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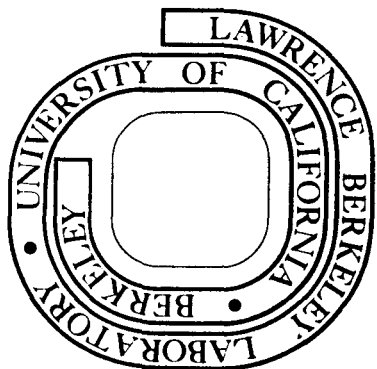
CHEMICAL EVOLUTION OF THE BIOPOLYMERS

Richard M. Lemmon

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## CHEMICAL EVOLUTION OF THE BIOPOLYMERS

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Approximately 3.5 billion ( $10^9$ ) years ago, the first living cells appeared on our planet. Their subsequent proliferations and mutations, leading to the development of diverse forms of life, are the results of Darwinian evolution. However, for our prebiological planet to have accumulated the necessary molecular "building blocks" for the first cells, Darwinian evolution had to be preceded by a kind of molecular evolution. That necessary-for-life chemistry, as well as the era in which it took place, has been called "chemical evolution", a term introduced by Melvin Calvin in 1953.<sup>1</sup> Chemical evolutionary studies are concerned with the development of a plausible "scenario" for the appearance of proteins, nucleic acids, carbohydrates, fats, etc., on the prebiological Earth. It is the purpose of this report to review our present understanding of how these biopolymers accumulated in an era before there were any living cells.

Accumulation of the Biomonomers

For the biopolymers to have appeared, their constituent units, the amino acids, nucleic-acid bases, sugars, fatty acids, etc., had to be available. Research over the past two decades has provided great understanding of the probable ways in which these biomonomers appeared on the primitive Earth.<sup>2</sup> This research was given great impetus by two particular events. First, in 1924 a Russian biochemist, A. I. Oparin, advanced the idea that a reduced atmosphere on the prebiotic Earth (mostly  $\text{CH}_4$ ,  $\text{CO}$ ,  $\text{NH}_3$ ,  $\text{N}_2$ ,  $\text{H}_2\text{O}$ ) was a key condition for the emergence of life. Second, in 1953 Miller and Urey showed that amino acids were easily produced in the laboratory when electric discharges (simulating lightning storms) were passed through gas mixtures resembling the presumed, reduced atmosphere of the early Earth. In the ensuing two decades laboratory experiments under plausible primitive Earth conditions have demonstrated the formations, in addition to those of most of the proteinaceous amino acids, of:

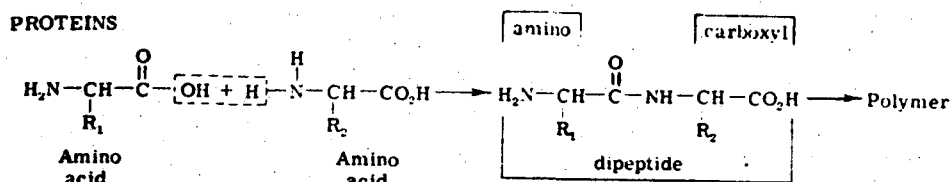
- ribose and deoxyribose (the sugar units of the nucleic acids)
- the nucleic-acid bases (adenine, guanine, cytosine, thymine, uracil)
- nucleosides (the base-sugar units of the nucleic acids)
- nucleotides (the base-sugar-phosphate units of the nucleic acids)
- fatty acids (such as acetic and lactic)
- porphyrins (the key organic grouping of heme and chlorophyll)

It is surprising and significant that these biologically key compounds are formed with this facility. It leads us to believe that our contemporary biology is based on these compounds because they were the ones selectively formed on the prebiotic Earth. We may also comment that the production of these compounds should have exceeded their destruction because of adsorption on the interstices of clay particles, protection from ultraviolet light by the overlaying waters of lakes and shallow seas, and transportation by rivers and oceans from locales of synthesis to locales of further reaction. In addition, it should be pointed out that recent findings of the radioastronomers have shown that our surrounding interstellar space is a vast reservoir of biologically important organic compounds.<sup>3</sup> It is quite possible that our planet, as it was being formed, was accumulating all the necessary biomonomers for the subsequent "chemical-evolutionary" pathways towards life.

