tions[51] concerning the above interpretation, it provides the most direct explanation of the experimental observations, and is preferred by the writer. Some support can be derived from the fact that related adducts do in fact undergo 6- and 8-bromination.[53] It is likely that similar reaction paths, available because of the easy reversibility of attachment of halogen to nitrogen, are to be found with related heterocyclic systems.

Reversibility in Aromatic Substitution: Applicability of Linear Free-Energy Relationships Involving Partial Rate Factors for Aromatic Substitution

The qualitative electronic theory of organic chemistry, together with attempts to put this theory in quantitative terms by using linear free-energy relationships or by making molecular orbital calculations concerning the relative availability of transition states, involves a number of assumptions which are not always stated explicitly. One of these is that the rate-determining transition states which are being compared have similar compositions and bonding characters. When this is not so, deviations from predicted rates and orientations are expected as possibilities, though they are not required. Thus it is by no means clear that two aromatic substitutions, one of which shows a primary H–D isotope effect and one of which does not, should give a reasonable linear free-energy relationship between their relative reaction rates. Quite good correlations are, in fact, found in a number of cases,[21] but Berliner and his coworkers[55] have illustrated how reversibility of the first stages of substitution (reactions b and c, Scheme I) can influence the orientation of the products in suitable cases. This provides one example of a number of ways in which the detailed chemistry of the carbocations and other intermediates leading to electrophilic substitution in unsaturated systems can be important in determining the relative rates at which particular products are formed.

This Account has surveyed a number of examples to the understanding of which the writers' coworkers have made contributions; it gives quite inadequate acknowledgment of his personal indebtedness to them, as it does also to the many other groups of workers contributing actively to our knowledge of these reactions.

(55) E. Berliner, J. B. King, and M. Link, *J. Org. Chem.*, 33, 1160 (1968); J. B. Kim, C. Chen, J. K. Krieger, K. R. Judd, C. C. Simpson, and E. Berliner, *J. Amer. Chem. Soc.*, 92, 910 (1970).

Prebiotic Chemistry and Nucleic Acid Replication

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The replication of nucleic acids is the central reaction responsible for the transmission of hereditary information in all contemporary organisms. It is widely believed that a similar process, some form of complementary replication of molecules related to nucleic acids, played an important part in the origins of life. In this Account we review our work on some reactions that we believe could have occurred on the primitive earth and played a part in the evolution of a self-replicating system.

Unfortunately, our picture of the conditions that prevailed on the primitive earth is incomplete, so no generally accepted rules can be given that determine whether or not a reaction condition is prebiotic. The following are the guidelines that we have followed in our work.

(a) All primary organic reagents must be derivable as significant products from a reducing atmosphere composed of a selection of the following simple gases: CH_4, CO, CO_2, NH_3, N_2, H_2O. Ultraviolet light, heat, or electric discharges may be used as sources of the energy involved in the synthesis of primary reagents from these elementary gases.

(b) No solvent other than water may be used. Reactions in aqueous solution must be carried out at moderate pH's, preferably between 7 and 9.

(c) Solid-state reactions must occur without excessive drying of the reactants. Solid-state reaction mixtures are preferably obtained by evaporating aqueous solutions that are initially at pH's between 7 and 9.

(d) All reactions must occur under conditions of temperature and pressure that occur on the surface of the earth today. We doubt that volcanos or thermal springs contributed much to the origins of life, so we prefer to carry out solid-state reactions at temperatures below 80°C (surface temperatures up to 90°C have been recorded in California deserts, and we have found that large areas of the desert surface are raised above 65°C for several hours on a typical

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Rolf Lohrmann was born in Germany in 1930, and studied at the Technische Hochschule in Stuttgart. He spent 2 years as Postdoctoral Fellow at the Genetics Foundation of the University of Texas, Austin, with H. S. Forrest, followed by work at the Institute for Enzyme Research, University of Wisconsin, with H. G. Khorana. Dr. Lohrmann has been Senior Research Associate at the Salk Institute since 1965.

summer day). We emphasize that other authors[1] believe that volcanos played a central role in the origins of life.

The conditions outlined above are so highly restrictive that they separate prebiotic chemistry from most other contemporary organic chemistry. Few organic chemists willingly undertake syntheses in an unsuitable solvent using unpromising starting materials, or attempt fusion reactions between unprotected, unactivated, multifunctional reagents at 65°C. Our work has not been motivated by the desire to discover novel or elegant methods of synthesis. Rather, it has grown out of the conviction that a primitive genetic system evolved on the earth and the hope that with patience and luck it may be possible to discover some of the organic reactions that made this possible.

The evolution of nucleic acids on the primitive earth was no doubt a lengthy process. It is likely that many of the very different types of chemical reactions that were to be important for the earliest organisms occurred simultaneously in the prebiotic soup. However, in the interests of simplicity, we shall discuss the transition from the primitive atmosphere to the simplest replicating molecules in the following four well-defined stages: the formation of a "prebiotic soup" of organic precursors, including the purine and pyrimidine bases and the pentose sugars; the condensation of these precursors and inorganic phosphate to form monomeric nucleotides and activated nucleotide derivatives; the polymerization of nucleotide derivatives to oligonucleotides; the complementary replication of oligonucleotides in a template-directed process that depends on Watson–Crick base pairing.

Before turning to the description of our experimental work, two more general questions must be discussed briefly, namely, the nature of the first nucleic acids and the priority of polypeptide synthesis or nucleic acid replication. We believe that nucleosides, deoxynucleosides, and derivatives of related sugars must have coexisted in the prebiotic soup, and copolymerized to form the first prebiotic nucleic acid like molecules; we do not think, therefore, that the question—which came first, DNA or RNA?—is a good one. We have used ribonucleoside-containing models extensively because ribonucleoside derivatives are readily available and undergo efficient template-directed condensations. Further work on derivatives of other sugars would be desirable.

The role of prebiotic polypeptides in the origins of life is unclear.[1] We have preferred to study the synthesis of polynucleotides in the absence of polypeptides in the first place because we were unable to guess which polypeptides were likely to be prebiotic catalysts for the reactions that interest us. Attempts to utilize small peptides or colloidal aggregates as catalysts for nucleic acid synthesis and replication are of considerable interest.

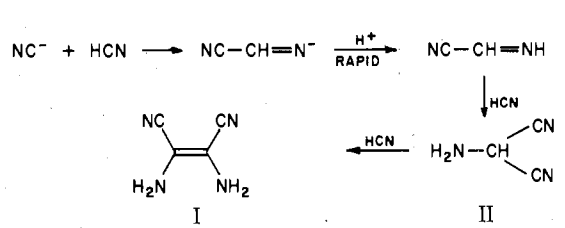
Prebiotic Formation of Sugars and Bases

In the course of his classic experiments on the prebiotic synthesis of amino acids, Miller confirmed that formaldehyde and hydrogen cyanide are abundant products of the action of an electric discharge on a gas mixture containing methane, ammonia, and water.[2] Formaldehyde and hydrogen cyanide are now known to be formed so readily under such a wide variety of conditions that they are considered likely precursors of a variety of important components of the prebiotic soup, including the sugars and purine bases.[1] The identification by radioastronomers of vast amounts of formaldehyde and hydrogen cyanide in dust clouds in outer space makes this suggestion all the more plausible.[3]

The Butlerow reaction, the synthesis of a complex mixture of sugars from formaldehyde by the action of alkaline catalysts such as calcium hydroxide, was discovered in the nineteenth century.[4] Analysis by paper chromatography has demonstrated that the pentose sugars, ribose and arabinose, are among the products.[5] Recent work on the prebiotic synthesis of sugars has shown that, in the presence of mineral catalysts, the polymerization of formaldehyde to sugars proceeds rapidly at 100°C, and presumably more slowly under milder conditions that could have occurred on the primitive earth.[6,7] We shall not discuss this reaction in detail, since little work on it has been undertaken in our laboratory.

