Integrating the Field of Prebiotic Chemistry into a Christian Worldview

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Abstract

This thesis will provide an overarching examination into the scientific field of prebiotic chemistry, the purported chemistry of the formation of life on earth. Prebiotic chemistry is a critical area of science particularly for Christian scientists to study since it is used by many evolutionists as evidence for the evolution of life, rather than the formation by an intelligent designer. Extensive literature-based research from previously published articles will be conducted on the formation of biologically necessary molecules, including amino acids. Primary focus will be placed on the formation of amino acids in a primordial earth environment, first demonstrated by the Urey-Miller experiment. The main reactants that are believed to be necessary to produce these various biomolecules were supposedly found in the earth's atmosphere, so attention will also be given to the possible theories of the atmospheric composition on an early earth. Following the literature review, experimental research will be conducted in a laboratory to attempt to produce amino acids using a similar experimental method published in 2008 by Cleaves et al. All the results produced by this experimental research will be discussed and implemented into a Christian scientific worldview.

Integrating the Field of Prebiotic Chemistry into a Christian Worldview

The origin of life debate has become a topic of great discussion among scientists from all branches, including biology, organic chemistry, and biochemistry. From an evolutionary perspective, the natural world existed before life was formed, and it is from elements of this natural world that life would eventually emerge. The formation of the earth is purportedly to have occurred about four and a half billion years ago, and life began just a mere billion years later (Wells, 2000). Amino acids are an essential part of life, as they are the basic units of proteins, which have a diverse amount of functions in all types of life. In developing a model for how life could have begun on earth, scientists had to propose what constitutes both the atmosphere of the earth and any bodies of water that would have been on earth at that time. The most infamous experiment done in this area of research was the Urey-Miller experiment (Wells, 2000). Through this experiment, amino acids were synthesized in what was believed to be the primordial environment on earth.

For Christian scientists, one of the greatest areas of needed growth is in the defense of their faith under the tremendous influence of the scientific community which pushes the agenda of the theory of evolution. By studying this area of research, Christian scientists can learn how to communicate the results from and problems with the experiment, and more importantly, how to integrate the experiment's conclusions into their worldview.

History of Prebiotic Chemistry

The question of where life began on earth has been one of great debate for scientists over the course of centuries. During the Renaissance, scientists and physicians were fascinated about the idea of the spontaneous generation of life. Before the idea of microbes and microscopes were mainstream science, scientists believed that parasites, flies, and other pests spontaneously appeared from nonliving materials (Wills & Bada, 2000). This was not the popular belief among the scientists of the day since the powerful Roman Catholic Church was against the idea of spontaneous generation (Wills & Bada, 2000). The Church's opposition to the theory was due to the belief that all of Creation was formed at one time, as described in the book of Genesis (Wills & Bada, 2000). Because of this, scientists were pressured into disproving spontaneous generation as a reasonable cause of producing living beings.

One physician that spent time disproving spontaneous generation was Francesco Redi in 1688 (Redi, 1688/1909). Redi developed an experiment to determine where maggots on decaying corpses or rotting meat came from (Wills & Bada, 2000). To the naked eye, these maggots seemed to appear spontaneously out of the meat, unlike other insects that would crawl to the meat. Redi solved this mystery by placing meat in a screened box and some other meat out in the open air (Wills & Bada, 2000). The meat in the protective box did not have maggots after a few days, as no flies were able to reach it. Redi deduced that the maggots were being left by the flies and not spontaneously forming from the meat (Redi, 1688/1909). Many more experiments were conducted like this one on a variety of organisms, but it was not until the discovery of single cell organisms and

the popular use of the microscope that the idea of spontaneous generation of life died down.

If what Redi did to disprove the theory of spontaneous generation was not enough, the famous scientist Louis Pasteur conducted a study in 1860 which dealt with the sterilization of microbes in flasks and broths. At the time, the spread of disease was not well established, and the growth of microbes was thought to come spontaneously from a life force found in the air (Wills & Bada, 2000). Pasteur wished to prove that there were no life-giving qualities in the air and that the air could not be "damaged" due to heat sterilization, as many proponents of spontaneous generation claimed (Wills & Bada, 2000). Pasteur was able to develop specialized glass flasks with very long necks and filled the flasks with a broth that was known to turn turbid with the growth of microbes (Wills & Bada, 2000). Pasteur boiled the broth in some of these flasks and left the remaining flasks with broth unboiled, as a control group. After a few days, the unboiled flasks showed growth of microbes as the broth became turbid, and the boiled flasks showed no growth, even though the boiled broth was exposed to the air through the neck of the flask (Wills & Bada, 2000). In fact, the necks were long enough to trap microbes, preventing them from entering the broth from the outside environment. This experiment had a tremendous impact on the scientific community as the idea of spontaneous generation was again denied legitimacy, and the germ theory of disease was established.

In the past two centuries, technology has advanced tremendously in the fields of microbiology, genetics, molecular biology, and biochemistry, allowing scientists to study

life at the molecular level. Incredible detail can be found in all forms of life, and with this new detail, more theories were developed about the development of life on earth.

In 1859, Charles Darwin published his infamous *Origin of Species* which gave scientists the mechanism by which complex organisms, such as humans, could have evolved from simpler one-celled organisms (Wills & Bada, 2000). The process he describes, natural selection, allows for simple organisms to form differences in some of the characteristics that they have (Darwin, 1872). These slightly different characteristics would either give the organism advantages or disadvantages in surviving over others of the same organism. This would cause the stronger organism to live over the weaker organism and thus pass its acquired characteristics to future generations (Darwin, 1872). This process must have occurred countless times for the complexity of humans to be achieved.

This new paradigm in the scientific community brought back the discussions of spontaneous generation to the forefront. If this process of natural selection were to have occurred, all living organisms must have come from a preexisting living organism that was able to reproduce and pass on its own characteristics to its offspring (Wills & Bada, 2000). The first living organism must have been formed from nonliving compounds on earth. It is from this problem that the field of prebiotic chemistry has emerged with the goal of explaining how the chemical compounds found in living organisms were formed, as well as describing how these compounds could have come together to eventually transition from a state of nonliving to living (Wills & Bada, 2000). Most of the well-

known experiments in prebiotic chemistry that have been completed revolve around the formation of amino acids from what is believed to be the atmosphere on a prebiotic earth.