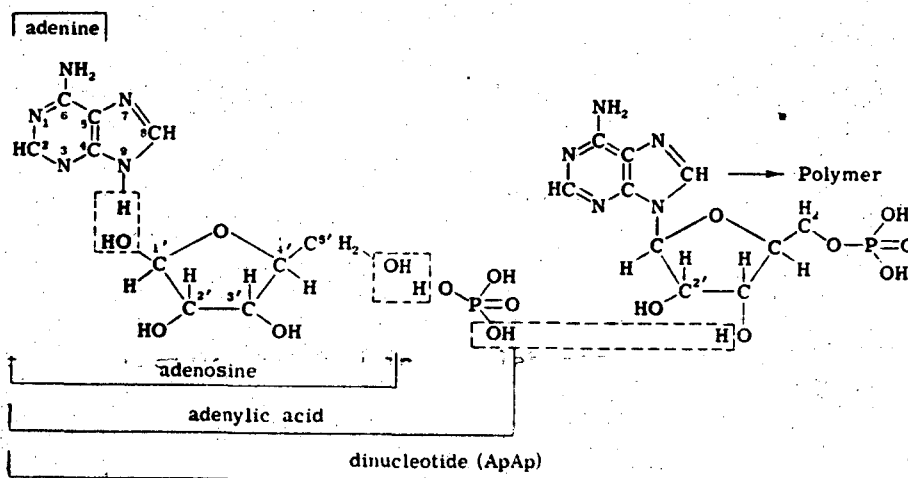
What now particularly interests students of chemical evolution, and is the subject of this report, are the possible routes by which the primitive Earth's biomonomers were joined to become biopolymers.

## Formation of the Biopolymers

Let us first consider the formation of the supremely important biopolymers, the proteins and the nucleic acids. For these to be formed from their monomers, the amino acids and the nucleotides, the elements of water must be removed. Additionally, the nucleotides are formed from three units, a base, sugar, and phosphate--again water must be removed in order that the nucleotide be formed. These water removals are illustrated by the dotted rectangles in the illustration below (taken from ref. 4).



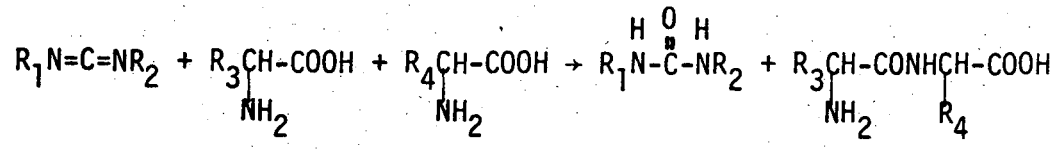
**NUCLEIC ACIDS (3 stages) RNA shown - DNA lacks OH on 2' position**



The necessary removal of water during the formation of the biopolymers has been, and is, a major problem in the study of chemical evolution. The trouble is that if the chemist wishes to remove the elements of water from two organic molecules he will not choose water as his solvent. Simple considerations of the equilibrium concentrations of reactants and products (often expressed as the "Le Chatelier principle") indicates that the absence of water will promote the biopolymer formation. In chemical evolution research, however, there is a widespread notion that the biologically-relevant chemistry of the prebiotic Earth took place in aqueous media. This notion is based, first of all, on paleontology, which strongly indicates that life arose in the seas. In addition, the bottoms of lakes and shallow seas appear the best locales for chemical-evolutionary processes. There, the biomonomers would be protected from the destructive effects of ultraviolet radiation; in addition, the water would serve as a vehicle to bring reactants together. Although there is reason to believe that some key biopolymer formations may have taken place at relatively dry environments (as will be discussed below), we shall first review the investigations into plausible ways in which the biopolymers might have been formed in aqueous environments.