In 1960 Oró and his coworkers discovered a remarkably simple synthesis of adenine.[8] They obtained this substance in 0.5% yield by refluxing concentrated ($\geq 1.0\ M$) solutions of ammonium cyanide. Our first studies in prebiotic chemistry were directed to the development of a related synthesis of adenine that could occur in more dilute solutions. This was necessary because the conditions used by Oró are not prebiotic; if all of the nitrogen in the atmosphere was converted to ammonium cyanide and dissolved in the oceans, the resulting solution would not exceed $0.2\ M$ in concentration. In fact, hydrogen cyanide hydrolyzes to formate so rapidly that it is unlikely that the cyanide concentration in the oceans ever exceeded $10^{-4}\ M$, although it could have been greater at special sites.[9]

A detailed study of the polymerization of aqueous solutions of hydrogen cyanide[9] showed that the only easily isolatable oligomer is the tetramer, diaminomaleonitrile (I). It is formed in the following sequence of reactions, *via* aminomalononitrile (II).



I II

(1) A selection of relevant literature references is given in S. L. Miller and L. E. Orgel, "The Origins of Life on the Earth," Prentice-Hall, New York, N.Y., 1974.

(2) S. L. Miller, *J. Amer. Chem. Soc.*, 77, 2351 (1955).

(3) M. D. Rank, C. H. Townes, and W. J. Welch, *Science*, 174, 1083 (1971).

(4) A. Butlerow, *Justus Liebigs Ann. Chem.*, 120, 295 (1861).

(5) R. Mayer, K. Runge, and H. Dreschel, *Z. Chem.*, 3, 134 (1963), and references therein.

(6) N. W. Gabel and C. Ponnamperuma, *Nature (London)*, 216, 453 (1967).

(7) C. Reid and L. E. Orgel, *Nature (London)*, 216, 455 (1967).

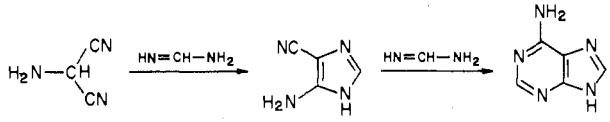
(8) J. Oró, *Biochim. Biophys. Res. Commun.*, 2, 407 (1960); J. Oró and A. P. Kimball, *Arch. Biochem. Biophys.*, 94, 217 (1961); 96, 293 (1962).

(9) R. A. Sanchez, J. P. Ferris, and L. E. Orgel, *J. Mol. Biol.*, 30, 223 (1967), and references therein.

It at first seemed likely that, in Oró's reaction, formamidine (III), formed from HCN and NH_3, reacted

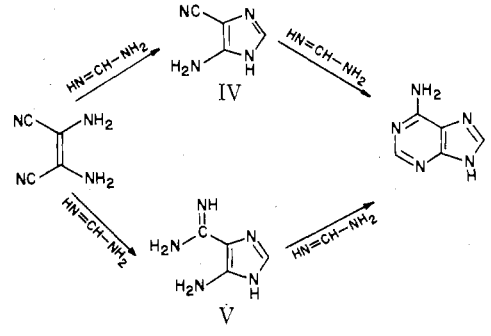
III

with II to give adenine. We synthesized II (at that time it was unknown, although a number of incorrect identifications had been reported) and showed that it does indeed react with formamidine (III) to give adenine.[10]



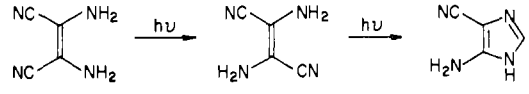
IV

However, a detailed kinetic investigation showed that this could not be the route by which adenine is synthesized in Oró's reaction, because II reacts so rapidly with HCN to give I that condensation with formamidine is prevented. In fact, adenine is formed by the reaction of formamidine with I, *via* 4-aminoimidazole-5-carbonitrile (IV) and the corresponding carboxamidine (V).[9] 4-Aminoimidazole-5-carboxamide (VI), a precursor of purines in the biosynthetic pathway, is formed as a side product by the hydrolysis of IV and V.



V

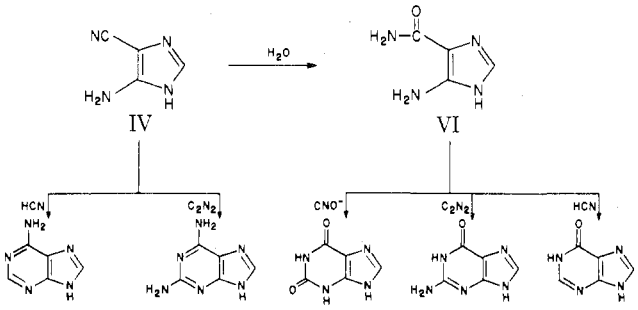
The synthesis discovered by Oró does not occur in dilute solution, since little formamidine is formed from a dilute solution of ammonium cyanide. Fortunately, it proved possible to isomerize I photochemically to give the desired carbonitrile (IV).[11] In the absence of oxygen, the yield in dilute aqueous solution is essentially quantitative.[12]



I IV

The purines adenine, hypoxanthine, diaminopurine, and guanine are obtained readily from IV, V, or VI by the action of cyanide, cyanogen, cyanate, etc.[13] Since these reactions proceed under prebiotic conditions, they complete the prebiotic synthesis of the biologically important purines.

(10) J. P. Ferris and L. E. Orgel, *J. Amer. Chem. Soc.*, 87, 4976 (1965); 88, 3929 (1966).
(11) J. P. Ferris and L. E. Orgel, *J. Amer. Chem. Soc.*, 88, 1074 (1966).
(12) J. P. Ferris, J. E. Kuder, and A. W. Catalano, *Science*, 166, 765 (1969).
(13) R. Sanchez, J. P. Ferris, and L. E. Orgel, *J. Mol. Biol.*, 38, 121 (1968).



IV VI

The reactions described above do not permit the synthesis of purines in appreciable yield from very dilute solutions of hydrogen cyanide; in 10^{-4} *M* solution, for example, formate is the main product. However, we were able to show that all of the component reactions proceed readily in the cold. If very dilute ammonium cyanide solutions are cooled to temperatures in the range −10°C to −22°C, ice separates out and a cold concentrated solution of ammonium cyanide is obtained. The tetramer (I) forms readily in such a solution[14] and rearranges to IV when exposed to ultraviolet irradiation. Furthermore, IV reacts slowly with HCN to give adenine, under these same conditions. Thus a synthesis of adenine starting from a dilute solution of HCN is possible, if the solution is first concentrated by freezing.

