effects of pressure, one can say that excitonic energy transport is of negligible importance for the thermal conductivity of mantle materials.

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Cyanovinyl Phosphate: A Prebiological Phosphorylating Agent?

Abstract. *Cyanovinyl phosphate may be prepared by the addition reaction of phosphate to cyanoacetylene. Kinetic studies have established that cyanovinyl phosphate undergoes a slow pseudo-first-order hydrolysis in aqueous solution. Cyanovinyl phosphate converts uridine to uridine monophosphate and phosphate to pyrophosphate.*

There have been several reports describing the synthesis of the pyrophosphate bond and the alkyl phosphate bond under potential prebiological conditions. Potassium cyanate effects the conversion of orthophosphate to pyrophosphate but only on an apatite surface (*1*). Pyrophosphate and nucleotides are produced by the thermal condensation of orthophosphate and nucleosides under anhydrous conditions (*2*). Unfortunately these syntheses proceed only under rather restrictive conditions. A more attractive mode for the prebiotic synthesis of alkyl phosphates and pyrophosphates is one which will proceed in dilute aqueous solution and as such may have taken place on the primitive earth in a large body of water. However, experiments designed with this goal in mind have yielded negative results (*3*).

Recently it has been proposed that cyanoacetylene has a central role in prebiological synthesis (*4*). It is one of the major products resulting from the action of an electric discharge on methane-nitrogen mixtures. Cyanoacetylene has been converted to cytosine on treatment with cyanate or cyanogen and it has been converted to asparagine and aspartic acid with cyanide and ammonia (*4*). The conversion of cyanoacetylene to cyanovinyl phosphate and the possible role of this high-energy phosphate compound as a prebiological phosphorylating agent are discussed in this report.

Cyanovinyl phosphate may be prepared in dilute solution by the addition of $10^{-4}M$ cyanoacetylene to $0.1M$ phosphate at *p*H 7 to 9. The product is obtained in over 90 percent yield as measured by the intensity of its absorption maximum at 225 mμ ($E =$ 14,000) (*p*H 7). Cyanovinyl phosphate may be synthesized on a preparative scale by heating 100 ml of a solution containing 0.1 mole of Na_2HPO_4 and 0.01 mole of cyanoacetylene at 60°C for 1 hour. The inorganic phosphate is then precipitated with 0.1 mole of barium acetate after dilution to 500 ml with water. The barium salt of cyanovinyl phosphate, (1.1 g, 35 percent) is then precipitated from the filtrate by addition of 350 ml of ethanol. Analysis: calculated for $C_3H_2NO_4PBa \cdot H_2O$: C, 11.91; H, 1.33; N, 4.63; P, 10.21. Found: C, 11.67; H, 1.55; N, 4.97; P, 10.69.

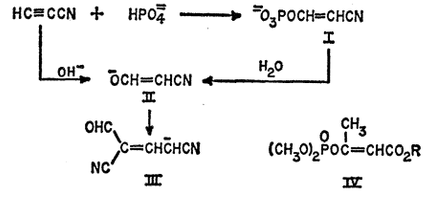
The cyclohexylamine salt was prepared from the barium salt by the procedure of Tener (*5*). Analysis: calculated for $C_{15}H_{30}N_3O_4P$: C, 51.86; H, 8.71; N, 12.10; P, 8.92. Found: C, 51.88; H, 8.81; N, 12.15; P, 8.58.

The structure of cyanovinyl phosphate follows from the method of synthesis, the elemental analyses, the ultraviolet spectrum (*6*), and infrared absorption (KBr) at 2235 cm^{-1} (CN) and 1650 cm^{-1} (C=C).

This appears to be a general synthesis of alkyl cyanovinyl phosphates. For example, the addition reaction of ethyl phosphate to cyanoacetylene may be observed by the appearance of an absorption maximum at 220 mμ.

