either phosphorylase *a* or *b* had replaced the original enzyme. This enzymically inactive protein had an $s_{20,w}$ of 5.6, compared to 13.2 for phosphorylase *a* and 8.2 for phosphorylase *b*[5]. The sedimentation pattern of this protein is shown in Picture A of Fig. 1, where an excess of PCMB is present. An experiment conducted by F. R. N. GURD is illustrated in Picture B, in which 10 moles of PCMB have been added per mole of protein, equivalent to 55% of the thiol groups. Area analysis of the sedimentation pattern indicates that 60% of the phosphorylase has been transformed into the lighter component. This experiment, and others of a similar nature, shows that the extent of conversion of phosphorylase *a* to the lighter protein component, like the extent of the enzymic inhibition, is proportional to the molar ratio of PCMB to protein.

When cysteine is used to reactivate the phosphorylase *a* which has been completely inhibited by PCMB, the enzyme has the usual sedimentation constant for phosphorylase *a*, and it crystallizes in the usual manner. The sedimentation pattern of such "reactivated" phosphorylase is shown in Picture C of Fig. 1.

Inactivation of phosphorylase *b* by PCMB is also accompanied by a change to a new molecular species which has a sedimentation constant similar to that of the PCMB inhibited phosphorylase *a*. On reversal with cysteine the new molecular species is converted back to phosphorylase *b* only. It should be noted that the molecular weight of phosphorylase *b* (242,000) is half that of phosphorylase *a* (495,000)[5].

Preliminary estimates of the molecular weight of the new molecular species suggest that when phosphorylase *a* is inhibited by PCMB the protein is split into four parts. The phosphorylase *b* molecule would therefore be expected to be split into two parts. Upon removal of the mercurial by cysteine the parts recombine to form a protein which is similar, if not identical, to the original enzyme.

Further investigations are in progress and it is hoped to publish a more detailed report later.

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CO_2 incorporation by extracts of *Thiobacillus thioparus*

Thiobacillus thioparus, an autotrophic, non-photosynthetic sulfur bacterium, derives its energy from the oxidation of inorganic sulfur compounds, and like photosynthetic organisms synthesizes its protoplasmic constituents from atmospheric CO_2. Chemosynthetic autotrophs present as a major problem the mechanism of CO_2 fixation.

Recent experimental work[1] has established that the first stable product formed during green plant photosynthesis is phosphoglyceric acid (PGA). CALVIN AND MASSINI[2] postulated that the primary mechanism of CO_2 fixation in photosynthesis involved a carboxylation of ribulose diphosphate resulting in the production of PGA. WEISSBACH *et al.*[3] and QUAYLE *et al.*[4], working with spinach and *Chlorella* extracts respectively, have shown that these extracts can synthesize PGA from ribulose diphosphate and CO_2. Thus far this reaction has been found only in photosynthetic systems. The data presented below demonstrate that extracts of *T. thioparus*, like those of photosynthetic organisms, can convert ribulose diphosphate and CO_2 to PGA.

Crude alumina-ground extracts of *T. thioparus* were incubated with various substrates plus radioactive CO_2. At the end of the incubation period the reaction mixture was deproteinized with trichloroacetic acid, and the supernatant fluid analyzed for total radioactivity (see Table I). Of the various substrates tested only ribulose diphosphate stimulated the incorporation of radioactive CO_2*.

To demonstrate the presence of ^{14}C in the formed PGA, this acid was isolated in the following manner. The supernatant solutions of the reaction mixtures were fractionated individually with $Ba(OH)_2$ at pH 8.2 after the addition of carrier PGA. PGA is precipitated by this procedure along with hexose diphosphate and various nucleotides. The precipitates were collected, washed

* Essentially similar results were obtained in an experiment carried out in conjunction with Dr. WILLIAM JACOBY.

TABLE I

EFFECT OF SUBSTRATES ON $^{14}CO_2$ INCORPORATION IN EXTRACTS OF *Thiobacillus thioparus*

Substrate added	*Total counts fixed in counts per minute*
None	0
10 μmoles Ribose-5-phosphate	750
5 μmoles Ribulose 1,5-diphosphate*	18,500
10 μmoles Citrate and 10 μmoles Pyruvate	1,500
10 μmoles Fructose-1,6-phosphate	1,660
10 μmoles Malate	1,210

Incubation mixture contained 0.3 ml crude extract, 0.3 ml 0.1 *M* Tris (hydroxymethyl) aminomethane buffer pH 7.2, 5 μM adenosinetriphosphate, 0.05 ml 10^{-2} *M* NaF, 1 μmoles $Na_2{}^{14}CO_3$ containing $7.4 \cdot 10^6$ cpm. Total volume, 2.0 ml. Reaction flask incubated at 30° for 12 minutes in air.

* No ATP present in this reaction flask.

Ribulose diphosphate was kindly supplied by Dr. ARTHUR WEISSBACH.

with water, and their radioactivity determined. The only precipitate which contained radioactivity was that derived from the reaction mixture which contained ribulose diphosphate. This precipitate was then dissolved in acid solution and placed on a Dowex-1 (200–400 mesh) anion exchange column in the chloride form. The eluting solvent was 0.15 *N* NaCl plus 0.05 *N* HCl, according to GOODMAN[5]. Fractions were analyzed for radioactivity and for inorganic phosphate after digestion of an aliquot of each fraction with $HClO_4$ and H_2O_2. Only one radioactive peak was eluted, and that peak coincided with the known elution pattern of PGA. Thus, these data suggest that all of the radioactivity was contained in the PGA.

The ^{14}C distribution in the carbons of the isolated PGA was also studied. Crude extracts of *T. thioparus* were incubated with ribulose diphosphate and $^{14}CO_2$ for one hour. The components of the reaction flask were the same as those described in Table I. The PGA was isolated by ion exchange chromatography, converted to glyceric acid with potato phosphatase*, and degraded with periodate according to BASSHAM *et al.*[6]. All of the radioactivity was contained in the carboxyl carbon; no counts were detected in the α- and β-carbon atoms.

Although extracts of *T. thioparus* can synthesize PGA from ribulose diphosphate and CO_2, it is not possible to conclude from these data alone that this is the initial carboxylation reaction in whole cells. Whole cell experiments in which the bacteria are exposed for very short time periods to radioactive CO_2 are in progress, and these experiments may provide evidence concerning the nature of the initial carboxylation reaction.

From a comparative biochemical standpoint it is interesting to point out that to date the synthesis of PGA from ribulose diphosphate and CO_2 has been found to occur only in autotrophic systems. Further work however is necessary to establish the validity of this correlation.

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