We must draw attention to a variation of this synthesis that may be prebiotic. In a sufficiently long time, adenine might be synthesized directly from dilute ammonium cyanide solution by eutectic freezing, without any photochemical step. Formamidine would no doubt be formed very slowly in cold, concentrated solutions of ammonium cyanide and might react with I, as in Oró's synthesis. The time required to test whether this reaction sequence is possible is a deterrent to laboratory investigation, but times of the order of decades may not have been prohibitive on the primitive earth.

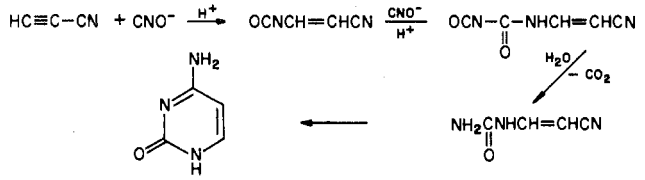
After completing our work on purine synthesis, we began to explore related pyrimidine syntheses. Since hydrogen cyanide seemed the most likely precursor of the purines, we hoped to find a molecule formed together with hydrogen cyanide in an electric discharge which would provide the three-carbon unit of the pyrimidines. Cyanoacetylene seemed the obvious candidate, since well-known syntheses of pyrimidines could be adapted to derive cytosine from cyanoacetylene and cyanate, cyanogen, cyanamide, or urea.

We had no difficulty in establishing that cyanoacetylene is a major nitrogen-containing product formed when an electric discharge is passed through a mixture of N_2 and CH_4; only HCN is more abundant.[15] Cyanoacetylene is also formed when simple gas mixtures containing carbon, hydrogen, and nitrogen are heated strongly.[16] The identification of cyanoacetylene in interstellar dust clouds makes it even more plausible that this molecule played a role in prebiotic synthesis.[3]

In aqueous solution cyanoacetylene reacts readily with a variety of nucleophiles. In particular, it reacts

(14) R. Sanchez, J. P. Ferris, and L. E. Orgel, *Science*, 153, 72 (1966).
(15) R. Sanchez, J. P. Ferris, and L. E. Orgel, *Science*, 154, 784 (1966); *J. Mol. Biol.*, 33, 693 (1968).
(16) L. J. Krebaum, *J. Org. Chem.*, 31, 4103 (1966).

with cyanate under mild conditions to give cytosine, probably by the following mechanism. Since cyanate can be derived, for example, from cyanogen by direct hydrolysis, this completes a prebiotic synthesis of cytosine. Uracil can be obtained from cytosine by aqueous hydrolysis under mild conditions.



It is a striking fact that biologically important sugars, amino acids, purine bases, and pyrimidine bases can all be obtained in aqueous solution, under mild conditions, from the small family of molecules, NH_3, CH_2O, HCN, HC≡CCN, NCCN, all of which, in turn, are derived from a mixture of N_2, H_2, CH_4, and H_2O. This same family of small, reactive molecules has recently been shown to be abundant in extraterrestrial dust clouds.[3] It is hard to believe that this web of connections is coincidental; it is far more likely that the amino acids, sugars, and nucleotide bases are important in contemporary biochemistry because they were prominent among the organic compounds that formed on the primitive earth.

Nucleoside Formation

The synthesis of nucleosides from purine or pyrimidine bases and ribose or deoxyribose has proved surprisingly difficult, and remains a major problem in prebiotic chemistry. We have failed in numerous attempts to obtain nucleosides in aqueous solution by the reaction of nucleoside bases with ribose either photochemically or in the presence of dehydrating agents. Attempts to displace a phosphate or pyrophosphate group in the 1 position of ribose or arabinose by a base have been equally unsuccessful. Early reports of successful syntheses of adenosine and deoxyadenosine in aqueous solution at moderate pH's turn out to be incorrect.[1,17]

Thermal syntheses from dry mixtures at first proved equally frustrating. Adenine reacts with ribose in the dry state on warming to give a mixture of isomeric 6-ribosylaminopurines, compounds that could not be isomerized to the nucleosides.[18] Cytosine and guanine behave similarly, while uracil and hypoxanthine do not give any products under these conditions.

In attempting to simulate, under prebiotic conditions, Schramm's synthesis of adenosine from adenine and ribose in "phenyl polyphosphate" or "ethyl polyphosphate,"[19] we heated adenine and ribose in the presence of a variety of inorganic polyphosphates. We obtained some β-adenosine from almost all of the reactions in which Mg^{2+} salts were used.[18] Further investigations showed that the polyphosphate anions are irrelevant; inosine, adenosine, and guanosine are formed when the corresponding bases are heated with ribose in the presence of a variety of inorganic salts (Table I).[20] The products formed include α and β derivatives of the furanose and pyranose forms of the sugars. The yields of the authentic β derivatives never exceeded 8%. We do not understand the mechanism of this reaction.

Table I
Effects of Various Substances on the Dry-Phase Synthesis of Inosine at 100°[a]

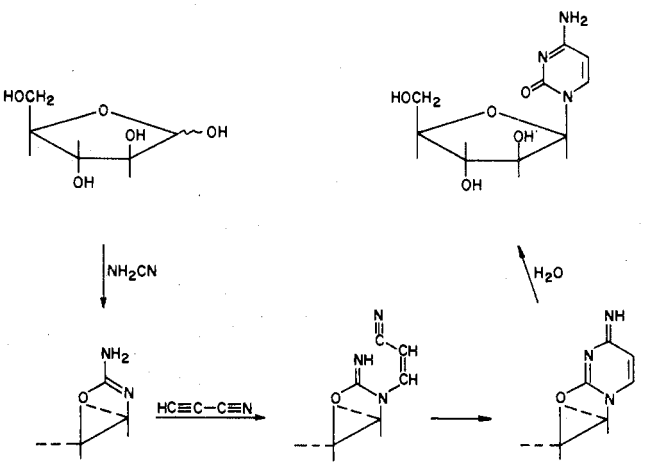
Good yields (10–20%)	Moderate yields	Poor yields, or negative (0–2%)	
HCl	$CaSO_4$	$(NH_4)_2HPO_4$	$CaCl_2$
$(NH_4)_2HBO_3$	Apatite	Na_2CO_3	Montmorillonite
H_2SO_4	$MgSO_4$	Na_2HPO_4	Kaolinite
p-$CH_3C_6H_4SO_3H$	$CaHPO_4$	H_3PO_4	Zeolite
$MgCl_2$	$(NH_4)_2SO_4$	$Ca(NO_3)_2$	Alumina
	$MnSO_4$	$Ca(OAc)_2$	Silica gel
	$MnCl_2$	$CaCO_3$	Sand
	$Mg(NH_4)PO_4$	$CaBr_2$	

[a] The yields are given for mixed inosine isomers. The salts are listed in a roughly decreasing order of efficiency. The order is very approximate since the quantitative results are not strictly reproducible.

It is a curious and perhaps significant fact that the mixture of inorganic salts obtained by evaporating seawater is as effective a catalyst as any other that we have discovered.[20] If tide pools contained purine bases and pentose sugars, purine nucleosides would have formed when the solids obtained by evaporations were heated, although the yields would have been small.

