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MOLECULAR MECHANISMS ON INTERACTIONS BETWEEN OXYGEN CO-ORDINATED METAL POLYHEDRA AND BIOCHEMICAL COMPOUNDS

by

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Part IV : Molecular Development of Mineralization Templates in Biological Systems (Matheja-Degens) (IN GERMAN) 14 p.

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Approved for Distribution Vaughan T. Bowen
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THE RÔLE OF PHOSPHATES IN BIOCHEMICAL REACTIONS

ABSTRACT

Information is presented on the structural and functional properties of phosphates in biochemical systems. Phosphates follow four structural formation principles:

(1) linkage of phosphate units via oxygen co-ordinated metal ion polyhedra,
(2) establishment of chains,
(3) cross linkages of chains resulting in corrugated layers, and
(4) cross linkages of layers resulting in distinct three-dimensional molecular networks.

One linkage type does not require nor exclude another linkage type.

On the basis of this concept on the associations of phosphate tetrahedra and metal ion oxygen polyhedra, a revised molecular model for DNA is proposed. Metal ion oxygen polyhedra excercise control on the shape of the DNA, and thus may introduce the stretching of the polymer chains This can be considered the ultimate reason why a single stranded DNA will associate itself readily with another single stranded DNA resulting in a double helix. In contrast, the coupling of a single stranded DNA by itself in making a sharp bend (loop) will not take place due to the stabilization of the structure by means of the metal ion oxygen polyhedra backbone. This model also explains the functional properties of the nucleic acids, for instance, the oxide chains and layers will favor proton jumps, and in the presence of a differential potential, they will form proton conduction bands.

The molecular organization, as introduced by the association of metal ion co-ordination polyhedra with the PO_4 groups, plays also a significant rôle in membrane dynamics. Analogous to the ion co-ordination interactions of polyphosphoric acids, the fixation of metal ions at the P-O surface and of membranes will result in a distinct molecular geometry as a whole. In this way, the membranes will act as dynamic molecular sieves, whereby the mesh size and the functional characteristics of these molecular sieves is determined by the flexible interplay of metals and the individual phospholipid compounds contained in the membranes.

TP (III-P) is characterized for its ability to form a co-ordination polyhedron with polyvalent cations. In this way it resembles polyphosphates and differs from I-P and II-P. Triphosphate exhibits two significant properties:

(1) terminal chain degradation, i.e. the release of terminal PO_4 groups and formation of PO_3 radicals which is controlled by external electrical forces, and
(2) affinity to all cations by means of metal ion oxygen polyhedra and the establishment of an exchange affinity series for all metal ions.

Concerning the biosynthesis of polysaccharides, proteins, and the nucleic acids, three conditions have to be fulfilled:

(1) acitvation of the reaction partner,
(2) maximum efficiency and minimum error, and
(3) well-defined control of the reactions in terms of kinetics and transportation mechanism.

All three requirements are most effectively executed by triphosphates. The controlled formation of the reactive PO_3 radical not only activates the reaction partner, but also eliminates by means of the phosphate formation, the OH and O groups from the reaction system and this with extraordinary efficiency and elegance. In biochemical reactions, this role is commonly exercised by ATP.

ORIGIN, DEVELOPMENT, AND DIAGENESIS OF BIOCHEMICAL COMPOUNDS

ABSTRACT

Biogeochemists are, inter alia, concerned with the origin of proteins and other polymeric building blocks of life during the primordial stage of the earth. From their point of view, it is reasonable to assume that mineral matrices participate in the synthesis of certain organic polymers.

To substantiate this supposition, the behaviour of amino acids in presence of mineral templates was investigated. About 60 different minerals, comprising representative members of silicates, oxides, phosphates, carbonates and sulfates were tested for their amino acid sorption characteristics. The selection of minerals was based so as to cover a wide range of crystallographically and chemically different minerals. It could be shown that during reorganization of the mineral phase (e.g. dehydration, recrystallization, and mechanical abrasion) coupling of amino acids occurred resulting in the formation of polypeptides with molecular weights up and exceeding 10,000. Hydrolysis of the polymer fractions separated by molecular sieving techniques resulted in the total recovery of the amino acids originally supplied to the mineral system.

In the light of these results and of previous investigations on the distribution of organic matter in the oceans, fresh-waters, and recent and ancient sediments, a concept on the origin, evolution, and diagenesis of biochemical matter has been developed. This concept illustrates the enormous variety of interactions that may take place in the natural habitat between inorganic and organic phases. The concept further outlines the way at which nucleic acids and the genetic code could possibly have come into existence.

CONCEPTS ON ABIOTIC DEVELOPMENT OF PHOSPHOLIPID-MEMBRANES, AND ORIGIN OF PRIMORDIAL METABOLISM

ABSTRACT

Molecular mechanisms leading to the formation of phospholipids under primordial conditions are discussed. Analogous to amino acids and simple organic acids, a variety of alcohols can be synthesized in atmospheric systems involving electric discharge, UV, or ionizing particles. Clay minerals can structurally accommodate preferentially glycol, glycerol and its homologous, relative to other alcohols.

Paraffins must be considered the principal precursor material for fatty acids. The formation of hydrocarbons is hereby related to high-temperature processes, which involve (a) the dissolution of carbon in silicate melts, (b) the production of submicroscopic graphite particles upon cooling, and (c) the hydrogenation of graphite. The required hydrogen is obtained by the hydration of silicates. Fixation of hydrocarbons on silicate surfaces may take place; their subsequent oxidation yields fatty acids.

The formation of lipids from fatty acids and glycerol is intimately connected to the formation of phospholipids. Principally, phosphate tetrahedra, linked to silicate surfaces, may collect fatty acids and glycerol. This will result in thermodynamically favorable transition structures between the hydrophobic elements and the ionic silicate network. Molecular processes such as ester formation are the chemical consequences. PO_4 and metal ion oxygen polyhedra will function as catalyzing agents.

The association of phospholipids produces distinct boundary layers at the contact zone between environment and coacervates. Such layers already posess the characteristics of a dynamic membrane system, i.e., flexible molecular sieve properties. This phenomenon is an essential requirement for a primitive metabolism.

The formation of an auto-catalytic phosphorylation cycle is the next step towards the origin of a primordial cell. This metabolic system must have evolved independent of the genetic translation apparatus. The performance of the genetic transcription machinery required the existence of a workable autocatalytic system.

An outline of the minimum requirements for origin and maintenance of living systems is presented in the concluding chapter of this article. The formation principles of polyphosphates are thoroughly discussed.
We emphasize: As a consequence of thermodynamic laws and probabilities the development of the phase-separating phospholipid membranes constitutes the physical basis for the development of life and thus represents the primary event. In contrast, metabolism constitutes the chemical basis which for the first time came into existence within the membranes; consequently, it constitutes a secondary event. The codon, placed in third position, eventually established the time-continuity of these fabrics by means of successive reaction cycles and represents a biological phenomenon which falls outside the field of experience of present-day experimental physics.

MOLECULAR DEVELOPMENT OF MINERALIZATION TEMPLATES IN BIOLOGICAL SYSTEMS

ABSTRACT

Attention is focused on a novel tool to study evolutionary trends in mineralized organisms. The article considers first the molecular mechanisms leading to the mineralization of organic tissues, and in second place outlines its phylogenetic implications.

Concerning the first question on the calcification mechanism it can be shown that protein and glycoprotein matrices act as templates for the fixation of anions and cations. Depending on the position of the sorption sites in the three-dimensional proteinaceous network, the nucleation of a mineral phase may take place. In the case of carbonate shells, calcium ions will associate themselves with the COO^- group of aspartic acid and will become co-ordinated with oxygen resulting in a $Ca^{++}O_6$ or a $Ca^{++}O_9$ co-ordination polyhedron. The increase in polyhedra will cause a structural reorganization of the glucosamine-and peptide chains. Thus, the metal ion oxygen polyhedra introduce a higher molecular order. The interaction of the polyhedra with the bicarbonate linked to, for instance, lysine or glucosamine by means of hydrogen bridges, results in an exchange of oxygen at the polyhedra, whereby the co-ordination of calcium to oxygen becomes more stable. The availability of oxygen for co-ordination purposes will determine whether calcite ($Ca^{++}O_6$) or aragonite ($Ca^{++}O_9$) will be the endmember of the calcification process. The resulting nucleation processes find their macroscopic expression in the texture and morphology of the calcareous shells.

In the case of phosphate systems, collagen always represents the mineralization template. It is suggested that hydroxyproline or serine are capable in forming phosphodiester bridges. A more comprehensive discussion on molecular mechanisms leading to the formation of bone structures is in preparation.

The phylogeny of calcified tissues is discussed in the second part of this article. It is proposed that the molecular structure of ancestoral shell proteins closely resembles the structure of molluscan periostraca and that the lack of fossilized shell materials in deposits older than 600 million years is a function of the non-existence of mineralized shell materials prior to Cambrian time. During the Precambrian, molluscs exclusively secreted an organic shell. The calcification aera started when functional sites in the ancestoral glycoprotein shell-matrix developed by the elimination of certain cross linkages, making, for example, aspartic acid available for the fixation of calcium ions. Slight changes in the environmental parameters, such as salinity, pH, temperature, or CO_2 partial pressure, may have actually caused a change in the overall shell secretion pattern of many organisms. In this way, the "population explosion" at the beginning of the Cambrian can reasonably be explained. Namely, organisms started to deposit minerals on an already highly evolved organic tissue. In the course of evolution, organisms progressively modified and improved their shell secretion apparatus, and this phenomenon finds its reflection in the biochemistry of the shell tissues which is species characteristic. In turn, this progressive biochemical change allows the construction of a phylogenetic treewhich conforms with the pattern of a phylogenetic tree constructed exclusively on morphological evidences.

THE RÔLE OF PHOSPHATES IN BIOCHEMICAL REACTIONS

by

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INTRODUCTION

P-O (phosphorus-oxygen) compounds play an essential part in biochemical processes. In biochemical shorthand they are known, for instance, as: ATP, DPN, TPN, FAD, or CoA*. They occur as intermediary products of low molecular weight, i.e. as mono-, di-, and tri-phosphates. Exceptions from this rule are the nucleic acids, where PO_4 tetrahedra are structurally incorporated in high-molecular weight polymers (Fig. 1). There is strong indication, that the specific properties contained in the structure of phosphates hold the key for the distinct rôle of phosphorus in biochemical processes.

It is the chief objective of this article, to focus attention on the unique structural characteristics of phosphates and to point out their special significance in biochemical reactions. Although we principally take the stand of an inorganic physical chemist, the biochemical implications will be discussed in some detail.

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* ATP = adenosine triphosphate
DPN= diphosphopyridine nucleotide
TPN = triphosphopyridine nucleotide
FAD = flavin adenine dinucleotide
CoA = coenzyme responsible for the transfer of acetyl groups

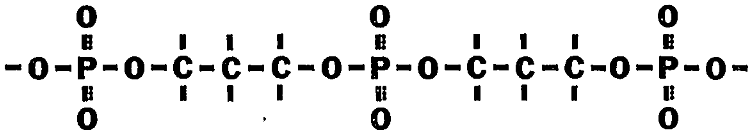
CO-ORDINATION STATE OF PHOSPHORUS

Phosphorus is outstanding in being able to form with other elements a variety of co-ordination polyhedra. The co-ordination number for phosphorus in phosphorus-chlorine compounds, for example, may range from three to six (1-10):

$$\begin{array}{cccc} 3\text{-}[p^3] & 4\text{-}[sp^3] & 5\text{-}[sp^3d] & 6\text{-}[sp^3d^2] \\ PCl_3 & PCl_4^+ & PCl_5 & PCl_6^- \end{array}$$

This flexibility in bonding state (Fig. 2) and the resulting chemical consequences (17), make phosphorus a perfect element for the functionally variable work assignments in the biochemical system.

The position of phosphorous in the periodic table of elements explains these characteristics as well as that phosphorus exhibits an intimate relationship to hydrogen, carbon, oxygen, and nitrogen which is unmatched by any other element (Fig. 3). This relationship is comparable and complementary to the functions exercised by nitrogen in biochemical reactions. Phosphorus differs from its neighboring elements carbon, nitrogen, and oxygen in that one of the 3s or 3p electrons can be easily transferred to the 3d orbital (3, 4, 10, 18, 19). Consequently, phosphorus in the 4-co-ordinated state (sp^3) has a pi bond in addition to the four sigma bonds. Based on their position in the periodic table of elements, the homologous elements nitrogen and phosphorus have complementary properties in relation to hydrogen and oxygen. This phenomenon is confirmed by biochemical research. One can conclude:



$$-\mathrm{O}-\underset{\underset{\mathrm{O}}{\|}}{\overset{\overset{\mathrm{O}}{\|}}{\mathrm{P}}}-\mathrm{O}-\underset{|}{\overset{|}{\mathrm{C}}}-\underset{|}{\overset{|}{\mathrm{C}}}-\underset{|}{\overset{|}{\mathrm{C}}}-\mathrm{O}-\underset{\underset{\mathrm{O}}{\|}}{\overset{\overset{\mathrm{O}}{\|}}{\mathrm{P}}}-\mathrm{O}-\underset{|}{\overset{|}{\mathrm{C}}}-\underset{|}{\overset{|}{\mathrm{C}}}-\underset{|}{\overset{|}{\mathrm{C}}}-\mathrm{O}-\underset{\underset{\mathrm{O}}{\|}}{\overset{\overset{\mathrm{O}}{\|}}{\mathrm{P}}}-\mathrm{O}-$$

FIGURE 1

Segment of phosphorus-oxygen-carbon backbone chain of the nucleic acids; nucleic acids constitute the only high-polymers, in which PO_4 units are structurally incorporated.

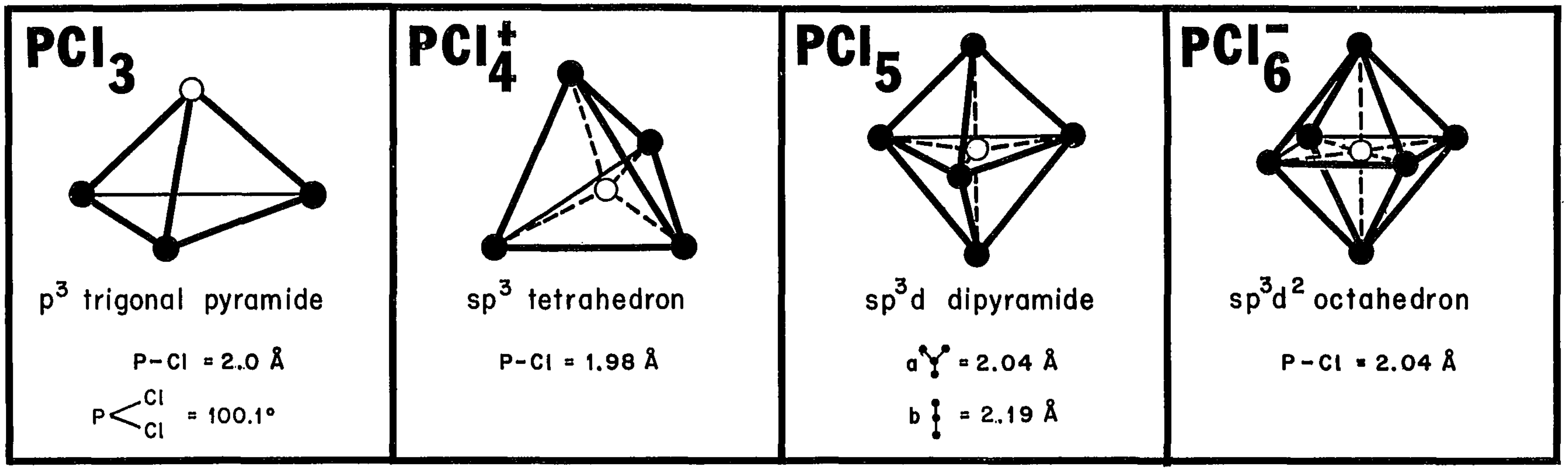


FIGURE 2

Bonding state and type of co-ordination polyhedra of phosphorus-chlorine compounds; atomic distances after (11).

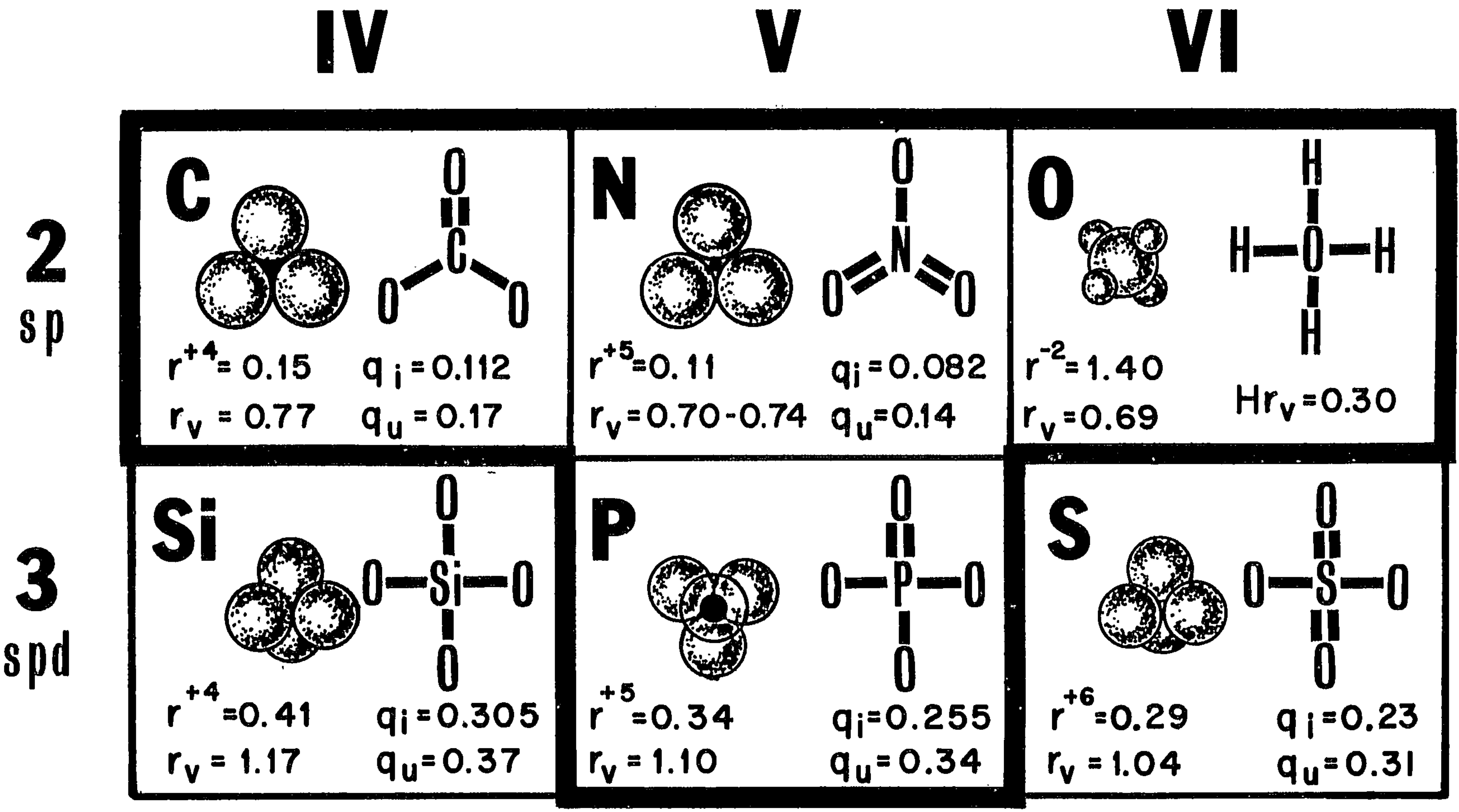

IV
V
VI
2
sp
3
spd
C
r+4 = 0.15
rv = 0.77
qi = 0.112
qu = 0.17
N
r+5 = 0.11
rv = 0.70-0.74
qi = 0.082
qu = 0.14
O
r-2 = 1.40
rv = 0.69
Hrv = 0.30
Si
r+4 = 0.41
rv = 1.17
qi = 0.305
qu = 0.37
P
r+5 = 0.34
rv = 1.10
qi = 0.255
qu = 0.34
S
r+6 = 0.29
rv = 1.04
qi = 0.23
qu = 0.31

Figure 3

FIGURE 3

Segment of periodic table of elements; biogenic elements enclosed by heavy lines. The ionic radii (r^{+n}) are reported in Å units; the individual atoms are assumed to be in the 6-co-ordinated ionic state (12).

r_V is the normal-valence-radius (13) which expresses the participation of the individual atoms at the intermolecular distance, should the number of bonds equal the normal valence. According to (13), the nitrogen r_V equals 0.70; based on the bonding distance: C - N = 1.51 Å (14), a revised nitrogen $r_V = 0.74$ is adopted. For the oxygen-valence-radius, a similar revision is applied ($r_V = 0.69$) based on the ester linkage distance C - O - C (C - O = 1.46 Å), e.g. in dioxane; the original value (13) is $r_V = 0.66$.

The corresponding r_V value for hydrogen (Fig. 3) is obtained after (15).

q_i is the ionic radii quotient for an oxygen polyhedron, whereby the corresponding corrections for the co-ordination number are applied:

$$q_i = \frac{r^{+n}}{r^{-2}_{ox.}} = \frac{\text{cation}}{\text{oxygen}}$$

q_u is the ratio of univalent radii$=\frac{\text{cation}}{\text{oxygen}}$ (16).

The oxygen co-ordinations of cations are shown as spheres to indicate that the co-ordination number is a function of the size of the cations. Phosphorus and silicon are too large in size to fit a triangle co-ordination, whereas nitrogen is too small for it. The minimum radii ratios (q) required for a stable co-ordination of a chemical molecule are listed in Table 1. A comparison of the q-values in Table 1, and the q_i and q_u values in this Figure indicates which of the molecules are stable.

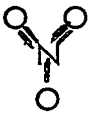
(1) the affinity of group V elements relative to hydrogen decreases with increasing atomic number; the formation of NH_3 represents a strong exothermic reaction, and that of PH_3 a weak exothermic one:

$$(94\ \mathrm{kcal})\quad N-H > P-H \quad (77\ \mathrm{kcal})$$

(2) the affinity of group V elements relative to oxygen increases with increasing atomic number; the formation of nitrogen oxides is a strong endothermic reaction, whereas that of phosphorus oxides is an exothermic one: (17, 20)

$$(\approx 60\ \mathrm{kcal})\quad N-O < P-O \quad (\approx 100\ \mathrm{kcal}).$$

These relationships become evident by considering the differences in ionic radii of the aforementioned elements*. Judged from the position of the elements in the periodic table, nitrogen (oxygen 3-co-ordinated), a priori, is expected to be more stable than carbon:



This, however, contradicts empirical data**. Yet, by looking upon NO_3 as a co-ordination polyhedron, a solution can be offered. A co-ordination polyhedron

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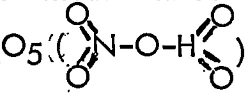
* It is emphasized that the ionic radii only represent characteristic numbers, since we are dealing with co-valent bonds. They were obtained by quantum mechanical considerations and can be used for comparative purposes (12).

** N_2O_5 ( $\mathrm{O_2N{-}O{-}HO_2}$) decomposes at room temperature; concentrated HNO_3 is easily decomposable. It oxidizes metals prior to dissolving them.

$$3Cu + 2HNO_3 \rightarrow 3CuO + 2NO + H_2O$$
$$3CuO + 6HNO_3 \rightarrow 3Cu\,(NO_3)_2 + 3H_2O.$$

Nitrates are unstable at moderate temperatures; they lose oxygen.

is obtained in the following way. The various ions are assumed to represent undistorted spheres and their radii quotient q_i is calculated:

$$q_i = \frac{r_a}{r_b} \qquad \text{where } r_a < r_b$$

A comparison of these data with the geometrically inferred minimum values results in the establishment of the co-ordination polyhedron. Based on q_i and q_u (Table 1), it is concluded that a 3-co-ordinated nitrogen oxide is chemically unstable. In contrast, phosphorus in tetrahedral co-ordination to oxygen represents a particularly stable polyhedron.

TABLE 1

MINIMUM RADIUS FOR STABLE CO-ORDINATION POLYHEDRA

Minimum radius ratio (9)	Co-ordination number and configuration.	Co-ordination polyhedron
< 0.155	2 $[sp, dp]$	linear
< 0.155	2 $[p^2, ds]$	angular
0.155	3 $[sp^2, dp^2, d^3]$	equilateral triangle
0.225	4 $[sp^3, d^3s, d^2sp, dp^3]$	tetrahedron
0.414	6 $[d^2sp^3]$	octahedron
0.528	6 $[d^4sp, d^5p]$	trigonal prism
0.645	8 $[d^5p^3]$	square antiprism
0.732	8 $[d^4sp^3]$	cube
1.000	12	cubo-octahedron and hexagonal prism

TABLE 2

Element	electro-negativity [x]	electron affinity [kcal]	I. excitation potential [ev]	I. ionization potential [ev]
O	3.5	88	9.0	13.6
C	3.0	31	7.5	11.3
N	2.5	1	10.2	14.5
P	2.1	3	6.4	10.9
H	2.1	16.4	10.1	13.5

Values after (21, 22).

The unique rôle of phosphorus in biochemical systems can be further underlined by referring to a series of empirical data (Table 2). The values for the electronegativity of phosphorus and hydrogen are identical*, a fact which attests to their competetive rôle in relation to oxygen:

$$H \rightarrow O \leftarrow P$$

On the other hand, nitrogen and phosphorus resemble each other with regard to their electron affinity which is a measure of the energy released by electron capture:

$$X + e^- = X^- + E \quad \text{(kcal)}$$

The values obtained for the two elements correspond to the electron affinity for alkali metals which is equal to zero. The two other columns in Table 2 are headed

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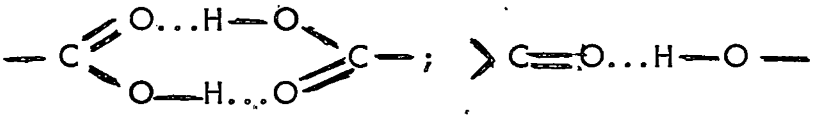
* The equation for bond energies, $D_{a-b} = 1/2 \left[D_{a-a} + D_{b-b}\right] + 23.06 \left[x_a - x_b\right]^2$, as proposed by Pauling (15) includes the electronegativity as a variable.

excitation potential and ionization energy. They are additional evidence for the complementary relationships of phosphorus and nitrogen to the elements oxygen and hydrogen. These relationships among the biogenic elements are illustrated in form of a relation diagram (Fig. 4).

In conclusion, the element pair phosphorus and nitrogen becomes incorporated in biochemical processes, because both elements introduce a higher biocrystallographical molecular order for the macromolecules. For example, only the substitution of a carbon for a nitrogen in the nucleic acids and peptides produces a well-defined three-dimensional molecular order (stereo-structure) via formation of hydrogen bridges:

- O-H....N   -O.....H-N   N....H-N

It is not unreasonable to assume that life processes would not exist, if the organization of macromolecules would depend only on O—H...O bridges like:



Analogues to nitrogen, the element phosphorus in the form of PO_4 has a similar significance in the biocrystallographical organization of molecules involved in the cell activities.

SiO_4 -- PO_4 ANALOGY AND PRINCIPLES OF PHOSPHATE STRUCTURES

In the forthcoming discussion, an analogue treatment is presented and PO_4 tetrahedra are compared to SiO_4 tetrahedra and their respective polymers. Such a

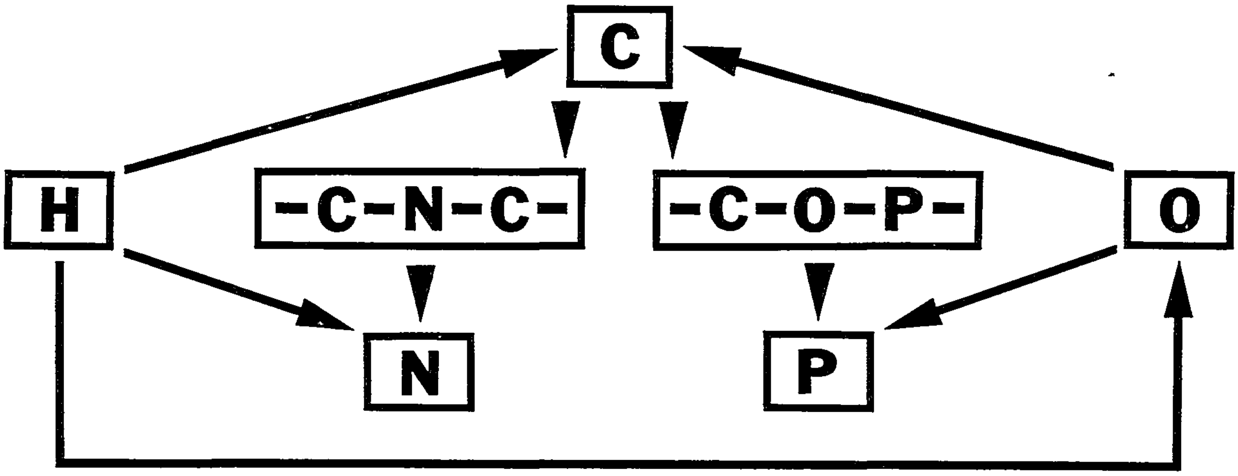


Figure 4

General interaction scheme of biogenic elements.

This diagram illustrates (a) the principal chemical functions, and (b) the relationships among the major biogenic elements.

Metabolism and energy production and release is shown by the outer arrows, involving e.g.:

$>C - C<$; $>C - H$; $>C - O -$; or O - H.

The dynamics of these reactions, which is maintained by the relationship H....O and P....O, is indicated by the arrows H → O and P ← O because both elements, H and P, are competitors in relation to oxygen.

The biocrystallographical functions are shown in the center of the diagram. By the substitution of carbon in a molecule by either nitrogen or O - P compounds, a molecule becomes capable to form cross linkages. The complementary nature of nitrogen and phosphorus in relation to hydrogen and oxygen possesses symmetry properties. The structural functions become operative via bridges:

N - H ... H; N- H ... O; N ... H - O; and PO_4 - metal ion - PO_4.

This will result in specific stereo-structures for biogenic macromolecules. In addition, hydrogen-oxygen linkages hold structural functions:

O ... H - O

Furthermore, nitrogen and phosphorus exhibit organisational properties. Namely, nitrogen has a static rôle in the form of information storage. In contrast, phosphorus has a dynamic rôle in the form of an activation element in metabolism. These relationships are indicated by the isolated positions of nitrogen and phosphorus, the arrows in the relation diagram come for a close at these two positions.

treatment appeared to be rather promising, for experimental data on metal-phosphate interactions are limited, while those on metal silicate interactions are plentiful. In this way, information on the interplay between metal ions and phosphate groups will be obtained which in turn can be used to throw more light on the function of metals and phosphates in biochemical systems.

The SiO_4 and PO_4 co-ordination polyhedra are iso-structural. Their main difference is the participation of one pi bond in case of the PO_4 tetrahedron. This results in certain restrictions regarding the three-dimensional linkage of the PO_4 units during polymerization. In contrast to SiO_4, one of the oxygens in PO_4 cannot directly function as a linkage element, except in the form of a hydrogen bridge:

$$P = O \ldots H - O - P$$

Consequently, PO_4 polymerizes in the form of chains and rings.

The comparison of structures containing PO_4 and SiO_4 units reveals that there is a surprising conformity in the way the individual tetrahedra units are stacked together (Table 3). Similar identities are established regarding their interactions with metal ions. These phenomena can be attributed to the correspondence in size of the PO_4 and SiO_4 groups (23-31) (Fig. 5). It is therefore proposed that the various metal ion co-ordination polyhedra, as well as the bonding strength:

$$Si - O \rightarrow metal \quad ; \quad P - O \rightarrow metal$$

are equivalent to each other.

The interactions of metal ions and oxygen can be described by the well-known electrostatic forces:

$$\varphi_1(r) = -\frac{z_1 z_2 e^2}{r} \qquad \varphi_2(r) = \frac{b}{r^n}$$

Coulomb attraction-potential

Bornian repulsion-potential

where z_1 and z_2 is the valence of the ions,
e the electron charge
r the ion distance
b and n constants.

In some approximation the attachment energies can be calculated.(32).

As a consequence of the sp^3 hybridization, the 4 sigma P-O bonds are equal, and distortions of the regular PO_4 tetrahedra only occur in case of an assymetrical overlap of the pi bonds. This phenomenon, i.e. the flexibility in the overlap of pi on sigma bonds (3, 4, 8, 10, 17, 18, 33-36), explains why PO_4 groups are capable in forming different but strongly bonded oxygen polyhedra with a variety of metals. For, a variable P-O distance and a variable  bonding angle will allow a PO_4 tetrahedron to respond to a metal ion with a specific geometry of the tetrahedron.

The variability in the shape of the PO_4 tetrahedron can also be explained by considering the differences in bonding energy of the P - O bond. The bonding energy is examined (Fig. 6) and shown as a function of its bonding distance:

$$E_{a-b} = \text{const.}\ d_{a-b}$$

Whereas the bonding energy of a C — O bond is strongly affected by a change in the bonding length, this is not so for the P - O bond, whose energy level stays nearly the same for all bonding lengths concerned. This fact further illustrates the unique properties of phosphorus which is unmatched by any other element, and explains why PO_4 is an optimal co-ordination partner. This flexibility

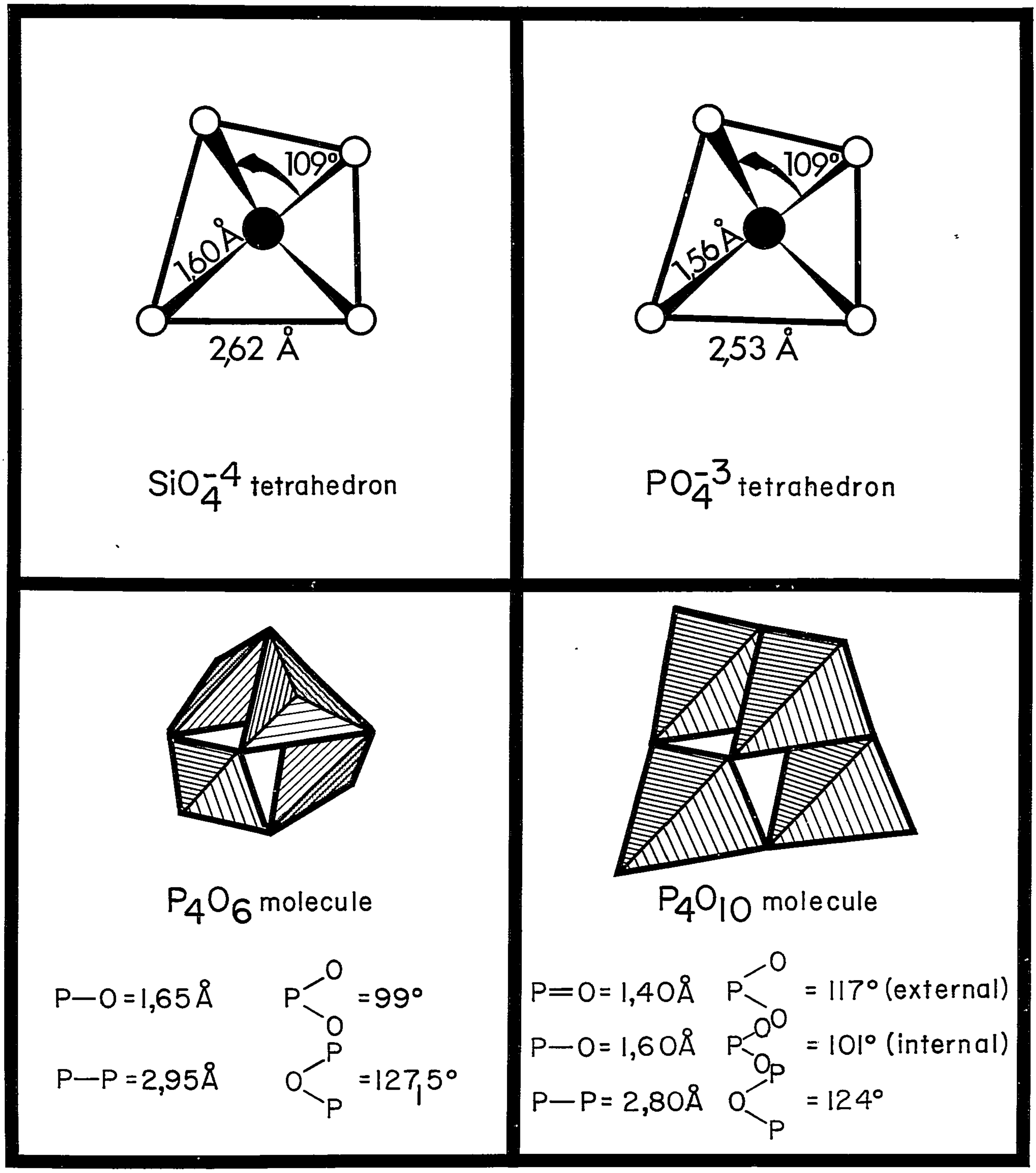


FIGURE 5

Structures of SiO_4^{-4} (25), PO_4^{-3} (24-28), P_4O_6 (29), and P_4O_{10} (29-31). In the case of P_4O_6, the phosphorus is positioned at the outer tips of the trigonal pyramids. In the case of P_4O_{10}, the phosphorus is located in the center of the tetrahedra; all other positions are occupied by oxygen.

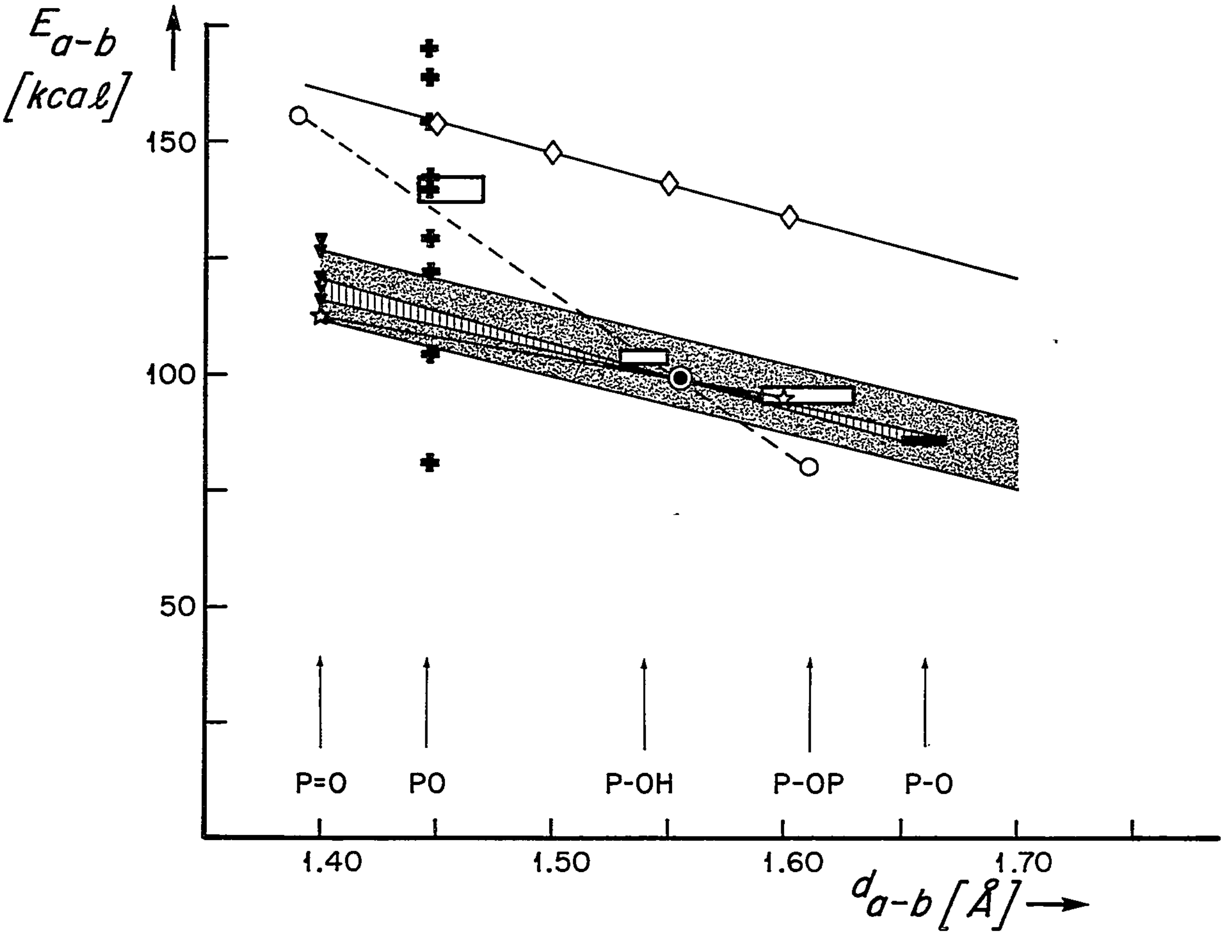


Figure 6a

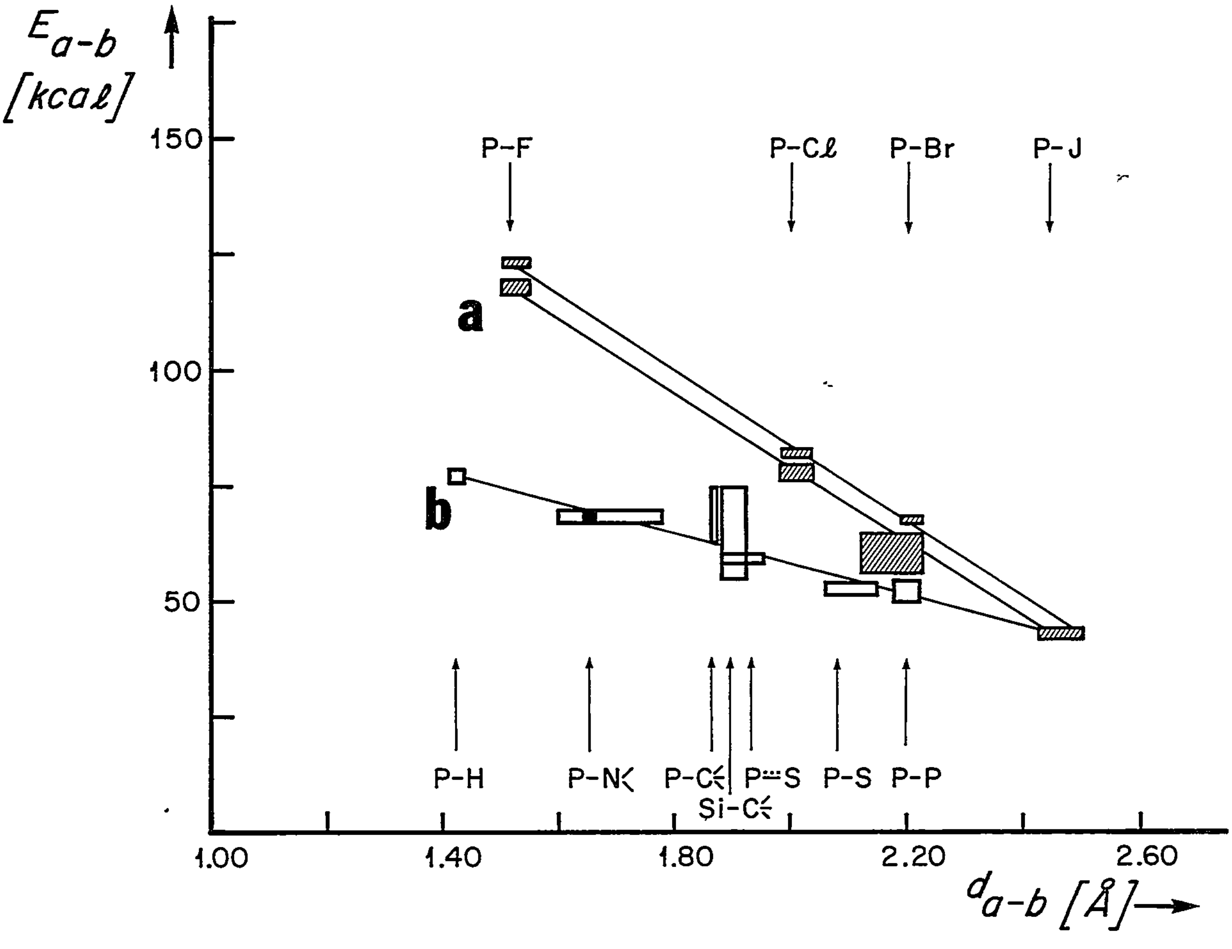


Figure 6b

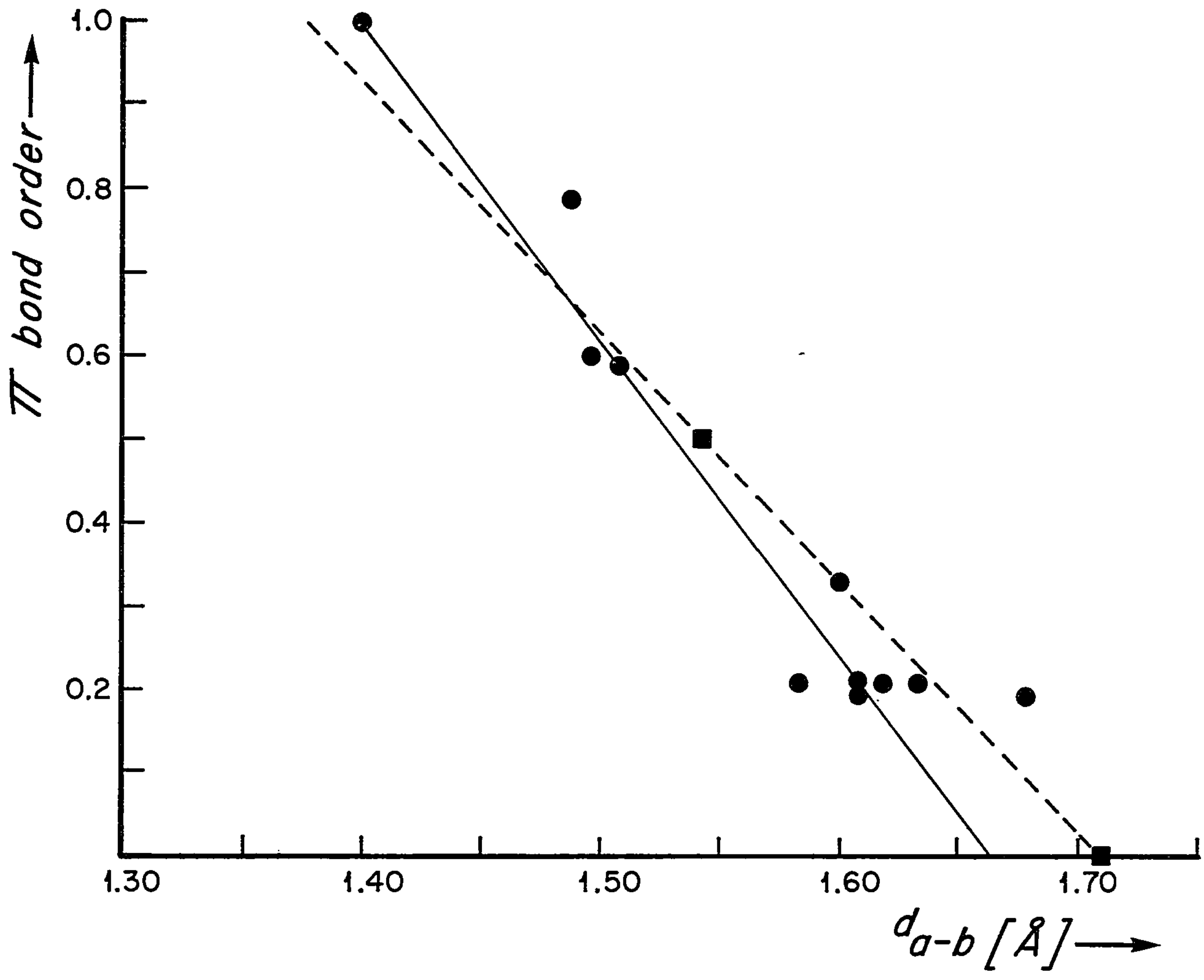


Figure 6c

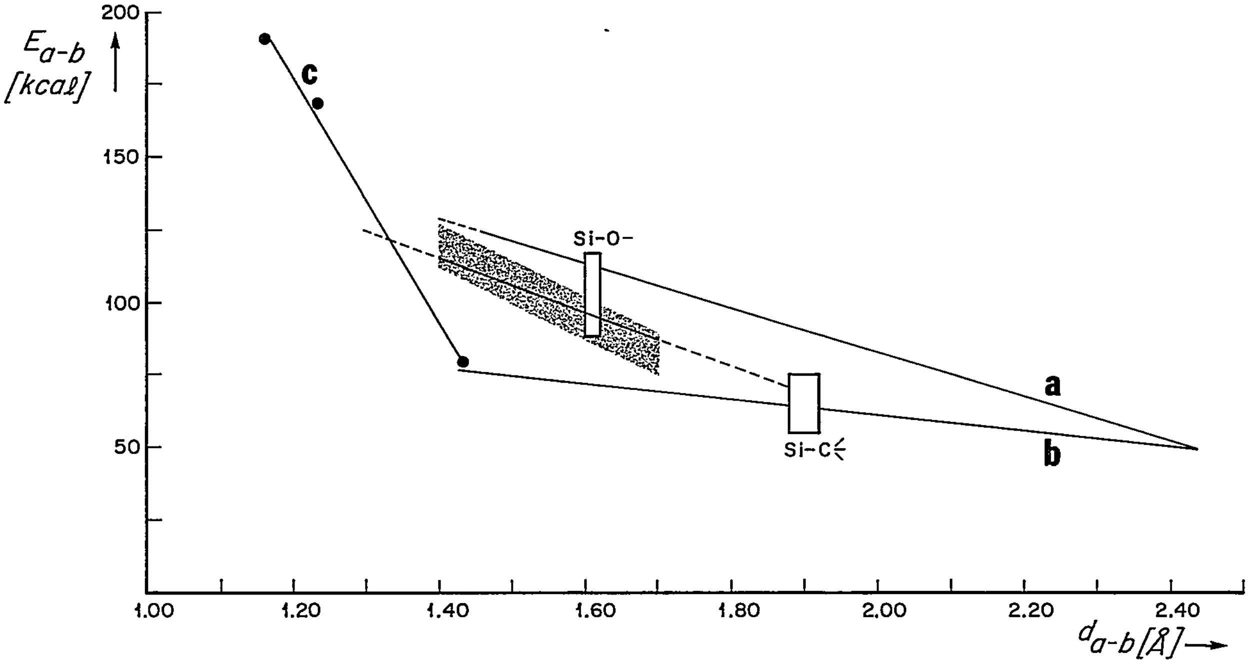


Figure 6d

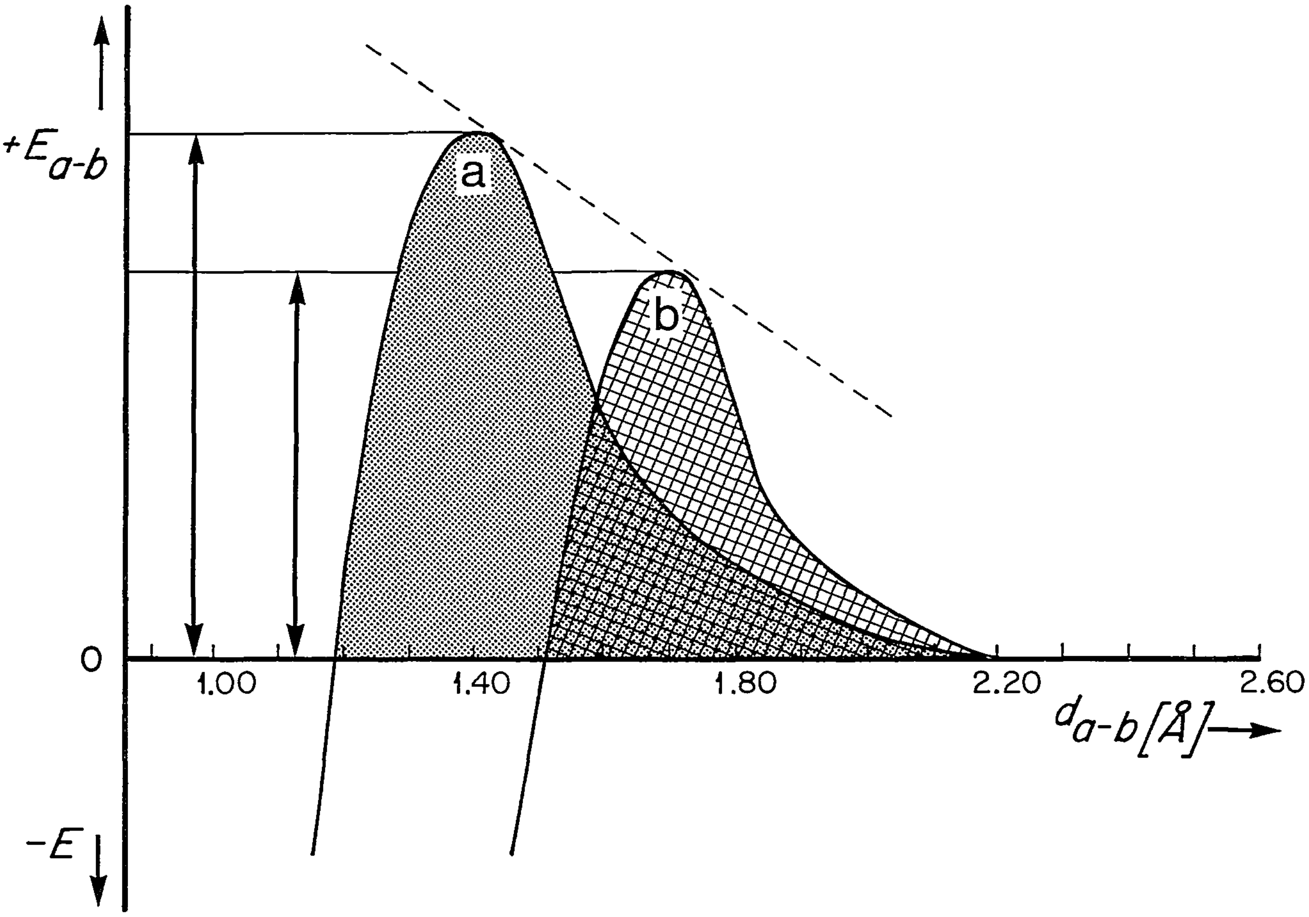


Figure 6e

FIGURE 6

The P - O bonding energies which confirm the assignment of the pi bonds is reflected in the flexible geometry of the PO_4 tetrahedron. The dashed line in Figure 6c shows the pi bond order as a function of the P - O bonding length (4). The solid line in Figure 6c illustrates the pi bond order dependence as determined by us. The dependence of bonding energy (E_{a-b}) from bonding length (d_{a-b}) of the P - O bond, is graphically presented in Figure 6a. To obtain a line segment expressing this relationship, two well-established reference values were used. The first reference point is allocated by the bonding energy of a single bond (P - O = 86 kcal) (20) in the P_4O_6 molecule with its distance (see Fig. 5) of P - O = 1.65 - 1.67 Å (11). The second reference point is calculated as follows. The heat energy of formation of the P_4O_{10} molecules is 1585 kcal both for theoretical and empirical reasons (37-39). This value is divided by the number of the P - O bonds (sixteen in P_4O_{10}) and plotted against the mean bonding lengths of 1.55 Å, i.e. $(3 \cdot 1.60 + 1.40) : 4 = 1.55$ Å (see Fig. 5). The interpolation of this line segment gives an E_{a-b} in the range of 116-120 kcal for the double bond (P=O) at 1.40 Å. These data agree with the observed values obtained upon oxidation of phosphorus compounds (e.g. P_4O_6 versus P_4O_{10} = 115 kcal (40). The observed (P=O) double bonding energies are shown as triangles in Figure 6a (17). On the basis of the suggested pi bond order and distance relation, the values of the in situ bonding energies (P=O = 112 kcal; and P - O = 95 kcal (20) fall completely within the range of the calculation error. These values are indicated as stars in Figure 6a and the connecting line intersects the "average point" at 1.55 Å.

The open boxes represent the values for the P - OH and P - OP bonds (41). Older concepts on P - O bonds and distance relation are shown as a dashed line P - O = 80 kcal, P = O = 155 kcal (42). The shaded area covers the range of the P - O bonding energy of all the data available and their standard deviation. This range characterizes the flexible geometry of the PO_4 tetrahedron. It is noteworthy that the calcualted P=O bonding energies, as a function of the bonding distance by means of the Morse-function (34), result in an identically shaped curve as the one determined by us. This curve is plotted in Figure 6a; the reason that this curve does not overlap ours is related to the different bonding energies of 156 kcal assumed for the P=O bond. The more probable value is 140 kcal (8, 20, 44). For comparison, the energy values of the PO molecule are included and shown as crosses (43). The most likely value amounts to 140 kcal and is indicated as an open box (20, 44).

In Figure 6b, the bonding energy, as a function of the bonding length, is plotted for the phospho-halogenides (curve a) and co-valent phospho-compounds (curve b) (8, 11, 20, 21, 41, 45, 46). This illustration is shown for comparative purposes and to further underline the linear relationships between bonding energy and bonding length.

A summary of the various bonding energies including those of Si - O and Si - C compounds (45 - 50) is shown in Figure 6d. One should point out that the PO_4 tetrahedron intersects the bonding area of the SiO_4 tetrahedron.

Rather informative is the comparison of the bond distance relationships between C - O and P - O bonds. In the case of the C - O bonds (line c), the bonding energy (E_{a-b}) is strongly dependent on the bonding distance (d_{a-b}), while this is not so for P-O bonds. Dependence of the phosphorus-oxygen bonding energy, in regard to the bonding length of the PO_4 tetrahedron, is indicated by a line which intersects the grey-shaded area at an angle. The values:

(a) P - O : d = 1.70 Å , E = 97 kcal ; (b) P = O : d = 1.40 Å, E = 115 kcal

In Figure 6e two curves are drawn (schematic) showing the interaction energies as a function of the bonding distances and the way these linear relationships (dashed line) come into existence.

in the geometry of PO_4 tetrahedra accounts for the fact that PO_4 polymers are able to bind metals of different valences and sizes. Unfortunately, this fact is frequently misinterpreted to be a result of a weak metal-phosphate bonding.

A representative series of isotype crystals* is listed in Table 3 to document the close relationship between silicates and phosphates. The fact that over a wide range of different cell dimensions, phosphates show such a striking resemblance to silicate structures supports the inference that the interactions between metal ions and phosphate and silicate tetrahedra are similar for both tetrahedra types concerned.

The two groups, i. e. SiO_4 and PO_4, exhibit differences in their reaction kinetics with metals. Whereas metal exchange in phosphate systems, e.g. ion exchange, is in the order of 10^3 sec., the corresponding value for silicate systems is about 10^7 sec. These differences can be linked to the flexibility of the PO_4 group which can adjust to different metals with a variable bonding state geometry. Such a flexibility does not exist for the SiO_4 tetrahedra.

To illustrate the molecular space linkage of PO_4 groups having metal ions as cross links, the crystallography of certain minerals is outlined. The common naturally occuring phosphate minerals represent isolated tetrahedra monomers which are linked together via metals and which form chains and layers. The unique

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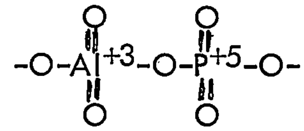
* isotype crystals are characterized by having the same space group, similar cell dimensions and equal proportions of the corresponding molecular units, e.g.
Libethenite $Cu_2[OH|PO_4]$ orthorhombic a = 7.47 Å, b = 8.31 Å, c = 5.83 Å
Andalusite $Al_2[O|SiO_4]$ orthorhombic a = 7.76 Å, b = 7.90 Å, c = 5.56 Å

TABLE 3

Phosphate	Formula	Type	Formula	Silicate	Literature
Triphyline	$Li(Fe, Mn)[PO_4]$	Monomer	$(Mg, Fe)_2[SiO_4]$	Olivine	51
Natrophilite	$Na(Mn, Fe)[PO_4]$	Monomer	$(Mg, Fe)_2[SiO_4]$	Olivine	52
Xenotime	$Y[PO_4]$	Monomer	$Zr[SiO_4]$	Zircon	53
Monazite	$(Ce, La, Pr, Nd)[PO_4]$	Monomer	$Th[SiO_4]$	Huttonite	54
Hurlbutite	$CaB_2[PO_4]_2$	3-dimensional network	$BaAl_2[SiO_4]$	Paracelsian	69, 70
Libenthenite	$Cu_2, OH[PO_4]$	Monomer	$Al_2, O[SiO_4]$	Andalusite	55
Herderite	$CaBeF[PO_4]$	Monomer	$Ca(OH)B[SiO_4]$	Datholite	56
Mg-Pyrophosphate	$Mg_2[P_2O_7]$	Dimer	$Sc[Si_2O_7]$	Thorveitite	57, 58
Pyromorphite	$Pb_{10}[Cl_2 \vert (PO_4)_6]$	Monomer/Dimer	$Pb_6Ca_4[Cl_2 \vert Si_2O_7)_3]$	Nasonite	59
Li-meta-Polyphosphate	$[LiPO_3]_n$	II-periodic chain	$[Ca, Mg(SiO_3)_2]_n$	Diopside	60
Rb-meta-Polyphosphate	$[RbPO_3]_n$	II-periodic chain	$Mg[SiO_3]_n$	Enstatite	61
Maddrell salt*	$[NaPO_3]_n$	III-periodic chain	$[CaSiO_3]_n$	Beta-Wollastonite	62
Kurrol salt	$[NaPO_3]_n$	IV-periodic chain	$[Na_2FeTi(SiO_3)_4]_n$	Neptunite	63-66
Kurrol salt	$[AgPO_3]_n$	IV-periodic chain	$[Na_2FeTi(SiO_3)_4]_n$	Neptunite	64, 66
Beryllonite	$NaBe[PO_4]$	3-dimensional network	$CaMn_2Be_3[SiO_4]$	Trimerite	67
Hurlbutite	$CaB_2[PO_4]_2$	3-dimensional network	$CaB_2[SiO_4]_2$	Danburite	68
Berlinite	$Al[PO_4]$	3-dimensional network	SiO_2	Quartz*	71
Al-Phosphate	$Al[PO_4]$	3-dimensional network	SiO_2	Tridymite	72
Al-Phosphate	$Al[PO_4]$	3-dimensional network	SiO_2	Cristobalite*	25
Ga-Phosphate	$Ga[PO_4]$	3-dimensional network	SiO_2	Cristobalite*	25

* high temperature form

position of three-valent (M^{+3}) metals such as Al and Ga within a three-dimensional PO_4 tetrahedra network, which is similar to that of silica, is a function of their valence (73) and ionic radius:



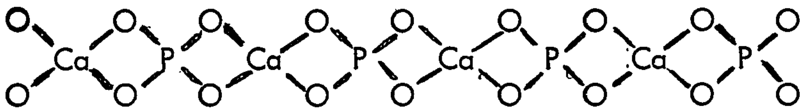
$$-O-\overset{\overset{O}{\|}}{\underset{\underset{O}{\|}}{Al}}{}^{+3}-O-\overset{\overset{O}{\|}}{\underset{\underset{O}{\|}}{P}}{}^{+5}-O- \quad ; \quad [M^{+3}P^{+5}] = [Si^{+4}Si^{+4}] \qquad \begin{matrix} M^{+3} = Al^{+3} & r = 0.50\ \text{Å}; \\ \text{and } = Ga^{+3} & r = 0.62\ \text{Å} \end{matrix}$$

a) ionic radius: condition, b) valence condition

The remarkable properties of Al-PO_4, which resembles crystalline silica in terms of phase relationships and polymorphism, can thus be understood (25, 69, 71, 72). Other three-valent elements with greater ionic radii, such as Sc (0.81 Å), In (0.81 Å), and Tl (0.95 Å), do not permit a 4-oxygen-co-ordination. The structures of $GaPO_4$ and $InPO_4$ are shown in Figures 7 and 8.*

In the case of other metals, the mono-, di-, and tri-phosphates form lattices, in which the phosphate groups are held together by means of metal oxygen polyhedra bridges and are arranged in the form of single, double, or triple chains. In contrast to silicates, the phosphates adjust to each metal ion and to a certain chemical environment with a specific space geometry.

The structure of $CaHPO_4[H_3PO_4 \cdot H_2O]$ consists of corrugated layers of $CaHPO_4$ and $H_3PO_4 \cdot H_2O$, in which the sheets of PO_4 tetrahedra are linked by oxygen co-ordinated calcium.



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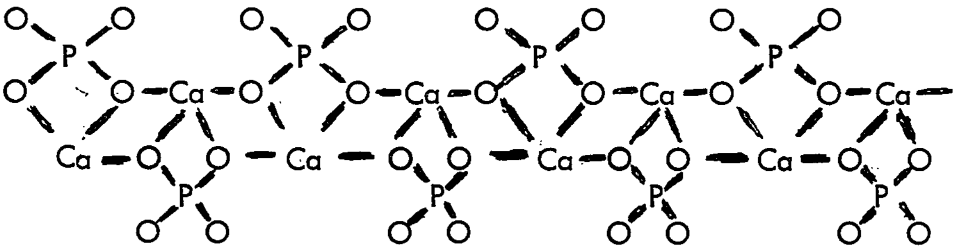
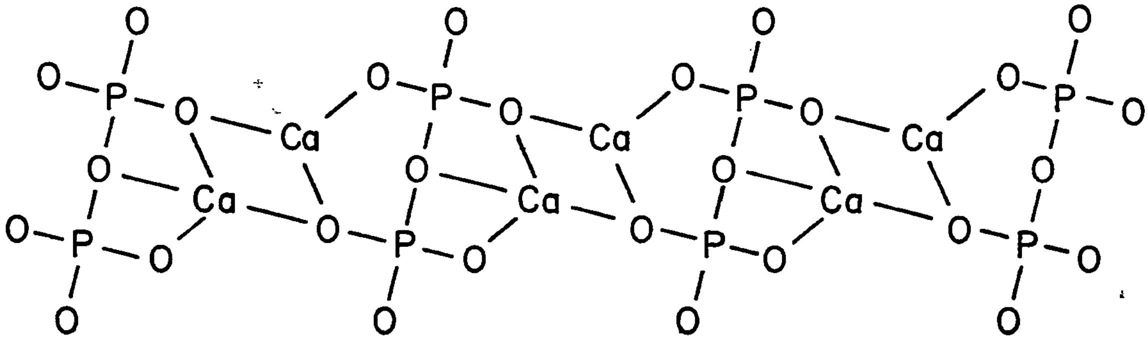
* Certain dynamic properties of membranes can be tested by means of the elements B^{+3}, Al^{+3}, and Ga^{+3} which should interact with the PO_4 tetrahedra of phospholipids and produce specific, and strongly cross-linked lattices.

The resulting chains become connected by the sharing of polyhedra edges (Figs. 9 and 10) and form corrugated layers which are joined by water and hydrogen bridges. The PO_4 tetrahedra and Ca^{++} ions produce double chains in case of the CaH PO_4 structure (76) which are linked by Ca-O bonds and result in a distorted sheet-type structure (Fig. 11):



Similar relationships are established for BaH PO_4 (77), or in the structure of pseudo-malachite $Cu_5(PO_4)_2(OH)_4$ (26), which exhibits two kinds of distorted octahedral chains cross-linked to layers by the sharing of oxygen at two edges of the copper octahedron. The elimination of hydrogen ions from the PO_4 tetrahedron, and consequently the lack of proton compensation for the oxygens, produces complicated molecular networks of which libethenite $Cu_2OH(PO_4)$ is an example. Equally complex is the structure of apatite $Ca_5F(PO_4)_3$ which consists of chains of oxygen polyhedra (78).

The diphosphate, <u>beta</u>-$Ca_2P_2O_7$, is characterized by Ca-P_2O_7 chains which are arranged in the form of corrugated layers (79):



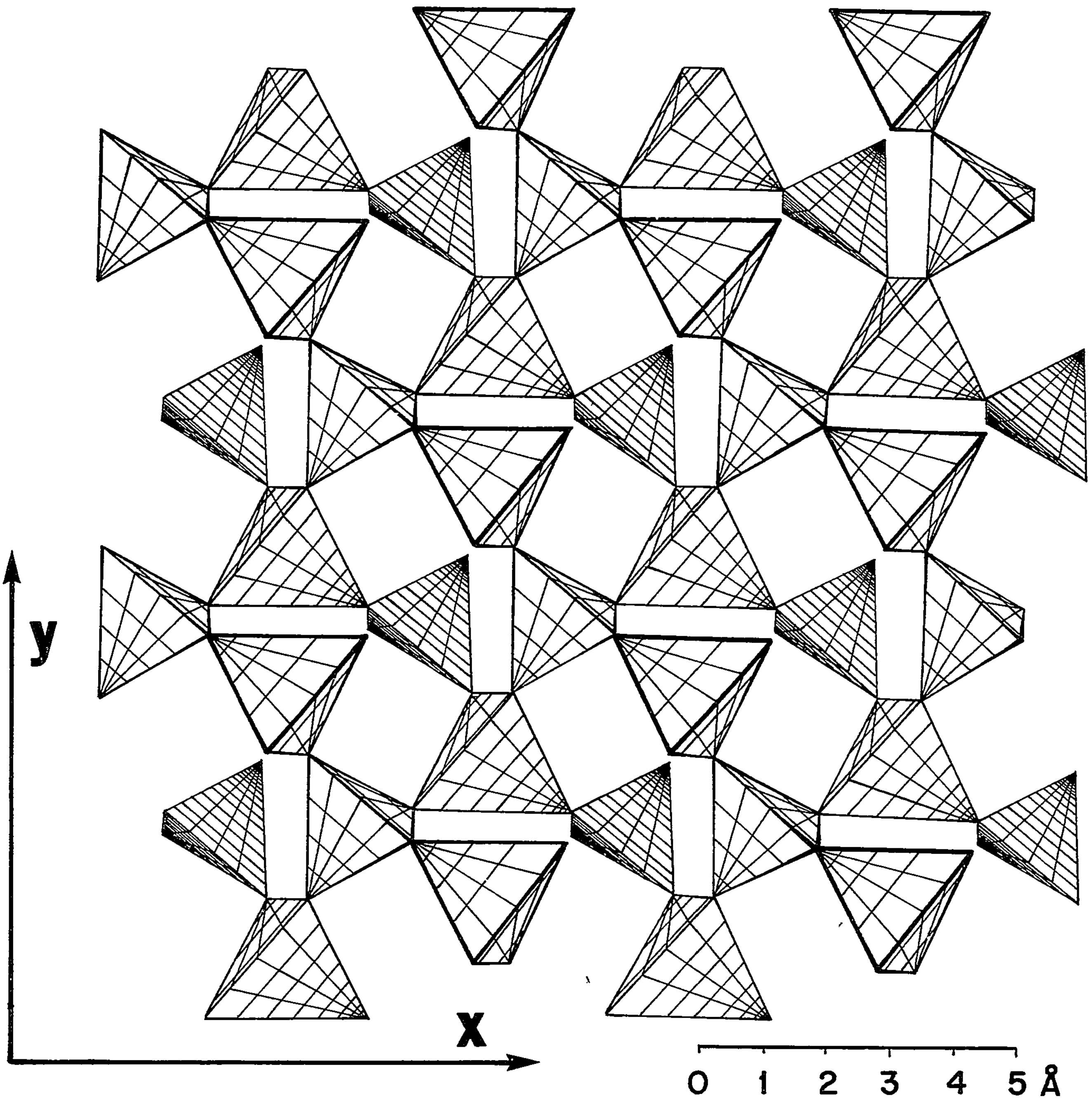


FIGURE 7

Gallium phosphate ($GaPO_4$);[001] projection of the original structure. Each PO_4 tetrahedron is joined by four additional tetrahedra, forming a three-dimensional network. The resulting helices are shown by a cross-line pattern and a spacial partition. These structures resemble SiO_4 structures. The gallium ion oxygen co-ordination tetrahedra are the larger ones. The cell dimensions for $GaPO_4$ are: a (=b)= 6.967 ± 0.003, and c = 6.866 ± 0.003 Å (25).

