orientations. Phase II contains tetrameric rings that do not invert but that are randomly distributed with respect to orientation in a static disorder. Phase III contains tetrameric rings that are in an orderly array with respect to orientation.

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6. The quantities R and R' are defined as follows:

$$R = \frac{\Sigma \mid F_o - F_c \mid}{\Sigma \mid F_o \mid}$$

$$R' = \frac{[\Sigma\, w(F_o - F_c)^2]^{\frac{1}{2}}}{(\Sigma\, w\, F_o{}^2)^{\frac{1}{2}}}$$

where F_o and F_c are the observed and calculated structure factors and w is the weight, in this case the set constant.
7. The use of the terms "right-handed" and "left-handed" needs qualification in point group $\bar{4}$. Two of the monomer components of a $\bar{4}$ tetramer must be right-handed and two left-handed, by definition of the symmetry operations. When a mirror plane operates on the tetramer, the right-handed and left-handed identities are interchanged. The transformed tetramer is not an enantiomorph of the original tetramer in the classical sense. It is formally equivalent to the original tetramer, being subjected to a compound rotation of 180° around an axis through the center of the tetramer and normal to the mirror plane combined with a rotation of 90° around the $\bar{4}$ axis. This qualification should be incorporated in the description of phase IV (*2*).
8. The complete Hermann-Mauguin space group symbol for this cell may be written as $A2_1/d''11$. The conventional symbol d for a diamond glide does not suffice in the monoclinic system. Here we use the symbol d'' for a "diamond glide of the second kind" with glide components of $b/4 - c/4$ ($0kl$ reflections extinguished unless $k - l = 4n$) and propose the symbol d' for a "diamond glide of the first kind" with glide components of $b/4 + c/4$ ($0kl$ reflections extinguished unless $k + l = 4n$).
9. The static disorder found in phase IV and proposed for phase II does not suffice to give good agreement between F_o and F_c for phase I (C. K. Johnson, personal communication).

23 September 1970

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Opal Precipitation by Marine Gastropods (Mollusca)

Abstract. *The high silicon content of certain radular sites of the Patellacea (Mollusca, Gastropoda) is shown by infrared absorption spectrums to be fixed in the form of the mineral opal. Opal and goethite mineralize the cusps and bases of the teeth. The presence of opalized base plates only in the families Acmaeidae and Lepetidae appears to be of taxonomic significance. Minimum values of the volume of fixation and of the turnover rate of opal by the Patellacea are calculated to assess the role of this previously neglected taxon in biological fixation of silica in the oceans. The significance of these organisms points to the much needed study of the silica transport system in tissue-grade Metazoa.*

The fixation of silica by marine organisms, apparently in the form of opal ($SiO_2 \cdot nH_2O$), is generally identified with certain unicellular algae, heterotrophs, and Protozoa and with cellular-grade Metazoa. They include the diatoms, some dinoflagellates, the silicoflagellates, ebridians, most radiolarians, the Hyalispongia, many Demospongia, and the coralline sponges (*1*). Determinations of elements present in invertebrates of the tissue grade long ago established that high amounts of silicon are also present in radular teeth of a few gastropod species of the superfamily Patellacea (*2, 3*). It has been suggested that the silicon-bearing compound of such teeth is opal on the basis of its specific gravity and optical properties (*2*). The teeth are also reported to contain high amounts of iron (*2, 3*). Therefore, the possibility of an iron silicate mineral has been considered (*4*). Most investigators have favored the idea that the iron and silicon are present in two separate minerals (*2, 3*). X-ray diffraction studies of mature teeth from four patellacean species have strengthened this concept, because goethite (αFeOOH) has been detected as the only crystalline compound (*5*). In the absence of the application of modern techniques, the mineralogic form of the silicon-containing compound has remained uncertain, consistently being referred to as the "silica" (*2, 3*).

Patellacea are found in all oceans. They are common on rocky shores in the intertidal zone where their populations may exceed 500 individuals per square meter. Smaller populations occur subtidally, mostly in mid- and high latitudes to depths of about 200 m. A few species of the family Lepetidae extend from shelf depths down to about 3600 m in the abyssal zone (*6*).

