Table 2. Factors influencing the yield of *Borrelia hermsi* in vitro. The yield is the number of organisms per milliliter as determined by the method of Magnuson *et al.* (*5*) after incubation at 35°C for 7 days.

Deletion	Inoculum	Yield
N-Acetylglucosamine	10^5	0.6×10^7
Proteose Peptone No. 2	10^5	2.5×10^7
Tryptone	10^5	1.2×10^7
Albumin	10^5	2.8×10^7
Gelatin	10^5	5.2×10^7
Gelatin	10^3	No growth
Complete medium	10^5	5.4×10^7
Complete medium	10^3	6.1×10^6

when cultures were exposed to more aerobic conditions in tubes filled only half full of medium. These results suggest that a small amount of oxygen was used by the organisms.

Evidence in further support of some consumption of oxygen was provided by the requirement for pyruvate in the medium. Growth was not obtained in medium lacking pyruvate, but organisms did grow in the absence of pyruvate if catalase was added to the medium. This suggests that the function of pyruvate was in the destruction of hydrogen peroxide produced by the autoxidation of flavoproteins (*4*).

The function of *N*-acetylglucosamine in the medium has not been determined. Glucosamine in equimolar concentration stimulated growth slightly. *N*-Acetylgalactosamine and *N*-acetylmannosamine were inactive.

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2. *Borrelia hermsi*, originally isolated from a patient with relapsing fever contracted at Lake Tahoe, California [E. M. Coffey and W. C. Eveland, *J. Infect. Dis.* **177**, 29 (1967)], was obtained in the form of frozen infected mouse tissues from Dr. Edith Coffey, California Department of Public Health, Berkeley. Organisms were recovered by inoculation of CF-1 mice with homogenates of the thawed tissue; they were maintained by serial passage of infected blood at 2- to 3-day intervals. For culture experiments, borreliae were isolated from citrated blood obtained by cardiac puncture of 15 to 20 infected mice. The pooled blood was centrifuged at 270*g* for 10 minutes. The supernatant plasma containing the spirochetes was removed and used for inoculation of culture tubes.
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6. I thank Dr. Edith Coffey for providing the isolate of *Borrelia hermsi* used in this study.

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Possible Prebiotic Condensation of Mononucleotides by Cyanamide

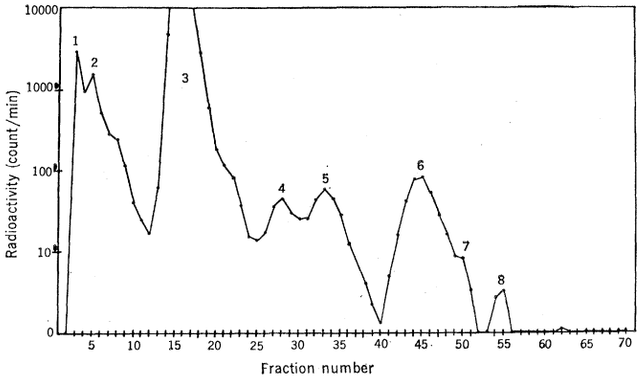
Abstract. *The condensation of mononucleotides has been carried out in aqueous solution at neutral* pH *in the presence of cyanamide. Oligodeoxyribonucleotides up to five units have been formed when montmorillonite was present.*

The investigations carried out during the past two decades make it quite clear that monomers such as amino acids, sugars, pyrimidines, and purines could have been formed under conditions that may have existed on the primitive earth (*1*). Once the monomers were formed, condensation reactions could have resulted in the formation of peptides, of nucleosides, and of sugar phosphates and nucleotides (in the presence of phosphate). Condensation of nucleotides to oligonucleotides and polynucleotides might have been initiated in a similar manner.

Formation of oligonucleotides in an anhydrous environment under prebiotic conditions has been reported; dinucleotides and trinucleotides have been produced in the presence of inorganic phosphate salts when nucleosides are heated in the absence of water (*2*). In addition, Schwartz and Fox report that a polymer (1 percent yield) is formed when cytidylic acid is heated in polyphosphoric acid (*3*). The product exhibited alkaline hyperchromicity after incubation in 0.1*M* NaOH at 37°C, and also indicated the presence of 2′,5′ and 3′,5′ phosphodiester linkage after enzymatic degradation.

Oligonucleotide formation has also been carried out in aqueous systems in the presence of a water-soluble carbodiimide. Sulston *et al.* (*4*), using a water-soluble carbodiimide, have shown that the formation of oligoadenylic acids from adenylic acid was facilitated by a polyuridylic acid template. Similarly, the condensation of guanylic acid was facilitated by a polycytidylic acid template (*5*).

