

# CHAPTER 4

## ONCE UPON A TIME THERE WAS A MOLECULE...

*“Primordial soup again?”*

— caveman to wife in a Gary Larson cartoon

*“I have made it my rule not to read this literature on pre-biotic evolution until someone comes up with a recipe that says ‘Do this and do that, and in three months things will crawl in there’”.*

Max Delbruck<sup>1</sup>

The explosive increase in understanding living matter directs our attention forward, toward the utilization of this great body of knowledge for curing mankind’s illnesses. At times, though, scientists glance backwards to gain a perspective on the present, and to ask, “How did this rich ecosystem of Earth come into existence?” The technical details of the answer vary from year to year, but the big picture has not changed for many decades. It is basically as follows.

The Earth formed from a spinning mass of gaseous matter — the “solar nebula” — by a process of accretion. Once this primitive, lifeless planet cooled down it was largely covered by an ocean. Its atmosphere contained nitrogen and carbon-containing substances, but was devoid of oxygen. Then a process called chemical evolution began on this primordial Earth. It resulted in the emergence of the first living cell and initiated biological evolution.

A recent text of biochemistry states these concepts succinctly:

*The origin of life probably occurred in three overlapping phases: Phase I, chemical evolution, involved the non-instructed synthesis of biological macromolecules. In phase II, biological macromolecules self-organized into systems that could reproduce. In phase III, organisms evolved from simple genetic systems to complex multicellular organisms.<sup>2</sup>*

Undergirding this scenario of life’s origin is an unstated, but very firm, conviction that under conditions where living organisms can exist, nature will bring life into existence. This notion arises from two indis-

putable facts: 1) on planet Earth conditions are favorable for life, and 2) our planet is teeming with a multitude of living organisms. Despite the circularity of this argument, if one *a priori* discounts the possible existence of a supernatural Creator, all that remain are naturalistic scenarios for the origin of life.<sup>3</sup> What follows here is a look at laboratory experiments and theoretical considerations that have been produced in support of evolutionary models.

Scientists are at their best when they study matter under well-defined conditions. While it is true that the time scope of chemical evolutionary postulates covers thousands or even millions of years (time segments unavailable for individuals), **the only function of time in these postulates is to increase the number of opportunities for interaction between substances.** Scientists can accomplish this in the laboratory by various means that compress the time scale of the chemical evolutionary events to manageable size.

We ought to be thankful for the efforts of stalwart chemists who have dared to test the validity of their postulates in the laboratory. Their work gives us a feel for the experimental challenges that confront chemical evolutionists. They are following standard practice in science, in contrast to some in a field of chemical evolution who appear content issuing theories, without a scintilla of experimental evidence.<sup>4</sup>

During the 1960s and 1970s, considerable experimental work was done on the behavior of matter under postulated primordial conditions.<sup>5</sup> These conditions were very diverse because a “primordial world” could be anything that was sterile and devoid of oxygen. The experiments were conceived to test the validity of the following theoretical transformation of matter on the primordial Earth:<sup>6</sup>

Stage 1 (early Earth atmosphere) → Stage 2 (hot dilute soup)  
→ Stage 3 (polymerization) → Stage 4 (protocell formation)  
Stage 5 (true cell formation).

### **CONSIDERATIONS OF THE EARLY EARTH'S ATMOSPHERE AND THE FORMATION OF THE PRIMORDIAL SOUP**

Reduced atmospheric carbon dioxide is the only potential source for the production of biologically relevant organic substances. The current atmosphere, which contains 20% oxygen, is very oxidizing. Under synthetic conditions with oxygen present, carbon dioxide yields

only biologically irrelevant materials. Moreover, in the presence of oxygen biologically relevant substances degrade.

Therefore it is axiomatic to any chemical evolutionary scenario that the primordial atmosphere was free of oxygen. Experiments with gaseous mixtures have varied from methane-ammonia-water (most reduced) to carbon dioxide-nitrogen-water (most oxidized).

