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EVOLUTION OF PHOTOSYNTHETIC MECHANISMS*

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INTRODUCTION

The planning of this discussion has turned out to be particularly difficult, perhaps the most difficult one that I have ever undertaken. The reason for this, I console myself, lies in the very nature of the evolutionary process itself. In physical science (and particularly in mathematical sciences) we are accustomed to a single sequence of events, in which each idea is precursor to the next, and one gradually develops a whole pattern of thought -- a whole notion from beginning to end -- in a single sequence. Those of you who are more familiar with the way biological material has evolved will know that this is not really the way the living organism can be described in its evolutionary history. The subject of this discussion, the problem of photosynthesis, is especially difficult to trace.

It turns out, as you will see as we go along, that the evolution of photosynthesis entails the fusion of a number of quite independent threads of evolution at some point in time to give rise to the modern process and the modern apparatus as we know it. In trying to describe that sequence of

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events, I find myself greatly increasing my respect for the novelist who writes historical novels. He has many apparently independent chains of events, giving rise to a particular incident at the end, or perhaps at the beginning of the novel, and he is very skillful at starting each of these threads and jumping from one thread to the next, bringing them along so they all come together at the right time and in the right place. I haven't yet been able to move smoothly among the various evolutionary threads that are involved here, which ultimately fuse together to give rise to the very complex process of photosynthesis. The story may appear, therefore, more confused than it really is, since I must jump back and forth between separate evolutionary threads and try to indicate their points of fusion.

MODERN PHOTOSYNTHETIC PROCESSES

With this apology over, let us begin our study of the evolutionary history of photosynthesis by first describing what we think we think we know of the modern process at which we must eventually arrive. The process of photosynthesis is <u>the</u> process by which living organisms are able to transform electromagnetic energy into chemical energy by inducing the reaction between carbon dioxide and water to evolve molecular oxygen and reduced carbon:

$$CO_2 + H_2O \xrightarrow{h\nu} (CH_2O)_n + O_2$$

This is the overall process of photosynthesis which has long been recognized as a process for transforming electromagnetic energy, here represented by the quantum, into chemical potential, represented by oxygen in the elementary form and the elements of carbon and hydrogen largely in the oxidation level of carbohydrate.[1,2,3]

If this were all we knew about the process of photosynthesis, we would be hard pressed to try and predict an evolutionary history which might give rise to this process. Fortunately, in the last decade or two we have learned perhaps more about the process of photosynthesis from this point on than in the previous one hundred years. This was the stage that was available to us roughly one hundred years ago. Only slow progress was made in increasing the chemical knowledge of photosynthesis until just prior to World War II -- beginning in the middle thirties and then going on after the war at an increasingly rapid rate.

What do we know today about the process of photosynthesis? Rather than try and give you a history of how the knowledge has evolved, I am going to (1) put down some of the established things that we know about photosynthesis, represented by the overall reaction, (2) examine the types of organisms which perform this process, (3) determine what the biological apparatus is within some of the organisms (as far as we can do it), and (4) finally go further on down to the molecular level. The question of the evolution of a process of this sort also raises others: What level shall we deal with? Shall we deal with photosynthesis at the level of the whole organisms, the level of the cell, the level of subcellular particles, the level of the macromolecules, or at the level of the small substrate molecules that are involved? We should, in fact, deal with all of these, if possible, but this is another complication which makes the organization of such a discussion as this extremely difficult.

I am going to try to pick up two aspects of it, the mechanism itself on the substrate, and possibly submolecular level, and the apparatus on the subcellular, or macromolecular level.

Nature of the Organisms

I hardly need review for you the nature of the organisms which are capable of performing the process of photosynthesis. Quite obviously, the higher green plants, such as a wheat field or a forest, do this on a grand scale. There is, however, a whole set of other organisms besides the higher green plants which are able to do this, or parts of it, and they represent an important part of the biological scheme of things to be examined in the course of our study. These are the marine algae; both the green and the red ones are important in terms of the amount of carbon which is turned over on the surface of the earth per year, as the algae represent the largest single plant family involved in this turn-over. Then, there is another group, the blue-green algae, which appear to be structurally more primitive organisms which are capable of doing the entire process of photosynthesis, that is, reducing carbon and evolving oxygen. And, finally, we come to the bacteria, both the green and the red, which are capable of performing part of this conversion process. The bacteria are capable of transforming electromagnetic energy into chemical energy, but not with the evolution of oxygen. They use ultimate reducing agents other than water in order to reduce the carbon and therefore they produce other oxidants than oxygen. But the photosynthetic bacteria are able to capture electromagnetic energy from the sun and transform it into chemical potential.

These are the classifications of organisms that can do all, or part, of this conversion (energy manipulation) process. These organisms really constitute the whole gamut of biological diversity, as far as I am aware of it, which can do all, or some, of this energy conversion process and they all can do the crucial part of it -- the quantum absorption and the quantum conversion.

MECHANISM OF THE PHOTOSYNTHETIC PROCESS

A The Path of Carbon in Photosynthesis

Let us see what we know about the mechanism of the process of photosynthesis itself. Part of this knowledge is a result of the tracer work which was mentioned earlier,[1,2,3] beginning before the war. My colleague, Sam Ruben, began this work, using radioactive carbon-11, but right after the war in 1945 we took it up again using carbon-14 labeled carbon dioxide to examine the sequence of events and determine the sequence of compounds involved in the transformation of CO_2 into carbohydrate. The answer to these questions is now available to us, and we can draw a rather complete road map of the reduction of carbon dioxide. (A simplified version of the carbon reduction cycle is shown in Figure 1.) The first step in the photosynthetic carbon cycle is the carboxylation of a sugar, ribulose diphosphate, to give phosphoglyceric acid, and this, in turn, can now be reduced to triose phosphate using some kind of reducing agent as well as some pyrophosphate-containing compound. The triose phosphate then goes through a series of rearrangements to produce ribulose diphosphate again, and the carbon cycle can continue.

The light is required to produce these two agents: a reducing agent, here represented by [H] and a particular (pyrophosphate containing) phosphorus compound to help the reducing agent in the reduction process. This particular phosphorus compound seems to be adenosine triphosphate (ATP) which contains a pyrophosphate linkage. This is of great importance and will be discussed in detail later on.

The major point that I want to introduce at this stage is the idea that the reduction of carbon dioxide through the carbon cycle and the whole sequence of enzymatic reactions that are involved in this reduction are dark reactions. Once we have available the products of the light reaction, namely, a reducing agent and some type of 'high energy' phosphate, the whole carbon cycle can be

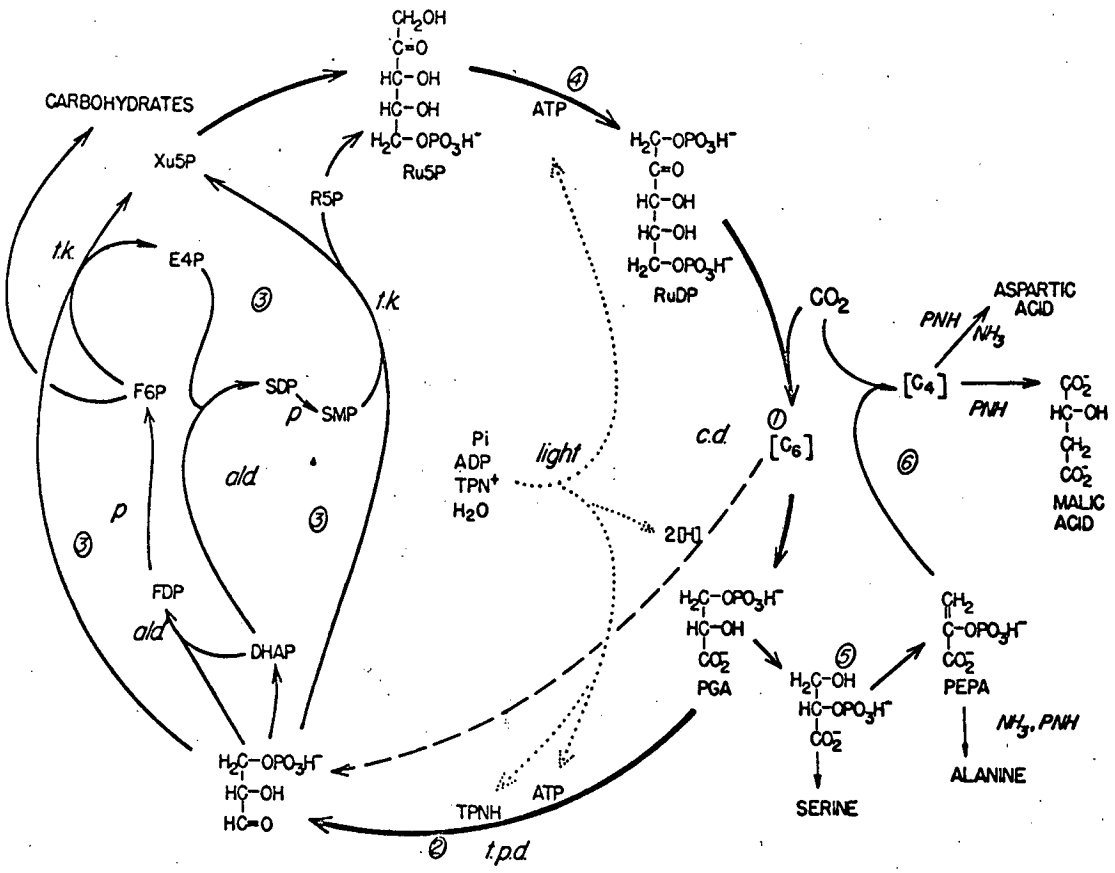

Figure 1. Carbon reduction cycle (simplified version). 1) Ribulose diphosphate reacts with CO_2 to give an unstable 6-carbon compound which splits to give two 3-carbon compounds, one of which is 3-phosphoglyceric acid. The other 3-carbon compounds might be either 3-PGA, as it is known to be in the isolated enzyme system, or some other 3-carbon compound such as a triose phosphate (dashed arrow). 2) PGA is reduced to triose phosphate with ATP and TPNH derived from the light reaction and water. 3) Various condensations and rearrangements convert the triose phosphate to pentose phosphates. 4) Pentose phosphate is phosphorylated with ATP to give ribulose diphosphate. Further carbon reduction occurs via conversion of PGA to phosphoenolpyruvic acid, 5, and carboxylation, 6, to form a 4-carbon compound (probably oxaloacetic acid). Reactions leading to the formation of some of the secondary intermediates in carbon reduction are shown by the arrows lettered a through e.

operated and carbon can be taken from CO_2 into a variety of compounds, among them sugar. The sugar can be taken out of the cycle. Every time the cycle turns six times, for example, we can take out a hexose sugar molecule and still have the cycle molecules left. This, indeed, is what happens.

