

View on Lipids of Microorganisms from the Standpoint of Prebiotic and Biological Evolution*

Leonid Andreev†

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Specificity of lipids as an object of molecular biological studies

Modern molecular biology incorporates a number of research areas dealing with substances and processes of general biological significance. Those areas of research, stimulated and inspired by the efficacy of the currently available physico-chemical methods, considerably differ in methodologies and – what is especially important – due to their specific developmental backgrounds, they have different levels of relationship with cellular biology.

When that relationship is lost not yet strong enough, it is often understood as an indication that some of the problems of physical chemistry of bioorganic molecules may be as broad and complex as the issues encountered with in the study of the functioning of live cells. This concerns particularly investigations of biological macromolecules that are functionally active outside the cells that synthesize them.

That kind of misconception is not accidental. The awareness of the fact that, despite a theoretically possible large variety of structural and functional organizations of biopolymers, the Nature has only a limited number of their variants, impedes the advance of researchers in physics and chemistry of the functionality of such molecules, as it makes them divert to working on problems which require qualitatively different approaches and expertise and force them to study such properties of organisms which require deep empirical knowledge.

This is a psychological reason that explains why many molecular biologists at least sympathize with, if not fully concede to, the thesis that the notion of ‘cell’ “has become a brake on the progress toward the understanding of live structures at the molecular level” and that “one may stop treating the cell as a biological unit but consider it as merely one of the stages of a complex chain of transformations” [16]¹.

In practice, such views may seem to be justified as the attempts not to limit oneself to the “barest necessities” of evolutionary biology and “biological purposefulness” and, instead, consider the cell to be “merely one of the stages of a complex chain of transformations” may often be helpful in extensive fundamental investigations in molecular biology, leading to innovative approaches to various aspects of the functioning, systematics and evolution of live organisms. A classical example of such fundamental works in molecular biology that already by now have significantly contributed into the progress in evolutionary biology, is the method of macromolecular chronometry developed a quarter of a century ago [34] based on physico-chemical, rather than biological, logic.

* Translated from the Russian. Original paper published in: *Voprosy Evolutsii Bakterij* (Evolution of Bacteria), USSR Academy of Sciences, Center for Biological Research, Institute of Biochemistry and Physiology of Microorganisms, Pushchino, 1984, pp. 93-119.
<http://www.matrixreasoning.com/pdf/OriginOfLifeRus.pdf>

† Current affiliation: Equicom, Inc., Scottsdale, AZ, U.S.A. Email: equicom@matrixreasoning.com

¹ Rendition is based on the Russian edition of the source book.

Notwithstanding the success in certain areas of molecular-biological studies concerned with the phenomenon of life, their independence from the general biological knowledge and cellular biology is only an illusion caused by the breathtaking new capabilities that look almost like a sudden materialization of something that only yesterday seemed to be not more than an idealized goal, set merely for justification of a fundamental research project. This is especially clearly exhibited in one of the areas of molecular biology – the study of lipids.

The structure of lipids is less complex as compared to proteins, nucleic acids, or polysaccharides, which is why the study of lipids of microorganisms has fairly quickly advanced in accumulating molecular-biological information, which, in its turn, helped to see the narrowness and, oftentimes, senselessness of investigations that are disconnected from the problems of cellular biology. Some ten years ago, O’Leary wrote to the effect that not long ago the studies on microbial lipids were basically boiling down to investigating almost exclusively the chemical nature of those compounds and their biosynthesis. Undertaken by many researchers within the past 20 years, those investigations unexpectedly appeared to be difficult, and the results they yielded were in many ways surprising. This field has already gathered sufficiently large amounts of information, and it continues to grow. Such a wealth of information and data on biosynthesis, although valuable in itself, is not much of an asset, as it says a lot but does not explain much. We have found ourselves in the role of observers solemnly gazing at the fascinating amount of data which shows how much we know about microbial lipids and how little we understand about them. It gets clearer to us what the lipids are and how they are organized, but – why are they needed? What does it all mean for the cell functioning? [12]²

Since the time these questions had been put, both the capabilities of theoretical and experimental lipidology and the information on lipids have dramatically expanded. But still again – in the preface to *Membrane Lipids of Prokaryotes* published in 1982 [20] – K. Blokh, a well-known specialist in the field, puts the same questions. He states that overabundance, rather than thrift, characterizes the composition of membrane lipids occurring in the Nature. In terms of the structural common denominator of all the components of the membrane lipid bilayer, it would suffice to have just one phospholipid that has an amphipathic structure and is capable of participating in formation of closed vesicles.

In the meantime, there is no cellular membrane in the Nature which would have only one phospholipid. “From the standpoint of compositional complexity of natural membrane lipids”, further writes K. Blokh, “it is remarkable that chemically homogenous liposomes imitate many of the properties of natural membranes, including transport, phase transitions, or the effect on membrane-bound enzymes. Clearly, there must be a great variety of membrane-dependent phenomena, expressed only in cells, which cannot be discovered by studying a single-component membrane model” [20].

Despite a relative simplicity of the lipid structure, in the Nature there are lots of structurally individual forms of lipids. For instance, bacteria-synthesized lipids consist of several classes, of which phospholipids are most widely occurring. There are over two dozens of various phospholipids that differ by a radical attached to the phosphate group. Rather than being an individual substance, each of them is a set of substances, which differs from others by the kind of fatty acid (some bacteria have up to several dozens of various fatty acids) with which the C1 and C2 hydroxyls of glycerol are esterified. Esterification of hydroxyl groups of glycerophosphate can be incomplete, in which case the so-called phospholipid lysoforms are produced. Hydroxyl groups of glycerophosphate can be bound to saturated and unsaturated alcohols, aldehydes and oxyacids.

There are two factors that contribute into diversity of the lipid composition of bacteria. Firstly, the number of structurally individual lipids and the ratio between them may vary – apparently, indefinitely – in bacterial species; and, unlike lab-synthesized lipids, bacterial lipids are not randomized. In paraphrase, each group of lipids has an unpredictably large variety of distributions of molecular species. Secondly, the group and fatty acid compositions of phospholipids of most of bacteria vary extremely widely and according to very complex patterns dependent on the physiological condition of a cell population, which, in its turn, depends on a variety of environmental factors. Oftentimes, such variations in phospholipids composition can be considered as qualitative [5].

For the said reasons, an exhaustive description of lipids of even one species of bacteria is quite complicated and uneconomic in terms of the time and effort involved, given that all those details and nuances have no practical application within the context of the currently existing theories on the functional role of lipids in bacterial cells.

