# **The Spark of Life:** *Where Did Organic Molecules Come From?*

*by* Annie Prud'homme-Généreux, Nicole F. Magill, and Tatiana N. Bliss Life Sciences Quest University Canada

## Part I – A Flash of Insight

#### I know how to test this!\*

It was the fall of 1951. Twenty-one-year-old Stanley had recently traveled from his native California to the University of Chicago to pursue a graduate degree in chemistry. At a departmental seminar, his imagination was captured by the presentation from a professor in his department, the Nobel Laureate Harold C. Urey.

"In the course of an extended study on the origin of the planets I have come to certain definite conclusions relative to the early chemical conditions on the Earth and their bearing on the origin of life," said Urey."

Stan listened intently while Urey continued to explain how the early Earth atmosphere was not as it is today.

"One sees that hydrogen  $(H_2)$  was a prominent constituent of the primitive atmosphere and hence that methane  $(CH_4)$  was as well. Nitrogen was present as nitrogen gas  $(N_2)$  at high temperatures but may have been present as ammonia  $(NH_3)$  or ammonium salt at low temperatures."

Stan was riveted. Urey went on to suggest, as had the Russian biochemist Oparin before him, that organic molecules (compounds containing carbon atoms) might have formed on the early Earth from inorganic gases. This was provocative because it suggested that the molecules of life (which are organic) could be created by simple chemistry, and it could explain how the building blocks of life were first created on our primitive lifeless planet.

"It seems to me that experimentation on the production of organic compounds from water  $(H_2O)$  and methane in the presence of ultra-violet light of approximately the spectral distribution estimated for sunlight would be most profitable. The investigation of possible effects of electric discharges on the reactions should also be tried since electric storms in the [Earth's early] atmosphere can be postulated reasonably."

*I know how to test this!* thought Stan again. Circumstances didn't allow him to approach Urey immediately, but a few months later, he asked Urey for the opportunity to test the idea that the conditions and atmosphere of early Earth were sufficient to create organic molecules, the building blocks of life. Urey thought the project was "too risky" for a graduate student since it was unlikely to yield interesting results in the time allowed to complete a PhD (after all, the process might have occurred over millions of years on Earth). At Stan's insistence, Urey gave him a year to experiment.

#### Question

1. What experiment did Stan have in mind? Propose an experimental design to test the hypothesis that organic molecules formed from inorganic ones under the conditions prevalent on the early Earth. Provide as many details as possible.

<sup>\*</sup> The events described in this case are inspired by the biography of Stanley Miller (Bada & Lazcano, 2003; 2012).

<sup>\*\*</sup> This "presentation" is constructed from a paper published by Urey a year later (Urey, 1952).

# Part II – Earth in a Bottle

Stan designed the glassware apparatus shown in Figure 1. He first sterilized all of his equipment to make sure there were no living things inside of it. He then created a vacuum inside the tubes to remove our atmosphere and inserted a mixture of the following gases: hydrogen (H<sub>2</sub>), methane (CH<sub>4</sub>), and ammonia (NH<sub>3</sub>). He filled the bottom flask with water (H<sub>2</sub>O) and placed it over a flame to heat it. In the top flask, he carefully inserted two electrodes and passed an electric current which created sparks. Between the top and bottom flasks, one of the connecting tubes (left connecting tube in Figure 1) was fitted with a condenser to cool any gas present at that location in the tube. Stan also placed a few valves to permit sampling of the chemicals inside his apparatus. After a few days, he noticed that the water inside the flask rapidly turned from clear, to yellow, to a darker shade of brown. These color changes indicated the creation of novel chemicals inside his apparatus.

#### Questions

- 1. This apparatus was meant to simulate the conditions of early Earth. Describe how each element mimics a component of the early Earth environment. Be sure to address the following:
  - a. The bottom flask that is heated
  - b. The top flask
  - c. The electric discharge
  - d. The condenser
- 2. What might constitute an appropriate control in this experimental design?
- 3. What data should Stanley measure and record in this experiment? What does he have to do to collect this data?
- 4. What are possible pitfalls of this experimental design?
- 5. Electric discharges simulate lightning, a source of energy to drive chemical reactions. What other sources of energy (identify at least two) might have been present on the early Earth? How might they be simulated in this experiment?
- 6. If some of life's organic molecules are created in Stan's experiment, what are the implications for the origins of life? (Will this tell us how life arose on Earth?)