The pyrimidine bases, uracil and cytosine, do not react with ribose to give nucleosides under the conditions described above, and no prebiotic condensation of pyrimidine bases and ribose to give nucleosides is known. However, a rather roundabout route *via* cytosine arabinonucleoside involves some interesting synthetic chemistry, even if its occurrence on the primitive earth is problematical.[21]

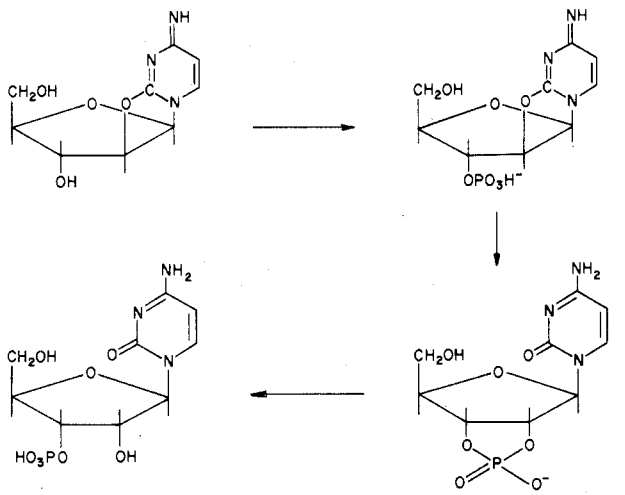
We have seen that cyanoacetylene reacts with cyanate or cyanogen in aqueous solution to give cytosine. If the reaction with cyanogen is carried out in the presence of ribose, α-cytidine is formed. The corresponding reaction of arabinose yields cytosine arabinonucleoside. Much better yields of these compounds are obtained when cyanamide is used in place of cyanogen. The mechanism of the syntheses



(17) C. Reid, L. E. Orgel, and C. Ponnamperuma, *Nature (London)*, 216, 936 (1967).
(18) W. D. Fuller, R. A. Sanchez, and L. E. Orgel, *J. Mol. Biol.*, 67, 25 (1972).
(19) G. Schramm, G. Lunzmann, and F. Bechmann, *Biochim. Biophys. Acta*, 145, 221 (1967).
(20) W. D. Fuller, R. A. Sanchez, and L. E. Orgel, *J. Mol. Evol.*, 1, 249 (1972)
(21) R. A. Sanchez and L. E. Orgel, *J. Mol. Biol.*, 47, 531 (1970).

has been determined. As required by the reaction sequence, only α-cytidine is formed from ribose and only β-arabinosylcytosine from arabinose. However, the isomerization of α-cytidine to β-cytidine occurs photochemically. The yield is poor, so this route provides only a marginal prebiotic synthesis of β-cytidine.[21]

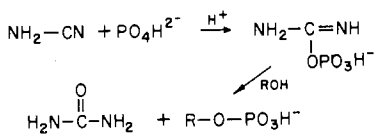
An alternative route to β-cytidine starts with arabinose and proceeds *via* $O,2'$-cyclocytidine. The phosphorylation of this substance, by methods to be described in the next section, gives $O,2'$-cyclocytidine 3′-phosphate,[22] which then opens to the ribonucleoside rather than the arabinonucleoside, *via* the cyclic 2′,3′-phosphate.[23] The accumulation of $O,2'$-



cyclocytidine on the primitive earth is not easily understood, since it hydrolyzes readily to cytosine arabinonucleoside.

Phosphorylation

(a) Solution Reactions. Two general methods of prebiotic phosphorylation have been proposed.[1] In the first, a molecule containing an activated double or triple bond, for example, cyanamide, is used as a condensing agent in aqueous solution.



In the second, phosphorylation is achieved by heating suitable dry mixtures that include inorganic orthophosphates.

A rather detailed investigation of a number of solution reactions led us to conclude that there is little prospect of achieving an efficient synthesis of nucleotides from nucleosides and inorganic phosphate with any of the reagents that have been proposed so far.[24] The attack of inorganic phosphate on activated double and triple bonds often proceeds so readily under prebiotic conditions that hydrolysis is not a serious competing reaction. However, there seems little selectivity in the subsequent reactions of the activated intermediate, so under all reasonable conditions hydrolysis through attack by the OH^- ion is much faster than esterification through attack by sugar hydroxyl groups.

Two partial exceptions to this general rule have been discovered. Nucleosides react with inorganic trimetaphosphate to give nucleoside 2′(3′)-phosphates in good yield.[25] However, this reaction does not resolve the problem of prebiotic phosphorylation, since the formation of the trimetaphosphate ion must be explained (see below). The phosphorylation of aldose sugars to sugar 1-phosphates is brought about efficiently by cyanogen,[26] but this reaction is very specific for the 1-OH group; the phosphorylation of nucleosides with cyanogen is not particularly efficient.

(b) Solid-State Reactions. It is possible that prebiotic condensing reagents exist that do not suffer from the drawback discussed above. However, rather than search for such reagents, we decided to explore the solid-state chemistry of inorganic phosphates. Of course, it has long been known that salts of the HPO_4^{2-} anion on heating are converted to pyrophosphates, and salts of the $H_2PO_4^-$ anion to a variety of "metaphosphates." The phosphorylation of alcohols and nucleosides by inorganic phosphates at elevated temperatures had also been described.[27] Our objective was to achieve these reactions efficiently at lower temperatures.

A survey of a variety of plausible prebiotic molecules indicated that certain amides catalyze the phosphorylation of nucleosides by inorganic phosphates, but that urea and those substituted ureas that retain a $-CONH_2$ group are much better catalysts.[28,29] Ammonium phosphate is the most reactive of the inorganic phosphates that we have used. Presumably, this is because phosphorylation occurs best under acidic conditions, and such conditions prevail in matrices which lose ammonia on heating.

We have investigated many aspects of the urea-catalyzed phosphorylation reaction. If ammonium phosphate, or a mixture of sodium phosphate and an ammonium salt, is heated with urea at moderate temperatures, pyrophosphate and other linear polyphosphates are formed in excellent yield.[30] The Mg^{2+} ion catalyzes pyrophosphate-bond formation; $MgNH_4PO_4$ is rapidly converted to the pyrophosphate at low temperatures in the presence of urea.[31] Hydroxylapatite and other calcium phosphates also yield pyrophosphate and small amounts of higher polyphosphates in the presence of urea and an ammonium salt, but the reaction requires longer times or somewhat higher temperatures.

