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CHEMICAL EVOLUTION

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Abstract

How did life come to be on the surface of the earth? Darwin himself recognized that his basic idea of evolution by variation and natural selection must be a continous process extending backward in time through that period in which the first living things arose and into the period of "Chemical Evolution" which preceded it. We are approaching the examination of these events by two routes.

One is to seek for evidence in the ancient rocks of the earth which were laid down prior to that time in which organisms capable of leaving their skeletons in the rocks to be fossilized were in existence. This period is sometime prior to approximately 600 million years ago. The earth is believed to have taken its present form approximately 4700 million years ago. We have found in rocks whose age is about 1000 million years certain organic molecules which are closely related to the green pigment of plants, chlorophyll. This seems to establish that green plants were already fluorishing prior to that time.

We have now found in rocks of still greater age, namely, 2500 million years, the same kinds of molecules mentioned above which can be attributed to the presence of living organisms. If these molecules are as old as the rocks, we have thus shortened the time available for the generation of the complex biosynthetic sequences which give rise to these specific hydrocarbons (polyisoprenoids) to less than 2000 million years.

The second approach is to attempt to reproduce in the laboratory those chemical processes induced by energy of various kinds — radiation from the sun, from radioactivity, from electrical storm, etc. — which could give rise to simple organic molecules and polymeric combinations of them, ultimately leading to systems which could be called alive. This attempt has also succeeded along a variety of lines, and many of the present day biologically important molecules have been constructed abiogenically from the primeval atmosphere, thus providing laboratory evidence for the hypothetical processes. The latest step in this approach has been to demonstrate the formation of polypeptides in dilute aqueous solutions through the agency of molecules formed in the primitive atmosphere of the earth.

Finally we must seek evidence for the same processes in material found elsewhere than on the earth, such as other parts of our solar system, e.g., the moon and Mars. We can expect to know whether such materials exist at all in the rocks of the moon within this decade. We may even know something more definite about the botany of Mars during this same period.

CHEMICAL EVOLUTION*

INTRODUCTION

The term Chemical Evolution is here used in a very specific sense to refer to that period of the evolutionary history of the earth during which the chemical components on its surface were changed from their primeval form into chemicals upon which living organisms, or from which living organisms, could develop. The idea that living organisms arose as a natural development in the course of the chemical transformation of the surface of the earth is not new. In fact, it was recognized by Darwin himself that the basic notions of evolution which he formulated were in fact continuous, not only throughout the appearance of living organisms and their varieties but continuing back through that stage of history into the period which preceded the existence of living organisms on the surface of this earth. This was recognized by him in a very famous remark which I thought it might be worth repeating now to make you more familiar with some of Darwin's chemical concepts held as early as 1874.

> He says (Royal Society, 1959): "You expressed quite correctly my views where you said that I had intentionally left the question of the Origin of Life uncanvassed as being altogether ultra vires in the present state of our knowledge, and that I dealt only with the manner of succession. I have met with no evidence that seems in the least trustworthy in favour of so-called Spontaneous Generation. I believe that I have somewhere said (but cannot find the passage) that the principle of continuity renders it probable that the principle of life will hereafter be shown to be a part, or consequence, of some general laws..."

The statement to which Darwin refers, and which he had forgotten, was written earlier, prior to 1871:

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* Bakerian Lecture of The Royal Society of London, June 17, 1965, London, England.

> "It is often said that all the conditions for the first production of a living organism are now present, which could ever have been present. But if (and oh! what a big if!) we could conceive in some warm little pond, with all sorts of ammonia and phosphoric acid salts, light, heat, electricity, etc. present, that a proteine compound was chemically formed ready to undergo still more complex changes, at the present day such matter would be instantly devoured or absorbed, which would not have been the case before living creatures were formed."

Darwin there exhibited two qualities: First, a remarkable perspicacity about the nature of chemistry and, secondly, an altogether characteristic conservatism about how much he knew, and how much chemists knew, at that time, about the nature of molecules. And he was quite right. In those days so little was known about the nature of molecules and their interactions and behavior that it was fruitless for him, and others like him, even to try to reconstruct the chemical evolutionary history of prebiotic times.

Today there are possible two approaches to gaining a concept, at least, if not direct unequivocal knowledge, of what this sequence of events might have been. One of these is to continue the Darwinian approach itself, namely, the examination of the record as it may exist in the rocks and surface formations of the earth. Darwin used only that part of the record — the fossil record — in which recognizable life forms existed; in which morphologically recognizable entities could be examined and described. Today, however, it is possible for us to go beyond this level of examination because of our biochemical knowledge and because of the evolution of analytical devices which permit us to not only determine that there are organic materials of various sorts present in ancient rocks which contain few or no morphological features which are recognizable, but to describe in significant detail the intimate molecular architecture of these substances. This is a kind of "fossil" examination and correlation

exactly analogous to that used by the paleontologists but it is in the hands of the organic and biological chemists.

The other approach is that of trying to reconstruct the possible sequence of chemical events that could have occurred prior to the existance of living things on the face of the earth, the effort that Darwin referred to as being conceptually possible but as yet not fruitful because, at that time, not enough information was available about the behavior of atoms and molecules under the influence of various physical-chemical forces. Today, however, this has become a significantly possible effort.

This afternoon I want to describe some of the things we have done along both of these approaches: (1) to examine the historical record beyond that of the morphologically recognizable forms -- "chemical fossils", if you like -- and (2) to quite independently see if we can find and reconstruct some of the chemical reactions which might have occurred among the primeval molecules on the surface of the earth which could give rise to biologically important substrates, leading ultimately (if we can trace it) to the structures and reactions which we know now are an essential component for the functioning of living organisms .(Calvin, 1961a, b, 1962b, Horowitz and Miller, 1962; 1964; Ehrensvard, 1963; Fox, 1960, 1965a, b; Gaffron, 1960; Keosian, 1964; New Biology, 1954; Oparin, 1957, 1959, 1961, 1964; Wald, 1964)

This kind of effort is more than just an exercise in detective work, because the possibility is now with us, within most of our lifetimes, of exploring other sites than the surface of the earth for a possible corroboration, or denial, of the kind of sequence with which we might come up, as a result of this study. What I am referring to is the iminent possibility that we will have pieces of the Moon (rocks) to examine for their organic constituents. To an organic chemist, looking for new sources of

strange and wonderful materials, to have a piece of the Moon seems like ".....asking for the Moon." In addition to that, shortly thereafter, the opportunity of having a close spectroscopic look at the surface of Mars also exists. (There will be a short look within the next two months, with not very sharp eyes. Within five to seven years that look should become longer and with sharper eyes.) Some undefined but quite finite time thereafter we may be able to set down some instruments on the Martian surface to see what the soil contains. Obviously the methods and concepts which are being developed now along the lines that I am about to discuss will be essential for understanding the messages which we may receive from these far places.

ORGANIC GEOCHEMISTRY

We shall now undertake the two exercises which I have described earlier, namely, looking at the "molecular fossils" on the earth's surface and seeing what we can find and then, after that, examining the possible chemical reactions that might give rise to important systems today.

Figure 1 gives us some clue to the geological history with which we have to deal. The age of the earth is approximately 5000 million years, and this figure has not changed much since this picture was drawn. Even the point of origin of organic evolution which was here intended to be an asymptotic thing is in approximately what seems to be the right place, although at the time we drew this figure we had no idea that this would be so nearly correct. The period of chemical evolution presumably begins with the formation of the earth in its present form and gave rise

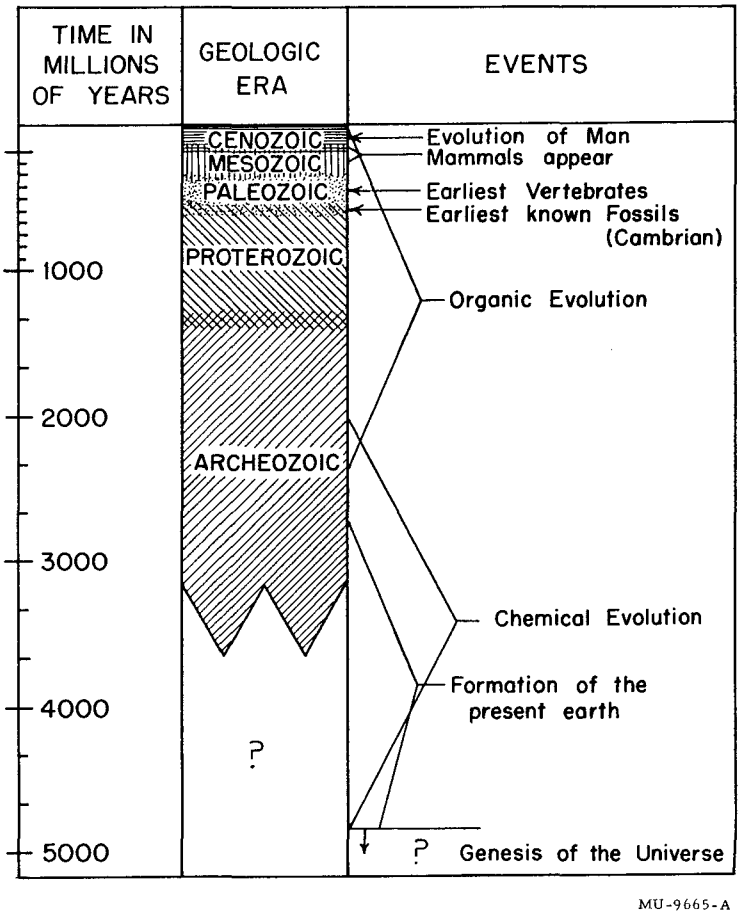


Fig. 1. Time scale for total evolution.

to more and more complex chemicals, and at some point in time organic evolution, that is, evolution based upon living systems as we know them today, began. I suspect that the asymptote as here drawn will probably have to be modified somewhat: with new knowledge we are pushing the beginning of organic evolution further and further back in time.

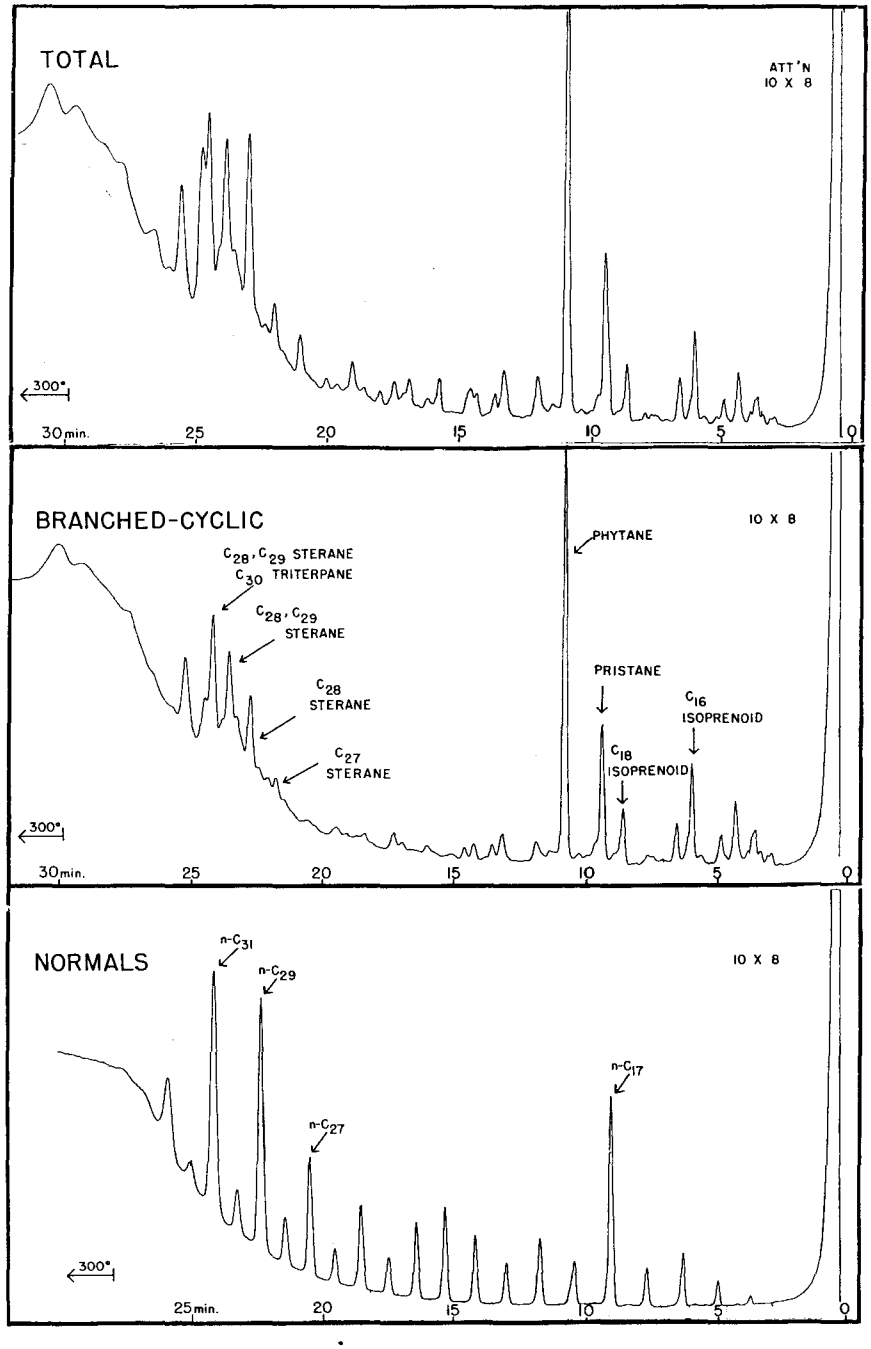
We are going to spend much of our time tracing organic evolution back insofar as we can trace it back in terms of "molecular fossils" from the earliest well recognized fossils of morphological form, approximately 600 million years ago, and we are going to talk about chemicals which we can find in rocks which are older than 600 million, some as old as 2700 million years.

