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.
16. N. S. Jones, *Proc. Trans. Liverpool Biol. Soc.* **56**, 60 (1948).
17. E. Fischer-Piette, *Ann. Inst. Oceanogr. N.S.* **21**, 1 (1941).
18. E. F. Ricketts and J. Calvin, *Between Pacific Tides*, revised by J. W. Hedgpeth (Stanford Univ. Press, Stanford, Calif., ed. 3, 1962), p. 367.
19. K. Isarankura and N. W. Runham, *Malacologia* **7**, 80 (1968).
20. P. H. Kuenen, *Marine Geology* (Wiley, New York, 1950), p. 106.
21. R. C. Harriss, *Nature* **212**, 275 (1966); A. P. Lisitsin, Y. I. Belyayev, Y. A. Bogdanov, A. N. Bogoyavlenskiy, *Int. Geol. Rev.* **9**, 604 (1967).
22. Contribution No. 1904 from the Division of Geological Sciences, California Institute of Technology. Supported by NSF grant GB-6707X1. I thank Drs. H. Lemche and J. H. Macpherson for samples of certain species; Dr. C. Hubbs or facilitating my participation in the Magdalena Bay Expedition; Dr. J. E. Smith for providing me with space and collection at the Plymouth Marine Laboratory; M. Dekkers and E. Bingham for technical assistance; and G. G. Rossman for taking the infrared spectrometer spectrums and for discussing their interpretation.

25 August 1970; revised 27 October 1970

## 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.

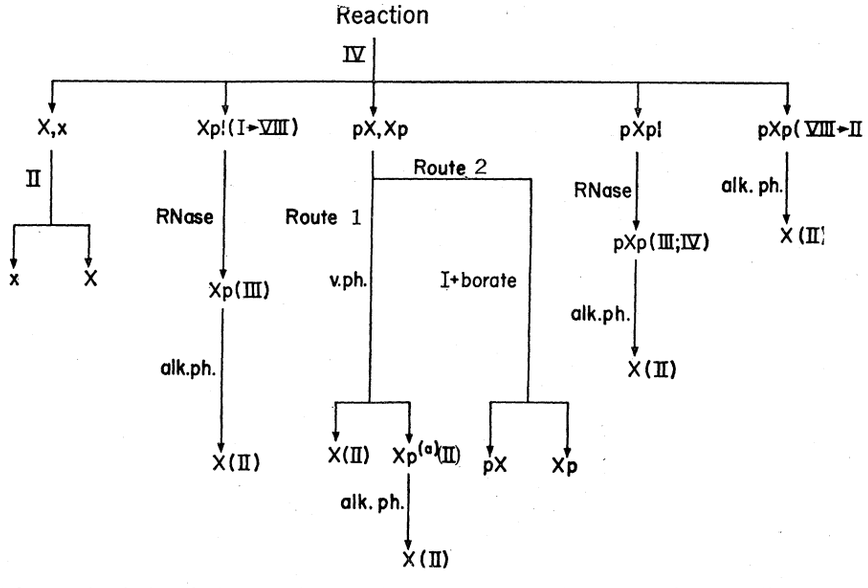


Fig. 1. Analysis of the reaction products from: (i) phosphorylations of $^{14}C$-labeled nucleosides (X) with inorganic phosphate in the presence of urea and ammonium chloride at 100°C (route 1); (ii) reaction of $^{14}C$-labeled uridine monophosphates with urea at 100°C (route 2). Roman numerals refer 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-

centrated ammonia, and water (55 : 10 : 35). Chromatography in the presence of borate was performed by first applying a band (2.5 cm wide) of 0.1*M* sodium borate at the origin of the chromatogram. After drying, the mixtures were spotted and developed in system I. We carried out paper electrophoresis on Whatman 3-MM paper at 3000 volts, with Varsol as the coolant. The buffers consisted of the following: system VII, 0.05*M* formic acid–ammonium formate, *p*H 2.7; system VIII, 0.03*M* potassium phosphate, *p*H 7.1; and system IX, 0.05*M* sodium borate, *p*H 8.5. The $R_F$ values and paper electrophoretic mobilities of the different compounds are listed in Table 1.

The characterization of reaction products was achieved as outlined in Figs. 1 and 2. In each case we identified the products by comparing the chromatographic and electrophoretic mobilities (Table 1) with those of authentic material, and by enzymatic degradations (Fig. 1).