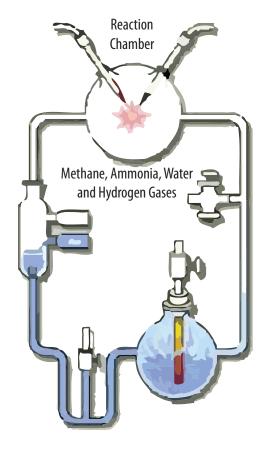
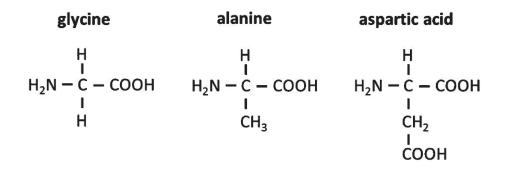


Figure 1. Stanley Miller's experimental set-up.

## Part III – Miller's Claim to Fame

Stan used chromatography<sup>\*</sup> to separate and identify each chemical in his apparatus. His experiment produced large quantities of the organic molecules glycine, alanine, aspartic acid, and gamma-amino butyric acid (GABA). The first three are amino acids used as building blocks to make proteins in all living organisms; the latter serves as a neurotransmitter in animals to facilitate communication between nerve cells. Figure 2 shows the chemical structure of these molecules.



### Gamma-amino butyric acid (GABA)

 $H_2N - CH_2 - CH_2 - CH_2 - COOH$ 

Figure 2. Chemical structure of the compounds produced in large quantity in Stan Miller's apparatus after a few days.

Stan's gamble had paid off and this finding made Stan famous. In short order, he was asked for interviews by newspapers and radio shows and his results graced the cover of *Time* magazine. Few graduate students receive this much attention. The idea that "life" could be created from primordial ooze quickly gripped the public's imagination.

Several researchers reproduced and confirmed his results. As the chemical detection methods improved, Stan's experiment was eventually shown to yield an impressive array of amino acids (33 different ones, including more than half of the 20 that are used by living organisms to make protein<sup>\*\*</sup>), as well as other organic molecules such as sugars, lactic acid, and the nitrogen bases of nucleic acids.

<sup>&</sup>lt;sup>\*</sup> The samples were spotted on filter paper and one edge of the paper was immersed in a liquid (e.g., phenol). The liquid rose up the filter paper by capillary suction. Each chemical in the spotted sample has physical properties that determine to what extent it dissolves in the liquid, how far it travels with the liquid, and the extent to which it stays bound to the filter paper. Thus each chemical travels a characteristic distance in the liquid and this can be used to identify it. This is how Stan separated and identified the chemicals present in his sample. Note that nowadays more sensitive techniques such as gas chromatography followed by mass spectrometry would be used and indeed Stan's 1953 samples were recently re-analyzed using this technique which yielded an impressive assortment of organic molecules (Johnson et al., 2008).

<sup>\*\*</sup> An amino acid is defined as a chemical on which an amino (-NH<sub>2</sub>), a carboxyl (-COOH), and a hydrogen (-H) are attached to a central carbon. Since carbon can attach to four other atoms, this central carbon can also make a bond to another group, labelled R (-R), which varies between amino acids. The identity of the R group determined the identity of the amino acid. For example, if R is the atom hydrogen (-H), then the amino acid is glycine; if R is a methyl group (-CH<sub>3</sub>), then the amino acid is alanine. A whole range of amino acids exists, but cells only encode and use 20 of them.

<sup>&</sup>quot;The Spark of Life" by Prud'homme-Généreux, Magill, & Bliss

#### **Ouestions**

- 1. Look at the chemical composition of the compounds that Stan originally discovered in his apparatus. Where does each of the atoms that make up these molecules come from? Particularly, where do the carbon (C), the nitrogen (N), and the oxygen (O) come from?
- 2. Most proteins begin their sequence with the amino acid methionine. A diagram of the chemical structure of methionine is shown in Figure 3. Is methionine likely to be among the 33 different amino acids discovered in Stan's flasks?

## methionine

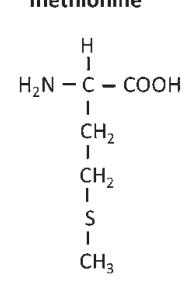


Figure 3. The chemical structure of the amino acid methionine.