While many rocks in which known fossils have been seen have been analyzed and the methods of analysis which were used for these rocks were just adequate, the determinationof the detailed molecular structures which are present in the younger rock in correlation with the recognizable fossil elements is only just beginning. This is partly because the analytical tools have only recently been refined to the point in which we can describe the intimate details of the molecular architecture thatis present. The various kinds of molecules that one can look for are obvious ones. One would look for amino acids and heterocyclic bases as representative of fossils of the proteins and nucleic acids. This latter has not been done to the same extent as the amino acid search because the analytical tools available to identify amino acids in trace amounts in the rocks were much better than for the nucleic acid bases. The third group of molecules which has been known for a long time as organic fossil material but whose intimate structures have not been analyzed with this in mind are the

hydrocarbons themselves, that is, molecules made up only of carbon and hydrogen in special architectural arrangements as represented by petroleum and the materials found in it.

We have chosen to examine the hydrocarbon composition of the ancient rocks to see if we could not find characteristic architectural features of the hydrocarbons which could be correlated in some way with the organisms which might have given rise to them. In order to get a date line for our work, we elected to examine some young rocks of recent origin whose biological precursors, at least, were well established. Using the modern analytical tools we undertook the examination of the Green River Shale which underlies a large part of the western North American continent. The Green River Shale is only 60 million years old and it has in it a high proportion of hydrocarbons; in fact, it is presumed to be one of the rich oil shales of the world. It was relatively easy to obtain samples and to undertake this analysis.

The Green River shale was analyzed by suitable extractions and fractionations (Eglinton, 1965) and figure 2 shows the vapor phase chromatogram of the alkanes from the Green River Shale. The upper chromatogram shows the total hydrocarbon extract after the removal of any non-hydrocarbon and aromatic components. This is clearly a complex mixture. It was possible to separate the hydrocarbon extract into two quite distinct components by means of molecular sieves (5 $\mathring{A}$). These sieves allowed the straight chain hydrocarbons to wend their way through the 5 $\mathring{A}$ holes, and to prevent the passage of any material with a branch or cyclic component. We were thus able to separate the straight-chain hydrocarbons from the rest of the materials which were present. None of the branched and cyclic hydrocarbons, which are shown by the center portion of figure 2, pass



GREEN RIVER SHALE (COLORADO), ~60X10^6 YRS. ALKANE FRACTIONS

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Fig. 2. Vapor phase chromatogram of alkanes from Green River Shale.

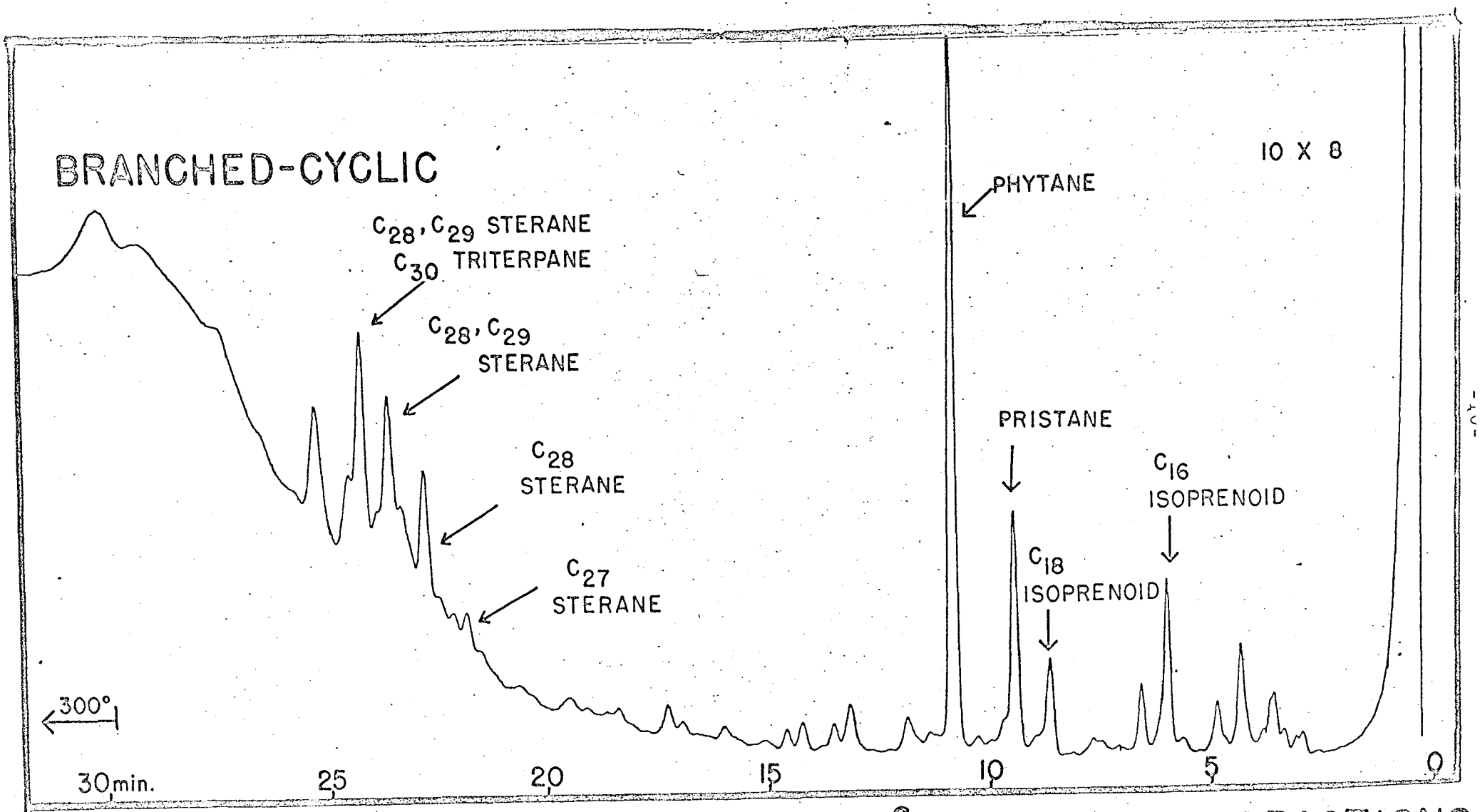
into the molecular sieve. In the bottom part of this figure is shown the fraction that entered the sieve. At least in the Green River Shale this fraction represents a nice, clean sequence of a normal saturated hydrocarbon homology.

We have since learned that these 5 Å sieves will also absorb straight-chain hydrocarbons with at least a terminal olefin in them. Whether they will absorb straight-chain hydrocarbons with an internal olefin or not remains to be determined. From an examination of models it seems as though the cis internal olefin should not enter the 5 Å hole while the trans might.

The branched cyclic components can thus be separated from the straight-chain hydrocarbons, and the non-thermodynamic distribution of the normal hydrocarbons can be seen -- the general dominance of the odd-numbered hydrocarbons represented here by the C_{17} and C_{19}, etc. There is no question about the biological origin of these straight-chain hydrocarbons; most of them come by the decarboxylation of the even-number saturated carboxylic acids.

There have been a few compounds labeled in the branched cyclic series. Among them are the C_{15}, C_{18}, C_{19} and C_{20} polyisoprenes; these were determined by cochromatography and by mass spectrometry, and there is no ambiguity about them. Phytane, of course, is the dominant one and pristane (one carbon atom less than phytane) next, and you will see the relationship between these in a moment. The absence of the C_{17} isoprenoid is also something to note, because I think it may give us some clue as to how these polyisoprenoids originated and how they may have been transformed.

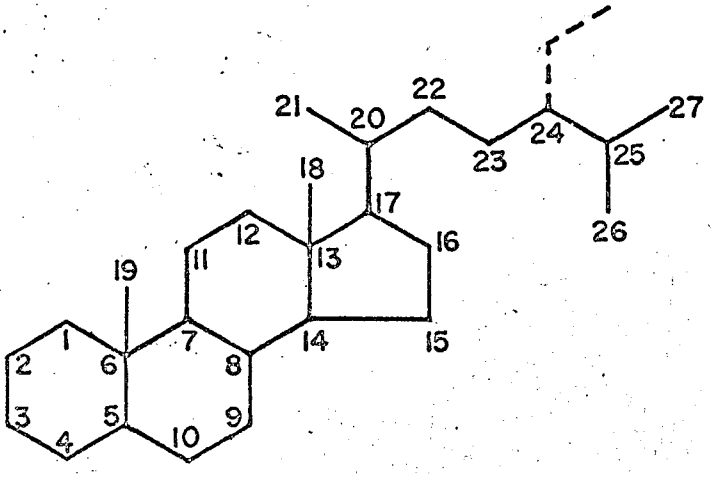
In addition, a number of cyclic polyisoprenes are present (see figure 2a); among these are the C_{27} sterane, cholestane, and probably coprastane,



GREEN RIVER SHALE (COLORADO), ~60 X 10^6 YRS. ALKANE FRACTIONS

Figure 2a. Vapor phase chromatogram of branched-cyclic fraction, Green River Shale

and the C_{28} and C_{29} saturated steroids, ergostane and sitostane. The presence of terpane, that is, C_{30} pentacyclic hydrocarbons, is also indicated (Burlingame, Haug, Belsky and Calvin, to be published). These are not simple peaks -- they are multicomponent peaks as the mass spectrometer has told us. At least two of them contain both the C_{28} and C_{29} steranes. The origin of these materials is probably the reduction of the corresponding unsaturated, or oxygenated, sterols. Here are shown the carbon skeleton of coprastane and cholestane:



C_{27} sterane (cholestane, coprostane)

C_{28} sterane (24-methyl C_{27} sterane, ergostane and isomers)

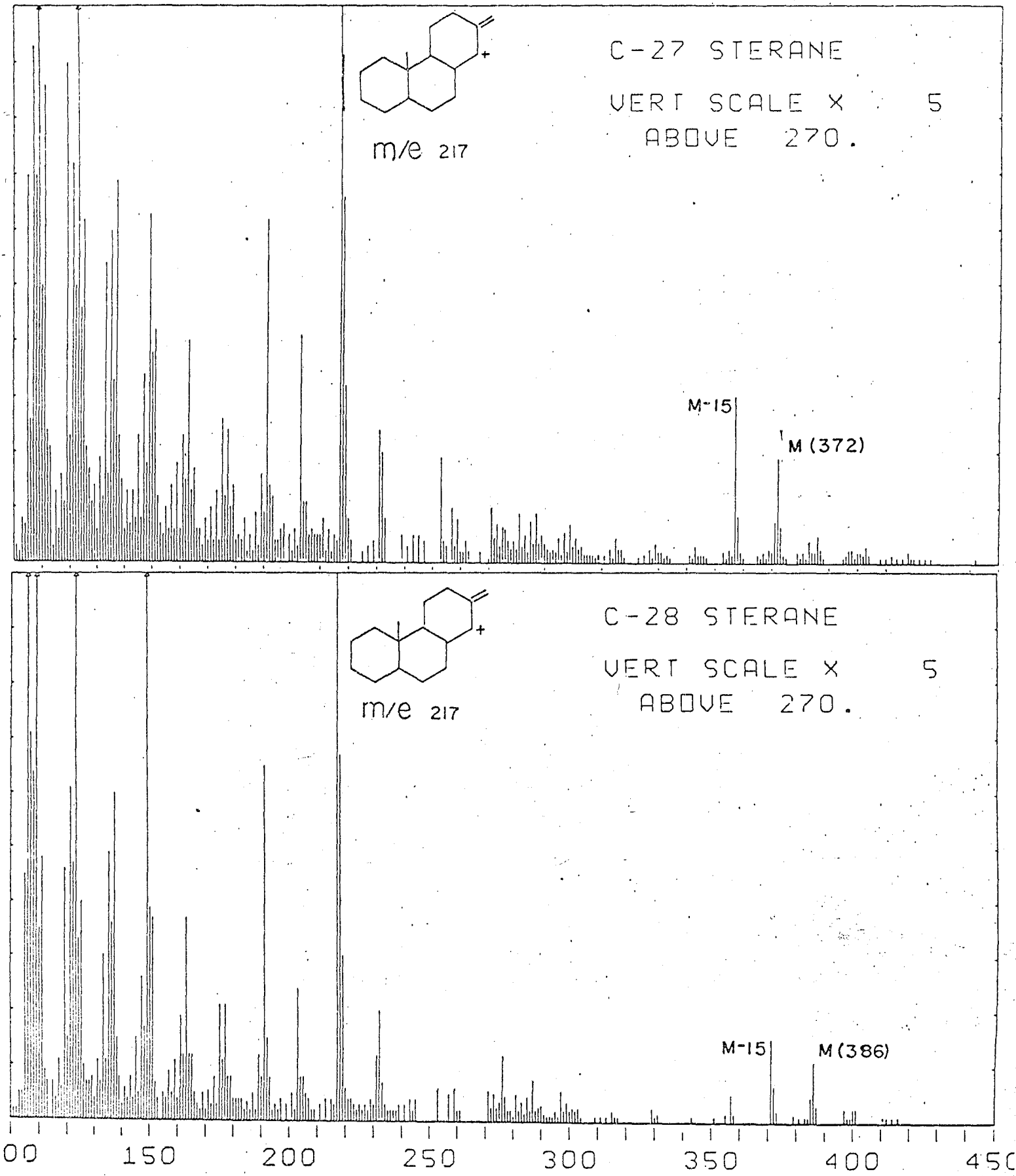
C_{29} sterane (24-ethyl C_{27} sterane, sitostane and isomers)

The ergostane has a C_{24} methyl and the sitostane a C_{24} ethyl group. In addition to that, the possibility exists in ergostane of four isomers because there can be cis-trans junction of various rings. The possibility

that there are four isomers of ergostane probably accounts in part for the multiple distribution of the ergostane mass spectrometry pattern in those four peaks, although this yet remains to be determined. The mass spectra of the three steranes and the pentacyclic triterpene which permitted their unambiguous identification in the five GLC peaks (figure 2) between 22 and 26 minutes are shown in figure 3.