We recognize also that all of the eleven enzymes (catalysts) that are involved in these transformations in the carbon reduction cycle are to be found very nearly everywhere very widely distributed in the biological world -- not limited solely to organisms which are converting solar energy, but also in organisms that have nothing whatever to do with the photosynthetic process. It therefore seems quite clear that at least this sequence, that is, the carbon reduction sequence, undoubtedly evolved in a separate chain of evolutionary events having little or nothing to do in the early stages with the electromagnetic energy conversion process itself.[4,5] The electromagnetic energy conversion process itself appears to produce in a primary act, or very close to it, two materials, a reducing agent and a pyrophosphate linkage, which can then run the carbon reduction cycle.

We can already see the two quite independent evolutionary streams which were joined only very recently in evolutionary history to produce the modern green plant.[6,7,8] The carbon reduction system was one independent stream. These streams will, of course, break up into finer parts as we go along, but this is our beginning.

B. Quantum Conversion in Photosynthesis

Let us now return to the photochemical process itself. Having separated out the carbon reduction system as a separate evolutionary stream, I am going to leave it since there is nothing unique about it for photosynthetic organisms except the combination of the product of the light reaction with a certain collection of enzymes, all of which can be found, either separately or in

various combinations, in nonphotosynthetic organisms.[9,10] Therefore, the carbon reduction cycle had a separate evolutionary history until the recent times.

Let us now see what more we can say about the quantum conversion process. We do not have anywhere near the detailed knowledge of the quantum conversion process as we do of the carbon reduction process. It is perhaps worthwhile to put down on paper before we start this discussion, the structural formulas of the two molecules which we believe to be essential for running the photosynthetic carbon reduction cycle. (There are undoubtedly others of which we are still unaware required for oxygen evolution as well.) To run the carbon cycle we need the reducing agent, which is a pyridine nucleotide in its reduced form. An adenine and pyridine moiety are tied together by two ribose sugars and a pyrophosphate link to give the molecule known as diphosphopyridine nucleotide. Actually, in photosynthesis it seems that there is a molecule very similar to this, but involving another phosphate group on one of the ribose molecules, and so I will actually use the triphosphopyridine nucleotide in its reduced form as the structural formula for the reducing agent which is required to run the carbon reduction cycle.

The possibility exists that still another, and perhaps more specific, reducing agent might be used by photosynthetic organisms in the reductive splitting of the initially produced carboxylation product (Figure 1, Step 1).[11] If so, it is almost certainly as good a reducing agent as TPNH and may or may not be structurally and kinetically related to it. If such a specific photosynthetic reducing agent functions in green plants, it will, in all probability, have been a late addition in the evolutionary development of a higher efficiency, since we already know that the cycle can operate through TPNH.

The other molecule that is essential for running the cycle and which clearly must come somewhere from the photochemical reaction is the adenosine triphosphate (ATP). Here, there are two pyrophosphate linkages, and the important one for our purposes is the terminal pyrophosphate link (Figure 2). These are the two molecules that are required in order to move the cycle around, and clearly these must be manufactured as a result of the photochemical transformation.

How much do we know about how the photochemical transformation manufactures those two substances? Here, we are not so thoroughly informed, but a good deal, nevertheless, is known and some of it is of considerable importance in guiding our thinking as to what the evolutionary relationships between the photosynthetic equipment and other equipment of living organisms might be.

1. Photoinduced Redox System

The principal photochemical reaction we now know is, first, the absorption of light by chlorophyll to produce some kind of an excited chlorophyll, either a molecule or molecular aggregate. (I don't mean this to be a separate chlorophyll molecule in solution, but simply the chlorophyll as it exists in the photosynthetic equipment of the organisms.) This electronically excited molecule must then undergo some kind of transformation -- for example, it may react with another molecule or molecules to produce a separation of an oxidant from a reductant.[12,13] I am using this language first because of a bit of confusion that has arisen in the meaning of these terms. In ordinary photosynthesis the oxidant will eventually become molecular oxygen; the reductant will eventually become a reduced compound, pyridine nucleotide. The pyridine nucleotide, together with the ATP, for which we have not yet described a formation mechanism, will then go on to drive the carbon cycle.

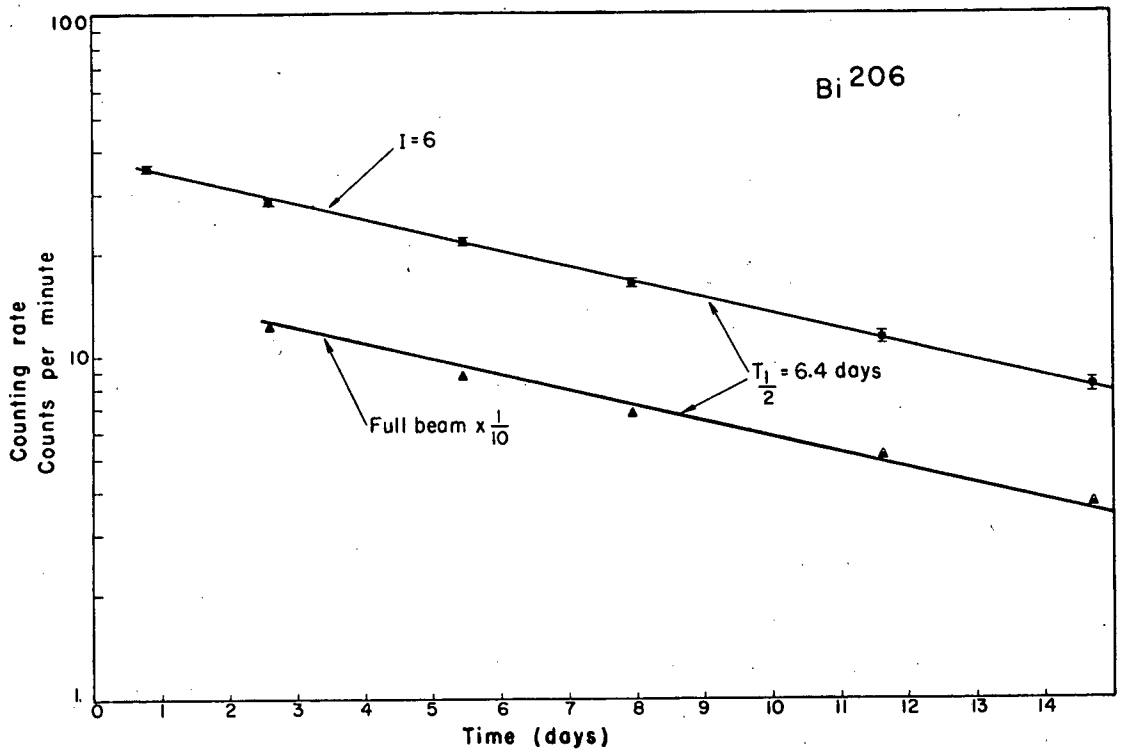

Figure 2. Structural formulas of triphosphopyridine nucleotide and adenosine triphosphate, two of the agents required to run the photosynthetic carbon cycle.

These terms, oxidant and reductant, are the chemists' terms for what happens after the excited chlorophyll loses its energy to some molecule, or collection of molecules, if any redox system is directly involved. The biologist has been accustomed to writing these two things in different terms. Following van Niel[14,15,16,17] he has generally associated the term M (Figure 3) with water and has generally called the oxidant ([O] in Figure 3) hydroxyl [OH], but he has been very careful, I must say, to put a bracket around it. (Those of you who know what the meaning of a bracket is, will understand the significance of this; when you see a biochemist putting a bracket around something of this sort it means that he doesn't really know what he is talking about. It is something we don't know and it is a general representation and not a chemical formula.) The reductant ([R] in Figure 3), according to the biologists, has been called [H] hydrogen, and this had led many to suppose that the primary process of quantum conversion involves the splitting of the water molecule itself. What is meant by the van Niel theory, at least in chemical terms, is the creation of a reductant of some general character, whose nature we do not know, and of an oxidant, also of some general character whose nature we do not know as yet. These two things must ultimately come from water as given by the stoichiometry of the primary reaction of photosynthesis in the first place.

In more recent years, still another terminology has entered into this discussion and it comes from quite a different source. The physicist has called the reductant the 'electron' and what is left after you take an electron away from a molecule is called a 'hole'.[18,19,20,21] These are the physicists' terms for the same phenomenon. You must not get confused about the terminology because all of these -- oxidant-reductant, hydroxyl-hydrogen, electron-hole -- all are different names for essentially the same thing. What we are trying to do

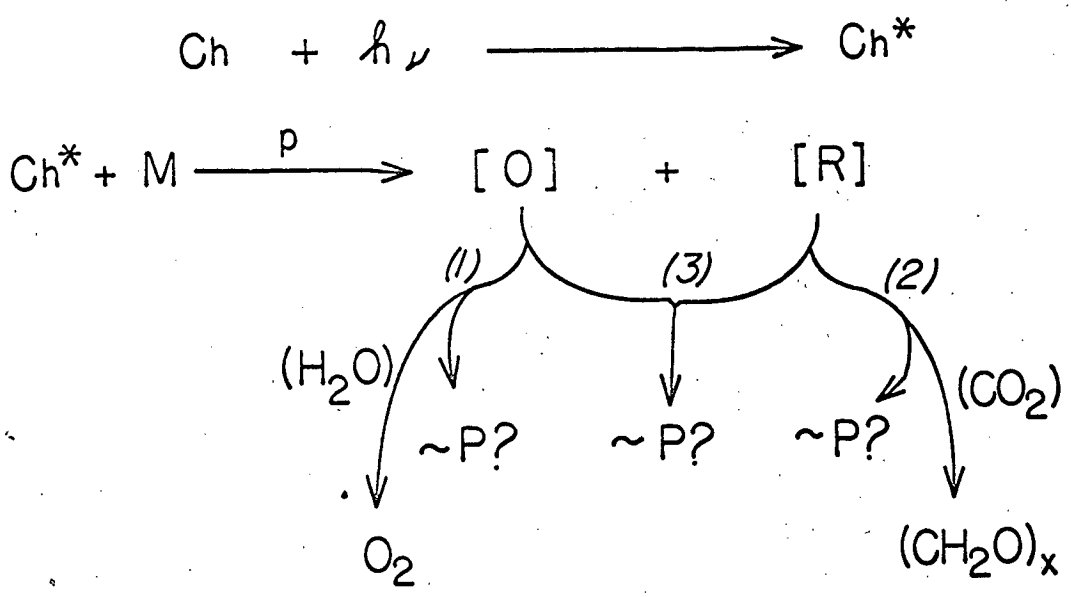

Figure 3. Simplified Photosynthesis Scheme.

