Diagnostic markers in the structures of human biological liquids

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ABSTRACT

Introduction: To date, morphology research in laboratory diagnostics has referred only to cell tissues and excluded biological fluids. Our new technology is based on the study of structures of biological fluids formed during transition from liquid to solid state by dehydration under special conditions. These structures are formed thanks to the special "mosaic" configuration of molecules and microaggregates of the substance (protein and others) dissolved in the studied fluids. It should be considered as well that the specific structure of protein molecules is a fundamental basis of anatomical and physiological peculiarities of an organism. All pathological processes are initiated by the changes in the spatial configuration of protein molecules.

Methods: We have developed a special method named "cuneiform dehydration of biological liquids" to obtain thin films which possess specific structures. During the transition of a biological liquid into a solid, organic molecules build specific macrostructures in conformity with their configuration.

Results: Studies of the solid phase structures of different biological liquids allow us to find specific markers of various pathological processes in the human organism. In blood serum, we discovered markers of inflammation, sclerosis, chronic intoxication; in tear – markers of different stages of glaucoma; and in synovial fluid – markers of different stages of osteoarthrosis. In our investigation of 143 healthy people and 1,419 patients, a high rate of compliance of the discovered markers with the results of routine clinical and laboratory methods were observed. As the suggested technology has a higher sensitivity, the results obtained by this technology and routine methods do not always coincide.

Conclusion: The developed technology gives new information about the state of the human organism and may be used in practice for diagnostics of various pathological processes and at pre-clinical studies of their development, as well as for the evaluation of the efficacy of medical treatment.

Keywords: biological fluid, cuneiform dehydration, dehydration, diagnostic markers of diseases

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INTRODUCTION

Morphology of biological fluids is a new scientific direction formed by the researchers from the Russian Scientific Research Institute of Gerontology of the Russian Ministry of Health. Till now, the notion "morphology" in biology only referred to cell tissues, while biological fluids were not included in morphology research. Cuneiform dehydration, a special method worked out by the authors, became the necessary methodical basis for the research on the morphological structure of biological fluids. With this method of dehydration, a very thin film (facia) or a fixed thin "section" of the studied fluid is obtained. The structure of the facia is a macroscopic and integrated figure of all existing biological fluid complex molecular interconnections.

This article explains the mechanisms of interaction of biological fluid molecules. It also highlights peculiarities of these interactions, which determine the ability of molecules-effectors to find exact object-targets in an extremely complicated and dynamic molecular surrounding. Morphology of biological fluids gives clinical medicine and biology in general a unique methodical complex that enables objective monitoring of the state of the organism with due respect given to the constantly changing parameters of the endomedium. The suggested methodical complex is characterised by technical simplicity, is highly economical and can be used in laboratories of all hospitals. Processes of inter- and intracellular metabolism are carried out using biological liquids of the organism. All protein-producing cells use some protein for their own needs and release the remaining into the liquid media of the organism. Biochemical, physical-chemical, immunological and other methods

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of research on biological liquids give only fragmentary information on the state of the organism, whereas their structural analysis gives us general information on the structure. To obtain complete information of the organism, we have developed a special method of dehydration of biological liquids with a given angle section of dehydrated mass. This method is cuneiform dehydration.

METHODS

A drop of biological liquid (0.02 ml) is placed on a transparent surface. It is evaporated at 20–25°C and 50%–60% relative humidity with minimum movements of the surrounding air for 18 hours. The fine film, which we named facia, thus formed is an analogue of a histological section. Facia has a complex morphological structure. We carried out studies on the facia morphological picture with a Leica MZ 12 microscope at ×10 – ×100 intervals under a natural, phase-contrast and polarised light.

