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# ALTERATION OF SUPERMOLECULAR STRUCTURE ON AGGREGATION

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Abstract Morphological peculiarities of supermolecular structure of native and model lyomesogenes on aggregation are discussed.

## INTRODUCTION

Peculiarities of supermolecular structure of native lyomesogenes, such as bile, serum and other liquids of living organism are tightly connected with state of health.[1–3] Thus it was shown[4] that the crystal nucleation and mesophase texture formation time in bile and its analogues depends on saturation with lecithin and on gall bladder function. So conventional phase diagrams for bile were built by Admirand and Small[5] and those for serum by Small and Shipley.[6] These diagrams in coordinates "lecithin–bile salt–cholesterol–water" and "lecithin–cholesterol–cholesterol esters–water" respectively are based on microscopical and X-ray analysis of lyomesogene structure and play an important role in

physico-chemical theory of pathogenesis.[7]

Later numerous papers devoted to structure of biomembranes and their components were published.[8] If the lyomesogene system "lecithin–water" is used as a basic model system for investigations of various effects produced by dopants and physical fields, then morphokinetic features of its aggregation are well known.[9] However, there is no phase diagram generally accepted for this system till now because of its inherent non-uniformity (heterogeneity). Thus the diagram has several versions.[10–12] Further considerations proceeded from the version based on diagram[12] modified by Popov et al.[13] It helps to interpret aggregation process peculiarities in native and model systems.

## STRUCTUROSCOPIC APPROACH

In studies of supermolecular structure alterations the optical polarizing microscopy method in its time-lapse modification is widely used. Elaboration of each new diagnostic test based on morphokinetic aggregation analysis includes:

– thorough microscopical investigations of samples;
– determination of optimal time and temperature conditions of the analysis;
– ascertaning the reference texture features;
– working out the code system which allows to use computer morphological data processing;
– computer analysis of structural and clinical data.

The whole procedure and its subsequent stages were developed

in our laboratory as the analytic system "MESOTEST". Physical aspects of morphokinetic approach are based on investigations of patterns formed in standard conditions, namely those of behaviour of linear defects (presumably disclinations) and texture transitions. Aggregation proceeds in isothermal conditions (at 295 or 310 $K$) in thin lyomesogene layer between two slides with initially homeotropic orientation and is due to evaporation from the edges. Thus we suppose that the basic system "lecithin–water" passes through all phases along the line corresponding to the temperature chosen on the diagram.[13] Discrepancies to the quasi-equilibrium diagram may be due to the influence of its composition and are revealed by formation of specific morphograms. Strongly non-equilibrium conditions (the temperature is lower than the physiological one; thin layer and changes of concentration) allows to reveal trends of texture formation rather quickly (nearly 24 hours) in comparison to isothermal (at 37.5°$C$) exposition of bulk samples (up to 21 days[14]).

On initial stages of elaboration of any diagnostic test we study reference model systems "lecithin–water" with dopes of salts (NaCl, KCl, $CaCl_2$, $MgCl_2$, $MgSO_4$) in physiological concentrations, main components (bile salts, cholesterol or cholesterol esters), bioactive agents (hystamine, acethylcholine chloride), vitamins, oxidation activators or inhibitors respectively, by means of microscopy, electron-positron annihilation and IR spectroscopy.[13,15,16] The model type is determined by the media analyzed.

For the universal morphokinetic study research the biomicroscope MBI-15-2 (USSR, 1989) together with the IBM-PC computer was used. Sample volume was about 0.05 $ml$, the lyomesogene layer thickness was 3–5 $\mu m$; sample exposition lasted from 15 minutes to 72 hours after preparation.

We used standard ampulled L$\alpha$-lecithin produced by Kharkov Factory of bacterial preparations and the standard procedure of basic system sample preparation described elsewhere.[12] The dopants required were chosen with purity guaranteed pharmacologically and checked-up analytically.