The compound reported to contain the high silicon concentrations in some species of these gastropods needs precise identification. One would like to know further whether all Patellacea precipitate a silicon-bearing compound. Currently there is disagreement on whether the low amounts of silicon in surface waters of the oceans is entirely or largely the result of biologic activity or largely due to inorganic processes (*7*). Petallacean gastropods, because they are common, should be included in reevaluating the total amounts of biologically fixed silicon and of the volume of siliceous hard parts transferred to oceanic sediments. The ability to metabolize silicon by a biochemical system of the tissue grade of Metazoa, such as the Patellacea, acquires greater significance in the light of the recent discovery that silicon may be important in the process of bone calcification in mammals (*8*).

In order to determine the mineralogy of the silicon-bearing compound of the Patellacea we initially studied samples of *Patella vulgata* from the family Patellidae and of *Lottia gigantea* from the family Acmaeidae (*9*). Radulae of adult individuals preserved in 75 percent alcohol were treated with Clorox to digest most of the organic fractions. The residue from the radulae of *P. vulgata* consisted of dark brown to brownish gray cusps and tooth bases; that of *L. gigantea* contained similarly colored cusps and tooth bases plus rectangular-shaped base plates which are embedded in pairs in the radular membrane underneath the tooth bases (Fig. 1). The rectangular base plates are colorless and translucent where they are thin, milkish-white in thicker portions, and iridescent in reflected light. When the radulae are stripped of their teeth and viewed in transmitted light under the microscope, the base plates are seen to be confined to the anterior part of the radula and to decrease in size and thickness posteriorly. The brownish tooth cusps and tooth bases of both species have been shown through x-ray diffraction studies to contain goethite (*5*). To remove this mineral, the cusps and tooth bases of both species were boiled in a solution of 38 percent hydrochloric acid for 2 days at 60°C. Under these conditions, the tooth cusps and their bases remain firm, retained their original shape, and acquired a colorless, milky-white appearance similar to that of the rectangular base plates of *L. gigantea* when extracted solely with Clorox. The tooth cusps and their bases were reduced in size and marginally in thickness. The teeth acquired a por-

