Speculations on the Origin and Evolution of Metabolism

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Summary. An autotrophic origin of metabolism is described, which requires clays, transition state metals, disulfide and dithiols, U.V. and cyanide ion. A general scheme is proposed, involving the fixation of CO_2 and N_2, for the evolution of intermediary metabolism based on the evolution of a complex system from a simple one. The basic conclusion is that metabolism could have evolved from a simple environment rather than from a complex one.

Key words: Evolution of Metabolism - Autotrophic Origin of Life.

In the speculations on the origin of life, the most difficult conceptual problems deal with the complexity of the first organism and the complexity of its environment. There are four possibilities: (1) the first organism was simple (e.g., RNA) and its environment was complex (heterotrophic origin); (2) the organism was simple (e.g., clays) and its environment was simple (autotrophic origin); (3) the organism was complex and its environment was complex (heterotrophic panspermia); (4) the organism was complex and its environment was simple (autotrophic panspermia).

The two panspermias will not be discussed in this paper; however, they cannot be ruled out. If the universe did have an origin, the first appearance of such organisms must have occurred on some planet, perhaps not the earth, and so the problem of the origin of life would be pushed back in time.

The men who first proposed in some detail the heterotrophic origin of life were Haldane (1928) and Oparin (1924). Haldane (1928) proposed that, in the absence of oxygen, U.V. acting on a mixture of carbon dioxide, ammonia, and water would yield a large variety of organic compounds, including amino acids and sugars. These compounds would have accumulated in the oceans until they reached the consistency of hot dilute soup. The first organisms were large molecules synthesized under the influence of sunlight and were capable of replication in this rich medium. By mutation and selection the first cell arose and then gradually evolved a photosynthetic capacity as the rich

medium became used up. Photosynthesis led to the production of oxygen and thus accounted for the present level of oxygen in our atmosphere.

This picture is the dominant view held today. The only modification was made by the experiments of Miller (1955), where the atmosphere was methane, ammonia, and hydrogen. The main problem which is left is the appearance of a molecule which is capable of self-reproduction. The best guess is that it was an RNA molecule (Crick, 1968; Orgel, 1968). This RNA molecule would then evolve by mutation and selection until it coupled with proteins by means of a primitive genetic code. The evolution of metabolism, anabolic and catabolic, is one of a gradual development of autotrophy from the initial heterotrophy, again by a process of mutation and selection as the soup became depleted (Horowitz, 1945).

A variant scheme has been proposed by Fox (1965). Thermal polymerization of amino acids led to the formation of microspheres which reproduced by budding. Evolution of microspheres led to a coupling with polynucleotides and so the first cell was born. The evolution of metabolism would then follow a similar course as outlined above.

The alternative hypothesis of an autotrophic origin has received far less attention than it deserves. The purpose of this paper is to consider the possibilities which open up if one considers that both the environment and the first organism were simple. The proposal made by Cairns-Smith (1965) that clays were the first simple organisms would push neo-darwinian evolution further back into the earth's past. The evolution of the clays would occur as soon as the planet had differentiated into crust, mantle, and core. In the process of this diffentiation the loss of the primary atmosphere (CH_4, NH_3, H_2) occurred and the evolution of a secondary atmosphere (CO_2, N_2, H_2O) began due to the outgassing of the evolving earth. Atmosphere, hydrosphere, and lithosphere began their intimate couplings driven by sun and radioactivity, resulting in the appearance of the biosphere (clays). The process of differentiation were sufficiently destructive so that any living system would be destroyed. It, therefore, can be conjectured that life began in the secondary atmosphere rather than in the primary one (Abelson, 1966).

This model of the secondary atmosphere has led geochemists to come into conflict with the present model of biopoiesis. In fact, the hypothesis of an early methane-ammonia atmosphere is contraindicated by the earliest sedimentary rocks which the geochemists have available (Abelson, 1966).

Since the secondary atmosphere would be dominated by carbon dioxide and nitrogen, the capacity of this atmosphere to sustain synthesis of amino acids and other biomonomers is limited. However, all models of the primitive atmosphere agree on the fact that no oxygen was present. Therefore, if the synthesis of biomonomers was severely reduced, it might be possible to accumulate a thin soup, given long times and no oxygen. The geochemists however, have come up with a greater objection to the "myth of the prebiotic soup". As a result of equilibrium calculations Sillen (1965) came to the conclusion that "equilibrium conditions seem to exclude the formation of the 'thin soup' in normal oceanic conditions, even under an anoxygenic atmosphere" (Rutten, 1971). The alternatives are either the soup developed in unusual circumstances (e.g., lakes or lagoons) or the primitive oceans cannot be described by an equilibrium model. The possibility that there was no soup of any complexity must, however, also be considered.

