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EVOLUTION OF ENZYMES
AND THE PHOTOSYNTHETIC APPARATUS

BERKELEY, CALIFORNIA

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Melvin Calvin

August 26, 1957

Printed for the U.S. Atomic Energy Commission

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Melvin Calvin

Department of Chemistry and Radiation Laboratory
University of California, Berkeley, California

August 26, 1957

ABSTRACT

A mechanism is proposed for the development of rudimentary catalytic functions into efficient ones leading to enzymes. By use of this development, an analysis is made of the time and stage in prebiological history at which photosynthesis appeared.

EVOLUTION OF ENZYMES AND THE PHOTOSYNTHETIC APPARATUS*

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Time does not allow, nor does this audience require, a summary, or introduction to the present-day conception of various stages that must have occurred in the origin of life on earth, as we now know it. The appearance of organic material on the surface of the earth has already been adequately discussed, and the discussion on the development of the cellular organism will come later.

It therefore seemed wiser for me not to attempt even a summary of the paper that appears in the publication of the proceedings of this conference, but rather to select one or two particular points which I thought might add something to the basic pattern as it has been developed by Oparin.¹ These fit well into the third, and perhaps the fifth, stage of the present conference. The first of these points that I would like to amplify somewhat might be termed the development of catalysts, that is, enzymes, and rudimentary synthetic sequences. The second will be a discussion of the possible mode of the development of what we now know as the photosynthetic apparatus. It so happens that in Berkeley we have been concerned with both of these problems rather directly and experimentally.²

With regard to the first point, that is, the development of catalysts and rudimentary synthetic sequences: Even the most cursory examination of what is now known about the nature of present-day enzymatic mechanisms cannot fail to impress one with the apparent identity between the enzymatic reactions and the reactions as they are known to the organic chemist in the laboratory. For example, glyoxylase, by which methyl glyoxal is converted to lactic acid, is nothing more or less than an internal Canizarro reaction that is catalyzed by bases. Almost all of the hydrolytic reactions--those of esterases, proteases, phosphatases--have their nonenzymatic counterparts in the form of generalized acid or base catalysis, or more specialized catalysis by metal salts. For example, again in the case of the phosphatases, the freshly precipitated trivalent metal hydroxides are extremely effective, or manganese ion is effective as a rudimentary phosphotransferase.

* Summary of a paper presented at the International Symposium on The Origin of Life on Earth, Moscow, U.S.S.R., August 19-24, 1957.

** The preparation of this paper was sponsored by the U.S. Atomic Energy Commission.

¹A.I. Oparin, The Origin of Life, third English edition (Oliver and Boyd, London, England, 1957.)

²M. Calvin, American Scientist, 44, 248 (1956).

One particular group of catalysts which is widely dispersed in present-day biological systems is that centered around the element iron--particularly catalase, peroxidase, and cytochrome. Here, a rather quantitative comparison can be made between the ability of the bare iron atom to perform some catalytic function and the ability of the iron atom to perform the same catalytic function as it has been developed in biological systems. Thus, in Fig. 1 one sees a comparison of the hydrated iron ion, the iron ion surrounded by a porphyrin as it is in heme, (haem), and the iron ion porphyrin, or heme, as it is incorporated into a protein, surrounded by two more coordinating groups of the protein to form catalase. The progressive increase in catalytic ability is from 10^{-5} for the aqueous ferric ion to 10^{-2} for the heme, to 10^5 for the complete enzyme. These are definitions of the catalytic function of the iron for the reaction involving the decomposition of hydrogen peroxide. However, we know that the iron has many other functions as well, most of which involve oxidation or reduction. For example, even catalase itself can function as a peroxidase, using hydrogen peroxide to oxidize organic substrates, provided the hydrogen peroxide is sufficiently dilute. Undoubtedly the iron is also involved in what we now recognize as oxidative phosphorylation--that is, the conversion of the energy liberated upon the passage of an electron from a highly reducing potential to an oxidizing agent, such as oxygen or other material, with the concomitant storage of some of that energy in the form of unstable (pyrophosphate) linkages which may be later used for other purposes.

