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Effects of Organic Matter on the Growth of *Thiobacillus intermedius*

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ABSTRACT

LONDON, JACK (University of California, Los Angeles), AND SYDNEY C. RITTENBERG. Effects of organic matter on the growth of *Thiobacillus intermedius*. J. Bacteriol. 91:1062–1069. 1966.—Yeast extract, glucose, glutamate, and other organic materials stimulate the rate and extent of growth of *Thiobacillus intermedius* in thiosulfate broth. Growth did not occur in glucose or glutamate mineral salts medium in the absence of thiosulfate, although a stable variant was obtained which grows on yeast extract alone. Cells harvested from media supplemented with organic matter have a reduced rate of thiosulfate oxidation (20 to 30% of autotrophic), oxidize the organic supplement, and have an additive rate of oxidation in the presence of both the organic substrate and thiosulfate. Carboxydismutase synthesis is repressed, and the incorporation of bicarbonate carbon into cell material is almost completely eliminated by the presence of organic matter in the growth medium. It is concluded that the availability of organic matter eliminates the autotrophic assimilatory mechanisms of *T. intermedius* but not its autotrophic energy-generating system. The data are discussed in relation to the existence of "obligate" chemoautotrophic bacteria.

The recently described facultative autotroph, *Thiobacillus intermedius*, is unusual in requiring both organic and reduced inorganic sulfur compounds for maximal growth rates and cell yields (6). A study of the interaction of the two classes of substrates was undertaken in the hopes of shedding some light on the nature of the autotrophic state. The present paper is concerned with the effects of organic matter on thiosulfate oxidation and carbon dioxide fixation, and attempts to delineate the role of the organic material in the development of *T. intermedius*.

MATERIALS AND METHODS

The basic autotrophic medium had the following composition: $Na_2S_2O_3 \cdot 5H_2O$, 0.5 to 1.0%; KH_2PO_4, 0.06%; NH_4Cl, 0.1%; $MgCl_2$, 0.05%; Pfennig's (10) trace salts solution, 4% (v/v); the *p*H was adjusted to 6.8 with $NaHCO_3$. Organic supplements were added to the desired concentration from sterile stock solutions. In some experiments, yeast extract, glucose, or glutamate was substituted for the thiosulfate.

Growth studies were done in 300-ml Erlenmeyer flasks with 14 by 130 mm side arms (Nephelo-Culture Flasks, Bellco Glass Inc., Vineland, N.J.). Increase in turbidity of the culture, measured at 540 mμ (Klett) was used to follow development. A standard curve was prepared relating optical density to dry weight of cells. Each flask contained 49 ml of medium plus supplement as desired and 1 ml of inoculum. Cultures were incubated at 30 C on a rotary shaker. In some experiments, the medium was periodically neutralized with sterile sodium carbonate during growth with chlorophenol red as an internal indicator. Growth was followed until the stationary period was reached, and growth yields were calculated from the maximal turbidity.

To determine CO_2 assimilation during growth, identical culturing procedures were used except that 20 mg of $NaH^{14}CO_3$ was added to the nephelometer flasks, which were then tightly sealed with sterile rubber stoppers instead of cotton plugs. In the stoppered flasks, growth was limited by acid production from thiosulfate and not by oxygen availability from the gas phase. Samples of cultures containing 6 to 8 mg (dry weight) of cells were treated with sufficient 50% trichloroacetic acid solution to give a final concentration of 5% and were filtered through Whatman fine-pore fiberglass filter pads. The filter pads were washed with two volumes of 5% trichloroacetic acid and one volume of distilled water, placed on planchets, dried, and counted in a Nuclear Chicago gas-flow counter.

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Cell-free extracts were prepared by ultrasonic disintegration as previously described (7).

