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Effect of Thiosulfate on the Photosynthetic Growth of *Rhodopseudomonas palustris*[1]

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Cell yields of *Rhodopseudomonas palustris* grown photoheterotrophically in pyruvate-mineral salts medium were increased by the photooxidation of added thiosulfate. However, thiosulfate had no effect on cell yields of cultures grown aerobically in darkness, although thiosulfate was also oxidized. The presence of thiosulfate increased photosynthetic cell yields on a variety of other organic substrates. Growth of cells in thiosulfate-containing medium, or the addition of thiosulfate to cells grown in thiosulfate-free medium, induced the formation of a thiosulfate-oxidizing system which quantitatively photooxidized thiosulfate to sulfate. *R. palustris* grew photoautotrophically with thiosulfate as an oxidizable substrate. Large amounts of supplemental bicarbonate carbon were incorporated when cells were grown photosynthetically in pyruvate-thiosulfate medium. Cells harvested after photoautotrophic or photoheterotrophic growth in fumarate-thiosulfate medium fixed $^{14}CO_2$ at an 8- to 10-fold greater rate when provided with thiosulfate. The evolution of $^{14}CO_2$ from pyruvate-*1*-^{14}C during photoassimilation by *R. palustris* was greatly suppressed by the presence of thiosulfate. The increase in photoheterotrophic cell yields of *R. palustris* caused by the oxidation of thiosulfate may result from assimilation of substrate carbon which is normally evolved as carbon dioxide.

Photosynthetic bacteria belonging to the *Chlorobacteriaceae* and the *Thiorhodaceae*, as well as *Rhodomicrobium vanniellii* (13), can grow photoautotrophically while oxidizing reduced inorganic sulfur compounds. In contrast, the *Athiorhodaceae* exhibit a photoheterotrophic metabolism at the expense of a variety of organic compounds. Van Niel (12) reported that *Rhodopseudomonas palustris*, a member of the *Athiorhodaceae*, was unique among the rhodopseudomonads in its ability to oxidize thiosulfate during photoautotrophic growth on bicarbonate. The present study was undertaken to determine the conditions under which thiosulfate is metabolized by *R. palustris* and the physiological consequence of thiosulfate oxidation on heterotrophic growth.

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MATERIALS AND METHODS

Organisms. R. palustris ATCC 11168 was obtained from the American Type Culture Collection, and the Foster strain was from S. Hutner. The strain of *Rhodospirillum rubrum* was S 1. Unless otherwise indicated, the ATCC strain 11168 of *R. palustris* was used.

Growth conditions. R. palustris and *R. rubrum* were grown in Hutner's medium as modified by Cohen-Bazire et al. (1), with the addition of 4.0 μg of *p*-aminobenzoate per liter (4) and the deletion of the Casamino Acids. The sodium salts of pyruvic, lactic, formic, malic, fumaric, succinic, benzoic, or 2-oxoglutaric acid were used for photoheterotrophic growth, and ammonium bicarbonate was the carbon source for photoautotrophic growth. The nitrogen source was 0.02 M ammonium chloride. Thiosulfate was added to the various media in many experiments, as described in the text. Ammonium bicarbonate and sodium pyruvate solutions were sterilized by filtration and added after the rest of the medium was autoclaved.

Photosynthetic growth studies were performed in tightly capped 16-oz (454-ml) bottles which were completely filled with medium after inoculation. Cells were grown photosynthetically under illumination of 2,000 ft-c in a water bath at 30 C. In one experiment cells were grown aerobically by being shaken in the dark in 200 ml of medium contained in 750-ml Erlenmeyer flasks. Rate of growth was determined by

measuring the optical density of the cultures at 680 mμ with a Beckman DU spectrophotometer. When the stationary growth period of the cultures was reached, estimates of total cell yield were made by triplicate dry-weight determinations and by cell counts with a Coulter counter.

