lization from the liquid state under high pressure is oriented along this plane.

By this method it was possible to follow the liquidus line of sulfur up to 60 kb (Fig. 1). The slope of the liquidus curve shows that the slope of the line changes from positive to vertical with increasing temperature and pressure. According to the Clausius-Clapeyron expression, vertical behavior means that the difference in specific volume between the solid and liquid sulfur disappears, that is to say, their densities are equal.

The quenching experiments were conducted in the area of the temperature-pressure diagram in which the solid sulfur is the stable phase. The x-ray diffraction patterns showed two crystalline forms. One form was the conventional rhombic sulfur and the other was a new cubic form, a = 13.66 Å (Table 1). The data plotted in Fig. 1 show that the triple point of cubic, rhombic, and liquid sulfur is at a pressure of 28 ± 4 kb and a temperature of 300° ± 5°C. The slope of the boundary line between cubic and rhombic sulfur is negative, which, according to the Clausius-Clapeyron expression, occurs because the density of cubic sulfur is higher than that of rhombic sulfur. The density of quenched samples of cubic sulfur was determined by the sink-float method in two experiments: the results were 2.18 and 2.19 g cm^{-3} at 30°C, respectively. The normal density of rhombic sulfur is 2.04 g cm^{-3}. The heat of transformation from rhombic to cubic sulfur was calculated as 49 ± 5 cal g^{-1}, based on a temperature of 30°C and a pressure of 1 atm. The heat of transformation would be different if the density difference changed significantly with increase in pressure. The cubic form is light yellow and insoluble in carbon disulfide; the unit cell contains 104 sulfur atoms.

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11 March 1965

Nucleotide Synthesis under Possible Primitive Earth Conditions

Abstract. *The nucleosides adenosine, guanosine, cytidine, uridine, and thymidine were each heated with inorganic phosphate. Nucleoside monophosphates were formed in appreciable yield. This result has a bearing on the hypothesis of chemical evolution.*

In our study of chemical evolution the main endeavor has been to reconstruct the path by which the constituents of the nucleic acid molecule could have arisen on the primordial earth before the appearance of life. The synthesis of the bases, adenine and guanine, and the sugars, ribose and deoxyribose, under simulated primitive earth conditions has been demonstrated earlier (*1*). Recent experiments have also shown that the nucleosides, adenosine and deoxyadenosine, could be formed in such an environment (*2*). Several attempts have already been made to synthesize nucleotides abiotically (*3*). Previously, we found that, when a dilute solution of adenine and ribose was irradiated with ultraviolet light in the presence of ethyl metaphosphate, the nucleotides AMP, ADP, ATP, and A4P (mono-, di-, tri-, and tetraphosphates of adenosine) were formed. Although the source of phosphorus used in this experiment was not one most likely to be found on the primitive earth, the result clearly established that the process could occur abiotically. We now find that the simple expedient of heating a nucleoside with a source of inorganic phosphate gives rise to the nucleoside monophosphates in appreciable yield.

In a series of experiments, the nucleosides adenosine, guanosine, cytidine, uridine, and thymidine were heated with sodium dihydrogen orthophosphate, NaH_2PO_4. Two sets of experiments were performed. In the first, the nucleosides were labeled with ^{14}C (specific activity of 1 mc/mmole). In the second, the phosphate was also labeled with ^{32}P. An aqueous solution, 100 μl, containing 2 μmole of a nucleoside and 2 μmole of the phosphate was placed in a 5-ml pyrex tube and lyophilized. By this method a film of solid material containing an intimate mixture of the nucleoside and the phosphate was deposited on the walls of the tube. The tube was then sealed and heated to 160°C for 2 hours. After the tube was cooled to room temperature the seal was broken, and the contents were dissolved in 200 μl of water. This solution containing the reaction products was then analyzed.

The analytical techniques used were electrophoresis, paper chromatography, electrophoresis combined with paper chromatography, and ion-exchange chromatography. In each one of these methods the identification of individual products was made with the coincidence technique of chromatography (*4*). This method, which had earlier been used by us for paper chromatography alone, was now extended to electrophoresis and ion-exchange chromatography.