Carboxydismutase (ribulosediphosphate carboxylase) was assayed by a modification of the method of Weissbach, Horecker, and Hurwitz (17). The usual two-step procedure, the production of 3-phosphoglyceric acid from ribulose diphosphate and its subsequent reduction to 3-phosphoglyceraldehyde, was condensed into one, which permitted direct observation of the kinetics of 3-phosphoglyceric acid formation by the extracts. The reaction mixtures contained 0.24 μmole of ribulose diphosphate, 250 μmoles of tris(hydroxymethyl)aminomethane (Tris) buffer (*p*H 7.8), 10 μmoles of $MgCl_2$, 37 μmoles of $NaHCO_3$, 5 μmoles of reduced glutathione, 3 μmoles of adenosine triphosphate (ATP), 0.14 μmole of reduced nicotinamide adenine dinucleotide ($NADH_2$), 150 μg of 3-phosphoglyceraldehyde dehydrogenase, 12 μg of 3-phosphoglyceric acid kinase, and cell-free extract (approximately 0.2 mg of protein) in a final volume of 1.01 ml. The rate of oxidation was negligible in the absence of added substrate, showing that the extracts contained little $NADH_2$ oxidase. Since it was shown that the dehydrogenase and the kinase were in excess, the rate of change in optical density at 340 mμ was a direct measure of the carboxydismutase activity of the extracts.

Cytochrome-linked reactions were demonstrated by difference spectra between reaction mixtures containing 1 ml of extract (8 to 10 mg of protein), 2 ml of 0.05 M potassium phosphate buffer (*p*H 6.9), and 0.1 ml of the appropriate substrate solution, and a reference solution lacking substrate.

Spectrophotometric measurements were made with a Cary model 15 recording spectrophotometer. Manometry was performed by the usual techniques. Protein was determined by the biuret procedure as modified by La Riviere (Thesis, Technische Hogeschool, Delft, 1958); glucose, by the anthrone technique; tetrathionate, by the method of Stamm et al. (14); and thiosulfate, iodometrically. 3-Phosphoglyceraldehyde dehydrogenase (Boehringer), 3-phosphoglyceric acid kinase (Boehringer), and ribulose diphosphate dibarium salt were purchased from Calbiochem.

RESULTS

Effect of yeast extract on growth and thiosulfate oxidation. It was previously shown (6) that *T. intermedius* grows relatively slowly in autotrophic medium and that both the growth rate and cell yields are markedly increased by addition of yeast extract to the medium. The effect on cell yield was reinvestigated in a quantitative manner by varying both the yeast extract and thiosulfate contents of the media. The concentrations of both substrates were kept relatively low so that the *p*H remained in the optimal range, 6.4–6.8, during the entire growth period, thus eliminating any independent effect of *p*H on growth. The results (Table 1) show that at a fixed yeast extract concentration cell yields increased in proportion to the added thiosulfate. It can be concluded that thiosulfate has a metabolic role, i.e., energy generation, in the presence of yeast extract.

Examining the data in another way, it can be seen that, at a fixed thiosulfate concentration, doubling the yeast extract doubles the cell yield. It will also be noted that the growth increment (Table 1) per unit of thiosulfate at the higher yeast extract concentration is about twice that at the lower. If the yeast extract were providing only carbon in the medium, then at a particular ratio of thiosulfate to yeast extract either one or the other should be growth-limiting, and only an increase in the limiting factor should increase the growth yield. Since this was not the case, the data indicate that yeast extract must be providing both carbon and energy in the presence of thiosulfate. Similar data were obtained when vitamin-free casein hydrolysate or peptone was substituted for yeast extract.

The addition of yeast extract to a cell-free extract of *T. intermedius* resulted in the appearance of absorption bands at 551, 520, and 422 mμ, which are typical of a *c*-type cytochrome (Fig. 1). The identical spectrum was observed when thiosulfate was added to cell extracts. The coupling of yeast extract oxidation to cytochrome reduction is additional evidence that it serves as an energy source.

As anticipated from results of the growth experiments, cells harvested from yeast extract-thiosulfate broth oxidized both substrates, with thiosulfate utilization being much more rapid (Fig. 2A). The rate of thiosulfate oxidation, however, was only 20 to 30% of that of autotrophically grown cells (Table 2). Cell suspensions fed both substrates simultaneously consumed oxygen at a rate that was at least additive as compared with the rates on individual substrates (Fig. 2A).