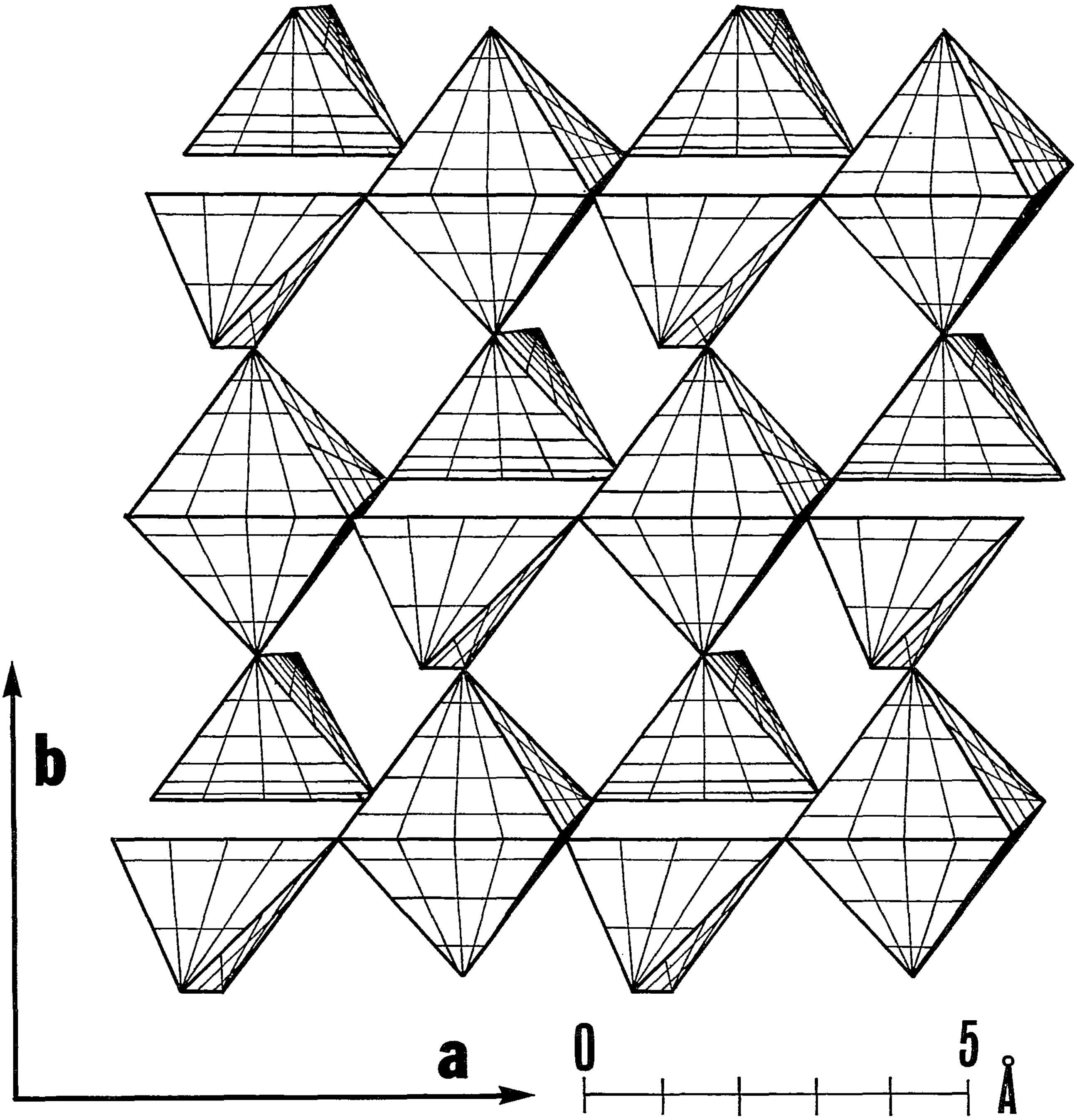


FIGURE 8

Indium phosphate ($InPO_4$); for the purpose of a better three-dimensional graphic pattern, the projection plane 001 is slightly distorted to reveal the tetrahedra structure. The indium is co-ordinated to 6 oxygens resulting in a distorted octahedron. The InO_6 octahedra have two edges with polyhedra from the alternate layers in common (parallel to a). The phosphate group represents, in some approximation, a regular tetrahedron having a P - O distance equal 1.56 A. The cell dimensions for $InPO_4$ are: a = 5.308, b = 7.851, and c = 6.767 A (24).

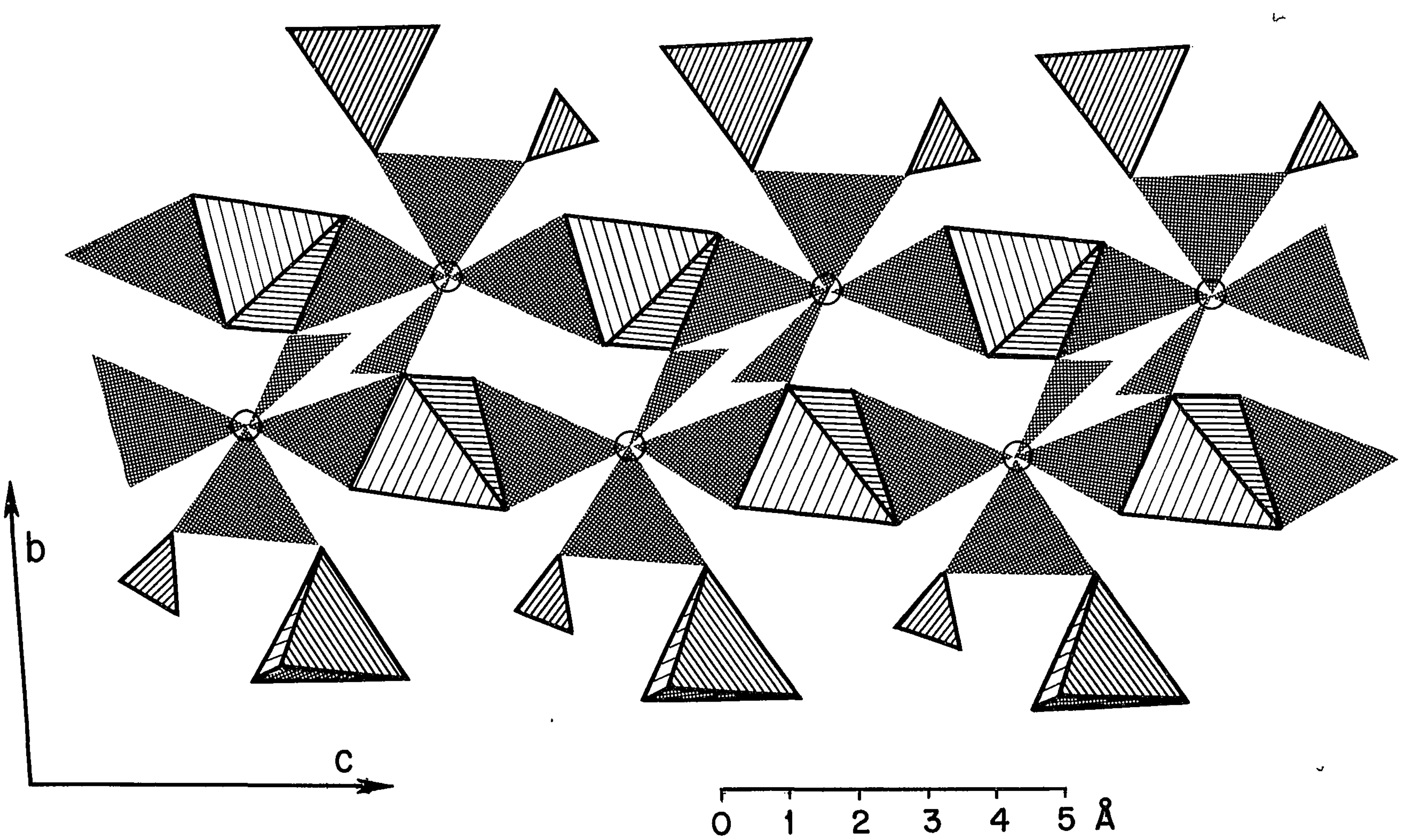


FIGURE 9

Two chains of $CaHPO_4 \cdot H_3PO_4 \cdot H_2O$ projected down the a axis are shown: the right chain is positioned higher than the left chain. By means of the shaded area it is shown how the $Ca^{++}O_8$ polyhedra within the chain join the PO_4 tetrahedra together; the calcium is positioned at the intersection of the shaded areas. They further promote cross-links between the individual chains and result in the formation of corrugated layers (001). The PO_4 tetrahedra in outer position alternate with H_2O molecules, which are drawn as small triangles. The layers are interconnected by means of hydrogen bonds. The cell dimensions are: a = 5.61, b = 11.89, and c = 6.46 Å.

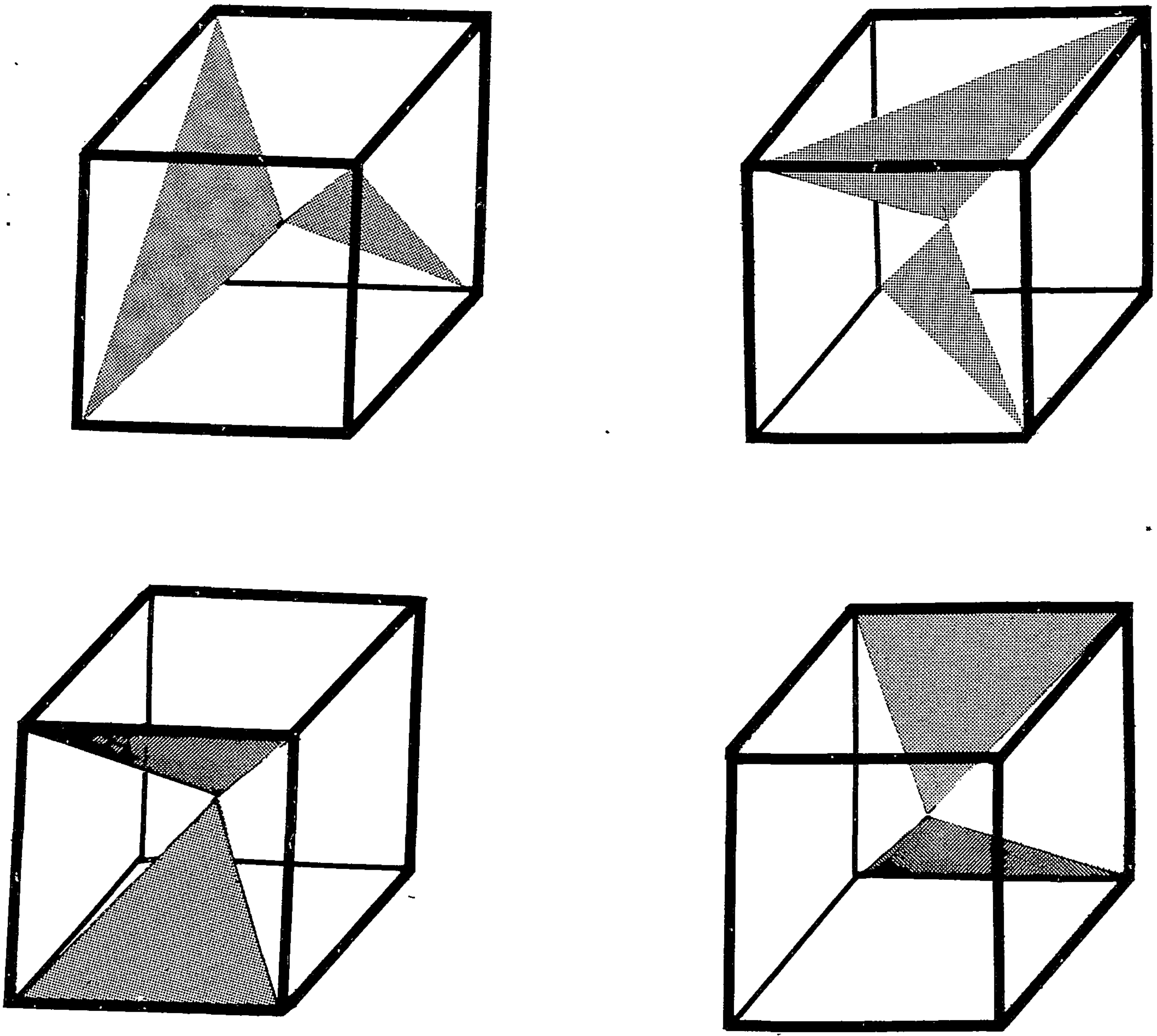


FIGURE 10

Metal ion co-ordination polyhedron $M^{+n}O_8$ (cube). The metal ion is positioned in the center, and the oxygens at the corners. The shaded areas which lead from the central cation to the co-ordinated oxygens, should assist to recognize the Ca^{++} co-ordination polyhedra in Figure 9 and 11.

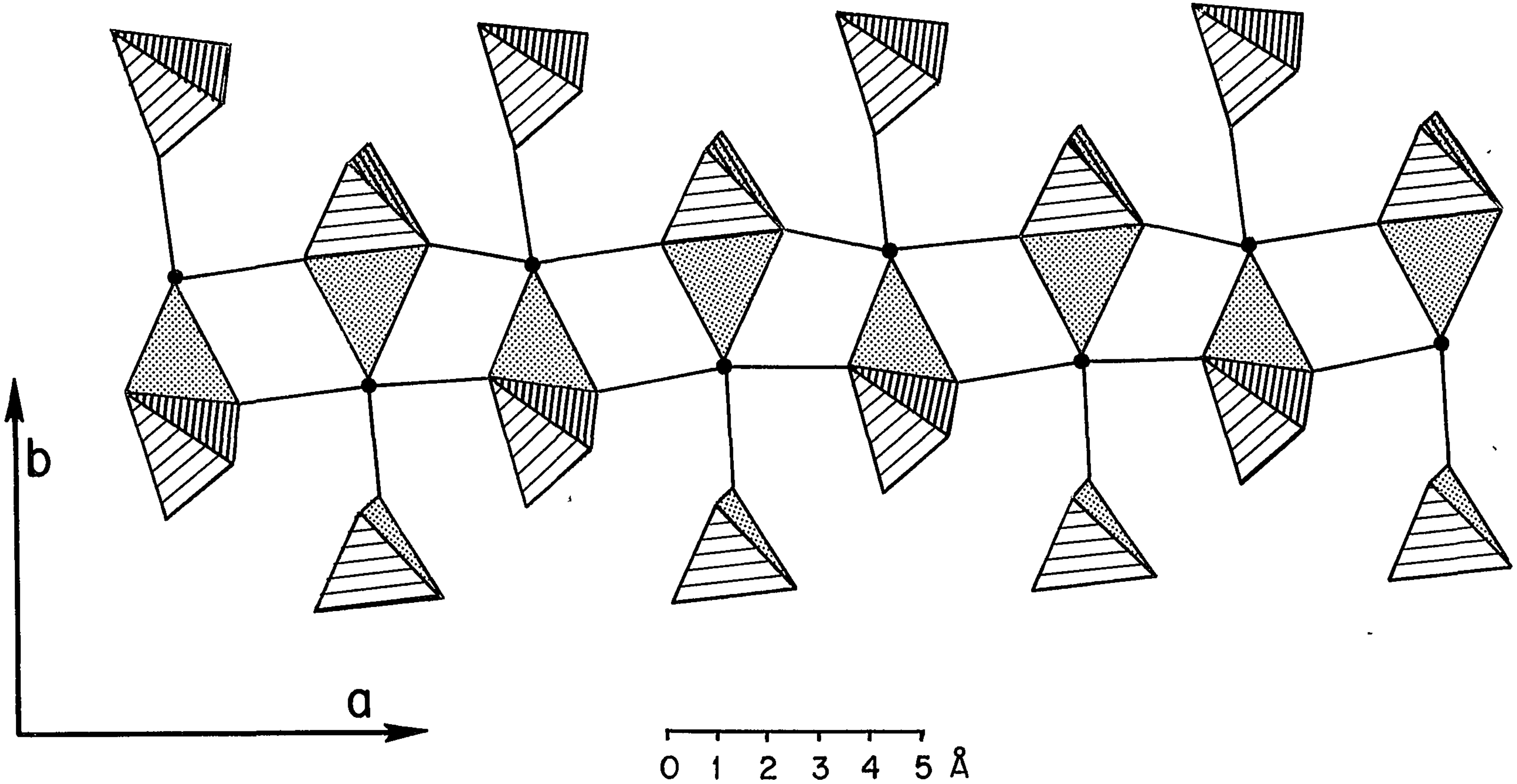


FIGURE 11

Double chain in $CaHPO_4$ structure, projected down the c axis. Connecting lines and shaded areas indicate the $Ca^{++}O_{7-8}$ co-ordination polyhedra which join the PO_4 tetrahedra, so that a double chain of Ca - PO_4 - Ca extends along the a-axis; Ca^{++} is a black dot. The individual chains, cross linked transversely in the b direction, result in the formation of a distorted sheet of atoms roughly in the (001) plane. The cell dimensions are: a = 6.90 ± 0.01, b = 6.65 ± 0.01, and c = 7.00 ± 0.01 Å (76).

The structure of sodium triphosphate $Na_5P_3P_{10}$ is that of a three-dimensional polyhedra packing which consists of cross-linked NaP_3O_{10} - Na chains (80, 81). The anions are linked end-on by the Na-octahedra and produce chains which have a continuous sheet-type arrangement*.

The PO_4 units can be linked together by sharing of their corners and thus can result in high-molecular chains of a distinct repeat pattern, e.g. two, three, etc.; the molecular arrangement can hereby be that of polyphosphate $[PO_3]_n$ helices (61, 62).

The phosphate structures selected for this discussion should be used as a demonstration for the diversity in metal-oxygen co-ordinated molecular networks. The PO_4 tetrahedra geometry may range from a regular PO_4 tetrahedron, for instance, $Cu_5(PO_4)_2(OH)_4$ or $GaPO_4$, to extremely distorted geometries. Even oxygen acting as a bridge, i.e. P — O — P can be co-ordinated with metal ions as shown for the beta $Ca_2P_2O_7$.

Bio-phosphates, such as adenosine-5-phosphate (82) or adenosine-3-phosphate dihydrates (83), exhibit linkages identical to those observed for inorganic structures. The PO_4 units linked to the ionar groups of aromatic rings give rise to zigzag chains which run through the crystal (Fig. 12). The intra molecular linkage of the alternating chains to a corrugated sheet is similar to the inorganic phosphate

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* Certain instructive relationships in the solubilities of two forms of triphosphate, as a function of the oxygen co-ordination state of sodium, are mentioned. Form I cakes readily when treated with water and dissolves only slowly. Its modification, form II, however, dissolves normally. Form I contains tetrahedrally co-ordinated sodium aside of the octahedral sodium. Sodium in tetrahedral co-ordinntion is easily hydrated, producing a solid phase of hexahydrate $Na_5P_3O_{10} \cdot 6\ H_2O$. In contrast, form II only contains octahedral sodium (60).

structures. They are linked by water molecules and by the hydrophobic bonding of purine units which form chains which follow crosswise to the zigzag chains. The alternating sheets, similar to $CaHPO_4 \cdot 2H_2O$, are held together by water molecules.

In case no polar structural units are present, the PO_4 groups are associated by themselves by means of:

$$P -- O....H -- O -- P$$

bridges, as shown for dibenzyl phosphoric acid (84) (Fig. 13).

In conclusion, the phosphates follow four structural formation principles:

(1) linkage of phosphate units via metal bridges,

(2) establishment of chains,

(3) cross-linkage of chains resulting in corrugated layers, and

(4) cross-linkage of layers resulting in distinct three-dimensional molecular networks.

These sequences of formation principles are additive in nature, but also independent from each other. That is, the occurrence of one linkage type does not require nor exclude another linkage type. In view of this flexible building pattern, it is not surprising that phosphates play an essential part in biocrystallographical structures in living systems. All four of the listed structural formation principles can be observed in biochemical reactions*.

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* There is no biological advantage by substituting PO_4 for SiO_4. First of all, the speed of metal exchange reactions in silicate systems is lower by a few orders of magnitude. Secondly, silicate polyhedra show no flexibility in terms of bonding state geometry, nor do silicates permit the formation of different structural units within the same molecular framework. Similar arguments can be listed which speak against a possible substitution of carbon for silicon in biochemical systems.

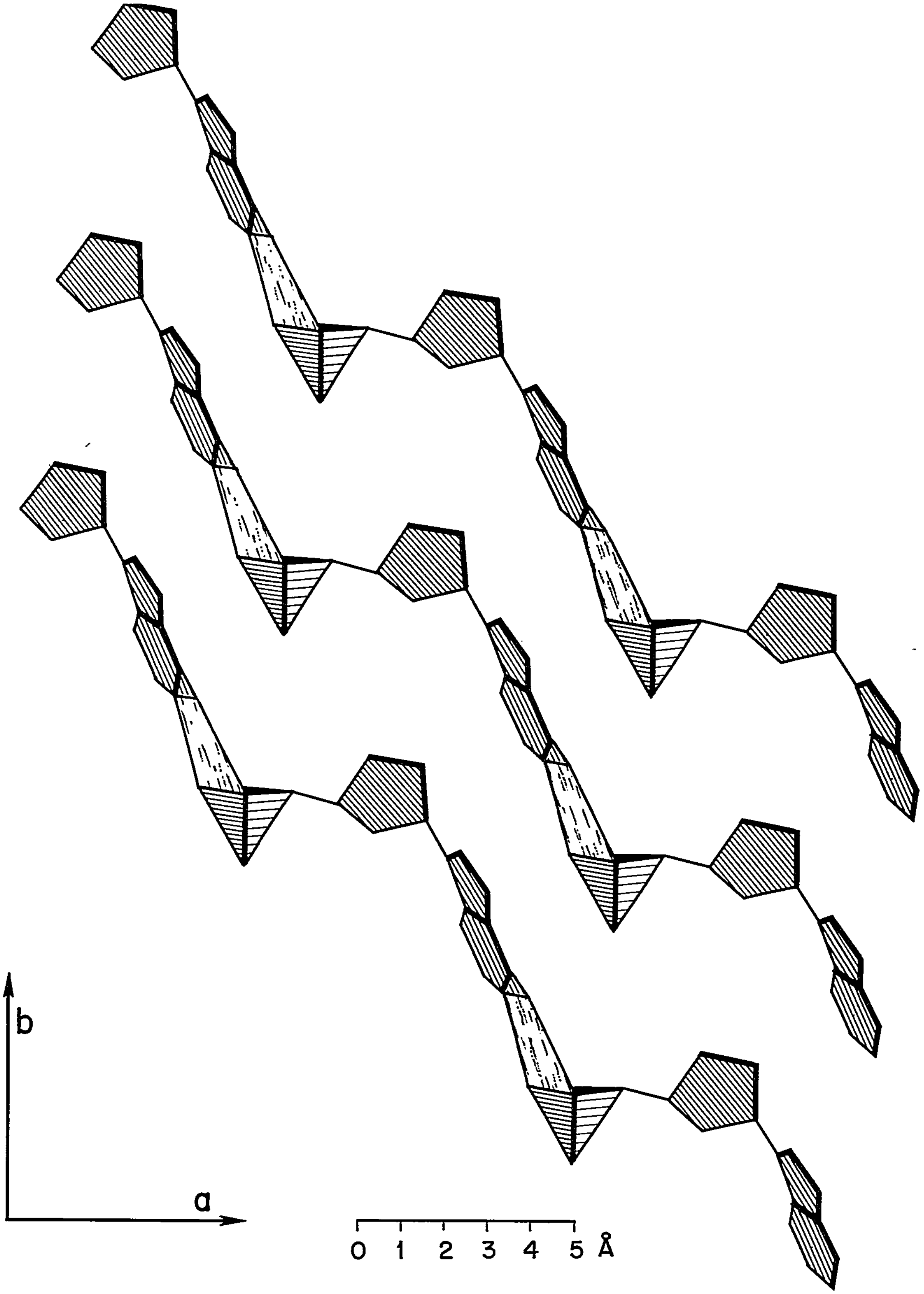

FIGURE 12

Molecular structure of adenosine 3 - phosphate dihydrate (adenylic acid b); the view of the structure is along the c axis. The phosphate oxygen atoms O_6 and O_8 are linked by means of hydrogen bonds to the purines (indicated by the broken-line stippled area), resulting in the constitution of chains running through the structure. The water molecules, interconnecting the individual chains, have been deleted for graphical reasons. The ribose ring oxygen points towards the adjacent base. The cell dimensions are:

a = 9.939 ± 0.005, b = 6.343 ± 0.002, and c = 11.896 ± 0.005 Å (83).

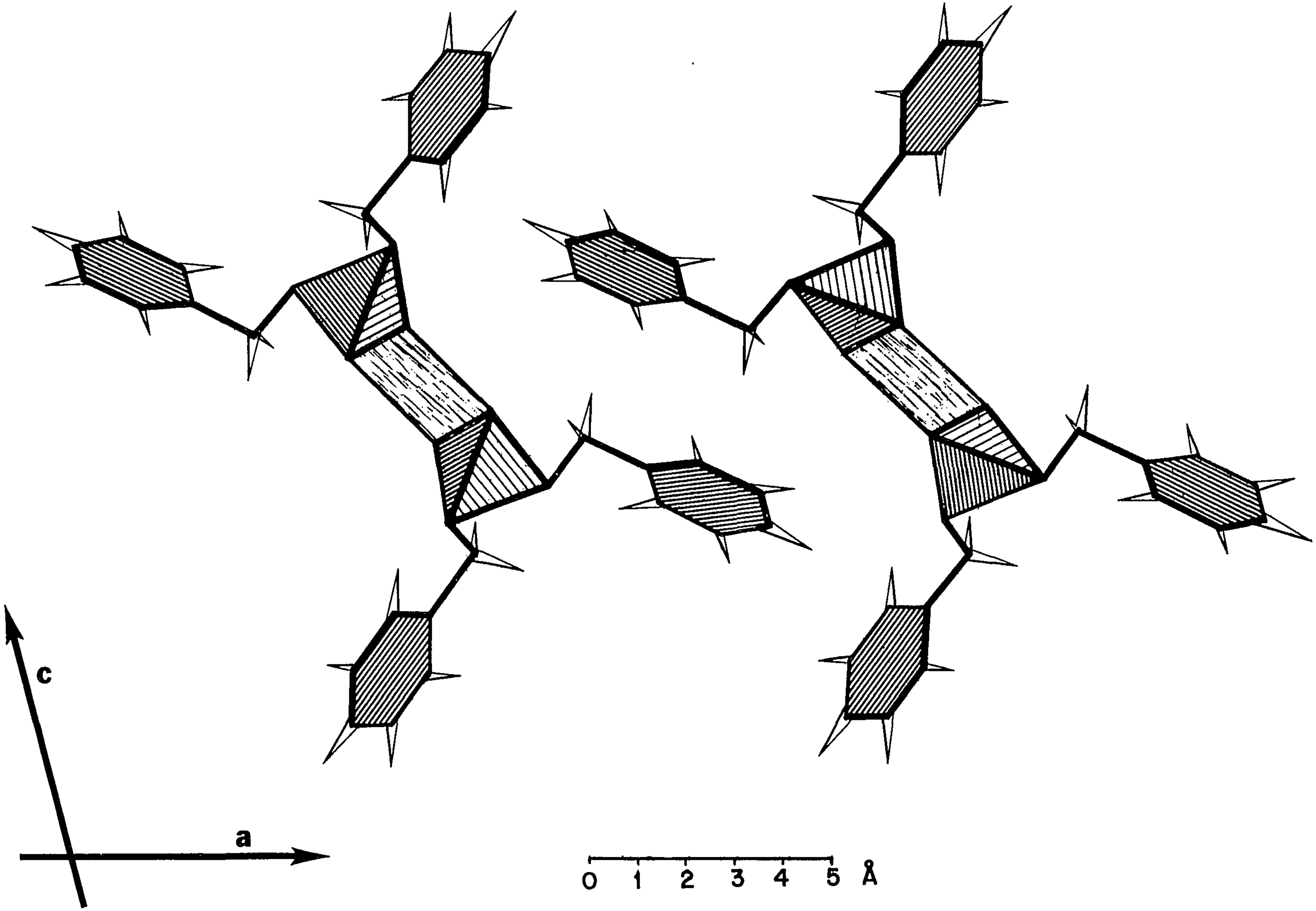

FIGURE 13

Molecular structure of dibenzyl phosphoric acid; the view of the structure is down the b axis. The two individual molecules are related to one another by the screw axis and the center of symmetry. The dihedral angles of two phosphate tetrahedra joined by hydrogen bridges (broken line/stippled areas) is near to 90°. The cell dimensions are: a = 20.29, b = 5.71, and c = 12.65 Å (84).

THE RÔLE OF THE PO_4 TETRAHEDRON AS BIOCRYSTALLOGRAPHICAL ELEMENT

To the extent as nitrogen increases the biocrystallographical order of organic molecules via hydrogen bridges, so does phosphorus in the form of PO_4. In biochemical systems, the oxygen in PO_4 tends to associate itself with metal ions and to fabricate an oxygen polyhedron having a cation in central position.(Fig. 14). The physical significance of this process is simply to alter the molecular order from a system containing randomly distributed particles to a system where the particles are present in a state of crystalloid assemblages. Inasmuch as these assemblages represent short-range order spacings, both well-defined stereo-structures and physical-chemical properties will be developed for these compounds. In this way, metal-oxygen co-ordinated polyhedra can act as functional elements in biochemical processes.

These viewpoints can be tested by examining empirical data. In case of the nucleic acids, where PO_4 groups play an essential part, two organization principles might be expected:

(1) the parallel bedding of aromatic molecules, with a minimum approach distance of 3.4 Å between adjacent planes, and which is governed by _pi_ electrons, is identical to that observed in the hexagonal network of carbon in graphite or in larger aromatic crystals, and

(2) a periodic distribution of PO_4 tetrahedra with a minimum approach distance of 6.7 Å and mean values of 7.0 to 7.5 Å depending on the kind of

metal-oxygen polyhedra involved. The molecular configuration of DNA, as inferred from X-ray diffraction studies, agrees with these values and thus supports our inferences (85-91).

A structural model of DNA in Na^{+1} co-ordination is presented (Fig. 15) in which the PO_4 tetrahedra and the $Na^{+}O_6$ octahedra are shown in their spacial dimensions. Metal ion-oxygen polyhedra exercise control on the shape of the macromolecules, and thus may introduce the stretching of polymer chains. This can be considered the ultimate reason, why a single stranded DNA will easily associate itself with another single stranded DNA resulting in a double helix. In contrast, the coupling of a single stranded DNA by itself in making a sharp bend (loop), and which is theoretically conceivable, will not take place due to the stabilization of the structure by means of oxygen co-ordinated metal ions.

The thermal transition temperature* of nucleic acids T_m is listed as additional proof of our concept. It has been shown (Fig. 16) that in case of synthetic homopolynucleotides (100) (e.g. inosinic acid in sodium salt solution), the transition temperature is linearly related to the logarithm of the ionic strength of the salt medium:

$$T_m = \text{const.} \times \log C_{Na}$$

Furthermore, divalent metal ions are more effective (generally in the order of 10^4) than monovalent metal ions in the preservation of the helical structure (100).

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* T_m represents the temperature at which structural changes of nucleic acids occur, e.g., the transition of a certain helical to another helical structure or to a randomly coiled state.

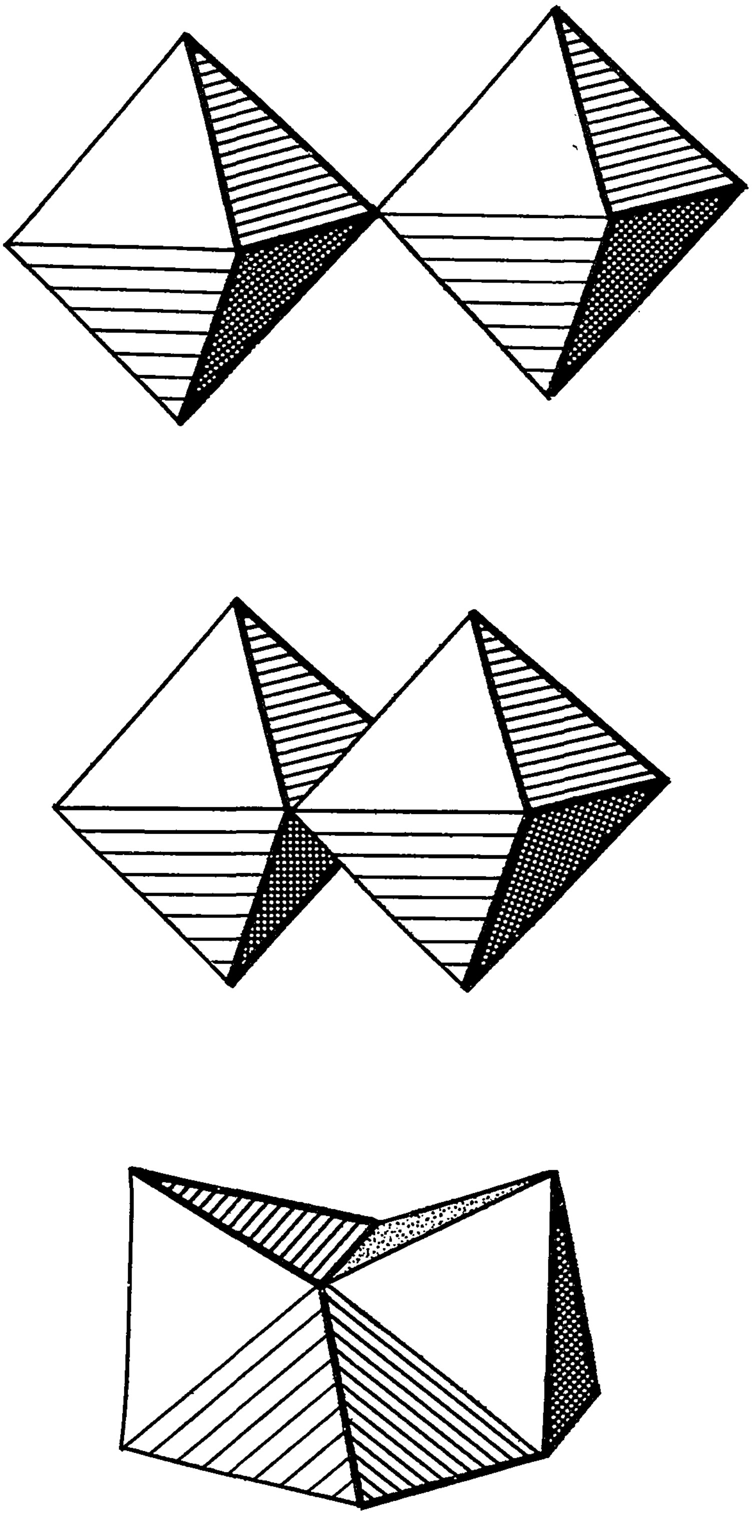


FIGURE 14

Schematic presentation of metal-oxygen polyhedra bridges involving (a) common edges, and (b) common corners as linkage elements. The smaller ions such as lithium, aluminum, magnesium, and sodium are octahedrally co-ordinated, whereas a cube is formed by larger ions such as potassium or barium. Calcium can occur in both, octahedron and cube (For comparison, see Figs. 10 and 18).

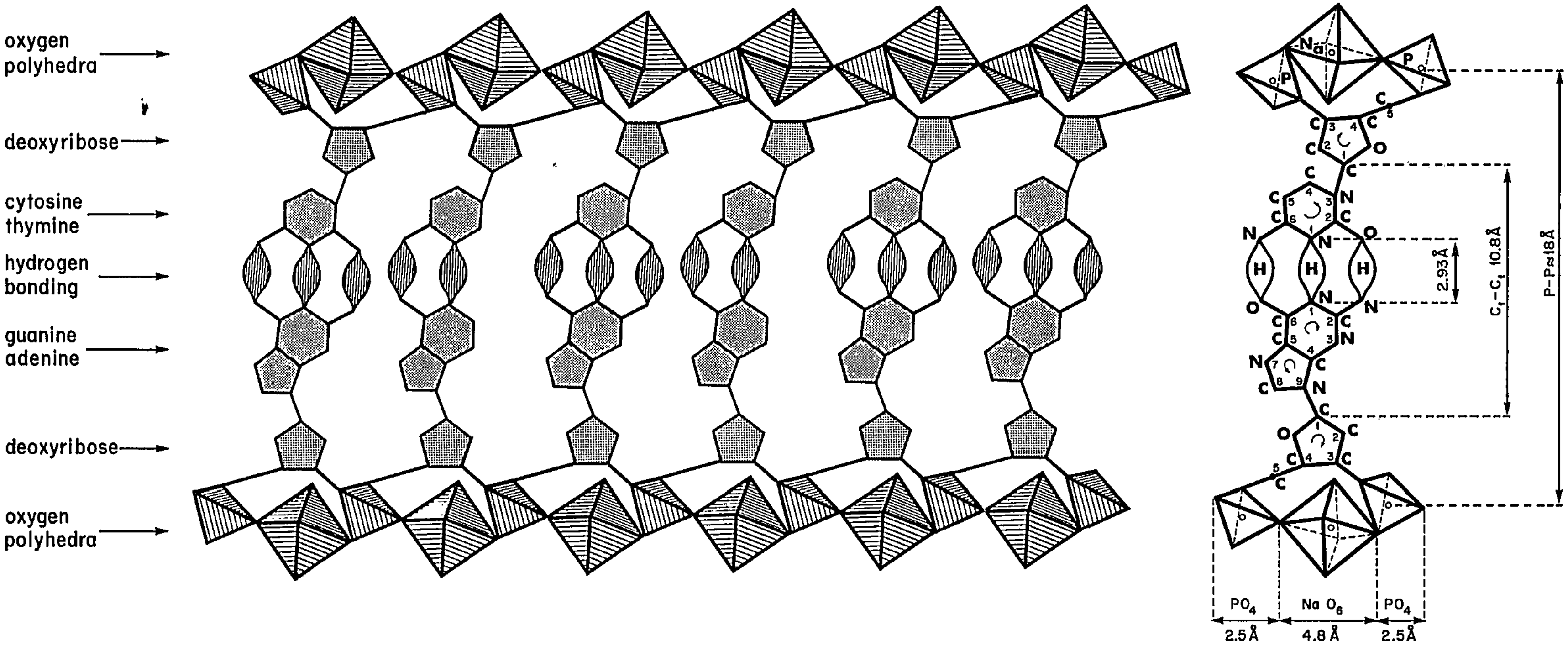

oxygen polyhedra
deoxyribose
cytosine thymine
hydrogen bonding
guanine adenine
deoxyribose
oxygen polyhedra
Na
P
P
H
H
H
2.93Å
C_1-C_1 10.8Å
P–P≈18Å
PO_4
Na O_6
PO_4
2.5Å
4.8Å
2.5Å

Figure 15

FIGURE 15

Model of DNA (schematic) showing metal co-ordinated phosphate groups. A sodium ion serves as linkage element between two phosphate tetrahedra by establishing an oxygen polyhedron. The intermolecular distance between (a) the hydrogen linked bases of the purines and pyrimidines, and (b) the glycosidic carbons are the most probable ones, and they have been determined following data in: (90, 91, 93-96). In addition, a weak C - H...O bonding could exist between thymine and adenine; its consequence for the electron bonding configuration in the aromatics would be considerable (83, 98, 99). It is also interesting to note that extremely short C - O distances (C_3 - O = 2.88 Å; C_2H - O_4 = 2.5 Å) are reported between deoxyribose and phosphate (89). The authors believe that the ultimate reason for the incorporation of sugars into the nucleic acids is related to the fact that the sugars will supply the oxygen necessary for the construction of the metal ion oxygen polyhedron (C_3 atom). It is theoretically conceivable that other biochemicals, such as hexane, could proxy for the sugars. Inasmuch as the OH groups are essential in the structural model for DNA as outlined by us, the logic of the sugar adoption becomes evident. Further support for the presence of metal ions in the DNA molecule can be found in electron spin resonance spectra (251-256). Not only do metal ions give DNA a structural order but interestingly enough they considerably reduce the photo damage caused by UV irradiation. Namely, ESR studies demonstrate that the yield of the thymine free radical in UV irradiated DNA is significantly quenched by paramagnetic metal ions linked to DNA (257-259). In contrast, the presence of water enhances the photo damage. Therefore, it is biochemically more logical to take advantage of metal ions rather than of water for radiation protection. That this inference is correct finds its ultimate proof in nuclear magnetic resonance measurements. These show that the amount of water linked to DNA must be neglibable and does not change even during denaturation (260).

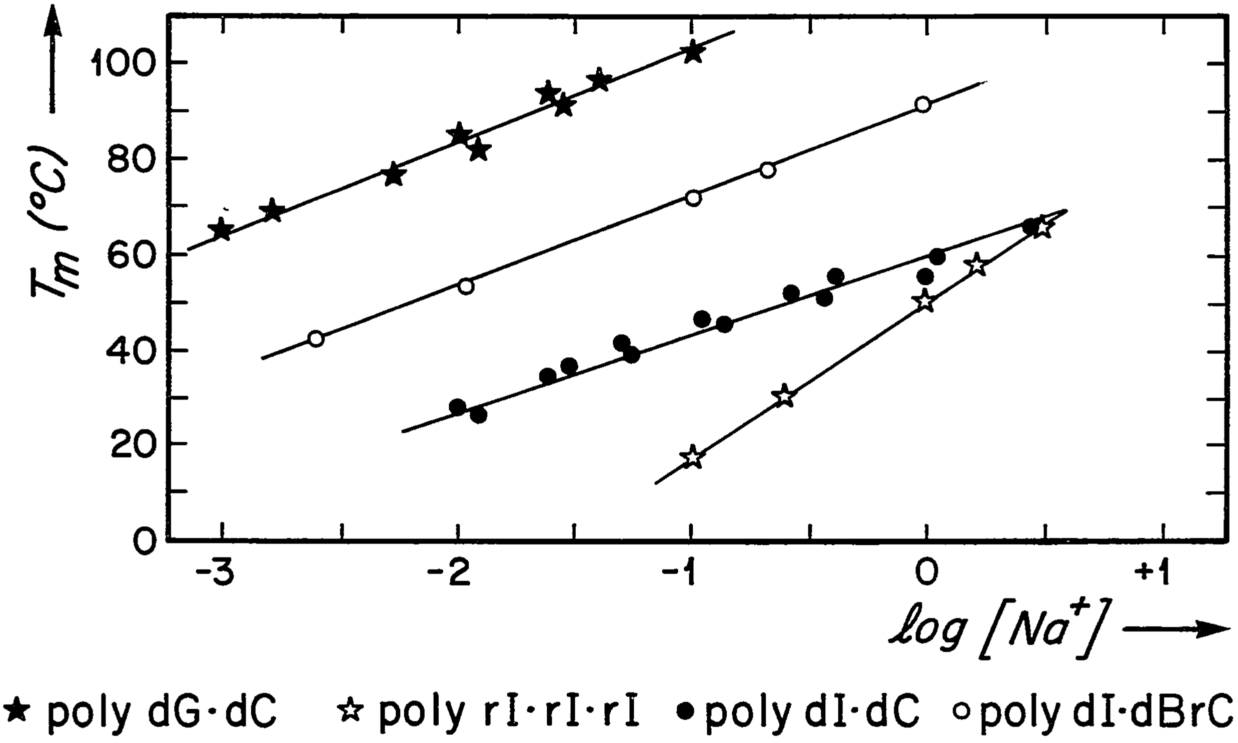


FIGURE 16

Variation of melting temperature (T_m) with ionic strength (Na^+) of various two-stranded polynucleotide complexes. All such polymers form hydrogen-bonded, multi-stranded, secondary structures (100).

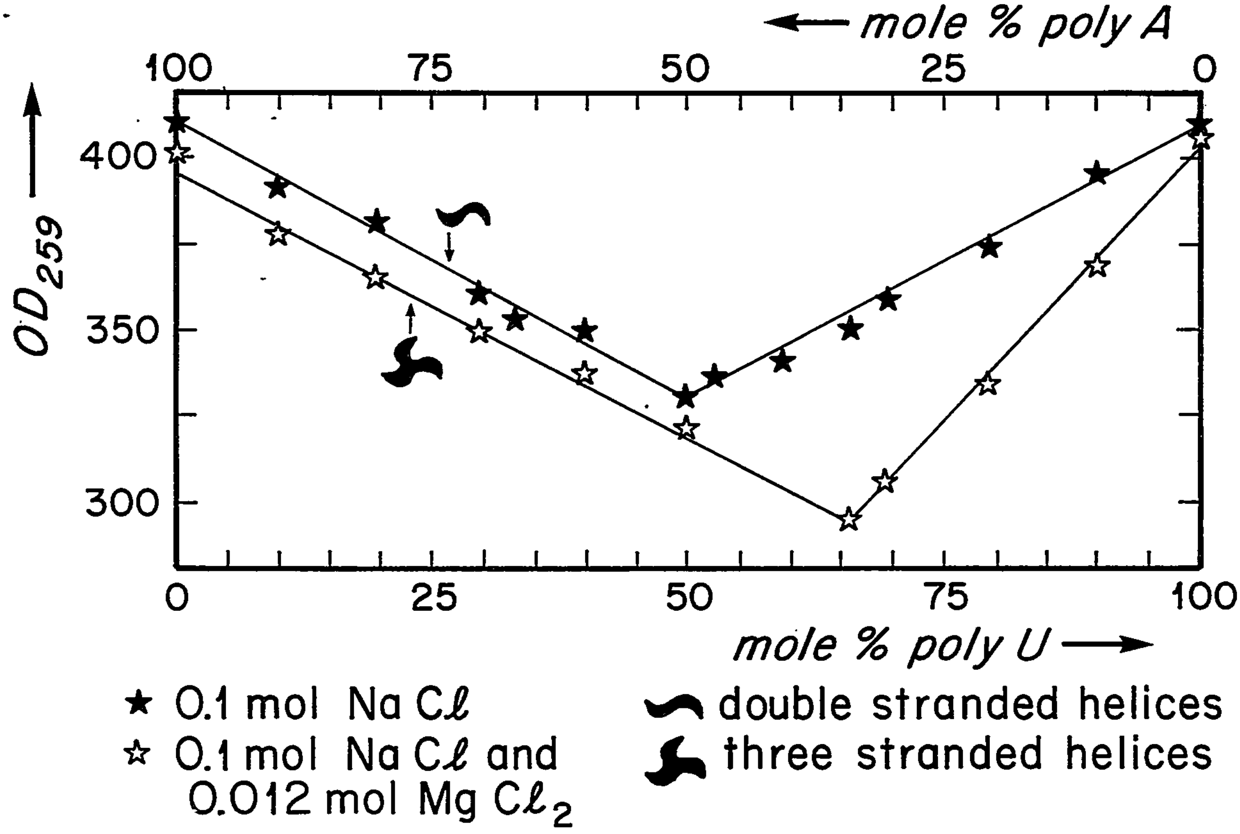


FIGURE 17

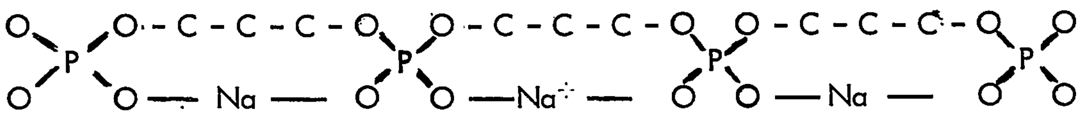
The optical density of various mixtures of polyadenylic acid and polyuridylic acid. Optical densities were measured two hours after mixing. All solutions contain 0.01 M glycylglycine; pH 7.4, T = 25° C. The addition of a small number of divalent cations induces the formation of a three-stranded molecule (103,104,105).

In the case of the RNA of the tobacco mosaic virus (TMV), Mg^{++} was found to be 2.5×10^4 more effective than Na^+ in stabilizing the helical regions of RNA (101). In comparing the valences and ionic radii of these two cations, the observed relationships become apparent. Magnesium, due to its smaller ionic radius, is co-ordinated in the form of an oxygen octahedron (Fig. 14), whereas

Polyhedra		MgO_6	NaO_{6-8}	CaO_{6-8}
Goldschmidt	(102)	r_i = 0.78 Å	r_i = 0.98 Å	r_i = 0.99 Å
Pauling	(12)	r_i = 0.65 Å	r_i = 0.95 Å	r_i = 1.06 Å

sodium, as a result of its radius and monovalence, forms a weaker bonded oxygen polyhedron. Consequently, the bonding strength of MgO_6 polyhedra to nucleic acids is more pronounced, because 1/3 of the oxygen is furnished from at least two PO_4 groups. By repeating the experiments on the RNA of the tobacco mosaic virus, but this time using Ca^{++} solutions, we can expect a 5×10^3 more effective stabilization of the helical regions of RNA relative to Na^+ solutions. This is so, because calcium and sodium have comparable ionic radii.

The nucleic acids exhibit a double chain backbone, one shown in Figure 1, the other represented by a chain of oxygen polyhedra. It is proposed that the



interactions of the individual enzymes with the nucleic acids, and the sequence of formation transfer takes place by exchange or elimination of the polyhedra metals. This is so, because the stereo-specifity of the nucleic acids is determined by these polyhedra. The experiments of Rich (103-105) can be listed in support of this stereo-specifity. Synthetic polyadenylic and polyuridylic acids form double helices only, in case the sodium concentration in solution exceeds a plateau value. This implies that a secondary structure is established via Na-O co-ordination polyhedra. The presence of Mg^{++} ions induces the formation of a three stranded molecule (Fig. 17).

Data on conductivity measurements of salt-free DNA solutions can be regarded a direct evidence in support of the metal ion-PO_4 co-ordination (106, 107). The critical DNA concentration at which denaturation occurs is extremely sensitive to type and amount of the salt ingredients. Most effective in lowering the critical concentration are the sodium ions.

Other experimental evidence involving the binding of messenger RNA to ribosomal RNA is mentioned to demonstrate the importance of metal ions. The attachment of polynucleotides to ribosomes only occurs, in case two valent metals are present in concentrations exceeding $M^{+2} \gg 10^{-2}M$ (108-111). For instance, the experiment on Escherichia coli has shown that polyU and polyC cannot be attached at the 10^{-3} M Mg-level to the ribosomes but require a ten-fold higher concentration. An increase in Mg^{++} to 2×10^{-2} has no further effect. Similar relationships are established for calcium or manganese (112).

The observation that (a) a certain magnesium level is required and (b) an increase in metal ions above this plateau will not promote an increase in bonding strength, and (c) magnesium can be replaced by either calcium or manganese, and (d) maximum attachment is observed in case all the phosphates of the nucleic acids are neutralized by the Mg^{++} ions (113, 114), can best be explained by the fact

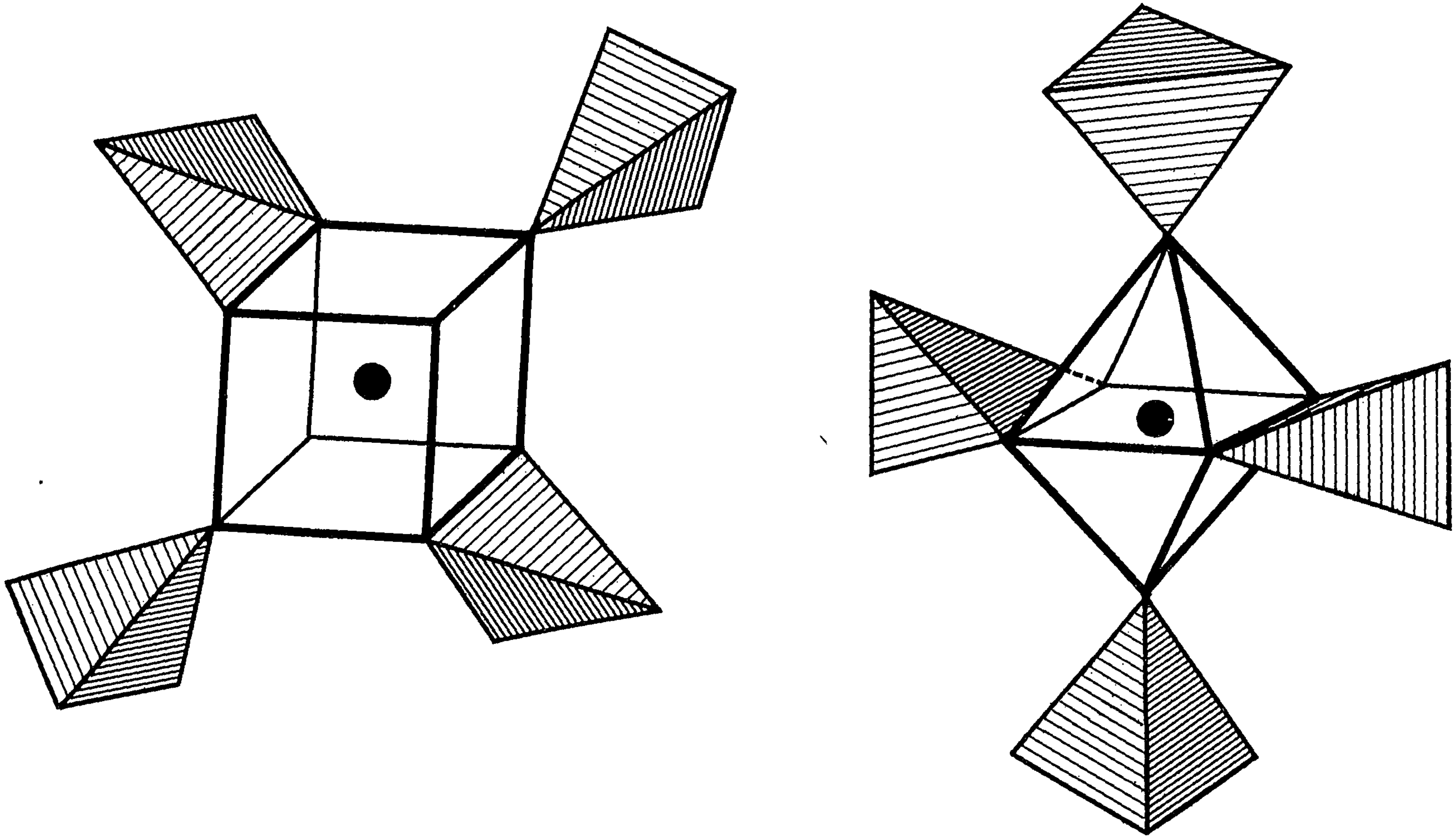
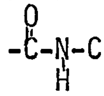


Figure 18

FIGURE 18

Linkage elements (bridges) of the type known from inorganic phosphate structures (schematic). The polyhedra bridges can be utilized for a number of biochemical work assignments such as the attachment of RNA to the ribosomes, the attachment of compounds necessary for the poly-U directed phenylalanine polymerization, and other operations. Principally, three kinds of bridges can be operative by sharing of oxygens (see also Fig. 14): (a) on the corners, (b) on the edges, and (c) on the polyhedron surface.

The oxygens positioned in the polyhedron may come from peptides  $-\overset{O}{C}-\underset{H}{N}-C$. The investigation of the attachment of ϕX 174 to *E. coli* C, and to the cell walls of *E. coli* C, may be interpreted as evidence for the participation of peptide oxygens in such polyhedra. It was shown that the linkage is an irreversible process (with an encounter efficiency close to one) should Ca^{++} be present (0.1 M $CaCl_2$). No linkage takes place in presence of Na^+ (0.1 M NaCl), nor does the addition of 0.1 M Na^+ affect the bonding in the presence of 0.1 M Ca^{++} (262). These results yield a consistent picture about the attachment, if one considers the striking aspect of the genetic map of T 4 virus that a large number of genes have morphogenetic functions and that little genetic information is needed for the assembly of synthesized building blocks (262). The assembly process of T 4 seems to be "coded" through the sequential order of events, i.e. in a molecular interpretation, through the *crystallographical structure of linkage elements*. Independent of this information on the T 4 virus, we derive the same conclusion of secondary codon elements from the experiments on ϕX 174, namely, that attachment occurs only in case the *crystallographical codon* element is present in the form of a defined *polyhedron* and in a specific concentration.

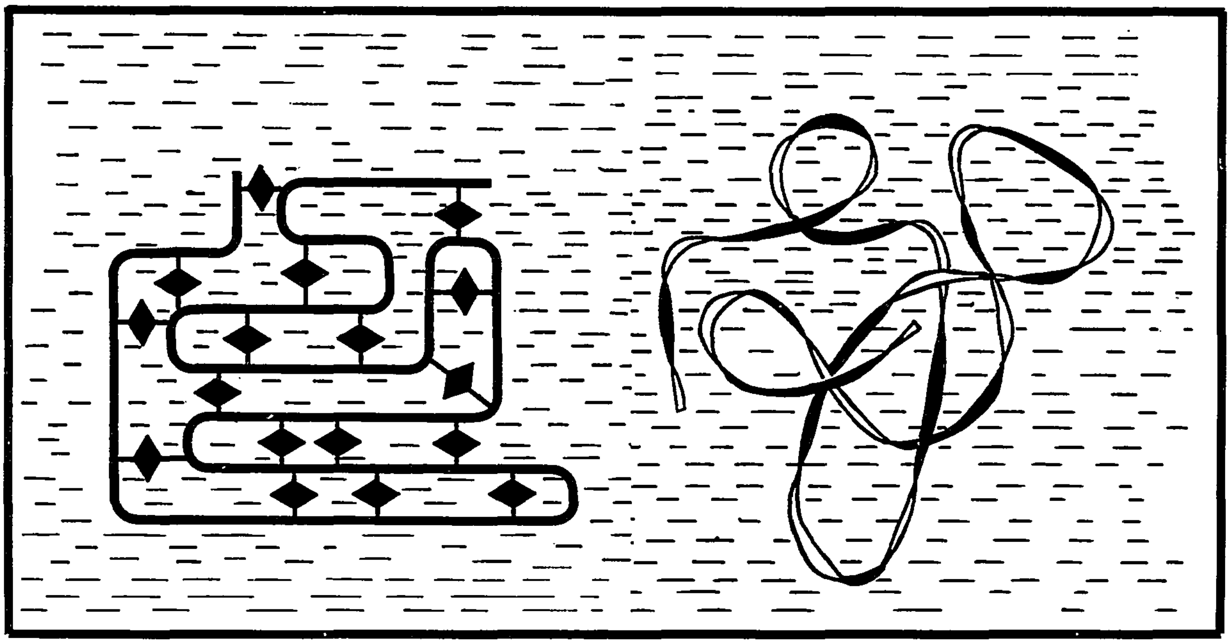


FIGURE 19

Randomly coiled chains (right-hand picture) will increase their molecular order through the association of PO_4 tetrahedra and metal ions (left-hand picture). This phenomenon is related to the fact that oxygen co-ordinated metals, due to their high co-ordination number, are linked to at least two PO_4 tetrahedra; the diamond-shaped figures (see Fig. 18) represent the linking elements, and the dashed line-pattern the solvent system. The stretching of the molecule is related to the formation of the polyhedra backbone; and the interplay between opposite polyhedra surfaces introduces a highly cross linked three-dimensional network. The increase in entropy, as a result of co-ordination processes of the type described above, is rather significant and comparable to that of solid crystalline phases. To list an example, the amorphous structure of SiO_2, known as quartz glass, has an entropy comparable to that of crystalline quartz.

that metal ions and PO_4 form oxygen polyhedra and thus, constitute a well-defined three-dimensional structure. This structure is immediately recognized by the ribosomes via spacial fit. It is also conceivable that the metal ion oxygen polyhedra of RNA serve in addition as a linkage element to the ribosomes by the sharing of the octahedra edges. This phenomenon is similar to the metal ion linkages which are known for the inorganic phosphate crystals:

$$\rightarrow P - O - M^{+2} - O - M^{+2} - O - P \leftarrow$$

The next two Figures, 18 and 19, show schematically (a) polyhedra-bridges between PO_4 units and (b) the way well-defined two-dimensional structures, involving polyhedra linkages, may come into existence. The investigation of the rôle of divalent cations in the polyU controlled phenylalanine polymerization (115) suggests that this influence is a consequence of the formation of a secondary structure and of linkages established by the sharing of oxygen (Fig. 10, 14, 18, 19).

In summary, in reactions where DNA and RNA participate (synthesis of peptides), the PO_4 tetrahedra and metal ions will form oxygen co-ordinated metal polyhedra. These polyhedra have three main functions:

(1) organization of randomly distributed compounds into a specific three-dimensional structure.

(2) control of interaction mechanisms and kinetics by introducing a specific reaction pattern, and

(3) incorporation of oxygens of different molecules into the polyhedra which will introduce intramolecular linkage elements.

SIMULATION OF SOLID STATE SURFACES AND FORMATION OF DYNAMIC MOLECULAR SIEVES BY MEMBRANES

In comparison to proteins and nucleic acids, only limited information is available regarding the physiological function of phospholipids. For instance, it has been assumed that phospholipids serve only one purpose, namely to give membranes physical structure and high impermeability. These viewpoints on the structural properties of membranes are strongly influenced by experimental data in the classical area of water chemistry. Recent work on membranes has disclosed that membranes also possess dynamic properties. We believe that the "polar" heads of fatty acids interact vividly with metal ions which, like water, have ionic properties and are polarizable.

Best studied is the membrane system of mitochondria. By adding alkali salts to a mitochondrial suspension, it was noticed that H^+ ions are liberated, and metal ions are simultaneously fixed (116) (Fig. 20). Measurements of the (H^+) protons exchanged by potassium indicate that the potassium content in the isolated pellets was equivalent to the H^+ content and that no significant gain or loss in water content of the mitochondrial pellets was observed during the K^+ exchange reaction (117). The uptake of metal ions by membranes hereby proceeds frequently at very low external concentrations and even against a concentration gradient (118, 119). Furthermore, divalent ions become more strongly adsorbed than monovalent ions as evidenced by the fact that an addition of Ca^{++} will release both H^+ and K^+ (116). This in turn suggests that membranes exhibit selective ion exchange characteristics. In order to fully understand ion exchange effects on membranes, information on inorganic poly-phospho-compounds are included.

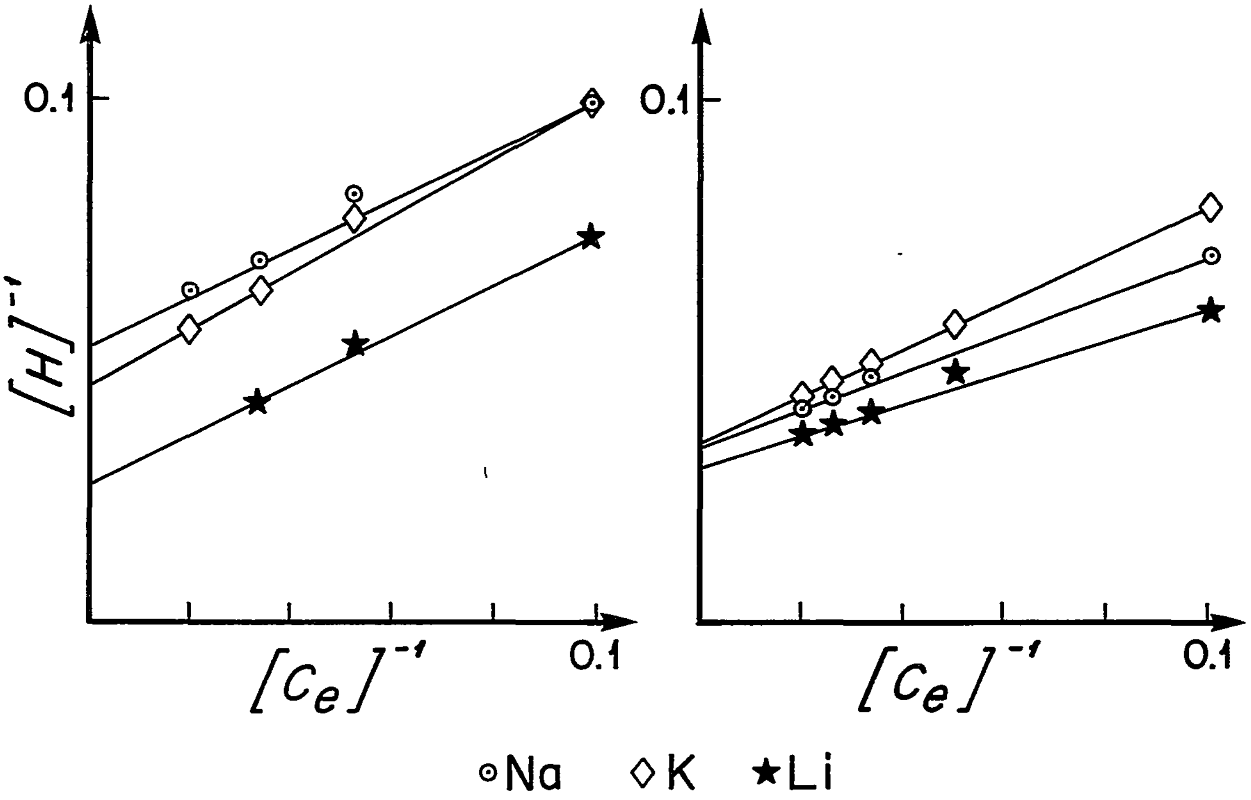


FIGURE 20

H^+ liberation from mitochondria in presence of various cations. The mitochondria were suspended in 0.25 molar sucrose; pH constant, T = 20° C. Mitochondria pretreated with 1% (v/v) n-butanol (right-hand picture); untreated mitochondria (left-hand figure). $(C_e)^{-1}$ is the reciprocal of the external cation concentration; $(H)^{-1}$ is the reciprocal of the liberated hydrogen in μeq/g. An affinity series of the alkalies is observed Li < K < Na in fresh mitochondria suspension, and Li < Na < K in butanol mitochondria suspension at corresponding $(C_e)^{-1}$ (116).

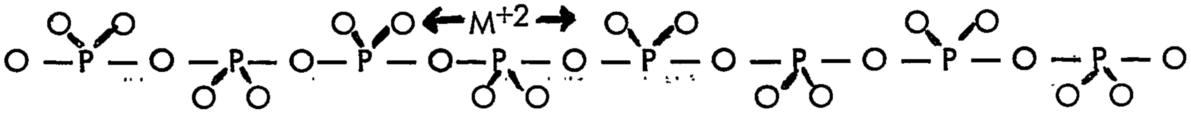
It is well established that among all naturally occurring minerals, the phosphates exhibit the highest ion exchange capacities (120). These outstanding ion exchange characteristics of phosphates are principally related to the enormous flexibility in the way the P-O bonds are arranged in phosphate structures (Fig. 6).

A series of experiments exist which suggest that polyphosphate chains promote ion exchange (120-128). Measurements indicate that chromatographically purified polyphosphate solution, in which the negative charge on PO_4 is compensated for by protons, will lower its pH upon addition of neutral salts. The lowering in pH is a function of the salt concentration. It will approach a minimum plateau value, at which all available H^+ ions are exchanged for metal ions. These phenomena are interpreted (60, 129, 130) to represent ion exchange processes; an altervative suggestion is metal complexing (128).

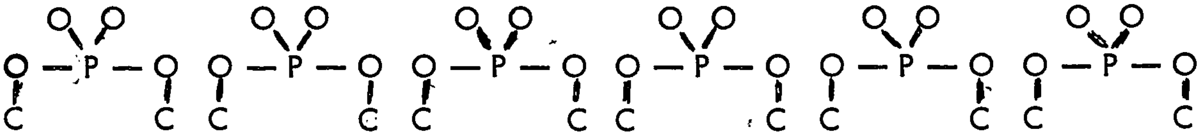
Ion exchange on polyphosphates follows the Rothmund rules (131) which are concerned with ion exchange on solid phases. The ease of proton substitution by alkali ions follows the sequence:

$$K^+ < Na^+ < Li^+ < M^{+2}$$

It is noteworthy that this series of ion exchange relationships has the same trend, as the series of hydration energies for the corresponding ions (22). The hydration energies were calculated on the basis of electrostatic attractions. This correlation indicates that metals are attracted by ionic forces to the PO_4 groups. The relationships between ionic radius and hydration energy are illustrated in Figure 21. Principally, these ion exchange mechanisms in polyphosphoric acid are similar to those observed on membranes, as can be expected on the basis of the structural pattern:



a) polyposphate chain



b) phospholipid ribbon

The investigation of the physical properties of polyphosphoric acids has shown a streaming double refraction and anisotropy in their electrical conductivity (132, 133). These data clearly show that the polyphosphate chain has a rod-like appearance and is only weakly coiled (60). The electrical conductivity (134, 135) and the transport measurements (136), on the other hand, are absolute proof for a strong bonding of the cations. The authors propose that the molecular organization, as introduced by the association of metal ion co-ordination polyhedra with the PO_4 groups, plays a significant rôle in membrane dynamics.

The concept of a cation-oxygen polyhedron formation is comparable to the ordering processes of fatty acids on a water surface in the direction to hexagonal lattices, and which are known since the beginning of physical chemistry. This phenomenon is just mentioned to illustrate our case. The difference rests only on the exchange of the organizing factors. In the case of the fatty acids/water

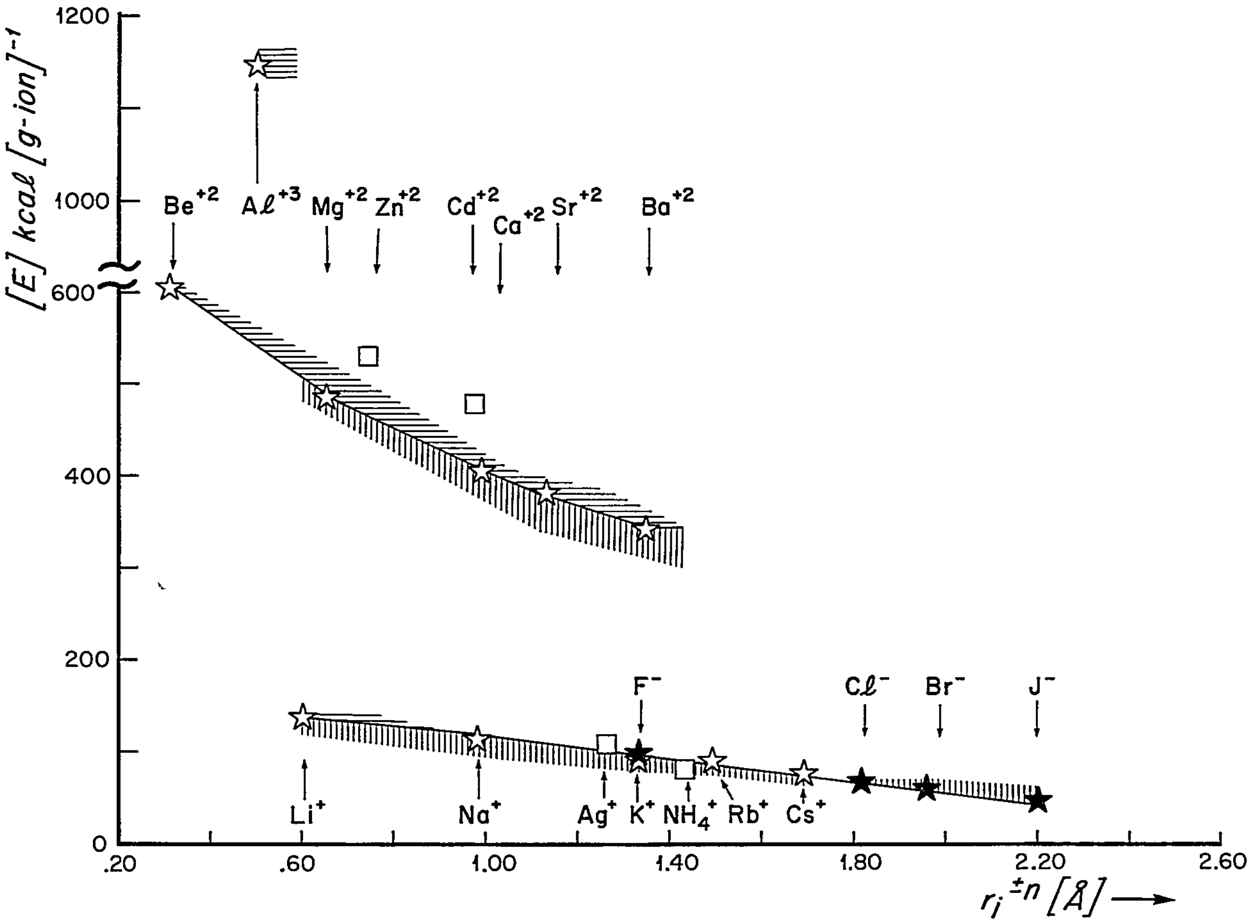


FIGURE 21

Relationships between ionic radius (12, 102) and hydration energy (22). The spread of data for an individual observation point is indicated by the extent of the horizontal (i.e. ionic radii), and vertical (i.e. hydration energies) line pattern. The fact that zinc and cadmium fall outside the established area for the alkaline earths may be related to metal complexing. The release of energy during hydration is a result of the electrostatic interactions between the ions and the water dipols.

surface phenomenon, these factors are the dipol-dipol interactions and the hydrophobic forces. In the case of the metal ion-oxygen polyhedron mechanism, these factors are the oxygen co-ordinated sodium or potassium, etc. atoms. A sort of crystalloid state of order will be the consequence*.

