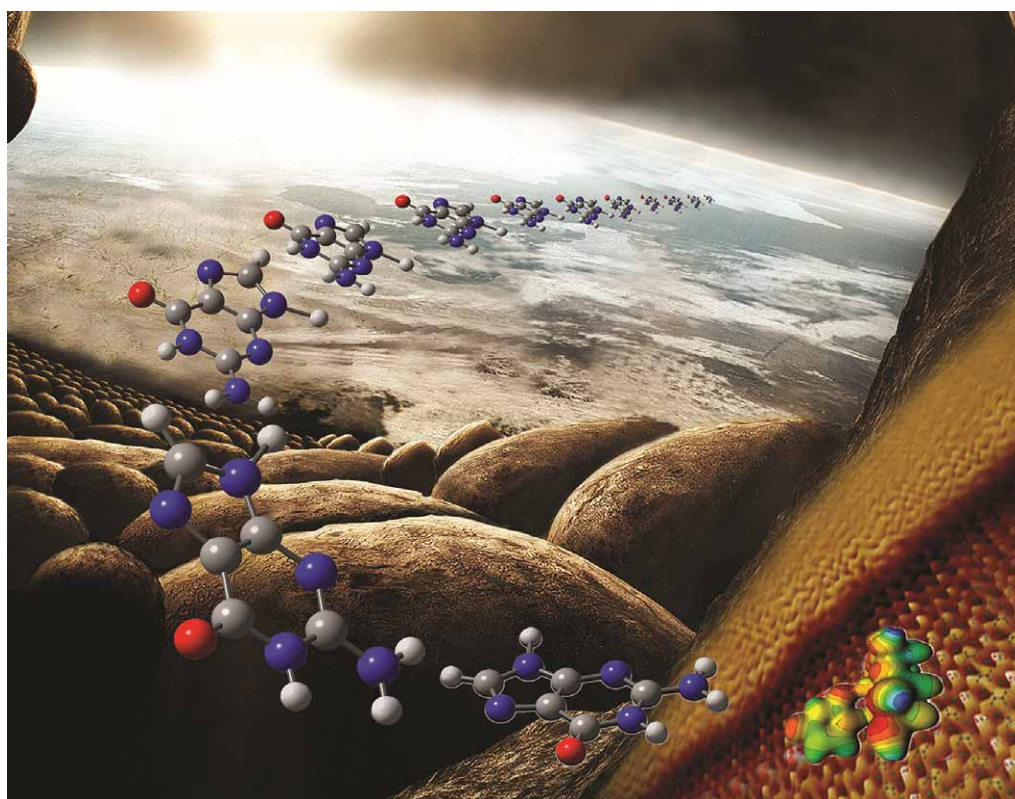


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REVIEW ARTICLE

New insights into prebiotic chemistry from Stanley Miller's spark discharge experiments†

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1953 was a banner year for biological chemistry: The double helix structure of DNA was published by Watson and Crick, Sanger's group announced the first amino acid sequence of a protein (insulin) and the synthesis of key biomolecules using simulated primordial Earth conditions has demonstrated by Miller. Miller's studies in particular transformed the study of the origin of life into a respectable field of inquiry and established the basis of prebiotic chemistry, a field of research that investigates how the components of life as we know it can be formed in a variety of cosmochemical environments. In this review, I cover the continued advances in prebiotic syntheses that Miller's pioneering work has inspired. The main focus is on recent state-of-the-art analyses carried out on archived samples of Miller's original experiments, some of which had never before been analyzed, discovered in his laboratory material just before his death in May 2007. One experiment utilized a reducing gas mixture and an apparatus configuration (referred to here as the "volcanic" apparatus) that could represent a water-rich volcanic eruption accompanied by lightning. Another included H₂S as a component of the reducing gas mixture. Compared to the limited number of amino acids Miller identified, these new analyses have found that over 40 different amino acids and amines were synthesized, demonstrating the potential robust formation of important biologic compounds under possible cosmochemical conditions. These experiments are suggested to simulate long-lived volcanic island arc systems, an environment that could have provided a stable environment for some of the processes thought to be involved in chemical evolution and the origin of life. Some of the alternatives to the Miller-based prebiotic synthesis and the "primordial soup" paradigm are evaluated in the context of their relevance under plausible planetary conditions.

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1. Introduction: simulating prebiotic synthesis in the laboratory

In May 1953, Stanley Miller working in the laboratory of Nobel laureate Harold Urey, reported in the journal *Science* the synthesis of amino acids from a gas mixture of H₂, H₂O, CH₄, and NH₃ using as an energy source a spark discharge to simulate lightning.¹ This experiment is now widely recognized as the first efficient abiotic synthesis of organic compounds under simulated primitive Earth conditions in the context of the origin of life. The reducing gas mixture used by Miller was at the time believed to be representative of the primitive terrestrial atmosphere.² Miller's choice of a spark discharge as an energy source was not an ad hoc selection. Beginning with the pioneering spark discharge experiments of Lord Henry

Cavendish on the how lightning might convert modern atmospheric gases into nitrous and nitric acids,³ this type of experimental set up had been widely used to simulate atmospheric chemical processes.

Today, some geoscientists favor an early atmosphere that was weakly reducing,⁴ containing N₂, CO₂, H₂O, CO and with only small amounts of reduced species such as H₂S, CH₄, and H₂, although there are counter arguments to this point of view.⁵ While early work examining amino acid synthesis from a neutral CO₂/N₂ atmosphere was generally disappointing,^{6,7} recent studies suggest amino acid synthesis was not as poor as previously thought.⁸ Lower yields in the earlier experiments appear to be the result of the aqueous solution, where synthesis of compounds like amino acids occurs, becoming highly acidic during the course of the experiment because of the production of nitrous and nitric acids.⁸ Using a CO₂-N₂ gas mixture and buffering the spark discharge experimental solution at pH 7 with calcium carbonate, the yields of amino acids was only about 2 times lower than that with a CH₄-NH₃-N₂ gas mixture.⁸

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If the aqueous solution is not buffered, the pH during the course of the CO₂/N₂ experiment drops to ~3 and the amino acid yield is greatly reduced. These results underscore the importance of natural water chemistry on the early Earth as a factor in prebiotic syntheses.

