PREBIOTIC CONDENSATION REACTIONS IN AN AQUEOUS MEDIUM: A REVIEW OF CONDENSING AGENTS

JOSÉE HULSHOF and CYRIL PONNAMPERUMA
Laboratory of Chemical Evolution, University of Maryland, College Park, Md. 20742, U.S.A.

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Abstract. Biopolymers are formed by dehydration-type condensation reactions. In aqueous solutions dehydration reactions are very unlikely to happen spontaneously. However, coupling of dehydration-condensation to the hydrolysis of condensing agents could facilitate the synthesis of biopolymers in an aqueous solution. The literature shows that the peptides, nucleosides, nucleotides and oligonucleotides can be formed in this way. A careful study of the literature pertaining to prebiotic condensing agents was conducted in order to determine the most plausible prebiotic synthesis of biopolymers. The condensing agents taken into consideration are cyanamide, carbodiimide, dicyanamide, dicyandiamide, hydrogen-cyanide-tetramer, cyanogen and the linear- and cyclic polyphosphates. From both a chemical as well as biological point of view the polyphosphates appear to be the most plausible general prebiotic condensing agent.

1. Introduction

During the past fifty years many experimental and theoretical studies have led us to believe that life may be the result of an evolutionary sequence in the universe. Step by step the molecules grow larger and more complex. They undergo prebiotic assembly and finally give rise to the most primitive replicating system. This process, called chemical evolution, can thus be delimited into several more or less separate steps: a gradual development from simple molecules to the emergence of life. Successively the following events probably occurred:

(1) The synthesis of the small molecules, among others the amino acids, the monosaccharides, the organic bases, and the fatty acids.

(2) The condensation of these small molecules into biomacromolecules – especially proteins, nucleic acids.

(3) The organization of the macromolecules into systems of increasing complexity.

(4) The emergence of life.

The initial step, the synthesis of the small organic molecules, has been most extensively studied. Many of the small molecules indispensable for life have been synthesized under presumably prebiotic conditions. For example, amino acids have been synthesized by electrical discharges in an atmosphere of methane, ammonia and water (Miller, 1953, 1955, 1957; Harada and Fox, 1964; Ponnamperuma and Woeller, 1967; and many others). Monosaccharides have been synthesized from formaldehyde (Gabel and Ponnamperuma, 1967). Purines and pyrimidines are also formed by plausible prebiotic reactions (Oró and Kimball, 1962; Ponnamperuma, 1965; Sanchez *et al.*, 1966a, 1966b). For more complete reviews see Lemmon (1970), Gabel and Ponnamperuma (1972), de Rosnay (1967a and 1967b) and Stephen-Sherwood and Oró (1973).

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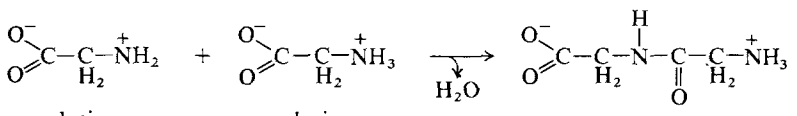
Since these investigations indicate that the biological monomers have been synthesized abiotically, this study is focussed on the next stage in chemical evolution, the formation of the macromolecules. From the following summary of the reactions involved in the synthesis of biopolymers, it is clear that all are dehydration-type condensation reactions.

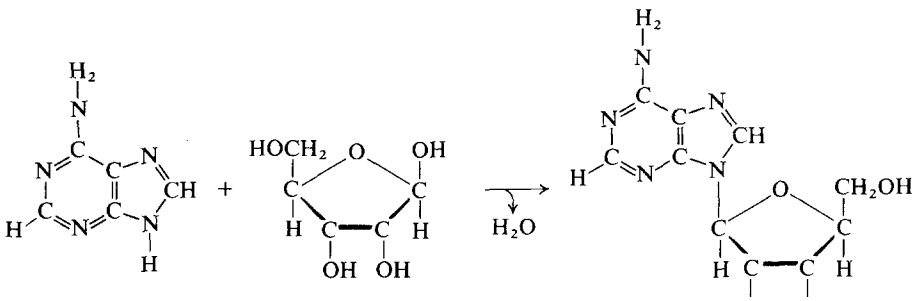
(A) The formation of the peptide bond occurs when the carboxyl function of an amino acid reacts with the amino group of another amino acid, with the production of one molecule of water. Thus all peptides and proteins are the product of dehydration condensation reactions of amino acids. Example:



glycine glycine diglycine

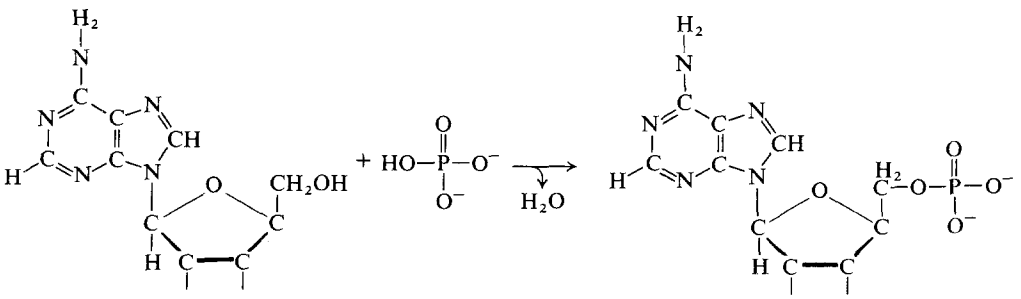
(B) Nucleic acid synthesis is the result of a number of reactions:

(a) Nucleoside synthesis. A purine or pyrimidine reacts with ribose or deoxyribose and forms a nucleoside with the formation of one molecule of water per nucleoside. Example:



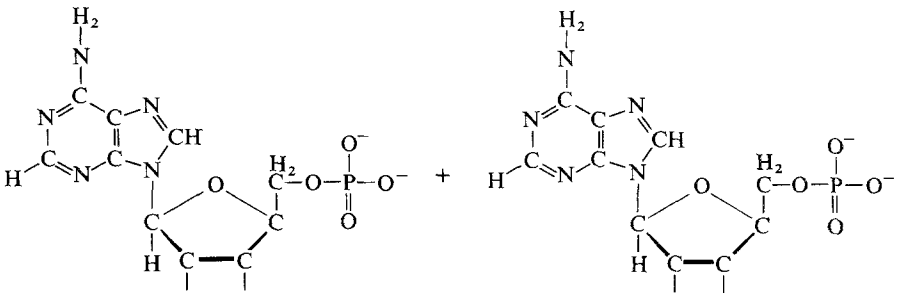
adenine ribose adenosine

(b) Phosphorylation of a nucleoside results in the synthesis of a nucleotide. The formation of this ester bond produces water.

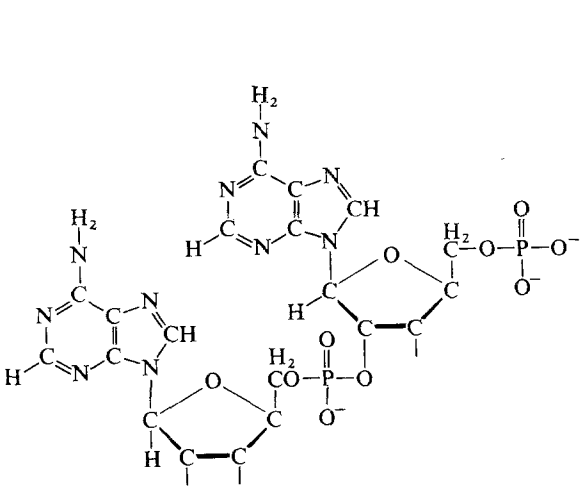


adenosine phosphate adenosinephosphate

(c) When nucleotides polymerize an ester bond is formed between the phosphate residue of one nucleotide and a hydroxyl group of the pentose residue. An example is shown below:

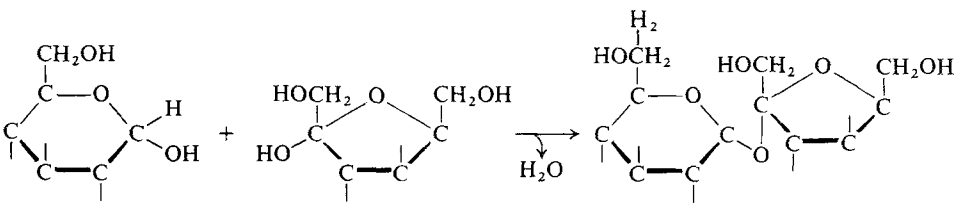


adenosine 5′ phosphate + adenosine 5′ phosphate



adenosinedinucleotide

(C) Polysaccharide synthesis occurs similarly when monosaccharides form acetal bonds with the production of one molecule of water per bond.