The quantum is first absorbed by the chlorophyll molecule; then something happens (p for primary) to the excited chlorophyll to produce two chemical species ([O] and [R], for example) which can go on, one of them [O] to become molecular oxygen in some way (1), and the other one [R] leading to the reduction of CO_2 to carbohydrate (2). Along these two routes, various other energy containing species may be created (ATP or ~P). ATP would be an energy storage product. This may be created on either, or both, sides. There may be back reaction (3) between the oxidants and reductants which also could create products of higher energy. The obvious one here is, of course, the pyrophosphate linkage in ATP.

now is to discover exactly the best way to describe these things in ultimate and intimate detail.

I introduce the terminology of the physicist because in the last few years we have learned a number of the reactions of excited chlorophyll and one of them is an electron transfer reaction which is observable spectroscopically. An electron is transferred from iron in the divalent state to give iron in the trivalent state,[22,23,24,25,26,27,28] with the electron located in an as yet unknown place. It is an important recognition that this phenomenon occurs and occurs very quickly after the chlorophyll absorbs the light.* The excited chlorophyll in some way is able to extract an electron from the ferrous iron compound, at present associated with the chlorophyll in modern organisms in the form of cytochrome, to produce the ferricytochrome and an electron in some molecules as yet undesignated.[24,30] This appears to be an important connection between a molecule that is unique to photosynthetic plants, namely, chlorophyll, and certain kinds of molecules which are not unique to photosynthetic plants, namely, the iron cytochromes (iron hemes). The iron hemes have universal distribution and this is an important fact to remember.

In addition, we now know that electrons must ultimately find their way to pyridine nucleotide. The oxidized iron, or something close to it, will eventually take electrons from water, giving rise to the ferrous iron and molecular oxygen and protons.

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* A recent modification[15] of the van Niel generalization inserts a ferrocytochrome ahead of the water molecule as the primary electron donor to the excited chlorophyll, but does not specify the primary fate of the excited electron which must be removed from chlorophyll. The oxidized cytochrome is presumed capable of oxidizing water to oxygen, with the concomitant formation of ATP, a suggestion similar to that of Bassham[29] and corresponding to reaction (1) in Figure 3.

At the same time that all of these things are happening, somewhere along the line, either on the way from the intermediate oxidant to oxygen (reaction (1), Figure 3), or on the way from the intermediate reductant to the pyridine nucleotide (reaction (2), Figure 3), or, perhaps, in a recombination reaction in which the electron falls back into the hole (reaction (3), Figure 3) we also create adenosine triphosphate. The ATP is designated by ~P?, which represents 'high energy phosphate' linkages. The reactions in Figure 3 indicate possibilities only, and not knowledge of three different ways (places) in which pyrophosphate could be created: (1) The fall of the intermediate oxidant toward oxygen; (2) the fall of the intermediate reductant (perhaps a sulfhydryl group) to the pyridine nucleotide which would, perhaps, give rise to pyrophosphate; or (3) perhaps the energy of recombination of the hydrogen-hydroxyl (electron-hole) could also give rise to a number of pyrophosphate linkages.

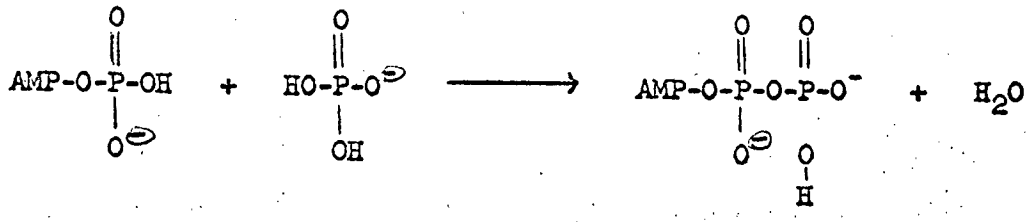
2. Photoinduced Dehydration

A more profound departure from the basic redox primary photo process is possible, particularly in the light of the recently indicated[31] reversibility of at least some of the steps of oxidative phosphorylation. Thus, there is evidence that in mitochondria it is possible to produce the reaction

$$Fe^{II} \text{ (cytochrome)} + 1/2 \text{ DPN} + (n)\text{ATP}$$

$$FE^{III} \text{ (cytochrome)} + 1/2 \text{ DPNH} + (n)\text{ADP} + n\text{Pi}$$

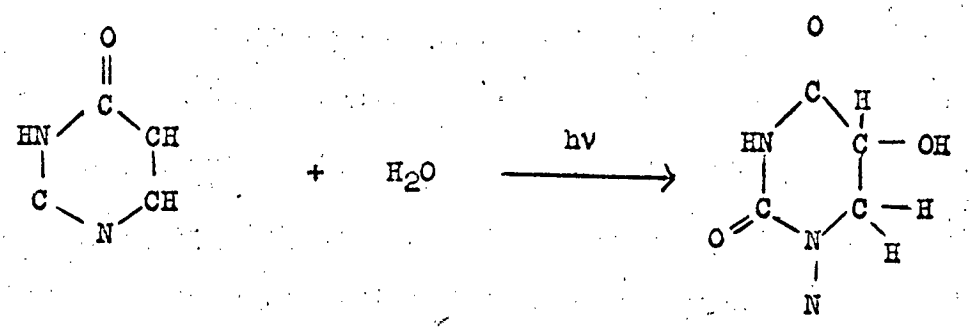
If an independent (non-redox) method of dehydration could be found for producing ATP according to the reaction,



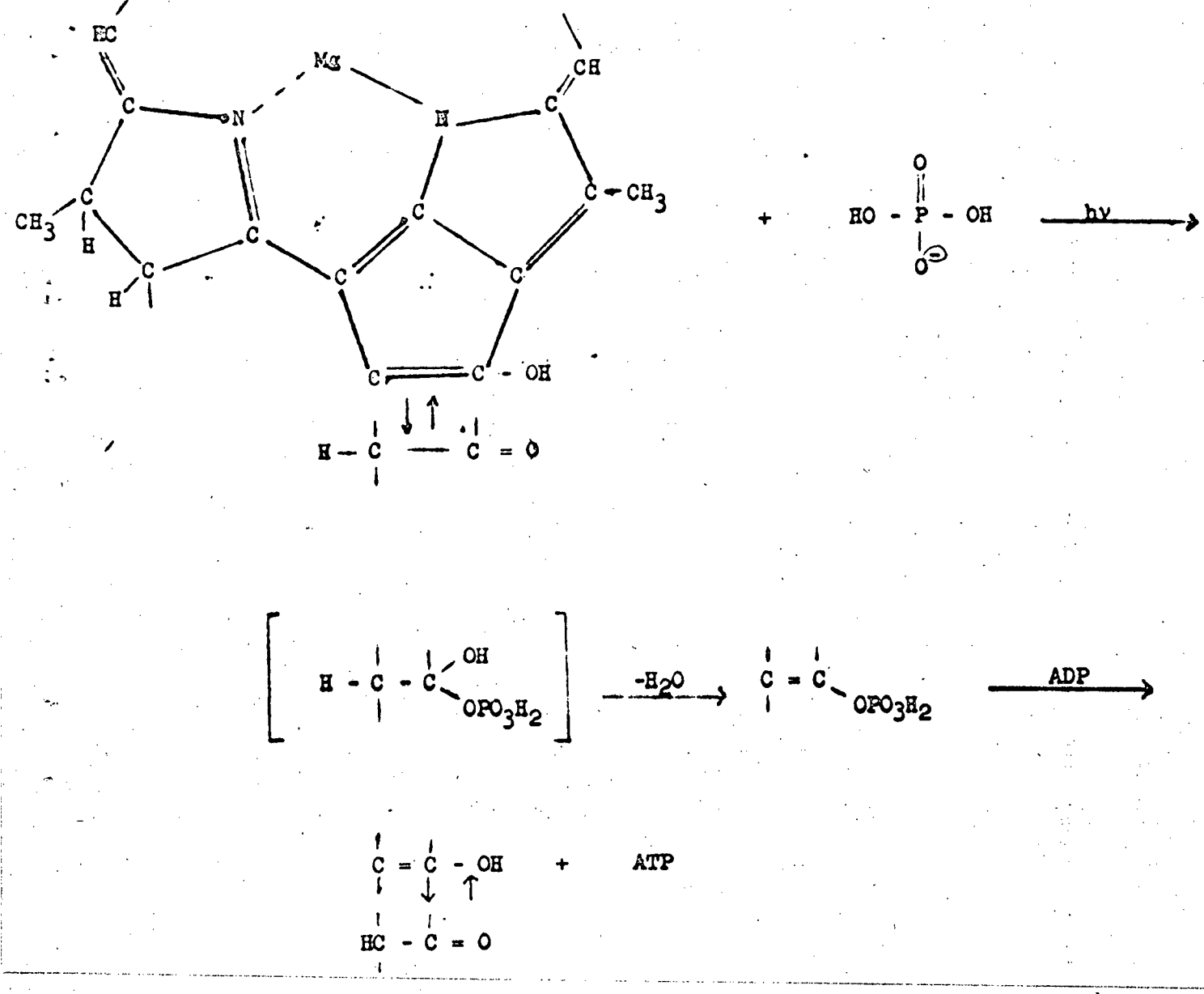
ADP Pi ATP

then both ATP and TPNH could be photoproduced without calling upon a photo-induced direct electron transfer reaction.

