A Production of Amino Acids Under Possible Primitive Earth Conditions

Stanley L. Miller

Methods and Logic - 2/24/15

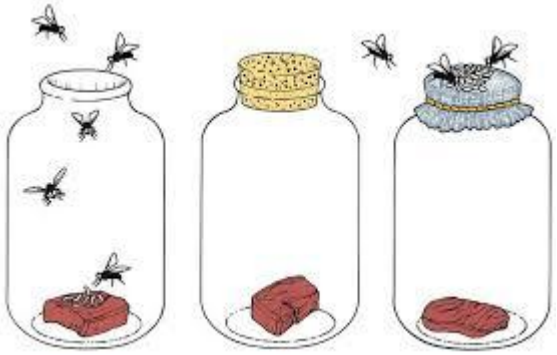
Outline for today's class

- The origin of life
- Stanley L. Miller and Harold Urey
- Background
- Landmark Paper
- Landmark Experiment
- Subsequent Studies

There are many theories regarding the origin of life

- **Theory of spontaneous generation:** living organisms can arise suddenly and spontaneously from any kind of non-living matter
  - Aristotle, ancient Egyptians
  - Popular until 1600s when it was disproved due to various experiments
    - Fransisco Redi (1665)
    - Louis Pasteur (1864)

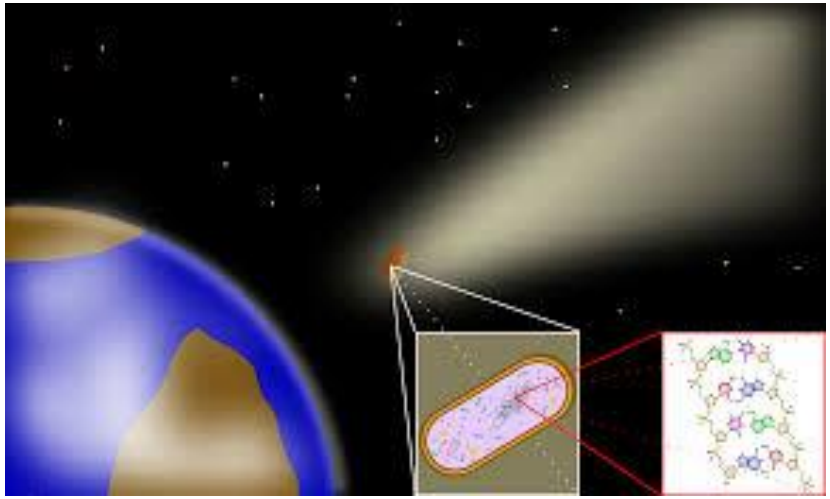
http://www.tutorvista.com/content/biology/biology-iii/origin-life/origin-life-theories.php#



http://bekarice.com/college-spontaneous-generation/

There are many theories regarding the origin of life

- **Cozmozoic theory (parpermia)**: life reached Earth from other heavenly bodies such as meteorites, in the form of highly resistance spores of some organisms
  - Richter (1865)
  - Arrhenius (1908)
  - Overall lack of evidence
  - Living matter cannot survive the extreme cold, dryness and ultra-violet radiation from the sun required to be crossed for reaching the earth.

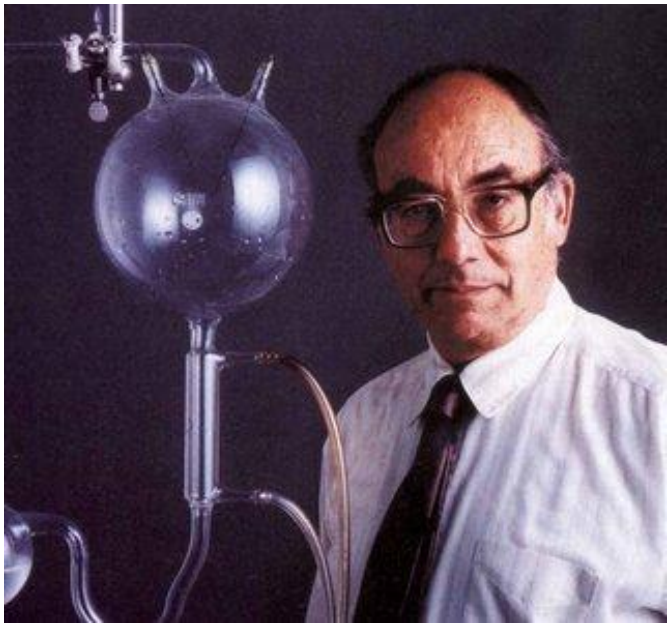


Wikipedia

There are many theories regarding the origin of life

- **Theory of chemical evolution:** Origin of life on earth is the result of a slow and gradual process of chemical evolution that probably occurred about 4 billion years ago
  - Oparin (1923)
  - Haldone (1928)
  - Early Earth atmosphere (mixture of gases and solar radiation/lightning)
  - Miller-Urey Experiment

Stanley L. Miller - Biography



National Academy of Sciences

Born: 1930 in Oakland, CA
Died: 2007 in San Diego, CA

High school nickname: “a chem whiz”
BS: UC Berkley - 1951
PhD: University of Chicago – 1954 (advisor: Harold Urey)

California Institute of Technology
Columbia University
UC San Diego (1960-2007)

Landmark Paper: (1953) Production of amino acids under possible primitive earth conditions". *Science* **117** (3046): 528–529

<u>Notable Awards:</u>

- Considered to be the “father of prebiotic chemistry”
- President of the International Society for the Study of the Origin of Life (1986-1989)
- Elected to National Academy of Sciences in 1973
- *Stanley L. Miller Award* for young scientists under the age of 37- International Astrobiology Society