There remains considerable uncertainty regarding when, where and how the raw materials needed for prebiotic reactions and molecular evolution were produced. The possibility remains that there were periods in early Earth's history prior to the origin of life in which reducing conditions prevailed,⁹ but even a neutral atmosphere could still have allowed for the synthesis of significant amounts of abiotic organic material. Organic compounds produced by plausible primitive Earth conditions would have contributed to the "primordial soup" that was thought to provide the raw material for the various reactions thought to be involved in generating molecular complexity and the first self-replicating entities.

Just prior to Miller's death on 20 May 2007, several boxes containing vials of dried residues were found in his laboratory material at the University of California, San Diego. Notebooks[‡] indicated that the vials came from his 1953–1954 University of Chicago experiments that used three different apparatus configurations as well from experiments conducted in 1958 while he was at Columbia University.^{10,11} In the 1953–1954-sample set,¹⁰ were ones from the original apparatus used in the classic 1953 paper.¹ Another set came from an experiment that used a differently configured apparatus that had an aspirating nozzle attached to the water-containing flask, injecting a jet of steam

and gas into the spark flask. A third apparatus design incorporated the aspirator device, but used a silent discharge instead of electrodes, an energy source that had been used by others in the early 20th century to study how organic compounds might have been synthesized in plants.¹² Although Miller repeated his experiment in 1972 using a single flask apparatus,¹³ he never again extensively tested the apparatus designs used in the 1950s.

Additional preserved samples from an experiment conducted in 1958 were part of those found in Miller's archived collection.¹¹ These were produced from experiments using the classic apparatus and a mixture of CH₄, NH₃, H₂S and CO₂. The original dried residues from the experiment had been collected, catalogued and stored by Miller. This experiment marks one of the first spark discharge experiment to which H₂S was added to the gas mixture imitating the primordial environment. For unknown reasons the residues from this experiment were apparently never analyzed and reported.

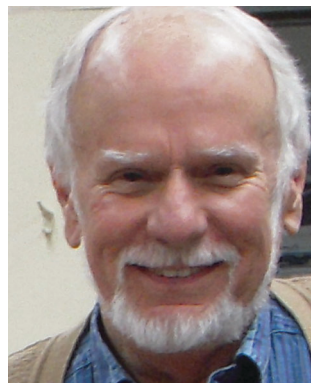
Because of the advances in analytical chemistry compared to those available to Miller in the 1950s, the original stored extracts from several of the 1953 and 1958 experiments were reanalyzed using state-of-the-art analytical methods.^{10,11} Although experiments with various gas mixtures and a spark discharge apparatus configured according to Miller's original designs can be readily carried out, the unique opportunity to investigate samples prepared by the pioneer in abiotic synthesis using state of the art analytical methods is of considerable historic interest, as well as providing new insights into the diversity of compounds that might be synthesized under a variety of simulated primordial Earth conditions.

This review addresses what has been learned about possible primordial synthesis on the Earth, and elsewhere, from the analyses of the archived 1950s Miller samples. In addition, the recent investigations of synthesis in a neutral CO₂/N₂ atmosphere are discussed with respect to their importance in providing the necessary compounds for the origin of life even if the global atmosphere was not reducing as originally assumed by Miller. Finally, the implications of these experiments as well as proposals for alternative prebiotic synthesis and molecular evolution routes involving hydrothermal vent systems are discussed in terms of their plausibility in the geochemical environments that likely existed on the primordial Earth. The results discussed here challenge claims that the "primordial soup" concept of the origin of life is "well past its sell-by date" and as such is null and void.¹⁴ In fact, the "primordial soup" theory as a model for the processes needed to produce the raw material used in subsequent reactions involved in the origin of life is very much alive and doing very well indeed!

2. The "volcanic" apparatus

One apparatus Miller tested in the 1950s incorporated an aspirating nozzle attached to a water-containing flask and a separate 5 L flask that housed the electrodes. The nozzle injected a jet of steam and gas into the spark (see Fig. 1). This experiment

[‡] Details of the experiments can be found in Miller's notebooks in The Register of Stanley Miller Papers (1952 to 2010) in the Mandeville Special Collection Library at the Geisel Library, University of California at San Diego, Collection Serial number 655, MSS642, Box 25.



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early Earth and other solar system bodies; and the detection and origin of organic compounds on Mars by in situ instrument-based analyses directly on the planet. Professor Bada is also affiliated with the Center for Chemical Evolution at the Georgia Institute of Technology, which is jointly supported by the National Science Foundation and the National Aeronautics and Space Administration Astrobiology Program. Photograph courtesy of Wes Newcome.

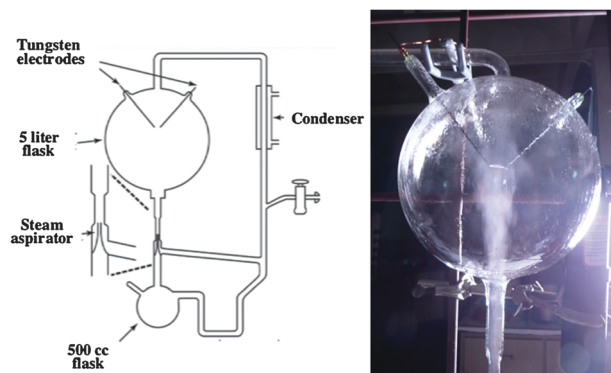


Fig. 1 Left: Diagram of the "volcanic" apparatus (taken in part from ref. 83); Right: photograph of the "volcanic" apparatus during a spark discharge experiment (courtesy David Brigg BBC Scotland).

thus simulated the spark discharge synthesis by lightning in a steam-rich volcanic eruption or geyser, although Miller never mentioned this possibility. Miller identified five different amino acids, plus several unknowns, in the extracts from this apparatus.¹⁵ Product yields appeared somewhat higher than in the classical configuration, although this was never confirmed. We analyzed the archived vials associated with this experiment in order to better characterize the variety of products synthesized in this "volcanic" apparatus simulation experiment.¹⁰