In order to convert the rudimentary catalytic functions which must exist in all the elements and their simple compounds into the highly efficient ones that we now recognize as enzymes, we must introduce two additional ideas, one from the realm of chemistry and the other from the realm of genetics, both of which, however, could be considered as manifestations of exactly the same phenomena. The first of these, from the realm of chemistry, is the idea of autocatalysis, that is, the basic notion that the product of a reaction may itself be a catalyst for the conversion of precursors into itself. This is a very common phenomenon in chemistry and perhaps is best illustrated in one of the simplest of cases, namely, the reaction of molecular hydrogen with cupric ion. This reaction leads, thermodynamically, to the formation of cuprous ion and oxidized hydrogen, either as acid or water, depending upon the anion with which the cupric ion is associated. It so happens that the reaction of molecular hydrogen with cupric ion, although thermodynamically possible, is an extremely slow one without catalysis. However, the product of this reaction, that is, cuprous ion, is an extremely good catalyst for the reaction between hydrogen and cupric ion. Therefore, one can imagine (and indeed we have experimentally realized this long ago) a system consisting of molecular hydrogen and cupric ion which would remain in this form for some period of time. However, should either a very slow noncatalytic reduction lead to cuprous ion, or some random electron transfer lead to the formation of an appreciable number of cuprous ions in the mixture, then immediately the entire reaction mixture would go over to the more stable system consisting of cuprous ion.

The other notion that we would like to introduce is the one developed by the geneticist Horowitz.³ He suggested that the very complex series of reactions that we are now finding to be responsible for the synthesis of

³N. Horowitz, Proc. Nat. Acad. Sci. 31, 153 (1945).

most of our existing biological material could have developed in a backward manner, beginning with the completely heterotrophic organisms. He postulated that the first organisms, the first living things, were complete heterotrophs and had available to them all possible precursors for their own duplication, and that their only function was to bring these together to produce themselves. One can then visualize a process in which at first one essential constituent of the mixture is depleted. Then, the particular organizational unit that has found a way of manufacturing the depleted item from remaining molecules will, of course, survive, whereas those units which are unable to do so will disappear, since they can no longer reproduce themselves. Such a process of gradual depletion of available substrates, with the evolution of continually longer and longer chains of synthesis, would then constitute the origin of the synthetic sequences that we are now finding in present-day living organisms.

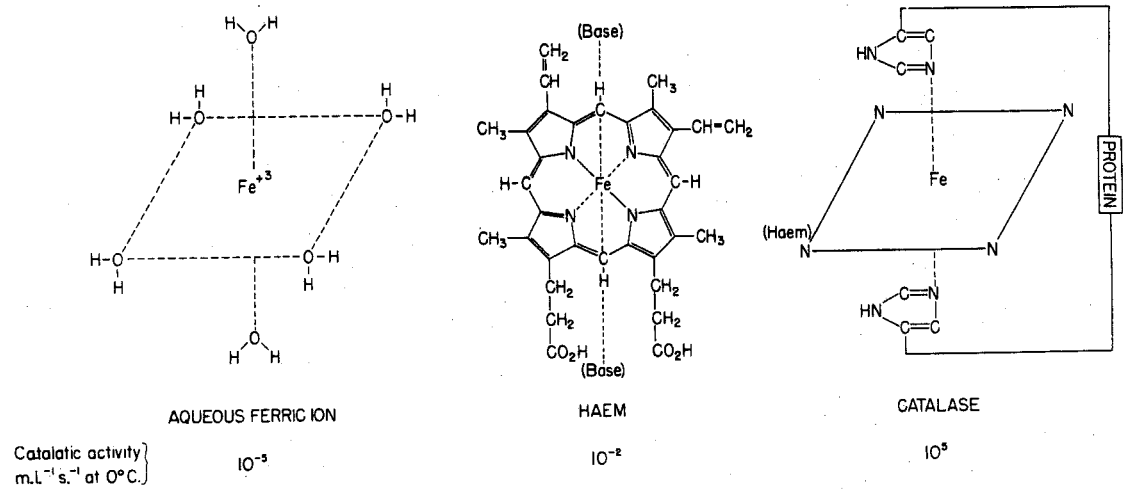
With these two ideas we can see how an enzyme, or, I should say, a highly efficient catalyst, could be built. It is perhaps worth while to trace such a development in a specific instance with which we might be familiar: for example, the iron porphyrin. Fortunately, the essential steps in the present-day biosynthetic route to porphyrin have been unraveled for us.⁴ One could begin with succinic acid and glycine, two compounds which we already know can be made by the various random methods that have been discussed earlier, such as gaseous discharge or ionizing radiation or ultraviolet light. From these (succinic acid and glycine) an alpha-amino-beta-keto acid is formed, followed by decarboxylation to delta-aminolevulinic acid, two of which then condense to form a pyrrole nucleus, porphobilinogen. This molecule then passes through a series of steps, involving a number of oxidations, leading finally to protoporphyrin 9. The skeleton of this sequence is shown in Fig. 2.