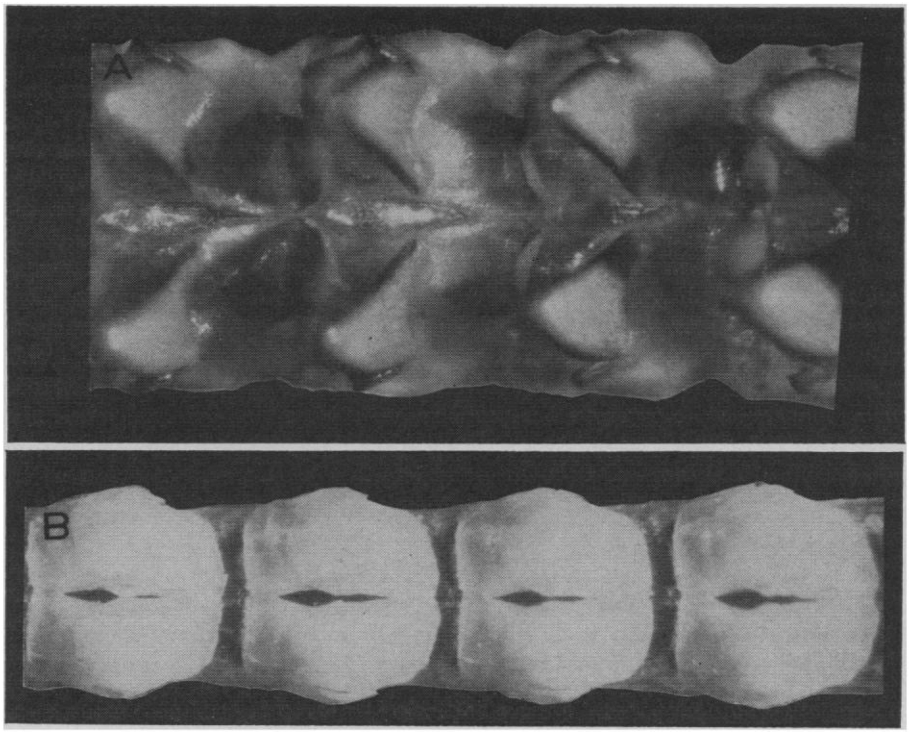


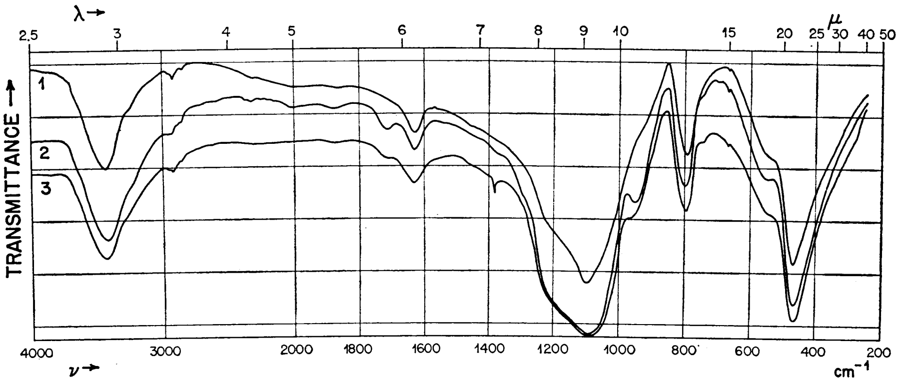
Fig. 1. Dorsal view of a portion of the radiula of *L. gigantea* showing (*A*) mature mineralized teeth (×160) and (*B*) paired base plates composed of opal embedded in the radular membrane (× 100).

ous appearance, more so for *L. gigantea* than for *Patella vulgata*. The colorless teeth recovered for both species after acid treatment account for 80 to 100 tooth rows corresponding roughly in *L. gigantea* to the number of pairs of base plates which were extracted with Clorox. The colorless teeth were present only in the anterior part of the radulae in the region where in *Lottia* similarly colored plates are developed (*10*). Therefore, the colorless compound is present only in mature, mineralized teeth of the anterior region of the radulae. X-ray diffraction patterns obtained from portions of the colorless tooth material of both species and from the base plates of *L. gigantea* show only one broad diffuse band at about 4.1 Å (*11*). Emission spectroscopic determinations carried out on another sample of the same material from the same sites of mineralization in both species showed that silicon is the only major constituent of the compound. Only traces of magnesium were detected, amounting to less than 0.05 percent by weight. These data indicate that the colorless material consists of a pure, amorphous silica mineral.

Infrared absorption spectrums uniquely identify amorphous minerals, including those of silica (*12*). Therefore, this method was used to identify the silica-bearing compound from the mineralization sites, where it occurs in the radulae of the two species. A sample of "fire" opal from Coober Pedy, Queensland, Australia, was used as a reference sample for comparison.

Comparison of the infrared spectrums of the silica-bearing compound from the mature teeth and of the radular embedded base plates of *L. gigantea* (Fig. 2) and of the mature teeth of *P. vulgata* (Fig. 3) with the spectrum of the Australian opal, particularly in the region from 200 to 900 cm^{-1}, which is sensitive to structural differences among silicates, demonstrates that the materials are identical. The water bands at 3440 cm^{-1}, 1630 cm^{-2}, and 950 cm^{-1}, together with the absence of the distinctive low-energy pattern of anhydrous quartz, confirm the presence of the hydrous form of silica, opal (*13*). Measurements of the refractive index of the material indicate that the water content of the base plates of *Lottia* amounts to about 12 percent, to about 5 percent for its tooth cusps, and to about 9 percent in the tooth cusps of *P. vulgata* (*14*).

By this unique method these data establish that opal is precipitated in the mineralized tissues of some tissue-grade metazoans. In two patellaceans the opal is localized in the mature lateral teeth and in some of the marginal teeth as a discrete mineral, together with goe-