Here are relatively stable hydrocarbon markers whose biological origin is pretty much unambiguous, and the probable origin of the phytane and polyisoprenoids is shown in figure 4. Phytane very likely comes from chlorophyll which has the phytol alcohol as an ester on one of the carboxyl groups. It is a tetraisoprenoid and has one double bond and one terminal hydroxyl group which, upon hydrogenation and hydrogenolysis, will give the C_{20} phytane. By hydrogenation and then oxidation of the terminal alcohol, followed by decarboxylation, the C_{19} polyisoprene pristane is obtained. The fact that the phytane is the dominant peak in the Green River Shale with pristane as the next one seems to be a significant fact, probably of the presence of chlorophyll in the biological material from which the Green River hydrocarbons arose. The presence of C_{16}, C_{18}, C_{19} and C_{20} polyisoprenes together with the unequivocal absence of the C_{17} compound would require two carbon-carbon bond cleavages and would, therefore, be highly improbable.

This work on the Green River Shale is, of course, only a preliminary exercise in learning how to perform the analysis and to read and understand the data which the analysis provides. What we are really trying to do is to go to much older rock formations in which the hydrocarbon content is the only evidence that we may have upon which to base our conclusions. We have done this, and have gone to several older rocks.


C-27 STERANE
VERT SCALE X 5
ABOVE 270.
m/e 217
M-15
M (372)
C-28 STERANE
VERT SCALE X 5
ABOVE 270.
m/e 217
M-15
M (386)
100
150
200
250
300
350
400
450

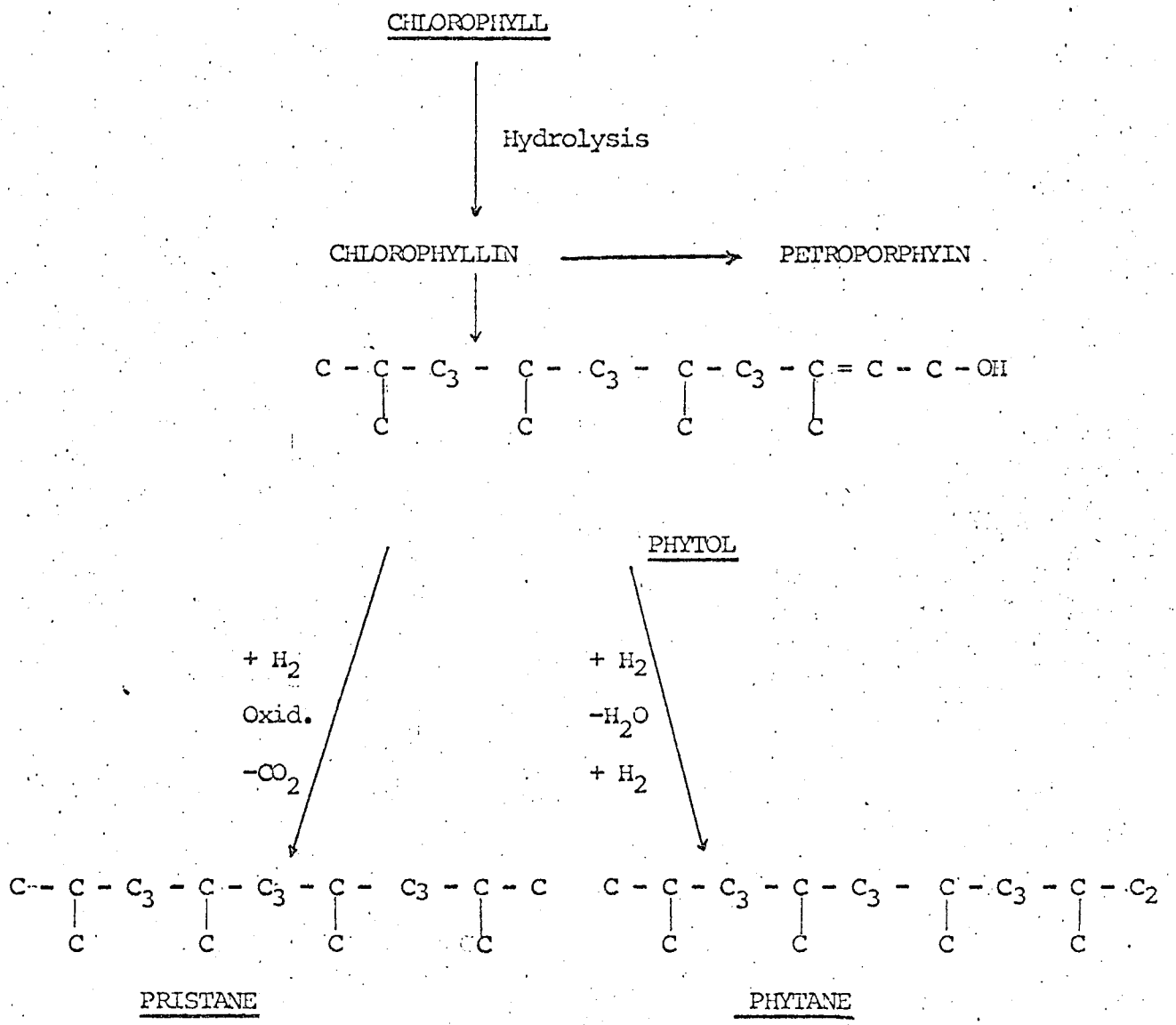
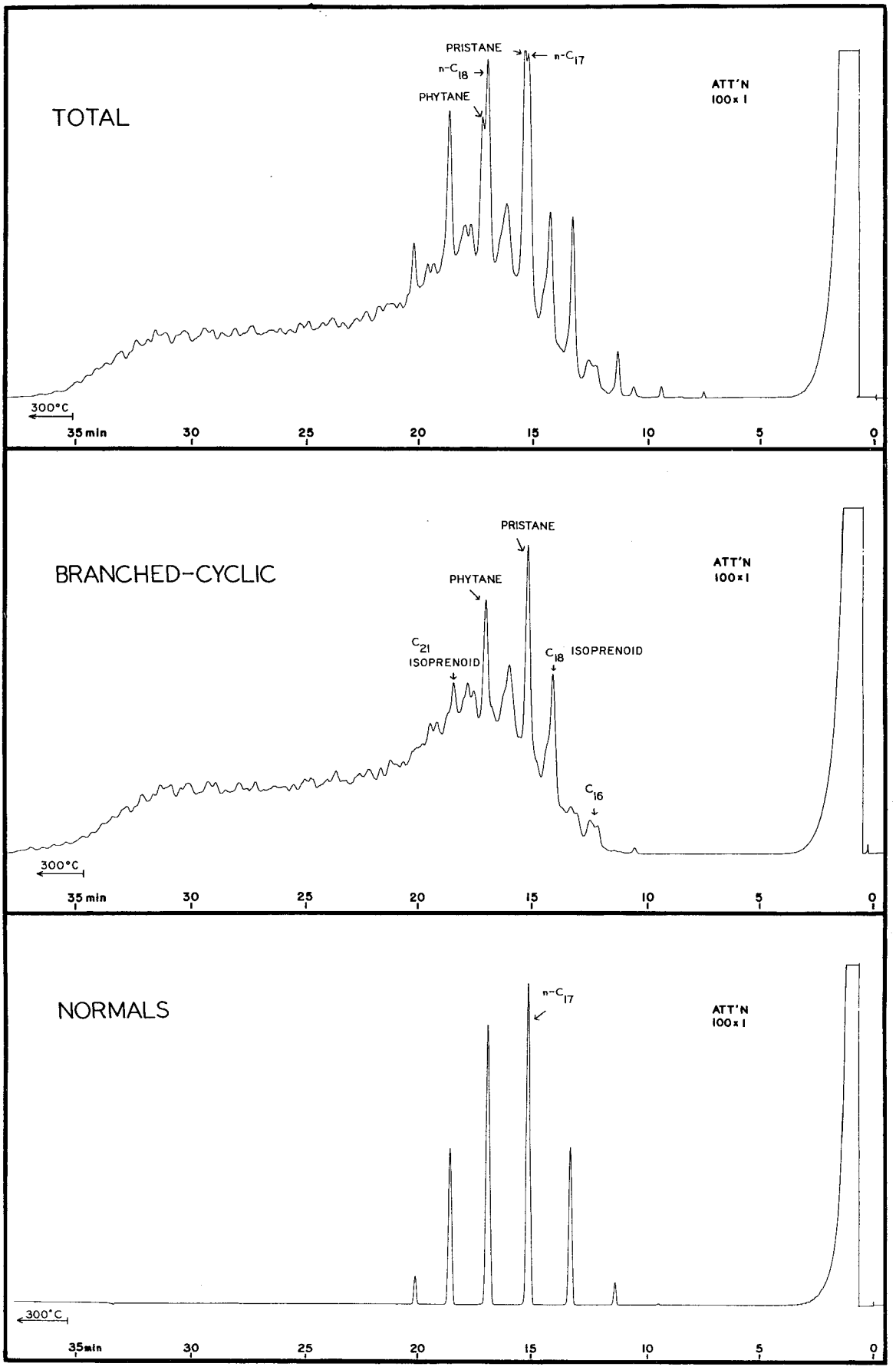


Figure 4. A possible source of pristane and phytane

(Bendoraitis et al., 1963)

We reported least year on the presence of the same two isoprenoids (phytane and pristane) in a rock of about 1000 million years, the Nonesuch Shale of Northern Michigan (Eglinton, et al., 1964). (Barghoorn, Meinschein and Schopf, 1965) The presence of both phytane and pristane in the Nonesuch Shale we took to mean that chlorophyll, or chlorophyllous materials, were already in existence as early as 1000 million years ago. That is to say, that the whole photosynthetic apparatus was functioning as early as 1000 million years ago (Calvin, 1962a).

Since that was reported roughly a year ago, we have gone still further back. We have obtained a piece of the Soudan Iron Formation of Minnesota which is dated at 2700 million years. (Cloud, Gruner and Hagen, 1965) If you remember the time scale that is more than half way back toward the origin of the present earth. The earth is 4700 million years and the Soudan is dated at $>$ 2700 million years. The analysis of the Soudan Shale alkane fractions is shown in figure 5. This is the same kind of analysis which I described a moment ago for the Green River Shale. However, as you can see, the Soudan which is 2700 million years old gives a different distribution of hydrocarbons: note that the straight-chain series is very much contracted and no longer has the sharp odd-even behavior that the normal hydrocarbon series had which we found in the very young oils. First of all, the Soudan has a very much narrower distribution over a very much narrower range, dominated by C_{17}. You can see that the C_{18} is really higher than it ought to be if we are going to have a C_{17}-C_{19} dominant series. However, the distribution is still far from anything that might be called thermodynamic in character. The presence in the 2700 million year old Soudan of the isoprenoid series, again (C_{18}, C_{19}, C_{20} and even the C_{21} isoprenoid) attests to the existence of the isoprene system even as early



SOUDAN SHALE ALKANE FRACTIONS

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Fig. 5. Vapor phase chromatogram of alkanes from Soudan Iron Formation.

as 2700 million years ago (Belsky, et al., 1965). What may be even more significant is the presence of the C_{27}, C_{28} and C_{29} steranes in the branched-cyclic fraction from this very ancient rock as well (Burlingame, Haug, Belsky and Calvin, to be published).