Condensation reactions could have been mediated by compounds that have been produced in reactions simulating a prebiotic environment. Cyanamide is one such compound. Cyanamide has been formed by ultraviolet irradiation of ammonium cyanide solutions and by electron irradiation of mixtures of methane, ammonia, and water (*6*). Cyanamide can tautomerize to carbodiimide ($H_2N-C\equiv N \leftrightarrow HN=C=NH$), the parent compound of the dialkyl-



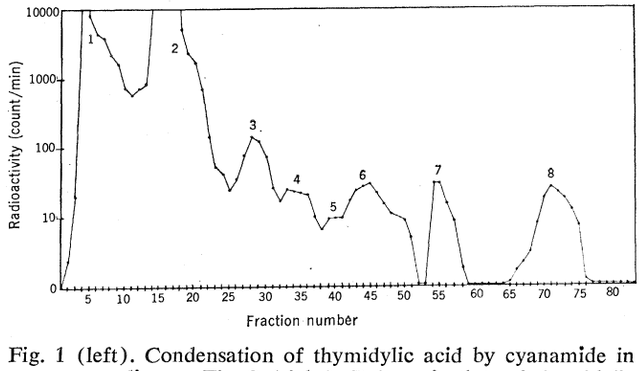


Fig. 1 (left). Condensation of thymidylic acid by cyanamide in aqueous media. Fig. 2 (right). Polymerization of thymidylic acid by cyanamide in the presence of montmorillonite.

Table 1. Formation of oligodeoxyribonucleotides by cyanamide in aqueous media.

Peak	Fractions	Yield (%)	Compound*
1	1–4	2.1	TdR
2	5–11	1.5	Cyclic TMP
3	12–25	92.3	TMP
4	26–30	0.9	TpT
5	31–39	1.3	$(pT)_2$
6	40–47	1.6	$(pT)_3$
7	48–51	0.02	Cyclic $(pT)_4$
8	52–56	0.003	$(pT)_4$

*Symbols: T, thymidine or thymine as appropriate; TdR, deoxythymidine; p on the left side indicates 5′, on right side 3′.

carbodiimides. This suggests that cyanamide could have similar chemical activity as the substituted carbodiimides. Oró has suggested that cyanamide could have been responsible for the formation of a number of polymeric compounds (*7*).

Ponnamperuma and Peterson (*8*) have reported peptide bond formation between amino acids in the presence of cyanamide. Dipeptides and tripeptides were produced from aqueous solutions of glycine and leucine (0.01*M*) at *p*H about 5. Steinman *et al.* (*9*) observed similar amino acid condensations in the presence of dicyandiamide.

In our studies, deoxyribonucleotides such as thymidine 5′-monophosphate (TMP) were allowed to react in aqueous solution (neutral *p*H) in the presence of cyanamide. In addition, similar experiments were carried out in the presence of montmorillonite. Clays such as montmorillonite are very effective catalysts in the condensation of amino acids and amino acid adenylates to peptides (*10*).

Radioactive thymidine 5′-monophosphate ([^{14}C]TMP; 0.50 μc, 50 μmole/μc) was dissolved in 100 μl of an aqueous cyanamide solution (1.0*M*, *p*H 7.3). The mixture was allowed to react at 90°C for 24 hours. The reaction mixture was analyzed on a diethylaminoethyl- (DEAE) cellulose column (7 by 0.9 cm) and eluted with a linear gradient of triethylammonium bicarbonate buffer (0.04*M* to 0.40*M*), *p*H 7.5. Fractions were collected, and the radioactivity of a portion from each fraction was counted on a scintillation counter (Packard model 3380). Samples having less than 100 count/min were counted for longer periods of time in order to provide the necessary statistical significance for all the chromatographic peaks. The average standard deviation did not exceed 3 percent in any case. The chromatographic pattern observed is shown in Fig. 1. Fractions corresponding to a particular peak were pooled and evaporated to dryness. The residues, as spots on Whatman No. 1 paper, were chromatographed in a solvent of isopropanol, ammonia, and water (7 : 1 : 2, by volume). This solvent system was used in the separation of oligothymidylic acids by Tener *et al.* (*11*). The products were tentatively identified by comparison of R_F values. In addition to the paper chromatographic identification, the peaks from the DEAE-cellulose column separation were also identified by comparing their elution pattern in this column to those of standard oligomers of thymidylic acids under the same conditions. The identification of the products formed in the presence of cyanamide is listed in Table 1.