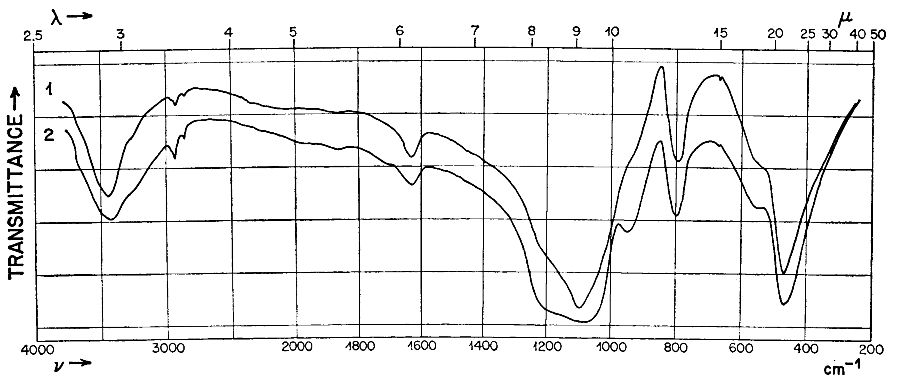


Fig. 2 (top). Infrared absorption spectrums: curve 1, reference opal; curve 2, radular base plates of *L. gigantea*; curve 3, acid-extracted residue of tooth cusps and tooth bases of *L. gigantea*. Fig. 3 (bottom). Infrared absorption spectrums: curve 1, reference opal; curve 2, acid-extracted residue of tooth cusps and tooth bases of *Patella vulgata*.

thite. In the acmaeid *L. gigantea* opal constitutes the sole mineralization agent of the radular plates underneath the mature teeth.

By treating teeth from a few species of the Patellacea belonging to the families Patellidae, Acmaeidae, and Lepetidae (*2, 3*), with hot hydrochloric acid, nitric acid, or aqua regia, two earlier investigators have isolated translucent, colorless toothlike forms. The description of the physical properties of these toothlike forms are similar to those for the teeth of the two species investigated here; hence, it would appear that opal precipitation is widespread among the Patellacea. Ten additional intertidal species taken from subarctic to tropical waters and an abyssal species were investigated (*15*). The procedure was limited to determinations of x-ray diffraction patterns of the mineralized tissues freed from the radulae with Clorox, treatment of a portion with hot hydrochloric acid, and determinations of x-ray diffraction patterns of the acid-treated extracts. Goethite was in all cases the only crystalline compound found. Colorless, translucent teeth, similar in appearance to those of *L. gigantea* and *P. vulgata* were recovered from all samples after acid treatment. Radular base plates, similar in physical appearance to those of *L. gigantea*, were found in all samples of the Acmaeidae and in the deep-water species of Lepetidae, but not in any of the Patellidae. The presence of base plates in the Patellacea appears to have taxonomic significance. The consistent presence of opal in radular sites of all Patellacea investigated to date strongly suggests that this mineral is a characteristic precipitate of this gastropod superfamily.

One would like to know the relative importance of the Patellacea as biologic agents of silica fixation in the oceans. There are some data on the density of *P. vulgata* and *L. gigantea* and the total Patellacea populations per square meter of rocky shores. On the Isle of Man, England, *P. vulgata* averages between 31 and 95 individuals per square meter and attains a maximum population density of 442 individuals per square meter (*16*). On the coast of northern Brittany, France, the mean population density of Patellacea averages between 48 and 532 individuals, and the maximum number found is 1004 individuals per square meter (*17*). At Pacific Grove, California, 997 individuals of unidentified Acmaeidae and 15 *L. gigantea* were counted within a square yard of rocky shore (*18*). We have found from 100 to 600 individuals of unidentified Acmaeidae and from 15 to 18 adult individuals of *L. gigantea* per square meter on the rocky coast of Punta Cabra, Baja California, Mexico.

The opal recovered from the entire radulae of adult individuals was determined to weigh 0.5 mg for *P. vulgata* and 1.5 mg for *L. gigantea*. The radulae of gastropods are continually replaced, and anterior teeth are shed in this process. *Patella vulgata* sheds a minimum of one to two tooth rows daily (*19*). If there were an average mean population density of 65 adult individuals per square meter, as at Port St. Mary, England, the opal tied up in the standing crop should amount to 32.5 mg, and the amount lost annually to the sediments should be 214.5 mg. Comparable figures for the northern Brittany coast of France, where the average population density is higher, are 120 mg of opal in the standing crop and 792 mg of opal lost annually. Several species of Patellacea commonly occupy the intertidal zone in overlapping ranges. Therefore, the amounts of opal in the standing crop and those lost to the sediments should be on the average in excess of those calculated for *P. vulgata*.