Stanley L. Miller – Doctoral Research

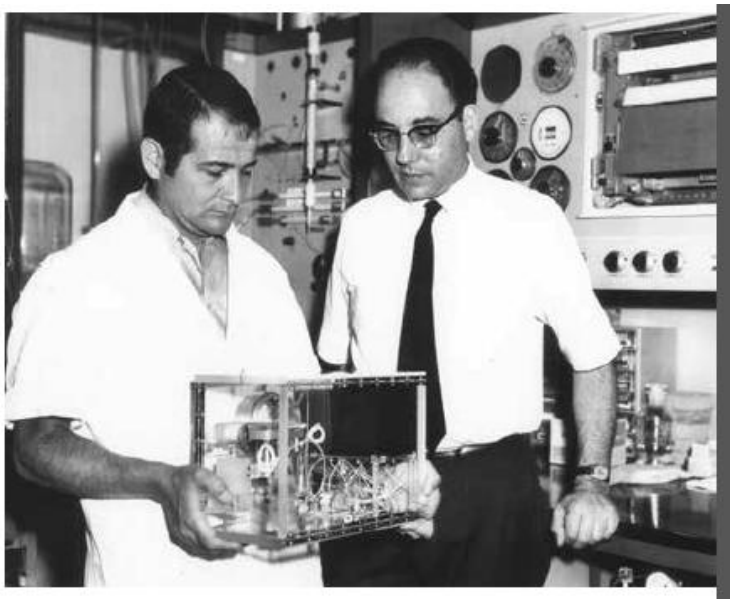
- Accepted into PhD program at University of Chicago in 1951 with a teaching assistantship
- He didn't initially want to do an experimental thesis. He claimed experiments tended to be "time-consuming, messy, and not as important" as theoretical research
- First joined Edward Teller's lab and worked on a project investigating how the elements were synthesized in stars
- After a year, he joined Harold Urey's lab and worked on a prebiotic synthesis experiment using a reducing gas mixture

National Academy of Sciences

He demonstrated the prebiotic synthesis of organic compounds (amino acids) under simulated primitive earth conditions with the context being the origin of life

Stanley L. Miller – Later research

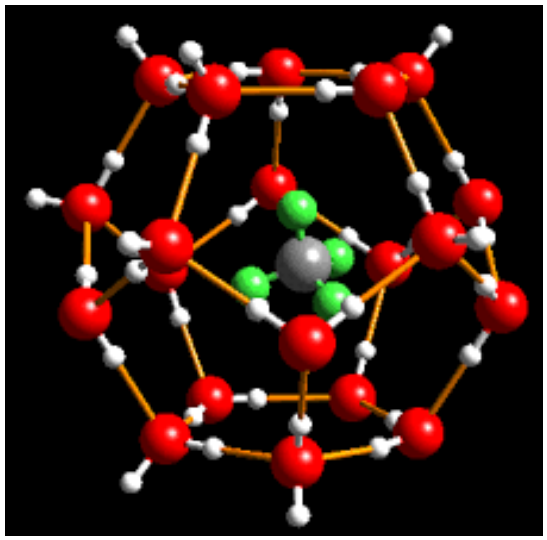
- He continued to investigate the origin of life through prebiotic chemistry research
  - Synthesis and stability of key biochemical components under early earth geocosmochemical environments
  - Transition from abiotic chemistry to biochemistry
- Was interested in the possibility of life on Mars
  - Received a grant from NASA to develop a miniaturized extraction system and amino acid analyzer to be deployed on a future mission
  - Device never got deployed



National Academy of Sciences

Stanley L. Miller – Later research

- He made significant understandings to the understanding of gas clathrates
  - Clathrates are icy solids made of water molecules that contain “cages” in which small gas molecules can be entrapped
- Predicted the presence of a naturally occurring air clatharate in the Antarctic Ice sheet
  - Named it “craigite” in honor of friend and fellow Urey grad student Harmon Craig
  - Was jokingly noted by colleagues that when craigite melts at atmospheric pressure, it spontaneously explodes into hot gas and water…in reference to Craig’s sometime volatile personality
- Investigated occurrence of the carbon dioxide clathrate on Mars
  - Should be a component of Martian polar ice



Harold Urey

Born: 1893 in Walkerton, Indiana
Died: 1981 in La Jolla, California

BS: University of Montana (Zoology) -1917

PhD: UC Berkley 1923 (advisor: Gilbert Lewis, known for discovery of the covalent bond and electron pairs)

Early Research: Awarded Nobel prize in in Chemistry in 1934 for the discovery of deuterium, played an instrumental role in development of the nuclear bomb

Niels Bohr Institute in Copenhagen
Johns Hopkins University
Columbia University
Manhattan Project

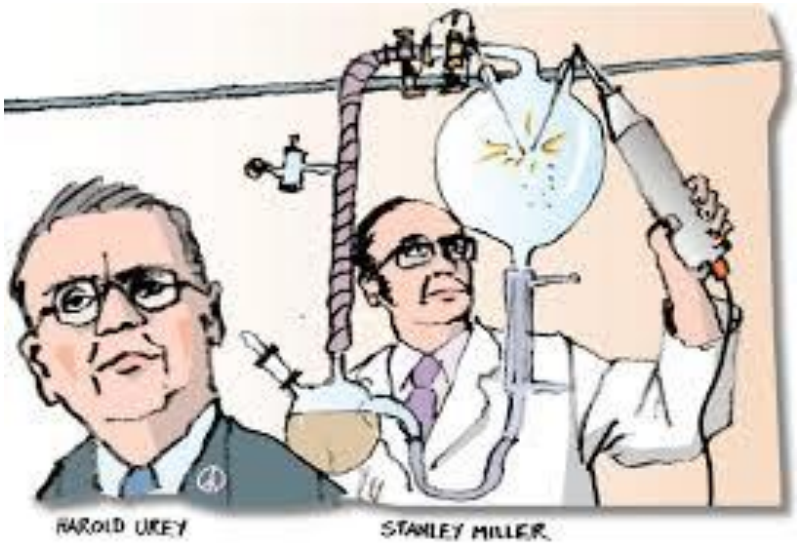
Later Research: Helped develop the field of cosmochemistry

University of Chicago
UC San Diego

Urey's motivation for Miller's Experiment

- Urey Mentioned in a lecture that it could be possible to synthesize organic compounds that in turn could have provided the raw materials needed for the emergence of life

- Urey was hesitant when Miller first approached him about doing such an experiment. He felt that "graduate students should only do experiments that have a reasonable chance of working, rather than taking a leap into the unknown"



- Miller Persisted and Urey reluctantly agreed to let Miller do the experiment, but specified that there must be signs of success within the first year, or the project would be abandoned