With the introduction of iron into the protoporphyrin--or perhaps a better way to view it would be the surrounding of the iron by the protoporphyrin grouping--the iron becomes a better oxidation catalyst, and, as you can see, there are several oxidation steps along the biosynthetic route as we now know it. Thus, one has only to suppose that one or more of the sequences of steps leading to protoporphyrin is dependent upon an iron-catalyzed oxidation, and this is almost certainly so, to arrive at the conclusion that once such an iron protoporphyrin is manufactured it will itself accelerate its own synthesis from such precursors as succinic acid and glycine and thus tend to build up the supply of the material and improve the iron catalyst that will be available for a variety of other functions as well.

The second point that I would like to make concerns the stage at which the development of the photosynthetic apparatus as we now know it might have occurred. In order to do this we review briefly what our present state of knowledge appears to be with respect to the existing mechanism by which the photosynthetic apparatus in the green plant and in the lower organisms can convert electromagnetic energy into chemical potential as reduced carbon and molecular oxygen. One need hardly do more than point out the essential features of the process to recognize its present-day separation into several rather distinct parts.⁵ The reduction of carbon

⁴D. Shemin, Harvey Lectures 50, 258 (1954-55).

⁵M. Calvin, J. Chem. Soc. 1956, 1895.



MU-9480

Fig. 1. Evolution of a catalyst for hydrogen peroxide.

