Chapter 5

Origin of Life: The Miller Experiment

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Reprinted from: Peifer, R. W. 1993. Origin of life: the Miller experiment. Pages 43-67, *in* Tested studies for laboratory teaching, Volume 7/8 (C. A. Goldman and P. L. Hauta, Editors). Proceedings of the 7th and 8th Workshop/Conferences of the Association for Biology Laboratory Education (ABLE), 187 pages.

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Introduction

The purpose of this lab exercise is to expose undergraduate biology students to the classic Miller experiment on the origin of life that is often referred to in general biology texts. The experiments are designed to run over two consecutive lab periods, each 2 hours long. During the first lab period, students collect samples from the Miller apparatus, spot their samples on chromatography paper alongside an amino acid standard sample, and develop chromatograms. They stain their chromatograms using ninhydrin, and then identify individual amino acids by calculating R_f values. In a second experiment, students investigate the phenomenon of chirality (handedness) by looking at the ability of the bacteria *Proteus vulgaris* to utilize stereoisomers of the amino acid phenylalanine.

These exercises have been used for several years with honors lab sections in a general biology course. No college-level chemistry is required, although a chemistry background is very helpful. Since no chemistry background is required of students, the technique used in the analysis of the Miller sample has been kept relatively simple, that is, students use paper chromatography. However, more complex analytical techniques, using a Dionex D-330 Amino Acid/Peptide Analyzer, are discussed, and the results of such analyses are put on display for the students to view and discuss. Reference reading on the origin of life is required of students prior to the lab, and students are also assigned to write a paper.

The apparatus is assembled by lab preparators because of time constraints and to guard against possible errors and accidents. However, it is feasible to involve the students themselves in the assembly of the apparatus, given sufficient space, care, and appropriate laboratory scheduling. The apparatus can be set up in approximately 4 hours, and is started 5 weeks before a sample is needed.

It took approximately 2 years to design and refine the apparatus so it would be suitable and reliable for student use. With routine maintenance, we now have a consistently functioning, problem-free design. Therefore, we recommend that if you want a safe and reliable design that you use the same items listed in our materials list.

Materials

Miller apparatus
Miller sample
Ninhydrin solution
Chromatography paper
Inoculating loop
D- and L-phenylalanine culture plates
Incubator
Ferric chloride solution, 2%
Proteus vulgaris culture
Hair dryers
Small vials
Pasteur pipets
Trivet
Chromatography developing jars
Plastic bags
Amino acid standard solution
Insulated gloves
Molecular models, D- and L-alanine
Bunsen burners
Glass atomizer (Fisher #05-719-5C)
Masking tape
Small vials with caps
Grease pencils
Hot plates
Pyrex glass plates
Pencils and metric rulers
Refrigerator
Disposable examination gloves
Compressed air source
Natural gas source
Whatman No. 3 chromatography paper
Liquid nitrogen

Student Outline

Introduction

The idea that life is extremely ancient and arose as a consequence of the physical and chemical evolution of our planet, was first proposed by the Russian biochemist A. I. Oparin in 1924 in a small monograph published in Russian (Oparin, 1924). Oparin's ideas remained little known to those outside the Soviet Union until he expanded his thoughts on the topic into a larger volume published in 1936. This work was eventually translated into English in 1938 (Oparin, 1938). Another eminent biochemist of the day, J.B.S. Haldane, also wrote about the origin of life from nonliving matter (Haldane, 1929). Both men's work on this topic received scant attention until the early 1950s. It should be noted that there were other ideas about the origin of life on earth that predated Oparin and Haldane's hypotheses. Many major figures of history such as Plato, Democritus, St. Augustine, St. Thomas Aquinas, Copernicus, Galileo, Harvey, Francis Bacon, and Descartes presented philosophical and theological discussions of the subject (Farley, 1977).

Oparin and Haldane conceived of an earth, recently consolidated, that had an atmosphere devoid of molecular oxygen, but rich in reduced gases, essentially the opposite of the current chemical state of the atmosphere. It was apparent to them that if the primitive atmosphere were reducing and devoid of oxygen, and the present atmosphere oxidative and rich in oxygen, the atmosphere of the earth must have gradually changed from its ancient state. The significance of this seemingly trivial fact cannot be over emphasized, for the very transition from a reduced atmosphere to an oxidative state created the conditions which allowed life to emerge. In fact, this transition has left its imprint in the form of oxidation/reduction reactions as the principal mechanism on which the biochemical pathways of all life are organized.

Somewhere along this transition, from a reduced to an oxidized atmosphere, the physical and chemical conditions must have been ideal for the generation of the basic molecular building blocks of life, the polymerization and self-assembly of these molecules into macromolecules, and finally,

the association and assembly of these molecules into volumes of space that preserved their integrity at a particular point on the redox scale of carbon.

The ideas of Oparin and Haldane addressed this ancient question of the origin of life without invoking the involvement of some vital force, which up to that time had been the hallmark of most origin of life hypotheses. Rather, the two men drew upon their knowledge of the physical sciences to formulate their ideas. Oparin and Haldane laid the foundation upon which all subsequent investigators of the origin of life have made their respective contributions.

It was not until the early 1950s that experimental investigations were undertaken at the University of Chicago, by Stanley L. Miller and Harold C. Urey, to test the hypothesis that the basic building block molecules of life could have been "spontaneously" generated in the primitive atmosphere of earth as proposed by Oparin and Haldane. An apparatus that could simulate the reduced atmosphere of earth was assembled by Miller, then a graduate student of Urey. The apparatus was a closed system that could be evacuated of any oxidative atmosphere, and then loaded with water and the reduced gases that were thought to be present some 3.8 billion years ago. A boiling flask and condenser were used to make the contents of the system circulate, while a high-energy spark discharge was passed through the simulated atmosphere to provide a source of free energy. Chemical products produced in the atmosphere of the apparatus would condense and collect in the boiling flask. The chemical compounds in the boiling flask would then undergo secondary reactions to produce more complex organic compounds. Periodically, samples could be drawn from the flask for analysis.

In the initial experiment (Miller, 1953), the gases used to produce the simulated primitive atmosphere were hydrogen, ammonia, and methane; the only other substance present was water. Within a single day of running the apparatus, some of the amino acids, the building blocks of proteins, began to appear in the boiling flask. As the experiment progressed, more kinds of amino acids began to show up in the contents of the boiling flask. The primary products identified in the first Miller experiment were amino acids, aldehydes, and hydrogen cyanide, but subsequent experiments, run with other types and combinations of gases, produced other classes of basic building block molecules, namely sugars and nucleotides. To date, all the major classes of organic compounds found in biological systems have been synthesized in experiments of this nature.

To appreciate the significance of Oparin and Haldane's hypothesis, that is, that life arose in a primitive atmosphere composed of reduced gases, one must understand the concept of *oxidation/reduction*, and what happens to the element carbon when it is exposed to different oxidative and reductive environments. The oxidation of a substance involves the removal of an electron from its structure, while reduction is the addition of an electron to a substance. When electrons are removed from a substance, they must be immediately transferred to another substance, since they do not exist as free entities. Therefore, we say that when one substance is oxidized another must simultaneously be reduced, hence the term *redox* to describe oxidation/reduction reactions. You cannot have one without the other.

The significance of all redox reactions lies in the energy transformations that take place during the course of the reactions. For example, a simple redox reaction is used to lift into orbit the enormous mass of NASA's Space Shuttle. Liquid hydrogen and oxygen are used as the main propellants for the main engines of the Shuttle. The reaction of hydrogen with oxygen releases a tremendous amount of energy. The reaction looks like this:

$$2H_2 + O_2 \text{ ---------> } 2H_2O + \text{energy}$$

On the surface, it is not readily apparent that this reaction involves the transfer of electrons, but if we look at how the reaction actually proceeds, we discover that electrons are indeed transferred from hydrogen to oxygen. The reaction takes place in three steps:

(1) $2H_2$ ---------> $4H^+ + 4e^-$

(2) $O_2 + 4e^-$ ---------> $2O^=$

(3) $2O^= + 4H^+$ ---------> $2H_2O$

The first reaction splits hydrogen into four protons (hydrogen nuclei) and four electrons. In the second step, the four electrons from hydrogen are transferred to the oxygen, thus forming two ions of a highly reactive form of oxygen. The oxygen ions then react with the four protons to form a molecule of water. Thus, the overall reaction can be described as the oxidation of hydrogen with the simultaneous reduction of oxygen to form water, and the concomitant release of energy. Or, in other words, hydrogen is "burned" in the presence of oxygen to obtain energy.