Urey's motivation for Miller's Experiment

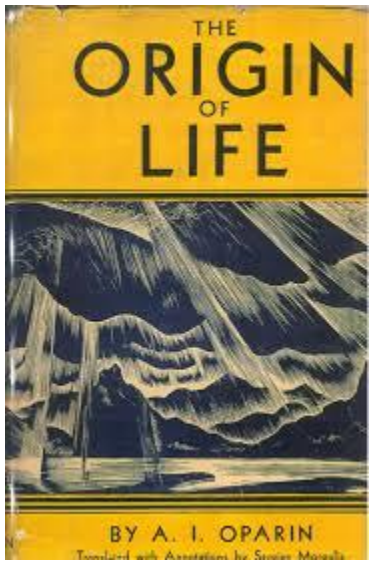
ON THE EARLY CHEMICAL HISTORY OF THE EARTH AND THE ORIGIN OF LIFE

By Harold C. Urey

Institute for Nuclear Studies, University of Chicago

Communicated January 26, 1952

Alexander Oparin

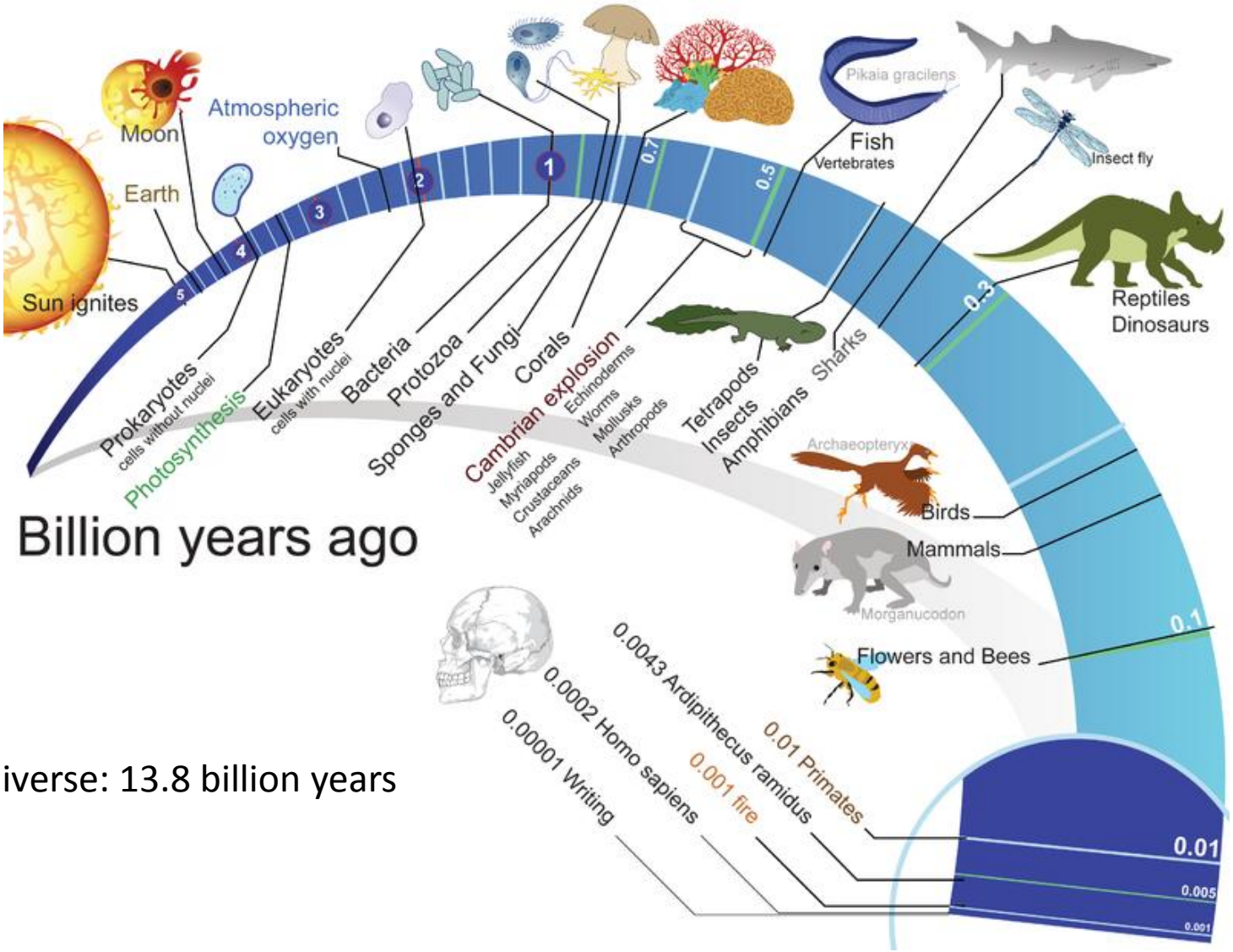


The Origin of Life.—The problem of the origin of life involves three separate questions in our present discussion: (1) the spontaneous formation of the chemical compounds which form the physical bodies of living organisms; (2) the evolution of the complex chemical reactions which are the dynamic basis of life; and (3) the source of free energy which alone can maintain the chemical reactions and synthesize the chemical compounds.

In order to estimate the early conditions of the earth, it is necessary to ask and answer the questions of how the earth originated, and how the primitive earth developed into the present earth.

Oparin speculated and Urey agreed that the early terrestrial atmosphere was probably composed of ammonia, methane, and hydrogen

