Biology of the Cell

Laboratory Explorations

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Color photography of a sugar crystal by Phillip A. Harrington.

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Preface

This laboratory manual is designed to be used in conjunction with *Biology of the Cell: An Evolutionary Approach* by De Witt, but the experiments are appropriate to any course in general introductory biology, cell biology, or evolution. The purpose of these experiments is to examine the structural and functional characteristics of cells by stressing evolutionary principles; experiments demonstrate various mechanisms that may have been involved in the origin and subsequent evolution of living cells. Beginning with Miller's classic experiment on the synthesis of amino acids from a mixture of simple gases thought to be present in the primitive atmosphere, these experiments examine the production of protein-like polymers (proteinoids) from amino acids, the formation of morphological aggregates (microspheres and coacervates) that serve as models for the origin of cellular organization, the establishment of intracellular symbiosis as a possible mechanism for the origin of eukaryotic cells, and various adaptations of cell structure and function.

Many of these experiments have never before been adapted to classroom use. We have designed all experiments to require a minimum of equipment and expense. In addition, a Notes to the Instructor section gives complete details for preparing solutions, constructing specialized equipment, and running the experiment.

We are grateful for the useful — and sometimes devastating — criticism of these experiments over the last six years by students taking introductory cell biology at Williams College. A few of the experiments were developed initially by Samuel A. Matthews, Professor Emeritus of Williams College, and we are particularly appreciative of his help. We also wish to thank Judy Counter for patiently deciphering and typing the manuscript.

William De Witt
Eleanor Brown

Contents

SECTION 1

The Origin of Life

Man has long been intrigued by the problem of life's origin on earth. Early beliefs in the spontaneous generation of living forms were dealt a serious blow in the seventeenth century by Redi's experiments, which proved that maggots, instead of arising spontaneously from rotting meat, originated from eggs laid by flies. But the idea that bacteria and other microscopic forms of life arose spontaneously from non-living matter still persisted until it was unequivocally disproved by Pasteur in 1862.

Pasteur's work led to the consideration of alternate mechanisms by which life may have arisen on earth. One of these was the mechanistic theory, strongly influenced by Darwin's concept of biological evolution. It contended that the first primitive living system arose from the chance aggregation of a variety of non-living chemical molecules, and that this organized structure, capable of reproducing, gradually evolved into more complex forms. This explanation is now generally considered unsatisfactory, because it depends upon one highly improbable event — the chance accumulation in one particular place and at one particular time of a large number of extremely complex organic molecules, followed by the aggregation of these molecules into a living form.

Most biologists currently favor the materialistic theory of life's origin, which was originally proposed independently by A.I. Oparin and J.B.S. Haldane in the 1920s. The materialistic theory contends that life arose as a result of a long process of chemical evolution, beginning with the formation of simple organic compounds from the gaseous components of the earth's primitive reducing atmosphere, using energy supplied by ultraviolet light, electrical storms, and other sources. It is proposed that these compounds accumulated in the oceans and, by reacting with one another, gradually increased in complexity, creating a "prebiotic soup" in which multimolecular aggregates formed. Some of the aggregates obtained a capacity for primitive reproduction, and eventually evolved into the first living cellular systems.

In recent years, biologists have attempted to test this hypothesis in the laboratory. Using experimental conditions that simulate those that may have existed on the primitive earth, they have performed a large number of experiments to see whether organic compounds characteristic of living systems can be formed. In addition, they have studied the tendency of organic compounds to aggregate into discrete entities that might have served as primitive precursors to the first cells. This section of our laboratory explorations will be concerned with some of these experiments.