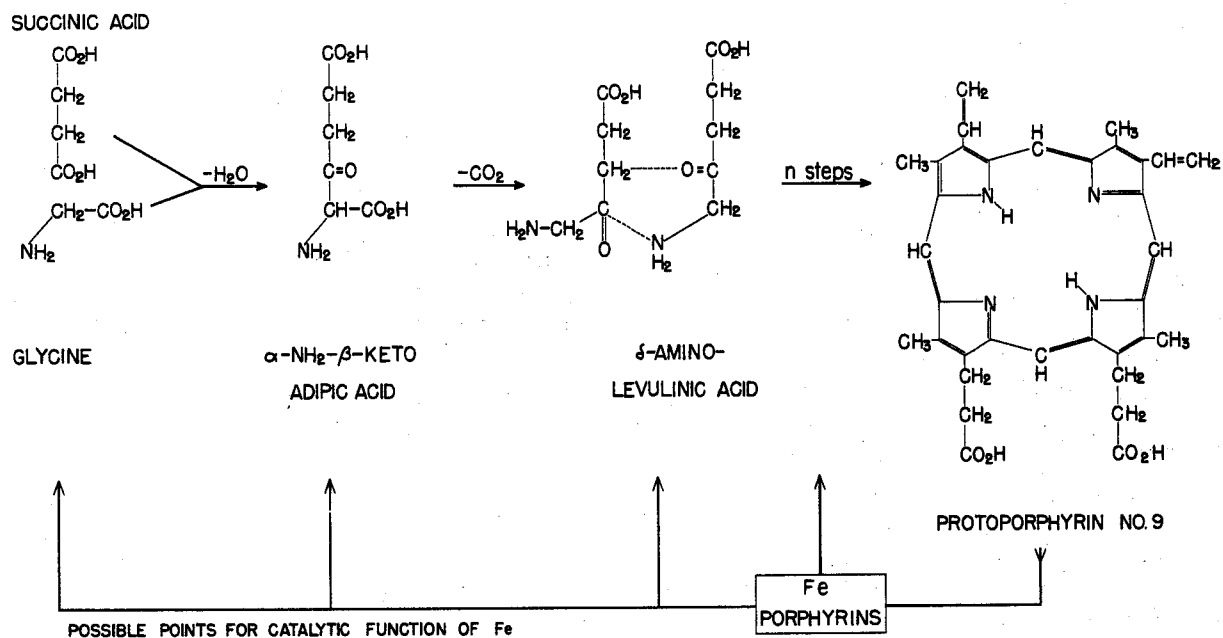


Fig. 2. Catalytic function of iron.

dioxide, we now have every reason to suppose, occurs in a series of reactions which can take place entirely in the dark. In fact, all the enzyme systems that we now know participate in the conversion of CO_2 to carbohydrate have been found in a wide variety of organisms, many of which are not photosynthetic. For example, the *Thiobacillus*⁶ contains very nearly all of the necessary enzymes, and *E. coli*⁷ grown in xylose contains not only carboxy-dismutase but also a number of other enzymes involved in the carbon-reduction cycle. The final step was indeed taken when Racker⁸ was able to make a mixture of all the requisite enzymes and the energy-storing compounds (reduced TPN and ATP), which produced hexose phosphate from carbon dioxide, all in the dark.

We can therefore follow van Niel⁹ and reaffirm the suggestion that the photochemical apparatus in the green plants is primarily concerned with the splitting of the water molecule and the generation of reducing agent and intermediate oxidant. The latter (intermediate oxidant) will eventually find its way into molecular oxygen. The former (reducing agent) will be used to reduce the compounds formed by combination of carbon dioxide with suitable intermediates in the cell. From our knowledge of the nature of the carbon cycle⁵ it is already clear that ATP is also required for this latter process, and this ATP may either be generated by oxidative phosphorylation or by a recombination of the intermediate oxidant with some intermediate reductant that has been formed by direct photolysis. Thus, we can suppose that the reaction sequence that is now used by green plants for the reduction of carbon dioxide was developed independently of the photosynthetic apparatus, and presumably later coupled to the energy-capturing and-transforming mechanism which the photosynthetic apparatus represents.

We must now examine the nature of the photochemical apparatus to see if we can discover something of its origin. The primary energy-trapping molecule is, of course, a porphyrin, chlorophyll. In recent years, the work of Granick¹⁰ has clearly indicated, if not conclusively demonstrated, that present-day synthesis of chlorophyll is a branch in the synthetic sequence leading also to the hemes. This branch seems to occur just before the insertion of the iron into the porphyrin ring (Fig. 3).