It is thus at least debatable whether or not the primitive atmosphere and hydrosphere with a random input of energy was able to build up and sustain a soup of biomonomers.

If one does not assume a soup, one cannot do better than heed the advice of the biochemist, Szent-Gyorgyi (1972): "Life has developed its processes gradually, never rejecting what it has built, but building over what has already taken place. As a result the cell resembles the site of an archeological excavation with the successive strata on top of one another, the oldest one the deepest. The older a process, the more basic a role it plays and the stronger it will be anchored, the newest processes being dispensed with most easily."

This idea was expanded by Bernal (1960) into a conceptual tool for studying evolutionary processes. The method described how a complex system evolves from a simpler system. "The pattern ...is one of stages of increasing inner complexity, following one another in order of time, each one including in itself structure and processes evolved at the lower levels." The system thus grows more complex, like an onion, and it must be possible to strip back the later layers of the onion to expose the simpler earlier stages.

It is, therefore, useful to look at the present-day building up of biomonomers by the biosphere, in other words, to examine intermediary metabolism. It was Lipmann(1965) who suggested that, in projecting backwards on the evolution of biosynthesis, one should look more attentively "for primitive evolutionary stages within the metabolic picture in the hope

to apprehend there surviving metabolic 'fossils'." In considering the various possibilities in a secondary atmosphere, Abelson (1966) came to the conclusion that HCN would be the dominant molecule produced; however, the resultant solution would be quite dilute. In order to facilitate the synthesis of the various building blocks, he appealed to the mechanisms of the present processes of biosynthesis. "The principal processes required to synthesize the 14 amino acids are condensations (such as those involving pyruvate, carbonate, and acetate in the Krebs cycle), hydrogenation, and transfer of NH_3. Furthermore, given condensation and hydrogenation, one has a mechanism for producing fatty acids. Formate would be a convenient source of hydrogen. Simple receptors for solar radiation might have helped speed the condensation reactions. Thus one can visualize that natural conditions might have favored synthesis of increasingly complex molecules from the simple but versatile substances available."

If we look at intermediary metabolism, in particular, anabolism, we are immediately struck by the central role played by the citric acid cycle. In Fig. 1 we see the anabolic functions of the citric acid cycle. If we apply the method of Bernal, it can be conjectured that the citric acid cycle came first and was followed by the amino acids, lipids, nucleotides, and carbohydrates. From a more detailed examination of metablism it is evident that some amino acids require fewer steps in their biosynthesis from the citric acid cycle. Glutamic acid, glutamine, proline and arginine are closely linked metabolically to ketoglutarate. Aspartic acid, asparagine, threonine are similarly linked to oxalacetate. Alanine, cysteine, serine, glycine are linked to pyruvate. Glycine is also made from glyoxalate.

There is a puzzle with respect to lysine for it is easier to make ornithine from the citric acid cycle. One might speculate that ornithine came into the biosphere first, later to be supplanted by arginine and lysine.

The amino acid histidine is closely associated with the biosynthesis of purines (Greenberg, 1969).