It has been demonstrated that oxidation and respiration processes in mitochondria are accompanied by ion exchange and ion transport on membranes. Based on the arguments developed in this article, we propose concepts pertinent for the understanding of the molecular structure of the polar (outer) layer of membrane surfaces. These concepts may also serve as the explanation of ion exchange mechanism and active particle transport phenomena in membrane systems. Analogous to the ion co-ordination interaction of polyphosphoric acids, the fixation of metal ions at the P-O surface causes a distinct molecular geometry of membrane surfaces as a whole**. Inasmuch as no work is available on the short-range order of membrane

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* Proof for the existence of a crystalloidal molecular order, of the type outlined here, can be obtained by reference to the physics of thin layers (137) and which finds its technical application in the form of interference filters and micro-electronical equipment of the type used in all laboratories.

**The listed molecular geometries of membrane surfaces can be described by a mechanical statistical formalism (138). We define: Complete short-distance order is achieved, in case the neighbors of a PO_4 unit (A) are exclusively B units; incomplete short-distance order is achieved in case C, D ... K units participate as neighbors of A. The medium number of B units is termed p; the medium number of the interfering C, D ... K units is designated q. A measurement of the degree of short-distance order can be expressed by the quotient:

$\eta = \frac{p - q}{p + q}$ where $p = n_0 \cdot B$ and $q = n_1 \cdot C + n_2 \cdot D + \ldots n_n \cdot K$; complete order $\eta = 1$

complete disorder $\eta = 0$

(footnote continued...)

fragments, examples of other phosphate structures are graphically presented. Figure 22 represents a corrugated layer which is composed of PO_4 tetrahedra linked via Ca^{++} polyhedra. These in turn are connected by means of water molecules to the alternate layers. In this way, stable geometries will be created, such as undulating surfaces, concave/convex surfaces with pores, etc. These deductions regarding the specific surface structures of phospholipid membranes, even including holes and surface granularity, are in concord with biochemical observations (electron micrographs), or studies on selective ion permeability.

The physical-chemical effect of an ion exchange mechanism in such structures is remarkable. The interchangeable nature of metal ions causes membranes to act as dynamic molecular sieves. The (a) pore size, and (b) sieving quality in

---

** Complete order has a value of 1 and complete disorder has a value of 0. The long-distance order can be described in similar terms. We project a defined grating pattern (=lattice) across the molecular building blocks, and allocate black lattice points (at the intersections) for the PO_4 units (A); the total number of points is designated as N. For differentiation, the B, C,...K units are indicated as white lattice points. Incomplete long-distance order is indicated, should only some (N_1) but not all of the original black lattice points be occupied by the elements A; the remainder ($N_2 = N-N_1$) will be taken by B, C...K particles. A measurement of the long-distance order is the expression:

$$\eta' = \frac{N_1-N_2}{N_1+N_2}\,; \quad N = N_1 + N_2$$

$\eta' = 1$ = complete long-distance order

$\eta' = 0$ = complete long-distance disorder

This treatment of the order phenomena in biological structures appears to be more appropriate than the energetic calculations frequently employed to elucidate this problem.

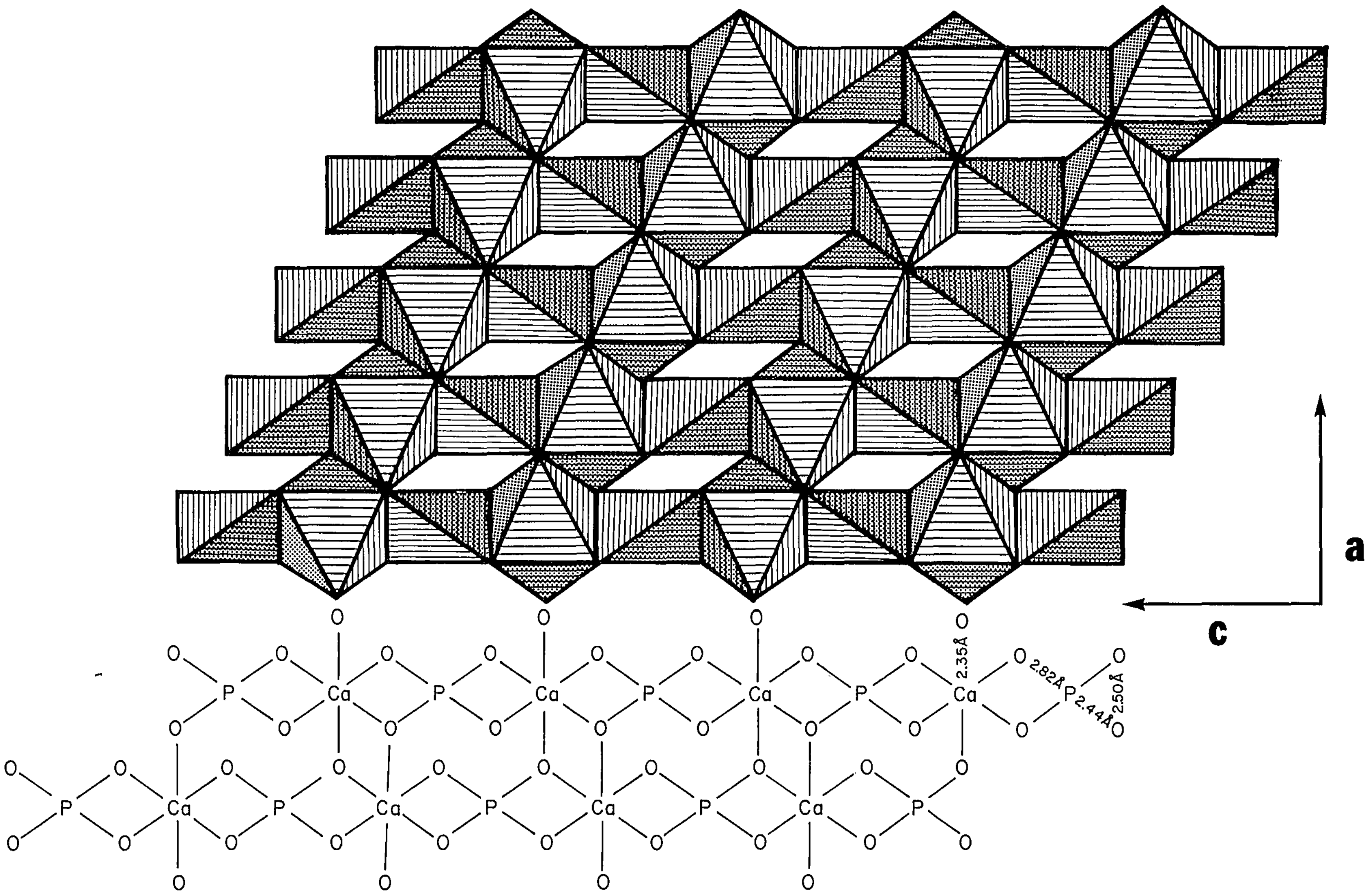


FIGURE 22

FIGURE 22

Schematic presentation of sheet composed of individual:

$= PO_4 = Ca = PO_4 = Ca = PO_4 = Ca = PO_4 = Ca = PO_4 = Ca$

chains which are cross-linked. Such structures are known from dicalcium phosphate dihydrate ($CaHPO_4 \cdot 2\ H_2O$) and monohydrate ($CaHPO_4 \cdot H_2O$) (74, 75).

The corrugated layers are shown in a simplified form because in reality, calcium is co-ordinated with 8 oxygens and not with 6, i.e., we have no octahedra. By using this type of graphical representation, we can emphasize the Ca - O - P bonding. In reality, the calcium is surrounded by two OH_2 or one OH_2 and $O - PO_3H$ (see Fig. 9). Water molecules enclose and link the $CaPO_4$ corrugated layers (sandwich structure). For comparative purposes, the bonding distances for dicalcium phosphate dihydrate are included (75).

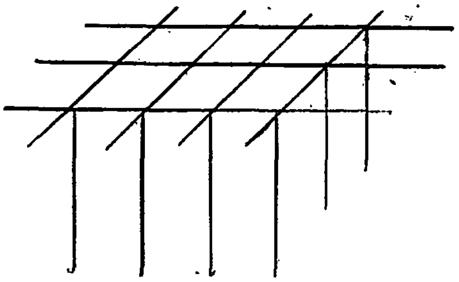
such dynamic molecular sieves is quite variable and dependent on the kind of metal ions. Under the assumption that ATP is capable to trap the metal ions, a periodic pulsation of the lattice is the consequence. To support these concepts, we refer to the oscillations of ionic constituents as was observed on mitochondria (139).

Inasmuch as no structural analysis is available, we refer to structures of inorganic phosphates to support our deductions (Figs. 23-25). These Figures illustrate that sieve-like space fabrics can be composed of PO_4 tetrahedra, in case structural water or hydroxyls are available. It is immediately obvious that these cage-type structures exhibit molecular sieve properties.

A more direct evidence in support of formation of molecular sieves are the X-ray diffraction studies on the swelling of myelin layers in hypertonic solutions (143, 144). The electron density distribution through a membrane exhibits a characteristic pattern which conforms only with the presence of metals between the lipid bileaflets (Fig. 26). This spectrum, on the other hand, excludes an association of PO_4 groups with water, in other words, a membrane hydrate structure.

Based on the here developed concepts, we offer a structural interpretation by means of a molecular-structural concept for the "molecular anonymous" thermodynamic Donnan equilibrium theory (145).

A l-dimensional cross-section through the membrane surface is shown in Figure 27. It represents a lipid-oxygen chain, and explains, aside of molecular dimensions, the space requirements for an oxygen co-ordinated metal polyhedron and for the hydrocarbon chains. As can be seen in Figure 28, the cross-section surface area of one hydrocarbon chain (=18.5 $\AA^2$) is identical to the cross-section surface area of a PO_4 tetrahedron (=16.5-19 $\AA^2$). These facts offer a simple explanation for the phospholipid formula, and demonstrate, why the diglycerides in particular are linked to PO_4 in membranes. This is so, because the cross-section surface of a single fatty acid is too small for a phosphate unit. On the other hand, three fatty acids linked to a 4-valent alcohol are too big.; an arrangement of this kind would result in the formation of a rigid and compact phosphate mineral layer, and the membrane would lose its elasticity. The introduction of a metal ion, the area of which averages about 4 $\AA^2$, will establish not only the optimal area conformation (39-40 $\AA^2$), but a fabric which is oriented vertical to the hydrocarbon chains and which supports simultaneously the membrane surface in a traverse manner:



The structural deductions are in full agreement with analytically derived facts such as the data on surface areas (146a, 146b). The interpretation of X-ray diffraction data require the presence of traverse fabrics in membranes, because a statistical thermodynamic analysis indicates that a pure lecithin molecule in water will aggregate to bilayers with a maximum disorder for reasons of the uncompensated charges (148). They represent non-stable fabrics, and the thickness

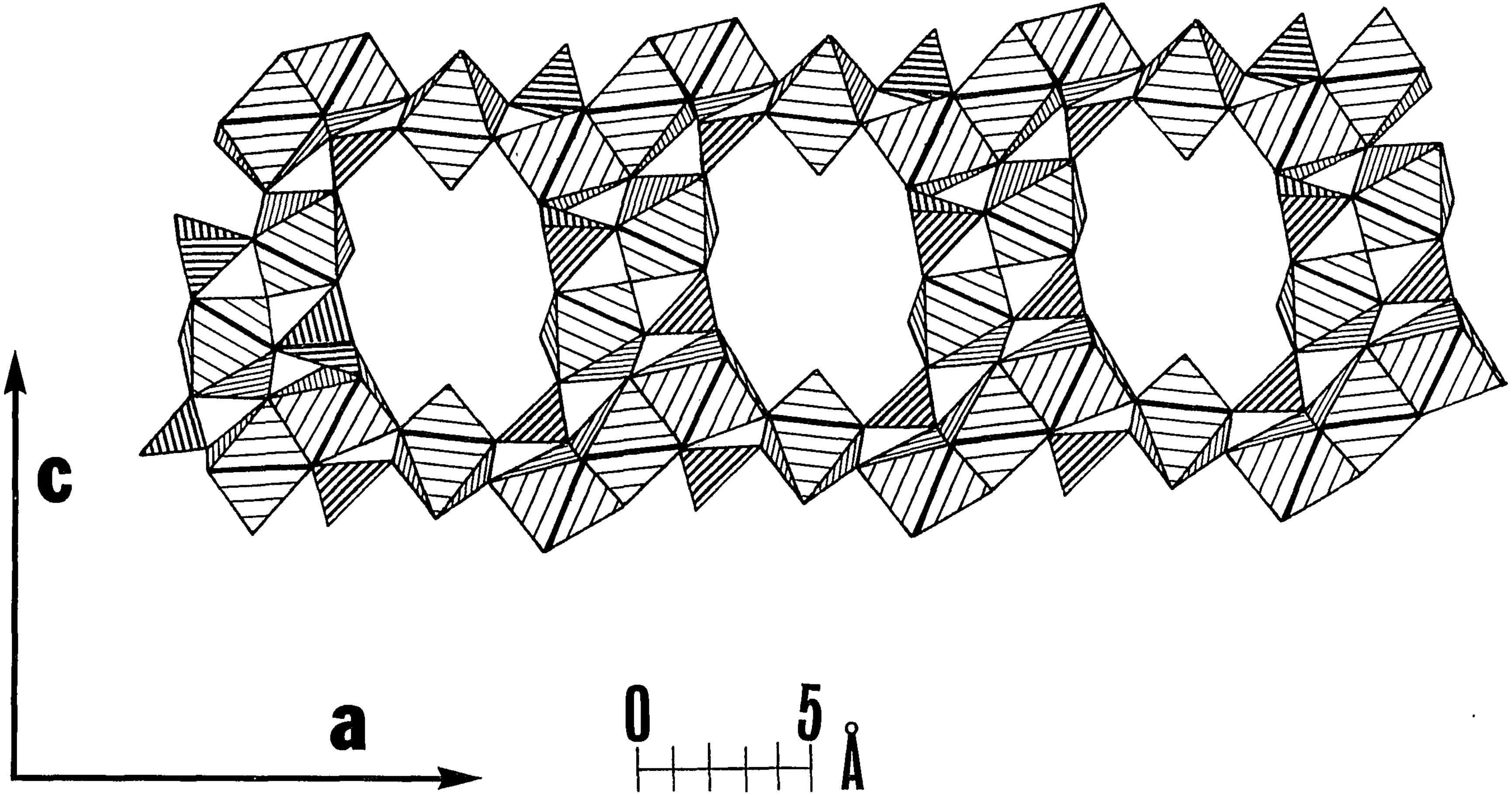


FIGURE 23

Molecular structure of beraunite, $Fe^{II}_{0.5}Fe^{III}_{2.5}(OH)_{2.5}(PO_4)_2 \cdot 3H_2O$. The crystal structure represents a three-dimensional network of Fe and P oxygen co-ordination polyhedra with empty channels along the screw axis, where free water molecules are located. For graphical reasons, the water molecules have been omitted. The cell dimensions are: a = 20.646 ± 0.005, b = 5.129 ± 0.007, and c = 19.213 ± 0.005 Å (142). The structural model requires that the oxygen on the the two-fold axis must be considered as belonging to an OH-group. Two possible sets of hydrogen atom configurations are hereby conceivable depending upon whether the hydroxyl ions are associated with the framework octahedra or the channel oxygens. The configuration of the hydroxyl ions located in the channels is of less residual entropy. This kind of configuration in biochemical systems may introduce an effective exchange along the channel structures and promote the transport of matter. For fluellite (Fig. 24), and vivianite (Fig. 25), similar relationships hold true.

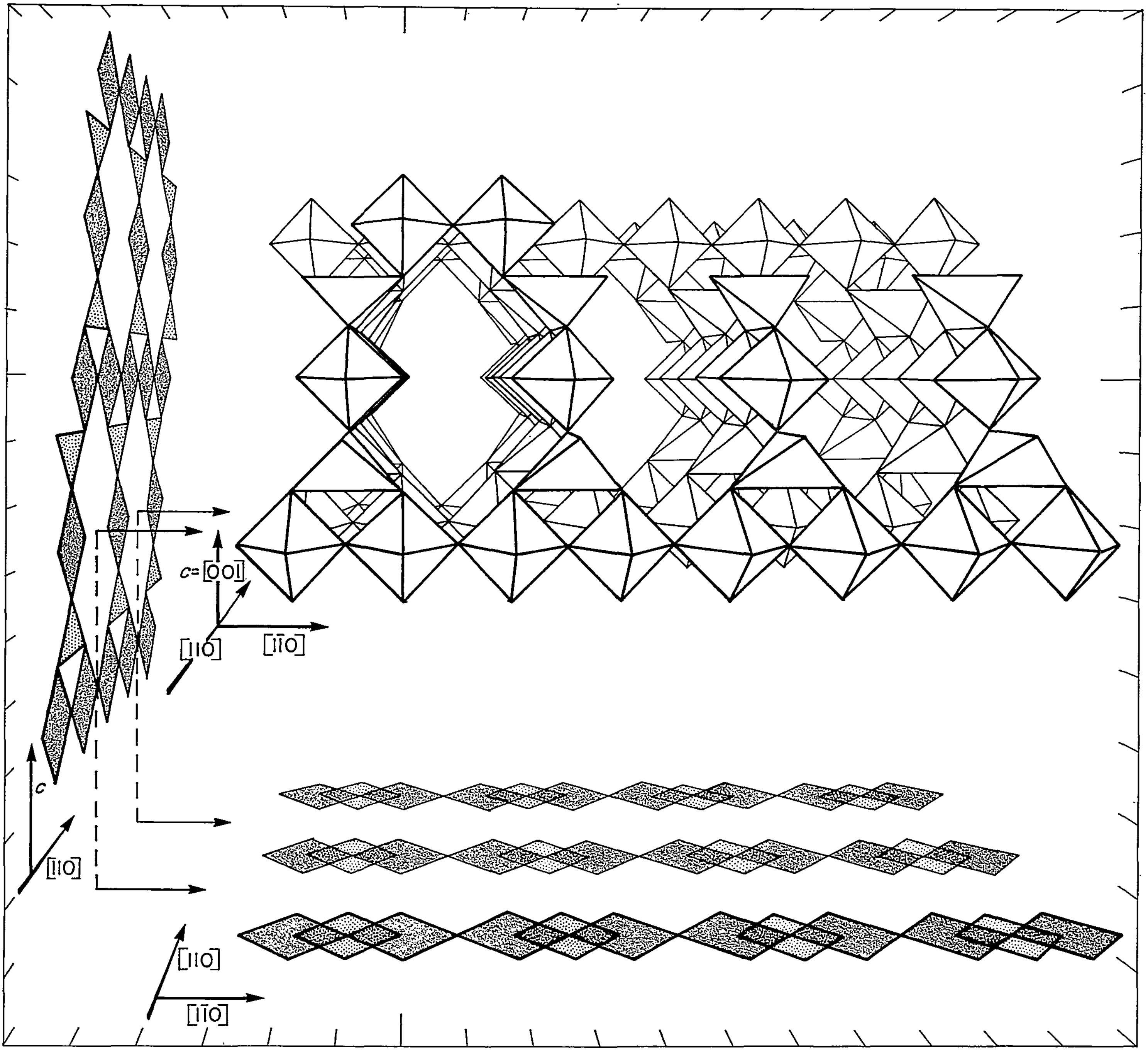


Figure 24

FIGURE 24

Molecular structure of fluellite, $Al_2PO_4F(OH) \cdot 7H_2O$. The aluminum atoms are positioned at centers of symmetry and are bonded octahedrally to two pairs of oxygen atoms, and one pair of fluoride ions. These octahedra are connected by corner-sharing at the fluorides to form chains along the [110] and [$\bar{1}$10] directions. Between the octahedrally and tetrahedrally co-ordinated cations (open framework arrangement) are distinct channels containing hydrogen-bonded water molecules which, in this illustration, have been deleted for graphical reasons. The cell dimensions are:
a = 8.546, b = 11.222, and c = 21.158 Å (141).

In order to reveal the three-dimensional structure of the polvhedra network in fluellite, a three-point perspective view, vanishing at the horizon line (projection box), is employed. The horizon line vanishes at infinity; the horizontal axis is along [$\bar{1}$10], c is the vertical axis, and the view of the vanishing axis is deviated around [110].

In the context of this work, we are particularly interested in the channeling system in terms of (a) channel width and cross pattern, and (b) the ways and means such a channel system can be controlled individually through molecular blocking of places within the channeling network, or through the exchange of individual octahedral building units. For this reason we include two projections, one vertically, and the other horizontally of the main figure. In this fashion we achieve a three-dimensional survey of the existing channel-system of fluellite which may give some idea of "how flexible channel networks and controlled valve systems" may arise from the biocrystallographical order of phospholipids in membranes.

The projections are constructed from the original data (141) by assuming that each octahedron is formed within a cube and that each tetrahedron represents half of a cube. The perspectively distorted octahedra and tetrahedra (left side figure) are plotted along c and [110]; this pattern is repeated in series along [110] and [$\bar{1}$10](basal figure). Every point in the main structure (central figure) is subsequently plotted from c, [110], and [$\bar{1}$10].

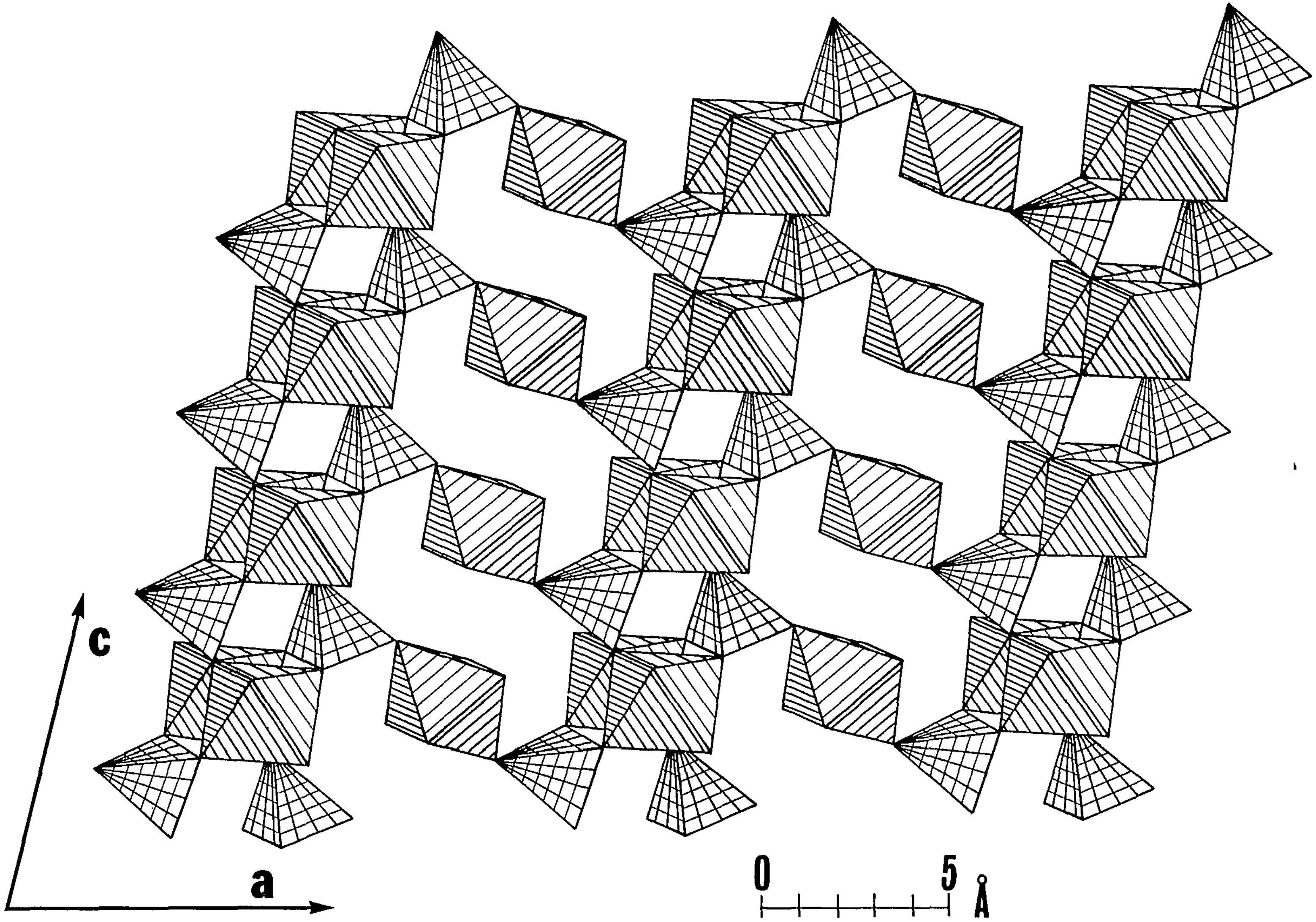


FIGURE 25

Molecular structure of vivianite, $Fe_3(PO_4)_2 \cdot 8H_2O$. The structure is built up of single and double octahedral groups of oxygen and H_2O around Fe. The double group, $Fe_2O_6(H_2O)_4$, is linked to two neighboring similar groups and four other single groups, $FeO_2(H_2O)_4$ by means of phosphate tetrahedra. This will result in a complex band parallel to (010); parallel bands are linked to each other by H_2O molecules. The cell dimensions are:

a = 10.08, b = 13.43, and c = 4.70 Å (140).

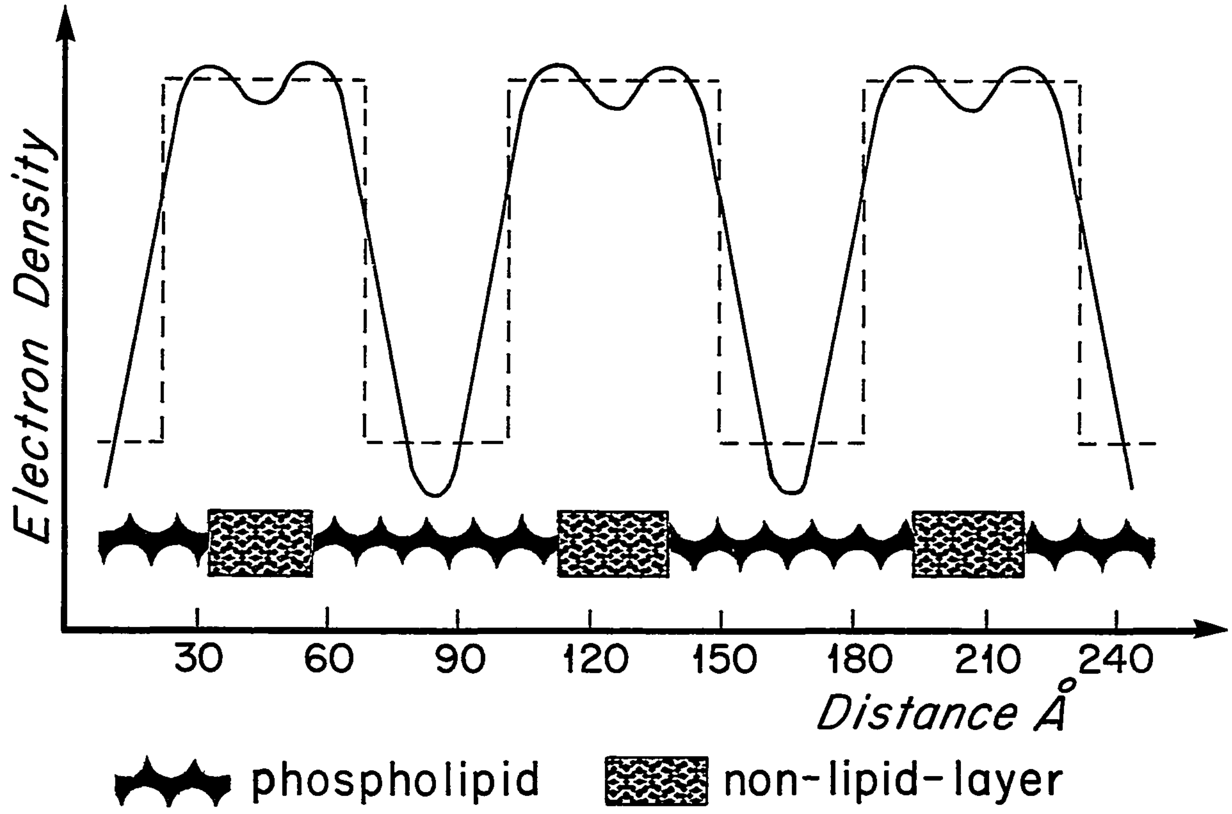


FIGURE 26

Diagram of electron density distribution curves through the myelin layers of rat optic nerves. The continuous line is the uncorrected curve, and the intermittent line shows the effect of maximum correction. Phospholipid bilayers are included as a chain, and the shaded areas indicate the probable locations of non-lipid components. The peaks of electron density represent the ionic ends of the lipid molecules together with the non-lipid components (metallic ions) (143, 144).

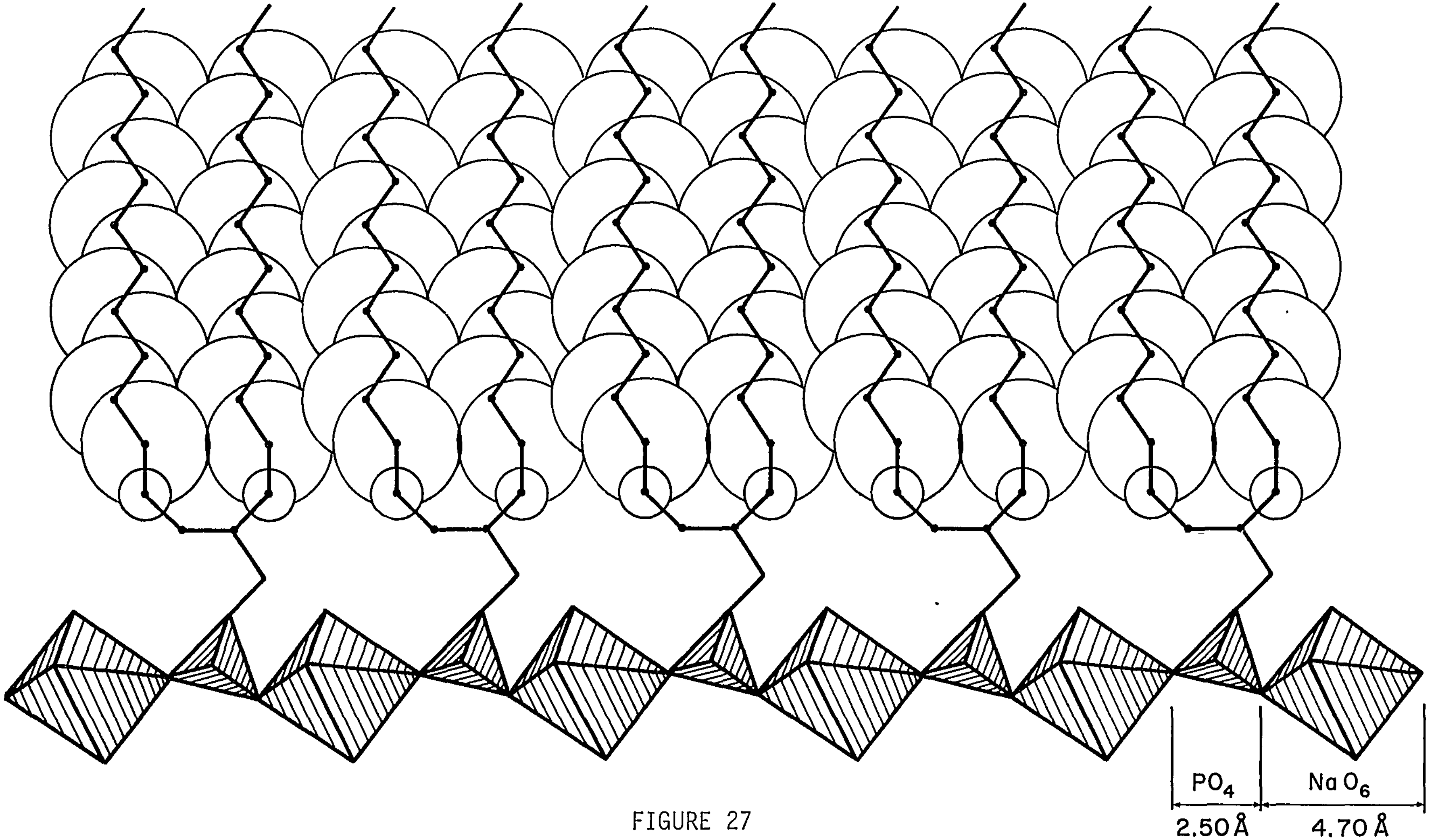
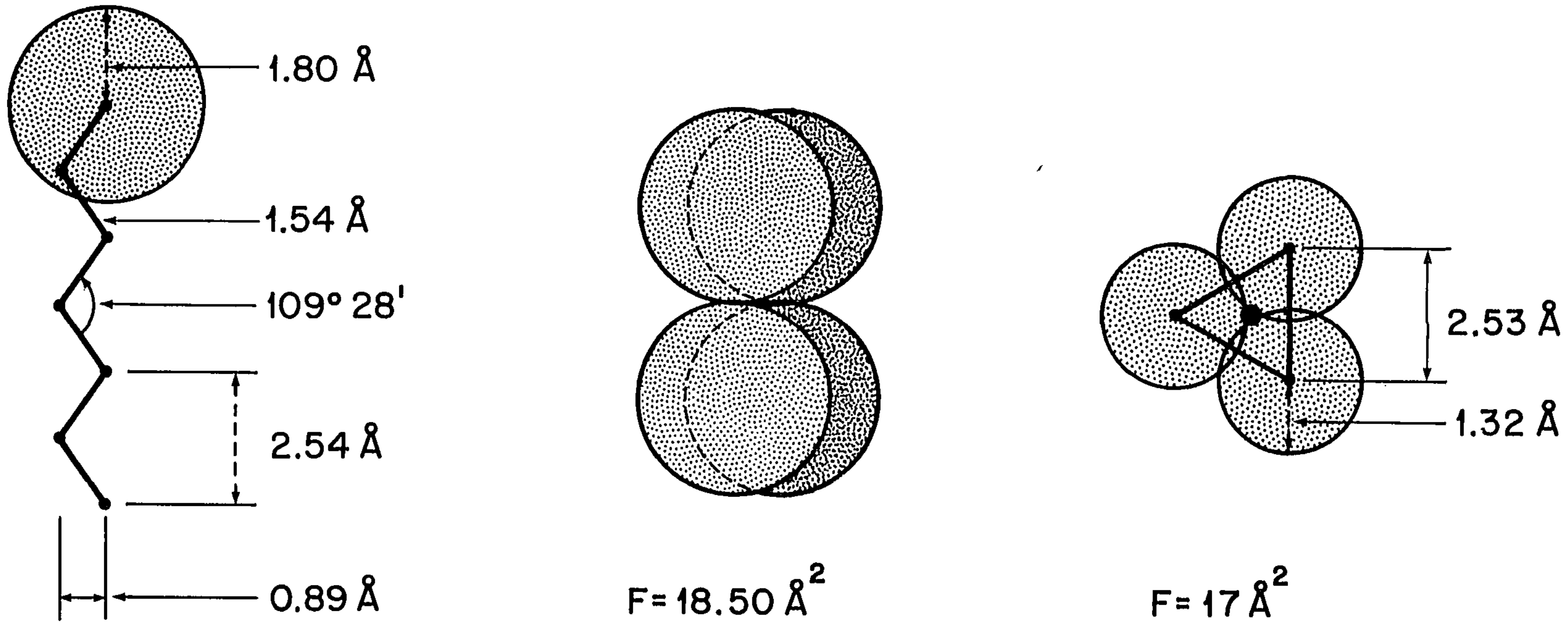


FIGURE 27

Membrane cross-section composed of double chain of lipid-oxygen polyhedra. Glycerol is only schematically represented; the carbonyl groups and the PO_4 residues have been omitted. The molecular dimensions are based on the NaO_6 octahedron. The molecular aggregation, as presented here, results in a corresponding interfacial area fit for both chains concerned. Assuming the surface area of a hydrocarbon chain is identical to the surface area empirically determined for fatty acids (20 Å^2 per molecule) (146a,146b), we obtain the same values for the surface area of a tetrahedron-octahedron unit. The cross-section surface for the minimum six oxygens falls in the range of 33-37.5 Å^2 with a most probable value of 35.5 Å^2. For metal ions, the mean surface area amounts to 4 Å^2. This would add up to a total surface area of 39.5 Å^2 for a polyhedron unit, a value which would conform with the surface area of the associated lipids. It is noteworthy, that for sphingomyeline the area, at the time of collapse on the water surface, amounts to 37 Å^2 which is identical to the minimum value observed in crystals of hydrocarbons (see Fig. 28).



ZIG-ZAG HYDROCARBON CHAIN

2 HYDROCARBON CHAIN CROSS-SECTIONS

PO_4 TETRAHEDRON CROSS-SECTIONS

FIGURE 28

Molecular dimensions of zig-zag hydrocarbon chains; dots are carbon atoms (left-hand picture). The CH_2 contact radius (≈1.8 Å), taken from the $n\cdot C_{29}$ $H_{6}0$ structure, (147) is incorporated. The cross-section of two hydrocarbon chains along the (001) plane of this structure is shown in the center picture. The surface area of both chains amounts to 37 Å^2. For comparison, these hydrocarbons are presented relative to the surface area of the PO_4 tetrahedron (right picture). The radius for oxygen is corrected to the 4-co-ordination state (original r=1.40 Å) (12). This would amount to a surface area of 17 Å^2 for the tetrahedron. The correction, of course, is up to criticism, because oxygen is also co-ordinated with the metals. Under such instances, the values will come out to be 18.5-19 Å^2 per unit PO_4.

of the bilayers will change with the amount of water present. In case metals are available, the laminae will be stabilized, as shown in studies on phospholipid/water systems (149). For instance, films of myristic acid and metal are solid up to a cross-section area of 50 $Å^2$ per molecule (150, 151). We also like to refer to the numerous phase transitions of pure phospholipid/water systems (146, 152) and the polymorphism of phospholipid crystals (143, 153) which suggest that even the pure phospholipid bilayers (in their true nature) do not represent stable structures.

A segment of the molecular structure of L-alpha-glycero-phosphorylcholine is shown in Figure 29 (154) to further underline this presentation. The structure confirms that PO_4 tetrahedra are linked in a zigzag manner via polyhedra and that they build up a chain. The molecular structure of barium diethyl phosphate (155) follows essentially the same construction principle. The Ba atoms form a chain of oxygen polyhedra via PO_4 tetrahedra linkages in the direction of the c-axis; the hydrophobic groups are turned outward and are held together by means of hydrophobic forces to other ethyl groups. It is emphasized that, as a function of metal ion size and valence, different surface networks will come into existence. The geometry of the individual membrane fragments is a result of the different phospholipid/oxygen-polyhedra ratios (Fig. 30). Namely, the physical-structural consistence of membranes is a consequence of two kinds of independently acting forces:

(1) hydrophobic bonding of hydrocarbon chains (156, 157)

(2) ionic bonding of metal oxygen co-ordination polyhedra.

The interaction of PO_4 tetrahedra and metal ions will yield a structurally well-ordered surface lattice. This surface lattice, in turn, will exercise control, through the aforementioned forces 1 and 2, on the composition of the phospholipid units contained in membranes. Consequently, units such as cephaline, lecithine, phosphatedynilosite, or sphingolipid will not be randomly distributed, but their two-dimensional arrangement will be a <u>functional</u> one analogous to the distribution of amino acids in peptides, or the bases of the purines and pyrimidines in DNA except that membranes will exhibit two-dimensional sequences. Similar viewpoints have been expressed by Lehninger (158), who considers a two-dimensional code device on membrane surfaces.

It has been shown (158-162) that individual cell membranes are characterized by a specific composition of the various lipids. In view of the fact that the spectrum of phospholipids in membranes cannot be altered by nutritional means, it is concluded, that the membrane system is genetically controlled. It has also been experimentally established that the specificity of membranes in terms of functional and physical properties is principally determined by the ratio of their individual phospholipids (144, 146, 152, 153, 163, 164).

The well developed molecular surface structure of phospholipid bileaflets will introduce a specific spacial geometry for the protein layer which is analogous to processes known under the heading (a) topochemistry (165), (b) epitaxial growth on crystals (166), or (c) mineral template phenomena (167).

The structurally well-organized surface of the phospholipid bileaflets will also affect the lattice organization of the protein layers. This is confirmed, for

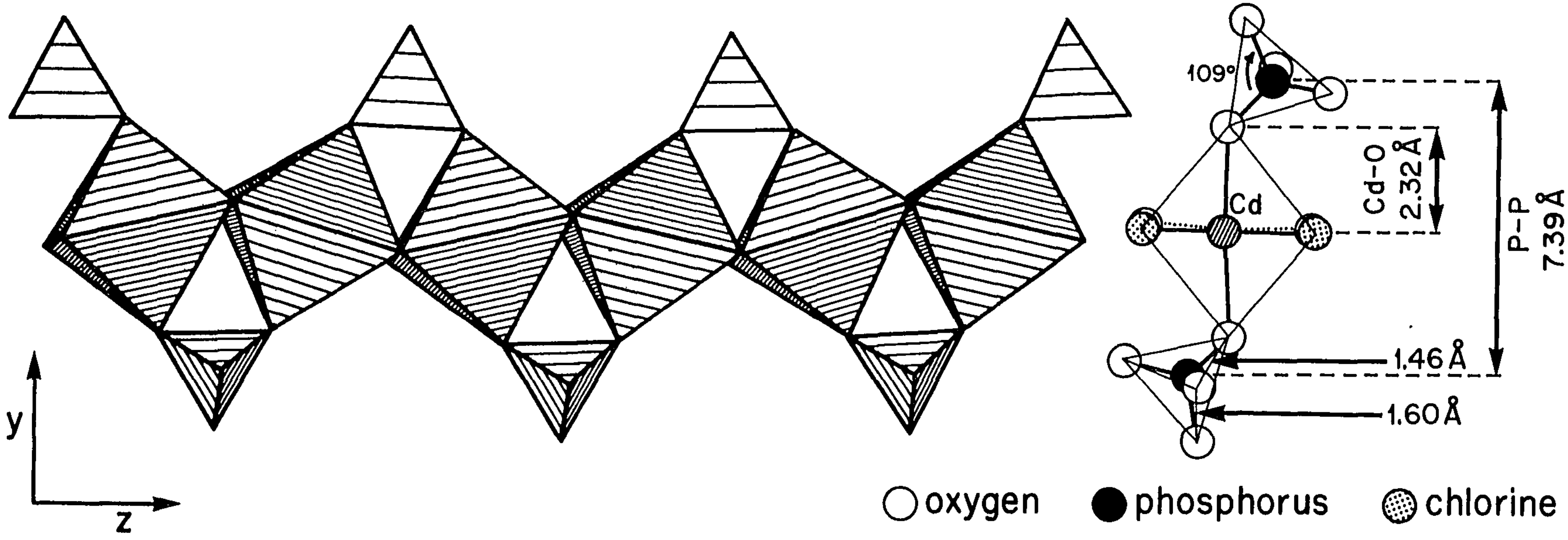


FIGURE 29

Molecular structure of L - α - glycerophosphorylcholine cadmium chloride trihydrate. The phospholipid component displays the characteristic gauche conformation for the choline residue and the gauche-gauche conformation for the glycerol moiety. Each phosphate is bound to two Cd atoms. The PO_4 tetrahedra are linked in a zigzag manner and they build up a chain (154).

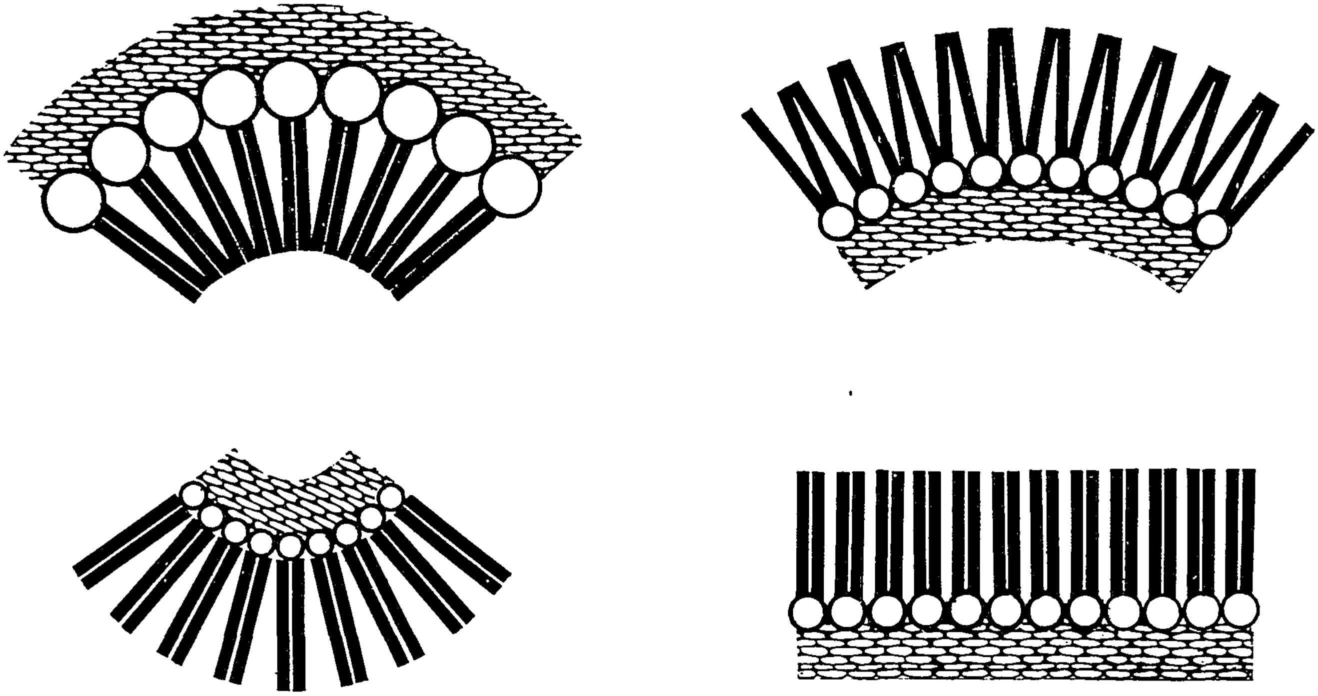


FIGURE 30

The drawings represent segments of a bilayer which illustrate in a schematic way the structural and operational principles involved in membrane systems. Open circles indicate oxygen polyhedra; black double-bars attached to an open circle represent a two-chain hydrocarbon. The ionic forces are contained in the oxygen co-ordination polyhedra; the hydrophobic forces are contained in the hydrocarbon chains.

Under condition one (left-hand picture), an exchange of metals in the oxygen polyhedra results in an inversion of the space geometry of the polyhedra. This will force the hydrocarbon chains into an inward or outward "fan pattern". In case the hydrocarbons are turned together, hydrophobic forces may become operative.

Under condition two (right-hand picture), no or only moderate changes take place in the space geometries of the oxygen polyhedra. However, hydrophobic elements in the hydrocarbon chains may differ and interact, causing the polyhedra surface to undulate. It is obvious that the structural properties of such a system is a consequence of the different phospholipid/oxygen polyhedra ratios. Chemo-elastic, and chemomechanical devices will come into existence, and molecular pumps will be established.

instance, by electron micrographs of cytomembranes (168), where a 40 Å periodicity (cubic lattice) for the structural proteins (cubic lattice) is established on the bileaflet surface. On the other hand, the crystalline order of the structural protein will introduce a specific spacing for the adjacent protein which is intimately connected with metabolic processes. It is most likely that this specific organization will operate in the way of enzymes or in the form of topochemical coding and information read-out device, as is suggested on empirical grounds (158, 163).

Oxygen co-ordinated polyhedra in particular may function as linkage elements between the structural proteins and the phospholipid layers*. A further implication is mentioned. Depending on the size of the cations involved in the co-ordination polyhedra, a membrane surface can exhibit mechano-chemical properties and thus may operate as an energy transfer device (Fig. 30). The hydrophobic bonding forces will be competitive and may counteract the ionic forces in case the oxygen polyhedron does not fit the outlined structure. Should cation exchange or proton transfer proceed, a chemo-elastic device would result.

In summary, the PO_4 units and the oxygen co-ordinated metals introduce a space order for the individual molecular building blocks which finds its

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* It is conceivable that in the interspace between the bilayers, as well as between the membrane and the protein, a three-dimensional oxygen polyhedra network exists which has a sieve- or cage-like appearance. In addition, metal chelates may participate in the composition of this fabric. It could be constructed by means of Al, Si, Mn and other metals, because these cations have been reported in considerable quantities in cell tissue analyses. Such a cage structure would exhibit a high but selective permeability and would not interfer with the transport of matter.

expression in a distinct geometry. This in turn causes the:

(a) formation of a traverse fabric perpendicular to the hydrocarbon fabric which will solidify the membranes as an entity,

(b) establishment of *dynamic molecular sieves* with variable mesh size and and sieving qualities,

(c) development of two-dimensional sequence (primary structure of membrane) of phospholipids and related compounds, resulting in a potential two-dimensional coding device for bonded proteins,

(d) well-ordered enzymatic activity as a consequence of (d), and

(e) origin of chemo-mechanical energy transfer device.

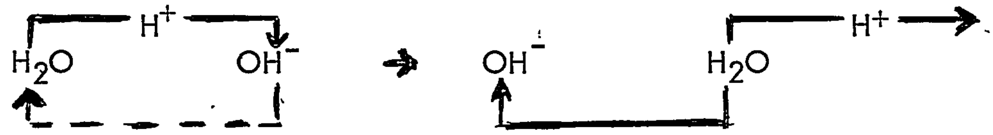
PROTON TRANSFER AND PROTON CONDUCTION PROCESSES

An implication of the structural concepts developed so far, and which may be pertinent for the understanding of certain reaction sequences in cells, is mentioned. It concerns the proton transfer, which frequently is discussed by the biochemists as charge transfer.

We have already seen that the nucleic acids contain an oxide backbone and that the membrane surfaces are covered by oxides. These oxide chains and layers will favor proton jumps. In the presence of a difference in electrical potential, they could act as proton conduction bands. We refer to water to illustrate the mechanism.

The electrical conduction of water systems is principally based on proton transfer which may take place in two different ways (169):

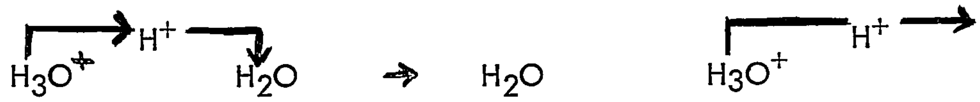
(1)



$$\overset{H^+}{H_2O \quad\quad OH^-} \rightarrow OH^- \quad\quad H_2O \;\; H^+ \rightarrow$$

i. e. proton jumps from water molecules to hydroxyl groups resulting in proton transfer chains; this will appear as an apparent migration of OH^-. This proton movement has to be assumed, because the mobility of an OH^- ion exceeds the mobility of a F^- ion by four*. Theoretically, we would expect similar values for both ions because hydroxyl and fluorine have a comparable ionic radius and mass.

(2)



$$\overset{H^+}{H_3O^+ \quad\quad H_2O} \rightarrow H_2O \quad\quad H_3O^+ \;\; H^+ \rightarrow$$

i. e. the electrical conduction represents proton jumps via water molecules. The mobility of such a proton transfer is approximately twice the mobility of the OH^- ions. By means of a potential barrier diagram, the types of proton transfer are illustrated (Fig. 32a, b). More thermal activation energy is required for a proton movement along the OH^- level relative to that along the H_2O level. This is reflected in the equivalent conductance. In turn, the binding of H^+ to OH^- is stronger than that of H^+ to H_2O.

Studies in recent years have confirmed and expanded the pioneering work of Gurney (169) and his model on proton transfer . It was revealed that

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* The equivalent conductance in aqueous solution at 18°C: $H^+ = 316.55$, $OH^- = 176.6$, $F^- = 46.65$, $Li^+ = 33.28$ (169).

proton migration proceeds in form of charge transfer; proton mobility was also observed on ice structures (172, 173). A number of new models on proton transfer processes have been proposed (174-179). On the basis of empirical data it became evident that the rate determining step of proton migration in water is the re-orientation of the water molecule itself; namely, it can receive a proton from an adjacent hydronium ion* (174, 175, 178, 180).

A smiliar type of proton migration, as is established for the water system, can also be assumed for the surfaces of metal co-ordinated oxygen polyhedra and PO_4. Since polyhedra, similar to crystal structures, are electrically neutral, their potential relief will be flat (Fig. 32e). The metal co-ordinated oxygen polyhedra associated with the phosphates will promote motion. The proton jumps will take place along the surface of the polyhedra:

$$HMO_n^+ \xrightarrow{\quad H^+ \quad} MO_n \rightarrow MO_n \quad HMO_n^+ \xrightarrow{\quad H^+ \quad}$$

Two such surfaces, one in octahedral (Fig. 32-4) and the other in tetrahedral (Fig. 32-1) co-ordination, have been drawn in such a manner, as they will effectively come into existence for proton migration, i. e. as they are seen by the protons. Because the polyhedra chains are electrically neutral:

$$[PO_4^-Na^+][PO_4^-Na^+][PO_4^-Na^+]$$

and the potential barrier is relatively flat, the H^+ ions will only become

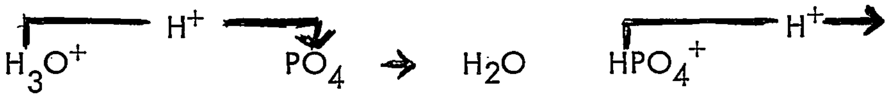
---

* The most probable concept for proton transfer in water has to do with (a) the location of protonic charge (H^+) within the $H_9O_4^+$ complex, and (b) the structural diffusion of such complexes by formation and breaking of hydrogen bonds with the surrounding water structure (176, 177, 181). We would like to propose a model on the molecular structure of the $H_9O_4^+$ complex, which is a consequence of our presentation (Fig. 31).

weakly attached (bonded) to the polyhedra surfaces. Consequently, the Brownian movement will transfer them easily from one oxygen polyhedron to the other (Fig. 32, e), in case interconnecting polyhedra chains or layers are available. With regard to the nucleic acids and membranes, chains and layers of polyhedra are proposed. These structural units will act as proton conduction bands stretched over macromolecular distance, should a difference in electrical potential or in the proton distribution exist. These two migration forces, i. e. electrical potential and pH differences, have been demonstrated to exist in cells. Our deductions will thus closely approach reality; there is no doubt that charge transfer conductors are present in organisms.

The formation of proton transfer bands on membrane surfaces can be considered a reason for the high oxygen content in form of fatty acids, ester linkages (-O-), or phosphatidyl inositol.

A proton transfer in absence of oxygen polyhedra:



i. e. via PO_4 and structural H_2O, is less effective, because higher potential barriers have to be passed (Fig. 32, f). The frequency of proton jumps would be reduced by a few orders of magnitude.

To mention biochemical processes, some information on the energetic position of the proton conduction bands are presented. The oxide polyhedra conduction surface will be positioned higher in comparison to the proton level of the H_2O groups or ATP, and these groups again higher than the level of the PO_4 groups (Fig. 32, e, f). Thus, proton jumps will especially and preferentially take place from the higher to the lower levels (Fig. 32, g). It is our belief that in the case of phosphorylation (ADP → ATP transformation), a proton generating process takes place, and protons are relaesed to the oxygen polyhedra conduction bands. In such bands, they will become distributed by means of thermal motion forces, and they will stay there, until they are transferred to a lower conduction level, or until they are extracted by proton traps.

In order to understand the rôle and the influence of proton trapping, we refer to conductivity measurements of alcohol systems, where these processes are well understood (169).

By adding a trace amount of water to an HCl-C_2H_5OH solution, the electric conductivity is appreciably reduced (Fig. 32, d). The electrical conduction of this system takes place because, analogous to water, the proton of HCl is transferred to an ethanol molecule:

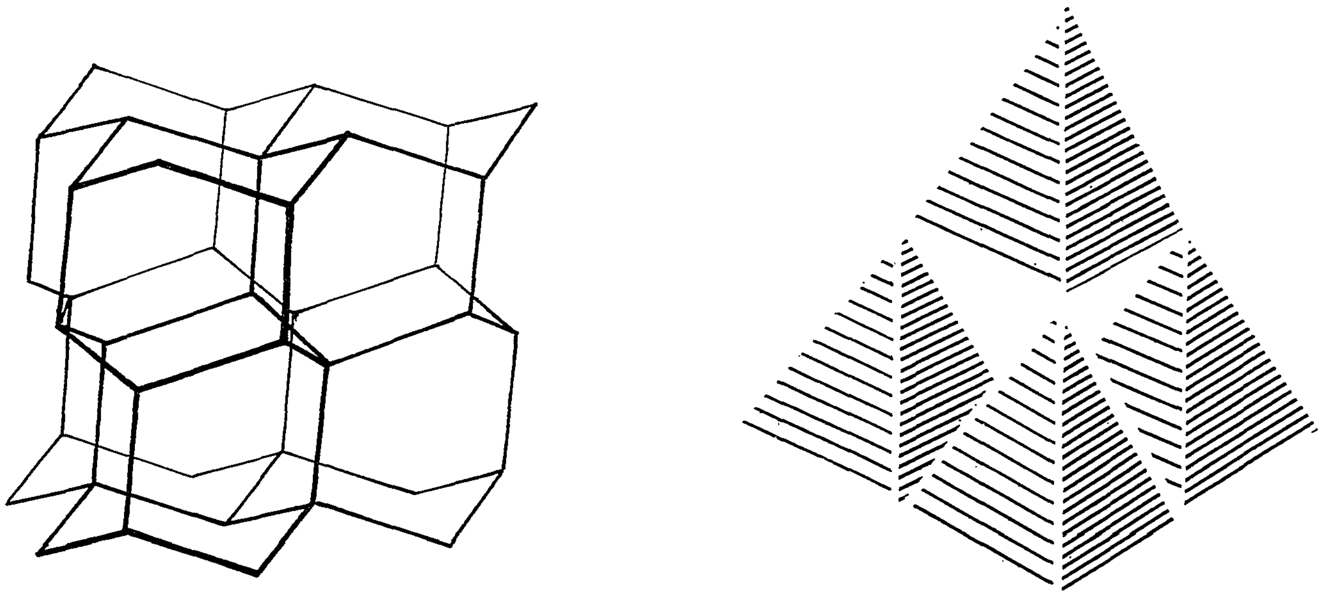
$$HCl + C_2H_5OH \rightarrow Cl^- + C_2H_5OH_2^+$$

and proton jumps between ethanol molecules occur:

$$\begin{tikzcd} C_2H_5OH_2^+ \arrow[r, bend left, "H^+"] & C_2H_5OH \arrow[r] & C_2H_5OH & C_2H_5OH_2^+ \arrow[r, bend left, "H^+"] & {} \end{tikzcd}$$

The large effect of the trace amount of water is caused by the trapping of protons on H_2O:

$$\begin{tikzcd} C_2H_5OH_2^+ \arrow[r, bend left, "H^+"] & H_2O \arrow[r] & C_2H_5OH & H_3O^+. \end{tikzcd}$$



Water structure

$O_4H_9^+$ complex

FIGURE 31

Molecular association of H_2O molecules (liquid phase). The oxygens form OH_4 tetrahedra (see Fig. 3) which via corner-linkages condense to 6-ring units and construct a three-dimensional network similar to that of the quartz lattice. The spacial cross-linkages of the 6-ring units is shown in the left picture (182). In view of the face that the hydrogen energy in H_2O can be considered a polization energy (183), $O_4H_9^+$ units will be the minimum structural building block for proton trapping, as is shown in the right picture. The proton is located in the center of the tetrahedra which are linked across corners; it is co-ordinated by four oxygens located in the center of the innermost tetrahedra surfaces. The charge units for the $O_4H_9^+$ complex amounts to:

4	O	atoms	(located in center of tetrahedra)	=	(-)	8
6	H	atoms	(edge position)	=	(+)	6
4	H	atoms	(located at the outer corners)	= ½	(+)	4
1	H	atom	(center position)	=	(+)	1
					(+)	1

This model is further supported by the fact that the phase diagram of ice includes lattices which contain ring-structures composed of cross-linked tetrahedra building four membered ring units.

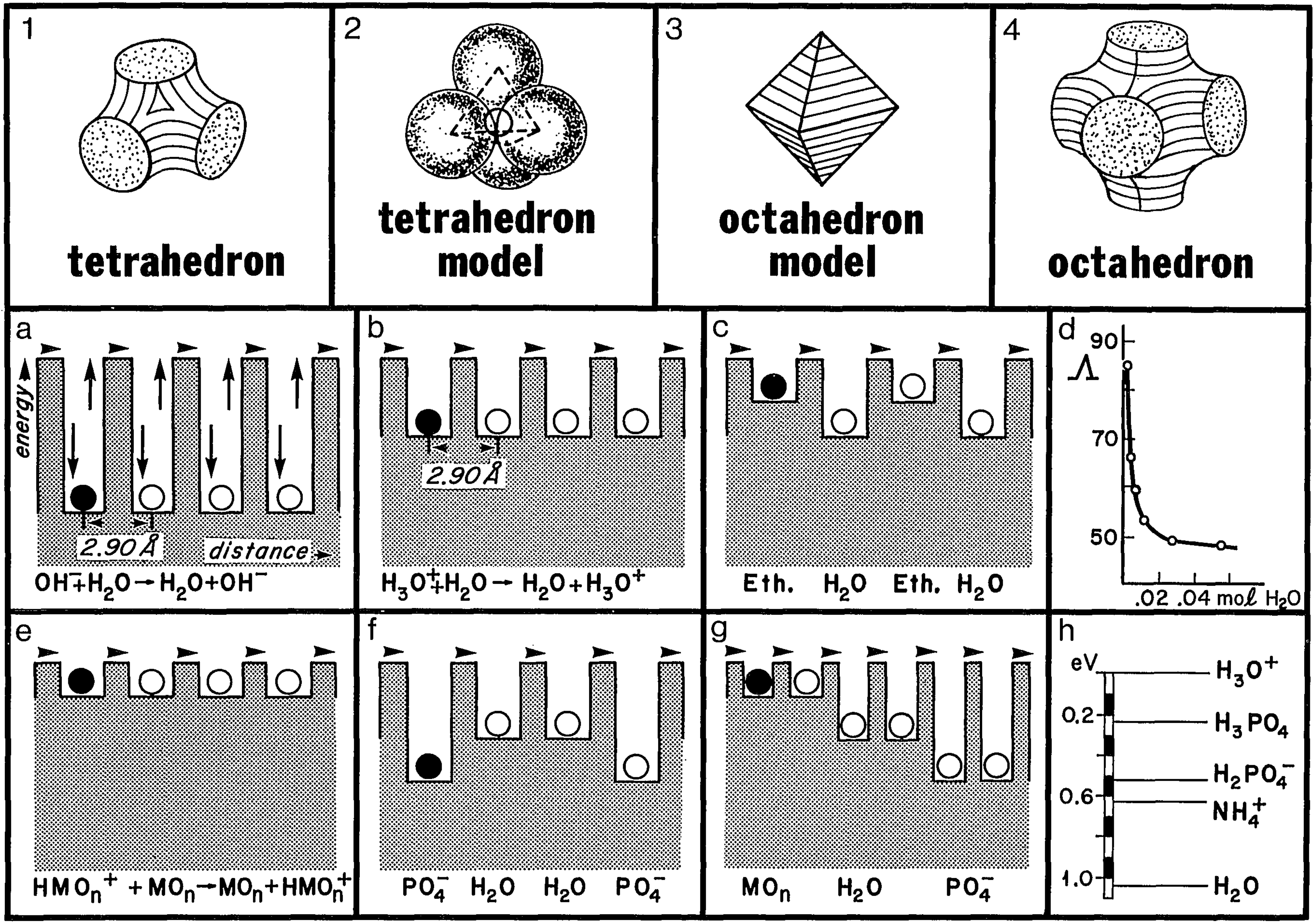

1
tetrahedron
2
tetrahedron model
3
octahedron model
4
octahedron
a
energy
2.90 Å
distance
OH⁻+H₂O → H₂O+OH⁻
b
2.90 Å
H₃O⁺+H₂O → H₂O+H₃O⁺
c
Eth. H₂O Eth. H₂O
d
Λ
90
70
50
.02 .04 mol H₂O
e
HMOₙ⁺ + MOₙ → MOₙ + HMOₙ⁺
f
PO₄⁻ H₂O H₂O PO₄⁻
g
MOₙ H₂O PO₄⁻
h
eV
0.2
0.6
1.0
H₃O⁺
H₃PO₄
H₂PO₄⁻
NH₄⁺
H₂O

Figure 32

FIGURE 32

Schematic energy level diagram illustrating the transfer of a proton from one energy level to the other by means of so-called "proton jumps" (Figs. a, b, c, e, f, g). The potential energy is plotted along the vertical axis; and a generalized configurational co-ordinate (distance) is plotted along the abcissa. An occupied level is indicated by a black dot, and an empty level by an open circle.

Proton migration for the electric conduction of water is shown in Figures a and b. The distance between two water molecules, within the hexagonal water network, amounts to about 2.90 A as determined by X-ray and neutron diffraction (182). The suppression of proton mobility by adding traces of water to acidified ethanol is illustrated in Figure c; the protons become trapped at the lower-positioned water levels. The drop in the equivalent conductivity ( ) of HCl, as a function of the mol-fraction of water added to ethanol, is shown in Figure d (169). A diagram of the energy level explaining the proton movement along a polyhedra chain is presented in Figure e. The respective polyhedra units are drawn in picture 1 to 4 in two different ways: (a) refined, for graphical reasons (picture 2 and 3) and (b) real in a three-dimensional design to illustrate the way the protons move along the polyhedra surfaces (picture 1 and 4); the dotted cross-sections are the cuts through the oxygen atoms. The proton migration mechanism along these polyhedra surfaces can be considered analogous to the polaron migration mechanism. In this instance, a self-trapping of the electron in the conduction band is caused by the polarization of the electron field (261). A similar self-localization of the protons can here be assumed and the flat potential barrier profile is the result.

Figure f illustrates proton migration in absence of metal ions in case single PO_4^- groups, as those of the nucleic acids and phospholipids, are bridged by structural water. Proof for the fact that in PO_4 containing systems proton-charge transfer processes preferentially take place and not those involving ions can be obtained by reference to electric conductivity measurements on H_3PO_4 (170, 171).

Figure g shows the action of H_2O and PO_4^- molecules upon the proton migration along the oxide chain. It causes a suppression in mobility via proton trapping or it can act as a means for proton suction.

In Figure h, the energy scale for a few of the calculated occupied proton levels is included (169). The values represent the energy distance relative to H_3O^+.

The potential relief for a proton transfer in acidified ethanol in the presence of water is schematically presented (Fig. 32, c). Suppression of proton migration on oxide polyhedra can be caused by the presence of isolated phosphates such as ATP or similar acting molecules (Fig. 32, g). It is evident that these processes represent an electro-chemical "liquid state" amplifier similar to the memistor device or the iodine-iodine cell. Electronically speaking, the proton traps within the oxygen polyhedra chain operate like the electrostatic control grid in a triode. In addition, such a device has an "on and off" switch by the elimination or introduction of polyhedra.

A further implication is briefly considered. In case jumps take place along the oxygen polyhedra chains of the nucleic acids by field induction phenomena, eddy-currents may come into existence due to the interaction of such a proton motion with the pi electrons of the heterocyclic compounds*. It appears to be that this fact plays a significant part in the molecular structure of the "memory".

FUNCTION OF ATP

ATP is an instructive example for the biochemical utilization of certain physical-chemical properties of inorganic molecules. The strong affinity of phosphorus to H_2O, and therefore the tendency of P-O-P linkages to hydrolyze, finds its expression in the extremely low vapor pressure for H_2O in presence of P_2O_5 (1.10^{-6} Torr at 10°C) (186) and that crystalline anhydrous $H_5P_3O_{10}$

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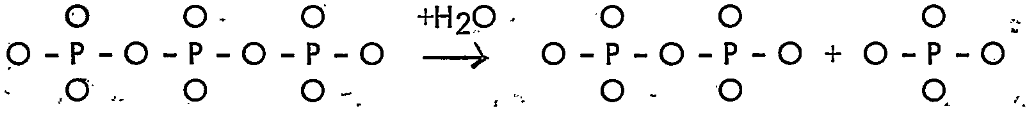
* Concerning the quantum mechanical properties of DNA, see (185).

(triphosphoric acid) is not known. All chain polymers of PO_4 are unstable in aqueous solution; hydrolysis starts at the terminal group. For triphosphates (TP), hydrolysis is a first order reaction; mono-and di-phosphates, in a molar ration of 1:1, are the hydrolysis products (187, 188):



$$O-\underset{O}{\overset{O}{P}}-O-\underset{O}{\overset{O}{P}}-O-\underset{O}{\overset{O}{P}}-O \xrightarrow{+H_2O} O-\underset{O}{\overset{O}{P}}-O-\underset{O}{\overset{O}{P}}-O + O-\underset{O}{\overset{O}{P}}-O$$

Di-phosphate yields mono-phosphate; again this is a first order reaction which proceeds without interference of III-P hydrolysis (189). This unique hydrolysis mechanism where:

(a) both hydrolytic degradations occur one after the other and independent of each other, and

(b) both processes are first order reaction,

is most certainly the physical reason for the key position of ATP in living cells. The empirically derived data reflect the "nature" of the PO_4 tetrahedron. The interpretation of the phenomenon that the hydrolysis of III-P is a first order reaction,* and that the order is unaffected by pH, (189, 192, 193), indicates the mechanism of degradation. The meaning of a first order reaction is that a structural

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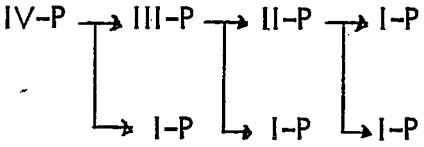
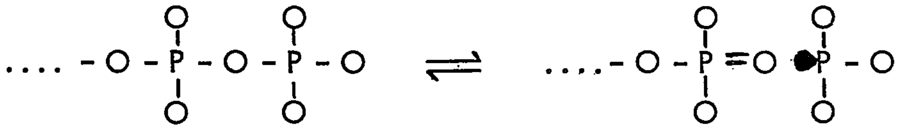
* Similar relationships are established for IV-P:
The hydrolysis of tetra-phosphates yields monophosphates which are released one after the other, starting from the chain terminal. Again, all reactions are first order ones and proceed independently from each other (190-192):



IV-P	tetra-phosphate
III-P	tri-phosphate
II-P	di-phosphate
I-P	mono-phosphate

transformation of the system takes place which is independent of the environment. The reason for the decomposition of the PO_4 polymers, therefore, lies within the phosphates and can be considered a reaction which is comparable to radioactive decay.

Consistent with this inference is the equilibrium process, which yields a $\bullet PO_3$ radical and a PO_4 tetrahedron having a P=O double bond:



$$\ldots\ldots -O-\overset{\overset{O}{|}}{\underset{\underset{O}{|}}{P}}-O-\overset{\overset{O}{|}}{\underset{\underset{O}{|}}{P}}-O \rightleftharpoons \ldots\ldots -O-\overset{\overset{O}{|}}{\underset{\underset{O}{|}}{P}}=O\ \bullet\overset{\overset{O}{|}}{\underset{\underset{O}{|}}{P}}-O$$

State A State B

The way the pi electron is allocated for in the tetrahedron (Fig. 6), can be considered the reason for this mechanism. The equilibrium between state A and state B is dependent on the electrostatic field developed by the particles or molecules which enclose the charged terminal group. In the presence of water, and when the individual water dipols are neutralized, the equilibrium between the state A and state B will be shifted in the direction of A; in contrast, the presence of cations will shift the equilibrium to the B side. That hydrolysis proceeds from the chain terminal is related to the absence of one charged oxygen in the centre group. The formation of a PO_3 radical is, or rather likely is, incidental, because many different phospho-oxides and the stable P_4O_6 molecules are known (Fig. 5).

The equilibrium process, as determined by the bond configuration, fulfills the condition of a first order reaction. In turn, the influence of the solution parameters is only reflected in the shift of the equilibrium between state A and state B. For, the rate determining step for hydrolysis is the transition from state A to state B. This is confirmed experimentally; a variable pH will only introduce a change in the rate constant $(k \cdot t^{-1})$ (191-193). The resulting PO_3 radical will immediately react with the OH^- groups and does not influence the reaction velocity.