The classic experiments of Stanley L. Miller, in the laboratory of Harold C. Urey,<sup>7</sup> circulated the gases methane, ammonia, and hydrogen in a closed system. The apparatus also included boiling a mixture of water and electrodes for spark discharge that simulated lightning. After a week, four of the twenty amino acids found in proteins were produced, along with other organic substances. Later this experiment was repeated using nitrogen and a trace amount of ammonia. With this gas combination, ten different amino acids were made along with other nonbiological amino acids and organic substances.

The Miller experiment was modified by other investigators, who used heat, ultraviolet radiation, and acoustic shock waves in place of sparks as an energy source. Additional gases — such as ethane, hydrogen sulfide, carbon monoxide, or hydrogen cyanide — were added. These efforts resulted in the production of most of the amino acids, except histidine, arginine and lysine.<sup>8</sup>

The synthesis of adenine, one of two purine components of nucleic acids (DNA, RNA), was reported from high concentrations of hydrogen cyanide in the presence of large amounts of ammonia.<sup>9</sup> Prebiotic scenarios that contain large concentrations of ammonia and hydrogen cyanide are difficult to postulate. Adenine can be formed from hydrogen cyanide, without ammonia, in the presence of sunlight.<sup>10</sup> The production of guanine — the second purine — under prebiotic conditions is less well-studied. However, it is known that guanine can be produced by reacting one of the intermediates of adenine synthesis with cyanogen, a substance derived from hydrogen cyanide.<sup>11</sup> The three pyrimidines needed for nucleic acid synthesis — uracil,<sup>12</sup> cytosine<sup>13</sup> and thymine — were synthesized by reacting selected substances (which could have been potentially available) under prebiotic conditions.

Another important class of ingredients — sugars — could have been produced in a primordial environment by the reaction of formaldehyde with alkaline substances.<sup>15</sup> Two types of sugars, ribose and 2-deoxyribose, are essential for building nucleic acids. These substances link up

with purines or pyrimidines and a phosphate to form nucleotides, which are the building blocks of nucleic acids (Table 2.2). The chemical linkage between sugars and a purine or pyrimidine occurs with the loss of two hydrogen and an oxygen, the equivalent of water. This process may not appear improbable, but thus far it has not been possible to achieve in a simulated primordial environment.<sup>16</sup>

In addition to amino acids, there has been successful laboratory synthesis of purines and sugars, short chain fatty acids, fatty alcohols and di- and tricarboxylic acids under simulated primordial earth conditions.<sup>17</sup> These achievements supported the postulate that necessary organic substances were collected in a primitive ocean, forming a “primordial broth”. Initially scientists estimated that about 3% of the primordial ocean by weight could have consisted of organic substances.<sup>18</sup> This estimate was made before the realization that on a prebiotic earth several factors would reduce the amounts of organic matter in the ocean. The very sources of energy that created organic compounds would also destroy some. In addition, other chemical interactions including water’s destructive effect would have diminished the amounts of organics in the primordial ocean by a factor of ten thousand less than originally proposed. This is the level we actually find today in the North Atlantic Ocean!<sup>19</sup> There is no geological evidence available for the existence of a primordial soup either worldwide or in smaller locations.<sup>20</sup>

Oxygen has been excluded from all prebiotic experiments, because its presence precludes the production of biologically useful substances. Yet no prebiotic scenario can rule out the presence of oxygen. High-energy ultraviolet light, which is now filtered out by a layer of ozone (a very reactive form of oxygen gas) high in the atmosphere, is capable of degrading water to oxygen and hydrogen. Hydrogen escapes from the atmosphere, whereas oxygen remains. On ultraviolet photographs of Earth taken from the Moon during the Apollo-16 mission, there is a large cloud of atomic hydrogen enveloping our planet, extending outward some 40,000 miles. The only conceivable source of this hydrogen is the extensive photodissociation of water vapor above the ozone layer.<sup>21</sup>

Thus laboratory simulations of the primordial production of biomonomers leave substantial unresolved problems that in any other field of science would result in the dismissal of the underlying theory. In this case, however, the attitude is: “since we know that chemical

evolution is true (we are here after all!), we just have to keep on looking for answers”.

An ingenious solution to a seemingly impossible predicament is the notion that many of the required biomonomers were delivered to Earth by interstellar dust, meteorites and comets.<sup>22</sup> This mechanism provides, in theory, unlimited amounts of starting material for chemical evolution to proceed.

