where M and N are metals (assumed univalent for simplicity) and the reaction involves the interchange of these metals between the essentially metallic state in an amalgam phase (Hg) and an ionic state in electrolytic solution. The standard Gibbs free energy change in this reaction is

$$\Delta F^\circ = -RT \ln [(N^+)\gamma_{N^+}(M)f_M/(M^+)\gamma_{M^+}(N)f_N] \quad (2)$$

in which the quantities in parentheses represent equilibrium molalities of the species indicated in the appropriate phase, the γ's are activity coefficients relative to hyp. 1 molal std. states in the same phase, and the f's are activity coefficients relative to the respective pure crystalline metals as standard states. Then ΔF° is also ΔF of the reaction

$$M^+(\text{hyp. 1 } M) + N(c) = M(c) + N^+(\text{hyp. 1 } M)$$

If concentration measurements are made in the equilibrium reaction (1), then the problem is reduced to finding the γ's and the f's. The latter may be determined by equilibration of the ternary amalgam with an aqueous solution[3] in which the free energies of formation and activity coefficients of the ions are known. The effect of the γ's in the non-aqueous solution may be eliminated by performing a series of amalgam partition experiments over a range of concentration and extrapolating to infinite dilution.

Preliminary results of the application of this method to the alkali metal ions in liquid ammonia solution are presented in Table I. The activity coefficients of the alkali metals in the dilute ternary amalgams exhibit simple concentration dependences, which greatly facilitates this work. In the experiments, the amalgams were equilibrated with aqueous solutions or anhydrous ammonia solutions at 0° and samples of the equilibrium phases analyzed for the alkali metals by flame photometry. $\gamma_{N^+}/\gamma_{M^+} = 1$ has been assumed in each case. This is justified by the conductivity data for these systems[4] and the constancy of the ΔF° calculated on this assumption.

TABLE I

N	M	NH_3(l) solution anion	milli-moles/l.	ΔF° kcal./mole Equation 1		
Na	K	I^-	1	3.22	3.27	3.30
		Br^-	5	3.24	3.27	
		Br^-	1	3.27		
Na	Rb	Br^-	3	2.9		
			7	3.0	2.9	
			15	2.9		

These results agree within one kcal. with those calculated by Latimer and Jolly[5] from early discordant solubility and e.m.f. data, but differ considerably more from the free energies which may be calculated from the cell data of Sedlet and DeVries.[6]

It has been verified that this method is suitable for these ions in acetonitrile solutions as well.

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(3) G. M. Smith and L. S. Wells, THIS JOURNAL, **42**, 185 (1920).
(4) V. F. Hnizda and C. A. Kraus, *ibid.*, **71**, 1565 (1949).
(5) W. M. Latimer and W. L. Jolly, *ibid.*, **75**, 4147 (1953).
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ENZYMATIC CARBOXYLATION OF RIBULOSE DIPHOSPHATE[1]

Sir:

Previous work[2] has indicated that ribulose-1,5-diphosphate acts as the primary carbon dioxide acceptor in photosynthesis. This conclusion was based upon observations with intact photosynthetic organisms. We now wish to report the demonstration of this carboxylation in a cell-free system.

A cell-free preparation was obtained from freshly harvested *Chlorella* by five-minutes treatment at 2–5° in a 9 kc. Raytheon oscillator and subsequent removal by centrifugation of cell wall material and remaining whole cells. It has proved necessary to perform all operations rapidly (20–25 minutes from harvest) and in the cold (<5°) to obtain good fixation. Ribulose diphosphate was isolated from *Scenedesmus* which were killed rapidly in ethanol after thirty seconds of nitrogen flushing following steady-state photosynthesis in 4% carbon dioxide in air. Such conditions lead to maximum concentrations of ribulose diphosphate.[2] It was isolated from the extract by phenol chromatography of strips of extract on oxalic acid-washed Whatman No. 4 filter paper, carrying spots of labeled ribulose diphosphate as markers.

Ribulose diphosphate or other substrates were added to the extract and $C^{14}O_2$ was introduced immediately. The results of one-minute exposure to $C^{14}O_2$ are embodied in the table.