It has also been demonstrated that cations, including H^+, strongly accelerate the hydrolysis of III-P (60). The affect of cations on III-P is similar to their affect in the hydrolytic degradation of high molecular polyphosphates (Table 4):

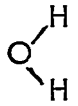
TABLE 4

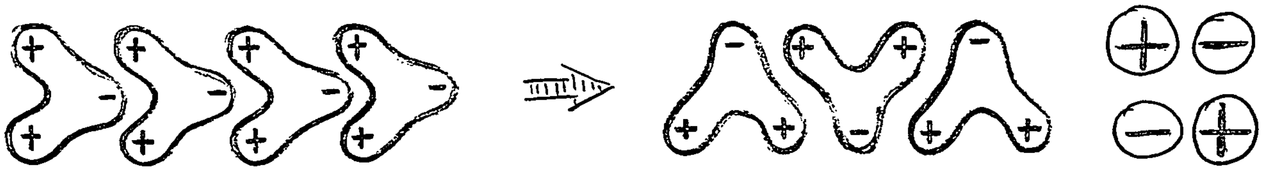
CATALYTIC ACTION OF VARIOUS CATIONS ON THE RATE OF HYDROLYSIS OF POLYPHOSPHATES

Cation	K^+	Na^+	Li^+	H^+	Ba^{++}	Sr^{++}	Ca^{++}	Mg^{++}	Al^{+++}
K/K_0 (194)	~1	~1	1.08	~1.4	1.56	1.69	2.78	3.52	7.50
Ionic Radius (12)	1.33	0.95	0.60	0.30	1.35	1.13	0.99	0.65	0.50

In this way the observation, that the catalytic affect of metal ions participating in the hydrolysis of phosphates increases upon lowering of the dielectric constant (by adding organic solvents), finds its interpretation (195). That is, through the elimination of the hexagonal water structure in presence of , e.g. dioxan, (Fig. 30), a dipol compensation of the H_2O molecules will be accomplished (Fig. 33). Consequently, the metal ions can become co-ordinated, without interference, to the III-P groups. For the formula shown in Figure 33, the molucular structure of hydrated TP is outlined (Fig. 34) to show the way, metal ions are co-ordinated to the triphosphates. In biochemical processes, ATP operates on these principles.

FIGURE 33-1

Association of  $O\begin{smallmatrix}H\\H\end{smallmatrix}$ dipols in water structure



large dipol momentum through chain association

small dipol momentum through dipol compensation

quadrupol

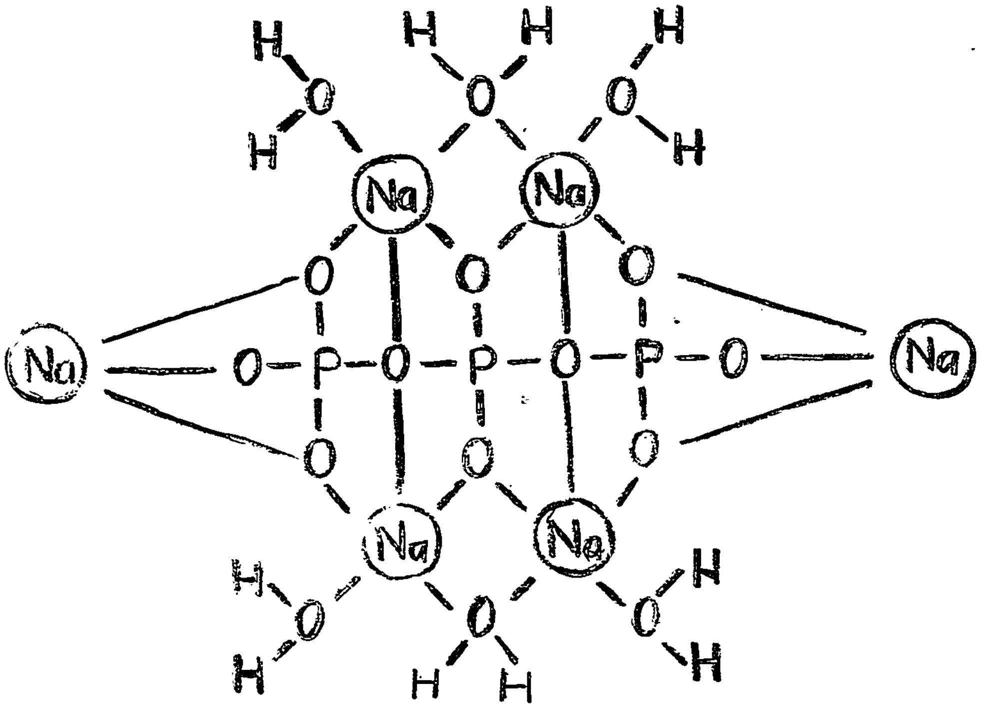


FIGURE 33-2

Attachment of the metal ions to the triphosphate; for polyhedra structure see Figure 34.

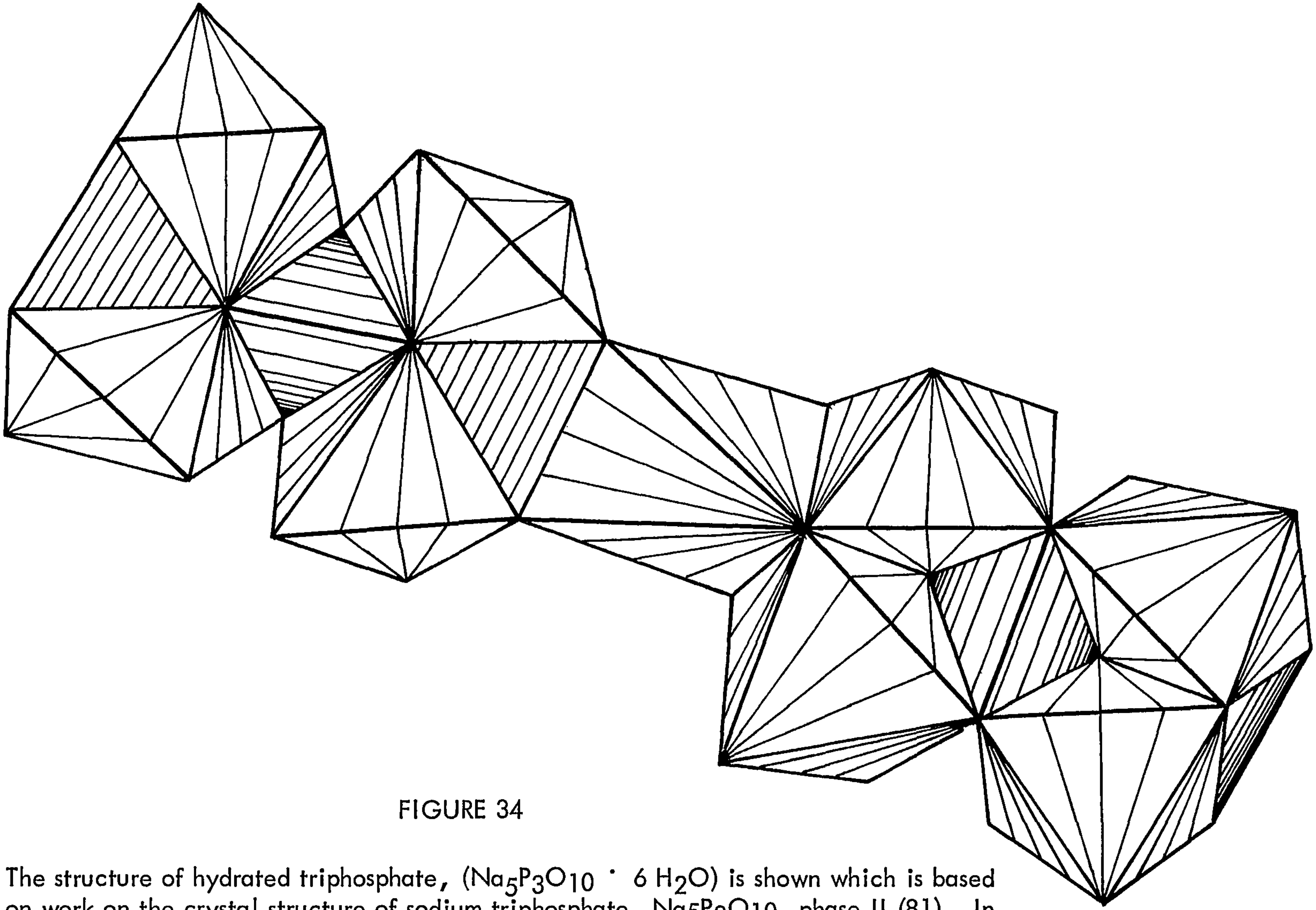
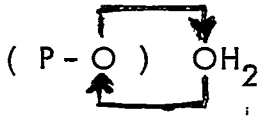


FIGURE 34

The structure of hydrated triphosphate, ($Na_5P_3O_{10} \cdot 6\,H_2O$) is shown which is based on work on the crystal structure of sodium triphosphate, $Na_5P_3O_{10}$, phase II (81). In the crystalline phase of the last mentioned phase, some of the sodium ions are co-ordinated in distorted octahedral arrangements, while the remainder are involved in 4-fold co-ordination. The sheet structure can be hydrated and yields the hexahydrate. The water molecules, of which the oxygens are co-ordinated to sodium, are located at the outer corners of the octahedra; hydrogen atoms have been omitted for graphical reasons. This drawing should illustrate the formation of metal-ion polyhedra in the case of the III-phosphates, and can further be used for the illustration of the interactions of metal ions and ATP.

To further underline our concepts on the hydrolysis mechanism of III-P, we refer to isotope exchange experiments. The exchange of ^{18}O in H_2O versus ^{16}O in $H_3P^{16}O_4$ follows a first order velocity equation (196, 197) which implies that the flow of oxygen is independent of the ^{18}O concentration and that the exchange process is not influenced by the neighboring reaction partner. Namely, the oxygen is tightly fixed to the phosphorus which is indicated below by parenthesis:



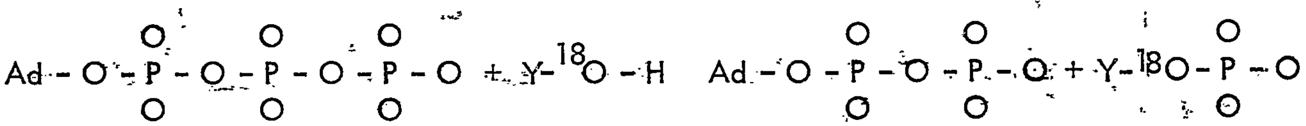
This phenomenon is also demonstrated by the electrolysis of H_3PO_4 solvent in $H_2{}^{18}O$. The oxygen released at the electrodes is exclusively derived from the solvent as shown mass-spectroscopically (196). Thus, external factors (environment) will not remove oxygens from the tetrahedra. In accordance with this phenomenon is the observation that no ^{32}P exchange takes place between H_3PO_3 and H_3PO_4 (198). On chemical grounds one has to assume that a solution of H_3PO_2 acid contains two tautomeric forms, i.e. a normal and an active one (Fig. 35). This has been confirmed by deuterium exchange experiments (200-203). Oxygen isotope exchange studies also favor the presence of a radical (tautomeric form b, Fig. 35) (203). Similar relationships have been postulated for H_3PO_3 on the basis of reaction kinetics; the existence of an active form, ●$P(OH)_3$, with an isolated electron-pair, has been suggested (204, 205). Deuterium exchange studies also favor the presence of the tautomeric form ●$P(OH)_3$ (206, 207) (Fig. 35).

In oxygen isotope analysis on carbonates, phosphoric acid (100%) is used for the liberation of CO_2 (208). It could be shown, that no exchange takes place between the CO_2 and the H_3PO_4 because the oxygen in the PO_4 tetrahedron is tightly bound.

It is interesting to note that no oxygen exchange takes place between H_3PO_3 and $H_2{}^{18}O$ in a solution of Na_2HPO_3 (209). This is so, because the oxygens of the PO_3 group are protected by the polyhedron and thus isolated from the solution. In case of alkali salts of H_3PO_4, similar supressions of oxygen exchange in presence of metals has been noticed (209, 210). The existence of an active PO_3 radical, with an isolated electron-pair, as we have postulated, agrees with analytical facts derived by others.

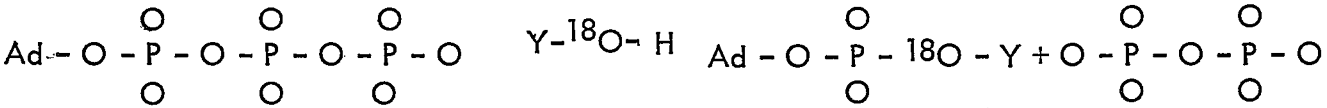
The existence of a radical-form is further supported by the analytically determined complex compounds such as $(Pt\ (OH_3)_4)\ Cl_2$, which contains a three-valent phosphorus, $-P\ (OH)_3$ in a stable form (211, 212).

The triphosphate cleavage mechanism, as mentioned here, finds its direct confirmation by ^{18}O exchange studies. The bond cleavage of ATP catalysed by myosin (213) or several other bond cleavage reactions of ATP (214-217) takes place between the terminal P atom and the oxygen:



$$Ad - O - \overset{O}{\underset{O}{P}} - O - \overset{O}{\underset{O}{P}} - O - \overset{O}{\underset{O}{P}} - O + Y - {}^{18}O - H \quad Ad - O - \overset{O}{\underset{O}{P}} - O - \overset{O}{\underset{O}{P}} - O + Y - {}^{18}O - \overset{O}{\underset{O}{P}} - O$$

This work further shows that the cleavage point is determined by type of enzyme. For instance, an enzyme of the snake venom, catalyzes the fission point in such a manner that a radical of adenosine monophosphate is obtained (218). This kind of reaction is commonly observed in biochemical reactions (219-221), ATP → AMP + DP.*



$$Ad - O - \overset{O}{\underset{O}{P}} - O - \overset{O}{\underset{O}{P}} - O - \overset{O}{\underset{O}{P}} - O \quad Y - {}^{18}O - H \quad Ad - O - \overset{O}{\underset{O}{P}} - {}^{18}O - Y + O - \overset{O}{\underset{O}{P}} - O - \overset{O}{\underset{O}{P}} - O$$

For further references on biochemical details or intermediary products involved in the hydrolysis of ATP, see (222).

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* Based on experiments on the enzymatic hydrolysis of inorganic high-molecular polyphosphates, we conclude that the enzymatic bond cleavage in phosphates represents a process which is well-adjusted to the individual organic products. In contrast to the hydrolysis in aqueous solutions, where the cleavage of phosphate groups proceeds from the chain terminal (60), the high-molecular polyphosphates are nearly exclusively cleaved enzymatically in the center of the chain (223) whereby metal ions excercise a significant influence (224).

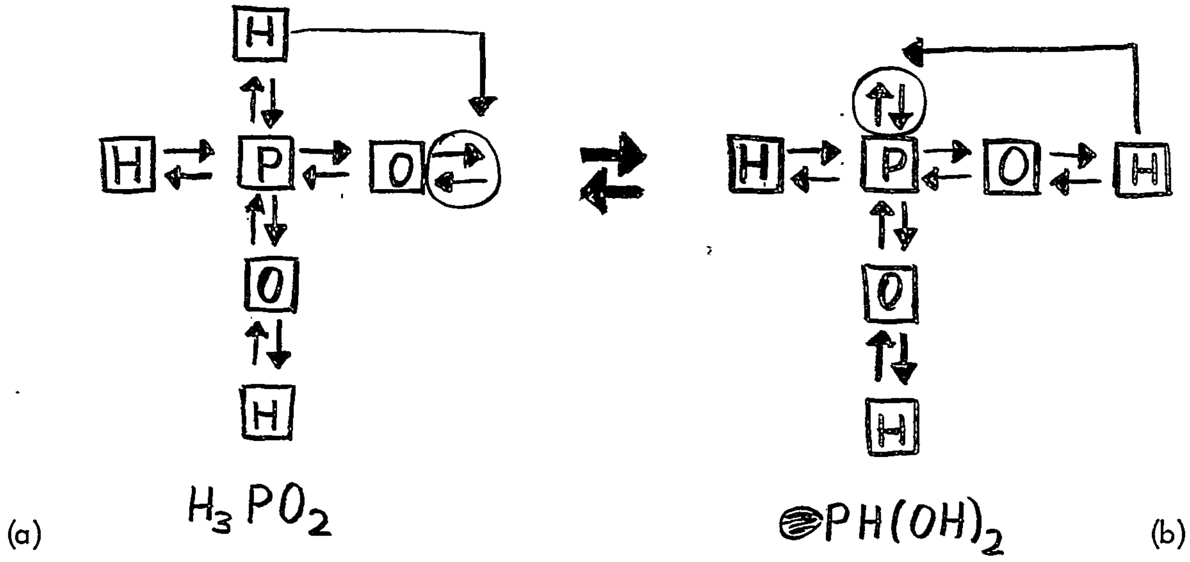


FIGURE 35-1

Equilibrium between the tautomeric forms (a) and (b). State (b) represents the active radical form, $PH(OH)_2$, having an isolated electron pair. Arrows indicate the bonding-electron pairs.

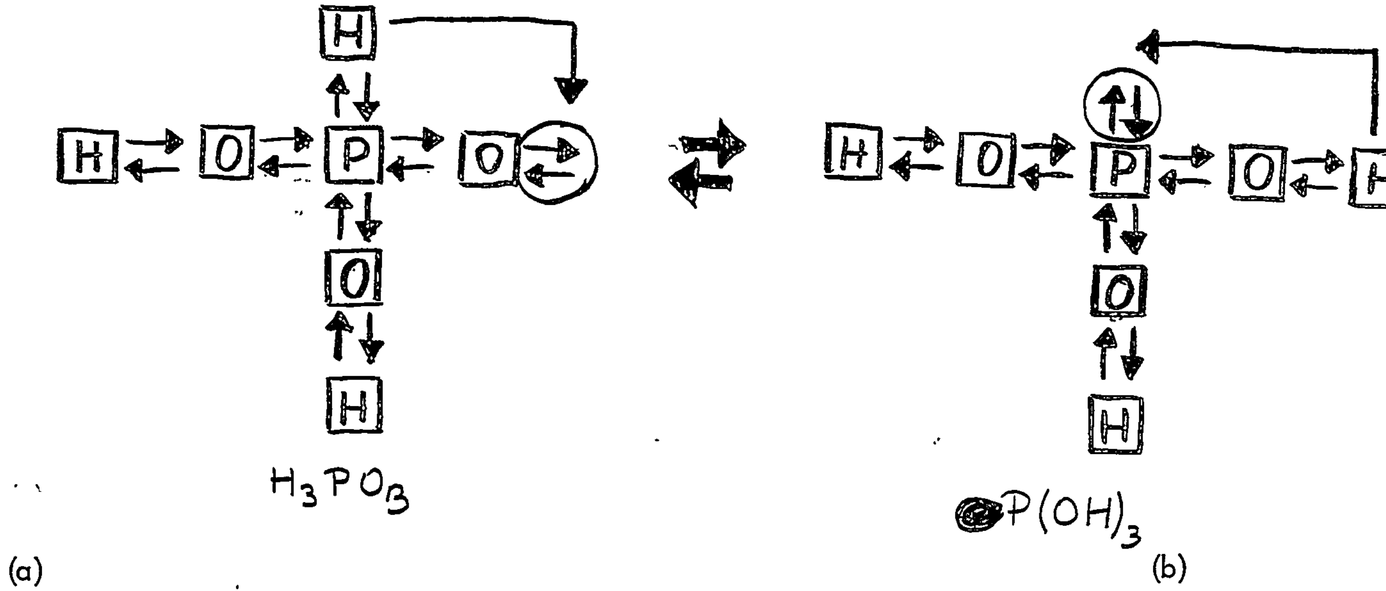


FIGURE 35-2

Equilibrium between the tautomeric forms (a) and (b). Arrows indicate the bonding-electron pairs. State (b) is the active radical form, $P(OH)_3$, having an isolated electron pair.

The III-P molecule is also characterized for its ability to form strongly bonded co-ordination polyhedra with cations. In this way, it resembles polyphosphates in the distinguished ability to bind polyvalent metals in such a manner, that they no longer can be determined by ordinary analytical precipitation agents (60, 225); this sets III-P apart from the mono- and di-phosphates. For a three-tetrahedron-chain, opposite to a di-phosphate, the formation of monomeric units with polyvalent ions (e.g. $Mg_2P_3O_{10}^{-1}$) is a reality; in contrast, di-phosphates will only produce metal-ion polyhedra with $M^{+2} \geqslant n$ in which at least two II-P groups participate (dimer units). They commonly end up as non-soluble salts which are cross-linked by the sharing of polyhedra, as is experimentally confirmed. A particular favorable bonding state of III-P is established for Ca^{++} in the presence of Na^{+} ions; it exceeds the capacity of the polyphosphates (123). It is most likely that this specific co-ordination of the $Ca_2NaP_3O_{10}$ units is utilized in biochemical reactions.

The stability constant pK for metals co-ordinated to III-P increases for the noble-gas configurated elements* in the order (226, 228):

$$Cs < K < Na < Li < Ba < Sr < Ca < Mg$$

This relationship strongly reflects the formation of metal ion oxygen polyhedra; for, the stability increases with the charge and the reduction in ionic radius.

* In view of the fact that non-noble gas configurated elements deviate from this rule (caused by chelation), the corresponding Δ pH values are presented:

Ion	Fe^{3+}	Pb^{2+}	Zn^{2+}	Cu^{2+}	Ni^{2+}	Co^{2+}	Cd^{2+}	Mg^{2+}	Ca^{2+}	Sr^{2+}	Ba^{2+}	K^{+}	Na^{+}
Ionic Radius	0.67	1.21	0.74	0.72	0.69	0.72	0.97	0.65	0.99	1.13	1.35	1.33	0.95
ΔpH (229)	4.37	3.80	2.20	2.15	1.98	1.95	1.90	1.35	1.10	1.10	0.98	0.01	0.01

ΔpH is a measure of the stability of complexes obtained by reacting $HP_3O_{10}^{-4}$ and metal ions $HPO_3O_{10}^{-4} + M^{n+} + MP_3O_{10}^{(5-n)^-} + H^+$. The formation constant K is an approximate function of $\Delta pH = 0.5 \ln (I + K \cdot [M])$ and the metal ion concentration $[M]$ (229).

In conclusion, triphosphate has two significant properties which are related but independant from eachother:

(a) terminal chain degradation, i.e., release of terminal PO_4 groups and formation of PO_3 radicals, which is controlled by external electrostatic fields and,

(b) affinity to all cations by means of metal ion oxygen polyhedra; and by virtue of this polyhedra formation, the establishment of a displacement order set among the metal ions.

Triphosphates are unique in the sense that they represent a convergation point of two physical properties (a and b), both of which are a function of the number of monomers in a chain:

(a) $\text{II-P} \gg \text{III-P} > \text{IV-P} > \text{V-P} \gg \text{VI-P} \gg \text{VII-P}$ hydrolysis order

(b) $\text{I-P} < \text{II-P} < \text{III-P} < \text{poly-P}$ metal bonding order

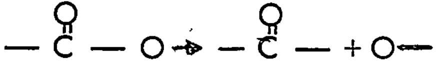
The stability against hydrolysis is reduced with increasing chain length (a), and the metal ion bonding strength increases with chain length (b). Consequently, III-P is a compromise between maximum metal ion affinity and minimum speed of hydrolysis. These two properties are biochemically utilized, since they permit the most efficient control on III-P hydrolysis. Namely, triphosphate carries, in the form of a metal ion, the tool along for its own cleavage.

It is logical that thriphosphate occupies a key position in cellular chemistry. For, the cleavage of the III-P bond represents a reaction which proceeds independantly from the concentration of the reaction partner:

$$\frac{d[\bullet PO_3 \text{ radical}]}{dt} = k\,[P_3O_{10}]\;;\; k = f(E);\; E = \text{local field strength}$$

i.e., the amount of particles in the solvent medium cannot influence the reaction. The rate constant (k) itself is a function of the local electrostatic fields, through which this I-order process is controlled.

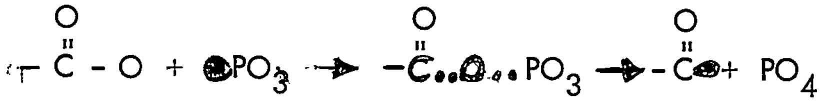
One can now ask, what kind of conditions have to be fulfilled for the biosynthesis of polysaccharides, proteins or the nucleic acids. Three principal requirements come to our mind:

(1) activation of the reaction partner, e.g. 

$$-\overset{\overset{O}{\|}}{C}-O \rightarrow -\overset{\overset{O}{\|}}{C}- + O-$$

(2) maximum efficiency and minimum error, and

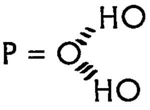
(3) well-defined control of the reactions in terms of kinetics and the sequence of singular events.

All three requirements are most effectively fulfilled by triphosphates. The metal ion dependant formation of the reactive PO_3 radical not only activates the reaction partner, e.g.,  $-\overset{O}{\overset{\|}{C}}-O + \bullet PO_3 \rightarrow -\overset{O}{\overset{\|}{C}}\bullet\bullet O\bullet\bullet PO_3 \rightarrow -\overset{O}{\overset{\|}{C}}\bullet + PO_4$ but also eliminates, by means of the phosphate formation, the OH or O groups from the reaction system. Due to the elimination of the OH or O group, the reaction proceeds only in one direction. In biochemical reactions, this rôle is exercised by ATP.

The condensation of mono-phosphates to di-phosphates appears to follow different ways. Analytical information are presented in support of this inference. In highly concentrated $H3PO_4$, a formation of $H_4P_2O_7$ takes place (171, 230, 231); in solutions greater than 85 weight per cent, the diphosphate can be determined by means of Zn^{+2} precipitation (232). The fact that the equilibrium constant K is practically unaffected by temperature variation (196, 231):

$$K = \frac{H_4P_2O_7 \cdot H_2O}{(H3PO_4)^2} \approx 5 \cdot 10^{-3}$$

and the ^{18}O exchange velocity on monophosphate increases rapidly with increase in concentration (196), suggests that the formation of diphosphate proceeds via ionic interaction of probably three PO4 tetrahedra. For, the isotope exchange at high concentrations can be explained satisfactorily if one assumes that it takes place via formation and hydrolysis of diphosphate and triphosphate (196).

Studies on the dehydrated melts of H_3PO_4 throw some light on the formation of polyphosphate. The H_3PO_4 structure, as determined by X-ray analysis (233, 234) indicates that the  $P = O \overset{HO}{\underset{HO}{<}}$ (P = O, d = 1.517 Å) double bonded oxygen is co-ordinated by two closely spaced HO-groups (0 - 0 distance = 2.84 A). This explains the presence of the auto-protolyse products (171) in melts:

$$2\,H_3PO_4 \rightleftharpoons P(OH_4)^+ + (H_2PO_4)^-$$

which were obtained by means of conductivity measurements. This equilibrium is rapidly established by a proton transfer (Fig. 36) and is in accordance with the correspondingly low bonding energy as caused by the P = O distance (the P-OH distance $\approx$ 1.57 Å) (see Fig. 6), and the measured proton mobility upon current transport. We like to point out that the form $P(OH)_4^+$ is most likely present as $P(OH)_4^+\ Cl^-$ (235) (in a compound with HCl and H_3PO_4), whereas $P(OH)_4^+\ ClO_4^-$ has been found in a compound with perchloric acid (236-238). Based on the type of electrical conductivity it has been shown that the complex-compound of H_3PO_4 with BF_3, dissociates into $P(OH)_4^-$ and $(H_2PO_4 \cdot (BF_3)_2)^+$ ions (239).

It is suggestive that the process of II-P formation takes place via $P(OH)_4^+$ as shown in Figure 36, because proton transfer and proton migration is unquestionably present in phosphoric acids (170, 171, 239). Just to give an idea in comparison to water, the ion concentration in a H_3PO_4 melt is about 10^6 times greater (171). In accordance with this mechanism are the observations that pyrophosphates are generated in a H_3PO_4 melt following this mechanism (232):

$$3\,H_3PO_4 \rightleftharpoons \left[H_3O^+ \cdot (H_2PO_4)^-\right] + H_4P_2O_7$$

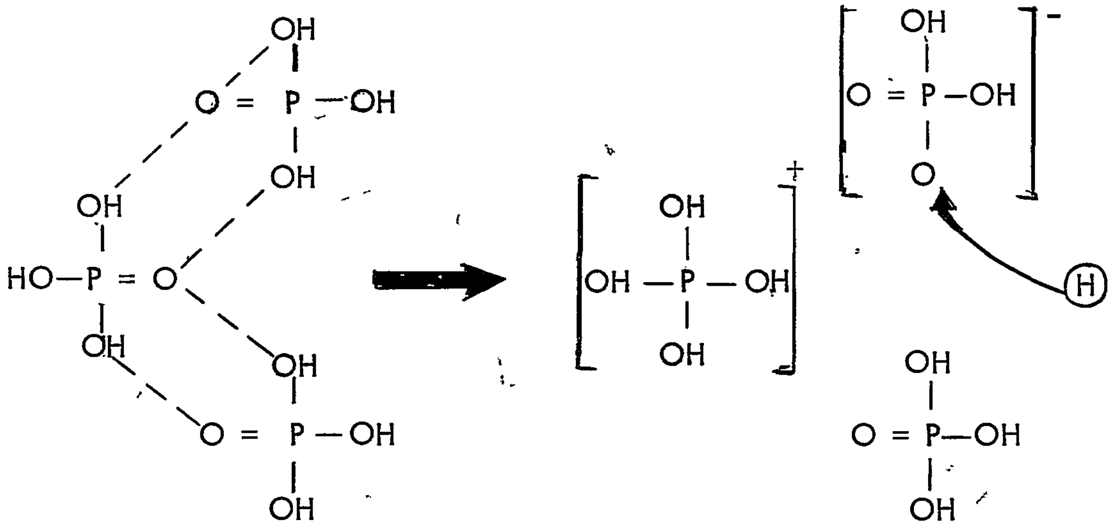
For crystal-structural reasons, this equilibrium does presumably involve H_3O^+, which is also indicated by the fact that water is not released during the formation of II-P (240). The same reaction mechanism will also lead to triphosphate with

FIGURE 36

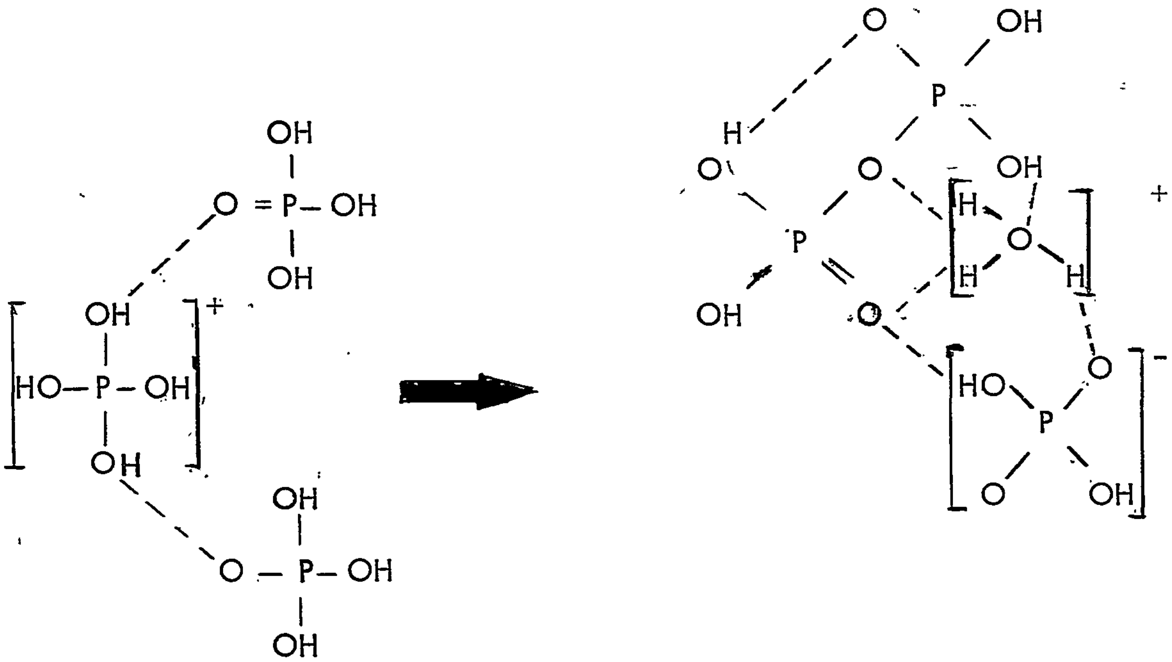
Formation of di-phosphate:

$$3\,H_3PO_4 \rightarrow [H_3O^+ \cdot H_2PO_4^-] + H_4P_2O_7$$

The configuration of the H3PO4 molecules is shown approximately to reality.



The form $[P(OH)_4^+ \cdot H_2PO_4^-]$ originates via proton transfer (auto-protolysis). In case the $H_2PO_4^-$ catches a moving proton, the $P(OH)_4^+$ complex becomes unstable and transformed into a more stable configuration.



Stabilization is achieved by the elimination of an OH^-, and a proton, from the $P(OH)_4^+$ molecule, and the joining to diphosphate, whereby the formation of $[H_3O^+ H_2PO_4^-]$ favors the newly generated form and allows a structural fit via O - H bridges.

a correspondingly lesser yield, of course. Chromatographic determinations give the ratio (241):

$$\text{I-P} : \text{II-P} : \text{III-P} = 3000 : 200 : 1$$

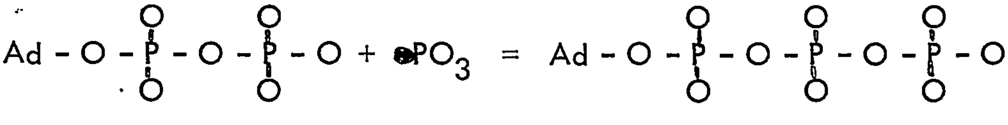
These data again illustrate the uniqueness of the phosphorus-oxygen compounds; it is the reversibility of the P – O – P bond cleavage and linkage which is utilized biochemically. The fact that the polymerization of PO_4 groups is a function of the PO_4 concentration, and that this process proceeds most likely via the ionar form $P(OH)_4^+$, permits certain conclusions regarding the biochemical execution of oxydative phosphorylation, such as involvement of ionic co-ordination neighbors and proton transfer, or the accumulation of PO_4 units in small reaction spots.

It is understandable that the inorganically self-propagating process of polyphosphate formation occurs in cells, whereby enzymes control and accelerate this process, to such an extent that even enzymes are capable to catalyze inorganic H_3PO_4 to $H_4P_2O_7$ (242), or II-P to $H_5P_3O_{10}$ (243).

To further underline the peculiarity of P – O compounds, an inorganic example is mentioned to show the way phosphorus compounds adjusted to the organic materials present in cells. Solid H_3PO_4 becomes only slowly esterified by alcohols such as methanol, glycerin, or phenol; it is noteworthy that the reaction is not proportional to the product of H_3PO_4 and alcohol but actually to the square of the acid $(H_3PO_4)^2$. It is thus concluded that the reaction proceeds via di-phosphate formation, and that the ester formation comes into existence by means of the cleavage of the P – O bonds (244) and not via linkage to the single PO_4 groups.

By the biochemical utilization of this property a distinct order of the reaction sequence in biochemical processes is maintained. For, only through the cleavage of the P-O-P groups the Y-OH groups can be esterified, whereby the process itself is controlled via the formation or supply of these P-O-P elements in the form of di-, and tri-phosphates.

Similarly, a distinct order in the cleavage of the P-O-C bond has been confirmed by ^{18}O-isotope exchange studies. The enzymatic hydrolysis of a phosphate ester results only in the cleavage of the P-O but not of the O-C bond (245-249). The ^{18}O studies further indicate that the phosphorylation reaction from ADP to ATP takes place via a $\bullet PO_3$ radical because the bridge-oxygen is supplied from the ADP group (250):



$$\mathrm{Ad} - O - \overset{O}{\underset{O}{P}} - O - \overset{O}{\underset{O}{P}} - O + \bullet PO_3 = \mathrm{Ad} - O - \overset{O}{\underset{O}{P}} - O - \overset{O}{\underset{O}{P}} - O - \overset{O}{\underset{O}{P}} - O$$

SUMMARY

Based on the fact that:

(a) phosphorus has a distinct relation to the common biogenic elements (Fig. 4),

(b) cell processes are thermodynamically maintained via oxidation,

(c) oxidation and other processes must be well-controlled, highly effective, and activated,

(d) PO_4 units introduce a biocrystallographical order, and

(e) the principle of economy requests that only a minimum number of different building blocks ought to be used,

it is finally concluded that phosphates represent the only meaningful solution for the kind of life processes as we know them. (264).

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ORIGIN, DEVELOPMENT, AND DIAGENESIS OF BIOGEOCHEMICAL COMPOUNDS

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INTRODUCTION

In recent years, much has been learned on the origin of life here on earth (1). The character of the primordial atmosphere, lithosphere, and hydrosphere, the geo-dynamics, and even cosmic events have been used for the elucidation of the phenomenon life. There is general agreement that the origin of life is a three step process: (a) formation of organic monomers, (b) synthesis of polymers, and (c) organization of cell material.

Thermodynamic equilibrium computation procedures have shown (2) that in pre-biological terrestrial atmospheres the key monomeric building blocks of life were present in concentrations of about 10^{-15} to 10^{-30} molar. Such minute amounts of, for instance, amino acids, will never accomplish polymerization effects. Of course, reaction yields will depend on the kind and degree of radiation energies. The observed photooxidation of organic matter in sea water by UV light (3), however, is independent proof for the unlikelihood of polymerization in atmospheric or sea water systems even if, due to a higher radiation dosis, the annual production rates for monomers could be increased by a few orders. The problem in the synthesis and development of abiotic polymers thus primarily requires a concentration mechanism for the extremely dilute monomeric systems.

Part one of our study is principally concerned with this problem. A concept is proposed in which silicates operate as concentration agent, ion exchanger, and polymerization template. The model is tested experimentally in part two of the work. Silicates are studied for their adsorption and polymerization efficiency. In the third part, we present information on the development of abiotic residues and which will lead to the origin of coacervates and eventually organized cell structures. The diagenetic fate of bio-residues in sediments is the subject matter of the last chapter.

In summary, it is the objective of the present work to enumerate and discuss some interactions in organic-inorganic systems as they relate to the origin, development, and diagenesis of biogeochemical matter.

ORIGIN OF POLYPEPTIDES IN SILICATE SYSTEMS

1. Theory

Essentially all monomeric building blocks of life can be synthesized in atmospheric systems using UV, ionizing particles, or electric discharge as activating energies. It is noteworthy that amino acids and the bases of the purines and pyrimidines can be generated upon radiation of an atmosphere containing only water vapor, nitrogen, and carbon dioxide and monoxide (4). These are in essence the same non-noble gases that are presently discharged by volcanoes. In turn this suggests a similar composition for the primordial terrestrial atmosphere (5). This viewpoint contrasts previous concepts which postulated an atmosphere in which the atoms of hydrogen, oxygen, carbon and nitrogen were in their fully reduced or hydrogenated state.

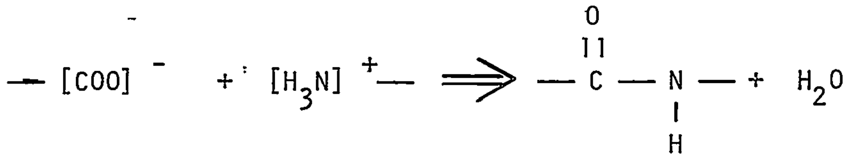
A second source of organic monomers are the volcanoes. The materials derived from this source can be grouped into two classes: (a) C-N-P-S (carbon, nitrogen, phosphorus-sulfur) compounds, which at temperatures of 1000 to 3000 °K cannot participate in the formation of silicate structures and are thus released during volcanic activities, and (b) metal carbides and metal nitrides that are trace constituents of high-temperature silicates; hydrolysis of carbides and nitrides should result in the production of a great number of organic compounds. Judged by the high abundance of carbon, nitrogen, phosphorus, and sulfur in the lithosphere (0.1-0.2 percent), the relevancy of this source should not be underestimated. Unfortunately, little is known on this subject.

Whereas the chemical processes leading to the formation of organic monomers in atmospheric systems are well understood (4), there is a lack of knowledge regarding the origin of polymers such as proteins or nucleic acids. It is true, chemists have successfully synthesized peptides by carboxyl activation involving, for example, acid chlorides, mixed anhydrides or active esters (6). However, these experiments have little information content regarding the abiotic synthesis of biopolymers in low-temperature natural habitats. Their principal objective was to produce biologically active peptides and in doing so, to obtain independent proof for the correctness of a peptide structure inferred from degradation studies.

The difficulties encountered in the synthesis of peptides under conditions normally encountered in natural environments are related to the following facts:

Amino acids do not polymerize in aqueous solutions because they build up a polymeric network with water as indicated by a rise of the dielectric constant of amino acids--water systems. Further supporting evidence is the greater solubility of a monomer relative to the equivalent dipeptide, for example, glycine *versus* glycyl-glycine. Polymerization will thus only take place in solvents in which amino acids do not form a strong network with the solvent.

In non-polar solutions such as benzene, amino acids do not dissolve readily; yet they build up crystals. This is a lower energy state because strong hydrogen-hydrogen bondings are developed, and dipol-dipol interactions occur. The problem, thus, is to find a system, where amino acids are in a state of molecular dispersion like in water but without solvent interaction. Under such circumstances a reaction would proceed analogues to the salt forming mechanism:

(a) 

$$-[COO]^- + [H_3N]^+- \Longrightarrow -\overset{\overset{O}{\|}}{C}-\underset{\underset{H}{|}}{N}- + H_2O$$

$$\text{(b)} \quad \begin{array}{llll} [Cl]^- & + & [Na]^+ & \\ [OH]^- & + & [H]^+ & \end{array} \Longrightarrow NaCl + H_2O$$

Certain mineral surfaces can act as such an ideal solvent system because the two requirements for polymerization, i.e. molecular dispersion and no solvent interference are realized. In Figure 1 the molecular state of amino acids in polar, non-polar, and mineral solvent systems are illustrated.

2. Silicate - Amino Acid Interactions

In order to understand the sorption characteristics of silicates and their function as polymerization template for amino acids, a few details on silicate structures are briefly mentioned. A full survey on silicates has been presented by Deer *et al.* (7).

Silicates are an ideal mineral solvent system for amino acids due to the intrinsic structural and chemical properties displayed on silicate surfaces. In Figures 2 to 5 a systematics for silicates is presented to illustrate the mineralogical disversities.

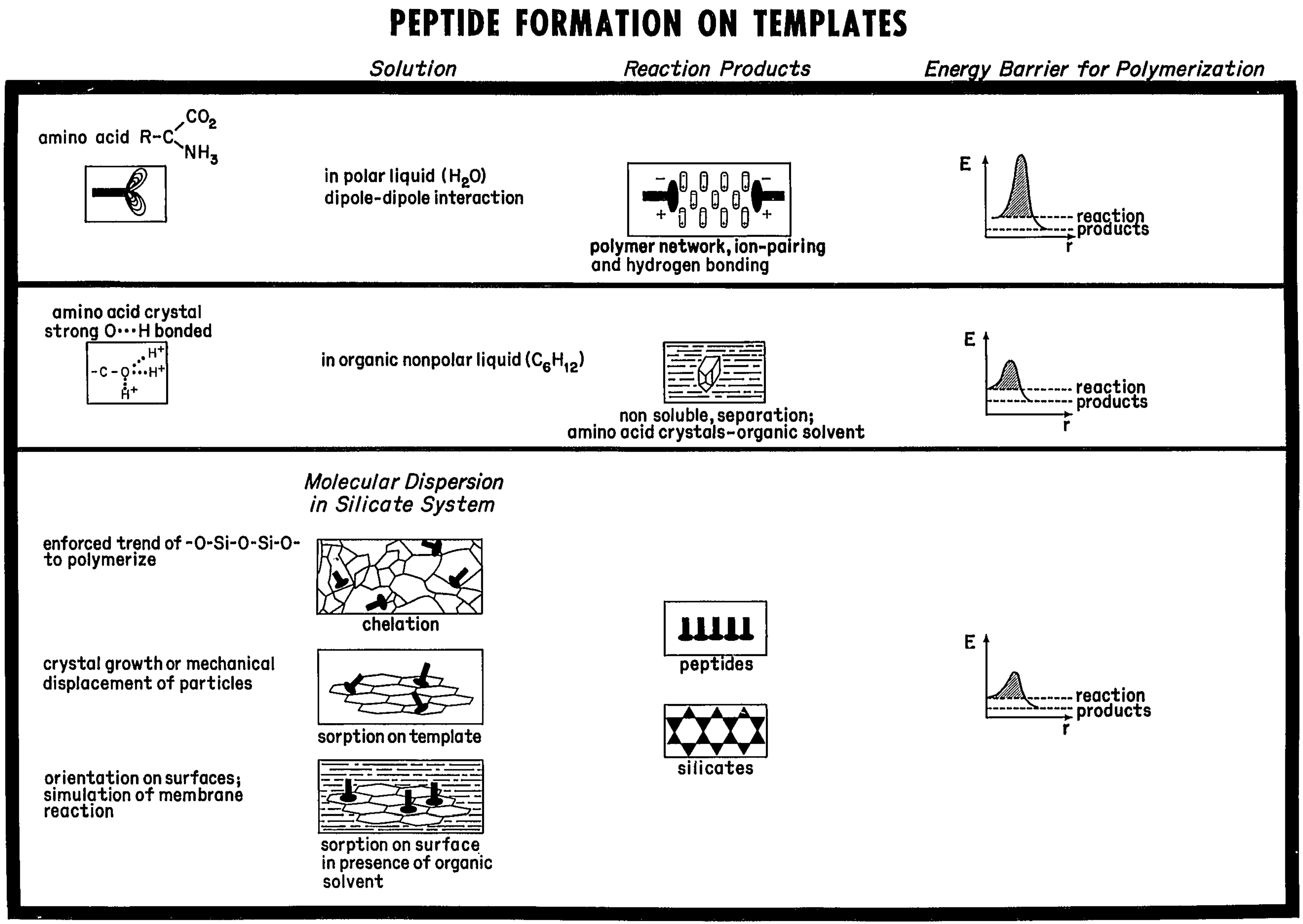


Figure 1. Molecular state of amino acids in polar, non-polar and mineral solvent systems.

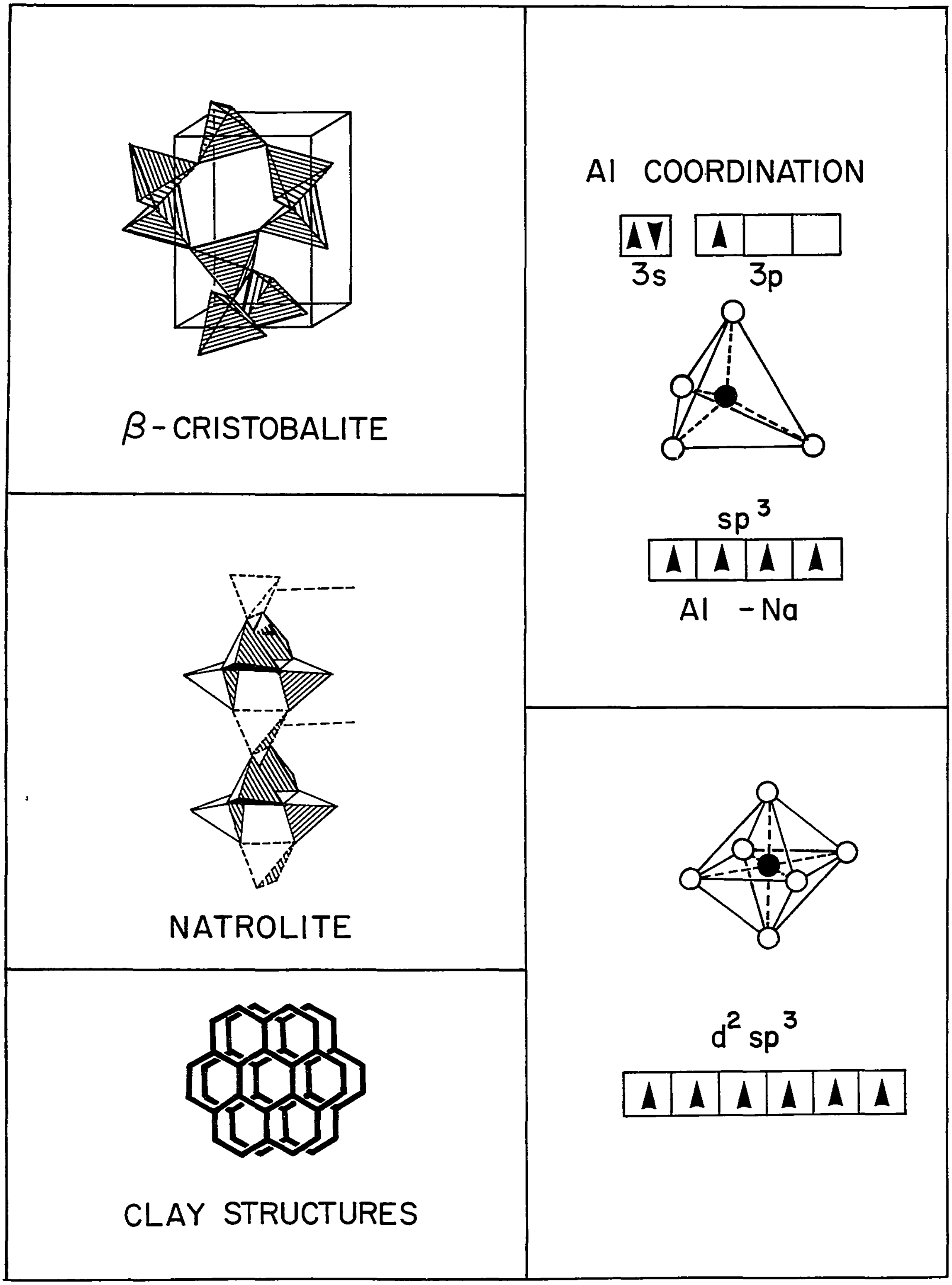


Figure 2. Molecular arrangement of SiO_2 molecules, and the coordination state of aluminum in silicates.

Mica Minerals

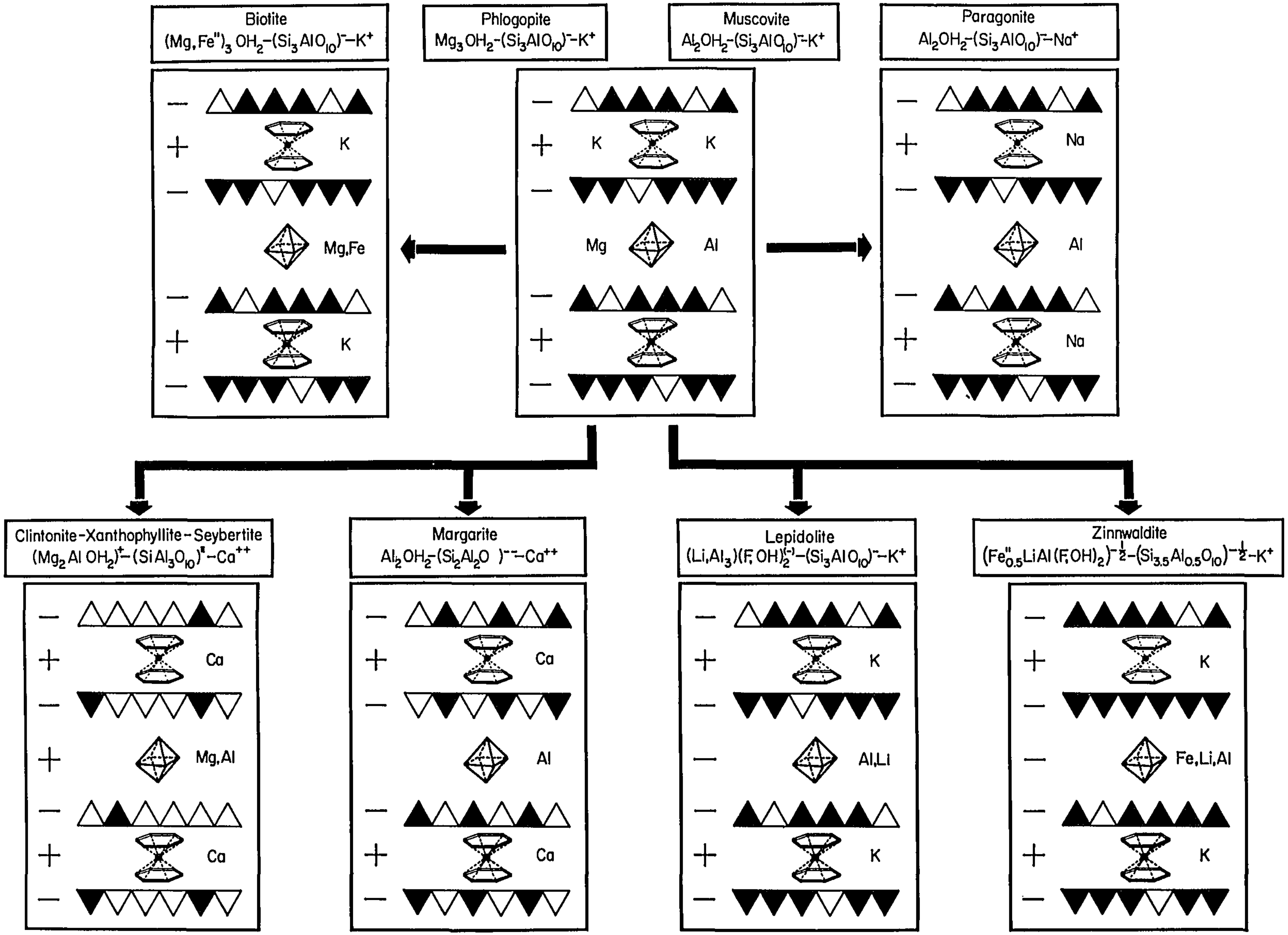


Figure 3. Mica structures.

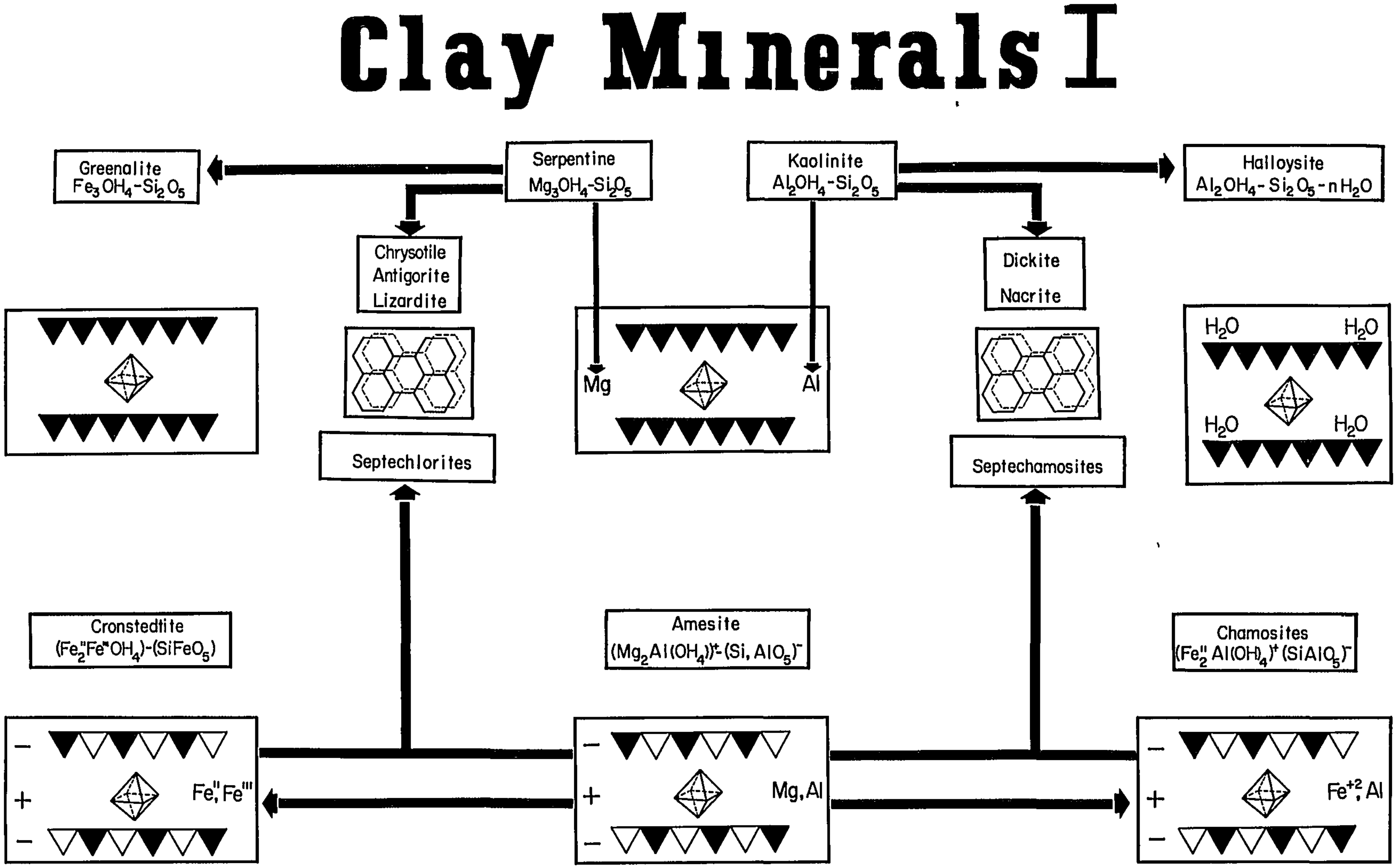


Figure 4. Clay mineral structures. I: kaolinite-serpentine group.

Clay Minerals II

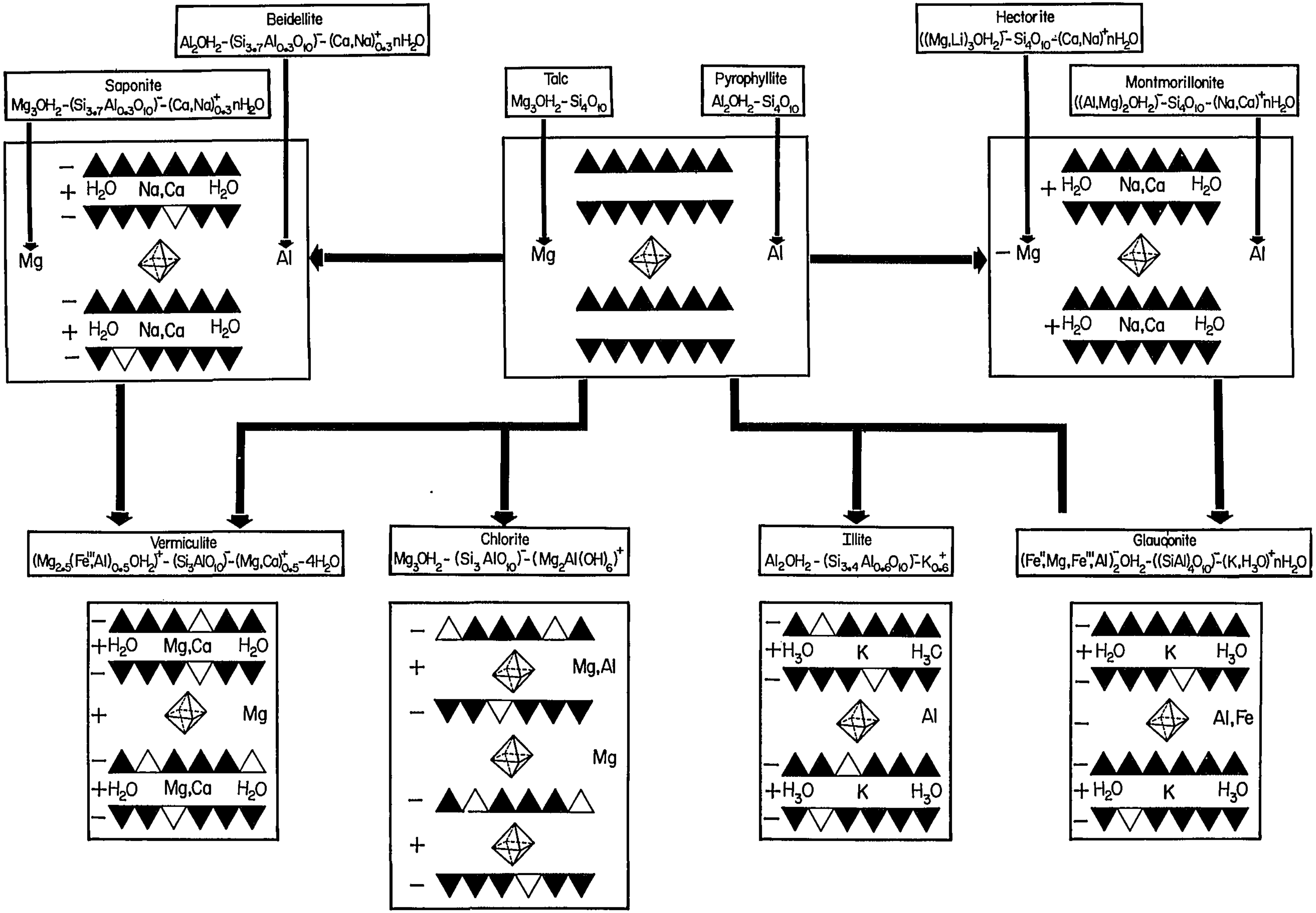


Figure 5. Clay mineral structures. II: talc-pyrophyllite group.

Pure SiO_2 molecules tend to form polymers. Changes in bond and rotation angles between two SiO_2 molecules will lead to the various structures of polymers like quartz, cristobalite and tridymite. Four-membered rings of SiO_2 molecules are only formed under high pressure/high temperature conditions and a representative member is coesite. In low-temperature environments, four-membered rings can only develop in case aluminum or three-valent iron will substitute for one of the silicon atoms. To balance the structure, elements such as sodium, magnesium, or calcium are introduced; a representative end-member is natrolite (Figure 2).

In low-temperature systems, aluminum, due to structural reasons, prefers to stay in the $\underline{d}^2\ \underline{sp}^3$ bonding state as a so-called aluminum octahedron:

$$(Al\ O_6\ H_6)^{-3}$$

and silicon tends to produce six-membered rings which are linked together in form of a layer. The various forms of clay and mica structures differ only in the way the different silica layers composed of silica tetrahedra:

$$(Si\ O_4)^{-2}$$

are stacked together (Figures 3 to 5).

Aluminum octahedra and silica tetrahedra are negatively charged which promotes sorption sites for compounds such as:

$$-NH_3^+ \quad ; \quad >NH_2^+ \quad ; \quad \text{or} \quad \geq NH^+ \ .$$

Positively charged sites may also be displayed as a consequence of substitution phenomena. It is the structural simplicity and chemical diversity that make clay minerals an excellent candidate for polymerization experiments involving amino acids. The sign of the electric charge (+) or (-) shown in Figures 3 to 5 refers to the kind of charge developed at the indicated structural site. Knowledge of the charge, whether it is positive or negative, will help to predict the degree and kind of interactions between the individual amino acids and the clay structures.

Thermodynamic considerations, aimed to estimate the reaction energies involved in combining two amino acids by an amide bond, are difficult to make is systems as complex as these. Yet, a straightforward approach is possible through a comparison of bond energies and bond distances in carbon-, oxygen-, and nitrogen-containing systems (8). The relationships are plotted in Figure 6. Two solutions can be presented: (a) the classic approach which assumes uncharged functional groups, i.e. NH_2 and COOH, and (b) the zwitterion approach, where NH_3^+ and COO^- functions are used. The classic approach yields a reaction energy of + 60 kcal/mol for the formation of an amide bond; the reaction energies calculated for the zwitterion approach are slightly less, namely about + 40 kcal/mol. Calculations based on the zwitterion approach vary within a certain range, because reaction energies will depend on the nature of the participating amino acids.

EXPERIMENTS

1. Logic

Our experiments to synthesize peptides were designed to meet the geological conditions that supposedly were established in a pre-biological environment at the sea water/sediment interface. There was no necessity to form monomers prior to the polymerization experiments, because many reports have already shown the feasibility of monomer synthesis in atmospheric systems. Since for reasons of

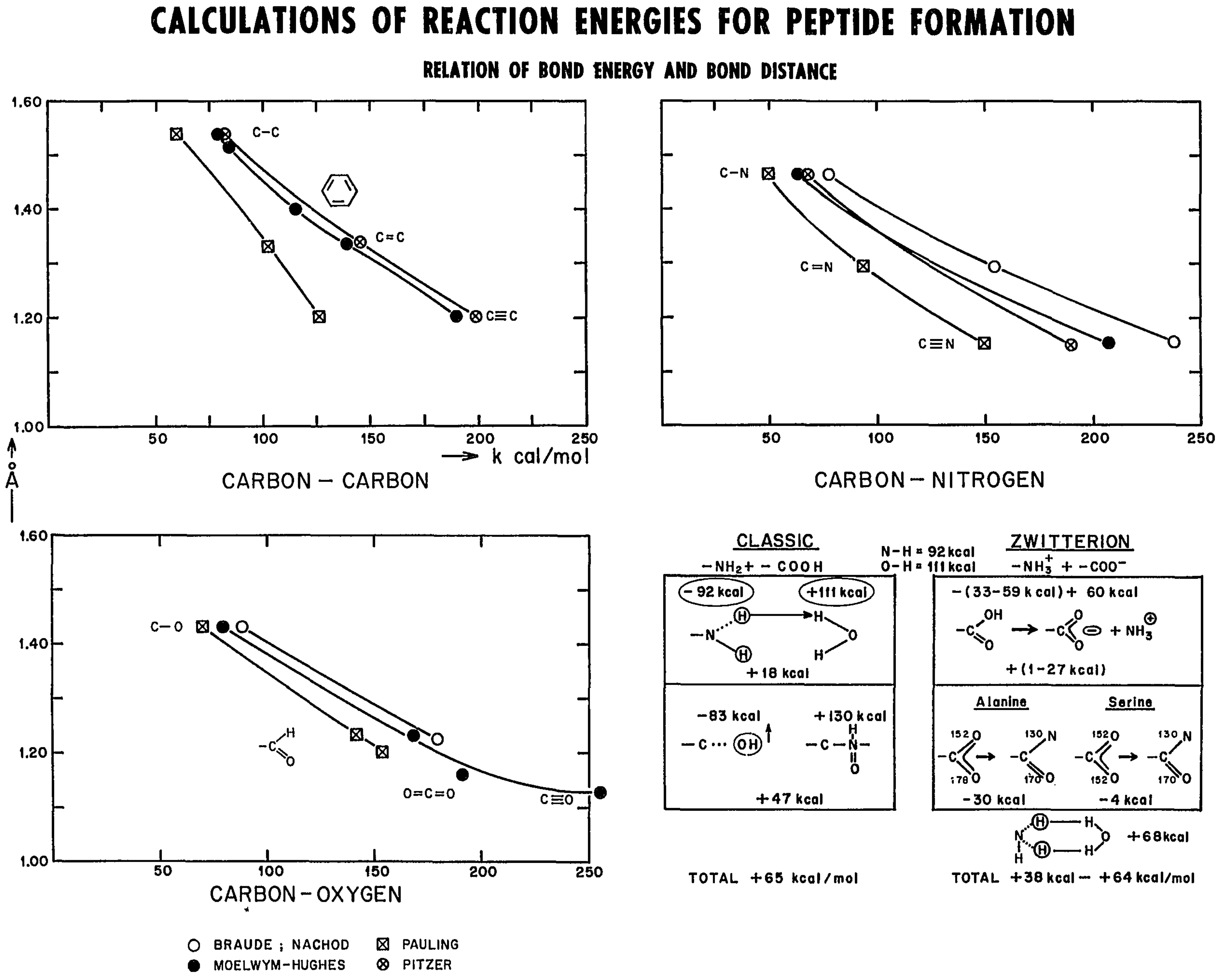


Figure 6. Calculations of reaction energies for peptide formation.

collision probabilities an initial concentration of monomers is required, sorption studies on various minerals preceeded the polymerization experiments.

The logic of our experiments aimed to synthesize peptides on mineral surfaces was basically to predict the efficiency of amino acid sorption by knowing the chemistry and structure of a mineral template. In varying the experimental conditions in terms of pressure and temperature gradients, wet and dry environments, coarse and fine mineral powders, grinding effects by crushing and mixing a sample in a mortar, or by adding salts, water or organic solvents to the system, the behaviour of the amino acids under the aforementioned circumstances could be followed and evaluated. Some 60 minerals have been examined. A comprehensive account on this part of the project, and including all the analytical data, has been presented elsewhere (9).

A representative analytical test for a given mineral included the following steps. The amino acids were added to the mineral system in concentrations ranging from 1 to 25 μmoles per individual amino acid. The total amount of mineral powder (<200 mesh) was 4 g. One set of sample was left at 25°C for 24 hours, another set at 60°C for two days, and a third one was subjected to high-pressures up to 60,000 psi for a few hours. Subsequently the amino acids were retrieved by first adding water in known amounts to the mineral system and by finally centrifugating the mineral slurry. Care was taken to remove all water-soluble amino acids from the system. This treatment was followed by extractions with 4 N ammonia, 6 N hydrochloric acid, formic acid and other solvents. The amino acids were subsequently analyzed by ion-exchange chromatography following a procedure that has previously been described. (10). The reproducibility of the ion-exchange system is better than 1% at the 10^{-8} molar level. In order to facilitate a better comparison between the various experiments, the original program for computing the amount of amino acids contained in a sample, and written in *Fortran II* for the GE 255 computer, has been expanded. The revised program allows to draw a bar graph by a Calcomp plotter which expresses the yield in amino acids in percent recovery (Figure 7).

Amino acid polymers contained in some of the extracts were separated by means of gel-filtration techniques using Sephadex G-25, G-50 and G-75, and by ultrafiltration membranes which are based upon polyion complex systems. Polymers with molecular weights up and exceeding 10,000 were subsequently hydrolyzed with 6 N Hcl and were studied for their amino acid composition. Another effective way to study polymerization effects was by comparing the amino acid pattern obtained before and after the extract had been subjected to acid hydrolysis. An increase in amino acid concentration in the hydrolyzed over the unhydrolyzed run could then be used as evidence for polymerization.

Up to 20 amino acids were simultaneously added to the mineral system. In this way, differences in sorption and polymerization rates between the individual amino acids on a given template could be used for the elucidation of the structural arrangement between and among amino acids and their mineral matrices.

2. Sorption and High-pressure Polymerization

The experimental treatment started with a study on the sorption probabilities of amino acids on naturally occurring silicates. Clay minerals were found to be one of the most effective scavenger of amino acids. Well-ordered structures such as those in micas were hereby considerably less efficient than their hydrated and less-ordered counterparts, e.g. the illites (Figure 7). Some amino acids were preferentially retained by certain silicates; others had greater affinity to different kinds of silicates.

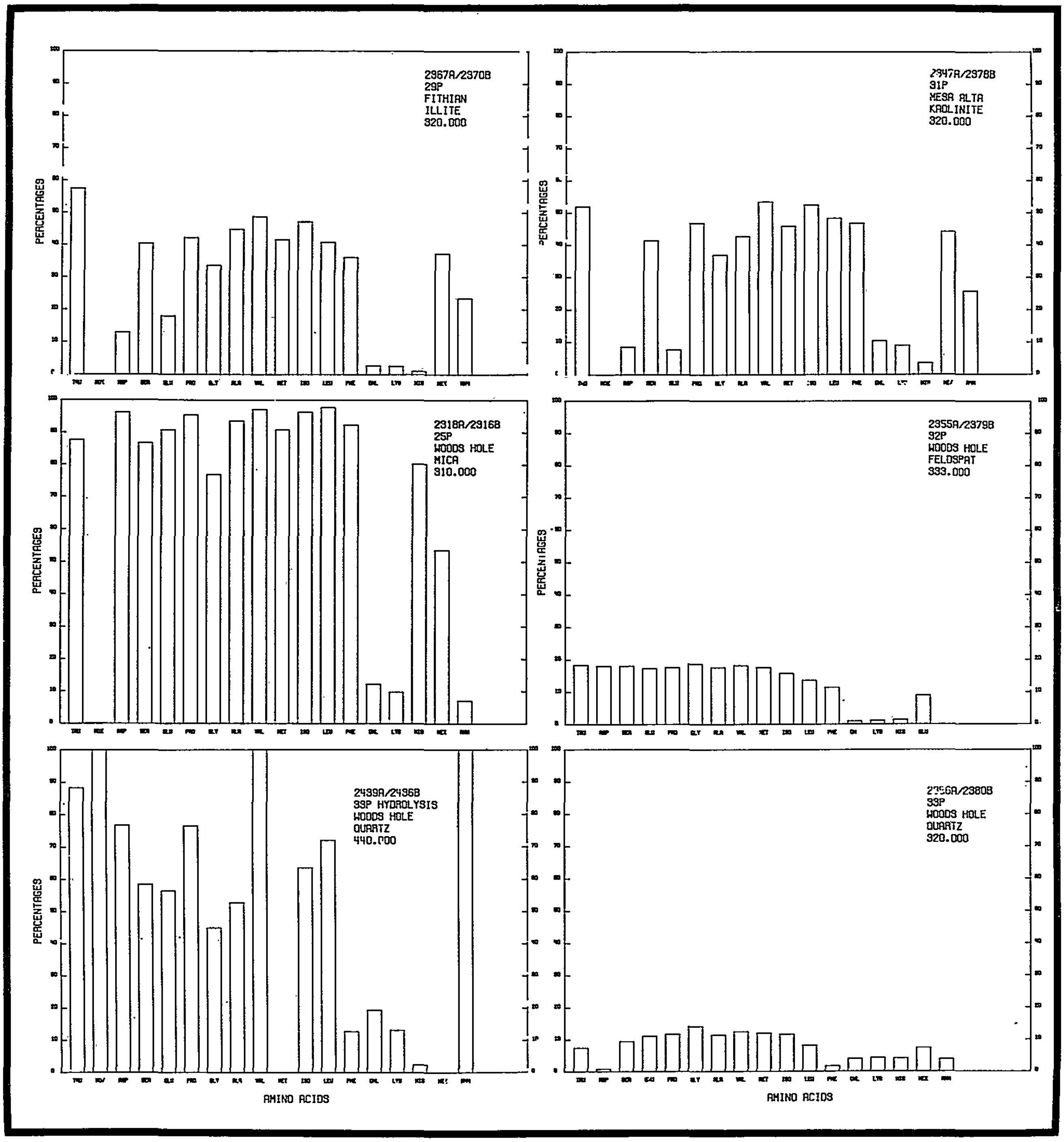


Figure 7. Bar graphs obtained by a *Calcomp* plotter; yield in amino acids expressed in percent recovery.