glucose + fructose → sucrose

These reactions show that the formation of the building blocks of life requires dehydration condensations. The abiotic synthesis of these macromolecules will therefore be greatly facilitated in an extremely dry environment. However, it is now generally considered that the evolution of life in the primordial oceans or in tidal areas would offer a more plausible explanation. In a large body of water the reactants can be transported easily and at the surface the reactions can occur with a continuous energy supply from solar irradiation. The products, at the same time,

can be protected against the damaging effect of the irradiation by storage in deeper water. A further suggestive indication that the oceans served as a prebiotic reaction medium is the fact that in contemporary living systems the cytoplasm still has a strikingly similar composition to seawater. That is why polymerization reactions in an aqueous medium have probably a greater prebiological significance than dry synthesis. However, dehydration-type condensation reactions are very unlikely to happen spontaneously in an aqueous solution, because the solvent shifts the reaction equilibrium towards the side of the reactant monomers.

During the preceding decade very interesting experiments have been conducted. They showed that if these dehydration condensations are coupled to the hydrolysis of certain condensing agents, then dehydrations in aqueous solutions are possible. Many compounds have been proposed as prebiotic condensing agents. Some of them seem to be very effective, the significance of others for chemical evolution may be doubtful. The prebiotic condensing agents that have been studied in recent years are the following:

Cyanamide $N\equiv C-NH_2$

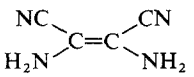
Carbodiimide $RN=C=NR$ (R is H or alkyl)

Dicyanamide $N\equiv C-\underset{\displaystyle H}{\underset{|}{N}}-C\equiv N$

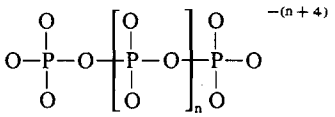
Dicyandiamide $H_2N-\underset{\displaystyle NH_2}{\underset{|}{C}}-\overset{\displaystyle H}{\overset{|}{N}}-C\equiv N$

Cyanogen $N\equiv C-C\equiv N$

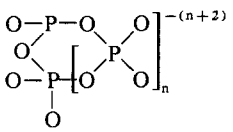
Hydrogen-cyanide-tetramer



Linear polyphosphates



Cyclic polyphosphates



This review will include the use of these condensing agents in the synthesis of peptides, nucleosides, nucleotides, and oligonucleotides. Some considerations will first be given to the most plausible prebiotic reaction conditions from a chemical point of view.

Reaction conditions in the environment of the primitive Earth are probably best duplicated by an aqueous solution with a pH value close to neutral or slightly basic, and a temperature within the range of liquid water, under approximately one atmosphere of pressure. The concentration of the reactants should be low. Finally, the selected condensing agent should meet the following requirements: (1) reactive under the conditions mentioned above, (2) available on the primitive Earth and

(3) general applicable to all types of dehydration condensations. Most of the information about the properties of condensing agents may be obtained from the literature on prebiotic peptide synthesis. Therefore, the relationship between condensing agents and the formation of the peptide bond will be discussed first. Considerable emphasis will be put on the mechanisms of the reactions since they may give an indication of the optimum reaction conditions.

2. Condensing Agents and the Peptide Bond

The condensing agents can be divided into two classes of compounds, the cyanide containing condensing agents and the condensed phosphates. The first class contains cyanamide, carbodiimide, dicyanamide and dicyandiamide, as well as cyanogen and the hydrogen-cyanide-tetramer.

A. CYANAMIDE

Cyanamide is the simplest of all condensing agents. Its structure is $NC{-}NH_2$, and it has been applied in the synthesis of diglycine (Halmann, 1968), triglycine, leucyl-glycine, and glycyl-leucine (Ponnamperuma and Peterson, 1965). Table I gives details about conditions and yields. From these results the conclusion can be drawn that at slightly acidic pH's some peptide formation occurs, but the yields are low. The current consensus of opinion in chemical evolution is that the pH of the primitive oceans were more likely to have been slightly basic rather than slightly acidic. This casts some doubt on the relevance of these results. Another important fact is that the availability of cyanamide on the primitive Earth is very doubtful. Studies (Schimpl *et al.*, 1965) have shown that cyanamide can possibly be synthesized by u.v. irradiation of a HCN-containing solution. But cyanamide dimerizes very rapidly into dicyandiamide. Dicyandiamide requires a much lower pH and is much less reactive (see dicyandiamide, Section D).

Unfortunately there are no experimental data available which may give direct information about the mechanism of the reaction. Studies conducted with carbodiimide do have this information, and this may very well be applicable to the case of cyanamide, since cyanamide and carbodiimide are tautomers.

$$\underset{\text{cyanamide}}{N{\equiv}C{-}NH_2} \rightleftharpoons \underset{\text{carbodiimide}}{HN{=}C{=}NH}$$

Carbodiimide was used to condense amino acids as early as 1955, but never under conditions simulating the primitive Earth. The data from the mechanistic study are sufficiently important for our understanding of cyanamide and the other condensing agents.

B. CARBODIIMIDE

In 1955 Sheehan and Hess (Sheehan and Hess, 1955) published the first use of a dialkylcarbodiimide. The solvents they used were tetrahydrofuran and later acetone, methylene chloride and dimethylformamide (Sheehan *et al.*, 1956). Table II gives a summary of the literature. A few months after Sheehan and Hess's first publication,

TABLE I

Cyanamide

Reactants	Products	Yield	Conditions	Remarks	Reference
glycine	diglycine	0.5%	pH 6.5, 65°C 13 days, 0.05 M gly 0.18 M NH_2CN	low yield, but first time that condensation occured at neutral pH	Halmann (1968)
glycine leucine	diglycine triglycine gly-leu leu-gly	1% 0.1 ? ?	u.v. irradiation 25°C 10^{-2} M gly pH 5.	low pH and small yields	Ponnamperuma and Peterson (1965)

TABLE II

Carbodiimide

Reactants	Products	Yield	Conditions	Remarks	Reference
Review and mechanism of carbodiimides, not from chemical evolutionary point of view					Bodanszky and Ondetti (1966)
Ibid.					Pharmacia Chemicals (1973)
serine threonine hydroxyproline	dipeptides	up to 80%	solvents are acetone, methylene chloride, dimethylformamide	nonaqueous, but in organic solvents thus not prebiotic	Sheehan *et al.* (1956)
glycine phenylalanine	dipeptides	87%	solvent is tetrahydrofurane	ibid	Sheehan and Hess (1955)
glycine phenylalanine	dipeptides	56%	dioxane, ether tetrahydrofurane chloroform during 4–18 hrs.	ibid, mechanistic study	Khorana (1955)

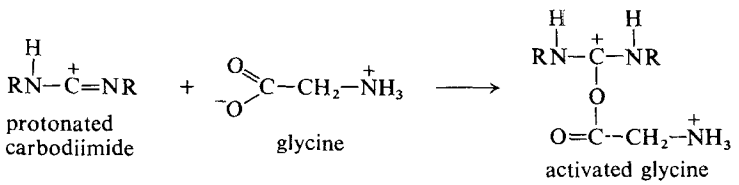
Khorana (1955) gave an mechanistic explanation of the observed phenomena. The central carbon atom in carbodiimides is partly positive due to electron delocalization and the adjacent nitrogen atoms are partially negative

$$RN{=}C{=}NR \leftrightharpoons R\bar{N}{-}\overset{+}{C}{=}NR \leftrightharpoons RN{=}\overset{+}{C}{-}\bar{N}R$$

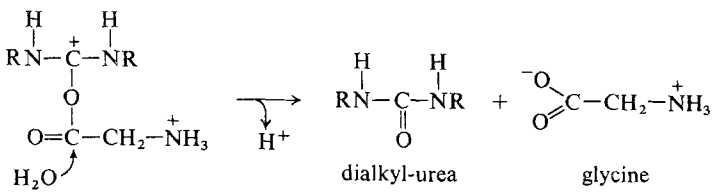
This means that, especially under acidic conditions, these canonical resonance structures make a greater contribution to the actual structure of the condensing agent, resulting in an even more electrophilic central carbon atom

$$RN{=}C{=}NR \xrightleftharpoons{H^+} RN{=}\overset{+}{\underset{H}{C}}{-}NR \rightleftarrows RN{-}\overset{+}{\underset{H}{C}}{=}NR$$

