

only monocalcium phosphate, a component of "superphosphate" fertilizer, applied at the highest dose level of about 600 lb/acre, effected a statistically significant reduction of Sr^{85} uptake in all four fractions of the wheat plant. Even at the very reasonable dosage of about 60 lb/acre, monocalcium phosphate gave a statistically significant reduction in Sr^{85} content in the grain and chaff. These results appear to indicate that a combined utilization of both the calcium effect (4) and the phosphate effect (5) may have some promise as a means of soil treatment to reduce plant uptake of radiostrontium.

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Vitamin B₁₂ Requirement of a Marine Blue-Green Alga

Abstract. A species of *Synechocystis* isolated from a marine mud has an absolute requirement for vitamin B₁₂. Analogues of B₁₂, including cobinamide and α -(adenyl) cobamide cyanide, satisfactorily substitute for vitamin B₁₂. Methionine alone or in combination with other compounds supports only a low level of growth.

Recent discoveries that many phytoplankton organisms require vitamins (1), notably B₁₂, point to vitamin cycles as significant determinants of the productivity of the sea. Blue-green algae are of widespread occurrence in marine environments and are undoubtedly important contributors to the productivity of these areas. Despite this, little is known of their growth and nutrition. A blue-green alga belonging to the genus *Synechocystis* (termed 17a in our laboratory), isolated from a mud sample from Long Island Sound, has been found to have an absolute requirement for vitamin B₁₂. Its B₁₂ requirement and possible sparing action of certain compounds were investigated in detail.

The ASP-2 medium of Provasoli (2), modified by increasing phosphate 10-

fold and nitrate 20-fold, initial pH 8.2, has been found suitable for the enrichment and isolation of many marine blue-green algae, and for subsequent growth studies. The *Synechocystis* was isolated in pure culture by repeated pour plates of the modified ASP-2 medium plus 2 percent agar.

Growth studies were carried out with a less elegant modification of the test-tube culture method of Myers (3). The organism was grown in test-tubes in 10 ml of modified ASP-2 medium in a thermostatted bath at 37°C with 1 percent CO₂ in air bubbling through the tubes. Growth was measured as optical density at 600 m μ , with a Bausch and Lomb Spectronic 20. The optical density was reasonably linear with cell concentration up to an optical density of 1.0. At this density the cell volume was 0.68 mm³/ml.

Growth is expressed in terms of the specific growth rate k with the dimensions of log₁₀ units per day (4), or as the optical density reached in an arbitrary time long enough to show effects of materials which become limiting, but which are present in sufficient amount to allow equivalent growth rates up to an optical density of 1.0. Optical density readings > 1.0 were obtained by diluting the suspension with fresh medium. The B₁₂ analogues were diluted from stock solutions (5). Table 1 shows the growth response of *Synechocystis* to B₁₂, B₁₂ analogues, and methionine.

Experiment 1 shows that the B₁₂ requirement is absolute and demonstrates the response to increasing B₁₂. Methionine alone supported only limited growth. Experiment 2 shows the response to various B₁₂ analogues. Growth was equally good on all the analogues tested except the two analogues with adenine substituted in the 2-position. With these two compounds the growth rate and the total growth in 40 hours were very low, possibly reflecting a steric effect which causes adenyl B₁₂ compounds substituted in the 2-position to be converted only with difficulty to the coenzyme form within the cell.

Several compounds and combinations were tried for sparing action of the B₁₂ requirement for *Synechocystis*. Deoxyribosides, S-3 vitamin mix (2), choline, sarcosine, RNA, DNA, and DNA hydrolyzed with formic acid allowed no growth. Casamino acids and yeast extract allowed only slight growth at 40 hours. Soytone was somewhat better.

With B₁₂ at a saturating level for growth, the addition of Casamino acids or yeast extract caused no increase in growth rate. The addition of Soytone with B₁₂ resulted in some inhibition of growth.

Table 1. Response of a species of *Synechocystis* (organism 17a) to B₁₂, methionine, and B₁₂ analogues. B₁₂ nomenclature is taken from *J. Am. Chem. Soc.* **82**, 5575 (1960). OD, optical density.

