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)
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.
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

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

Notable Awards:
• 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

He demonstrated the prebiotic synthesis of organic compounds (amino acids) under simulated primitive earth conditions with the context being the origin of life
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
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 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/
Earth's atmosphere is constantly changing

Prebiotic earth:

\[
\begin{align*}
\text{NH}_3 \\
\text{CH}_4 \\
\text{H}_2\text{O} \\
\text{H}_2
\end{align*}
\]
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$_2$O, 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$_2$O (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

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$_2$SO$_4$ 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.
Miller noted the appearance of: glycine, $\alpha$-alanine, $\beta$-alanine, aspartic acid, and $\alpha$-amino-$n$-butyric acid.

(T.E. Parry, 1957)
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 crater, 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 H₂, 20 cm of CH₄, and 20 cm of NH₃. The water in the flask was
boiled, and the discharge was run continuously for a week.

A Production of Amino Acids Under Possible Primitive Earth Conditions

Stanley L. Miller

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 CH₄, NH₃, H₂O, and H₂ 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 quanta absorbs wavelengths short enough to cause photo-disassociation 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, mixed with the gases in the flask, circulates past the electrodes, condenses, and empties back into the boiling flask. The U tube prevents circulation in the opposite direction. The acids

References
1. METCALF, R. L., and MARCH, R. W. To be published.

Manuscript received September 15, 1952.
Conclusion from Paper

- NH$_3$, CH$_4$, H$_2$O, 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

\[ \text{CO}_2 \rightarrow \text{CO} + [\text{O}] \text{ (atomic oxygen)} \]
\[ \text{CH}_4 + 2[\text{O}] \rightarrow \text{CH}_2\text{O} + \text{H}_2\text{O} \]
\[ \text{CO} + \text{NH}_3 \rightarrow \text{HCN} + \text{H}_2\text{O} \]
\[ \text{CH}_4 + \text{NH}_3 \rightarrow \text{HCN} + 3\text{H}_2 \]

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

\[ \text{CH}_2\text{O} + \text{HCN} + \text{NH}_3 \rightarrow \text{NH}_2\text{-CH}_2\text{-CN} + \text{H}_2\text{O} \]
\[ \text{NH}_2\text{-CH}_2\text{-CN} + 2\text{H}_2\text{O} \rightarrow \text{NH}_3 + \text{NH}_2\text{-CH}_2\text{-COOH} \text{ (glycine)} \]
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 Meyerhoff’s offer and contacts editor of Journal of the American Chemical Society, stating “A mistake was made in sending this to you.”

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.

  - Same electric discharge experiment + hydrogen sulfide (H2S)
  - Samples analyzed by liquid chromatography mass spectrometry
  - A total of 23 amino acids were reported in these samples
  - Simulated early volcanic plume chemistry?
Two additional studies were done on unreported 1950s Stanly Miller experiments

1958 experiment: Miller sparked a gas mixture of CH\textsubscript{4}, NH\textsubscript{3}, and H\textsubscript{2}O, 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 today's 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 Earth's atmosphere is predicted to have been made up primarily of NH₃, CH₄, H₂O, and H₂

• Stanley Miller’s 1953 experiment showed that a mixture of NH₃, CH₄, H₂O, and H₂ 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?