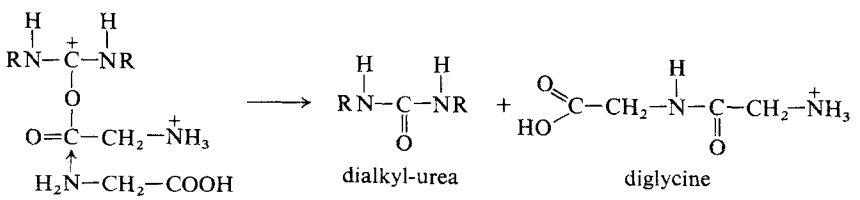
This protonated carbodiimide is then susceptible towards a nucleophilic attack by the carboxyl group of the amino acid. A highly reactive, very unstable, intermediate will be formed



In this activated glycine molecule the carboxyl carbon is more strongly positive than in unactivated glycine, since the carbodiimide residue is electron withdrawing. Therefore this intermediate can be attacked easily by another nucleophile such as water, resulting in hydrolysis of the intermediate. This reaction produces the hydrolyzed carbodiimide, or dialkylurea, and glycine.



The other possibility is a nucleophilic attack by the lone pair of electrons on the amino group of another amino acid, resulting in the desired dipeptide and the hydrolyzed condensing agent.



The conclusion that can be drawn from this mechanism is that for the first step, the formation of the activated glycine, a highly acidic medium is desirable, whereas for the second step a neutral to basic solution would be preferable, because

that would increase the availability of the lone pair of electrons on the amino group. This mechanism has been investigated over a number of years (Bodanszky and Ondetti, 1966; and Separation News 1973) and is very readily applicable to cyanamide. Cyanamide gives upon protonation

$$N{\equiv}C{-}NH_2 \xrightarrow{H^+} H_2N{-}\overset{+}{C}{=}NH$$

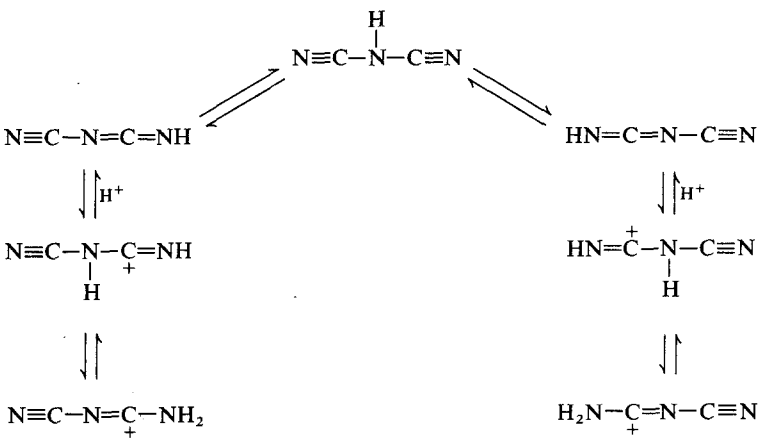
which is the very same starting material as protonated carbodiimide (R is H).

The low yields may be explained by the fact that for the first step a low pH is required, which deactivates the lone pair of the amino group, required for the second step of the reaction.

Another condensing agent that presumably reacts according to the same mechanism is dicyanamide.

C. DICYANAMIDE

Dicyanamide has the following structure $N{\equiv}C{-}\overset{H}{\underset{|}{N}}{-}C{\equiv}N$. This structure can be considered as cyanamide in which a hydrogen atom is substituted by a cyanide group. Thus far it has not been synthesized under primordial conditions. This compound has mainly been studied by Steinman *et al.* (Steinman, 1967; Steinman, Kenyon and Calvin, 1966, 1967; Steinman and Cole, 1968; Steinman, 1971). Table III shows that the pH must be very low in order to obtain observable yields. Steinman, Kenyon and Calvin (1966) describe the reaction mechanism that occurs when a mixture of glycine and dicyanamide are allowed to react at a pH of 1.4. Dicyanamide is in equilibrium with tautomeric carbodiimides, that are themselves protonated



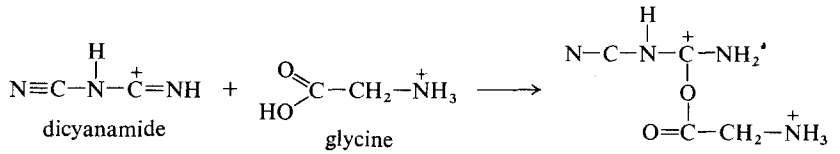
These structures show that both carbon atoms can undergo a nucleophilic attack, especially in an acidic environment. This mechanism is basically the same as in the case of carbodiimide, after protonation a nucleophilic attack is performed by the carboxyl group of an amino acid, resulting in an activated amino acid.

TABLE III

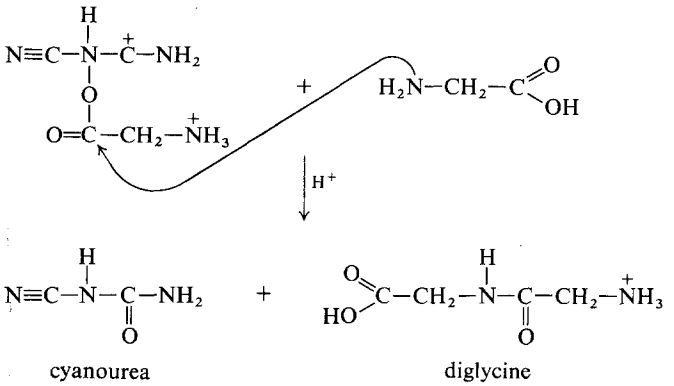
Dicyanamide

Reactants	Products	Yield	Conditions	Remarks	Reference
glycine leucine	selective polymerization	? ?	poly leu template ?	very short abstract no conditions given	Steinman and Cole (1968)
glycine leucine alanine	leu-gly gly-gly gly-ala, etc.	only relative yields given	0.01 M gly 0.1 M DCN[1] pH 1.0	very low pH no real yields	Steinman (1967)
glycine	diglycine	8.9% 3.8%	pH 1.4 pH 2.6 0.12 M	low pH, but interesting mechanistic study	Steinman *et al.* (1966)
Review of conditions and mechanisms					Steinman (1971)
glycine alanine valine-leucine isoleucine phenylalanine	many dipeptides	only relative yields	pH 1 0.1 M DCN[1] 0.01 am. acid	low pH, selectivity as function of pK	Steinman and Cole (1967)
alanine	dialanine	1.58%	pH 2 0.02 M 25°C 20 hrs		Steinman, Kenyon, and Calvin (1965)

[1] DCN = Dicyanamide

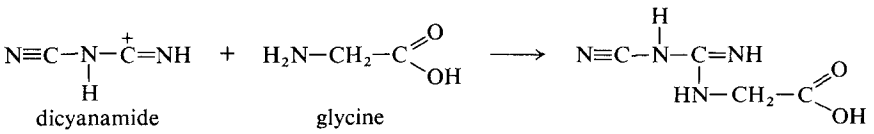


The activated amino acid can be hydrolyzed, so that the net result is just a deactivation of the condensing agent, or it can be attacked by the lone pair on the amino groups of another amino acid molecule, resulting in diglycine and cyanourea.



This mechanism explains again why an acidic medium is required. The hydrogen ion concentration is much higher than possibly can be expected on the primitive earth. For this reason serious doubts have arisen about the value of this compound as a primordial condensing agent.

Another reaction that may be relevant for the origin of life is a direct attack of the amino group on the protonated dicyanamide.



The product is representative of the guanidinium compounds, from which guanidine and creatine are the most important in contemporary biological systems. These compounds are much more stable than the activated amino acids. They can not be intermediates in the dipeptide synthesis, since the amino group is deactivated instead of activated.

A fourth representative in the class of condensing agents mentioned is dicyandiamide.