We already have a precedent for the idea that an optically excited pi-electron system can have an increased affinity for water leading to its hydration by an only very slowly reversible process so that energy may be trapped in this manner.[32,33]



For example, if the 9-10 enol in chlorophyll were to add orthophosphate (when excited) an enol phosphate could be produced, which presumably would be capable of phosphorylating ADP to make the required ATP.[34] Part of this would then be used to reverse the DPNH-cytochrome reduction to produce the ultimately necessary separation of oxidant and reductant (water splitting) required for O_2 production and CO_2 reduction.



The not inconsiderable difficulty with such a plan as this is the necessity for producing a good many more than one ATP for each quantum absorbed by chlorophyll. Even if a way of circumventing this difficulty were found, it remains fairly clear that such a device would be a rather recent evolutionary addition to an already highly developed biosynthetic energy manipulating system.

PYROPHOSPHATE LINKAGE IN NONPHOTOSYNTHETIC PROCESSES

The appearance of pyrophosphate linkage in a variety of organisms is well known. In practically all organisms, there are mechanisms for producing ATP which do not involve photosynthetic mechanisms at all. One of them is a reversal of one reaction in which ATP is used in the photosynthetic cycle (triose phosphate dehydrogenase). By running the reaction backwards (Step 2, Figure 1) one can make ATP. A more important source is a reaction which apparently involves iron -- the cytochromes, involving also the oxidation and reduction of the pyridine nucleotide. The two reactions together are involved in the creation of ATP in nonphotosynthetic organisms. This process of the oxidation of pyridine nucleotide by the passage of electrons from pyridine nucleotide back to oxygen through the iron cytochromes with the concomitant formation of ATP is known as oxidative phosphorylation. It leads to the creation of more ATP than does the substrate oxidation process. The return of a photoexcited electron of chlorophyll through all or part of a similar chain could produce the necessary ATP (see reaction (3), Figure 3).

Thus the creation of both the reduced pyridine nucleotide and the ATP are not unique to photosynthetic processes. These processes also occur in nonphotosynthetic organisms.[7] We know something about how pyridine nucleotide is created, but we know relatively little about how ATP is created in oxidative phosphorylation in which the electrons pass from reduced pyridine nucleotide through iron back to oxygen. This is one of the major problems of energy transformation in all biological organisms.

We have now split up the photo process of photosynthesis into two other streams of evolutionary development, the stream which gave rise to pyrophosphate (ATP_3) and the stream which gave rise to pyridine nucleotide.

Neither of these necessarily involves the photo process directly. This leads us to the conclusion that the appearance of the photo reaction, or the coupling of the photo reaction, with the creation of ATP and of reduced pyridine nucleotide was a very late thing in the evolutionary scheme.[4,5] You see that we are forced, now, to consider the question of the origin of life in discussing the origin of photosynthesis. We cannot dodge that issue, and we are indeed considering it and doing so in a much more sophisticated way than has been possible up until recent times. This has been discussed more thoroughly elsewhere,[42,43] so I shall not dwell on it in any great detail.

I shall simply pass through some of the states that we need in order to try and focus your attention on the separate evolution of mechanisms for making ATP, mechanisms for making the molecules which are involved in the creation of ATP today, mechanisms for creating pyridine nucleotide, and, finally, at the very end, how the light capturing molecule, chlorophyll, may have appeared and was coupled to the other energy transforming processes. This is really the story in principle, and I now want to go through it quickly and try to give you some idea of how I think these things might have occurred.

EVOLUTION OF THE PHOTOSYNTHETIC APPARATUS IN THE GREEN PLANT

Figure 4 shows the apparatus (the chloroplasts) in the green plant which is responsible for performing the process of photosynthesis. I have not discussed in detail the visible features of the photosynthetic apparatus, but it is perhaps necessary to say a few words here about the relationship of the tangible physical material that performs photosynthesis as it can be seen on the subcellular, but still visible, level. I will then discuss the macromolecular level (where this apparatus cannot yet be seen), and, finally, go to the substrate level where we can again deal with things in a chemical way.

Three different kinds of chloroplasts are shown in Figure 4, illustrating the highly ordered array of layers in all of the three types of organisms: a unicellular green alga, a blue-green alga which does not have a chloroplast (the layers are still present, however, winding their way in and out through the entire cell), and a chloroplast from a higher plant (tobacco) showing the layering of the green material very cleanly. The layers (lamellae) themselves are constructed of arrays of macromolecular subunits which we now think we can see.[35] Figure 5 gives a model for chloroplast lamellar structure and Figure 6 is an electron micrograph of frozen dried spinach chloroplast supernatant purporting to show the substructure of the lamellae.

Figure 4 shows the high degree of order in the chloroplasts, and, furthermore, that this high degree of order exists in other elements in the cell, such as the mitochondria, which perform other functions (formation of ATP by oxidative phosphorylation of pyridine nucleotide).[36] The

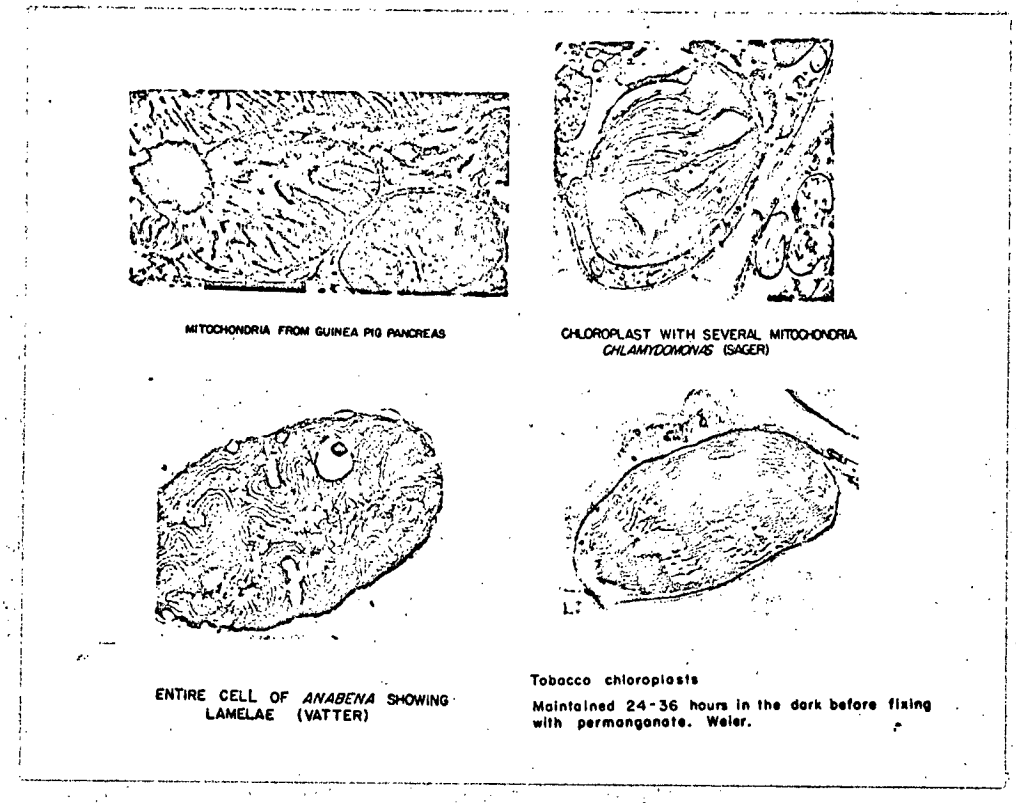


Figure 4. Chloroplasts from a unicellular green alga, from a blue-green alga, from tobacco, and mitochondria from guinea pig pancreas

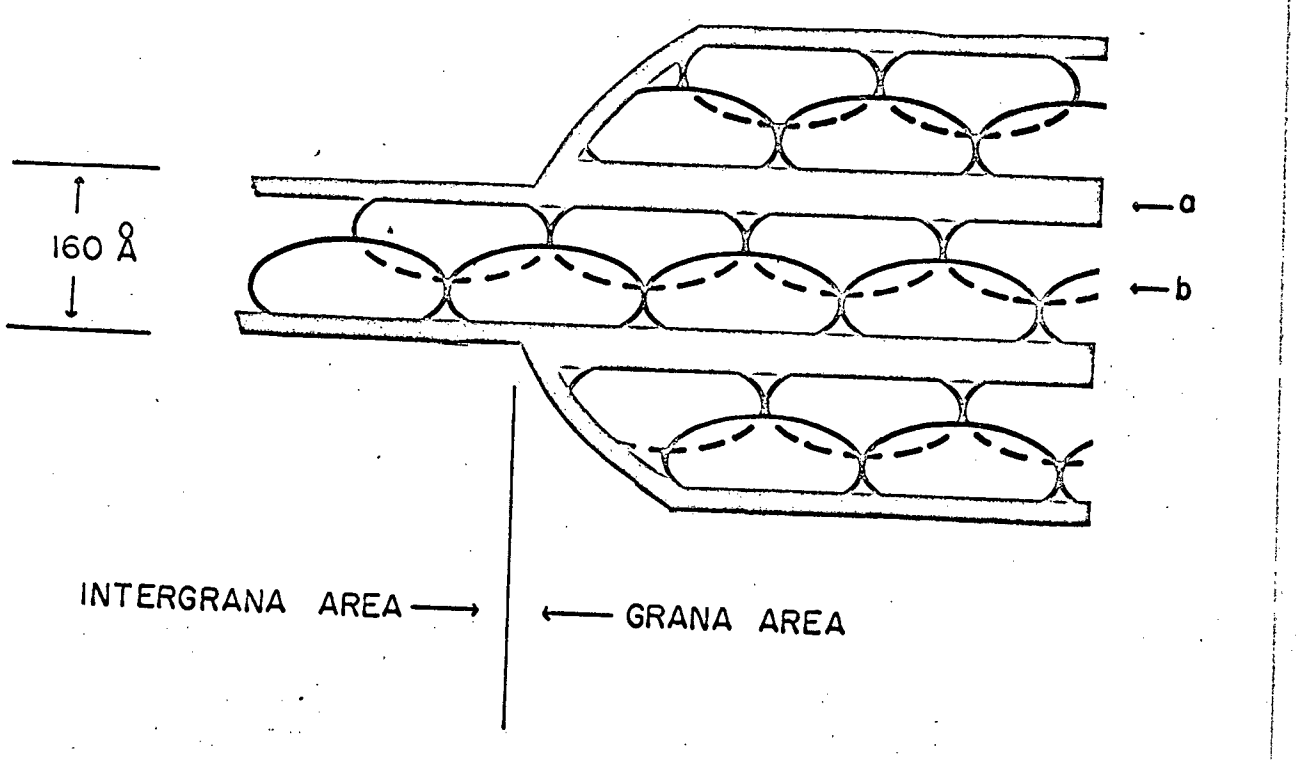


Figure 5. Model for lamellar structure within a spinach chloroplast

a. Osmium-staining layer of the lamellar structure. Thickness 30 Å in the intergrana regions and 60 Å in the grana regions.

b. Particles forming the granular inner surface of the two layers making up the lamellar structure. The packing of oblate spheres would not be as simple as illustrated in the figure since the central axis of both layers would not be in the same vertical plane shown here.