Purine rings are constructed from glycine, formate, glutamine, aspartic acid, and carbon dioxide (Buchanan, 1960). Pyrimidine rings are constructed from aspartic acid, carbon dioxide, and glutamine (Hartman, 1970). Thus it can be conjectured that the synthesis of purines and pyrimidines began as soon as glycine, formate, aspartic acid, and glutamine came into the biosphere.
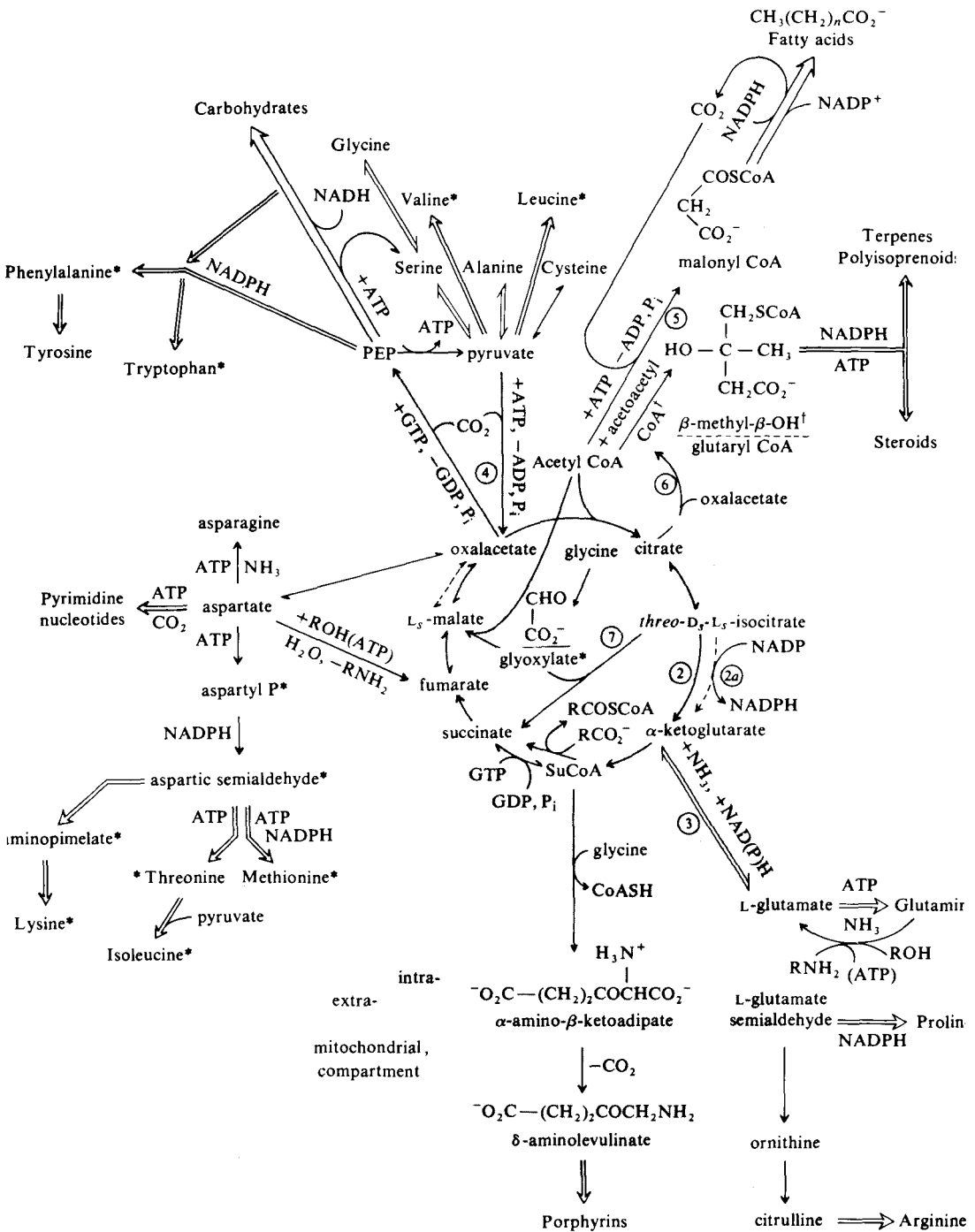
Fig. 1. Biosynthetic functions of the citric acid cycle. From: Biological chemistry, by H.R. Mahler and E.H. Cordes. Reprinted by permission of Harper & Row Publishers Inc.

The amino acids valine, leucine, isoleucine, methionine, tryptophan, phenylalanine, and tyrosine require many more metabolic steps for their synthesis and hence would have entered the biosphere later than the other amino acids.

The first polymerization would have been the synthesis of fatty acids from acetyl-CoA.

Thus, from the citric acid cycle, by counting the number of metabolic steps necessary for their biosynthesis, the amino acids would have entered the biosphere in three groups:
(1) glycine, alanine, aspartate, glutamate;
(2) glutamine, asparagine, proline, serine, cysteine, threonine, "ornithine", arginine (histidine);
(3) valine, leucine, isoleucine, methionine, tryptophan, phenylalanine, tyrosine, "lysine".
The synthesis of purines and pyrimidines would have started after the entry of the first two groups of amino acids into the metabolic scheme.