D. DICYANDIAMIDE

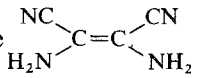
Dicyandiamide or cyanoguanidine, $H_2N-\underset{\underset{NH}{|}}{C}-\overset{\overset{H}{|}}{N}\equiv C\ N$, is the dimer of cyanamide,

and is easily synthesized prebiotically, when an aqueous solution of HCN is irradiated with ultra-violet light (Schimpl *et al.*, 1965). The compound is so similar to cyanamide and dicyanamide that along with the experimental result that a low pH is required the conclusion seems reasonable that the mechanisms will be identical. There are no data to support this contention however. Table IV gives a summary of the reaction conditions and yields.

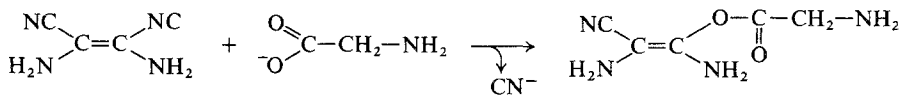
E. CYANOGEN

Cyanogen has never been described as a condensing agent in peptide synthesis.

F. HYDROGEN-CYANIDE-TETRAMER

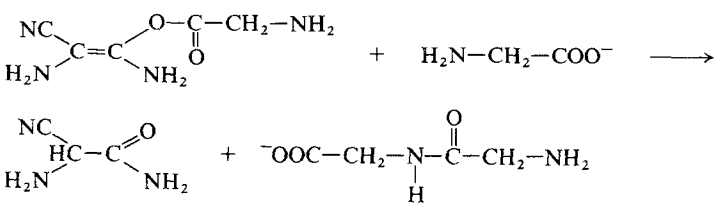
Hydrogen-Cyanide-Tetramer has the structure  The polymerization of HCN, resulting in the synthesis of the tetramer among many other products, has been intensively studied by Sanchez *et al.* (1967). They showed that the HCN-tetramer, cis-diaminomaleonitrile, can be easily formed by u.v.-irradiation of an aqueous solution of HCN, at a pH close to the pK-value of this compound (9.2 at 25°C).

The behavior of this compound as a condensing agent has been studied by Chang *et al.* (1969, Table V) for the synthesis of diglycine. The mechanism of this dehydration reaction differs from the others in that not a nitrogen atom, but a cyanide group is presumably the electron acceptor. Therefore a neutral to basic pH is preferable. The mechanism of this reaction can be represented as follows



HCN-tetramer glycine activated amino acid

Once more an unstable amino acid-condensing agent complex is formed. Since a low pH is not necessary the second step can proceed more easily, because the lone pair of electrons on the amino group becomes more available with increasing basicity, explaining why the yields increase with increasing basicity.



hydrolyzed HCN-tetramer diglycine

This makes the HCN-tetramer strongly favored over the previously described condensing agents, since those were only reactive in an acidic medium. The disadvantage of the HCN-tetramer is its instability. It polymerizes quite quickly, especially at elevated temperatures, to an unreactive tarry polymer. Its possible role

TABLE IV

Dicyandiamide

Reactants	Products	Yield	Conditions	Remarks	Reference
alanine	dialanine trialanine	1.2% trace	pH 2, 25°C 0.01 M DCDA[2] 0.01 M alanine, 20 hrs	very low pH, and low yields	Steinman, Lemmon, and Calvin (1965)
alanine	dialanine	0.51%	ibid	ibid	Steinman, Kenyon, and Calvin (1965)

[2] DCDA is Dicyandiamide

TABLE V

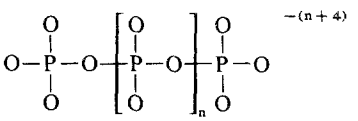
Hydrogen-Cyanide-Tetramer

Reactants	Products	Yield	Conditions	Remarks	Reference
glycine	diglycine	2% 4.8%	pH 8 pH 9 85°C, 0.025 M	reactive at neutral pH	Chang *et al.* (1969)

in the synthesis of peptides which occur in the mixtures resulting from the action of electrical discharges on simulated primitive atmospheres has been suggested (Ponnamperuma and Flores, 1966; Flores and Ponnamperuma, 1972).

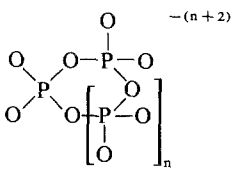
G. CONDENSED PHOSPHATES

Several kinds of condensed phosphates can be distinguished, linear and cyclic polyphosphates being the most important for our purpose. The linear polyphosphates can be represented as the following (Van Wazer, 1958; Gabel, 1971)



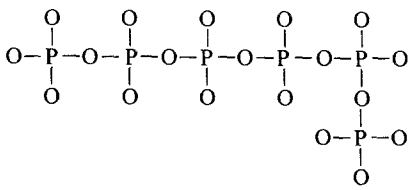
The phosphorus-oxygen bonds not involved in the anhydride linkage are all equivalent, which implies that the negative charge as well as the double bond character is equally spread over those oxygen atoms. Therefore no double bonds are drawn and just an overall charge is shown. The end groups can be distinguished from the middle groups by both chemical and spectroscopic properties.

The cyclic polyphosphates can be represented by this structure



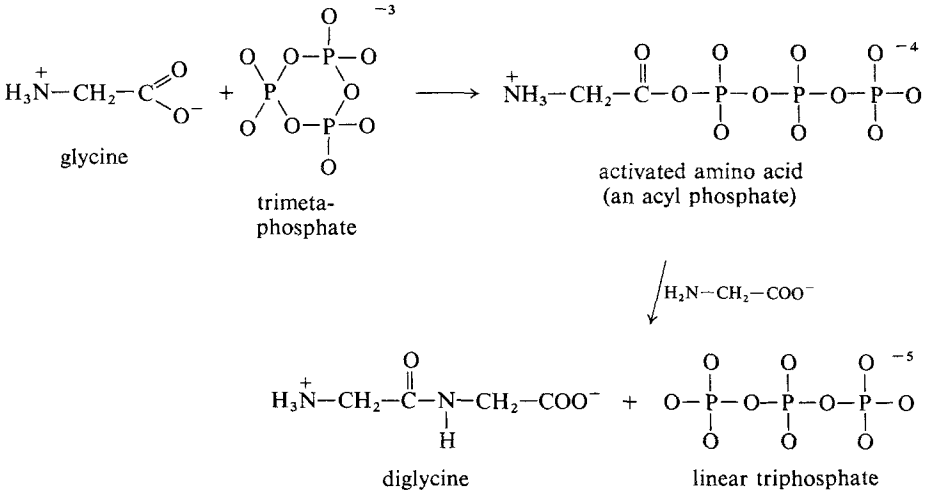
Again the double bond character as well as the negative charge are equally shared by the oxygen atoms not participating in the ring structure.

A third group of phosphoanhydrides are the branched structures



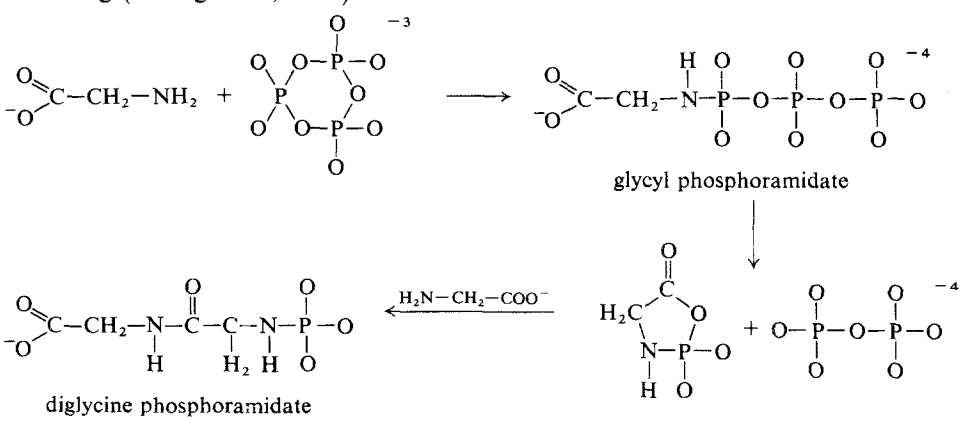
The last group is extremely reactive, but has a very short hydrolytic halflife. This means that it can only have been important in chemical evolution if it were continuously synthesized with our present knowledge of primitive earth conditions such a situation seems highly unlikely. Therefore only the linear and the cyclic polyphosphates will be described. The origin of the polyphosphates is still not entirely clear. The ubiquity of phosphorus in biological processes leads one to believe