This situation is clearly a result of the overestimation of the capability of exact sciences to solve the problems of cellular biology at the molecular level. Contemporary theories on the functional role of lipids in bacterial membranes are mostly of the physico-chemical, rather than biological, nature, and are based on interpreting such integral properties of lipids as the high degree of reduction (lipids as energy accumulators), spatial dissociation of hydrophilic and hydrophobic groups, capability for phase- isolation in aqueous environment, dependence of the degree of spatial freedom of fatty acid radicals on their structure, etc.

The physico-chemical approach to the study of natural lipids has helped to solve a number of important problems, including, first of all, production and utilization of artificial biological membranes. As well, it significantly contributed into the progress of technology of lipid studies, and many of the results obtained by physico-chemical methods are successfully used in biology and medicine. Nevertheless, it would be fair to say that, in the long run, the study of biological role of lipids has suffered serious damage as a result of preoccupation with physico-chemical methods.

This has become especially clear when physico-chemical lipidology, having failed to offer a more or less credible explanation to the astonishing diversity of the lipid spectra in living organisms, has changed its position toward downplaying the significance of this crucially important fact. There came a new wave of studies whose authors were determining the melting temperature of lipids isolated from various microorganisms, assuming that the cell lipid metabolism, as intricate as it is, is responsible mainly for maintaining a required level of membrane liquidity [21, 30]. For quite a long time, this inappropriately naïve idea kept attracting many researchers as

² Back translation from the Russian edition of the source book.

an affordable way to obtain integral quantitative characteristics of a cell lipid pool, although absolutely helpless in terms of explaining the uniqueness of lipid spectra in bacteria which avail themselves of thousands of ways to create a same physical effect. This phenomenon is an indication that phase transitions in bacterial membranes are by no means the main cause of purposeful changes in lipid composition – instead, they are one of the effects of those changes.

The idea about the defining role of the physical condition of membranes is closely connected with the works on physiology and biochemistry of bacteria artificially rendered unable to synthesize certain lipids [28]. It was shown, for instance, that bacteria can grow and develop even when their membranes are artificially supplied with mixtures of lipids that they cannot not naturally synthesize.

It runs through all of those studies that specificity of lipid composition of bacteria is not life's necessity. However, as that idea was further developing, it eventually has led to an absolutely opposite result – a realization that there must be lots of membrane-dependent phenomena that are exhibited only in cells and cannot be detected by experimenting with physico-chemical models, even those that imitate a live cell.

Proteins and nucleic acids can be studied in respect of their functionality, without going deep into fine details of the biology of organisms they are isolated from. With lipids, such an approach seems to be practically senseless, and that is why it is not surprising that, despite the tremendous progress in deciphering the fine mechanisms of functioning of proteins and nucleic acids, the function of lipids remains as unclear as it was decades ago. Functionally, lipids are inseparable from organisms that synthesize them. This is the main peculiarity of lipids as objects of molecular-biological studies, which requires the whole methodology of lipid studies to be revised so as to restore its connection with the cellular level.

Cellular level of lipid studies

The cellular approach to the study of lipids means the establishing of functional relations between the lipid composition and its regulation, on the one hand, and biological (morphological, physiological, ecological, and other) characteristics of organisms that synthesize them, on the other.

Taking a full advantage of that approach involves two serious but surmountable challenges. The first one is the need of solid knowledge in biology of microorganisms under study, which can be resolved through research cooperation. The second challenge comes from is a virtually complete lack of an adequate theoretical basis. As a minimum, we need hypotheses explaining the fantastic diversity of lipid spectra of bacteria, let alone the capability to predict, based on bacteria known properties, lipid compositions of specific bacteria.

Properties of lipids should be studied in close connection with biological peculiarities of cells of bacteria that synthesize them, as this will help to bridge the gap between theory and experiment. Functional integrity of the lipid pool of living cells is especially valuable in the context of this problem.

Evolution of bacteria was accompanied with the restructuring of their membrane system with its inherent integrity of the structure and functions. As well, integrity is characteristic of lipids of live cell membranes, which can be proved on various examples, some of which are discussed in [2, 4-7, 13]. Primarily, it is expressed in the character of adaptational changes in membrane lipids in the course of bacterium growth and development, in response to changes in the environment parameters. In such cases, the lipid pool of live cells responds as an integral system, acting by fairly complex rules that are certainly impossible to figure out based on lipid physico-chemical properties solely.

Integrity of the lipid pool is also expressed in the fact that evolutionary modifications of certain biological properties of bacteria were accompanied with coordinated changes in lipid compositions, which were similar in different bacteria, irrespective of their systematic position and ecology.

Functional integrity of the lipid pool of live cells and functional inactivity of lipids isolated from the cell, as well as the diversity of lipid spectra, should be taken into account when designing a model claiming to explain the biological role of lipids.

The concept of polylipids

According to this concept, phospholipids of cell membranes form cellular polymeric structures [1, 5]. In the ideal case, a polylipid of the bacterial cytoplasmic membrane can be considered a molecule of the same size as a bacterial cell. Phospholipids form the skeleton of the native membrane – a so-called polylipid membrane. Monomer phospholipids of the polylipid membrane are bonded mainly by principal valence forces.

Fig. 1 shows a schematic view of a polylipid membrane built from molecules of phosphatidyl ethanolamine (PE) and cardiolipin (CL). The basic monomer unit in such a membrane is a dimer in which PE molecules are connected through a metal atom in such a way that fatty acid residues of both PE molecules are oppositely oriented. Another type of bond between monomer phospholipids (PE dimers) is formed by substitution of protons of enolized ester carbonyls by ions of polyvalent metals (mostly, Mg). Such bonds, particularly with the involvement of Mg, have low conformational freedom and can be broken by the attachment of protons.

Two layers of polylipids, organized in the way shown in Fig. 1, form the membrane's polylipid bilayer whose stability is determined by potassium and sodium atoms in the intra-bilayer space. These atoms interact with ester carbonyls of phospholipids of both monolayers. This interaction involves also amino groups of phosphatidyl ethanolamine, whose degree of involvement determines the ratio between Mg and Na in the inter-bilayer space.