It is also clear from biosynthetic pathways that "most of the consecutive reactions of intermediary metabolism involve the sequential transfer of amino, acyl, phosphate, methyl, formyl, or carboxyl groups, or of hydrogen atoms" (Lehninger, 1970).

These transfers are carried out by co-enzymes in association with apo-enzymes (proteins). It is the co-enzymes which serve as carriers of specific functional groups such as hydrogen atoms, amino groups, methyl, formyl, and acetyl groups. The co-enzymes are listed in Table 1.

In the building up of the biomonomers the co-enzymes are the organic chemists of the cell. It was pointed out by Lipmann (1965) that "in at-tempting to map evolutionary steps, I prefer to assume that poly-nucleotides and proteins are late developments". The apo-enzymes being proteins might, therefore, have evolved after the proto-coenzymes. We might here speculate that the co-enzymes came before the enzymes. In fact, the co-enzymes must have had an evolutionary history of their own. In the various model studies of the co-enzymes, only fragments are used (e.g., nicotinamide, flavin, or thiazolium ring) (Bruce & Benkovic, 1966). Much of the co-enzymes is not used in the actual reactions and must relate to their binding by proteins.

It was pointed out by Snell (1965) that an area in prebiotic chemistry which had been neglected was the formation of "the primitive co-enzymes... which

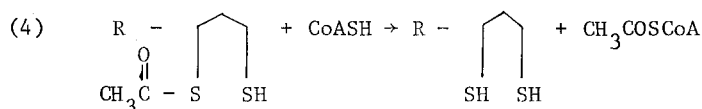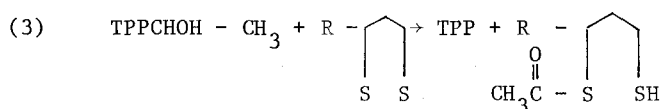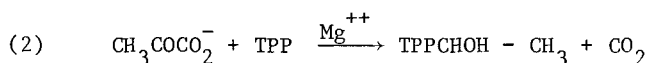Table 1, Co-enzymes in group-transferring reactions

Entity transferred	Co-enzyme
Electrons	Heme
Hydrogen atoms	Nicotinamide adenine denucleotide (NAD)
Aldehydes	Thiamine pyrophosphate (TPP)
Acyl groups	Co-enzyme A
Acyl groups	Lipoamide
Alkyl groups	Cobamide (corrinoid)
Carbon dioxide	Biocytin
Amino groups	Pyridoxal phosphate
Methyl, methylene, formyl, or formimino groups	Tetrahydrofolate

might, by their presence, greatly reduce the activation energies needed for the formation of other essential compounds and, therefore, promote their formation under conditions much milder than those that have so far been used." This field, although it has been intensively studied by the organic chemist, has not been taken up by the prebiotic chemists.

The start of the citric acid cycle comes from the condensation of acetyl-CoA and oxalacetate to form citrate. The main source of oxalacetate comes from the condensation of carbon dioxide and pyruvate mediated by biotin.

(1)   pyruvate + CO_2 $\xrightarrow{\text{biotin}/\text{ATP}}$ oxalacetate
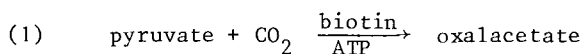
The main source of acetyl CoA is NAD-lipoate-linked Pyruvate Dehydrogenase (Lowenstein, 1971).

(2) $CH_3COCO_2^-$ + TPP $\xrightarrow{Mg^{++}}$ TPPCHOH $-$ CH_3 + CO_2

(3)   TPPCHOH $-$ CH_3 + R $-$ [dithiolane, S S] $\rightarrow$ TPP + R $-$ [ring with $CH_3\overset{O}{\overset{\|}{C}}-S$ and SH]

(4)   R $-$ [ring with $CH_3\overset{O}{\overset{\|}{C}}-S$ and SH] + CoASH $\rightarrow$ R $-$ [ring with SH SH] + $CH_3COSCoA$
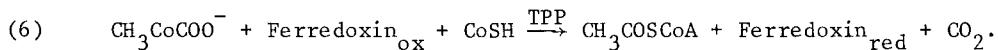
The reactions which follow are the oxidation of the dithiol by a Flavo-

protein and the oxidation of the Flavoprotein by a NAD-enzyme. The over-all reaction is