The coastlines of all continents have been estimated to amount to about 400,000 km (*20*). Let us assume that only half of the coastlines have rocky shores and that per linear meter of shoreline there is only 1 m^2 of intertidal area. Given the low value of the mean average density of *P. vulgata* per square meter at Port St. Mary, one finds that the opal fixed by the standing crop of Patellacea in the intertidal zone of the oceans amounts to 6.5×10^6 g, and that the volume of opal annually contributed to marine sediments equals about 4×10^7 g. These minimum values for annual opal production by Patellacea are significant and deserve consideration in spite of the fact that they may appear small compared to the estimates of annual opal fixation by the combined standing crops of marine diatoms and silicoflagellates, which range from 0.77×10^{16} g to 16.1×10^{16} g (*21*). There still remains the question whether the opal of the Patellacea, transferred in the form of shed radular segments, becomes stabilized in the subtidal sediments or is largely dissolved by the surface waters, which are undersaturated with reference to dissolved silica in most coastal areas. Investigation of rapidly accumulating sediments composed of clay minerals and other silicates should provide an initial clue to this question. The recent discovery that silicon seems to be involved in the initiation of mineralization of preosseous tissue in mammals (*8*) suggests that silicon metabolism, contrary to previous concepts, is likely to be widespread among tissue-grade Metazoa, even though mineralization of hard tissues by silica, as in Patellacea, may be the exception.

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9. *Patella vulgata* was included in the study because most earlier investigations dealt with the silicon content of its teeth.
10. The distribution of the silicon-bearing teeth on the radulae was determined by dividing the radulae into a series of sections and boiling each separately in hot acid.
11. The x-ray diffraction patterns match those for opals published by J. B. Jones, J. V. Sanders, E. R. Segnit, *Nature* **204**, 990 (1964), figure 1, g and h.
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13. The spectrums were taken with 1.0 mg of sample in KBr pellets on a Perkin-Elmer 225 infrared spectrophotometer. The variations in intensity of the small band at 950 cm^{-1} represent minor differences in the degree of hydration of the materials, which come about from different extraction procedures used with the samples, as noted earlier.
14. The estimated values of the water contents serve solely to show that the compound is hydrous. We have experimental data to show that different preserving fluids and different extraction procedures result in different water contents of the same material, and hence we do not know the original concentration of nonessential water in the silicon-bearing compound in question.
15. The species sampled were: *Patella intermedia* and *P. aspera*, Plymouth, England; *P. (Ancistromesus) mexicana*, Mazatlan, Mexico; *Cellana solida* and *C. tramoserica*, Victoria, Australia; *Amaea mitra*, Juneau, Alaska; *A. limatula*, Corona del Mar, California; *A. (Collisellina) sarcharina*, Palau, Caroline Islands; *Nomeopelta dalliana*, Puertecitos, Baja Cali-

fornia, Mexico; and *Lepeta* sp. dredged from 24°30.5′N, 113°28.7′W to 24°37.3′N, 113°0.88′W, recovered from 3565 to 3576 m.

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Urea–Inorganic Phosphate Mixtures as Prebiotic Phosphorylating Agents

Abstract. *Previous attempts to phosphorylate nucleosides by heating with inorganic phosphate succeeeded only when acid phosphates such as $Ca(HPO_4)_2$ were used. The addition of urea and ammonium chloride to the reaction mixture permits phosphorylation in high yield with neutral or basic phosphates at temperatures in the range of 65° to 100°C. Since the abundant mineral, hydroxylapatite, is a satisfactory substrate for this reaction, we believe that this procedure provides a plausible model for prebiotic phosphorylation.*

The phosphorylation of nucleosides under potentially prebiotic conditions has been attempted in two ways. In the first an aqueous solution containing the nucleoside and an inorganic phosphate is treated with a "prebiotic" condensing agent such as cyanamide or cyanogen (*1, 2*). In the second a dry mixture of the nucleoside and a suitable inorganic phosphate is heated (*1, 3*).

The main obstacle to effective phosphorylation in aqueous solution is the difficulty of obtaining efficiencies in excess of a few percent, even in relatively concentrated phosphate solutions. At present, the concentration of phosphate in the ocean and most other surface waters is very low ($10^{-7}M$ to $10^{-6}M$), owing to the precipitation of basic calcium phosphate, hydroxylapatite. If the same was true on the primitive earth, phosphorylation efficiencies in solution must have been negligible.