The reason for subjecting the mineral-amino acid system to high pressures (60,000 psi) was principally to simulate pressure conditions established in diagenetic environments. The significance of the high-pressure experiments lies in the reorganization of mineral matter, both physically and chemically. As shown in Figure 1 coupling of amino acids may occur; our subsequent chemical analyses have indeed confirmed this inference.

High-pressure experiments on quartz are rather informative. Only small quantities of amino acids were recovered in the aqueous extract afterwards attesting to the apparent efficiency of amino acid retention on quartz. Hydrolysis of the aqueous solution, however, appreciably increased the recovery yield (Figure 7). As confirmed by molecular sieve techniques, this increase is due to polymerization. The portion of amino acids not recovered during this test is still retained in a water insoluble form on the quartz surface. This particularly involves the basic amino acids, whose molecular status in terms of a monomeric or a polymeric arrangement is not yet known.

3. Reaction Mechanisms in Silicate Systems

Encouraged by the positive results of the sorption and high-pressure experiments, parallel studies were performed to delineate stepwise the molecular mechanisms involved in these reactions. Three different avenues of approach were followed: (a) formation of organo-silicate glasses, (b) chelate polymerization, and (c) synthesis of peptides on clay proto-structures. Inasmuch as a full report on these studies has already been presented, only a few details should be mentioned here.

(a) Amino acids heated at 60°C in presence of colloidal silica will produce glass structures. The addition of proline and leucine will promote a particularly stable glass configuration. Absorption spectroscopy and UV-luminescence enable us to draw conclusions about the bonding state that exists between silica and amino acids. By varying the chemical composition of the silica matrix, for instance, by using colloidal Na-silica solutions, their effect on the bonding state in the amino acid-silica system can be examined.

(b) In presence of colloidal silica and $AlCl_3$, amino acids will become organized into stable chelate compounds with molecular weights up and exceeding 10,000. Addition of certain electrolytes, such as citrate buffers commonly used in amino acid ion-exchange chromatography, will de-polymerize the chelates and release their amino acids. If peptide formation will take place under these conditions, the yield must be extremely small.

(c) The molecular mechanisms leading to the formation of minerals such as quartz, clay minerals, or dolomite in low-temperature environments are only poorly understood. This is so, because the kinetics of the reactions is very slow. Interpretations on the origin of these phases are, therefore, frequently based on experiments performed in the temperature range 150 to 500°C. There is indication, however, that the formation of clay minerals in low temperature habitats is strongly influenced by organic compounds. For kaolinite, the reaction mechanisms are schematically illustrated in Figure 8. Amino acids become adsorbed to the kaolinite proto-structure. Reorganization during early diagenesis will result in polymerization, stripping of the peptide, and the simultaneous formation of the kaolinite structure. In this way, amino acids actually promote the synthesis of kaolinite and epitaxial growth is observed. The frequent association of kaolinite with sediments rich in organic matter may be related to this chain of events. For further illustration, the basic structural elements involved in a silicate-amino acid system are schematically presented in Figure 10.

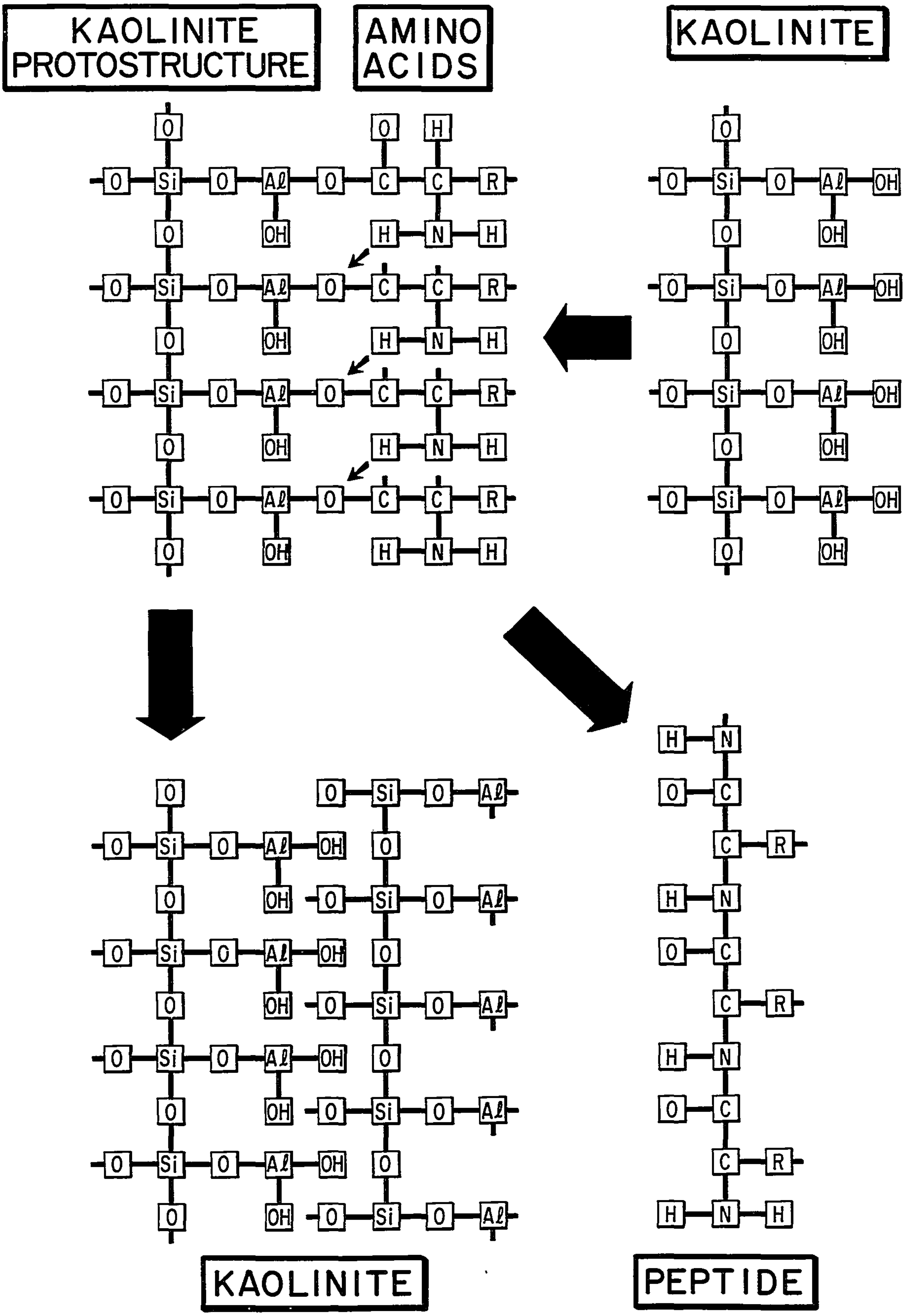


Figure 8. Structural relationships in an amino acid-peptide-kaolinite system (schematic).

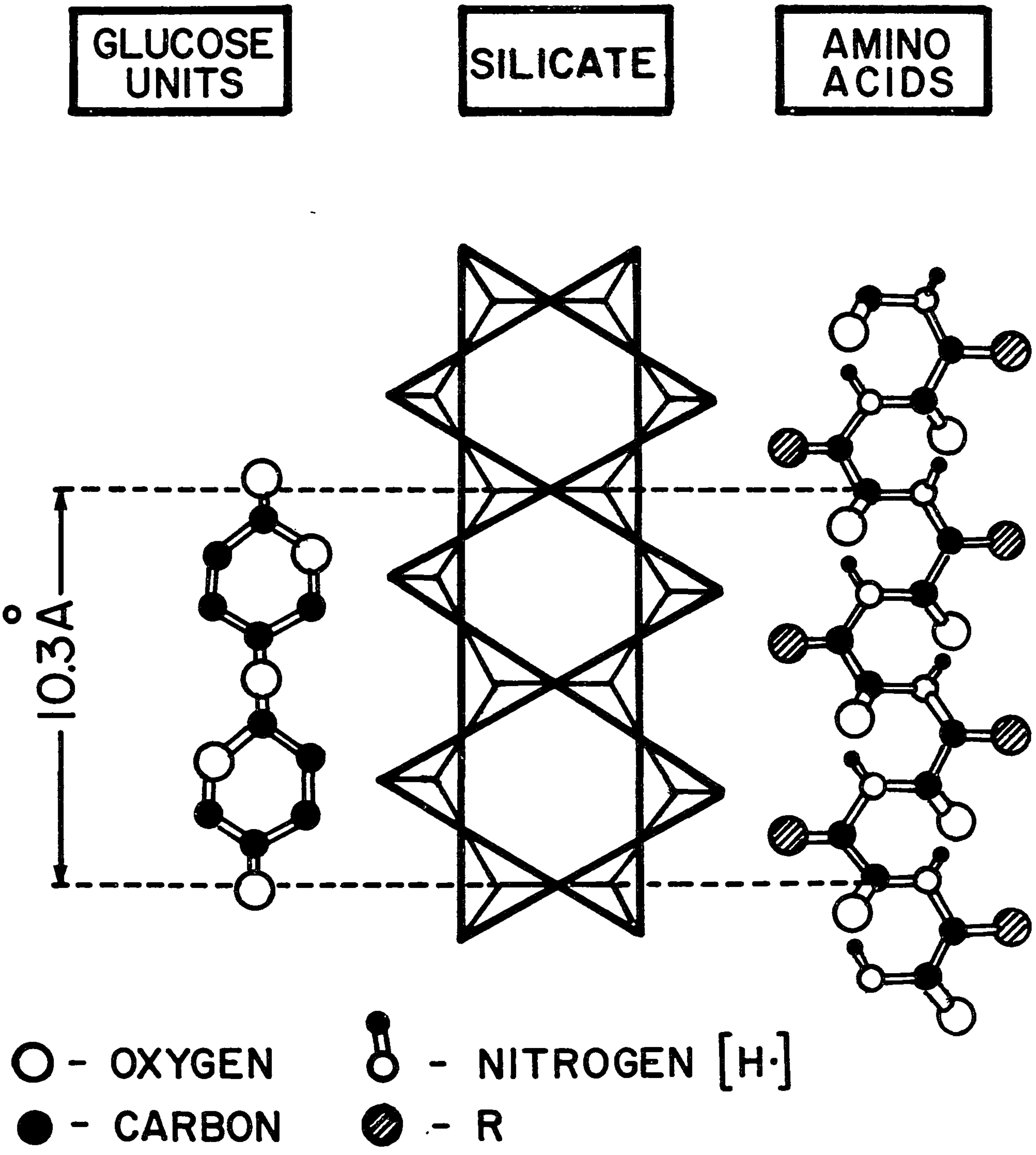


Figure 9. Structural relationship in a silicate-organic system (schematic).

In conclusion, minerals can be considered the principal agents for synthesizing peptides in low-temperature natural environment. This process is an exothermic one and does not require outside activation energies as is commonly assumed. Since protective groups are abundant in such systems, peptide chains of high-molecular weight can be obtained.

DEVELOPMENT OF ABIOTIC ORGANIC MATTER

1. Coacervates

Intermediary products between the unorganized abiotic organic matter and the first living cell are frequently termed coacervates (11). These compounds already contain a number of well-ordered structural elements and may exhibit membrane characteristics. It is highly suggestive that the formation of coacervates is linked to the diagenesis of sediments and involves interactions between aqueous, organic, and mineral phases.

After the synthesis of the major monomers in atmospheric systems *via* hydrogencyanide and simple carboxylic acids of the type still involved in the reductive pentose phosphate cycle of photosynthesizing organisms, or after their synthesis by volcanic events, the formation of high-molecular weight products of biological interest requires the participation of minerals in order to concentrate, purify, and polymerize the complex mixture of biochemicals originally synthesized in atmospheric or volcanic systems. Most of these precursor materials will end up in the sea from where they are efficiently extracted by mineral scavengers such as clay minerals or hydroxides. The minerals will eventually settle to the bottom of the ocean, and natural chromatography of the type described here will result in separation, concentration, and polymerization of certain biochemicals. It is interesting to note that in a sedimentary environment of this kind, phosphorus, sulfur, magnesium and other metals essential in living systems are highly abundant and in a sort of flux in response to changes in environmental parameters as compaction of the sediment material proceeds. In this way, the upper few meters of sediments will contain not only those organic compounds that can be mobilized by natural chromatography but in addition elements that have been generated in the course of diagenesis. This is the environment, we propose, that will initiate the formation of coacervates.

The association of hydrocarbons, peptides, hetero-polycondensates, and phosphosulfo-chelates will produce a particularly stable conformation between the various structural elements.

Most essential during this part of biopoesis are fluctuations in salinity. Such changes can be brought about during compaction of sediments or by membrane activities within the coacervates. It is well established, that a salinity gradient is a rather effective mechanism in the solubilization and chromatography of a number of organic compounds including the bases of the purines and pyrimidines (12).

2. Chromatography in Sediments

The formation of specific coacervates is intimately related to diagenetic processes in sediments. Structural rearrangements in mineral systems play an essential part in these reactions, because metal ions will be mobilized, polymerizations of organic and inorganic monomers take place, and compounds unfit to become part of the *in situ* generated stable phases will migrate. Let us examine these processes closer.

Recent sediments contain up to 90 percent water, whereas the water content in ancient sediments is generally in the order of 5 to 10 percent. This implies that during compaction of the rock formation the water will gradually be released. The amount of water transportation per unit time is hereby most pronounced in the upper few meters of deposition. Yet, under steady-state conditions, none of the interstitial water will escape into the overlying water column. Instead, to match the loss of water to the ancient sediments, one has to postulate a small but continuous supply of new water which will be added to the top of the sediment column.

In studies on marine sediments off the coast of southern California (13) it was estimated that over the last 3.8 million years, roughly 30 liters of water have passed through each square centimeter of a sediment layer burried at a depth of 1500 meter below the present sea water/sediment interface. It is reasonable to assume that such a vast flushing of water has left a considerable chemical imprint both on the water and the sediments.

Chemical alterations can be brought about, for instance, by dissolution and precipitation processes. In the context of this work, however, the most effective mechanism for the concentration and separation of organic compounds appears to be natural chromatography along mineral surfaces. Its efficiency will depend on factors such as the type, grain size, and orientation of the mineral substrate, the overall chemical and physical parameters of the aqueous phase, and the kind and distribution of the participating organic compounds. Natural chromatography may involve effects such as adsorption, differential solubility, ion exchange, and emulsion transfer.

As already pointed out, virtually no water is lost to the overlying hydrosphere during compaction except in those cases where the sediment strata becomes stirred up; for example, by the action of wind, waves, erosion, or biological activities. In view of this, a chromatographic pattern showing distinct layers of chemically well-defined biochemicals, is expected.

Todays such patterns are generally masked or destroyed by the action of microorgansims and burrowing animals. Nevertheless, in strongly reducing sediments with no or only traces of life, a chromatographic stratification for amino acids and sugars has been observed (14). The pattern for the amino acids can be seen in Figure 10. To explain this phenomenon as a result of specific microbiological activities can be ruled out for a number of reasons. These include the geological settings in terms of a strongly reducing environment and the virtual absence of microbial activities. The rate of sedimentation for this profile is about 1 meter per 1,000 years (13).

There are certain shortcomings in relating the chromatographic pattern observed in sediments to similar patterns that might be produced in analytical ion-exchange columns. Distinct separations can analytically be only achieved if the input of the organic mixture is a discrete and not a continuous event. In contrast, contribution of organic debris to the sediments endures for the whole length of deposition. Thus, to explain the stratification pattern of the amino acids in a meaningful way, one has to assume that well-defined chemical barriers exist in the sediment column allowing the passage of some but not necessarily all migrating organic molecules. Since the chemistry of interstitial waters has a determining influence on the migration pattern of many organics, a chemical alteration of the aqueous phase in the course of diagenesis may mobilize materials from a strata that once has acted as a chemical barrier. In this fashion, a sediment column will produce results comparable to an analytical ion-exchange column regarding separation and purification effects for organic and inorganic compounds.

3. Chemistry of Primordial Sea

Interstitial waters in recent sediments are chemically similar to the sea above. Since the chemical nature of the aqueous phase has a decisive influence

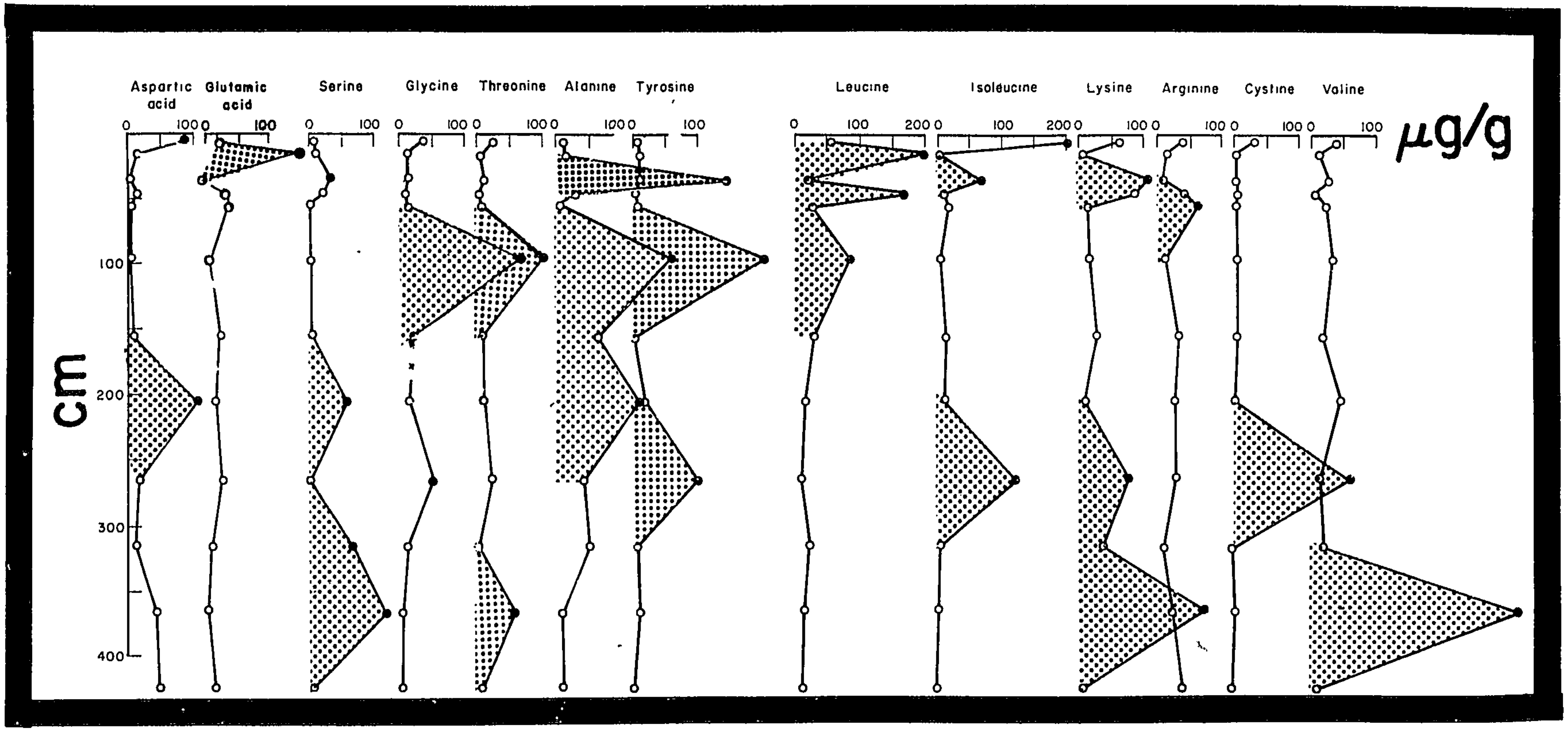


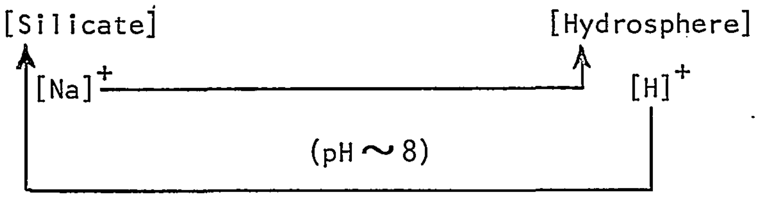
Figure 10. Depth distribution of amino acids in marine sediments of Santa Barbara Basin, California (µg/g dry weight).

on the efficiency of natural chromatography, informations are offered bearing on the origin and composition of the early ocean.

The origin of oceans is a matter of controversy among geochemists. The problem has principally to do with the size and chemistry of the primordial sea and its physical and chemical development up to the present. An excellent formulation and discussion of the problem has been given by Rubey (15) and Sillen (16).

Rubey's concept is based on the assumption that the amount of water released by volcanic activities throughout geological history is sufficient to account for all the water in lithosphere and hydrosphere. The concept thus implies that the water has gradually accumulated with time. Uncertainties in the calculations, however, are introduced due to the fact that no informations are presently discernable of how much of this water has directly come from juvenile magmatic sources and how much is simply activated interstitial water. One should mention that most of the water presently discharged by volcanoes or in thermal areas is meteoric in origin.

Concerning the chemistry of the Precambrian sea, a major breakthrough in thinking has been made by Sillen (16). He strongly objects against the well-rooted theory that the pH of the sea is mainly controlled by the carbonate system: H_2CO_3; HCO_3^-; and CO_3^{-2}. Instead, he proposes that a dynamic equilibrium exists between hydrosphere and lithosphere and that pH is principally a function of the exchange equilibria between silicates and the solutes in the sea; as an example:



In this way, silicates act as an effective buffer system which implies that the pH of the ancient oceans was always very close to the present value of about 8 to 9 and that the exchange equilibria in the atmospheric/hydrospheric carbonate system had a much less influence on the pH of the oceans. The pH values at the immediate interface of hydrolyzing minerals and water, and called the abrasion pH, have been determined for the principal rock forming minerals (17)).

A similar dynamic buffer system can be assumed for interstitial waters. Consequently, there are experimental facts available to interpret the chromatography of organic molecules in simple terms, and one has no longer to rely exclusively on tentative suggestions.

4. Origin of Nucleic Acids and of the Genetic Code

The concepts developed in this paper allow us to elaborate on the origin of the genetic code from a biogeochemical point of view. It is surprising that the results on the physical chemical properties of the codons (18) can be regarded as experimental evidence for the correctness of the deductions made in the present work.

The structure of the specific set of codon assignments represents the fundamental building block of the genetic code. The translation apparatus, however, does not represent a straight line between triplets and amino acids but many intermediary steps are involved in the genetic transcription process. The macromolecules participating in this translation mechanism include the activating enzymes, the sRNAs, the mRNA, and the ribosomes. The reason for a special codon assignment for a given amino acid is not known and little experimental work has been done on this subject.

Studies aimed to synthesize nucleic acids under primordial conditions are generally considered to be of greater significance than those that elaborate on the formation of peptides and proteins. This viewpoint rests on the fact that the conventional route of biosynthesis of peptide chains on the ribosomes of the cells involves nucleic acids as templates. In turn this argument implies that, if we have nucleic acids, one can form peptides. Advocates of this theory, however, should bear in mind that the translation apparatus embodies a great variety of proteins of which the ribosomes contain more than thirty. It is most likely that protein participation in the genetic transcription process existed ever since the first living cell was formed. There is no evidence in support of a drastic change of the translation apparatus in the course of time.

On theoretical grounds, the bases of the purines and pyrimidines are no likely candidates for polymerization on mineral surfaces. They are apt to degradation as a result of charge transfer if they become adsorbed on clay minerals, for example. Also structural considerations speak against possible polymerization effects. Instead we propose that peptides acted as templates for the abiotic synthesis of nucleotides. It is suggested that the synthesis of nuleotides took place during the migration of peptides through the sediment column. Peptides, adsorbed on polar mineral surfaces, promoted the sorption of other polar constituents; this in turn resulted in the formation of larger molecules (Figure 11).

In many minerals, phosphorus-oxygen tetraeder (PO_4) are isostructural to the SiO_2 polymers and the silicates. It is inferred that during organic polymerization the (PO_4) groups were linked to the SiO_2 structure. The additional components of the nucleotides were supplied by peptides. It is concievable that the end groups of the amino acids played an active part in these reaction processes. Work on the configurations of amino acids and their corresponding codons shows the stereochemical feasibility of such structures (18, 19). Further evidence in support of this inference can be drawn from the chemistry and structure of sRNA which, in many aspects, is more reminiscent of a protein than of a nucleic acid (e.g. aliphatic substitution on many of its bases; - S - S - bridges).

Inasmuch as the synthesis of nucleotides in sediment systems was achieved in presence of pure organic phases, specific chemical relationships between the participating reaction partners should exist. Due to chromatographic processes, the internal relationships between codon and amino acid must involve a third reaction partner. The third partner is the sediment environment, i.e. the medium which brought about the isolation and concentration of the individual molecules.

Experimental evidence for the possible relationship displayed in the system: *amino acid-codon-medium*, has recently been presented (18). Principally, the experiments tested the chromatographic behaviour of amino acids in a pyridine-water solvent system. Since all heterocyclic bases are expected to produce a similar chromatographic pattern for the amino acids, the results obtained for pyridine chromatography should be applicable to the genetic code and its development. The ordering of amino acids according to R_f-value or polar requirement, respectively, bears a striking relation to the ordering by codon assignment. For instance, all amino acids whose codons differ from one another in the third position only have nearly identical polar requirements; and all amino acids with codons containing U in the second position constitute a closely related group, as do those amino acids with C_{II} codons (18).

The subsequent development of abiotic systems and leading to an intact genetic code mechanism are speculative due to lack of empirical data.

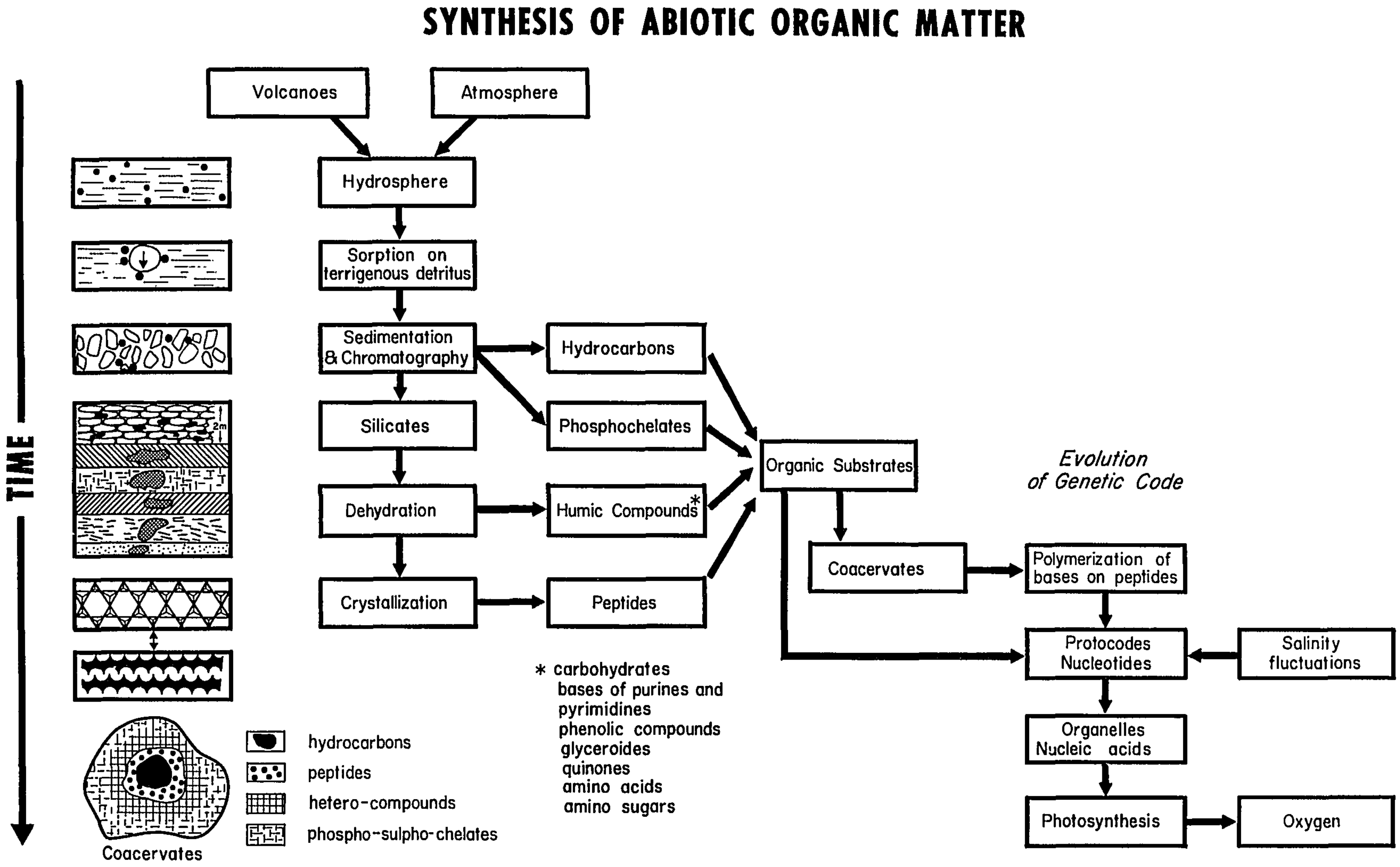


Figure 11. Synthesis of abiotic organic matter (flow diagram).

However, insight into the general mechanism of peptide synthesis in living matter may be obtained by reference to studies on calcification in biological systems (10, 21). Protein templates, responsible for the deposition of a solid mineral phase in molluscs, show a striking evolutiary development in the sense that the amino acid composition of the shell tissues can be described by a limited number of amino acid factors which are related to both phylogeny and environment. The most interesting relationship is observed in a series of eight *Haliotis* species (ancestoral gastropods). Here, the high-molecular weight proteinaceous matter of the shells and the periostracum (>100,000 MW), is essentially composed of five groups of amino acids which are characterized by nearly identical codon assignments or polar requirements. For instance, there is a linear relationship between serine and alanine, and between valine, leucine, isoleucine, and methionine. The codon assignments read as follows:

a)	serine	UCU	UCC	UCA	UCG
	alanine	GCU	GCC	GCA	GCG
b)	valine	GUU	GUC	GUA	GUG
	leucine	CUU	CUC	CUA	CUG
	isoleucine	AUU	AUC	AUA	
	methionine				AUG

The interpretation is offered that the formation of the high-molecular weight proteins represents actually a two-step process, whereby in the cells of the mantle-epithel of the molluscs at least five groups of soluble peptides are secreted which subsequently are rendered insoluble as a result of intermolecular reactions between the individual peptides. Only the last incident will produce the high-molecular weight proteins present in the shells and the periostracum. The extreme differences observed in the amino acid composition between these

Haliotis specimens are simply a reflection of the relative proportions of the five amino acid groups. It is noteworthy that changes in environmental parameters such as salinity and temperature fluctuations will alter the factor structure. As specific cells in the mantle-epithel will secrete only a well-defined peptide, similarly, we might expect that identical sites developed on templates involved in the biosynthesis of proteins, will only produce a specific peptide chain. The biosynthesis of peptides may thus be looked upon as a submicroscopic chromatographic mechanism.

Nucleotides are qualified to polymerize, because this reaction is linked to the formation of phosphate esters. In turn, their incorporation into coacervates originating in the upper sediment layers should have started an abiotic evolution of the organic matter which was characterized by membrane activities, chromatography, polymerization, in other words by differentiation processes. In this way, multitudes of chemically different coacervate types were developed on the ocean floor, probably none resembling each other.

The origin of the first "living coacervate," or to be more specific, of the genetic code able for genetic transcription, must be considered a single incident. The local geologic environment supplied the essential building blocks of life in a sequential manner uncommon to other sea-bottom environments where the order of chromatographic elution was different in some way. We conclude: *The order of chromatographic elution of molecules from a sediment column must represent a feasible physiological cycle.* This cycle has been maintained ever since by the organisms for reproductive purposes in terms of genetic codon assignments and type of translation apparatus. Thus, the most remarkable feature of the genetic reproduction mechanism is its conservatism. The reason for this conservative attitude is easy to understand: The organisms did not invent the codon by themselves, but the codon came into existence by chromatographic differentiation processes, and was offered to the organisms in that state. The organisms started to live on the basis of the genetic codons and had no longer access to their modification.

The codon assignments, as presently established in the living cell, does not necessarily represent the only and exclusive form of a biogenic reproduction apparatus. Other mechanisms are conceivable. However, the moment the genetic code arose in a prebiological coacervate and became adopted for genetic transcriptions, the chances for the development of other code systems were erased simply due to the radical and speedy consumption of the abiotic food sources by the living populations.

DIAGENESIS OF ORGANIC MATTER

1. Geochemical Cycle

Organic matter has been isolated from sediments as old as three billion years (21). Most of it, namely 3.6×10^{15} metric tons, and which represents 95 percent of the total fossilized organic matter, is present in shales (22). For comparison, the coal deposits of the world have been estimated at about 6×10^{12} metric tons; this is just 1/500th of the amount that occurs in a finely disseminated state in shales. Estimates of the primary petroleum reserves run to 0.2×10^{12} metric tons which represents only 1/16000th of the total biochemical matter incorporated in sediments.

Inasmuch as the concentration of bio-residues in shales is generally in the order of 1 to 3 percent and well above the concentrations generally observed in limestones (0.2 percent) and sandstones (0.05 percent), clay minerals appear to be of special significance for studies concerned with organic geochemistry (23).

The annual production rate of organic matter has been estimated at 3.6×10^{10} metric tons for the sea and at 5.2×10^{10} metric tons for the land areas. This adds up to a total production for the biosphere of 8.8×10^{10} metric tons per year (24). From the comparison of the present production rate figure for the biosphere and the total yield of organic matter from ancient sediments, it is apparent that only a minute quantity of living matter becomes fossilized.

Principally, the organic materials embedded in sediments can be grouped into compounds that are *survivors of diagenesis* and *products of diagenesis*. The first group includes all those organic molecules which are chemically similar or identical to living biochemical matter. Compounds that arose during diagenesis from the organic debris, but are generally not part of plants or animals, belong to the second group.

Although a number of biochemical fossils such as porphyrins, fatty acids, or amino acids have been isolated from sediments as old as the Precambrian (21), the bulk of the organic matter has lost its biological identity in the course of diagenesis. Polymers break up into their monomeric building blocks, and these in turn can be severely modified by elimination of functional groups, e.g. $-COOH$; $-OH$; $-OCH_3$; $-C=O$; $-NH_2$, hydrogenerations and isomerizations, cleavage reactions, and, in general, processes which destroy the ordered building pattern of biochemical compounds.

In the early stages of diagenesis, the bulk of the biochemical macro-molecules, for instance, proteins, polysaccharides, lipids, or the nucleic, are rapidly eliminated. This is largely a result of the activities of microbial populations in Recent sediments and soils. Metabolic breakdown products are, of course, legion and a few are listed at random: amino acids, amines, phenols, quinones, indoles, sugars, the bases of the purines and pyrimidines, fatty acids, hydrocarbons, or partially degraded polymers. These can interact and give rise to new polymers which for lack of a better term we will name heteropolycondensates to emphasize their complex origin. Inasmuch as these compounds are no longer of nutrional value, they may accumulate as *bio-residues* even in the zone of microbial activities. Interactions with the surrounding mineral matter may stabilize the organic complex or may catalyze certain reactions.

Although plants represent the main precursor for the organic matter in sediments, microorganisms have extensively modified the organic debris in the early stages of diagenesis. It is thus a matter of opinion whether plants or microbial degradation products should be considered the chief source of the organic matter in sediments.

After termination of bacterial action, largely a result of the depletion in available food materials, the diagenetic history of the organic matter is that of slow inorganic maturation and redistribution as a result of natural chromatography or of simple migration processes. Loss in functional groups may result in the formation of hydrocarbons, phenols, and other constituents, and may cause a reduction in aliphatic side chains in former bio-residues. The resulting coal or kerogen material will become more and more aromatic in nature. The number-one factor causing diagenetic alteration is thermal degradation.

It is inferred that practically all former biochemical matter, independent of chemical nature, origin, or environment of deposition, is diagenetically reduced to either coal, kerogen or the various crude oil components. If given sufficient time, the organic residues leading to coal or kerogen will gradually acquire the structural characteristics of graphite, whereas the petroleum will become more and more paraffinic. In its final stage, all organic matter will eventually end up as graphite, methane, carbon dioxide, ammonia, and water. An illustration of the main path of diagenesis for the principal organic constituents is presented in Figure 12.

The fact that cellulose, peptides, and in particular the glycoproteins are occasionally found in sediments of Mesozoic and Paleozoic age makes oneself wonder what kind of syngentic and diagenetic environments will favor the preservation of these bio-polymers. The most essential factor in the early stages of diagenesis is an effective protection against microbial degradation. This may be achieved in three ways: (a) strongly reducing environments, (b) fast rate of deposition to move the organic debris rapidly through the zone of bacterial activity, and (c) incorporation into minerals, e.g. shell carbonates, or other protective compounds, such as asphalt or salts. The main requirements for preservation during the subsequent stages of diagenesis are low-temperature conditions for the geologic periods concerned. It is of special interest that among the bio-polymers the glycoproteins rank first in diagenetic stability. Cellulose and proteins follow next, whereas hemicellulose, lipids, and most of the heterocyclic polymers are virtually unstable as far as fossilization is concerned. No information is discernable on the diagenetic fate of the lignins due to the difficulties encountered in their isolation and identification. In this way the presence of biopolymers may serve as a clue for the thermal history of a rock formation.

There have been periodic claims in the literature that the presence or absence of certain amino acids in ancient rocks can also be used as a geothermometer (25). These claims were based on the differences in stability of free amino acids when subjected to thermal degradation. This is not the place to review such inferences critically. The fact, however, that amino acids in ancient

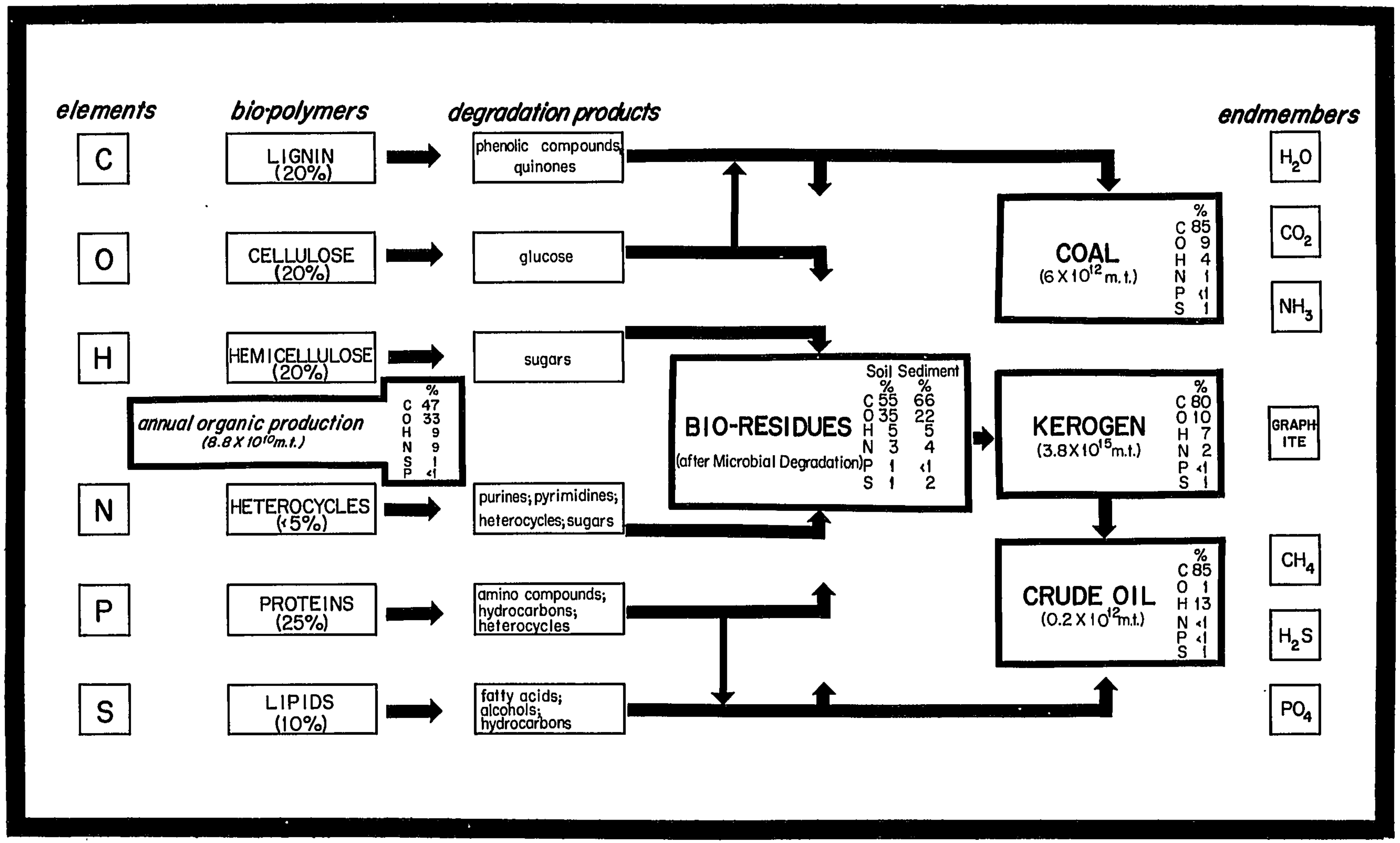


Figure 12. Diagenesis of biochemical compounds (flow diagram).

sediments do not occur in a free state but are part of highly cross-linked hetero-polycondensates, casts sincere doubts as to the applicability of such experiments to geology. Furthermore, serine, one of the amino acids predicted to be relatively short-lived, is rather common in ancient sediments (14).

2. Chemistry

The chemical reaction mechanisms involved in the degradation of living matter and leading to the formation of bio-residues in Recent sediments and soils are related to the activities of microorganisms and burrowing animals in the rock strata. Such transformations from bio-polymers to hetero-polycondensates is accompanied by a reduction in structural order. The principal molecular building blocks for the hetero-polycondensates are hereby derived from a pool of metabolic waste materials generated by the living population in sediments and soils. Thus, the chemical nature of the bio-residues is determined by: (a) the effeciency of biological degradation, and (b) the interactions between minerals and metabolic products.

A comparison of the distribution pattern of carbon, oxygen, hydrogen, and nitrogen between living matter and the various bio-residues generated during diagenesis is rather informative, (13, 14, 26, 27). In the early stages of diagenesis, the levels of hydrogen and nitrogen drop by about 50 to 75 percent and the amount of oxygen is slightly reduced (Figure 12). The loss is matched by an increase in carbon content. These relationships are related to the preferential elimination of proteins, carbohydrates and fats and the corresponding reduction in NH_2 and alcoholic OH groups. In contrast, the aromatic compounds principally derived from lignins or aromatic amino acids, the quinones and some heterocycles such as indoles or furans, are retained in the reaction system leading to the hetero-polycondensates. Aside from these products, fatty acids, amino acids, sugars, and some low-molecular weight polymers can be released in the process of biological degradation and thus may also become incorporated into the bio-residues of Recent soils and sediments.

Based on the elemental composition and on the knowledge of principal biochemical building blocks in the hetero-polycondensates, a clathrate-type structure for this complex mixture of molecules is suggested. As a result of EDA (electron-donator-acceptor) interactions, polar OH-groups form hexagonal ring structures. In this way, neutral and polar compounds can coexist within the same molecular framework. A hypothetical segment of a typical molecule in a hetero-polycondensate is illustrated in Figure 13 together with other examples for clathrate cross links and the cage structure of quinol. It is of significance that the OH-groups of sugars and amino acids, as well as the carbonyl-groups can actively participate in the formation of the clathrate network and the relative high abundance of serine in ancient sediments can be attributed to this phenomenon. Furthermore, the plentiful intramolecular space permits the incorporation of small chain polymers and hydrophobic molecules. By the latter incident, a more stable conformation of the structure is achieved.

Clathrates exhibit striking similarity to the molecular network of zeolites. These silicates are known as discrete ion exchangers and molecular sieves. It is most likely that clathrates have identical properties and the association of specific elements in the various biogenic residues may be the consequence of these characteristics. On the other hand, ions, including Ca, Ba, PO_4, CO_3 or SO_4 may act as stabilizing elements in the cage structures; furthermore noble gases, carbon dioxide, ammonia and methane may become incorporated into the clathrate network. Phenolic acids such as salicylic acid, have the tendency to form stable dimers (Figure 13) and are consequently not suitable for clathrate cross-linkages.

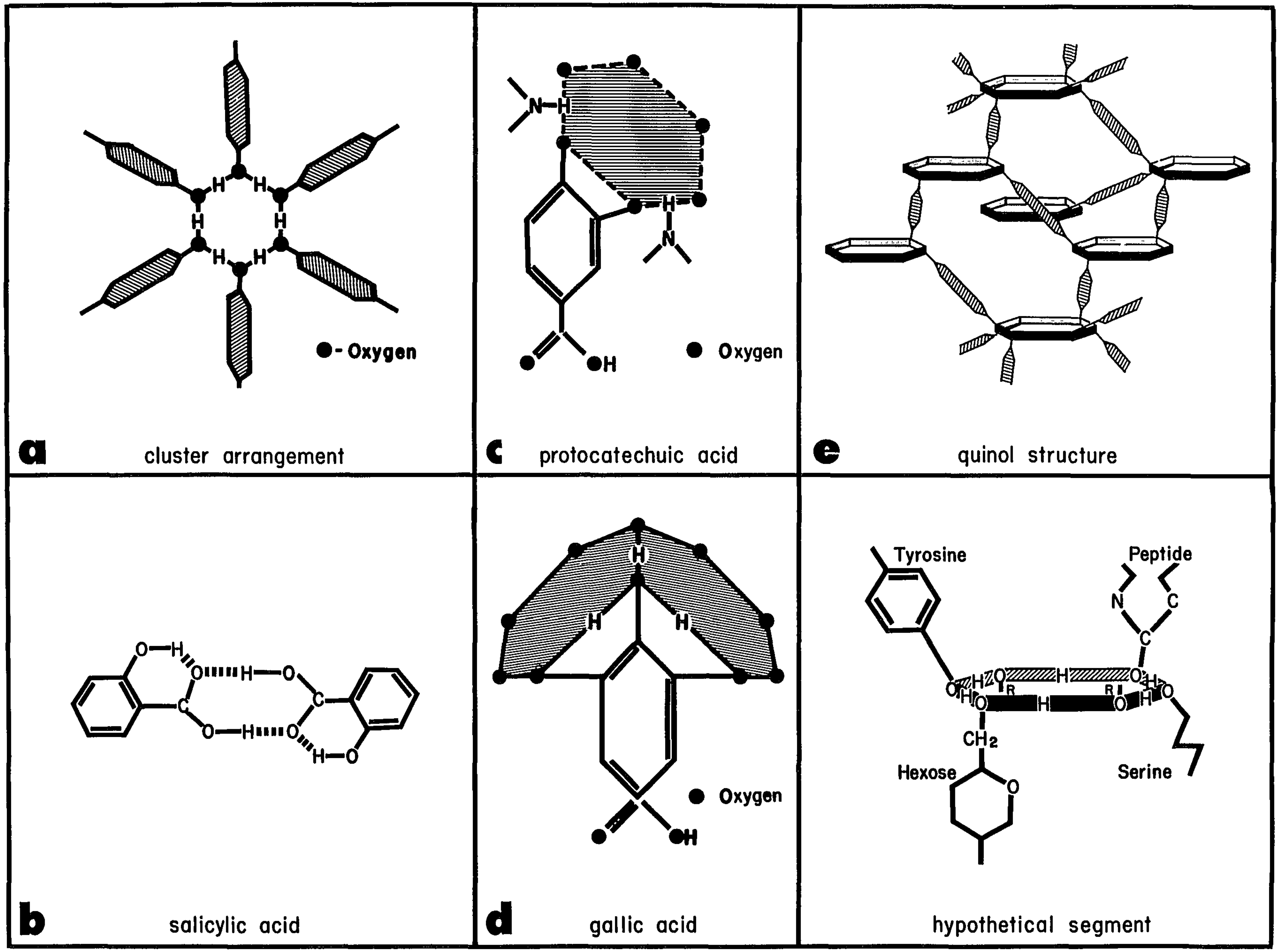


Figure 13. Clathrate structures.

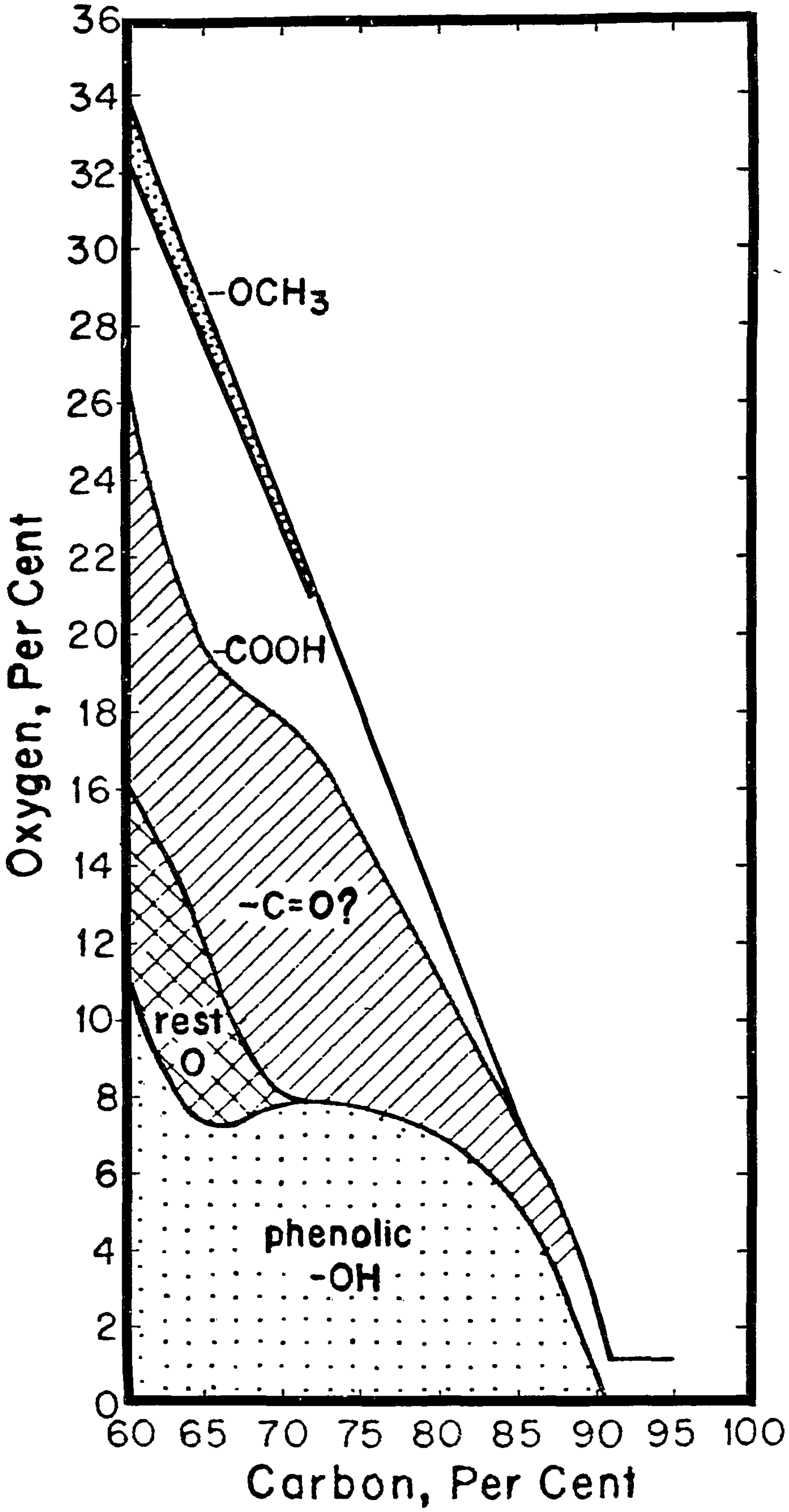


Figure 14. Carbon/oxygen relationships during coalification.

After cessation of microbial activity, most of the elemental changes in the bio-residues concern carbon and oxygen. The gradual drop in oxygen in the course of diagenesis has to do with the elimination of oxygen functions, i.e. methoxyl, carboxyl, carbonyl, and hydroxyl groups. Eventually, the clathrate network will collapse resulting in a fusion of aromatic nuclei. The final product of thermal degradation will be graphite.

The diagenetic changes associated with coalification are well understood (27). The carbon/oxygen relationships in Figure 14 reveal the sequence of events. Methoxyl functions are the least and carbonyl and hydroxyl functions the most stable configurated groups. Whereas the main precoursor of coal is lignin, kerogen is mainly derived from protein-carbohydrate-lipid degradation products. In turn, more aliphatic units will become incorporated into the clathrate network of the kerogen relative to the coal progenitors. This relationship is reflected in the nitrogen and hydrogen values which are higher in kerogen by a factor of two or three when compared with coals of the same concentration of carbon. It is for this reason that kerogens may generate hydrocarbons by the thermal degradation of fatty acids and amino acids, whereas coals under the same diagenetic circumstances will only produce carbon dioxide, methane, and water. It is of significance that hydrocarbons as well as some of the so-called O-N-S- compounds (oxygen-nitrogen-sulfur heterocycles) contain no functions that would permit a clathrate-type stabilization. In turn, these products can be flushed from the rock by means of formation waters and accumulate as crude oils in geologic traps.

In summary it is concluded that the formation of fossil organic matter is a three-step process: (a) the microbial degradation of biopolymers, (b) the association of metabolic products in form of complex hetero-polycondensates, and

(c) the slow inorganic maturation with thermal degradation being the number one alteration factor. Thus, given sufficient time, all fossil organic matter is eventually reduced to graphite, water, methane, ammonia, and carbon dioxide. The fact that chemical fossils such as pristane, fatty acids, and amino acids form an indigenous part of sediments that measure in billion of years is proof that organics, if kept in low-temperature diagenetic environments, are rather stable (18).

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LIST OF FIGURES

CONCEPTS ON ABIOTIC DEVELOPMENT OF PHOSPHOLIPID-MEMBRANES, AND ORIGIN OF PRIMORDIAL METABOLISM

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INTRODUCTION

The functional rôle of phosphates in biochemical reactions is understood (1). Principally, the oxygens of PO_4 tend to associate themselves with metal ions which results in the formation of an oxygen polyhedron having a cation in central position. Such a co-ordination will introduce a higher molecular order for phosphorus-containing polymers such as the nucleic acids, and phospholipids. Aside of its physical significance in stabilizing molecular structures, the participation of phosphates in biochemical reactions is an essential prerequisite of life.

In knowing the principles behind the flexibility of the molecular geometries of phosphate structures in cells, insight can be gained on the abiotic development of phospholipid membranes. Information on the reaction kinetics of phosphates in biochemical systems, i. e., the physical-chemical control of linkage and cleavage of the P-O-P bond, on the other hand, may throw light on the origin of the primordial metabolisms.

In Figure 1, we illustrate schematically the individual abiotic events necessary to create a primordial cell and simultaneously outline our methodological approach. The content in this Figure has a direct bearing on concepts previously outlined in Figure 11 of (2).

PROBLEM ANALYSIS

Previous work on the origin of life has principally been concerned with the dynamic aspects of such a development, for instance, the formation of enzyme systems, or the interactions between peptides and the nucleic acids regarding the translation machinery (see list of references in 3 and 4). However, little attention has been paid concerning the origin of the molecular fabric that houses and supports these key biochemical components. In living systems the rôle of the molecular fabric is exercised by the phospholipid membranes. It was accepted that the activity of functional elements such as enzyme processes in colloidal micelles, and which were physically held together by hydrophobic forces and hydrogen bridges, and being in this way separated from the surrounding environment, were sufficient to produce life.

As presented in the discussion on the structure of micelles (Fig. 20), the type of micelles contained by such kind of boundary surface phenomena represents a rather instable fabric. Fluctuations in salinity (ionic strength) and the kind of ions present may invert these micelles so that the total "cell" content will become ejected. Due to the environmental sensitivity and in turn instability of this type

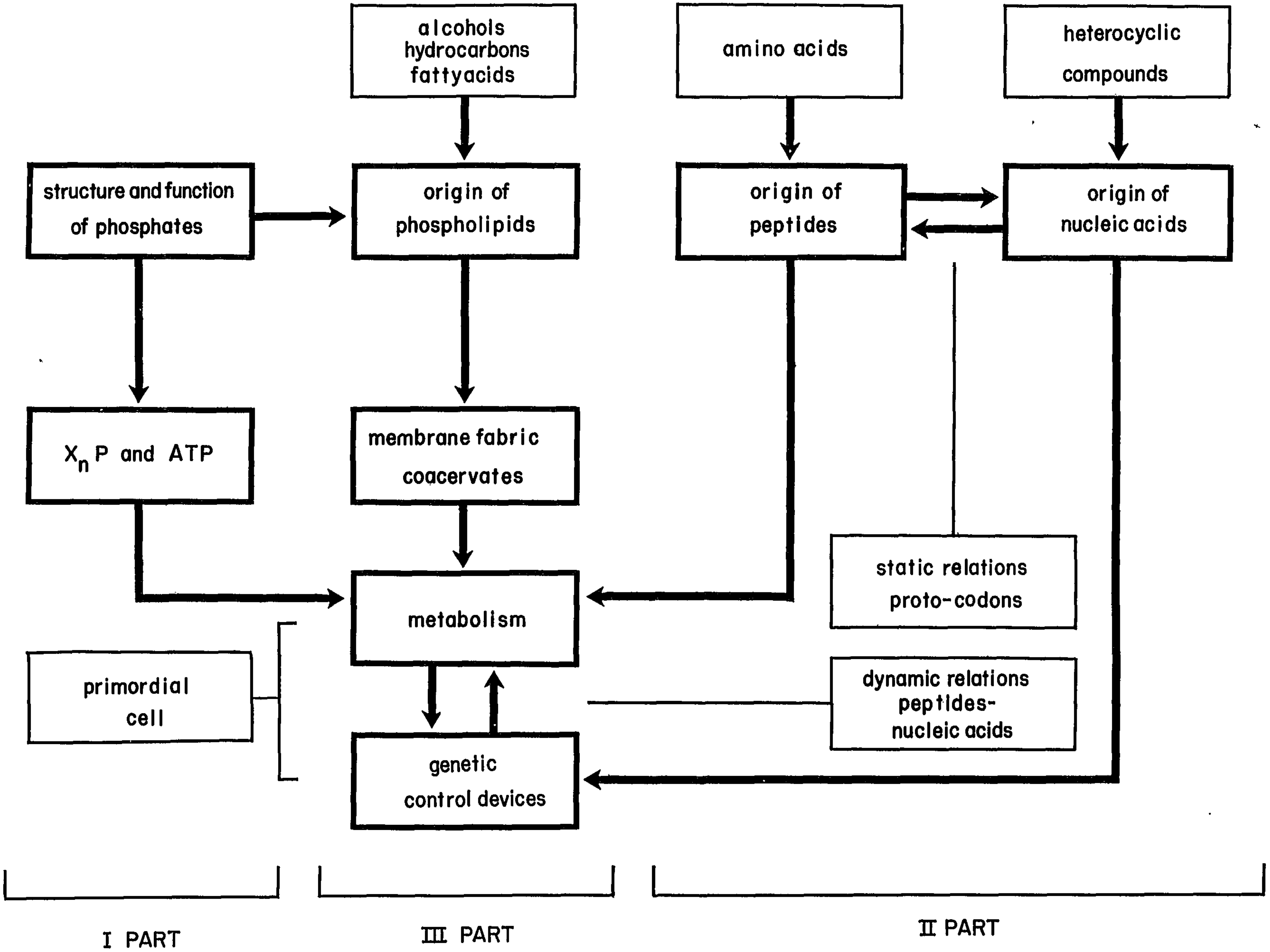
alcohols hydrocarbons fattyacids
amino acids
heterocyclic compounds
structure and function of phosphates
origin of phospholipids
origin of peptides
origin of nucleic acids
X_n P and ATP
membrane fabric coacervates
static relations proto-codons
metabolism
primordial cell
dynamic relations peptides-nucleic acids
genetic control devices
I PART
III PART
II PART

Figure 1

of organic micelles, it is rather unlikely that a development in the direction of the primordial cell took place in this manner. Namely, such processes require an effective protection from the environment by means of isolation through the formation of a stable fabric, and this for perhaps millions of years. This isolation, however, is not guaranteed by the aforementioned bonding forces. We are thus confronted by the question of what is the nature of these minimum and stable fabrics we have to postulate for a meaningful coacervate system; we attempt to find a constructive answer.

Self-reproducible and auto-catalytic systems follow the laws of thermodynamics, and we know that only in case the maintaining environment is included, these systems will become physically realizable. That is, to define an auto-catalytic system, we have to be familiar with the kind of energy source and its transport from the environment into the system as well as with the utilization of this energy. The problem thus centers around the question as to the minimum requirements, both for the environment and the system, in order to produce and maintain a "living" entity. In view of these requirements, we must also consider the external layers of partition boundaries, i. e., the membranes, as part of the environment.

One way to elucidate this problem is to enumerate basic essentials and to disclose the way they possibly are interrelated; yet, the number of facts would be enormously high. Because of this, we offer a solution which is based on a heuristic theorem which implies that physical-chemical phenomena symbolized on

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** It is a logical conclusion that only the hydrosphere is the ultimate reason for the kind of life processes as they occur here on earth. For if no hydrosphere is present, but only structural water, the aforementioned phase separation does not take place. Under those kinds of conditions, a different sort of phase separation will proceed and the organics will be associated in a different manner in response and adjusted to the respective environmental circumstances. It is theoretically conceivable that these processes also may yield living entities.

the boundary between two different states, characterize these two states.*

We follow this principle in the forthcoming discussion and we try to develop the minimum requirements. One should mention the successive steps:

(a) the separation of water insoluble organic molecules from the environment; concerning the dynamics of this phase separation, a wealth of empirical information is available (5-7). Nevertheless, the well known experience that oil can be separated from water and form micelles has considerable consequences (see page 3)**;

(b) the collection of additional organic compounds which are soluble in water (e.g., C - N and C - OH compounds) by the micelles, and their subsequent accumulation within the micelles; analytical chemical separation and extraction procedures are based on this phenomenon;

(c) that these aforementioned processes of collection and concentration represent a contribution of matter and thus will initiate chemical reactions which will extend up to the moment a new equilibration state is reached; and

(d) that here the concept, we have followed, has its natural limitation; even if we would assume a multiple collection of organics by these micelles, in the last instance, they would converge against a "dead" equilibrium with its surroundings.

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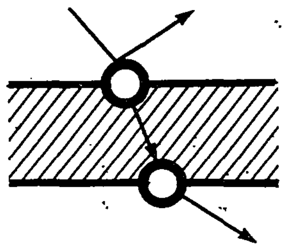
* In this context the theorem implies that the physical-chemical properties of the environment and the respective auto-catalytic system are "written" on the boundary layer, i. e., on the membranes.

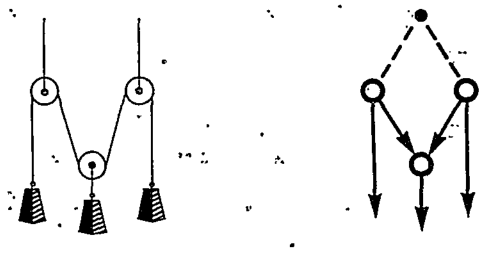
Two examples are listed to illustrate this theorem:

(1) refraction and reflection of a light ray proceeds between two different states. Both states are characterized by boundary surface phenomena which are indicated as circles;

(2) the parallelogram of forces that is established between the boundary points of individual states (vectors) will describe the physical systems of a pulley:



(1)



(2)

In summary, this concept deals with self-propelled phase separation and as a consequence the establishment of a boundary layer. On the other hand, if we would co-ordinate certain properties to this phase boundary in the sense that:

(e) an integration of the inorganic and organic phases is achieved resulting in compatibility of both phases, and thus would represent a physical-chemical stability,

(f) the boundary layer posesses molecular sieve properties which consequently results in that the organic and inorganic phases are not in thermodynamic equilibrium, but in that each individual phase, but only for itself, is in equilibrium with the corresponding parts of the membrane,* and

(g) this boundary layer is capable of mapping both the phases within the micelles as well as of the surrounding environment by physical-chemical means, (i.e., in the physical-chemical structural configuration of the boundary the processes developed in these phases will be reflected as a sort of image on the membrane).**

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* Inasmuch as this point at issue touches upon fundamental problems in physics, we extend the discussion. It was argumented from a thermodynamic point of view (8) that only via the realization of a semipermeable membrane a discrimination between molecules can be achieved, that is, a "go no-go" choice and by this a departure from true equilibrium. Molecular-kinetic considerations, which under the term "Maxwell's demon" have been introduced into physics, (9, 10) are additional proof for (f). The problem under consideration is basically the same, and the question of a semipermeable membrane is treated in the form of "that a being, who can see the individual molecules, opens and closes this hole" (after Maxwell; 10), or in the form of valves and pistons. The mathematical treatment of this problem of the question of semipermeable membrane ("go no-go" choice) shows that the operation of such a membrane is consistent with thermodynamics and does not constitute a "perpetuum mobile," because each physical measurement as an information receipt causes a corresponding increase in entropy (10-17). The question on: information, entropy, and irreversibility was discussed in a generalized form in (18). There are even criteria that indicate to what extent a certain equation describes an irreversible process, i.e., we can distinguish between processes that are (a) deterministic (if T is a hermitian operator), and (b) non-deterministic (eigenvalues with a negative imaginary part) (19).

** This property concerns the question that a semipermeable membrane can forget its information about its own function. Loss of information and a repetitive computation, on the other hand, increases the entropy. This problem is treated by Brillouin: "the gas progressively forgets the information" (10), as well as by von Neumann (20), and Jordan (21). Morrison (8) treats this complex in the form of a shunting system introduced into the membrane.

We obtain a system which not necessarily converges against a "dead" thermodynamic equilibrium with the environment, as is the case in crystal growth (case b), but we obtain a system which is potentially capable to evolve and to gradually become transformed into an autocatalytical and self-maintaining system in case the environment permits this energywise (case e, f, and g).

We have to deal with the question, what kind of fabric exhibits the properties e, f, and g in form of a boundary layer, and more specifically, what kind of fabric is capable to store information which in this instance can only be stored in the form of a structural order information.

Based on the aforementioned properties we can outline a physical-chemical picture of membranes. We postulated that information about the "status" of the phases-(I)-is transferred to the boundary layer -III-. Inasmuch as we have to postulate carriers -II- for the information, it is most reasonable to recognize as carriers those molecules which are released in the course of the chemical processes:

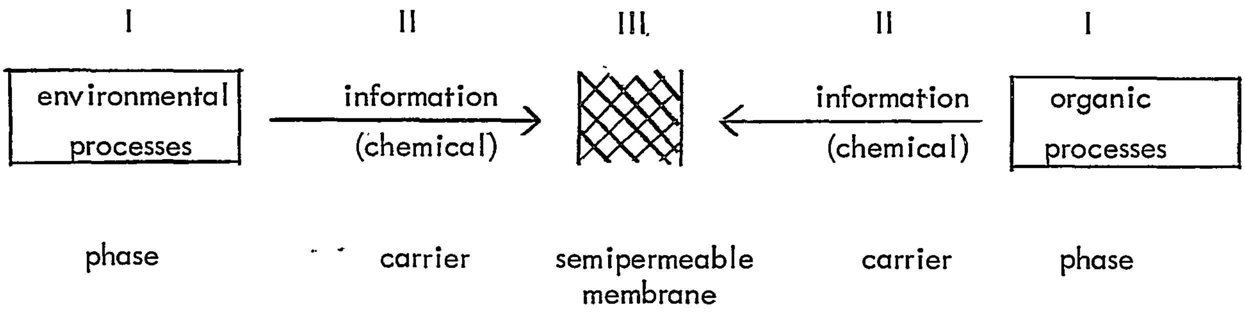

FIGURE 2

Furthermore, the membrane can only receive information more than once, if she can interact reversibly with the carriers, i.e. with the molecules. An irreversible bonding would not fulfill the properties outlined in case-g-. In chemical terms, the boundary layer must permit molecular exchange processes for both the molecules and the ions.

We schematically illustrate these relationships in Figure 2. One should point out that only through this kind of assembly the processes in the micelles can utilize the free energy supply and overcome the "probability barrier."*

For, the physical-chemical properties of the phase boundary will be altered, when the information-carrying molecules associate themselves with the membranes; and it follows that the selectivity, regarding the molecular transport across the membranes, is changed. Depending on the kind of micelle, this may supress or support the development of the micelle. To illustrate the last mentioned process, the time sequence of changes in a molecular population (8), which describes the gradual passage across the probability barrier, is shown in Figure 3:

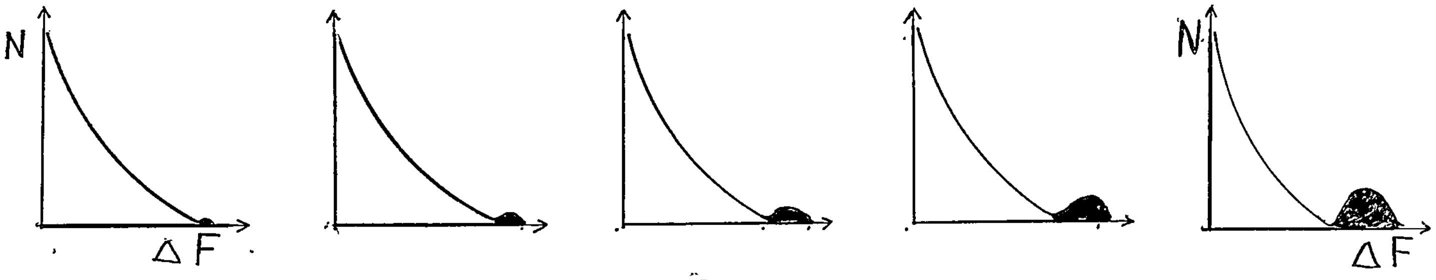

FIGURE 3

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* We describe by the term "probability barrier" the fact that the interplay of these minimally required compounds for a self-maintaining device represents a thermodynamically non-probable event. This is so, because the density of molecular forms (N) must principally be considered as a Boltzmann-distribution, $N = f(\exp -\Delta F/kT)$.