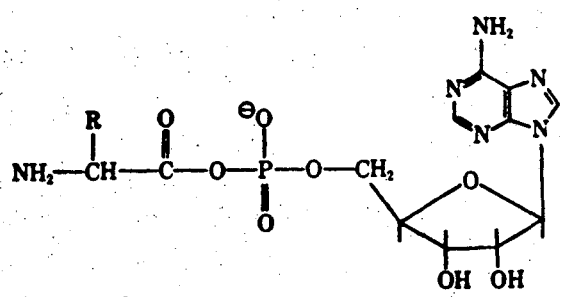
Formation of Peptides (Aqueous Environment). Some progress has been achieved in forming peptides in dilute aqueous solution at room temperature. This has been done by using simple high-energy analogs of the carbodiimides ( $\text{RN}=\text{C}=\text{NR}'$ ), reagents that have been used for some time to promote dehydration condensations in nonaqueous solvents. One example is cyanamide,  $\text{H}_2\text{N}-\text{C}\equiv\text{N}$ , a known "primitive Earth" compound, that is, one that

appears on irradiation of  $\text{CH}_4\text{-NH}_3\text{-H}_2\text{O}$  mixtures. It is a tautomer of the unstable parent of the carbodiimides series ( $\text{HN=C=NH}$ ), and it has been used to prepare simple di- and tetra-peptides.<sup>5</sup> The carbodiimide reagent can selectively remove H and OH from two amino acids, even in the presence of water, to form a peptide bond (the carbodiimide becoming a substituted urea):

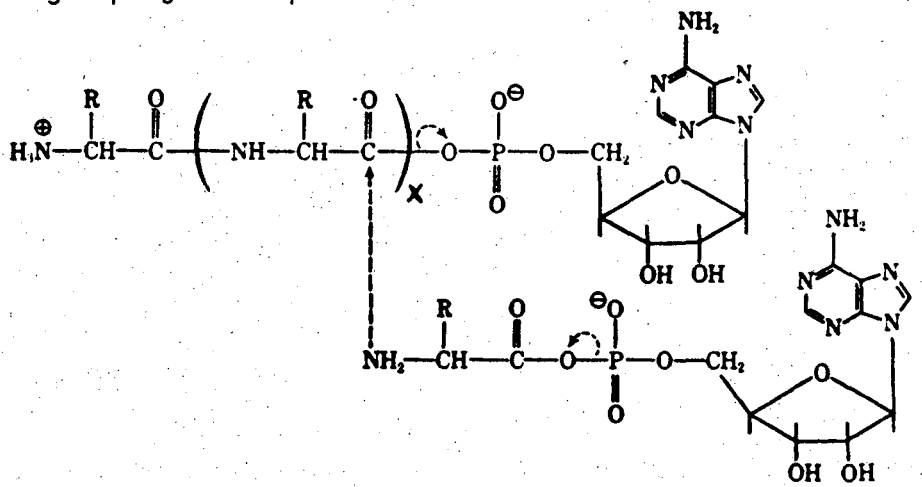


It has also been shown that such condensations may be promoted by the presence of natural clay-mineral surfaces; the increased peptide formation appears to be the result of concentration effects on the surfaces. Another interesting result is that dicyanamide shows considerable selectivity in dipeptide formation--those peptide bonds that are the most common in contemporary proteins (e.g., glycine-glycine) are the ones most easily formed by dicyanamide.

Another interesting, "chemical-evolutionary" way to form peptides in a watery environment has been demonstrated by scientists at the Weizmann Institute in Israel.<sup>6</sup> They made use of amino-acid adenylates, whose general formula is:



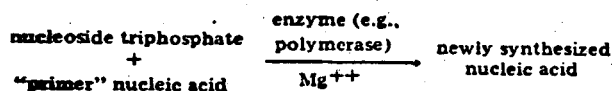
In these compounds the amino acid is attached to a phosphate group of a nucleotide (in the above example, adenylic acid, exactly as it is in transfer RNA). That is, these adenylates are just the compounds employed in the protein building processes of contemporary living cells. The Israeli group found that in water solution, and in the presence of a clay mineral (montmorillonite), the adenylates gave polypeptides with up to approximately 50 amino acid units. What happens is that the amino-acid  $\text{NH}_2$  group of one adenylate attacks the acyl group of another, with the simultaneous release of adenylic acid, to give a dipeptide coupled to one adenylic acid. It is the repetition of this process that gives one, finally, several polypeptides; these may either be free or attached to one adenylic acid grouping. The process is shown below in a sketch adopted from reference 6:



A number of discrete molecular-weight groupings were found; probably this is related to some not yet understood feature of the mineral surface.

