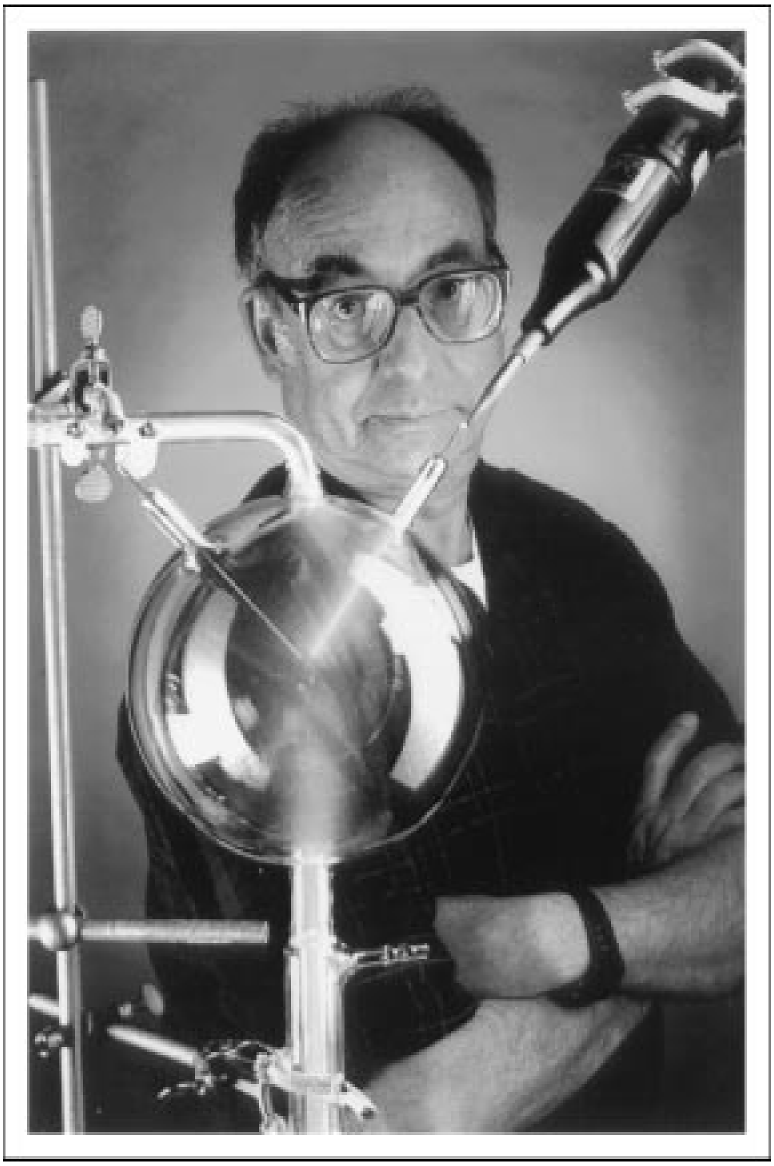
STANLEY MILLER'S 70TH BIRTHDAY



Stanley L. Miller in 1998. Photo by Jim Sugar/Corbis.

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Stanley L. Miller turned 70 years old on March 7, 2000. Remarkably, his 70th birthday is only three years shy of the 50th anniversary of his classic experiment that demonstrated how amino acids and other simple organic compounds could be synthesized under plausible primitive Earth conditions. To mark the occasion of the celebration of these events, we thought it would be appropriate to recap the story of his monumentous discovery.

The concept that life began in a primordial soup on the early Earth was proposed by Oparin and Haldane in the 1920s. However, how the organic compounds in the soup could have been synthesized on the early Earth was first experimentally demonstrated in spring of 1953 during a seminar given by Stanley in Kent Hall at the University of Chicago. In the audience were some of our century’s greatest scientists, who had come to the University as part of the Manhattan project and stayed on after the war.

Only the most distinguished scientists, many who either had or would eventually be awarded Nobel prizes, were usually invited to present these distinguished Department of Chemistry seminars. To have a 23 year old graduate student give one was unprecedented. Nonetheless, the room was full because the word had spread that some new Earth-shaking results were going to be presented. Needless to say, Stanley was a bit nervous.

The news that Stanley reported is of cource now world famous-passing an electric spark through a sealed glass apparatus containing a mixture of methane, ammonia, hydrogen, and water vapor had resulted in the synthesis of some of the amino acids found in proteins. Perhaps, he suggested, this was how organic compounds were made on the ancient Earth before life existed.

While Stanley was confident of his results, the rows of famous faces in his audience were, to say the least, intimidating. He was bombarded with questions. Were the analyses done correctly? Could there have been contamination?

James Arnold, who is now an emeritus professor at the University of California at San Diego, was in the fourth row. He remembers sitting between two other chemistry professors who – before the lecture – were convinced that what Stanley was going to report was eiher an artifact or caused by contamination. After the lecture they sat in silence, unable to find any flaws in what Stanley had done.