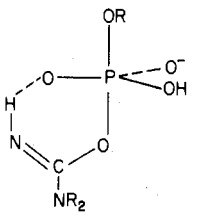
When nucleosides are heated with inorganic phosphates and urea they are first converted to a mixture of the nucleoside 5′-phosphate with smaller amounts of the 2′- and 3′-phosphates.[28] On more prolonged

(22) R. Lohrmann, unpublished results.
(23) C. M. Tapiero and J. Nagyvary, *Nature (London)*, 231, 42 (1971); J. Skoda, J. Moravek, and J. Kopecky, *Fed. Eur. Biochem. Soc., Meet., 6th, 1969*, Abstr. 433 (1969).
(24) R. Lohrmann and L. E. Orgel, *Science*, 161, 64 (1968).
(25) R. Saffhill, *J. Org. Chem.*, 35, 2881 (1970); A. W. Schwarz, *Chem. Commun.*, 1393 (1969).
(26) M. Halmann, R. A. Sanchez, and L. E. Orgel, *J. Org. Chem.*, 34, 3702 (1969).
(27) C. Ponnamperuma and R. Mack, *Science*, 148, 1221 (1965).
(28) R. Lohrmann and L. E. Orgel, *Science*, 171, 490 (1971).
(29) M. J. Bishop, R. Lohrmann, and L. E. Orgel, *Nature (London)*, 237, 162 (1972).
(30) R. Osterberg and L. E. Orgel, *J. Mol. Evol.*, 1, 241 (1972).
(31) G. J. Handschuh, R. Lohrmann, and L. E. Orgel, *J. Mol. Evol.*, 2, 251 (1973).

heating the nucleoside cyclic 2′,3′-phosphate is the major product. In addition a variety of dinucleoside phosphate derivatives and nucleoside polyphosphates are formed.[32]

In this system, the Mg^{2+} ion depresses the rate of phosphorylation of the OH groups of nucleosides and greatly accelerates the rate of pyrophosphate bond formation. The major product formed from a nucleoside 5′-phosphate and inorganic phosphate in the absence of Mg^{2+} is the 2′(3′),5′-bisphosphate; in the presence of Mg^{2+}, the nucleoside 5′-diphosphate is the major product. We have been able to obtain uridine 5′-diphosphate and small amounts of uridine 5′-triphosphate from uridine and inorganic phosphate by an appropriate manipulation of the reaction conditions.[31]

Our attempts to discover the mechanism of the urea-catalyzed reaction have met with no success. We were unable to detect either carbamoyl phosphates or phosphoramidates in the reaction mixture, under conditions that would probably have permitted their survival. We suggest, very tentatively, that the reaction involves a hydrogen-bonded pentacovalent intermediate formed by the attack of the carbamoyl oxygen of urea on the phosphate group, since this hypothesis enables us to rationalize the activity of amides and unsymmetric dialkylureas as catalysts for the reaction, and the inactivity of symmetric dialkylureas.[29]



However, a mechanism involving general acid–base catalysis rather than nucleophilic attack by urea is also possible.

The urea-catalyzed reactions of inorganic phosphates could probably have occurred on the primitive earth. They would have led to the formation of a variety of inorganic polyphosphates, nucleotides, and nucleoside polyphosphates. It is our present working hypothesis that a mixture of polyphosphates, formed in this way or by some related process, provided the energy necessary for polynucleotide and polypeptide synthesis on the primitive earth.[33]

Polymerization of Nucleotides

We assume that long polynucleotides were first formed on the primitive earth either from mononucleotides or from short oligonucleotides. Other alternatives, for example, the addition of bases to a preformed polyribose phosphate backbone, seem less likely. The evolution of self-replicating molecules must clearly have involved two different types of polymerization. In the first, activated intermediates combined directly, either in solution or in the solid state, to form "primary" templates. In the second, these "primary" templates directed the synthesis of complementary molecules in aqueous solution according to the Watson–Crick pairing rules. Possibly the two polymerization processes collaborated in the generation of polynucleotide strands containing self-paired regions.[34,35]

We have used the same kinds of activated precursors in our experiments on direct synthesis and in our experiments on template-directed synthesis, namely nucleoside cyclic 2′,3′-phosphates and activated nucleoside 5′-phosphates. Almost all activated nucleoside 2′,(3′)-phosphates cyclize readily in aqueous solution to the cyclic 2′,3′-phosphates, compounds which are themselves activated and, therefore, capable of polymerizing in the solid state or in solution. Thus, if we neglect the remote possibility of a secondary activation step in which an activating agent attacks the already cyclized nucleotide,[36] the only 2′,(3′)-activated derivatives we need consider are the cyclic 2′,3′-phosphates themselves. These compounds are formed readily under prebiotic conditions, for example, directly in the urea-catalyzed phosphorylation reaction, or by the cyclization of nucleoside 2′,(3′)-phosphates.

Activated nucleoside 5′-phosphates of many different kinds could be considered as possible precursors of prebiotic oligonucleotides. Stable derivates include, of course, the nucleoside 5′-polyphosphates which are so important in biochemistry. Various stable N–P derivatives derived from amines, amino acids, and imidazoles have been synthesized under prebiotic conditions in our laboratory and utilized as substrates for template-directed synthesis. Another possibility is that the reactive intermediates were formed in aqueous solution by the attack of condensing agents on nucleoside 5′-phosphates. A good deal of work has been done with water-soluble carbodiimides, and some studies with potentially prebiotic condensing agents such as cyanamide have been reported.[37]

All our attempts to synthesize oligonucleotides directly in aqueous solution without the help of enzymes have been unsuccessful. In the absence of a template we obtain at most a few per cent of the dinucleotide. Our failure cannot be attributed to the inadequate activation of our starting materials since nucleoside 5′-diphosphates and nucleoside 5′-triphosphates form high molecular weight polymers in the presence of suitable enzymes, and nucleoside 5′-phosphorimidazolides have comparable free energies of hydrolysis. Even the nucleoside cyclic 2′,3′-phosphates form short oligomers in the presence of appropriate ribonucleases.

An efficient prebiotic procedure for the synthesis of polynucleotides from an aqueous solution of activated monomers would be extremely important, so it may be worth discussing the various approaches that have been proposed, even if none has yet proved successful.

(a) It is possible that among the minerals present on the primitive earth was one on which activated nucleotides adsorbed from solution and condensed,

(32) R. Osterberg, L. E. Orgel, and R. Lohrmann, *J. Mol. Evol.*, 2, 231 (1973).
(33) R. Lohrmann and L. E. Orgel, *Nature (London)*, 244, 418 (1973).
(34) F. H. C. Crick, *J. Mol. Biol.*, 38, 367 (1968).
(35) L. E. Orgel, *J. Mol. Biol.*, 38, 381 (1968).
(36) A. M. Michelson, "The Chemistry of Nucleosides and Nucleotides," Academic Press, New York, N.Y., 1963, Chapter 7.
(37) J. D. Ibanez, A. P. Kimball, and J. Oro, *Science*, 173, 444 (1971).

in situ, to form polynucleotides. The few experiments that we have carried out using the minerals hydroxylapatite and montmorillonite produced little or no condensed material.

(b) Oparin suggested that coacervates played an important role in the origins of life.[38] It seems possible that a positively charged colloid might adsorb negatively charged polyphosphates on its surface and catalyze internucleotide bond formation. Small yields of polynucleotides are said to be formed on "protein microspheres."[39]

(c) A recurrent theme in the speculative literature concerns a small organic molecule that acted as a catalyst for polynucleotide synthesis on the primitive earth, and later "evolved" into an enzyme. Unfortunately, the nature of this catalyst is rarely specified. We have carried out a few experiments with such obvious "catalysts" as polylysine, relatively simple derivatives of imidazole, Zn^{2+}–amino acid complexes, etc., but without success. A systematic search for an active catalyst is probably justified.