You might be wondering what all of this inorganic chemistry has to do with the chemistry of organisms? As it turns out, the chemistry of life is largely the chemistry of oxidation/reduction reactions of carbon compounds. The significance of these redox reactions is, like the reaction described above, the energy transformations that occur during the process. It is the free energy liberated by redox reactions that is harnessed by organisms to build, maintain, and ultimately perpetuate themselves.

To better understand the redox processes of organisms, and how oxidation/reduction emerged as the predominant method that organisms use to obtain and handle energy, let us consider the different redox states that can be assumed by the element carbon. Because carbon is a tetravalent element, that is, because it has four electrons in its outermost energy level, the element can form a maximum of four single chemical bonds. For example, the chemical bonding requirements of carbon could be met by forming four single bonds with four atoms of hydrogen, thus forming a molecule of methane. In this state, the carbon atom is said to be reduced because hydrogen has been added to it. The addition of hydrogen or the removal of hydrogen from carbon is equivalent to the addition or removal of electrons. Hydrogen, composed of only a single proton and electron is simply an electron in disguise. When a single proton and a single electron are in close proximity to each other, their respective charges disappear to the observer. Methane, CH_4, therefore represents the most reduced state of carbon because no further electrons may be added to it, while carbon dioxide, CO_2, is the most oxidized form of carbon. These two molecules form the extremes of a scale of redox potential for carbon. In between these two extremes, other forms of carbon compounds are thermodynamically possible.

Many of you are probably familiar with the process that converts methane to carbon dioxide. The process is used to cook our food and heat our homes, just to name a few of its uses. The reaction proceeds as follows:

$$CH_4 + 2O_2 \text{ ---------> } CO_2 + 2H_2O + \text{energy}$$

Notice that in this redox reaction, electrons are removed from methane, and then placed on oxygen. Therefore, we say that the methane has been oxidized while the oxygen has been reduced. Again, the important point to remember is that energy is released by the oxidation of a reduced substance.

Now that some basics have been covered, we can consider the significance and the implications of Oparin and Haldane's ideas. Oparin first pointed out that the primitive atmosphere of the earth must have been much different from the present atmosphere. Evidence for this assertion comes from many disciplines, such as geology, astronomy and cosmology. He hypothesized that the atmosphere would have been composed primarily of reduced gases, such as hydrogen, methane, and ammonia. The oxygen on the planet would mostly have been locked up in nonvolatile silicate minerals or water, therefore no free oxygen would have been present.

The significance of the absence of oxygen can be appreciated if we consider the redox scale of carbon, Figure 5.1. The diagram illustrates that in the most reduced, primitive atmosphere (left side of scale), the only thermodynamically stable form of carbon would have been methane. That is, if any other forms of carbon were subjected to these conditions, the substances would have been unstable, and eventually would have been converted to methane. The right hand portion of the diagram illustrates that in the present atmosphere, the only stable form of carbon is carbon dioxide. In the contemporary atmosphere, any carbon compound, other than carbon dioxide, that is exposed to the oxidizing effects of oxygen, will eventually be converted to carbon dioxide. Methane and carbon dioxide therefore represent the extremes of the redox scale of carbon.

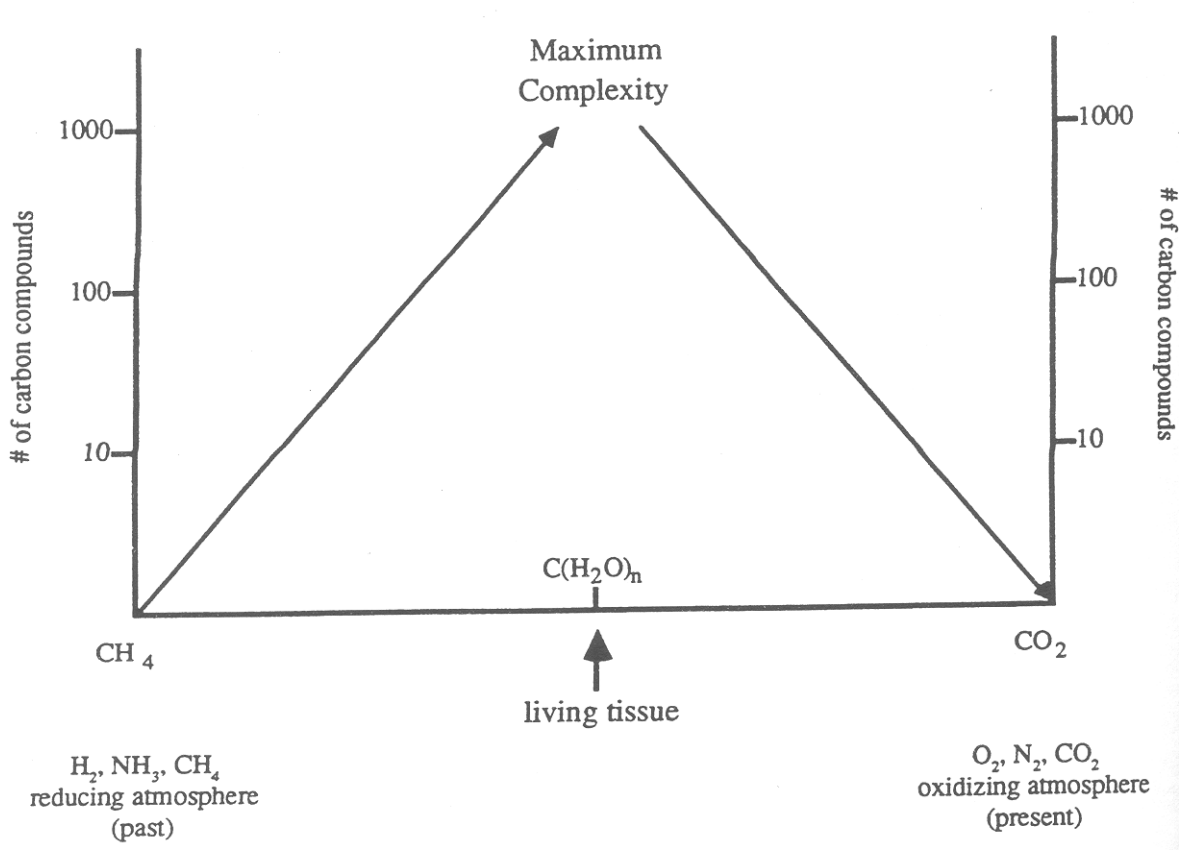

Figure 5.1. The redox scale of carbon and its relationship to the redox state of the atmosphere.

What other forms of carbon, if any, lie between these extremes? As you move from the extreme left to the right on the redox scale, notice that the number of different forms of carbon that are thermodynamically stable increases to a point that is approximately at the center of the scale. Oparin realized that the transition along the redox scale suggested a possible mechanism for the generation of the basic building block molecules of all life forms. He knew that as the atmosphere of the earth lost the lighter gases, such as hydrogen, the atmosphere would have evolved to a more oxidized state. The biochemist hypothesized that if the gases in the atmosphere were subjected to sources of energy, such as U.V. light or lightning, during this gradual transition of the chemical composition of the atmosphere, then chemical reactions would have occurred that could have generated many different types of carbon compounds. Since the atmosphere would have been devoid of strong oxidants, such as oxygen, the compounds would have been thermodynamically stable and could have accumulated in the water basins on the earth's surface. As J. B. S. Haldane

(1929) stated in *Rationalist Annual*, without free oxygen to destroy these newly generated compounds, "the primitive oceans reached the consistency of hot dilute soup."