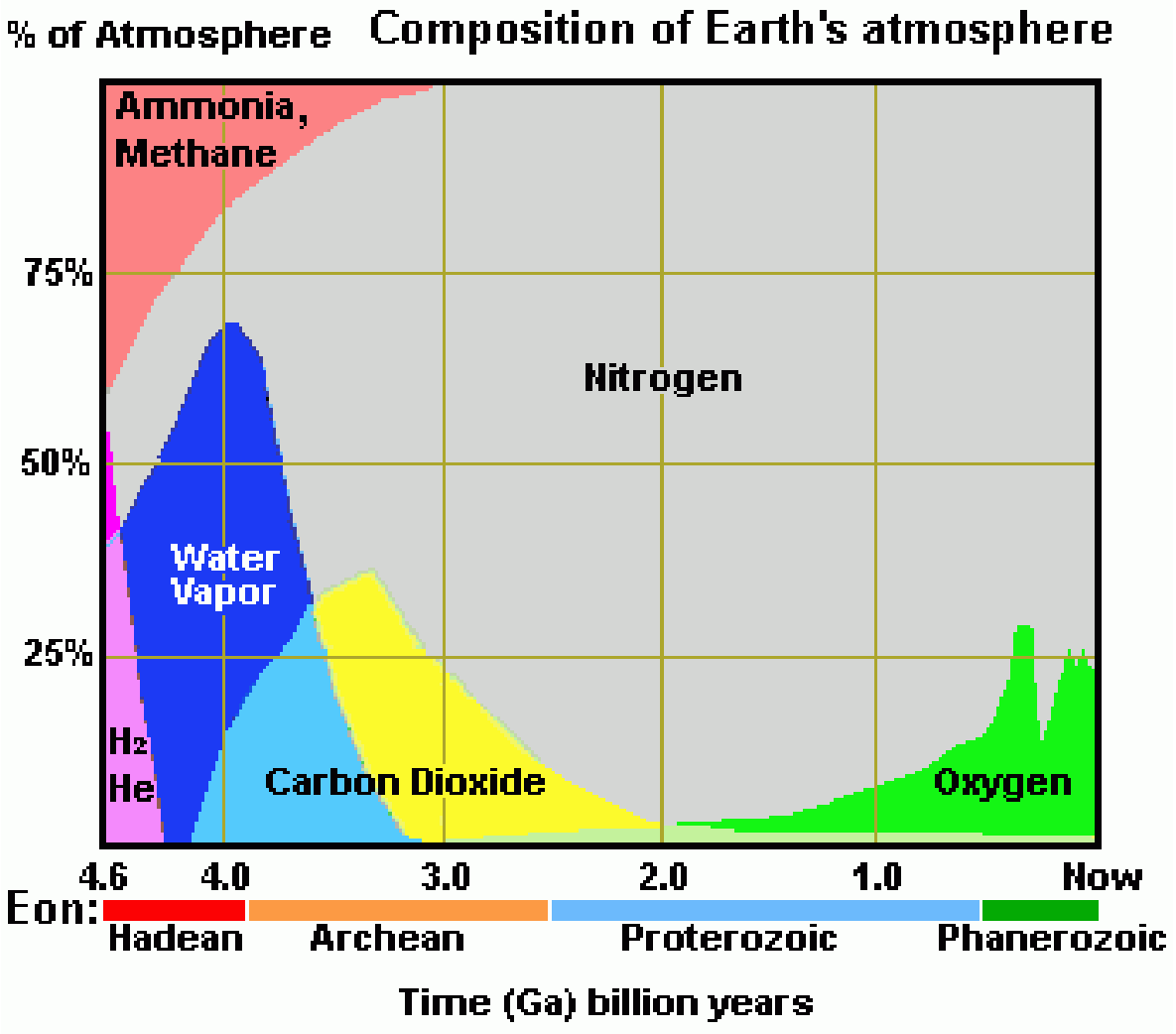
Earth is ~4.5 billion years old



universe: 13.8 billion years

http://www.ck12.org/book/CK-12-Biology/r23/section/1.2/

Earths atmosphere is constantly changing



Prebiotic earth:

NH_3

CH_4

H_20

H_2

http://www.scientificpsychic.com/etc/timeline/atmosphere-composition.html

Production of amino acids under possible primitive earth conditions

Hypothesis: Organic compounds that serve as the basis for life were formed when the earth had an atmosphere of methane, ammonia, water and hydrogen

How can this hypothesis be tested?

Build an apparatus to mimic early earths Atmosphere
Requirements: NH_3, CH_4, H_20, and H_2 must be circulated past an electric discharge

Components:

- A sterile 5-liter glass flask with NH_3, CH_4, and H_2 gasses
- A 500ml sterile glass flask with H_20 (heated to induce evaporation)
- Two electrodes used to generate electrical sparks

Method:

- Two flasks were connected in an apparatus to allow constant cycling of components
- Apparatus was run continuously for a week

Miller-Urey Experiment Apparatus

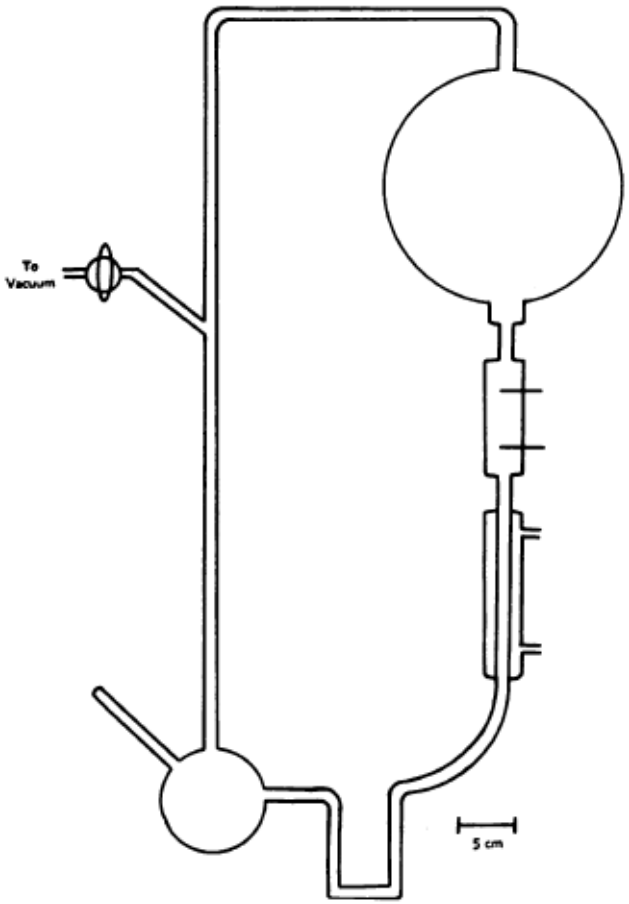
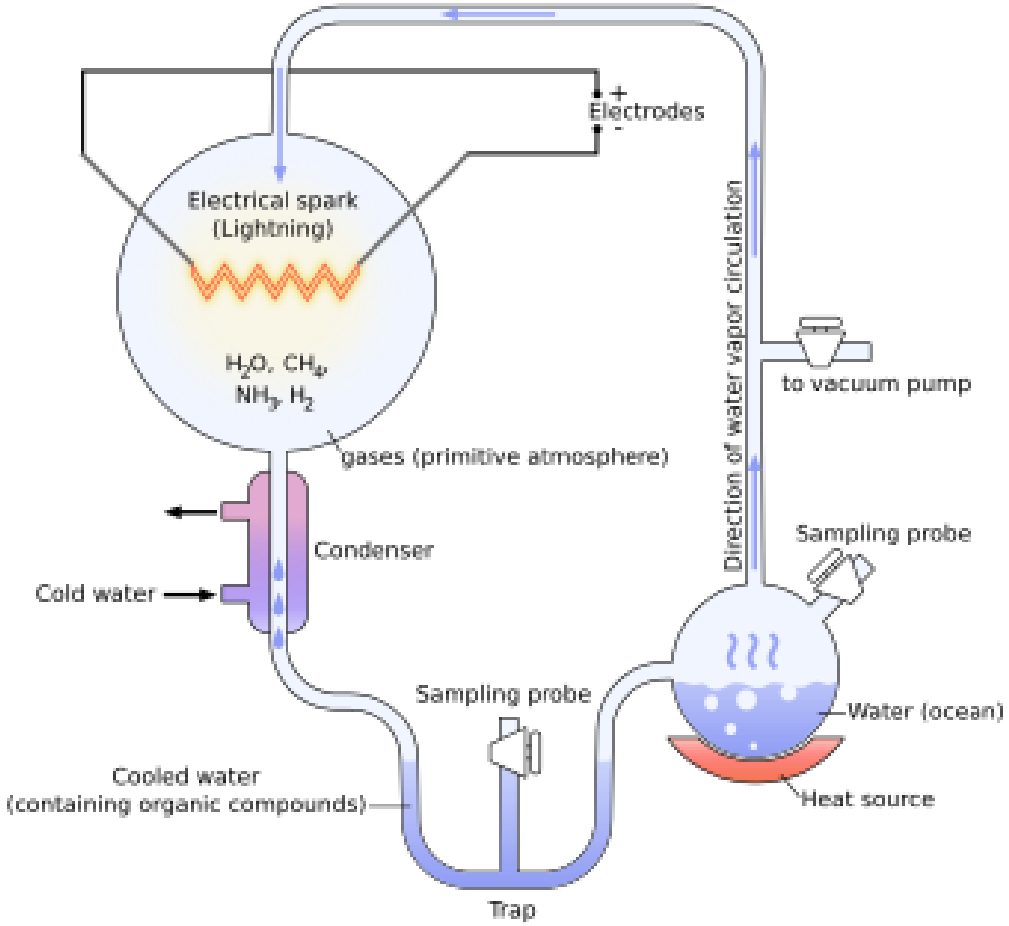


