THE 1953 STANLEY L. MILLER EXPERIMENT: FIFTY YEARS OF PREBIOTIC ORGANIC CHEMISTRY

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Abstract. The field of prebiotic chemistry effectively began with a publication in *Science* 50 years ago by Stanley L. Miller on the spark discharge synthesis of amino acids and other compounds using a mixture of reduced gases that were thought to represent the components of the atmosphere on the primitive Earth. On the anniversary of this landmark publication, we provide here an accounting of the events leading to the publication of the paper. We also discuss the historical aspects that lead up to the landmark Miller experiment.

Keywords: prebiotic chemistry, reducing atmosphere, Strecker synthesis, electric discharges

1. Introduction

Fifty years ago, *Science* published in its 15 May 1953 issue the short, less than two-page, paper by Stanley L. Miller titled 'A production of amino acids under possible primitive Earth conditions' (Miller, 1953). In it, Stanley reported the stunning results he had achieved by the action of an electric discharge on a mixture of the reducing gases CH_4, NH_3, H_2O, and H_2 that simulated what was viewed at the time as a model atmosphere for the primitive Earth. The result of this experiment was a substantial yield of a mixture of amino acids, together with hydroxy acids, short aliphatic acids, and urea. One of the surprising results of this experiment was that the products were not a random mixture of organic compounds; rather, a relatively small number of compounds were produced in surprisingly high yields. Moreover, with a few exceptions, the compounds were of biochemical significance, thus providing support for the primitive soup heterotrophic theory proposed in the 1920's by Oparin and Haldane. With this landmark experiment the modern era in the study of origin of life began.

2. The Hectic Story Behind Publication

Although the primitive soup theory had attracted considerable attention among biologists, it had gone largely unnoticed in other fields of science. In order to buttress

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his intuition, Oparin needed to demonstrate that organic compounds could form in the absence of living beings. Although he did not perform any actual experimental simulations of the primitive milieu, several important pieces of information supported his claim, including the universality of anaerobic fermentation and the existence of extraterrestrial organic compounds in meteorites, that the first organisms were more likely to have been heterotrophic (Oparin, 1938).

One attempt to study the possibility of organic compound synthesis under primitive Earth conditions begun in 1950, when Melvin Calvin's group at the University of California, Berkeley, irradiated a gas mixture of CO_2, H_2O, H_2 and a solution of Fe^{2+} with 40-meV helium ions in an attempt to simulate the radiation environment in the terrestrial crust (Garrison *et al*., 1951). The results, however, were not encouraging: only small amounts of formic acid and formaldehyde were obtained, which is similar to results obtained in experiments done since the 1920s by several other researchers (see Rabinowitch, 1945).

Harold C. Urey, who had been involved with the study of the origin of the solar system and the chemical events associated with this process, would later consider the origin of life in the context of his proposal of a highly reducing terrestrial atmosphere. Urey first presented his ideas in a lecture at the University of Chicago in the fall of 1951, and the next year he published a paper detailing his model of the Earth's primitive atmosphere (Urey, 1952). In September 1952, almost year and a half after attending Urey's seminar, Stanley L. Miller, then a graduate student in Chemistry at the University of Chicago, approached Urey about the possibility of doing a prebiotic synthesis experiment using a reducing gas mixture (Miller, 1974). After overcoming Urey's initial resistance, they designed three different spark discharge apparatus to be used in the experiment (Figure 1). The apparatus was meant to simulate the ocean-atmosphere system on the primitive Earth. Water vapor produced by heating would be like evaporation from the oceans, and as it mixed with methane, ammonia and hydrogen, it would mimic a water vapor-saturated primitive atmosphere. The apparatus shown in Figures 1a and b was the one most extensively used in the original experiments, and is the design most widely known today. The apparatus in Figure 1c led to a higher inner pressure, and an important aspect of this design is that it generated a hot water mist that could be considered similar to a water vapor-rich volcanic eruption. The apparatus shown in Figure 1d used a so-called silent discharge instead of a spark, a concept that had been used previously in attempts to make organic compounds from CO_2 in order to try to understand photosynthesis (Rabinowitch, 1945).