The electrophoresis was performed at 1500 volts with a Pherograph (*5*). With a *p*H 3.5 buffer and Whatman 3MM paper, a satisfactory separation was effected in 1 hour (*6*). A 50-μl sample containing approximately 0.5 μc of the material to be analyzed was streaked at the origin with 10 μg of each of the 2′-, 3′-, and 5′-monophosphates and cyclic 2′,3′-phosphate as nonradioactive carriers. The radioactive spots were located as dark areas on an autoradiograph, and the nonradioactive carriers appeared on a shadowgram as bright spots on a dark background. There was coincidence between the light area on the shadowgram and a dark area on the autoradiograph. This indicated that material having the same electrophoretic mobility as the carriers at *p*H 3.5 had been synthesized in the experiment. Figure 1 illustrates such an analysis for five different experiments starting with adenosine, guanosine, cytidine, uridine,

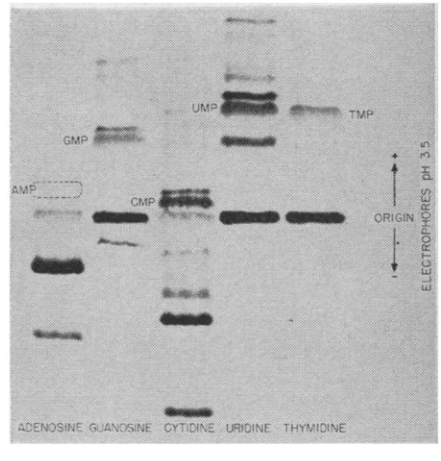

Fig. 1. Reaction between ^{14}C-nucleosides and NaH_2PO_4; autoradiograph shows electrophoretic separation of products at *p*H 3.5. The monophosphates were indentified by the coincidence technique. Several unidentified bands are present.

Table 1. Total uridine monophosphate formed with different phosphates.

Phosphate used	Monophosphate obtained (%)
$NaH_2PO_4 \cdot H_2O$	16.0
$Na_2HPO_4 \cdot 7\ H_2O$	0.6
$Na_3PO_4 \cdot 12\ H_2O$	0.6
$NaNH_4HPO_4 \cdot 4\ H_2O$	13.1
$NH_4H_2PO_4$	5.9
$(NH_4)_2HPO_4$	13.4
H_3PO_4	8.3
$Ca(H_2PO_4)_2 \cdot H_2O$	10.5
$Ca_3(PO_4)_2$	0.1

and thymidine. At *p*H 3.5, the 2′-, 3′-, and 5′-nucleotides did not separate from each other. When the electrophoresis was performed at *p*H 8, the 2′- and 3′-nucleotides moved together apart from the 5′-monophosphate (*7*). A more definite identification was obtained for each of the nucleosides by two-dimensional electrophoresis, at *p*H 3.5 in one direction and at *p*H 8 in the other. Here again coincidence between autoradiograph and shadowgram was used as the criterion of identity.

For paper chromatography we used the method described (*4*). The monophosphates and cyclic phosphates from the electrophoretic separation were eluted and chromatographed on Whatman No. 11 paper, with isobutyric acid and 0.5*N* ammonium hydroxide (5 : 3 by volume) in one direction, and with butanol-1, propionic acid, and water (14 : 9 : 10 by volume) in the other. A two-dimensional chromatogram was prepared for the reaction products of each of the nucleosides. Nonradioactive carriers were present in the material

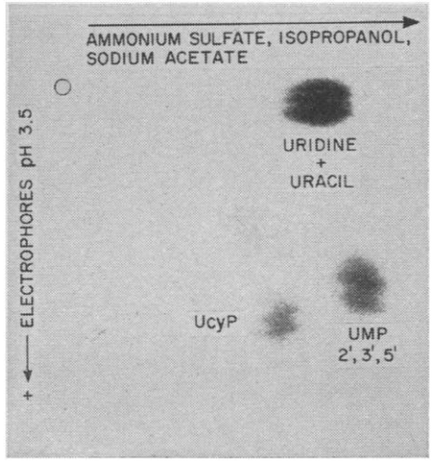

Fig. 2. Autoradiograph illustrating separation of ^{14}C-uridine monophosphates by paper . chromatography in one direction and electrophoresis in the other.

eluted from the electrophoregram. The coincidence technique established the presence of the 5′-, the cyclic 2′,3′- and the 2′- or 3′-monophosphates. In the chromatographic system used the 2′- and 3′-monophosphates traveled as a single spot. Hydrolysis of the cyclic 2′,3′-phosphate with 0.1*N* HCl for 4 hours at room temperature yielded only 2′- and 3′-phosphates, establishing the nature of 2′,3′-linkage (*8*).