Primordial Earth

For the evolutionary theory of how life developed on earth to be true, the basic components or building blocks of life would need to be synthesized during the early days on earth after its formation. The exact type of environment that the earth had at that point would be needed to form a hypothesis on the mechanism behind the formation of life. This was the goal of the Russian chemist Alexander Ivanovish Oparin, concurrently with the British scientist J. B. S. Haldane (Wills & Bada, 2000). In 1936, Oparin published his famous book *The Origin of Life*, which explained his theory of abiogenesis, or life coming from the natural, nonliving environment. Oparin (1938/1953) suggested that when carbon was first present in the atmosphere, it was in the form of reduced hydrocarbons, particularly methane. Carbon dioxide was considered to be in low concentrations and would have come from inside the earth's crust to the atmosphere (Oparin, 1938/1953). Also, nitrogen would have been in its reduced form of ammonia and formed a large portion of the atmosphere (Oparin, 1938/1953). These gases would make a good combination for a primordial atmosphere as the reduced forms of these elements are reactive and can form organic compounds with an adequate amount of energy input (Oparin, 1938/1953). Oparin (1938/1953) also suggested that the most likely place on the surface of the earth for the formation of organic molecules would be in a warm, shallow pond where gases could easily diffuse into the water, and the energy

needed for the reaction to occur would be easily accessible, possibly from electrical discharges.

Since the number of molecules and atoms that could possibly be involved with forming these organic compounds are so abundant, Oparin (1938/1953) concluded that enough organic molecules would be synthesized no matter how unlikely or unfavorable the reaction might have been. Also, the amount of time between the formation of the earth and the estimated emergence of life is incredibly large that polymers such as proteins would have been formed even though their formation would be unbelievably unfavorable without the use of functional enzymes to facilitate the reaction (Oparin, 1938/1953).

To fit Darwin's theory, Oparin (1938/1953) conjectured that before cellular life was formed, the organic compounds must have been formed first and concentrated in a small area for polymers of some of the organic molecules to form on their own. This mixture of organic molecules is referred to as a colloidal system (Wills & Bada, 2000). A colloidal system is a mixture of small particles homogenously mixed within another substance, which cannot be separated easily (Wills & Bada, 2000). An example of a colloid system is homogenized milk, and the mixture of organic molecules that Oparin pictured would have had a cloudy appearance like the milk (Wills & Bada, 2000). Oparin also gave these mixtures the name *coacervates* (Wills & Bada, 2000). These coacervates would have looked like a simpler cell with no organelles and a membrane-like boundary (Wills & Bada, 2000). Some of these would have formed stronger boundaries than others which would have allowed them to last longer in the environment and to presumably

survive until a means of replication was developed to preserve the structures of its components such as proteins and nucleic acid structures (Wills & Bada, 2000). Once a system of replication for the cell was developed, Darwin's theory of natural selection would then have implications on the development of future organisms.

The First Experiment

Based on the hypothesis on how the earth existed when life began, two scientists began making strides to prove this theory by developing an empirical experiment that would mimic the conditions on a prebiotic earth. At the University of Chicago chemistry laboratory, Harold Urey was a professor of chemistry and had an ambitious graduate student, Stanley Miller. Both researchers wished to continue their study of Oparin's conjectures into a laboratory setting by attempting to synthesize organic compounds found in living organisms using the supposed atmospheric conditions.

To accomplish this goal, Urey and Miller constructed an apparatus that would hold the gases, as well as a flask with boiling water that would allow the water vapor to circulate in a loop and be condensed back into liquid (Miller, 1953). Also, they included two electrodes in the apparatus that would carry an electrical discharge to form free radicals from the gases, which was to simulate possible electrical discharges happening on a primordial earth (Miller, 1953). After all the air in the apparatus was evacuated using a vacuum, 200 mL of water was placed in the flask, with 100 torr of hydrogen gas (H_2), 200 torr of methane (CH_4), and 200 torr of ammonia (NH_3) (Miller, 1953). The electrical discharge was run for a week with the circulation of water vapor (Miller, 1953).

Running the apparatus caused the water in the flask to turn a turbid, red color, which was ascribed to the formation of organic compounds that adsorbed with the silica from the glass reactor (Miller, 1953). The compounds were then concentrated using a series of reagents including barium hydroxide ($Ba(OH)_2$) and sulfuric acid (H_2SO_4). Also, mercury (II) chloride ($HgCl_2$) was added after the run to prevent any living organisms from growing in the water (Miller, 1953).

The products formed from the reaction were analyzed using two-dimensional paper chromatography. This technique requires that a sample of a mixture of compounds be spotted onto a piece of paper. This paper is then placed in a jar filled with a small amount of a solvent that run up the paper to the other end. Once the solvent front is close to the top of the paper, the paper is removed, turned 90 degrees, and placed in another jar with a different solvent. The paper is removed after the solvent front reaches the top, and any compounds that were separated can be visualized. Ninhydrin is specifically added to visualize amino acids. Urey and Miller used a n-butanol-acetic acid-water solution for one solvent and a phenol solution for the other (Miller, 1953). The amino acids that were produced in the experiment were visualized using ninhydrin, and the Rf values were calculated from the chromatogram, as seen in Figure 1 (Miller, 1953). A Rf value is the ratio of the distance the sample travelled over the distance the solvent front travelled.

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Figure 1. Urey and Miller's TLC of the amino acids produced in their apparatus (Miller, 1953)

The amino acids that were produced were determined by comparing the Rf values from the sample to known Rf values for amino acids using those particular solvents. These included glycine, α-alanine, β-alanine (Figure 2), aspartic acid, and α-amino-n-butyric acid (Miller, 1953). The total amount of amino acids produced was around 1 mg, which is very small for the amount of energy that was used to produce constant electrical discharges for a week (Miller, 1953). To the many evolutionary scientists that hold to life being formed on a primordial earth, this experiment seemed to provide a huge leap into confirming their beliefs, but as scientists continued to work in this field, ideas on what a prebiotic earth looked like changed.