As the atmosphere continued to undergo its transition to a more oxidized state, a point would have been reached where the reactions that produced the "dilute hot soup" would no longer have been possible. Also, as the atmosphere became more oxidative, the compounds themselves would have become thermodynamically unstable. It is at this juncture that Oparin said life must have originated. That is, some of organic compounds must have associated and assembled themselves in some collective manner in a restricted volume of space that offered protection from the oxidizing effects of the atmosphere. By assembling in such a manner as to create an environment that had a redox potential that was the same as their molecular structures, but different from the surrounding environment, these molecular assemblages could in fact exploit the gradual transition to a more oxidized state.

At first glance, a move to a more oxidized atmosphere appears to be a dead end proposition because the molecular assemblages would literally be "burned" to simpler carbon compounds. Obviously this did not occur. Rather, these primitive molecular systems, confined in small volumes of space, must have been able to obtain adequate free energy from their surroundings to maintain their redox states, and thus their chemical and physical structure. By taking reduced compounds (food) from the surrounding environment, and by exposing these compounds to more oxidized molecules, they could control the rate at which these food substances "burned". In this way, they could provide a constant supply of free energy to maintain the status quo, that is, the reduced state of their structures. It was the thermodynamic considerations of the redox state of carbon that led Oparin to suggest that organisms are "living fossils," stuck at the midpoint on the redox scale of carbon, capitalizing on a 3.8 billion year old transitional event that allowed life to emerge, and that continues to allow life forms to build, maintain, and perpetuate themselves.

The process described above should sound familiar, for it is this very principle that runs the industrial nations of the world. Reduced carbon compounds are exposed to the atmosphere (O_2) and ignited. The resultant oxidation of these reduced compounds to CO_2 and H_2O liberates free energy which is used to build and maintain the structure of modern life. Just as we keep these types of fires under control by regulating the rate at which oxygen is exposed to the fuel source, our cells, and their enzymes, regulate the rate at which oxygen is allowed to interact with our food.

The experiments of Stanley Miller, and scores of other experiments by investigators that followed, have all confirmed that the basic molecular units of life could have been generated in the primitive atmosphere of earth. Other individuals have demonstrated that many of the basic building block molecules can polymerize, and then "self-assemble" in the appropriate chemical and physical environment. Additionally, hypotheses on the origin of the genetic code, and its connection to protein synthesis, also look promising. Many questions still remain to be answered, and still more asked, but a firm foundation has been established from which others may build.

During the next two lab periods, we will try and duplicate certain aspects of Stanley Miller's experiment that was first conducted in 1953. Because of the duration of the experiment (4 weeks) and the complexity of the apparatus, it was not possible for you to participate in the initial set-up and run of the apparatus, but you will have the opportunity to collect and identify the contents of the collection vessel. Also, you will investigate another phenomenon that relates to the origin of life — chirality.

Part 1: The Miller Experiment

In 1953, Dr. Stanley Miller assembled an apparatus that would attempt to test the Oparin/Haldane hypothesis that the basic building block molecules of life could be generated in a

reduced gaseous atmosphere with natural free energy sources. The experiment was elegant in its theoretical simplicity and experimental design.

Basically, the experiment consisted of a simulated primitive atmosphere of reduced gases circulated in an enclosed glassware system while a high energy spark was passed between two electrodes. As chemical products were produced in the area of the spark discharge, they would condense and fall into the liquid water contained in the system. The concentration of the various products would continue to increase as the experiment progressed.

You are directed to Stanley Miller's original papers (1953 and 1954) for a description of the apparatus and experimental procedures he used. Because the apparatus must run for approximately 4–5 weeks to obtain sufficient yields of amino acids, and because it is a fairly complicated apparatus to assemble, you will only be concerned with the analysis of the contents of the collection vessel. But, you will have a chance to view the apparatus, and also extract from it the sample you will analyze.

The principle component of the Miller apparatus is a custom piece of glassware consisting of a boiling flask, condenser, and reacting chamber with electrodes (Figure 5.2). A gas manifold is attached to the main glassware via a vacuum stopcock. Each gas line of the manifold is attached to a specific gas tank (H_2, CH_4, or NH_3). Also incorporated into the gas manifold is a vacuum line used to evacuate the atmosphere of the Miller apparatus, and all gas lines. A liquid nitrogen trap between the gas manifold and the vacuum pump is used to trap water vapor, so it does not mix with the oil of the vacuum pump. An ammonia resistant vacuum/pressure gauge is used to monitor the internal pressure of the apparatus while the experiment is running, and it is also used to load the gases in the particular ratio desired. The boiling flask is outfitted with a vacuum stopcock spigot for extracting samples. A heating mantle is used to bring the system to a boil which causes the gaseous atmosphere to circulate. The spark between the electrodes is the source of free energy for the various reactions, and it is produced by a solid state induction coil. A water flow regulator is used to maintain a constant flow of water through the condenser.

The first step in loading the apparatus once the system has been assembled is to evacuate the atmosphere of the main apparatus plus all gas lines. When the system has been evacuated and checked for vacuum leaks, double distilled water may be added to the system. Next, the water must be degassed of oxygen by bringing the system to a boil. Again, the system must be vacuumed to remove any oxygen.

Once the system has been completely depleted of oxygen, the experimental atmosphere may be loaded into the apparatus. The vacuum gauge may be used to monitor the amount of each gas added to the apparatus. Thus, any desired ratio of gases may be easily selected. When all gases have been loaded, the heating mantle and the water condenser may be turned on. A protective shield of acrylic should be mounted in front of the apparatus before any spark is introduced to the reaction chamber.

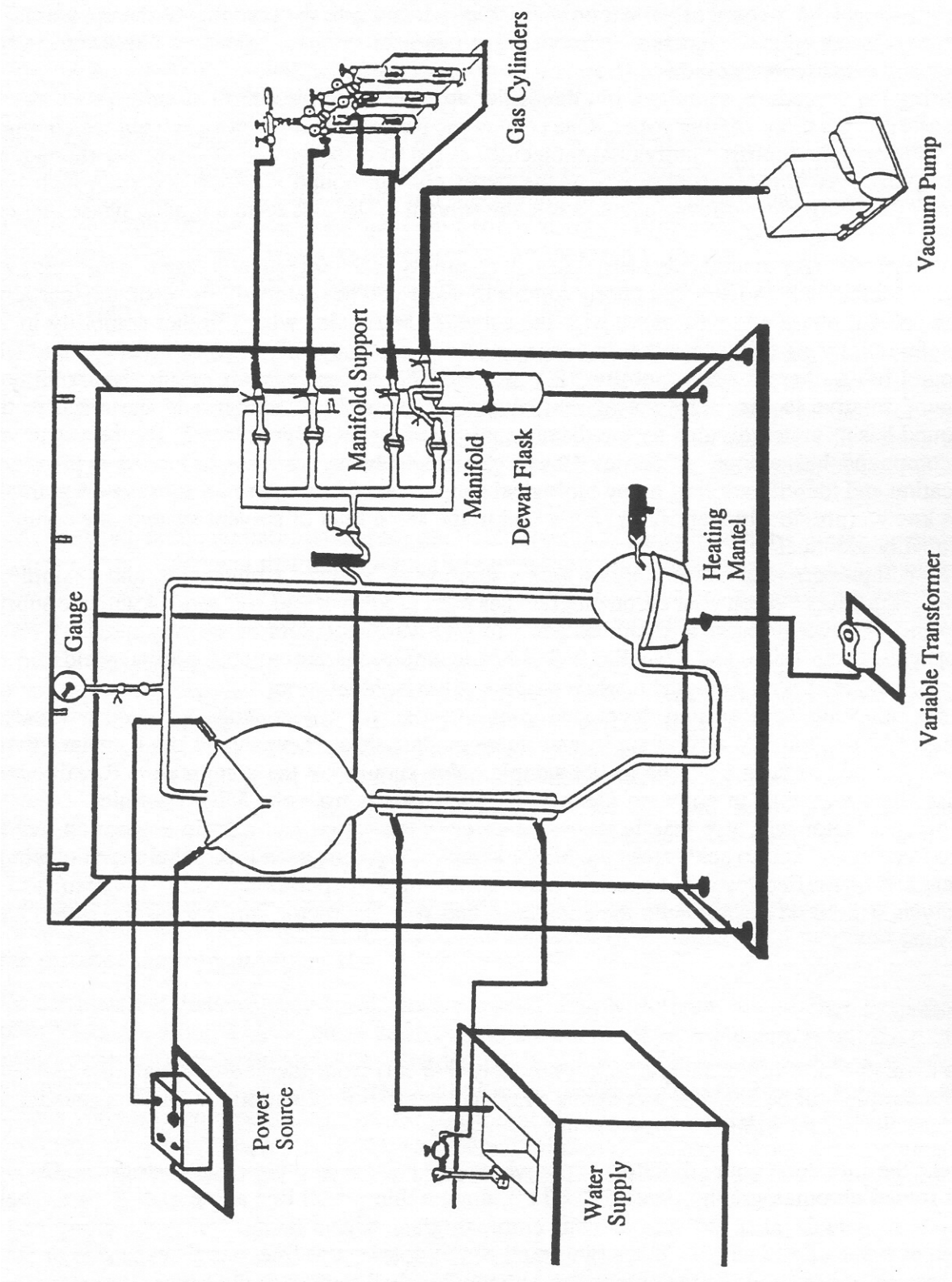

Figure 5.2. An assembled Miller apparatus.