- 3. What is the significance of discovering a neurotransmitter (GABA) in Stan's apparatus?
- 4. In 1909 a German chemist by the name of Walter Löb showed that it is possible to synthesize the amino acid glycine by using spark and a gas mixture.\*\*\* His findings were reported in a chemistry journal in German. Unfortunately, Löb died in 1916 and never saw the outcome of Stan's experiment. Given that his experiment is essentially identical to Stan's, and that it was performed nearly half a century earlier, why is it that it never received the attention that Stanley's experiment enjoyed? (Note: there is no indication that Stan was aware of this experiment when he designed his own).

<sup>\*\*\*</sup> This finding is reported on pages 90 and 262 of the book: Hazen, R (2005). Genesis: The Scientific Quest for Life's Origins. Joseph Henry Press.

## Part IV – Fly in the Primordial Soup

What if Urey got it wrong? Here's a summary of some of the evidence suggesting that the early atmosphere of Earth differed from Urey's proposed model (Abelson, 1966; Wells, 2002).

- *Geological Evidence:* As the planet cooled and solidified into a rocky surface, the atmosphere reacted with the minerals to affect the chemical composition of the rocks. Thus, the chemical composition of the planet's oldest rocks can serve to infer what the early atmosphere was like. Such studies do not support that the atmosphere was as proposed by Urey.
- *The Presence of Volcanoes on Earth:* There were many volcanoes on early Earth. Volcanoes eject gases which can affect the composition of the atmosphere. Assuming that the chemical composition of the interior of the planet is not much changed, volcanoes are expected to expel the same gases now as they did then. This includes gases not envisioned by Urey such as carbon dioxide (CO<sub>2</sub>), carbon monoxide (CO), and nitrogen gas (N<sub>2</sub>).
- *Greenhouse Gases for a Liquid Ocean:* For the ocean to be liquid, which is often proposed as a condition for the origins of life, there had to be greenhouse gases in the atmosphere to warm up the Earth. Stan assumed the atmosphere contained methane and ammonia, which are greenhouses gases, but these are such strong greenhouse gases that they would cause the planet to warm up to the point where the oceans evaporated. Carbon dioxide's effect on global warming is not as pronounced and its presence in the atmosphere would ensure liquid oceans.
- *A Lack of Hydrogen:* Stan assumed hydrogen was present in the atmosphere. Hydrogen is very light, and because Earth's gravity would not retain it, it would be lost to space.

#### Questions

1. The relative electronegativity<sup>\*</sup> of the atoms of life is as follows: H < C < N < O. Consider simple gases that contain these atoms and can therefore serve as building blocks to create organic molecules ( $CH_4$ , CO,  $CO_2$ ,  $NH_3$ ,  $N_2$ ,  $H_2O$ ,  $O_2$ ,  $H_2$ ). For each one, determine whether the atom of life is reduced (attracts the electrons from its bonding partner) or oxidized (donates its electrons to its bonding partner). In a bond between two identical partners (e.g. H-H), place the gas in either the reduced or oxidized column by comparing it to other gases that contain the atom. In other words, if the central atom is oxidized in the other gas (it donates its electrons), then the gas where the electrons are shared equally is relatively more reduced and should be written down in the reduced column. The last row of this table, which considers the reduced or oxidized state of H in some gases, has been filled-in as an example.

Gases containing the central atom	Reduced Gas (gases in which the central atom attracts electrons from bonding partner)	Oxidized Gas (gases in which the central atom donates electrons to its bonding partner)
Carbon (C) (CH <sub>4</sub> , CO, CO <sub>2</sub> )		
Nitrogen (N) (N <sub>2</sub> , NH <sub>3</sub> )		
Oxygen (0) (0 <sub>2</sub> , H <sub>2</sub> 0)		
Hydrogen (H) (H <sub>2</sub> , H <sub>2</sub> O)	H <sub>2</sub>	H <sub>2</sub> 0

<sup>\*</sup> Electronegativity is a property of an atom. It refers to how much it "pulls" on the electrons in a covalent bond with another atom. This value is provided in the Periodic Table of Elements. To determine which atom hogs the electrons in a bond, the electronegativity of each partner is compared. If they are the same, the atoms share the electrons equally. If one atom has a greater number, it pulls in a larger share of the electrons in the bond and is considered *reduced*. If the atom has a lower electronegativity, it lets go of the electrons to its partner and is considered *oxidized*.