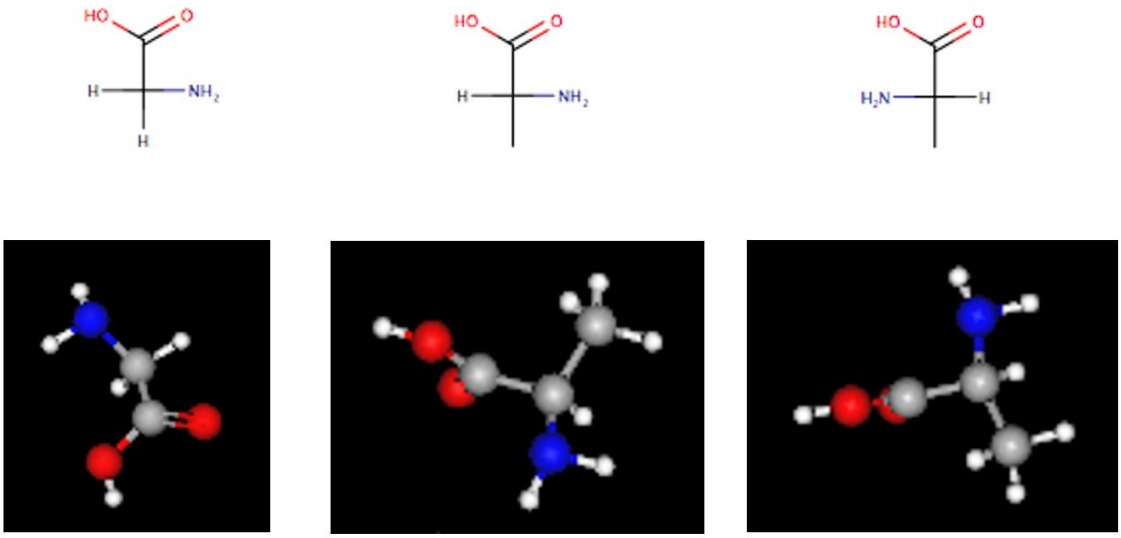

Figure 2. Three of the amino acids produced in the 1953 Urey-Miller experiment included glycine (left), D-alanine (middle), and L-alanine (right), with the structural formula seen on top and ball-and-stick structures on the bottom.

Later Experiments

After completing their first experiment, Stanley Miller and Harold Urey continued their research in synthesizing organic compounds from what they believed were prebiotic earth conditions. In 1959, Miller and Urey published a paper with an in-depth discussion on the mechanism of how the organic compounds would have been formed, as well as a defense for some of the assumptions that they made in developing their experiment. One of these assumptions deals with the composition of the atmosphere on earth, which they believed was reducing, as opposed to oxidizing conditions (Miller & Urey, 1959). This meant that the atmosphere was composed of hydrogen, methane, and ammonia gases, which was what was used in their previous experiment. One of their reasons behind choosing these gases was that during the formation of earth from the "cosmic dust clouds," hydrogen gas was present in excess (Miller & Urey, 1959; Tian, Toon, Pavlov, & De Sterck, 2005). Also, other planets in the solar system, such as Jupiter and Saturn,

have methane and ammonia in their atmospheres, from which scientists infer that earth had a similar composition at some point (Miller & Urey, 1959). This also meets the qualifications that Miller and Urey (1959) state as "one must show that reactions known to take place will not rapidly change the atmosphere to another type" (p. 245). Since the development of as single-celled organism is expected to occur very slowly, the atmosphere is assumed not to change rapidly as well.

For the current atmosphere on earth to have formed with the major constituents being nitrogen and oxygen, the hydrogen that was on the early earth slowly escaped the upper atmosphere on earth also allowing the rest of the atmosphere to be oxidized to their current states (Miller & Urey, 1959). Based on the current rate of escape for hydrogen gas in the upper atmosphere, which is 10^7 atoms of hydrogen per square centimeter per second, the current atmosphere would have been formed in 2.5 billion years with an initial hydrogen pressure of $1.5x10^{-3}$ atm (Miller & Urey, 1959). While the current loss of hydrogen can be empirically measured, the rest of this data is based on speculation and assumption of the starting and end conditions on earth.

The carbon compounds and nitrogen compounds in the atmosphere would have been in a state of equilibrium, according Miller and Urey (1959). While the hydrogen pressure on earth was high, the state of carbon would have been pushed the following equilibrium:

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$

Based on this equilibrium, the reducing atmosphere needed to produce organic compounds is only present due to the presence of hydrogen gas in the atmosphere, to lower the presence of carbon dioxide and carbon monoxide (Miller & Urey, 1959).

Also, the presence of carbon dioxide near the oceans was kept low due to the formation of limestone in the ocean. Calcium silicate ($CaSiO_3$) would have been present in the ocean and would have reacted with the carbon dioxide in the air to form limestones ($CaCO_3$) and silica (SiO_2), as seen in the following reaction (Miller & Urey, 1959; Sleep & Zahnle, 2001):

$$CaSiO_3 + CO_2 \rightarrow CaCO_3 + SiO_2$$

These two reactions kept the amount of carbon dioxide low, until the amount of hydrogen in the atmosphere dropped below 10^{-6} atm due to loss in the upper atmosphere (Miller & Urey, 1959).

The ammonia in the atmosphere would have also been controlled with an equilibrium system with the hydrogen present in the atmosphere. Since ammonia is very soluble in water, it would have existed mostly in its ionized form in the oceans, as seen in this reaction (Miller & Urey, 1959):

$$\frac{1}{2} N_2 + \frac{3}{2} H_2 + H^+ \rightarrow NH_4^+$$

Once the hydrogen in the atmosphere lowered below 10^{-5} atm, the ammonia in the ocean would have decomposed, into nitrogen gas (Miller & Urey, 1959). While the qualities of a prebiotic atmosphere on earth could never be completely proven, the reasoning behind why Miller, Urey, and other scientists believed in a reducing atmosphere is detailed and explainable.

Another assumption that Miller and Urey had to make about the possible conditions on a prebiotic earth was the source of free energy needed to synthesize the organic compounds from the atmospheric gases. Currently on earth, there are many sources of energy found naturally that could have provided the energy to form amino acids needed to form life on earth. The largest source of energy is radiation given off from the sun, which is estimated to be 260,000 cal/(cm^2*yr) (Miller & Urey, 1959). Although, Miller and Urey (1959) ruled out radiation from the sun as a cause for producing small organic molecules since the sun gives off little energy with wavelengths less than 200 nm, which is the range of wavelengths that methane and ammonia can absorb. Also, the ultraviolet light that is given off from the sun was not likely the cause of synthesis of organic molecules since most reactions with UV light occur in the upper atmosphere (Miller & Urey, 1959). The molecules most likely would not have made it to the surface of the earth before they would have been decomposed by longer UV wavelengths (Miller & Urey, 1959). Other sources of energy that did not offer enough energy to form free radicals in a reaction included cosmic rays, radioactivity from decomposing radioactive elements, and even volcanic energy and heat (Miller & Urey, 1959). None of these sources would have offered a continuous source of energy that would be needed to form the organic molecules.