The standard oligothymidylic acid series was prepared and products were identified according to the procedures of Khorana *et al.* (*12*). The products consisted of the homologous series of linear oligomers up to eight units in length and the cyclic series up to six units.

Furthermore, the above experiment was repeated for enzymatic characterization of the products. Radioactive thymidine 5′-monophosphate was reacted with cyanamide (1.0*M*, *p*H 7.3) under the exact conditions as described above. Separation of the products on a DEAE-cellulose column showed the same chromatographic pattern as shown in Fig. 1. Peak 5 (dinucleotide) and peak 6 (trinucleotide) were treated with snake venom phosphodiesterase in order to determine the extent of 3′,5′ phosphodiester linkage. Peak 5 showed 44 percent degradation to TMP and peak 6 showed 86 percent degradation to TMP after enzymatic treatment for 2 hours at 37°C. As these enzymatic tests show, peak 6 consists almost entirely of a trinucleotide with 3′,5′ phosphodiester linkage, and peak 5 contains a dinucleotide which largely consists of the natural biological 3′,5′ phosphodiester linkage (44 percent).

A similar reaction mixture was allowed to react in the presence of montmorillonite. Radioactive [^{14}C]TMP (0.50 μc, 50 μmole/μc), montmorillonite (35 mg), and 100 μl of a 1.0*M* cyanamide solution were allowed to react at 85° to 90°C for 24 hours. The products were analyzed as described above. The separation of products on a DEAE-cellulose column is shown in Fig. 2, and identification of the products is listed in Table 2.

Table 2. Formation of oligonucleoides by cyanamide in the presence of montmorillonte.

Peak	Fractions	Yield (%)	Compound*
1	1–10	13.3	TdR and cyclic TMP
2	11–24	86.1	TMP
3	25–31	0.14	TpT
4–5	32–40	0.06	$(pT)_2$
6	41–52	0.06	$(pT)_3$
7	53–58	0.05	$(pT)_4$
8	65–77	0.05	$(pT)_5$

* Symbols as in Table 1.

Our studies indicate that deoxyribonucleotides such as thymidine 5′-monophosphate are condensed to oligodeoxyribonucleotides in the presence of cyanamide. Oligomers up to four units in length can be formed in aqueous solution at neutral *p*H (Table 1). It is significant that yields of 1.3 and 1.6 percent are observed for dinucleotides and trinucleotides, respectively. Although concentrations of 1.0*M* cyanamide were used in these experiments, condensation of mononucleotides is possible at cyanamide concentrations of $10^{-2}M$ (*13*). In the presence of montmorillonite, the yields of the dinucleotides and trinucleotides are lower (Table 2). However, pentanucleotides are produced in the presence of montmorillonite. Thus, the clay surface allows for chain elongation at the expense of lowering formation of smaller oligodeoxyribonucleotides.

Our studies suggest that simple compounds such as cyanamide are plausible condensing agents in promoting oligodeoxyribonucleotide formation. They also indicate that longer oligomers can be formed in the presence of inorganic surfaces such as clays.

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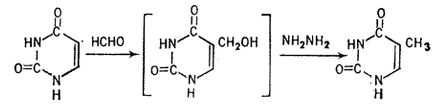
Thymine: A Possible Prebiotic Synthesis

Abstract. *A possible prebiotic synthesis of thymine has been achieved via the methylation of uracil with formaldehyde and hydrazine.*

Thymine is the only nucleic acid base that has not been synthesized under possible prebiotic conditions. We report here the successful synthesis of thymine under conditions presumably like those present on the primitive earth. The abiotic synthesis of the other nucleic acid bases, in particular the pyrimidines, uracil (*1, 2*), and cytosine (*3*), have been accomplished from simple precursor molecules. In addition, uracil could have arisen from cytosine by way of deamination (*3*). Attempts to synthesize thymine in a similar manner have not proved successful, and we therefore decided to investigate the methylation of uracil as a means of synthesizing thymine abiotically.

The catalytic addition above *p*H 7 of formaldehyde to uracil (*4, 5*), uridine (*4*), or its 5′-mononucleotides (*6*) has been reported. The 5-hydroxymethyl derivatives are obtained almost exclusively in each case. 5-Hydroxymethyluracil is found in the DNA of a group of *Bacillus subtilis* bacteriophages in the place of thymine (*7*). It is also a probable intermediate in the biological synthesis of thymine nucleotides (*8*). 5-Hydroxymethyluracil may be reduced to thymine, 5,6-dihydrothymine, or 5,6-dihydro-5-hydroxymethyluracil by catalytic hydrogenation (*4, 9*). Therefore, the reaction of uracil and formaldehyde in an ammoniacal solution in the presence of a reducing agent was investigated as a possible prebiotic method for producing thymine, as shown below.