- 2. Consider the gases included in Stan's flasks. Are the atoms of life present in these gases in their reduced or oxidized form?
- 3. Consider the gases that seem to have been present on the early Earth (as per the evidence presented above). Are the atoms of life present in these gases in their reduced or oxidized form?
- 4. The distribution of electrons in a bond affects its reactivity (proneness to react in chemical reactions). When oxidized gases (which are now thought to have been present on the early Earth) are included in Stan's experiment, few organic compounds are produced. Given this data, why might Stan's experiment still be considered one of the most important for the origins of life?
- 5. Life's building blocks (which are organic molecules) are essential for the creation of the first life. If Stan's experimental conditions did not mirror those found on the surface of early Earth, it is possible they were found elsewhere. Where might this be? Suggest at least two possibilities.

## Part V – Deep Sea and Deep Space Prebiotic Soup

Stan strongly favoured and promoted the idea that life arose on the surface of the planet. However, the difficulties of explaining how this occurred in light of the evidence that the atmosphere was oxidized have led some researchers to look elsewhere.

One possibility is deep sea hydrothermal vents (Figure 4). These sites, located kilometers beneath the ocean's surface, are underwater volcanoes. They are very hot (the water can reach temperature anywhere from 60°C to 464°C) and the depth causes pressures to be as high as 300 atmospheres (compared to our one atmosphere at sea level). When seawater interacts with the rising magma and hot rocks, it becomes superheated, dissolving minerals (such as iron) out of the Earth's crust. Reduced chemicals such as hydrogen sulfide (H<sub>2</sub>S), ammonia, methane, and hydrogen radiate from these vents. When these reduced compounds arrive in the ocean, they encounter a comparatively oxidized environment. That is because the sun's UV light can split the ocean's H<sub>2</sub>O into H<sub>2</sub> and O<sub>2</sub>. H<sub>2</sub> is light and floats out of the atmosphere, leaving behind the oxidized O2 gas. The highly electronegative oxygen atoms in O<sub>2</sub> in the ocean environment will seek to react with the reduced atoms coming out of the vents (so that they can gain their electrons). This serves to drive chemical reactions near the vents. Thus, the vents provide a steady supply of chemicals and a driver for chemical reactions (the difference in reduced and oxidized state of the vent materials and the ocean, respectively). Because these sites are deep below the surface, they may have protected emergent life from meteorite bombardment, as well as from UV light which is damaging to biological molecules such as RNA and DNA. So the vent environment may have offered a relatively stable environment to favor the development of organic

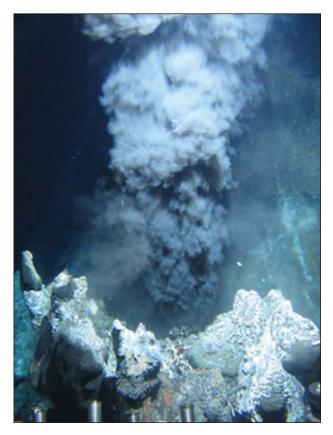


Figure 4. The "smoke" rising from a deep sea hydrothermal vent. Source: https://commons.wikimedia.org/wiki/ File:High\_Rise\_black\_smoker.jpg.

molecules and eventually the emergence of living organisms. Today, hydrothermal vents harbour life, with an ecosystem based on bacteria that can extract energy from the vents' chemicals.

There's at least one other possibility. Organic molecules may have formed in outer space. In this scenario, ice-covered mineral dust picks up stray atoms of carbon, hydrogen, oxygen, and nitrogen, and while floating in space these particles are subject to UV radiation from nearby stars. The radiation makes molecules more reactive by stripping off their electrons, and the atoms interact with each other, forming larger organic molecules. Over time, the forces of gravity coalesce the dust particles into ever larger rocks. Some of these were not included in the formation of our solar system's planets and moons and continue to roam the solar system. They are called comets. They sometimes crash to Earth, potentially delivering their organic cargo. This mechanism may have been particularly significant on the early Earth which was subject to frequent meteorite impacts during its first billion years.

What did Stan think of this prospect? Commenting on the possibility that organics may have been brought to Earth from outer space, he had the following to say: "Organics from outer space? That's garbage, it really is." (Quoted in Radetsky, 1992.)