According to Urey and Miller (1959), the only reasonable source of energy would have come from electric discharges, such as lightning. These discharges would have occurred close to the surface of the earth, which would allow the amino acids to be produced in the air above water, such as the ocean (Miller & Urey, 1959). This is also

important since the majority of ammonia that would be found on earth would be dissolved in the ocean (Miller, 1955).

Finally, Miller and Urey (1959) list a few of the remaining steps in chemical synthesis to produce all the molecules needed in a living organism. These include the synthesis of peptides (chains of amino acids), purines, pyrimidines, high-energy phosphate bonds, nucleotides, and functional enzymes (Miller & Urey, 1959). Miller and Urey were able to successfully synthesize some amino acids, but there still remained a great deal of problems and undiscovered steps for those who assumed that life developed from nonliving elements found on the earth. After Miller and Urey, scientists continued to put efforts into researching the prebiotic synthesis of molecules, particularly amino acids.

In 1983, Gordon Schlesinger conducted a follow-up study on the results of the 1953 Miller-Urey experiment. Schlesinger used slightly different gas mixtures to determine if the amounts and types of amino acids that were produced were similar to the original experiment. This included using the original gases used; methane, ammonia, and hydrogen; along with nitrogen gas (N_2) and water, but with different ratios of hydrogen to methane (Schlesinger & Miller, 1983). This differing experiment showed no significant change in amino acids produced related to the hydrogen-methane ratios, even if ammonia was present or not, with a yield of 1.2 to 4.7% of amino acids (Schlesinger & Miller, 1983). Also, Schlesinger analyzed the amino acids produced using carbon dioxide or carbon monoxide instead of methane and determined that the yield was around 2%, even with different CO/H_2 and CO_2/H_2 ratios (Schlesinger & Miller, 1983). When using either

CO or CO_2, there was hardly any variety of amino acids that were produced, compared to using methane. The only amino acid produced in a significant amount using CO or CO_2 was glycine, the simplest amino acid (Schlesinger & Miller, 1983). Since the variety of amino acids was limited using these sources of carbon rather than methane, Schlesinger concluded that an atmosphere with methane would more likely have existed on an early earth since it was necessary to produce the amino acids that led to the beginning of life on earth (Schlesinger & Miller, 1983).

A Neutral Atmosphere

For the most part, the scientific consensus remained unchanged regarding the composition of the prebiotic atmosphere and it having reducing characteristics, although some scientists did theorize that the earth actually had developed a non-reducing, neutral composition much earlier than originally estimated, possibly even before any organic molecules were synthesized (Cleaves, Chalmers, Lazcano, Miller, & Bada, 2008). The theory is based on the possibility of the hydrogen gas that was present in the atmosphere escaped into space more quickly than estimated (Cleaves et al., 2008). Based on this new theory, a new experiment was devised that determined what organic molecules were produced when the gases in the atmosphere were exposed to electrical discharges. As was seen in the Schlesinger experiment, the amount of amino acids produced in a neutral environment was much less than what was produced in a reducing atmosphere (Schlesinger & Miller, 1983), but in 2008, H. James Cleaves and others published their research in neutral atmospheres, which involved slightly altering the environment to increase the amount of amino acids produced (Cleaves et al., 2008).

Cleaves set up his experiment to use the same apparatus that Schlesinger used in his 1983 experiment, which was filled with 100 mL of water and 100 torr of both CO_2 and N_2 (Cleaves et al., 2008). The gases were then exposed to an electrical discharge continuously for 48 hours at 23℃. The amount of amino acids that was produced was analyzed using high-performance liquid chromatography (HPLC) separation techniques and detected using fluorescence after workup of the amino acids with a fluorescent reagent (Cleaves et al., 2008; Zhao & Bada, 1995). Yields of amino acids were approximately 10^{-2}% based on the N_2 reactant (Cleaves et al., 2008). Also, after the reaction, the pH in the water was very low (3.2) which would have inhibited the Strecker amino acids synthesis from originally formed amino-acid precursors (Cleaves et al., 2008). Cleaves solved this problem by modifying the experiment using a calcium carbonate buffer that kept the pH near 7 (Cleaves et al., 2008). This change in the experiment caused the amino acid yields to increase by 2 to 15-fold to a total amino acid yield of 0.19% (Cleaves et al., 2008).

During the analysis of the produced amino acids, nitrate and nitrite that are also products of the reaction can interfere with the amino acids and oxidize them into nitrosoamines which would alter the final amino acid yield (Cleaves et al., 2008). Cleaves also attempted to solve this problem by adding an oxidation inhibitor, ascorbic acid, to the experiment (Kuwahara et al., 2012). This also increased the amino acid yield by another 10 to 100 fold (Cleaves et al., 2008). After the analysis, the amino acids that were produced included glycine, alanine, serine and glutamic acid (Cleaves et al., 2008). Small amounts of aspartic acid, α-aminoisobutyric acid, γ-aminobutyric acid, and β-alanine

were also detected (Cleaves et al., 2008). These results are similar to the results from the 1953 Urey-Miller experiment as the major products in both included glycine and alanine, amino acids with the simplest side group structure (Miller, 1953).

After completing these experiments in a neutral atmosphere without oxygen, Cleaves tested the effect that a small amount of oxygen in the atmosphere would have had on the production of amino acids. His experimental team prepared a mixture with 2 mmol $CaCO_3$ in 100 mL of water with 100 torr of both CO_2 and N_2 and 10 torr of O_2 (Cleaves et al., 2008). They exposed this mixture to 48 hours of electrical discharge, just as in the previous experiments (Cleaves et al., 2008). While it was considered at the time that amino acids could not be produced in the presence of oxygen, Cleaves determined that a small amount of glycine and alanine were produced (Cleaves et al., 2008). Although, this has little effect on when evolutionary scientists believe that life began, which was before there was any significant oxygen in the atmosphere three billion years ago (Cleaves et al., 2008).

Some other conclusions that Cleaves made from his experiment was that there was no noticeable polymerization of the hydrogen cyanide (HCN) that was produced. This was contrary to other experiments that had been done previously including the 1959 Urey-Miller experiment, but Cleaves determined that this was most likely due to the decomposition of the cyanide to nitriles (Miller & Urey, 1959; Cleaves et al., 2008). Cleaves did note that there remains the possibility of cyanide polymers to have formed below the detection limits (Cleaves et al., 2008).