According to our theoretical ideas, in cuneiform dehydration, special mechanisms work, and provide for building a system and a subsystem with solid phase structures. The effect of these mechanisms is illustrated in Fig. 1. The scheme shows a drop of a biological fluid (located on a horizontal plane) in a frontal section along the diameter. The given scheme shows that vaporisation of the fluid is uniform across the entire open surface of the drop. Since the thickness of a half-sphere differs in the centre and on the periphery (a wedge), the concentration of dissolved substances during water vaporisation is non-uniform in the examined drop. In particular, the concentration in the thin (peripheral) parts increases far more quickly than in the central (thick) part. Given these changes, osmotic and oncotic forces start manifesting. Since the power of osmotic forces is about 200 times higher than that of oncotic forces, salts begin moving rapidly towards the centre of the drop, the area with a lower concentration of dissolved substances. At the same time, they take water from proteins and other molecular substances with a high molecular weight. As water vaporises, the solution becomes saturated and salts start "squeezing out" organic substances from water, transforming them into the solid phase. However, due to the very complicated composition of biological fluids, this process takes place in stages with the formation of concentration waves (areas) of the solid phase, which are formed at the expense of the appropriate components on the biological fluid with certain physical and chemical parameters (Fig. 1).

During cuneiform dehydration of biological liquids, molecules and supermolecules formed under the influence of osmotic and convector forces, intermolecular interaction and other physical and chemical processes are distributed into strictly determined places along the surface of the dehydrated drop in the shape of concentrated waves. The dynamics of the formation of a blood serum facia during cuneiform dehydration are shown in Fig. 2. A thin film (facia) is thus formed and its specific structure is determined by the whole complex of qualitative and quantitative parameters of biological liquids.

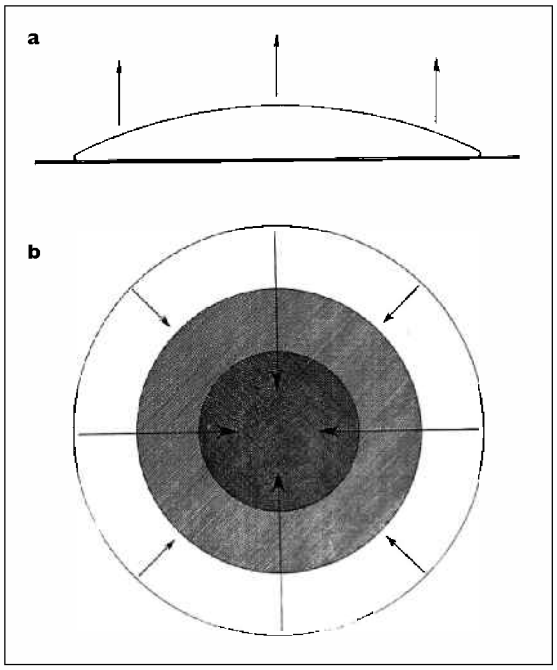

Fig. 1 A drop of a biological fluid on a plane (scheme): (a) sagittal section; (b) plan.

RESULTS

Study method of the structural organisation of a solid phase of biological liquids is rather simple. At the same time, the picture of facia of biological liquid is extremely complicated. The facias of different biological liquids of a healthy man are shown in Fig. 3.

Facia of a biological liquid is a standard thin "section" of a highly mobile tissue, structures of which are organised during dehydration. Structural information contains data not only about the concentration of elements that constitute a biological liquid, but mainly about the character of their interaction. The developed method enables a selective search for molecular disorders that occur in the organism at early developmental stages of a pathological process, when cell structures still keep their physiological forms, i.e. at the pre-clinical stage of the disease. Peculiarities of system organisation of blood serum were studied in 143 clinically-healthy persons and in 1,419 patients with different types of pathology, tear in 127 clinically healthy persons and in 52 glaucoma patients, synovial fluid in four patients without any clinical signs of osteoarthrosis and in 14 patients with different stages of osteoarthrosis (Table I). All patients were examined by clinical, laboratory and instrumental methods.

