Committee I
The Evolution of Man

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THE IMPORTANCE OF PREBIOTIC PORPHYRINS IN BIOPOIESIS

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THE IMPORTANCE OF PREBIOTIC PORPHYRINS IN BIOPOIESIS

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Abstract

After the formation of our planet, on the primordial Earth, small molecules, such as aminoacids, porphyrins or mononucleotides and macromolecules, might have had the chance to interact with each other, polymerize to a larger degree, form an enclosed space and find themselves assembled into an organized living system, the one individual ancestor cell of all cellular organisms that have come afterwards.

Study of the abiogenic formation of biologically important compounds under primitive Earth conditions has led to the demonstration that porphyrin-like substances can be produced under simulated geochemical conditions of primordial life. All forms of life except viruses and certain prokaryote bacteria require porphyrins and it has been proposed that porphyrin-peptide or other like-prebiotic complex might have been of significant importance during the transition period of the primeval anaerobic atmosphere of the Earth to an oxidant one.

Along the path of chemical evolution, porphyrins enhanced the process of photosynthetic phosphorylation, providing a high energy source as ATP and the efficiency of redox processes; making posible the transition of the primary

reductive atmosphere to an oxidative one. Then following biological evolution, porphyrins became vital for living organisms. All these statements are obviously asigning to porphyrins an essential role in the origin of life.

Therefore, the search for the formation, evolution and role of the porphyrins during the chemical and subsequent biological evolution is of greatest importance.

Evolutionary Stages - General view:

All forms of life, excepted viruses and certain prokaryotes require porphyrins, in addition to nucleic acid and proteins. Those of us who love and so place porphyrins on the highest pedestal, do think that to live without porphyrins should be a sluggish, zestless and truly undesirable way of life. The existence of porphyrinless organisms such as viruses and a few prokaryote bacteria does not argue against an essential role of porphyrins in the origin of life, because there are multiple and solid evidence that they have played a key role in biopoiesis; so such organisms depleted of porphyrins may very likely represent a kind of retrograde evolution form of primeval especies which eventually developed a means of survival without requiring porphyrins (1).

Therefore, due to the vital role of porphyrins for all living organisms a number of experimental and theoretical studies have been performed in the last decades to elucidate the facts concerning both the abiogenesis and the function they played in the course of emergence and evolution of biosystems. These studies would reveal that porphyrins appeared in the earliest stages of molecular evolution.

For a long time the theory of spontaneous generation of living things, have not been doubted by anyone and was indeed supported by people like Aristotle, Plotinus, Descartes and Christians, reflecting the ancient myth that man was originally formed from clay. However, the more and more research accumulated on living nature, the actual authenticity of such vivid poetic image for life appearance was shaken. Nevertheless, many scientists do think today that clay minerals are likely to have been involved in the origins of life; helping to initiate an evolutionary process that would in turn led to the development of the first proto-organisms; they must have arisen from some sort of stockpile of the necessary and essential molecular components, the so-called "primordial soup". Those first things made of much the same materials of organisms now, were able to evolve by Darwin's mechanism of natural selection; however, this evolution process must have been preceeded by a "chemical evolution" to bridge the gap between the simple compounds such as methane, ammonia hydrogen, nitrogen and water, assumed to have been present in the early atmosphere of the primitive Earth and the more complexe amino and nucleic acids, proteins and porphyrins, which can be identified as the "*molecules of life*".

Stanley Miller classic and famous experiment (2) was indeed and encouraging beginning. Simulating primordial energetic and atmospheric conditions in the laboratory by passing sparks through a mixture of methane ammonia, hydrogen and water; they obtained a "broth" containing some of the aminoacids essential to life. It seemed as if the gap between non-life and life might be bridgeable. Yet thirty five years later the gap is still huge.

However, to establish the steps leading to the appearance and evolution of contemporary living systems, scientists have divided this process into three big stages: 1) abiogenic formation of essential macromolecules; 2) spontaneous phase-separation of such macromolecules followed by self-organization and 3) biological evolution after the formation of the first living system (3). These three stages about the evolution of matter are being studied in different laboratories, gaps among them are still untouched, nevertheless, are expected to be filled by future research, from which the unified understanding of life might be achieved. At present it is still of value to approach the study of each step separately.