Addition	k	OD at 40 hr
<i>Experiment 1</i>		
No B ₁₂	0	0.027
B ₁₂ , 0.05 $\mu\text{g/lit.}$	1.82	1.52
B ₁₂ , 0.1 $\mu\text{g/lit.}$	2.18	3.62
B ₁₂ , 0.5 $\mu\text{g/lit.}$	2.10	7.45
B ₁₂ , 1.0 $\mu\text{g/lit.}$	2.20	7.70
DL-Methionine, 20 $\mu\text{g/ml}$		0.147
DL-Methionine, 200 $\mu\text{g/ml}$		.268
<i>Experiment 2</i>		
No B ₁₂	0	0.007
α -(5, 6-Dimethylbenzimidazolyl) cobamide cyanide, 1.0 $\mu\text{g/lit.}$	2.30	5.09
α -(2-Methyladenyl) cobamide cyanide, 2.0 $\mu\text{g/lit.}$		0.96
α -(Adenyl) cobamide cyanide, 2.0 $\mu\text{g/lit.}$	2.36	7.21
Cobinamide, 2.0 $\mu\text{g/lit.}$	2.20	5.09
α -(5-Hydroxybenzimidazolyl) cobamide cyanide, 2.0 $\mu\text{g/lit.}$	2.36	6.20
α -(2-Methylmercaptoadenyl) cobamide cyanide, 2.0 $\mu\text{g/lit.}$		0.27
α -(5-Methyladenyl) cobamide cyanide, 2.0 $\mu\text{g/lit.}$	2.20	5.53
α -(Benzimidazolyl) cobamide cyanide, 2.0 $\mu\text{g/lit.}$	2.36	4.95

Various 6-substituted pteridines known to occur in blue-green algae (6), including pteridines isolated from *Synechocystis*, were tested singly and together with methionine for possible sparing action. None had any marked effect except 2,6-diamino-4-hydroxy-pteridine (7), which, in the presence of methionine, allowed twice as much growth in 40 hr as methionine alone.

In common with many other marine algae and protozoa (8), it appears that marine blue-green algae will also show a requirement for exogenous B₁₂. The red pigmented marine blue-green alga, *Phormidium persicinum*, requires B₁₂ (9). An examination of the vitamin requirements of other marine blue-green algae recently isolated in this laboratory has shown that more than 50 percent also require B₁₂. Whether this requirement for a vitamin and possibly utilization of other organic materials reflects a greater degree of heterotrophy in marine blue-green algae than in the fresh water blue-green algae presently known remains to be seen (10).

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Synthesis of Uracil under Conditions of a Thermal Model of Prebiological Chemistry

Abstract. Uracil has been formed under thermal conditions which yield other materials of theoretical prebiological significance. The chemical evidence for the identity of uracil rests on chromatography, spectrography, color tests, and melting points and analyses of isolated material. The place of uracil in a scheme of biochemical origins, including carbon dioxide as an intermediate, is discussed.

A thermal model of biochemical origins has yielded a network of chemical pathways resembling a flowsheet of biosynthesis (1), amino acid copolymers having many of the properties of proteins (2), and microspherical units with some of the properties of bacterial boundaries (1). Part of the uniqueness of this model lies in the fact that these phenomena emerge in a continuum of physical conditions (1). One of the first products in the thermal experiments was ureidosuccinic acid (3), an intermediate in the biosynthesis of pyrimidines (4). This report describes the direct synthesis of uracil itself under conditions consistent with the other physical details of the model and of its recapitulationist emphasis on malic acid (5) which is in turn derivable from simpler compounds (Fig. 1).

In the light of the recent finding that polyphosphoric acid promotes the thermal condensation of amino acids (1) and the recognition of the many biological activations by polyphosphate, earlier studies of the reaction of malic acid and urea were extended by testing polyphosphoric acid as a reactive solvent. In the first experiments hypothetical hydrogen acceptors such as nicotinamide were included, but controls showed that the reaction proceeded in the presence of polyphosphoric acid alone. No chromatographic evidence of uracil was obtained in numerous experiments in which ureidosuccinic acid or oxaloacetic acid was included instead of malic acid.

Chromatography was carried out with ascension in one dimension on 3 MM Whatman chromatography paper with butanol-1 saturated with 10-percent urea or butanol-1 saturated with 7N NH_4OH . The R_F values of authentic uracil and of one spot was 0.32 in the former solvent and 0.16 in the latter solvent. The spots were identified by fluorescence in the rays from a Mineralight R-51 lamp. Uracil was isolated from a simultaneous series of bar chromatograms by elution with 0.1N HCl.