EXPERIMENT I

Synthesis of Simple Organic Compounds under Primitive Earth Conditions

In 1953, Stanley Miller, a graduate student of Harold Urey, published a paper that described the production of some simple organic compounds under environmental conditions that may have existed on the primitive earth. Miller subjected a mixture of gases (methane, ammonia, water vapor, and hydrogen) to spark and silent discharges for periods of about a week in order to determine whether organic compounds would be synthesized. Analysis of the products demonstrated small amounts of the amino acids glycine, alanine, aspartic acid, and glutamic acid, as well as a wide variety of other simple organic compounds including formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, urea, and others. Since amino acids are the building blocks of proteins, constituents of all living cells, Miller's results demonstrated a feasible mechanism by which biologically important organic compounds may have been synthesized on the lifeless prebiotic earth.

This exercise involves the characterization of amino acids obtained in an experiment similar to that described by Miller.

THEORETICAL BACKGROUND AND EXPERIMENTAL DESIGN

Spark Discharge Apparatus

The spark discharge apparatus used in this experiment (Fig. 1-1) is in principle similar to that of Miller, but is of simpler design. It consists of a glass chamber which can be made airtight by closing the access port (*A*). Two electrodes (*B*), between which a spark can be continuously generated by means of a Tesla coil, are situated at the upper end of the chamber. The chamber can be filled with any desired mixture of gases, and, in addition, water vapor can be generated inside the chamber by boiling water in the bottom of the chamber (*C*). Excess water vapor is condensed in the region of the condenser (*D*).

The spark discharge apparatus will be operating during the week prior to your laboratory period; you should observe its operation during that time. The apparatus was set up by your instructor in the following way. Prior to use, the apparatus was thoroughly cleaned and sterilized to prevent internal bacterial contamination, which would introduce organic compounds into the apparatus. The chamber was evacuated with a mechanical vacuum pump to remove air, because organic compounds are destroyed in the presence of oxygen. Through the access port, 100 ml of sterile distilled water, containing a few sterile glass beads to serve as boiling chips, was introduced. This was done by simply immersing the glass tube of the access port in the water; because there was a vacuum in the chamber, opening the access port sucked water and beads into the chamber, without letting any oxygen enter. Hydrogen, methane, and ammonia were then added at partial pressures of 100:200:200 mm Hg, again without letting oxygen into the chamber. Cool water

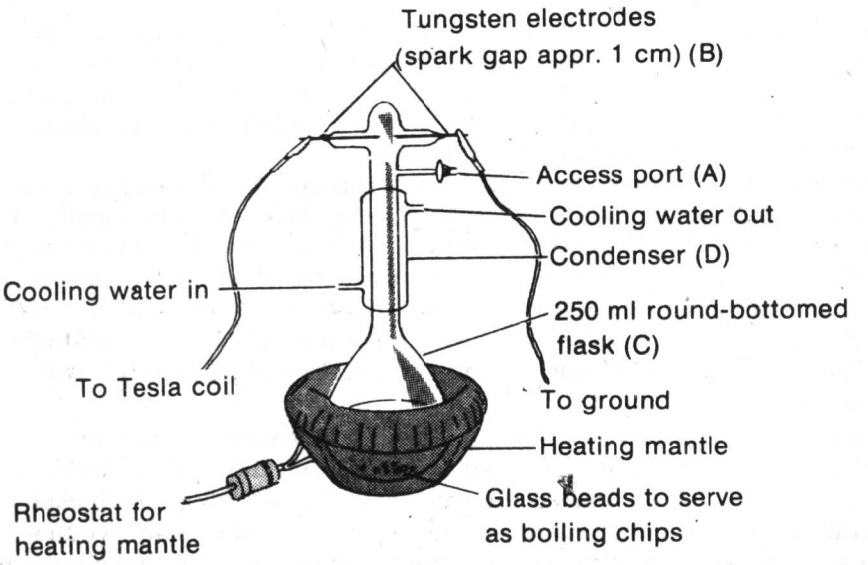

Figure 1-1. Spark discharge apparatus used in this experiment. (Adapted from C.L. Strong [1970], The amateur scientist. *Scientific American* 222:130.)

was circulated in the condenser, the water in the chamber was heated to boiling, and sparking was begun.