In experiments with resuspended cells, *R. palustris* was harvested (after 24 hr of photosynthetic growth) by centrifugation at 8,000 × *g* for 15 min. Cells were washed twice in one-tenth the original culture volume of 0.02 M potassium phosphate buffer (*p*H 6.8) and resuspended in 0.1 M potassium phosphate buffer (*p*H 6.8). Cell suspensions were incubated at 30 C either under photosynthetic conditions or in darkness without aeration. Components of the systems are given under each experiment.

Measurement of radioactivity. Samples of suspensions were removed and collected on membrane filters (type HA, 0.45 μ; Millipore Corp., Bedford, Mass.) to determine the incorporation of radioactive carbon into cells. The cells were washed with distilled water and the filter pads were dried. All radioactivity measurements were made in a Nuclear-Chicago model 181B scintillation spectrometer (50% efficiency) by immersing the filters in scintillation liquid: 0.25 g of 1,4-bis-2(5-phenyloxazolyl)-benzene and 4 g of 2,5-diphenyloxazole in 770 ml of toluene and 230 ml of absolute ethyl alcohol.

Manometry. The conventional Warburg manometric technique was used. The reactions were run at 30 C; the reaction flasks were illuminated by two water-cooled 150-w reflector flood lamps placed 2 ft (60 cm) from the vessels.

Chemical determinations. Bacteriochlorophyll was determined by the method of Cohen-Bazire et al. (1); pyruvate, by the method of Straub (10); sulfate, by the method of Gleen and Quastel (3); and thiosulfate, iodometrically.

Special chemicals. Sodium pyruvate-*1*-^{14}C and ^{14}C-labeled sodium bicarbonate were purchased from Volk Radiochemical Co., Chicago, Ill.

RESULTS

Effect of thiosulfate on growth of R. palustris. The addition of 0.01 M thiosulfate to 0.005 M pyruvate medium increased the final photosynthetic cell yield of *R. palustis* by 40 to 60% (Tables 1 and 2). The increased photoheterotrophic cell yield, as estimated by optical density at 680 mμ, was correlated with two independent measurements of cell yields: dry-weight determinations and total cell counts which were determined by use of a Coulter counter (Table 1).

The presence of thiosulfate caused no increase in cell yield when cultures were grown aerobically in the dark, although more thiosulfate was oxidized during aerobic growth in the dark than during photosynthetic growth (Table 2). In all these growth experiments pyruvate was completely utilized by cells grown either with or without thiosulfate.

TABLE 1. *Correlation of growth parameters of photoheterotrophically grown Rhodopseudomonas palustris*

Growth medium[a]	Absorbancy at 680 mμ	Cell yield[b]	Cells/ml[c]
Pyruvate	0.283	190	9.1×10^8
Pyruvate plus $Na_2S_2O_3$	0.432	330	1.6×10^9

[a] Cells were grown photosynthetically in modified Hutner's medium with 0.005 M pyruvate and with or without 0.01 M $Na_2S_2O_3$.

[b] Milligrams (dry weight) of cells formed per 5 mmoles of pyruvate utilized by cells during growth.

[c] Determined by use of a Coulter counter.

TABLE 2. *Effect of thiosulfate and light on the cell yield of Rhodopseudomonas palustris*[a]

Strain of *R. palustris*	Growth conditions[a]	Cell yield[b]	Thiosulfate oxidized[c]
11168	Photosynthetic minus $Na_2S_2O_3$	258	—
	Photosynthetic plus $Na_2S_2O_3$	335	3.4
	Aerobic in darkness minus $Na_2S_2O_3$	119	—
	Aerobic in darkness plus $Na_2S_2O_3$	127	9.4
Foster	Photosynthetic minus $Na_2S_2O_3$	250	—
	Photosynthetic plus $Na_2S_2O_3$	363	5.4
	Aerobic in darkness minus $Na_2S_2O_3$	109	—
	Aerobic in darkness plus $Na_2S_2O_3$	102	8.6

[a] Cells were grown in modified Hutner's medium with 0.005 M pyruvate with or without 0.015 M $Na_2S_2O_3$.

[b] Milligrams (dry weight) of cells formed per 5 mmoles of pyruvate utilized by cells during growth.

[c] Millimoles of $Na_2S_2O_3$ oxidized per 5 mmoles of pyruvate utilized by the cells during growth.