(5) $CH_3COCOO_2^- + NAD^+ + CoSH \xrightarrow[TPP\ Mg^{++}]{lipoic} CH_3COSCoA + NADH + CO_2$
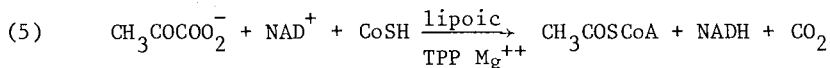
In anaerobic organisms the over-all reaction is (Mortenson et al., 1962)

(6) $CH_3CoCOO^- + Ferredoxin_{ox} + CoSH \xrightarrow{TPP} CH_3COSCoA + Ferredoxin_{red} + CO_2.$

In recent years the reverse reaction has been found in anaerobic bacteria (Buchanan, 1972).

(7) $acetyl - CoA + CO_2 + Ferrodoxin_{red} \rightarrow Pyruvate\ CoA + Ferrodoxin_{ox}$
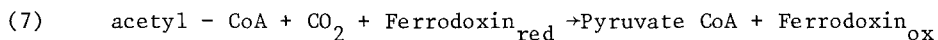
Therefore, starting with acetyl-CoA by two carbon dioxide fixations, the citric acid cycle may begin. Furthermore, Ljungdahl & Wood (1969) have shown that carbon dioxide fixation can lead to the formation of acetate. The co-enzymes involved are tetrahydrofolate and a corrinoid (cobamide). This is a model system in which a C_2 skeleton is constructed in net fashion from two one-carbon units. If such a system were coupled to the citric acid cycle, the total carbon necessary for biosynthesis would come from carbon dioxide. Thus, if the proper proto-coenzymes (e.g., Co-Chelates) were available, then Goldschmit's (1952) hypothesis that carbon dioxide was the main primary material in the origin of life would be satisfied.

The problem, in general, of carbon dioxide fixation has not been elucidated. The reaction mechanisms have not been worked out by the organic chemists as carbon dioxide is not a molecule used by organic chemists in their synthesis of complex molecules. One suggestion that might be a starting point for further experimentation is the spontaneous reaction of carbon dioxide with ammonia to form carbamate. The reaction of carbon dioxide with ammonia and the primary and secondary amino groups of aliphatic amines and amino acids is rapid and requires no catalyst (Edsall & Wyman, 1958). This formation of carbamates might be a primitive mechanism for carbon dioxide fixation. Therefore, if CO_2 were the dominant source of available carbon it would be possible by means of carbon dioxide fixation to build all the carbon skeletons needed for metabolism.

Given the carbon skeletons, the next problem is the source of nitrogen. If the atmosphere was dominated by molecular nitrogen, some method of nitrogen

fixation would be necessary. Today nitrogen fixation is mediated by a nitro-
genase which consists of two metalloprotein components:
(1) azoferrodoxin and (2) molybdoferredoxin (Dalton & Mortenson, 1972). In
model studies Schrauzer et al. (1971) showed that molybdenum complexes with
sulfur containing ligands, in the presence of $Na_2S_2O_4$ as reducing agent,
could serve as good models of nitrogenase. They suggested that the iron in
nitrogenase would function as an electron transfer catalyst.

Nitrogen fixation was therefore possible if the formation of molybdenum-
thiol complexes occurred in the primitive organism and if a strong reductant
was available.

It was suggested by Bernal (1951) that in the absence of O_2 the main source
of free energy needed to drive the metabolic machine would be provided by
two inorganic reactions - "the oxidation and reduction of ferrous to ferric
iron and of sulphydryl - SH to disulfide - S - S -. Early life in the
absence of atmospheric oxygen must have proceeded almost entirely by the
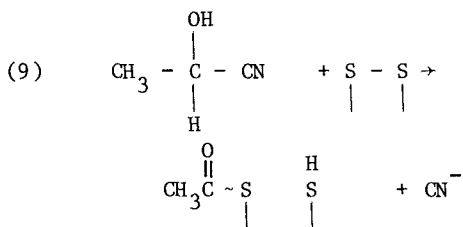enzymic utilization of these transformations".

Today the metabolic machine is driven by two "high energy" compounds, ATP
and acetyl - CoA, one a high-energy phosphate and the other a high-energy
thiol ester. It has been pointed out by Racker (1965) that "in anaerobic
glycolysis, probably a very primitive process of energy production, a thiol
ester serves as a precursor of ATP. Thiol esters may possibly represent the
evolutionary ancestors of 'high energy' ATP."