When the apparatus is operating, the four gases — hydrogen, methane, ammonia, and water vapor — surround the sparking electrodes. The spark, simulating the electrical activity of lightning storms on the primitive earth, provides an energy source for chemical reactions between the gases in the chamber. Condensation of the water vapor serves to wash the products of the reaction into the boiling liquid.

The sparking apparatus is run for approximately one week. At the end of this time, the reaction products are removed through the access port and refrigerated prior to use in this experiment.

Separation of Amino Acids by Paper Chromatography

Chromatography is a widely used technique for separating a mixture of organic compounds into its individual components. Separation by chromatography depends upon a process of differential migration of the various components of a mixture through a porous medium (stationary phase). There are many types of chromatography, but the principle is similar in each. In the simplest form of chromatography, a gas or liquid containing the mixture to be separated (mobile phase) is passed through the stationary phase, which has a different affinity for each of the components in the mixture. Hence, the component with the greatest affinity for the stationary phase is retarded by the stationary phase to a greater degree than is a component with less affinity. A separation of the components in the mixture is thus achieved.

In paper chromatography, which will be used for the separation of amino acids in this experiment, the stationary phase is a layer of water surrounding the cellulose fibers of a piece of filter paper (chromatography paper). The mobile phase consists of an organic liquid that is not miscible with the aqueous, stationary phase. The mixture to be separated is spotted on the chromatography paper and the mobile

phase is then allowed to migrate over the stationary phase by capillary action. Since the components in the mixture have different affinities for the two phases, the individual components will migrate through the stationary phase at different rates and hence will separate from one another. The principle of paper chromatography is illustrated in a simplified manner in Figure 1-2.

The liquid used in paper chromatography is called the *chromatographic solvent.* When the technique of paper chromatography was first developed, two-phase solvents were used, as mentioned above. The paper was first saturated with the aqueous phase, and the immiscible organic phase was then allowed to pass over the paper by capillary action. More recently, one-phase solvents consisting of the organic liquid saturated with an aqueous substance have been used with essentially similar results. Such a solvent, consisting of *n*-butanol, acetic acid, and water, will be used in this experiment.

In practice, a small volume of the mixture to be separated is usually spotted on a piece of clean cellulose chromatography paper and thoroughly dried. For good separation it is preferable to keep the spot small (about 0.5 cm in diameter). If the concentration of the components in the mixture is low, small volumes of the mixture are repeatedly spotted on the chromatogram and thoroughly dried between spottings. This prevents the area of the spot from increasing with each application.

The mixture to be separated (consisting of the liquid from the spark discharge apparatus) and each of five amino acid standards are spotted on the chromatography paper at intervals along a line drawn parallel to one edge of the paper (Fig. 1-3). The paper is fastened into a cylindrical shape and placed in a sealed glass chamber containing a small amount of chromatography solvent in the bottom of the chamber (Fig. 1-4). The chamber is covered with a black cloth to help prevent temperature fluctuations during the course of the chromatography. The solvent will move up the