TABLE 1. *Effect of yeast extract and thiosulfate concentrations on growth yields of Thiobacillus intermedius*

$Na_2S_2O_3 \cdot 5H_2O$ (%)	Dry wt of cells*			
	Yeast extract, 0.05%	Δ†	Yeast extract, 0.1%	Δ†
0‡	2.8		5.0	
0.005	4.0	1.2	7.6	2.6
0.0075	4.6	1.8	8.9	3.9
0.01	5.1	2.3	10.0	5.0

* Milligrams per 50 ml of medium.

† Increase in yield relative to the thiosulfate-free medium.

‡ Data for thiosulfate-free media obtained with the yeast extract variant (see text); all other results are for the parent strain.

In flasks containing both substrates, total oxygen uptake at the time of thiosulfate exhaustion was greater than that in control flasks containing equivalent amounts of thiosulfate only (Fig. 2A). It can be concluded, therefore, that both substrates are oxidized simultaneously.

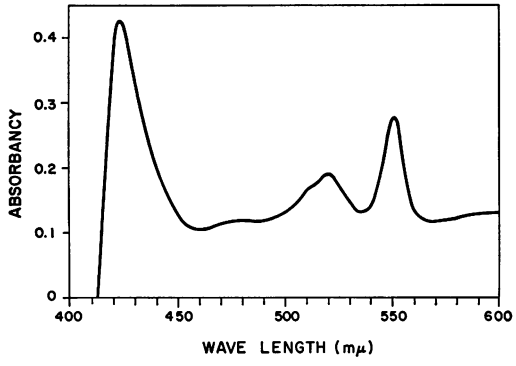

FIG. 1. *Reduction of cytochrome in Thiobacillus intermedius extracts by yeast extract. Difference spectrum after 20 min between experimental cuvette containing yeast extract and reference cuvette lacking yeast extract.*

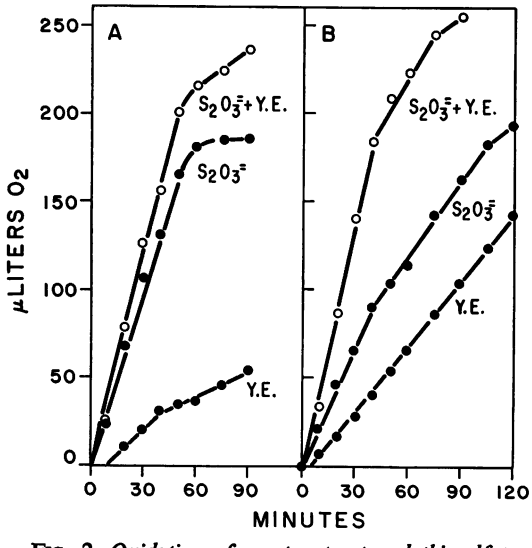

FIG. 2. *Oxidation of yeast extract and thiosulfate by cell suspensions of Thiobacillus intermedius. Data corrected for endogenous respiration; gas phase, air; 30 C. (A) Parent strain cultured in yeast extract-thiosulfate broth. Flask contents: 1 ml of cell suspension (2.1 mg of protein) in 0.01 M potassium phosphate buffer (pH 6.9); 0.8 ml of 0.05 M potassium phosphate buffer (pH 6.9); 0.1 ml of 20% KOH in center well; 5 μmoles of $Na_2S_2O_3$ or 1.0 mg of yeast extract, or both; and water to 2.1 ml. (B) T. intermedius variant cultured in yeast extract broth. Conditions as in A except cell suspension contained 1.6 mg of protein.*

TABLE 2. *Effect of yeast extract in the growth medium on the oxidation of thiosulfate by cell suspensions of Thiobacillus intermedius*

Culture medium	Oxidation rate*	
	A	B
Autotrophic, 0.5% $Na_2S_2O_3 \cdot 5H_2O$....	15.0	100
plus 0.05% yeast extract.....	5.3	36
plus 0.10% yeast extract.....	2.9	19
Yeast extract, 0.5%, 1st generation...	4.0	26
Yeast extract, 0.5%, 2nd generation..	3.3	22
Yeast extract, 0.5%, 6th generation...	4.6	30

* A = micromoles of O_2 per hour per milligram of protein; B = per cent of autotrophic rate.