We estimated the yields of radioactively labeled nucleosides and nucleotides by cutting the paper into strips and passing it through a radiochromatogram scanner with integrator (Baird Atomic RSC 363). Subsequently, the radioactive zones were cut out, and the radioactivity was counted more accurately in a Beckman liquid scintillation counter. We calculated the yields by taking the counts in the radioactive zones as a percentage of the total number of counts on the paper, after allowing for background. When unlabeled uridine and [$^{14}$C]urea were used (Table 2), we determined the yields by elution of the ultraviolet-absorbing spots from the chromatography paper and measurement of their optical densities against blanks in a spectrophotometer (Zeiss PMQ2).

Bacterial alkaline phosphatase (220 unit/ml) (Worthington Biochemical Corp.) was used for the hydrolysis of primary phosphate ester groups. The incubation mixtures contained about 5 optical density units (O.D.U.) of substrate dissolved in 100 $\mu$l of a solution, 0.05*M* in tris-HCl and 0.02*M* in $MgCl_2$, *p*H 8.2, to which 10 $\mu$l of enzyme suspension was added.

Crude snake venom (*Crotalus adamanteus*) (Sigma Chemical Company) was used to hydrolyze the 5′-linked primary phosphate groups while the 3′-linked groups were left unchanged. A stock solution in 0.1*M* tris-HCl (*p*H 9) containing 10 mg of venom per 1 ml of buffer was prepared. Incubation

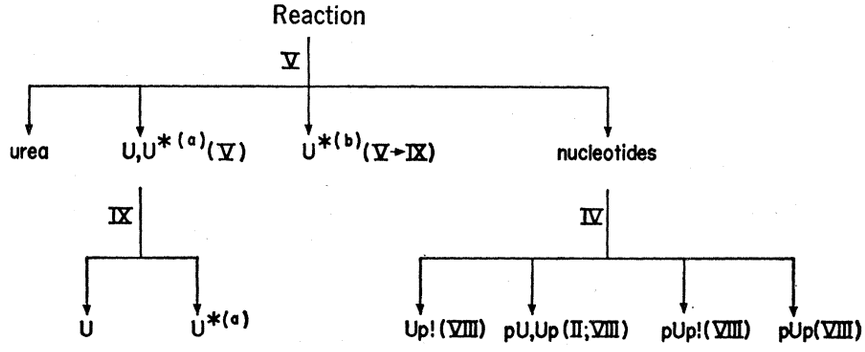


Fig. 2. Analysis of the reaction products from a mixture of uridine, inorganic phosphate, ammonium chloride, ammonium bicarbonate, and [$^{14}$C]urea after heating at 100°C. U*(a) is 2′(3′)-carbamyluridine; U*(b) is 5′-carbamyluridine.

mixtures containing 5 O.D.U. of the substrate, 0.1*M* tris-HCl with a *p*H of 9.0 (75 $\mu$l), and the enzyme (10 $\mu$l) were kept at 37°C for 2 hours.

Pancreatic ribonuclease (Boehringer Mannheim Corp.) was used for the hydrolysis of pyrimidine–nucleoside cyclic 2′,3′-phosphates. The incubation mixtures contained 5 O.D.U. substrate, 0.05*M* tris-HCl with a *p*H of 7.5 as buffer (100 $\mu$l), and enzyme (50 $\mu$g). They were kept for 2 hours at 37°C.

Ribonuclease $T_1$ (Calbiochem) was used to hydrolyze cyclic guanosine 2′,3′-phosphate. The stock solution contained 1000 unit/ml of 0.1*M* tris-HCl buffer; 1 O.D.U. of substrate was dissolved in 50 $\mu$l of tris-HCl buffer (0.02*M*, *p*H 7.0) and incubated with 10 $\mu$l of enzyme solution at 37°C for 1 hour.

Ribonuclease $T_2$ was used for the hydrolysis of cyclic adenosine 2′,3′-phosphate. The stock solution contained 0.4 unit of enzyme per milliliter in 0.1*M* ammonium acetate (*p*H 4.5) (*10*). The digestion was carried out with 2 O.D.U. of substrate and 20 $\mu$l of enzyme solution, at 37°C for 1 hour.