Cyanovinyl phosphate is an exceptionally stable enol phosphate. The kinetics of its hydrolysis at *p*H 7, 9, and 11 were followed spectrophotometrically at 60°C and 100°C. When the hydrolyses were performed at *p*H 7 and *p*H 9, compounds II and III could be detected by absorption maxima at 248 mμ and 310 mμ, respectively. The same compounds are produced by the action of base on cyanoacetylene (*4*). At *p*H 11 only a small level of II was apparent. This is to be expected, since compound II is readily cleaved at high *p*H and also it does not condense to III in strong base (*4*).

The hydrolysis was pseudo-first-order and *p*H-independent in the *p*H range 7 to 11 (the kinetic data at 100°C are plotted in Fig. 1) with half-lives of 0.049 day ($K_1 = 14.1$ day^{-1}) at 100°C and 4.9 days ($K = 0.14$ day^{-1}) at 60°C. Half-lives for hydrolysis of 300, 10^3, and 10^4 days were obtained by extrapolation of a log K versus $1/T$ plot to 30°, 20°, and 0°C, respectively. These data may be contrasted with the hydrolysis of IV (R = H), which has a half-life of 2 days at *p*H 11 and 30°C (*7*).



Since the rate of hydrolysis is pseudo-first-order and *p*H-independent over a wide range, the reaction mechanism probably involves the attack of a water molecule on the cyanovinyl phosphate anion.

Enol phosphates are known to be efficient phosphorylating agents in nonaqueous systems. However, the high reactivity of enol phosphates precludes their use in aqueous systems (*8*). The stability of aqueous solutions of cyanovinyl phosphate prompted our investigation of its properties as a possible prebiological phosphorylating agent.

We found that orthophosphate is converted to pyrophosphate, and uridine to uridine-5′-phosphate, by an aqueous solution of cyanovinyl phosphate. A 2 to 4 percent yield of pyrophosphate is obtained when 0.3 ml of $0.2M$ ammonium cyanovinyl phosphate is heated for 18 hours at 85°C with $0.25M$ orthophosphate in the *p*H range 6 to 8 (*9*). Addition of 0.025 to 0.05 g of calcium phosphate, a catalyst for some phosphorylation reactions (*1*), did not enhance the yield of pyrophosphate. Uridine-5′-phosphate (UMP) was synthesized in 3.9 percent yield (based on

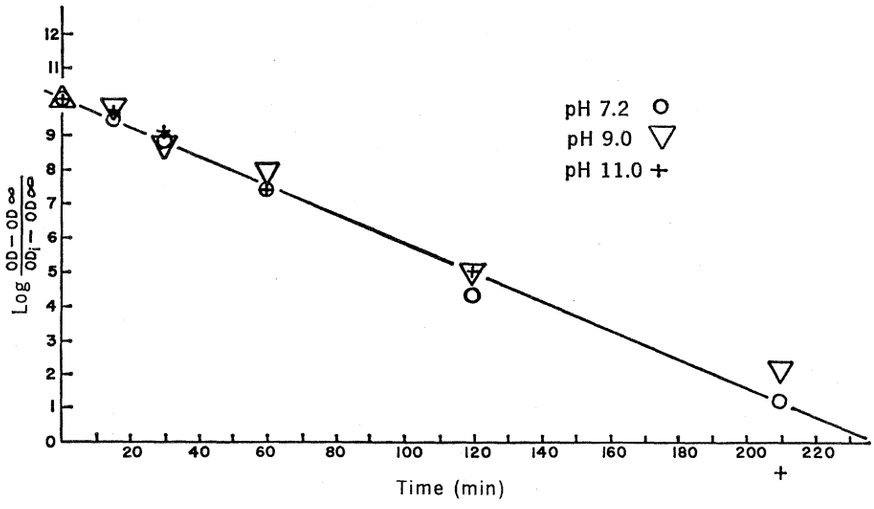


Fig. 1. Kinetics of the hydrolysis of cyanovinyl phosphate at 100°C.

cyanovinyl phosphate) by heating 0.7 ml of 0.75*M* sodium cyanovinyl phosphate and 1.6*M* uridine (containing 0.1 curie of C^{14}-uridine) at 60°C for 18 days. The UMP was detected by the coincidence of the radioactivity with that of an authentic UMP sample on paper chromatography (*10*). The ultraviolet spectrum of a sample eluted from the paper chromatogram was identical with that of UMP (λ_{max} 260 mμ).