In a further scheme of analysis we combined paper chromatography with electrophoresis. Figure 2 illustrates such a separation in the case of uridine. The electrophoresis at *p*H 3.5 was performed under the same conditions as before. Paper chromatography in the second dimension was effected with saturated ammonium sulfate, isopropyl alcohol, 1*M* sodium acetate (80 : 2 : 18 by volume) (*9*). By the use of appropriate standards, the 2′-, 3′-, and the 5′-monophosphates and the cyclic 2′,3′-phosphate were identified. Here again the 2′-monophosphate was not separated from the 3′-monophosphate.

As neither electrophoresis nor paper chromatography was able to separate 2′- and 3′-monophosphates from each other, we turned to ion-exchange chromatography. A Dowex-1 formate column (1 by 15 cm) was used for this purpose (*10*). The separation of the cytidine monophosphates is shown in Fig. 3. The sample was added in 0.5 ml of water; 0.02*M* formic acid was used as the eluant. Known nonradioactive carriers, 5′-, 2′-, and 3′-cytidine monophosphates, were added to the column. The eluant from the column passed through an ultraviolet analyzer which continuously recorded the ultraviolet absorption at 2600 Å. The three distinct peaks shown in Fig. 3 were produced by the 5′-, 2′-, and 3′-monophosphates. After passing through the ultraviolet analyzer, the radioactivity in the eluant was monitored with the aid of a flow-cell scintillation counter (*11*). A second pen on the recorder traced the radioactivity. The radioactive trace coincided with that of the ultraviolet absorption, showing that material identical with 5′-, 2′-, and 3′-nucleotides was synthesized from the starting material, ^{14}C-labeled cytidine and ^{32}P-labeled phosphate.

The sodium dihydrogen orthophosphate was a reasonable choice as a source of phosphate for our first experiments since it may have been present on the primitive earth. In subsequent experiments, we used a number of other sources of phosphorus which may also have occurred on the prebiotic earth. Among these were disodium monohydrogen, trisodium, sodium ammonium monohydrogen, ammonium dihydrogen, diammonium monohydrogen, monocalcium and tricalcium orthophosphates, and phosphoric acid. In every case monophosphates were formed. A comparative study for uridine monophosphates is shown in Fig. 4. Table 1 expresses the same result quantitatively. The second column gives the total phosphate formed based on the percentage of the uridine used. The presence of a large amount of water of crystallization appears to lower the yields. However, the highest yield is in the case of $NaH_2PO_4 \cdot H_2O$ which had one molecule of water of crystallization. In order to assess the role of water, we studied the effect of added water on the reaction. In the case of uridine (2 μmole) and $NaH_2PO_4 \cdot H_2O$ (2 μmole), the yield of 2′-, 3′-, and 5′-phosphates was not affected even with the addition of 50 μmole of water, although the amount of cyclic phosphate formed decreased. When more water was added, the total yield diminished. In the presence of 500 μmole of water, the reaction still took place, although the yield was an order of magnitude less.

A study of the reaction at 160°C over a 24-hour interval indicated that

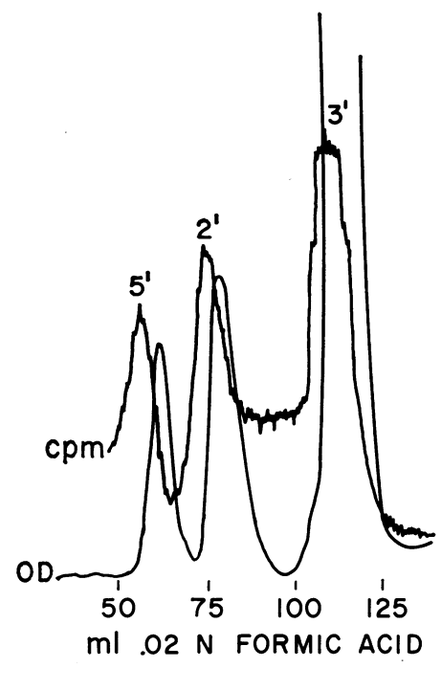

Fig. 3. Cytidine ^{32}P-monophosphates separated on Dowex-1 formate column. The slight shift in the radioactive trace is due to the displacement of the second pen of the recorder.