Nucleosides and nucleoside monophosphates were purchased from

Table 1. The $R_F$ values and electrophoretic mobilities.

| Compound | $R_u$† | | | | | | $R_m$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | I | I + borate | II | III | IV | V | VII‡ | VIII§ | IX‖ |
| U | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 0 | 0 | 0.50 |
| U* (5′) | | | | | | 0.75 | | | |
| T | 1.42 | 3.47 | 1.82 | 1.15 | 1.16 | 2.44 | | 0 | 0 |
| A | | | 1.24 | 0.88 | 0.88 | 0.12 | 0.78 | | 0.32 |
| Adenine | | | 1.55 | .93 | .88 | 2.02 | 1.25 | | 0 |
| C | | | 0.73 | .93 | .93 | 0.67 | 1.0 | | 0.38 |
| Cytosine | | | .95 | .93 | .92 | 1.21 | 1.58 | | |
| G | | | .75 | .82 | .79 | 0.60 | | | 0.40 |
| Guanine | | | .92 | .70 | .68 | .80 | | | |
| Up! | 0.82 | | | .87 | .82 | | | 0.63 | 0.70 |
| Ap! | 1.01 | | | .73 | .73 | | | .46 | .54 |
| Cp! | 0.88 | | | .78 | .74 | | | .58 | .65 |
| Gp! | .59 | | | .70 | .68 | | | .51 | .57 |
| pU | .13 | 0.19 | .16 | .35 | .50 | | | .95 | 1.10 |
| Up | .22 | .55 | .20 | .37 | .50 | | | 1.0 | 1.0 |
| pT, Tp | .26 | | | .51 | .72 | | | 0.94 | |
| pA | | .23 | .23 | .27 | .41 | | | .84 | |
| Ap | | .95 | .33 | .31 | .38 | | | .92 | |
| pC | .12 | .17 | .17 | .22 | .51 | | | .92 | |
| Cp | .22 | .59 | .22 | .33 | .44 | | | .96 | |
| pG | | .12 | .14 | .23 | .30 | | | .85 | |
| Gp | | .32 | .16 | .27 | .31 | | | .92 | |
| pUp! | | | | | .33 | | | 1.28 | |
| pAp! | | | | | .26 | | | | |
| pCp! | | | | | .24 | | | | |
| pGp! | | | | | .22 | | | | |
| pUp | | | .053 | | .19 | | | 1.35 | |
| pTp | .04 | | .031 | | .28 | | | 1.34 | |
| pAp | | | .056 | | .12 | | | 1.26 | |
| pCp | | | .048 | | .14 | | | 1.35 | |
| pGp | | | .050 | | .10 | | | 1.27 | |
| Urea | | | 1.37 | | | 1.32 | | | |

† The $R_u$ values given are relative to uridine. ‡ The $R_m$ values given are relative to cytidine (at 3000 volts for 30 minutes; migration is toward the cathode). § The $R_m$ values given are relative to Up (at 3000 volts for 30 minutes; migration is toward the anode). ‖ The $R_m$ values given are relative to Up (at 3000 volts for 60 minutes; migration is toward the anode).

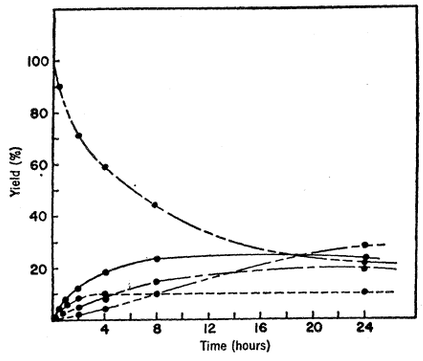


Fig. 3. Reaction of pU with a mixture of urea and ammonium chloride at 100°C. U, ————; pU, — - - —; pUp!, — - —; pUp, - - - - - -; Up!, — - - - —.

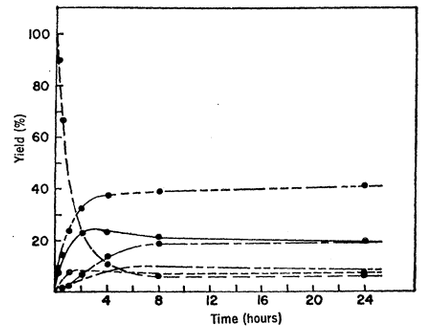


Fig. 4. Reaction of Up with a mixture of urea and ammonium chloride at 100°C. U, ————; pU, — - - —; pUp!, — - —; Up!, — - - - —; Up, — — —; pUp, - - - - - -.