Finally, Cleaves made some conclusions about how the ocean chemistry must have been like for amino acids to be produced in neutral atmospheric conditions. The pH of the ocean is naturally close to a neutral pH, and acids in the ocean, including carbonic acid, nitrous acid, and nitric acid, would have slowed down the production of amino acids (Cleaves et al., 2008). In the laboratory, Cleaves was able to correct for the formation of these acids by buffering the mixture and adding ascorbic acid as an oxidation inhibitor (Cleaves et al., 2008). Ascorbic acid would not have been likely found in high concentrations in the ocean, so other oxidation inhibitors must have been present in order for a significant amount of amino acids to be produced (Kuwahara et al., 2012). Cleaves was able to test possible oxidation inhibitors that could have been in the ocean, and the only ones that had a significant impact on the oxidation of products were pyrites and iron (III) sulfate ($FeSO_4$) (Cleaves et al., 2008).

The research that Cleaves and his team completed establishes a new view on the field of prebiotic chemistry. The results that he published brought into question the necessity of a reducing atmosphere for any organic molecules to be formed, and even brings into question the conclusiveness that many evolutionary scientists ascribe to the 1953 Urey-Miller experiment on the source of life on earth. If the atmospheric composition and thus the mechanism of the formation of amino acids is in question, the conclusion that life was formed by natural occurrences on this earth is considerably weaker.

Christian Responses to Prebiotic Chemistry

As Christians, we must carefully approach the field of prebiotic chemistry, especially when it is presented to us as conclusive evidence for the formation of life from nonlife. Many Christian scientists have discussed the problems with prebiotic chemistry, specifically relating to the original 1953 Urey-Miller experiment. One main argument that scientists make is that the atmosphere that Urey and Miller used in their experiment is not a realistic representation of what a prebiotic atmosphere would have looked like on earth. In his book, *Icons of Evolution*, Jonathan Wells builds a case for various flaws in evolutionary theory informed by his studies in molecular biology. He writes that the original atmosphere that would have been on earth when it was formed would have been lost in space (Wells, 2000). This is based on the fact that compared to other planets and the sun, the earth has a lower proportion of noble gases in its atmosphere, which would mean that the earth would not have had a methane-ammonia atmosphere that was similar to other planets' atmospheres (Wells, 2000).

This conclusion that the earth did not have an atmosphere composed of methane and ammonia for a long time begs the question for evolutionists of where did the current atmosphere come from. After the earth was formed, there would have been a large amount of volcanic activity, which includes the expulsion of gases and vapors from inside the earth's crust (Bergman, 2004). Volcanoes today release a large amount of carbon dioxide, nitrogen, hydrogen, and water vapor into the atmosphere (Bergman, 2004). If the volcanoes observed today would have been similar to volcanoes billions of years ago, the atmosphere would contain a large amount of carbon dioxide and nitrogen

(Wells, 2000). This would have eventually produced a neutral, non-reducing atmosphere, which refutes the work done by Urey and Miller in reducing atmospheres (Wells, 2000). It would, however, complement the work done by the Cleaves research team in producing amino acids in neutral atmospheres (Cleaves et al., 2008).

Another problem that scientists bring up about the Urey-Miller experiment is that the only way they were able to synthesize amino acids was when there was no free oxygen (O_2) in the atmosphere. As was seen in the Cleaves experiment, the presence of free oxygen in the atmospheric system significantly decreases the amount and variety of amino acids that are produced (Cleaves et al., 2008). The 1953 Urey-Miller experiment did not have any free oxygen in the experiment (Miller, 1953), which would have been very likely due to presence of water vapor in the atmosphere. Some of the water molecules in the air would have been decomposed into hydrogen and oxygen gas through a process called photodissociation (Wells, 2000). Photodissociation is caused by the ultraviolet light (100-320 nm wavelengths) that is emitted from the sun to excite the hydrogen and oxygen atoms in the water molecule (Wells, 2000). The excited atoms then gain enough energy for them to break their bonds within the molecule and form new bonds with another atom of their type (Wells, 2000). The hydrogen gas formed is very light and would have eventually escaped earth's atmosphere and been released into space (Wells, 2000), as previously discussed. The oxygen gas would have been much heavier and stayed closer to the surface of the earth, which would have effected any type of synthesis reactions that were hypothesized by Miller and Urey (Wells, 2000). By not including even a small amount of oxygen in their experiment, Miller and Urey did not

accurately represent the primordial atmosphere of earth, which allowed them to produce more amino acids than would be realistic based on other theories of atmospheric composition.

Another problem that scientists have pointed out about the results from the 1953 Urey-Miller experiment relates to the molecular characteristic of chirality. Chirality, also called handedness, of a molecule occurs when two molecules are chemically identical, but the three-dimensional connections between the atoms are in a different order at a chiral center (Bergman, 2004). Two molecules which are chiral to each other are non-superimposable, mirror images of each other and are either labeled left-handed or right-handed (Bergman, 2004). In the laboratory, if a molecule is synthesized that is chiral, a 50/50 mixture of both left and right-handed molecules will be produced, which is called a racemic mixture (Bergman, 2004). The only way to just produce one type of chiral molecule is to perform a synthesis reaction using a chiral reagent.

In the 1953 Urey-Miller experiment and other subsequent experiments, the amino acids that were produced existed in a racemic mixture. This is a problem because in nature, only left-handed amino acids are used in proteins (DeWitt, 2007). Right-handed amino acids are harmful to living systems as all enzymes are shaped to work only with left-handed amino acids (Bergman, 2004). Only half of the amino acids that would have been formed from a prebiotic atmosphere would have been able to react with each other to form the proteins that are seen in all living organisms today. Also, all the right-handed amino acids must have been degraded before any organism could try to incorporate the

amino acids into any proteins it would try to make, which is another area of research that evolutionists must embark upon.

Some evolutionary researchers have realized that the synthesis of amino acids according the theory set by Oparin and tested by Urey and Miller might not be a feasible explanation for the beginning of life on earth. Some have suggested that rather than amino acids and proteins forming first on earth, it was rather the nucleic acid RNA that formed first. RNA (ribonucleic acid) is a nucleic acid that consists of a nitrogenous base, which usually has a pyrimidine or purine ring structure, a phosphate group, and a ribose sugar (DeWitt, 2007). It is used in all living organisms mostly as an intermediate in the process of protein synthesis (DeWitt, 2007). The genetic material is stored in the form of DNA (deoxyribonucleic acid) in an organism, and enzymes in the cell copy the information stored in the DNA to a complementary strand of messenger RNA (mRNA), through a process known as transcription (DeWitt, 2007). Other enzymes then take the information in the mRNA and use it to produce a protein or enzyme that would be used in the cell from amino acids (DeWitt, 2007).