Solid-state reactions carried out under moderately dry conditions provide an alternative approach to the synthesis of polynucleotides from activated monomers. We have studied the polymerization of a few activated 5′-nucleotides in the solid state, but so far with little success. We have also examined the polymerization of the nucleoside cyclic 2′,3′-phosphates in some detail.[40,41]

If the salts of nucleoside cyclic 2′,3′-phosphates are heated strongly, oligonucleotides are formed in fairly good yield.[23] However, in the absence of catalysts, little reaction occurs at temperatures below 100°C. A number of amines act as excellent catalysts for the polymerization of adenosine cyclic 2′,3′-phosphate. Yields of polymer up to 68% have been obtained using ethylenediamine under very dry conditions. Under more plausibly prebiotic conditions, yields of up to 25% of oligomers (mainly dimer) have been obtained.[40]

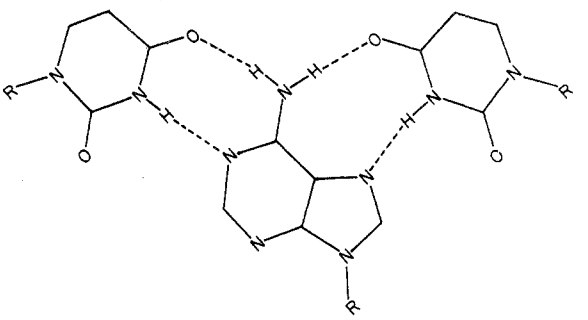
A study of the di- and trinucleotides produced in typical condensations showed that the naturally occurring 3′–5′ linkage predominates over the 2′–5′. This is in marked contrast to the results obtained in template-catalyzed reactions, including those of cyclic 2′,3′-phosphates. Template-directed reactions always yield an excess of the unnatural 2′–5′ isomer.

The products from a reaction carried out at room temperature under very dry and, therefore, nonprebiotic conditions, in the presence of ethylenediamine were analyzed in some detail.[41] Up to 8% of material larger than the hexamer was obtained. The highest molecular weight fraction obtained by gel permeation chromatography contained 0.67% of the starting material and had an average chain length of 13.4 residues.

Template-Directed Synthesis

Organized helical structures are formed by poly(uridylic acid) (poly(U)) with adenosine or with monomeric adenosine derivatives such as adenosine 5′-phosphate. These helical structures are held together by hydrogen bonds between the uracil and adenine ring systems. However, the bonding is significantly different from that which occurs in the double-helical nucleic acids.

The helices formed by adenosine derivatives with poly(U) are triple helices involving two poly(U) strands and one column of monomers.[42] Adenosine is bound to a residue of one of the poly(U) chains by the standard Watson–Crick hydrogen bonds and to a residue of the second poly(U) chain by a different system of hydrogen bonds.



Derivatives of guanine form similar triple helices with poly(cytidylic acid) (poly(C)) under most conditions, but it is possible to obtain a double helical structure from guanosine and poly(C) at pH's close to 8. The situation is completely different when attempts are made to use poly(adenylic acid) (poly (A)) or poly(guanylic acid) (poly (G)) as a template. Neither of these polymers will form stable helices with monomeric derivatives of the complementary pyrimidine nucleoside. This is almost surely due to the very limited tendency of pyrimidine derivatives to form stacks.

Organized helices involving monomeric nucleoside derivatives are much less stable than nucleic acids. The 2poly(U)·pA triple helix melts at low temperatures, for example at 12°C under conditions of our first experiments,[43] while the poly(U)·poly(A) double helix and the 2poly(U)·poly(A) triple helices melt at much higher temperatures.

The organization of monomeric purine nucleoside derivatives on polypyrimidine nucleotide chains shows some specificity. Poly(U) does not form organized structures with guanosine derivatives and poly(C) does not interact with adenosine derivatives. This suggests that a specific, template-directed synthesis of oligonucleotides would occur if monomeric nucleotide derivatives organized in a double or triple helix, while still attached to the template, could be induced to form internucleotide bonds.

The first template-directed organic synthesis to be reported was the condensation of two hexathymidylic acid residues to the dodecanucleotide on a poly(A) template in the presence of a water-soluble carbodiimide.[44] Our own first experiments also utilized

(38) A. I. Oparin, "The Origins of Life on Earth," Academic Press, New York, N.Y., 1957; Proiskhozhdenie Zhizn, Izd. Moskovski, Rabochii, Moscow 1924.

(39) See S. W. Fox, J. R. Jungck, and T. Nakashima, Proceedings of the First Meeting of the International Society for the Study of the Origins of Life, Barcelona, 1973.

(40) M. S. Verlander, R. Lohrmann, and L. E. Orgel, *J. Mol. Evol.,* 2, 303 (1973).

(41) M. S. Verlander and L. E. Orgel, *J. Mol. Evol.,* 3, 115 (1974).

(42) F. B. Howard, J. Frazier, M. F. Singer, and H. T. Miles, *J. Mol. Biol.,* 16, 415 (1966); S. Arnott and P. J. Bond, *Nature (London), New Biol.,* 244, 99 (1973).

(43) J. Sulston, R. Lohrmann, L. E. Orgel, and H. T. Miles, *Proc. Nat. Acad. Sci. U.S.,* 59, 726 (1968).

(44) R. Naylor and P. T. Gilham, *Biochemistry,* 5, 2722 (1966).

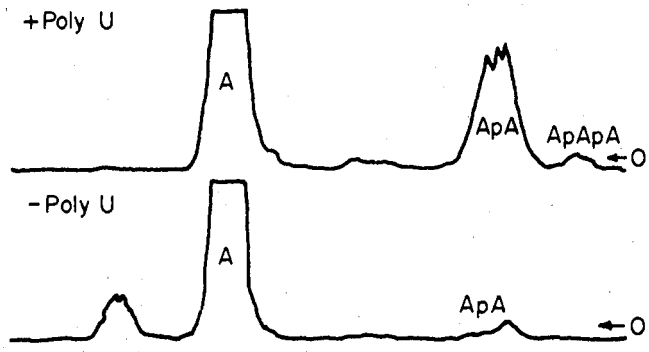


Figure 1. Paper chromatography of the products formed from [^{14}C]adenosine and adenosine 5′-phosphate in the presence of a water-soluble carbodiimide, with or without poly(U). The trace represents counts measured directly with a strip scanner. The extra peak in the lower trace corresponds to an adduct formed by the carbodiimides with adenosine when no poly(U) is present.

water-soluble carbodiimides, at first to link together adenosine derivatives on a poly(U) template.[43] We found that the condensation of adenylic acid with either adenosine or adenylic acid is greatly enhanced in the presence of poly(U) at temperatures below the melting point of the 2poly(U)·pA triple helix (Figure 1). On the other hand, poly(U) had no influence on the condensation of adenylic acid with uridine, cytidine, or guanosine.[45] In a precisely parallel series of experiments we found that poly(C) enhances the carbodiimide-induced condensation of guanylic acid with guanosine, but has no effect on the combination of guanylic acid with uridine, cytidine, or adenosine.[46]

These reactions establish that the Watson–Crick rules apply to template-catalyzed nonenzymatic condensation reactions. However, a detailed analysis of the products revealed a startling difference between the chemical condensation and the enzymatic reaction. The condensation of adenylic acid with adenosine or adenylic acid on a poly(U) template yields a product which is predominantly 2′–5′ linked,[43] while the enzymatic reactions of RNA polymerases yield exclusively the 3′–5′ isomers. In an exactly similar way the nonenzymatic condensation of guanylic acid with guanosine, whether in a double or triple helix, yields a mixture of the 2′–5′ and 5′–5′ isomers and only a small amount of the naturally occurring 3′–5′ dinucleotide.[46]

Despite many attempts to circumvent this difficulty, we do not as yet know any nonenzymatic system in which an activated 5′-nucleotide or oligonucleotide condenses on a template with a monomer or an oligomer to give the 3′–5′ oligonucleotide as the major product. Furthermore, attempts to force the formation of 3′–5′ bonds by working with 2′-deoxynucleosides have failed. When a water-soluble carbodiimide is used as condensing agent, 2′-deoxyadenosine condenses with adenylic acid to give the 5′–5′ dinucleoside phosphate as the major product.[47] The condensation of two molecules of 2′-deoxyadenosine 5′-phosphate yields a 5′–5′ pyrophosphate. It appears that the lower nucleophilic reactivity of the 3′-OH group, perhaps augmented by steric factors, militates against the formation of 3′–5′ internucleotide bonds in these reactions.

