ruvate-pyruvate phosphokinase system coupled to the DPNH-lactic dehydrogenase system.[5,6] More direct evidence for equations (1–5) was obtained by ion-exchange chromatographic analysis of the reaction products in 2 experiments. In the reaction of UTP and A5P (C^{14}-labeled) (Table I),[7] roughly equal concentrations of the mono-, di- and triphosphates of uridine and adenosine were obtained. The identity and quantity of each component was determined by spectrophotometric measurements and, where indicated, by radioactivity measurements and enzymatic assays. No reaction occurred when either UTP or A5P was omitted from the incubation mixture.

TABLE I: TRANSPHOSPHORYLATION BETWEEN ADENINE AND URACIL NUCLEOTIDES

The reaction mixture (2.05 ml.) contained 0.2 ml. of glycylglycine buffer (0.5 *M*, *p*H 7.5), 0.1 ml. of $MgCl_2$ (0.1 *M*), 0.5 ml. of 2-C^{14}-A5P (0.002 *M*, 82,000 c.p.m./μmole), 0.25 ml. of UTP (0.004 *M*), and 1.0 ml. of the heated enzyme preparation (fraction I heated in a boiling water-bath for 3 minutes at *p*H 1.5; 0.57 mg. of protein). After incubation at 36° for 1 hour, the reaction mixture was heated in a boiling water-bath for 3 minutes.

	0 min. μmoles	60 min. μmoles	Δ μmoles	Δ Total c.p.m.
A5P[a,b]	0.89	0.30	−0.59	−45,260
UTP[c]	.83	.32	− .51	
ADP[d]	.00	.33	+ .33	+26,800
ATP[e]	.00	.28	+ .28	+23,100
U5P[f]	.00	.31	+ .31	
UDP[g]	.00	.35	+ .35	

[a] Anion-exchange chromatography of the reaction mixture yielded A5P and UTP free from each other and the other nucleotides; ADP and U5P were eluted together; ATP and UDP were separated except in a few fractions. [b] Estimated spectrophotometrically at 260 mμ and by radioactivity measurements. [c] Estimated spectrophotometrically at 260 mμ. [d] Estimated by radioactive measurements and with pyruvate phosphokinase (0.29 μ mole found). [e] Estimated by radioactivity measurements. Fractions assayed with hexokinase and glucose-6-phosphate dehydrogenase (A. Kornberg, *J. Biol. Chem.*, **182**, 779 (1950)), gave results in agreement with the radioactivity measurements. [f] Estimated spectrophotometrically at 260 mμ correcting for the ADP present. [g] Estimated spectrophotometrically at 260 mμ correcting for the ATP present. Fractions assayed with pyruvate phosphokinase gave results in agreement with the spectrophotometric data.

A second experiment demonstrates the stoichiometry of the reaction between UTP and U5P (C^{14}-labeled) (Table II).[7] In the absence of either UTP or U5P, no UDP was formed. UDP, isolated chromatographically, was identified by its absorption spectrum (max. at 262 mμ, λ280/λ260 = 0.37 in *N* HCl) and by the molar ratios of uracil:acid-labile phosphate:total phosphate:phosphate acceptor (in the phosphopyruvate-pyruvate phosphokinase system) of 1.00:0.97:1.94:0.94.

The enzyme activity responsible for reactions (2–5) is relatively stable to heating at acid *p*H (47% loss after 3 min. at 100° at *p*H 1.3). Thus it resembles the myokinase (adenylate kinase) of yeast[5] in this respect; its behavior on fractionation is also similar. While these findings suggest that a single enzyme is responsible for the mixed myokinase reaction described in equations (1–5), further work is necessary to establish this point.[8] The absence of the nucleoside diphophoskinase of Berg and Joklik[9] in our preparation was demonstrated spectrophotometrically by the failure of ATP to react with inosine diphosphate.