The main reason researchers are interested in the possibility of RNA being the first molecule that would have started the development of life is that in some instances RNA can catalyze its own reactions, just like an enzyme (Wells, 2000). RNA molecules that function as an enzyme are called ribozymes (DeWitt, 2007). Ribozymes are used in reactions with other RNA molecules, specifically splitting other RNA (DeWitt, 2007). If RNA molecules were able to be synthesized and then would be able to self-replicate,

evolutionist scientists believe that RNA would be a plausible substitute for amino acids as the first molecules of life.

There are many problems with RNA acting by itself, including its own synthesis and chirality, but one of importance is the limited reactions that RNA can catalyze. Ribozymes are only used in the breaking down, or cleavage of RNA molecules, not in reproduction or polymerization (DeWitt, 2007). RNA molecules in living organisms today are only polymerized through the use of preexisting, complex enzymes along with a significant amount of energy input (DeWitt, 2007). Also, the main role of RNA and DNA is to store and transfer information. This information is found in the specific order of the RNA or DNA monomers, or nucleotides. Every three RNA nucleotides form a codon, which is used in protein synthesis to instruct the protein-building enzymes, ribosomes, to select a specific amino acid (DeWitt, 2007). These codons follow a standardized code that is found in all living organisms (DeWitt, 2007). For any type of RNA to be useful, the RNA nucleotide must be in a proper sequence for a protein to be formed, and only through the assistance of preexisting enzymes (DeWitt, 2007). Finally, RNA molecules would have been very difficult to synthesize in large amount on a prebiotic earth, as they are much more complicated structures compared to amino acids (Wells, 2000). A simple electric discharge in a mixture of gases would not be enough to synthesize a complex RNA molecule.

Experimental

Being able to educate young scientists about the origin of life debate is very important for any school of higher learning to do. The ultimate goal of this research

project is to establish a simple laboratory experiment that organic chemistry students would be able to perform or observe to assist them in learning the details of the Urey-Miller experiment. Another goal is to reproduce the Cleaves experimental model to confirm or reestablish what products were formed, or possibly alter the experimental parameters to demonstrate what products would or would not form under different environmental conditions.

Construction of the Aparatus

To complete the apparatus, several types of equipment was acquired. A three-liter flask with removable tungsten electrodes was used, as it was used in the Cleaves study (Figures 3 & 4) (Cleaves et al., 2008). A vacuum pump is necessary to evacuate any gases that are in the inside the apparatus. To confirm that all the gases have been evacuated and that the appropriate amount of nitrogen and carbon dioxide have been added, a vacuum gauge is added to measure the pressure in the flask and manifold. A Welch vacuum gauge was used, and the pressure was lowered to 0.002 millibar. Also, thicker vacuum hosing was purchased to allow for better seal of the vacuum.

Figure 3. The current apparatus that will be used for future research at Liberty University.

Figure 4. Three-liter flask model used by the Cleaves 2008 model paper, including removable tungsten electrodes and electric discharge source (Schlesinger & Miller, 1983).

Development of Analytical Techniques

Materials.

The TLC sheets used for the 2D-TLC were Cellulose F (Merck KGaA). The polarimeter was a Jasco P-2000 polarimeter. The HPLC instrument used was a Agilent Technologies 1260 Infinity, and the column used was a Fortis Technologies 5 µm H2o, 150x2.1 mm.

2D-TLC.

To establish a simple means of determining what amino acid are produced by the reaction, several different methods of two-dimensional thin-layer chromatography (2D-TLC) were tested to determine which would be the best to use in an organic chemistry teaching laboratory experiment. The following compounds were used: L-serine (Acros Organics, 99%), L-alanine (Eastman Chemical Company), glycine (Sigma-Aldrich Chemical), 2-aminoisobutyric acid (Acros Organics, 99%), and 4-amiobutyric acid (Acros Organics, 99%). Several solvents were used to make the TLC mobile phase, which included acetonitrile, methanol, n-propanol, ethyl acetate, and tap water.

A 1:1:4 water: acetonitrile: methanol mobile phase was used. Alanine, glycine, serine, aminobutyric acid, and aminoisobutyric acid were run in one dimension on the cellulose TLC plate to determine the respective Rf values. A 2D-TLC with alanine, glycine, and serine was run with the 1:1:4 water: acetonitrile: methanol for the first mobile phase and 7:3 n-propanol: water as the second mobile phase (Bhawani et al., 2012). Another 2D-TLC was performed with the 1:1:4 water: acetonitrile: methanol and 3:1:1 ethyl acetate: methanol: water mobile phases (Bhawani et al., 2012). All the TLC plates were visualized using ninhydrin.

Polarimeter.

Some of the analytical techniques were also examined, including use of a polarimeter. A polarimeter allows for the determination of the amount of optical rotation that a chiral substance undergoes when exposed to plane-polarized light. A known concentration L-proline (0.050 g/mL) was measured as control experiments. The path length of the polarimeter cell was constant at 1 dm.

HPLC.

A high performance liquid chromatography (HPLC) instrument with fluorescence and UV/Vis detectors (Figure 5) was used to determine the unique amino acids produced by the Cleaves method. In order to do so, various columns and mobile phases were compared to test the effectiveness of the different HPLC methods. The amino acids, L-alanine (Acros Organics) and L-proline (Sigma), were chosen, and solutions containing 1mM of each were prepared. A solution containing both of these amino acids was also prepared.

Figure 5. The current HPLC instrument at Liberty University, which was used in the conduction of this experiment.

A method of HPLC separation presented in a technical poster published by Fortis Technologies was used (Butchart & Woodruff, 2012). This involved preparing two mobile phase mixtures. Mobile phase A had 1000mL of deionized (DI) water, 1.8 mL triethylamine (TEA) (pH 7.3) (Acros), and 3 mL of tetrahydrofuran (THF) (Acros Organics), with 2.72 g of sodium acetate (Fisher). Mobile phase B had 200 mL DI water, 400 mL of methanol (MeOH) (Pharmco-Aaper), and 400 mL of acetonitrile (Fisher), with 2.72 g of sodium acetate (Fisher). The mobile phase gradient started at 2% B at 0.45 mL/min, 2% B at 1 min, 60% B at 17 min, 100% B at 18 min, 0.8 mL/min at 18.10 min, 0.8 mL/min at 23.90 min, 100%B at 0.45 mL/min at 24 min, 2% B and 0.45 mL/min at 25 min, and equilibrated at 30 min. The UV/Vis detector was set at 260 nm.