In this context, we like to mention an additional problem which has to do with the extraction of the "consumed" chemicals from the system.* By means of a hydraulic scheme (Fig. 4) we would like to emphasize this problem:

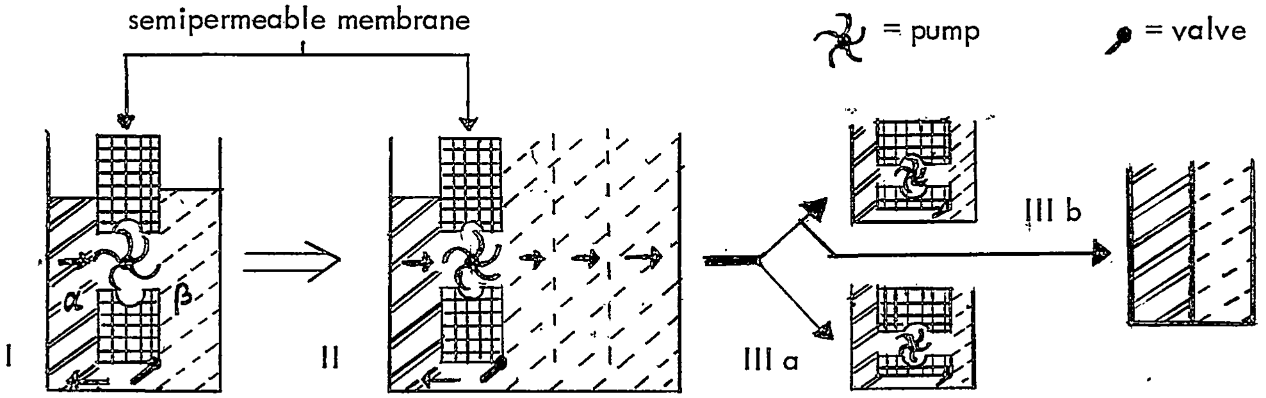

FIGURE 4

In Figure 4 - I -, the boundary layer is represented as a pump which, as already shown, is capable to shift certain materials from phase α to phase β and thus maintains a stationary disequilibrium. We have to postulate a reverse flow via the programmed valve (i.e. case f and g, Fig. 4 - I -) because otherwise an expansion of the cell would take place (Fig. 4 - II -) and result in micelle division. The hereby created daughter cells must share the "active" constituents of the membrane (Fig. 4 - IIIa), should we not assume the rather unlikely incident that only one of the daughter cells retains all of the active parts of its precursor (Fig. 4 - IIIb). That these relationships do exist can be inferred from empirical data (5-7, 22). For, the synthesis of the constituents present in the boundary layer within the micelle can be excluded at this stage of development.

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* Discussed thermodynamically in (8) as the extract of free energy from the reaction box.

The loss of membrane components through the fracturing of the active membranes will simply produce micelles which do not fulfill the conditions requested by -e, f, g -. Therefore, the properties listed in - g - represent an intrinsic issue of our considerations. This is the reason, why we have to postulate a reception and storage ability of information as a minimum requirement (case - g -) whereby simultaneously a monitoring of the valve system (outflow), and a synchronization to the pump is pursued (Fig. 4 - I -). The information flow is assymetric (one way), and it does not constitute a circulation pattern* (Fig. 2). Namely, the establishment of a cycle implies that the boundary layer participates in chemical reactions and "transmits" the hereby liberated molecules as information carrier. Under such circumstances, the membranes would consume themselves, and consequently this type of boundary layer would vanish.

We have postulated specific properties in order to obtain a consistent picture of the boundary surface. In turn, we know about the minimum physical-chemical characteristics which have to be fulfilled. If we examine on the other hand, the phospholipids, we will notice that they contain all the properties requested for a membrane structure. These characteristics and their relation, based on results in (1), are shown in Figure 5. It is thus most plausible to consider the phospholipids the fundamental elements for the biotic development. Only this kind of boundary

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* A one-way information transfer can establish a self-regulatory transport of molecules. A reverse flow of information, like in this system, is not necessary. The membrane, acting on order only, translates the messages into crystallographical structures which in turn determine a certain molecular transfer. This determination of the molecular flow into the micelle does not constitute an information flow. For, information is connected with uncertainties and is a result of a choice from a set of probable events.

layer permits the crossing of the "probability barrier", and will lead to the organic cell.

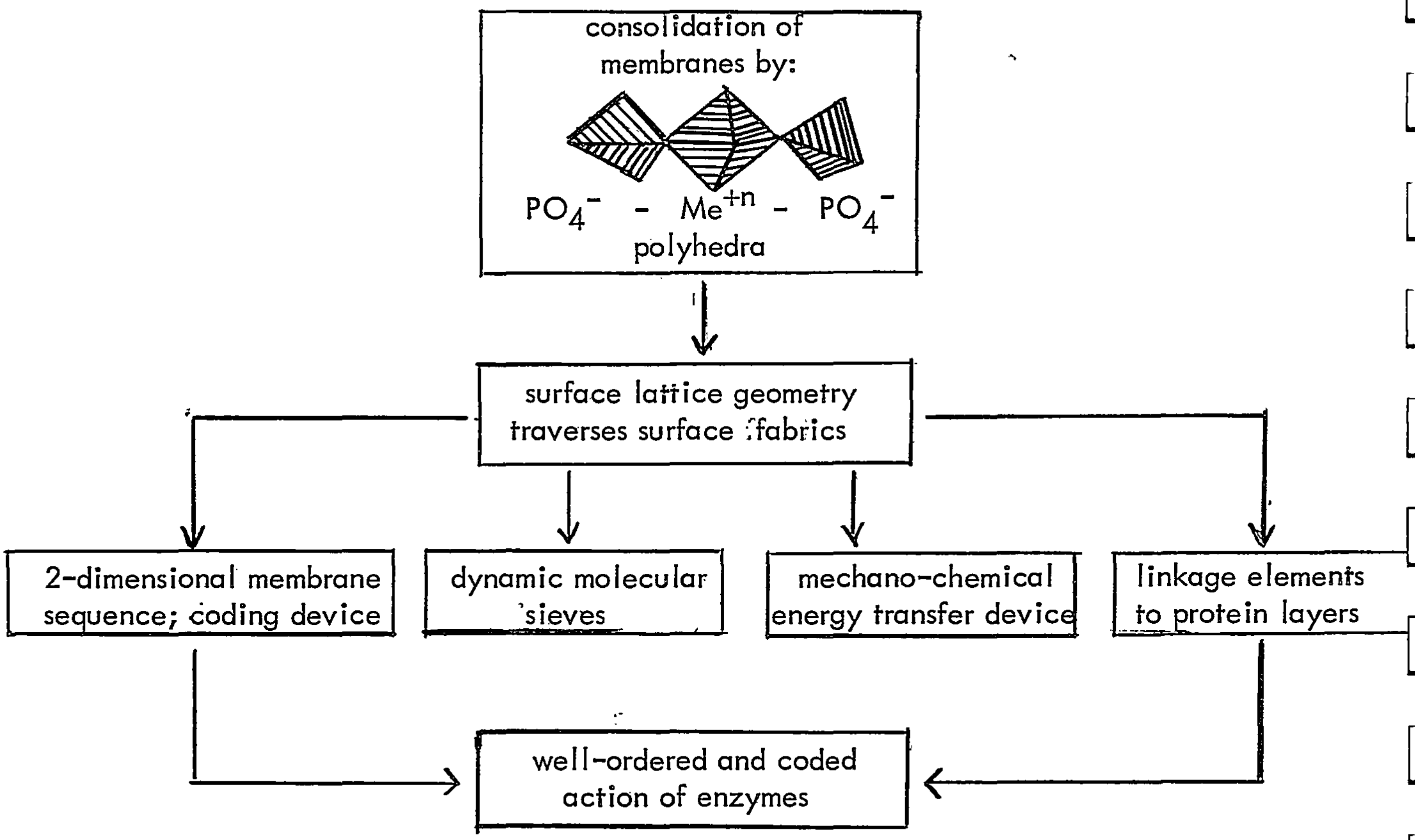

FIGURE 5

The principle dealing with the properties of boundary layers are coherent with empirical observations; it has been shown that the so-called "active membranes" which exhibit abnormal osmosis phenomena (23-29) can be constructed of inorganic materials such as magnesium silicates (24), which implies, that this kind of property is by no means an invention of organisms. These membranes, in dependence of solution parameters, can show (a) normal osmosis, i.e. the transport of the solvent from the diluted to the concentrated phase, and (b) negative osmosis, i.e. a volume transport from the concentrated to the diluted phase. The same implies for migration of ions which may proceed in the direction or against the concentration gradient; the last phenomenon is referred to in biochemistry as molecular pump. This behavior of membranes was explained theoretically (30-36) to be a result of the electrically charged fabric of the membrane matrix. An elaborate treatment of this phenomenon is given in (36). It is emphasized that the charged fabric of the boundary layer, by interaction with the surrounding phases, develops potential and pressure gradients which act as driving force. Other work from a different area is listed in support of the potential-model of interfacial layers. It has been shown (37) that the interfacial tensions between water and organics is linearly related to the logarithm of the degree of miscibility of these systems. It is thus implied that the interfacial forces are the determining factor for the boundary layer. In accordance with this conception is also a potential-model of a double layer which is capable to interpret empirically the capacity-charge relation that previously could not be interpreted (38).

Phospholipid membranes do indeed represent a charged fabric and these abnormal operation capabilities appear to be related to the anisotropic composition pattern.* By considering the thermodynamics of the molecular and ionic transport involved in this abnormally operating membrane system, it becomes evident, that salts move against the concentration gradient on the expense of free energy which is released during the reverse

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* In this context we would like to remind that just recently the coupling between metabolic processes and membrane permeability has received its mathematical consistency. For, on the basis of the Curie principle (39, 40) which forbids the coupling between a vectorial diffusion flow and a chemical reaction flow (i.e. tensor of zero order) and which implies that coupling is achieved only between flows of the same tensorial orders, one had to regard active transport in bio-membranes as a transport mechanism being disconnected from the metabolism (41). Analytical considerations, however, have recently shown that this kind of coupling principle does not exist in case of anisotropic membrane media (42, 43), and a coupling coefficient could be defined (34, 44).

movement of the solvent.* The theoretical treatment of this phenomenon further shows that the efficiency may be equal to one, i.e. the total amount of free energy of the solvent transport could be transformed into motion against a concentration gradient (36).

The here quoted facts - as applied to the micelle - must mean that a selective transport of matter requires a chemical degradation and in turn a production of "solvent media" in the cell, because only via the expense of free energy at the outflow valve (Fig. 4), the pump would maintain an abnormal input-flow. One thus has to postulate a chemical turnover in the cell in order that a linkage of two irreversible processes and which for themselves would proceed in opposite directions, can be stationary maintained for a certain length of time. It is logical that among the various kinds of interface layers present in abiotic micelles there were also those with boundary surfaces following the principle of negative osmosis (different types are mentioned in (23-29)). Therefore, the reason for the election of phospholipids has to do with the remarkable functional properties of (a) phosphorus in cell processes and (b) the geochemical parameters.

Inorganically speaking, the phospholipids - opposite to the proteins - represent rather simple structures. Similarily, the structural composition of the nucleic acids

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* Investigations on bio-membranes have shown that the aforementioned coupling between "metabolism" and ion migration is indeed established (45-49). For instance, the uptake of potassium by E. coli does only proceed in presence of glucose (48); and another example, the energy utilized for the active transport of sodium ions through isolated frog skin by means of glucose degradation (burning) is linearly related to the ion transport that has taken place with an efficiency of about 1/3 of the total input (49). The coupling of such a degradation can be considered a controlled production of the "volume" fraction that can be transported.

also follows a primitive scheme because only the sequence of four building block can be permutated.

Geochemically speaking, the PO_4 units are rather common; so are the hydrocarbons and their oxidation-derivatives, the fatty acids. By now taking biological facts into consideration which we have not mentioned so far that:

(a) in biologically performing systems a cellular organization structure is established, and

(b) all biological systems construct their membranes on the basis of the bilayer scheme*

the abiotic results – as presented here – are in accordance with these biological invariance principles. Namely, to avoid a lack of consistency, we have to assume a "reaction box" (cell) that is furnished with a distinct boundary layer. We believe to understand the reasons behind the conservative and invariant structure for the membranes; only a boundary layer which is chemically inert, that is, which does not participate in metabolic activities could have promoted the development of micelles. Analogously, we have expressed this characteristic as a one-way information flow based on information viewpoints (Fig. 2). Consequently, this type of membrane – in contrast to enzyme systems which are capable to pick-up and release information – could not participate in a chemically active way during the first developmental stages of the coacervates, because this would have represented a chemical reaction and thus would have ended in self-destruction.** It is remarkable that this kind of membrane system – once

---

* "The unit membrane is, however, the only one that is universally present and appears to be an irreducible minimum bounding element" (after Morowitz, 50).

** Perhaps this feature can be considered the reason for the protection of the hydrocarbon chains in form of the membrane bilayer from the "reaction box." In other words, those coacervates which did not invent an effective protection for the paraffins, perished through their own metabolism by consuming the hydrocarbons.

✓

established - was no subject to further evolution which implies that the cellular nature of the organisms was maintained ever since in its primordial habitus.

It is logical, when we concern ourselves with the concrete question as to the development of the phospholipid membranes, and when we consider this question a separate chapter in the development of life. The formation of phospholipids represents the primary event; from here, the boundary layers of coacervates were constituted. This finally initiated the development of an auto-catalytic metabolic system.

ORIGIN OF PHOSPHOLIPIDS

In comparison to the straightforward synthesis of peptides (1) the origin of phospholipids is a rather complicated process. It not only requires the formation of alcohols, fatty acids, and lipid units as single events (Fig. 6):

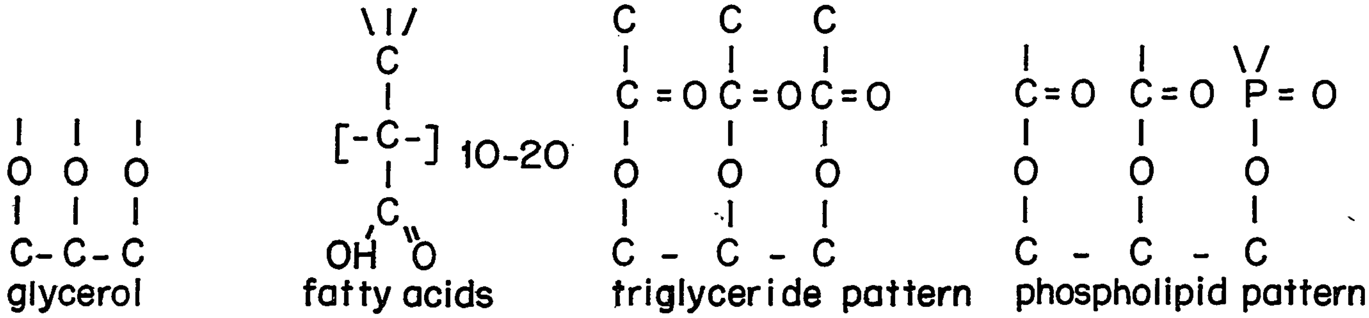
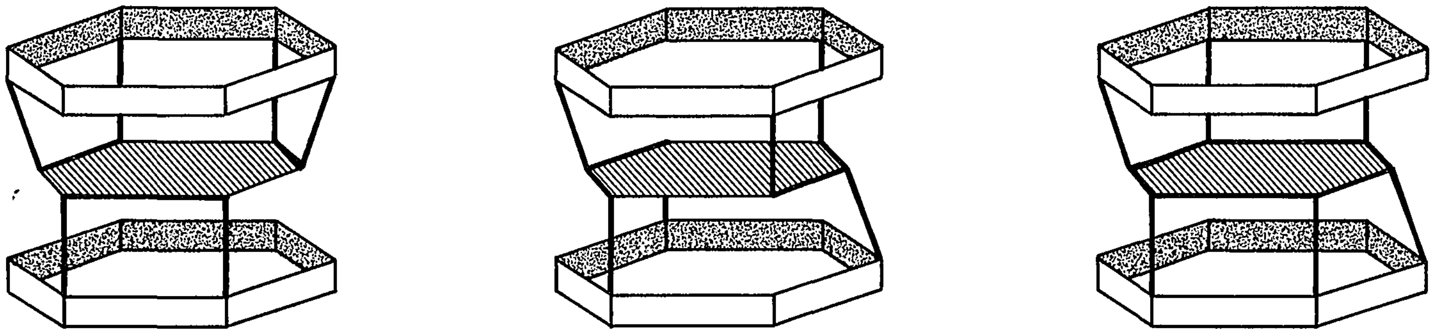


FIGURE 6

but also the assemblage of the last structure into phosphate-containing lipid compounds.

Alcohols

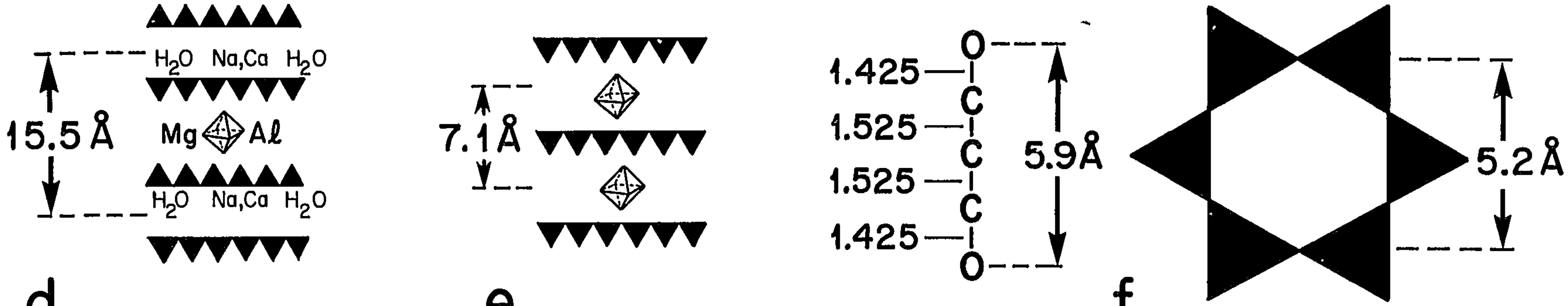
The synthesis of amino acids under conditions that were supposedly present in primordial terrestrial atmospheres and involving electric discharge, UV, or ionizing



a glycol

b glycerol

c inositol



d montmorillonite

e kaolinite

bonding distances

f silicate layer unit

Figure 7

FIGURE 7

Schematic presentation of the structural model of (a) glycol, (b) glycerol, and (c) inositol in order to show the stereochemistry and spacial fit with regard to their adsorption onto (d) montmorillonite and (e) kaolinite. The basal spacing (d_{001}) of montmorillonite is dependent on the number of water layers per cell and may range from 10 to about 21 Å (79). Most commonly in nature are the Ca-montmorillonites having a basal spacing of ≈15.5 Å which corresponds to two water layers per cell unit. By administering glycol, montmorillonites will expand to 17 Å which corresponds to two layers of glycol, and to 17.7 Å should glycerol be used. Other organics such as amines or proteins can become incorporated and give rise to an interplanar spacing up to 48 Å (80). In contrast, no expansion has been observed for kaolinite in presence of polyvalent alcohols (78, 80); the basal spacing for kaolinite amounts to 7.1 Å (76, 81).

A comparison of the alcohols in their adsorbed state reveals that glycol is attached via two O - H - O linkages to the SiO_4 tetrahedra ring units (Fig. a), whereas the glycerol and inositol association is maintained by three (b) or six (c) O - H - O bridges. In addition, glycol contributes six, glycerol four, and inositol two CH_2 groups per 6-ring unit layer of the silicate. As smaller the number of CH_2 groups as better the space fit. Similar relationships exist for other homologous with a higher carbon number; the probability of their abiotic formation, however, is less by a few orders of magnitude. The distances involved in the C-C and C-O bond (Fig. f) have been obtained from studies on the crystal structures of inositol, δ-L-sorbose and β-DL-arabinose (82) and they are compared to the hexagonal unit of a clay mineral (Fig. f).

particles, also results in the formation of simple carboxylic acids such as HCOOH, CH_3COOH, and other organic acids of the type presently involved in the TCA-cycle (51-53). It is reasonable that alcohols ($- CH_2 - OH$) might also be formed in these reactions (54-65) should milder oxidation conditions be applied. As we have previously outlined (2), the alcohols, dispersed in an aqueous environment, may become concentrated in silicates. For structural reasons, a preferential adsorption of glycol, and glycerol and its homologous is expected to occur in sheet silicates as has been verified experimentally (Fig. 7)* (66-80).

However, under primordial conditions, the formation probability for the homologous alcohols with higher carbon numbers is less by a few orders of magnitude. Among the alcohols, glycerol may thus be regarded as a "mean" between probability of formation and number of carbons in the molecule (Fig. 8). These are the chief reasons why - with regard to polyvalent alcohols - glycerol will be present in silicate structures, and why - among its homologous - it becomes enriched (Fig. 7) and thus protected against degradation. Inositol, erroneously called muscle sugar, and which forms ester-type linkages with phospholipids (82), is further evidence in support of this inference. This alcohol, both stereochemically and bonding-wise, fits exactly the layered structure of montmorillonite and

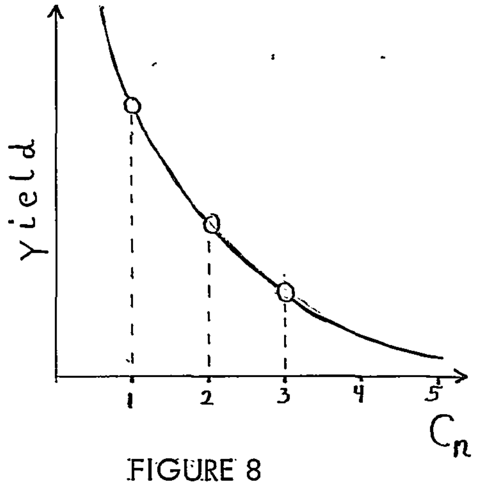


FIGURE 8

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* The determination of the uptake of glycol or glycerol has been found to be a rather reliable technique for the gravimetric determination of the montmorillonite content in clay mineral mixtures (68-73). In X-ray studies on clay minerals glycol is most commonly used as an analytical tool to determine the swelling properties of the sheet silicates (74-80).

related clays and may thus especially become enriched. For this reason we consider phosphatityl inositol an "archaic chemical fossil" which still carries traces of its origin.

Hydrocarbons

On physical-chemical grounds, paraffins must be considered the source of fatty acids $CH_3(CH_2)_nCOOH$; (n = 6-30). Unlike the formation of amino acids and the bases of the purines and pyrimidines, fatty acids cannot be synthesized in terrestrial atmospheric systems because:

(a) their source material, the paraffins in the appropriate carbon range, have a boiling temperature* greater than that of water, and

(b) they would be decomposed - this argument actually represents the chief objection.

On geochemical grounds we have to assume that prior to the condensation of a hydrosphere, a great variety of hydrated minerals were present in the lithosphere, having a dehydration temperature in the range of 300 to 800°C. The hydration of, for instance, the feldspars - a mineral group comprising about 2/3 of the present lithosphere - yields mica structures and six hydrogens per molecule:

$$\underset{\text{(feldspar)}}{(Na, K)\,AlSi_3O_8} + 4\,H_2O + 2\,Al \rightarrow \underset{\text{(mica)}}{(Na, K)(AlOH)_2(AlSi_3)O_{10}} + 6\,H$$

Furthermore, it is known from metallurgical experience that carbon dissolves readily in silicate melts and upon cooling produces sub-microscopic carbon and graphite particles (83-85). These two facts combined:

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* The boiling temperature for C_7H_{16} is approximately that of water. Following the Kopp-rule, the addition of one CH_2 unit increases the boiling temperature by $\simeq$ 20°C and for the compound $C_{17}H_{36}$ we have a boiling temperature of 303°C.

(a) hydration of silicates, and

(b) presence of carbon and graphite

may explain why saturated and unsaturated hydrocarbons could be produced in large quantities during this stage of development. Additional proof for the hydrogenation mechanism is the fact that in contrast to other metal oxides like those of copper, zinc, or iron, the carbon - at higher temperatures - cannot reduce the strong electropositive alkali, alkaline-earths, and aluminum oxides (86-88). Consequently, carbon could not become oxidized to CO_2 because the non-reducible elements predominate. In turn, carbon will become preferentially associated with hydrogen**.

Starting from carbon, a great number of high-molecular weight hydrocarbons should come into existence as a consequence of the thermodynamic equilibrium, because:

(a) carbon is respectably abundant (0.12 M/percent in the earth's crust), and

(b) the amount of hydrogen is limited; (one should bear in mind that the addition of an additional carbon-carbon bond safes two hydrogens.)

In the case of graphite, reactions will preferentailly proceed from the outer molecular layers and here from the atoms at the edges of the benzenoid layers (89) and will result in a subsequent removal of hydrogenated chain- and layer fragments*. The process of hydrogenation is thermodynamically a rather effective one, for, the bond energies are:

$$C - C = 58 \text{ kcal} \quad ; \text{and} \quad C - H = 98.2 \text{ kcal}$$

---

* The molecular mechanism starts from lattice defects (89-92) and thus explains the formation of higher molecular aromatics and chains. Regarding this phenomenon of autocatalysis by structural defects we refer to (93).

** The addition of hydrogen to a carbon-iron-silicate system - at higher temperatures - will result in a lowering of the graphite content upon cooling (85).

A great number of articles have been published which indicate that the hydrogenation of carbon is effectively catalyzed by metal oxides (94, 95). This suggests that carbon hydrogenation itself may introduce the hydration of certain silicates at moderate temperatures, whereby the minerals catalyze these reactions. For further support we like to refer to the presence of saturated and unsaturated hydrocarbons in meteorites (96, 97)*. It has been suggested - on the basis of conclusive evidence - that a few hydrocarbon deposits here on earth are inorganic in origin (111, 112)**.

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* The origin of hydrocarbons in carbonaceous chondrites is a subject of controversy. On the basis of gas chromatographic and mass spectrometric analysis three principal viewpoints have been expressed:

(a) the hydrocarbons are derived from a biological source (97-99);
(b) the hydrocarbons are of inorganic origin (100-103); and
(c) the data are inconclusive, i.e. hydrocarbons can be either (a) or (b) (96).

Concerning (a), hydrocarbons are regarded as evidence for extraterrestrial life (97) or may represent simply terrestrial contamination (96).

Concerning (b), hydrocarbons may form under conditions of thermodynamic equilibrium or near equilibrium (in the absence of graphite) in a solar nebula, or in planetary atmospheres at moderate temperatures and low pressures (100, 101); or alternatively, hydrocarbons are built up on mineral surfaces by the reaction of methyl radicals with that surface (102, 103). To us, the last reaction mechanism seems to be the most convincing one. The co-existence of hydrated minerals, water, and hydrocarbons which is uncommon for other types of meteorites (e.g. normal chondrites, achondrites, or iron meteorites) may be regarded as an evidence for the simultaneous hydration of silicates and the hydrogenation of a carbon source. The forms of carbon present in chondrites include: carbonate (104), diamond (105), carbide, Fe_3C (106), extractable organics (96, 97), graphite (107, 108), and polymeric organics (109). A subsequent oxidation of hydrocarbons will introduce the formation of fatty acids (see chapter on fatty acids). The presence of fatty acids in meteorites (110) may thus simply be the consequence of this reaction scheme.

** It is true, of course, that the vast majority of the world's crude oil deposits are derived from organisms. This inference is based on geological and chemical facts. Namely, the productive oil fields are nearly exclusively found in shallow water marine deposits (e.g. geosynclines, continental shelf areas), whereas fresh water deposits and crystalline rock formations rarely contain crude oil (113). In addition, the carbon isotope pattern of these marine oil field hydrocarbons is identical to that of its anticipated biological

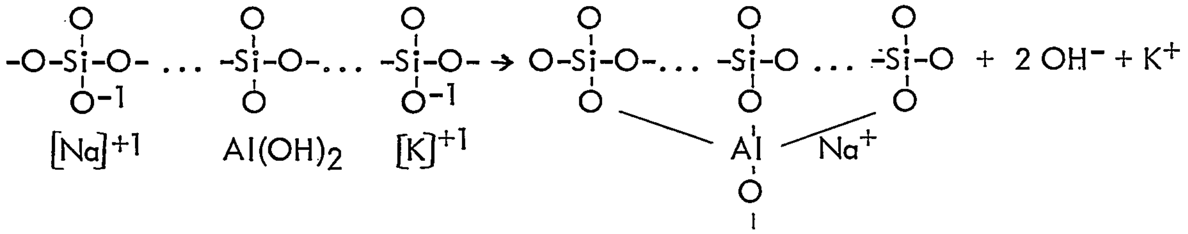
(footnote continued on next page)

Fatty Acids

Analytical data in the aerea of flotation techniques may offer some insight into the reactions involved in the generation of fatty acids. During the processing of ore minerals one takes advantage of the fact that sulfides and metals can be wetted by oil emulsions; the hydrocarbons are hereby tightly bound parallel to the surface of the sulfides and metals (117). In contrast, silicates, oxides, and carbonates, due to their hydrophilic nature, do not allow this kind of wetting (117, 217a). Namely, in this case the hydrocarbons are oriented perpendicular to the surface of the minerals resulting in a minimum surface energy (Fig. 9). In geological environments hydrocarbons are expected to behave similarly regarding their interactions with silicates. That is, we should find them in spots and oriented perpendicular to the silicate surfaces (Fig. 9).

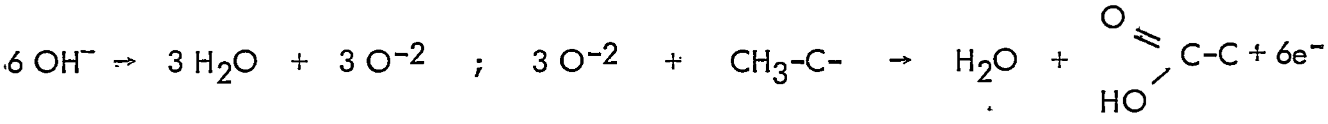
The next step required for the formation of fatty acids is the oxidation of paraffins (118), which are positioned vertical to the surface of the silicates. The following oxidation mechanisms are conceivable:

1. During the formation of clay minerals such as montmorillonite, illite, or kaolinite, the hydrated Al-octatahedron becomes incorporated into the Si_2O_5 network by substituting for Si atoms:



** precursor material (114), and isoprenoid hydrocarbons such as phytane and pristane — which are considered an evidence of life - are commonly present (115). Little is known regarding the geochemistry of hydrocarbons occurring in crystalline rocks (116). Unless proven otherwise, it is more in line that they are indigenous and thus inorganic in nature. Recent work (116) on the genesis of hydrocarbons in igneous and metamorphic rocks seems to support the last statement.

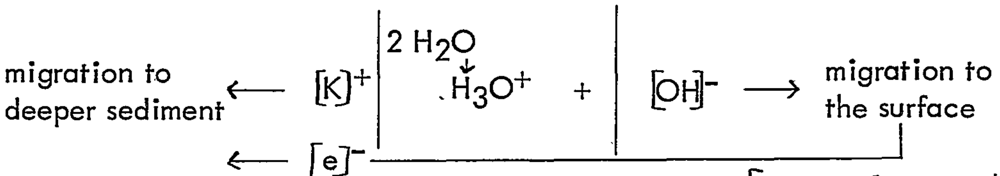
i.e. a transposition of AlO_6 into an AlO_4 tetrahedral co-ordination. This process is accompanied by the release of two hydroxyl groups. The process takes place at lower temperatures. Considering the fact that the ratio of tetrahedral Si^4:Al^4 is at least in the order of 100 to 1, the amount of reactive hydroxyl groups reaches astronomical figures. These OH groups will leave the lattice and may oxidize the hydrocarbons (Fig. 9):



$$6\,OH^- \rightarrow 3\,H_2O + 3\,O^{-2} \;;\quad 3\,O^{-2} + CH_3\text{-}C\text{-} \rightarrow H_2O + \overset{O\,\searrow\!\!\searrow}{\underset{HO\,\nearrow}{}}C\text{-}C + 6e^-$$

It is most likely that traces of certain minerals such as Al_2O_3 will catalyze the reactions. In view of the fact that this process represents a combustion phenomenon, it is favored thermodynamically.

2. An equally important mechanism to initiate the oxidation of hydrocarbons is ion-exchange and ion-migration in argillaceous sediments. A concentration gradient of alkali ions in sediments and in interstitial waters which confirms with empirical data (119-120) will also promote the production and release of OH^- ions:



because metal ions compensate the charge deficit of the $[(AlO_4)^{-1} \cdot K^{+1}]$ unit. Therefore, the migration of potassium has to go parallel to the production of an hydronium ion $(H_3O)^+$ which substitutes for potassium and thus compensates for the charge of the AlO_4^- tetrahedron (Fig. 9).* It is a known fact that hydronium ions compensate for a significant part of the charge deficit in illites and glauconites present in aqueous sediments (126, 127).

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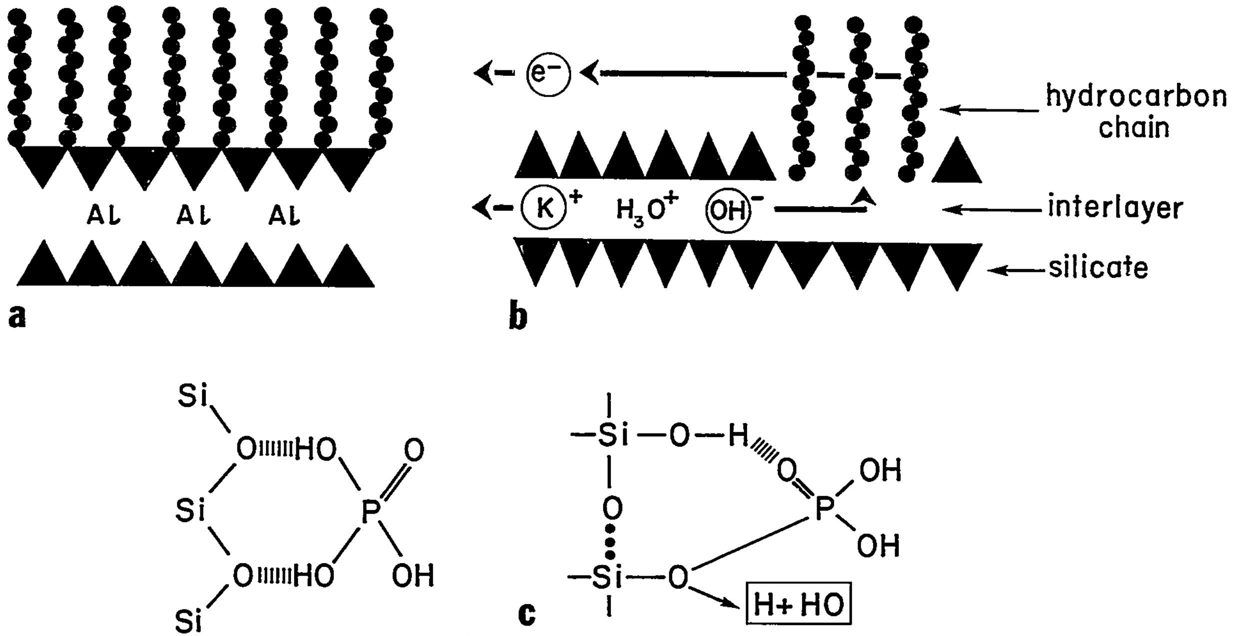
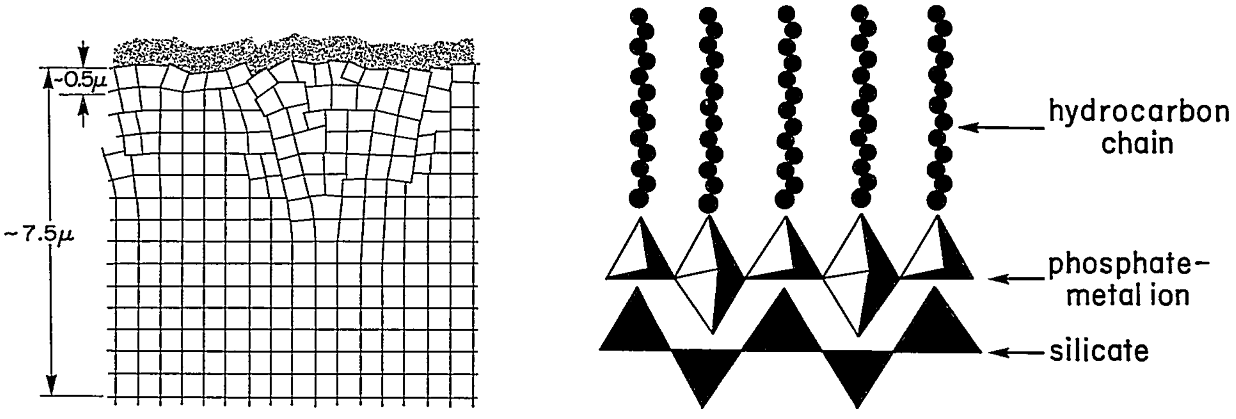
* For empirical evidence of H_3O^+ in chemical processes see (121-125).



types of PO_4 bonding



d crystal surface

e transition structure

Figure 9

FIGURE 9

Formation of phospholipids.

The oriented attachment of paraffins on layers of. montmorillonite (Fig. a) has been confirmed by calculations on the density of surface films (217a). The oxidation of hydrocarbons is presented in Figure b. The alkali ion leaves its lattice position upon thermal activation; and this place becomes substituted by an hydronium ion $(H_3O)^+$ at the AlO_4^- tetrahedron. The resulting hydroxyl ion $(OH)^-$:

$$K^+(AlO_4)^- \rightarrow K^+ + AlO_4^- \ ; \ AlO_4^- + 2H_2O \rightarrow (AlO_4)^-H_3O^+ + OH^-$$

will migrate in the next proximity of the migrating alkali ion; upon hitting another hydroxyl ion, an H_2O molecule and a free oxygen atom will be produced. The electrons hereby released will draft in the next proximity of the alkali ion; thus, the mono-atomic oxygen relieved from its negative charge will diffuse easier through the silicate lattice and can oxidize hydrocarbons attached to the surface of silicates. This process may result in the formation of fatty acids:

$$3\,O + H_3\text{-}C\text{-} = \text{-}\,COOH + H_2O.$$

The reaction is catalyzed by the silicates themselves due to their metal "oxide" nature. The oxidation of hydrocarbons does of course not necessarily stop at the fatty acid level; it may continue and its final endmember will be CO_2.

The PO_4 bonding on a SiO_4 surface is shown in Figure c. There are two alternatives,

(a) formation of hydrogen bridges (upper figure), or
(b) co-ordinative sharing of oxygens and cleavage of H + OH (lower figure).

The schematic drawing of a silicate surface is presented in Figure d (217). X-ray studies show that the surface energy is stored in the form of structural irregularities; it will become stabilized by a monomolecular surface film. Additional, but indirect evidence for the storage of surface energy can be obtained by reference to (153, 218, 219). It can be demonstrated that the isoelectric point of Al and Si oxides can be strongly influenced be adsorbed impurities (219). The surface energy storage may result in structures shown in Figure d; furthermore, it is favorable energywise to cover the distorted surface with a monomolecular film (shaded area in Fig. d).

A transition structure incorporating hydrocarbons, a chain of phosphate/metal ion polyhedra and a silicate surface are shown in Figure e. Due to the structural flexibility of the phosphate tetrahedra in response to the kind of metal ion co-ordination, a smooth surface film can be created on top of the silicate structure.

These oxidation processes for hydrocarbons may take place at temperatures ranging between 10 and 50°C and predominantly in the upper most layers of the sediment strata. At greater depths, a kind of stationary state ion equilibrium has already been achieved because silicates are already organized as pseudo-crystalline and crystalline particles. The chances for hydrocarbons to become oxidized are greatest for those that, within the temperature range of 10 to 50°C, are (a) mobile and (b) adsorbed on silicate surfaces. Using these criteria, the hydrocarbons starting with $C_{15}H_{32}$ and having a melting temperature of 10°C, and closing with $C_{24}H_{50}$ with a melting temperature of 51°C represent the most likely candidates for the fatty acid formation. Hydrocarbons with a lower carbon number are too mobile, and hydrocarbons having higher carbon numbers are too inert to become frequently attached in form of monomolecular layers along silicate surfaces.

Lipids and Phospholipids

The oxidation products of hydrocarbons - fatty acids - will considerably increase the surface adsorption because hydrogen bounding will attach these molecules to the SiO_4 tetrahedra. In addition, the melting temperature of a fatty acid is about 40°C higher than that of its paraffin precursor. This will also reduce the migration mobility and will support the reaction mechanism discussed below.

The formation of lipids from fatty acids is intimately connected to the formation of phospholipids. The proposed reaction scheme is as follows: The phosphate tetrahedra linked to the surface of the Si-O hexagons* do represent condensation centers for the fatty acids via PO_4 units because a thermodynamically favorable transition structure between the

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* Experiments have shown (see text in Fig. 9), that the formation of a covering layer on silicate surfaces is energetically favorable and, therefore, will be present.

hydrophobic hydrocarbons and the ionic silicate network is created*. In the course of the building of this sandwich structure, the reaction of fatty acids with alcohols and the formation of ester bonds to the phosphates are catalyzed by metal ion polyhedra. Among the various types of esters, involving for instance, glycol, and tri- and tetra-alcohols, those of the diglycerides represent unique compounds. This is so, because two paraffin chains occupy the same surface area (40 $Å^2$) as a PO_4 tetrahedron associated with a M^+ oxygen co-ordinated polyhedron (Fig. 10, 11). For this reason, they will be subjected to a continuous assemblage on phosphate films, whereas other compounds become chemically degraded. The processes leading to the common phospholipids are outlined below on the basis of empirical facts.

It has been observed that the silicate surface of glassware readily adsorbs $H_2PO_4^{-1}$ tetrahedra (132-134). The strong bonding of PO_4 to the surface of SiO_4 structures are shown in experiments involving colloidal silica and clay minerals (135-141). In addition, it has been observed, that PO_4 groups can be adsorbed onto AlO_6 polyhedra which is rather significant in view of the fact that Al-octahedra are present in sheet silicates (142-146). On the basis of the spacial dimensions of the PO_4 tetrahedron and the SiO_4 tetrahedron, substitution phenomena become plausible (1). The way these H_2PO_4 groups are hereby linked to the surface of the SiO_4 tetrahedra is graphically illustrated in Figure 9. This inference is supported by the occurrence of silica-apatites (147, 148) where PO_4 groups are substituted by SiO_4 tetrahedra. The presence of Si in these structural positions is common for the naturally occurring phosphates like monazite, xenotim, and apatite and may reach concentrations up to 1 per cent (149). Analogously, silicate minerals accommodate PO_4 groups always in a ratio of:

$$P : Si = 1 : 10^{-3} \text{ to } 10^{-4}$$

in their lattice (149).

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* Experiments on polyphosphates indicate that phosphates are capable to form unique transition structures on solid surfaces. Their sorption on surfaces induces hydrophilic properties (128-131).

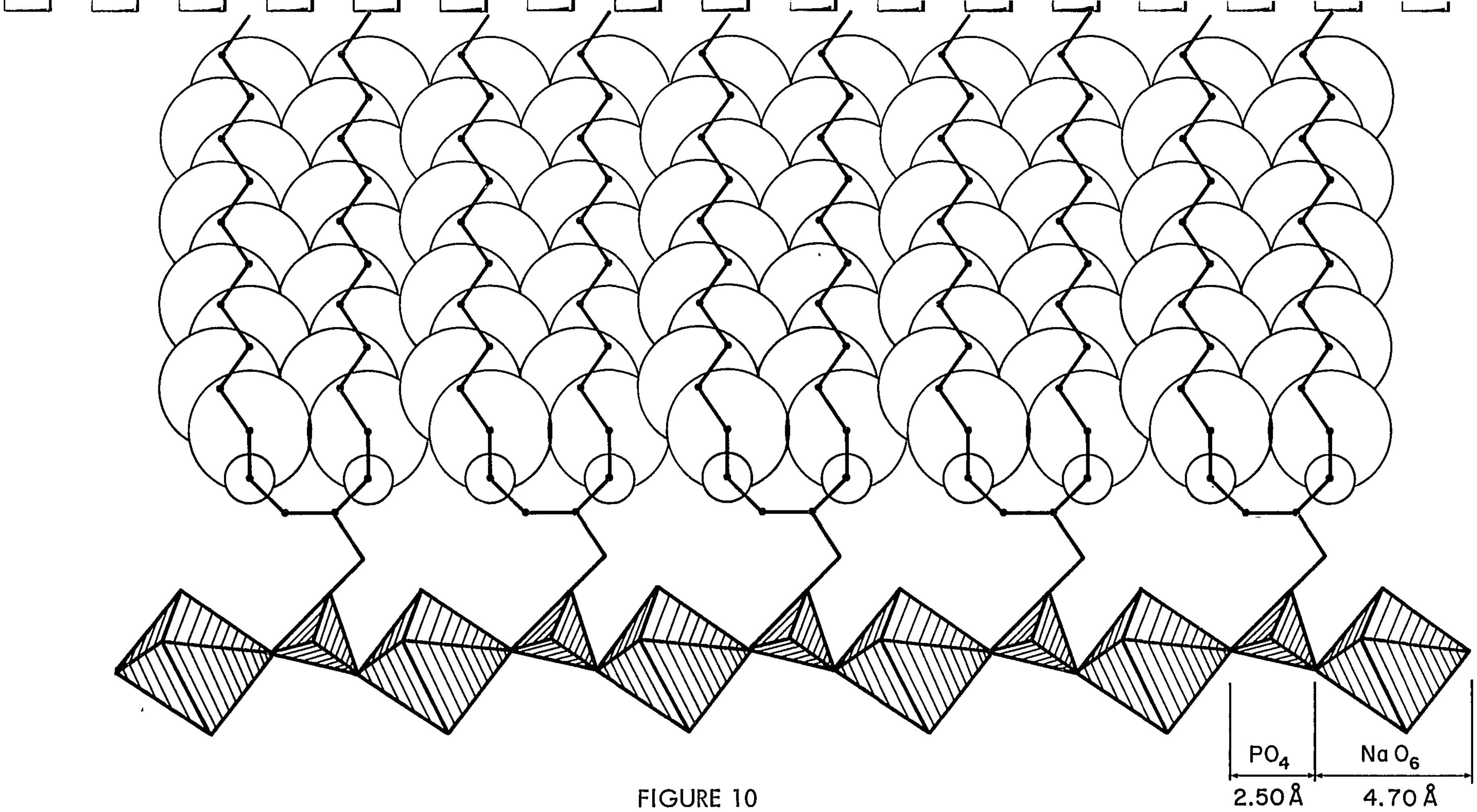
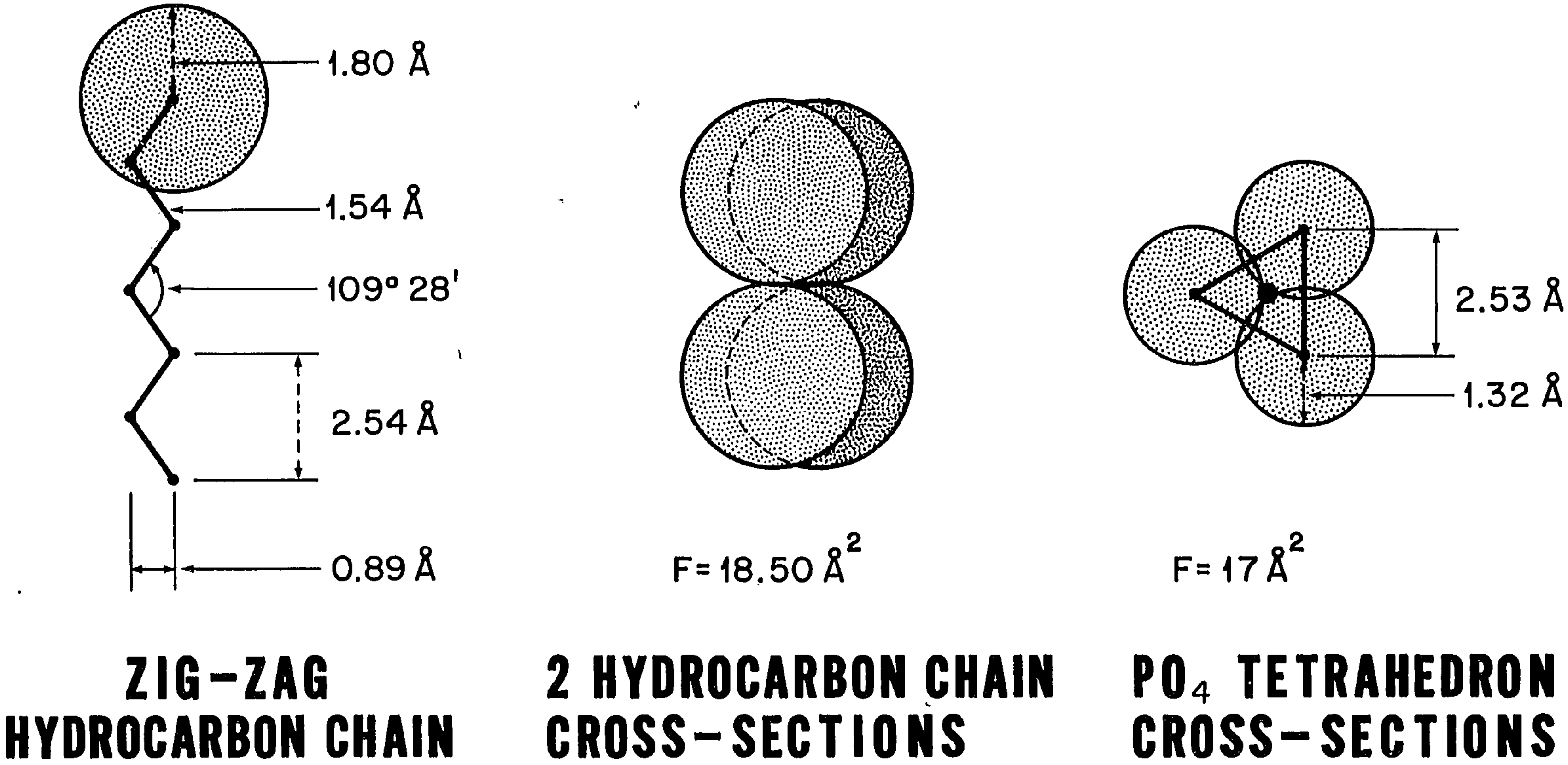


FIGURE 10

Membrane cross-section composed of double chain of lipid-oxygen polyhedra. Glycerol is only schematically represented; the carbonyl groups and the PO_4 residues have been omitted. The molecular dimensions are based on the NaO_6 octahedron. The molecular aggregation, as presented here, results in a corresponding interfacial area fit for both chains concerned. Assuming the surface area of a hydrocarbon chain is identical to the surface area empirically determined for fatty acids (20 Å^2 per molecule) (208-211), we obtain the same values for the surface area of a tetrahedron-octahedron unit. The cross-section surface for the minimum six oxygens falls in the range of 33-37.5 Å^2 with a most probable value of 35.5 Å^2. For metal ions, the mean surface area amounts to 4 Å^2. This would add up to a total surface area of 39.5 Å^2 for a polyhedron unit, a value which would conform with the surface area of the associated lipids. It is noteworthy, that for sphingomyeline (213) the area, at the time of collapse on the water surface, amounts to 37 Å^2 which is identical to the minimum value observed in crystals of hydrocarbons (see Fig. 11).



ZIG-ZAG HYDROCARBON CHAIN

2 HYDROCARBON CHAIN CROSS-SECTIONS

PO_4 TETRAHEDRON CROSS-SECTIONS

FIGURE 11

Molecular dimensions of zig-zag hydrocarbon chains; dots are carbon atoms (left-hand picture). The CH_2 contact radius (=1.8 Å), taken from the $n \cdot C_{29} H_6O$ structure, (214) is incorporated. The cross-section of two hydrocarbon chains along the (001) plane of this structure is shown in the center picture. The surface area of both chains amounts to 37 $Å^2$. For comparison, these hydrocarbons are presented relative to the surface area of the PO_4 tetrahedron (right picture). The radius for oxygen is corrected to the 4-co-ordination state (original r = 1.40 Å) (215). This would amount to a surface area of 17 $Å^2$ for the tetrahedron. The correction, of course, is up to criticism, because oxygen is also co-ordinated with the metals. Under such instances, the values will come out to be 18.5-19 $Å^2$ per unit PO_4.

In silicates such as nagatelite (150) and oyamalite (151), the phosphorus content reaches the surprising value of 3 percent. These data indicate that it is reasonable to assume that phosphate groups - realeased during weathering processes - will cover the surfaces of clay minerals as molecular films* in epitaxial order. Evidence for this supposition is the presence of films of $Ca_3(PO_4)_2$ on glauconite surfaces (156).** These molecular surface films of phosphates which in nature are quite common because phosphorus is a major element in the lithosphere,*** represent a key element for the generation of phospholipids and in turn the active membranes.

At this point begins the significant interaction of PO_4 groups with organic molecules such as alcohols and fatty acids; and in biological membranes these artifacts of a template**** origin can still be observed. For, the PO_4 structures actually copy the silicate templates by joining them in epitaxial order.***** The structural principles of phosphates do permit an optimal epitaxial attachment to silicates. The phosphate films will have structures similar to those observed in phosphate minerals, i.e. networks of PO_4 units which are associated with metal ions.

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* Regarding the evidence for the activity of a phosphate film, it has been shown that traces of sodium polyphosphate, $(NaPO_3)_n$, can effectively supress the growth of $CaCO_3$ nuclei (152). This again demonstrates that small crystallites - by themselves - have a high surface energy (153) and that, therefore, they must change into a lower energy state by developing a transition structure (surface film). The carbonate chain in $CaCO_3$ permits an epitaxial growth of a polyphosphate chain, whereby - as a transition structure - a poly-bicarbonate chain linked by hydrogen bonds similar to that in $NaHCO_3$ (154) most certainly is established.

** Recent glauconite analyses indicate the presence of PO_4 groups ($P_2O_5 = 0.34\%$) (155).

*** The phosphorus content of the major rock types is in the order of 0.1 to 0.9% P_2O_5 (157).

**** A similar template copying principle has been mentioned (158). It was suggested that the organic macromolecules may represent an image of a topologic well-defined silicate surface.

***** The evidence for the template-copying relationship becomes apparent by considering the isotopy relations between silicate and phosphate structures. These relationships are shown in Table 3 in (1).

Polar organic molecules will become preferentially attached onto these layers - which can be assumed a few molecules thick - for reasons that the variable network geometries of phosphate causes a stronger bonding of polar organics to them rather than to the rigid and nonflexible silicate networks. Since time is abundant in geological environments, organophosphate molecules of the type of phosphatidic acid (Fig. 9) can eventually develop.

We believe that ester formation involving the fatty acids and glycerol and PO_4 is catalyzed* by the metal ions present in the polyhedra.

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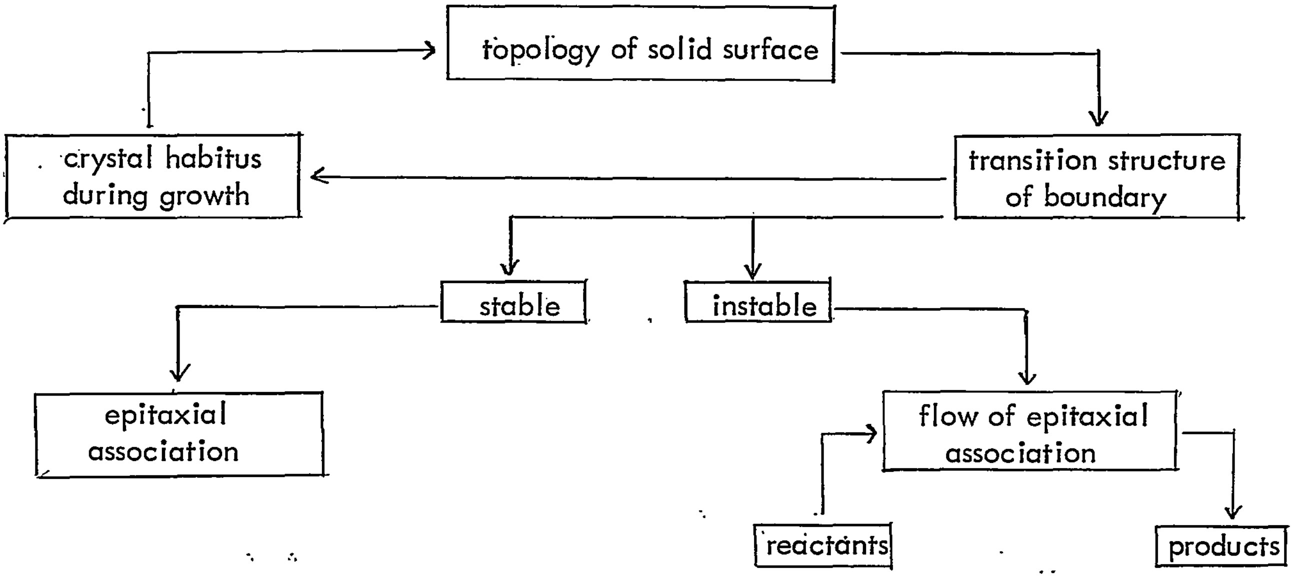
* This catalysis represents a rather involved phenomenon and cannot be treated appropriately in this article. Therefore, we would like to stress certain general features of catalysis in order to show that - whenever we mention catalysis in the context of this work - we are always in accordance with the general principles of catalysis. Based on data on the molecular configuration of catalysis processes on solid state phases it is evident that catalysis represents a process by which a solid state surface "tries" to establish a thermodynamically favorable boundary transition structure with the adsorbent. Independent of models describing catalysis such as potential energy curves (159-161), geometric factors (162), or electron transfer (163-165), we can outline the phenomenologics of catalysis in the following way:



CATALYSIS

(footnote continued next page)

Expressed in Å^2 units, the surface area of a PO_4 group amounts to almost 20 Å^2 which is identical to the surface area of a hydrocarbon cross-section (19-21 Å^2).

By assuming:

(a) closest packing, and
(b) stable structures for both, the hydrocarbons and the PO_4 units within the transition boundary layer,

it is impossible that a PO_4 group will be co-ordinated with just one hydrocarbon chain.** For, due to the need for an identical surface area for the hydrocarbons and phosphates, this would cause the formation of polyphosphates, which can be empirically ruled out for reasons of the hydrolysis of the - P - O - P - bridges (212-214). The PO_4 groups will surround themselves with at least 3 H_2O molecules as bridging elements between them; this can be inferred from the hygroscopic behavior of phosphoric acid. Consequently, an increase in surface area, corresponding to the addition

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(continuation of footnote)

* This scheme is in agreement with empirical facts. For instance, the crystal habitus is catalyzed through the solution participants by the formation of boundary structures (166-170). In contrast, the specific topology of a solid state surface will yield specific boundary transition structures (177-187). In case these transition structures are chemically stable, we are dealing with epitaxial co-ordination (188-199), i.e. oriented intergrowth. Should the building of these transition structures cause a chemical alteration of the adsorbent, e.g. through polymerization (200-201) or hydrogenation and dehydrogenation (202-204) we have the fact termed catalysis processes. The principles of catalysis are in accordance with biochemical observations (205-207).

** For comparative purposes we present the arrangement of tetrahedral networks in silicate layers (Fig. 12), showing various anion packing arrangements (216).

of the water molecules, is the result. In turn, the incorporation of metal ions such as $Na^+ \simeq 4\,Å^2$ will produce - for reasons of good accordance - a particularly stable co-ordination which amounts to a surface area of about $40\,Å^2$. In this way it is understandable, that

(a) two hydrocarbon chains are co-ordinated to only one phosphate - metal unit (closest packing), and

(b) di-glycerides as minimum units will yield stable structures

because the traverse connection of the hydrocarbons by glycerol constitutes an additional linkage element to their own hydrophobic bounding. Further stabilization is achieved by the presence of glycerol as an oxygen transition element from the ionic PO_4 to the non-ionic paraffins.

MEMBRANE SYSTEMS

There is no doubt that a variety of organic systems, and separated by different boundary layers, were in existence during certain low-temperature stages in the development of the earth's surface (220). Among these were agglomerates containing phospholipids as boundary layers and which we will refer to as coacervates. Based on the wealth of publication* dealing with various sorts of micelles and emulsions, it is possible to enumerate the probability of development for the individual micelles in terms of a "taxonomy game" (221) plotted along a time scale. For, the interplay and its result is determined by the

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* The literature embraces practically the whole field of natural sciences and includes phenomena such as emulsions, foams, surface physics (electric charge and capacity, tension, capillary, and others), lubrication research, colloidal surfactants, detergents, interfacial chemistry, soaps and tensides, petroleum research, thin films technology and many other phenomena).

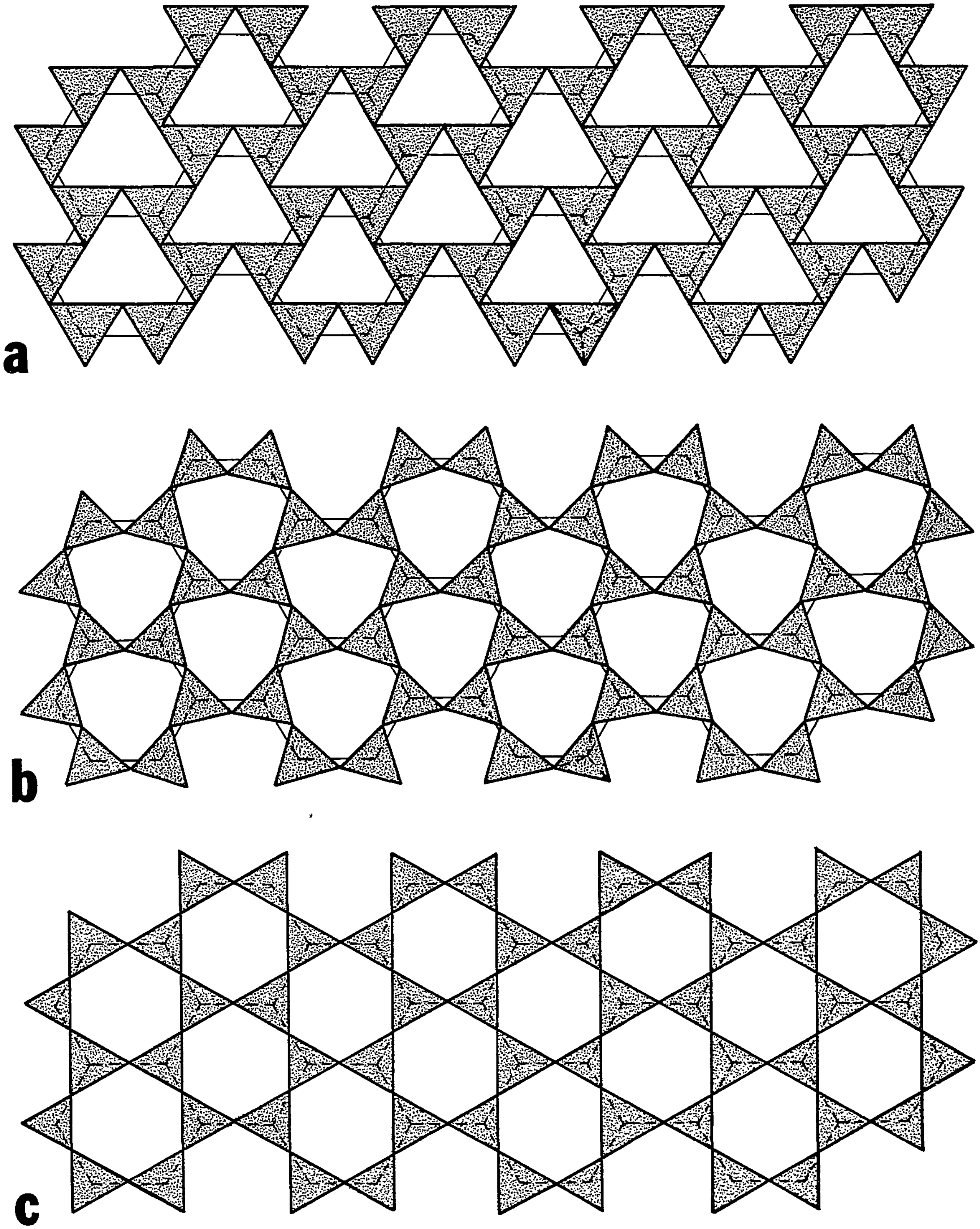


FIGURE 12

The various arrangements of the tetrahedral networks (represented by the connected tetrahedron bases) are shown. The closests packing for anions is shown in the upper figure (a), and the regular arrangement is shown in the lower figure (c), where the anions take up 3/4 of the close-packing position. A transitional arrangement of the anion packing is presented in the intermediate structure (b) (216).

kind of boundary structure. In general, we can distinguish between:

	components			
membrane structure	non-ionic		ionic	
	neutral	polar		
isotropic	benzene			
anisotropic	ether	peptides	fatty acids	phospholipids

The various cases will be discussed individually. Inasmuch as this topic is intimately connected with the sub-structure of a primordial cell, we will briefly present the physical-chemistry of micelle formation in order to estimate to what extent cell organelles can possibly be considered a self-propelled assemblage and under which circumstances genetic information is required to create organelles (222).

Liquid water-organic phases

Hydrocarbons and their derivatives are generally characterized by structural polymorphism which is determined by a variable three-dimensional geometry of single chains and its rotational energy. The weak bonding forces - van derWaals forces and hydrogen bridges - which interconnect these molecules in the form of a crystal network, is the reason that across:

(a) a narrow temperature range (Δt = 1-10°C), and
(b) a narrow concentration range of the added compound (Δc = 0.1-10%)

structural alterations will take place. This phenomenon contrasts the similarly sophisticated polymorphism established in silicates where due to the strong intermolecular bonding these structural modifications are distributed over a range of 1000°C and a concentration gradient of 10 to 100 percent of the added substance.

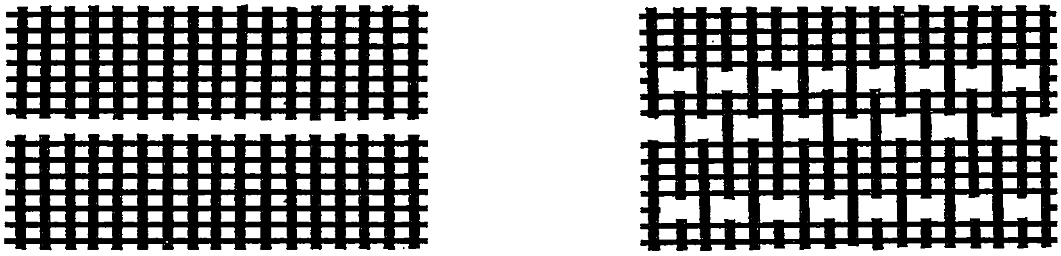
Inasmuch as the range of structural changes for the organics is lower by a factor of 100, more sensitive analytical techniques and a high purity of the compounds is required which - unfortunately - is generally not the case. In turn, knowledge on the interphase question has not yet received a consistent presentation. We intend to elucidate this problem by closely following the informations on silicates and on this knowledge we will base concepts on the development of coacervate systems.

Even solid crystalline normal hydrocarbons show complicated modifications, e.g. reversible changes of the cross-section of the unit cell (223-225). Consequently, one can infer that the number of these structural changes will be accelerated in the liquid or semi-fluid state. Yet, it is possible to deduce general structural principles which concern the area of liquid crystals and colloids. Namely, on the basis of detailed investigations it has been established, that the properties of the liquid phases of organic compounds are principally determined by two types of cross linkages (226) (Fig. 13a-c). These two linkage orders are characterized by the number of co-ordination neighbors and the distribution of bonding forces regarding these co-ordinated molecules. In the case of the liquid phase, we can distinguish between:

(a) isotropic cross-linkages (Fig. 13a) which come into existence in case the number of neighboring molecules is small, i.e. should the co-ordination number represent a minimum value. The organic chain molecules will be contained in macromolecular stacks of layers.

(b) anisotropic cross-linkages (Fig. 13b) which come into existence in case the co-ordination number is larger than the minimum number, i.e. no layer assemblages (stacks) will result, but three-dimensional cristallites.

It is emphasized that these kinds of phase transitions are abrupt and occur at well-defined



a isotropic crosslinkage

b anisotropic crosslinkage

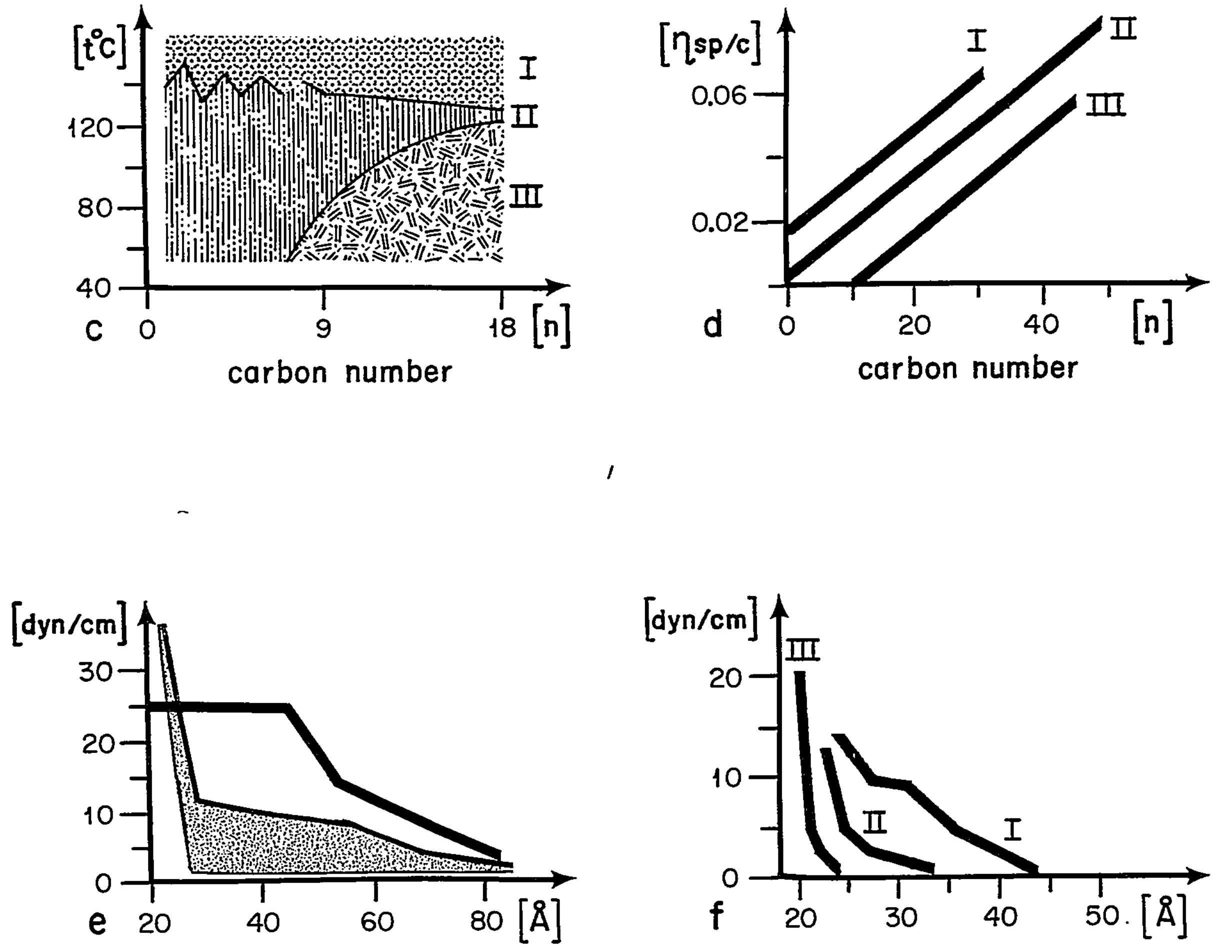


Figure 13.

FIGURE 13

Relation between co-ordination and chemical constitution of liquid organic compounds. In Figures a and b, two co-ordination types between hydrocarbon chains are shown. Figure a represents the isotropic cross linkage between individual hydrocarbon chains; the chain molecules are held together in form of stacks. In contrast, Figure b illustrates the anisotropic cross linkage between chain molecules. In the case of structure a, each molecule has two co-ordination neighbors; in structure b, four co-ordination neighbors are present in the two-dimensional drawing. It is logical that the number of co-ordination partners determines the properties of the liquid phase (226a), because a bonding in three dimensions (anisotropic cross linkage) , relative to that in two dimensions (isotropic cross linkage), will differently influence the macro-molecular order of the liquid phase. The precise location of the individual molecule within a stratification network will determine the strength of the lateral and planar attachment forces.

For illustration, a diagram showing the transition temperature between three different liquid phases (I, II, and III) of the 2-p-n-alkaxbenzylideneaminofluorene as a function of chain length is shown in Figure c (226).

The relationships between specific viscosity and chain length for paraffins and paraffin derivatives up to 500 Å in 1.4% CCl_4 is given in Figure d; the upper curve (I) shows the relationships for n-fatty acids; n-paraffins and n-fatty acid esters are presented in the central curve (II); and n-alcohols (III) are shown in the lowest curve; we can write

$$\frac{n_{sp}}{c} = K_m \cdot M$$

Normal paraffins are hereby considered as polymers of CH_2 group (molecular weight = 14). A solvent containing 14 g CH_2 per liter has a strength of 1.4 percent (= 1 basic molar). The term n_{sp} is the specific viscosity, i.e. the increase in viscosity obtained upon dissolution of a compound in a solvent; c = concentration of solvent; in basic molarities; K_m = constant for homologous polymers; and M = molecular weight. In contrast to Einstein's relation, the specific viscosity of equally concentrated solutions is proportional to the molecular weight, i.e. proportional to the increase in chain length. This explains why a dissolved chain molecule requires a larger volume than would correspond to its own volume (231).