You will use the method of paper chromatography to analyze the contents of the apparatus. The procedure is based upon the fact that different organic molecules have different solubilities in various aqueous and nonaqueous solvents.

During the procedure, samples from the Miller apparatus and a solution of amino acid standards are absorbed into a piece of filter paper. One edge of the paper is inserted into a solvent which migrates through the sample deposit. Individual molecules begin to dissolve and move in the (nonaqueous) solvent. However, the cellulose fibers of the paper contain bound water molecules which form a stationary (non-moving) aqueous phase, while the solvent molecules form a mobile phase that is less soluble in the water phase.

Each type of organic molecule has a different solubility in the solvent and water. Molecules which are highly soluble in the solvent but poorly soluble in water will be preferentially separated (partitioned) into the solvent phase and will move with the solvent. Molecules with a higher solubility in water will preferentially partition into the stationary water phase and will move at a slower rate. Each compound has a characteristic mobility (R_f) in a specific solvent system. R_f is the mobility of a compound relative to the migration of the solvent. That is, R_f is the ratio of the distance that a compound has migrated divided by the distance migrated by the solvent front. The observation that each compound has unique properties (R_fs in various solvent systems) has been applied to the purification and identification of many biological substances. If an unknown substance has the same R_f as a known (previously identified) compound in the same kind of solvent system, the compounds are probably identical.

You will prepare your chromatogram with a solution of "known" amino acids, and a sample from the Miller apparatus. When your chromatogram has been produced, you will spray it with the substance ninhydrin. Ninhydrin reacts with amino acids to give various colors of varying intensity for each type of amino acid (Hais and Macek, 1963). For example, the amino acid proline yields a yellow color when reacted with ninhydrin, while alanine gives a violet color.

After you have sprayed and developed your chromatogram with ninhydrin, you will calculate R_f values for the "known" amino acids, and then compare these R_f values to the R_f values that you calculate for the products from the Miller sample. You should use the comparative R_f value results, and also the similarities in color, to identify the amino acids from the Miller sample.

Your instructor will also briefly discuss a column separation and atomic absorption technique used to identify the amino acids from the Miller sample. We have used this technique to obtain more accurate and quantified results at specific intervals during an experimental run. The results of such an analysis will be on display with the apparatus, and your instructor will discuss the technique and the results.

Procedure

1. In a small vial, draw a sample (approximately 3–5 ml) from the Miller apparatus. *Be careful.* The sample will be hot, and it contains a high concentration of ammonia which is caustic. Also, when closing the stopcock do so gently.
2. Take the sample to your laboratory room where you will prepare your chromatogram. On a piece of folded chromatography paper (3" × 11"), draw a thin pencil line about 1/2" from the bottom. Also, in pencil, label the side of your chromatogram with a name from your group and your laboratory section number. Place two small pencil dots on this line, one on each side of the fold. Exercise care in handling the chromatogram; touch only the edges of the paper. From your sample vial extract some liquid with a pulled pasteur pipet. Spot this sample onto one of the dots. Blow dry the spot with a hair dryer until all water has evaporated. Repeat 10–15 more times, blow drying between each application. Label this side "sample". On the other pencil dot,

spot a single drop of a mixture of amino acid standards (this mixture should be labelled and at your table), and blow dry. Label this side of the paper “standard”.

3. Once the sample and amino acid standard mixture have been applied to the paper and dried, use a pencil to label the paper at the top with the names in your group. Your instructor will collect the chromatograms from each group and place them in a large plastic bag. They will then be placed in a refrigerator until your next lab period, at which time you will complete the analysis. Because it takes over 5 hours to develop the chromatogram, the lab preparators will start the chromatogram for you before you come to lab next period. The developing solvent is an 85% ethanol/water solution.
4. The first thing you should do at the beginning of the second lab period is to remove the chromatogram from the developing jar. The jars are located in metal wire baskets in the fume hood. Remove the chromatogram from the jar, and immediately mark the leading solvent edge with a pencil. You will need to know this position so you may calculate R_f values for the individual amino acids. To dry the chromatogram, hang it from the ceiling in the fume hood for approximately 15 minutes.
5. The next step in the preparation of your chromatogram is to spray it with the substance ninhydrin. Ninhydrin is a chemical that reacts with all free-amino groups of amino acids to give blue/violet colored products. Each type of amino acid reacts with ninhydrin to give a characteristic color. These specific colors should aid you in identification of the amino acids.

   Note: Your instructor will demonstrate how to spray your chromatogram. Be careful with the atomizer — it's fragile. All spraying should be done in the fume hood. Let the chromatogram air dry while hanging from clips provided on the drying rack of the fume hood. When it is dry to the touch, remove it from the clips. Place the chromatogram on a warm hot plate at a setting from 2 to 3, then set a pyrex dish on top of it. Note the order of spot development, and the spot colors. When a medium violet color is obtained with the standard, remove the chromatogram from the hot plate. Place the hot pyrex dish on an asbestos trivet when not in use. Use insulated gloves when handling hot glassware.
6. Calculate R_f values for the amino acid standards, and also for the spots produced from your Miller sample. Use the following formula: R_f equals the distance an amino acid migrated divided by the distance the solvent migrated.

To calculate the distance each amino acid migrated, simply measure the distance from the point of application, along a common vertical axis, to the center of each pigment spot. Each of these values (one for each spot) should be divided by the distance the solvent migrated, that is, from the point of sample application to the leading edge of the migrated solvent. Table 5.1 lists R_f values for some common amino acids you might find in your Miller sample, and the colors produced when those amino acids are reacted with ninhydrin. These values were obtained from running chromatograms on known amino acids using the same technique just outlined. List your results in Table 5.2.

Table 5.1. R_f values and ninhydrin stained colors of some common amino acids.

Amino acid	R_f value (EtOH)	Ninhydrin product color
Alanine	0.28	Violet
Aspartic acid	0.05	Blue-violet
Glycine	0.14	Red-violet
Proline	0.33	Yellow
Valine	0.47	Violet

Table 5.2. R_f and ninhydrin stain results from your Miller sample.

Amino acid	R_f value (EtOH)	Ninhydrin product color

Part 2: Chirality and the Role of Enzyme Specificity

If we examine the proteins of living organisms, we find that many of the amino acids that they contain are optically active. That is, when subjected to a plane of polarized light, the plane of light is rotated in a specific direction. But, if we subject a mixture of amino acids extracted from the Miller apparatus to the same polarized light, no optical activity is observed. How can we account for this difference between the two mixtures of amino acids?

The answer lies in the spatial configuration of the molecules. Some substances, referred to as chiral structures, can exist in two forms whose structures are nonsuperimposable mirror images of each other. The word chiral is derived from the Greek word *cheir* which means hand, hence we say molecules of this type display the property of handedness. When equimolar concentrations of each type are present, no rotation occurs because one form rotates the light in the dextro (right-handed) direction and the other in the levoro (left-handed) direction, thus cancelling the observable rotatory effect. If only one form, dextro or levoro, is present, then rotation occurs in only one direction, and the rotatory effect can then be observed. This phenomenon of optical activity is related to the stereochemistry of the compounds, that is, to the absolute configuration of the four different constituents in the tetrahedron around an asymmetric carbon atom.