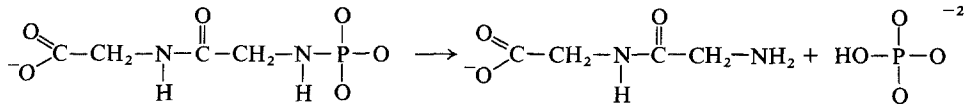
that the element was readily available during the early stages of chemical evolution on Earth. Some very plausible theories have recently been developed to bolster this hypothesis. Rabinowitz, Woeller *et al.* (1969) showed that by electrical discharges on an atmosphere containing phosphine (PH_3), ammonia and water, polyphosphates are formed. The question arises then, whether phosphine was available on the primitive earth. Recent discoveries on the atmosphere of Jupiter have indicated that phosphine is a detectable trace constituent of Jupiter's (Ridgway, 1974; Combes *et al.*, 1974) and Saturn's (Gillet and Forrest, 1974) present atmosphere. The Earth's primary atmosphere is supposedly very similar to Jupiter's present atmosphere, which may suggest that phosphine was available on the primitive Earth. Another possibility that accounts for the availability of polyphosphates is the following sequence of reactions proposed by Griffith (Griffith *et al.*, 1975, in preparation). Iron (III) phosphate, $FePO_4$, can be reduced by carbon monoxide, producing iron (II) phosphate and carbon dioxide. Iron (III) phosphate gives pyrophosphate in the presence of H_2S, and pyrophosphates polymerize spontaneously at moderately elevated temperatures to linear and cyclic polyphosphates plus orthophosphate. This chain of reactions is plausible under prebiotic conditions. Geophysical considerations are supportive of the idea that scribesite, a meteorite mineral containing phosphorus may have reached the primitive ocean during the stage of late accretion of our planet (Holland, 1961). Phosphides reacting with water could release phosphine, which in turn would yield the water soluble phosphates. Experiments with polyphosphates as condensing agents have given very promising results, although their reproducibility has not been very good. Before the experiments with well-described polyphosphates were initiated, reactions with indistinct phosphoanhydrides were performed by Schramm and Wissmann (1958). These experiments were not intended to be a simulation of prebiotic reactions because they were conducted in dry organic solvents. Nooner and Oró (1974) recently obtained similar results with this polyphosphate-ethyl-ester. The yields are high, but are not very relevant for chemical evolution. Schramm's experiments prompted the use of phosphoanhydrides in more carefully designed experiments. The polyphosphates were no longer a poorly described mixture, called polyphosphate-ethyl-ester, but cyclic or linear polyphosphates with a distinct number of phosphate residues. Rabinowitz's work especially (see Table VI) has produced very significant results. Rabinowitz succeeded in obtaining very high yields of oligo-peptides under reasonably plausible prebiological conditions (slightly basic pH, low temperatures, low concentrations, Rabinowitz, 1969a, 1969b, Rabinowitz, Flores *et al.*, 1969, Rabinowitz, 1970, Rabinowitz, 1971). In these investigations the reported data varied widely under approximately the same conditions. A more comprehensive study followed in 1971, by Chung *et al.*, in which all the preceding articles about cyclic polyphosphates were combined. This comprehensive paper reports the yields under many conditions, as well as a mechanistic study in which Rabinowitz's 1969b mechanism was altered. The two mechanisms differ in so far as that in 1969b a nucleophilic attack by the carboxyl group is proposed (a mechanism very similar to the mechanisms described so far), whereas in the 1971 study a nucleophilic attack by the lone pair of electrons in the amino function is described. If the carboxyl group is the nucleophile, the mechanism can be represented by these reactions.



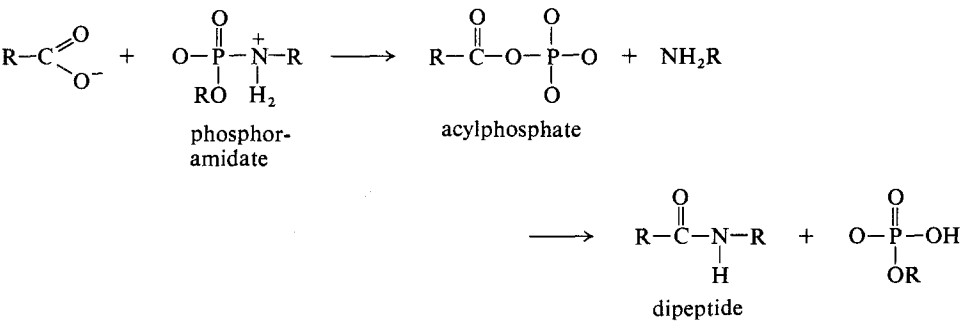
Through the nucleophilic attack of the carboxyl group on a phosphorus atom the ring is opened and the activated amino acid is formed. This activated amino acid is very similar to the activated amino acids in the biochemical pathways in all contemporary living systems. The only difference is that, in the latter, the third phosphoanhydride bond is replaced by a phosphate-ester bond with adenosine. This activated amino acid can undergo a nucleophilic attack by a water molecule, resulting in the net hydrolysis of one phosphoanhydride bond, or it can undergo a nucleophilic attack by the amino group of another amino acid, resulting in the net production of a peptide bond and the hydrolysis of one phosphoanhydride bond. The experimental results show that this reaction occurs most readily in a slightly basic environment. This is understandable since in a basic environment both the carboxyl group and the amino group are stronger nucleophiles.

This mechanism was only based on theoretical assumptions, there were no experimental data to support it. The fact that the carboxylic acids do not attack the cyclic polyphosphates, and also the fact that phosphoramidates are very common compounds, caused these investigators to change the previous mechanism into the following (Chung *et al.*, 1971)



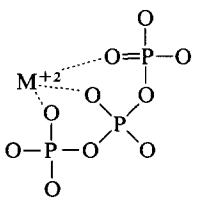


Here it is the amino group that performs the nucleophilic attack. This mechanism is supported by the confirmation of glycine-N-phosphate, diglycine-N-phosphate and triglycine-N-phosphate. In this way the formation of glycylphosphoramidates is rationalized. The succeeding step, the conversion of glycylphosphoramidate is more difficult to rationalize. Clark *et al.* (1966) and Preobrazhenskaya (1972) describe a nucleophilic substitution in which the phosphoramidate is replaced by an acylphosphate, rather than the formation of the ring structure. This acylphosphate then forms orthophosphate and an amide.



Both articles show that the above reaction chain is capable of producing high yields for the synthesis of oligopeptides. Therefore, the nucleophilic attack by both the carboxyl and the amino group seems plausible, but in either case the acylphosphate seems to be the activated amino acid, and probably not the ring compound. The activated amino acid is also very labile in an acidic solution and much more stable in a slightly basic solution.

An examination of Table VI shows that there is a fairly large discrepancy in the yields of oligopeptide under exactly the same conditions. One factor that may account for the large discrepancy is the possible presence of trace amounts of cations in the water. In none of the reported studies has this factor been considered. In our laboratory (Hulshof, preliminary results) a pertinent relationship has been demonstrated between the yield of oligopeptide and the availability of divalent cations. This is not surprising since the phosphoanhydrides are known to be excellent chelating agents. By coordination of a phosphoanhydride and a cation, the phosphoanhydride becomes more susceptible to nucleophilic attack because the phosphorus atom is now much less shielded by partially negative oxygen atoms. This can be represented by the following figure. M^{+2} stands for a divalent cation.

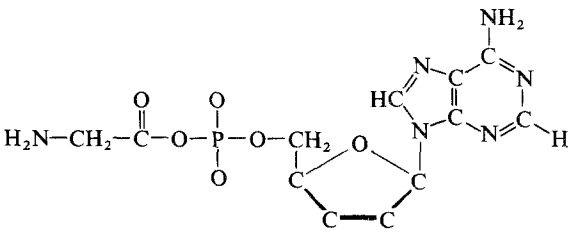


The negative charge on the oxygen atoms is partially withdrawn by the cation, causing a larger positive charge on the phosphorus atom, combined with a greater availability of this charge. This effect facilitates the attack by nucleophiles and forms more stable intermediates. Since the availability of cations in the water, in the experiments of Table V, was not specifically studied, a difference in cation concentration may be put forward as the reason for the discrepancy in yields of oligopeptides.