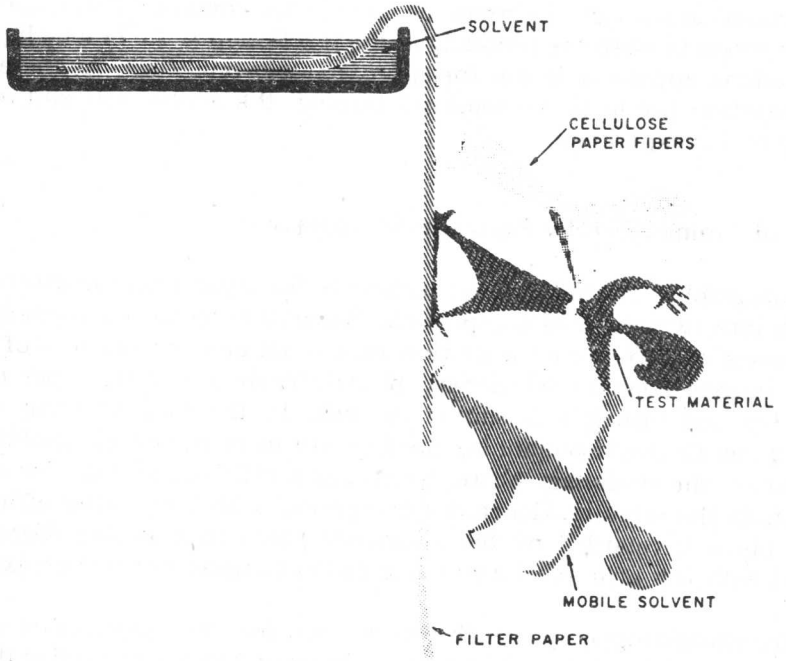

Figure 1-2. An extremely simplified, although not inaccurate, representation of the factors involved in paper chromatography. (*From* J. Sherma and G. Zweig [1971], *Paper Chromatography and Electrophoresis*, Vol. II, *Paper Chromatography*, Academic Press, Inc., New York, p. 9.)

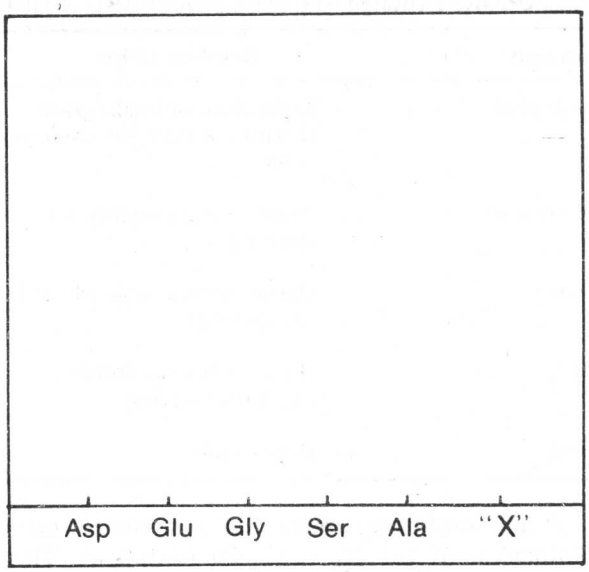

Figure 1–3. Preparation of the chromatography paper for spotting.

paper by capillary action and will separate the components of the mixture according to the principles described above.

After a specified period of time, the paper is removed from the chamber and dried. The amino acids are detected by spraying the paper (known as the *chromatogram* following chromatography) with a ninhydrin-cupric nitrate solution. This solution reacts with amino acids, producing a characteristic color for each amino acid. Table 1–1 on the following page shows the colors of the spots formed by the five standard amino acids used in this experiment.

The spots appearing on the chromatogram may be identified by this method in amounts as low as 1 to 2 μg. By comparing the spots obtained from the liquid in the spark discharge apparatus with those from the amino acid standards, one should be able to identify, at least tentatively, some of the amino acids produced in the spark discharge apparatus.

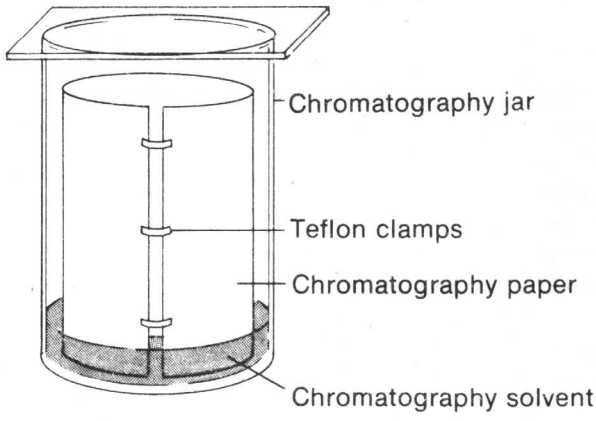

Figure 1–4. Sealed glass chamber containing chromatography paper fastened into a cylinder.