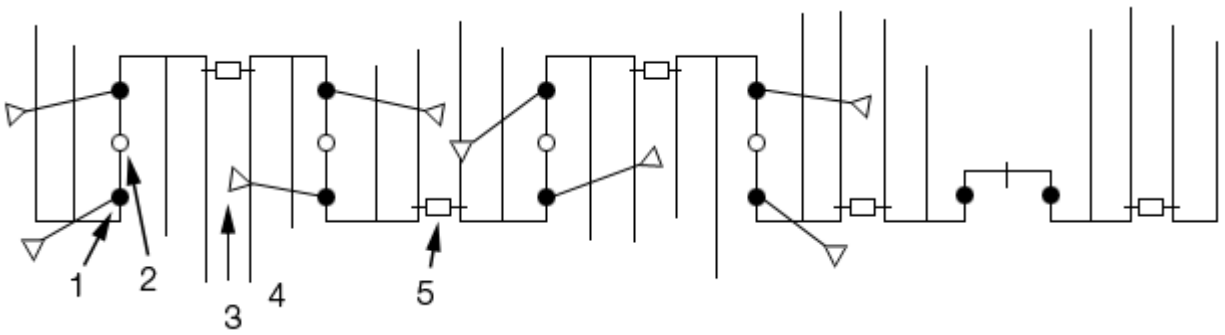


Fig. 1. Schematic view of a polylipid bilayer of the cytoplasmic membrane of Gram-negative bacteria: 1, phosphoric acid residues; 2, bivalent metal atoms; 3, amino groups of phosphatidyl ethanolamine; 4, fatty acid radicals of phospholipids; 5, the bond formed by substitution of two protons of enolized ester carbonyls of phospholipids by a polyvalent metal (mainly, Mg) ion.

A bilayer whose polylipid structure suffers a damage can transform itself into a lamellar-type bilayer in which phospholipids are positioned back-to-back with their nonpolar radicals, whereas their polar heads are directed outward, according to the Gorter-Grendel model proposed in 1925 and further developed by Dawson and Danielli. A lamellar-type bilayer is not a polylipid since it is organized according to physical, but not chemical, interaction of monomer units.

The biological membrane of eubacteria is structured by the polylipid layer both lengthwise and crosswise. Qualitative composition and proportions of monomer phospholipids determine to the large extent mechanical and physico-chemical properties of the polylipid skeleton of the membrane and underlie the diversity of their molecular organization. Both in Gram-positive and Gram-negative bacteria, diphosphatidyl glycerol (or cardiolipin (CL)) is the cross-agent that splices individual polylipid chains. As it follows from the general principles of the model, polylipid monolayers can change their positions relative to each other, using alkali metal ions as a lubricant. However, the transfer of monomer phospholipids from one polylipid monolayer to another occurs only if the polylipid skeleton is destroyed and a lamellar-type bilayer is constructed.

Interaction between polylipids and proteins

The concept of polylipids, even in the above-presented form, allows the understanding of the cause of the qualitative and quantitative diversity of lipid spectra of such organisms as bacteria whose cytoplasmic membrane performs practically all of the membrane-dependent functions; it also explains the diversity of the forms of bonds between proteins and membranes. Based on the model of molecular organization of the biological membrane, we will discuss a possible mechanism for protein embedding into the membrane's polylipid skeleton.

When contacting the surface of the bilayer polylipid membrane, protein interacts with it through weak van der Waals forces emerging when the protein non-polar amino acid radicals come into contact with short fragments of fatty acid radicals "sticking-out" on the surface of the polylipid layer (cf. Fig. 1). When the cumulative amount of that interaction reaches a certain critical value, the pressure, directed onto the surface of the polylipid skeleton, destroys it in the site of protein embedding. This effect is determined by the limitedness of conformational freedom of the metal (magnesium)-lipid complexes, due to which even a small deformation of the lipid skeleton causes a dramatic increase of reactive capacity of M-O (metal – oxide) bonds whose disruption results not only in the embedding of proteins but also in their covalent bonding with lipids.

Thus, a biological membrane polylipid layer serves as both an accumulator and amplifier of weak interactions that are determined by the spatial structure of the embedding proteins, on the one hand, and the lipid composition at the embedding site, on the other. Anisotropy of protein embedding into the membrane is determined by the asymmetry of the polylipid bilayer. During the embedding process, the weak quantum-mechanical interactions between lipids and proteins are transformed into interactions with the breaking and formation of primary valence bonds. The strength of the both types of primary valence bonds between monomer phospholipids is determined by numerous factors, including the concentration of cations – primarily, protons. However, it should be taken into consideration that the polylipid membrane is a dynamic structure wherein primary valence bonds are being broken and restored. The structure of the polylipid skeleton is determined by not only the quality and quantity of individual lipids, but also – and maybe even to a higher extent – by the qualitative and quantitative composition of proteins and other components of biomembranes.

At the initial stage of protein embedding into the membrane polylipid skeleton, non-polar interactions between amino acid and fatty acid residues induce the restructuring of the polylipid skeleton to achieve a thermodynamically advantageous state of the maximal contact. Prior to the contact with a macromolecule to be embedded, the embedding site represents, due to high lateral flexibility of monomer phospholipids, a polymer with randomized distribution of monomers. However, after initiation of the contact, the distribution becomes more or less specific. Regardless of the extent of specificity – hence, the energy of non-polar interactions for protein embedding with the breaking of primary valence bonds – the protein contact with the polylipid membrane results in the decrease of the contents of individual phospholipids that are "complementary" to a given protein and the increase of those that are "non-complementary". As a result, the changes in the individual lipid contents in the membrane appear to be closely connected with the changes in the protein composition of the membrane.

Role of fatty acid residues

The proposed model of polylipids brings us closer to solving the problem of the purposefulness of bacterial biosynthesis of fatty acids with different structures of fatty acid residues.

Calculations indicate that at the dimer configuration of phospholipids in the membrane, only the terminal sections of fatty acid chains containing more than 12 carbon atoms are actually accessible for direct contact with amino acid residues of proteins. In most Gram-negative bacteria, fatty acids contain 14 to 18 carbon atoms. With the lamellar structure of the phospholipid layer, this difference range is negligible, and, therefore, it is unclear why a 5-15% change of the average length of fatty acid residues should correlate with substantial changes in morphology, physiology and biochemistry of bacteria. Based on the proposed model, however, the increase from 14 to 18 carbon atoms means a many-fold expansion of the area for lipid-protein contacts.

The polylipid model demonstrates a great extent of rationality in the organization of lipid-protein interactions in Gram-positive bacteria whose fatty acid residues are in structural accord with the residues of such non-polar amino acids as valine, leucine, isoleucine, and alanine. The same amino acids are biosynthetic predecessors of iso- and anteiso-fatty acids with 14 -17 carbon atoms [27], and their addition to the growth medium typically results in the increase of the contents of respective fatty acids [26]. According to the proposed model, an increase of the share of those amino acids in the amino acid spectrum of the average statistical protein should cause same changes in fatty acid spectra of lipids.