TABLE I

PRODUCTS FROM ONE MINUTE $C^{14}O_2$[a] FIXATION BY CELL-FREE PREPARATION FROM *Chlorella*

	Substrate added 0.1 μmole				
Products	None	Ribulose-di-P[c,d]	Ribulose-5-P[f]	Ribose-5-P	Fructose-di-P
Phosphoglyceric acid	0[b]	320	0	0	0
Phosphoenol pyruvic acid	0	60	0	0	0
Alanine	0	60	0	0	0
Malic acid	750	80	600	720	900
Aspartic acid	100	10	50	50	40
Citric acid[e]	200	60	250	200	210

[a] Specific activity, 4.8 × 10^6 c.p.m./μmole. [b] Counts per minute measured on paper chromatogram (self-absorption ~0.6) fixed during the first minute in μ.2 ml. solution containing contents of 10 mg. (wet weight) *Chlorella* cells. [c] Isolated by water elution from ether-washed paper chromatograms of known amounts of *Scenedesmus* extracts. The amount, 0.1 μmole, was calculated assuming a cellular concentration of 10^{-3} mole. [d] It will be noted that fixation with this substrate is markedly lower than with the others. Eluates of a similarly treated blank chromatogram also inhibited fixation due to toxic constituents remaining on the paper (*e.g.*, phenol, quinones, oxalic acid). [e] In longer fixation periods radioactivity became incorporated in other tricarboxylic acid cycle intermediates (succinic, fumaric and glutamic acids). No sugar phosphate labeling was observed. [f] We are indebted to Dr. B. L. Horecker for a sample of ribulose-5-P.

Complementary experiments with labeled ribulose diphosphate and this preparation demonstrated its rapid conversion to free sedoheptulose and a variety of normal metabolic intermediates which suggests a short lifetime for this substrate,

(1) The work described in this paper was sponsored by the U. S. Atomic Energy Commission.

(2) J. A. Bassham, A. A. Benson, L. D. Kay, A. Z. Harris, A. T. Wilson and M. Calvin, THIS JOURNAL, **76**, 1760 (1954).

limiting its availability for carboxylation. Similarly, labeled phosphoglycerate was largely converted to alanine in times longer than five minutes at room temperature.

Phosphoglycerate was identified by its chromatographic coördinates and cochromatography of the phosphatased compounds with authentic glyceric acid in four solvent systems. The degradation of the labeled glyceric acid showed 100% radioactivity in the carboxyl carbon,[3] and no (<2%) detectable activity for the β carbon.

It is clear that the extracts contain an enzyme (or enzymes) capable of catalyzing the carboxylation of ribulose diphosphate, specifically, to form phosphoglyceric acid. No intermediates between these compounds have been detected by this method which would have detected as little as an amount corresponding to 5% of the phosphoglyceric acid formed.

(3) We wish to thank Dr. J. A. Bassham of this laboratory for doing the degradations of glyceric acid samples.

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PENTOSE PHOSPHATE AND CO_2 FIXATION WITH SPINACH EXTRACTS

Sir:

Studies with photosynthesizing plants have shown that $C^{14}O_2$ fixed in this process is first detected in the carboxyl group of phosphoglyceric acid (PGA).[1] It has recently been postulated that the mechanism of this carbon dioxide fixation involves a carboxylation of ribulose diphosphate.[2,3] We have obtained a soluble extract from spinach leaves which catalyzes the fixation of carbon dioxide into PGA in the presence of ribose-5-phosphate. Light is not required by this system. Ribose-5-phosphate is more effective in stimulating carbon dioxide fixation than any other substrate tested (Table I).

TABLE I

EFFECT OF SUBSTRATES ON CARBON DIOXIDE FIXATION

Additions[a]	Total counts fixed
None	<600
+20 μmoles Ribose-5-PO_4	11,800
+20 μmoles Sedoheptulose-di-P	<600
+18 μmoles Fructose-di-P	<600
+100 μmoles Glucose-6-P	1,200
+100 μmoles Phosphoglyceric acid	<600
+100 μmoles Sodium pyruvate	<600

[a] The incubation mixtures contained 1.0 ml. of extract, equivalent to 0.2 g. of fresh spinach, 1 μmole of TPN, 50 μmoles of $PO_4^{=}$ buffer, *p*H 7.1, 35 μmoles of $K_2C^{14}O_3$ (2.15×10^7 c.p.m.), + substrate in a total volume of 2.2 ml. Incubations were at 30° for 12 minutes with carbon dioxide as the gas phase.