In the presence of oxygen the need for reducing power became necessary; it
is provided by NADH today.

If one now puts together the ideas of Bernal and Racker and one looks at
pyruvate dehydrogenase, one may propose the following scheme. Assuming that
acetate is available and can be reduced to acetaldehyde (see Ljungdahl &
Wood, 1969), then the following reactions become possible.

(8) $\quad CH_3CHO + CN^- \rightarrow CH_3\overset{\displaystyle OH}{\underset{\displaystyle H}{C}} - CN$
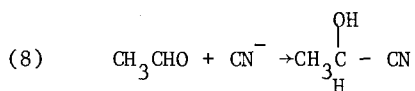
Here the use of CN^- instead of TPP is suggested by the studies of Breslow
(1959), who proposed that catalysis by thiazolium salts is analogous to
catalysis by the cyanide ion.

(9)

$$CH_3 - \overset{\overset{\displaystyle OH}{|}}{\underset{\underset{\displaystyle H}{|}}{C}} - CN \quad + \quad \overset{S}{\underset{|}{}} - \overset{S}{\underset{|}{}} \rightarrow$$

$$CH_3\overset{\overset{\displaystyle O}{\|}}{C} \sim \underset{|}{S} \quad \overset{H}{\underset{|}{S}} \quad + \quad CN^-$$

The use of disulfide is suggested by the reaction of 1 - hydroxyethyl - TPP and lipoic acid. We now have a thiol ester which is a high-energy compound which can now be used along with carbon dioxide fixation to begin the citric acid cycle.

The problem which now remains is the oxidation of the dithiol to a disulfide. This could be accomplished as follows.

(10)

$$\overset{H}{\underset{|}{S}} \quad \overset{H}{\underset{|}{S}} \quad + \text{ Ferricyanide}$$
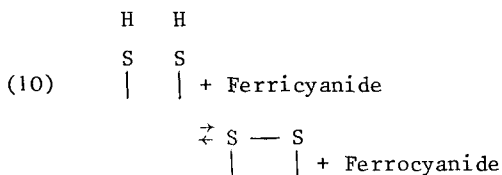
$$\overset{\rightarrow}{\leftarrow} \underset{|}{S} - \underset{|}{S} \quad + \text{ Ferrocyanide}$$

This is a reaction which has been studied by protein chemists (Herriott, 1947).

If, however, one assumes that there is no oxygen the dominant species of the iron cyanides would be ferrocyanide; however, a well-studied reaction in photochemistry is (Calvert & Pitts, 1966):

(11)   Ferrocyanide + U.V. light $\rightarrow$ Ferricyanide + e^- (aq)
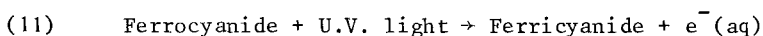
This reaction at a wave-lenght of 2537 $\overset{o}{A}$ has a quantum yield of 1. Thus, in U.V. light, which would be the dominant source of energy in an atmosphere which did not have an ozone layer, the ferrocyanide would be oxidized to ferricyanide.

The fate of the solvated electron may have been to reduce a thio-molybdenum chelate for the fixation of atmospheric nitrogen.

Given these reactions or a similar set, it is thus possible to imagine the appearance of an early metabolism which, driven by light (U.V.), fixating N_2 and CO_2, eventually evolved into the metabolism which we know today.

From the view of the autotrophic origin of life, it is not surprising that the paleontological record should show the early appearance of blue-green algae (Schopf, 1970). The blue-green algae are the most selfsufficient cells known. These organisms obtain their energy from sunlight, their carbon from CO_2 and their nitrogen from atmospheric N_2.

The outstanding problem left is to justify the evolution of metabolism in terms of an advantage to an evolving living system. If one assumes that clays were the original living organisms, the ability to synthesize poly-carboxylic acids would be an advantage as "these kinds of molecule can increase the solubility of silicia - they might thus act on the surrounding rocks to provide an immediate source of food" (Cairns-Smith, 1971).

An autotrophic origin thus requires clays, transition state metals (Fe, Co, Mo), disulfide and dithiols, U.V. and CN^-. Given these ingredients a plausible scheme for the fixation of CO_2, N_2, and the evolution of metabolism can be conjectured.

The final decision between the four possibilities concerning the origin of life must await further experimentation and a deeper understanding of the first billion years of the earth' history.

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