cogenic viruses are stimulated by double-stranded RNA and DNA but not by single-stranded RNA (*13*). Transfer RNA, which has been shown to bind ethidium bromide (*14*), stimulates the reaction (*15*). Perhaps the strong inhibition by ethidium bromide indicates that transcription of the RNA of these viruses must involve double-stranded regions. There is now evidence that ethidium bromide inhibits the growth of tumors in mice and interferes with the replication of MSV(M) in cell cultures (*16*).

SHALOM Z. HIRSCHMAN
Mount Sinai School of Medicine, New York 10029

References and Notes

1. H. M. Temin and S. Mizutani, *Nature* **226**, 1211 (1970); D. Baltimore, *ibid.*, p. 1209; M. Green, M. Rokutanda, K. Fujinaga, R. K. Ray, H. Rokutanda, C. Gurgo, *Proc. Nat. Acad. Sci. U.S.* **67**, 385 (1970); M. Rokutanda, H. Rokutanda, M. Green, K. Fujinaga, R. K. Ray, C. Gurgo, *Nature* **227**, 1026 (1970); M. Hatanaka, R. J. Huebner, R. V. Gilden, *Proc. Nat. Acad. Sci. U.S.* **67**, 143 (1970); E. M. Scolnick, S. A. Aaronson, G. J. Todaro, *ibid.*, p. 1034; S. Spiegelman, A. Burny, M. R. Das, J. Keydar, J. Schlom, M. Travenicek, K. Watson, *Nature* **227**, 563 (1970); *ibid.*, p. 1029; S. Mizutani, D. Boettiger, H. M. Temin, *ibid.* **228**, 424 (1970); J. Riman and G. S. Beadreau, *ibid.*, p. 427; J. P. McDonnell, A. Garapin, W. E. Levinson, N. Quintrell, L. Fanshier, J. M. Bishop, *ibid.*, p. 433.
2. J. Schlom, D. H. Harter, A. Burny, S. Spiegelman, *Proc. Nat. Acad. Sci. U.S.* **68**, 182 (1971).
3. E. M. Scolnick, S. A. Aaronson, G. J. Todaro, W. P. Parks, *Nature* **229**, 318 (1971).
4. R. C. Gallo, S. S. Yang, R. C. Ting, *ibid.* **228**, 927 (1970).
5. C. Gurgo, R. K. Ray, L. Thiry, M. Green, *Nature New Biol.* **229**, 111 (1971).
6. M. Waring, *J. Mol. Biol.* **54**, 247 (1970).
7. A. Sippel and G. Hartmann, *Biochim. Biophys. Acta* **157**, 218 (1968); E. D. Mauro, L. Snyder, P. Marino, A. Lamberti, A. Coppo, G. P. Tocchini-Valentini, *Nature* **222**, 533 (1969).
8. J. H. Subak-Sharpe, M. C. Timbury, J. F. Williams, *Nature* **222**, 341 (1969).
9. H. Diggelmann and C. Weismann, *ibid.* **224**, 1277 (1969).
10. A. Nagayama, B. G. T. Pogo, S. Dales, *Virology* **40**, 1039 (1970).
11. R. Saffhill, H. Schneider-Bernloehr, L. E. Orgel, S. Spiegelman, *J. Mol. Biol.* **51**, 531 (1970).
12. L. S. Lerman, *ibid.* **3**, 18 (1961); M. Waring, *ibid.* **13**, 269 (1965).
13. S. Spiegelman, A. Burny, M. R. Das, J. Keydar, J. Schlom, M. Travnicek, K. Watson, *Nature* **228**, 430 (1970); R. Gallo, personal communication.
14. T. Tao, J. H. Nelson, C. R. Cantor, *Biochemistry* **9**, 3514 (1970).
15. R. Gallo, personal communication.
16. S. Z. Hirschman, *Trans. N.Y. Acad. Sci.*, in press; C. Friend and S. Z. Hirschman, unpublished data.
17. I thank Dr. C. Friend for encouragement in this work. I thank Gruppo Lepetit, Italy, for the AF/AP and AF/APB and Dr. J. Cuttner for the daunomycin hydrochloride. Supported by a grant from the New York Chemotherapy Foundation.