The age of the Earth is calculated to be about 2 billion years old, although its existence as planet goes further back to 4.5 billion years. The age of the Precambrian rocks, the oldest ones, is between 1.7 - 2.8 billion years, indicating that consolidation of the Earth occurred by them. However, from the 2 billion years of Earth history, only 550 million years keep a good fossil record, meaning that at that time, development of life had reached such a level that the deposition of plant and animal rests on the rocks could be preserved as fossils.

The discovery of the amazing uniformity of most if not all the fundamental metabolic reactions sustaining life in all organisms, allowed to preclude a rational scientific approach for studying the problem of the origin of life, because it pointed out to the appearance on Earth of one single proto-cell, the one individual ancestor cell of all organisms that have come afterwards (4).

As we have already stated, there is still a gap between the stage of prebiotic formation of the molecules of life and the unique historical event of the appearance of the most primitive system that might be qualified as living. However, our task will not be to reach stage three and describe the first appearance of the one special and only successful protocell but to understand the previous stages which led to the formation of an entire class of life-like phenomena.

As we all know, the theory of the chemical evolution of life began with the original experiments of Oparin and Haldane fifty years ago. In Table 1 an outline of the hypothesis on the course of organic evolution is given (4), it considers certain phases in a hypothetical history of evolution of life. This theory obviously is not based on the spontaneous generation of living things but on the progressive evolution and self-organization of organic compounds initially produced in an absolutely anaerobic or non-oxidizing atmosphere as that existing in the primitive Earth environment.

It is perhaps interesting to point out, from the point of vew of the evolution of scientific thought, that the origins of the ideas of Oparin, Haldane and others, go back to a misconception of the origin of petroleum due to Mendelejeff. He believed that petroleum was derived from the chemical hydrolisis of metallic carbides. So, being petroleum related in its chemical composition to that of living organisms, if it was generated geochemically from natural Earth products, why not the organic matter from which the living cells evolved could have also arisen by analogous reactions in the Earth's past?

The reconstitution of the whole evolutionary chain will need the elucidation of the various keys stages of the evolution, which are dependent on the major ecological changes which occurred on the primitive Earth. These stages took place under specific conditions, in constant changing, giving rise first to the appearance of some more and more organized systems and then, to a rigorous selection of organisms and to their adaptions to the enviromental changes.

Table 1: Scheme proposed for the succession of Evolutionary Stages (*)

Stage	Environmental conditions	Main sources of energy	Outcome
I	Anaerobic, strongly reducing	UV; heat; electrical discharges	Simple radicals. Accumulation of simple organic molecules in the oceans
	Gradual loss of hydrogen		
II	Anaerobic; traces of O_2	UV; heat Visible radiation (VR) (400-800nm)	Complex organic substances (carotenes, nucleotides, peptides, polyphosphates, pigments, *porphyrins*). Organo-metallo and photocatalysis. Intermolecular oxidoreductions
	Ozone layer; loss of UV		
III	Mainly anaerobic; traces of CO_2 and O_2	VR	Evolution of "synthetic cycles". Replication. Specific catalysis and *photochemistry*. Primitive enzymes. Genes
	First organisms		
IV	As in III	VR via photoreduction Fermentation	Replication of metabolic units. *Photophosphorylation. Photoconversion of acetate. Photoreduction* of CO_2 at the expense of hydrogen donors
	Appearance of oxygen in large amounts		
V	Mainly aerobic with anaerobic pockets (first more, then less, CO_2	VR via photosynthesis Respiration	Autotrophic plants. Evolution of respiring organisms. Autoxidation, photooxidation. Plants & animals completely dependent upon the photosynthesis with free CO_2. Equilibration to present conditions. Continuous turnover of a nearly constant volume of organic matter

(*) Adapted from Graffon (4).

Darwin's mechanisms of natural selection, must have begun deep within the anaerobic era IV. The origin of life should then lie much further back, still even before the time photosynthesis with the release of oxygen from water made its appearance; that is in its anaerobic form which is called photoreduction.

On the other hand, the photochemical core, that is the pigment complex with a chlorophyll-like compound, in all probability was functioning efficiently, transforming light energy into chemical action, long before the first cell or even the first true enzyme was completed. What we are trying to say is that we asume, as indicated in Table 1, that porphyrin photochemistry has not only accompanied but firmly influenced the results of evolutionary reactions from earliest times and became very important during stage II.