Formation of Polynucleotides (Aqueous Environment). The monomeric unit of the nucleic acids, the nucleotides (adenylic acid in the example shown earlier in this report) consists of a nucleic-acid base (e.g., adenine), a sugar (e.g., ribose), and a phosphate group. The bases and sugars are known products of chemical-evolutionary experiments, and in the presence of water the predominant chemical form of phosphorus is as phosphate. The cyanamide reagents have also been used to form, in dilute aqueous solution at room temperature, the necessary base-sugar and sugar-phosphate bonds. Consequently, the nucleotides would have accumulated on the prebiological Earth. What progress has been made in understanding possible routes for their prebiotic polymerizations?

The first research that should be mentioned here is that by Goulian, Kornberg, and Sinsheimer.<sup>8</sup> They showed that the following transformation will take place:



If the "primer" is left out of the reaction mixture, nucleic acid synthesis still occurs, but at a slower rate. The nucleoside phosphates (including triphosphates such as ATP) have been synthesized under more or less prebiotic-Earth conditions, and magnesium ion has always been abundant. Although the above reaction will not take place at any measurable rate in the absence of enzyme, perhaps some day a chemical-evolutionary catalyst (such as the proteinoids or microspheres mentioned above) may be found that can be substituted for the enzyme.

In more typical chemical evolution experiments, Sulston et al. have shown that carbodiimides induce in dilute water solution (about 0.01 M in all reactants), at room temperature, the formation of di- and trinucleotides from mixtures of nucleosides and nucleotides.<sup>9</sup> The yields are quite good--a few percent. Furthermore, these reactions are aided by the presence of synthetic polynucleotides, and the Watson-Crick pairing rules seem to apply. For example, the adenosine-adenylic acid condensations are aided by the presence of polyuridylic acid, and the guanosine-guanylic acid condensations are aided by polycytidylic acid. Although unnatural (2'-5') as well as the natural (3'-5') sugar linkages are formed, this work demonstrates a likely route for polynucleotide, or nucleic acid, synthesis on the prebiological Earth.

It has also been shown that imidazole, the five-membered ring that is present in the amino acid, histidine, and in the nucleic-acid purines, is a catalyst for the oligomerization of the mononucleotide, thymidylic acid.<sup>10</sup> The same catalyst, which forms in chemical-evolution experiments (from HCHO and ammonia), has also been shown to promote the formation of peptides from amino acids.<sup>11</sup>

The intimate association of the proteins (enzymes) and the nucleic acids in the living cells' reproductive processes leads to the belief that these biopolymers were closely associated together in their syntheses in the prebiotic world. Perhaps they served as cross-catalysts: polypeptides catalyzing polynucleotide synthesis, and vice versa. In our laboratory we have recently developed a system wherein oligopeptides and oligonucleotides appear simultaneously.<sup>12</sup> We were led to this system by the observations reported from other laboratories, and cited above, that imidazole is a catalyst in separate peptide- and oligonucleotide-forming systems. We have found small yields (a few percent) of both alanine oligomers and adenylic-acid oligomers from an aqueous solution of alanine, ATP, imidazole, and MgCl<sub>2</sub>. The solution was heated for about four hours at 60-80° until a thick, clear gel was formed. The presence of the oligomers was established by a combination of ion-exchange, paper, and gas-liquid chromatographies. The temperature employed, and the need to form the gel, make this experiment less than ideal from the chemical-evolutionary viewpoint. However, we believe that, in time, suitable surface catalysts will be found so that simultaneous polypeptide and polynucleotide formation may be demonstrated in dilute solution at room temperature.

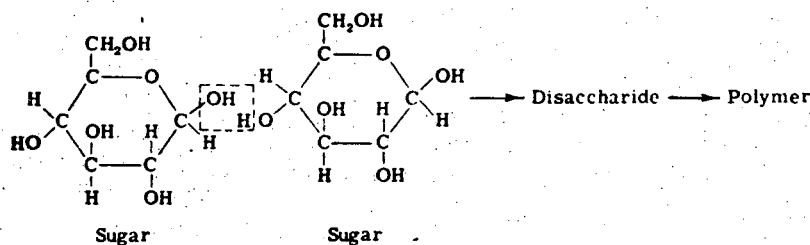
0 0 0 0 4 3 0 4 9 1 7

Formation of Peptides (Non-aqueous Environment). Here we need to comment that, even granting that the first living cells probably appeared in the water, key biopolymer-forming steps might have been taken in a relatively non-aqueous environment. Consider the following possible scene on the prebiotic Earth: at high tide, ocean waves deposit their solution of dissolved amino acids in pools on a shore where there is geothermal activity. The tide then recedes, the water evaporates, and the amino acid deposit heats up to 100°C or above. The amino acids are converted to protein-like polymers. The tide comes in again, the waves dissolve the polymers and/or cause them to be adsorbed on clay particles--in either case, the stage is set for further chemical evolutionary progress.