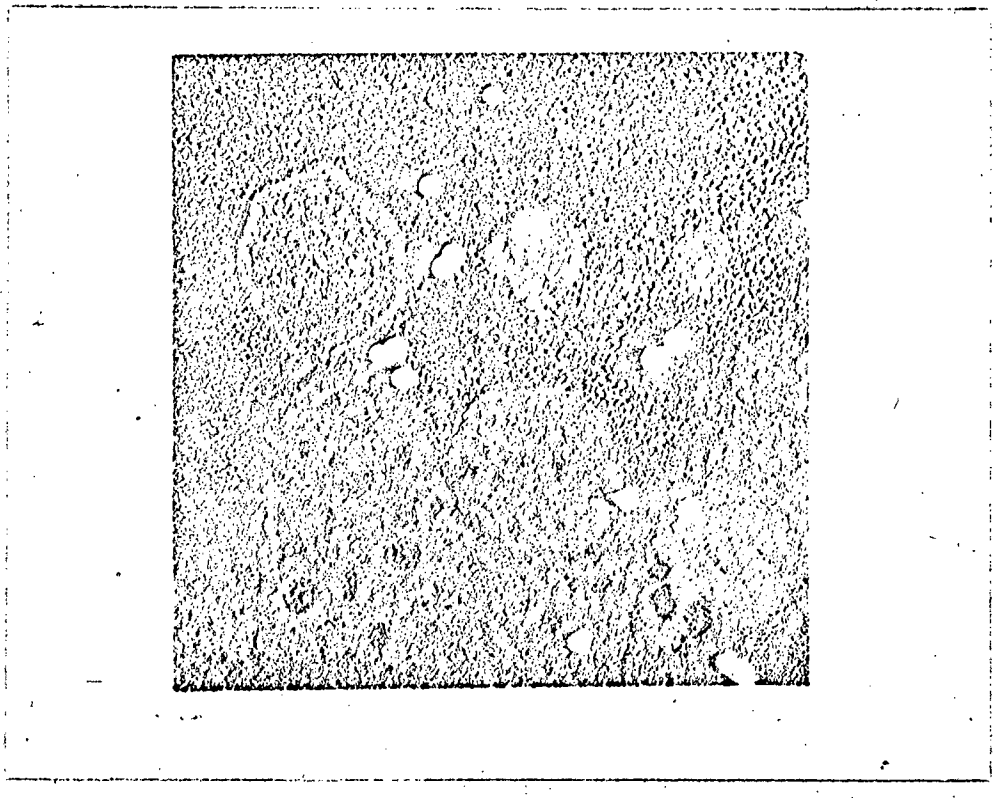


Figure 6. Frozen dried spinach chloroplast sonicate; 880 Å diameter PSL (polystyrene latex) markers.

purpose of Figure 4 is to show the similarity of structure between the photosynthetic apparatus and material which is not photosynthetic, and to show also that it is a highly ordered array in all cases. This highly ordered array must be achieved in some systematic way from molecules which themselves are ordered by virtue of the atoms of which they are made.

Chlorophyll Structure

The actual detailed structure of the one molecule unequivocally associated with the capture of light and its transformation, i.e., chlorophyll, is shown in Figure 7. This shows the structure of some of the different kinds of chlorophyll that are known: The first is protochlorophyll which appears in etiolated plants grown in the dark. When such plants are placed in the light, the protochlorophyll is converted to chlorophyll.[55] The principal difference between protochlorophyll and chlorophyll is the addition of two extra hydrogen atoms at the double bond in ring D. Bacteriochlorophyll is the molecule which is responsible for the capture and conversion of light in the purple and green bacteria, and differs from green plant chlorophyll in having a second, dihydropyrrole ring in it.

We must devise some way of making those ordered chloroplast structures which were seen in Figures 4, 5 and 6, and we must envisage some way of evolving this particular molecule, chlorophyll, belonging to the general class of tetrapyrrollic substances known as porphyrins. These two things -- ordered array within the cells and the development of chlorophyll itself -- are two essential features of our evolutionary scheme for the process of photosynthesis.

The structural feature, the appearance of order and structure, is something common to the evolution of all living organisms, and belongs to the general discussion of how ordered structures may be evolved from nonliving material. This is really part of the problem of chemical evolution and the origin of life.

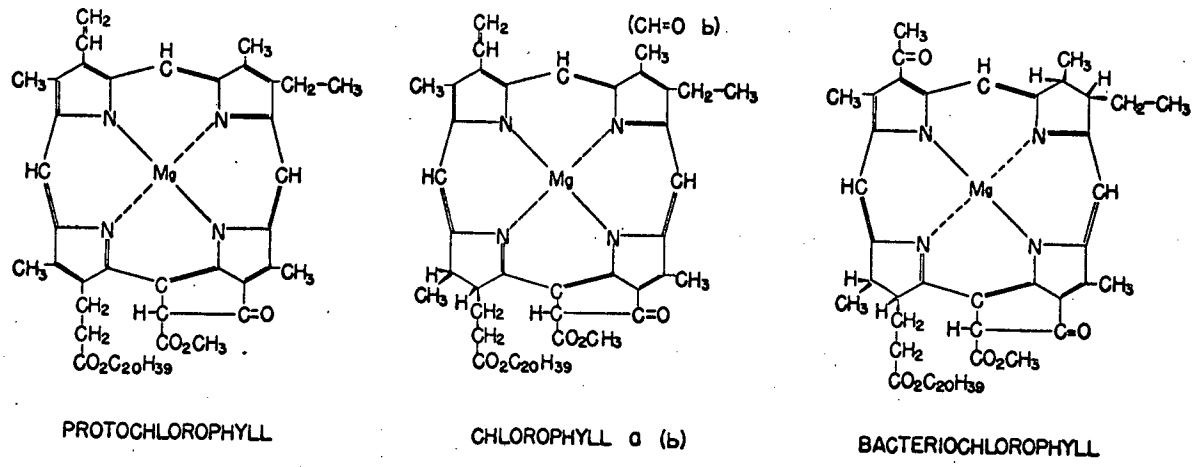


Figure 7. Structure of protochlorophyll, chlorophyll and bacteriochlorophyll

CHEMICAL EVOLUTION

I wish to discuss briefly the beginnings of chemical evolution, starting with the molecules of a primitive atmosphere, which you heard about earlier, being subject to a primitive photosynthesis using the far ultraviolet or radiation from the radioactivity of the earth's crust to transform them. The earliest molecules on the surface of the earth are presumed to be those shown in Figure 8 (top row), particularly methane, ammonia and water. If these molecules are subjected to radiation of energy great enough to break the bonds of carbon-carbon, carbon-hydrogen, hydrogen-hydrogen, nitrogen-hydrogen, hydrogen-oxygen, which can be done by ionizing radiation,[37] such as the beta-rays of potassium-40 which are plentiful in the earth's crust or with ultraviolet light of wavelengths shorter than 2200 Å,[38] then the atoms which are so formed may reorganize to form more complex molecules, a few of which are shown on the bottom row of Figure 8. You already recognize these molecules as being the present-day substrate materials (formic acid, acetic acid, succinic acid and glycine) upon which all living organisms operate. Glycine, shown here, is the only nitrogen-containing compound in the bottom row of Figure 8, and it is the simplest of the amino acids, of which the proteins are constructed. By exchanging one of the carbon-bound hydrogen atoms of the glycine for any of a group of other atoms, some twenty different amino acids can be built up.

In the first experiment of this type in 1950 in which we used the cyclotron as a source of ionizing radiation,[37] we started with CO_2, hydrogen and water, and were able to get, by random transformation processes, reduced carbon compounds such as formic acid, acetic acid and succinic acid. In