Cell yields of *R. palustris* grown under photosynthetic conditions in pyruvate or pyruvate-thiosulfate media increased in a direct relationship to pyruvate concentration up to 0.02 M (Fig. 1). For any pyruvate concentration up to 0.02 M, the growth increment caused by the addition of thiosulfate to pyruvate medium represented a constant percentage of the total cell yield. Increasing pyruvate concentrations above 0.02 M caused little increase in total cell yield in

either thiosulfate-containing or thiosulfate-free medium although pyruvate was completely consumed. There was a direct relationship between the growth increment caused by the addition of thiosulfate and the amount of thiosulfate consumed by the cells during growth (Fig. 1).

The addition of thiosulfate to growth media containing a variety of carbon substrates increased the photosynthetic growth yields of *R. palustris* (Table 3). The concentration of carbon substrates was kept at 0.005 M to insure their complete utilization. *R. rubrum*, which did not metabolize thiosulfate, was grown in similar media as a control. The data in Table 3 show that, for substrates of the same carbon number, the photosynthetic cell yields of *R. palustris* and *R. rubrum* increased as the reduction level of the carbon substrate increased. With the addition of thiosulfate to the various media, the cell yields of *R. palustris* increased, but those of *R. rubrum* were not affected. The difference between cell yields of *R. rubrum* and *R. palustris* (with thiosulfate) on several substrates may reflect the differences in the intermediary photometabolism of these organic acids. The addition of thiosulfate resulted in greater growth increments of cells grown on oxidized substrates (fumarate) than with comparable, reduced substrates (succinate). During photoheterotrophic growth in thiosulfate-containing media, 80 to 90% of substrate carbon was incorporated into cell material, compared to 50% for cultures grown photoheterotrophically in thiosulfate-free medium on more oxidized substrate.

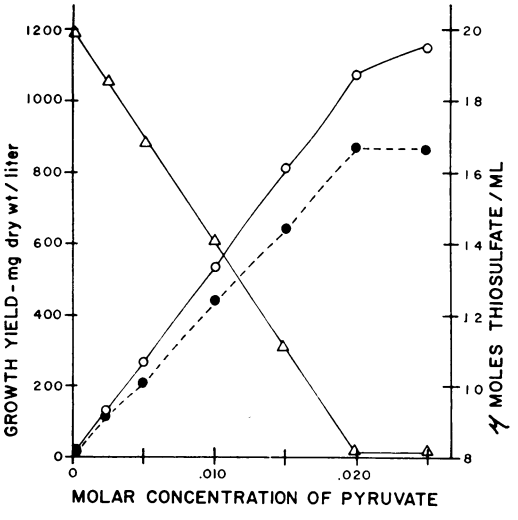

FIG. 1. *Photosynthetic cell yields of Rhodopseudomonas palustris in pyruvate or pyruvate plus 0.02 M thiosulfate media. Broken line indicates cells grown in pyruvate. Solid line indicates cells grown in thiosulfate-pyruvate media. Growth yields* (○, ●) *and thiosulfate* (△) *disappearance are shown.*

TABLE 3. *Effect of thiosulfate on the cell yield of Rhodopseudomonas palustris grown photosynthetically on various carbon substrates*[a]

Substrate	Cell yield of *R. palustris*[b]		Thiosulfate utilized[c]	Cell yield of *R. rubrum*[d]
	$-Na_2S_2O_3$	$+Na_2S_2O_3$		
Formate	50	96	1.6	93
Acetate	108	172	0.6	173
Ethyl alcohol	117	178	0.5	193
Pyruvate	201	318	3.6	195
Lactate	275	314	1.3	280
Fumarate	222	444	5.5	242
Malate	225	398	4.6	223
Succinate	337	430	1.8	340
Butyrate	415	421	1.8	387
2-Oxoglutarate	329	469	5.7	337
Benzoate	688	735	1.5	722

[a] The concentration of the carbon substrates was 0.005 M in modified Hutner's medium.

[b] Milligrams (dry weight) of cells formed per 5 mmoles of substrate utilized by cells during growth.