Figure 6 gives a comparison of a straight-chain set as a function of age (Antrim, Nonesuch and Soudan Shales). I hesitate to draw any very significant conclusions from these relative distributions because not only do the ages of the rocks differ but the nature of the deposits from which they come are different. One of them is a fresh water deposit and the others appear to be salt water deposits, and we are not yet certain of what the significance of that difference may be. We really don't know how to read the message which is contained in this as yet; there is information there, but what it means is not yet clear.

I neglected to mention that there were really four different homologies present in the ancient rocks: the normal homology of the straight chain, the isoprene homology, the anteiso series and the iso series. Figure 7 gives the structural relationship between the homologies. The ones with the numbers under certain atoms are the ones, except for the C_{17} isoprenoid, which represent the dominant compounds in each of these series. Among the isoprenoids formed from phytane, the C_{19} is undoubtedly made by stripping off the terminal hydrocarbon atoms (C_{20}), the C_{18} by breaking the bond between the C_{17} and C_{19} atoms. I think you can see that the next one can be formed by splitting off between C_{16} and C_{17}. In order to get the C_{17} isoprenoid we would have to make two breaks -- the one between C_{17} and C_{19} and the one between C_{17} and C_{18}. As we have previously suggested,

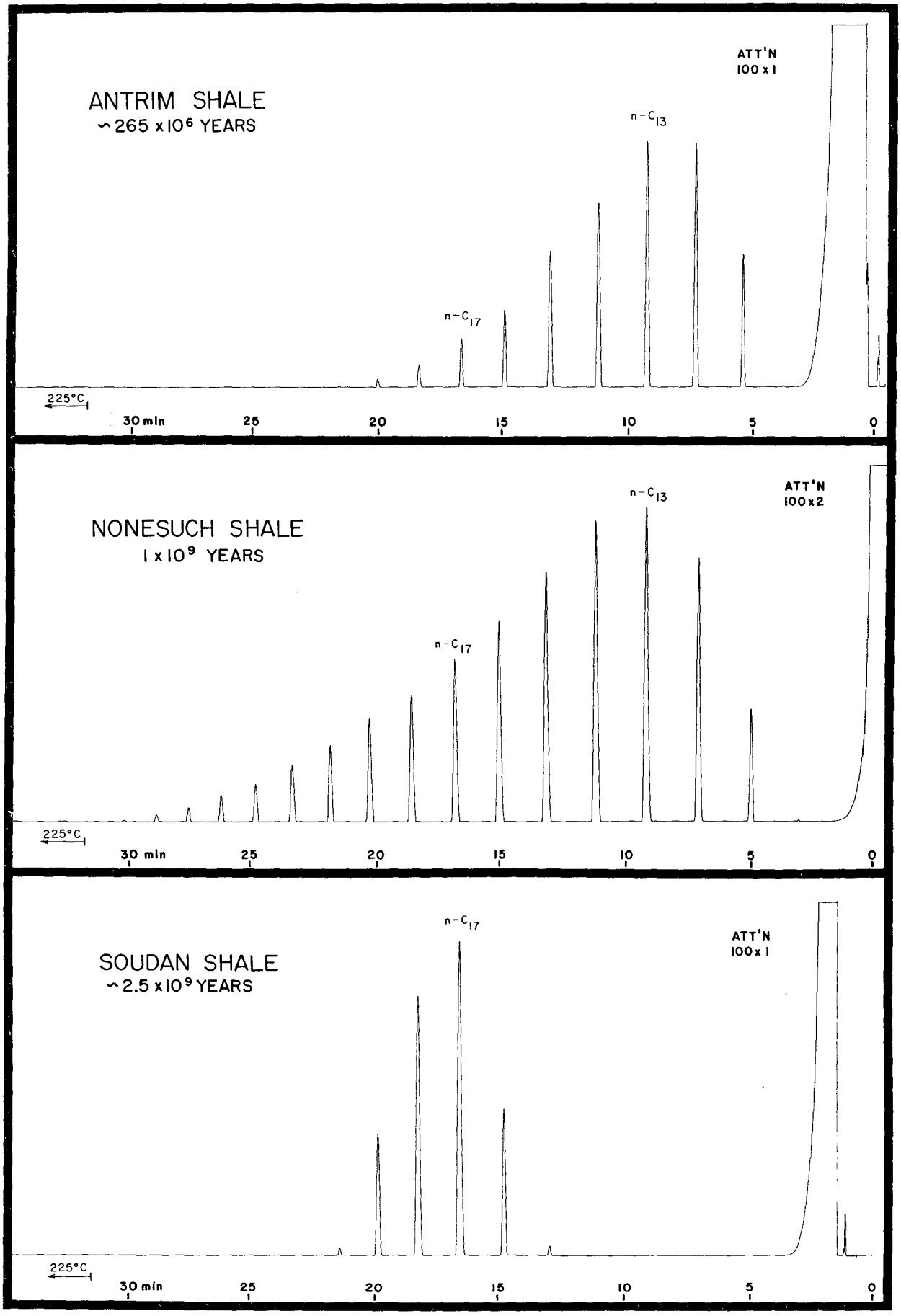

Fig. 6. Vapor phase chromatogram of staight-chain hydrocarbons in three shales of different geological ages.

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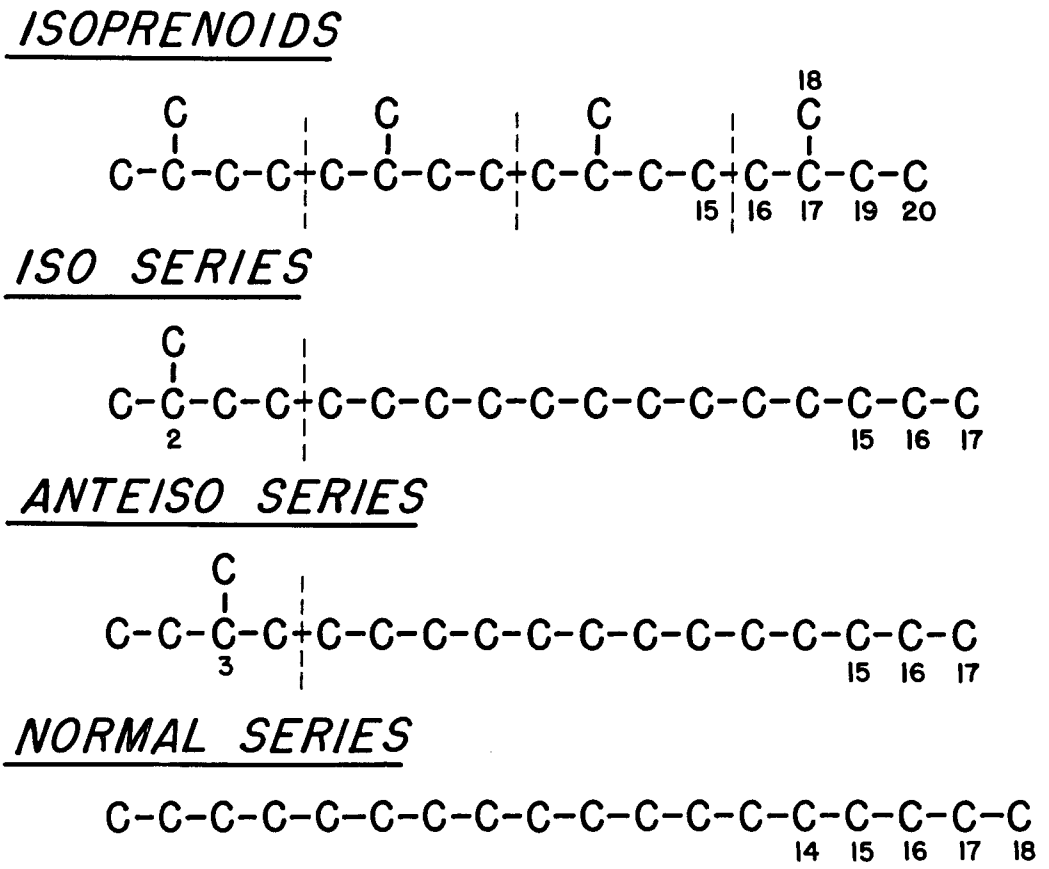

Fig. 7. Structural relationship between homologies in ancient rocks.

that is probably the reason why the C_{17} isoprenoid is not present in the ancient rocks, but the others are.

What are the structures of the polyisoprenes and how do we believe they came into being? What does it mean that the presumed biological apparatus for their synthesis was already fully developed, or well developed, as early as 2700 million years ago? How are these homologies formed in the first place? Figure 8 gives a brief summary of the potential isoprenoid reactions. First of all, we get Δ^3-isopentenyl pyrophosphate by a series of reactions through mevalonic acid from acetyl Coenzyme A. The pyrophosphate can then react with more acetyl CoA to give rise to the anteiso series. The condensations (with isopentnyl pyrophosphate) initiated by the incipient carbonium ions arising from the dimethallyl pyrophosphate give rise, of course, to the polyisoprenoids. The condensation of this same incipient carbonium ion with the acetyl CoA, and followed by further acyl CoA condensation, will give rise to the iso series. The acetyl CoA would itself give rise to the normal series. We have here a rather complex sequence of chemical reactions which are required to produce the unsaturated pyrophosphate (isopentenyl and its isomer, dimethallyl) which is the common precursor to all of them with branches and also to produce the acetyl CoA. These are to be followed by all of the reactions which are required to make these four homologies. The number of reactions is considerable, and the specificty is high.

The interpretation which I want to make of this is that as early as 2700 million years ago the whole biosynthetic apparatus for producing these intermediates had evolved, thus giving rise to the very specific isoprenoids as well as the peculiar distribution of the straight-chain homologies which we now find as the molecular fossil evidence of the early existence of this apparatus.

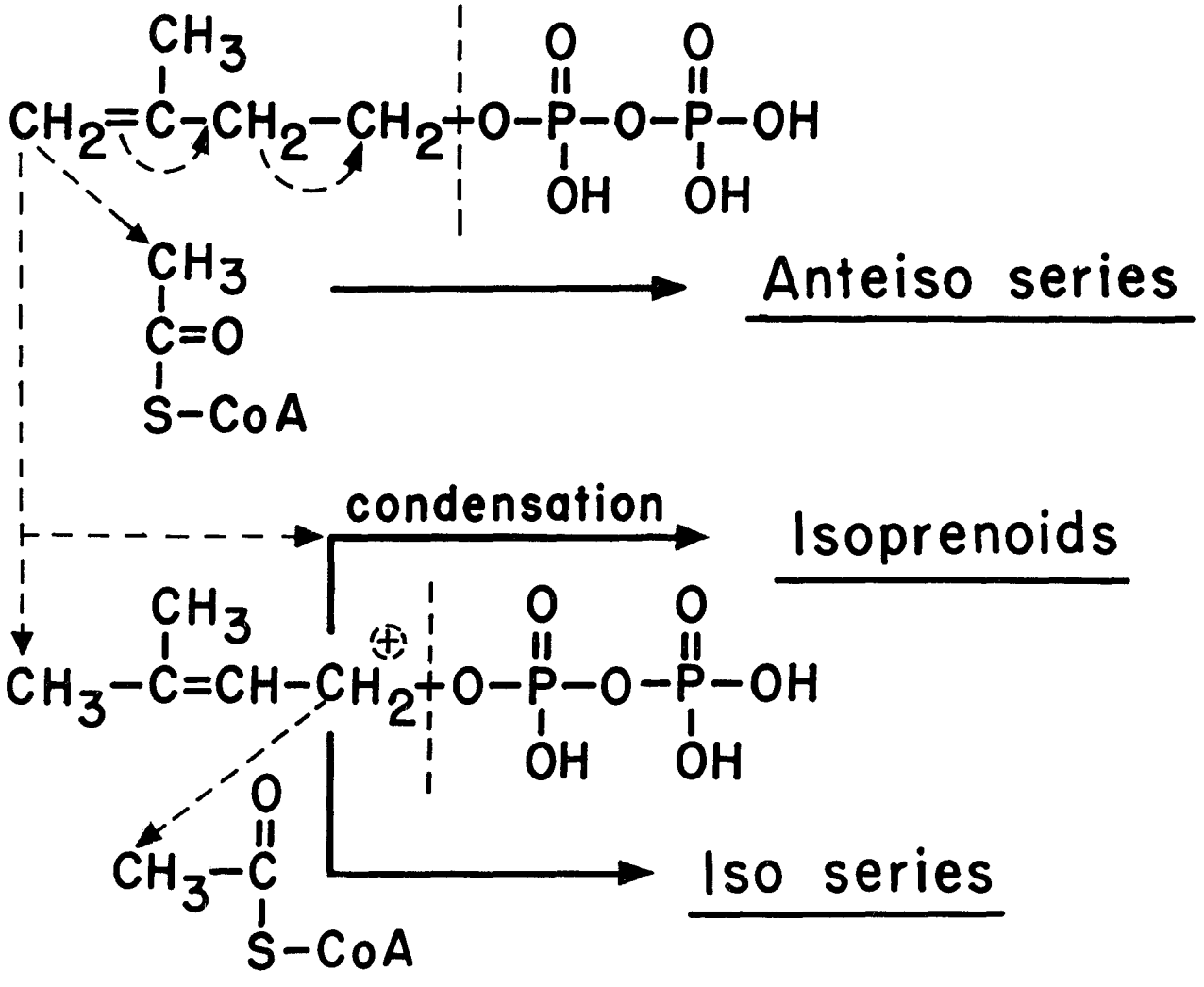

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Fig. 8. Summary of potential isoprenoid reactions.