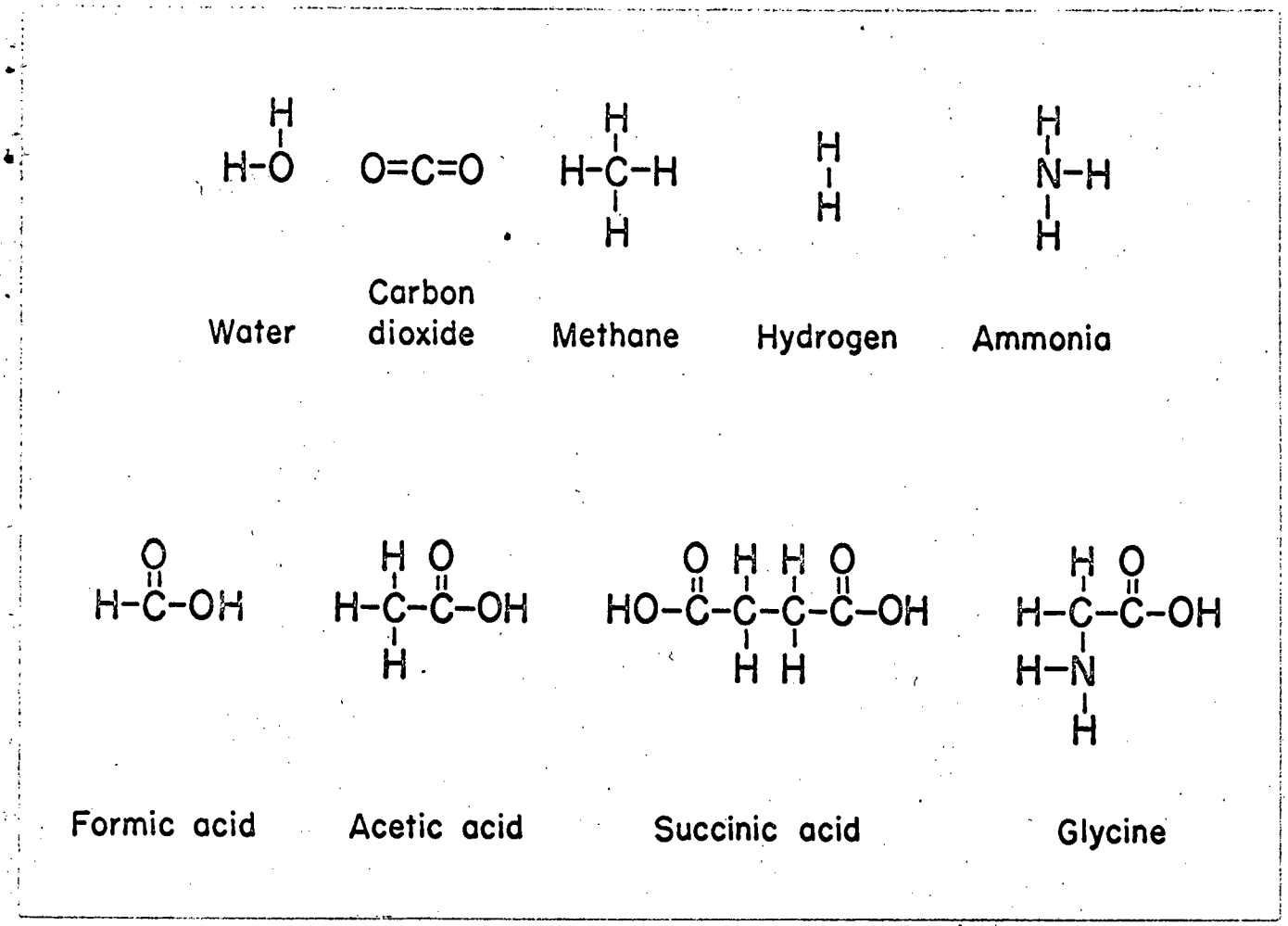


Figure 8. Primeval and primitive organic molecules

later experiments, in which ammonia was added to the initial mixture following Miller,[39,40] glycine was obtained. Still more recently (in the last three or four months) we have performed this experiment again, but instead of depending upon ordinary analytical methods to find these randomly occurring compounds, we have used carbon-14 labeled methane in the primitive gas mixture, thus providing radioactive carbon atoms which could be followed around. The discharge from a 5 mev electron linear accelerator was passed through the mixture of methane, ammonia and water, and we took the water solution containing the product from this bombardment and spread it out on a piece of filter paper in a systematic way.[41]

Figure 9 shows the results of one of these bombardment experiments. It is a photograph of the darkened x-ray film which results when a paper chromatogram containing radioactive products is placed on top of an x-ray film. Wherever there is a black spot on the film a particular compound has been located. We can tell what the nature of the compound is by where it is located on the film with respect to its origin. All of the different nonvolatile radioactive compounds which result from one particular bombardment are shown in Figure 9, and about a dozen compounds have separated out.

We have been able to identify in this way some half-dozen compounds,* including glycine, alanine and various other amino acids and sugars, some fatty acids and some hydroxy acids -- the very things of which today's living matter is composed. One of the compounds, representing about sixty percent of the total, is urea. We find in neutral and acidic fractions a large number of compounds, including lactic acid and sugars. You can also see that alanine and glycine (amino acids) represent a very small amount of the total. There

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* HCN was identified in the aqueous solution by a separate procedure.

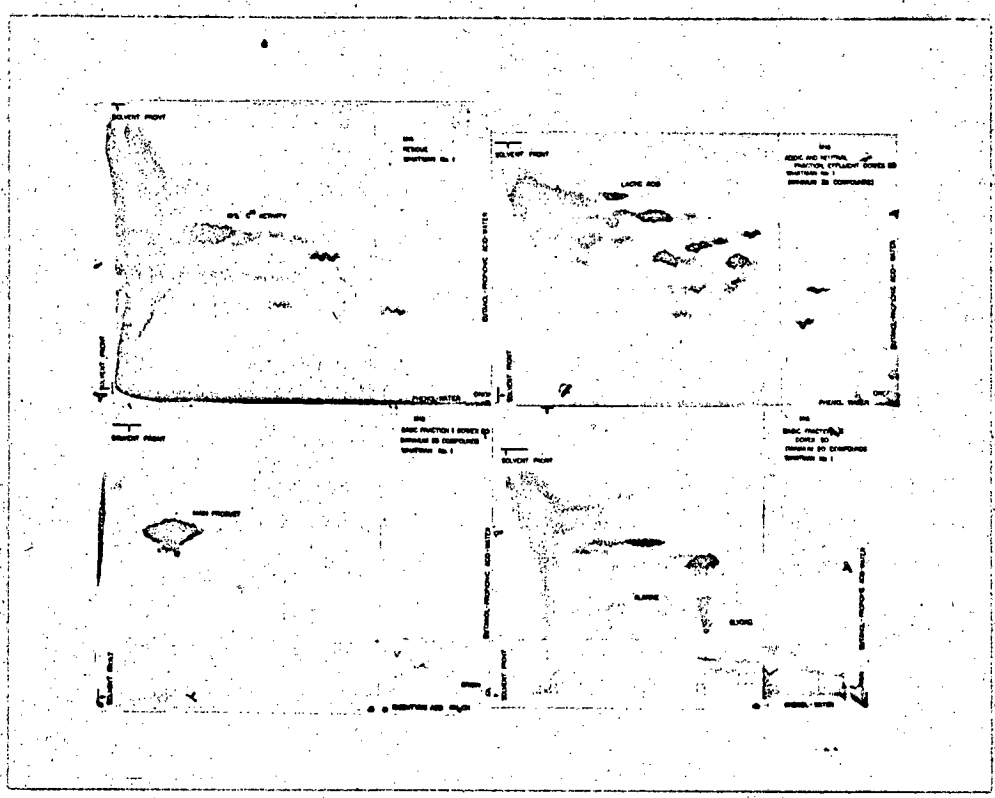


Figure 9. Radioautograph of paper chromatogram showing compounds which result after irradiation of a mixture of $C^{14}H_4$, ammonia and water with 5 mev electrons

appears to be present in this irradiated mixture a number of undetermined bases, including heterocyclics. Thus, such random processes as these may give give rise to all of the simple compounds that are needed by present-day living organisms.[42,43,44]

Having made these simple compounds (particularly the amino acids) by the random methods, we can build them up into proteins in various ways. Aside from the more or less laborious and specific methods involving special protective or activating groups, at least two simpler methods, possibly applicable to primitive conditions, have been successfully demonstrated in the laboratory recently. The first involves heating amino acid mixtures in molten glutamic acid together with some polyphosphoric acid to produce a mixed polypeptide resembling protein.[45,46] The second involves heating the amino acid in an aqueous ammonia solution to produce a polypeptide of intermediate size.[47]

The proteins themselves can take on a specific structure which is shown in Figure 10. The helical structure is built-in into the linear array of the amino acids because of the particular arrangement of carbon, hydrogen, nitrogen and oxygen atoms in such a chain. Figure 11 shows how the helical structure can take on visible order. The upper photograph is an electron micrograph of a protein which is a component of collagen. When the protein filaments are aggregated, as shown in the lower photograph, they do so in a specific ordered array because of the particular arrangement of amino acids in the proteins. Here you can begin to see the appearance of the visible order that must be generated to create mitochondria, chloroplasts and other subcellular particles. This generation of order is, of course, common to all living things, and is not unique to photosynthesis. One can generate order, beginning from the primitive molecules (figure 8) of an early earth's atmosphere, through proteins (Figures 10, 11) into the subcellular material itself (Figure 4).

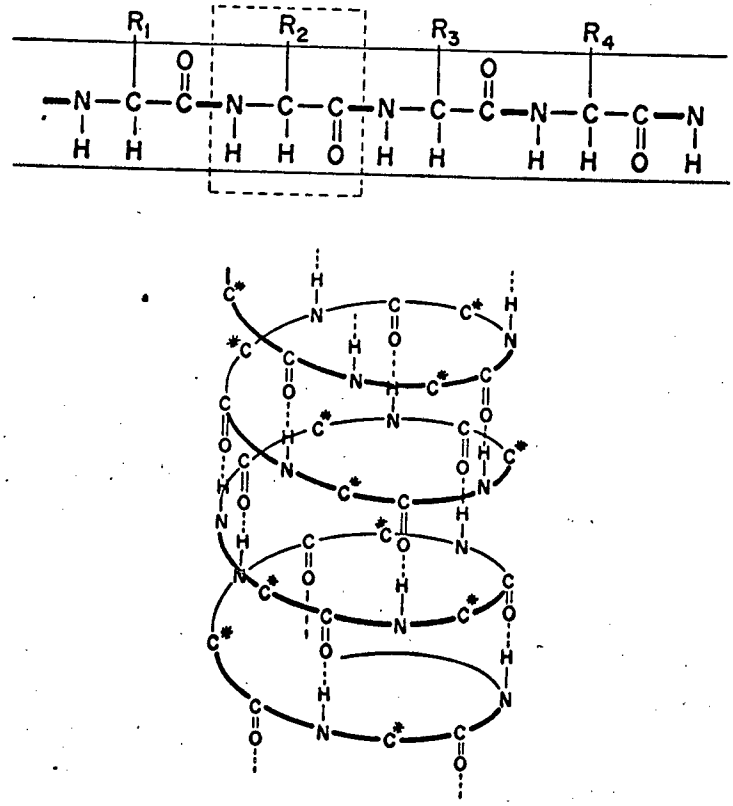
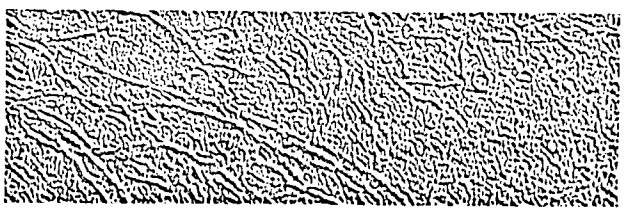
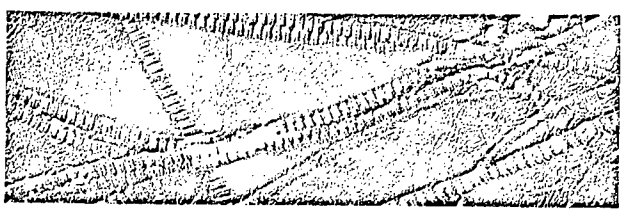


Figure 10. Protein structure



FILAMENTS OF COLLAGEN, a protein which is usually found in long fibrils, were dispersed by placing them in dilute acetic acid. This electron micrograph, which enlarges the filaments 75,000 times, was made by Jerome Gross of the Harvard Medical School.



