Photosynthesis in *Rhodospirillum rubrum*
I. Autotrophic Carbon Dioxide Fixation

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Summary. The incorporation and distribution of activity from $^{14}$CO$_2$ was investigated under autotrophic conditions in the facultative photoautotroph, *Rhodospirillum rubrum*, with cells cultured on hydrogen, carbon dioxide, and ammonium sulfate. In 1 second $^{14}$CO$_2$ fixation experiments essentially all of the activity was found in 3-phosphoglyceric acid; plotted against time percent incorporation into phosphate esters has a strikingly negative slope. These results suggest that under autotrophic conditions the reductive pentose phosphate cycle or the key reactions of the cycle play a major role in carbon metabolism in this photosynthetic bacterium. Incorporation into amino acids and into intermediates of the tricarboxylic acid cycle was quite low.

The photosynthetic bacterium, *Rhodospirillum rubrum*, one of the Aithiorrhodaceae, can be grown under a number of dissimilar conditions: photoheterotrophically, when provided with a suitable carbon source; heterotrophically in the absence of light, and aerobically, on the same medium; or photoautotrophically on molecular hydrogen, CO$_2$, and nitrogen gas or another inorganic nitrogen source. This organism is a truly facultative photoautotroph, as distinct from those photosynthetic bacteria, such as *Chromatium* and *Chlorobium*, which are able to grow photoheterotrophically or photoautotrophically, but are not able to grow in the absence of light. It is, therefore, ideally suited for an investigation of the variability in, and control of, carbon metabolism accompanying heterotrophic and autotrophic growth.

Photosynthetic CO$_2$ fixation has been shown to occur through the reductive pentose phosphate cycle in plants, algae, and chemoautotrophic bacteria. Since the rate of photosynthetic CO$_2$ fixation is much lower in the photosynthetic bacteria (9) than in green plants or algae (11), strictly analogous experiments using short term kinetic analysis to show the operation of the reductive pentose phosphate cycle under either photoautotrophic or photoheterotrophic conditions in the bacteria have not been performed previously. After 30 seconds both 3-phosphoglyceric acid (3-PGA) and aspartic acid appeared to be primary products of CO$_2$ fixation in autotrophically cultured *Chromatium* (7). The key enzymes of the reductive pentose phosphate cycle are present in this organism (7). Recently a ferredoxin-dependent CO$_2$ fixation cycle distinct from the reductive pentose phosphate cycle has been proposed to account for autotrophic growth and bacterial photosynthesis in *Chlorobium* (6). The purpose of the present experiments, with *Rhodospirillum rubrum*, was to investigate, using chromatographic techniques, the kinetics of $^{14}$CO$_2$ incorporation under strictly autotrophic conditions in short term experiments. The results obtained are consistent with the operation of the reductive pentose phosphate cycle (or of the key reactions of the cycle) in such cells under strictly photoautotrophic conditions.

Materials and Methods

Growth of Bacterium. *Rhodospirillum rubrum*, strain S-1, was obtained from Dr. Howard Gest. The medium was 2.5 mm in (NH$_4$)$_2$SO$_4$ and contained the same levels of minerals, trace elements, and biotin, as that employed by Ormerod, et al., (10), except that the phosphate concentration was 10 mm. The inoculum was cultured and transferred as described by Ormerod, et al.: a 10% inoculum was used for autotrophic growth. Cells were grown in Roux bottles in a water bath at 30° and were bubbled continuously with 1% CO$_2$ in H$_2$. The light source consisted of 3 lumiline 60

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3 For simplicity the term "autotrophic" is used here to describe photosynthotrophic growth; "Photoorganotrophic" to describe photoorganotrophic growth; "Heterotrophic" to describe dark, aerobic, organotrophic growth. Biotin is required for growth in all experiments.
yielding light. Growth was measured by turbidity at 650 

**Experimental Procedure.** Cells were harvested by centrifugation and re-suspended in a pH 7 buffer solution consisting of the minerals, trace elements, and phosphate used in the original media, diluted 10-fold. For experiments over 10 seconds in duration Warburg flasks, gassed with hydrogen (except as indicated), were used. For shorter term experiments 125 ml Erlenmeyer flasks with a small side opening closed with a rubber cap which permitted the injection of radioactive bicarbonate from a syringe were employed. The depth of the cell suspension was the same in either case. Cells were preincubated for 30 minutes in the light with NaH$^{14}$CO$_3$ prior to the addition of radioactive NaH$^{14}$CO$_3$. Temperature was controlled at 30°. The light source consisted of 10 photoenlarger bulbs, General Electric number 212, 150 watt, 115 to 125-volt. Experiments were terminated by the addition of sufficient bioling absolute ethanol to bring the final alcohol concentration to 80%. Extracts were analyzed chromatographically by the methods of Benson, Bassham, and Calvin (5).