In just this way, by heating mixtures of dry amino acids, protein-like polymers called "proteinoids", have been formed by S. W. Fox and his collaborators.<sup>7</sup> These polymers show many of the properties of biological proteins--for example, peptide (or natural protein) bonds, molecular weights in the thousands, hydrolysis by proteolytic enzymes, and some enzyme-like activity. The proteinoids also show selectivities with respect to which amino acids are predominantly incorporated, and toward the production of favored molecular-weight classes. In addition, they display some interesting self aggregation properties; these are described below under "Biopolymer Structures and Aggregations".

Formation of Polynucleotides (Non-aqueous Environment). Little work has been done, and little success attained, in inducing the formation, under dry conditions, of polynucleotides from the mononucleotides. However, some interesting results have been obtained in work with nucleoside cyclic 2',3'-phosphates. First, it has been shown that these compounds could have been formed under possible prebiotic conditions; for example, the heating of uridine (100° for 24 hrs) with  $\text{NH}_4\text{H}_2\text{PO}_4$  and urea gives a 37% yield of the uridine cyclic 2',3'-phosphate. Second, it has been observed that, on heating (25-85°C) in the presence of amine catalysts, these cyclic phosphates give yields of up to 25% of oligomers (mostly dimer).<sup>13</sup> Perhaps, like the peptides, dry conditions may have contributed to, or even been necessary for, the appearance of the first polynucleotides on the prebiotic Earth.

Formation of the Polysaccharides. As in the cases of peptide and polynucleotide formations, water must be removed from the monomeric unit (e.g., glucose) to form the polymer (e.g., starch):



As yet no "prebiotic-Earth" experiment has demonstrated the formation of polysaccharides from sugars in aqueous solution. However, as anyone who has prepared caramel knows, the sugars polymerize rather easily on application of mild heat. Extensive research has been done on the variety of polysaccharides that are formed on the mild (ca. 100°C) heat treatment of glucose and other sugars.<sup>14</sup> With respect to the monomeric units, the sugars themselves, it is easy to see how they were formed in prebiological times. More than a century ago the Russian chemist Butlerov showed that formaldehyde (one of the products formed in "primitive Earth" atmospheres) condenses easily in dilute aqueous alkali to a complex mixture of sugars. Consequently, the resultant appearance of the polysaccharides on the prebiological Earth is easy to visualize.

Formation of Fats. The fatty (also called aliphatic or carboxylic) acids is another class of biopolymers whose appearance on the prebiological Earth was likely. It has been demonstrated that a wide variety of hydrocarbons, with molecular weights in the hundreds,



are formed on the passage of ionizing radiation through methane. It has also been shown that ionizing radiation causes the direct addition of  $\text{CO}_2$  to a hydrocarbon to form the corresponding fatty acid ( $\text{RH} + \text{CO}_2 \rightsquigarrow \text{RCO}_2\text{H}$ ) and to amines to form amino acids. In view of these results, we should remark here that, even in the predominantly-reduced atmosphere of the prebiotic Earth, the presence of some  $\text{CO}_2$  would be expected.

It has also been demonstrated that exposure of methane and water to a semicorona discharge results in the formation of monocarboxylic acids from  $\text{C}_2$  to  $\text{C}_{12}$ .<sup>15</sup> Products identified were acetic, propionic, butyric, isobutyric, valeric, isovaleric, and isocaproic acids; mass spectroscopic evidence indicated that the  $\text{C}_6$ - $\text{C}_{12}$  acids were predominantly branched-chain.

Since the fats are esters of glycerol, it should be mentioned that glycerol is a minor product of the Butlerov reaction referred to above.<sup>16</sup> The synthesis of fatty acids and of glycerol under conditions that probably prevailed on the prebiological Earth leads us to believe that there was a steady production of fats at that time.