The conditions studied in the synthesis of uracil included temperatures in the range of 100° to 140°C, heating periods of from ¼ to 2 hr, and a range of ratios of polyphosphoric acid (Victor Chemical Co.) to equimolar proportions of malic acid and urea. Uracil was obtained over the entire range of temperature. Increase in proportion of polyphosphoric acid had the most pronounced effect of the factors tried. The variation in one experiment is shown in Table 1. The yield was determined by elution after chromatography and estimation against a standard curve at 260 $\text{m}\mu$ on a Beckman DU spectrophotometer.

From one of these samples was isolated by bar chromatography 86 mg of white recrystallized (H_2O) uracil of melting point and mixed melting point, 335°C, decrease uncorrected. Calculated for $\text{C}_4\text{H}_4\text{N}_2\text{O}_2$: C, 42.85; H, 3.59; N, 24.99. Found (by the Mikroanalytisches Laboratorium, Bonn, Germany): C, 42.84; H, 3.71; N, 24.84.

The uracil synthesized in the presence of polyphosphoric acid had a melting point and mixed melting point with authentic material of 335°C, decrease uncorrected. The Wheeler-Johnson color test with bromine-water and barium hydroxide (6) was positive

Table 1. Yields of uracil from varying amounts of polyphosphoric acid with 0.010 mole of malic acid and 0.015 mole of urea at 130°C for 60 min.

Amount of polyphosphoric acid (ml)	Yield of uracil (mmole)
0.5	Negligible
1.0	0.04
2.0	0.06
4.0	0.4
8.0	1.2
16.0	1.4

Ultraviolet absorption spectra of the newly synthesized material in acid, in neutral, and in alkaline solution were identical with the corresponding spectra of authentic material. The infrared absorption in Nujol mull of the new material was also identical with that of authentic uracil.

The thermal formation of urea from the inorganic substances ammonia and carbon dioxide is well documented as an industrial reaction and can thus explain the primordial origins of urea (7). Malic acid can be visualized as producible in the primitive context from acetic acid (8) which could arise in turn from methane, water, and hydrogen (9) or from glycine by radiolysis (10) or in other ways (11). Although the presence of carbon dioxide in the original atmosphere has been a controversial issue (11), it should be recognized that carbon dioxide would probably become available as the result of reactions of other compounds—for example, Miller has found substantial proportions of carbon dioxide in a sparked atmosphere which contained none at the outset (9).

The results reported lead to a revised thermal flowsheet, as shown in Fig. 1. The now enlarged picture of prebiological chemical reactions, including synthesis

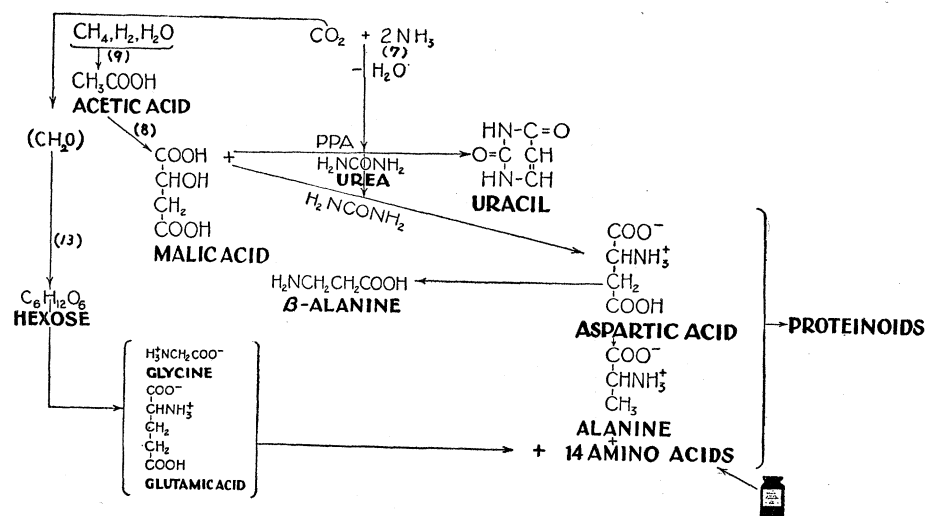


Fig. 1. Flowsheet, predominantly thermal, of suggested prebiological reactions. Unnumbered reactions are documented in (1).