After the residues in the vials from the "volcanic" experiment had been re-suspended in 1 ml aliquots of doubly distilled deionized water, analyses of the OPA-NAC (*o*-phthalaldehyde-*N*-acetyl-L-cysteine) fluorescent amino acid derivatives were characterized by a combination of high performance liquid chromatography with fluorescence detection, and liquid chromatography-fluorescence detection-time of flight mass spectrometry.¹⁰ Twenty-two amino acids and five amines (Fig. 2) were detected, some of which have never been previously identified in spark discharge experiments. For comparison, we analyzed the archived vials from the classical apparatus. The yield and diversity of amino acids synthesized in the "volcanic" experiment in general exceeds that found in the original experiment with the classic apparatus.¹⁰ In both experiments, HCN and aldehydes/ketones were produced by

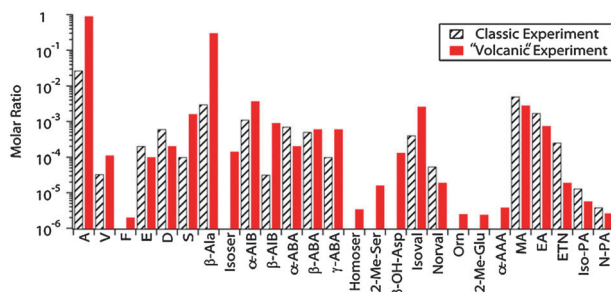
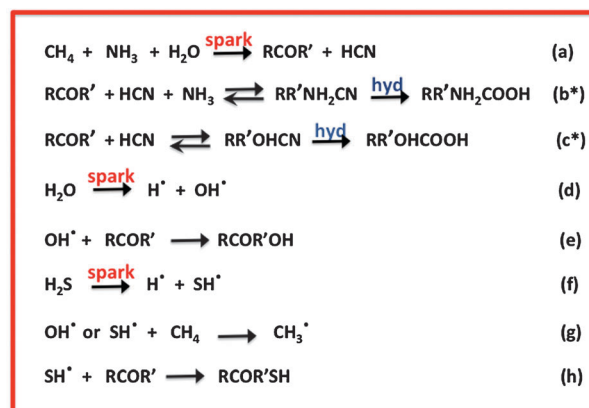


Fig. 2 Abundances (relative to glycine = 1) of amino acids produced in the "volcanic" spark discharge experiments taken from ref. 10. Amino acids are designated by either their one-letter abbreviation (for protein amino acids), or the abbreviations are given in ref. 10.



Scheme 1 Based on ref. 16 and 84–87. The symbol * denotes reactions that take place in aqueous solution. All others are assumed to be gas phase reactions.

the action of the spark on the reduced gases (Scheme 1a). These then dissolved into the aqueous phase and amino acids were then synthesized by the Strecker reaction pathway (Scheme 1b). Hydroxy acids are also synthesized in this experiment¹⁵ *via* the cyanohydrin pathway (Scheme 1c). However, because Miller separated the amino acids from the reaction mixture by cation-exchange chromatography,[†] these compounds were not isolated and saved with the archived amino acid samples.

Hydroxylated compounds appear to be preferentially synthesized in the "volcanic" experiment. This suggests that the steam injected into the spark apparently split water into H^\bullet and OH^\bullet radicals (Scheme 1d).¹⁶ The OH^\bullet radicals then likely reacted with aldehydes and ketones (Scheme 1e), which in turn reacted *via* the Strecker reaction (Scheme 1b) to produce the hydroxylated amino acids detected in the extracts.

As noted, a criticism of the original Miller experiment is a reducing gas mixture is not considered by some to be a realistic representation of the primitive atmosphere,⁴ which is now claimed to have consisted of CO_2 and N_2 . However, there are compelling arguments suggesting that reducing atmospheric gases could have prevailed on the early Earth as a result of the formation of a Titan-like atmospheric organic haze UV shield protecting gases such as methane and ammonia from photochemical degradation.¹⁷ Additional sources of both reduced gases and reagents needed for efficient prebiotic synthesis could also have existed on the primordial Earth. Serpentinization reactions associated with oceanic hydrothermal circulation could have resulted in the release of significant quantities of reduced gases that contributed to maintaining a steady state reduced atmosphere.¹⁸ Impacts of bolides rich in organic matter could have supplied reagents like HCN and formaldehyde, and there is evidence that even organic poor bolides could have generated abundant HCN from an N-rich and relatively oxidizing atmosphere.¹⁹

The "volcanic" apparatus experiment indicates that localized prebiotic synthesis could have also been effective even if the overall atmosphere was not reducing in composition. Reduced gases and lightning associated with volcanic eruptions in hot spot, island arc type systems could have been prevalent



Fig. 3 The Great Eruption of Vesuvius, August 8, 1779 by Pietro Fabris (© Stapleton Collection/Corbis with permission).

on the early Earth before extensive continents formed.²⁰ It is now well established that lightning is a ubiquitous process that occurs during volcanic eruptions although the mechanisms responsible for lightning production are only beginning to be understood.²¹ One of the first detailed accounts of volcanic lightning were made by Sir William Hamilton during the 1779 eruptions of Mt Vesuvius (see Fig. 3): “but the black smoke and ashes issuing continually from so many new mouths, or craters, formed an enormous and dense body of clouds over the whole mountain, and which began to give signs of being replete with the electric fluid, by exhibiting flashes of that sort of zig-zag lightning, which in the volcanic language of this country is called *ferilli*, and which is the constant attendant on the most violent eruptions.”²²

Recent advancements in our understanding of volcanic lightning indicate that it is primarily associated with water, fine ash, rich eruptions, by processes somewhat akin to those that produce lightning in “dirty thunderstorms”.²¹ Although lightning flashes can occur at both the volcano vent and in the immediate vertical area directly above it, most of the lightning takes place in the plume at two discrete heights of 1–4 km and ~10 km above the volcano vent.²¹ The volcanic plume tends to partially retain its initial chemical composition although there is some mixing with the surrounding atmosphere. Thus, any gases



Fig. 4 Depiction of a eutectic pond in the proximity of a lightning-rich eruption on a hot-spot type volcanic island. The top part is based on a photograph of volcanic lightning associated with the Eyjafjallajökull eruption in the spring of 2010 (©Lucas Johnson/Reuters with permission). The bottom part was derived from a drawing provided by Professors Nicholas Hud and Thom Orlando, Georgia Institute of Technology.

ejected during the eruption are still entrained within the plume and could undergo lightning induced chemical reactions. If the surrounding atmosphere was also reducing, this would have enhanced the overall synthesis yield.