It should therefore be accepted that porphyrin-like compounds have a significant importance during the transition of the primeval reducing atmosphere of the Earth to an oxidating one. Along the path of chemical evolution, porphyrins enhanced the efficiency of redox process and, following biological evolution, they became vital for living organisms.

Today we all know that these so-called pigments of life are involved in the two essential processes of life:

- *Photosynthesis*: with particular importance for keeping life on Earth, the sole source of free oxygen and the most important supplier of organic matter as a consecuence of trapping and then converting solar energy into potential energy stores in plant material.

- *Cellular respiration*: the potential energy stored in plants is converted after aerobic oxidation in the process of respiration, into dynamic forms of ATP type energy.

Different porphyrin-protein complexes actively participate in the two fundamental processes described above as well as in others. Thus, chlorophylls, bacteriochlorophylls, chromopeptides, hemoenzymes take part in the photosynthesis reaction; cytochromes, peroxidases, catalases in respiration; hemoglobins, myoglobins, chlorocuprins... in oxygen uptake and so on.

By analogy with the structure they have today, it is believed that porphyrins were the first organic compounds absorbing the energy of visible light acting as photosensitizers, although open-chain tetrapyrryl pigments such as phycobilins of the blue and red algae might compete for that honor.

On these grounds the interest in the search for the appearing evolution and role of porphyrins during the chemical and subsequent biological evolution is largely justified.

Models of precellular evolution. Primordial biogenesis.

Many kinds of small molecules and macromolecules accumulated in the primordial Earth since the Earth had formed. Under simulated geochemical conditions of primordial type, the formation of a number of simple organic and inorganic compounds has been demonstrated (5).

Among these compounds many investigations emphasize the importance of HCN in primary chemical evolution (3,6), which can polymerize easily when it is in an aqueous solution or liquid with a base.

Several other compounds can be obtained from HCN and it is now quite clear that HCN-aqueous ammonia reaction mixtures have a prominent role in the prebiotic synthesis of biochemical compounds. In addition to one-carbon compounds, aminoacids, aminoacid amides, malonic and maleic acid derivatives, imidazole derivatives, purines, pyrimidine and pteridine derivatives and some polymeric molecules can be found in this reaction mixture, a real prebiotic Pandora's box (7).

The formation of formaldehyde and pyrroles from methane, ammonia and water has been obtained and in this environment, porphyrins has also been observed to form upon irradiation, heating or electrical discharge (8).

Therefore, there is extensive evidence of the prebiotic genesis of organic compounds of potential biological utility (1).

Along with the active species generated by the available energy, these organic substances were absorbed on the frozen surface of the primary ocean, which preserve them against the destructive action of strong energy sources; playing the role of the earliest matrix for the formation and the accumulation of both biologically useful and useless organic compounds.

The slow thaw of the frozen ocean surfaces accelerated and diversified the chemical reactions and gave rise to the appearance of the so-named "*primordial soup*". At the same time, the contact between the solid surfaces of rocks and clays and the primordial ocean allowed its enrichment with new elements such as mineral salts and metals, producing a diversification of the matrices; which in turn increased the complexity of the possible chemical reactions leading to the formation of biomonomers and biopolymers.

During this stage of molecular evolution a rigorous selection of those compounds thermodynamically more stable took place.

The next step on the way to life was the appearance of an enclosed space, because that is one of the characteristics of life. Substances were concentrated in an enclosed space or coacervates. Initially such enclosed spaces could have been isolated portions of the primordial soup, able to interact with each other generating the first multimolecular aggregates which could lead to more and more organized biostructures. Experimentally, it was demonstrated that concervate droplets enclosing different compounds could be easily formed by blowing air on drops of lipids and proteins. Within these coacervate interactions of substances encapsulated were more efficient and they even might then reproduce themselves and grow.

Moreover, coacervates were separated from their environment which was their mother liquid, and protected from the unfavorable changes in the environment, and so the mean life of various compounds might be longer than they had been in solutions. This has been one of the most important steps of chemical evolution.

The competition of chemical, energetical and environmental factors determined the self-assembling of the first *protocells* or *probionts*.

The above statements constitute the main ideas of the known *cold theory* about the appearance of the first living forms.