Schwarz BioResearch or Boehringer Mannheim. All four cyclic ribonucleoside 2′,3′-phosphates (*11*), the ribonucleoside 2′(3′),5′-diphosphates pAp, pUp, pCp, pGp (*7*, *12*), and thymidine 3′,5′-diphosphate (*13*) were synthesized by established methods. The following labeled compounds, [2-$^{14}$C]-thymidine, [2-$^{14}$C]cytidine, [8-$^{14}$C]adenosine, [8-$^{14}$C]guanosine, [2-$^{14}$C]uridine 5′-phosphate, and [2-$^{14}$C]uridine 2′-(3′)-phosphate, were purchased from Schwarz BioResearch.

The nucleosides were phosphorylated as follows: A solution (1 ml) containing 0.04*M* $^{14}$C-labeled nucleoside (0.075 mc/mmole), 0.4*M* urea, 0.2*M* $NH_4HCO_3$, 0.4*M* $NH_4Cl$, and 0.04*M* $Na_2HPO_4$ was applied to squares of Whatman GF 82 glass-fiber paper, 0.1 ml of solution per square (1 by 1 cm). A series of control experiments for the reaction of uridine was carried out in which the following components were omitted: (i) urea, (ii) $NH_4Cl$, and (iii) urea and $NH_4Cl$.

The squares were dried by exposure overnight to air, and then they were irradiated for 20 minutes with an infrared light so as to maintain a temperature of approximately 65°C. Each square was then kept at the bottom of an individual open test tube, standing on a heating block usually at 100° ± 1°C. In a few experiments we used a temperature of 66°C. Samples were withdrawn from time to time, eluted with water, and analyzed as shown in Fig. 1.

In the cases where hydroxylapatite was used in the place of glass-fiber paper, 40-mg samples of analytical reagent-grade $Ca_5(PO_4)_3OH$ (Mallinckrodt) were washed repeatedly. The precipitates were kept for a short time at 100°C to remove excess water and then mixed thoroughly with 0.1-ml portions of a solution containing 0.02*M* [2-$^{14}$C]-uridine (0.15 mc/mmole), 0.2*M* urea, 0.02*M* $Na_2HPO_4$, 0.2*M* $NH_4Cl$, and 0.1*M* $NH_4HCO_3$. In a series of control experiments the following components were omitted: (i) $Na_2HPO_4$, (ii) urea, or (iii) urea and $Na_2HPO_4$. The resulting viscous suspension finally covered the lower end of the test tubes (11 by 100 mm) in the form of a thin film. The wet samples were heated for varying times in nonstoppered tubes at 100° ± 1°C. The radioactive materials were eluted with 20 percent formic acid containing 0.12*M* EDTA and analyzed as above (Table 3).

The reaction of uridine phosphates with urea was carried out as follows: Solutions (1 ml) containing 0.04*M* [2-$^{14}$C]uridine 5′-phosphate, or [2-$^{14}$C]-uridine 2′(3′)-phosphate (0.025 mc/mmole), plus 0.4*M* urea and 0.4*M* $NH_4Cl$, were adjusted to *p*H 8.5 with concentrated $NH_4OH$, and applied to glass-fiber paper as above. Similar solutions, in which the urea or $NH_4Cl$, or both, were omitted were used in control experiments. In other respects these experiments were identical to those described above (Figs. 3–5).

We repeated the phosphorylation of uridine with the complete mixture of phosphate, ammonium chloride, and urea at 100°C, as described above, but with the use of $^{14}$C-labeled urea (0.1 mc/mmole) and nonradioactive uridine. Samples were taken from time to time and chromatographed as in Fig. 2. The bands migrating most rapidly in system V contained uridine and carbamyluridine, respectively. They were cut off and eluted just before they reached the end of the paper (after about 4 days). The phosphorylated products, which had stayed at the origin of the chromatogram, were now chromatographed in system IV on the same paper. Elutions of the bands and comparison of their optical densities at 262 nm gave the percentage yields as shown in Table 2. The bands were then further purified: the nucleosidic bands by chromatography in system V, followed by electrophoresis in system IX; the nucleotide bands by chromatography in system II or electrophoresis in system