As Huang *et al.*²³ have noted, the Earth’s outer core appears to be oxygen depleted, possibly implying a more reducing primitive lower mantle. Thus, volcanic emissions linked to hot spot deep-seated mantle plumes could be rich in reduced gases. As has been recently suggested, localized prebiotic synthesis occurring in the lightning-rich gas emissions associated with these types of volcanoes could have been a robust source of organic compounds on the early Earth.^{10,11,20}

Amino acids formed in volcanic island arc systems could have been locally rained out onto the volcano flanks and then been washed into ponds and tidal areas, where they could be polymerized by reagents such as carbonyl sulfide, a simple volcanic gas that has been shown to facilitate peptide formation under mild conditions.²⁴ Another condensation reagent that may have been common on the early Earth was cyanamide,²⁵ or its dimer 2-cyanoguanidine, which has been shown to promote the formation of simple peptides in spark discharge simulation experiments.[§]

Prebiotic syntheses such as these could have been common in partially frozen eutectic water pools in proximity to hot-spot type volcanoes on a ice covered early Earth (see Fig. 4), which might have existed because of the dimmer Sun and insufficient levels of carbon dioxide, or other greenhouse gases, needed to raise surface temperatures above freezing.^{5,9,26} In the type of environment shown in Fig. 4, periodic freezing could have taken place producing eutectic mixtures containing concentrated solutions of the reagents for robust prebiotic synthesis.^{27,28}

Another process that would concentrate both reagents and synthesized compounds was evaporation of shallow ponds,

§ J. L. Bada *et al.*, “A plausible simultaneous synthesis of amino acids and simple peptides on the primordial Earth”, presented at Astrobiology Science Conference, Atlanta, Georgia, April 2012 (manuscript in preparation).

and lakes, estuaries and tidal lagoons.^{29,30} Evaporation would produce eutectic brines concentrating reagents that would have then readily reacted to produce simple compounds, which in turn could have been converted into ones with increasingly complexity. These types of processes may have provided the material needed to eventually produce complex molecules with the properties of information storage and replication prone to random mutations, the hallmark of both the origin of life and evolution.

On the modern Earth, hot spot island arc-type volcanic systems such as the Hawaiian Islands have ages that can range from several hundreds to several millions of years.³¹ Today, these islands gradually become submerged as plate movements transport the islands into deeper waters and their relatively long geologic life time and isolation offers a unique opportunity to estimate molecular evolutionary rates.³¹ On the primordial Earth before the appearance of modern plate tectonics,³² island arc volcanic systems could have had even longer lifetimes before becoming submerged below the ocean surface. Thus, island arc volcanic systems could have provided significant time for chemical evolution to take place, leading first to increasing molecular complexity and then eventually primitive self-replicating entities. Given the large numbers of such systems on the early Earth, it is conceivable that the origin of life may have taken place at several locations, with ultimately one living entity out competing the others to become the ancestor of the modern DNA-protein world.

Models of the primitive Earth tend to make global descriptions of the prebiotic environment,^{4,5,9} but more realistic schemes should as include the role of energy-rich localities and local environments in prebiotic chemical syntheses.

3. Synthesis in a reducing atmosphere containing H₂S

Another set of preserved samples from an experiment conducted in 1958, this one with H₂S in the gas mixture, was also found in Miller's archived collection.¹¹ Analyses of the H₂S experiment extracts were carried out using the procedure described for the extracts from the "volcanic" apparatus.¹⁰ The compounds detected included one- and two-carbon amines and two- to six-carbon amino acids.¹¹ Both protein amino acids, such as aspartic and glutamic acids, and non-proteinogenic amino acids, such as isovaline and β -aminobutyric acid, were found to be racemic within experimental error, indicating they were produced in the experiment and are not products of contamination associated with sample storage. In total, 23 amino acids and 4 amines, including 6 sulfur-containing amino acids and 1 sulfur-containing amine,^{11,33} were found (Fig. 5). There was also evidence indicating that several other higher carbon amino acids were also generated, but at much lower relative amounts. In addition, the 1958 H₂S experiment also generated amino acids such as threonine, leucine, and isoleucine, which were not detected in Miller's other spark discharge experiments.

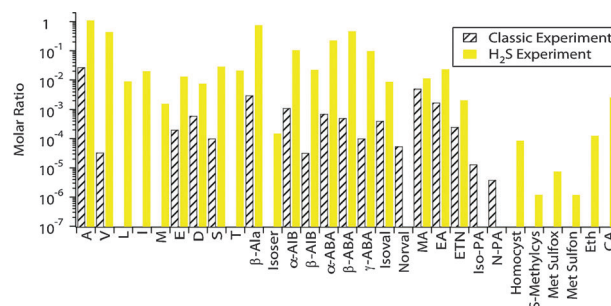


Fig. 5 Abundances (relative to glycine = 1) of amino acids produced in the spark discharge experiment with H₂S taken from ref. 11. Amino acids are designated by either their one-letter abbreviation (for protein amino acids), or the abbreviations are given in ref. 11.

These results represent the earliest example of the production of a variety of organosulfur compounds, including *S*-methylcysteine, methionine, ethionine, methionine sulfoxide, and methionine sulfone from a spark discharge experiment designed to mimic possible primitive Earth conditions. Because Miller himself never analyzed the 1958 sample extracts he was unaware of this breakthrough.