Results were produced almost as soon as Stanley begun the experiments in the fall of 1952. Although by comparison with contemporary analytical tools the methods available to Stanley were crude, he was able to demonstrate that after only two days of sparking the gaseous mixture glycine could be detected. After repeating the experiment sparking the mixture for a whole week, the inside of the sparking flask was coated with an oily material and the water had a yellow-brown color. When paper chromatography was used to analyse the compounds that had

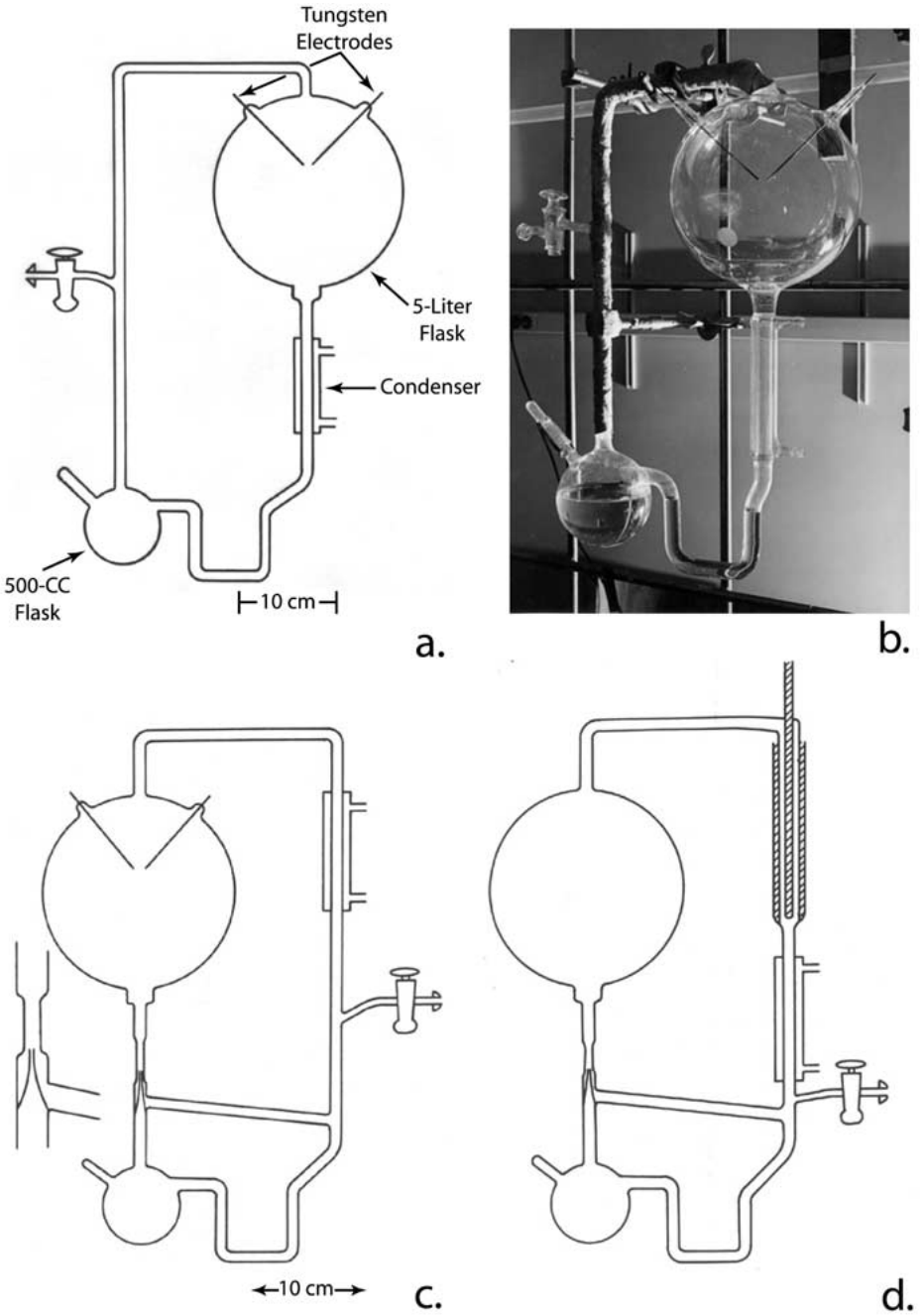

Figure 1. The various apparatus used in the Miller experiment (Miller, 1954; the photograph is courtesy of Stanley L. Miller). The design shown in (a) and (b) is the one that was used for the experiments published in *Science* on 15 May 1953. The apparatus shown in (c) was also tested and in general yielded similar results to those obtained with the one in (a). The apparatus in (d) used a silent discharge that was generated by delivering ~97 watts of power to copper electrodes placed in 50% H_2SO_4 that filled the shaded area of the apparatus.

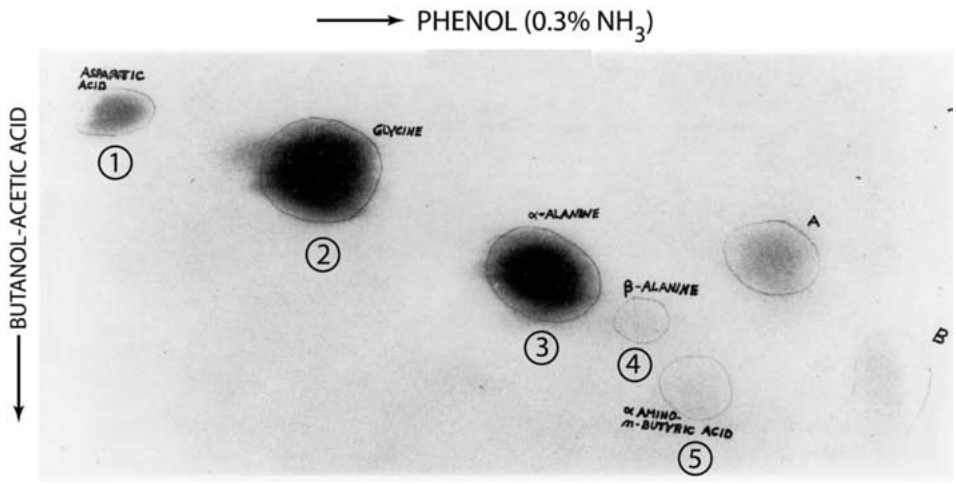

Figure 2. The two-dimensional paper chromatogram of the amino acids produced from the sparking experiment using the apparatus shown in Figures 1a and b (courtesy of Stanley L. Miller). The spots corresponding the various amino acids were produced by spraying the chromatogram with ninhydrin. The amino acid labels are Stanley's original writing. We have added numbers to help identify the various spots. 1 = Aspartic acid; 2 = glycine; 3 = α-alanine; 4 = β-alanine; and 5 = α-amino-n-butyric acid. Spots labeled A and B where not identified.

formed, the glycine spot was much more than intense and spots corresponding to several other amino acids were also detected (Figure 2).

Experiments with the apparatus in Figures 1a and b as well as the one in Figure 1c produced in general a similar distribution and quantities of amino acids and other organic compounds. In contrast, experiments with the apparatus in Figure 1d showed lower overall yields and a much more limited suite of amino acids: essentially only sarcosine and glycine were produced (Miller, 1955).

After Miller showed the impressive results to Urey, they decided that it was time to get them published, preferably in a leading journal such as *Science*. Urey contacted the editors and asked for the paper to be published as soon as possible. He also declined Stanley's offer to coauthor the report because otherwise Stanley would receive little or no credit. In the meantime, Urey became so enticed by the outcome of the experiment that he began mentioning in his lectures the results achieved by Stanley. As shown by the articles published on 24 November 1952 issues of both *Time* and *Newsweek*, the news attracted considerable attention not only from scientists but also from the media. Could it be that the origin of life could finally be understood?