Table 2. Reaction products formed from a mixture of uridine, $Na_2HPO_4$, $NH_4Cl$, $NH_4HCO_3$, and [$^{14}$C]urea at 100°C. The percentage of carbamate indicates the degree of carbamylation of each of the individual compounds; for instance, after 4 hours of heating, when 11.6 percent total Up! had formed, 0.8 percent (6.9 percent of the total Up!) was carbamylated and 10.8 percent was uncarbamylated.

| Product | After heating for | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 hours | | | 4 hours | | | 7 hours | | | 24 hours | | |
| | Yield (%) | Carbamate (%) | | Yield (%) | Carbamate (%) | | Yield (%) | Carbamate (%) | | Yield (%) | Carbamate (%) | |
| | | 5′ | 2′(3′) | | 5′ | 2′(3′) | | 5′ | 2′(3′) | | 5′ | 2′(3′) |
| Uridine | 51.9 | 4.3 | 1.2 | 36.8 | 9.2 | 1.8 | 33.4 | 12.0 | 2.1 | 28.9 | 29.0* | 2.4 |
| Uridine cyclic 2′,3′-phosphate | 7.0 | 3.8 | | 11.6 | 6.9 | | 19.4 | 9.9 | | 23.9 | 27.5 | |
| Uridine monophosphate | 28.8 | 3.0† | | 30.9 | 4.4† | | 24.0 | 10.3† | | 21.0 | 22.4† | |
| pUp! | 4.5 | 0 | | 10.0 | 0 | | 14.9 | 0 | | 18.7 | 0 | |
| pUp | 7.8 | 0 | | 9.1 | | 1.5 | 8.4 | | 3.1 | 7.5 | | 6.1 |
| Phosphorylation | 60.4 | | | 80.7 | | | 90.0 | | | 97.3 | | |

* In addition, 1.8 percent of the 5′,2′(3′) dicarbamylated product was formed. † The 5′- and 2′(3′)-phosphates were not separated.

VIII. We evaluated the degree of carbamylation of the individual reaction products by determining their specific activities (Table 2).

Figure 6 summarizes the yields of a number of "crude" phosphates obtained by heating an equimolar mixture of uridine and inorganic phosphate with a tenfold molar excess of urea and ammonium chloride at 100°C and then analyzing in system IV (Fig. 1). Under these conditions 97 percent of the phosphate is incorporated into nucleotides after 24 hours, mainly as Up! and pUp! When the ammonium chloride is omitted, phosphorylation is much less extensive and gives 23 percent Up! together with small amounts (2.5 percent) of uridine monophosphates in 24 hours. In the absence of urea, no matter whether ammonium chloride is added to the reaction mixture or not, very little phosphorylation occurs.

A more detailed examination of the products of the above reactions shows that many of them are mixtures of a nucleotide with small amounts of carbamylated derivatives that are not resolved in system IV. A rather complex analysis of the products from one sequence of reactions with uridine was undertaken in order to separate the different isomers (Fig. 2). The results (Table 2) indicate that extensive phosphorylation occurs with only minor complicating side reactions; after 4 hours we found 81 percent phosphorylation, but only 6 percent carbamylation.

The phosphorylation of the other nucleosides with a mixture of ammonium chloride and urea proceeds similarly. Cytidine incorporates 96 percent of the available phosphate, but adenosine and guanosine incorporate only 80 percent and 71 percent, respectively (Table 4). Under the same conditions thymidine gives 24.8 percent Tp, 19.7 percent pT, and 19.8 percent pTp (Fig. 7). In each case some carbamylation occurred on the sugar and in some cases carbamylation occurred on the base. We did not carry out a detailed quantitative analysis.