FIG. 1.

Figure 1 from paper



Wikipedia

Sample Analysis

- After 1 week of cycling, the boiling flask was removed and $HgCl_2$ was added
- Ampholytes (amphoteric molecules that contain both acidic and basic groups) were separated
  - Addition of $Ba(OH)_2$ and in vacuo evaporation to remove amines
  - Addition of H_2SO_4 and in vacuo evaporation to remove acids
- Paper Chromatography used to identify amino acids

Paper Chromatography was used to identify amino acids

- Chromatography is an analytical tool used for distinguishing different biomolecules based on their chemical properties
- In paper chromatography, sample is placed on cellulose filter paper (hydrophobic)
- Hydrophobic sample is drawn up the paper via capillary action
- Different R-groups cause amino acids to move at different rates (R_f value)
- Spraying with ninhydrin labels amine groups
- solvent mixtures are prepared by mixing of butanol, acetic acid, and water in the ratio of 4:1:5 and phenol and ammonia

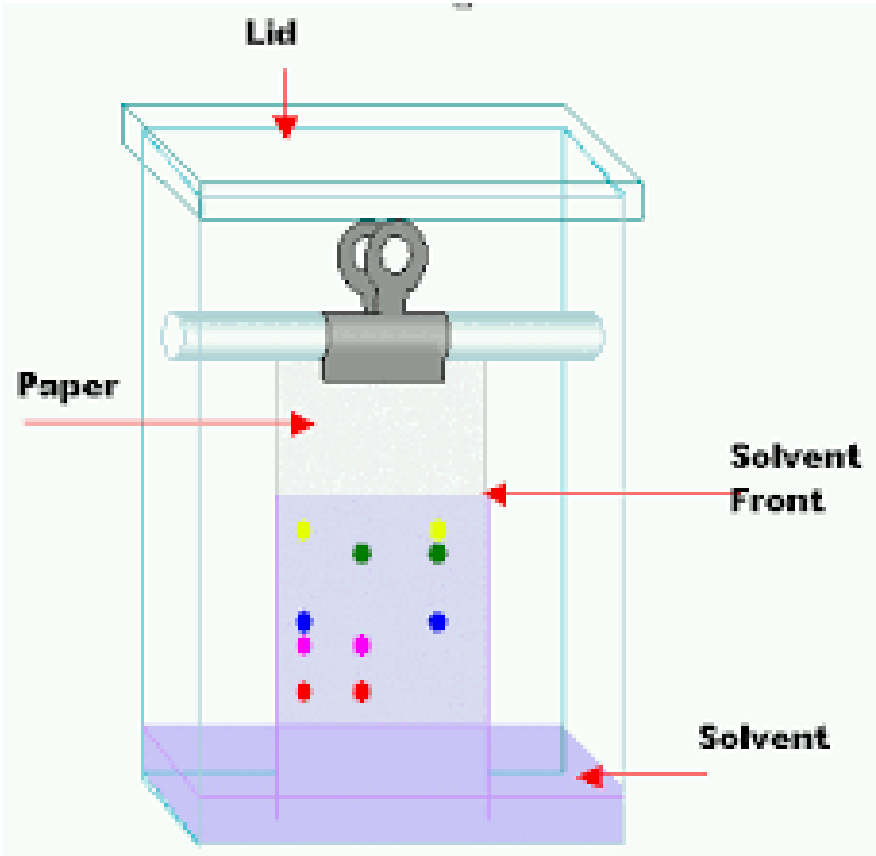


Figure 2: Paper chromatograph

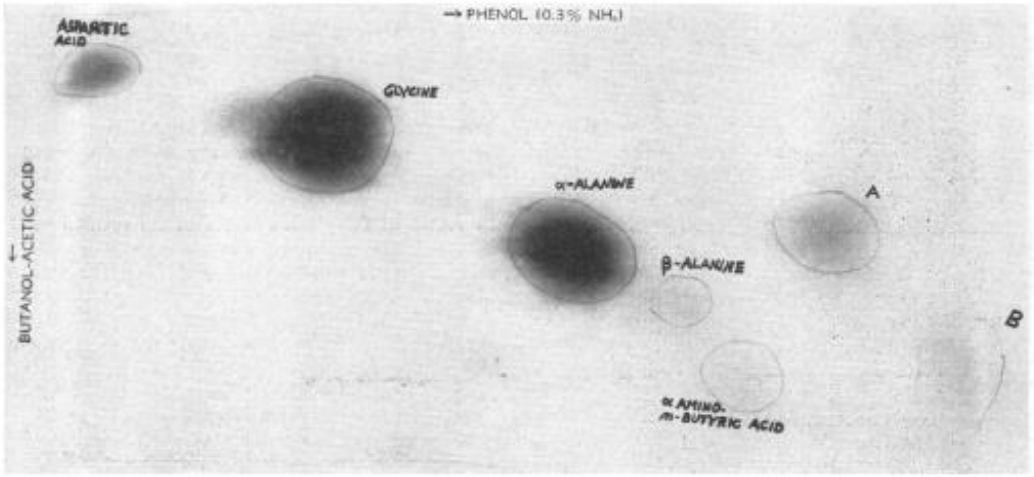
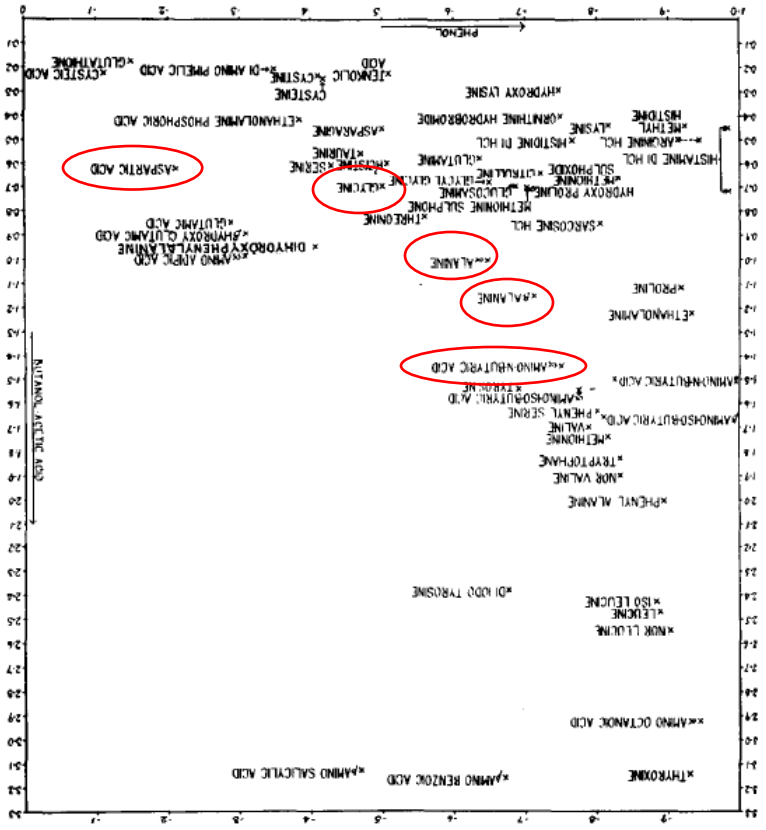


FIG. 2.

Miller noted the appearance of: glycine, α-alanine, β-alanine, aspartic acid, and α-amino-n-butyric acid



(T.E. Parry, 1957)

ties of the compounds manometrically (*5*). In the other, the material is applied to the paper along 8 cm of the base line rather than as a spot and, after resolution, areas 8 × 5 cm containing the various compounds are cut from the paper and rolled in shell vials. Ten anesthetized houseflies are then introduced into each vial, and the toxicity of the compounds is characterized by rate of knockdown and 24-hr mortality.

The paper chromatographic method is useful in studying the metabolism of phosphorus insecticides in plants, mammals, and insects. With it, for example, we have been able to demonstrate the conversion of parathion and its methyl analog to the corresponding phosphates by an enzyme system found in *Periplaneta americana* (L.) (*2*). Further studies are in progress. The method has also been of value in studying the action of heat on purified parathion and methyl parathion and in isolating the compounds formed and in studying their biological properties (*1*).