At one point, someone – according to Arnold, Enrico Fermi – politely asked if it was known whether this kind of process could have actually taken place on the primitive Earth. Harold Urey, Stanley’s research advisor, immediately replied, saying ‘If God did not do it this way, then he missed a good bet’. The seminar ended amid the laughter and, as the attendees filed out, some congratulated Stanley on his results.

The original idea for the landmark experiment had actually been suggested eighteen months earlier, in the fall of 1951. Urey had given a seminar, in the very same room where Stanley later presented his extraordinary results, dealing with the origin of the Solar System. He discussed how the process of planetary accretion must have given rise to certain chemical conditions on the early Earth, and how these conditions were likely to have been important in the origin of life. He explained that, given recent advances in the understanding of geochemistry and cosmochemistry, it was very reasonable to suppose that the primitive Earth had a reducing atmosphere. After the seminar someone called Urey's attention to the book by Oparin, *Origin of Life*, which had been translated into English in 1938 (Macmillan, New York). Although the book had generated quite an impact among biologists, it had gone largely unnoticed among scientists in other fields.

A reducing atmosphere, as Urey and Oparin envisioned it, would have constained energy-rich gases such as hydrogen, ammonia and methane. These gases could have reacted together to produce organic compounds. Such an atmosphere would have stood in vivid contrast to the oxidizing atmosphere that our planet possesses at the present time, with an abundance of free oxygen that destroys rather than builds up organic materials.

Up until that time, Urey pointed out, few experiments had been carried out to mimic prebiotic organic synhesis. One of them had been performed by Melvin Calvin, who would later win the Nobel Prize in 1961 for working out (with Andrew Benson, who did not share the prize) the mechanism of photosynthesis. In a paper published in 1951, Calvin and his co-workers tried to see whether photosynthesis-like reactions could have taken place in the absence of life. They irradiated carbon dioxide and water with high-energy helium ions generated by a cyclotron at Berkeley, and managed to obtain formic acid and tiny amounts of formaldehyde, a first step in the abiotic formation of sugars.

Urey remarked acerbically that 'if you have to go to these measures to get organic compounds, then perhaps a new idea is needed'. He suggested that someone needed to try to synthesize organic compounds using reducing conditions, which so far had never been done, even though Oparin had suggested the idea some 25 years earlier.

Stanley was just beginning his graduate studies in the Department of Chemistry in the fall of 1951, and the attended Urey's seminar. Fascinated, he still remembers aspects of the lecture in great detail. But he did not immediately jump at the opportunity to do the experiment that Urey had suggested. According to Stanley, he 'found experiments to be time-consuming, messy and not as important', and as a result, he decided to concentrate on theoretical work.

He began a project with Edward Teller, the father of the hydrogen bomb, to determine how chemical elements might have been synthesized in the early Universe. After nearly a year had gone by without much progress (the problem was actually in the process of being solved in elegant detail by Geoffrey Burbridge, Margaret Burbridge, Edward Fowler and Fred Hoyle), he started to have doubts about the project. Moreover, Teller left for California, and it did not seem like like a good idea for a graduate student to have his advisor halfway across the country. Faced with this problem, Stanley began to think about Urey's talk.

Stanley approached Urey in September of 1952 about the possibility of doing a prebiotic synthesis experiment using a reducing gas mixture. Urey was not very enthusiastic. He felt, with some justification, that graduate students should do experiments that had a reasonable chance of working, rather than taking a leap into the unknown. He suggested instead that Stanley work on determining the amount of the element thallium in meteorites, a safe and pedestrian topic. But Stanley was persistent. Urey finally relented and let him try some experiments, but specified that there must be signs of success within a year or the project should be abandoned.

The first challenge was to design an experiment. The mix of water and gases that Stanley wanted to try was unlikely to do anything interesting if it just sat there in a flask. He needed some sort of high-energy imput to encourage chemical reactions.

Stanley knew that chemists had been experimenting with electric sparks in gas mixtures since the end of the 19th century, sometimes producing interesting syntheses, but it seemed that no one had though about how this might relate to prebiotic syntheses and the origin of life. More often than not, these early experiments had been designed as attempts to understand the biological flixation of carbon and nitrogen. It was believed at the time that the first organisms had been photosynthetic microbes and very few, if any, scientists considered the need for the prebiotic synthesis of organic compounds as a prerequisite for the origin of life.

In contrast, Stanley started with Oparin's idea of heterotrophic origin of life and the need for an energy source to assist in the abiotic formation of organic compounds. He realized that electric discharges were probably common on the early Earth. The atmosphere at the time must have been filled with lightning flashes, and with auroral displays that were far more spectacular and energetic than the present-day northern lights.

Calvin had focused on trying to synthesize carbohydrates, but Stanley thought amino acids would be much more interesting. One could not make a living organism entirely out of carbohydrates, whereas the amino acids were the components of proteins, some of the most important components of the biochemistry of living cells.