In other experiments pU was heated with ammonium chloride and urea. Extensive transphosphorylation occurred (Fig. 3), and after 24 hours 28 percent Up! and 18 percent pUp! were obtained. The same system with urea alone gave a similar pattern of products, but the reaction was much slower. In the absence of urea little reaction occurred. When Up was heated with urea and

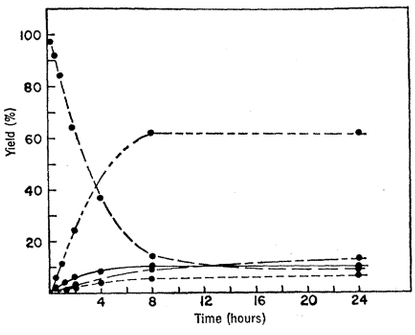


Fig. 5. Reaction of Up with urea at 100°C. U, ————; pUp!, — — —; Up!, — — — —; Up, — — —; pUp, - - - - - -.

ammonium chloride (Fig. 4), the main product was 41 percent Up! together with 19 percent pUp!; when Up was heated with urea alone about 62 percent Up! was obtained together with 13 percent pUp! (Fig. 5). In no case did we obtain substantial quantities (>5 percent) of di- or oligonucleotides.

We may summarize our results as follows:

1) Very little phosphorylation or transphosphorylation occurs when nucleosides are heated at 100°C for 24 hours with ammonium phosphate, and virtually none occurs when nucleosides are heated with sodium phosphate.

2) Urea and sodium phosphate together form a moderately good phosphorylating mixture. Transphosphorylation is relatively slow but cyclization of uridine 2′-phosphate or uridine 3′-phosphate is rapid.

3) A mixture of urea, ammonium chloride, and sodium phosphate is an excellent reagent for the phosphorylation of nucleosides. This mixture also causes extensive transphosphorylation. The major product from nucleosides is always the cyclic 2′,3′-phosphate, but a good deal of the 5′-phosphate is also formed. Thymidine gives large amounts of both the 3′- and the 5′-phosphates. Ammonium chloride causes the removal of the purine portion of adenosine under some conditions.

4) Under the conditions of our experiment the yields of di- or polynucleotides is small.

5) Carbamylation always occurs in parallel with phosphorylation, but it is easy to find conditions in which carbamylation is only a minor side reaction. In particular, the presence of

Table 3. Reaction products (percentages based on the starting material) formed by heating [2-$^{14}$C]uridine for different times at 100°C with a mixture of $NH_4Cl$, $NH_4HCO_3$, and urea on hydroxylapatite (a) in the presence of or (b) in the absence of $Na_2HPO_4$. After 24 hours the control solutions containing all of the above components except urea and all of the above components except urea and $Na_2HPO_4$ contained 3.7 percent and 1.7 percent uridine monophosphate, respectively.

| Product | After heating for | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 2 hours | | 6 hours | | 14 hours | | 24 hours | |
| | a | b | a | b | a | b | a | b |
| Uridine monophosphate | 6.0 | <1 | 15.3 | 7.7 | 22.8 | 14.0 | 24.8 | 16.1 |
| Uridine cyclic 2′,3′-phosphate | 4.4 | 0 | 4.9 | 3.0 | 6.5 | 3.7 | 8.9 | 4.4 |
| Radioactivity staying near origin | 1.6 | 0 | 1.7 | <1 | 3.3 | 1.3 | 4.9 | 2.0 |

Table 4. Reaction products (percentages based on the starting material) from the phosphorylation of $^{14}$C-labeled cytidine, adenosine, and guanosine with a mixture of $Na_2HPO_4$, $NH_4Cl$, $NH_4HCO_3$, and urea at 100°C.

| Product | [$^{14}$C]Cytidine | | | [$^{14}$C]Adenosine | | | [$^{14}$C]Guanosine | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 hr | 4 hr | 24 hr | 1 hr | 4 hr | 24 hr | 1 hr | 4 hr | 24 hr |
| X | 67.8 | 35.4 | 22.9 | 70.0 | 36.7 | 13.8 | 83.0 | 67.8 | 45.2 |
| x | | | 2.0 | 3.1 | 6.9 | 24.2 | | | 2.0 |
| Xp! | 3.8 | 13.9 | 31.8 | 3.6 | 10.1 | 23.7 | 2.5 | 4.4 | 11.4 |
| Xp[a] | 9.5 | 7.6 | 6.2 | 7.3 | 6.3 | 4.3 | 4.8 | 7.1 | 5.7 |
| pX | 13.9 | 22.8 | 8.3 | 13.0 | 21.7 | 6.7 | 6.1 | 9.3 | 7.9 |
| pXp! | 1.0 | 10.6 | 18.1 | | 7.9 | 15.7 | | 4.3 | 12.9 |
| pXp | 3.9 | 9.7 | 6.6 | 3.2 | 8.3 | 7.0 | | 7.2 | 10.2 |
| Phosphate incorporation | 37.0 | 84.9 | 95.7 | 30.3 | 70.5 | 80.1 | 13.4 | 43.8 | 71.2 |

[a] This fraction also contains 2′(3′)-carbamylnucleoside 5′-phosphates, which are not degraded by venom phosphoesterase.