Prebiotic formation of porphyrins

Porphyrin-like compounds can be obtained under conditions simulating the primary atmosphere of the Earth, they could have evolved at stage II of the chemical evolution as shown in Table 1 and their abiogenesis can be studied in model experiments.

Among the evidence of the prebiotic formation of porphyrins, we should mention the studies of Kolesnikov & Egorov (9) who demonstrate the presence of metalloporphyrins and a linear tetrapyrrole pigment, similar to phycobilin 655 in samples of Precambrian rocks 2.6 billion years old from USSR.

Evidence of the presence of complex organic molecules, including six biological aminoacids as well as porphyrins in carbonaceous condrites and strong evidence for a specific non-biological porphyrin in the interstellar medium has been proposed by Johnson (10).

Blue-green algae are the most primitive organisms capable of photosynthesis. Their photosynthetic apparatus involves chlorophyll and plycobili-proteins, secondary pigments facilitating migration of excitation energy towards chlorophyll. It is probable that in the course of evolution blue-green algae emerged from a primitive photoautotrophic organism which existed at an early stage of the evolution of life on the Earth. Then the evolution of the photosynthetic apparatus in plants developed in such a way that chlorophyll became the major photosensitizer.

On the other hand we should recall again the pioneer experiments of Miller (2) on the formation of primeval compounds essential to life under abiogenic conditions. Among the aminoacids formed, glycine was dominant in the mixture, following alanine and aspartate.

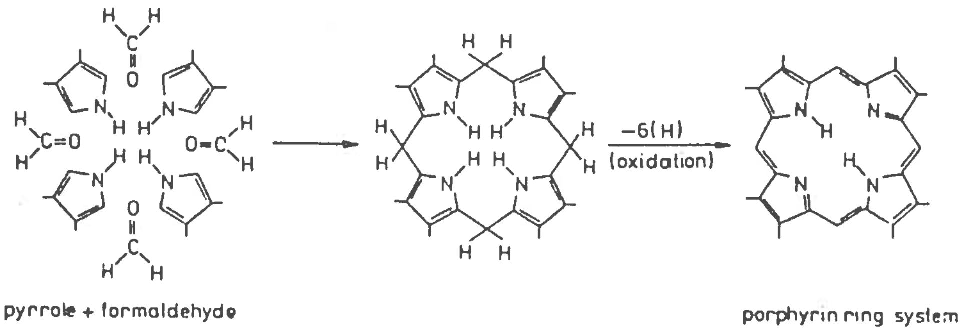
Fisher and Fink (11) observed that acetylacetic, acetic and succinic acid could also be obtained abiotically. This was of special interest, since, with succinate, glycine is essential for the biosynthesis of porphyrins leading to delta-aminolevulic and porphobilinogen (12) and Graffon (4) has suggested that porphyrins which are excellent photosensitizers in laboratory experiments, should have played a decisive part early in evolution, practically the same they play at the present time.

If porphyrins can be formed spontaneously from the component of Miller's brew it is obvious that evolution must have been speeded up considerably by these fluorescent pigments, particulary because daylight is available in much greater amounts than UV. At the same time the action of visible radiation is much milder, less destructive, less sterilizing and yet sufficient to promote any number of electron and hydrogen transfers, oxidoreductions and outright oxidations between small or large organic molecules. This is not likely but a necessary hypothesis to account for the speed of evolution.

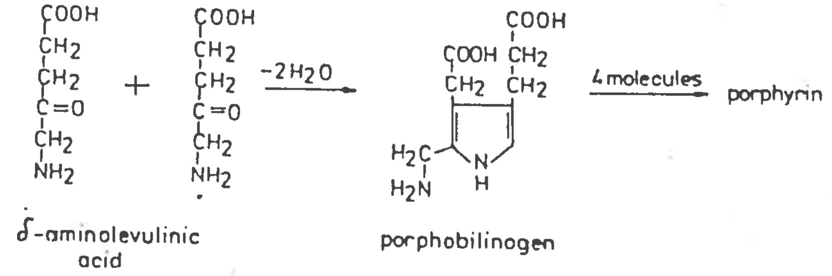
Several groups in the world are carrying out studies in model experiments to validate these theories and results obtained so far confirm the hypothesis of the genesis of tetrapyrrolic compounds in abiotic conditions.