If we now have to produce these complex molecules and the whole apparatus for making them in such a short evolutionary period of something less than 2000 million years, the whole sequence of evolutionary events must be much faster than most of us had originally supposed (Calvin, 1965). I am afraid that this time will become still shorter as we finish our present series of observations. As we go back in our examination of these ancient rocks it would seem reasonable to find a period in time at which these hydrocarbons in the rocks begin to get simpler; in which, for example, the entire isopentenyl pyrophosphate sequence is no longer present but something more elementary in the way of biosynthetic reactions would have been occurring. I would like to find that point in time, and I suspect that it will bring us so close to what is now believe to be the age of the formation of the earth that we are going to face some kind of a revolution in our thinking about this early evolutionary history (Cloud, 1965). The possibility that many of the organic compounds now believed to be the proximate substrate for organic evolution might have been present in the original cosmic dust which gave rise to the earth itself has recently received added support (Studier, Mayatsu and Anders, 1965).

We are searching for still older rocks, rocks which have ages beyond 3000 millions of years and which contain carbon. The amounts of carbon we need are, of course, getting smaller as our analytical tools are becoming more sophisticated, and I believe that we will be able to determine the nature of the carbon-containing molecules present in even the oldest rocks.

There is at least one ambiguity in this whole question, and that is whether the rocks and the hydrocarbons in them are of the same age. It is conceivable that some of the hydrocarbons may have settled into

the rocks long after the rocks were formed. In discussing this with my geological friends they have informed me that a shale, for example the Soudan rock, compacts relatively quickly after it is settled in the bottom of the lake or ocean bed. All their experience with the rocks as a function of age suggests that these particular types of rocks are compacted in something less than 100 million years, which, for the present purpose, is a very short time. At present I would not be too concerned whether the oil was a hundred million years more or less aged than the rocks in which it is found, which are dated at least 2700 million years. So much, then, for the conclusions we can draw from the geochemical examination in the ancient rocks.

PREBIOTIC CHEMISTRY

Let us now take the other approach to the problem of Chemical Evolution, namely, that of starting with the primitive earth, at the other end of the evolutionary sequence. Let us begin with what the astronomers tell us was the nature of the primitive earth. They tell us that the primitive earth was formed by a cold aggregation of dust and gases, and that it was originally dominated by hydrogen. Therefore, the primitive earth's atmosphere was dominantly populated by reduced molecular species shown in the first row of figure 9, where the carbon, nitrogen and oxygen are all attached primarily to hydrogen to give methane, ammonia and water. This is presumed to be the population of the primeval atmosphere of the earth's surface.

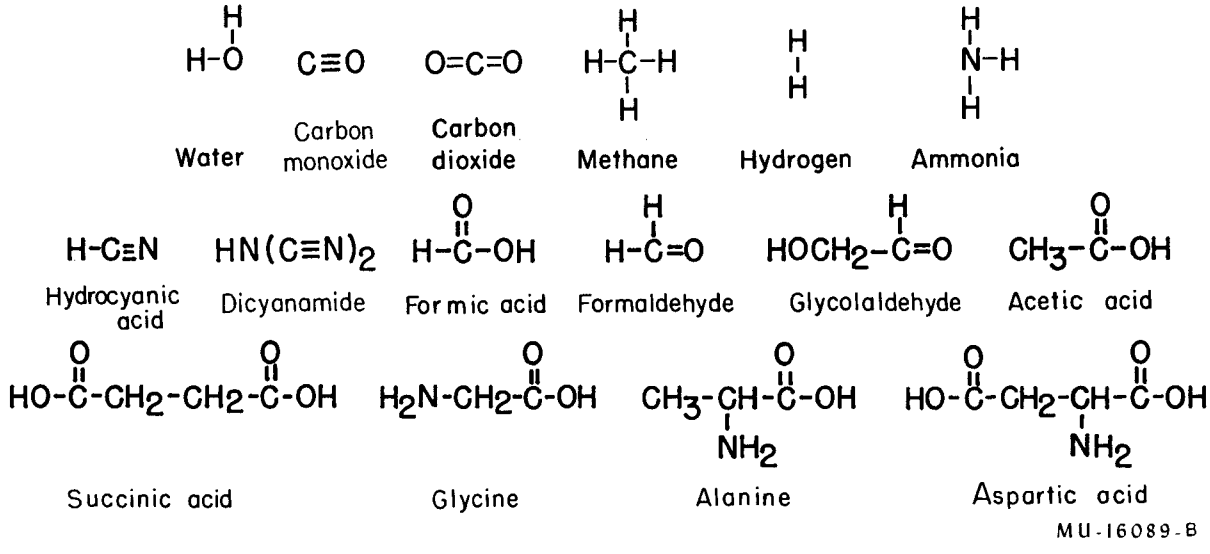

Fig. 9. Primeval and primitive organic molecules.

The question is: Can we, by introducing energy into such an aggregation of molecules, achieve any kind of evolutionary development of molecular structures of any significance? The answer lies in the fact that such a question is subject to experimental test. We can set up such molecular compositions and subject them to a variety of energy sources, such as ionizing radiation in the form of either particulate or gamma radiation; or we can introduce energy in the form of ultraviolet radiation or in the form of electrical discharge (such as lightning might produce). All of these have been done (Garrison, et al., 1951; Calvin, 1956; Miller, 1955; Miller and Urey, 1959; Oro and Kimball, 1961, 1962; Ponnamperuma, et al., 1963 a,b,c; 1964; 1965 a,b. Steinman, Lemmon and Calvin, 1964, 1965; Steinman, Kenyon and Calvin, 1965). Indeed when such high energy sources are brought to bear on such molecular aggregates, the molecules do indeed break -- the carbon-hydrogen, hydrogen-hydrogen, nitrogen-hydrogen and oxygen-hydrogen bonds break -- and the radicals, or ionic fragments, which result from such breakage recombine not necessarily in their original forms but in other metastable forms. We have been able to demonstrate the formation of all the compounds in the second and third rows of figure 9 as formed from the primeval molecules in the top row. Note that these primitive secondary molecules are the very same small molecules upon which present day living organisms are based, both as metabolites and as structural elements.

The experiments have been carried much further than this would seem to indicate. Sugars have been formed (Miller, 1955; Miller and Urey, 1959); heterocycles have been formed (Oro and Ponnamperuma, previously cited); and other compounds are formed from the HCN first produced and the ammonia initially present (Schimpl, Lemmon and Calvin, 1965). This is

an important comment which at the time figure 9 was initially prepared we did not appreciate. We have since looked for compounds which can be formed from ammonia and HCN, namely, cyanamide (Schimpl, Lemmon and Calvin, 1965) and its various relatives, such as the dimer of cyanamide, dicyandiamide (Schimpl, previously cited), melamine (Hayatsu, 1961), and, of course, dicyandiamide.* All of these molecules which have been sought have been found after we specifically sought them, and the reason for this search will become apparent.

The important results of these experiments is that the introduction of energy into the primeval reduced molecular system does indeed convert the system into a more complex one, and the direction of that conversion seems to be toward the molecules which are today the real substrates of living organisms, both in structure and in function. Note that the amino acids, the hydroxy acids, the dicarboxylic acids, the sugars and related substances are the common materials upon which the present day living organisms can operate.

How does a living organism operate on these materials? As you well know, this is accomplished by virtue of its structural features. Many of these features, as well as the enzymes which are required for modern living organisms, are made up of polypeptides and protein molecules. The energy conversion apparatus is very sharply dependent upon highly structured features of the specific catalytic systems which the linear array of polypeptides contain, or can develop; the informational transfer aspect of living organisms is contained in another kind of linear array, the polynucleotides, and there is a complex interrelationship between these

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* Dicyanamide is formed in greater than 1% yield upon ultraviolet irradiation for a few hours of ~ 0.01 M solution of NH_4CN, as observed in our laboratory by Steinman, Kenyon and Calvin (1965).

various types of substances. All of these properties and behaviors are dependent upon structural features which are built into the molecules and which show in the next stage of their evolution.

Dehydration condensation reactions

In order to achieve the next stage of the evolution of the biologically important molecules, the necessity for hooking the small molecules together must be established. When you examine the biological macromolecules which constitute the structural and much of the catalytic basis for living organisms, you can see that they are derived by a reaction common to all of them. They are made from the primitive molecules (amino acids, sugars, phsophoric acid, hydroxy acids and the like) by a single kind of reaction, namely, a dehydration condensation. Figures 10 and 11 show the dehydration condensation reactions in principle, at least. Figure 10 shows the dehydration condensation of the amino acids; the carboxylic acid and the amino group interact to give the peptide linkage and then, of course, the molecule can grow from either end (the amino end or the carboxyl end) to make the polypeptides, which ultimately become large enough to have catalytic and structural properties which we now recognize as characteristic of proteins. The formation of polysaccharides is also a dehydration polymerization. (It is drawn in figure 10 as a glucosidic linkage between the semiacetal structure of one glucose molecule and the 4-hydroxyl group of another, but there are other types of dehydration reactions which would give rise to polysaccharides.) The lipids also are the result of dehydration condensation reactions, the alcohol and the carboxylic acid giving rise to ester linkage, and this occurring on several of the hydroxyl groups of the glycerine will, of course, give rise to the ordinary lipid type of structure.

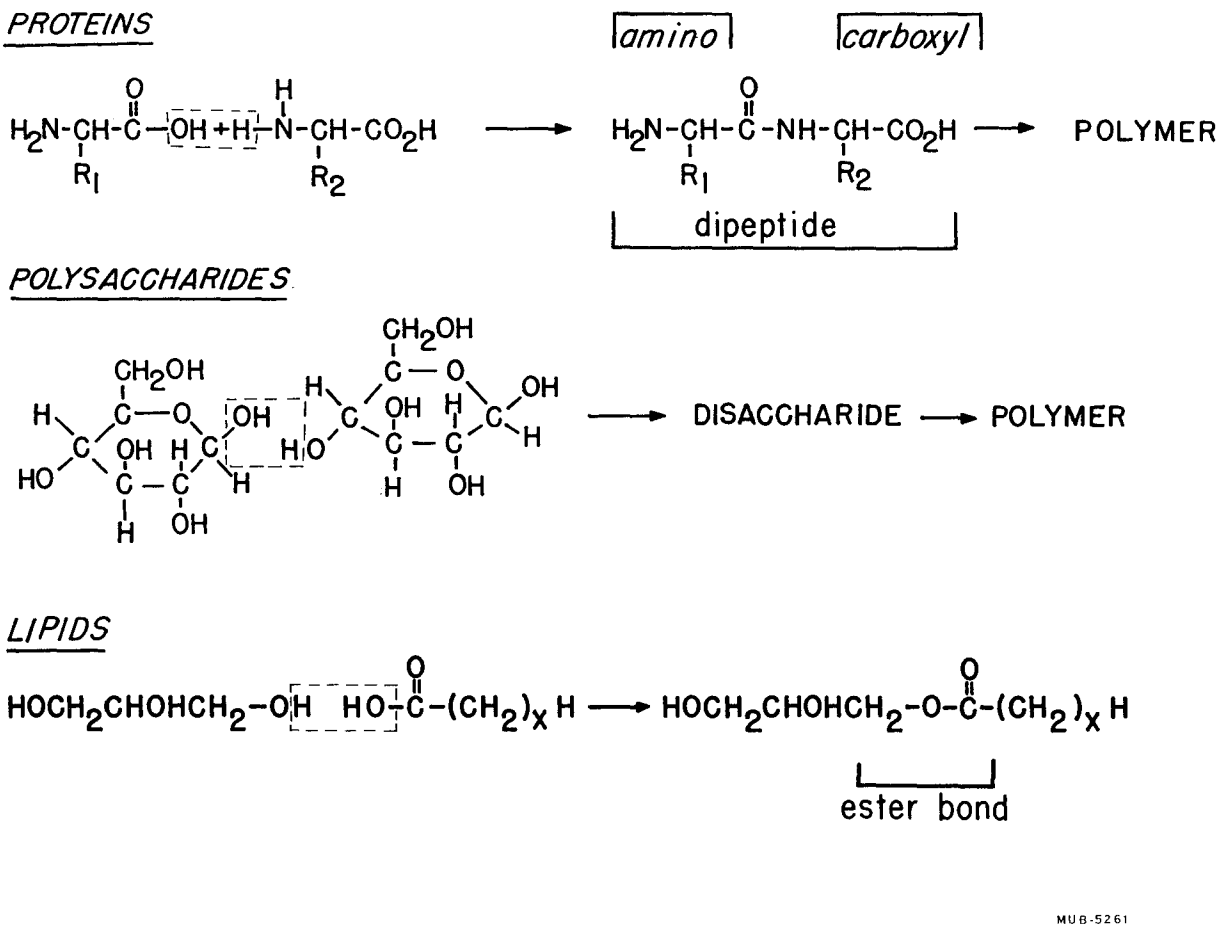


Fig. 10. Dehydration condensation of amino acids.