TABLE 1-1. COLORS FORMED BY FIVE STANDARD AMINO ACIDS

Amino acid	Reaction Color
Aspartic acid	Light blue, or bright green if removed from the oven too soon
Glutamic acid	Purple, fading slightly on standing
Glycine	Orange brown, with a bright orange ring
Serine	Greenish brown, forming a ring on standing
Alanine	Dark purple

Another means of differentiating among various compounds on a paper chromatogram is by determination of the R_f value for each spot. This value represents a ratio between the distance traveled by the compound divided by the distance traveled by the solvent front.

$$R_f = \frac{\text{distance traveled by spot}}{\text{distance traveled by solvent}}$$

Materials

Chemicals

L-aspartic acid (chromagraphically pure; 2 mg/ml in 0.3 N HCl)
L-glutamic acid (chromagraphically pure; 2 mg/ml in 0.3 N HCl)
Glycine (chromagraphically pure; 2 mg/ml in 0.3 N HCl)
L-serine (chromagraphically pure; 2 mg/ml in 0.3 N HCl)
L-alanine (chromagraphically pure; 2 mg/ml in 0.3 N HCl)
Liquid obtained from spark discharge apparatus (made 0.3 N in HCl)
Chromatography solvent (*n*-butanol:water:glacial acetic acid, 4:5:1)
Ninhydrin-cupric nitrate indicator solution

Equipment

Whatman #1 chromatography paper (8¾ × 8¾ inches)
Waxed paper
Ruler
Capillary tubes
Filtered-air jet
Teflon clamps
Chromatography jar and cover
Vaseline
Metal clamp
Plastic gloves

Shared Equipment (Available in the Laboratory)

105°C oven
Atomizer in hood for spraying chromatograms with indicator solution

EXPERIMENTAL PROCEDURE

1. Obtain a piece of Whatman #1 chromatography paper measuring 8¾ inches square. *Use extreme care in handling this paper in order to avoid contaminating it with amino acids from your hands.* The chromatography paper should be handled only by its edges, and the top of your laboratory bench should be covered with a piece of waxed paper before you set the filter paper down.

2. On the filter paper, lightly draw a starting line in pencil (not ink) one inch from and parallel to the bottom edge of the paper. Mark a dot along the starting line 1¼ inches from the left hand edge of the paper; then, moving to the right, mark successive dots every 1¼ inches along the starting line for a total of six dots.

3. Obtain solutions of the following five amino acids: aspartic acid (asp), glutamic acid (glu), glycine (gly), serine (ser), and alanine (ala).

4. With a pencil, label the dots on the filter paper according to the amino acid to be spotted.

5. Use a capillary tube to spot the chromatogram with the amino acid solutions. Make three applications of each amino acid, drying each application thoroughly with a filtered-air jet before respotting. It is essential that each spot be very small for good resolution (approximately 0.5 cm in diameter).

6. On the one remaining dot, spot the solution from the spark discharge apparatus. Make five applications, again being sure to dry the spot thoroughly after each application.

7. Using Teflon clamps, bring the side edges of the paper together so that they abut one another, but do not quite touch. The chromatogram should form a cylinder with the starting line downward. The bottom edge of the cylinder must be even, so that it will stand perfectly upright without tilting.

8. Stand the cylinder on its edge, starting line downward, in a chromatography jar containing about $^3/_8$ inch of chromatography solvent. **Plastic gloves should be worn when handling organic solvents.**

9. Place the lid tightly on the chromatography jar, using a light layer of Vaseline to make an airtight seal, and then cover the jar with a black cloth. Allow the solvent to migrate for 2 to 3 hours, or until the solvent front is about 1½ inches from the top of the paper.

10. *Wearing plastic gloves*, remove the chromatogram from the jar, being careful not to contaminate the paper with Vaseline from the lip of the jar. *Handle the chromatogram only by its edges.* Remove the clamps from the chromatogram and place it on your laboratory bench on top of a piece of waxed paper. Immediately mark the position of the solvent front with a pencil. Put a metal clamp on the top edge of the chromatogram and hang it to dry for 5 minutes in a 105°C oven.