Typical force (=surface pressure) vs. area (occupied by one molecule) curves for octadecyl sulfate monolayers are shown in e. The dotted area indicates the contraction of a monolayer as a function of Na^+ ions in the range of 5 M NaCl (lower curve) to 10^{-3} M NaCl. The fact that the increase in Na^+ ions introduces a condensed monolayer is proof for the isotropic cross linkages by means of ionic metal polyhedra at the interface. In contrast to the contraction introduced by Na^+ ions at 20 $Å^2$, the addition of 1 M HCl (upper curve) causes a collapse of the monolayer at about 50 $Å^2$ (232). This is so, because the H^+ ion cannot build up a polyhedra network.

Analogously, the addition of cholesterol to myristic acid films (I) has a similar contraction effect (Fig. f). Even one molecule in four of cholesterol (II) causes appreciable contraction of the film, while with one in two (III) the acid is completely reduced to the state of a condensed film; in the last instance, the breaking point is at about 20 $Å^2$ which is identical to that of NaCl -octadecyl sulfate (Fig. e). Other fatty acids exhibit similar relationships (232). Again this demonstrates that within the organic part of the monolayer the isotropic cross linkage can be enhanced.

temperatures.* This contrasts the observations made for glass systems, where a diffuse (gradual) softening takes place over a certain temperature interval. It is plausible that these two kinds of cross-linkages are temperature dependent and that the probability for the development of an anisotropic cross-linkage pattern (type b) will increase with increase in temperature and chain length; the empirical proof is shown in Figure 13c. The same structural principles apply to mixtures of organic compounds as well as to organic-inorganic liquid systems but more complex relationships are the consequence. Two empirical relations are mentioned which give us a better idea regarding co-existing liquid phases:

1. The additivity of molecular cohesion (228-230), i.e. the additivity of the various increments in bonding energy. This concerns the successive increase in length for chain molecules (intermolecular bonding as a function of chain length).
2. The observation that in diluted solutions of paraffins and paraffin derivatives the increase in viscosity - different to the Einstein relation - proceeds proportional to the chain length (231), i.e. to the molecular weight (Fig. 13d).

This result shows that a dissolved chain molecule requires a larger volume than its own volume, and this effective sphere increases with the square of its chain length. The sphere is schematically shown in Figure 14, whereby the height of the cylinder represents the cross section of the chain molecule, and the cylinder diameter is the chain length. Consequently, the effective volume of a chain molecule increases with the square

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* For instance: 5-chloro-6-n-heptyloxy-2-naphtoic acid (227)

$$\text{solid} \overset{165.5°\text{C}}{\rightleftharpoons} \text{smectic} \overset{176.5°\text{C}}{\rightleftharpoons} \text{nematic} \overset{201°\text{C}}{\rightleftharpoons} \text{isotropic}$$

of the carbon number:

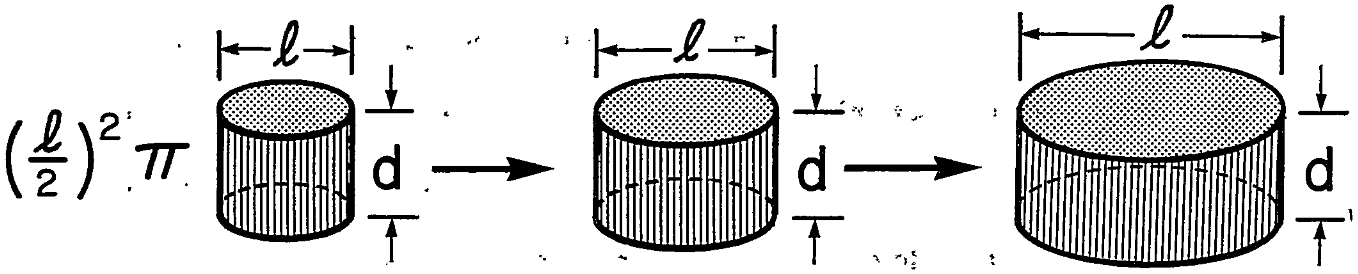


FIGURE 14

Based on this information we can immediately understand the condensation of monomolecular layers of fatty acids induced by the presence of ionic salts or cholesterol (Fig. 13e and f).

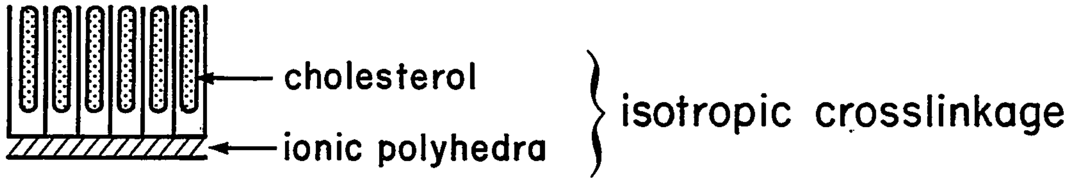


FIGURE 15

Metal ions as well as cholesterol are capable to reduce the aforementioned spheres of chain molecules, and to initiate a contraction of the monomolecular film to a condensed state. The mechanism of this process is the following that:

(a) the bonding to metal-oxygen polyhedra, or
(b) the interlayering of cholesterol

will dampen the thermal motions of the chain molecules. For cholesterol it has been directly shown that its great size and inertia obstructs the tilting motions and that no bounding of cholesterol to the hydrocarbons takes place as can be inferred from vapor pressure measurements of the surface films (233).

Regarding the dissolution of organics in aqueous systems these molecular structural viewpoints can be applied. It is established that organics can continuously dissolve in water up to a critical concentration value, from whereon a phase separation will come into existence. In turn, a spontaneous formation of aggregated molecules, known as micelles, will result (225, 226, 234-236). This micelle formation, which physically can be considered the formation of a liquid eutectic, is reflected by a discontinuous change in the physical-chemical properties of the solution (Fig. 16a and b). The point at which this change occurs is designated as Krafft's point (247) or the critical micelle concentration (CMC). Based on the hexagonal water structure and its distortion by dissolved molecules, one can understand that this spontaneous phase separation is a function of chain length and ionic or polar properties of these molecules. The ratio for molecules with carbon numbers up to 20 is about:

(a) water molecules : non-ionic organic molecules = 10^7-10^5 : 1

(b) water molecules : ionic organic molecules = 10^5-10^2 : 1

The number of aggregated molecules within the micelles falls in the range of:

$$\text{number of aggregated molecules} \approx 10^1 \text{ to } 10^5$$

which will yield a micelle radius in the neighborhood of:

$$\text{micelle radius} \approx 10 \text{ to } 100\,\text{Å}$$

The self-propelled separation process which trips at a certain concentration is highly affected by additives inasmuch as the effective volume (Fig. 14) of a dissolved molecule is influenced by these (Fig. 13e and f). By considering the kind of cross-linkage forces which come into existence by these additives, the influence of the solvent participants upon CMC can be understood (Fig. 16c). There are two alternatives, either the:

(a) cross linkage of the organic phase is enhanced, or

(b) reduced by the co-solvents

since the interfacial forces between the liquid-liquid system of water and organics can be considered a summation of bonding forces of the single dissolved organic molecule to the water molecules. This is derived from the linear function of interfacial tension (37) shown in Figure 16d. This explains why the critical concentration of micelle formation is lowered with increase in chain length, because an increasing number of CH_2 groups also enhances the probability of cohesional bonding (Fig. 16e and f).

The ionic and non-ionic organic compounds are clearly distinguished by their behavior in aqueous solutions. Whereas cohesional cross linkage forces are the predominant ones for the polar molecules, weak cohesional and strong adhesional cross linkage forces are competitively operating in the case of the ionic molecules such as the fatty acids. For this reason we observe a so-called "clouding" with increase in temperature in micelles formed by polar components. The term clouding hereby refers to the cross linkage among individual micelles which ultimately may result in a complete phase separation. When dealing with high molecular compounds, this clouding is frequently referred to as coagulation and denaturation.

The clouding process caused by links between micelles has been verified by measurements. Namely, the cloud temperature point is independent of the concentration

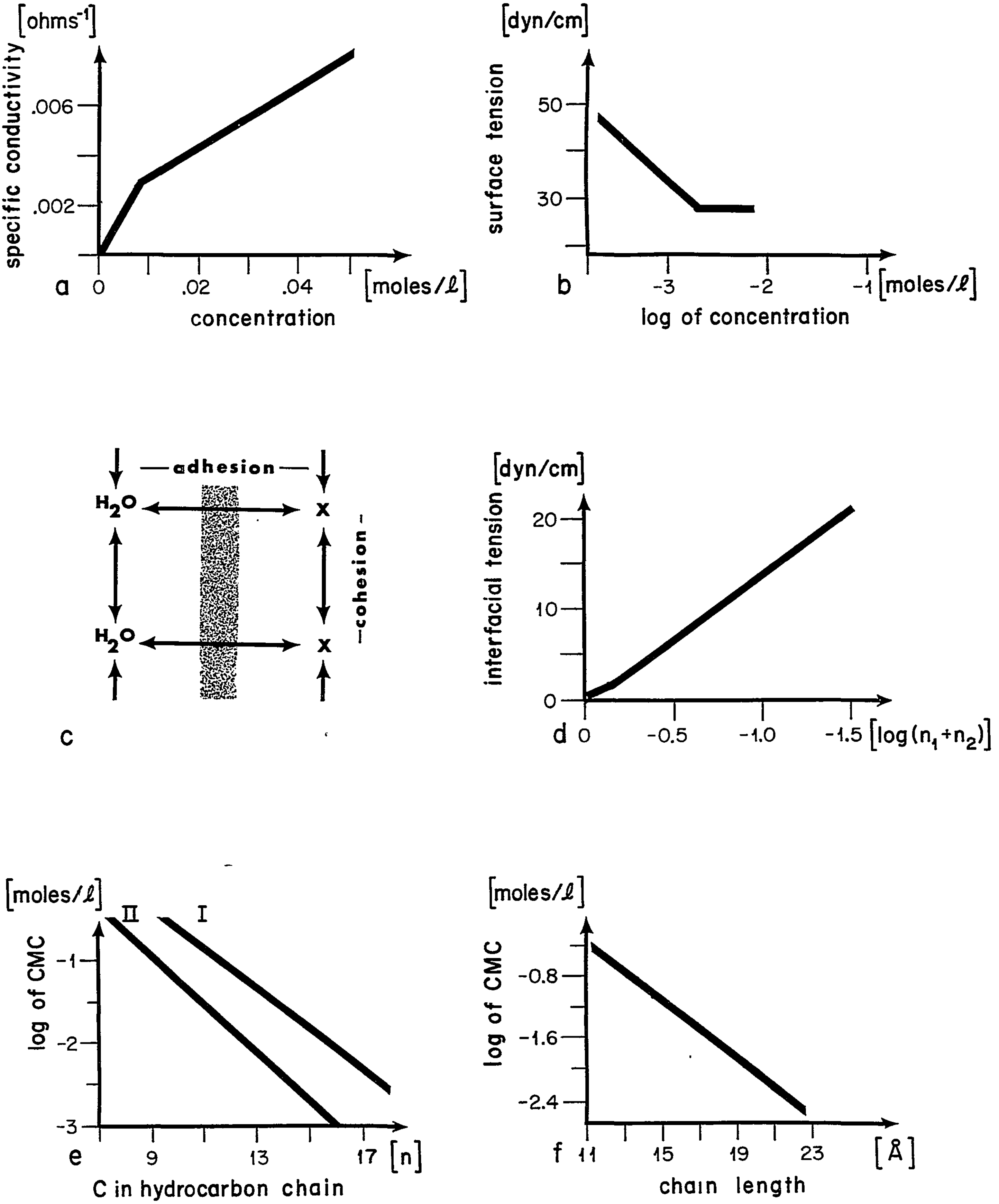

[ohms⁻¹]
specific conductivity
.006
.002
a
0
.02
.04
[moles/ℓ]
concentration
[dyn/cm]
surface tension
50
30
b
-3
-2
-1
[moles/ℓ]
log of concentration
adhesion
H₂O
X
H₂O
X
cohesion
c
[dyn/cm]
interfacial tension
20
10
0
d
0
-0.5
-1.0
-1.5
[log(n₁+n₂)]
[moles/ℓ]
log of CMC
II
I
-1
-2
-3
e
9
13
17
[n]
C in hydrocarbon chain
[moles/ℓ]
log of CMC
-0.8
-1.6
-2.4
f
11
15
19
23
[Å]
chain length

Figure 16.

FIGURE 16

Dissolution properties of organics in aqueous systems.

The micelle formation in an aqueous solution of an ionic compound is shown by the abrupt change in electrical conductance at a certain concentration (CMC) (Fig. 2) (237). The surface tension of a nonionic compound (decyl glycosides) in aqueous solution as a function of concentration at 25°C is presented in Figure b (238). Again, a sharp break at a certain concentration shows the micelle formation. Nonpolar organic compounds tend to form anisotropic cross linkages, whereas ionic compounds such as the polar heads of fatty acids form isotropic cross linkages (see Figs. 13 a and b).

The behavior of ionic and polar organics can be understood by the competitive interaction between cohesional and adhesional forces between molecules (Fig. c); the interfacial tension of water-organic liquids increases linearly with the logarithm of the degree of inmiscibility of water and organic liquids (Fig. d) (239-242). The forces shown in Figure c are the factors which determine the magnitude of interfacial tension. This relation indicates that in the case of fatty acids - where the adhesional forces are strongly oriented - micelle surfaces are formed, whereas in the case of weakly polar compounds - where the cohesional forces are strong - a phase separation most likely occurs (anisotropic cross linkage).

In Figure e, the logarithm of the CMC (moles/liter of potassium mono- and dicarboxylates is shown as a function of hydrocarbon chain length at 25°C (237). The increase in one ionic group (di-carboxylate; upper curve) indicates that the adhesional forces determine the micelle formation since the aggregation to a micelle only proceeds at high concentrations. The effect of chain length of paraffins and paraffin derivatives in A on CMC is shown in Figure f (243-246). Each additional carbon atom in the carbon diminishes the CMC by a factor of approximately 2 (246). This shows - as is expected - that the cohesional forces increase with increase in chain length.

(Fig. 17a) and the pH of the solution (Fig. 17b). Furthermore, the micelle radius increases with temperature (Fig. 17c) in contrast to ionic micelles whose weight is lowered with increase in temperature (Fig. 17d). The presence of metal ions support the cross linkage, i.e. metal ions lower the cloud temperature (Fig. 17 b and e); the same implies for organic additives (Fig. 17f)

These processes find their interpretation by considering the action of metal ions in the interface layer (Fig. 13e) or the action of organic additives (Fig. 13f) upon the structural order of molecules within the micelle (Fig. 15 and Fig. 16c). The same can be implied for metal ions and oo-solvents for the micelles formed by ionic molecules (Fig. 17a and b); an enhancement of the structural order will be achieved this way.

On the basis of the here presented characteristics, the general rules can be summarized as follows:

1. Micelles which are composed of ionic materials will constitute a co-existent phase system with the aqueous media, because the stability of micelles is principally determined by the ionic boundary layer. In contrast
2. Polar micelles such as peptides, which basically represent retarded liquid eutectica, constitute potential nucleation centers which are mainly determined by internal cohesive cross linkage.

It is logical, if we restrict ourselves in the discussion on the development of a primordial cell to the ionic micelles, because they can collect polar and neutral organic molecules. This can be accomplished by solubilization effects, i.e. these micelles act as a co-solvent for water inmiscible or water insoluble solid organic compounds. Metal ions (salinity) will excercise a particular rôle during this solubilization process, because they lower the critical value of micelle formation, whereby the suppression is a function of the metal valence (Fig. 18a); the same effect is accomplished by dissolved organic molecules (Fig. 18b). We thus have to consider the collection of organics in ionic environments a physical-chemical consequence of liquid water - organic systems. In turn, it is justified to conclude that organic compounds were collected in the form of micelles at the earth's surface during the pre-biological and low-temperature stages.

DEVELOPMENT OF GLOBULES AND COACERVATES

The development of micelles to macro-micelles (globules), and to coacervates depends on the surface boundary layer, i.e. on the kind of membrane. This is in accordance with experimental data on emulsification and foams (5, 265) which indicate that micelles equipped with anisotropic membranes are able to grow on the expense of other micelles, i.e. by consuming the other micelles via surface attachment. This process is generally known as emulsification. On thermodynamic grounds, these globules, -or to use other terms, for instance, soaps or emulsion particles - will exhibit an optimal critical diameter (266-268) which is in the order of:

$$\text{optimal critical diameter} \approx 10^3 \text{ to } 10^4 \text{ Å } (= 0.1 - 1\,\mu)$$

Measurements on emulsions confirm this range (269-286). A typical size distribution curve is shown in Figure 18e which indicates that liquid globule systems tend to approach an optimal particle diameter. The macro-cell structure of such an emulsion system is shown in Figure 18f. On the basis of theoretical considerations it follows that the structural order of this cell aggregate is determined by the packing of the cells and not by diffusion

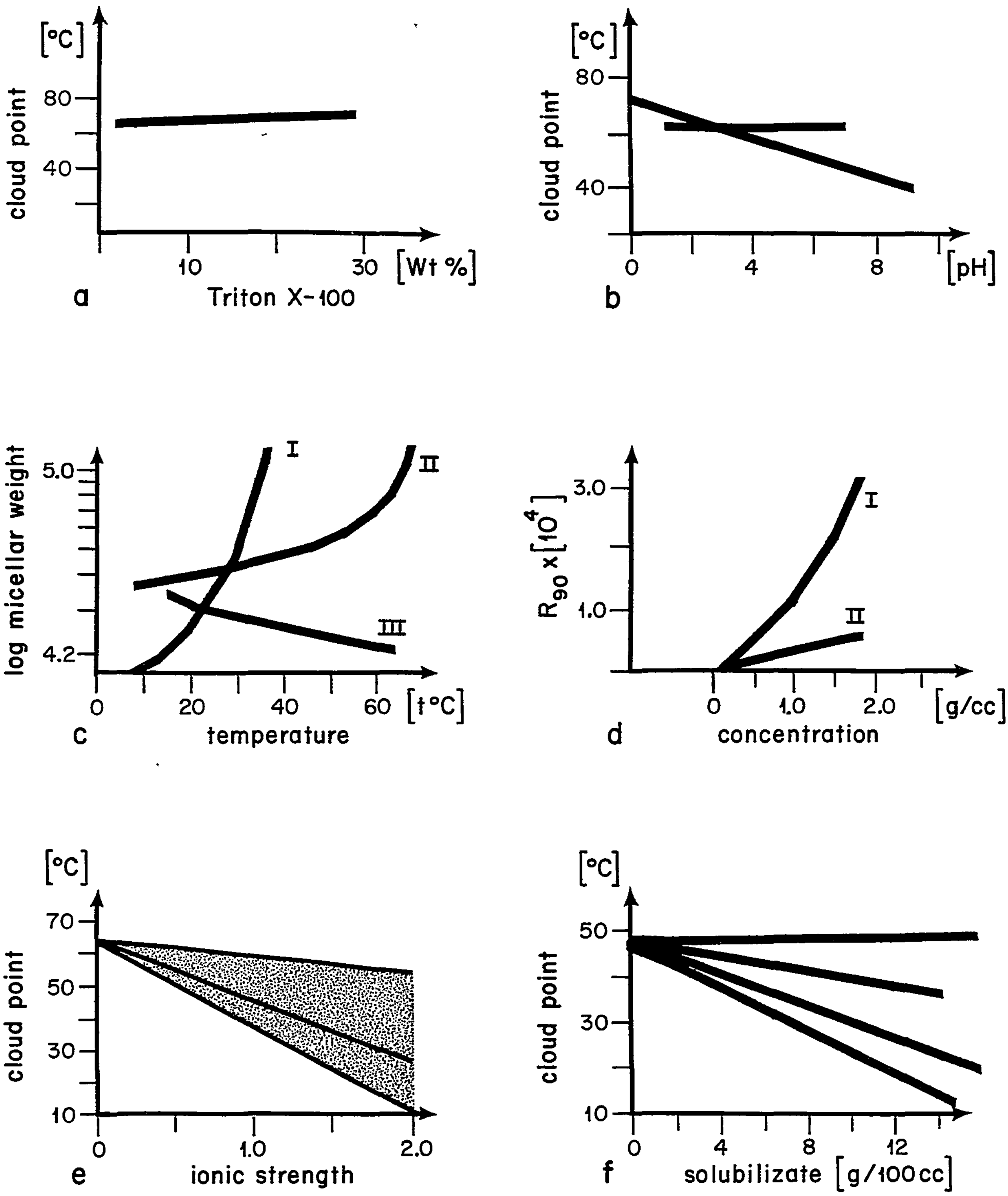

[°C]
cloud point
80
40
10
30
[Wt %]
a
Triton X-100
[°C]
cloud point
80
40
0
4
8
[pH]
b
log micellar weight
5.0
4.2
I
II
III
0
20
40
60
[t °C]
c
temperature
$R_{90} \times [10^4]$
3.0
1.0
I
II
0
1.0
2.0
[g/cc]
d
concentration
[°C]
cloud point
70
50
30
10
0
1.0
2.0
e
ionic strength
[°C]
cloud point
50
30
10
0
4
8
12
f
solubilizate [g/100cc]

Figure 17.

FIGURE 17

Brief presentation of physical-chemical basis of micelle formation.

1. Non-polar systems.

For instance, CCl_4 and water mixture; separation will take place within short periods of time; this system is thus of no interest in the context of this work.

2. Polar (non-ionic) systems.

The cloud point - where the solution starts to separate into two phases upon rise of temperature - is virtually not affected by the concentration of the solutions; the case of Triton X-100 is shown in Figure a (248, 249).

pH does not affect the cloud point; an unbuffred 2% Triton X-100 solution upon addition of HCl does not change the clouding temperature (Fig. b, horizontal curve) (248), whereas the increase in total electrolyte concentration (Fig. b, sloped curve) represents a significant variable. This can be explained by the competition:

$$H^+ \rightleftharpoons Me^+$$

i.e., H^+ and H_3O^+ ions are introduced into the structure determined by the metal ions.

The difference between the effect of temperature on the micellar weight of ionic and nonionic surface active agents is shown in Figure c. The relationships for methoxydodecaoxyethylene decyl ether, $C_{10}H_{21}O\ (CH_2CH_2O)_{12}CH_3$ (curve I), and methoxypolyoxyethylene octanoate, $C_7H_{15}COO\ (CH_2CH_2O)_{7.6}CH_3$ (curve II) in water are presented in Figure c. (250, 251). This increase in micelle weight with a rise in temperature contrasts the observation made for ionic micelles whose weight drops with increasing temperature (e.g. sodium dodecyl sulfate in 0.1 N NaCl (curve III, Figure c) (252). The temperature dependence of micellar weight measured by light scattering (256) for methoxydodecaoxyethylene decyl ether at 75°C (curve I) and 9.7°C (curve II) as a function of R_{90} and concentration is shown in Figure d.

The cloud point is depressed in case electrolytes are added; in Figure e, Na_2SO_4 (lower curve), Li_2SO_4 (central curve), and $AlCl_3$ (upper curve) were added to a 2 percent Triton X-100 solution (see also Fig. 17b) (248); the curve for NaCl and KCl follows approximately that of Li_2SO_4. This effect - known as salting out - can be regarded as metal bridge formation between the micelles; and the strongly hydrated multivalent cations are hereby less effective than monovalent cations which are not so strongly hydrated.

The addition of solubilizates will also affect the cloud point of solutions (253-255), for instance, that of polyoxyethylene dodecyl ether, $C_{12}H_{25}O(CH_2CH_2O)_6H$, (422 g/100cc) Fig. 17f). The depression is rather remarkable for aliphatic alcohols or fatty acids (lower curves), whereas hydrocarbons have very little effect (upper curve). The depression of the cloud point is related to the fact that forces within the micelle entrance are cohesions, and a subsequent separation takes place.

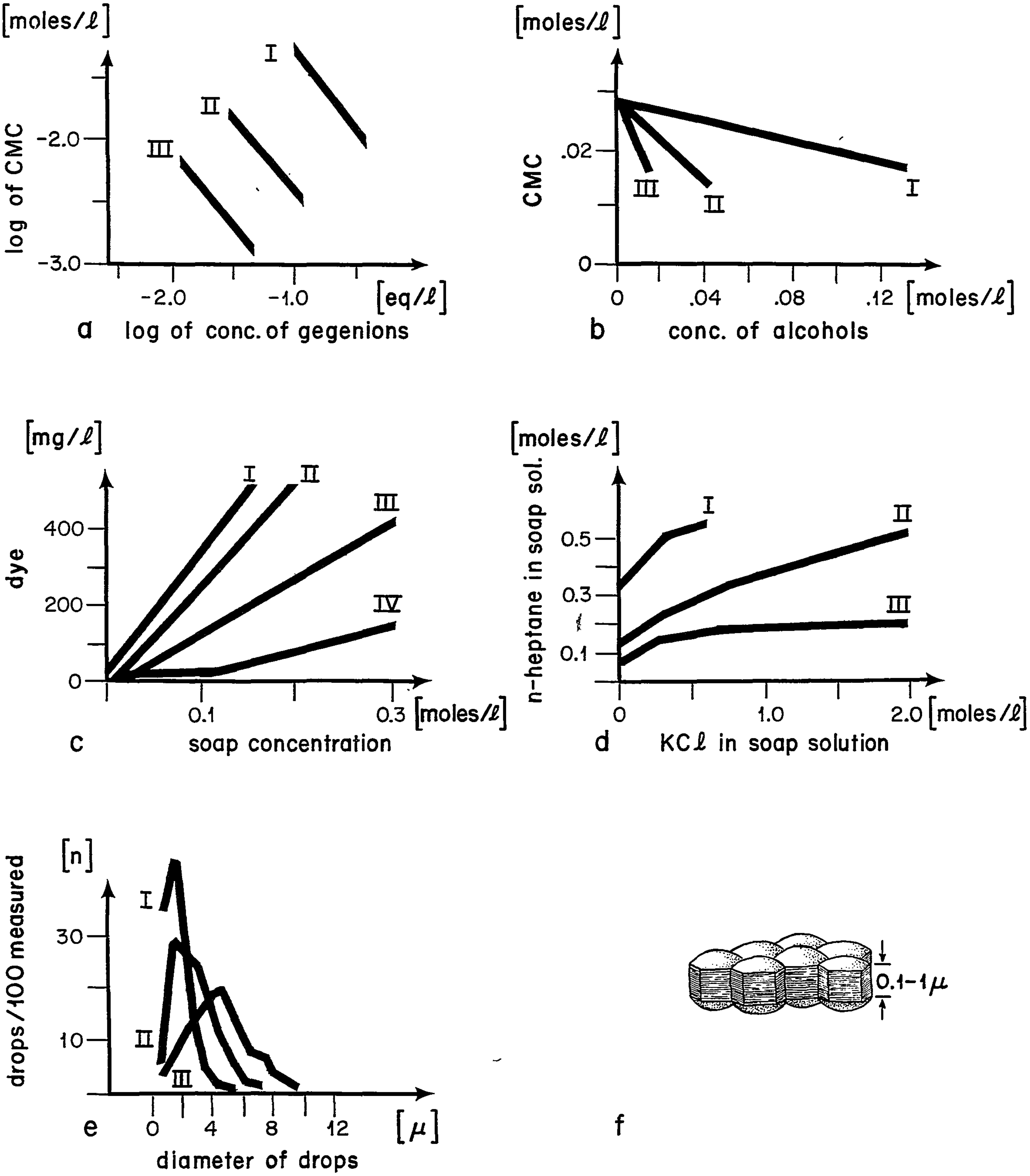

[moles/ℓ]
log of CMC
-2.0
-3.0
I
II
III
-2.0
-1.0
[eq/ℓ]
a
log of conc. of gegenions
[moles/ℓ]
CMC
.02
0
III
II
I
0
.04
.08
.12
[moles/ℓ]
b
conc. of alcohols
[mg/ℓ]
dye
400
200
0
I
II
III
IV
0.1
0.3
[moles/ℓ]
c
soap concentration
[moles/ℓ]
n-heptane in soap sol.
0.5
0.3
0.1
I
II
III
0
1.0
2.0
[moles/ℓ]
d
KCℓ in soap solution
[n]
drops/100 measured
30
10
I
II
III
0
4
8
12
[μ]
e
diameter of drops
0.1-1μ
f

Figure 18.

FIGURE 18

The logarithm of the CMC is a linear function of the logarithm of the concentration of gegenions of $R_{12; 14; 16}CH(COOK)_2$ as is shown in Figure a (I-III) (261).

The effect of isopentanol (I), hexanol (II), and heptanol (III) on the CMC of K-dodecanoate at 10°C is presented in Figure b (258-260).

Solubilization of dimethylaminoazobenzene (DMBA) in fatty acid soap solutions at 30°C (Fig. c); K-oleate (I); K-myristate (II); K-laurate (III); and K-caprate (IV) (262).

Effect of added KCl on solubility of n-heptane in 0.25 M K-tetradecanoate plus 0.301 (I), 0.064 (II), and 0 mole octanol-1 per liter, respectively (Fig. d) (246).

Change in the number of drops as a function of ageing for 1/200 M soaps (caesium oleate and octane); 1st day (I), 3rd day (II), 7th day (III) (Fig. e) (257). The size of the drops and the area decreases, whereas the amount of soap per unit area increases with time. Similar relationships are established for sodium oleate and octane emulsions. The macro-cell structure of an emulsion system is shown in Figure f.

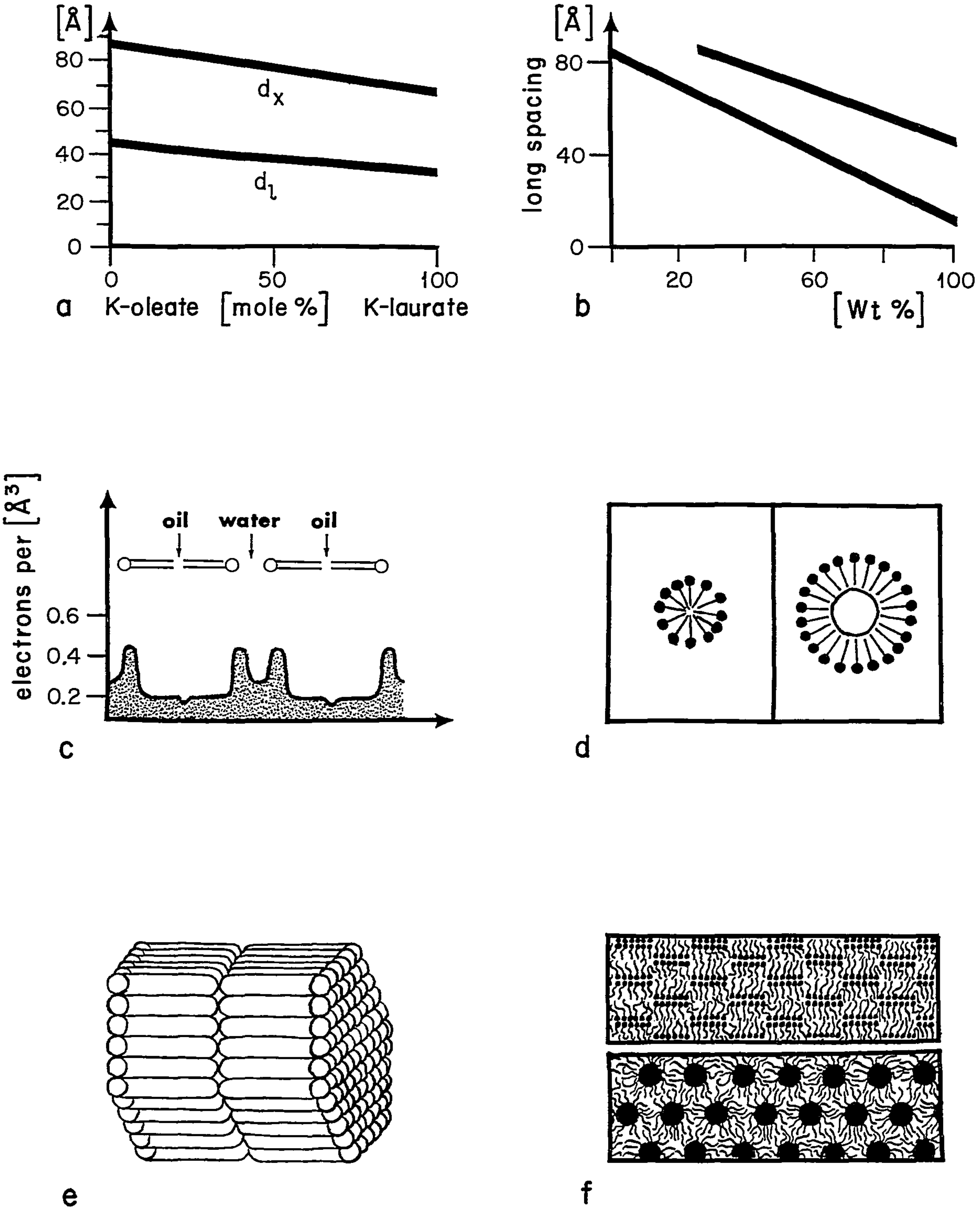

[Å]
80
60
40
20
0
d_x
d_l
0
50
100
a
K-oleate [mole %] K-laurate
[Å]
long spacing
80
40
0
20
60
100
b
[Wt %]
electrons per [Å^3]
oil
water
oil
0.6
0.4
0.2
c
d
e
f

Figure 19.

FIGURE 19

X-ray diffraction studies (295-300) of mixed crystals; the diffraction maxima of the K-oleate/K-laurate mixture does not show X-ray diffraction maxima of the pure (individual) substances but intermediate maxima for the interplanar distances d_1 and d_x (Fig. a) (298).

The relationship of spacing as a function of concentration of an aqueous solution of Detergent 'X' (lower curve), and Emulphor 'O' (upper curve) (Fig. b).

The variation of electron density within a lamellar micelle of potassium laurate (295, 296). The highest electron density is located at the ionar group ($-CO_2K$); the water is positioned in the central valley. Should a hydrocarbon become solubilized, it is localized at the lowest electron density point (Fig. c).

The expansion of a micelle, based on X-ray data of an 18% K-laurate solution in water before and after benzene was added (Fig. d). The benzene is included between the ends of the hydrocarbon chains of a lamellar micelle.

Structure of lamellar micelle (Fig. e) (299). Schematic structure of some liquid-crystalline phase of anhydrous soaps (Fig. f). The polar groups (alkaline soaps) form ribbons (upper Fig.); the hexagonal phase of divalent soaps (lower Fig.); the polar groups are localized in the cylinders which are viewed along their long axis (300).

GLOBULE DYNAMICS

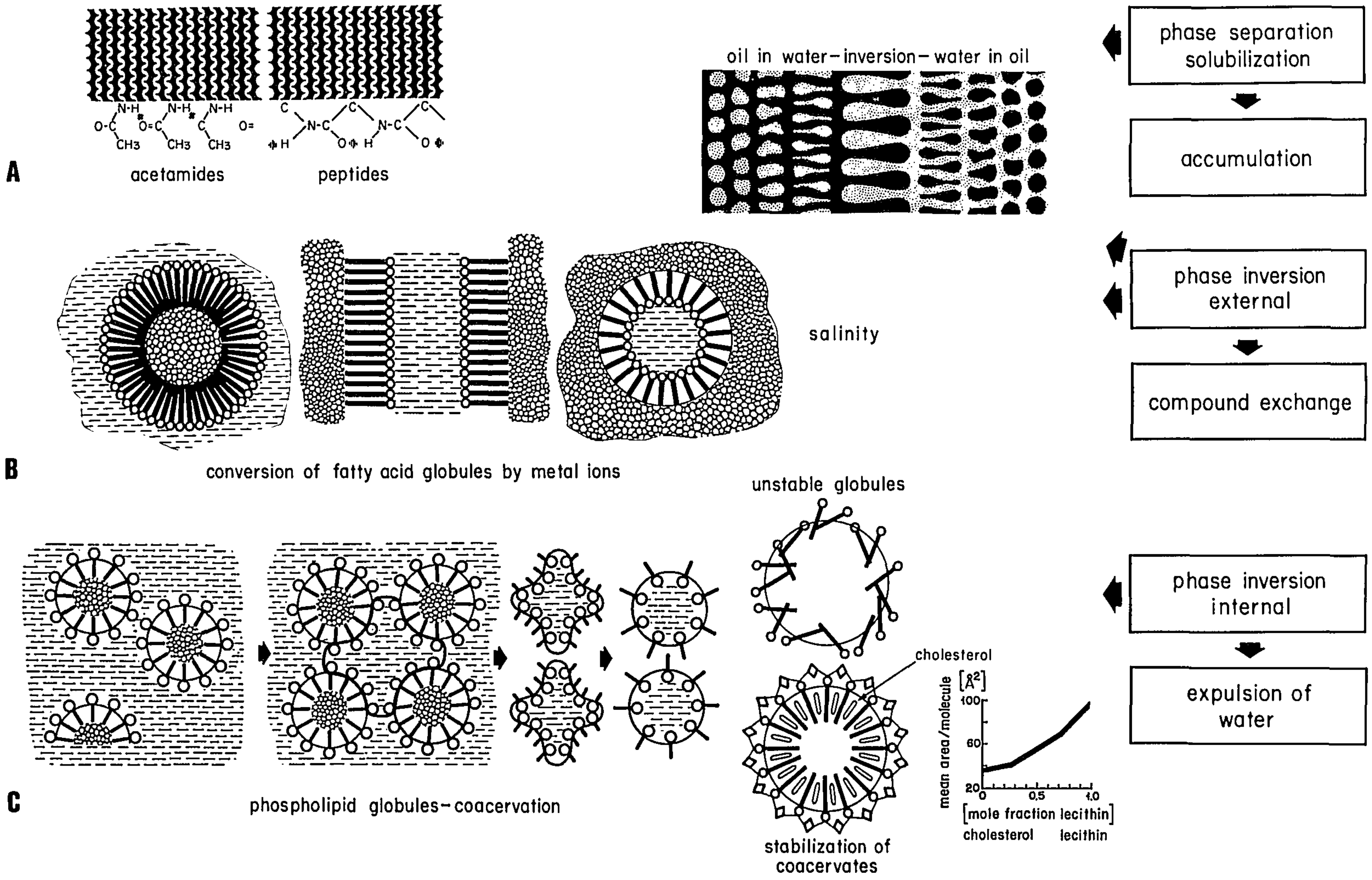


Figure 20..

because the ordering process proceeds at a faster rate than could be achieved by diffusion processes (287).

X-ray diffraction data confirm the experimentally inferred condition of stable emulsion systems such as the existence of an anisotropic boundary layer (288-294), i.e. a charged interface having structural order (e.g. orientation of paraffin molecules) (Fig. 19). This Figure illustrates that hydrophobic organics are collected at the side of the paraffins; this has also been observed in the classic experiments (301, 302). The main requirement for this water-organic system is the presence of an anisotropic and charged boundary layer. Many articles (303-311) have emphasized the need of an ionic fabric for the existence of stable colloids.

The metamorphosis of a regular micelle (Fig. 19e and f) into an emulsion micelle (Fig. 18f) - which we assume to have taken place during the first developmental stages at the earth's surface - is compatible with the thermodynamic deduction that the water vapor increases with the decreases in the diameter of a droplet. Thus if time is abundant, the larger cells will grow on the expense of the smaller ones as a function of this vapor pressure dependence.

On the basis of the previous discussion on the relations between liquid phases, it is possible to outline the development of coacervates. A summary on the formation of globules (emulsion cells) is shown in Figure 20. The concept on the kind of primordial boundaries frequently mentioned in the literature, and which is also shared by Oparin (3), is illustrated on the upper left of Figure 20; hydrophobic chains of paraffins become separated from the water, and boundary layers are formed, whereby the hydrophobic stacks are transformed into membranes by the uptake of polar films such as acetamide (R-NH-CO-CH_3) (330).

or peptides. It is obvious that this kind of association is out of question for the origin of life because it will not lead itself to stable membrane systems. Analogously, stable soap cells formed by fatty acids (Fig. 20B) and metal ions do geologically represent rather instable fabrics which – as a function of metal ion concentration – can easily invert and by this eject the cell content (269, 314–322). The influence of metal ions (293 and 324) is graphically presented in Figure 21.

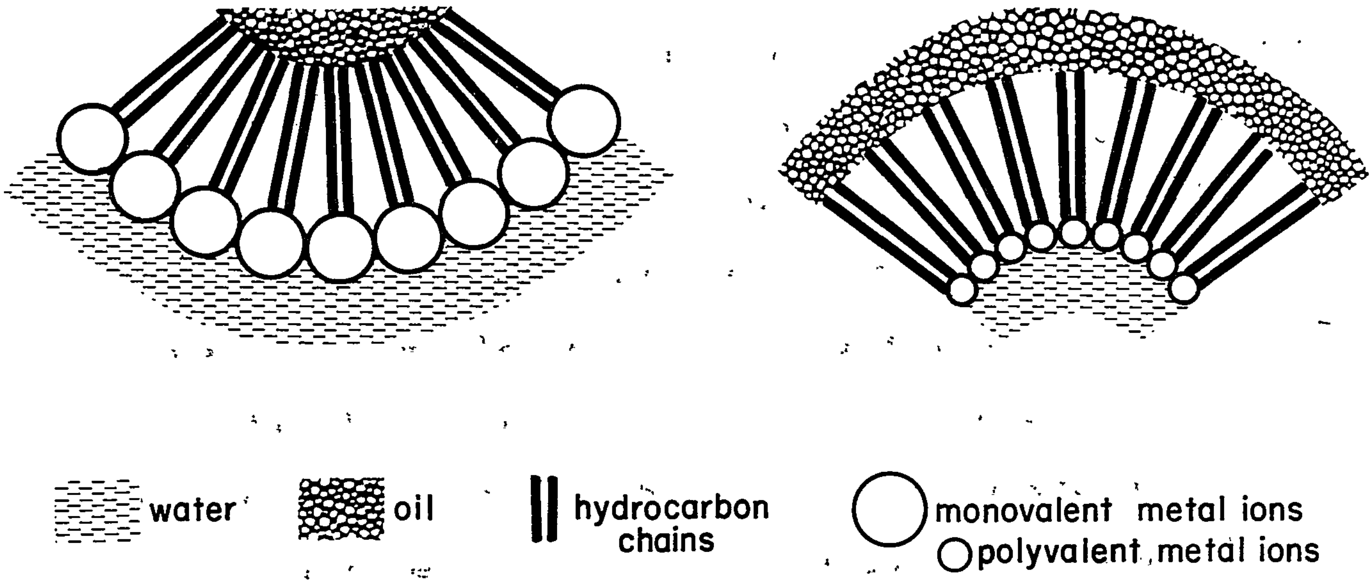


FIGURE 21

As a function of ionic radius and valence, a contraction of ionic boundary layers (hydration sphere) will come into existence, so that polyvalent metals form water-in-oil emulsions. In the presence of phospholipids as boundary material this reaction will not take place. As shown in Figure 10 and 11, PO_4 tetrahedra and metal ions will construct stable fabrics which remarkably contrast the expandable hydration layer at the ionar "heads" of fatty acids.

The developmental stages of globules which were separated from the environment by means of anisotropic membranes composed of phospholipids, do exhibit an evolutionary trend which was only indirectly influenced by environmental parameters. Such a metamorphosis – the coacervation – is shown in Figure 20 C; the water becomes expelled by the joining of phospholipid micelles. The resulting capability of phase transformation represents the most remarkable consequence of coacervation. Namely, subsequent to the concentration process, this coacervate system will not maintain a stable equilibrium, but successive alterations will take place such as the internally caused phase inversion (Fig. 20C). Water droplets can become enclosed by membranes and the original micelle or globule content can become exchanged. These aforementioned processes are based on the fact that phospholipid membranes can be condensed to a rigid membrane layer by the addition of, for instance, cholesterol (325, 326) (Fig. 20) or metal ions (Fig. 13 e and f); and the expulsion of water proceeds during the inter-cellular attachment by virtue of oxygen co-ordinated metal bridges. With the assistance of the ionic fabric closely attached to the coacervate surface it is possible to precipitate the organic compounds dissolved in the surrounding environment such as peptides, carbohydrates or nucleotides (Fig. 17). Their subsequent consumation via internal phase inversion can take place in a similar fashion as is illustrated in Figure 20 C. In biochemistry, this step is known as "complexing" and has been discussed in the chapter on micelle formation. Thus, there is no problem for the assumption that coacervates, enriched in certain organics, could come into existence.

A major point, which concerns the physical foundation for the development of the metabolism is mentioned, i.e. the formation of the bilayer membrane type, and the herewith connected variable topology of boundary layers. The way bileaflets originate is schematically drawn in Figure 22. A double layer membrane is only weakly bounded in the center because only one cross link per chain is established, whereas in perpendicular direction more than 10 bonds interconnect the layers (Fig. 23a). In turn, the individual membrane layers could glide upon each other and produce pouches (Fig. 23b). These pouches can be produced by certain metal ions or by attached proteins; this has its analogy in the well-known structure of mitochondria and is in accordance with the properties of phospholipids (328-331), for instance, their polymorphism. This gliding effect of an individual layer has considerable consequences; hence different micro-reaction places come into existence through the subdivision of the coacervate into chambers (Fig. 23c and d). This will introduce fluctuating concentration profiles in case a reaction is accomplished. An example is presented to illustrate this phenomenon:

A chemical reaction starts at X and molecules a, b, c, d. . . are obtained. At Y - separated from X by a membrane pouch, - the simultaneoulsy produced molecules a, b, c, d. . . will not arrive at the same time because:

(a) their diffusion rate through the membranes is different, or
(b) they have to bypass the membrane to come to Y.

The implications are obvious; a micro-chromatographic pattern will be obtained and well-defined and ordered molecular and ionic sequences will arrive at certain spots within the coacervate. As a consequence, a well-developed concentration profile for the whole cell will come into existence, because the chromatographic pattern within the cell will differ at any place.

This physical state is not far away from a coupling of the consecutive reactions. It is thus implied that a primitive metabolism, self-propelled, -regulated and -controlled, will come into existence. Mitochondria may represent such a case; judged by the conservative nature of the structure of mitochondria it appears that the mitochondrial system still carries traces of its abiotic origin. The development of such a self-controlled reaction system agrees with thermodynamic conclusions. It has been shown that a stable cyclic process is possible in the vicinity of a stationary state and which may operate repeatedly an infinite number of times without ever passing through the stationary phase itself (332). For further support we refer to permeability measurements of molecules passing through

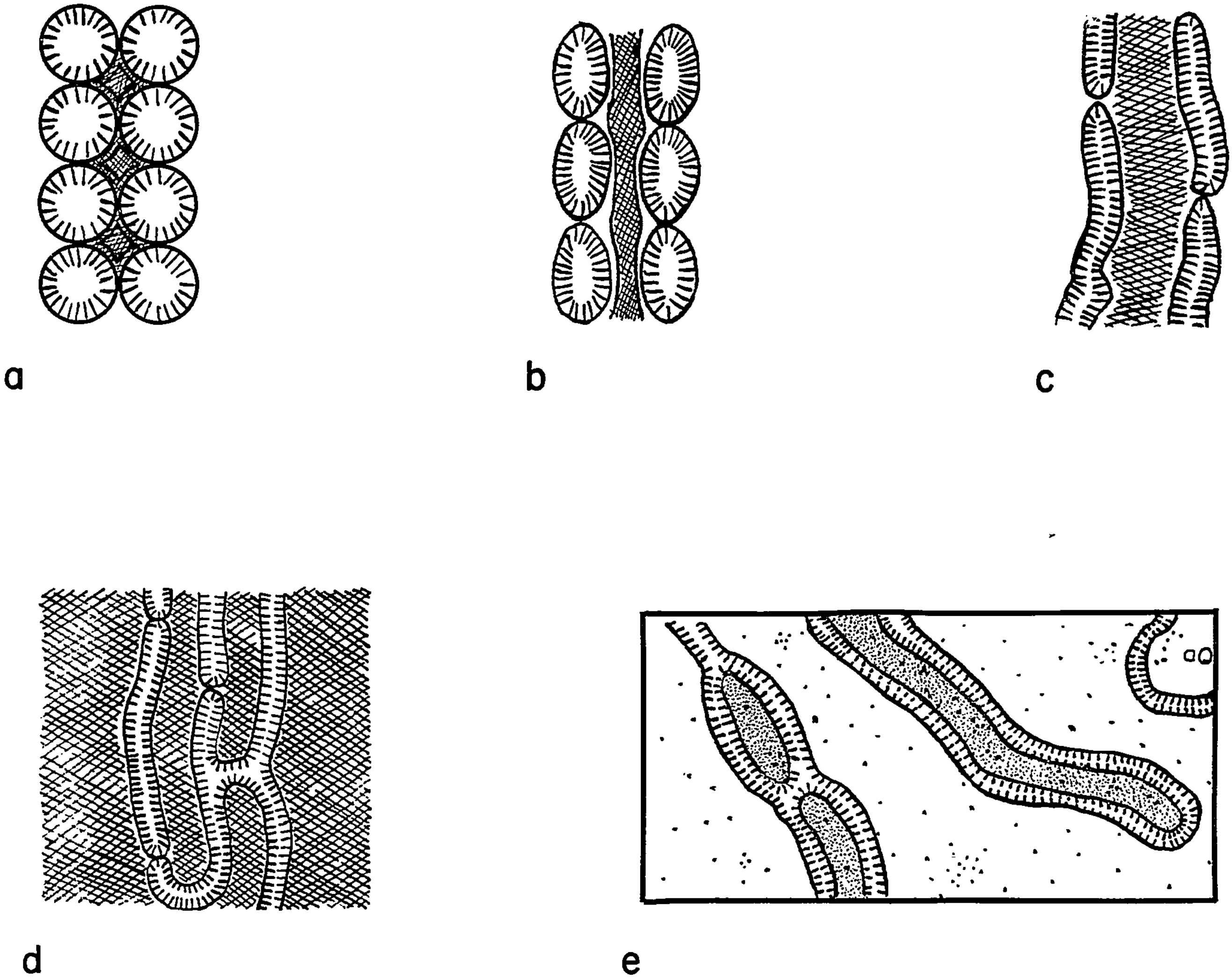


FIGURE 22

In the course of the coacervate development, structures will come into existence by its own which are enclosed by a double phospholipid "skin." Such a development is shown in Figures a–c. The most likely reason for this transformation is the change in the kind of metal ions. Alternatively, hydrophobic forces may combine hydrocarbons in the form of double layers, should these coacervates be exposed to aqueous conditions. In case double layers are formed, i.e. phosphate groups are oriented towards the aqueous phase, these double layers may also combine to multi-layered stacks. The formation of a two-fold double stack is shown in Figures d and f. The creation of such stacks will be enhanced in presence of polar-ionic compounds such as peptides. It is known that phosphates can precipitate proteins dissolved in water.

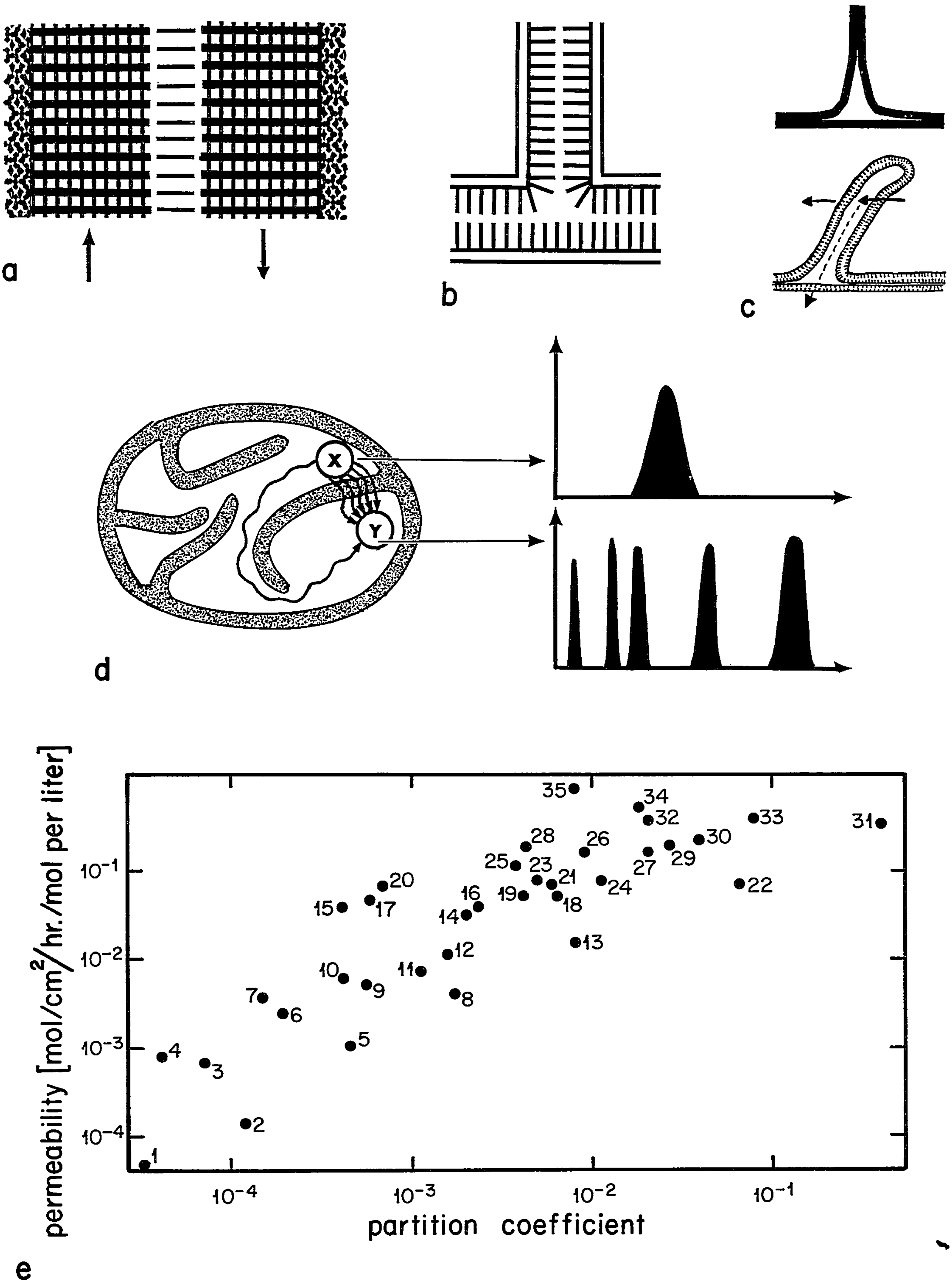

a
b
c
X
Y
d
permeability [mol/cm²/hr./mol per liter]
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-4}
10^{-3}
10^{-2}
10^{-1}
partition coefficient
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
e

Figure 23.

FIGURE 23

Schematic drawing of a double layer membrane to show

(a) the bonds that interconnect the layers (Fig. a), and
(b) the way membrane pouches can come into existence (Fig. b).

This will result in the formation of multi-chamber coacervates which structurally bear a striking resemblance to mitochondria (Figs. c and d). Fluctuating concentration profiles are shown to the right of Figure d (chromatographic pattern). The permeability values for a number of biochemical compounds through Chara cells (334) are graphically represented in Figure e. The rate at which a substance penetrates through the cell walls depends on its solubility in lipids and the size of the molecule. For compounds with equal solubility in lipids, the smaller molecules penetrate at a faster rate. The numbers in Figure e refer to the following compounds listed in order of increasing permeability:

1. Erythritol
2. Malonamide
3. Glycerol
4. Methylolurea
5. Dicyandiamide
6. Urothropin
7. Urea
8. Diethyl malonamide
9. Lactamide
10. Methylurea
11. Thiourea
12. Ethylurea
13. Monacetin
14. Dimethylurea
15. Glycol
16. Glycerol methyl ether
17. Acetamide
18. Diethylurea
19. Succinimide
20. Formamide
21. Glycerol ethyl ether
22. Diacetin
23. Propylene glycol
24. Monochlorhydrin
25. Propionamide
26. Butyramide
27. i-Valeramide
28. Cyanamide
29. Antipyrin
30. Trimethyl citrate
31. Triethyl citrate
32. Urethylan
33. Urethane
34. Ethyl alcohol
35. Methyl alcohol

The corresponding permeability values for arabinose are 3.1×10^{-4}, and for glucose, lactose, and maltose smaller than 3×10^{-5}.

membranes; cations diffuse less rapid than anions (333); and each individual compound has a distinct permeability value (334) (Fig. 23e).

This concept on the origin of the primordial cell in marine surface sediments finds support by a comparison of the distribution profiles of ions in extra-cellular fluids of various organisms (335-341) and sea water (342) (Fig. 24). Lower organized marine forms live in stationary exchange with sea water which suggests that initially the living cells could not chose or discriminate against particular ions to optimize a given biochemical function; they simply picked up the ions in the water as they were offered to them by the sea. Organisms on the other hand had to accept that ion "climate" and in turn were in equilibrium with the surrounding environment. Discrimination against ions, and enrichment of others for the purpose of a more efficient biochemical operation was only achieved later in the course of evolution (Fig. 24). The development of the organisms is thus also reflected in their ion budget.

ORIGIN OF PRIMORDIAL METABOLISM

A comparison of the two homologous elements, nitrogen and phosphorus, reveals that nitrogen functions only as a static element, whereas phosphorus represents a dynamic element in cell activities (1). This statement finds support, for :

(1) only PO_4 groups but not NH_4 groups are released during the biosynthesis of macromolecules;
(2) free PO_4 groups interact with membrane surfaces; and the
(3) membrane surfaces are able to respond to PO_4 with the establishment of different oxygen polyhedra geometries; and in turn the
(4) different geometries will affect the permeability properties of a membrane; and furthermore
(5) di- and tri-phosphates are continuously regenerated in the course of metabolic processes.

In considering the reduplication mechanism by various nucleic acids, codon assignments, and a selected number of enzymes it is implied that a sort of "metabolism" must have been functionally alive prior to the formation of the genetic transcription apparatus, because this apparatus requires a functionally well-operating metabolism. By taking

(a) the facts mentioned under 1 to 5 into consideration, and
(b) the polymerization experiments involving biochemical monomers in presence of polyphosphoric acid (345-348, 323, 312, 313),

we conclude that the metabolism of a coacervate was most likely maintained via a reversible phosphorylation cycle. In consequence, the origin of metabolism is in no way linked to the development of the genetic transcription apparatus; it must be considered an independent formation process.

For this reason, the abiotic origin of, for instance, phosphorylation, must be considered an equally important step towards the creation of a primordial cell, as is the development of the genetic code. The problem, therefore, centers around the question of how to polymerize the common mono-phosphates into , di-, tri, or tetra-phosphates, since polyphosphates are unstable in natural aqueous environments (212). The high-temperature synthesis can be excluded because in low-temperature aqueous environments such compounds would hydrolyze again. The only reasonable choice left is to place the polymerization event into the coacervates; indeed, empirical data on polyphosphate formation support this inference (1, 349-352).

It has been shown that aqueous solutions of $Na_3H_2P_3O_{10}$ and containing acetone will promote the formation of tetra- and penta-phosphates if kept at room temperature for a few days (358, 359). The influence of:

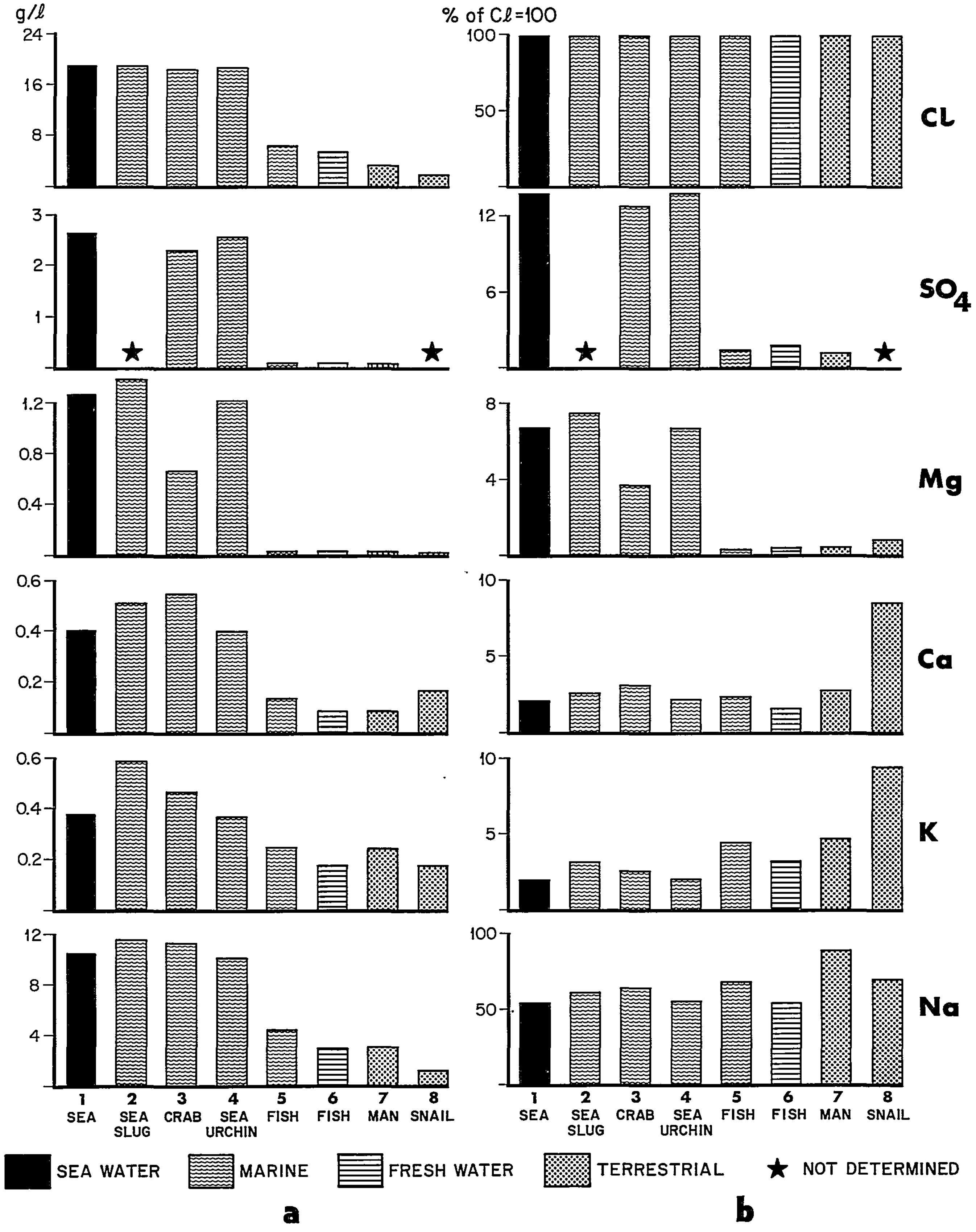

g/ℓ
24
16
8
3
2
1
1.2
0.8
0.4
0.6
0.4
0.2
0.6
0.4
0.2
12
8
4
% of Cℓ=100
100
50
12
6
8
4
10
5
10
5
100
50
CL
SO4
Mg
Ca
K
Na
1 SEA
2 SEA SLUG
3 CRAB
4 SEA URCHIN
5 FISH
6 FISH
7 MAN
8 SNAIL
SEA WATER
MARINE
FRESH WATER
TERRESTRIAL
★ NOT DETERMINED
a
b

Figure 24.

FIGURE 24

Distribution of principal ions in body fluids of selected marine, fresh-water and terrestrial organisms; the ionic composition of sea water is included for comparison. The data are reported in g/l (Fig. a), and in percent of chlorine equal 100 (Fig. b).

Marine invertebrates, in particular the sea urchin, show a striking resemblance, both in absolute and relative terms, to sea water. This phenomenon is a result of an effective regulatory and exchange mechanism along membranes; the ambulacral system of echinodermata appears to be especially efficacious. In contrast, the blood of a marine vertebrate (No. 5) is distinctively different from sea water, both in total concentration and the relative abundance of ions, except for the sodium-chlorine relationships (Fig. b). This pattern in marine fish is quite similar to that in fresh-water fish and man. It is implied that (a) relatively early in the history of evolution organisms learned to discriminate against magnesium and sulfate, whereas potassium and to a lesser degree calcium became relatively enriched, and (b) that in the course of evolution this pattern was handed over conservatively from one species to the other. One should mention that for potassium the degree of enrichment is even more remarkable in cellular fluids (337). This phenomenon is most likely related to the fact that potassium is required for muscle activities. Similarily, the high abundance of calcium in a terrestrial pulmonate (No. 8) must be related to the need for calcium in calcification processes; such a precaution (i.e., storage) is not necessary for marine organisms because of (a) their dynamic exchange with sea water, and (b) the high calcium content in the sea.

In view of the distinct role of metal ions for various work assignments in biological systems (a) structurally (e.g., biocrystallographical order of macromolecules; mineralization processes) and (b) functionally (e.g., ATP cycle; membrane activities), a re-evaluation of the published record on the chemistry of body fluids (e.g., 337, 343, 344) and a more intensified research program in this area, appears to be rewarding. Description of sample materials:

(1) sea water (major constituents; standard chlorinity = 19 ‰) (342)
(2) *Doris tuberculata* (341)
(3) *Cancer pagurus* (335)
(4) *Echinus esculentus* (341)
(5) *Lophius piscatorius* (338)
(6) *Cyprinus carpio* (339)
(7) *Homo sapiens* (336)
(8) *Helix pomatia* (340)

* A comparison of metal ion content and negatively charge PO_4 groups in cells seems to be rather promising. This would perhaps allow to estimate to what extent metal ions have other work assignments in biological systems, for instance, with proteins.

(a) metal ions, and

(b) organic compounds

in the synthesis of ATP and ADP is well recognized (360, 361); for instance, the inorganic triphosphate formation catalyzed by myokinase and in presence of Mg^{++} and Na^{+} (362):

$$ADP + PP \rightarrow A5P + PPP$$

This experiment is rather helpful to unravel the development of the phosphate polymerization. It has also been observed that acetyl -poly-phosphate acids can be generated should H_3PO_4 be gently heated in presence of acetylchloride or acetic acid anhydride (362a). These data suggest that polyphosphates* could evolve within the coacervates, whereby the catalytic power of metal ions and polypeptides may have played a significant part. For, it is reasonable to assume that among the polypeptides synthesized on mineral templates (2) or by other means (353-355) there were those that exhibited enzyme properties. In case such catalyzing elements become incorporated into the coacervates, considerable consequences might be anticipated. Namely, due to the multi-chamber structure of coacervates (Fig. 23) significant concentration differences and orders of reaction sequences are expected. A reversible polymerization and degradation of phosphates could possibly develop as a result of the molecular and ionic flow to or from the various chambers whereby the reactions themselves are distinctively influenced by the compounds present in the chambers (348, 349, 356, 357).

We have already given some examples that the poly-phosphate formation can be accomplished in inorganic reaction media and that these processes - proceeding at room temperature - are not a privilege of biological systems (1).

---

* We mention phosphates in particular and pay less attention to the kind of ester formation such as adenosine, since it is conceivable that aside of adenosine other phosphates can play a similar rôle in certain biochemical systems. For instance, it appears that a hexa-meta-phosphate in Neurospora functions similarly to ATP (375).

The establishment of an interconnected and integrated chemical reaction pattern for the coacervate system as a whole will become operative in case a phosphorylation cycle can be maintained over prolonged periods of time without interference. This, however, requires a constant supply of organics and metal ions consumed during this development. In this manner, a certain "modus vivendi" for coacervates is accomplished.

The thermodynamics of these reactions have previously been considered. The diffusion against a concentration gradient and the question regarding the transport of compounds through membranes are in accordance with thermodynamic considerations.

Regarding:

1. the type and source of consumed compounds, and
2. the mechanics of the energy transfer,

it is most likely that chemical energy in the form of oxidizable organic compounds was the source of energy supplied from the surrounding environment to the coacervates. The main contribution was represented by organics which were chromatographically separated along silicates in sediments; they were supplied in distinct sequences to the coacervates. Such a well-ordered supply may also have stimulated and regulated the coacervate chemism. In addition, changes introduced by chemical or physical variations in the overall geological environment such as kind and abundance of metal ions, may have caused an instantaneous development.

For comparative purposes, the rather informative oxidation process of H_3PO_2, catalyzed inorganically, is presented to illustrate the mechanism of energy transfer that may have been involved in coacervate systems.

The aqueous solution of H_3PO_2 is stable in the presence of free oxygen and will not be oxidized (363). In case Fe^{+2} is present in solution, oxygen will be picked up, whereby a simulataneous oxidation of iron and H_3PO_2 proceeds (364, 365):

$$Fe^{+2} \rightarrow Fe^{+3} \text{ ; and } H_3PO_2 \rightarrow H_3PO_3$$

The reaction comes to an end as soon as all the two valent iron is oxidized, since Fe^{+3} is inactive. However, a complete inversion in the functions of iron can be observed by administering small quantities of organic acids such as dioxymaleic acid. Under these circumstances the auto-oxidation of H_3PO_2 proceeds at high speed and for many hours (365-367). The most remarkable aspect of the catalytic action of iron is the fact that the presence of traces of diphenylamine and hydroquinone will essentially inhibit the reaction (364). Should Cu^{+2} – which in itself is inactive – be added to the system, it will inhibit the action of Fe^{+2} (366, 367). Again the addition of organic acids will control the action of Cu^{+2} and the blocation of Fe^{+2} and Cu^{+2} is lifted. The controlled interrelationships are summarized in the form of an action diagram (Fig. 25) to emphasize the complexity of oxidation in a simple H_3PO_2 solution, and to point out that complicated and regulated oxidation mechanisms could have been developed in a chemically straightforward fashion by coacervate systems. The variability of chelate dynamics (368) is listed as additional support; or the iron metabolism in microorganisms (369).

In this context it is mentioned that auto-catalysis has been observed even during the oxidation of H_3PO_3. Aqueous H_3PO_3 solutions are not oxidized in presence of oxygen (370). Should metals be present, oxidation can be achieved; for instance, $AgNO_3$ will be reduced to Ag. The oxidation process for the last mentioned reaction will be significantly enhanced in case H_3PO_4 is added to the solution (371). Autocatalysis even persists at a ratio of:

$$H_3PO_3 : H_3PO_4 = 10^6 : 1$$

With regard to autocatalytic reactions known from chemistry, there is a gradual transition to processes having auto-replicating characteristics. In this case, the formation of a compound will only take place, should this compound be present in certain quantities; an example is mentioned (22). Upon addition of PCl_5 to acetic acid, we can observe:

$$2\,CH_3COOH + 2\,PCl_5 \rightarrow 2CH_3OCl + 2\,PCl_3O + 2\,HCl:$$

by administering zinc ethyl, we obtain:

$$2\,CH_3COCl + Zn(CH_3CH_2)_2 \rightarrow 2\,CH_3CH_2COCH_3 + ZnCl_2$$

and by adding oxygen, the methylethylketone will yield four acetic acid molecules:

$$2\,CH_3CH_2COCH_3 + 6O \rightarrow 4\,CH_3COOH$$

This reaction unquestionably is a self-replication, which of course is far away from the replication phenomena observed in cells. Yet, this reaction can still be regarded as an evidence that self-replicating processes represent ordinary reactions. The logic of such systems - physically considered the systems with hysteresis - is discussed in (372, 373), whereby the momentary state is a function of the previous history of:

(a) the surroundings, as well as
(b) the system itself.