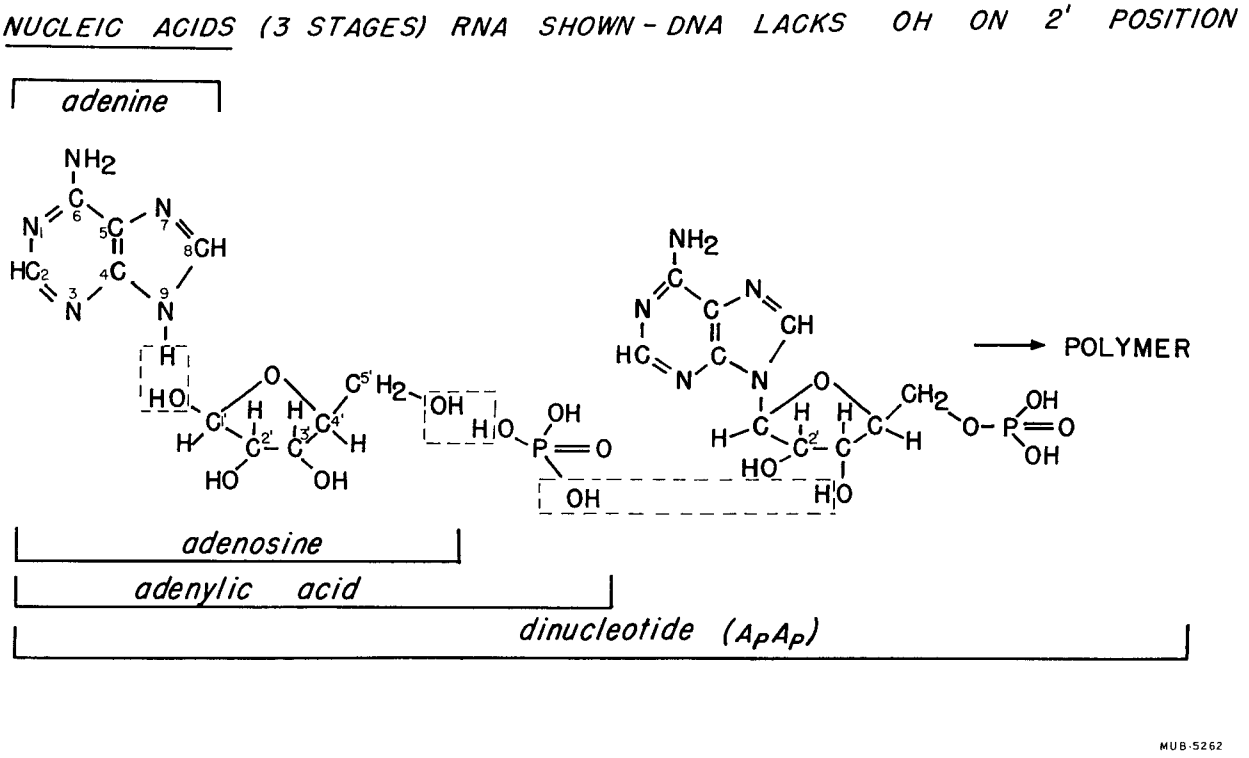


Fig. 11. Dehydration condensation of nucleic acids.

The fourth of the biological macromolecules of great importance is the group of nucleic acids, and figure 11 shows how nucleic acids may be formed as the result of several different kinds of dehydration condensation. First is the dehydration condensation which gives rise to the glycosidic linkage between the heterocyclic NH group and the glycosidic hydroxyl of the sugar; second, the ester linkage between the primary hydroxyl (5') of the sugar and the phosphoric acid, and, finally, a second esterification between the second hydroxyl of the phosphoric acid and another hydroxyl group (3') of another sugar molecule on a second mononucleotide. This, of course, can go on to another sugar, etc., giving rise, finally, to a polynucleotide.

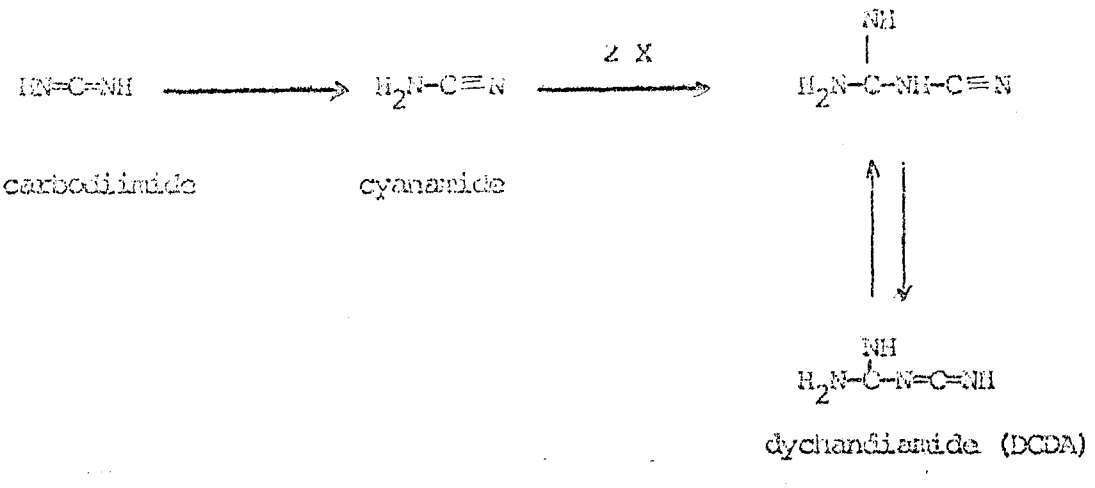
All of these, as you can see, are reactions of the same kind --- dehydration condensations --- and various methods have been conceived as routes to achieving these hydration condensations in an abiological system. The first one which the chemists would immediately consider is to put the monomeric material into an anhydrous situation --- into a medium in which the activity (thermodynamic) of the water is somehow depressed --- such that the dehydration will proceed spontaneously. This has been done. One can, as you know, simply dissolve amino acids in a suitable non-aqueous medium and get dehydration condensation reactions which can be carried to such an extent as to give polypeptides of high molecular weights. Fox (Harada and Fox, 1964) has been the principal protagonist of this approach.

There are other ways, and one could imagine that the hydroxylic and amino containing compounds could be absorbed on a specific type of clay or mineral surface in which the activity of the watr is in some specific way reduced. Thus the dehydration condensation would be made to take place

dehydrating
on the/surface of a particular kind of mineral. The chief protagonist of this approach to the formation of these polymeric materials has been Bernal (1959), and very early he proposed the clay mineral surface as the principal site for the formation of these polymeric materials; however, the experiments which might demonstrate this on any significant scale have not yet been carried to the point where we can presume all of the kinds of/the dehydration reactions under those circumstances (Miller and Parris, 1964).

We have taken a still different approach. We felt that since these monomeric units are formed primarily in dilute aqueous media, we should try to find ways of inducing the dehydration condensation in such dilute aqueous media. To chemists this might seem like something that is a foolish thing to try, i.e., to try to induce a dehydration in water solution, but it turns out that this can be done.

We took our cue from the presence of HCN in the reaction mixture in the first instance (Miller, 1957), recognizing that even though HCN has in it the capacity for absorbing the elements of a water molecule to form formamide, it does not do it very readily in water itself. Then, coupling this idea with the knowledge of the use of a multiple carbon-nitrogen bond in a specific dehydration condensation which exists in the use of the carbodiimides in the accomplishment of these dehydration condensation reactions (Khorana, 1961), we took the next step and asked, "Why can't we use the parent carbodiimide?" The tautomer of carbodiimide is, of course, cyanamide. Cyanamide in aqueous solution does not remain as cyanamide very long but dimerizes to form dicyandiamide:



Although we started with cyanamide we very quickly moved our efforts to dicyandiamide (DCDA) because of its greater stability in aqueous media. It turns out that dicyandiamide can indeed achieve these dehydration condensations, all of them, in dilute aqueous solution (Steinman, Lemmon and Calvin, 1964, 1965; Steinman, Kenyon and Calvin, 1965). By dilute I mean 0.01 M in dicyandiamide, 0.01 M or 0.001 M in amino acids, phosphates, sugars and ribosides. All of these reactions have been accomplished with dicyandiamide, with varying degrees of efficiency.

Figure 12 shows the types of dehydration condensation reactions promoted by dicyandiamide -- peptides, phosphate ester, pyrophosphate and acetate ester; all of these have been done with DCDA. Figure 13 shows a likely mechanism by which the DCDA makes the dipeptide, and you can see that we use it in the form of the carbodiimide. The carbodiimide amidinium form will add the carboxylic acid to form the intermediate, which has not yet been isolated and which would undergo a nucleophilic substitution of the incipient carbonium ion to form the peptide and guanylurea. I might add that this quantitative relationship between peptide formation and guanylurea formed has been established, at least

Figure 12

TYPES OF CHEMICAL BONDS PROMOTED BY DICYANDIAMIDE

PEPTIDE

a) alanylalanine (from alanine)
b) alanylalanylalanine (from alanine)

PHOSPHATE ESTER OF A PRIMARY ALCOHOL

a) glucose-6-phosphate (from glucose)
b) ribose-5-phosphate (from ribose)
c) adenosine-5'-phosphate (from adenosine)
d) O-phosphoserine (from serine)
e) glycerol-1-phosphate (from glycerol)

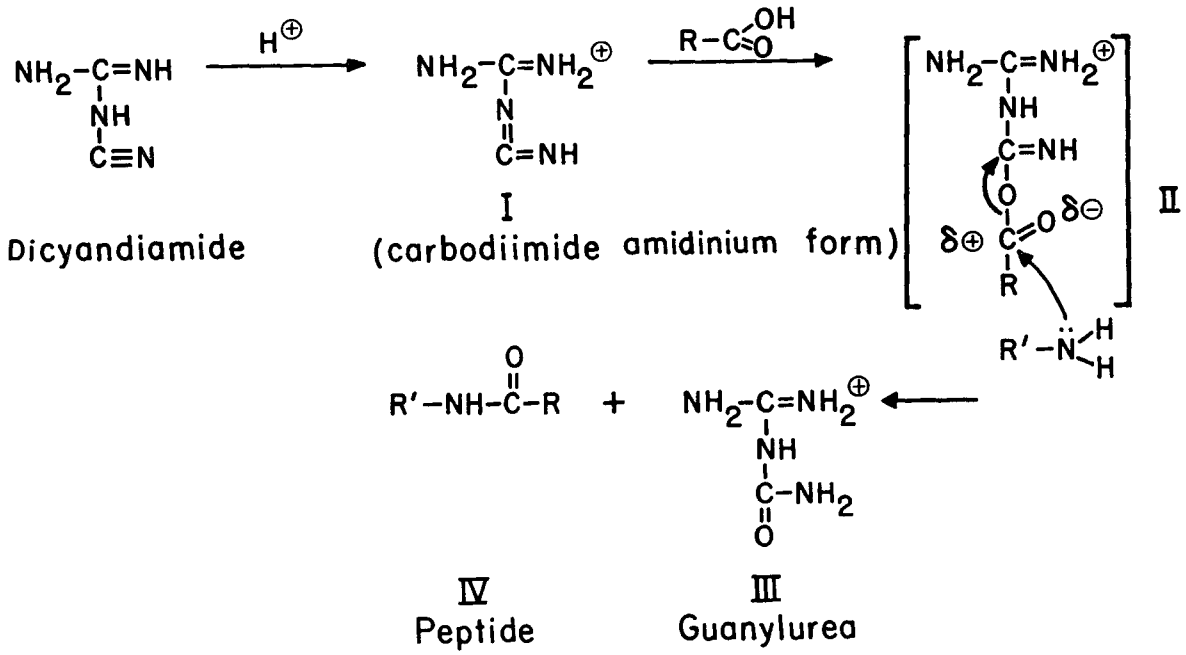
PYROPHOSPHATE (ACID ANHYDRIDE)

a) adenosine diphosphate (from AMP)
b) adenosine triphosphate (from ADP)
c) pyrophosphoric acid (from orthophosphoric acid)

ACETATE ESTER OF A PRIMARY ALCOHOL

a) glycerol-1-acetate (from glycerol)

PRESUMED MECHANISM FOR PEPTIDE FORMATION BY DICYANDIAMIDE



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as far as dicyandiamide is concerned. We took this step about six months ago, and although there was a quantitative relation between the amount of peptide formed and the amount of guanylurea formed (and a similar relationship of guanylurea to phosphate anhydride, or ester formed), this was not very satisfactory because the yields were most often less than 10% and frequently less than 1%. It should be kept in mind that we have not yet studied the mechanism of this reaction in detail.