Very closely related to these types of reactions are the experiments with amino-acyl-adenylates as starting materials.

H. THE AMINO-ACYL-ADENYLATES

This group of compounds is almost identical to the amino-acyl-phosphates, the activated amino acids in the last section. The only difference is that the phospho-anhydride is not an inorganic phosphate but one of the acidic groups is involved in an ester bond with adenosine



glycyladenylate

It is the condensation product of glycine and adenosine triphosphate. The amino-acyl-adenylates are highly reactive. The amount of free energy released upon hydrolysis is approximately 10 kcal/mole, compared with an energy of hydrolysis of the peptide bond of approximately 3 kcal/mole. The consequence is that the amino-acyl-adenylates undergo spontaneous formation of the peptide bond with other amino acids at slightly alkaline pH. Under acidic conditions hydrolysis predominates. Table VII shows that this is indeed what is observed. Since this is a spontaneous process it does not provide any additional information about prebiotic condensation reactions and condensing agents. Therefore I will not go into any detail about this spontaneous amino acid polymerization.

One comment needs to be made about the amino-acyl-adenylates, compounds which are found in contemporary living systems as precursors of polypeptides. In so far as the reaction sites of these molecules are similar to the amino-acyl-phosphates, they provide supporting evidence for polyphosphates as generalized prebiotic condensing agents.

In the preceding sections the current status of the oligomerization of amino acids has been discussed. The oligomerization of amino acids has been the most extensively studied part of prebiotic dehydration-type condensation reactions. Another important series of condensation reactions has been the synthesis of nucleosides, phosphorylated pentoses, nucleotides, and oligonucleotides. In the following sections these reactions will be reviewed.

TABLE VI

Polyphosphates

Reactants	Products	Yield	Conditions	Remarks	Reference
glycine + trimetaphosphate	diglycine	0% 23%	pH 7 pH 9 0.1 M, 4 days	mechanistic study very high yields	Chung *et al.* (1971)
Mechanistic study and evaluation					Lohrmann and Orgel (1973)
Polymeta-phosphate-ester DL-alanine l-arginine L-cystine l-glutamic acid glycine DL-leucine DL-phenylalanine DL-serine DL-valine	oligopeptides up to 12 units	up to 57%	50–70°C 1 gr in 2 ml DMF 24 hrs	in dimethyl-formamide very concentrated poorly analyzed for such recent work	Nooner and Oró (1974)
glycine + trimetaphosphate	diglycine	36%	0.01 M–0.1 M 70°C; 25°C pH 8		Rabinowitz (1969a)

glycine trimetaphosphate	diglycine	15% 3%	pH 7; 70°C pH 7; 20°C	contradicting first reference in this table	Rabinowitz (1969b)
glycine trimetaphosphate	diglycine	12% 3%	pH 7–8; 70°C pH 7–8; 20°C		Rabinowitz, Flores, Krebsbach and Rogers (1969)
trimetaphosphate glycine alanine serine	diglycine dialanine no diserine, but serine-P	40% 12.2% 0% 4%	pH 11, 20°C		Rabinowitz (1970)
trimetaphosphate glycine + HCN glycine, no HCN	diglycine	20% 12%	pH 11, 20°C	under same conditions yields vary widely	Rabinowitz (1971)
Review and mechanisms					Rabinowitz (1972)
polymeta-phosphate ester	peptides	up to 50%	solvent is diethylphosphite	not prebiotic, but first use of phosphoanhydrides	Schramm and Wissmann (1958)

TABLE VII

Amino-acyl-adenylates

Reactants	Products	Yield	Conditions	Remarks	Reference
adenylates of 16 different L-amino acids	non-random polymerization	5%	0.0016 M pH slightly basic		Banda and Ponnamperuma (1971)
Careful thermodynamic study					Katchalski and Ailam (1967)
alanyladenylate	alanine		pH 7		Lewinsohn, (1967)
	diala triala tetraala	60%	pH 10 0.025 M		
prolyladenylate	proline		pH 7		Paecht-Horowitz and Katchalski (1967)
	dipro tripro tetrapro pentapro hexapro	?	pH 8.5 0.025 M		

3. Condensing Agents and the Formation of Oligonucleotides and their Constituents

In Section 1, it was shown that the synthesis of oligonucleotides consists of several steps. Again a survey will be given on the effect of condensing agents on these reactions. There is a great paucity of literature on these reactions, and reaction mechanisms are poorly described.

A. CYANAMIDE

Cyanamide does not induce any adenosine formation under many conditions (Fuller *et al.*, 1972). However, it can produce an activated pentose β-ribosephosphate from ribose and orthophosphate (Halmann *et al.*, 1969, see Table VIII). The prebiological significance of the latter reaction is dubious though, since orthophosphates are quite insoluble in solutions containing divalent cations. Only if these cations are bound by strong chelating agents the orthophosphate concentration can rise to the required concentration of 10^{-3}M. A solution containing large amounts of ammoniumoxalate is able to dissolve apatite to a phosphate concentration of 10^{-3}M. (Schwarz, 1971; Schwarz and Deuss, 1971; Schwarz *et al.*, 1975). It is questionable if these large amounts of oxalate were available on the primitive Earth. Polyphosphates are far more soluble (Van Wazer, 1958) and do not require the intermediate step of cyanamide, so that the activated sugars are more likely to be formed by reactions with condensed phosphates.

A reaction that cyanamide does seem to catalyze to some extent is the oligomerization of a nucleotide, thymidine 5'phosphate (Ibanez *et al.*, 1971). Surprisingly the oligomerization occurs even at a pH of 7. Another reaction, the phosphorylation of uridine is also facilitated by cyanamide (Lohrmann and Orgel, 1968), but here again the question arises as to whether orthophosphate was available in sufficiently high concentrations in seawater.

B. CARBODIIMIDE

Carbodiimide enhances the phosphorylation of uridine (Table IX), as well as the oligomerization of adenosine and adenosine 5'phosphate. Interestingly, in this case reasonable yields are obtained at neutral pH. Another phenomenon in the oligomerization of nucleotides is a strong catalysis by the biochemically reciprocal polymer. This cannot explain the formation of the first nucleotide, but represents an autocatalytic reaction of the type that may have been very important in biochemical evolution. Unfortunately none of these studies goes into any detail about the reaction mechanisms.

C. DICYANAMIDE AND DICYANDIAMIDE

Neither one of these two condensing agents has been studied for its effect on the synthesis of oligonucleotides and/or their constituents.

D. CYANOGEN

Cyanogen seemed only successful in the phosphorylation of ribose with orthophosphate (Halman *et al.*, 1969). The insolubility of orthophosphate in seawater makes questionable relevance of the results of this experiment. Cyanogen was

TABLE VIII

Cyanamide

Reactants	Products	Yield	Conditions	Remarks	Reference
adenine + ribose	no reaction	0%	0.1–0.0001 M 30°C–100°C pH 2–pH 11 with or without u.v.		Fuller *et al.* (1972)
D-ribose orthophosphate	ribose-phosphate	8%	pH 7; 65°C		Halmann et al. (1969)
thymidine 5'-phosphate	dinucleotide trinucleotide tetranucleotide	1.3% 1.6% 0.0023%	pH 7.3 1 M cyanamide 90°C		Ibanez *et al.* (1971)
uridine + Orthophosphate	uridine 5'phosphate	2%	pH 7; 65°C	intermediates isolated	Lohrmann and Orgel (1968)

TABLE IX

Carbodiimide

Reactants	Products	Yield	Conditions	Remarks	Reference
uridine + orthophosphate	uridine 5' phosphate	3.6%	pH 7; 65°C		Lohrmann and Orgel (1968)
deoxyadenosine deoxyadenosine-5'phosphate	dinucleotide trinucleotide	18% 1.4%	pH 5.7 0°C, 8 days 0.05 M poly U	poly U template greatly increases yield	Schneider-Bernloehr *et al.* (1968)
deoxyadenosine-5'phosphate	dimer AppA trimer AppApA dimer ApAp	71% 5.5% 1.9%	pH 5.7 0°C; 8 days 0.05 M poly U	ibid	ibid
adenosine adenosine 5' phosphate	dimer ApA trimer ApApA dimer ApA	10% 1.4% 1%	as above as above, but without poly U.	as above	Sulston *et al.* (1968a)

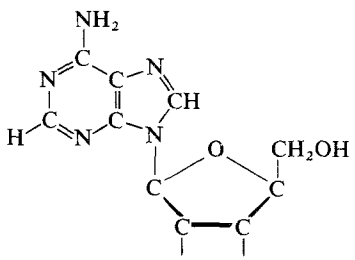
unable to cause any other dehydration-type condensation reaction, even with a reciprocal polynucleotide template as catalyst (see Table X). This casts serious doubts on the relevance of cyanogen as a general prebiological condensing agent.