The manuscript was mailed to *Science* on 10 February 1953, and was received at the editorial office on 14 February (a detailed record of the submission and subsequent correspondence with *Science* is in the Urey papers in the Mandeville Special Collection at the University of California at San Diego library). On 27 February 1953, Urey wrote Howard Meyerhoff, chairman of the Editorial Board,

complaining about the lack of progress in publication of the manuscript. He stated 'If *Science* does not wish to publish this promptly we will send it to the *Journal of the American Chemical Society*'. He closed the letter saying 'I would appreciate an immediate reply so that we can make a decision in this matter'.

In the meantime, on Sunday 8 March 1953, the *New York Times* published a rather cryptic short article titled 'Looking back two billion years', wherein the experiments of Wollman M. MacNevin and his associates at the Ohio State University were described. It was reported that MacNevin and his team had performed a number of experiments simulating the primitive Earth, including a discharge experiment in which a spark was sent through methane producing 'resinous solids to complex for analysis'. MacNevin also reported the production of porphyrin from the heating of a mixture of CO_2, H_2O and NH_3. The next day Stanley sent Urey a copy of the clipping together with a note in which he wrote 'I am not sure what should be done now, since their work [MacNevin and his group] is, in essence, my thesis. As of today, I have not received the proof from *Science*, and in the letter that was sent to you, Meyerhoff said that he had sent my note for review'.

Infuriated by what he believed to be an unfair delay, Urey telegrammed Meyerhoff on 10 March asking that *Science* return the paper. He then submitted the manuscript for Stanley to the *Journal of the American Chemical Society* on 13 March. In the meantime, Meyerhoff, obviously frustrated with Urey, wrote directly to Stanley on 11 March telling him that he wanted to publish the manuscript and that he was 'unwilling to accept Dr. Urey's orders, unless it is your personal wish that the manuscript be returned to you and not used as a lead article in *Science*'. Stanley promptly accepted Meyerhoff's offer to publish the manuscript and telegrammed the Editor of *Journal of the American Chemical Society* asking that the manuscript be returned, stating 'A mistake was made in sending this to you'. The paper appeared 2 months later in the 15 May issue of *Science*.

Interestingly, while Stanley's manuscript was under review at *Science*, another paper by Kenneth Wilde and co-workers, on the attempted electric arc synthesis of organic compounds using CO_2 and water was also under review. This manuscript was actually received on 15 December 1952, before Stanley's was submitted. In the Wilde *et al*., manuscript, it was reported that no interesting reduction products, such as formaldehyde, were synthesized using the CO_2/water mixture. This result nicely supported the surmise of Miller and Urey that reducing conditions were needed in order for effective organic syntheses to take place. The Wilde *et al*. (1953) paper was published in *Science* on 10 July 1953, and made no mention of Stanley's paper although they did mention that their experiments had 'implications with respect to the origin of living matter on earth'.

3. Earlier Laboratory Syntheses of Amino Acids

Friedrich Wöhler's report in 1828 on the synthesis of urea from silver cyanide and ammonium chloride represented the first synthesis of an organic compound from inorganic starting materials (Wöhler, 1828). Although it was not immediately recognized as such, a new era in chemical research had begun: in 1850 Adolph Strecker achieved the laboratory synthesis of alanine from a mixture of acetaldehyde, ammonia and hydrogen cyanide (Strecker, 1850). This was followed by the experiments of Alexandr M. Butlerov (1861a, b) showing that the treatment of formaldehyde with strong alkaline catalysts, such as sodium hydroxide (NaOH), leads to the synthesis of sugars.