[c] Millimoles of $Na_2S_2O_3$ per 5 mmoles of substrate utilized by cells during growth.

[d] Since thiosulfate had no effect on the cell yields of *R. rubrum*, the cell yields were averaged from thiosulfate-containing and thiosulfate-free media. Cell yields are expressed as milligrams (dry weight) of cells formed per 5 mmoles of substrate utilized by cells during growth.

The ability of cells to assimilate supplemental bicarbonate carbon while growing under photoheterotrophic conditions was investigated. Table 4 shows that *R. palustris*, grown photoheterotrophically in a medium of 0.005 M pyruvate-thiosulfate with the addition of 0.015 M ammonium bicarbonate, had a cell yield double that of *R. palustris* grown in pyruvate-bicarbonate medium without thiosulfate. Bicarbonate carbon present in the medium during photoheterotrophic growth of cells is apparently assimilated at the expense of thiosulfate utilization.

With thiosulfate present in the medium, *R. palustris* can be grown photoautotrophically with ammonium bicarbonate as the sole carbon source except for traces of *p*-aminobenzoate (Table 4). This photoautotrophic growth rate of *R. palustris* was approximately one-seventh that of photoheterotrophically grown cells. Cells did not grow aerobically in the dark in thiosulfate-bicarbonate medium.

TABLE 4. *Effect of thiosulfate on the ability of Rhodopseudomonas palustris to assimilate bicarbonate carbon during photosynthetic growth*

Growth medium[a]	Cell yield[b]	Thiosulfate utilized[c]
Pyruvate	208	—
Pyruvate, NH_4HCO_3	221	—
NH_4HCO_3, $Na_2S_2O_3$	221	4.5
Pyruvate, $Na_2S_2O_3$	258	1.8
NH_4HCO_3, pyruvate, $Na_2S_2O_3$	423	7.4

[a] Modified Hutner's with the following as noted: pyruvate, 0.005 M; NH_4HCO_3, 0.015 M; $Na_2S_2O_3$, 0.02 M.

[b] Milligrams (dry weight) of cells formed per liter of growth medium.

[c] Millimoles of $Na_2S_2O_3$ per liter of growth medium.

Metabolism of thiosulfate by R. palustris. Cell suspensions of *R. palustris* harvested from pyruvate-thiosulfate medium and incubated under photosynthetic conditions consumed thiosulfate immediately (Fig. 2). The rate of thiosulfate disappearance was 40 μmoles of thiosulfate per mg of bacteriochlorophyll per hr. Thiosulfate was not metabolized by cells incubated without aeration in the dark at 30 C. The thiosulfate consumed was converted into sulfate beginning 6 hr after the start of thiosulfate consumption (Fig. 2). Once it began, sulfate accumulation increased rapidly until the levels of thiosulfate consumed were accounted for quantitatively by the amount of sulfate formed. When the maximal rate of sulfate formation was reached, the rate or sulfate accumulation remained twice the rate of thiosulfate consumption until thiosulfate uptake stopped. The initial lag in formation of sulfate remains unexplained, but it may represent the intracellular accumulation of an intermediate in thiosulfate oxidation, e.g., tetrathionate, followed by the oxidation of the tetrathionate to sulfate.

Cells harvested from thiosulfate-free medium and resuspended with thiosulfate did not metabolize thiosulfate during a 12-hr period (Fig. 2). Cells grown in thiosulfate-free medium could be induced to utilize thiosulfate by resuspending these cells with thiosulfate, ammonium chloride, and either ammonium bicarbonate or sodium pyruvate as a carbon source (Fig. 3). During the induction period, there was less than a twofold increase in the number of cells with either carbon substrate. Thiosulfate disappearance began after 5 hr with pyruvate as a carbon source and after 12 hr with ammonium bicarbonate. The consumed thiosulfate was oxidized to sulfate; sulfate