#### Questions

- 1. Design an experiment to test the hypothesis that deep sea vents provided the necessary conditions for the synthesis of complex organic molecules.
- 2. An analysis of the microbes found near hydrothermal vents today shows that they are among the most ancient life forms on Earth (nearest the root of the tree of life). Does this mean that life arose at deep sea vents? Propose two scenarios about the location of the origins of life that would be compatible with these results: one that would support the idea that life arose at deep sea vents and one that proposes another site of origin.
- 3. The panspermia hypothesis is the idea that the universe is teeming with life and that Earth was seeded by comets and asteroids. Compare and contrast the panspermia hypothesis with the outer space hypothesis presented above.
- 4. Why might Stan be so pessimistic about the prospect that organics were delivered to Earth from outer space?
- 5. What evidence would convince you that the type of chemical reactions that occurred in Stan's apparatus happened in outer space and that these are the original source of organic molecules on Earth?

## Part VI – An Extra-Terrestrial Origin?

There are several ways to test the hypothesis that organic molecules form in space. Spectral analysis can be conducted on the light that passes through dense molecular clouds in space (each atom absorbs characteristic wavelengths of light and this can be used to confirm the molecular composition of the clouds). This has been done and more than 140 different compounds have been identified including organic molecules with dozens of atoms or more.

There is also another way to investigate this idea. If this hypothesis is true, then we might expect to find organic compounds in meteorites (pieces of comet or asteroid that periodically fall to Earth from space). In 1969, while the US had its eye on the moon with the imminent landing of the Apollo mission, a meteorite fell near the town of Murchison, Australia. There were many witnesses who reported a strong solvent smell at the time of the event. This smell is often associated with organic compounds. Several fragments were recovered shortly after impact (in fact some of them were collected while they were still hot!) and analyzed for their content (Figure 5). The Murchison meteorite contained many amino acids. The relative abundance



*Figure 5.* A piece of the Murchison meteorite. Photo by Jon Taylor, CC BY-SA 2.0, https://commons.wikimedia. org/wiki/File:Murchison\_meteorite\_0.459g.jpg.

of amino acids found in the Murchison meteorite is shown in Table 1 on the next page. For the sake of comparison, the relative abundance of the amino acids produced in Stan's experiments is also shown in the table.

#### Questions

- 1. How does the actual amount of the amino acid glycine found in Stan's apparatus compare to the amount of glycine in the Murchison meteorite?
- 2. What do you conclude from the data provided in Table 1? What aspects of the data support and do not support your conclusion?
- 3. What other evidence would convince you that the types of chemical reactions that occurred in Stan's apparatus are occurring in outer space and that they are the source of some organic molecules on Earth? What else would you like to know?
- 4. If this is a plausible mechanism for the creation of life's building blocks, then what is the "next step" in the formation of life?

Amino acid	Murchison Meteorite	Stans Experiment
Amino acids commonly found in the proteins of living	organisms	
Glycine	4	4
Alanine	4	4
Valine	3	2
Proline	3	1
Aspartic acid	3	3
Glutamic acid	3	2
Amino acids NOT commonly found in the proteins of l	iving organisms	
lpha-amino-n-butyric acid (GABA)	3	4
lpha-aminoisobutyric acid	4	2
Norvaline	3	3
Pipecolic acid	1	<1
$\beta$ -alanine	2	2
$\beta$ -amino-n-butyric acid	1	1
$\beta$ -aminoisobutyric acid	1	1
$\gamma$ -aminobutyric acid	1	2
Sarcosine	2	3
N-ethylglycine	2	3
N-methylalanine	2	2

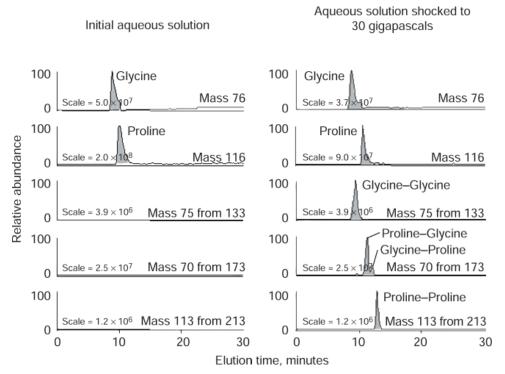
Table 1. Relative abundance of amino acids in the Murchison meteorite and the Miller-Urey experiment.\*

\* The abundance of each amino acid was compared to the amount of glycine (a simple amino acid) in each sample. In other words, the results are standardized against the concentration of glycine in each sample. Those found to be equally abundant to glycine (defined as 50–100% of the quantity of glycine in the sample) were given a value of "4" in the table. Those present as 5–50% of the amount of glycine were labeled "3". Those present as 0.5–5% of the amount of glycine were labeled "2". Those present as 0.05–0.5% of the amount of glycine were labeled "1". (Data adapted from: Wolman Y, Haverland WJ, Miller SL (1972). Nonprotein amino acids from spark discharges and their comparison with the Murchison meteorite amino acids. *PNAS* 69(4): 809–811.)