Concerning the minimum requirements for self-maintenance, the following sequences of reactions are established:

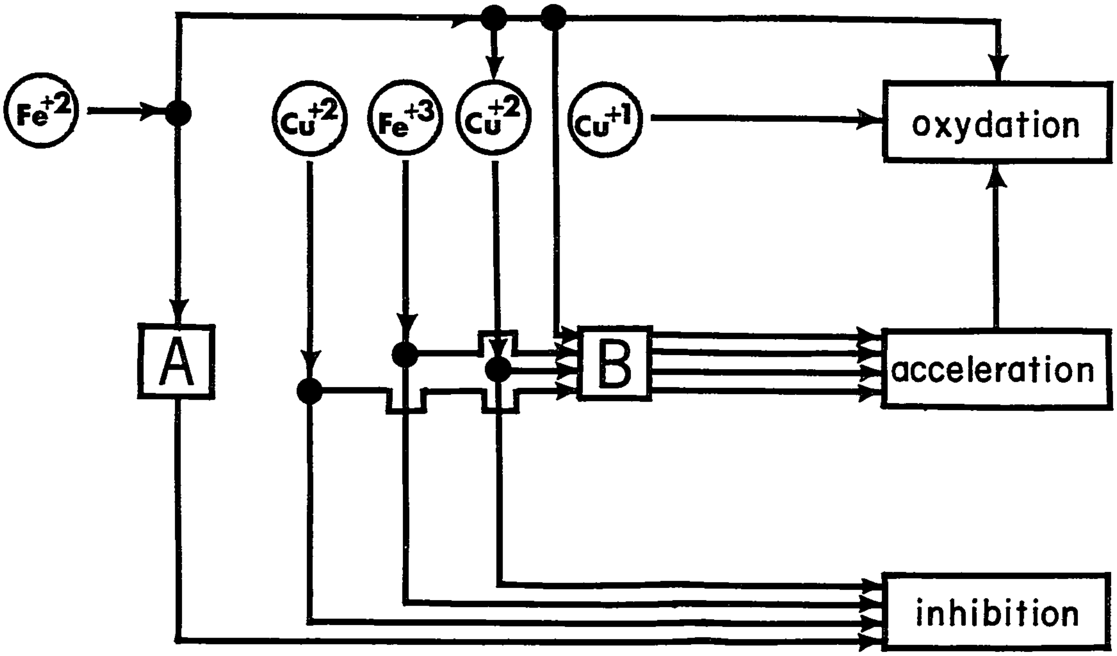
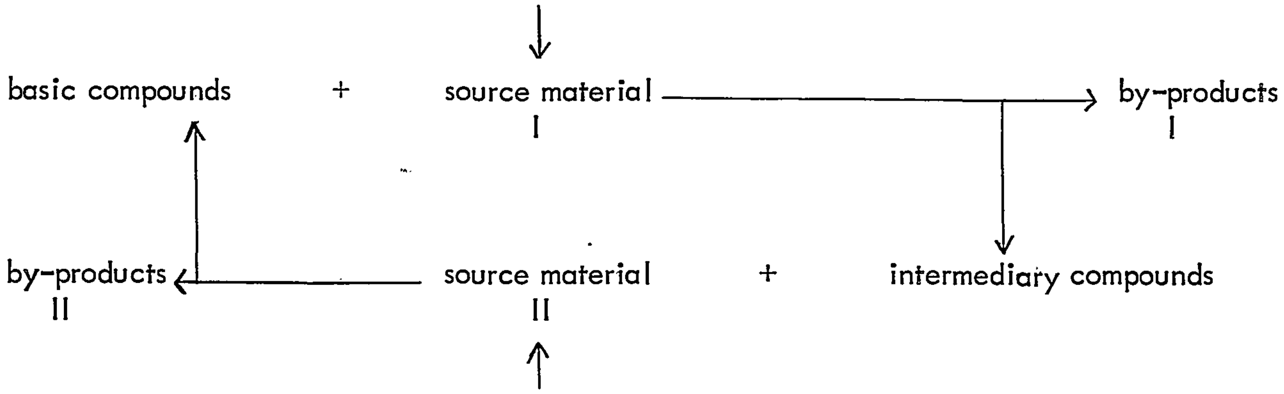


FIGURE 25

Catalytical oxidation of H_3PO_2. The action diagram should be read as follows:

Fe^{+2} catalyzes (upper line of Fe^{+2} to box); alternatively, Fe^{+2} catalysis is blocked by the addition of hydroquinone or diphenylamine (compounds A). The lower line of Fe^{+2} has to go via A to the lowest box, etc...

Compound B represents certain organic acids such as dioxymaleic thioglycolic, oxalic acids and others, which can introduce inversion of the function of, for instance, iron, etc... (363-367).



The here developed concept on the metabolism of coacervates in the form of utilization of energy released by the oxidation of organic compounds during a phosphorylation cycle, also agrees with concepts on the primitive physiology of organisms. Heterotrophic metabolism is emphasized by (374)*, who derived this conclusion through a comparison of heterotrophic and autotrophic cells. Genetic considerations lead (376) to postulate a heterotrophic metabolism.** Glycloysis, which is similar for all organisms investigated, is used (375) as evidence, to regard the anaerobic metabolism as the most primitive one. We conclude that a primitive metabolism could form in abiotic coacervates, and that this metabolism progressively improved. Its development took place independently of the evolution of the genetic code and the nucleic acids (2). By superimposing the two separately developed entities, the primordial cell came into existence. The primitive metabolism of coacervates - kept alive via phosphorylation processes - was explorated and embodied by the self-reproducing cycles. Namely, the nucleic acids could adopt the available peptides (enzymes) for their own reproduction. In turn, the physical-chemical relationships between peptides and nucleic acids were gradually transformed into an operating codon (2). The link between the two independently developed events:

(a) primitive metabolism, and
(b) genetic reproduction process

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* "The primitive metabolism of energy was entirely anaerobic and dependent on the interaction of organic substances with molecules of water" (374).

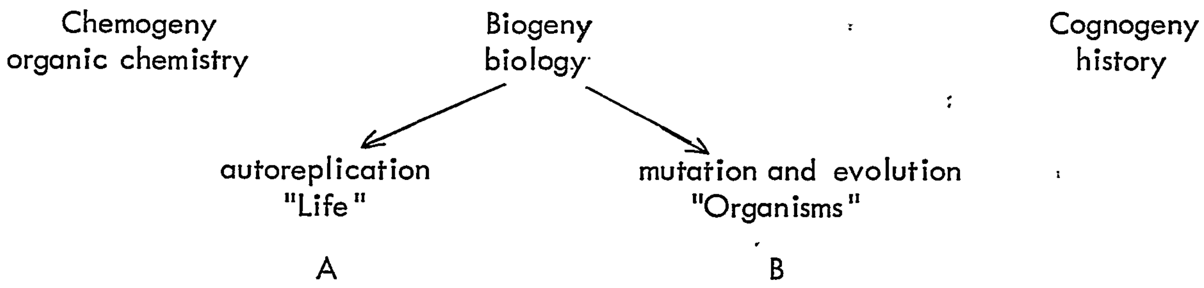
** "The first living entity was a completely heterotrophic unit, reproducing itself at the expense of prefabricated organic molecules in its environment " (376).

is represented by the peptides. They must be considered the essential tool by which the coacervate metabolism – for the purpose of the nucleic acid replication – was utilized. In the course of time, the reproduction cycle became progressively reshaped regarding the enzymatic information transfer*; so that presently even the most ancestoral organisms do not constitute an analogy for the primordial reproduction cycle. Artificial co-acervates would allow the reconstruction of the abiotic metabolism and by this throw some light as to the minimum chemical and structural conditions needed for a workable coacervate unit.

ORIGIN AND MAINTENANCE OF LIVING ENTITIES

A series of articles has been published (374–383) concerning the origin of life and which outline the minimum physical-chemical requirements for the creation and maintenance of living systems. In this chapter, we utilize recent data of molecular biology to verify, expand, or reject previous concepts on this subject.

Lederberg (377) clearly formulates an analogy of evolution; three scientific categories are distinguished:



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* In contrast, the codon assignments – once adopted for reproductive purposes by the primordial cell – remained unaltered throughout evolution.

A wealth of empirical information has been gathered on Chemogeny (3, 4, 378). Research on Biogeny - although intensive - has not yet reached conclusive results. Biogeny (attribute: A) is concerned with the processes that lead to well-ordered sequences of events (chemical), and which posess an auto-catalytic character, and which are capable to maintain themselves (autoreplication) within a given physical-chemical space and time unit*. This can be regarded as a definition of life.

An organism must be considered a part of Biogeny (attribute: B). Namely, an organism, contains - aside of the autoreplication - the attributes:

1. mutation,** and
2. evolution.

Living forms are known which over generations have remained unchanged. A living entity, thus, not necessarily includes the attribute mutation. Mutation is an independent event and can be regarded a discrete thermodynamic disorder phenomenon. In addition, the

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* "The central problem in the origin of life is to account for the origin of material combining these properties" (378);
"There is only one dominant chemical pattern of life. If more than one exists the others must be obscure, as are some of the red algae, and unnecessary to the survival of the dominant;"
"... is the chemical and structural conservatism or inertia of once established patterns of structure or activities;" and
"We may define life, for the sake of this discussion only, as the embodiment within a certain volume of self-maintaining chemical processes" (379).

** "An organism, to be called living, must be capable of both replication and mutation; such an organims will evolve into higher forms" (378) .

attribute mutation only implies alteration, but does not mention anything regarding the direction of change. For this reason we have to introduce the attribute evolution, i.e. a directed and oriented development, because a highly advanced (derived) organism will never mutate back into an ancestoral form.

The central problem of the origin of life is focussed on the minimum requirements for the formation and maintenance of a workable living system. In this context, questions regarding the controlling elements of evolution and mutation, or the mechanism of perception and computation (Cognogeny), have no significance.

We have approached this problem by trying:

(a) to understand the complex organization and maintenance network of a living cell, and
(b) to enumerate and to define the individual physical-chemical events leading to this pattern.

Starting from the axiom that life is derived from ordinary chemical reactions - as is generally accepted (374, 379-386)*- we make the heuristic assumption that an autoreplicating system is an outgrowth of its environment and thus can be described - and only be described - in connection (co-ordination) with its environment. Concerning the question of living and non-living forms, we agree with (388) that such a discussion represents an unfruitful scientific "Scheinproblem." Whether or not an isolated chromosome or nucleus is alive cannot be answered clearly as was stated (389). The question exists regarding the physical meaning, i.e. the interpretation of the copying process as well as the autonomous nature of living entities.

The minimum requirements for an autoreplication system are shown in Figure 26. They can be grouped into the following categories:

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* This supposition is questioned in (387).

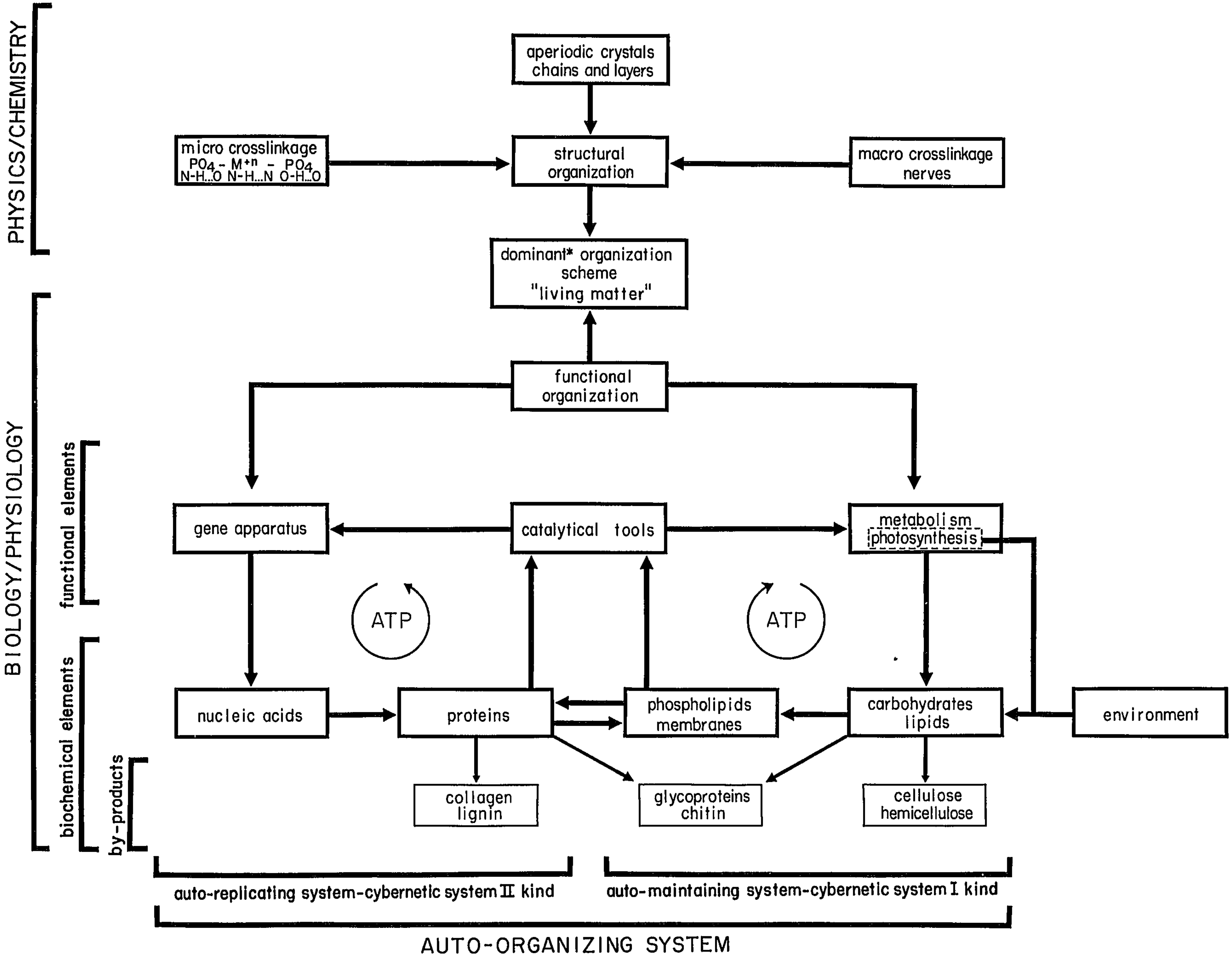
PHYSICS/CHEMISTRY
aperiodic crystals chains and layers
micro crosslinkage PO4 - M+n - PO4 N-H...O N-H...N O-H...O
structural organization
macro crosslinkage nerves
dominant* organization scheme "living matter"
BIOLOGY/PHYSIOLOGY
functional organization
functional elements
gene apparatus
catalytical tools
metabolism photosynthesis
ATP
ATP
biochemical elements
nucleic acids
proteins
phospholipids membranes
carbohydrates lipids
environment
by-products
collagen lignin
glycoproteins chitin
cellulose hemicellulose
auto-replicating system-cybernetic system II kind
auto-maintaining system-cybernetic system I kind
AUTO-ORGANIZING SYSTEM

Figure 26.

(a) structural organization, and
(b) functional organization.

The structural organization reflects the physics and chemistry as a carrier of the system. The functional organization is represented by two feed-back controlled systems:

(c) metabolism, and
(d) genetic translation apparatus,

both of which are well integrated.

This scheme summarizes the concepts which we regard as essentials for the origin and maintenance of life and which conform with the viewpoints of other authors. The discussion of the phenomenon of "aperiodic crystals" and their significance for the structure of macromolecules has been pointed out (390). The importance of hydrogen bonds in biopolymers has been shown (391). The significance of the phosphate/metal ion co-ordination for the structure arrangement in cellular compounds has been treated (1). These facts are the molecular-structural basis on which the auto-catalytic reproduction cycle is built upon. The principles of the structural organization can be described and comprehended by physical-chemical laws and thus have no speculative character.

More difficult are questions concerned with the constitution of a self-replicating entity and the resulting autonomy of biological forms. In agreement with results in the area of molecular genetics, which consider the codon as an independent category, we principally distinguish between two systems, the genetic transcription apparatus, and the self-maintaining system of metabolism*. By definition, the second system (c) is

---

* "A system that tends to improve its performance while pursuring its task or goal and does so without explicit outside help is called self-organizing" and "A goal-seeking system is called cybernetic if it pursues its goal while interacting with an environment. A special case is the homeostat which tends to maintain a prescribed state in the face of perturbations from the environment" (392).

independent of the first system (d), because the performance of the genetic replication apparatus requires the presence of a metabolism. The concept of an independent development of the metabolic system is also shared by (386). The proteins and phospholipid membranes were the tools and driving force in the metabolic processes. They were maintained by organic substrates available in the sediment strata and its energy utilization via phosphates. Considering the organization of this system the incident of photosynthesis has only peripheral significance although – with respect to phylogeny – the start of photosynthetic processes is an essential turning point of evolution because of the production of the "correct" biochemicals.*

Although no concepts have been advanced regarding the coupling of the genetic translation apparatus onto the metabolic device, empirical observations cast no doubt that this integration process represents the decisive step in the formation of the primordial cell. Namely, a novel type of information storage device, capable of information output via peptide synthesis, started to act upon the metabolic system. The two properties

(a) information storage, and
(b) information output

controlled the metabolic reaction cycle by maintaining a precise sequence of physiological events.

NATURE OF REPLICATION

One of the intrinsic problems connected with the evolution of organisms has to do with the origin of the codon responsible for membrane synthesis; in this respect the codon

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* It is of interest to note that the reductive pentose phosphate cycle is not restricted to photosynthesizing organisms. Certain anaerobic bacteria, and which utilize ammonia as source of energy, also follow this cycle (393).

of the metabolic system is only of secondary significance. As was shown throughout this work, one can distinguish between two different entities:

1. A self-replicating system which can keep itself in a stationary phase over periods of time but which is principally a dependent of the environment; namely, this system will stay intact as a self-replicating unit only as long as the environment permits (e.g. viruses and coacervates).
2. A self-replicating system which can maintain itself relatively independent from the environment (e.g. bacteria).

The question arises what is the difference between the self-replicating systems 1 and 2? We have shown in the chapter on "Problem Analysis" that a system can cross the probability barrier and maintain a thermodynamic disequilibrium, and as a function of interplay, this system can evolve in case the environment permits. It can even develop to such a metabolic state that it only utilizes the true basic components, i. e. CO_2, H_2O, PO_4, and N and some metals and trace constituents. A system capable for photosynthesis would be an example for such a case.

It is justified to ask the question, whether the last mentioned system is truly independent from the environment; even if it contains a nucleic acid codon? Furthermore, is it really sufficient if it generally possesses a DNA codon? We must conclude that a true continuity in time is not guaranteed simply by the ownership of a workable codon device. Proof that the presence of an operative codon is not sufficient can be obtained from the example of the virus. By means of the codon, a virus can duplicate itself but it cannot guarantee the existence of its host. Without a well-equipped host system, no continuity in time is maintained for the virus. Consequently, it is implied, that there has to be a difference between a virus and its host; but where are the differences? To answer this question we refer to the known bacteriophage:

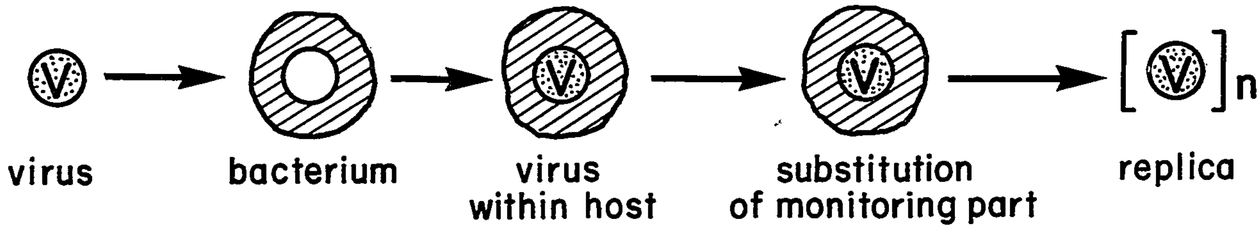

FIGURE 27

In essence, a viral infection of a bacterium implies that the virus substitutes for part of the bacterial system - the monitoring part - because no emulation between the bacterial cell and the virus takes place. The viral DNA substitutes the cellular program. It is logical that a monitoring capability does not represent the essential difference between a bacterium and a virus. Based on information (see: Problem Analysis) one can say that the difference rests on the fact that a bacterium is capable to produce its own boundary layer, whereas the also-codon-equipped virus cannot do this. We are thus forced to state that the true physical-chemical fundaments of biological auto-replicating systems are based on the program - code - for the synthesis of membranes. The statement also implies that this part of the genetic codon which holds the instruction for the synthesis of phospholipids, is the biochemical basis of living organisms.

Information transfer

In the chapter on "Problem Analysis", we have presented evidence than an asymetric information flow is established from the cell to the membrane. The membrane is able to receive chemical information (Fig. 28). However, she is out of question as a source of information because this would imply a chemically active participation in the metabolism which in turn would consequently lead to the self-destruction of the membrane.

An asymetric information flow of a similar kind is also established for the nucleic acids (codon). Based on observational knowledge it is not possible to put information into the codon (Fig. 26) from the side of the metabolism:

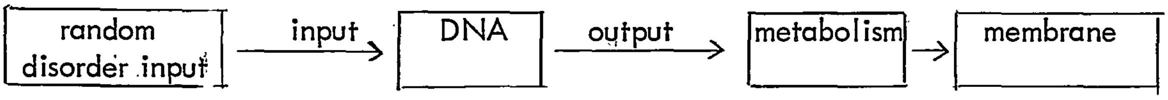

FIGURE 28

This shows that a cell is not capable to develop by its own power; a cell can only repeat the same life pattern over and over again. The information input into the genetic code takes place only accidentally via triplet alterations which may lead to mutation (394-397). Figure 29 presents examples for the initiation of mutation by X-ray irradiation.

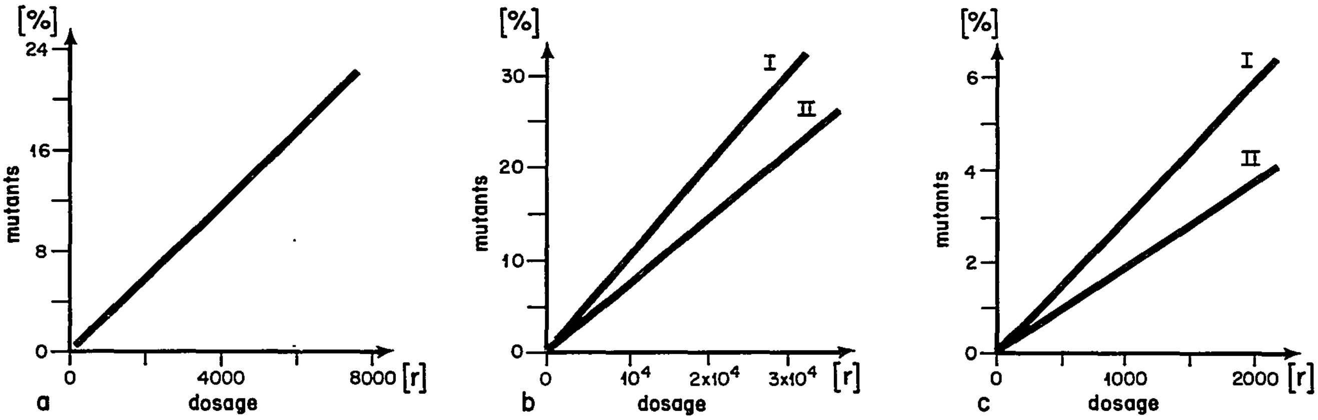

FIGURE 29

Effect of dosage (r units) on mutation rate (degree of fertility) for Drosophila melanogaster (Fig. a; source :X-rays) (395); irradiation experiments on Neurospora crassa (Fig. b; source :X-rays) using low intensities (II) (240 r per minute), and high intensities (I) (5.400 r per minute) (394); comparison between effect of dosage of X-rays (I) and neutrons (II) for Drossophila melanogaster (Fig. c) (396). The induced mutation rates are directly and linearly proportional to the ionization rate of the irradiation (395).

By considering the elements involved at the start and at the end in the course of the asymetric information flow (Fig. 28) it becomes apparent that both the codon and the structure of the membrane are archaic elements - biochemists generally use the term universal elements instead - because they were not modified in the course of evolution (50).

The random input of information into a living cell which also may contain nonsense information, will eventually look, as if an information flow from the environment into the cell is established (Fig. 30). For, only those organisms survive in the course of evolution where the random input did represent a favorable information. In turn, an apparent communication circle between the cell and its environment becomes established. The question remains, whether this communication circle represents an optimal working system in natural environments and over geological periods of time; or alternatively, whether this coupling only reflects accidental events on the primordial earth surface.

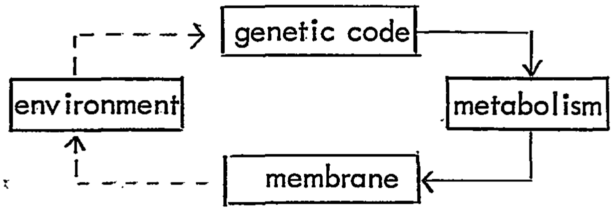

FIGURE 30

Apparent communication circuit

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MOLEKULARE ENTWICKLUNG MINERALISATIONSFÄHIGER ORGANISCHER MATRIZEN

von

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EINLEITUNG

Molekulare Wechselwirkungen von biologischen Hochpolymeren mit anorganischen Ionen finden sich in allen biologischen Reaktionssystemen. Anorganische Kationen treten in vielen Enzymreaktionen als katalytische Elemente auf; u.a. spielen Kaliumionen eine besondere Rolle in der Muskelmechanik. Metallkationen sind beteiligt an allen Membranvorgangen in der Zelle. Ueber die spezifischen Funktionen einzelner Kationen in den Zellprozessen und ueber deren molekularkinetische Eigenschaften herrscht manche Unklarheit, denn diese Wechselwirkungen entziehen sich allgemein einer direkten und diskreten Beobachtung, weil sie einerseits als dynamische Vorgaenge mit kurzen Reaktionszeiten vorliegen und andererseits sich nur in Mikroreaktionsraeumen ereignen.

Dagegen bieten die Vorgaenge in biologischen Systemen, die zur Mineralisation von organischem Gewebe fuehren, experimentellen Zugang. Eine intensive Untersuchung der molekularen Mechanismen von Kristallkeimbildungen auf anorganischen Matrizen wird auch Verstaendnis fuer die Rolle der Ionen in Membransystemen erbringen. Haeufig sind diese Membranen mit einer schwerloeslichen Proteinhaut bedeckt und Wechselwirkungen dieser Eiweisse mit anorganischen Ionen koennten aehnlich verlaufen wie Prozesse der Kondensation auf Proteinmatrizen. Erwaehnenswert erscheinen in diesem Zusammenhang periodische Phaenomene, die durch veraenderliche Quervernetzungen von Eiweisspolymeren erklaert werden. Entsprechend resultiert die Muskeltaetigkeit durch veraenderliche Quervernetzung der Eiweissfibrillen Myosin und Actin.

Es ist das Anliegen der vorliegenden Arbeit, die analytisch leicht fassbaren molekularen Beziehungen im Bereich der verschieden Verkalkungs- und Entkalkungsprozesse bei Mollusken und Crustaceen, die wir untersuchten, in ihren molekularen Mechanismen zu deuten und auf ihre phylogenetischen Implikationen hinzuweisweisen.

HOCHMOLEKULARE PROTEINE UND GLYCOPROTEINE ALS KRISTALLISATIONSMATRIZEN

Von geologisch-palaeontologischer Seite ist die Frage nach der Ursache des ploetzlichen und weltweiten Auftretens von Kalkschalen bildenden Organismen an der Wende des Praekambrium/Kambrium vielfach gestellt und unterschiedlich beantwortet worden. Eine Diskussion der einzelnen Vorstellungen findet sich in den einschlaegigen Lehrbuechern der Geologie und Palaeontologie. Die vorliegende Untersuchung greift u.a. diese Fragestellung auf. In ihrer Beantwortung stuetzt sie sich auf neue Ergebnisse aus dem Gebiet der Vergleichenden Biochemie.

Die Bildung von Kalkschalen und Knochen im Tierreich wird durch organische Matrizen gesteuert (1). Diese sind chemisch gesehen Eiweiss- (Abb. 4,a) oder Chitin-Eiweiss (Abb. 4,b) Hochpolymere. Es konnte gezeigt werden, dass im Falle der Invertebraten die organische Matrix des Schalengeruestes artspezifisch ist (2). Es erwies sich ferner, dass die eigentliche Ausscheidung fester Phasen an das Auftreten freier Karboxylgruppen in Asparaginsaeure und freier Aminogruppen in Lysin und Glucosamin gebunden ist. Die Lage dieser aktiven Funktionsgruppen im dreidimensionalen Netzwerk der Eiweissmatrize bestimmt demnach Struktur und Morphologie von Schalenkarbonaten und Knochen. Sollten diese aktiven Gruppen jedoch inaktiviert worden sein, etwa durch Vernetzung als Ester oder durch Peptidbrueckenbildung, so unterbleibt die Anreicherung von Ionen und damit die Ausscheidung fester Mineralphasen auf organischen Matrizen.

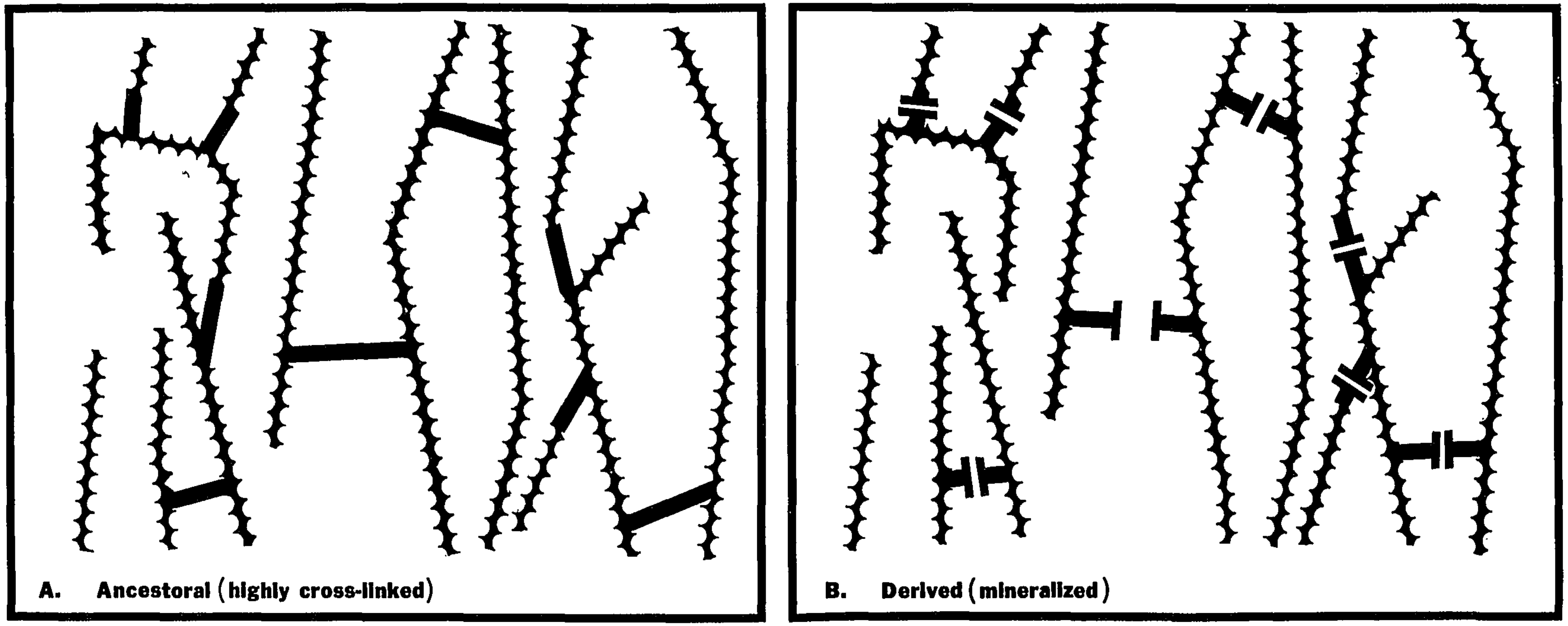
Fuer die vorliegende palaeontologische Fragestellung ergibt sich die Schlussfolgerung, dass mineralbildende Organismen urspruenglich ein rein organisches Schalenmaterial ausgeschieden haben, das zwar reich an Aminozuckern und Asparaginsaere war, in welchem aber die zur Karbonat- oder Phosphatbildung erforderlichen Kondensationszentren infolge von Quervernetzungen nicht frei zur Verfuegung standen.

Aus den angefuehrten Untersuchungen (2) ist weiterhin bekannt, dass eine Veraenderung der Salinitaet, des pH-Wertes oder der Wassertemperatur den Sekretionsmechanismus, der zu der organischen Matrix fuehrt, massgeblich beeinflussen kann. Eine Veraenderung des Umweltmilieus kann damit ohne weiteres ihren biochemischen Niederschlag in der Eiweissmatrize finden., d.h. auf einer zunaechst mineralfreien organischen Schale konnten die zur Kristallbildung notwendigen diskreten Kondensationszentren freigestellt werden. Wir vermuten, dass besonders Schwankungen in der Salinitaet und Wassertemperatur zu dieser Aktivierung fuehrten.

Dieser Sachverhalt wird schematisch in Abbildung 1 zum Ausdruck gebracht. Das ancestorale Glycoprotein-Netzwerk der Schalensubstanz, bestehend aus einzelnen Peptid- oder Aminozuckerketten, ist ueber Seitengruppen quervernetzt und unfaehig, eine Mineralisation einzuleiten (Bild a); dagegen stellt dieses Netzwerk nach Aufhebung der Quervernetzung eine Mineralisationsmatrize dar (Bild b). Die nun in ionarer Form vorliegenden Querbrueckenglieder treten als Kondensationszentren in Erscheinung.

ENTWICKLUNG DER SCHALENMATRIZE

Wir halten als Arbeitshypothese zusammenfassend fest, dass die allmaehliche Umstellung von einem rein organischen Schalenmantel zu einer Schalenkarbonatbildung durch die teilweise Eliminierung von Vernetzungskomponenten in der organischen Matrix hervorgerufen wurde. Der Verlauf der phylogenetischen Entwicklung organischer Matrizen zeichnet sich durch den steigenden



A. **Ancestoral (highly cross-linked)**

B. **Derived (mineralized)**

DEVELOPMENT OF GLYCOPROTEIN MATRIX

Abbildung 1 Entwicklung der Glycoprotein-Matrizen in Schaleneiweissen.

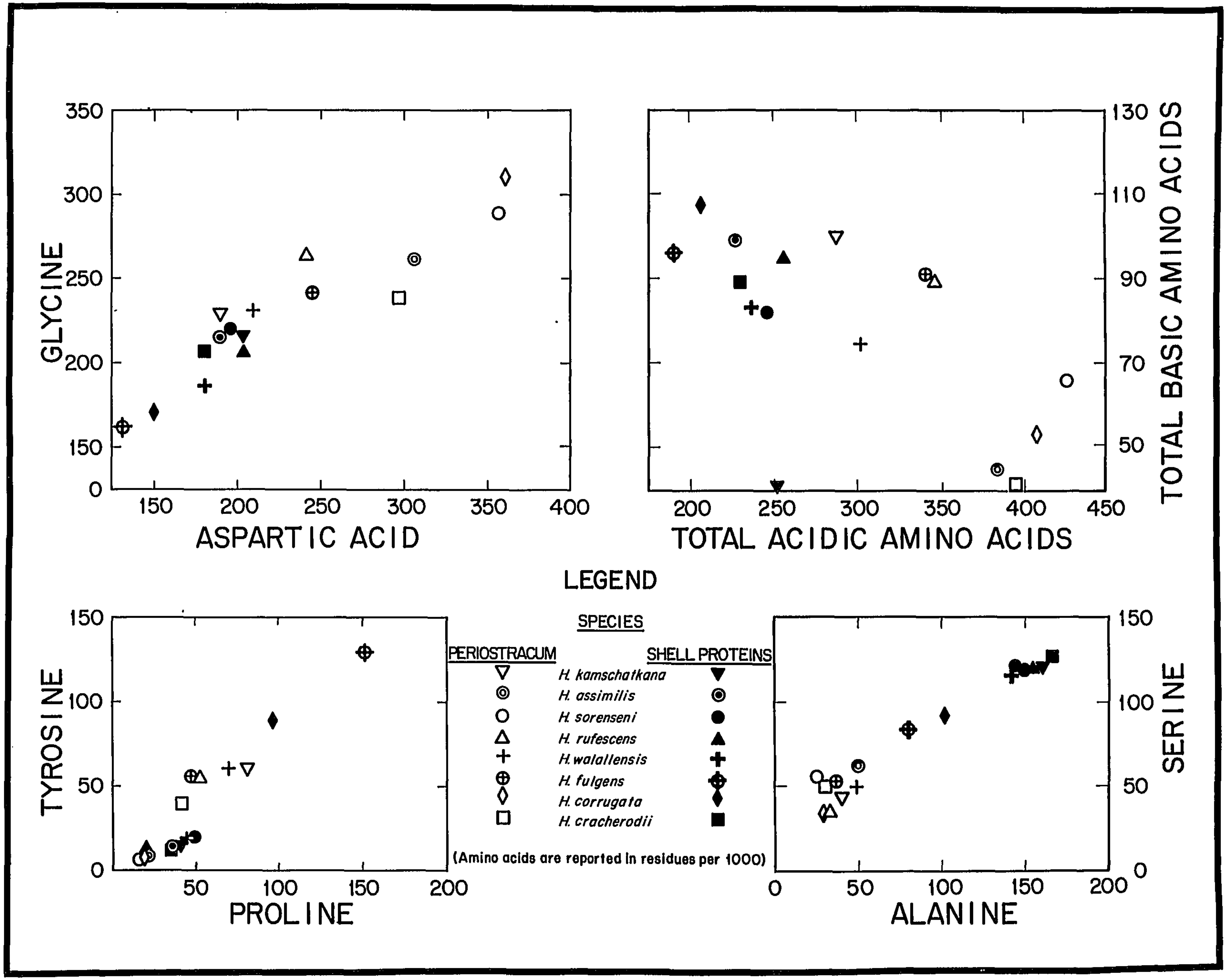


Abbildung 2 Beziehungen von Aminosäuren in verschiedenen Schaleneiweissen von *Haliotis sp.* (6).

Anteil ionarer Gruppen aus. Dadurch bildeten sich immer mehr Keimbildungszentren fuer die Ausscheidung fester Mineralphasen. So erklaert sich die Abnahme der Eiweissfraktion in den Karbonatschalen im Verlauf der Evolution. Primitive oder ancestorale Mollusken haben mehrere Prozent organische Substanz im Schalengeruest eingelagert, waehrend hochentwickelte Formen oft nur 0.01% Eiweiss fuer die Schalenbildung benoetigen.

Die molekularen Vorgaenge, die sich im Verlauf einer derartigen Entwicklung abspielen, lassen sich aus dem Vergleich der Aminosaeurezusammensetzung zwischen mineralisierten und nicht-mineralisierten Schaleneiweissen ableiten. Die fuer diesen Zweck besonders geeigneten Analysen einer aus acht Spezies zusammengesetzten Haliotis-Serie sind in Abbildung 2 dargestellt. Die Beziehungen Serin/Alanin, Tyrosin/Prolin und Glycin/Asparaginsaeure deuten an, dass die Biosynthese der verschiedenen Schalenproteine ein Zweistufenprozess ist. Zunaechst werden in den Zellen des Mantelepithels spezifische aber einfach aufgebaute Peptide gebildet. Im vorliegenden Fall sind es wenigstens vier verschiedene Peptide, d.h. ausser den drei schon genannten eine an Lysin, OH-Lysin, Histidin und Arginin angereicherte Peptid-Fraktion. Der Beweis fuer die Annahme derartiger Fraktionen besteht darin, dass ausser den hier vorliegenden Beziehungen sich keine weiteren linearen Verhaeltnisse zwischen den einzelnen Aminosaeuren aufstellen lassen.

Die in den Vakuolen der Zellen aufgespeicherten Peptide werden anschliessend in geloester Form sekretiert und durch intermolekulare Vorgaenge, wie z.B. durch Vernetzungen, in Hochpolymere ueberfuehrt. Mineralisationsmatrizen, sowie das Periostracum sind die biochemischen Endprodukte dieser Reaktionen. Der wesentliche biochemische Unterschied zwischen diesen beiden Eiweisstypen ist lediglich durch die Verhaeltnisanteile der einzelnen Peptidkomponenten bestimmt.

Daraus laesst sich ableiten, dass der Sekretionsmechanismus der verschiedenen Mantelepithelzellen gut abgestimmt sein muss, um eine charakteristische Aminosaeurezusammensetzung fuer die Mineralisationsmatrize oder das Periostracum zu erreichen. Aendert sich das dynamische Gleichgewicht dieser Sekretionsabfolge, so schlaegt sich dieses Verhalten im Chemismus der Glycoproteine nieder.

Es ist interessant festzuhalten, dass rezente Formen, die seit dem fruehesten Palaeozoikum bekannt sind, eine Mineralisationsmatrize besitzen, deren Aminosaeurenzusammensetzung gegenueber einer Veraenderung des Umweltmilieus unbeeinflusst bleibt. Im Unterschied dazu erwies sich, dass bei geologisch kurzlebigen Formen die Aminisaeurenhaeufigkeiten der Schalenmatrize sehr empfindlich auf Veraenderungen im Environment reagieren. Milieuempfindlichkeit ist besonders ausgepraegt bei den Cephalopoden, deren Proteinmatrix eine starke Aehnlichkeit aufweist zu der organischen Matrix, die in den Kalkablagerungen des Exoskeletts von Crustaceen eingebettet ist (3). Sie befaehigt die Crustaceen wachstumsbedingte und periodische Verkalkungs- und Entkalkungsprozesse physiologisch zu steuern. Drei Moeglichkeiten sind erwaehnt (4), um derartige Entkalkungen zu erklaeren: (a) Aufloesung der Mineralkomponente durch organische Saeuren, (b) Chelatbildung und (c) Austauschvorgaenge durch Anreicherung bestimmter Ionen wie Magnesium, Karbonat oder Citrat. Unsicherheit besteht, ob zunaechst die organische Matrix oder die Minerale resorbiert werden. Vieles spricht dafuer, dass Aminozucker an diesen Umsetzungen stark beteiligt sind (3). Im Falle der Cephalopoden jedoch darf man annehmen, dass diese spezifischen Eigenschaften der Proteinmatrize sowohl die Formfuelle wie auch die Kurzlebigkeit etwa bei den Ammoniten bedingte. Das Phaenomen dieser einzigartigen Matrize, wie sie heute noch in den Crustaceen vorliegt und fuer die Ammoniten anzunehmen ist, sollte es ermoeglichen, die Bedingungen experimentell nachzuweisen, die zum Aussterben der Ammoniten gefuehrt haben, nachdem alle Moeglichkeiten der biochemischen Entwicklung ihrer Mineralisationsmatrize erschoepft waren.

Diese Betrachtungen sind belegt durch mehr als 500 Analysen der Aminosaeurezusammensetzung von Mineralisationsmatrizen. Durch die Faktorenanalyse der Untersuchungsergebnisse, die nur mit

Hilfe eines Computers durchfuehrbar war, konnte die Komplexitaet des biologischen Mineralisationssystems so reduziert werden, dass einfache und uebersehbare quantitative Aussagen ermoeglicht wurden. Diese Ergebnisse finden ihren Ausdruck in der hier dargestellten Entstehung und Entwicklung von Mineralausscheidungen auf Glycoproteinmatrizen. Es ist von Interesse zu erwaehnen, dass die Beziehungen, welche sich zwischen den untersuchten Mollusken allein auf Grund der chemischen Zusammensetzung ihrer Schalenmatrix herausstellten, eine Aufstellung eines phylogenetischen Relationsdiagrams erlaubten (Abb. 3). Dieses Relationsdiagram, dass auf Grund von Zahlenwerten eines Rechengeraetes komponiert wurde und mit dem durch klassische Methoden gewonnenen phylogenetischen Baum prinzipiell uebereinstimmt, zeigt uns, dass die Mineralisationsmatrizen nicht als Zufallsprodukt dastehen, sondern in die phylogenetische Entwicklung eingereiht werden koennen.

MINERALISATIONSMECHANISMEN

Um das Verstaendnis von Mineralisationsprozessen in biologischen Systemen zu erleichtern, haben wir die wesentlichen chemischen Grundlagen und molekularen Vorgaenge graphisch zusammengefasst und kurz erlaeutert.

Die physikalischen und chemischen Eigenschaften von Peptiden und Eiweissen werden durch die Aminosaeureabfolge in einer Polymerkette bestimmt (Abb. 4,b). Besonders wichtig fuer die vorliegende Fragestellung ist das Verhaeltnis hydrophober ($-CH_2$; $-CH_3$) zu hydrophilen (COO^-; NH_3^+) Seitengruppen. Waehrend die Hauptmasse der Eiweisse, z.B. Kollagen und Albumin, nur 5% bis 20% Aminosaeuren mit ionaren Gruppen enthalten, so haben die Schaleneiweisse im Durchschnitt 50% Aminosaeuren mit diesen Seitenketten. Die Bedeutung der ionaren Gruppen ist eine doppelte; einmal dienen sie als Vernetzungskomponenten (Abb. 1), zum anderen treten sie in Wechselwirkung mit anorganischen Ionen und damit werden sie, *inter alia*, Keimzentren fuer die Ausscheidung fester Phasen. Einige der dabei auftretenden Vernetzungsglieder der Glycoproteine bringt Abbildung 5. Sie wurden in verschiedenen Biopolymeren nachgewiesen. Es ist wichtig festzuhalten, dass nicht nur Aminosaeuren untereinander als Brueckenbildner auftreten, sondern dass auch Aminozucker oder Hexosen die dreidimensionale Konfiguration der Eiweissmatrix bestimmen koennen. Die Teilnahme von Phosphatgruppen, etwa im Fall der Vernetzungen zwischen OH-Prolin und Glucosamine, ist eine interessante Variante speziell im Hinblick auf die Apatitausscheidung in Kollagensystemen.

Die molekulare Seite derartiger Kondensationen ist mannigfach und nur mit vereinfachenden Ansaetzen behandelbar. Die vorliegenden Analysenergebnisse sprechen gegen eine Kristallisation aus uebersaettigten Loesungen, sie sind jedoch vereinbar mit den in Abbildung 6 dargestellten Keimzentren auf biologischen Matrizen. Kalziumionen werden von der COO^- Gruppe der Asparaginsaeure eingefangen und bilden unter Hinzuziehung weiterer Sauerstoffe ein Koordinationspolyeder (Bild a). Eine Zunahme von Polyedern fuehrt zwangslaeufig zu einer strukturellen Umgruppierung der Peptid- und Glucosaminketten. Die Sauerstoffpolyeder bedingen somit ein organisches Netzwerk mit hohem molekularen Ordnungsgrad, dessen Struktur durchaus mit der organischer Glaeser vergleichbar ist. Damit wird der Glycoproteinkomplex aus einer zunaechst reinen Adsorptions- in eine Mineralisationsmatrize ueberfuehrt.

Die Wechselwirkung der Polyeder mit den an Lysin oder Glucosamin angelagerten $-O-\overset{\overset{O}{\|}}{C}-O-$ Gruppen (Abb. 6,b,c) fuehrt zum Sauerstoffaustausch am Polyeder, wodurch Kalzium gegenueber Sauerstoff stabiler koordiniert wird. Daraus ergibt sich entweder ein Calcit (CaO_6) oder ein Aragonit (CaO_9) Koordinationspolyeder. Die nun einsetzenden Keimwachstumsprozesse finden ihren Niederschlag in der Textur der Kristallphasen und liegen bereits im Erfahrungsfeld der Kristallphysik.

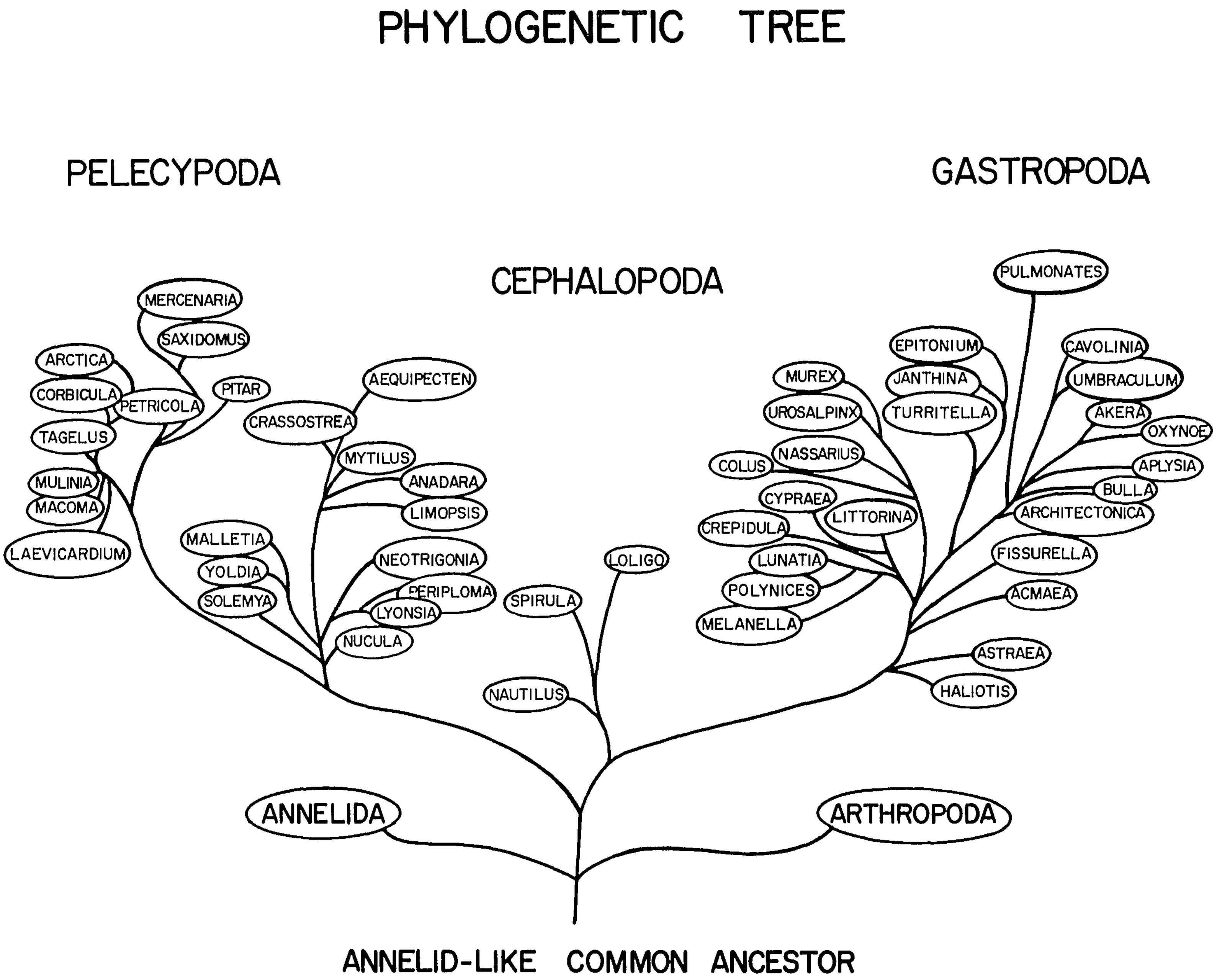


Abbildung 3 Phylogenetisches Relationsdiagram der Mollusken.

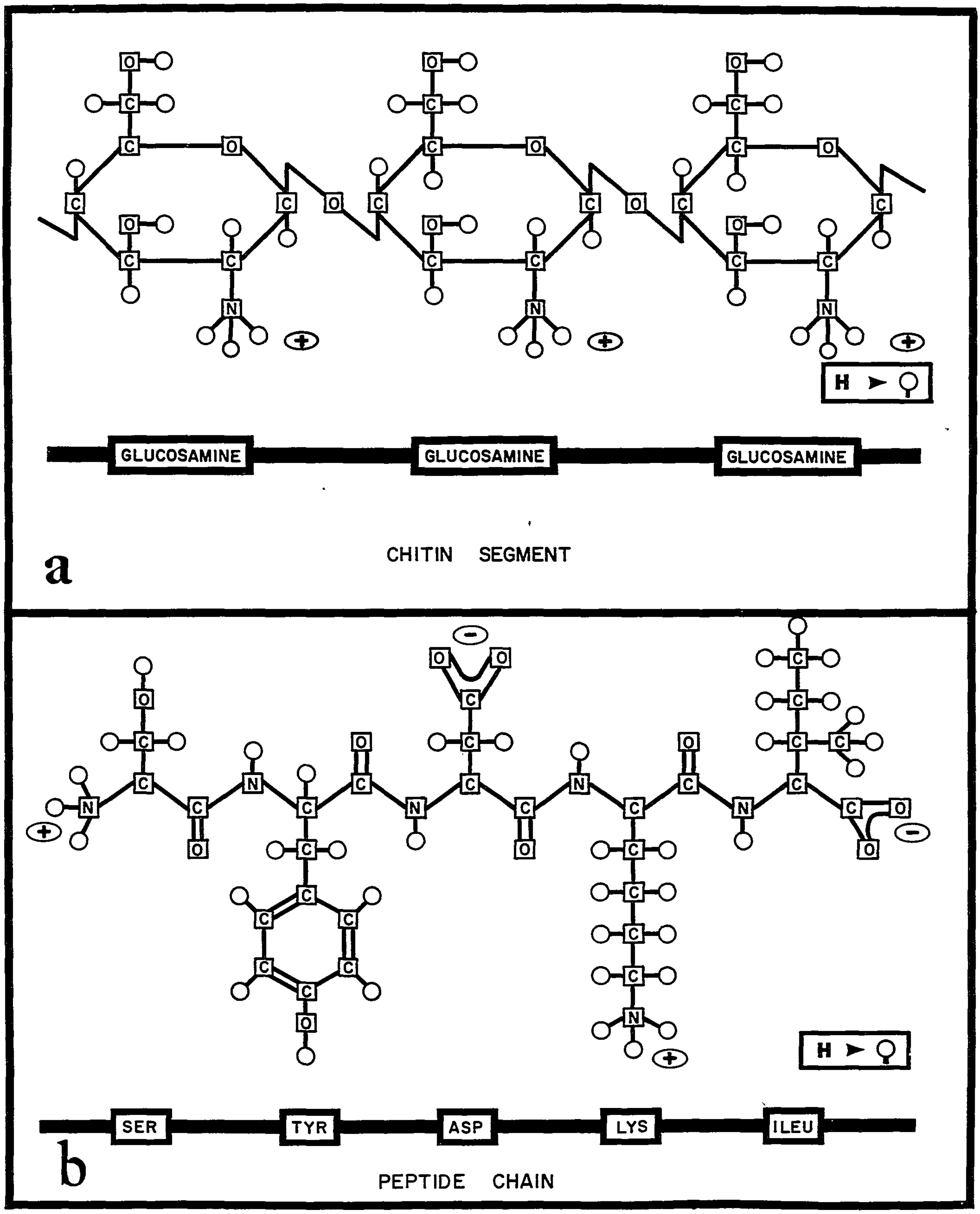


Abbildung 4 Grundeinheiten der Chitin- (a) und Eiweiss-Strukturen (b).

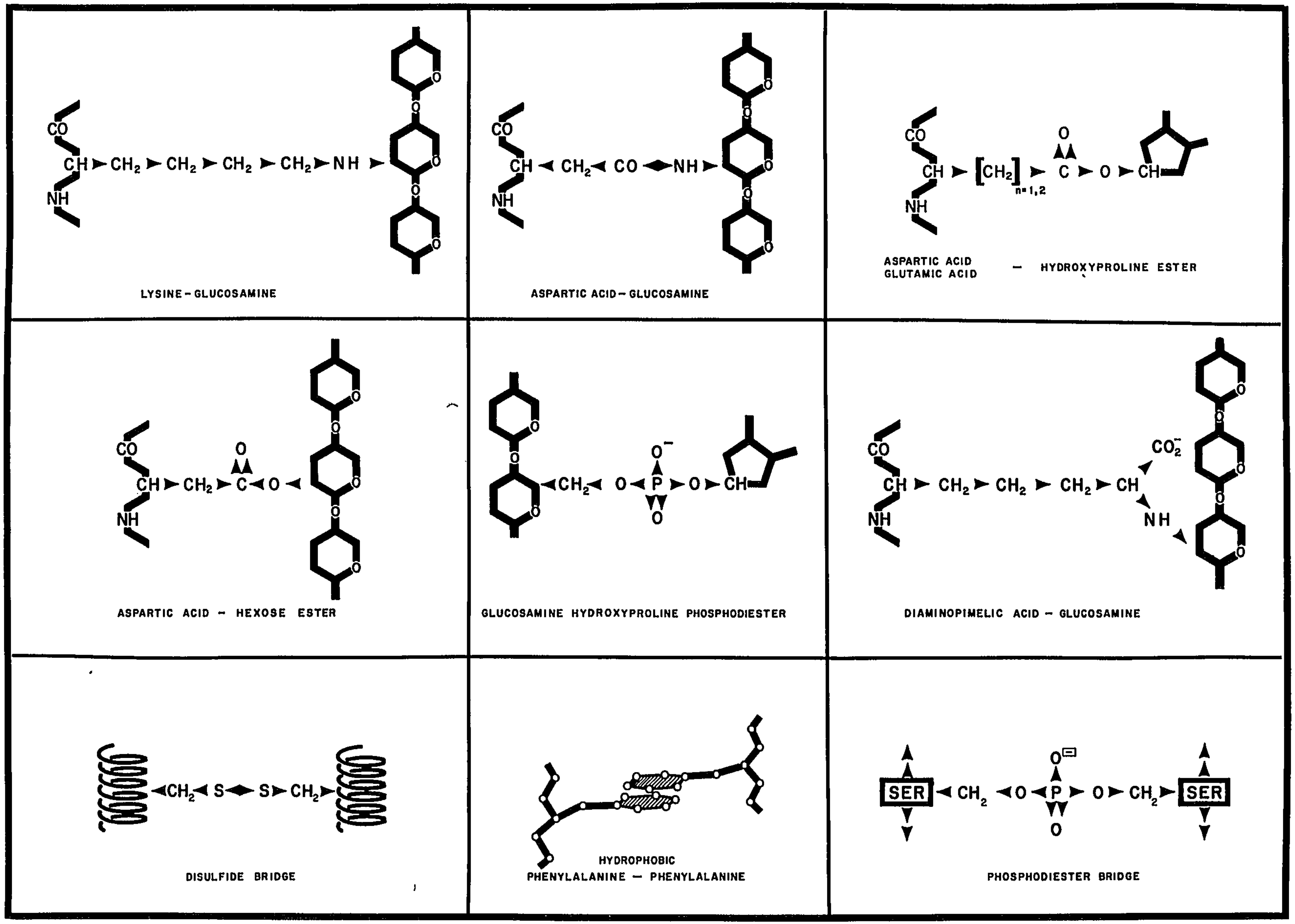
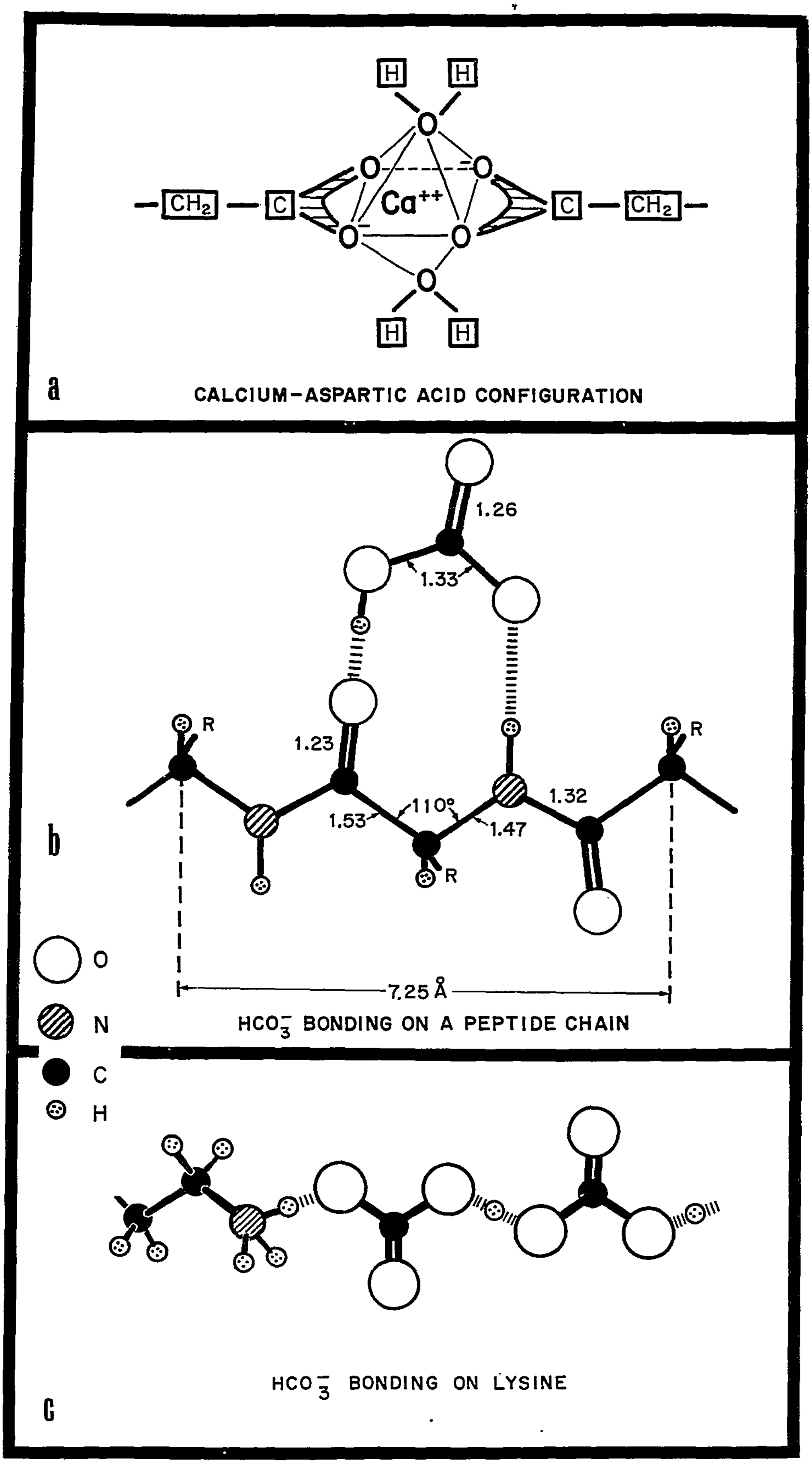


Abbildung 5 Quervenetzungselemente in Schaleneiweissen.

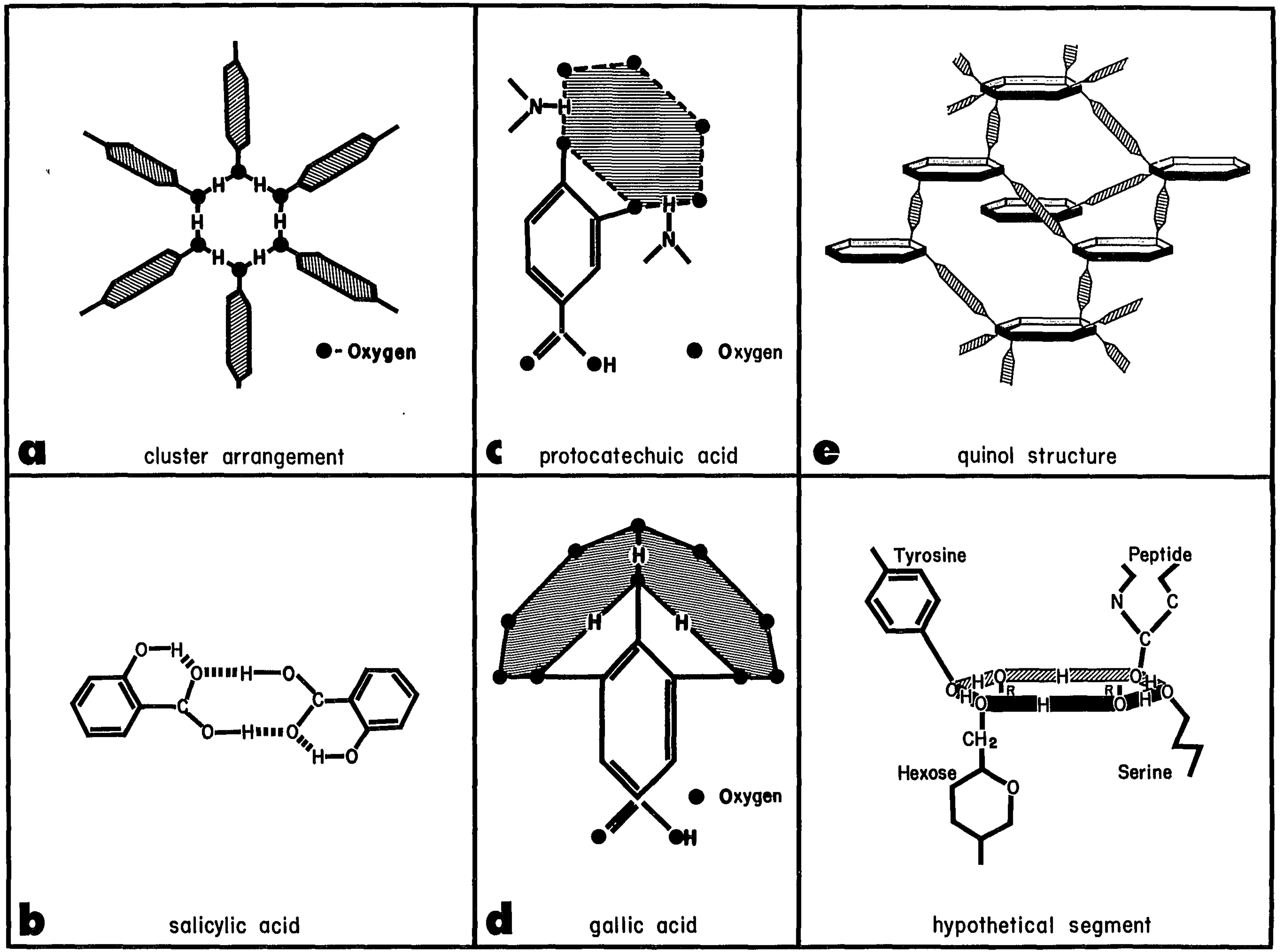


CALCIUM-ASPARTIC ACID CONFIGURATION

HCO_3^- BONDING ON A PEPTIDE CHAIN

HCO_3^- BONDING ON LYSINE

Abbildung 6 Kalzium Koordinationspolyeder (a) und Bikarbonatbindung an Peptidkette (b) und Lysin (c) auf Mineralisationsmatrizen.


a cluster arrangement

b salicylic acid

c protocatechuic acid

d gallic acid

e quinol structure

hypothetical segment

Abbildung 7 Bauprinzipien von Clathraten.

Die Frage nach der physiologischen Herkunft der einzelnen Karbonatkomponenten ist schwierig zu beantworten. Es ist moeglich, dass das Karbonation katalytisch aus CO_2 vermittels der Carbonic-Anhydrase hervorgegangen ist. Aus der Verteilung stabiler Sauerstoff- und Kohlenstoffisotopen in Schalenkarbonaten mariner Organismen darf man jedoch annehmen (5.), dass die Hauptmasse der Karbonationen dem Meerwasser selbst entstammen. Anders lassen sich die isotopischen Gleichgewichtsbeziehungen zwischen dem biologischen Karbonat- und dem Meerwassersystem nicht in Einklang bringen. Hinsichtlich der Herkunft von Ca^{++} ist zu vermuten, dass bei der Zulieferung die OH-Gruppen im Zucker einen massgeblichen Einfluss ausueben; und zwar erwirken einmal die Sauerstoffaffinitaet des Kalziums sowie sein grosser Ionenradius chelatartige Sorptionsbindungen, wobei ein Ca^{++} von zwei Hexosaminringen umgeben sein sollte. Die NH_2-Gruppen der Aminozucker sind sowohl aktiv an den Chelaten beteiligt gemaess den Umlagerungen: $Ca^{++} + 2H_2O \rightarrow Ca^{++} + 2H^{+} + 2OH^{-} \rightarrow Ca(OH)_2 + 2NH_3^{+}$. Haben die NH_2 bereits Protonen angelagert, so stellen sie eine wirksame Potentialbarriere dar, welche die Sorption und Diffusion von Ca^{++} unterbindet.

STRUKTUR ANCESTORALER SCHALENEIWEISSE

Im Rahmen dieser Betrachtungen moechten wir noch Stellung nehmen zu der ursprueglichen biochemischen Zusammensetzung des organischen Schalenmaterials und einen Vorschlag ueber dessen dreidimensionale Struktur zur Diskussion stellen.

Neben der Karbonatschale scheiden viele Mollusken zum Schutz der Mineralphase das Periostracum aus, welches hauptsaechlich aus Eiweiss und Aminozuckern besteht. Es ist interessant festzuhalten, dass bei vielen ancestoralen Formen wie Neopilinia oder Solemya, chemisch kaum Unterschiede bestehen zwischen den Eiweissen in Schalenkarbonaten und denen im Periostracum (6). Mit zunehmender Entwicklung jedoch verschwinden die Aehnlichkeiten. Man darf demnach annehmen, dass die Organisman urspruenglich, d.h. vor der Kalkschalenaera, die vor rund 600 Millionen Jahren begann, eine rein organische Schale ausgebildet haben, die chemisch aehnlich aufgebaut war, wie das Periostracum.

Chemisch gesehen ist das Periostracum durch einen hohen Anteil aromatischer Aminosaeuren gekennzeichnet. Daraus leiten sich verschiedene Phenolkomponenten und Chinone ab (7). Strukturell bieten sich zwei Bauprinzipien fuer derartige Polymere an: (a) Depside und (b) Clathrate. Im ersten Fall liegen Polyester der Phenolcarbonsaeuren vor unter Teilnahme der OH-Gruppen von Zuckern und Aminosaeuren. Die zweite Vorstellung zieht Clathrat-Raumvernetzungen ueber EDA (Elektronen-Donator-Acceptor Wechselwirkungen) Molekuelverbindungen in Betracht (8).

Fuer die Bildung von Clathrat-Raumnetzwerken sprechen folgende Argumente. Die Struktur erlaubt eine Anordnung von polaren und neutralen Bestandteilen zu stabilen gebilden. Das Ordnungsprinzip, bestehend aus einer Seperation und Wechsellagerung von Baueinheiten ist in Abbildung 7, Bild a gezeigt. Polare OH-Gruppen treten infolge der EDA-Wechselwirkung zu hexagonalen Ringeinheiten zusammen. Phenolcarbonsaeuren wie die Salicylsaeure sind zu dieser Raumvernetzung nicht befaehigt, da sie eine ausgesprochene Neigung zur stabilen Dimerenbildung zeigen (Abb. 7, Bild b). Die Abwesenheit solcher Bestandteile im Schalenmaterial kann als weiteres Argument fuer dieses Bauprinzip angesehen werden. Im Unterschied dazu sind die zur Clathratbildung befaehigten Molekuele (Abb. 7, Bild c und d) im biologischen Schalenmaterial haeufig nachgewiesen worden. Besonders interessant ist hierbei das bei Insekten nachgewiesene Resilin (9).

Die hier vorgeschlagene Raumanordnung von Molekuelen entspricht Kaefignetzwerken in der Art wie sie bei Zeolith-Raumverknuepfungen in Silikatsystemen vorliegen. Letztere sind besonders als Ionenaustauscher oder Molekularsiebe bekannt geworden. Die kaefigbildende dreidimensionale Bauweise der Clathrate ist am Beispiel des Quinols in Abbildung 7, Bild e illustriert (10). Durch derartigen Aufbau werden die bereits quervernetzten Glycoproteinpolymere im Periostracum eine weitere Steigerung der Raumvernetzung erfahren. Denn sowohl die OH-Gruppen der Zucker und Aminosaeuren, wie auch Carbonylgruppen koennen aktiv an der Vernetzung teilnehmen (Abb. 7, Bild f). Der reichlich vorhandene Zwischenraum unterstuetzt einerseits die Unterbringung von Kettenpolymeren und ermoeglicht andererseits den Einbau hydrophober Molekuelverbindungen. Letzteres traegt wiederum zur Erhoehung der Stabilitaet des Netzwerkes bei; die Ligament-Eiweisse der Mollusken besitzen beispielswise bis zu 30% Methionin und 60% Glycin und die extreme Kontraktionsfaehigkeit und Flexibilitaet der Ligamente beruht wohl auf diesem Umstand.

Die Resistenz solcher Polymerordnung gegenueber chemischen und physikalischen Einfleussen laesst sich am Beispiel des Kerogens veranschaulichen. Kerogen ist die Hauptmasse der in den Sedimentgesteinen in stabiler Form vorliegenden organischen Substanzen. Auf Grund der chemischen Zusammensetzung muessen Clathratstrukturen angenommen werden, nur ist der aromatische Anteil hier wesentlich hoeher als im Periostracum. In beiden Faellen, dem Kerogen und dem Periostracum, werden Molekularstrukturen benutzt, die gegenueber dem jeweiligen Umweltsmilieu den wirksamsten Schutz bieten. In diesem Zusammenhang ist erwaehnenswert, dass Chitin- und Periostracum-Strukturen aus kambrischen Gesteinen (500 - 600 Millionen Jahre) isoliert wurden.

Abschliessend seien die spezifischen Permeabilitaetseigenschaften dieser Strukturen erwaehnt; und zwar koennten sie als extrem selektive Molekularsiebe auftreten und damit ausser Schutzfunktionen auch Bedeutung fuer die Physiologie der Organismen besitzen (11).

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LISTE DER ABBILDUNGEN

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MOLECULAR MECHANISMS ON INTERACTIONS BETWEEN OXYGEN CO-ORDINATED METAL POLYHEDRA AND BIOCHEMICAL COMPOUNDS by Egon T. Degens and Johann Matheja. 314 pp. August 1967. Contract Nos. NSR-22-014-001 and PRF 1943-A2.

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