However, it occurred to one of the students that if cyanamide is good for dehydration in aqueous solutions, dicyanamide might be better. (I don't know whether that is a very logical argument, but that isn't always the way science progresses anyhow.) It turned out tht dicyanamide (DCA) is indeed better (Steinman, Kenyon and Calvin, 1965). In fact, one of the reasons we had originally been disappointed with cyanamide was that we could not build the polypeptide very large. We had to run the concentration of amino acids up very high before we could get the polymerization to go very far, and this was another reason for seeking better agents. It turns out that dicyanamide is remarkable. Not only does it make the dipeptide but its reactivity with a dipeptide appears to be greater than it is with the amino acids. When you start with an amino acid you won't get much dipeptide; the second reaction is faster than the first. For example, we can get 6% yield of tetraglycine with only 2.5% yield of diglycine, and this is a very short reaction time. A whole sequence of investigations now is opening up: The examination of this reaction in terms of mechanism, in terms of its significance for dehydration polymerization in general, the conditions that are required for surface catalysis and pH are just now being explored.

GENERATION OF ORDER AND NEW INFORMATION

The next step in the generation of structure is to recognize that once having obtained these biopolymers they contain within their linear sequence structural instructions, in fact, thermodynamically stable structural possibilities. There follows now a series of four illustrations which show something with which most of you are familiar: that the linear array of polypeptides has in it structural information given rise to this second order structure (alpha helical structure) by virtue of the hydrogen bonding between every third or fourth amino acid carbonyl group (figure 14). The interaction of the side chains (indicated here by the stars) also plays an important role not only in the stabilization of that structure but also, of course, in its catalytic and other properties. The evidence for the fact that this is a thermodynamically stable structure, one that is the result of the polypeptide sequence itself, is shown by the fact that after we have destroyed that structure by suitable means (either temperature or pH adjustment) we can, by reversing either the temperature or the pH change, recover that structure. It is thermodynamically built in and is part of the atomic arrangement and amino acid sequence. The evidence for that reversibility is a spectroscopic one, as shown in figure 15. This is for polyglutamic acid, a synthetic polypeptide. When the terminal carboxyl group is ionized by raising the pH, the charge repulsion between the negative carboxyl group is ionized by raising the pH, the charge repulsion between the negative carboxyl groups is sufficiently great to break

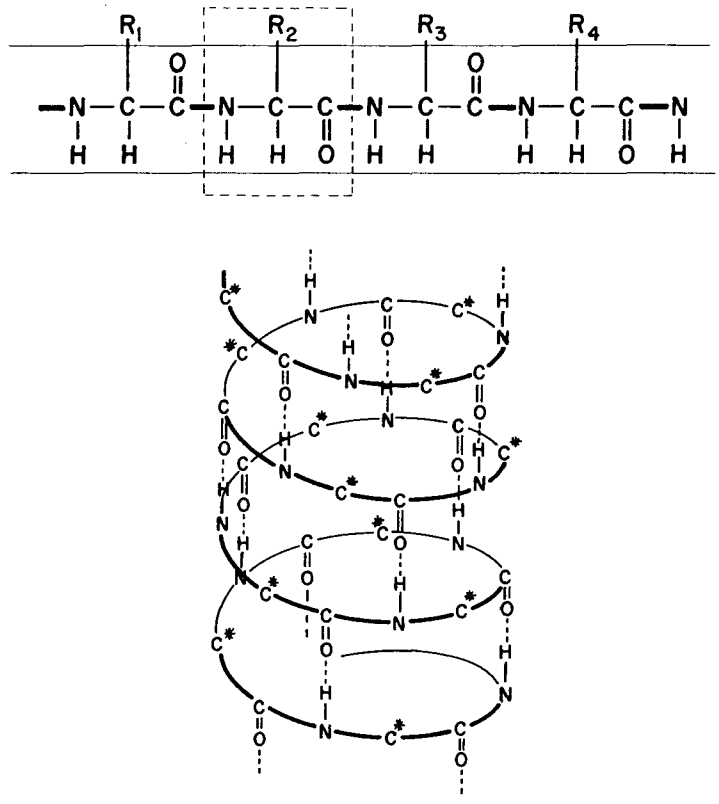

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Fig. 14. Protein structure.

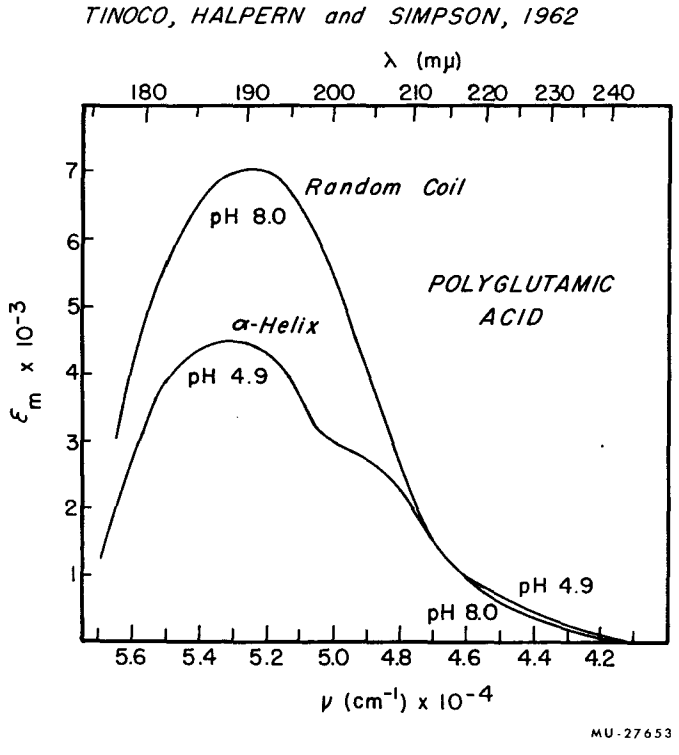


Fig. 15. Absorption spectrum of polyglutamic acid in both helical and random coil forms.

down the helical structure, and you get a random coil; when the terminal carboxyl groups are neutralized, which they would be at pH 4.9, the alpha helix structure returned, as is evidenced by this reversible abosrption spectrum.

The same kind of evidence is available for the double helix structure of the sequence of bases which is a result of base pairing and hydrogen bonding of the polynucleotides (DNA). The structure of the molecular components of DNA is shown in figure 16. Here are shown the sugar phosphate chains as a pair of ribbons from which is hung a series of bases (guanine, adenine, cytosine, thymine). Two such chains are paired in this particular manner to give rise to a helical structure which one can visualize as resulting from the two strips being twisted, thus turning the base pairs flat-on to each other in an aromatic type of stacking. The aromatic type of stacking in addition to the hydrogen bonding holds the chains together; the aromatic stacking helps to stabilize the helical structure. The helical structure makes itself apparent in many ways, among which is a change in the ultraviolet absorption of the base pairs. In Fig. 17 is shown the spectrum of the helical structure, as well as that of the random coil. The reversible transition between them is also demonstrated as a sort of melting and crystallization phenomenon. As the temperature is raised, the helix is melted into the coil, and upon slowly lowering the temperature the helix comes back again. All that is shown here is that the secondary helical structure of the polymers is built right into the linear array of the units of which they are made.

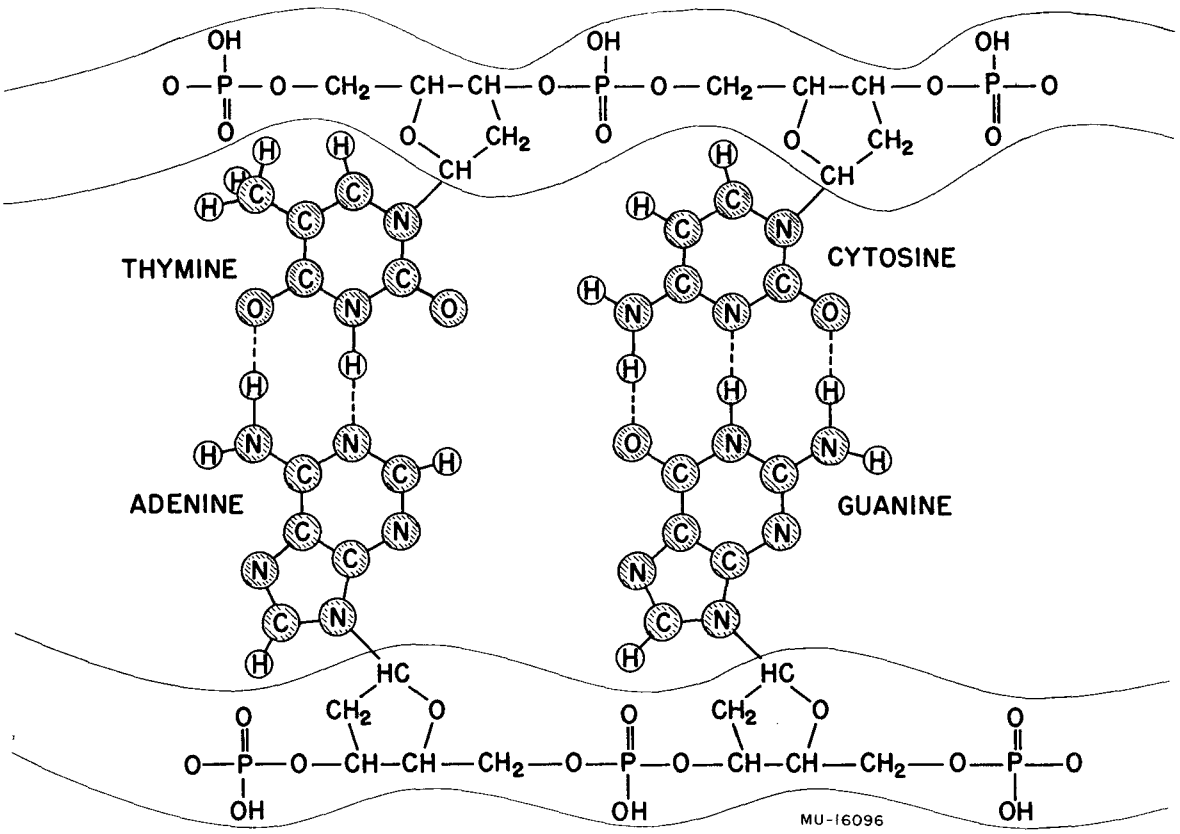


Fig. 16. Molecular drawing of components of DNA.

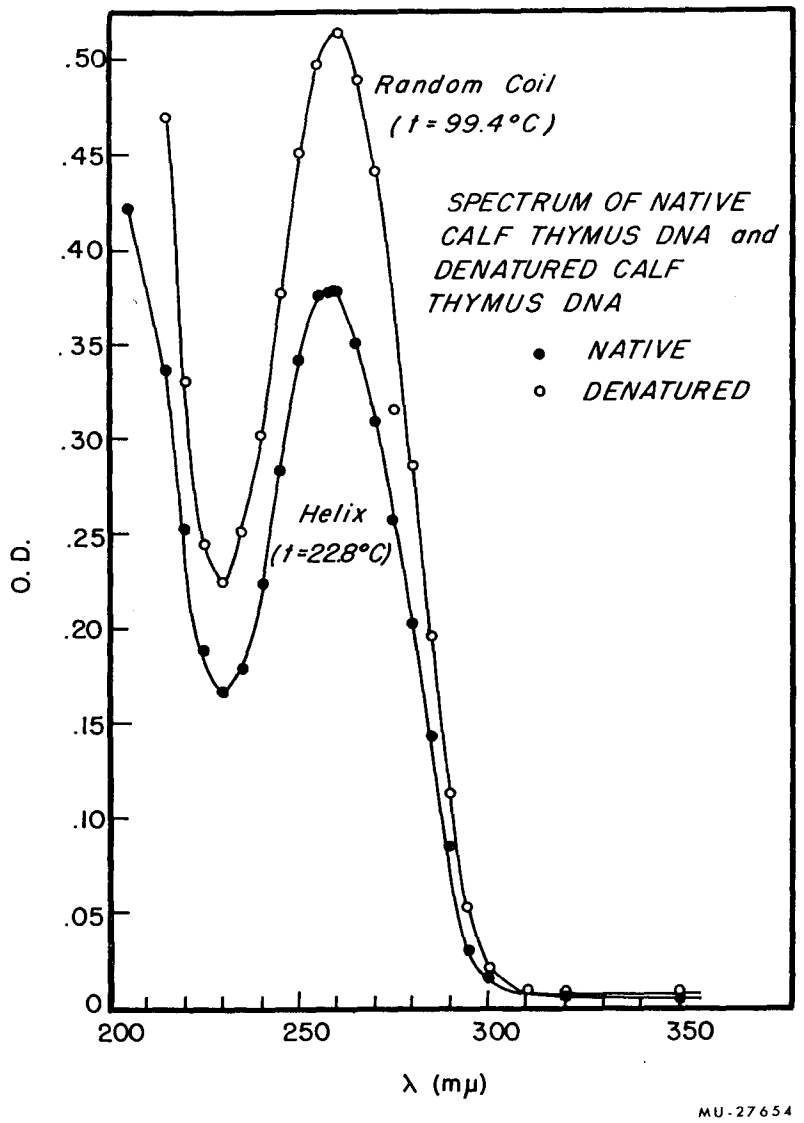
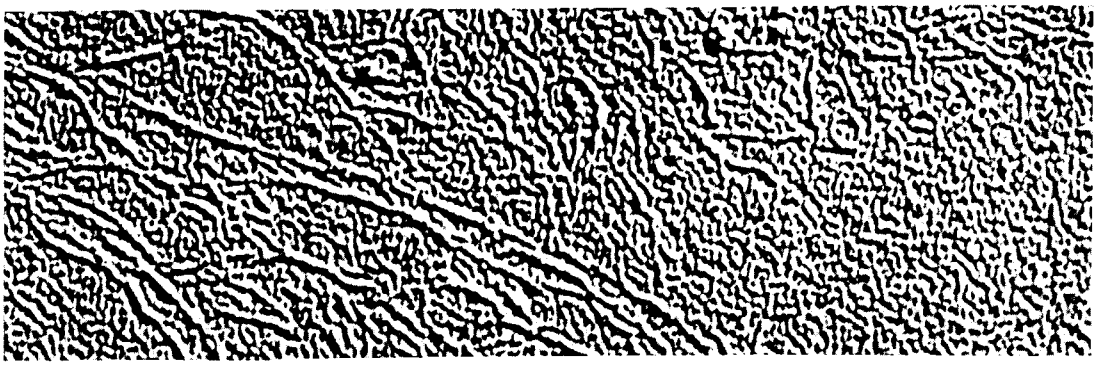