The concept of polylipids taken out of the context of other aspects of regulation of lipid spectra of bacterial cells, cannot answer the numerous questions that modern bacteriology has for lipidology. For instance, it is hard to explain why there are distinctive groups of bacteria that have fundamentally different fatty acid metabolism. Examples of such groups are: (1) all Gram-negative bacteria except for very few genera and species, e.g. *P. maltophilia* and *P. putrefaciens* of *Pseudomonas* genus; *Thermus* genus which belongs to the following group; and some others; (2) Gram-positive bacteria with fatty acids of iso- and anteiso-structures, as, for example, such phylogenetically different groups of bacteria [25, 29] as *Bacillus*, *Propionibacterium*, and *Arthrobacter*; (3) mycobacteria, nocardia, rhodococci, and animal-pathogenic corynebacteria; (4) lactobacilli and alike. These groups differ also in cytostructural organization, metabolism, physiology, and ecology.

A notable fact is that eubacteria of different groups differ in lipid metabolism more drastically than, for instance, all eukaryotes do. The cause of those differences may partially be explained by a controversy in the functioning of the prokaryote membrane system [3].

Enzymes and coenzymes

The membrane system of prokaryotic organisms lacks an expressed anatomic compartmentalization of membrane enzymatic processes, which is the key difference between eukaryotic and prokaryotic organisms. The prokaryotic type of membrane system organization causes tremendous difficulties for the cellular level regulation of the membrane enzymatic processes which involve same coenzymes. Strangely, this peculiarity of bacteria has been getting very little attention [3].

Based on molecular weights of the most widely distributed coenzymes that supply membrane-immobilized enzymes of small reacting molecules, such as protons, C1- and C2- compounds, etc., it can be easily established, by the Einstein diffusion equation, that at physiological temperatures, those enzymes cover within a second the distances that several times exceed the length of a bacterial cell. Knowing that, it is hard to understand, for instance, the secrets of coordination of the activity of dozens of types of enzymes, whose functioning requires pyridine nucleotide carriers in specific concentrations strictly determined by a current physiological state of the cells.

As to eukaryotic organisms, the above problem is solved by the presence of partially autonomous membrane systems which can provide necessary conditions for physiologically adequate functioning of enzymatic systems – firstly, by selective distribution of enzymes in intracellular membrane systems; and, secondly, by creating optimal concentrations of coenzymes and cofactors in the membrane's closed compartments. Prokaryotes must possess other mechanisms, one of which probably consists in mostly allosteric regulation of the activity of membrane enzymes by changing the composition of lipids that surround the enzymes. In its turn, changes in lipid composition can be done through changing the concentrations of coenzymes involved in lipid biosynthesis.

Principle of quasi-equilibrium of biosynthesis of bacterial lipids

The concept of polylipids sheds light upon the role of lipids in coordinating the activity of membrane-immobilized enzymes with the activity of coenzymes and cofactors dissolved in the cellular fluid. Changes in individual lipid compositions influence the conformational changes in enzymes and their location in biological membranes, which, in its turn, changes their activity. It seems that for such changes to be in accord with coenzymes three main conditions must be met: (1) there have to be two types of lipid biosynthesis control by coenzymes and cofactors – strict and non-strict; (2) the control has to be provided in the near-equilibrium area; and (3) allosteric regulation of enzymes of a given particular organism or group of organisms must be specifically adapted to the effect of the lipid environment.

The first of the three conditions is required because, along with the problem of regulation of individual enzymes, there is a problem of coordination of the activities of enzymes in enzymatic systems. In enzymatic systems, the roles of same coenzymes can be drastically different. The third condition is determined by the well-known fact that the mechanisms for allosteric regulation of the membrane's homologous enzymes, isolated from different organisms, are different and use different spectra of isoenzymes. We will focus more closely on the second condition as its practical realization in bacteria can be easily proven.

All eubacteria synthesize saturated normal fatty acids. Their biosynthesis occurs due to cooperation of seven enzymatic systems: acetyl-CoA carboxylase, acetyl-CoA-ACP transacylase, malonyl-CoA-ACP transacylase, β -ketoacyl synthetase, β -ketoacyl reductase, β -hydroxyacyl dehydrase, and enoyl reductase [28]. The last four stages occur upon each successive bonding of the C_2 unit.

All partial reactions are reversible – however, it does not mean that the anabolic and catabolic processes involved in regulation of biosynthesis of normal saturated fatty acids differ only in the reaction direction. Differences between direct and reverse reactions become more expressed in case of fatty acids with C_1 -modified fatty acid residues, such as cyclopropanic, monomethyl-substituted.

In view of the above, the equilibrium of biosynthesis of fatty acids in bacteria exists not due to but in spite of the known molecular mechanisms. Nonetheless, and irrespective of whether or not we are ready to understand the general and fine mechanisms of this phenomenon, equilibrium is the fundamental principle of biosynthesis of lipids – both whole molecules and parts of molecules, i.e. fatty acid residues – in bacteria. Clearly, the means for maintaining such equilibrium are very much different from those used in non-biological systems, and therefore the term ‘quasi-equilibrium’ is more appropriate for this phenomenon.

Indeed, in bacterial populations, there are certain barriers that prevent the establishing of equilibrium by regular means known in thermodynamics of chemical processes: intercellular, intermembrane, axial, and intramembrane lateral barriers, of which only the latter seems to be more or less surmountable. As well, it is hard to explain how the equilibrium constants stay unchanged in a bacterium population where the ratio between live and dead cells varies according to certain laws that are both complex and unknown to us.

The (quasi-) equilibrium of lipid biosynthesis can be easily discovered based on the law of mass action. Under (quasi-) equilibrium, the ratio of the mathematical product of concentrations of synthesis products to the mathematical product of initial substrates’ concentrations raised to the power that equals their stoichiometric coefficients, at a given constant temperature, must remain constant at changing concentrations of individual substances. Examples of equilibrium of biosynthesis of phospholipids and fatty acids in various species of bacteria were previously provided by us in [2, 4-7, 19]. In the context of this paper, it should be important to discuss one of the examples demonstrating that the effect of environmental physical factors on the lipid composition of bacterial membranes can be explained and predicted without the recourse to biophysics-based simplifications such as “melting and solidification of membrane lipids”.