### Part VII – Where Do Peptides Come From?

The production of simple organic molecules from inorganic ones is the best understood step among the ones thought to lead to the origins of life. Researchers have adapted Stan's experiment to mirror the conditions of deep sea hydrothermal vents, the cold vacuum of space, and even Saturn's moon Titan. All experiments yield an impressive assortment of organic molecules. Creating molecules such as amino acids is relatively easy, but amino acids are not life. Once created, they must be assembled in the correct manner into larger molecules (peptides) that have the potential to carry out chemical reactions that are essential for life.

Jennifer Blank is a geologist interested in the hypothesis that amino acids were delivered to Earth by meteorites. Curious as to whether amino acids could survive the forces of a meteorite's entry into the atmosphere and collision with Earth, she designed an experiment to mimic these conditions. Working with engineers, she built a 6.2 meter-long gun that can launch a projectile at a speed of Mach 6 (six times the speed of sound or almost 2 km per second). In this gun, the bullet is aimed at a dish containing a sample of the amino acids glycine and proline. At the moment of impact, the solution is exposed to pressures 400,000 times the atmospheric pressure and to temperatures ranging from 500°C to 800°C. When she opened her amino acid vial, she noticed that her initially clear solution was now yellow. She analyzed the samples before and after the "impact"; the results are shown in Figure 6.



*Figure 6. Results from Jennifer's gun experiment to mimic the effects of a meteorite impact on the fate of amino acids.* These graphs show the composition of her amino acid samples before (left graphs) and after (right graphs) impact. Jennifer first separated all compounds in her samples using a method called gas chromatography. This method relies on the different affinities of each chemical in her sample for the column material (i.e., if a chemical has physical properties that make it "stick to" the column material, it will stay in the column longer than other chemicals in the sample). On the graphs, the abscissa indicates time since the sample was applied to the column, and the ordinate indicates the abundance of the chemical coming out of it at each time point. Each peak represents one chemical coming out of the column. To identify each chemical they were subjected to mass spectrometry, which provides a very accurate measure of the mass of each compound, which helps to identify it. Typically, all chemicals in a sample are shown on one graph (which shows as many peaks as there are compounds in the sample), but here they are broken up into graphs based on their mass to make the results clearer.

Source: A hitchhiker's guide to early Earth. Science & Technology Reviews September 2002: 4–9. Retrieved December 26, 2014 from: https://www.llnl.gov/str/September02/Blank.html

#### Questions

- 1. What proportion of amino acids present in the original sample survived the violent shock of impact?
- 2. What new chemical(s) was (were) found? How much was found?
- 3. What is the significance of this finding for the origins of life on Earth?
- 4. What would you want to know next?
- 5. Recently, a group in Japan conducted a similar experiment and found tri-peptides (three amino acids linked together) at the end of their experiment (Sugahara and Mimura, 2014). This is provocative but still not "life." What are the next steps in the formation of life?

## **Ongoing Epilogue**

From Jennifer's work, we know that meteorite impact is one possible mechanism for the formation of peptides from simpler amino acids. There are other ways to achieve this. For example, clays such as Montmorillonite (a weathering product of volcanic ash) have been shown to attract amino acids and to catalyze their polymerization into peptides. Clays do the same with nucleotides to form RNA (Ferris, 2006). Therefore, clays are a tantalizing area of research to understand how the macromolecules of life arose. Another possibility is that peptides may form under conditions simulated by Stan's experiment. Watch this brief video to learn more about this possibility.

• *Stanley Miller's Forgotten Experiments, Analyzed.* Produced by Georgia Tech. Running time: 2:13 min. Date: 2014. http://youtu.be/As-B4kOfHNE

From abiotic chemicals, to simple organic molecules, to the macromolecules of life: our understanding of the origins of life, which began more than 50 years ago with Stan's simple experiment, continues to grow...

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