References

1. Metcalf, R. L., and March, R. B. To be published.
2. ———. *Ann. Entomol. Soc. Amer.* (in press).
3. Kritchevsky, T. H., and Tiselius, A. *Science*, **114**, 299 (1951).
4. Hanes, C. S., and Isherwood, F. A. *Nature*, **164**, 1107 (1949).
5. Metcalf, R. L., and March, R. B. *J. Econ. Entomol.*, **42**, 721 (1949).

Manuscript received September 15, 1952.

A Production of Amino Acids Under Possible Primitive Earth Conditions

Stanley L. Miller[1, 2]

G. H. Jones Chemical Laboratory, University of Chicago, Chicago, Illinois

The idea that the organic compounds that serve as the basis of life were formed when the earth had an atmosphere of methane, ammonia, water, and hydrogen instead of carbon dioxide, nitrogen, oxygen, and water was suggested by Oparin (*1*) and has been given emphasis recently by Urey (*2*) and Bernal (*3*).

In order to test this hypothesis, an apparatus was built to circulate CH_4, NH_3, H_2O, and H_2 past an electric discharge. The resulting mixture has been tested for amino acids by paper chromatography. Electrical discharge was used to form free radicals instead of ultraviolet light, because quartz absorbs wavelengths short enough to cause photo-dissociation of the gases. Electrical discharge may have played a significant role in the formation of compounds in the primitive atmosphere.

The apparatus used is shown in Fig. 1. Water is boiled in the flask, mixes with the gases in the 5-l flask, circulates past the electrodes, condenses and empties back into the boiling flask. The U-tube prevents circulation in the opposite direction. The acids and amino acids formed in the discharge, not being volatile, accumulate in the water phase. The circulation of the gases is quite slow, but this seems to be an asset, because production was less in a different apparatus with an aspirator arrangement to promote circulation. The discharge, a small corona, was provided by an induction coil designed for detection of leaks in vacuum apparatus.

The experimental procedure was to seal off the opening in the boiling flask after adding 200 ml of water, evacuate the air, add 10 cm pressure of H_2, 20 cm of CH_4, and 20 cm of NH_3. The water in the flask was boiled, and the discharge was run continuously for a week.