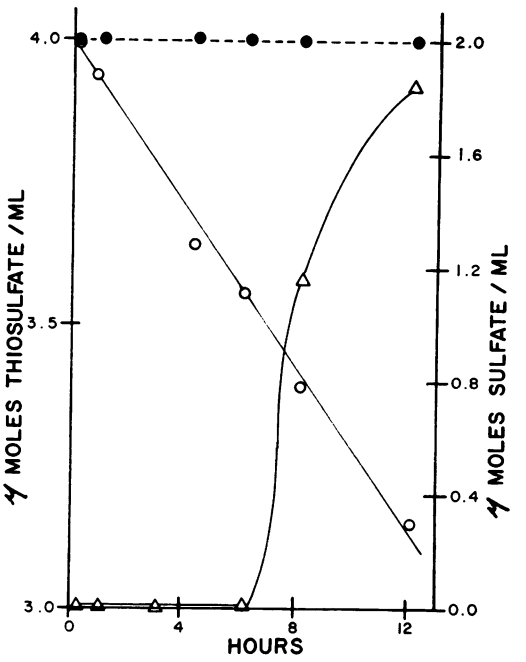

FIG. 2. *Effect of thiosulfate in the growth medium on the metabolism of thiosulfate by cell suspensions of Rhodopseudomonas palustris under photosynthetic conditions. Cells were harvested after photosynthetic growth either in 0.02 M pyruvate (broken line) or in pyruvate-0.010 M thiosulfate (solid line). Flask contents: 110 mg (dry weight) of cells, 1.2 mmoles of $Na_2S_2O_3$; 12 mmoles of phosphate buffer (pH 6.8); and water to 300 ml. Thiosulfate (○, ●) disappearance and sulfate (△) production are shown.*

accumulation started between 6 and 8 hr after thiosulfate disappearance began (Fig. 3).

Cell suspensions harvested from photosynthetic growth in pyruvate-thiosulfate medium exhibited a fourfold increase in rate of O_2 uptake when thiosulfate was added to illuminated cells. Cells were illuminated to reduce the amount of endogenous metabolism exhibited by cells harvested from pyruvate medium and incubated under aerobic conditions in the dark (6). Addition of thiosulfate had no effect on the rate of O_2 uptake by cell suspensions harvested from thiosulfate-free medium. These data indicated that the presence of thiosulfate in the growth medium, or the addition of thiosulfate to suspensions of cells grown in thiosulfate-free media, induced in *R. palustris* the formation of a thiosulfate-oxidizing system.

Effect of thiosulfate on metabolism of pyruvate-1-^{14}C and on carbon dioxide fixation. Morita (6) showed that during the photometabolism of pyruvate by *R. palustris* one-half of the radioactive

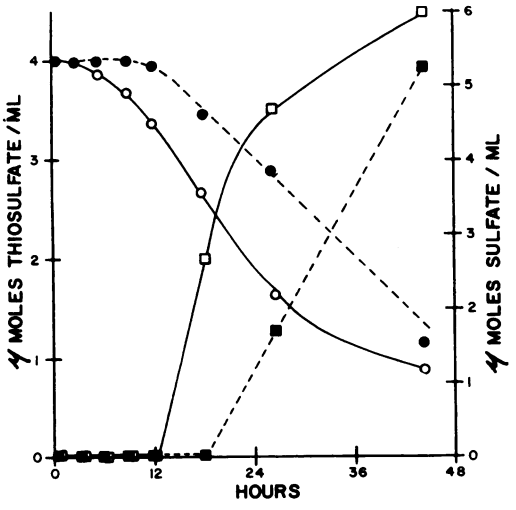

FIG. 3. *Inducible formation of the thiosulfate-oxidizing system in cell suspensions of Rhodopseudomonas palustris incubated under photosynthetic conditions. Suspensions of cells grown photosynthetically on pyruvate were incubated with NH_4HCO_3 or pyruvate as carbon sources. Flask contents: 138 mg (dry weight) of cells; 1.5 mmoles of pyruvate or 4.5 mmoles of NH_4HCO_3; 12.0 mmoles of phosphate buffer (pH 6.8); 1.2 mmoles of NH_4Cl; 1.2 mmoles of $Na_2S_2O_3$; and water to 300 ml. Cells incubated with NH_4HCO_3 are indicated by a broken line. Cells incubated with pyruvate are indicated by a solid line. Thiosulfate (○, ●) disappearance and sulfate (□, ■) production are shown.*