## EXAMPLE OF APPLICATION

Because morphological approach is the most attractive one for media which may be obtained by simple non-invasive method and is also attractive for developing visual methods of monitoring in hospitals and in medico-ecological control we worked out special instruments for the optical microscope–computer videoanalysis. They include recognition of typical textures, filtration (simplification) and quantitative analysis of images. From this point of view oral cavity liquid is of great interest. As a matter of fact, it is a diluted water solution of salts with small amounts of mucus and cell elements which does not possess any lyomesogenic properties if not additionally treated. Under such treatment we understand sedimentation or stratification or imbedding into 10% ethanol solution of the lecithin standard matrix with known morphokinetic aggregation characteristics, as it is proposed in this paper. Data

on the salt dopes influence are published elsewhere.[13,15,16] For this system the $Na^+$ and $K^+$ effect is of primary interest.

It was shown earlier that physiological $Na^+$ concentrations do not cause significant morphology changes[17] while $K^+$ changes strongly the texture transition sequence by widening the area of myelin forms and narrowing that of confocal textures on the morpho-kinetic diagram.[16] This fact is of great diagnostical value because an individual ability of the patient to adaptation correlates with the $Na^+/K^+$ balance in his oral cavity liquid.[18] Statistical data obtained on large groups of children with various adaptability according to respiratory disease index show that the texture differences are reliable. Additional studies according to the procedure described showed that morphological "signs" based on the texture behaviour of the "lecithin solution–oral cavity liquid" system may be used in gastroenterology and allergology as well.

## PECULIARITIES OF AGGREGATION

We found the following sequence of supermolecular aggregation changes typical for normal native probes and model systems with physiological salt concentrations:

| | | | | |
|---|---|---|---|---|
| isotropic texture with homeotropic orientation | – – single myelin forms | – – oily streaks or complex configurations of myelin forms and isolated confocal domains | – – confocal texture | – – fan texture |

Polygonal textures consisting of metastable parabolic domains[9]

are usually absent. Confocal textures originate primarily from end cups of myelin forms and due to fusion of areas with initially isolated domains. Oily streaks are rows of confocal domains with tilted general axes. Their reorientation leads also to formation of fields of confocal domains. Fan textures are rarely formed, beginning in confocal texture, and consist of small "morphological units".

Reorientation of large molecular complexes observed at optical magnifications is initiated by layer relaxation due to varying thickness during evaporation from the edges.

If the composition differs from the normal one then specific morphological features appear. Some sequence stages may not take place. Thus at large concentrations of $K^+$ ions the stage of myelin forms is prolonged and this texture is predominant. Bundles consisting of 2–20 parallel myelin forms are formed. Confocal textures are rarely formed while fan textures are much more developed. Their formation starts from the appearance of a set of radial lines in the bundles regions of maximal curvature. These lines can be considered as rows of edge disclinations in the system of conjugated bilayers (Figure 1). Hence stresses near the head of the row are high and the configuration consisting initially of continual tubes is cut into segments. Further relaxation towards new continual tubes is cut into segments. Further relaxation towards new conditions, accompanied by interaction with dislocations, leads to formation of fan-shape "morphological units". This texture transition type is never observed in "normal" systems.

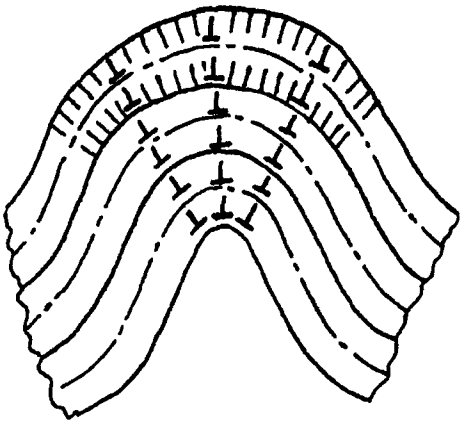

FIGURE 1 Dislocation scheme of transition myeline forms–fan texture.

Another example of specific textures is an "expanded fan texture" appearing as isolated regions bordered by a row of small confocal domains and occupied by rather regular, nearly radial oily streaks with dark iso- and homeotropic areas between them. One can suppose that formation of these "atypical" textures is due to arrangement of files of disclinations which through the texture visualizes areas with different elastic properties. Detailed description of this texture will be published in further papers.

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