Results and Discussion

2D-TLC

When the five amino acids were run in one dimension with a 1:1:4 water: acetonitrile: methanol mobile phase, alanine had Rf values of .44 and .45, as seen in

Figure 6. Glycine had an Rf value of .26. Serine had an Rf value of .27. Aminobutyric acid had an Rf value of .35, and the aminoisobutyric acid had an Rf value of .67. The only two amino acids that were difficult to distinguish were glycine and serine, so a 2D-TLC was used to determine if the amino acids could separate better.

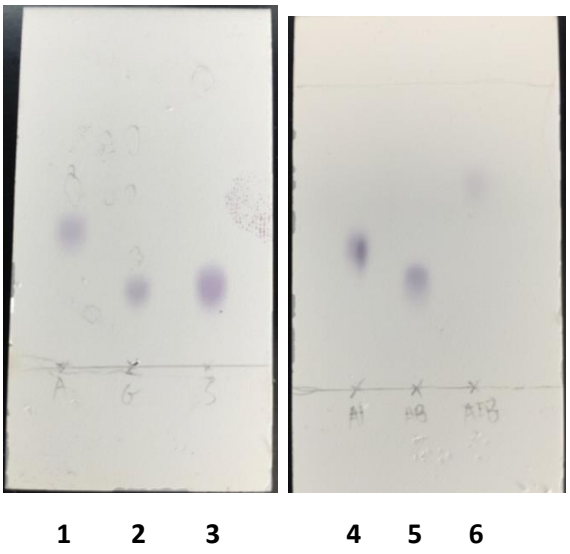

Figure 6. One-dimensional TLC (cellulose) of alanine (1 and 4), glycine (2), serine (3), aminobutyric acid (5), and aminoisobutyric acid (6) with a mobile phase of 1:1:4 water: acetonitrile: methanol, after treatment with ninhydrin.

With the two mobile phases, 1:1:4 water: acetonitrile: methanol (up) and 7:3 n-propanol: water (right), alanine had Rf values of .48 and .24 for upward and rightward movement, respectively. Glycine had an upward Rf value of .29 and a rightward Rf value of .19. Serine had an upward Rf value of .31 and a rightward Rf value of .17. Again, the glycine and serine were hard to distinguish due to their similar Rf values, which can be seen in column 4 which had glycine and serine on the same spot, as seen in Figure 7.

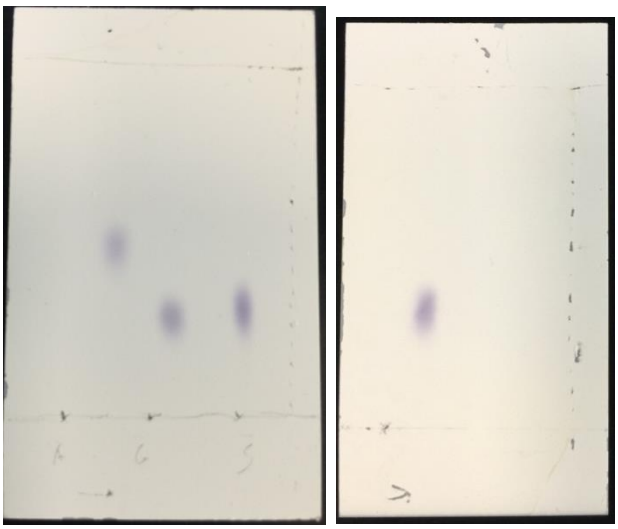

Figure 7. Two-dimensional TLC (cellulose) of alanine (1), glycine (2 and 4), and serine (3 and 4) with an upward 1:1:4 water: acetonitrile: methanol mobile phase and a rightward 7:3 n-propanol: water mobile phase, after treatment with ninhydrin.

Another combination of mobile phases, 1:1:4 water: acetonitrile: methanol (up) and 3:1:1 ethyl acetate: methanol: water (right), was used to determine if glycine and serine would have significantly different Rf values on a 2D-TLC. Alanine had Rf values of .53 and .29 for upward and rightward movement, respectively. Glycine had an upward Rf value of .30 and a rightward Rf value of .17. Serine had an upward Rf value of .34 and a rightward Rf value of .14. Glycine and serine were placed on the same spot again (column 4), and again the two amino acids did not separate enough to distinguish themselves, as seen in Figure 8.

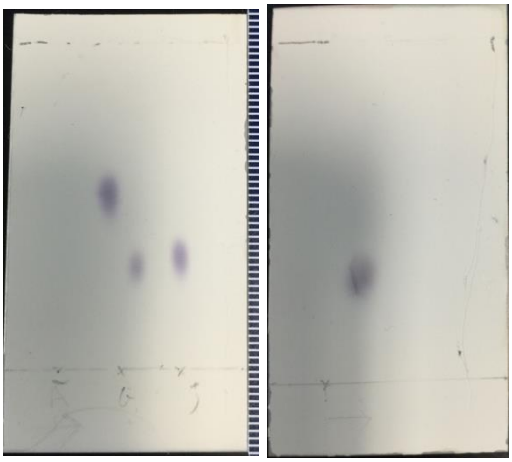

Figure 8. Two-dimensional TLC (cellulose) of alanine (1), glycine (2 and 4), and serine (3 and 4) with an upward 1:1:4 water: acetonitrile: methanol mobile phase and a rightward 3:1:1 ethyl acetate: methanol: water mobile phase, after treatment with ninhydrin.

Polarimeter

The sample of authentic L-proline had a measured optical rotation of -4.3º. After correcting for the concentration and pathlength, the specific optical rotation was -86º, with the literature value being -85º. These tests confirm that the polarimeter is functional and is able to be used to determine the optical rotation or lack thereof once the amino acid synthesis reaction will be performed.

HPLC

The chromatogram, using the Fortis column, showed multiple small peaks in the blank as well as the samples (Figure 9). For the alanine chromatograms (Fig. 9b & d), a small peak was seen around 8.4 min. For the proline chromatograms (Fig. 9c & d), no clear peak was seen.