All optically active organic substances may be related stereochemically to a single compound that has been arbitrarily selected to act as a standard for stereoisomers (substances whose molecules possess an identical structure but different arrangement of their atoms in space). Glyceraldehyde, the smallest 3-carbon sugar to have an asymmetrical carbon, is the standard reference molecule. The two possible forms of glyceraldehyde are, by convention, designated D and L. All amino acids

that are capable of optical activity in living organisms, excluding some of those found as constituents of cell walls and antibiotics, are of the L configuration.

An apparent contradiction seems to be emerging from the previous statements. If the original mechanism that produced the basic building block molecules of life resulted in an equal mixture of stereochemical forms, then why do we find only the L form of amino acids in organisms? What caused or brought about the original selection of L forms of amino acids from the "primeval soup", and what process maintained the original choice?

The phenomenon of chirality produces some of the most intellectually stimulating and challenging questions and ideas about the origin of life. Some investigators have suggested that chance played the major role in the original choice of one stereoisomer form over its counterpart. Others have taken a point of view that is 180° from the idea of chance, and believe that the original selection was preordained by the physical and chemical environment in which the selection occurred. Whatever the reason, whether chance, deterministic chemistry, or something in between, it is clear to all sides of the argument that once the original choice was made, and there were proto-life forms that carried out chemistry linked to a genetic code, that natural selection was the process that amplified the choice and maintained it. The question of which point of view is correct is beyond the scope of this course, but we will look briefly at how enzymes, acting through the process of natural selection, can maintain that original choice made some 3.8 billion years ago.

In the exercise today, you will investigate enzyme specificity as it relates to chirality, or in other words, the ability of an enzyme to specifically utilize the stereoisomeric forms of a compound. The reaction you will investigate is the bacterial deamination of the amino acid phenylalanine (an amino acid that is optically active) to phenylpyruvate (Figure 5.3). A deamination reaction simply removes ammonia from a substrate compound. The enzyme that brings about the reaction is L-amino acid oxidase. As its name implies, the enzyme works on amino acids that have the L configuration of the stereoisomers.

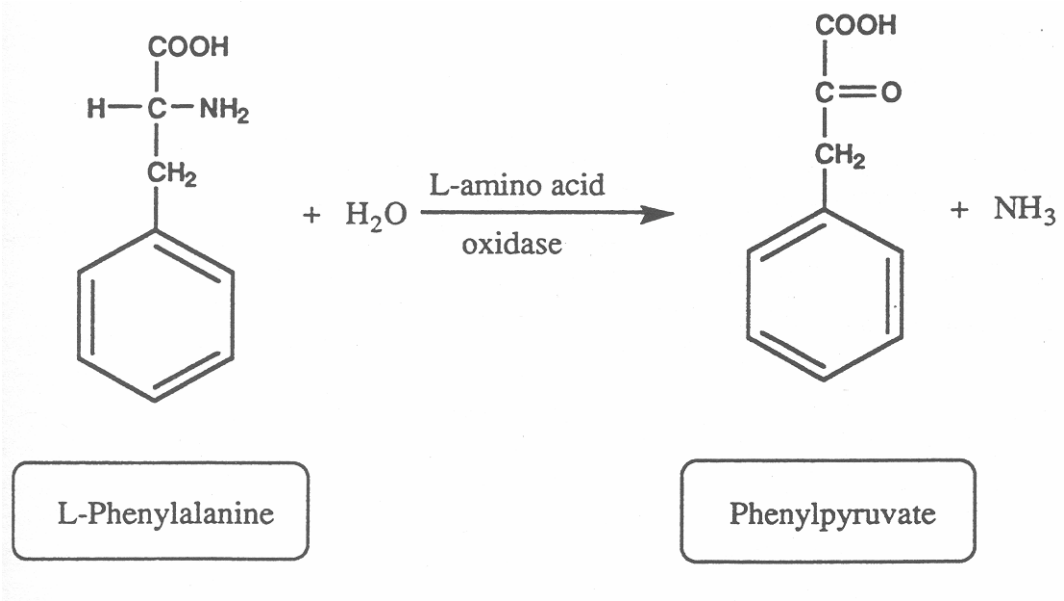

Figure 5.3. Deaminization of L-phenylalanine to phenylpyruvate by the enzyme L-amino acid oxidase.

Rather than do this reaction in a test tube using chemical extracts made from organisms, we will allow the enzymes inside a living bacterium, *Proteus vulgaris*, to catalyze the reaction. To do this, it is necessary to grow the bacteria on a substrate that contains food. One of the foods that *Proteus*

vulgaris uses is phenylalanine. This suggests that we should be able to prepare bacterial substrates that contain the two optical forms of phenylalanine, and then grow the bacterium on these plates to see if the organism can utilize (i.e., catalyze) the different stereoisomeric forms of phenylalanine. The test to check whether or not the reaction was carried out by the bacterial enzymes is a rather simple one. A few drops of a 2% solution of ferric chloride ($FeCl_3$) are placed on the bacterial plates after the bacteria have had a chance to grow. If deaminization has occurred, the drops of ferric chloride will turn green.

Procedure

Note: Bacterial culture plates have been prepared for you in advance of your lab. There are three types of plates: one with L-phenylalanine, one with D-phenylalanine, and the remaining one with a combination of D-phenylalanine and L-phenylalanine. The types of plates are labeled with the numbers 1, 2, or 3. You will not be told which number corresponds to which type of substrate; that is for you to discover.

1. Each group of four students should obtain two culture plates of each type of substrate. *Caution:* Do not remove the covers from the culture plates; doing so will contaminate the plates.
2. Your instructor will demonstrate the sterile technique that is necessary for you to follow while inoculating your plates. Failure to follow these directions carefully will most surely result in a botched experiment. You will be using an open flame so use caution.
3. After your group has inoculated your plates, stack them and bundle with tape. Label the tape clearly with your name, laboratory section, time inoculated, and date. Place your plates in the culture rack holders from which you originally obtained your plates.
4. Your plates will be refrigerated for one day and then transferred to an incubator set at 37°C. The plates will then be incubated for 24 hours to promote growth.
5. When you return for your next laboratory class, collect your plates from the culture rack holders. Remove each cover from your plates, and add a few scattered drops of ferric chloride solution to the areas of growth you observe. After you have added the drops of ferric chloride, watch for a color change. A green color indicates that deaminization has occurred. No color change means no reaction has taken place.

Table 5.3. Color change on experimental bacterial plates after the addition of ferric chloride.

Plate type	Color change (+ or -)	Type of substrate (D, L, or DL)
1		
2		
3		

Notes for the Instructor

General Description of the Miller Apparatus

The Miller apparatus is a device that allows water vapor and reducing gases to circulate through a closed glass system while passing a high-energy spark through the gas atmosphere (see Figure 5.2). A heating mantle supplies the thermal energy needed to cause circulation of gases within the system. Heated water vapor and gases are driven up glass tubing from the boiling flask and around to a reaction vessel. When the gases reach the reaction chamber, they pass near a high energy spark, which is generated between two electrodes. Organic compounds are produced in the area of the spark, and are subsequently dissolved into a condensed liquid phase with the aid of a water condenser. The condensed organics fall into a U-shaped area of the apparatus, the purpose of which is to permit circulation in only one direction. The organic compounds eventually flow into the boiling flask where they will be concentrated, and heat-induced secondary reactions may occur. Paper chromatography or column separation may be used to assay for the presence of amino acids in the boiling flask.

The glass portion of the Miller apparatus must be custom-made by a skilled glass blower. It will be necessary for a craftsman to anneal the glassware and coat the electrodes with uranium glass. Usually, only a university chemistry or physics glass shop is equipped to handle such tasks. The apparatus may be assembled in any reasonable physical size or dimension.

Critical to the proper function of the apparatus are the types of stopcocks used to completely seal the apparatus. We recommend a stopcock that seals first with a teflon surface and has a single O-ring as a secondary seal. Stopcocks that have an O-ring as their primary seal tend to swell, and eventually crack the glassware due to a reaction with ketones produced in the apparatus. In addition, the type of pressure gauge used can be critical to the function of the apparatus. We recommend a stainless steel, ammonia-resistant gauge, which is attached to a Covar-Pyrex gauge connector. Stainless steel couplings should be used throughout the connector sleeve, as they do not react with ammonia gas or organic products.