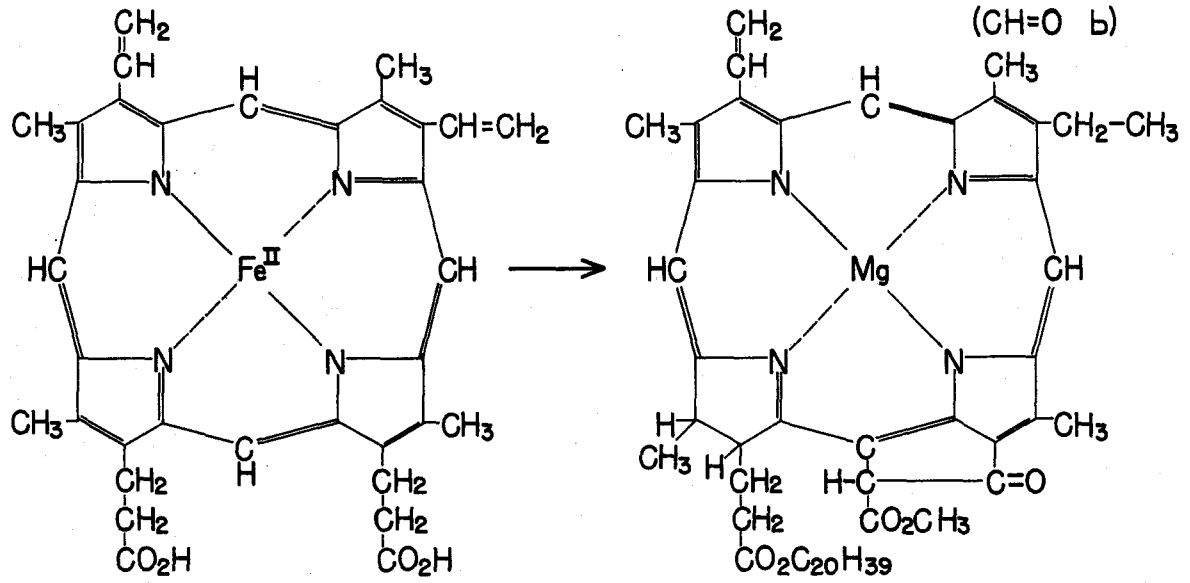
⁶P. A. Trudinger, *Biochem. J.*, 64/274 (1956); G. Milhaud, J. P. Aubert, and J. Millet, *Compt. rend.* 243, 102 (1956).

⁷R. C. Fuller and M. Gibbs, *Proc. Amer. Soc. Plant Physiol.*, Storrs, Connecticut. *Plant Physiol.* 31 xxxi, Aug. 1956.

⁸E. Racker, *Nature*, 175, 249 (1955).

⁹A. J. Kluyver and C. B. van Niel, 'The Microb's Contribution to Biology,' Harvard University Press, Cambridge, Massachusetts (1956). Chapter 6-- Evolution as Viewed by the Microbiologist.

¹⁰S. Granick, *Harvey Lectures* 44, 220 (1948-49).



HAEM (as in Haemoglobin and
Cytochrom)
Fe -PROTOPORPHYRIN NO.9

CHLOROPHYLL a

MU-13770

Fig. 3. Structural relationships between heme and
chlorophyll a.

We can thus see that the most likely course for the evolution of the photosynthetic apparatus involved the prior existence of the porphyrin type of catalytic structure. This catalytic structure was evolved not in response to the existence of sunlight, but rather in response to the other evolutionary driving force, which we have already seen operative in the development of the porphyrin nucleus, namely, the oxidative catalysis at which the iron compounds of the porphyrins are so efficient. This leads to the suggestion that the development of the porphyrin either was not directly dependent upon the existence of oxygen in the primordial atmosphere but occurred in response to its function in other oxidative dismutations not involving molecular oxygen, or that there was molecular oxygen in the earth's atmosphere prior to the advent of the photosynthetic apparatus that now produced it.

The development of such highly organized structures as are now found in the organelles of the cells, such as the chloroplasts and mitochondria,¹¹ seems to me to be incompatible with the penetration of very much of the ultraviolet radiation from the sun in the wavelength around 2500 Å which would have occurred had there been no oxygen in the atmosphere. Today, of course, the oxygen in the upper atmosphere is photodissociated by this very ultraviolet light and is thus converted into quantities of ozone which, in turn, strongly absorbs in the region between 2200 Å and 3000 Å. While it is possible to conceive of the development of heterotrophic organisms in the shelter of deep water and thus not exposed to the destructive ultraviolet, it is difficult to conceive of the evolution of a photosynthetic apparatus, even a primitive one, also in the same environment, since there would be little visible light available, either, for the evolutionary selective mechanism.