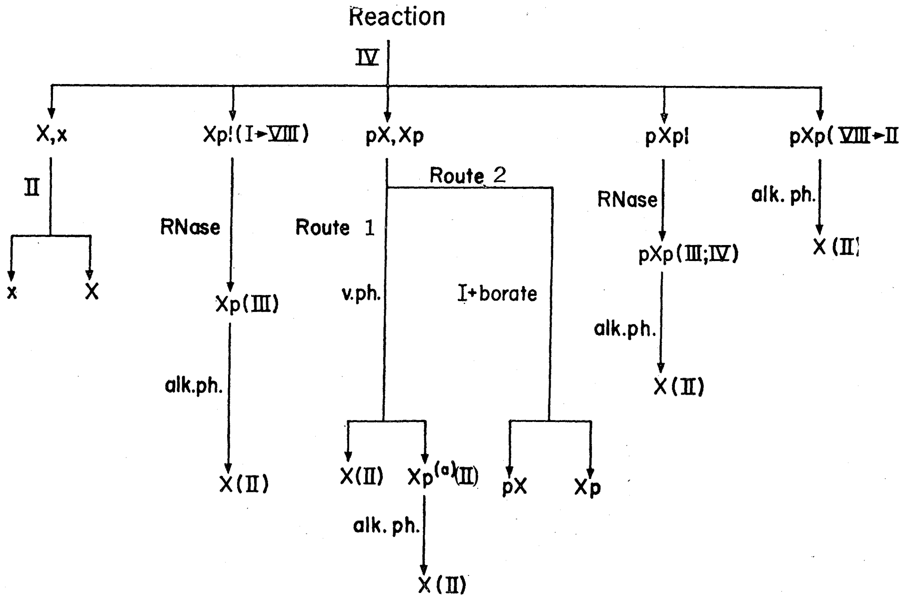

Fig. 1. Analysis of the reaction products from: (i) phosphorylations of ^{14}C-labeled nucleosides (X) with inorganic phosphate in the presence of urea and ammonium chloride at 100°C (route 1); (ii) reaction of ^{14}C-labeled uridine monophosphates with urea at 100°C (route 2). Roman numerals refer to chromatographic or electrophoretic systems; *v.ph.*, crude rattlesnake venom (*Crotalus adamanteus*); *RNase, ribonucleases* (pancreatic T_1 or T_2); *alk.ph.*, bacterial alkaline phosphatase. The numerals in parentheses indicate systems in which individual compounds were further characterized. See (a) in Table 3 for meaning of the superior (a).

Two highly specific phosphorylation reactions in aqueous solution are known. Cyanogen brings about a quite efficient synthesis of β-ribofuranose 1-phosphate from ribose and inorganic phosphate (*4*). Inorganic trimetaphosphate reacts with *cis*-glycols in alkaline aqueous solution to give monophosphates (*5*); with nucleosides a mixture of 2′- and 3′-phosphates is obtained in excellent yield (*6, 7*). Neither of these reactions provides a promising route to nucleotides under prebiotic conditions, since the cyanogen reaction occurs only at the 1-position of a free sugar and inorganic trimetaphosphate is unknown in nature.

Some of the solid-state reactions give quite good yields of nucleotides, but unfortunately they require the use of soluble acidic phosphates such as KH_2PO_4 and $Ca(H_2PO_4)_2$, which are very uncommon in nature, and probably could not have existed in quantity on the primitive earth. We have suggested elsewhere that the thermal reactions of ammonium phosphate are somewhat more promising since ammonium phosphates formed on evaporating neutral or alkaline solutions lose ammonia when heated and then provide an acidic environment in which phosphorylation occurs more readily (*3*).

There are scattered references in the literature to the use of urea to facilitate phosphorylation reactions. Condensed phosphates are obtained from ammonium phosphate and urea at temperatures between 60° and 150°C (*8*); alcohols are phosphorylated very effectively at 140° to 150°C by means of a urea–phosphoric acid mixture (*9*). We show here that the main obstacles to prebiotic phosphorylation can be overcome if urea is present.

Paper chromatography was carried out by the descending technique on Whatman 3-MM paper. The solvent systems were mixtures containing the following proportions (by volume): system I, isopropanol, concentrated ammonia, and water (7 : 1 : 2); system II, *n*-butanol and $5M$ acetic acid (2 : 1); system III, 95 percent ethanol and $1M$ ammonium acetate, *p*H 7.5 (7 : 3); system IV, 95 percent ethanol and $1M$ ammonium acetate, made up to $2 \times 10^{-3}M$ with ethylenediaminetetraacetic acid (EDTA) and adjusted to *p*H 5.0 with glacial acetic acid (7 : 3); system V, *n*-butanol, saturated with water; and system VI, *n*-propanol, con-