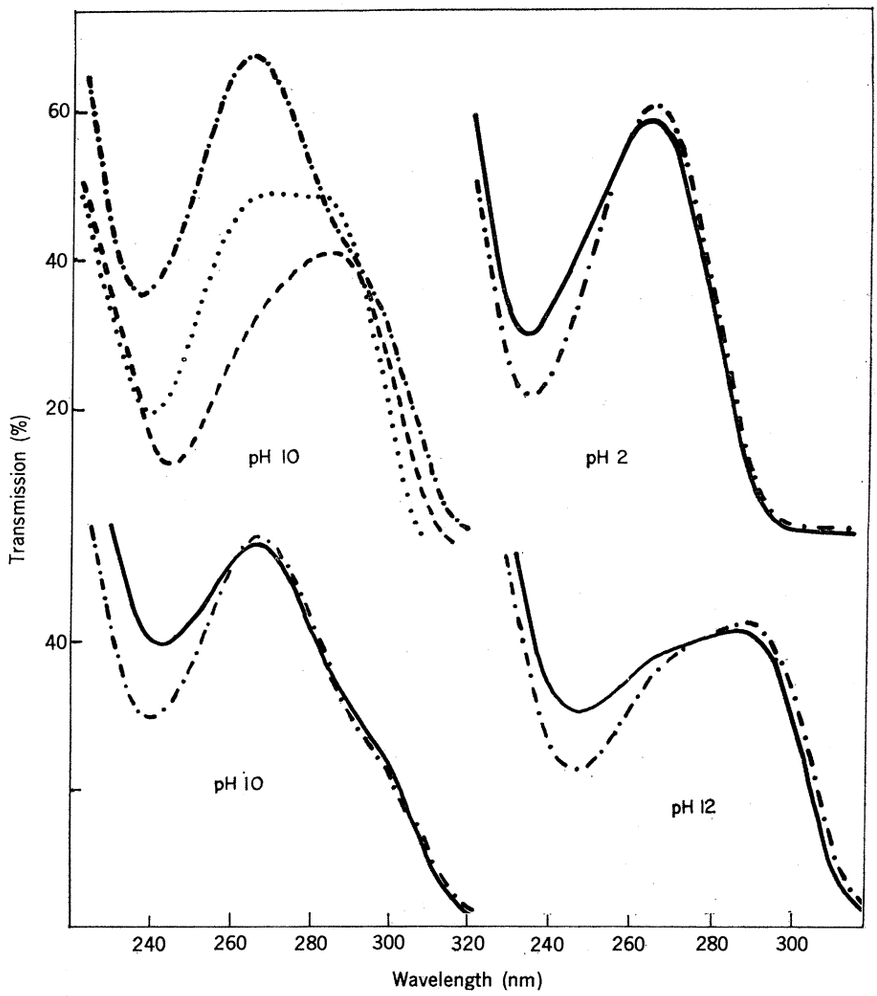


Fig. 1. Ultraviolet absorption spectra of the unknown compound (———), thymine (–·–·–), uracil (·····), and 5-hydroxymethyluracil (– – – –).

Hydrazine was chosen as the reducing agent, since this compound is formed by the action of electric discharges on ammonia (*10*). Also hydrazine will reduce the hydroxymethyl group of pyridoxine hydrochloride to a methyl group yielding 4-deoxypyridoxine (*11*).

In a typical experiment, thymine was obtained when uracil, paraformaldehyde, and hydrazine (0.005 mole, respectively) were heated in an ammoniacal solution (100 ml, *p*H 9) for 3 days at 70°C. The reaction mixture before heating had a *p*H of 8.5. On completion of the reaction, the solution was stirred with freshly washed Dowex 50 (H^+ form, 100 to 200 mesh, 3 g), filtered, and concentrated under reduced pressure at 40°C to a final volume of approximately 0.5 ml. The unreacted uracil, which precipitated out of solution during the concentration process, was removed by filtration. The thymine was isolated by means of two-dimensional preparative paper chromatography with two different solvent systems; the isolated product was finally purified by thin-layer chromatography on polyamide plates (*12*). Descending paper chromatography on Whatman No. 1 paper was used; the solvent system for the first dimension was 2-butanol saturated with water (thymine,