The laboratory synthesis of biochemical compounds was soon extended to include more complex experimental settings. By the end of the 19th century a large amount of research on organic synthesis had been performed, and had led to the abiotic formation of fatty acids and sugars using electric discharges with various gas mixtures (Rabinowitch, 1945). This work was continued into the 20th century by Klages (1903) and Ling and Nanji (1922), who reported the formation of glycine from formaldehyde and potassium cyanide, probably as a result of a Strecker synthesis and by Herrera (1942), who reported two uncharacterized amino acids using the same starting material. Moreover, Walther Löb, Oskar Baudish, and others worked on the synthesis of amino acids by exposing wet formamide ($CHO\text{-}NH_2$) to a silent electrical discharge (Löb, 1913) and to UV light (Baudish, 1913).

Löb did indeed report the synthesis of glycine by exposing wet formamide to a silent discharge. He suggested that because of either the ultraviolet light or the electrical field generated by the silent discharge, formamide is first converted to oxamic acid, which in turn is reduced to glycine. He also claimed that glycine is produced when wet carbon monoxide and ammonia are subjected to the silent discharge; he proposed formamide as the intermediate in this synthesis. Löb theorized that glycine might also be produced from wet carbon dioxide and ammonia in a pathway wherein formamide was again the intermediate, but he did not demonstrate this directly.

Although Löb apparently did produce glycine from formamide, this cannot be considered a prebiotic reaction because formamide would not have been present on the primitive Earth in any significant concentrations. It is also possible that the wet carbon monoxide and ammonia led to the formation of HCN, which would have produced glycine on polymerization and hydrolysis. From a careful reading of Löb's 1913 paper it is clear that his motivation for doing the experiment was to try to understand the assimilation of carbon dioxide and nitrogen in plants. There is no indication that he had any interest in the question of how life began on Earth, or in the synthesis of organic compounds under possible prebiotic conditions. This is not surprising. Since it was generally assumed that that the first living beings had been autotrophic, plant-like organisms, the abiotic synthesis of organic compounds

did not appear to be a necessary prerequisite for the emergence of life. With the exception of Herrera (1942), who tried to demonstrate the likelihood of an autotrophic origin of life, these organic syntheses were not conceived as laboratory simulations of Darwin's warm little pond, but rather as attempts to understand the autotrophic mechanisms of nitrogen assimilation and CO_2 fixation in green plants.

Quite surprisingly, in his extensive review Oparin (1938) did not mention neither Strecker's synthesis of alanine or Löb's work with electric discharges, which may have been forgotten by then. To the best of our knowledge, the work of Löb and other 19th century chemists was first discussed within the context of prebiotic chemistry by Stanley L. Miller in his 1954 Ph.D. Thesis and in an article following the publication of his 1953 *Science* paper (Miller, 1955).

3.1. 1953: ANNUS MIRABILIS

On 29 May 1953, Sir Edmund Hilary and his sherpa Tenzing Norgay, reached the summit of the Sagarmatha, as the Nepals call Mount Everest, the world's highest mountain. They thus achieved something that had seemed impossible. Other major peaks were also reached during the first several months of 1953, which, in retrospect, have had a tremendous impact on our understanding of the origin and nature of life: Stalin died on 5 March, finally liberating genetics research in the USSR from the grasp of Trofim D. Lysenko; also in March, the first report on part of the amino acid sequence of a protein (insulin) was published by Sanger and Thompson (1953); on 25 April the double-helix model of DNA was published in *Nature* by Watson and Crick (1953); and of course Miller's publication on 15 May on the prebiotic synthesis of amino acids and organic compounds under plausible primordial conditions. These were major intellectual events whose importance cannot be underscored.

The tremendous impact of the 1953 Miller experiment almost overnight transformed the study of the origin of life into a respectable field of inquiry, at a time in which not only the molecular nature of the genetic material was being elucidated by the models of Watson and Crick, but also the demonstration that amino acids are not randomly located in a protein was also shown. The times were ripe, as not only the effort of Wilde, Calvin and others to attempt organic synthesis under primitive conditions show, but also because by then, evolutionary biology was rapidly becoming an established, properly recognized field of scientific enquire (Ruse, 1999) that could accommodate quite easily the study of the origin of life, and by the development of space programs which would soon open new perspectives for those interested in the appearance of life in the Universe (Wolfe, 2002).