Yeast extract will support growth of *T. intermedius* in the absence of thiosulfate (6). There is, however, a long lag (16 to 28 days) prior to the initiation of growth when cells grown either autotrophically or in thiosulfate-yeast extract broth are transferred to yeast extract broth. Serial transfer in the latter medium resulted in the selection of a stable variant capable of relatively rapid growth in the organic medium (Fig. 3). The variant retains the ability to grow autotrophically, and no extended lag occurs upon its transfer from autotrophic conditions to yeast extract broth. Like the parent strain, its growth is more rapid and abundant in the presence of both thiosulfate and yeast extract (Fig. 3).

Cell suspensions of the variant harvested from yeast extract broth oxidized thiosulfate without a lag (Fig. 2B). The stoichiometry shows that thiosulfate is converted quantitatively to sulfate. In the experiment presented (Fig. 2B), the cells employed were harvested from the sixth consecutive transfer in yeast extract broth. As observed with the parent strain, thiosulfate was oxidized more rapidly than yeast extract. When the two substrates were present simultaneously, the rate of oxidation was somewhat more than additive. The rate of thiosulfate oxidation by heterotrophically grown cells was about 20 to 30% of the rate shown by autotrophically grown cells. Continued cultivation under heterotrophic conditions did not further reduce the capacity for thiosulfate oxidation (Table 2).

Effect of glucose on growth and thiosulfate oxidation. It was shown that the addition of glucose to autotrophic medium also stimulated growth of *T. intermedius*. Cell yields in unneutralized cultures increased at a constant increment over the range of 0.1 to 0.7% added glucose (Fig. 4). The stimulatory effect of glucose was several-fold greater in cultures maintained near neutrality by

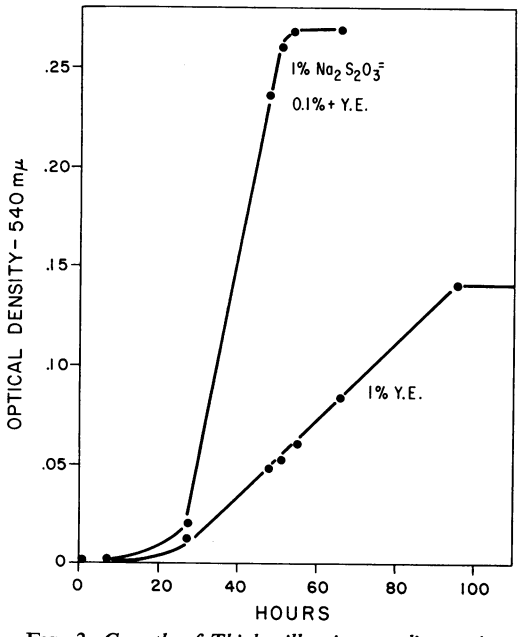

FIG. 3. *Growth of Thiobacillus intermedius variant in thiosulfate-yeast extract broth and in yeast extract broth. Inoculum was grown in 1% yeast extract broth.*