Our attempts to discover potentially prebiotic reagents that could be used in place of the carbodiimides have not been successful. Condensing agents such as cyanamide react so slowly at temperatures below the melting points of polymer–monomer helices that they cannot easily be studied in the laboratory. Cyanogen, although it reacts rapidly with nucleotides and other phosphates in aqueous solution, does not bring about the synthesis of di- or oligonucleotides. Cyanogen halides are fairly effective condensing agents, but we have not been able to generate them efficiently under plausible prebiotic conditions.[48]

Most of our more recent work has utilized preactivated nucleotide derivatives. The most obvious preactivated monomer, ATP, does not undergo efficient self-condensation on a poly(U) template, alone or in the presence of Mg^{2+}, but hydrolyzes slowly to ADP and AMP. Adenosine cyclic 2′,3′-phosphate, on the other hand, does give the dinucleotide (up to 20%) and a trace of the trinucleotide.[49] The major products are adenosine 2′- and 3′-phosphates produced by direct hydrolysis. These reactions are very slow in the absence of catalysts, but in the presence of di- or polyamines, for example, in the presence of propylenediamine, reasonable yields of the dinucleotide can be obtained in a few days. In a few typical cases the nature of the internucleotide bond was examined and the proportion of the 2′–5′-linked product found to exceed 97%.

The cyclic 2′,3′-phosphates are likely to have been among the most abundant nucleoside derivatives on the primitive earth. Nonetheless, we think it unlikely that they were the substrate of the first template-catalyzed polymerization that led to the formation of long oligonucleotides. Our own experiments never yield appreciable amounts of trimers or higher oligomers. Equilibrium calculations suggested that, even if hydrolysis of the cyclic phosphates to the 2′- and 3′-phosphates could be suppressed completely, the yield of high molecular weight material would not exceed a few per cent.

We have studied the reactions of the imidazolides of nucleoside 5′-phosphates more extensively than any other derivatives.[50] They can be obtained under prebiotic conditions and they react, although fairly slowly, to form oligonucleotides in high yield. In typical experiments, internucleotide bonds are formed from imidazolides with an efficiency in excess of 50%. In our most recent experiments we have characterized penta- and hexanucleotides in the products formed from ImpA after about a week at 0°C in the presence of poly(U) and Mg^{2+}. The linkage in these products is almost exclusively 2′–5′.

The condensation of ImpA with a variety of nucleosides and analogs has shown that the reaction is fairly specific with respect to the nature of the ac-

(45) J. Sulston, R. Lohrmann, L. E. Orgel, and H. Todd Miles, *Proc. Nat. Acad. Sci. U.S.*, 60, 409 (1968).
(46) J. Sulston, R. Lohrmann, L. E. Orgel, H. Schneider-Bernloehr, B. J. Weimann, and H. T. Miles, *J. Mol. Biol.*, 40, 227 (1969).
(47) H. Schneider-Bernloehr, R. Lohrmann, J. Sulston, B. J. Weimann, L. E. Orgel, and H. T. Miles, *J. Mol. Biol.*, 37, 151 (1968).
(48) R. Lohrmann and L. E. Orgel, unpublished results.
(49) M. Renz, R. Lohrmann, and L. E. Orgel, *Biochim. Biophys. Acta*, 240, 463 (1971).
(50) B. J. Weimann, R. Lohrmann, L. E. Orgel, H. Schneider-Bernloehr, and J. E. Sulston, *Science*, 161, 387 (1968).

ceptor.[51] Thus ImpA does not condense efficiently with α-adenosine or with arabinosyladenosine on a poly(U) template, although organized helices are formed in both cases.

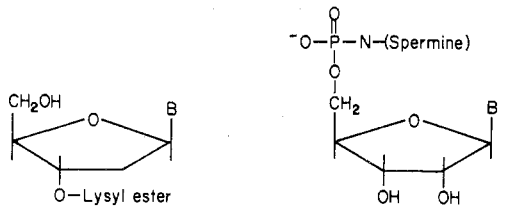
The optical specificity of the reaction is particularly interesting. D-Adenosine and L-adenosine form triple helices of comparable stability with poly(U) (poly(D-uridylic acid)). Nonetheless ImpA (D isomer), which condenses very efficiently with D-adenosine, reacts poorly if at all on the template with L-adenosine. This reaction can be used to achieve a partial resolution of racemic D,L-adenosine; a substantial proportion of the D isomer is incorporated into oligonucleotides, leaving behind a mixture containing an excess of the L isomer.[52]

This observation is important in connection with the evolution of optical activity in biological systems. It suggests strongly that oligonucleotides that are made up of monomers having the same enantiomeric configuration can undergo template-directed replication, while "mixed" oligomers cannot. Of course, D and L oligomers would replicate equally well, so our result shows only that the components of the primitive nucleic acids must either all have been L or all have been D isomers. It does not explain why only all D nucleic acids are important in biology. (See ref 2 for a discussion of this question.)

Work in Progress[53]

Our objective is to discover conditions under which short polynucleotides (say up to 20 residues in length) can be used as templates for the syntheses of their complements. To this end we are attempting (1) to incorporate pyrimidine nucleosides into polymers on purine-containing templates; (2) to increase the proportion of high molecular weight product obtained in template-directed condensation; (3) to increase the rate and efficiency of template-directed condensation.

We have used two approaches to the problem of incorporating pyrimidines. First, we have prepared a number of derivatives of adenosine which contain one or more cationic groups, for example

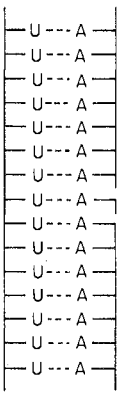


We hoped that positively charged groups would interact with the phosphate backbone of the polynucleotide and so stabilize the helices. Since none of the adenosine derivatives formed particularly stable helices with poly(U), there seemed little chance that the corresponding pyrimidine derivatives would form helices with polypurine nucleotides. We have, therefore, abandoned this project.

Our second approach was to utilize the *N*-phosphorimidazolides of the dinucleotides pUpG and pCpA. These do undergo condensation reactions on appropriate templates. In the case of ImpUpG the major products seem to be oligonucleotides. Thus, ImpUpG condenses with GpG on a poly(C, A) template containing about 6 cytidine residues for each adenosine residue to give a good yield of GpGpUpG. Since little or no product is formed on a poly(C) template and ImpU does not condense with GpG on a poly(C, A) template, it appears that a neighboring G·C base pair stabilizes an A·U base pair sufficiently to allow ImpUpG to form internucleotide bonds. However, not all cases that we are studying are straightforward, and a great deal of further analysis will be required if we are to understand the products found in the template-catalyzed condensations of dinucleotides in any detail.