It was also found that the H₂S experiment produced a greater relative abundance of many amino acids in comparison to the other spark discharge experiments Miller carried out.^{10,13} The yields of the non-protein amino acids found in the H₂S samples are remarkable. The combined yields of non-protein amino acids, α -aminobutyric acid in particular, in the original, volcanic, and silent experiments were at least hundred times smaller than what was detected in the H₂S samples.^{10,13} The production of HO \cdot and HS \cdot radicals (Scheme 1d and f) in the spark discharge enhance the production of H₃C \cdot radicals (Scheme 1g) that in turn promote an increase in aldehyde/ketone alkylation and thiyl radical addition (Scheme 1h) prior to reaction *via* the Strecker synthesis pathway to produce amino acids. In addition, the higher production of alkyl amino acids may arise from the degradation of aspartic and glutamic acids.³⁴ In the presence of H₂S, aspartic and glutamic acids may form their respective cyclic thioimides, which can be hydrolytically opened to produce α - and ω -thioacids. Dethio-carboxylation of these thioacids may have then formed some of the alkyl amino acids detected in the spark discharge experiment with H₂S.³⁴

A detailed investigation of the prebiotic synthesis of methionine was carried out by Van Trump and Miller in 1972,³⁵ who used an electric discharge acting on a simulated primitive Earth atmosphere containing CH₄, N₂, NH₃, H₂O, and H₂S or methane thiol (CH₃SH). The finding of acrolein (propenal, CH₂=CH-CHO) as a product of the discharge and the demonstration of its likely involvement in the abiotic formation of methionine led to the suggestion that acrolein had played a central role as a precursor in the prebiotic synthesis of a number of amino acids that included methionine, glutamic acid, homocysteine (HSCH₂CH₂CHNH₂COOH), homoserine (HOCH₂CH₂CHNH₂COOH) and α,γ -diaminobutyric acid.³⁵ A similar reaction synthesis pathway was probably responsible

for the sulfur-containing compounds identified in the 1958 H₂S experiment.

The relative amino acid yields produced by the H₂S spark discharge experiment and the relative amino acid abundances detected in the carbonaceous chondrites (CCs) are intriguingly similar.¹¹ This suggests that Miller-type reactions involving reduced gas including H₂S produced some of the organic compounds found in CCs. Thus, H₂S likely played an important role in prebiotic reactions not only on the early Earth, but in early solar system environments in general.

Volcanoes are major modern natural sources of atmospheric H₂S on the Earth today.^{36,37} Much of the atmospheric sulfur on the early Earth was also likely derived from volcanism and H₂S may have been a significant sulfur species in the primitive atmosphere.²

In summary, the combined results of the analyses of both Miller's 1953 volcanic experiment and the 1958 study that included H₂S serve as models for the chemistry that may have occurred on the early Earth, especially in volcanic plumes.^{10,11} The presence of gases that were likely commonly emitted during early volcanic eruptions, including H₂S, NH₃, CH₄ and CO₂, in conjunction with ubiquitous volcanic lightning, could have provided a powerful mechanism for driving the synthesis of numerous prebiotic biomolecules. The occurrence of these reactions on long-lived volcanic island arc systems would have provided a stable environment for the processes involved in the origin of life to take place.

4. Prebiotic synthesis in a neutral CO₂/N₂ atmosphere and the role of pH in amino acid production

In the Strecker synthesis of amino acids (Scheme 1b), the overall yield is dependent on the pH at which the aqueous phase is buffered.^{8,38} Neutral to slightly basic values favor amino acid synthesis by the Strecker mechanism, while lower pH values favor hydroxy acid synthesis by the cyanohydrin mechanism (Fig. 6). Using a CO₂-N₂ gas mixture and an unbuffered spark discharge experimental solution,⁸ the pH during the course of drops to <3. Electric discharges acting

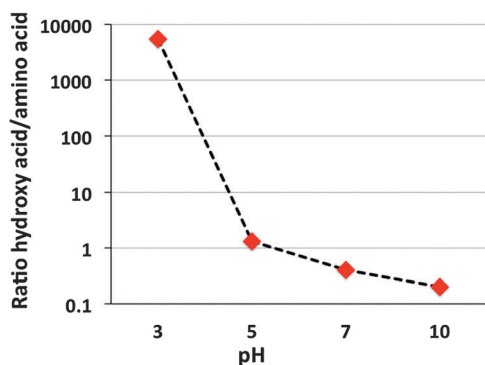


Fig. 6 The pH dependence of the relative yields of hydroxy and amino acids at 100 °C. Based on ref. 38.

on CO₂ + N₂ gas mixtures over liquid water, and on N₂ + H₂O (g) mixtures, produce large amounts of HNO₂ and HNO₃ (along with lesser amounts of ammonia and hydrazine (H₂NNH₂)).^{39–41} This suggests that H₂O vapor present in the experimental setup undergoes disproportionation to hydrogen and hydroxyl radicals (Scheme 1d). The latter, in turn acts as oxidizing agent (hydrogen peroxide is probably the oxidation agent). N₂ is partly converted to both ammonia plus nitrite, which are then oxidized to nitrate.^{39–41} As can be seen in Fig. 6, at low pH the amino acid yield is greatly reduced with the major product being hydroxy acids. In a similar experiment in which the aqueous phase is buffered at neutral pH with calcium carbonate, the yields of amino acids increase dramatically and are only about 2 times lower than that with a CH₄-NH₃-N₂ gas mixture.⁸ With the neutral gas mixture, the main amino acids synthesized were serine, glutamic acid, glycine and alanine, along with traces of aspartic acid, α -aminoisobutyric acid, γ -aminobutyric acid and β -alanine. The relative amounts of amino acids produced with CO₂/N₂ appear to be less diverse than with a reducing atmosphere (see Fig. 2 and 5), although this result needs to be verified with more rigorous characterization using the combination of high performance liquid chromatography with fluorescence detection, and liquid chromatography-time of flight mass spectrometry utilized in the recent analyses of Miller's old archived experiments.^{10,11} Also, the relative amounts of hydroxy acids synthesized in comparison to amino acids with a CO₂-N₂ gas mixture have not yet been investigated.

Recently, Kuwahara *et al.*⁴² have claimed that the amino acids reported in the CO₂/N₂ experiments of Cleaves *et al.*⁸ were artifacts derived from the use of ascorbic acid to prevent nitrosamine formation when amino acids are acid hydrolyzed in the presence of nitrate and nitrite. However, analyses by Cleaves *et al.*⁸ of the ascorbic acid used in their experiments did not detect any amino acids above blank levels. Moreover, the Kuwahara *et al.* experiments were not buffered and as a result the pH at the end of the experiment was <1.⁴² As discussed, at this pH only very low amounts of amino acids would have been produced, implying the amino acids found by Kuwahara *et al.* are possibly contaminants associated with the experimental protocol.

These experiments demonstrate that the amino acid yield in spark discharge experiments is critically dependent on the pH of the aqueous phase and underscores the importance of pH in the synthesis of prebiotic compounds in natural waters on the early Earth and elsewhere.

