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.

RADIATION LABORATORY AND   J. R. QUAYLE
  DEPARTMENT OF CHEMISTRY   R. C. FULLER
UNIVERSITY OF CALIFORNIA   A. A. BENSON
BERKELEY, CALIFORNIA   M. CALVIN

PENTOSE PHOSPHATE AND CO₂ 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-PO₄	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 PO₄⁼ buffer, pH 7.1, 35 μmoles of K₂C¹⁴O₃ (2.15 × 10⁷ 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

| C¹⁴ Substrate | Relative specific activity | | |
	COOH C atom	α C atom	β C atom
C¹⁴O₂[a]	10,300	820	660
1-C¹⁴-ribose-5-PO₄[b]	2,100	470	6500

[a] The incubation mixture contained 1.0 ml. of spinach extract, 50 μmoles of PO₄⁼ buffer, pH 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 K₂C¹⁴O₃ (2.46 × 10⁷ 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 PO₄⁼ buffer, pH 7.1, 1 μmole of DPN, 5 μmoles of ATP, 200 μmoles of glucose, 400 units of glucose dehydrogenase, 50 μmoles of K₂CO₃ and 100 μmoles of 1-C¹⁴-ribose-5-PO₄ (1.47 × 10⁶ 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 × 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 PO₄⁼ buffer, pH 7.1, 35 μmoles of K₂C¹⁴O₃ (2.46 × 10⁷ c.p.m.), 96 μmoles of ribose-5-PO₄ + 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.

Since the spinach extracts contain pentose phosphate isomerase, the immediate precursor for carbon fixation may be ribose-5-phosphate or ribulosephosphate. ATP may enter the reaction either before or after pentose phosphate is cleaved. The formation of PGA from carbon atoms 1 and 2 of pentose phosphate and carbon dioxide requires a reductive step which may be linked by TPN to the oxidation of glyceraldehyde-3-phosphate arising from carbon atoms 3, 4 and 5. The green parts of plants have been shown to contain a triose phosphate dehydrogenase which is active with TPN.[6]

NATIONAL INSTITUTE OF ARTHRITIS AND
  METABOLIC DISEASES A. WEISSBACH[7]
NATIONAL INSTITUTES OF HEALTH P. Z. SMYRNIOTIS
UNITED STATES PUBLIC HEALTH SERVICE B. L. HORECKER
BETHESDA, MARYLAND
RECEIVED MAY 29, 1954

(6) M. Gibbs, Nature, 170, 164 (1952).
(7) Aided by a fellowship from the National Foundation for Infantile Paralysis.

ELECTRON TRANSFER BETWEEN NAPHTHALENE NEGATIVE ION AND NAPHTHALENE[1]

Sir:

We have investigated the electron transfer reaction between naphthalene negative ion and naphthalene dissolved in tetrahydrofuran by a spectroscopic method.

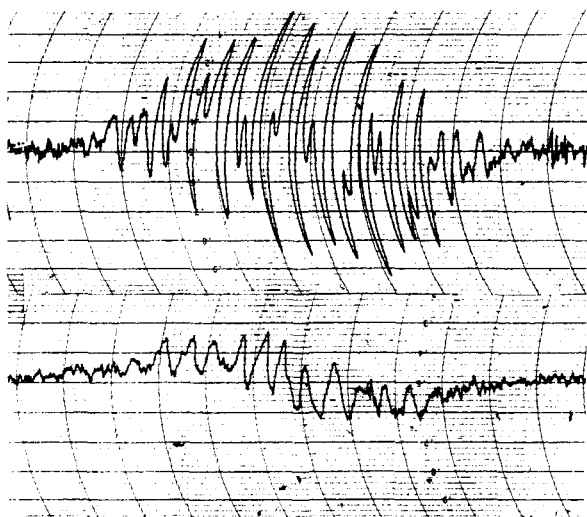Naphthalene negative ion, in dilute solution in

Fig. 1.—First derivative with respect to magnetic field versus magnetic field of the paramagnetic resonance absorption of naphthalene negative ion: upper curve $(C_{10}H_8^-) = 5 \times 10^{-4} M$, $(C_{10}H_8) = 0.0$; lower curve $(C_{10}H_8^-) = 5 \times 10^{-4} M$, $(C_{10}H_8) = 0.35 M$.

tetrahydrofuran, possesses a complex paramagnetic resonance absorption spectrum of twenty-eight lines.[2] The lines are hyperfine components arising from interaction between the magnetic moment of the unpaired electron and the magnetic moments of the protons in the naphthalene negative ion.

For our purposes it is convenient to describe the spectrum by means of the reciprocal line breadths and reciprocal intervals. The reciprocal breadth of the individual lines, in dilute solutions of the sodium salt of naphthalene negative ion, is 6×10^{-7} seconds; the reciprocal intervals are in the neighborhood of 3×10^{-7} seconds.

When naphthalene is added to a dilute solution of naphthalene negative ion, the paramagnetic resonance spectrum of the latter is altered. Addition of a small amount of naphthalene leads to broadening of the individual hyperfine components. As larger amounts of naphthalene are added the hyperfine components merge into a single peak with broad tails extending beyond the region encompassed by the original hyperfine pattern.

A representative pair of spectra taken at 30°, one with the naphthalene negative ion at a concentration of $5 \times 10^{-4} M$ and with no added naphthalene, the other with naphthalene negative ion at $5 \times 10^{-4} M$ and naphthalene at $0.35 M$ are given in Fig. 1. (The spectra are displayed as first derivative of absorption with respect to magnetic field versus field.) The broadening of the individual lines may be observed by direct measurement, by the merging together of close components, and by the decrease in amplitude between maxima and minima.[3]

The line broadening in the presence of naphthalene we ascribe to the transfer of electrons from naphthalene negative ions to naphthalene molecules. Such transfer limits the lifetimes of the quantum states responsible for the hyperfine pattern and consequently broadens the lines. According to this interpretation of the line broadening the mean lifetime of an individual naphthalene negative ion in the presence of 0.8 M naphthalene is 1.2×10^{-6} second. Under the assumption that the electron transfer follows a second order rate law, the rate constant at 30° is 1.0×10^6 liter mole^{-1} sec.$^{-1}$.

The method here described does not depend, as do most rate determinations, on some method of distinguishing reactants from products. The observations reveal directly the quantity of interest—the mean time during which a particular configuration persists undisturbed.

DEPARTMENT OF CHEMISTRY
WASHINGTON UNIVERSITY R. L. WARD
ST. LOUIS, MISSOURI S. I. WEISSMAN
RECEIVED MAY 24, 1954

(2) D. Lipkin, D. E. Paul, J. Townsend and S. I. Weissman, Science, 117, 534-535 (1953); S. I. Weisman, J. Townsend, D. E. Paul and G. E. Pake, J. Chem. Phys., 21, 2227 (1953); D. E. Paul, Ph.D. Thesis, Washington University, 1954.
(3) Under the conditions prevailing in this experiment the amplitude is proportional to the square of the reciprocal line breadth.