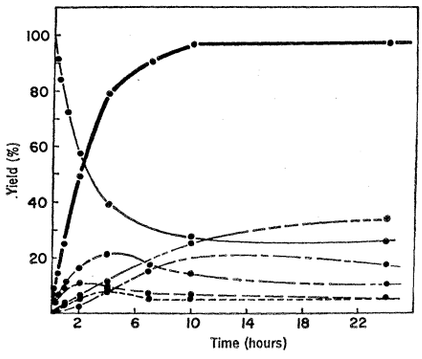


Fig. 6. Phosphorylation of uridine with a mixture of inorganic phosphate, ammonium chloride, ammonium bicarbonate, and urea at 100°C. U, ————; Up!, — - - - —; pU, — - - —; Up, — — — —; pUp!, — - —; pUp - - - - - -; total incorporation of inorganic phosphate, ————.

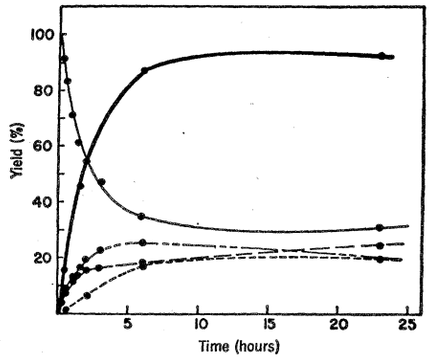


Fig. 7. Phosphorylation of thymidine with a mixture of inorganic phosphate, ammonium chloride, ammonium bicarbonate, and urea at 100°C. T, ————; pT, — - - —; Tp, — — — —; pTp - - - - - -; total incorporation of inorganic phosphate, ————.

ammonium chloride limits the extent of carbamylation.

It is doubtful that temperatures as high as 100°C occurred at many points on the primitive earth. We therefore carried out a few experiments at lower temperatures. The rate of reaction was then much decreased but still quite significant. For example, after 4 days at 66°C we were able to detect 9.6 percent combined uridine phosphates and 4 percent Up! from the ammonium chloride–urea mixture. Surface temperatures above 70°C are quite realistic since they are reached today in many desert areas and also in loose-packed beach sand.

Finally, we carried out experiments in which phosphate is present as hydroxylapatite, since this is the form of phosphate found most often in nature. After 24 hours at 100°C 16 percent combined uridine phosphates and 4 percent Up! were obtained (Table 3).

The rates of solid-state reactions are extremely sensitive to the physical state of the reactants. Hence the quantitative details described here are probably not important. Reactions on inert materials with larger surface areas would probably be more efficient. Reactions on the surface of a glass tube are slower but go to completion after a few hourly cycles of moistening and reheating to 100°C. The incomplete conversion of guanosine to phosphates may well be due to its low solubility. The main point we wish to make is that high yields can certainly be obtained under mild conditions.

We have preliminary evidence that condensed inorganic phosphates are formed in good yield from inorganic phosphate, ammonium chloride, and urea under the reaction conditions that we employed. However, we have no evidence that condensed inorganic phosphates play any part in the reaction. Organic phosphates such as carbamyl phosphate or phosphoramidates may be the true active intermediates.

We believe that the reactions described here constitute a completely plausible model of prebiotic phosphorylation. They proceed at temperatures of 100°C in a few hours or 66°C in a few days. Yields of a few tens of percent can be obtained from an equimolar mixture of soluble phosphate and a nucleoside. Hydroxylapatite can be substituted for sodium phosphate with only a moderate (fivefold) decrease in yield. The synthesis we describe could easily have occurred either on beach sand or in desert areas where temperatures in excess of 70°C are encountered.

The synthesis of urea under prebiotic conditions has been reported many times (*14*). Irradiation of ammonium cyanide solutions gives yields of up to 30 percent (*15*). Thus the requirement for urea is no obstacle to this proposed prebiotic reaction sequence.