(1) J. G. Buchanan, J. A. Bassham, A. A. Benson, D. F. Bradley, M. Calvin, L. L. Daus, M. Goodman, P. M. Hayes, V. H. Lynch, L. T. Norris and A. T. Wilson in W. D. McElroy and B. Glass, "Phosphorus Metabolism," Vol. II, The Johns Hopkins Press, Baltimore, Md., 1952, p. 440.

(2) M. Calvin and Peter Massini, *Experientia*, **8**, 445 (1952).

(3) J. A. Bassham, A. A. Benson, L. D. Kay, A. Z. Harris, A. T. Wilson and M. Calvin, THIS JOURNAL, **76**, 1760 (1954).

PGA formed in various incubation mixtures was isolated, following addition of carrier PGA, by precipitation of the water-insoluble barium salts and subsequent purification of the barium fraction by weak acid hydrolysis to decompose hexose and heptose diphosphates, followed by ion exchange chromatography on Dowex-1. The PGA recovered, after removal of the phosphate group with potato phosphatase, was degraded with periodate in a manner similar to that described for the degradation of serine.[4] The distribution of label in the PGA isolated from incubation mixtures with either $C^{14}O_2$ or 1-C^{14}-ribose-5-phosphate is shown in Table II. With $C^{14}O_2$ as the precursor, essentially all of the radioactivity is found in the carboxyl group of PGA. When 1-C^{14}-ribose-5-phosphate is the precursor, over 70% of the C^{14} is in the β-carbon atom with most of the remaining activity found in the carboxyl carbon atom. The appreciable incorporation of the 1 carbon of ribose into the carboxyl carbon atom of PGA may be explained by reactions which form 1,3 labeled triose phosphate from 1-C^{14}-ribose-5-phosphate in leaf extracts.[5]

TABLE II

DISTRIBUTION OF C^{14} IN PHOSPHOGLYCERIC ACID

C^{14} Substrate	Relative specific activity: COOH C atom	α C atom	β C atom
$C^{14}O_2$[a]	10,300	820	660
1-C^{14}-ribose-5-PO_4[b]	2,100	470	6500

[a] The incubation mixture contained 1.0 ml. of spinach extract, 50 μmoles of $PO_4^{=}$ buffer, *p*H 7.1, 100 μmoles of ribose-5-phosphate, 200 μmoles of glucose, 400 units of glucose dehydrogenase, 2 μmoles of DPN, 2 μmoles of ATP, 83 μmoles of $K_2C^{14}O_3$ (2.46×10^7 c.p.m.) in a total volume of 2.44 ml. Glucose and glucose dehydrogenase, added in the early experiments, were later found to have no effect on the extent of carbon dioxide fixation. [b] The incubation mixture contained 1.0 ml. of spinach extract, 50 μmoles of $PO_4^{=}$ buffer, *p*H 7.1, 1 μmole of DPN, 5 μmoles of ATP, 200 μmoles of glucose, 400 units of glucose dehydrogenase, 50 μmoles of K_2CO_3 and 100 μmoles of 1-C^{14}-ribose-5-PO_4 (1.47×10^6 c.p.m.) in a total volume of 2.5 ml. Both incubations were for one hour at 25° with carbon dioxide as the gas phase.

TABLE III

TPN AND ATP STIMULATION OF CARBON DIOXIDE FIXATION

Additions[a]	Total counts fixed $\times 10^{-2}$
None	4
+1 μmole DPN	28
+1 μmole TPN	97
+2.5 μmoles ATP	23
+1 μmole DPN + 2.5 μmoles ATP	39
+1 μmole TPN + 2.5 μmoles ATP	161

[a] The incubation mixtures contained 1.0 ml. of charcoal-treated spinach extract, 50 μmoles of $PO_4^{=}$ buffer, *p*H 7.1, 35 μmoles of $K_2C^{14}O_3$ (2.46×10^7 c.p.m.), 96 μmoles of ribose-5-PO_4 + additions in total volume of 1.7 ml. The mixtures were incubated for ten minutes at 30° in an atmosphere of carbon dioxide.

Carbon dioxide fixation by the crude extract is stimulated by TPN and ATP (Table III). In other experiments it was found that the ATP effect is markedly enhanced by Mg^{++}. Isolation of PGA from an incubation mixture with ATP, TPN, and Mg^{++} has shown that the carbon dioxide fixed in these experiments is accounted for largely as PGA.

(4) W. Sakami, *J. Biol. Chem.*, **187**, 369 (1950).

(5) M. Gibbs and B. L. Horecker, *J. Biol. Chem.*, in press.