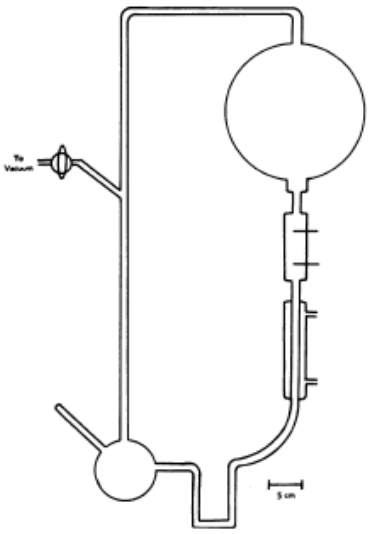


Fig. 1.

During the run the water in the flask became noticeably pink after the first day, and by the end of the week the solution was deep red and turbid. Most of the turbidity was due to colloidal silica from the glass. The red color is due to organic compounds adsorbed on the silica. Also present are yellow organic compounds, of which only a small fraction can be extracted with ether, and which form a continuous streak tapering off at the bottom on a one-dimensional chromatogram run in butanol-acetic acid. These substances are being investigated further.

At the end of the run the solution in the boiling flask was removed and 1 ml of saturated $HgCl_2$ was added to prevent the growth of living organisms. The ampholytes were separated from the rest of the constituents by adding $Ba(OH)_2$ and evaporating *in vacuo* to remove amines, adding H_2SO_4 and evaporating to remove the acids, neutralizing with $Ba(OH)_2$, filtering and concentrating *in vacuo*.

The amino acids are not due to living organisms because their growth would be prevented by the boiling water during the run, and by the $HgCl_2$, $Ba(OH)_2$, H_2SO_4 during the analysis.

In Fig. 2 is shown a paper chromatogram run in *n*-butanol-acetic acid-water mixture followed by water-saturated phenol, and spraying with ninhydrin. Identification of an amino acid was made when the R_f value (the ratio of the distance traveled by the amino acid to the distance traveled by the solvent front), the shape, and the color of the spot were the same on a known, unknown, and mixture of the known and unknown; and when consistent results were obtained with chromatograms using phenol and 77% ethanol.

On this basis glycine, α-alanine and β-alanine are identified. The identification of the aspartic acid and α-amino-*n*-butyric acid is less certain because the spots are quite weak. The spots marked A and B are unidentified as yet, but may be beta and gamma amino acids. These are the main amino acids present, and others are undoubtedly present but in smaller amounts. It is estimated that the total yield of amino acids was in the milligram range.

In this apparatus an attempt was made to duplicate a primitive atmosphere of the earth, and not to obtain the optimum conditions for the formation of amino acids. Although in this case the total yield was small for the energy expended, it is possible that, with more efficient apparatus (such as mixing of the free radicals in a flow system, use of higher hydrocarbons from natural gas or petroleum, carbon dioxide, etc., and optimum ratios of gases), this type of process would be a way of commercially producing amino acids.

A more complete analysis of the amino acids and other products of the discharge is now being performed and will be reported in detail shortly.

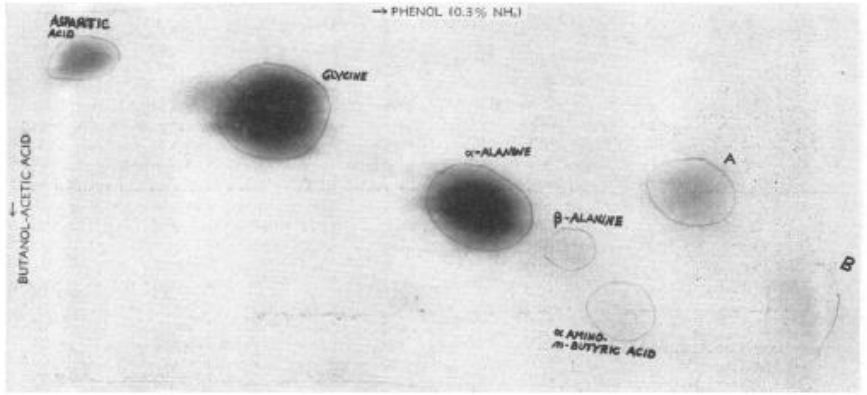


Fig. 2.

References

1. Oparin, A. I. The Origin of Life. New York: Macmillan (1938).
2. Urey, H. C. *Proc. Natl. Acad. Sci. U. S.*, **38**, 351 (1952); *The Planets*. New Haven: Yale Univ. Press Chap. 4 (1952).
3. Bernal, J. D. *Proc. Phys. Soc. (London)*, **62A**, 537 (1949); **62B**, 597 (1949); *Physical Basis of Life*. London: Routledge and Kegan Paul (1951).

Manuscript received February 13, 1953.

A Vacuum Microsublimation Apparatus

John R. Maher[1]

Chemistry Branch, Sixth Army Area Medical Laboratory, Fort Baker, California

The analytical biochemist is frequently confronted with the task of isolating microquantities of substances in a chemically pure state from small quantities of tissues or biological fluids. Kofler (*1*) edited a book covering the use of microsublimation, melting point, eutectics, etc., in identifying microquantities of organic material. The advantages of sublimation over other methods of purification have been discussed by Hubacher (*2*). Many types of vacuum sublimation apparatus have been described (*1–3*). The equipment described here is inexpensive and can be assembled readily by any laboratory worker with a modicum of glassblowing skill.

To a thick-walled, round-bottom, Pyrex test tube, 30 × 200 mm, is attached a glass side arm about one in. from the bottom. Using a suspension of very fine emery in glycerin or fine valve-grinding compound, the open end of the test tube is ground against the aluminum block of a Fisher-Johns melting point apparatus (Fisher Scientific Co., St. Louis, Mo.) until it makes a vacuum-tight seal when dry. This is the vacuum hood. Microbeakers are prepared from flat-

[1] The author is indebted to Robert Puckett, of this laboratory, for technical assistance in preparing this apparatus.

[1] National Science Foundation Fellow, 1952–53.

[2] Thanks are due Harold C. Urey for many helpful suggestions and guidance in the course of this investigation.