Although the chemical reactions involved in their prebiotic synthesis are not yet elucidated, it was established that the intermediates are formaldehyde and pyrroles (13). The reaction can occur even at high dilution and is catalyzed by metal ions, which chelate the nitrogens of the pyrrolic rings.

Thus, using different radiations as energy sources, Szutka (14) first demonstrated that pyrrole and benzaldehyde condense to a porphyrin derivative. Thermal condensation of pyrrol with formaldehyde into porphyrin pigments in the presence of minerals was also achieved by Hodgson & Baker (15) (Graph 1). An easy formation in yields of metalloporphyrin was also demonstrated (5). All these assays were carried out under geologically simulated conditions.



Graph 1



Graph 2

Intermediates of porphyrin synthesis were also identified in abiotic experimental systems (Graph 2): pyrroles can be formed from aminolevulic acid (ALA); Scott (16) reported the synthesis of porphobilinogen in anaerobic conditions and aqueous medium. Szutka (17) achieved the direct photochemical conversion of ALA to porphyrin pigments by the influence of UV radiation under primeval-like Earth conditions. And ALA had been obtained by Choughuley (18) under prebiotic conditions upon the exposure Urey's reductive atmosphere to accelerate electrons (β-rays).

The analytical criteria used, allowed to establish that the abiotically synthesized tetrapyrrolic compounds have an identical behaviour to that of authentic porphyrins.

So, porphyrin-like compounds very likely emerged at a quite early stage of the chemical evolution, still in the conditions of primeval reductive atmosphere and due to a mechanism quite different from that known in contemporary organisms without enzyme intervention.

The metalloporphyrins obtained at the stage of chemical evolution could perform effective catalysis of certain reactions. The chemical energy thus released was further used to satisfy the energy requirements of the first living entities.

The incorporation of porphyrins into the early organisms determined more and more effective participation, as sensitizers, of photoreactions to obtain chemical energy. The process providing the useful energy for organisms developed in time, giving rise to the complex bioenergetic reactions of today.

Practically, almost the same set of chemical reactions is used in all organisms to perform vital functions, regardless whether primitive bacteria or man are considered. Not just the mechanisms and reactions are the same, but even the molecules taking part in these metabolic pathways are identical and found in most biological species.

Actually, the fundamental identity of the metabolic processes in millions of known biological species constitutes the best proof for the common origin and further evolution of all living organisms. The energy of all life processes comes from sun, being captured by photochemical reactions, through the photosynthesis in plants, where it is stored as bond energy in carbohydrates.

The animal energy comes from sequential and controlled combustion of plant carbohydrates leading to carbon dioxide and water. The energy released in these reactions is used to form ATP, the principal molecule in metabolism to store and transfer energy.

Plants and animals are therefore mutually dependent organisms in the big reaction cycle that distributes sunlight energy to the process of life and keeps equilibrium between oxygen and carbon dioxide in the today biosphere. And porphyrins are the key compounds in these processes fundamental to life.