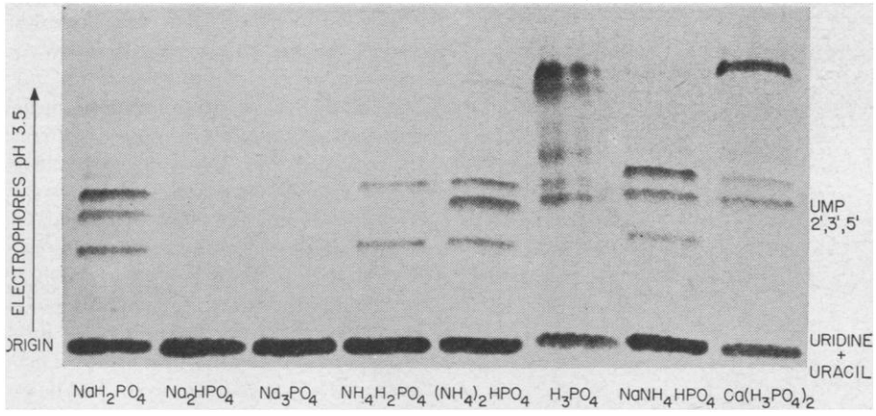

Fig. 4. Autoradiograph showing reaction between ^{14}C-uridine and different sources of phosphorus. Electrophoresis of products at *p*H 3.5.

the total monophosphate formed reached a peak around 4 hours and remained fairly constant, with a gradual decline after 8 hours. After 2 hours, the cyclic phosphate was present in greater amount than the noncyclic. This established that some cyclization process was taking place. The 2′-, 3′-, and 5′- were perhaps being converted into cyclic forms. We have so far identified only the cyclic 2′,3′-monophosphates.

The percentage yields of monophosphate of different types of nucleosides were adenosine, 3.1; guanosine, 9.8; cytidine, 13.7; uridine, 20.6; thymidine, 6.3. Thus uridine monophosphate was obtained in highest yield and adenosine monophosphate in lowest. The pyrimidine nucleosides gave higher yields than the purine nucleosides.

We also have preliminary evidence for the presence of dinucleoside phosphates ApA, GpG, UpU, CpC, and TpT (A, adenosine; G, guanosine; C, cytidine; U, uridine; T, thymidine). Their presence was indicated by the relative rate of migration at *p*H 3.5 (*6*) and separation by paper chromatography with a mixture of 95 percent ethanol and 1*M* ammonium acetate (5:2 by volume) (*7*). Further examination of this product is necessary before its identity can be definitely asserted. There is also an indication from the electrophoretic migration that the nucleoside diphosphates and nucleoside triphosphates are formed in this reaction.

It has been successfully demonstrated that methane, ammonia, and water can, by the action of various forms of energy, give rise to some of the constituents of the nucleic acid molecule and of the protein molecule. Different solutions to this problem have been proposed. Amino acids have been copolymerized to give compounds of high molecular weight by heating them in the absence of water (*12*). Dehydrations have also been effected in dilute aqueous solutions (*13*). In our laboratory several possibilities have been studied—dry conditions, a dilute aqueous milieu, an environment with a relative absence of water, and reactions in contact with the surface of a clay bed (*14*).

We have presented the results of reactions in an environment with a relative absence of water. Since water is not incompatible with this reaction and does not hinder it unless present in large excess, the conditions under which the reaction proceeds may be described as hypohydrous. The maximum temperature was 160°C. Whereas we obtain a yield of about 20 percent at that temperature in 2 hours, experiments at 80°C have given us a yield of monophosphate of about 3 percent in 12 days. The 3 percent was made up of 2′-, 3′-, and 5′-monophosphates and cyclic 2′,3′-phosphate. At this temperature the yield of dinucleoside monophosphate was about 2 percent. At a temperature lower than 80°C the reaction may still take place, but at a much slower rate. We do not know how catalytic or surface reactions could accelerate this process. Preliminary evidence from our own experiments suggests that the surface of clay can promote such a reaction. Our report establishes very clearly that the five nucleotides present in RNA and DNA can be prepared in good yield under conditions which may be considered to be genuinely abiotic and which could reasonably have existed on the primitive earth.

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29 March 1965

Late Glacial Ice-Wedge Casts in Northern Nova Scotia, Canada

Abstract. *Ice-wedge casts in northern Nova Scotia and the relation of the casts to the outwash that contains them indicate that the ice wedges formed in a permafrost environment after the accumulation of the outwash. This permafrost environment is tentatively correlated with pollen zone L-3 of the Gillis Lake deposit, Cape Breton Island, Nova Scotia, and with the Valders time of the midcontinental sequence.*

My observations indicate that the last Pleistocene ice sheet to cover northern Nova Scotia dissipated primarily by downwasting, probably by downmelting (*1*). When the crest of the Cobequid Mountains (Fig. 1) became exposed, the ice to the south between the mountains and Minas Basin stagnated and separated. Rivers of meltwater deposited valley trains south of the mountains. These valley trains merged into outwash fans where the valleys broadened and into deltas where the outwash reached the sea.

Casts of ice wedges are common in the outwash and are particularly well-