FIBRILS OF COLLAGEN formed spontaneously out of filaments such as those shown above when 1 per cent of sodium chloride was added to the dilute acetic acid. These long fibrils are identical in appearance with those of collagen before dispersion.

Figure 11. Electron micrograph of collagen filaments

DEVELOPMENT OF RUDIMENTARY CATALYSTS

Let us now turn to the question of the generation of the porphyrins which seem to be central not only in the capture of light, as represented by chlorophyll, but to the appearance of adenosine triphosphate in present-day organisms and perhaps to the appearance of ATP in primitive organisms as well.

Figure 12 shows that starting with the primitive function of iron for the decomposition of hydrogen peroxide, which will be formed in the seas either by ultraviolet radiation or by K^{40} radiation, the iron catalysis can be improved by a factor of a thousand if it is built into a porphyrin. If we now transform this iron further by encasing the heme into a folded protein and make the molecule of catalase, the catalytic function is improved by another factor of ten million for this particular peroxide decomposition reaction.[44]

This fact is of great importance because I believe that peroxide appeared in the primitive seas of the earth at the very earliest stages as a result of both the ultraviolet radiation at the top of the atmosphere and of the potassium-40 radioactivity in the earth's crust. This peroxide can now serve as an evolutionary selection pressure[48] to improve the catalytic function of iron from the bare iron to the iron heme to the iron heme-protein combination.

The way in which this can occur is shown by having a look at the way in which hemes are synthesized by modern living organisms (Figure 13). We start with succinic acid and glycine, which were made by random synthesis from the primitive earth's atmosphere, and by combining these two substances, we make the alpha-amino-beta-keto adipic acid which then decarboxylates to give the delta-aminolevulinic acid, two of which can combine to form the heterocyclic pyrrole ring. Then there follows a series of oxidation and condensation

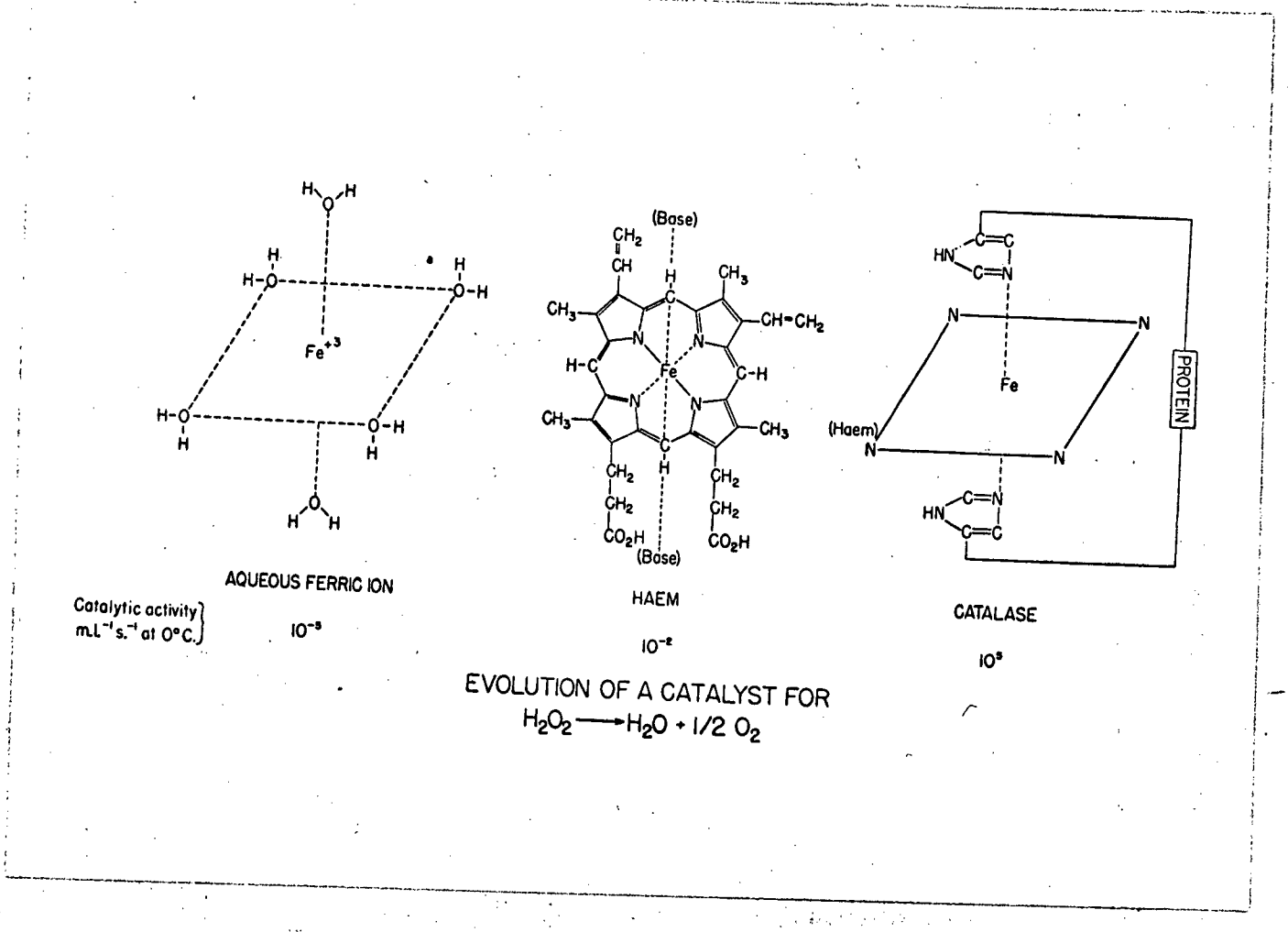


Figure 12. Evolution of a catalyst

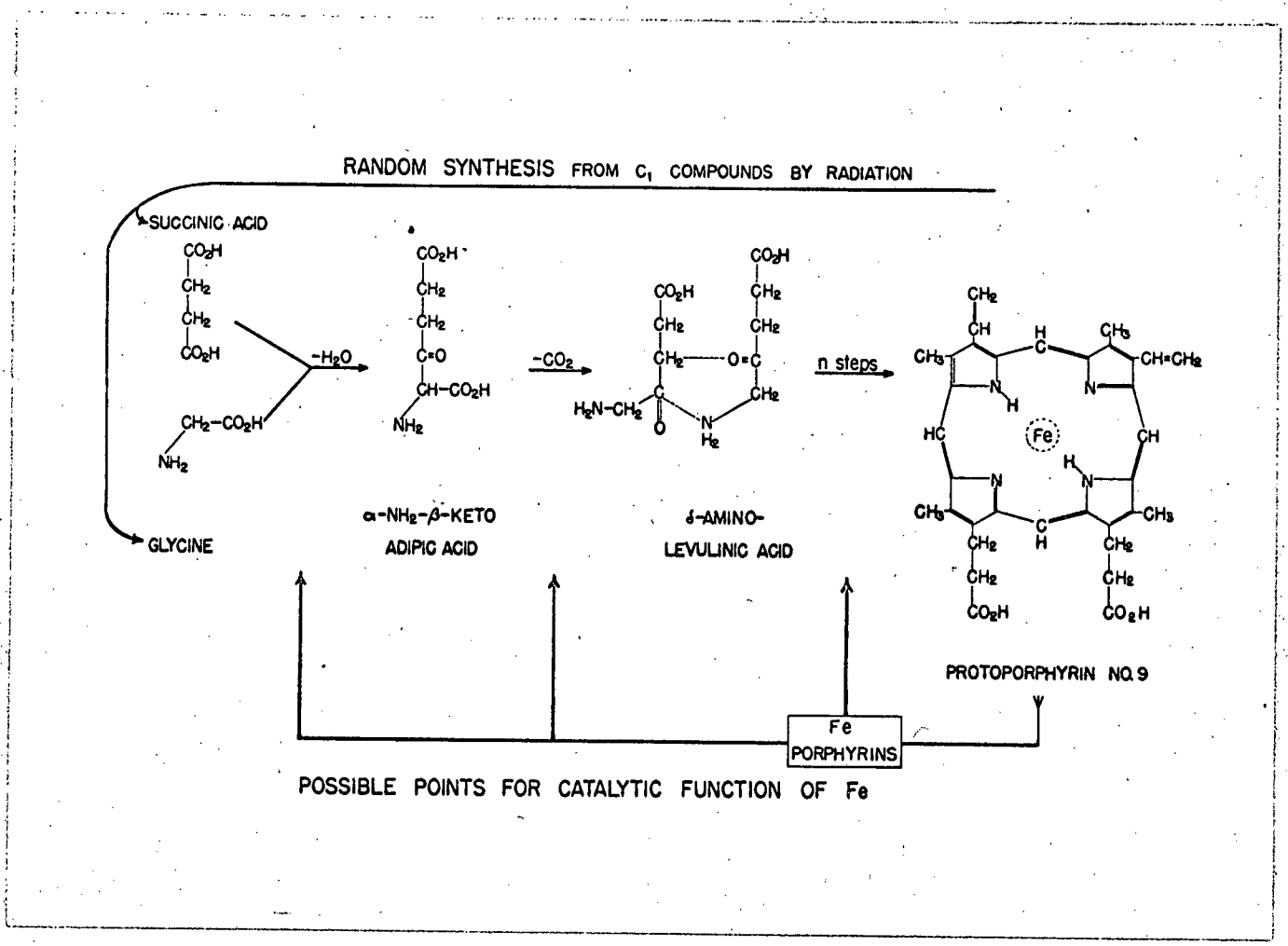
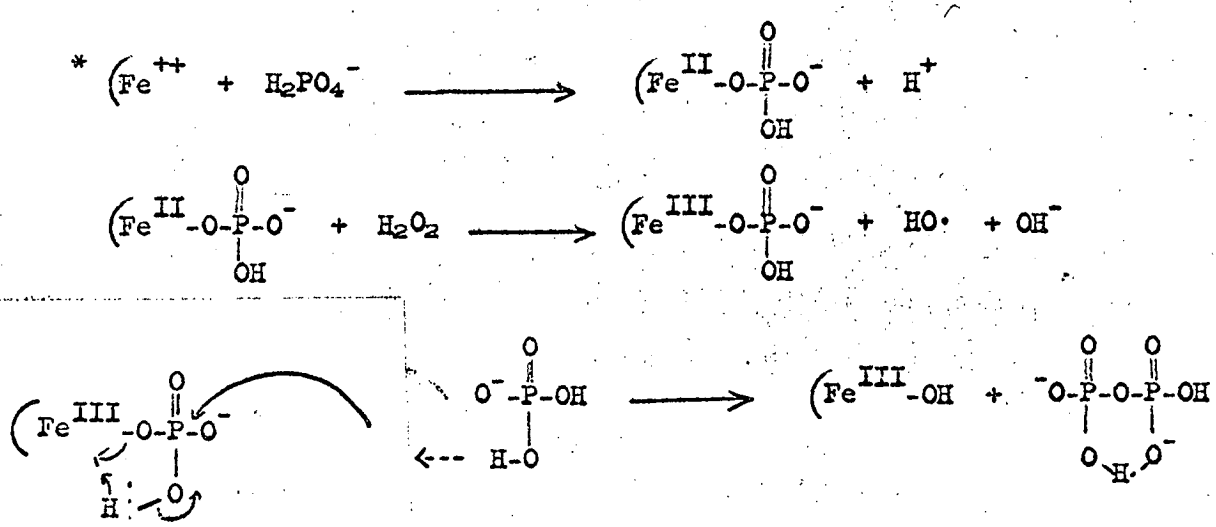