5. Prebiotic synthesis and plausible geochemical conditions

It is apparent from the various results summarized above that prebiotic syntheses could have taken place in a variety of geochemical environments that may have existed on the primitive Earth. Variables such as atmospheric gas composition, natural water pH and temperature are of central importance in determining in which environments robust synthesis may

have taken place. In addition, variables such as water salinity and composition may have also been significant factors in the efficiency of some synthetic processes.

The results in the experiments simulating a neutral CO₂/N₂ atmosphere have shown the critical importance of pH. The pH of natural waters is controlled by many factors, with the presence of certain atmospheric gases being of paramount importance. The ocean pH today is ~8 and is regulated by the amount of CO₂ in the atmosphere according to the equation:^{43,44}

$$P_{\text{CO}_2} = \frac{[\text{H}^+]^{3/2}}{K_1 K_{\text{H}}} \left(\frac{2K_{\text{sp}}}{K_2} \right)^{1/2}$$

where

$$K_{\text{H}} = \frac{[\text{H}_2\text{CO}_3]}{P_{\text{CO}_2}}; K_1 = \frac{[\text{H}^+][\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3]};$$

$$K_2 = \frac{[\text{H}^+][\text{CO}_3^{2-}]}{[\text{HCO}_3^-]}; K_{\text{sp}} = [\text{Ca}^{2+}][\text{CO}_3^{2-}].$$

Values of the various equilibrium constants as a function of temperature and salinity are readily available in the literature.⁴⁴ This relationship shows that as the partial pressure of CO₂ the atmosphere increases, the pH of ocean water decreases. This relationship is the cause of the present day acidification of the oceans associated with increasing atmospheric CO₂ from fossil fuel combustion.

On an early Earth with a neutral CO₂/N₂ atmosphere with *p*CO₂ supposedly many times the present day preindustrial values in order to compensate for the faint-young sun and keep surface temperatures above freezing,⁴ ocean pH could have been as low as ~5 (this would correspond to a CO₂ partial pressure of 10 atm).⁴⁴ At this pH value, as shown in Fig. 6, the yields of amino acids and hydroxy acids *via* the Strecker/cyanohydrin pathways (Scheme 1b and c) would have been about equal at 100 °C. However, temperature would also play a central role in the relative synthesis of amino acids and hydroxy acids. The change to dominance of hydroxy acids shown in Fig. 6 below pH ~6 is due to the decreasing concentration of free NH₃. Because NH₃, not NH₄⁺, is involved in the Strecker synthesis of amino acids (Scheme 1b), as pH becomes less than the ammonia–ammonium *pK*_a, the amount of free NH₃ decreases and thus the yield of amino acids relative to hydroxy acids decreases. The ammonia–ammonium *pK*_a is highly sensitive to temperature and decreases as temperature increases (9.4 at 25 °C and ~7 at 100 °C at the salinity of seawater⁴⁵). Therefore, although the results in Fig. 6 at 100 °C show an increase in the relative amounts of hydroxy acids relative to amino acids for pH <5, at lower temperatures this transition would take place at higher pH values. This indicates that at high atmospheric CO₂ partial pressures which would yield oceanic pH values of ~5 at 25 °C,⁴⁴ the synthesis of hydroxy acids would be favored over amino acids.

In contrast, with a reducing atmosphere containing NH₃ and H₂S with only trace amounts of CO₂, the atmospheric levels of these gases and the dissolved amounts of NH₄⁺ and SH[−] would have determined ocean pH, although phyllosilicate mineral

buffering may have played a minor role.[¶] The possible levels of atmospheric NH₃ and H₂S in the primitive atmosphere are not easily constrained. The level of NH₃ in the atmosphere is generally considered to have been low because of rapid photochemical degradation,⁴⁶ although there are alternative views.¹⁷ Moreover, the release of ammonia by submarine hydrothermal vents into the early oceans and other water bodies,¹⁸ and because of the high ammonia solubility, there could have been significant concentrations of dissolved NH₃. This in turn would have promoted the synthesis of amino acids. With a pH of 7 and a concentration of NH₄⁺ ~0.001 M (corresponding to an atmospheric *p*NH₃ of ~10^{−4} atm if the ocean–atmosphere were in equilibrium), the ratio of synthesis of amino acids to hydroxy acids would be around unity or greater. The concentration of dissolved SH[−], which could have been also directly added to the oceans by hydrothermal discharges, would have been regulated by the concentration of dissolved Fe⁺² and the solubility of FeS. It is unlikely that Fe⁺² was ever higher than ~0.1 mM⁴⁷ and thus the concentration of SH[−] and dissolved H₂S would have probably been too low to have a significant influence on ocean pH.

The overall effect of the ammonia, and to a lesser extent hydrogen sulfide, buffers would have been to regulate the pH of the oceans and other water bodies in the range 6–9, with the actual pH depending on both atmospheric levels and the solubility of the gases. It is interesting to note that in the Miller spark discharge experiments with NH₃ and H₂S as components of the gas mixture, pH values were ~8 whereas with only ammonia in the gas mixture the pH is 8–10.^{||} As is apparent in Fig. 6, at these pH values amino acid synthesis is the favored reaction pathway and this would have been the case over a temperature range from 0 °C to 100 °C.

The role of salinity of natural waters on the primordial Earth in prebiotic syntheses has only partially been investigated.⁴⁸ With only minor areas of continental crust on the primitive Earth, there would have been no place to store evaporites and thus the total amount of the dissolved salt in the ocean could have been more than twice what it is today.⁴⁹ With few continents, non-saline, large fresh water accumulations may have been scarce.

6. Hydrothermal syntheses as an alternative source of prebiotic organic compounds

Highly acidic or highly alkaline conditions such as found in hydrothermal vent discharges have also been proposed as

[¶] It has been suggested⁷⁸ that phyllosilicates play an important role in the regulation of the ionic composition of seawater including possibly H⁺. Phyllosilicates today are mainly formed by the weathering of continental silicate rocks while on the early Earth they may have been formed by the weathering of deep ocean hydrothermal deposits. Whether these minerals would have played an important role in regulating the pH of the early oceans is uncertain.