24 February 1971

Cultivation of Borrelia hermsi

Abstract. *A medium has been developed which permits the isolation and growth of* Borrelia hermsi, *an organism that causes relapsing fever.*

Although relapsing fever borreliae may be maintained in the laboratory in experimental animals or in embryonated eggs, they have not been cultivated in artificial media (*1*). Recently in this laboratory, studies on the effects of various chemical and physical factors on the survival of *Borrelia hermsi* in vitro led to the development of a growth medium for this organism. *Borrelia hermsi* (*2*) has been maintained in continuous cultivation for 8 months (36 subcultures) and has retained its ability to infect mice.

The growth medium which evolved from the preliminary studies is shown in Table 1. The maximum yield of organisms, 3×10^7 to 5×10^7 per milliliter, was obtained after 7 days of incubation. The calculated generation time for *B. hermsi* in culture was 18 hours.

The contribution of individual components in the medium to the yield of organisms is shown in Table 2. Absence of *N*-acetylglucosamine from the medium reduced growth by 90 percent. Deletion of Proteose Peptone No. 2, Tryptone, or albumin also reduced the yield, but to a lesser extent. Eliminating gelatin from the medium did not influence the yield of organisms when tubes were inoculated with 10^5 organisms; however, growth was not obtained in the absence of gelatin when a smaller inoculum was used (10^3 organisms).

Organisms were tested at the 15th and 26th subculture for infectivity in mice by the intraperitoneal inoculation of .02 ml of medium from 5-day-old cultures. In both instances, maximum spirochetemia was observed in the blood 48 hours after inoculation. Organisms from the 26th subculture were maintained in mice through four passages and then reisolated by culturing blood from the fourth animal.

During incubation, the *p*H of the culture medium dropped from an initial value of 7.8 to 6.8 at 7 days after inoculation. In studies on the carbohydrate metabolism of purified suspensions of *Borrelia duttoni* prepared from infected rat blood, Fulton and Smith (*3*) found that glucose was rapidly metabolized to lactic acid. This was not accompanied by any detectable utilization of oxygen. In culture, *B. hermsi* was found to require high amounts of glucose, and growth was limited by acid production unless a high concentration of phosphate buffer (.05 mole/liter) was included in the medium. These culture requirements suggest a similar metabolic pathway for glucose catabolism in *B. hermsi*; the spirochete, however, would not grow in the total absence of oxygen. Culture tubes filled completely to the top with medium did not sustain growth. Conversely, growth was poor

Table 1. Composition of growth medium for *Borrelia hermsi*. Basal medium and bovine albumin solutions were stored at −20°C. The gelatin solution was autoclaved at 115°C for 15 minutes and stored at 4°C. Sodium bicarbonate solution was freshly prepared at the time of use. Borosilicate screw-cap tubes with Teflon liners (13 by 100 mm) were used as culture vessels. The tubes (9 ml, total capacity) were filled with medium to a final volume of 8.5 ml. Complete medium was prepared from the stock solutions by addition of 4 ml of sodium bicarbonate solution, 34 ml of bovine albumin (fraction V, 10 percent solution adjusted to *p*H 7.8 with NaOH), and 2 ml of distilled water to 80 ml of basal medium. The mixture was sterilized by filtration under pressure through a 0.22-μm membrane filter and dispensed in 6-ml portions per culture tube. The sterile gelatin solution (7 percent) was liquefied by immersion in warm water, and 2 ml was added to each tube. Five-tenths milliliter of sterile rabbit serum was added and the tubes were mixed by inversion. Tubes were inoculated with .05 ml of *B. hermsi* (subculture or a suspension of organisms obtained from infected mouse blood) and incubated at 35°C after the caps were securely tightened.