Several other major pieces of equipment are needed to operate the system. A heating mantle, which must be regulated by a variable transformer, is needed to circulate the gases in the system. The high energy spark between the electrodes is produced by a solid state spark generator. We do not recommend the use of Tesla coils to generate the spark because they do not withstand the 4–5 weeks of continuous use. High voltage wires, threaded through Tygon tubing for added insulation, are used to connect the spark generator to the electrodes. A water flow regulator and backflow

valve must be used with the water condenser. A vacuum pump that is driven by a sealed explosion-proof motor is used to evacuate the system of oxidizing gases.

Materials for Constructing a Miller Apparatus

Glassware

Boiling flask: 500-ml with round bottom

Condenser: standard, water-jacketed

Electrodes: 0.080 tungsten wire, uranium glass-coated, spaced from 1/2" to 3/4" apart at the tips

Gauge connector: Covar/Pyrex seal

Joints: 18/9 ball and socket joint

Liquid nitrogen trap: standard

Manifold: custom-designed with 4-mm pressure valves

Reaction chamber: 3000-ml flask with round bottom

Stopcocks: Ace 0-8 teflon valve with single O-ring

Glass tubing: 5/8", medium-walled

Accessory Parts

Aluminum frame: 1/2" aluminum flexframe rod, supports glassware, holds shield in place

Clamps: (1) ball joint 18/9 (used to secure glass ball and socket joints, Fisher #05-885D); (2) chain, adjustable (used to mount the Dewar flask around the trap); (3) extension ring support (holds heating mantle beneath the boiling flask); (4) fork, versatile, medium-sized (supports glassware within the aluminum frame); (5) lattice connectors (hold aluminum rods together in frame, anchors fork clamps in place)

Dewar vacuum flasks: one mounted to enclose liquid nitrogen one used for transporting liquid nitrogen

Fiberglass insulation for boiling flask and glass tubing: prevents premature vapor trap, for boiling flask and glass tubing: prevents premature vapor condensation.

Gases: anhydrous ammonia (99.99% min), hydrogen (Prep. 99.99% min), and methane (C.P. 99.0% min), dispensed from lecture bottles with appropriate flow rate gauges/regulators (Matheson gauge regulator model 3320 for hydrogen and methane, and model 3332, for ammonia)

Gauge (Industrial Duralife Pressure Gauge): stainless steel, ammonia resistant, (Ashcroft, size: 2 1/2", type: 1009 S02L, range: -30 IMV to 60 PSI), threaded onto a sweat tube-to-pipe connector, previously silver soldered onto the covar piece, and made air tight with teflon pipe tape, and further sealed by melting Apeazon W into joint

Grease: silicone lubricant, high vacuum grease, Dow-Corning (Fisher Scientific)

Heating Mantle: sized according to boiling flask, connected to the voltage regulator.

Manifold support: custom designed, 1/4" clear acrylic sheet with drilled holes, springs and 1/2" plastic dowel ears, springs and manifold valves secure manifold in support, support held in aluminum frame with fork clamps clamped to dowel ears

Mounting board: board for mounting the aluminum framework with flex frame support flanges

Shield: custom designed, 1/4" clear acrylic sheet with hooks, hung on face of aluminum frame, held in place at the bottom with wing nuts and metal dowel

Spark generator: solid state induction coil (Electro-Technic Products Company #ID 200), produces 1/2" to 2" spark and plugs into 115 volt line

Temperature regulator: variable autotransformer (Staco Energy Products Co. type 3PN1010) input 120V, 50/60 Hz; output 0–120V/140V

Tubing: (1) amber 1/4" ID × 1/8" wall, short length (for loading distilled water through lower port); (2) black 3/8" ID × 3/32" wall (for connecting condenser to water supply, draining condenser); (3) Tygon 5/16" ID × 3/32" wall (for additional insulation of current-carrying wires); (4) vacuum 3/8" ID × 1/4" wall (connects gas tanks to manifold and vacuum pump to liquid nitrogen trap).

Vacuum pump: sealed, explosion-proof motor, capable of evacuating apparatus to -30 inches of mercury (Hg)

Water Flow regulator: Watts model 3-26A

Wire: 16 gauge, rubber insulated high voltage wires connecting spark generator to electrodes, threaded through 5/16" Tygon tubing for additional insulation

Assembling the Apparatus

The apparatus is assembled and loaded with gases 5 weeks in advance of the lab exercise. One week is allowed for trouble shooting, and the remaining 4 weeks are required to obtain adequate quantities of amino acids for assay.

The first step in assembling the apparatus is the construction of an aluminum frame in which to secure the glassware. It should be built on a stable platform, and built in such a fashion as to surround the glassware from all sides. No specific dimensions for the frame are given because it will have to match the overall dimensions of your customized glassware. The Miller apparatus must be clean before securing it to the frame. Attach all fork clamps to the frame and open them as wide as possible. Two people may be needed for this operation. Carefully insert the glassware in the center of the frame, and close the fork clamps gently. When the glassware is supported, ease the heating mantle under the boiling flask and support it with an extension ring support. *Caution: Never use excessive force while assembling or securing the apparatus.*

Next, assemble and secure the gas manifold. Grease and assemble the four pressure valves of the manifold, using care to ensure that grease does not clog the valve ports. Mount the manifold into the acrylic support by stretching and securing small springs over the glassware.

Now, attach and secure both the gas manifold and the pressure gauge to the apparatus in the aluminum frame. Grease the male ends of the ball and socket joints with a thin film of grease. Attach the ammonia resistant gauge to the upper stopcock, and secure the connection with a pinch clamp. Next, carefully move the manifold support with its attached glass manifold into place. Anchor the manifold support to the frame with fork clamps which attach to the plastic dowel ears on the acrylic support. Secure the ball and socket joint with a pinch clamp. Grease each ball and socket joint on the gas manifold and attach the gas lines, securing each with a pinch clamp. To eliminate strain on the manifold, each gas line should be supported by a fork clamp prior to actual attachment of the lines to the manifold. Now, attach all gas lines to their respective gas sources.

Attach an adjustable chain clamp around the Dewar flask, and secure it to the frame in a position near the vacuum line of the gas manifold. Connect a short glass spacer, with greased ends, to the vacuum line of the manifold, and secure it with a pinch clamp. Next, insert the liquid nitrogen trap into the Dewar flask and attach and secure it to the short glass spacer. All ball and socket

connections should be adequately greased. Before attaching the vacuum line to the nitrogen trap, secure it to the frame with a fork clamp to prevent undue stress on the glassware. The water lines for the condenser may be attached next. The water flow regulator and back flow valve are used to ensure a constant flow of water through the condenser.

Thread the high voltage wires through Tygon tubing, for additional insulation. Attach two fork clamps to the aluminum frame, above the reaction chamber, in alignment with the electrodes. These clamps are used to secure the wires prior to attaching them to the electrodes. This is a critical step because any undue stress on the electrodes will almost assuredly crack the glassware. We also suggest that a short section of vacuum hose be added as an extra layer of insulation between the high voltage wires and the fork clamps. Gingerly attach the wires to the electrodes. Attach a ground wire from an electrical outlet to the aluminum frame. Finally, wrap fiberglass insulation around the top of the boiling flask and the glass tubing leading to the pressure gauge. This helps prevent premature condensation and facilitates

Filling the Apparatus

When the apparatus is properly connected to the gas cylinders and vacuum pump, the system is ready to be loaded with water and gases. Begin by closing the lower and middle stopcocks and all of the pressure valves of the manifold. Temper the Dewar flask with a small quantity of liquid nitrogen. When the flask has been tempered, fill it with liquid nitrogen. Turn on the vacuum pump and *slowly* evacuate each section of the apparatus, beginning with the manifold and gas lines. Do not allow moisture-laden air to pass too quickly through the trap, as moisture and corrosive gases may harm the pump. Evacuate the system until the pump quiets, and the gauge needle has ceased its downward movement (about -29 to -30 inches mercury). Close all stopcocks and valves. Hold the system in this state for at least 2 hours to be certain that no leaks exist.