Thus, we are constrained to believe that not only was the evolution of higher organisms delayed until the appearance of appreciable amounts of oxygen in the atmosphere, but also the evolution of the porphyrin catalysis itself and some of the more complex structural elements of living cells had to wait this protection. Presumably, it came about by virtue of the photodissociation of water into hydrogen and oxygen, followed by the escape of the hydrogen from the gravitational field of the earth, leaving behind an appreciable quantity of oxygen, even though it is now entirely photosynthetic in origin. We can thus envisage the development of the highly organized structural units which we see today in the form of mitochondria prior to the development of the chloroplasts and of the photochemical apparatus. The similarity in the structure of these two organelles is very striking, and it is not unreasonable to suppose that the latter had its origin in the former (Fig. 4).

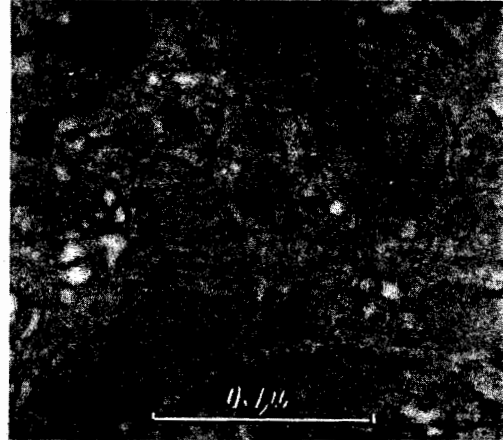
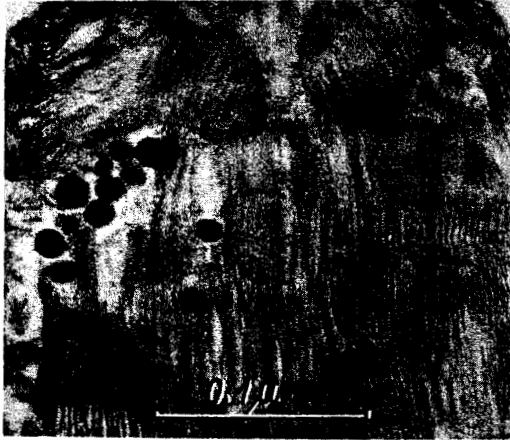
Thus, the porphyrin molecule and the structural unit into which it is built had an independent evolution. Only at a much later time did the coupling of the optical properties of the porphyrin molecule to the energy demands of the carbon-reduction cycle take place. This presumably occurred at a time when

¹¹F. Sjostrand, Die Umschau 4, 751 (1954).

there may have already been a rudimentary photosynthetic energy-yielding apparatus in the near ultraviolet, perhaps using sulfur compounds as direct absorbers. The more efficient energy-capturing molecule, which the porphyrin is, could not be used in energy conversion until it was divested of the iron atom which was its principal reason for being. The reason for this is that the requirements for an efficient energy conversion carry with it the requirement for a long-lived excited state after the capture of the quantum. The iron porphyrin, although absorbing a good deal of visible light, cannot have a very long life because of the presence of the magnetic iron atom in the molecule. The inhomogeneous magnetic field surrounding the iron atom breaks down the triplet-singlet selection prohibition and does not allow a long-lived triplet state of porphyrin. However, if the iron is replaced by magnesium, or some other diamagnetic divalent cation such as zinc, then this molecule can have a long-lived triplet state which is essential for energy migration and conversion with any high degree of efficiency.

I suspect that the rudimentary beginnings of a chlorophyll type of photosynthesis may have occurred in the precellular period, in a period that had already seen the appearance of lipid-type coacervates and in which the disc-like molecules of chlorophyll could assume a pseudocrystalline arrangement, facilitating the energy transport and conversion. In all probability, it was at this point, or very near this point, that the cellular habitat of life took shape. Finally, the generation of the oxygen-evolving mechanism, perhaps requiring the participation of the carotenoids and other such materials, occurred, leading to the higher plants.

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SJÖSTRAND

ZN-1772

Fig. 4. Comparison of structure of mitochondria and chloroplasts. (From "Sjöstrand.")