E. HYDROGEN-CYANIDE-TETRAMER

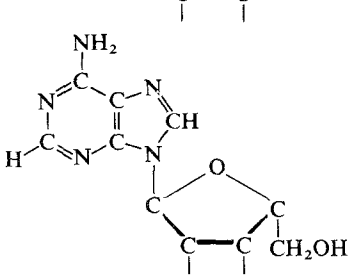
This condensing agent has only been applied to the phosphorylation of uridine, an attempt that was unsuccessful (Lohrmann and Orgel, 1968). No other condensation reactions with hydrogen-cyanide-tetramer have yet been described in the literature.

F. CONDENSED PHOSPHATES

Condensed phosphates were the first condensing agents used in the formation of nucleotides by Schramm *et al.* in 1962. They used the polyphosphate-ethyl-ester. Polyphosphate-ethyl-ester is a phosphoanhydride with an indistinct chain length and with some of its acidic groups esterified with ethanol. Since it has the same characteristics as unesterified phosphoanhydrides, it may be relevant for the studies in the origin of life, although polyphosphate-ethyl-ester itself has no prebiological relevance. Table XI gives a survey of the literature about this compound. Carbon's results (1963 and 1964) with the adenosine synthesis are quite interesting, since he obtained all six possible adenosine isomers. Only one out of the six isomers has been selected by life processes. The six isomers result from the following stereochemistry: Sugars can form acetal bonds in two directions-above the plane, resulting in the β-bond, and beneath the plane, resulting in the α-bond.



β-adenosine



α-adenosine

The adenine molecule has three sites that can possibly undergo a condensation reaction with sugars. These are the nitrogen atoms 9 and 7, and the primary amine adjacent to carbon 6. The only isomer found in living systems is the N_9,C_1'-ribofuranosyladenine.

TABLE X

Cyanogen

Reactants	Products	Yield	Conditions	Remarks	Reference
D-ribose orthophosphate	β-ribose-phosphate	20%	pH 7-8.8 25°C, overnight	carefully analyzed	Halmann *et al.* (1969)
D-deoxyribose orthophosphate	no reaction	0%	as above		as above
adenine + ribose	no reaction	0%	pH 2–pH 11 30 °C–100°C with or without u.v.		Fuller *et al.* (1972)
adenosine adenosine 5′ phosphate	no reaction	0%	poly U template pH 8 0°C, 14 days		Weiman *et al.* (1968)

TABLE XI

Polyphosphates

Reactants	Products	Yield	Conditions	Remarks	Reference
adenine + ribose polyphosphate-ester	deoxyadenosine	13–19%	in dimethylformamide	six isomers identified not prebiotic	Carbon (1963)
adenine + ribose + polyphosphate-ester	no reaction	0%	30°C–100°C pH 2–11 *with or without* u.v.		Fuller *et al.* (1972)
as above	adenosine ?	0.01% 0.01%	phosphoric acid polyphosphate-ester 40°C, u.v.irr.	base-sugar product confirmed, but no structural analysis	Ponnamperuma, Mariner and Sagan (1963)
adenine + deoxyribose polyphosphate-ester	deoxyadenosine	up to 30%	in dimethylformamide 50°C, 20 hrs	no structural analysis, criticized by Carbon, J.A. 1963.	Schramm *et al.* (1962)
adenine + ribose + orthophosphate polyphosphate-ester	AMP ADP ATP A4P	0.08% 0.06% 0.05% 0.04%	u.v. irradiation	no structural analysis	Ponnamperuma, Sagan and Mariner (1963)
adenosine + triphosphate	adenosine-5′,3′ or 2′-phosphate	1.1%	pH 7.5–8.0 100°C, 4–6 hrs		Schwarz and Ponnamperuma (1971)

Carbon (1964) observed also adenosine synthesis in a boiling aqueous solution without any condensing agent. Ponnamperuma, Mariner and Sagan (1963), however, could not synthesize any adenosine under those conditions. They did obtain minute yields after addition of poly-phosphate-ethyl-ester. These latter results could not be reproduced by Fuller *et al.* (1972), who did not obtain even the slightest amount of adenosine after addition of polyphosphate-ethyl-ester. Linear polyphosphate was applied successfully to the phosphorylation of adenosine by Schwartz and Ponnamperuma (1971).

Considering the findings reported in the last sections, one must conclude that still very much is not yet known about the aqueous synthesis of the nucleosides, nucleotides and oligonucleotides.

4. Evaluation and Conclusions

From the results in the peptide synthesis the conclusion can be drawn that, from the chemical point of view, hydrogen-cyanide-tetramer and the polyphosphates are more likely to have played a role in the second stage of the process of the emergence of life on Earth, more than cyanamide, dicyanamide, dicyandiamide and carbodiimide. The reason for this is two-fold. First, the fact that the last four condensing agents can only produce results under conditions unlikely to have occured on the primitive Earth, since the pH should be 5 or lower. Even under optimum conditions the yields are still quite low, due to the fact that amines are not very strong nucleophiles in an acidic environment. The second reason is that the availability of the cyanamide-like condensing agents on the primitive earth is not very certain, except for the least successful dicyandiamide.

The other condensing agents, hydrogen-cyanide-tetramer and the phospho-anhydrides appear to be much more plausible from the point of view of the prebiotic environment, considering their reactivity in neutral to slightly basic surroundings.

Currently the general idea is that the prebiological reactions are primitive precedents of present-day biochemical processes. A cogent argument for the relevance of polyphosphates is therefore the finding of inorganic polyphosphates in many organisms, from micro-organisms (Harold, 1966) to mammals. Inorganic poly-phosphates were discovered in rat brain and rat liver as well as several other mammalian tissues (Gabel and Thomas, 1971). Very recently, inorganic condensed phosphates have been shown to occur in intact metabolizing human erythrocytes via ^{31}P-NMR spectroscopy (Glonek, 1975). Thus, whereas condensed phosphates are still functional compounds in contemporary organisms, the cyanide containing compounds by contrast are deadly poisons for contemporary living systems. The strongest biological argument, however, for the importance of phosphoanhydrides as prebiological condensing agents is the fact that the only condensing agents used by living systems are the organic phosphoanhydrides of which adenosine tri-phosphate is the most common. This shows that the phosphoanhydride bond has proven through an evolution of many billions of years to be a very successful condensing agent under the conditions in which the biochemical reactions take place.