Conclusion from Paper

- NH_3, CH_4, H_20, and H_2 circulated past an electric discharge is sufficient to generate amino acids
- These amino acids could serve as the building blocks for life

Later Studies

- English Research Group led by Hough and Rogers was first to replicate results in 1956. They saw the exact same compounds.
- Subsequent experiments modified the conditions/energy source and led to the identification of nine different amino acids
- In 1961, Joan Oró found that the nucleotide base adenine could be made from hydrogen cyanide (HCN) and ammonia in a water solution (precursors found in prebiotic reaction system)

Chemical Reactions Justifying Miller-Urey Experiment

Atmospheric Components

$CO_2 \rightarrow CO + [O]$ (atomic oxygen)
$CH_4 + 2[O] \rightarrow CH_2O + H_2O$
$CO + NH_3 \rightarrow HCN + H_2O$
$CH_4 + NH_3 \rightarrow HCN + 3H_2$

The formaldehyde, ammonia, and HCN then form amino acids and other biomolecules:

$CH_2O + HCN + NH_3 \rightarrow NH_2\text{-}CH_2\text{-}CN + H_2O$
$NH_2\text{-}CH_2\text{-}CN + 2H_2O \rightarrow NH_3 + NH_2\text{-}CH_2\text{-}COOH$ (glycine)

Paper Publication Controversy

After the experiment, Miller showed Urey the results and Urey decided it was time to write a manuscript and publish in a leading journal

Urey declined to be co-author, stating Miller would receive "little or no credit"

- February 10, 1953: Manuscript was mailed to Science
- February 14, 1953: Manuscript received
- February 27, 1953: Urey wrote Howard Meyerhoff (chair of editorial board), complaining about the lack of progress in publishing the manuscript
- March 10, 1953: Urey sent Meyerhoff a telegram asking Science to return the paper
- March 11, 1953: Meyerhoff replies that he wants to publish the manuscript
- March 13, 1953: Urey submits manuscript to Journal of the American Chemical Society
- Miller decides to accept Meyerhoffs offer and contacts editor of Journal of the American Chemical Society, stating "A mistake was made in sending this to you"
- May 15, 1953: The paper appears in Science

Source: Stanley L Miller Biographical Memoirs. 2012. National Academy of Sciences

Two additional studies were done on unreported 1950s Stanley Miller experiments

- Following a severe stroke in 1999, Jeffrey Bada (one of Stanley Miller's previous graduate students) inherited boxes of experimental samples from Miller's lab.

- While sorting through the boxes, Bada saw "electric discharge sample" in Miller's handwriting on the outside of one box.

- He opened it up and saw samples corresponding to Miller's 1953 experiments in addition to several more previously unreported samples

- Primordial synthesis of amines and amino acids in a 1958 Miller H2S-rich spark discharge experiment. PNAS (2011). Parker et al.
  - Same electric discharge experiment + hydrogen sulfide (H_2S)
  - Samples analyzed by liquid chromatography mass spectrometry
  - A total of 23 amino acids were reported in these sample
  - Simulated early volcanic plume chemistry?

Two additional studies were done on unreported 1950s Stanly Miller experiments

A Plausible Simultaneous Synthesis of Amino Acids and Simple Peptides on the Primordial Earth**

Eric T. Parker, Manshui Zhou, Aaron S. Burton, Daniel P. Glavin, Jason P. Dworkin, Ramanarayanan Krishnamurthy, Facundo M. Fernández, and Jeffrey L. Bada**

Dedicated to Stanley L. Miller and Joan Oró

2014 Paper

- 1958 experiment: Miller sparked a gas mixture of CH_4, NH_3, and H_2O, while intermittently adding the plausible prebiotic condensing reagent cyanimide.
- Liquid chromatography- and mass spectrometry-based analyses and found that the reaction samples contained di-peptides in addition to amino acids
- “Everybody who would have been there and could verify this is gone, so we’re just left to scratch our heads and say ‘how’d he get this idea before anyone else,’” – Jeffrey Bada

Miller's 1958 Sample Vials

Scripps Institution of Oceanography, UC San Diego

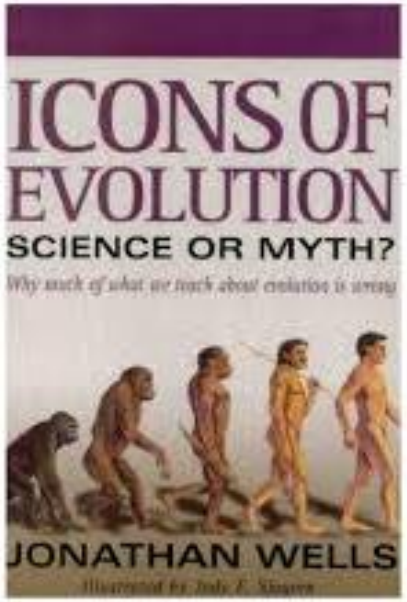
Original 1953 Experiment Apparatus is on display at the Denver Museum of Nature and Science

Criticisms of the Miller Experiment

- Miller ignored the presence of oxygen, which destroys the desired products. Wells explains that oxygen was likely abundant due to photodissociation of water in the atmosphere. The oxygen would remain, while the hydrogen would quickly escape to space.

- Even if trace amounts of ammonia or methane and other reducing gasses were present, they would have been rapidly destroyed by ultraviolet radiation

- The amino acids produced were racemic (mixtures of left- and right-handed forms). Except in rare exceptions, life uses only the left-handed form.
    - Astrobiologists need to explain how the first replicator isolated one hand out of the mixture, or obtained function from mixed-form amino acids initially, then converted to single-handed forms later. Neither is plausible for unguided natural processes -- especially when natural selection would be unavailable until accurate replication was achieved.



Criticisms of the Miller Experiment

- Amino acids tend to fall apart in water, not join. Under the best conditions with cyanamide, Bada and Parker only got dipeptides. Repeated cycles of wetting and drying would need to be imagined for polymerization, but many astrobiologists today think life originated at deep sea hydrothermal vents.

- The desired reagents would be extremely dilute in the oceans without plausible concentrating mechanisms. Even then, they would disperse without plausible vessels, like cell membranes, to keep them in proximity.

- Lifeless polypeptides would go nowhere without a genetic code to direct them.

Additional Discussion Topics

How does synthesis of precursors of life justify an origin of life?

What came first: RNA, DNA, or amino acids?

If you had to make a similar pre-biotic simulating device with todays technology, how would you design it? How would this differ from Miller's experiment?

Is your scientific notebook labeled clearly enough for someone 50 years in the future to identify various samples/experiments?

What other discussion questions can you think of?

In Conclusion

- The early Earths atmosphere is predicted to have been made up of primarily of NH_3, CH_4, H_20, and H_2
- Stanley Miller's 1953 experiment showed that a mixture of NH_3, CH_4, H_20, and H_2 circulated past an electric discharge is sufficient to generate amino acids
- This experiment supports a possibility for the origin of life through chemical evolution

Chicken or the egg?