolution from carbon atom four of glucose was calculated as described in the text. Symbols:* O, $^{14}CO_2$ *from glucose-1-*^{14}C; ●, $^{14}CO_2$ *from glucose-4-*^{14}C; △, $^{14}CO_2$ from glucose-2-^{14}C; □, $^{14}CO_2$ from glucose-3-^{14}C; and ▲, $^{14}CO_2$ from glucose-6-^{14}C.

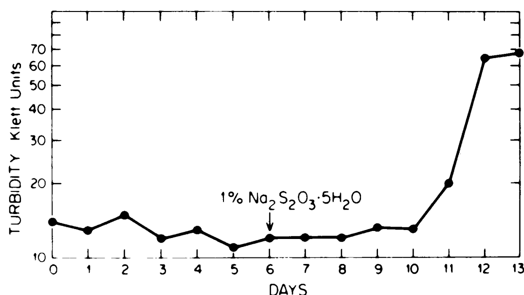

FIG. 3. *Attempted cultivation of T. neapolitanus in glucose-mineral salts medium under conditions of continuous dialysis. See Methods for apparatus and medium composition. Ten Klett units are approximately equal to 1.4* $\times$ *10^8* cells/ml.

of glucose, and from its low and relatively constant levels of fructose diphosphate aldolase and phosphogluconate dehydrogenases.

The failure to detect phosphogluconate dehydrase in cells grown in a glucose medium eliminates the Entner-Doudoroff pathway as a significant route of glucose catabolism in *T. novellus*. In agreement with the enzyme data, the radiorespirometric measurements show a marked lack of equivalence of C-1 and C-4 in glucose respiration by this organism. The organism does exhibit a high KDPG aldolase activity, but it is not clear what role this enzyme plays in the absence of phosphogluconate dehydrase. It may be noted here that *T. novellus* is not unique in this respect. A high KDPG aldolase activity in the absence of the dehydrase has also been reported in glucose-grown *Escherichia coli*, *Enterobacter aerogenes*, and *Salmonella typhimurium* (7); in glucose- or gluconate-grown *Erwinia carotovora* and *Serratia marcescens* (7); and in *Brucella abortus* (26), and the wild-type strain of *Rhodopseudomonas spheroides* (28).

The Embden-Meyerhof pathway is likewise eliminated as a major route of glucose catabolism in *T. novellus* by nonequivalence of C-3 and C-4 in glucose respiration, and by low and relatively constant levels of fructose diphosphate aldolase.

The rapid and extensive release of C-1 of glucose in the radiorespirometric tests points to a major role for the pentose phosphate pathway in this organism. This inference is strengthened by enzyme data, which show high specific activities of glucose-6-phosphate and phosphogluconate dehydrogenases, especially in glucose-grown cells. However, the low levels of fructose diphosphate aldolase and the higher respiration rate of glucose C-4 compared to C-2 and C-3 are inconsistent with any extensive cyclic operation of this pathway. Since the specific activity of phosphoglyceraldehyde dehydrogenase in glucose-grown cells is high, it is possible that phosphoglyceraldehyde generated from glucose is converted mainly into pyruvate which is channeled into the Krebs cycle. The high specific activities of isocitrate dehydrogenase (NADP-linked) and NADH oxidase, and the correlation of these activities with growth rates in various media suggest that the Krebs cycle is important in energy generation by *T. novellus*. The respiration patterns of C-4, C-2, and C-3, as well as delayed release of C-6 of glucose as CO_2 are also consistent with the operation of the Krebs cycle.

Some interesting similarities between *T. novellus* and *B. abortus* may be noted here. In both organisms glucose is apparently dissimilated by pentose phosphate pathway along with the Krebs cycle, and phosphogluconate dehydrogenase activity is predominantly NAD-linked (25, 26); in both, fructose diphosphate aldolase is present at relatively low levels (26); and, as has been noted above, in both organisms KDPG aldolase but not phosphogluconate dehydrase is present (26). There are, however, major differences in the radiorespirometric patterns of the two organisms (25).

The failure of glucose to support or stimulate growth suggests that glucose does not function as an energy substrate for *T. neapolitanus*, *T. thiooxidans*, or *T. thioparus*, at least for the particular strains of these organisms investigated. It is not surprising, then, that compared to the mixotrophic and heterotrophic thiobacilli, the presence of glucose in the growth medium exerts relatively little influence on the enzymatic constitution of these organisms. Our data show that these organisms lack the Entner-Doudoroff pathway. A complete Embden-Meyerhof pathway is apparently also absent from *T. thioparus* and *T. neapolitanus* since Johnson and Abraham (11) found that these organisms lack phosphofructokinase. However, a complete pentose phosphate pathway can operate in these organisms. Evidence for the presence of this pathway in *T. thioparus* and *T. neapolitanus* has been presented by Johnson and Abraham (11), and our data corroborate these findings for *T. neapolitanus* and extend them to *T. thiooxidans*.

The presence of the pentose phosphate pathway in these organisms makes it difficult to understand why glucose fails to support growth, even if it is assumed, as has been suggested (11), that substrate-level phosphorylation is the only means by which these organisms can generate ATP during heterotrophic metabolism. The pentose phosphate pathway enzymes should enable these organisms to generate phosphoglyceraldehyde from sugars, and further conversion of

phosphoglyceraldehyde into pyruvate should make substrate level phosphorylation possible. Evidence for the presence of most of the enzymes required for the conversion of phosphoglyceraldehyde to pyruvate in *T. thioparus* and *T. neapolitanus* has been presented (11).

Apart from glucose metabolism, the obligately chemolithotrophic thiobacilli also differ from the other thiobacilli in the predominance of their NAD-linked over their NADP-linked isocitrate dehydrogenase. Since the obligate chemolithotrophs have an incomplete Krebs cycle (27) which apparently functions only in biosynthesis, it appears that the NAD-linked isocitrate dehydrogenase is involved in biosynthetic reactions. This is somewhat unexpected since most biosynthetic enzymes generally are linked to NADP rather than to NAD (12). Conversely, the predominance of the NADP-linked isocitrate dehydrogenase in the mixotrophic and heterotrophic thiobacilli, which under heterotrophic conditions need the Krebs cycle for energy generation, indicates its functioning in energy-generating reactions. This inference is supported by the finding that in *T. intermedius* it is the NADP-linked enzyme which is markedly increased under heterotrophic conditions of growth (19). The NADP-linked enzyme also predominates in *Hydrogenomonas* species (30).

The presence of an inducible Entner-Doudoroff pathway in *T. intermedius* (19), *T. ferrooxidans* (F. R. Tabita, and D. G. Lundgren, Bacteriol. Proc., p. 125, 1970), and *T. perometabolis*, when considered with other common physiological (19) and morphological features, suggests a close taxonomic relationship between these organisms and the hydrogenomonads. It is perhaps pertinent in this connection that *Hydrogenomonas eutropha* (strain ATCC 17697) can oxidize thiosulfate to sulfate (A. Matin, and S. C. Rittenberg, *unpublished data*).

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