These results and reports of natural occurrence of nucleoside di- and triphosphates,[9] make it likely that enzymes with comparable functions to this yeast enzyme are widespread in nature.[10]

(5) A. Kornberg and W. Pricer, Jr., *J. Biol. Chem.*, **193**, 481 (1951).

(6) With ATP and various nucleoside monophosphates, this assay system was used to study the specificity of the enzyme. Phosphate transfer from ATP was measured by the appearance of pyruvate. In addition to U5P and A5P, desoxyadenosine-5′-phosphate and guanosine-5′-phosphate were active. Apparently inactive were the 5′-substituted phosphate esters of inosine, desoxycytidine, desoxyguanosine and nicotinamide riboside, and adenosine-3′-phosphate.

(7) The substrates were purified by anion-exchange chromatography.

TABLE II

STOICHIOMETRY OF UDP SYNTHESIS FROM U5P AND UTP

The reaction mixture (2.0 ml.) contained 0.2 ml. of glycylglycine buffer (0.5 *M*, *p*H 7.5), 0.1 ml. of $MgCl_2$ (0.1 *M*), 0.2 ml. of 2-C^{14}-U5P (0.01 *M*, 17,000 c.p.m./μ mole), 0.45 ml. of UTP (0.004 *M*), and 0.8 ml. of the enzyme preparation (fraction III, containing 0.26 mg. of protein). After incubation at 36° for 45 minutes, the reaction mixture was heated in a boiling water-bath for 3 minutes.

	0 min. μmoles	45 min. μmoles	Δ μmoles	Δ c.p.m.
U5P[a]	2.20	1.81	−0.39	−9270
UTP[a]	1.64	1.27	−0.37	+5220[c]
UDP[a,b]	0.00	0.82	+0.82	+5330[c]

[a] Estimated spectrophotometrically at 260 mμ after chromatography on Dowex-1 anion-exchange resin. [b] Estimated with pyruvate phosphokinase. [c] The specific activity of the UTP is relatively high and that of the UDP, low.

(8) NOTE ADDED IN PROOF.—Further studies have revealed that heating of the enzyme preparation at neutral *p*H (100°, 3 min.) results in a recovery of 14.6% of the adenylate kinase activity (equation (5)) but less than a 1% recovery of the uridylate kinase activity (equations (2) and (3)). This evidence indicates the presence of two distinct enzyme activities in our preparation.

(9) R. Bergkvist and A. Deutsch, *Acta Chem. Scand.*, **7**, 1307 (1953); H. Schmitz, V. R. Potter, R. B. Hurlbert and D. M. White, *Cancer Research*, **14**, 66 (1954).

(10) NOTE ADDED IN PROOF.—Dr. A. Munch-Petersen and Dr. D. R. Sanadi have observed reactions similar to those described in this report (personal communications) in yeast and animal tissues, respectively.

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RECEIVED JUNE 1, 1954

THE AMALGAM PARTITION METHOD FOR THE DETERMINATION OF IONIC FREE ENERGIES IN NON-AQUEOUS SOLUTIONS[1]

Sir:

The standard free energies of formation of ions in solution, although important to an understanding of the properties of electrolytic solutions, are practically unknown except for aqueous solutions. The only widely applicable methods previously available for obtaining such data are measurements of the potentials of reversible e.m.f. cells, of the solubility equilibria of pure electrolytes of known free energy, and of the partition equilibria between immiscible aqueous and non-aqueous electrolyte solutions.[2] A new method is described here which leads to the relative free energies of formation of metallic ions in suitable non-aqueous solvents.

This method involves equilibria of the type

$$M^+ + N(Hg) = M(Hg) + N^+ \qquad (1)$$

(1) This work was supported by the Atomic Energy Commission.

(2) H. L. Friedman and G. R. Haugen, THIS JOURNAL, **76**, 2060 (1954).

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).
(6) J. Sedlet and T. DeVries, *ibid.*, **73**, 5808 (1951).

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).