References

Banda, P. W. and Ponnamperuma, C.: 1971, *Space Life Sci.* **3**, 54.
Beck, A. and Orgel, L.: 1965, *Proc. Nat. Acad. Sci. U.S.* **54**, 664.
Bodanszky, M. and Ondetti, M. A.: 1966 *Peptide Synthesis*, p. 116, New York Intersci Publ.
Carbon, J. A.: 1963, *Chem. Ind.* 529.
Carbon, J. A.: 1964, *J. Am. Chem. Soc.* **86**, 720.
Chang, S., Flores, J., and Ponnamperuma, C.: 1969, *Proc. Nat. Acad. Sci. U.S.* **64**, 1011.
Chang, S., Williams, J., Rabinowitz, J., and Ponnamperuma, C.: 1970, *Space Life Sci.* **2**, 14.
Chung, N. M., Lohrmann, R., Orgel, L. E., and Rabinowitz, J: 1971, *Tetrahedron* **27**, 1205.
Clark, V. M., Macrae, A. R., Richter, J. F. P., and Lord Todd: 1966, *Tetrahedron* suppl. 7, 337.
Combes, M., Encrenaz, T., Vapillon, L., Zeau, Y., and Lesqueren, C.: 1974, *Astron. Astrophys.* **34**, 33.
De Rosnay, J.: 1967a, *Ann. Chimie* **2**, 57.
De Rosnay, J.: 1967b, *Ann. Chimie* **2**, 153.
Flores, J. and Ponnamperuma, C.: 1972, *J. Molec. Evol.* **2**, 9.
Fuller, W. D., Sanchez, R. A., and Orgel, L. E.: 1972, *J. Molec. Biol.* **67**, 25.
Gabel, N. W.: 1971, in *Chemical Evolution and the Origin of Life* (ed. by R. Buvet and C. Ponnamperuma) North Holland Publ. Co. p. 369.
Gabel, N. W. and Ponnamperuma, C.: 1967, *Nature* **216**, 453.
Gabel, N. W. and Ponnamperuma, C.: 1972, in *Exobiology* (ed. by C. Ponnamperuma) North Holland Publ. Co., p. 95.
Gabel, N. W. and Thomas, V.: 1971, *J. Neurochem.* **18**, 1229.
Gillet, F. C. and Forrest, W. J.: 1974, *Astrophys. Letters* **187**, 137.
Glonek, T.: 1975, in preparation.
Griffith, E., Gabel, N. W., and Ponnamperuma, C.: 1975, in preparation.
Halmann, M.: 1968, *Arch. Biochim. Biophys.* **128**, 808.
Halmann, M., Sanchez, R. A., and Orgel, L. E.: 1969, *J. Org. Chem.* **34**, 3702.
Harada, K. and Fox, S. W.: 1964, *Nature* **201**, 335.
Harold, F. M.: 1966, *Bact. Rev.* **30**, 772.
Holland, H. D.: 1961, *J. Geophys. Res.* **66**, 2356.
Ibanez, J. D., Kimball, A. P., and Oro, J.: 1971, *Science* **173**, 444.
Katchalski, A. and Ailam, C.: 1967, *Biochim. Biophys. Acta* **140**, 1.
Khorana, H. G.: 1955, *Chem. Ind.* 1087.
Lemmon, R. M.: 1970, *Chem. Reviews* **70**, 95.
Lewinsohn, R., Paecht-Horowitz, M., and Katchalsi, A.: 1967, *Biochim. Biophys. Acta* **140**, 24.
Lohrmann, R. and Orgel, L. E.: 1968, *Science* **161**, 64.
Lohrmann, R. and Orgel, L. E.: 1971, *Science* **171**, 490.
Lohrmann, R. and Orgel, L. E.: 1973, *Nature* **244**, 418.
Miller, S.: 1953, *Science* **117**, 528.
Miller, S.: 1955, *J. Amer. Chem. Soc.* **77**, 2351.
Miller, S.: 1957, *Ann. N.Y. Acad. Sci.* **69**, 260.
Nooner, D. W. and Oro, J.: 1974, *J. Molec. Evol.* **3**, 79.
Orgel, L. E. and Sulston, J.: 1971 in *Prebiotic and Biochemical Evolution* (ed. by A. P. Kimball and J. Oro), p. 89.
Oro, J. and Kimball, A. P.: 1962, *Arch. Biochem. Biophys.* **96**, 293.
Paecht-Horowitz, M. and Katchalski, A.: 1967, *Biochim. Biophys. Acta* **140**, 14.
Pharmacia Chemicals: 1973, *Separation News*, September.
Ponnamperuma, C.: 1965, in *The Origin of Prebiological Systems* (ed. by S. W. Fox), p. 221.
Ponnamperuma, C. and Flores, J.: 1966, 152nd Nat. Meeting of the Am. Chem. Soc., New York, C 33.
Ponnamperuma, C., Mariner, R. and Sagan, C.: 1963, *Nature* **198**, 1199.
Ponnamperuma, C. and Peterson, E.: 1965, *Science* **147**, 1572.
Ponnamperuma, C., Sagan, C., and Mariner, R.: 1963, *Nature* **199**, 222.
Ponnamperuma, C. and Woeller, F.: 1967, *Curr. Mod. Biol.* **1**, 56.
Preobrazhenskaya, N. N.: 1972, *Russian Chem. Reviews* **41**, 54.
Rabinowitz, J.: 1969a, 158th A.C.S.-meeting.
Rabinowitz, J.: 1969b, *Helv. Chim. Acta* **52**, 2663.
Rabinowitz, J.: 1970, *Helv. Chim. Acta* **53**, 1350.
Rabinowitz, J.: 1971, *Helv. Chim. Acta* **54**, 1483.
Rabinowitz, J.: 1972, *Chimia* **26**, 350.
Rabinowitz, J., Flores, J., Krebsbach, R., and Rogers, G.: 1969, *Nature* **244**, 795.
Rabinowitz, J., Woeller, F., Flores, J., and Krebsbach, R.: 1969, *Nature* **244**, 796.

Ridgway, S. T.: 1974, *Astrophys. J. Letters* **187**, L 41.
Sanchez, R. A., Ferris, J. P., and Orgel, L. E.: 1966a, *Science* **153**, 72.
Sanchez, R. A., Ferris, J. P., and Orgel, L. E.: 1966b, *Science* **154**, 784.
Sanchez, R. A., Ferris, J. P., and Orgel, L. E.: 1967, *J. Molec. Biol.* **30**, 223.
Sanchez, R. A. and Orgel, L. E.: 1970, *J. Molec. Biol.* **47**, 531.
Schimpl, A., Lemmon, R. M., and Calvin, M.: 1965, *Science*, **147**, 149.
Schneider-Bernloehr, H., Lohrmann, F., Sulston, J., Weiman, B. J., and Orgel, L. E.: 1968, *J. Molec. Biol.* **37**, 151.
Schneider-Bernloehr, H., Lohrmann, R., Sulston, J., Weiman, B. J., and Orgel, L. E.: 1969, *J. Molec. Biol.* **47**, 257.
Schramm, G.: 1965, in *Origin of Prebiological Systems* (ed. by S. W. Fox), p. 299.
Schramm, G., Grötsch, H., and Pollmann, W.: 1962, *Angew. Chemie* (Int. Ed.) **1**, 1.
Schramm, G. and Wissmann, H.: 1958, *Angew. Chemie* **91**, 1073.
Schwartz, A. W.: 1971, in *Chemical Evolution and the Origin of Life* (ed. by R. Buvet and C. Ponnamperuma), p. 207.
Schwartz, A. W. and Deuss, H.: 1971 in *Theory and Experiment in Exobiology* (ed. by A. W. Schwartz) vol. I, Publ. Wolters-Noordhoff, p. 73.
Schwartz, A. W. and Ponnamperuma, C.: 1971, in *Prebiological and Biochemical Evolution* (ed. by Kimball and J. Oro), p. 78.
Schwartz, A. W., Van der Veen, M., Bisseling, T., and Chittenden, G. J. F.: 1975, *Origins of Life* **6**, 163.
Sheehan, J. C. and Hess, G. P.: 1955, *J. Amer. Chem. Soc.* **77**, 1067.
Sheehan, J. C., Goodman, M., and Hess, G. P.: 1956, *J. Amer. Chem. Soc.* **78**, 1367.
Steinman, G.: 1967, *Arch. Biochim. Biophys.* **121**, 533.
Steinman, G.: 1971, in *Prebiotic and Biochemical Evolution* (ed. by A. P. Kimball and J. Oró), p. 31.
Steinman, G. and Cole, M. N.: 1967, *Proc. Nat. Acad. Sci. U.S.* **52**, 735.
Steinman, G. and Cole, M. N.: 1968, *Fed. Proc.* **27**, 765.
Steinman, G., Kenyon, D. H., and Calvin, M.: 1965, *Nature* **206**, 707.
Steinman, G., Kenyon, D. H., and Calvin, M.: 1966, *Biochim. Biophys. Acta* **124**, 339.
Steinman, G., Lemmon, R. M., and Calvin, M.: 1965, *Science* **147**, 1574.
Stephen-Sherwood, E., and Oró, J.: 1973, *Space Life Sci.* **4**, 5.
Sulston, J., Lohrmann, R., Orgel, L. E., and Miles, T.: 1968a, *Proc. Nat. Acad. Sci.* **59**, 726.
Sulston, J., Lohrmann, R., Orgel, L. E., and Miles, T.: 1968b, *Proc. Nat. Acad. Sci.* **60**, 409.
Van Wazer, J. R.: 1958, *Phosphorus and its Compounds*, Vol. 1, Intersci. Publ.
Weiman, B. J., Lohrmann, R., Orgel, L. E., Schneider-Bernloehr, H., and Sulston, J.: 1968, *Science* **161**, 387.