Together, Stanley and Urey sketched a diagram of the now famous spark discharge apparatus, which showed two glass flasks connected by two glass tubes. The small flask would contain water, which could be heated. The larger flask was designed to have two electrodes projecting from the outside into the interior, which could be used to introduce an electric spark into the gas mixture.

This was not an *ad hoc* design, but was meant to simulate the ocean-atmosphere system on the Earth. Water vapor produced by heating the main flask would be like evaporation from the oceans. The gases and water vapor in the flasks would act to mimic the atmosphere, and the sparks between the electrodes would simulate lightning. A condenser added to connect the two flasks would collect any newly synthesized compounds in the miniatmosphere, allowing them to be washed into the 'ocean' (the flask with the water).

It took about a week for the glassblower to build the apparatus and the stage was set for the first experiments. After pumping all the air out of the apparatus, Stanley introduced a mixture of methane, ammonia and hydrogen. he started the spark and began to heat the water flask gently. After two days, the water had turned pale yellow and a tarry residue coated the inside of the 'atmosphere' flask around the electrodes. Anxious and unable to wait any longer, he stopped the experiment at that point and analyzed the water for amino acids.

The analytical methods that Stanley had available to him were very primitive by present day standards. He first separated the compounds in the water flask using paper chromatography. Next, he dried the paper chromatography sheet and sprayed it with ninhydrin, which was known to react with different amino acids to give a variety of purple, red and yellow tones. To his great excitement, a single faint purple spot showed up on the paper, just in the position where glycine, the simplest amino acid, should have been.

Urey was out of town at the time on a lecture tour. During one of his lectures he mentioned that he had a student who was in the process of testing the reducing atmosphere prebiotic synthesis idea. Someone in the audience then asked, evidently not too politely, 'And what do you except to get?' Urey replied, shortly, 'Beilstein'. He was referring to the 100-plus volume compendium begun by Friedrich Beilstein, *Beilsteins Handbuch der Organische Chemie*, which describes all the organic compounds that have been synthesized. And that one-word answer showed that Urey was now very optimistic about the chances for success with the experiment, a big change from only a few weeks earlier.

When Urey returned from his trip he was naturally very pleased when informed about the glycine spot. Stanley repeated the experiment, this time sparking the apparatus for a week and boiling he water rather than heating it gently. At the end of the week, the inside of the sparking flask was coated with an oily scum and the water solution was yellow-brown. Now the glycine spot on the paper chromatogram was far more intense, and spots corresponding to several other amino acids were showing up as well.

Stanley estimated that he had made a few milligrams of these amino acids, a surprising amount considering that the synthesis was thought at the time to be non-specific for any particular class of organic compound. He had been working on the project for only a little over three months, well within the time limit given by Urey.

After he showed the new impressive results to Urey, they decided that it was time to get them published, preferably in a leading journal such as *Science*. Urey called the editor, Howard Meyerhoff, and asked that the paper be published as soon as possible – an approach that is open to Nobel Laureates but to few others. Meyerhoff replied that this could be done in about six weeks. Stanley wrote a draft of the paper and when he showed it to Urey he was surprised by his immediate and generous response. Urey said his name should not be on the paper because if it was, Stanley would receive little or no credit.

The paper was submitted in mid-December 1952. After waiting for the promised six weeks, Stanley still had heard nothing from the editor about the status of the paper. When Urey found out about this, he was furious and had Stanley withdraw the paper and submit it to the *Journal of the American Chemical Society*, whose editor promised immediate publication. However, after Meyerhoff called frantically promising to get it out right away, Stanley resubmitted the paper and it was published in *Science* on May 15, 1953. The delay was caused by a reviewer who had refused to believe the results, and who had put the paper aside without sending any comments back to Meyerhoff. The reviewer later identified himself to Stanley and apologized for the delay. Even Oparin himself, when he found out about the paper, could not believe at first the ease by which amino acids and other important biochemical monomers had been synthesized.

In retrospect, 1953 was a remarkable year for evolutionary biology. Within a span of only a few months Watson and Crick published their double helix model of DNA, Sanger and co-workers reported the first amino acid sequence of a protein, thus opening the way for sequence comparisons, and Stanley demonstrated how organic compounds could be synthesized under plausible primitive Earth conditions.

So began the field of prebiotic organic chemistry. In the ensuing nearly 50 years since Stanley's first experiment many of the organic compounds considered necessary for the origin of life have been synthesized using conditions which, in many cases, have been very similar to those first used in Stanley's experiment. Because we have no geologic record of the conditions on the primitive Earth at the time life arose, it is not surprising that some of the assumptions underlying the 1953 experiments have been questioned. However, the significance of experiments and scientific observations depend on their specific historical framework. Even if one disagrees with the idea of a reducing atmosphere or on the ultimate source of organic compounds, Stanley's work deserves full recognition not only because of its intrinsic merits, but also because it opened new avenues of emperical research about the origin of life.

No wonder then, that at the 1993 ISSOL meeting in Barcelona, newspaper accounts of the meeting referred to Stanley as 'le padra chemie prebiotic'. Happy 70th Stanley!

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