R. LOHRMANN
L. E. ORGEL
*Salk Institute for Biological Studies, San Diego, California 92112*

**References and Notes**

1. See D. H. Kenyon and G. Steinman, *Biochemical Predestination* (McGraw-Hill, New York, 1969), chap. 5.
2. R. Lohrmann and L. E. Orgel, *Science* **161**, 64 (1968).
3. A. Beck, R. Lohrmann, L. E. Orgel, *ibid.* **157**, 952 (1967).
4. M. Halmann, R. A. Sanchez, L. E. Orgel, *J. Org. Chem.* **34**, 3702 (1969).
5. V. W. Feldmann, *Chem. Ber.* **100**, 3850 (1967).
6. A. W. Schwartz, *Chem. Commun.* **1969**, 1393 (1969).
7. R. Saffhill, *J. Org. Chem.* **35**, 2881 (1970).
8. K. O. Knollmueller, U.S. Patent 3.333921; *Chem. Abstr.* **67**, 74990c (1967); F. O. Lundstrom and C. W. Whittaker, *Ind. Eng. Chem.* **29**, 61 (1937).
9. G. C. Daul and J. D. Reid, U.S. Patent 2.609360; *Chem. Abstr.* **47**, 920h (1953).
10. Ribonuclease $T_2$ was supplied by Dr. G. W. Ruchizky.
11. M. Smith, J. G. Moffatt, H. G. Khorana, *J. Amer. Chem. Soc.* **80**, 6204 (1958).
12. Abbreviations: U, uridine; T, thymidine; A, adenosine; G, guanosine; C, cytidine; Xp!, ribonucleoside cyclic 2′,3′-phosphate; pX, nucleoside 5′-phosphate; Xp, nucleoside 2′(3′)-phosphate; pXp!, 5′-phosphonucleoside cyclic 2′,3′-phosphate; pXp, nucleoside 5′,2′(3′)-diphosphate; x, base of the corresponding nucleoside X; and U*, carbamylated uridine.
13. An anhydrous solution containing thymidine (0.2 mmole), pyridinium cyanoethylphosphate (0.8 mmole), and 2,4,6-triisopropylbenzene sulfonylchloride (1.6 mmole) in pyridine (4 ml) was kept for 3 hours at room temperature. Subsequently, the mixture was diluted with water (4 ml), and left standing for another 15 minutes. After addition of 10*N* $NH_4OH$ (30 ml), the mixture was maintained under pressure at 80°C for 18 hours. It was then evaporated under vacuum and chromatographed in system VI. Elution of the strong ultraviolet-absorbing band gave the ammonium salt of pTp (74 percent yield).
14. M. Labadie, R. Jensen, E. Neuzil, *Bull. Soc. Chim. Biol.* **49**, 673 (1967); J. E. Varner and R. C. Burrell, *Euclides* **15**, 1 (1955); *Chem. Abstr.* **49**, 11735h (1955); C. U. Lowe, M. W. Rees, R. Markham, *Nature* **199**, 219 (1963); C. Ponnamperuma, *Proceedings of the Third International Radiation Research Congress*, G. Silini, Ed. (North-Holland, Amsterdam, 1967), p. 700.
15. R. Lohrmann and L. E. Orgel, in preparation.
16. We thank S. Khoury for technical assistance. Work supported in part by NASA grant NGR 05-067-001 and NIH grant 13435.

10 November 1970

## Radioimmunoassay for Prostaglandins

Abstract. *Antibodies to prostaglandin were obtained by immunization of rabbits with $PGA_1$, $PGA_2$, and $PGE_1$ protein conjugates of prostaglandins. The antibodies demonstrated specificity toward both the cyclopentane ring and the aliphatic side chains. With the use of these antibodies a highly sensitive radioimmunoassay capable of measuring less than picomolar amounts of $PGA_1$, $PGA_2$, and $PGE_1$ has been developed.*

The prostaglandins are 20-carbon aliphatic carboxylic acids with a cyclopentane ring; their molecular weights range from 332 to 356. They are widely distributed in biological systems, and physiologic studies have indicated diverse effects on smooth muscle tone (*1*), blood pressure (*2*), lipolysis (*3*), platelet aggregation (*4*), and central nervous system function (*5*)—which