Compound	Stock solution (g/liter)	Final concentration (mg/ml)
Basal medium		
$Na_2HPO_4 \cdot 7H_2O$	26.52	12.40
$NaH_2PO_4 \cdot H_2O$	1.03	0.48
NaCl	1.20	0.56
KCl	0.85	0.40
$MgCl_2 \cdot 6H_2O$	0.68	0.32
Glucose	12.75	6.00
Proteose Peptone No. 2*	5.95	2.80
Tryptone*	2.55	1.20
Sodium pyruvate	1.06	0.50
Sodium citrate-dihydrate	0.47	0.22
N-Acetylglucosamine	0.53	0.26
$NaHCO_3$ (4.5 percent)		1.06
Bovine albumin		20.00
Gelatin		16.50
Pooled rabbit serum		6 percent

* Difco Laboratories.

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.

RICHARD KELLY
Department of Pathology,
Baptist Memorial Hospital, and
University of Tennessee
Medical Units, Memphis 38103

References and Notes

1. P. H. Hardy, Jr., in *Manual of Clinical Microbiology*, J. E. Blair, E. H. Lennette, J. B. Truant, Eds. (American Society for Microbiology, Bethesda, Md., 1970), p. 236.
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.
3. J. D. Fulton and P. J. C. Smith, *Biochem. J.* **76**, 491 (1960).
4. K. V. Thimann, *The Life of Bacteria* (Macmillan, New York, ed. 2, 1963), p. 222.
5. H. J. Magnuson, H. Eagle, R. Fleishman, *Amer. J. Syph. Neurol.* **32**, 1 (1948).
6. I thank Dr. Edith Coffey for providing the isolate of *Borrelia hermsi* used in this study.

1 March 1971; revised 20 May 1971

Possible Prebiotic Condensation of Mononucleotides by Cyanamide

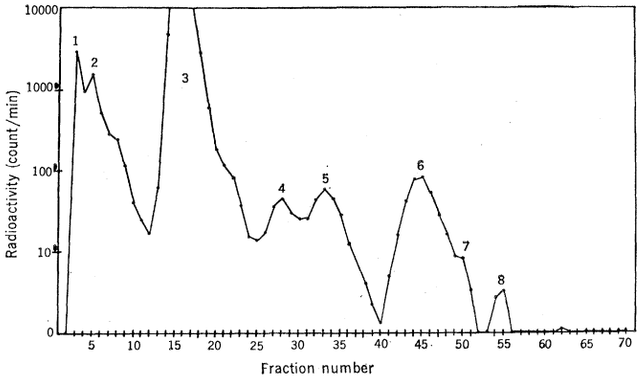
Abstract. *The condensation of mononucleotides has been carried out in aqueous solution at neutral* pH *in the presence of cyanamide. Oligodeoxyribonucleotides up to five units have been formed when montmorillonite was present.*

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-



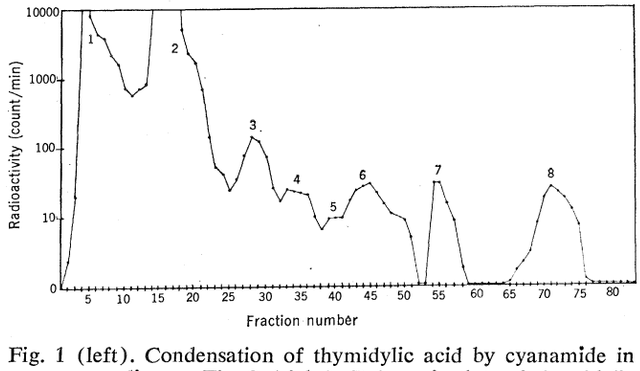


Fig. 1 (left). Condensation of thymidylic acid by cyanamide in aqueous media. Fig. 2 (right). Polymerization of thymidylic acid by cyanamide in the presence of montmorillonite.