carbon from the carboxyl group of pyruvate-*1*-^{14}C was not assimilated into cellular material but was evolved as $^{14}CO_2$. To determine what effect thiosulfate metabolism has on metabolism of pyruvate-*1*-^{14}C, cells were grown photoheterotrophically in thiosulfate-pyruvate medium, harvested, and resuspended in the presence of pyruvate-*1*-^{14}C with and without thiosulfate. Samples were removed until incorporation of radioactive carbon stopped. The data in Table 5 show that cells incubated without thiosulfate assimilated only one-half the available radioactive carbon from pyruvate-*1*-^{14}C, thus confirming the results of Morita (6). In contrast, the cells which were supplied with thiosulfate photoassimilated most of the available radioactive carbon from pyruvate-*1*-^{14}C.

The effect of thiosulfate on the ability of *R. palustris* to fix carbon dioxide was determined by growing cells photoautotrophically or photoheterotrophically with fumarate as a carbon source in thiosulfate-containing or thiosulfate-free media. The data in Table 6 show that cells harvested, after growth under either photoautotropic conditions or under photoheterotrophic conditions in thiosulfate-containing fumarate medium, fixed carbon dioxide at an 8- to 10-fold greater rate than either cells grown in thiosulfate-

TABLE 5. *Effect of thiosulfate on the evolution of $^{14}CO_2$ from pyruvate-1-^{14}C during photoassimilation by Rhodopseudomonas palustris*[a]

Incubation time (hr)	Strain	^{14}C incorporated by cells (counts/min)	
		$-Na_2S_2O_3$	$+Na_2S_2O_3$
14	11168	3.0×10^5	5.1×10^5
	Foster	2.2×10^5	7.0×10^5
64	11168	4.4×10^5	7.8×10^5
	Foster	4.2×10^5	8.6×10^5

[a] Flasks contained either 18 mg (dry weight) of Foster cells or 14 mg (dry weight) of 11168 cells plus 400 μmoles of phosphate buffer (*p*H 6.8), 500 μmoles of NH_4Cl, 10 μmoles of unlabeled pyruvate, 0.08 μmoles of pyruvate-*1*-^{14}C (8.80×10^5 counts/min), and with or without 200 μmoles of $Na_2S_2O_3$. The final volume was 20 ml. Incubation was under photosynthetic conditions.

TABLE 6. *Effect of thiosulfate in the growth medium on the ability of cell suspensions of Rhodopseudomonas palustris to fix CO_2*

Growth conditions of cells[a]	Incubation condition[b]	Rate of $^{14}CO_2$ fixation[c]
Photoautotrophic	Plus $Na_2S_2O_3$	500
Photoautotrophic	Minus $Na_2S_2O_3$	50
Photoheterotrophic plus $Na_2S_2O_3$	Plus $Na_2S_2O_3$	335
Photoheterotrophic plus $Na_2S_2O_3$	Minus $Na_2S_2O_3$	46
Photoheterotrophic minus $Na_2S_2O_3$	Plus $Na_2S_2O_3$	47
Photoheterotrophic minus $Na_2S_2O_3$	Minus $Na_2S_2O_3$	42

[a] Cells were grown photoautotrophically on 0.03 M NH_4HCO_3 with 0.020 M $Na_2S_2O_3$. Cells were grown photoheterotrophically on 0.015 M fumarate with or without 0.02 M $Na_2S_2O_3$.

[b] Systems contained 16.1 mg (dry weight) of photoautotrophically grown cells or 18.6 mg (dry weight) of photoheterotrophically grown cells. The cells were incubated photosynthetically for 1 hr with 500 μmoles of NH_4Cl, 100 μmoles of NH_4HCO_3, 2 μmoles of $NaH^{14}CO_3$ (specific activity, 6.0 μc/μmole), 400 μmoles of phosphate buffer (*p*H 6.8), and with or without 200 μmoles of $Na_2S_2O_3$. The final volume was 20 ml.