In order to produce a large proportion of high molecular weight material it is essential to work under conditions that permit the elongation of preexisting oligomers, but do not permit the formation of new dimers from monomers. The work of Pitha and Ts'o suggests how this can be done.[54]

The melting point of the triple helix formed by poly(U) with the heptamer of A is much higher than that of the 2poly(U)·pA helix. At temperatures between these two melting points, a single pA residue will "stack" at the end of each oligo(A) chain.



This suggested to us that ImpA might condense preferentially with oligo(A), if the temperature is held above the melting point of the 2poly(U)·ImpA triple helix, but below the melting point of the 2poly(U)·oligo(A) helix.

We prepared a solution containing poly(U) (0.05 *M*), pentaadenosine tetraphosphate (0.56×10^{-3} *M*) and a large excess of [^{14}C]ImpA (0.25 *M*, specific activity 2 mCi/mmol). Aliquots were maintained at a series of temperatures spanning the melting points of the 2poly(U)·ImpA and 2poly(U)·oligo(A) helices. In Table II we give the ratio of the rates of elongation of pentamer and monomeric pA that we observed. The result is an anticipated; the relative yield of hexamer increases greatly at higher temperatures. Even higher ratios could probably be achieved at higher temperatures, but at the cost of a lowered efficiency.

These results suggest that there is a relatively narrow temperature range in which the nonenzymatic replication of oligonucleotides can proceed only if a primer is supplied. At higher temperatures, no tem-

(51) H. Schneider-Bernloehr, R. Lohrmann, J. Sulston, L. E. Orgel, and H. T. Miles, *J. Mol. Biol.*, 47, 257 (1970).

(52) H. Schneider-Bernloehr, R. Lohrmann, L. E. Orgel, J. Sulston, and B. J. Weimann, *Science*, 162, 809 (1968).

(53) R. Lohrmann, J. Ninio, L. E. Orgel, and J. Shim, unpublished results.

(54) P. M. Pitha and P. O. P. Ts'o, *Biochemistry*, 8, 5206 (1969).

Table II
Relative Rates of Chain Initiation and Chain Elongation on a Poly(U) Template at Various Temperatures[a]

$$R = \frac{[(Ap)_4A] + 2[(Ap)_5A]}{[ImpApA] + [pApA] + 2[pApApA]} \frac{[ImpA]_{initial}}{[(Ap)_4A]_{initial}}$$

Temp, °C	Time, hr	ImpA	pA	ImpApA	pApA	pApApA	$(Ap)_5A$	$(Ap)_6A$	R
0	56	61.5	13.8	18.0	5.0	1.20	0.56		2.0
				(9.0)	(2.5)	(0.8)	(0.56)		
5	56	52.9	18.2	17.0	7.6	2.60	1.50		4.7
				(8.5)	(3.8)	(1.9)	(1.50)		
10	24	69.0	14.1	11.4	3.9	0.90	0.83	Tr[b]	4.5
				(5.7)	(1.95)	(0.60)	(0.83)		
15	24	68.2	18.5	8.1	2.70	1.04	1.50	Tr	11.0
				(4.05)	(1.35)	(0.69)	(1.50)		
25	24	57.0	32.5	6.5	1.53	0.30	1.74	0.44	23.0
				(3.25)	(0.77)	(0.1)	(1.74)	(0.22)	

[a] The solutions (pH 7.5) contained poly(U) 0.05 *M*, [8-^{14}C]ImpA 0.025 *M*, $(Ap)_4A$ 0.56 × 10^{-3} *M*, $MgCl_2$ 0.10 *M*, NaCl 0.20 *M*, and imidazole buffer 0.20 *M*. The yields are given as percentages of the total radioactivity on the chromatogram. The values in parentheses are proportional to the molecular yields. *R* is a rough measure of the ratio of the efficiency of chain elongation on $(Ap)_4A$ to chain initiation on pA. [b] Tr, trace.

plate-directed synthesis occurs, and at lower temperatures low molecular weight material is formed in large amounts. On the primitive earth, short primers could have formed at low temperatures, and have "grown" at somewhat higher temperatures.

We are attempting to accelerate the synthesis of oligomers in two ways (neither is prebiotic). In the first, we utilize a concentrated 1-methylimidazole buffer at pH's between 7 and 9. The following equilibria are attained quickly.

$$ImpA + H^+ \rightleftharpoons (HIm)^+-pA \qquad pK_a \approx 6.5$$

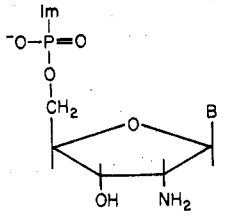
$$(HIm)^+-pA + MeIm \rightleftharpoons (MeIm)^+-pA + Imidazole \qquad K \approx 1$$

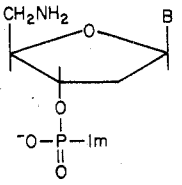
Examination of these equilibria shows that ImpA can be converted extensively to $(MeIm)^+$-pA, if the concentration of 1-methylimidazole is increased sufficiently. Thus at pH of 8.5 only about 1% of ImpA is present in the protonated form, but if 0.25 *M* 1-methylimidazole is added to 0.025 *M* ImpA, about 10% of $(MeIm)^+$-pA is formed.

The above considerations are important because ImpA itself is unreactive, while $(HIm)^+$-pA, and equally $(MeIm)^+$-pA react readily with nucleophiles. Thus the presence of 1-methylimidazole greatly increases the rate of hydrolysis of the imidazolide and also the rate of the template-directed formation of internucleotide bonds. Very recently we have shown that it is possible to form oligomers of pA up to the pentamer in a single day in a template-directed reaction at 0°C in a 1-methylimidazole buffer.

Our second approach is very different. We are attempting to use nucleotide analogs containing strategically placed nucleophilic substituents in place of simple nucleotides. We hope in this way to use templates to direct the synthesis of nucleic acid like molecules in which some of the O atoms of the phosphate backbone are systematically replaced by nitrogen or sulfur. Our initial experiments show that aminonucleosides react with phosphorimidazolides at least 100 times more rapidly than do nucleosides. When 0.01 *M* ImpA is incubated with 0.1 *M* 2′-amino-2′-deoxyuridine (U_{2N}) it is converted into a dinucleotide analog in greater than 80% yield. Similar results are obtained with 3′-amino-3′-deoxynucleosides, and we anticipate that the 5′-amino-5′-deoxynucleosides will behave similarly.

The synthesis of large quantities of activated precursors such as





has proved unexpectedly difficult when B is a purine, so we have not yet attempted template-directed condensations. However, we do know that a 0.2 *M* solution of $ImpU_{2N}$ yields oligomers up to the decamer in about 70% yield after 15 days at 0°C; the corresponding uridine derivative gives at most 1% of dimer. If template-directed syntheses are accelerated by amino substitution as much as reactions in free solution, this work could lead to the synthesis of novel self-replicating molecules.

The work described in this paper has been supported by grants from NASA, National Institutes of Health, and National Science Foundation.