To assess the prebiotic significance of cyanovinyl phosphate as a phosphorylating agent, the relative ease with which it transfers orthophosphate to uridine and water must be determined. If the selectivity for uridine is high then cyanovinyl phosphate may have prebiotic significance. If it is low then cyanovinyl phosphate will only undergo hydrolysis in dilute solution without effecting any phosphorylation. This selectivity factor, which was first defined by Lohrmann and Orgel (*11*), may be formulated as shown, with M_u the molarity of uridine, X the percent yield of UMP, and assuming the molarity of water to be 55:

$$\text{selectivity}\left(\frac{\text{uridine}}{\text{water}}\right) = \frac{55}{M_u}\left(\frac{X}{100 - X}\right)$$

This selectivity factor is only 1.3 for uridine, suggesting that very little UMP was formed prebiotically in homogeneous solution from uridine and cyanovinyl phosphate. This result is in agreement with the usual observation that water and alcohols are phosphorylated at about the same rate (*11, 12*).

However, orthophosphate is phosphorylated 4.5 to 9.2 times as efficiently as water, suggesting that cyanovinyl phosphate may have been the prebiotic source of the pyrophosphate bond. For example, 1 percent and 0.1 percent conversions to pyrophosphate would be obtained starting with 0.1*M* and 0.01*M* phosphate, respectively, and assuming a selectivity factor of 5.5.

A potential unified synthesis of pyrimidines, amino acids, and a high-energy phosphate compound from one source makes cyanovinyl phosphate attractive as a potential prebiological phosphorylating agent.

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Transplantation of Marrow to Extramedullary Sites

Abstract. *Autologous fragments of transplanted marrow have survived in various extramedullary sites in the rat, rabbit, and dog. Survival of the fragments occurs with a complete reconstitution of the hemopoietic and adventitial structures. The process originates from a network of surviving reticular cells which proliferate and differentiate into osteoblasts and give rise to trabecular bone. Later, the reticular cells reconstruct the marrow's microcirculation. Hemopoietic repopulation of the marrow implant takes place only after its sinusoidal microcirculation has been established.*

The failure of implants of autogenous bone marrow to survive in extramedullary sites has led to a conclusion that hemopoiesis cannot be sustained except in the marrow cavity. This concept is supported by indications that hemopoiesis requires the unique microcirculation of the marrow. The work of Knospe, Blom, and Crosby (*1*) demonstrates the relation between the two major elements of bone marrow, the blood forming elements and the adventitial elements which comprise the microcirculation of marrow. Hemopoiesis cannot be sustained except when sinusoidal blood vessels are present and functioning normally. In earlier attempts to transplant fragments of marrow or aspirates, with one exception (*2*), this specialized adventitia was evidently not retained (*3, 4*). Our success in transplanting the bone marrow of rat, rabbit, and dog into extramedullary tissues evidently requires the use of a relatively large piece of undisrupted marrow to permit a reconstruction of the sinusoids.

More than 200 Wistar albino rats (300 to 500 g) of both sexes were used. A window was cut by a dental drill in the anterior plate of the tibia, the sequestrum was removed, and the marrow was lifted out on the tip of a spatula; by this method we obtained a relatively large piece of marrow without disrupting the tissue. These fragments were implanted into splenic, renal, subcutaneous, hepatic, muscular, and omental tissues. They were removed periodically; after obtaining touch imprints on slides for cytological and cytochemical examinations, we fixed the tissue in 10 percent buffered formalin for at least 3 days and decalcified it in Prenny's solution. Sections were stained