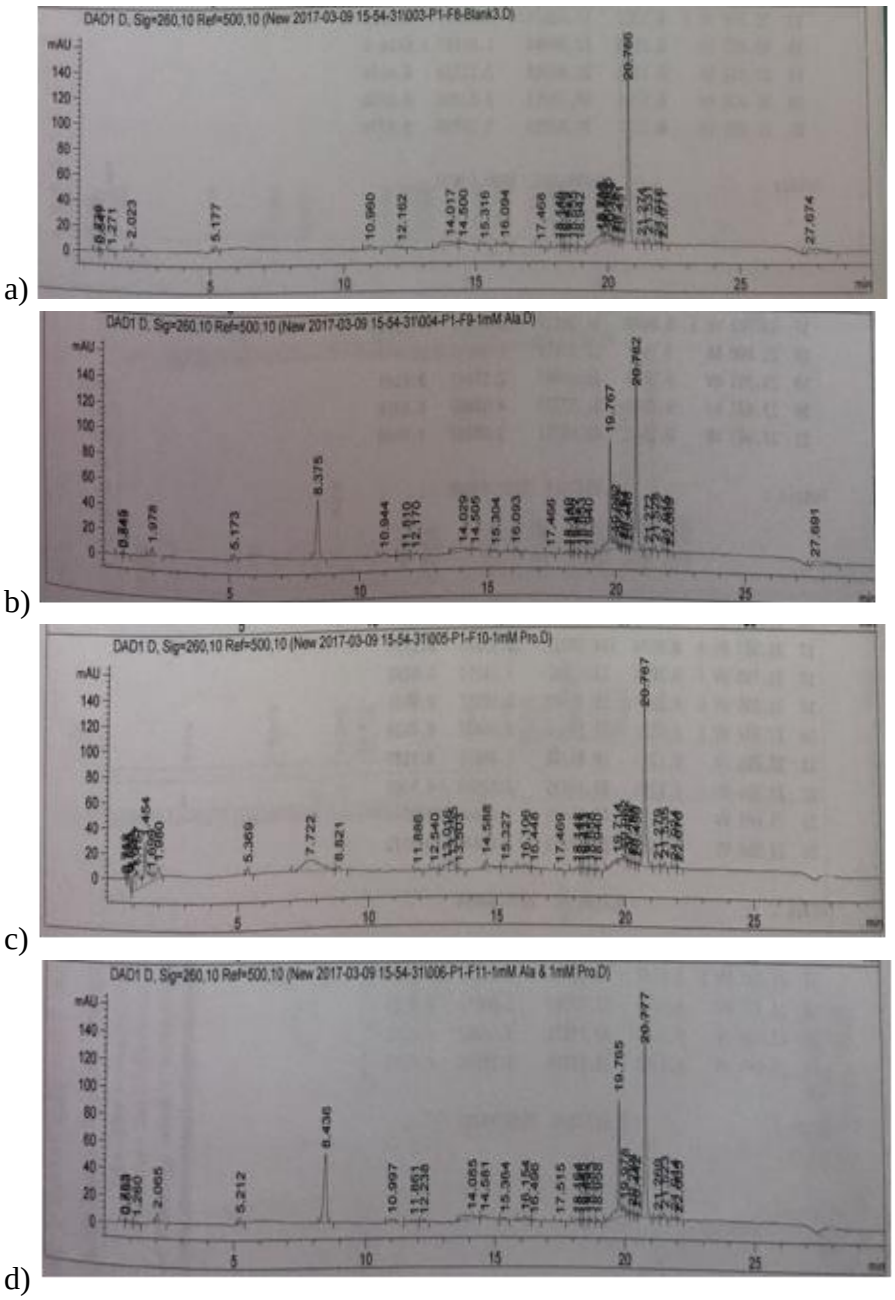

Figure 9. HPLC chromatogram of the blank sample (top) and 1mM Alanine and 1mM Proline (bottom).

Future Work

Preparations are being made to continue this research by derivatizing the mixture of amino acids so that they would be able to fluoresce and be detected in the HPLC.

Amino acids are not naturally very responsive to traditional HPLC detection methods, so attaching an additional agent to amino acids make them more easily detected. These methods include o-phthalaldehyde (OPA) and dansyl chloride methods, using the reagents seen in Figure 10. Also, a new HPLC column, which is polar-endcapped which is supposed to allow good resolution of amino acids without need for derivatization. Also, research into the reevaluation of Urey-Miller experiment will continue with the goal of incorporation into an organic chemistry laboratory curriculum.

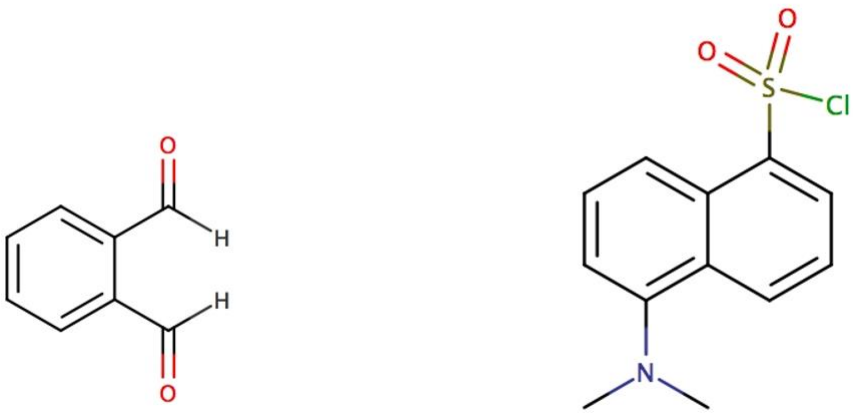

Figure 10. Derivatizing agents OPA (left) and dansyl chloride (right).

Conclusion

Through the examination of previous research and review of ongoing research at Liberty University, the importance of understanding the science and chemistry behind the creationism and evolutionism dialogue can be better understood. If Christians wish to be good apologists of their faith and belief that the earth and all of life was created in six literal days, they must be willing to understand some of the science behind the evolutionary claims and the inherent flaws within them. The Urey-Miller experiment and the conclusions that evolutionists draw from it are not enough to support the theory of

abiogenesis, so it should not be continually used as evidence in textbooks and classrooms around the world for the source of life on earth. Logically, the only remaining solution to this problem is that life was formed not naturally, but rather supernaturally.

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