When the system has no leaks, distilled water may be added. Water is drawn into the system through amber tubing connected to the port on the barrel of the lower stopcock. Fill the boiling flask until the water is level with the top of the U-shaped trap. Turn on the heating mantle and boil the distilled water for a few minutes to release any dissolved gases. *Note: Boiling occurs at a lower temperature when the system is under vacuum.* Turn the mantle off and allow the water to cool before slowly reevacuating the apparatus. *Make certain that the liquid nitrogen Dewar is full each time the vacuum pump is used.*

The gases may now be loaded into the system. The desired gas ratio is 20% hydrogen, 40% methane, and 40% ammonia. *Note: This process may require two people.* Begin by evacuating the hydrogen line. Close off the vacuum line at the pressure valve and open the main valve on the tank, then the regulator valve, followed by the pressure valve on the manifold. Slowly open the middle stopcock while monitoring the pressure gauge. Assuming that the original vacuum was -30 inches mercury, load hydrogen until the gauge reads -25 inches mercury, and then close the stopcock. Close off the main hydrogen valve, and reevacuate the manifold and the hydrogen gas line. In a similar manner, add methane next, then add ammonia. Load the equivalent of 10 inches mercury for these two gases. It is important that the manifold and gas lines be evacuated before and after each gas is loaded. A filled system should register approximately -5 inches mercury. This slight vacuum at the onset of the run helps prevent excessive pressure build-up during the run.

For added safety, a protective acrylic shield is hung on the aluminum frame. It is secured with a metal dowel and two wing nuts anchored to the lower aluminum rod.

Starting the Apparatus

When the water and gases are loaded, the system may be turned on. The variable transformer should be adjusted so that a continuous, gentle boil is achieved, Generally, a setting of 45–60% of maximum is sufficient. The flow of tap water through the condenser should be adjusted for maximum condensation. When the water in the boiling flask reaches a boil, clouds of vapor should be seen pulsating through the reaction chamber. Also, small rivulets of condensate should be seen running down the reaction chamber and through the condenser into the U-shaped trap. When the system has fully warmed and the pressure of the system has stabilized, the spark may be started. *Caution: Make certain that the aluminum framework is grounded prior to turning on the spark.* Adjust the output on the spark generator to the minimum voltage capable of maintaining a continuous spark across the electrodes. The polarity should be switched regularly to prevent disproportionate wear of the electrodes.

After a few hours, the water in the U-tube should take on a faint yellow color. This indicates that organic compounds are being formed. Within a few days, glycine and alanine should be present in detectable amounts. In approximately 1 week, a brownish-black liquid may collect at the stopcocks. The upper stopcock should be kept clear of liquid because liquid may interfere with accurate pressure readings. After a few weeks, the liquid in the boiling flask should be amber to dark brown.

The pressure of the system may vary during the course of a run. From a starting pressure of -5 inches mercury, the pressure may rise to nearly 15 psi and then recedes to about 10 psi for the remainder of the run. *Caution: The pressure should not be allowed to exceed 15 psi because the apparatus is not designed for higher pressures.*

Extracting a Sample

At the end of a 4-week run, our students tap a sample from the boiling flask. The shield need not be removed for this purpose, nor is it necessary to turn off the spark. Simply connect a short length of amber tubing to the port on the barrel of the lower stopcock, and open the stopcock by a partial turn. A few milliliters of liquid sample are ample for analysis using paper chromatography. At the end of each run, we freeze about 200 ml of sample for further analysis with an Amino Acid/Peptide Analyzer.

Cleaning the Apparatus

After a run is completed and samples have been collected, the apparatus should be promptly cleaned. First, drain all remaining liquid from the boiling flask, keeping the lower stopcock open until the pressure gauge reads close to zero. Next, carefully detach all glass joints and loosen clamps. Remove the main glass piece from the frame, and carefully rinse it with water. We then take the main glass piece to a glass shop where it is cleaned with 10% hydrofluoric acid solution, followed by an annealing process in an oven. The annealing process removes any remaining hydrofluoric acid and residual organic compounds. A final rinse with distilled water may be required to rid the apparatus of dry particulate residue. The manifold pressure valves can be degreased with acetone. *Caution: Do not use acetone near the stopcock O-rings because it will degrade the rubber.* Finally, empty the liquid nitrogen trap of any condensed liquid. The apparatus is now ready for reassembly and a fresh run.

Trouble Shooting

1. *Aluminum frame charged:* This occurs when the wires from the power source rest on the frame, the wires are inadequately insulated, or aluminum foil is used to insulate the boiling flask. Correct the problem first by grounding the frame. To further insulate the wires, thread them through Tygon tubing, and hold them away from the frame with fork clamps. The Tygon-clamp contact may need to be further insulated with a short length of vacuum hose.
2. *Apparatus humming:* This may indicate a crack in the glass of the apparatus and should be checked. If the system is under a vacuum, a Tesla coil can be used. A bright white line will illuminate the pathway of molecules into the system. The most likely area for cracks to develop is the area around the electrodes, because the metal and glass expand at different rates with temperature fluctuation. When the system is loaded and under a vacuum, the situation is potentially dangerous, as hydrogen and in-rushing oxygen will explode in the presence of a spark.
3. *Spark not jumping/power supply buzzing*: This may indicate that the gap between the electrodes is too large, or that the power supply is weak or wearing out, or that there may be a short in the system. A likely location for a short is at the glass-electrode junction.
4. *Vacuum not holding:* The inability to hold a vacuum suggests a leak in the system. First, determine which area(s) is at fault. Begin by pulling a vacuum on the entire system and then close off each stopcock and valve. Leaks in the glassware should be detectable with a Tesla coil. If a stopcock is at fault, replace the O-ring and/or grease the tapered end. However, greasing stopcock ends does introduce a contaminant into the system, and, thus, should be avoided whenever possible. A leak at the lower stopcock inlet may be corrected by plugging the port. If the gauge region is at fault, it may be that the threaded connection is not air-tight, or that the soldering has become corroded. If the former is true, wrap one end with teflon tape, re-thread the connection, and then drip hot Apeazon W into the crack.
5. *Gauge connector fills with liquids:* This is easily corrected by screwing the upper stopcock into and out of the barrel. It is not advisable to let these liquids build up near the gauge, because it makes it impossible to measure the pressure accurately.
6. *Pressure is excessive:* The pressure may be moderated by increasing the water flow through the condenser, or by turning down the variable transformer. If this fails, water vapor and gases may be released into the trap through the middle stopcock. However, this undoubtedly alters the chemical make up of the system, and should be done only as a last resort. It is advisable to fill the system with less gas at the onset if excessive pressure is a recurrent problem.

Cautionary Notes

1. Before loading the gases and turning on the system, make absolutely certain that there is no gaseous oxygen present, otherwise an explosion might occur. Follow the instructions for evacuating the system and loading the water and gases carefully.
2. Do not let the system pressure exceed 15 psi. The glassware and joints are not designed to withstand high pressures.
3. Do not use stopcocks that use an O-ring to provide the first seal. Though this type of stopcock may provide an adequate seal at the onset, the O-rings will react with ketones produced during a run, causing the O-rings to swell and crack the glass barrels of the stopcocks.

General Notes for Part 1

Lab preparators pre-cut the chromatography paper into 3" × 11" strips and crease the paper, lengthwise, to provide the dividing line between the sample and the standard when the chromatograms develop. The crease also provides a means of holding the chromatogram upright during the developing process.

Fire-pulled 9" pasteur pipets are used for spotting the sample and the standard. These are provided in numbers of 2 per lab station (12 per room). The pipets are prepared by heating the tips over a bunsen burner flame until the glass is melted. The tip is then extruded to a very fine tip. Pipet bulbs should not be used to draw up solution. Capillary action should be sufficient to load the pipet. It is crucial that students dry the chromatography paper between application of sample spots. For this purpose, we provide one hair dryer per lab station (six per lab room).