Fig. 2 shows a correlative dependence between elongation indexes of fatty acids of four strains of bacteria of the genera *Arthrobacter*, *Curtobacterium* and *Microbacterium*, grown for 63 hours on slanted meat-peptone agar medium at 19° and 29° C. According to the principle of (quasi-) equilibrium of lipid biosynthesis, the elongation index A of a series m fatty acid with the n number of C atoms equals:

$$A = \ln \frac{[m_n + 2]}{[m_n]} = \ln K_{m_n} + \ln \frac{\bar{a}}{a}$$

where a and \bar{a} are concentrations of the coenzyme involved in the transfer of a C_2 unit to fatty acid m_n in free (CoA) and activated (malonyl-CoA, acetyl-CoA) forms, respectively; and K_{m_n} is the constant of quasi-equilibrium of the reaction of elongation of fatty acid m_n ; concentrations of the substances are indicated in square brackets.

The plot shown in Fig. 2 demonstrates that the quasi-equilibrium principle is strictly observed in the acids that not only have different melting point temperatures but also their melting point temperature dependences are different. It appears that the pattern of the fatty acid chain elongation in the course of raising the cultivation temperature by 10° does not depend on the concentration of each acid at the initial temperature of 19°C. For instance, 19°C the content of fatty acids a15:0, a17:0, i15:0 and i17:0 in *Arthrobacter oxydans VKM 663* was 72.57, 9.95, 3.63, and 0.14%, respectively; whereas at 29°C the amounts of the same fatty acids were: 63.71, 16.43, 4.58 and 0.45%, respectively. The assessment of temperature-based fluctuations of individual fatty acids’ presence in a bacterium population does not reveal any specific pattern, and at first glance those fluctuations seem to be chaotic. The linearity of the curve shown in Fig. 2 is a result of (a) quasi-equilibrium and (b) identical temperature-dependences for the quasi-equilibrium constants and coenzyme concentrations, which can be explained by the physiological and biochemical affinity between the coryneform bacteria strains under study.

The quasi-equilibrium principle helps to understand the origin of the diversity of fatty acid spectra in eubacteria and its relationship with environmental factors. First of all, within the limits of the four groups of the afore-mentioned bacteria, it sheds light upon certain general regularities that are common for the genera and families represented by each of the four groups. For instance, in Gram-negative bacteria, *Mycobacteria* and alike, as well as *Lactobacilli*, the main reactions occurring during the quasi-equilibrium modification of fatty acids are dehydration, methylation, and elongation; and in all cases, without any exceptions, growth and development consist of two stages. During the first stage of bacterial growth and development, lipids in general and fatty acids in particular undergo demethylation; whereas the second stage is accompanied with methylation. There have been found no exceptions from this rule. Lipids act as a depot of freely convertible methyls that are released at the peak of a bacterium’s physiological activity. In Gram-positive bacteria that synthesize branched fatty acids (group 2), no lipid methylation occurs at all. Dehydration occurs in only some of the bacteria of this group and most often is a species or strain feature. In such bacteria, the fatty acid composition is regulated by their predecessors – mainly, amino acids – which, upon their deamination into ketoacids, initiate the biosynthesis of various branched acids [26, 27].

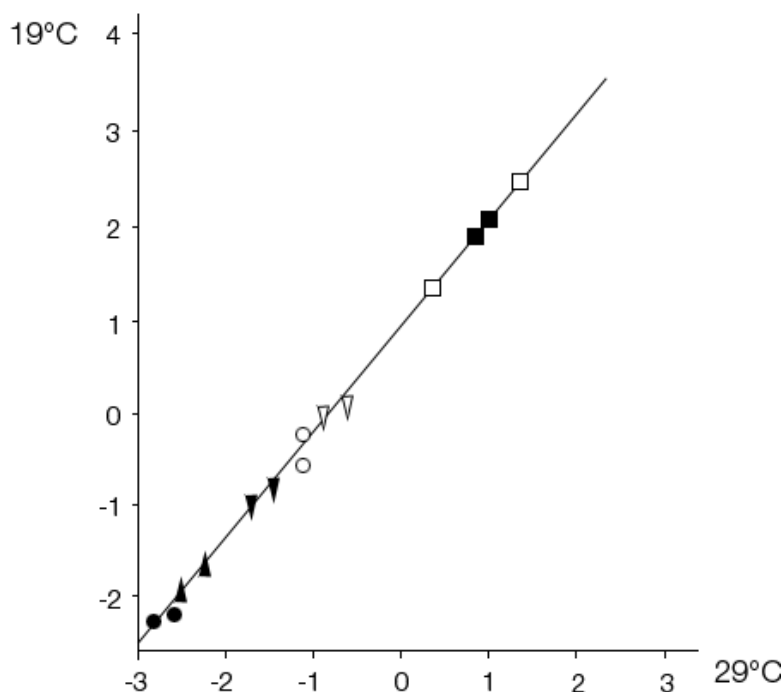


Fig. 2. Correlative dependence between elongation indexes of fatty acids of bacteria grown for 63 hours on slanted meat-peptone agar medium at 19° and 29° C. White color symbols are used at data points for *Arthrobacter oxydante* VKM 663 and *Arthrobacter nicotinae* VKM 803; black color symbols, for *Curtobacterium testaceum* VKM 1229 and *Microbacterium laevaniformans* VKM 1215. Symbol shapes indicate fatty acids: iso- even -▼- (n=14); iso- odd -▲- (n=15); anteiso-odd -●- (n=15); saturated normal -■- (n=14).

Thus, the quasi-equilibrium principle³ provides the understanding of physiological and biochemical significance of the diversity and specificity of lipid composition of bacteria. Obviously, one can speak of the morphological role of the lipid composition, as in many bacteria morphological differentiation seems to be designed to provide compartmentalization of membrane enzymatic processes as a solution for resolving the conflicts between enzymes and coenzymes.

As was mentioned above, groups of bacteria which have distinctive peculiarities of lipid metabolism, have also distinctive specifics of their cytostructural organization, general metabolism, physiology and ecology. No doubt that these factors – as complex as all the phenomena of the biological level of complexity – had been the determining factors in natural selection and evolution of the ways for lipid metabolism organization. Therefore, no matter how thoroughly and scrupulously we explore the lipid metabolism of live organisms, the questions of why it is organized in a certain particular way and not in any other, which of its features are common in all organisms, and why it is different in different groups of organisms – will remain unanswered as long as we do not have a sufficiently clear understanding of how the life had emerged and evolved on Earth.