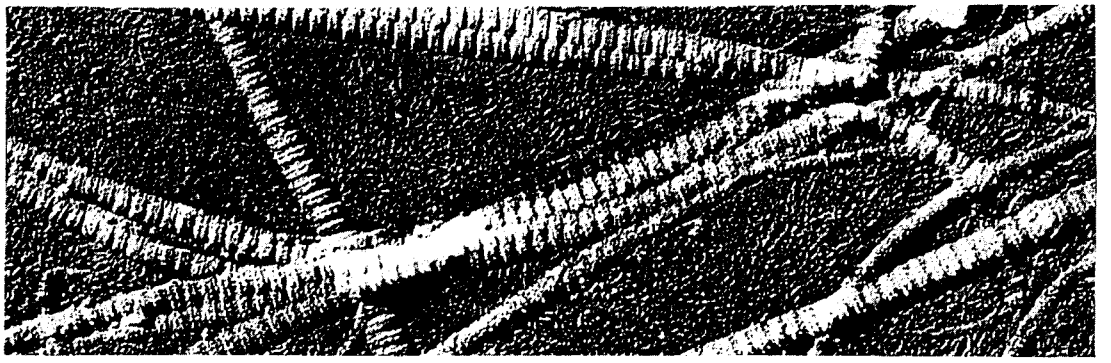
Fig. 17. Hyperchromism on nucleic acid.

Beyond this, there is a third degree of order which is again thermodynamically controlled. If we now take some of the helices, for example, the helix of protein or polypeptide, and put them into a solution of suitable ionic strength and pH, the helices themselves can begin to aggregate in a thermodynamically controlled fashion, and we will find that certain kinds of polypeptides will aggregate to give certain types of structure, and only certain types of structure. The evidence for this is again manifold. I have picked only one case because it is a rather well known one and a rather spectacular phenomenon. This is the case in which one takes apart a collagen fibril (a natural polypeptide) into single helices -- single protein molecules -- and then, by suitably adjusting the salt concentration and pH, allows them to reaggregate and to get back the original microscopically visible biological structure, as shown in figure 18. In the upper half we can see the single collagen molecules, and in the lower half they are reaggregated from the single collagen molecules. The collagen fibrils shown here appear to be identical with the naturally isolated original collagen fibrils. This is a higher degree of order than the previous one (which was the second degree of order) which is built right into the linear array of polypeptides. Here, in the collagen, is exhibited a third degree of order which is again the result of the helical structure of the polypeptides which we have already seen is built into the linear array of the amino acids. We have now reached something which is visible -- a visible structure built into the molecules as a result of the atoms of which they are made.

We can go one step further and say that similar kinds of structural features in two dimensions can be built in, and have been observed



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.

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with lipid type molecules, giving rise to the two-dimensional structure of surface films. This is a much less developed field of work, in these terms at least, and is only just beginning to explode as a possible area for organic, physical and biological investigation; it is one of the areas which I think will develop very quickly in the next few years (Luzzati and Husson, 1962). Figure 19 shows, using chlorophyll as the lipid, the spontaneous aggregation of chlorophyll in monolayers at a water-air interface, to show that the two-dimensional array is a thermodynamically controlled phenomenon (Trurnit and Colmano, 1959).

Finally, I want to pass on from that to the next higher level of biological structure shown in figure 20 which is a collection of microscopically visible things which we know play an important role in biological phenomena -- in energy transfer and information transfer, the two major kinds of things which a living organism has to be able to do. The chloroplasts here (upper left) show the lamellar array, and upper right shows the structure of one of these lamella looking flat-on. You can see that it is made up of particles roughly 100 to 200 Å in diameter, shown in greater detail in figure 21(Park, 1965). It is beginning to be evident that even the quantasomes can be resolved into what appear to be subunits (perhaps four) with an approximate dimension of 60 Å. Since the major dimension of the porphyrin head of the chlorophyll molecule is of the order of 15 to 20 Å it is evident that there cannot be many in each of these subunits and that they are not likely to be randomly arranged therein.

We are now coming down from the biological level to the molecular level, and within the not too distant future I believe we will be able to reconstruct this chlorophyll-containing structure (quantasomes

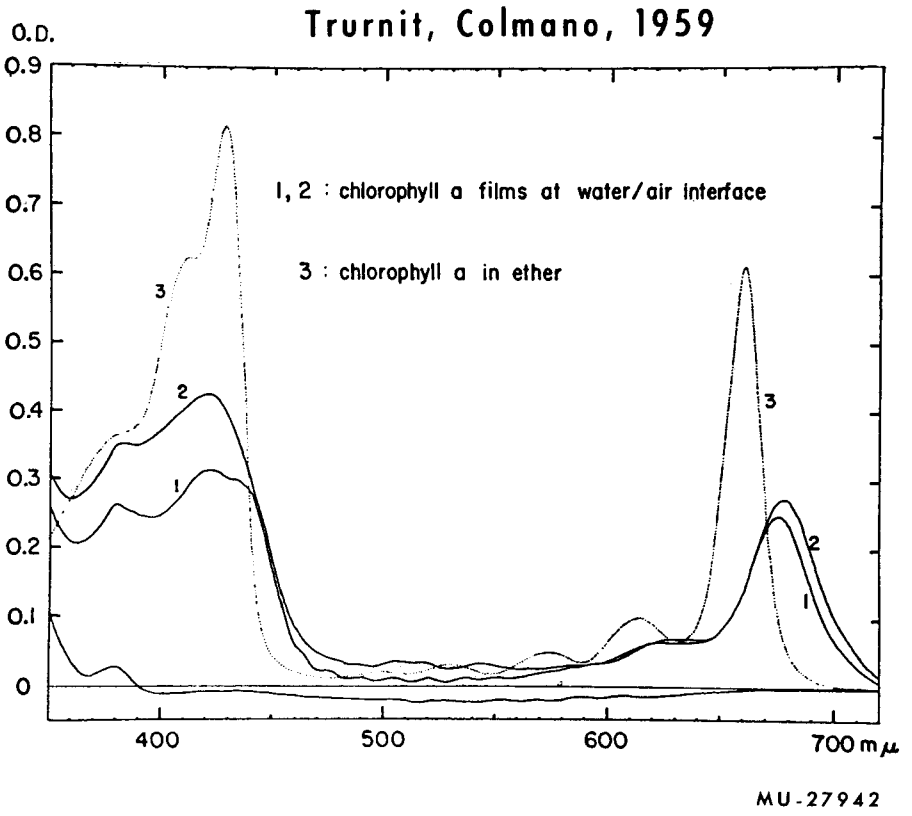
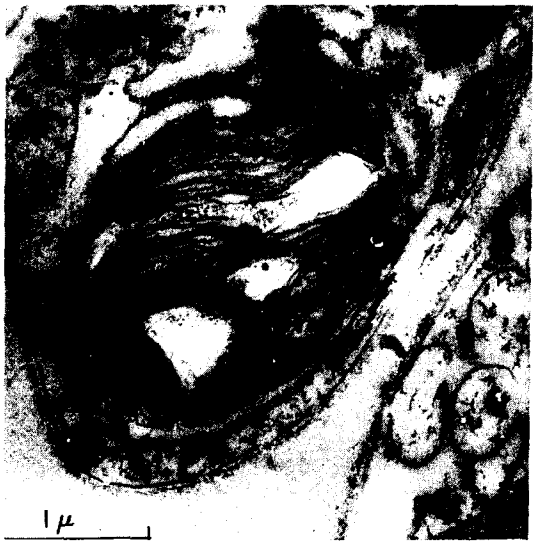
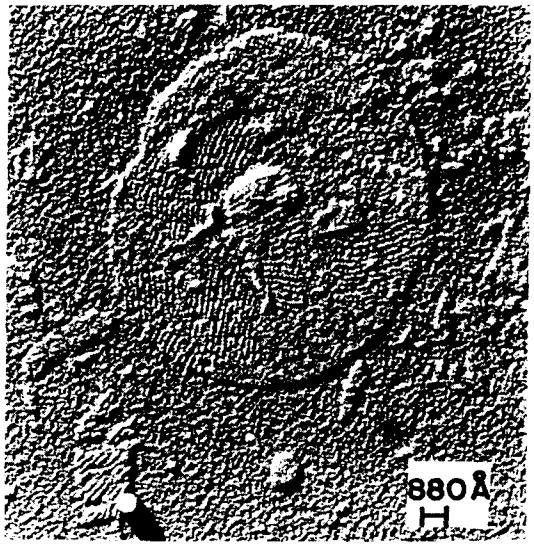


Fig. 19. Film spectra of chlorophyll at water-air interface, and solution spectrum.

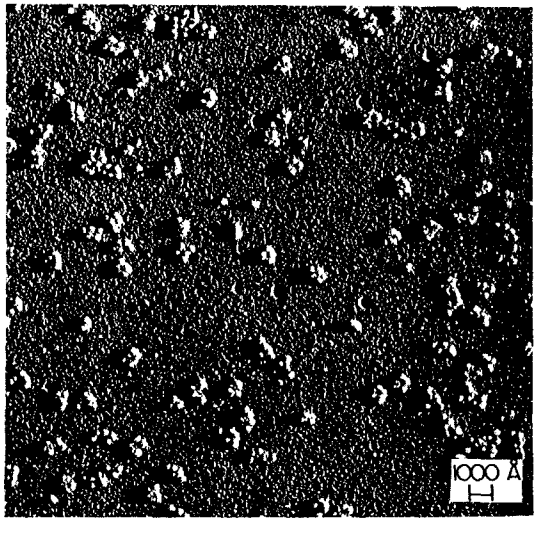


CHLOROPLAST WITH MITOCHONDRIA
CHLAMYDOMONAS (SAGER)



QUANTASOMES FROM SPINACH
(PARK and HEALEY)

NEG.-STAINED MITOCHONDRIA
(PARK and PACKER)



POLYSOMES MAKING HEMOGLOBIN
(WARNER, RICH and HALL)

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Fig. 20. Electron micrograph showing the "fundamental particles" of biology: ribosomes, electron transport particles of the mitochondria, quantasomes of the chloroplasts and unit lipoprotein membrane.

ZN-5021

Fig. 21. Quantasomes from spinach chloroplast lamellae. Shadowed paracrystalline quantasome array (3000,000 X); quantasome with contained subunits is circled.

of figure 2. from its component molecular parts. When we can do that we will have carried out the whole structural evolution from the atoms of which the molecules are made up, to the visible, biological functioning structure.

CONCLUSION

We have, I believe, reached a level of understanding of the nature of biological structure and function in molecular terms which allows us to suggest a reasonable sequence of events from the primeval molecules of the earth's surface to the structural units which constitute the functioning living organism. Because we have been able to do this in terms of a chemistry we think we understand, we are prone to take the next step. It seems like an obvious one, but it could be quite wrong. That is to suggest that, given a starting environment anywhere which resembles what we think was the primeval environment of the earth's surface, the same kind of sequence of events is likely to have occurred — in fact, it would have been inevitable. The exciting thing about this point in time, especially for the young students who have the future ahead of them, is that we (they) will be able to find out whether this notion, which is really a fundamental notion in all human thinking, is so or is not so.

ACKNOWLEDGMENT

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