Once the chromatography papers have been spotted, dried, and labelled, students place them in a plastic bag for storage until Part 2 of the exercise. Because the developing process requires approximately 5 hours, the lab preparators collect the chromatography papers and place them in a cold room (2–4°C) until they are needed. The papers are stored until 5 hours prior to the next laboratory period. At that time, preparators start developing the chromatograms by placing the spotted chromatography papers in the developing chambers.

The second activity during this first period is an investigation into the ability of bacteria to utilize stereoisomers of phenylalanine. More specifically, students inoculate bacterial culture plates with *Proteus vulgaris* bacteria. For this, we provide three inoculation stations per room, including: bunsen burners, inoculating loops in holders, capped tubes of *Proteus vulgaris* culture (in broth) in test tube racks, and a flint (or matches). We also supply three types of plates for inoculation: D-phenylalanine plates, L-phenylalanine plates, and D,L-phenylalanine plates. For each lab section, preparators set out 12 of each plate type using a total of three petri plate racks as holders. (*Note*: Do not label plates with the type of phenylalanine it contains. Instead, label them 1, 2, or 3 so that the students themselves must determine the phenylalanine stereoisomer(s) present in each plate.) Three additional petri plate racks are provided for collecting the plates following inoculation. The preparators collect the inoculated plates following the first lab period, then store them in a refrigerator until 24 hours before the second lab period. At that time, they are transferred to an incubator set at 37°C.

General Notes for Part 2

During Part 2 of the exercise, student groups obtain their chromatograms from the developing chambers, hang them up to dry in the fume hood, spray them with ninhydrin solution, and heat the chromatograms to accelerate the ninhydrin color reaction. Students then use the chromatograms to calculate R_f values for each amino acid. In addition, students collect incubated plates and examine them for ferric chloride reactivity.

One basket of six developing jars is used per lab section. To each developing jar is added approximately 25 ml of solvent (85% ethanol). Five hours prior to Part 2, preparators remove the spotted chromatography papers from the cold room, staple the top corners, and carefully place them in the developing jars. The developing jars (plastic, wide-mouthed jars, 12" tall × 2-3/4" in diameter) have been previously wired upright into a metal basket for greater stability.

When students come into the lab, they are instructed to proceed directly to the fume hood, remove their chromatogram, mark the solvent front, and hang the chromatogram from the drying rack in the fume hood. The drying rack consists of a metal ring stand, to which a metal dowel crossbar, lined with hooks, is clamped. Several pinch clamps are hung on the hooks of the metal crossbar. It is crucial that the chromatograms are dried completely both before and after they are sprayed with ninhydrin.

Next, students spray their chromatograms with a 2% ninhydrin solution (in acetone). *Note: Ninhydrin is a poison; extreme caution should be used during this step.* We provide disposable gloves for students to use during spraying. Also, prior to this second lab period, preparators tape sheets of brown packaging paper onto the fume hood surfaces. This is done to minimize ninhydrin contamination in the fume hood area.

A two-piece, glass atomizer is used to spray the chromatograms. We use a long segment of amber tubing to connect the atomizer with a compressed air source. In order to prevent accidents, preparators secure the entire length of tubing with tape. This reduces the risk of students pulling on the tube and breaking the glass atomizer. Two students should cooperate in the spraying process, one to hold the atomizer and one to control the air flow. Best results are obtained when a steady, even spray is used and the chromatogram is completely wetted down, but not totally saturated. After each chromatogram has been sprayed, it is essential that they be hung up on the drying rack until they are completely dried.

When the chromatograms are dry (approximately 15 minutes), they can be moved to the hot plate developing station. We provide two such stations per room: hot plate, pyrex dish (8" square), asbestos trivet (approximately 10" square), and a pair of insulated gloves. When bright colors are visible, students may use metric rulers (one per student station, six per room) for measuring the migration of individual amino acids.

For the chirality experiment, we provide a 2% aqueous ferric chloride solution in 250-ml glass-stoppered bottles with holstered syringes (two bottles per room). In addition, the previously incubated culture plates (24 hours at 37°C) are set out in petri plate racks. Since the total amount of each stereoisomer of phenylalanine was held constant when the culture media were prepared, the predicted results are:

L-phenylalanine medium: green color reaction
D-phenylalanine medium: no color reaction
D,L-phenylalanine medium: pale green color reaction (1/2 the concentration of L-phenylalanine)

At the end of the lab, the bacteria should be killed before they are discarded. The bacteria in culture broth are put into a water bath and boiled for about 10 minutes before discarding. The bacteria on the plates are destroyed by autoclaving for 10 minutes at 15–20 psi.

Acknowledgements

I would like to thank Bill Radmer, Kim Person, Amy Mundelius, and Don Anderson for helping with the design of the Miller apparatus and development of the experimental protocol.

Literature Cited

Oparin, A. I. 1924. Proiskhozhdenie Zhizni (The origin of life). Izd. Moskovskii Rabochii (State Scientific Press Moscow Workers), Moscow. (In Russian)

———. 1938. The origin of life. Translation with annotations by Sergius Morgulis. Macmillan, New York, 270 pages.

Haldane, J. B. S. 1929. The origin of life. Rationalist Annual, 148:3–10.

Farley, J. 1977. The spontaneous generation controversy from Descartes to Oparin. John Hopkins University Press, Baltimore, 225 pages.

Miller, S. L. 1953. A production of amino acids under possible primitive earth conditions. Science, 117:528–529.

———. 1954. Production of some organic compounds under possible primitive earth conditions. Journal of the American Chemical Society, 77:2351–2361.

Hais, I. M., and K. Macek. 1963. Paper chromatography: A comprehensive treatise. Third edition. Publishing House of the Czechoslovak Academy of Sciences, Prague; Academic Press, New York, 955 pages.

APPENDIX A
Media and Solution Recipes

Amino Acid Standard

0.75 g glycine (Sigma #G-2879)
0.89 g alanine (Sigma #A-7627)
1.17 g valine (Sigma #V-0500)
1.33 g aspartic acid (Sigma #A-9256)
1.15 g proline (Sigma #P-0380)

Stir until dissolved. Refrigerate until use.

Solvent (85% Ethanol)

Combine 890 ml 95% ethyl alcohol with 110 ml distilled water. Stir.

Ninhydrin Solution

In a fume hood, dissolve 2g ninhydrin (Sigma #N-4876) in 1 liter acetone. Keep refrigerated.

Ferric Chloride Solution (2%)

Add 20 g $FeCl_3 \cdot 6H_2O$ (analytic grade) to 980 ml distilled water. Stir until dissolved.

Phenylalanine Plates

To 1 liter of distilled water add:

1 g phenylalanine*
3 g yeast extract
1 g Na_2HPO_4
5 g NaCl
20 g agar

Swirl until mostly dissolved. Autoclave this mixture for 20 minutes at 20 psi. Swirl again after autoclaving in order to thoroughly mix the ingredients. When cooled to approximately 60°C, pour into sterile plastic petri plates. Once the plates have cooled completely and the agar has set, store the plates in plastic bags in a refrigerator until needed. The plates can be stored for up to 1 year, if they are not contaminated.

*D,L-phenylalanine (Sigma #P-1876), label plates as #1
D-phenylalanine (Sigma #P-1751), label plates as #2
L-phenylalanine (Sigma #P-2126), label plates as #3

APPENDIX B
Addresses of Suppliers

Stopcocks, pressure valves: Ace Glass Incorporated, Vineland, NJ 08360, (609) 692-3333.

Ammonia resistant pressure gauge: Ashcroft, 400 West Lake St., Suite 318, Roselle, IL 60172, (312) 980-9030

Power source: Electro-Technic Products Co., 11222 Melrose Ave., Franklin Park, IL 60131, (312) 451-0150. Distributed through Central Scientific Co.

Atomizer, ball joint clamps, Dewar flasks, silicone grease, variable transformer: Fisher Scientific, 1600 West Glenlake Ave., Itasca, IL 60143, 1-800-942-4543.

Lecture bottles and flow rate gauges: Matheson Gas Products, P.O. Box 96, Joliet, IL 60434, (815) 727-4848

Chemicals: Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178, 1-800-325-3010

Variable transformer: Staco Energy Products (Dayton, Ohio); distributed through Fisher Scientific.

Water flow regulator: Watts Regulator Co., P.O. Box 628, Lawrence, MA 01842-1328.