On the origin of life on Earth

In an article on the origin of life on Earth, Woese – criticizing Oparin’s prebiotic broth hypothesis – theorizes that life could have emerged in microdroplets of water that was present, in the aerosolized state, in the prebiotic atmosphere containing hydrogen and carbonic acid [32]. Per Woese, microdroplet water suspension was that very biochemical reactor in which the basic metabolism could have emerged, which, in its turn, should have resulted in the emergence of methanogens, the first possible life form according to Woese. This original theory of Woese’s shares at least one of the points of Oparin’s theory [13, 15]: that life must have first emerged in a phase-separated system, i.e. the medium in which the main events were developing was characterized by a higher ratio of phase interface to phase volumes. Phase interface have higher levels of free surface energy, hence an increased capability to concentrate

³ For more information about theoretical and practical applications of the quasi-equilibrium principle, see, e.g.:

L. Andreev. Quasi-equilibrium as a general principle of regulation of lipid composition of eubacteria. In: *Environmental regulation of microbial metabolism*. I.S. Kulaev, E. A. Dawes, D. W. Tempest (Eds.). Academic Press, London, pp. 161-185, 1985.

L. Andreev. Taxonomic calculations based on fatty acid spectra of bacteria. Requirements for chromatographic analysis of fatty acids. In: *Rapid Methods and Automation in Microbiology and Immunology*. K.-O. Habermehl (Ed.), Springer Verlag, Berlin, Heidelberg, New York, pp. 265-273, 1985.

L. Andreev et al. Grouping of *Staphylococcus* species based on their fatty acid spectra. In: *The Staphylococci*. J. Jeljaszewicz (Ed.). Gustav Fisher Verlag, Stuttgart, New York, pp. 151-155, 1985.

substances from the volumes of adjacent phases and thus facilitate the occurrence of chemical reactions. Phase-separated systems have a number of peculiarities that are significant in the context of scientific reconstruction of the prebiotic evolution processes [10]. The examination of those peculiarities can help to narrow the action arena of the forces that have produced Life and to propose a certain general scenario whose implementation in any of the countless possible ways could have led to the emergence of live organisms. However, by the modern science standards, this kind of substantiation is not sufficient for asserting that life on Earth has spontaneously self-emerged on Earth and not as a result of panspermia or the six-day creation by God.

The majority of contemporary scientists tend to maintain that the emergence of life was a pre-determined result of the substance and energy properties exhibited in the course of progressive evolution of the more and more complex carbon compounds and macromolecular open systems formed from them [15]. This idea has inspired much optimism and created many believers in the feasibility of discovery of the origin of life by exploring into molecular foundations of the functioning of current forms of life. However, the concept of spontaneous self-emergence of life on Earth remains to be more of an article of faith than a subject of strong scientific proof. To many scientists, the life origin scenarios which explain both the most important and the least probable processes by referring to predetermination, spontaneity, and various tunnel effects are no longer credible, and some of the researchers turned to believe that terrestrial life could not get started on Earth⁴.

In live organisms, various substances – from metal ions to huge macromolecules – are involved in an incredibly complex network of interrelations, in which the subordination is both strict and flexible. The cause of such duality may lie in the conflict between the historic order of formation of the substances that have a general biological importance and their functional roles in the currently existing live organisms. Even though the probability of spontaneous emergence of macromolecules with a biologically purposeful structure is estimated as a decimal fraction with hundreds of zeros at the right of the numerator, most scientists agree that it was the formation of functionally active biopolymers that gave the impetus to the emergence of life, whereas a major point of disputes is: which of the two – proteins or nucleic acids – were formed first? Pre-biological self-development of macromolecules is viewed upon as a certain harmony between the physico-chemical nature of substances and the environment, which eventually leads to self-assembly of a functionally holistic structure out of parts that have emerged and reached structural and functional perfection outside that structure. If the emergence of life was thrust by the organization of a system of such macromolecules as DNA, RNA, or a protein, which had previously become “almost live” due to purely physico-chemical mechanisms, then it should be perfectly logical to look for such mechanisms which, of course, must be capable of spontaneous self-implementation.

There is a great deal of publications describing possible scenarios of the emergence of life. Some of the scenarios are based on certain pre-defined initial conditions – cf. hypotheses by Oparin [13-15], Fox [17], Woese [32]. Others focus on certain general laws of physics and chemistry which could provide the foundation for self-development of functionally active biopolymers and their self-assembly into something similar to a live cell – e.g. the principle of kinetic perfection proposed by Shnol [18], a hypercycle according to Eigen [23, 24], etc. Following those discussions, the notion of natural selection of macromolecules has become popular but has not provided any clarity as to a definite purpose of such selection. The purpose is being set by the proposing researchers based on retrospective analysis of the known properties of the living matter. Here is, for instance, a plan for animation of inorganic substances, proposed by Eigen and Schuster in their trilogy, *Hypercycle* [23, 24]. The whole process consists of six stages (the quotes below are from a discussion by Volkenstein [9]):

“1. The emergence of macromolecules is determined by their structural stability and monomer contents. The early stage polymers were protein-like chains and a few RNA-like polymers capable of replication.

2. The composition of primary polynucleotides was determined by concentrations of monomers. Sequence reproduction depends on the accuracy of copying, which is higher in GC sequences. Reproduced sequences formed the quasi-species distribution.”

After the next three points, there follows:

“6. That organization further developed by taking advantage of favorable phenotypic changes. It appeared that a fair selection of specific genotypes required spatial separation”.

The above plan is a good example of transformation of logical cognition techniques through gradual replacement of induction by deduction, which is getting increasingly widespread in the contemporary science about the origin of life. As of now, there is a lot of established truths that raise no doubt – such as, for instance, the role of DNA as a carrier of genetic information – which leads many scientists to believe that the deduction of such truths from other, more general, truths is more productive than the formulation of scientific laws based on concrete experimental data and models.

The said tendency is clearly seen in theoretical studies into the origin of life. However realistic the verification of the heretofore proposed hypotheses may seem to be, as of today they are as dogmatic as creationism, as, in the essence, they boil down to the postulate about spontaneous self-formation of biologically purposeful macromolecules. In reality, a discussion – at whatever high scientific level – of the process of self-formation of such macromolecules can be scientific only in the part concerning the *post factum*, not prior, events. Therefore, scenarios like *Hypercycle* are regarded by serious researchers in the origin of life as “The Game of Life”, even if it is played by talented physicists, chemists, and mathematicians.