11. Wearing plastic gloves, remove the dried chromatogram from the oven. Hold the chromatogram in a fume hood and *thoroughly* saturate the paper with the indicator solution containing ninhydrin and cupric nitrate. Your instructor will show how to do this. **Be sure to wear plastic gloves when you spray the chromatogram, as the indicator solution will stain your hands very badly.**

12. After spraying, place the chromatogram in an oven for 2 to 5 minutes at 105°C until colors are evident.

13. With a pencil, outline the spots on the chromatogram and note their colors, as the spots may fade with time. Then store the chromatogram between clean pages of a notebook to protect it as much as possible from light.

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LABORATORY REPORT SHEET

Experiment I

Name _____

Date _____

Laboratory Section _____

Part I: Synthesis of Simple Organic Compounds Under Primitive Earth Conditions

Data

1. Attach your original chromatogram, or a traced copy of it, to this report. Be sure to indicate the positions and colors of all spots, as well as the line of origin and the position of the solvent front.

2. Calculate the R_f values for the five amino acid standards used in the experiment. To determine the distance travelled by each spot, it is established procedure to place a dot in the estimated geometrical center of the spot, and to measure from the base line to this dot.

Aspartic acid

Glutamic acid

Glycine

Serine

Alanine

Discussion Questions

1. Do the R_f values of the standard amino acids differ enough so that, by this criterion alone, the amino acids may be unambiguously distinguished from one another? Explain.

2. By the characteristic colors and R_f values of the standard amino acids, determine which amino acids (if any) are present in the solution taken from the spark chamber. Explain.

3. R_f values tend to be a more reliable criterion for identifying amino acids in this experiment than colorimetric differences. Propose a reason for this fact.

EXPERIMENT II

Preparation of Proteinoids by Thermal Polymerization of Amino Acids

The experiments of Miller and others have yielded a wide variety of biologically important organic compounds including amino acids, carbohydrates, and nucleic acid derivatives (nitrogenous bases, nucleosides, and nucleotides). Using these compounds as starting materials, other researchers have tried to synthesize more complex molecules, again under conditions likely to have existed in prebiotic times. By heating mixtures of dry amino acids, S.W. Fox and his coworkers have produced protein-like amino acid polymers. They termed these compounds *proteinoids* to differentiate them from biologically synthesized proteins. Fox has postulated that the formation of proteinoids could have occurred on the primitive earth if water from the ocean, thick with abiotically produced organic compounds, had splashed onto hot lava, thus concentrating, drying, and heating amino acids at high temperatures.

This experiment is designed to demonstrate the production of proteinoids by thermal polymerization of amino acids.

THEORETICAL BACKGROUND AND EXPERIMENTAL DESIGN

The principles and use of spectrophotometry in biological research are summarized in Appendix A, and should be reviewed before you attempt to perform this experiment. In addition, you should know how to make solutions of specified concentrations by diluting a stock solution (Appendix B), and you should be familiar with the principles of buffering and pH maintenance (Appendix C).

Preparation of Proteinoids

Neutral amino acids (those possessing no net charge at physiological pH) will decompose if they are heated to temperatures greater than 150°C. However, in the presence of an excess of aspartic acid and glutamic acid (acidic amino acids which are negatively charged at physiological pH), dry mixtures of 16 other amino acids common to protein may be heated to temperatures of up to 200°C with a minimum of thermal decomposition. Proteinoids containing all 18 amino acids are produced, as well as some tars and other compounds. The proteinoids resemble naturally occurring proteins in a number of ways. Both proteinoids and proteins are composed of amino acids, display similar types of chemical bonding between amino acids (peptide and disulfide bonds), are susceptible to proteolytic (protein-digesting) enzymes, and have similar molecular weights and solubility characteristics. Some proteinoids have also been shown to possess a small amount of catalytic activity.

In this experiment, two parts of glutamic acid, two parts of aspartic acid, and one part of an equimolar mixture of 16 other amino acids are heated together in the