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	Ozone layer; loss of UV		
III	Mainly anaerobic; traces of CO_2 and O_2	VR	Evolution of "synthetic cycles". Replication. Specific catalysis and *photochemistry*. Primitive enzymes. Genes
	First organisms		
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	Appearance of oxygen in large amounts		
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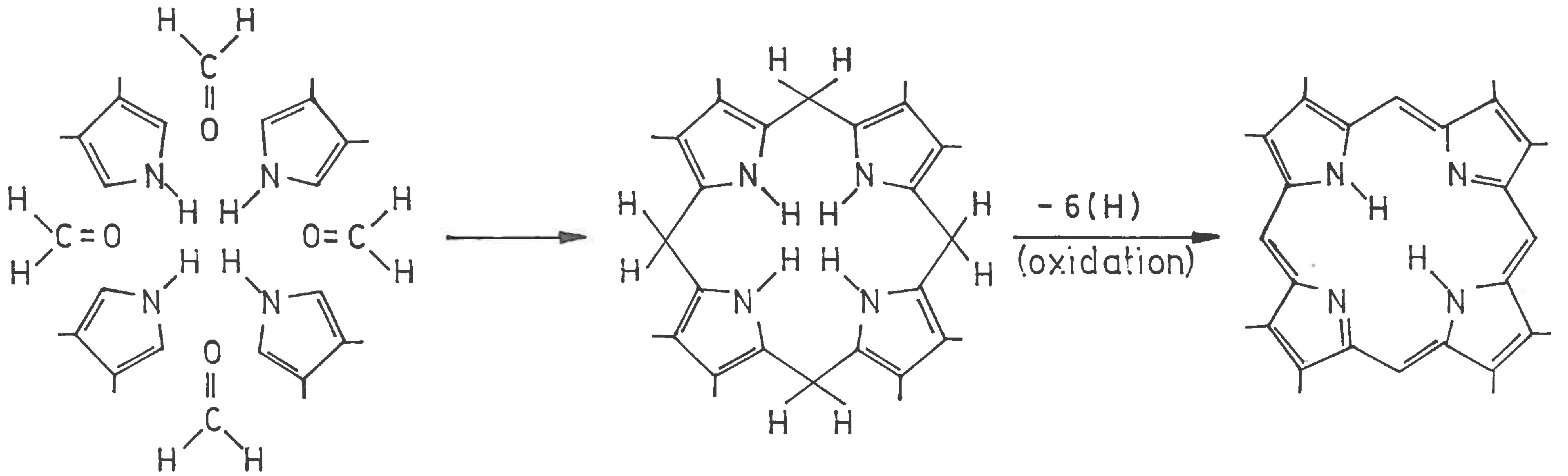
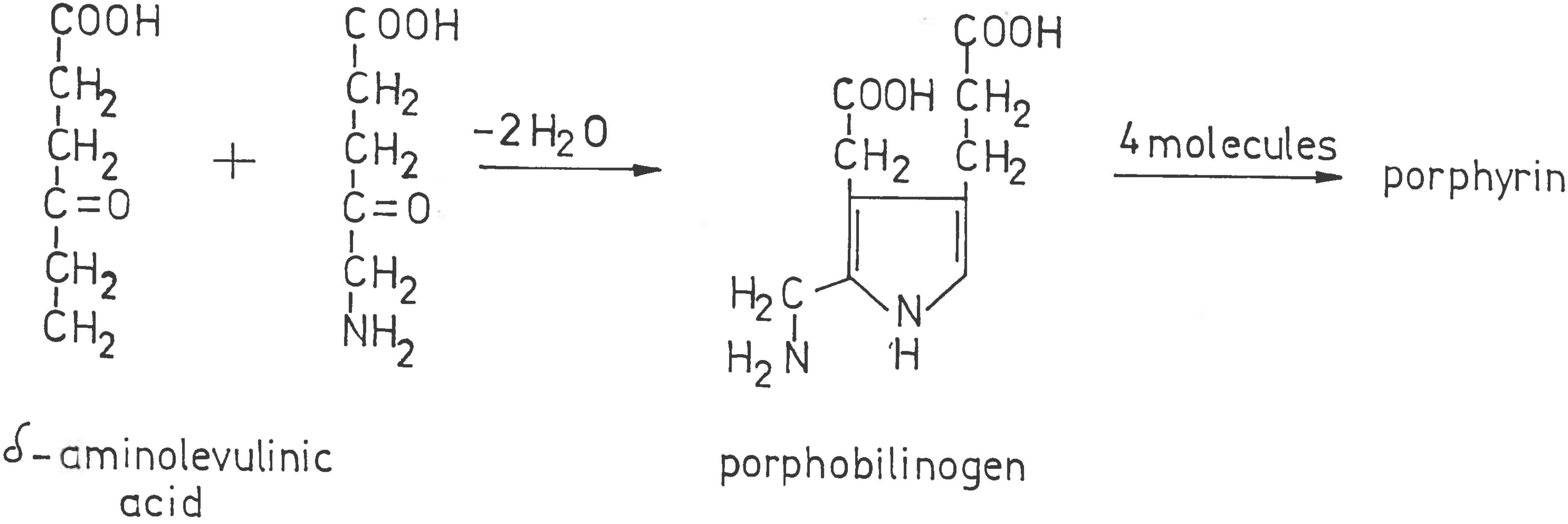
(*) Adapted from Graffon (4).



pyrrole + formaldehyde

porphyrin ring system

Graph 1


COOH
CH2
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C=O
CH2
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COOH
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CH2
C=O
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NH2
−2 H2O
COOH
COOH
CH2
CH2
CH2
H2C
H2N
N
H
4 molecules
porphyrin
δ-aminolevulinic acid
porphobilinogen

Graph 2