^{||} Miller measured the pH in some of his experiments and the values can be found in his notebooks.[‡] Also in recent repeats of the Miller spark discharge experiments at the Georgia Institute of Technology with methane and ammonia, pH values are in general close to 8–9 (Bada, Cleaves, Parker, Zhou and Fernández, unpublished results).

plausible sites for prebiotic chemistry and thus a source of the raw material considered necessary for the origin of life.^{14,50–53} These extreme environments present severe limitations with respect to their potential for prebiotic chemistry by the reaction routes discussed here because of stability and synthetic pathway issues associated with temperatures and pH.^{54,55} It has been claimed that autocatalytic metabolic-like reactions can overcome these limitations,^{14,53} although this has never been demonstrated using plausible geochemical conditions.

In the case of hot ($T > 250\text{ }^{\circ}\text{C}$), low pH systems such as those found in Black smoker vent systems⁵⁶ characterized by blackish metal sulfide chimneys, reagents such as HCN would be extremely unstable and have a life time of seconds.^{44,57} Thus the HCN steady state concentration would likely be too low for effective synthesis of amino acids and other compounds *via* the Strecker synthesis pathway. Even if HCN somehow attained a steady state concentration sufficiently high to react with aldehydes and ketones (assuming they too were sufficiently stable for a significant steady-state concentration to exist), in these low pH vent systems the Strecker/cyanohydrin pathways overwhelmingly would favor the synthesis of hydroxy acids (see Fig. 6).

White smoker vent systems are characterized by vent waters with lower temperatures (T 60–90 $^{\circ}\text{C}$) and alkaline pH values usually >9 .⁵⁸ Because these vent waters are rich in barium, calcium, and silicon they tend to have dull whitish chimney-like structures, in contrast to black metal sulfide chimneys characteristic of Black smokers. Although the stability problems associated with White smoker vent conditions could be less of an issue than in Black smokers because of the lower temperatures, efficient prebiotic syntheses are nonetheless problematic. HCN has a half-life at 75 $^{\circ}\text{C}$ and pH 10 of <1 day.^{44,57} If the steady state concentration of HCN could be maintained high enough to support the Strecker synthesis, at alkaline pH values amino acid synthesis would indeed dominate over hydroxy acid synthesis (Fig. 6). This is supported by experiments done with aqueous KCN solutions at ~ 13 pH in the presence of high pressures of carbon monoxide (CO) and nickel catalysts at temperatures between 100–200 $^{\circ}\text{C}$.⁵⁹

Although both Black and White smoker systems have been modeled and investigated with respect to their potential for prebiotic synthesis, serious questions remain about whether the conditions used in these theoretical calculations and laboratory-based simulations are indeed geochemically plausible. For example, using 0.1 to 0.2 M KCN in the presence of metal sulfides and 75 bars of CO, lactic acid and glycine have been produced.⁵⁹ One likely mechanism of formation was the Strecker/cyanohydrin pathways with the necessary formaldehyde being formed by metal catalyzed reduction reactions of formate generated by cyanide hydrolysis and the direct hydration of CO. Nevertheless, the high concentrations of KCN used are unreasonable because this would require that the entire surface inventory of the Earth's nitrogen be converted into cyanide.⁶⁰ In addition, the high pressures of CO used in the experiment are not plausible under any conditions that might have existed on the early Earth.⁶⁰

Analyses of both Black and White hydrothermal fluids have not yielded any evidence for the synthesis of important compounds needed to produce a rich inventory of the raw material needed for the steps thought to be involved in the origin of life. Although amino acids have been detected in some vent fluids, their enantiomeric composition indicates they are of biological origin and not the products of abiotic chemistry.⁶¹ On the other hand, some hydrocarbons have been detected in vent fluids and these appear to be indeed abiotic and not derived from the biota associated with hydrothermal vent systems.⁶²

The potential importance of hydrothermal vents reactions should not be underestimated, however. Some of these systems, *via* serpentinization reactions, could have played an important role in providing some components of the prebiotic soup.⁶³ In addition, if concentrations of reagents used in hydrothermal synthesis experiments were more typical of these expected in natural systems perhaps these systems could potentially provide some prebiotic compounds. In this sense, the Miller-type prebiotic and hydrothermal vent based syntheses could then be viewed as synergistic and complementary. These combined sources, as well as the possible infall of organic rich CCs,⁶⁴ could have produced a rich “primordial soup” on the early Earth and this then in turn provide the raw material for subsequent steps involved in the origin of life.

7. The transition from prebiotic to biotic chemistry

With the “primordial soup” theory, temperature, pH, and ocean–atmospheric composition would have been of utmost importance in determining what compounds could have been synthesized and thus been available to undergo further reactions to produce the molecular complexity needed to develop an entity capable of self-sustaining replication. The composition of the atmosphere would have been important in regulating whether simple organic compounds might have been directly globally or locally synthesized, and in what relative amounts. As has been noted, pH is important in determining whether amino acids or hydroxy acids are synthesized. Temperatures would need to be low enough to permit the accumulation of compounds to a point where they could be concentrated by processes such as eutectic freezing and evaporation. In addition, localized environments surrounding volcanic islands with eutectic ponds and evaporating tidal lagoons may have played a bigger role in the transition from abiotic to biotic chemistry than the ocean itself. All these factors suggest a relatively cool early Earth with waters near neutral pH, with few, if any, major continental areas, but rather a series of small volcanic island oases that allowed for the continuum of the steps thought to be involved in the origin of life to take place.

Another important parameter is the time needed for the transition from abiotic to biotic chemistry. Although this is basically an unknown there are some interesting indicators of the pace of some processes. For example, CCs provide a potential time constraint for at least the transition from the

prebiotic epoch of simple compounds to molecules with increasing complexity. CCs contain abundant amino and hydroxy acids that appear to have been synthesized by Strecker–cyanhydrin type processes during a phase when aqueous solutions percolated through the meteorite parent body.⁶⁴ However, complex molecules such as peptides have not been detected in CCs with the exception of low amounts of some dipeptides.⁶⁵ Thus, there is no indication that during the active organic synthesis phase in carbonaceous meteorites that simple molecules evolved into more complex ones.** The aqueous alteration phase in the meteorite parent bodies is generally considered to have lasted $>10^3$ to $<10^6$ years⁶⁶ although recent studies have suggested much shorter, episodic aqueous alteration periods of only 1–10 years.⁶⁷ This suggests the progression from simple molecules to ones with increasing complexity has a rough time constraint of greater than the aqueous alteration phase on carbonaceous parent bodies of between 1–10 to $<10^6$ years.