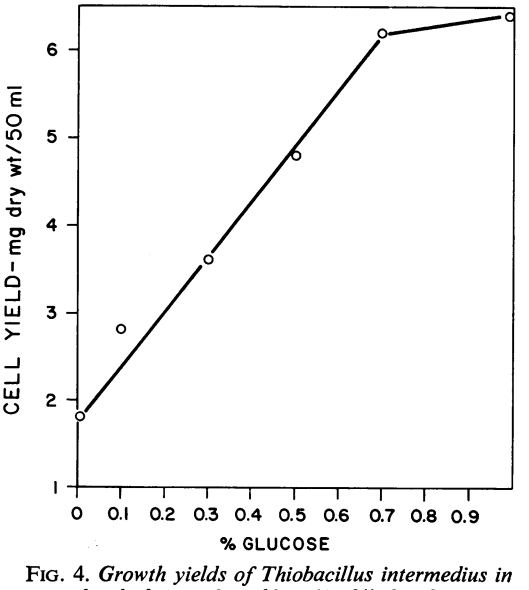

FIG. 4. *Growth yields of Thiobacillus intermedius in unneutralized glucose-thiosulfate (0.5%) broth. Inoculum was grown in thiosulfate (0.5%)-glucose (0.25%) broth.*

the periodic addition of carbonate (Table 3). Although thiosulfate was completely oxidized under these conditions, only about 10% of the added glucose had disappeared from the medium at the termination of growth. Data presented in the next section show that glucose serves as the major carbon source when added to the autotrophic medium (Table 5). Whether its metabolism also contributes to energy generation has not yet been determined; however, the failure of neutralized cultures to utilize the bulk of the added glucose appears to preclude this possibility.

Glutamate was similar to glucose in enhancing cell yields. On a weight basis, these compounds were about 50% as effective as yeast extract and the other complex supplements in augmenting cell crops.

Cell suspensions harvested from glucose-thiosulfate broth oxidized glucose at a rate approximately one-tenth that of thiosulfate oxidation, and an additive rate was observed when both substrates were fed simultaneously (Table 4). However, the absolute rate of thiosulfate oxidation was much lower than that of cells grown autotrophically (Table 4). With increased glucose in the growth medium, there was an increase in the cells' capacity to oxidize glucose and a decrease in thiosulfate-oxidizing activity (Table 4). Both effects were small and are probably not significant.

Total oxygen uptake by cell suspensions fed

TABLE 3. *Utilization of glucose in neutralized cultures of Thiobacillus intermedius**

Addition to 0.5% thiosulfate medium	Glucose (mg/50 ml)		Glucose utilized	Cell yield†
	Initial	Residual		
			%	
None	—	—	—	2.6
Glucose, 0.3%	150	140	6.7	7.0
Glucose, 0.5%	250	228	8.9	14.0
Glucose, 0.7%	350	320	8.5	14.0

* No residual $S_2O_3^{-2}$ or $S_4O_6^{-2}$ was found.

† Milligrams (dry weight) of cells per 50 ml of culture.

TABLE 4. *Effect of glucose in the growth medium on the oxidative activity of suspensions of Thiobacillus intermedius*

Glucose in medium	Rate of oxidation*			
	Thiosulfate (1)	Glucose (2)	1 + 2	Endogenous
%				
0	15.0	Nil		Nil
0.2	4.7	0.31	5.05	0.58
0.5	3.9	0.45	4.19	0.62
1.0	3.4	0.53	3.90	1.4

* Micromoles of O_2 per hour per milligram of protein, corrected for endogenous.

glucose alone averaged 3.5 μmoles per μmole, or 58% of that required for complete oxidation. Whether the difference resulted from oxidative assimilation, from the accumulation of an unoxidized fragment, or from residual glucose was not directly investigated. It was observed, however, that increasing the amount of glucose in the growth medium increased the rate of endogenous respiration of cell suspensions, which suggests the accumulation of an oxidizable reserve material (Table 4).

Although heterotrophic growth occurred in yeast extract broth, neither the parent strain of *T. intermedius* nor the variant grew in glucose or glutamate mineral salts broth without thiosulfate.

Effect of organic matter on carboxydismutase levels and on CO_2 fixation. Cell-free extracts were prepared from late log phase cells cultured in thiosulfate-mineral salts medium with and without organic supplements and were assayed for carboxydismutase activity. Figure 5 shows the results of one experiment in which an extract of autotrophically grown cells was used. Except for the slope of the linear portion, the curve is typical of those obtained with the other extracts.