[c] Rate of increase in counts per minute incorporated into cells per minute per milligram (dry weight) of cells.

containing media and incubated without thiosulfate or cells grown in thiosulfate-free medium and incubated with or without thiosulfate. Again, the presence of thiosulfate and competent cells resulted in much greater rates of CO_2 fixation.

DISCUSSION

Growth of *R. palustris* in thiosulfate-containing medium or exposure of cells grown in thiosulfate-free medium to thiosulfate induces the formation of a thiosulfate-oxidizing system. Truper and Pfennig (11) reported that the thiosulfate-oxidizing system of another photosynthetic bacterium, *Chromatium* 1121, was inducible. They observed that *Chromatium* 1121 grown photoautotrophically in sulfide-containing medium did not utilize thiosulfate immediately, as did cells grown in thiosulfate medium. In contrast to *R. palustris* and *Chromatium* 1121, the thiosulfate-oxidizing systems of many other photosynthetic bacteria, including *Chromatium* D (8) and *Thiocapsa floridans* (11), are constitutive systems.

Thiosulfate greatly affects the carbon assimilation of *R. palustris*. The presence of thiosulfate in bicarbonate medium allowed photoautotrophic growth of cells, but the addition of thiosulfate to media containing various organic substrates increased photoheterotrophic cell yields. The increased photosynthetic cell yields on various carbon sources in the presence of thiosulfate were correlated with an increase in the number of cells, thereby eliminating the possibility that the cell mass increase was caused by the intracellular accumulation of intermediates of thiosulfate oxidation, as was found with *Chromatium* D (8).

A possible reason for the increase in photoheterotrophic cell yield of *R. palustris* grown in the presence of thiosulfate is found in considering the pathway of organic carbon assimilation during photosynthesis in the *Athiorhodaceae*. Stanier et al. (9) showed that in bacterial photosynthesis organic substrates are converted into readily assimilable sources of carbon for the cell. Members of the *Athiorhodaceae*, e.g., *R. rubrum* (2) and *R. palustris* (7), photoassimilate many organic substrates by first converting them into acids which then enter the tricarboxylic acid cycle. Large amounts of carbon dioxide are evolved from the carboxyl groups of these acids during photoassimilation, and this carbon dioxide forms a reservoir of potential carbon that would increase cell yields if its loss could be prevented.

Evidence exists that the evolution of carbon dioxide from the carboxyl groups of organic acids during photoassimilation can be prevented by the addition of excess reducing power. Kikuchi et al. (5) showed that the addition of reduced organic acids, e.g., succinate or lactate, to cell suspensions of *Rhodopseudomonas spheroides* during photometabolism of malate-*1,4*-^{14}C or pyruvate-*1*-^{14}C caused a significant suppression of the loss of $^{14}CO_2$ from the more oxidized substrates. The reducing power supplied by succinate or lactate might prevent loss of carbon dioxide either by supplying electrons for the direct reduction of the carboxyl groups of the organic acids to form compounds which are not subject to decarboxylations, or by supplying electrons to fix the carbon dioxide after its evolution. Evans (2) showed that the reduced nicotinamide adenine dinucleotide generated by *R. rubrum* during photoassimilation of succinate was utilized to fix carbon dioxide evolved during the process of conversion of succinate into phosphoenol-pyruvate. It is probable that the reducing power derived from the photometabolism of organic substrates by photosynthetic bacteria circumvents much of the loss of carbon dioxide during assimilation of organic acids by serving as a reductant for the refixing of evolved carbon dioxide.