Although some of Löb's results as well as those of other 19th century organic chemists may have some bearing on our understanding of prebiotic syntheses, part of the significance of Miller's experiment lies not only in the production of amino acids and other compounds, but in their formation under what was viewed at the time as plausible primitive Earth conditions. Few, if any, scientific ideas are the

product of spontaneous thoughts most theories, experiments and interpretations have been preceded by many others, and the same is true of Miller's experiment. Even if one disagrees with the assumptions underlying the simulation by Stanley L. Miller and Harold C. Urey of the primitive Earth, it deserves recognition not only because of its intrinsic merits, but also because it opened new avenues of empirical research into the origin of life.

There are huge gaps in our understanding of the origin and early evolution of life, and it is not clear that a sufficient variety of organic compounds could have been synthesized on the primitive Earth. Other possible sources for organic molecules likely included meteorites, whose indigenous amino acids are due to reactions involving ammonia, hydrogen cyanide, and aldehydes/ketones just like in Miller's experiment. It is possible that the primitive atmosphere was not as reducing as Oparin, Urey and Miller believed. Nevertheless, for all our uncertainties regarding the emergence of life, a proper assessment of the significance of Stanley L. Miller 1953 experiments implies that it is part of the classics that have shaped contemporary science.

References

Baudisch, O.: 1913, *Angew. Chem.* **26**, 612–616.
Butlerow, A.: 1861a, *Liebig's Ann. Chem.* **120**, 295–296
Butlerow, A.: 1861b, *Compt. Red. Acad. Sci.* **53**, 145–147
Garrison, W. M., Morrison, D. C., Hamilton, J. G., Benson, A. A. and Calvin, M.: 1951, *Science* **114**, 416.
Herrera, A. L.: 1942, *Science* **96**, 14.
Klages, A.: 1903, *Ber. Dtsch Chem. Ges.* **36**, 1506.
Ling, A. R. and Nanji, D. R.: 1922, *Biochem. J.* **16**, 702.
Lob, W.: 1913, *Ber. Dtsch Chem. Ges.* **46**, 684.
Miller, S. L.: 1953, *Science* **117**, 528.
Miller, S. L.: 1954, *Ph.D. Thesis*, University of Chicago, Chicago, Ill, pp. 112.
Miller, S. L.: 1955, *J. Am. Chem. Soc.* **77**, 2351–2361
Miller, S. L.: 1974, in J. Neyman (ed.), *The Heritage of Copernicus: Theories 'Pleasing to the Mind'*, The MIT press, Cambridge MA, pp. 228–242.
Oparin, A. I.: 1938, *The Origin of Life*, MacMillan, New York.
Sanger, F. and Thompson, E. O. P.: 1953, *Biochem. J.* **53**, 353–366.
Rabinowitch, E. I.: 1945, *Photosynthesis*, Vol. I, Interscience, New York, pp. 61–98.
Ruse, M.: 1999, *Mystery of Mysteries: Is Evolution a Social Construction?*, Harvard University Press, Cambridge.
Stent, G.: 1969, *The Coming of the Golden Age*, Natural History Press, Garden City, NY.
Strecker, A.: 1850, *Liebigs Ann. Chim.* **75**, 27–31.
Urey, H. C.: 1952, *Proc. Natl. Acad. Sci. USA* **38**, 351–363.
Watson, J. D. and Crick, F. H. C.: 1953, *Nature* **171**, 737.
Wilde, K. A., Zwolinski, B. J. and Parlin, R. B.: 1956, *Science* **118**, 43–44.
Wöhler, F.: 1828, *Ann. Physik* **12**, 253.
Wolfe, A. J.: 2002, *ISIS* **93**, 183.