### Biopolymer Structures and Aggregations

The preceding discussions indicate that there is some understanding of how the biopolymers may have accumulated on the prebiological Earth. However, the biopolymers in our contemporary biology have precise structures, or conformations, and they aggregate in rather precise ways. Recent research in biochemistry and molecular biology strongly supports the notion that these structures and aggregations are the expected result of the intrinsic characteristics of the biopolymers themselves.

If we think of the successive attaching together of, for example, amino acids under prebiological Earth conditions, we see that the end result might be a simple random coil. Its only structure (the "primary" structure of the biochemist) would be that of its individual constituent amino acids. In contrast, natural proteins, including the enzymes, have an ordered three-dimensional structure or conformation. The principal manifestation of this structure is the helical form that the proteins assume; it is an example of "secondary" structure. In addition, there is a "tertiary" structure, a regular folding of protein helices back upon themselves.

The helical, secondary structure is held together principally by hydrogen bonds, and this structure may be disrupted by a change in external conditions, such as the temperature or the pH of the protein's solution. However, such changes, or loss of structure, are reversible; a restoration of the original temperature or pH will restore the helix. It is also of note that polymerization studies have shown that the rate of lengthening of a polypeptide chain increases as soon as a helical configuration is reached.<sup>17</sup> In other words, a helical polypeptide acts as a kind of catalyst for the reactions that increase its own chain length. It is also known that we can partially disrupt and reform a hydrogen-bonded tertiary structure while leaving the secondary structure essentially intact. These changes can be followed by changes in various protein spectra (ultraviolet absorption, nuclear magnetic resonance, circular dichroism, and optical rotatory dispersion). For elaborations of these techniques for determining protein structure the reader is referred to a recent discussion by Mahler and Cordes.<sup>18</sup>

The need for precise shapes (conformations) of proteins in contemporary biology is most apparent in the mechanisms of enzyme actions. The enzymatic proteins must have precise conformations in order that reactive centers are in precise juxtapositions. This is necessary in order that the substrate molecule "fits" perfectly onto the enzyme's reactive site. Losses of enzymatic activity has been well correlated with losses of the particular shapes of enzymes. It is quite sure that our life would not be possible without the existence of a high degree of spatial organization in proteins.

Since our biological proteins have an inbuilt tendency to regain a disrupted conformation, we have reason to believe that an abiogenetically produced, random-coil polypeptide, or protein, would do the same thing. The appearance on the prebiological

Earth of proteins with many of the same properties, including the conformations, of contemporary proteins, appears likely, if not inevitable.

Much of the same may be said about the nucleic acids. Their conformations are necessary for replication, that is to say, for the transmission of genetic information. The nucleic acid change from a random coil to the helical structure may also be followed both by spectral changes and by altered behavior during centrifugation. The random coil-helix changes are reversible; again we are led to the supposition that, on the prebiotic Earth, polynucleotides would tend spontaneously to assume the conformations that we find essential for contemporary life.

The living cell is an extraordinarily complicated assemblage of biopolymers and metabolites. Merely to say that we can envisage the formation of the biopolymers and of many metabolites on the prebiological Earth is a long way from saying that we understand how the first living cell was assembled. Yet there are some intriguing hints. For example, the "proteinoids" mentioned above show a surprising tendency to aggregate into forms that are curiously similar to the gross morphology of the living cell. This effect is manifest when proteinoids are dissolved in warm water, and the solution then allowed to cool. When the resultant solution (and suspension) is viewed under the microscope, it is seen to contain a great number of small globules, or "microspheres". These are fairly uniformly sized, about 2 microns in diameter (in the size range of many living cells). They have internal substructure, or compartmentalization, and they have a double-walled outer boundary or membrane. They even show a kind of growth by accretion from the solution, followed by the development of spherical protuberances that break off and form new microspheres. Many other properties suggestive of living cells have been observed (staining characteristics, catalytic activity, and motility). In the formation and morphology of the microspheres we appear to be seeing in organic polymers an intrinsic tendency to aggregate and form detailed structures.

This same tendency is seen between nucleic acids and proteins. This has been dramatically shown in the spontaneous partial reassembly of viruses (including a return of infectivity) that have been disrupted into their nucleic acid and protein constituents.<sup>19</sup> Since viruses are usually considered to be near the center of the living-nonliving continuum, these results are particularly important for chemical evolution.

The emergence of life undoubtedly depended on the intrinsic properties of the biopolymers that caused them to "shape up" and to form orderly aggregations.

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