Evolution of $^{14}CO_2$ during the photoassimilation of pyruvate-*1*-^{14}C by *R. palustris* is suppressed almost completely by the addition of thiosulfate to cell suspensions (Table 5), thus suggesting a similarity between the effect of thiosulfate and the effect of reduced acids on the photoassimilation of carbon from the carboxyl group of pyruvate. Although not rigorously proven in this investigation, the fixation of evolved carbon dioxide is favored as the means of increasing the photoheterotrophic cell yields of *R. palustris* in the presence of thiosulfate for the following three reasons: first, the addition of thiosulfate to pyruvate medium allowed incorporation of large amounts of supplemental bicarbonate carbon into cellular material during growth under photo ynthetic conditions (Table 4); second, cultures failed to show an increase in cell yield on pyruvate-thiosulfate medium when grown under aerobic conditions in the dark (Table 2), the same conditions under which *R. palustris* did not grow autotrophically in bicarbonate-thiosulfate medium; third, the presence of thiosulfate in the growth media increased greatly the ability of illuminated cell suspensions to fix carbon dioxide (Table 6). The manner in which the loss of carbon dioxide during photoassimilation of organic substrates is suppressed seems to be the same whether the excess reducing power is provided by the oxidation of organic substrates or of thiosulfate. We do not know whether this reducing power results from a direct photoreduction of pyridine nucleotide or of ferredoxin, or from a reverse electron flow involving an intermediate adenine nucleotide.

These data show that *R. palustris* can be distinguished from the other members of the *Athiorhodaceae* not only by its ability to grow photoautotrophically with thiosulfate as an electron donor, but also by its ability to utilize thiosulfate to increase photoheterotrophic cell yields. This increased capacity to photoassimilate available carbon because of thiosulfate oxidation may give *R. palustris* environmental advantages over the other members of the *Athiorhodaceae* and may indicate that *R. palustris* is an intermediary bioform between the *Athiorhodaceae* and the *Thiorhodaceae*.

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LITERATURE CITED

1. COHEN-BAZIRE, G., W. R. SISTROM, AND R. Y. STANIER. 1957. Kinetic studies of pigment synthesis by non-sulfur purple bacteria. J. Cellular Comp. Physiol. **49**:25–68.
2. EVANS, M. C. W. 1965. The photoassimilation of succinate to hexose by *Rhodospirillum rubrum*. Biochem. J. **95**:669–677.
3. GLEEN, H., AND J. H. QUASTEL. 1953. Sulphur metabolism in soil. Appl. Microbiol. **1**:70–77.
4. HUTNER, S. H. 1946. Organic growth essentials of the aerobic nonsulfur photosynthetic bacteria. J. Bacteriol. **52**:213–221.
5. KIKUCHI, G., S. ABE, AND A. MUTO. 1961. Carboxylic acids metabolism and its relation to porphyrin biosynthesis in *Rhodopseudomonas spheroides* under light-anaerobic conditions. J. Biochem. **49**:570–577.
6. MORITA, S. 1955. The effect of light on the metabolism of lactic acid by *Rhodopseudomonas palustris*. J. Biochem. **42**:533–554.
7. MORITA, S. 1961. Metabolism of organic acids in *Rhodopseudomonas palustris*. J. Biochem. **50**:190–196.
8. SMITH, A. J. 1966. The role of tetrathionate in the oxidation of thiosulfate by *Chromatium* sp. strain D. J. Gen. Microbiol. **42**:371–380.
9. STANIER, R. Y., M. DOUDOROFF, R. KUNISAWA, AND R. CONTOPOULOU. 1959. The role of organic substrates in bacterial photosynthesis. Proc. Natl. Acad. Sci. U.S. **45**:1246–1260.
10. STRAUB, F. B. 1936. Quantitative methoden zur Untersuchung der Fumarsaure Katalyse. Z. Physiol. Chem. **244**:117–127.
11. TRUPER, H. G., AND N. PFENNIG. 1966. Sulphur metabolism in *Thiorhodaceae*. III. Storage and turnover of thiosulfate sulphur in *Thiocapsa floridans* and *Chromatium* species. Antonie van Leeuwenhoek J. Microbiol. Serol. **32**:261–276.
12. VAN NIEL, C. B. 1944. The culture, general physiology, morphology, and classification of the non-sulfur purple and brown bacteria. Bacteriol. Rev. **8**:1–118.
13. VAN NIEL, C. B. 1963. A brief survey of the photosynthetic bacteria, p. 459–467. *In* H. Gest, A. San Pietro, and L. P. Vernon [ed.], Bacterial photosynthesis. Antioch Press, Yellow Springs, Ohio.