Figure 13. Biosynthesis of porphyrin and the evolution of the catalytic functions of iron

steps to give rise to the tetrapyrrole ring.[49] This reaction is a spontaneous one which involves a number of oxidation steps, several of which are almost certainly catalyzed by iron. The oxidation is achieved either by oxygen or peroxide under the influence of iron and presumably better achieved by iron in a porphyrin than by bare iron. Therefore, once the porphyrin is formed, more of it will be formed because of this autocatalytic self-selection mechanism.[4,5,8]

Pyrophosphate Formation

This idea is important because the mechanism of the formation of pyrophosphate seems to involve the oxidation of iron. In the last month or so, we have been able to demonstrate that one can generate pyrophosphate in aqueous media by simply allowing hydrogen peroxide to oxidize ferrous iron in the presence of orthophosphate.[50] In this reaction, a certain amount of orthophosphate is converted into pyrophosphate. The reaction may be written as follows:



I believe this to be evidence of the primitive way in which the highly evolved oxidative phosphorylation which takes place today began. The complexing of phosphate by ferrous iron, followed by the withdrawal of an electron from the ferrous

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* The half-circle around the iron symbol is introduced to represent any other coordinated atoms or groups.

iron to make ferric iron, the elimination of a water molecule to make pyrophosphate, and reduction of the ferric iron to ferrous, completes a cycle for the formation and the liberation of the pyrophosphate linkage. This is now demonstrated in a simple system, and I think it will not be long before we will be able to demonstrate it in the highly evolved iron systems that are used in oxidative phosphorylation, both in plants and in animals, and which are also used in photosynthetic phosphorylation, probably in a similar manner.

You can see here a driving force which will give rise to the porphyrin molecule. The driving force is the peroxide present in the ocean and the usefulness of transforming orthophosphate to pyrophosphate in aqueous solutions so the pyrophosphate can then be used to assist the combination of amino acids to make proteins. This was the evolutionary sequence which gave rise first to the porphyrin and second to a mechanism for manufacturing pyrophosphate.

COUPLING

As yet we have suggested no mechanism for using light to perform these processes. All that would be required in the later stages was to find a way of removing the electron from the iron, not with hydrogen peroxide but with light, in order to couple the photochemical reaction to what we now know to be nonphotochemical processes.

I think this event happened very late in the evolutionary scheme, and the evidence for it lies in the fact that the chlorophyll molecule is today manufactured by a sequence of reactions almost identical with the sequence of reactions used to manufacture the heme,[51,52,53,54] but just before the iron is put into the heme (Protoporphyrin IX), a branching occurs leading to the chlorophyll molecule in which magnesium is situated (Figure 14). I think the reason for that reaction is, first, that the light absorbing ability of the heme itself is very poor.

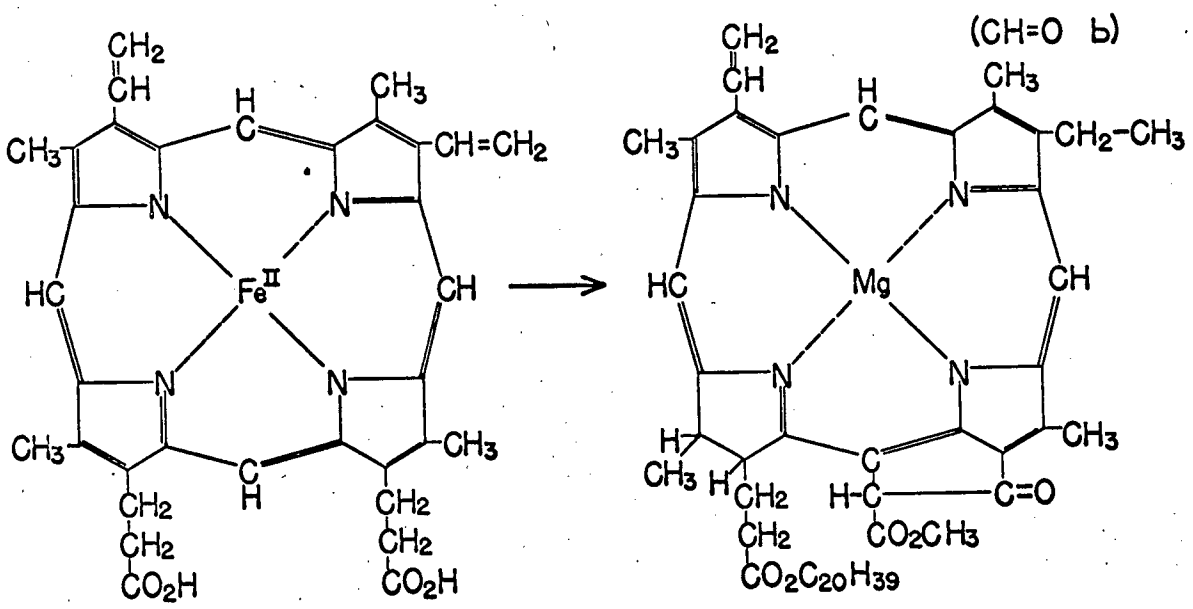

Figure 14. Structural relations between heme and chlorophyll

Although heme is red, it does not have anywhere near the light absorbing capacity of chlorophyll, and one of the reasons for the evolutionary selection of magnesium chlorophyll (magnesium chlorin) is the fact that the absorption of light by a magnesium chlorin is several thousand times greater than that of the iron porphyrin. Secondly, something very special about the electronic structure of the magnesium and of the packing together of the chlorophyll molecules in a crystal lattice, leading to the separation of electrons from the chlorophyll,[12] is better achieved by the chlorin than it is by the porphyrin. If the dehydration-phosphate activation idea (by the 9-10 enol of chlorophyll) turns out to play a role, we would then have a third powerful selective factor favoring the chlorophyll structure.

The emerging liklihood that the products of two different quantum conversion acts can collaborate to produce the products of photosynthesis more efficiently than either one alone must be considered.[56-60] One of these processes seems to be electron transfer from reduced cytochrome.[12,13] It has been suggested that the other is electron transfer to oxidized cytochrome.[59] An alternative pair of transfers would be to chlorophyll (from cytochrome) and from chlorophyll (to quinone or disulfide).[61] The experimental question as to whether either one of these two different quantum acts alone could accomplish the whole of photosynthesis, albeit at reduced efficiency, has yet to be unequivocally answered. In any case, the collaboration is surely a late addition.

The mechanism and the detailed chemical and physical reasons for the advantage of the chlorophyll over the porphyrin remains for the future to discover. It is of interest to examine the paleontological record to see if it might be possible to (1) confirm the notion that heme (and its oxidative function) preceded the appearance of large amounts of oxygen in the earth's atmosphere for whose presence oxygen-producing photosynthesis seems to be the only competent

geochemical process; and (2) if confirmed to date, the appearance of chlorophyllous pigments. The presence of both heme and chlorophyll fossil molecules in petroleum and other organic minerals has long been known.[62] The principal hope of distinguishing between these two origins lies in the possible presence of a carbon substituent on the delta-carbon atoms of these substances derived from chlorophyll with its icocyclic ring. The relative stability of other possible distinguishing features, and even the structure of some of the bacterial chlorophylls (Chlorobium), are not yet known to us. Presumably bacterial photosynthesis, producing as it does only ATP (no oxygen), is a more primitive process and, therefore, the pigments there involved might be expected to have appeared earlier. As yet, no porphyrin at all has been unequivocally found in Pre-Cambrian formations although the presence of fossil forms strikingly resembling in morphology the blue-green algae have been described by Barghoorn.[63]

As early as 1937 Hans Fischer, in discussing chlorophyll, said: 'In historical development we regard hemin as the older dyestuff,'[64] but he did not give explicit reasons. These were undoubtedly based on structural chemical relationships, and in view of our modern knowledge of the present-day biosynthetic relationship, he will probably turn out to be right.

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