## POLYMERIZATION

Although there are some positive laboratory results showing how biomonomers may have arisen in a primordial setting, there is an almost complete meltdown in experimental efforts to show how biopolymers may be formed. If one stipulates the availability of an unlimited supply of primordial biomonomers, three major obstacles surface on the way toward producing proteins and nucleic acids. (The other two classes of biopolymers — polysaccharides and lipids — are not considered here, because they could be formed enzymatically if the correct proteins were available.)

The obstacles to forming proteins and nucleic acids are:

- 1) How do amino acids and nucleotides link up into proteins and nucleic acids in an aqueous environment, when the linkages involve the **loss of water**?
- 2) In the process of joining biomonomers together, how are only the “left-handed” amino acids selected for proteins and the “right-handed” sugars for nucleic acids, when at the start there is an equal mixture of both?<sup>23</sup>
- 3) How are the amino acids and nucleotides arranged in meaningful sequences?

Our biotechnology is at such a level now that we can manufacture protein and nucleic-acid fragments at will in the laboratory. These processes involve chemical activation of the linkage groups of the building-block substances, meanwhile protecting the rest of the molecule from participating in the linkup. The joining of these modified building blocks occurs in the total absence of water.<sup>24</sup> The order of amino acids and nucleotides is determined by the experimenter.

Formation of a peptide bond (see Table 2.2) between two amino acids is not favored thermodynamically. [It has been calculated that if

one started with a very concentrated solution of amino acids (a concentration of “one mole per liter”), it would be necessary to have a volume  $10^{50}$  times the Earth, in order to form spontaneously a single protein molecule, 100 amino acids long<sup>25</sup>!

Therefore it is not reasonable to suppose that amino acids would ever link up into chains while in the primordial soup. Heating pure solutions of amino acids to 200°C for 6-7 hours has led to the formation of random protein-like polymers.<sup>26</sup> But many of the peptide bonds between amino acids were unnatural in these “proteinoids”, and the sequence of amino acids reflected the composition of the initial mixture. Moreover, no suggestion exists to explain how a catalytically active proteinoid could be reproduced. Most recently it was shown that up to six residues of the amino acid glycine could be linked under high pressure in a simulated thermal vent.<sup>27</sup> The authors offer a reasonable theory that the chain elongation occurs through a cyclic intermediate (diketopiperazine). However, this mechanism implies that other amino acids may not be able to elongate, because their structures prevent the formation of this cyclic intermediate.

Biomonomers (amino acids or nucleotides) may be linked to each other in the presence of a chemical condensing agent which traps the water molecules that are split out between the monomers. But in an aqueous environment these agents will interact preferentially with the large amount of water in the environment. Thus, condensing materials work only in nonaqueous environments.

The other two grave difficulties with primordial polymer formation — the exclusive use of only “left-handed” or “right-handed” monomers for protein or nucleic-acid synthesis, and the source of information that resides in the sequences of biomonomers in proteins and nucleic acids — have not been satisfactorily addressed.

The synthesis of either proteins or nucleic acids under prebiotic conditions has yet to be accomplished. These necessary processes can be considered the end of the chemical evolutionary road paved only in scattered patches with experimental results. If one wishes to proceed beyond, it is necessary to traverse on the rocky terrain which consists mostly of speculations.

### **PROTO CELL AND TRUE CELL FORMATION**

Using Zubay’s terminology, it is envisioned that the first phase of chemical evolution consisted of the “non-instructed” phase,<sup>2</sup> where

the emphasis was on the synthesis of random polymers of either proteins or nucleic acids, or both. Current thinking is leaning toward a random synthesis of ribonucleic acid, because it was discovered recently that some ribonucleic acids have catalytic activity.<sup>28</sup> This discovery led to the speculation that the first biopolymers had both catalytic and genetic capacities.

The second phase of chemical evolution is the “instructional phase”, where macromolecules would self-organize into autocatalytic systems. That is, self-replicating systems would form from mixtures of random RNA or protein molecules. Somehow such systems would be wrapped into protective membranes, forming “protocells”, the precursors of true cells.