Radioactive spots on duplicate chromatograms were counted using a thin-window Geiger tube. The total activity used in computation of the percent of incorporation into any specific compound was the sum of the radioactivity measured in the spots on the paper and not the activity of the origin before development of the chromatogram, since the origin was self absorbent with respect to counting. Occasionally a few counts remained on the origin after development. These were not included in the tabulation of counts.

**Reagents.** High specific activity (30 to 46 mc/mg) Ba$^{14}$CO$_3$ was obtained from New England Nuclear Corporation; NaH$^{14}$CO$_3$ was formed by trapping, in NaOH, $^{14}$CO$_2$ which had been generated by the addition of concentrated H$_2$SO$_4$ to the barium salt. Phenol, used in the development of chromatograms, was freshly redistilled. All other chemicals were analytical reagent grade.

**Results**

The highest level of carbon dioxide fixation was found with young cells in the log phase of growth. There was a sharp decline in fixation as the stationary phase was approached. In preliminary experiments the relationship of culture age to rates of CO$_2$ fixation was a critical factor in obtaining optimal and reproducible results (fig 1). Under the conditions used in these experiments fixation was linear for 10 minutes indicating that steady state conditions prevailed.

The distribution of activity in the photosynthetic of autotrophically cultured *R. rubrum* fixing $^{14}$CO$_2$ under an atmosphere of H$_2$ is presented graphically in figures 2a and b.

When the percent incorporation of isotope into phosphate esters is plotted against time the slope of the curve is strongly negative (fig 2a). The first products noted on the chromatograms were 3-PGA and sugar diphosphate. 75% of the activity at 1 second was in 3-PGA. After 1 second several phosphate esters became labelled. In 11 seconds there were 8 spots in the phosphate ester portion of the chromatogram containing 129 counts (70% of the activity fixed into the soluble portion): 1 spot corresponding to sugar diphosphate, 5 to monophosphates, and 2 to P-enolpyruvate and 3-PGA. Percent incorporation into 3-PGA versus time was negative between 1 and 30 seconds (75%–20%); percent incorporation into the diphosphate area was positive.

The initial increase in labelling in alanine and glutamate (fig 2b) corresponds to incorporation
The reductive pentose phosphate cycle is clearly implicated in the pattern of photosynthetic CO₂ fixation observed in this organism. The negative slope of incorporation into phosphate esters, the observation that 3-PGA is labelled prior to other compounds, the secondary labelling of the diphasate ester area of the chromatogram are all consistent with the operation of the key steps of the cycle. In addition recently we have observed extremely high levels of ribulose-diphosphate carboxylase activity in extracts of autotrophically grown R. rubrum and have demonstrated the presence of several of the enzymes of the reductive pentose phosphate cycle. These experiments are reported in the third paper in this series (4).

Since the incorporation of radioactivity into tricarboxylic acid cycle intermediates is low, an anaerobic tricarboxylic acid cycle as proposed by Gest et al. (8) for photoheterotrophic metabolism in R. rubrum probably does not operate under strictly photoautotrophic conditions.

In addition to the above observation, the positive slopes of incorporation, and the low activity in alanine and glutamate rule out any important participation in this organism of the ferredoxin-dependent cycle proposed recently by Evans, Buchanan, and Arnon (6) to account for photosynthetic CO₂ fixation of Chlorobium thiosulfatophilum. The only known or postulated pathway for net CO₂ fixation consistent with these data is the reductive pentose phosphate cycle.

In R. rubrum cultured on l-malate, glycolic acid appears to be the first stable product of carbon dioxide fixation under photoheterotrophic conditions (2, 3). Apparently 2 pathways for the uptake of CO₂ occur in R. rubrum, one, involving 3-PGA, which accounts for the major part of CO₂ fixation in autotrophically grown cells, and the other, involving glycolic acid, in photoheterotrophically grown cells.

Clearly the nature of the carbon source during growth controls and influences both the kinetic pattern of fixation and the photometabolism of CO₂ in this photosynthetic bacteria.
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Literature Cited