This conclusion presents a problem for the time available in hydrothermal systems for prebiotic reactions to generate first simple compounds, then to ones with increasing complexity and finally the molecules essential for the transition to biochemistry. Both Black and White smoker vent systems tend to be short-lived with respect to the geologic time scale. Black smokers are episodic and transient systems with relatively short lifetimes.⁵⁶ White smokers last longer, but their lifetimes are still only between 10^2 to 10^4 years.⁶⁸ An important issue is whether these timescales are compatible with the time required for the transition from abiotic to biotic chemistry. As noted, in carbonaceous meteorites the timescale for amino acid production is between $>10^3$ to $<10^6$ years, perhaps even as short as 1–10 years, and there is no evidence that the prebiotic chemistry that took place on the meteorite parent bodies during this aqueous alteration period produced anything beyond simple monomeric compounds.** It has been suggested that catalysts such as metal sulfides present in vent systems would promote more rapid synthesis,^{53,69} but by the principle of microscopic reversibility, these must also catalyze the decomposition of any synthesized compounds. Moreover, if this was the case there are certainly potential metal catalysts present on meteorite parent bodies and, as has been indicated, nothing beyond simple molecules were apparently synthesized.

The chimneys associated with White smoker vent systems have been claimed to “fit the bill as a hatchery of life” (ref. 70).†† What the unique conditions are that would facilitate the transition from abiotic to biotic chemistry in White smoker systems are not elucidated, however. Given the rapid flow of hydrothermal solutions through these systems and their relatively short life-times, to go from the starting reagents, to the synthesis of simple compounds, to the synthesis of complex

molecules and finally to biotic chemistry similar to life as we know it on short time scales seems essentially akin to spontaneous generation. It is uncertain whether in hydrothermal systems the transition from simple abiotic compounds to biotic chemistry as we know it is feasible.

An additional issue regarding the hydrothermal systems as they exist on Earth and their potential role in prebiotic chemistry and the origin of life is that these systems are associated with tectonic processes. Therefore, on planets on which the process of plate tectonics never began or existed, and if the hydrothermal vent based prebiotic reactions leading to the formation of simple molecular entities are indeed feasible, life could be limited to only those types of planets with geologic processes similar to the Earth.⁵² In contrast the “primordial soup” theory for the formation of prebiotic molecules and their chemical evolution to molecules of increasing complexity requires only the presence of water and an environment where there are sufficient energy sources available to maintain that water in a liquid state.

8. Conclusions

The seminal experiments that Miller carried out over a half a century ago continue to provide insights into the processes that can give rise to abiotic compounds in various environments both on the Earth and beyond. These insights in turn have provided a gauge of the diversity and the inventory of the organic compounds that would have been available for the chemical evolution of simple molecules into ones with increasingly complex functions and eventually the establishment of primitive self-replicating entities, the benchmark of the origin and evolution of life. Given that the gases needed for effective prebiotic syntheses are wide spread in the Universe, and the robust nature of the Miller-type synthetic reactions, simple prebiotic compounds are likely common in the cosmos. It is likely that the formation of more complex molecules, including one with rudimentary capabilities of imperfect replication, could also be common throughout the Universe. However, whether these simple entities would follow an evolutionary pathway that first yields a RNA World and eventually a DNA-World Earth-like biota (see Table of Contents figure) is much less certain.

With respect to the environments where prebiotic syntheses might occur, there is an important note of caution that ensues from this overall discussion: Just because certain reagents can be used to synthesize a variety of organic compounds by elegant pathways in prebiotic simulation experiments, or predicted to be synthesized based on theoretical calculations and models, the conditions and proposed syntheses are only relevant if they are compatible with the plausible geochemical conditions that likely existed on the early Earth. Examples of this are the use of unrealistically high concentrations of starting amino acids in hydrothermal vent peptide synthesis simulation experiments⁷¹ and the use of the alternative solvent formamide to promote polymerization and condensation reactions that are unfavorable in water.⁷² In the former example, using realistic amino acid concentrations results in negligible yields of peptides.⁷¹

** CCs do contain a significant amount of water insoluble macromolecular carbon^{79,80} but other than providing possible source material for the formation of primitive vesicles,⁸¹ it has no apparent major content of important complex biomolecules.

†† Lane⁷⁰ (p. 23) attributes this statement to M. Russell *et al.*,⁸² but there is no such statement in this publication. Rather Russell *et al.* refer to low-temperature vent systems as “stable culture chambers”.

In the latter, a process for the generation of pure formamide in a plausible primitive Earth environment has never been demonstrated and thus its use as an alternative solvent seems highly unlikely.

The ultimate goal of Miller's pioneering studies to help define the processes that provided the prebiotic chemical inventory needed for chemical evolution and the origin of life has been realized. Nonetheless, there are still considerable unknowns in how simple molecules could have been transformed into more complex ones and in what environments these transformations could have occurred. However, these unknowns are part of an intense area of research and the prospects of further advances in the near future are great. The role of mineral catalysts in promoting some of the essential reactions such as condensation and polymerization is an area of emerging research that offers the prospect of new insights into various processes.^{73,74} Recent work on the development of novel, new alternative synthetic genetic systems that can undergo Darwinian evolution have prospects for enhancing our understanding to the range of possibilities that could have arisen from a prebiotic assortment of organic compounds.⁷⁵ As noted in the recent book "Regenesi: How synthetic biology will reinvent nature and ourselves" by Church and Regis,⁷⁶ "Synthetic genomics has the potential to recapitulate the course of natural genomic evolution".

Although the "primordial soup" concept discussed throughout this review has many attributes in its favor, alternatives such as hydrothermal based syntheses and their role in the origin of life are nevertheless likely to remain popular in some quarters. However, the micro-conditions in the hydrothermal systems that supposedly drive abiotic processes towards their transition into biotic processes on short time-scales, may be restricted to only those few planets or planetary bodies in the Universe that have tectonic systems similar to those on Earth.

In Philip Ball's book "*Elegant Solutions: Ten beautiful experiments in the chemistry*",⁷⁷ Miller's seminal work is selected along side such notable achievements of Cavendish, Pasteur, Rutherford, Curie and Woodard. This is certainly a fitting recognition of Miller's classic research.

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