Experimental models of protocells include coacervates (microscopic droplets) of proteins and nucleic acids,<sup>29</sup> proteinaceous microspheres,<sup>30</sup> and lipid vesicles.<sup>31</sup> These structures have been synthesized in the laboratory from preformed biopolymers, or from protein-like substances that were obtained by heating pure amino acids at high temperatures. Without entering into detailed considerations of each, it can be said that none of these complexes manifest the essential qualities of living cells. They were aggregates of polymers, predictably held together by physical forces. As such, they represent experimental dead-ends, without any promise of shedding light on the mysteries of abiogenesis.

But having come this far in our hypothetical journey on a primordial Earth, it seems a pity to stop. If self-replicating systems of proteins and nucleic acids could be found, could these serve as precursors to modern cells? Evolutionary theoreticians posit that the Darwinian “descent with modification”, along with “survival of the fittest”, concept of biological evolution may have been at work even during chemical evolution.<sup>32,33</sup> Thus, through a process of continual modification, different biopolymers would have been formed, and those with useful properties would have been retained.

There is an enormous amount of information stored in the structures of nucleic acids and proteins in modern cells. It has been estimated that one cubic micrometer (one thousand billionth of a cubic centimeter) of deoxyribonucleic acid (DNA) encodes 150 megabytes of information. This is more than an order of magnitude greater than the current CD-ROM optical storage density.<sup>34</sup> The complete nucleotide sequence of the genetic material of *Escherichia coli*, printed in a standard book

form, takes up about 1,100 pages. A similar effort for the human genome would fill one thousand volumes of 1,100 pages each.

The rules of grammar define the correct spelling of words and the proper usage of each word in a sentence. **They do not determine the choices of words.** Similarly, although the rules of chemistry determine how biomonomers may be linked into polymers, they are silent on the order in which these should be linked so as to have biological significance.

The question is, by what processes would self-replicating systems of proteins and nucleic acids select for and accumulate biologically valuable polymers? In abiotic systems **no selection pressure exists in favor of biologically useful polymers!** The potential usefulness of biopolymers is manifested only in living cells. In nonliving matter the biological potential of molecules is of no consequence. This point is generally ignored by chemical evolutionary theoreticians. A salient example is the “obcell” hypothesis by T. Cavalier-Smith. He proposes that in the prebiotic era, membrane-protein complexes known as “inside-out cells” (or “obcells”) formed as precursors of true cells. These structures contained transport proteins, as well as nucleic-acid replication machinery and ribosomes,<sup>35</sup> and somehow had the ability to harness light energy. He gives no indications whether his “obcell” is alive or dead.

Albert Lehninger, however, clearly states that the hypothetical protocells should have been alive:

*...the first structure possessing ‘life’ was not necessarily a modern cell, complete with a membrane, a chromosome, ribosomes, enzymes, a metabolism, and the property of self-replication. The minimum requirement is that it could potentially evolve into a complete cell.*<sup>36</sup>

If a cell possessed life, then its molecular components had to be in steady state non-equilibria. Such a state could be maintained only if the individual chemical reactions in the protocell were kept from reaching equilibrium. In modern cells this is accomplished by the linkages of chemical reactions into biochemical pathways and by coordinating the chemical activities of pathways through regulation of key enzymes. **Since protocells were supposed to have risen from random encapsulation of compounds and their catalysts, it would follow that their chemical reactions were neither linked into pathways nor regulated.** Thus, chemical reactions in protocells would

have been expected to reach equilibria some time after encapsulation, resulting in dead protocells.

These considerations apply to all postulates of chemical evolution, regardless whether they approach modern life from the inorganic<sup>37</sup> or from the organic world,<sup>4</sup> whether the first postulated biopolymers were RNA<sup>38</sup> or proteins,<sup>30</sup> and whether life supposedly evolved on this planet or somewhere in outer space.

#### SUMMARY OF CHAPTER 4

1. Theories about the abiotic origins of life have been tested extensively in the laboratory.
2. The feasibility of abiotic synthesis of many biomonomers in simulated primordial settings has been demonstrated.
3. Simulated primordial syntheses of functional biopolymers have been unsuccessful.
4. It has not been possible to show, even in theory, how living matter may arise from hypothetical “protocells”.

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