Incorporation of single organic compounds or complex mixtures into the growth medium reduced the carboxydismutase activity of the resulting cells (Table 5). Cells grown in the presence of 0.05% yeast extract showed a 74% decrease in enzyme activity. Growth in the presence of higher yeast extract concentrations further reduced carboxydismutase activity to barely detectable levels. Similar marked reduction of enzyme activity was observed with tryptone and casein hydrolysate as supplements. The addition of glucose or glutamate to the growth medium also lowered the carboxydismutase activity of the cells, but to a lesser degree than that observed with the complex mixtures. The decrease in activity was not enhanced by increasing the concentration of these compounds. The addition of inactive extracts to active ones did not reduce the carboxydismutase activity of the latter.

The specific activities of carboxydismutase in autotrophically grown *T. intermedius* and in *T. thioparus* grown under comparable conditions are approximately the same, and are somewhat higher than in *T. thiooxidans*. The difference may be related to the presence of an active pyruvate carboxylase which is responsible for 30% of the CO_2 fixed in the latter organism (16).

The relative contribution of CO_2 and the organic additives to cell carbon of *T. intermedius* was investigated by comparing the extent of $H^{14}CO_3^-$ incorporation into the trichloroacetic acid-insoluble fractions of cells growing with and without organic supplements. As would be predicted from the carboxydismutase activities, little of the cell carbon was derived from bicarbonate when organic compounds were present (Fig. 6). The relative incorporation of bicarbonate carbon was reduced to between 3 and 22% of the autotrophic control by addition of 0.05% organic

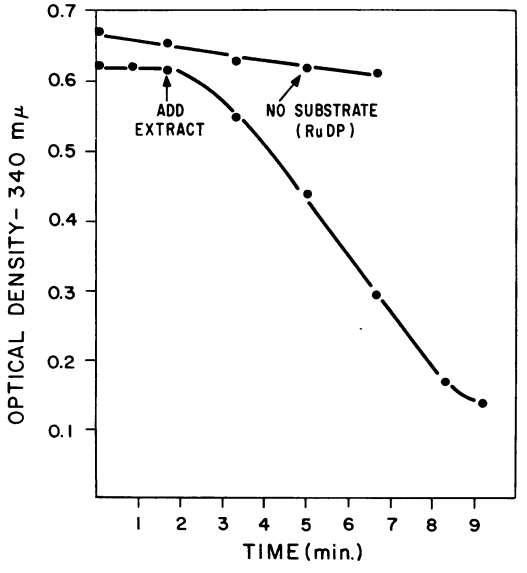

FIG. 5. *Spectrophotometric assay of carboxydismutase activity of an extract of Thiobacillus intermedius. Cuvette contents as per text, with the use of an extract (0.2 mg of protein) of autotrophically grown cells.*

TABLE 5. *Carboxydismutase activity[a] of Thiobacillus intermedius grown in thiosulfate broth with organic supplements*

Addition to medium	Carboxydismutase		Cell carbon from CO_2[d]
	Activity[b]	Per cent[c]	
			%
None	56	100	100
Glucose, 0.2%	19	35	—
Glucose, 0.5%	24	44	3
Glucose, 1.0%	24	44	—
Yeast extract, 0.05%	14	26	7
Yeast extract, 0.25%	3	0.6	2
Yeast extract, 0.5%	5	0.9	1
Tryptone, 0.25%	14	26	2
Casein hydrolysate, 0.25%	6	12	14
Glutamate, 0.05%	43	77	22
Glutamate, 0.5%	39	71	12
None, *T. thiooxidans*	41	74	—
None, *T. thioparus*	54	94	—

[a] See Fig. 5 for assay procedure.
[b] Millimicromoles of $NADH_2$ oxidized per minute per milligram of protein.
[c] Relative to autotrophic activity.
[d] See Fig. 6.