Volkenstein notes that the model by Eigen and Winkler obviously does not claim to be a re-enactment of actual events that had been occurring on Earth at the time when life was emerging but aims at proving the possibility of self-organization of the matter, based on the known principles of physics [9]. So, it would appear that the main road leading down has been cleared, and all what is left to be done is to solve particular problems – for instance, to find the right place for DNA, the “soul” of the biological cell, so it should be able to continue the process of spontaneous development. But this, too, appears to have

⁴ The original Russian text includes a quote from an article by Ch. Wickramasinghe in UNESCO Courier [8].

been theoretically solved, and the path from the general to particulars seems to be even cleaner – this, of course, refers to phase-separated systems, such as, for instance, coacervate droplets as proposed by A. Oparin. In one of his latest works, he wrote: “Initially, at the molecular level, only protein- and nuclein-like polymers, devoid of any “biological purposefulness” in their intramolecular structure, could emerge... Only when combined in multi-molecular phase-separated systems, those polymers, interacting with each other, were capable of mutual coordination of their intramolecular structures and functions within a system. Natural selection of phase-separated systems had also determined the emergence of biological “purposefulness” and specificity of polymer structures, as well as the life-specific form of information transfer (heredity) [15].

In fact, all of the known hypotheses about the origin of life differ from each other only in the part concerning the view on the kind of setting that is most likely to have been proper for a sudden materialization of the key “figure” – DNA. The main requirement to such a setting is that it should represent a structure of the live cell type but made of inorganic matter.

Phase-separate systems certainly seem to be by far more natural as an environment for the emergence of life, than, for instance, simple water solutions. Nevertheless, natural selection, by itself, of phase-separated systems can result in neither the “biological purposefulness” and specificity of polymer structures, nor the emergence of the “life-specific form of information transfer (heredity)”. That would be possible only if phase-separated systems themselves had a biologically purposeful structure or were capable of performing biological functions at least at the primordial, maximally reduced level. To assume that the first of these two conditions was met would equal the assumption that a biologically purposeful structure of the primitive phase-separated systems had spontaneously emerged by itself – which is even less probable. As to the functionality, it could have developed only in the presence of an adequate structure.

Macromolecular chronometry

It would seem that this logical deadlock could be resolved by comprehensive investigation of regularities in the evolution of those macromolecules which perform same functions in evolutionarily distant contemporary organisms. With the fundamental capabilities created by molecular biology methods, it is possible now not only to investigate evolutionary relations between various organisms but also to estimate a relative time point of their divergence, i.e. evolutionary distances. The idea of the “molecular evolutionary clock” was first proposed by Zuckerkandl and Pauling [34] in 1965. It is based on the fact of existence of a great variety of macromolecules that, having different sequences of monomers, are capable of performing same functions. Consequently, mutational changes in proteins and nucleic acids can provide a measure of the evolution time.

Woese [33] recently made a detailed analysis of the results obtained with the use of the molecular chronometry method and discussed the ways for further improvement of the method and overcoming its limitations. For instance, one of the major limitations of the method is caused by the fact that the relative speed of homologous macromolecular clocks can be different in different organisms. Also, in bacteria, an intensive interspecies transfer of genes with totally different evolutionary backgrounds may contribute into different genealogy of macromolecules of one and the same organism. Both of those limitations can be resolved in one or another way. For instance, it was shown that the use of two independent molecular clocks – cytochrome c and ribosomal RNA – provided consistent data on purple synthesizing bacteria [22].

Phylogenetic structures based on molecular chronometry studies [25, 29] attract a lot of interest in biologists of various areas of specialization, and there is a hope that this method can help to develop objective criteria for evaluation of evolutionary relations between various groups of organisms. Nonetheless, the areas in which molecular chronometry is either ineffective or insufficiently effective include, first and foremost, the issues of prebiotic evolution, emergence of life and the functioning of early life forms. It is also important to realize that the discovery of the paths of evolution of life on Earth is not an answer to the question about the reasons for the choice of those paths.

Interpolation of the regularities of evolutionary changes in the structure of most conservative macromolecules to the possibly distant past has resulted in a seemingly substantiated concept of progenote, the last common ancestor of urkaryotes, eubacteria, and archaeobacteria – the “forefathers” of the three contemporary kingdoms of life forms [29, 31]. However, the question of where that proto-ancestor came from will send us back to the idea of spontaneously emerging biologically purposeful macromolecules. Comprehensive studies of the fine structure of biological macromolecules have not added anything new into the understanding of how such molecules could have emerged in the ancient Earth atmosphere, and, if that did occur, then how exactly it could have led to formation of systems with some signs of life and sufficient for the start of evolution towards living organisms.

Molecular model of a protobiont

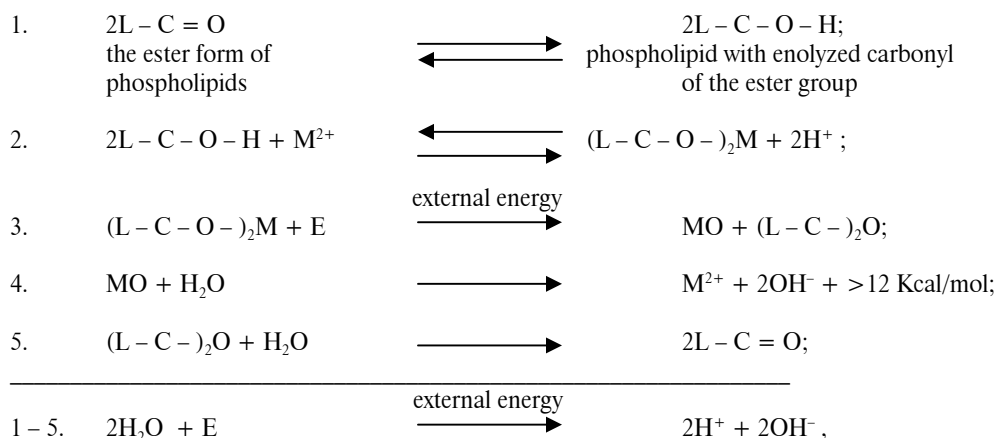
Previously, we proposed a molecular model of a hypothetical structure capable to accumulate energy in certain conditions as the earliest structure that has the properties of a biologically purposeful organization, or – more precisely – as a structure that could have evolved into something very similar to the biological cell [1]. To a certain extent, this hypothetical model represents a molecular model of a protobiont, although it does not touch upon the reproduction issues.

In the proposed model, the role of a phase-separated system evolving into a protobiont is played by coacervate formations, built by the principle of the polylipid membrane. Supposedly, in the process of evolution, most of the mechanisms underlying the self-development of phase-separated polylipid formations had been replaced by significantly more efficient mechanisms, and therefore it is practically impossible to locate them in the contemporary forms of life. Particularly, most of the processes that were dependent on physical environment had acquired a highly efficient regulation factor, such as a large assortment of structural and catalytic proteins.

Other mechanisms, in particular, membrane oxidative phosphorylation and photosynthesis, which had achieved the highest level of evolutionary improvement as far as their respective underlying principles are concerned, have been kept unchanged by most of the contemporary forms of life.

Phase-separated systems organized by the above-described principle of the polylipid membrane could spontaneously emerge at the very early stages of chemical evolution as a result of physical factors – for instance, high compression as a result of impact of ultrasound waves that cause conformational changes in the polylipid structure. Lipids that were required for their emergence were available from fatty acids, glycerin and phosphoric acid which were present in the primordial soup. Formation of the polylipid membrane involved ions of Mg, Ca, K and Na, most widely distributed in the Universe.

Under the influence of mechanical or thermal energy from external sources, the polylipid membrane could provide for formation of primary macroergic compounds – oxides of bivalent metals (Mg, Ca) which are known to release considerable amounts of energy (more than 12 Kcal/mol) when interacting with water. Formation of metal oxides involves the dissociation of the bidentate complex produced by substitution of protons of enolyzed phospholipids by a metal ion. This whole hypothetical process includes several stages:



where L is an abstract phospholipid.

Thus, the overall effect of the above-shown hypothetical process is dissociation of water. However, if during this process, the metal oxide was transferred to the opposite part of the phospholipid membrane, it would cause the separation of charges, placing the protons on the membrane outer side, and hydroxyls on the inner side. Hydroxyls could recombine into hydrogen peroxide whose decomposition (for instance, under the influence of iron salts) was releasing atomic hydrogen. The latter could interact with the gradient-driven protons from across the membrane, as well as participate in oxidation of the dissolved organic substances.

Already in this version, the proposed hypothetical process of energy concentration, which – as is seen from the above formulae – does not require free oxygen, has all the necessary prerequisites to allow the primitive phase-separated systems, within which it could supposedly develop even at a negligibly small scale, to evolve into systems with the signs of biological purposefulness. This is an example of a quite concrete model of the emergence of life, which excludes a “voodoo” element that is present in all of the heretofore proposed hypotheses on the origin of life.

Within the context of this model, photosynthesis, for instance, can be viewed upon as an improved variant of the primitive energy production process in prebiotic systems. As is seen from the formulae of the known chlorophylls, all of them contain enolyzed carbonyl at the 9-position of the porphyrin ring. Substitution of protons of enolic hydroxyl groups in chlorophyll molecules by a Mg atom from the porphyrin ring of other chlorophyll molecules could result in formation of aggregates, which is a known [11] but unexplained fact. The influence of light could cause the decomposition of such aggregates by the same mechanism that, according to the model of primitive energy production, provided the metal oxide formation. That means the formation of chlorophyll molecules containing highly active magnesium oxide, coordinated in the porphyrin ring, which in that form can participate in various syntheses and, in particular, interact with carbon dioxide. Thus, from the standpoint of the proposed model, photosynthesis as a method for transformation of the energy of light into the chemical energy could in general be implemented in primitive systems that did not yet know the synthesis of bio-purposefully organized proteins and nucleic acids.

As was earlier noted, formation of MO (metal oxide) – the earliest macroergic compounds – was occurring when the rigid, asymmetrical structure of the polylipid membrane was exposed to a mechanical impact. In the course of time, the response to occasional environmental impacts developed into efficient and organized conformational changes in the membrane-embedded proteins. Thus, polylipid membrane was serving as a sort of template for the purposeful selection of proteins whose spontaneous synthesis was occurring under direct energetic control. Evolution of proteins was developing in two directions. One group of proteins, by building-in to the polylipid membrane, was improving the membrane structure to facilitate energy concentration from external sources. Another group of proteins which were capable to change their conformation due to certain factors – for instance, interaction with protons – was used to increase the efficiency of the mechanism of MO production based on utilization of the energy of chemical transformations.

The application of MO as a macroergic compound was restricted to polylipid-protein membranes of the earliest phase-separated systems, as metal oxides could not survive in water systems. Therefore, there had been developed and maintained a primitive mechanism for transformation of the primary macroergic compounds – metal oxides – into secondary macroergic compounds, which are supposed to have been polyphosphates, as well as the third generation macroergic compounds – nucleoside phosphates – which became possible as the metabolism system was developed. This should explain the macroergic properties of these compounds: it is apparently due to their history of being the mediators of that critically important role that metal oxides played in the emerging metabolism.

As was previously proposed [5, 8, 12, 25], the transition from primary to secondary and tertiary macroergic compounds involved cardiolipin, one of the most easily synthesized phospholipids, which consists of two residues of phosphoric acid, bonded with three molecules of glycerin, two of which are esterified with fatty acids. The role of the phosphate group donor was played by a cardiolipin salt produced at cardiolipin interaction with MO. After elimination of the phosphate group, cardiolipin was turning into phosphatidyl glycerin, no longer capable of linking the polylipid chains, which made that area of the polylipid membrane incapable of producing MO until the cardiolipin re-synthesis from phosphatidyl glycerin by connecting it with the phosphate group and diglyceride.

To produce macroergic oxides, the early polylipid membrane needed high energy, which, in its turn, demanded that the system had a highly cooperative structural-functional organization – one of the main properties of the live matter. Had the protobiont an easier way for accumulating the energy from outside, it would probably never had a chance to evolve into a live biological cell.

It is also possible to visualize a model of the early primitive mechanism that could further provide for information accumulation and reproduction in the form of nucleic acids. It could consist in condensation of phosphoric acid residues in those areas of the polylipid membrane where MO was produced. In the same areas, under the influence of MgO, the synthesis of carbohydrates from formaldehyde could occur. Thus produced oligomers were able to carry the information about the optimal topography of monomer phospholipids most helpful in MO production and, therefore, to become a template of the optimal structure of the polylipid membrane.

Conclusions

We have presented the outlines of a few hypotheses concerning the biological role of lipids. Some of positions of these hypotheses are extremely hard for experimental proof or disproof, at least at present time. By presenting those hypothetical mechanisms, we mostly wanted to draw the attention of researchers in the origin of life to the fact that there are other logical roads that can take to solving the problem of the emergence and evolution of the Earth-based life on the molecular level, than those where every significant event starts with the miraculous appearance of “live” macromolecules. In the proposed model, both the “enlivening” and evolution of functionally active macromolecules occur in the course of the protocell “enlivening” and the cell evolution. This principle – of the primary unity and inseparability of the molecular and cellular levels of life processes – is obviously an advantage in designing a model of the origin of life.

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