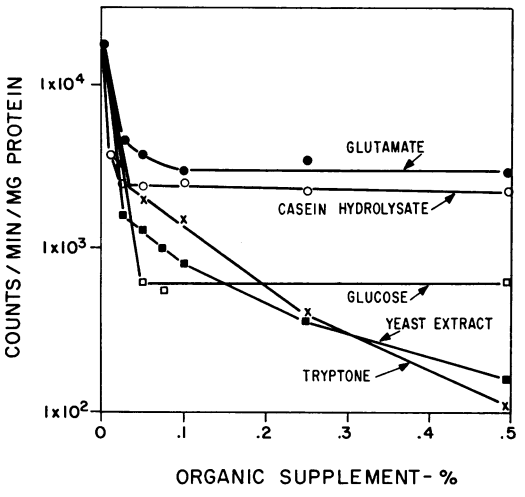

FIG. 6. *Uptake of C^{14} from $H^{14}CO_3^-$ by Thiobacillus intermedius grown in thiosulfate broth supplemented with organic compounds.*

matter to the medium. At higher concentrations of yeast extract and tryptone, incorporation of $C^{14}O_2$ was almost completely inhibited; with glutamate and casein hydrolysate, the level of CO_2 fixed plateaued at a value about 15% of that in the autotrophic control. It should be noted that the decrease in CO_2 fixed was much greater than the decrease in carboxydismutase activity when either glucose or glutamate was the supplement (Table 5).

DISCUSSION

It is apparent from the data that the presence of diverse organic compounds in the growth medium influences key aspects of the autotrophic metabolism of *T. intermedius*, i.e., energy generation and synthesis. This is evidenced by a decreased rate of thiosulfate oxidation as measured with cell suspensions, and by a decreased activity of carboxydismutase as measured with cell extracts. Since both measurements were made in the absence of added organic matter, it is reasonable to assume that repression of the corresponding enzymes by the organic supplement in the growth medium occurred. The alternate hypothesis, inhibition of enzyme activity by endogenous inhibitors in the cells or their extracts, is not critically excluded by the experiments. This explanation, however, is rendered very unlikely by the observation that cell suspensions fed thiosulfate and organic matter simultaneoulsy consume oxygen at a rate equal to or greater than the sum of the rates with the individual substrates. Further, the addition of inactive cell-free extracts to active ones, did not reduce the carboxydismutase activity of the active extract.

Assuming that repression occurs, the effect is more pronounced for carboxydismutase synthesis than for the synthesis of the rate-controlling enzyme in thiosulfate oxidation. Under no situation investigated was the rate of thiosulfate oxidation reduced below 20% of the autotrophic rate. Even after numerous generations in the absence of thiosulfate, the variant retained a constitutive level of 20 to 30% of the autotrophic rate. The situation is similar in some respects to that observed in the facultatively autotrophic hydrogenomonads which may retain 10 to 20% of their autotrophic hydrogenase activity over long periods of cultivation in heterotrophic medium (18; Goodman and Rittenberg, *unpublished data*).

In contrast, under certain conditions, carboxydismutase synthesis was completely repressed by organic matter. Similar observations have been made for a *Hydrogenomonas* species in the presence of lactate (Goodman and Rittenberg, *unpublished data*), for a formate-utilizing pseudomonad grown on oxalate (11), and for the photosynthetic organisms, *Thiopeda* sp. and *Chromatium* sp. grown with pyruvate or malate (3). Since a variety of organic materials appear to inhibit carboxydismutase synthesis, but to different degrees, one might assume a multiplicity of repressors with different efficiencies or a single repressor arising from the catabolism of the organic supplements. In the latter case, the pool size of the repressor would vary with the different organic compounds. Although the postulate of a single repressor is the more appealing alternative because of similarities to other repressible systems (8), there is no experimental basis for choosing between the alternatives.

The data not only suggest repression of carboxydismutase synthesis in growing cultures, but also effective inhibition of its activity by organic matter. In the presence of glucose and glutamate, the relative contribution of CO_2 to cell material decreased much more than the decrease in carboxydismutase. Actually, part or all of the CO_2 assimilated in the presence of organic matter could have been fixed in reactions not involving carboxydismutase, since the range of incorporation, 3 to 22% cell carbon, is similar to that observed in heterotrophic bacteria lacking this enzyme. The data could also be explained without postulating an inhibition of carboxydismutase if the organic matter effectively repressed synthesis of phosphoribulokinase. This possibility was not investigated. Regardless, one can conclude that the presence of organic matter triggers regulatory processes which eliminate the autotrophic assimilatory mechanisms of *T. intermedius*.

The autotrophic energy-generating mechanisms are not similarly excluded. Although reduced in

quantity, the thiosulfate-oxidizing system continues to function in the presence of organic matter. This is shown by the respiratory rates in the presence of both organic matter and thiosulfate, by the increased growth yield with increasing thiosulfate concentrations at low levels of yeast extract where growth is not limited by *p*H changes, by the necessity of thiosulfate for growth with glucose, and by the cessation of growth with the exhaustion of thiosulfate in media supplemented with glucose or glutamate. In summary, *T. intermedius* can utilize inorganic energy sources to assimilate and grow on organic carbon. It is thus similar to *D. desulfuricans* (9).

It is probable that in this respect it differs only quantitatively from the putative strict autotroph. The pioneering investigations of Winogradsky and Omeliansky (21) showed that relatively high concentrations of organic matter inhibited growth of the nitrifying bacteria; similar data were obtained by Starkey (15) for *T. thiooxidans*. In neither instance do the data exclude the possibility of assimilation of organic compounds when present at noninhibitory concentrations. Such utilization of organic matter by "strict" autotrophs is implicit in the observed growth stimulation of *T. thiooxidans* by glucose (15), of *Nitrosomonas* by Nahrstof-Heyden (5), and of a mutant of *T. thiooxidans* by yeast extract (12). More directly, growth on glucose has been reported for *Ferrobacillus ferrooxidans* (Remsen and Lundgren, Bacteriol. Proc., p. 33, 1963) and for *T. thiooxidans* (Borichewski and Umbreit, Bacteriol. Proc., p. 92, 1964). The incorporation of labeled amino acids, acetate, and other organic molecules, singly or in combination, has been shown for *T. denitrificans* (van Niel, *unpublished data*), *Nitrobacter agilis* (1, 4), *T. thiooxidans* (Butler and Umbreit, Bacteriol. Proc., p. 84, 1965), and for a "strictly chemoautotrophic thiobacillus" (Kelley, Proc. Soc. Gen. Microbiol., p. v, 1965). Similarly, photoassimilation of organic matter occurs in organisms once considered as strict photoautotrophs (2, 13). It is clear that energy generated by chemo- or photolithotrophic mechanisms is in general available for the assimilation of organic compounds. In nature, the autotrophs flourish in soil and water, environments usually containing significant quantities of organic matter. It is a reasonable presumption that the energy-sparing ability to utilize preformed organic compounds from the environment for synthesis would have an evolutionary advantage, thus making the existence of "strict" autotrophs an anachronism. Regardless of such speculation, there remain no critically documented examples of the anorgoxidant or strict autotroph as defined by Winogradsky (20, 21), and as the term is still widely used.

The question remains, nevertheless, as to why some chemosynthetic autotrophs fail to grow at the expense of organic matter for both carbon and energy. Paradoxically, this question arises and also remains unanswered for *T. intermedius*, variants of which grow heterotrophically on yeast extract but not on glucose, even though both materials can serve as essentially the sole carbon source and both are respired. In this connection, one further peculiarity of *T. intermedius* deserves comment, and that is its relation to yeast extract. As the data show, the parent strain is incapable of growth on this material except after a long lag of several weeks. The duration of the lag suggests that its termination requires a mutational change, and indeed simple selection procedures resulted in the isolation of a stable variant which does not exhibit the extended growth lag on yeast extract. One might expect that a comparison of the variant and the parent strain would reveal some difference which could explain the inability of some autotrophs to grow heterotrophically. This did not prove to be the case for the parameters examined: both strains oxidized yeast extract at essentially the same rate; both were equally stimulated by the addition of yeast extract to thiosulfate medium; and both were provided carbon and energy by yeast extract in such cultures. The nature of the change remains unknown.

ACKNOWLEDGMENT

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