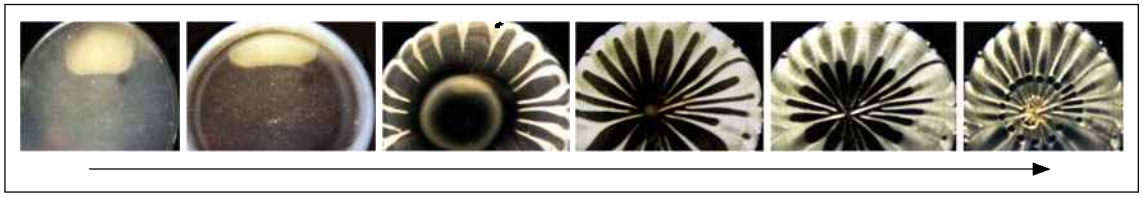

Fig. 2 Dynamics of the formation of a blood serum facia during cuneiform dehydration. (Magnification ×15).

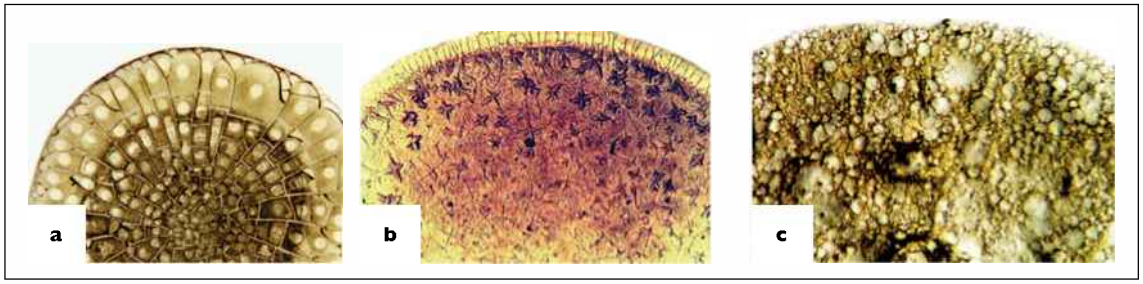

Fig. 3 Facias of human biological liquids: (a) blood serum; (b) tear; (c) synovial fluid (Magnification × 25).

Studies of the morphological picture of blood serum of clinically-healthy patients showed presence of different signs of pathology in 50 patients. Tear studies of 127 patients without any clinical signs of glaucoma showed presence of early stage glaucoma marker in two patients. Further observation of these patients by an ophthalmologist confirmed presence of the disease. Synovial fluid was studied in four patients with an open trauma of joints. Studies of the morphological picture of blood serum in 1,419 patients with different diseases showed presence of a sclerosis marker in 51 patients, a chronic intoxication marker in 206 patients, an inflammation marker in 27 patients without any clinical signs of the corresponding pathology. Divergence of morphological and clinical data is due to the higher sensitivity of cuneiform dehydration.

In all healthy patients, blood serum facias were similar and were characterised by symmetry of radial "cracks", numerous rounded formations on the whole surface of the facia (Fig. 3a). Tear facias had two zones: peripheral – protein, and central – crystal (Fig. 3b), and synovial fluid facias were characterised by a small pore structure (Fig. 3c).

It was the first time that the following specific markers of biological liquids of the organism had been determined:

- "leaf structure" is an index of tissue sclerosis,
- "tongue structures" is an index of inflammatory processes,
- "three-ray cracks" is an index congestion changes,
- "block cracks" is an index of acute disorders in the blood supply in the brain and heart,
- "crested structures" in the peripheral zone of a facia show disturbances in microcirculation at hypertensive crises,
- "patch structures" in facia periphery are markers of acute and chronic intoxication.

Table I. Correlation of morphological (M) and clinical (C) signs of a disease in the group of "healthy" people and in the group of patients.

Type of biological liquid	Type of pathology	Group of "healthy" people		Group of patients			
		n	M (+) C (-)	n	M (+) C (+)	M (+) C (-)	p (χ^2)
Blood serum	Sclerosis	143	14 (9.8%)	1419	875	51 (5.8%)	0.046
	Acute heart insufficiency		0		27	0	–
	Chronic intoxication		31 (21.7%)		414	206 (49.7%)	0.007
	Inflammation		5 (3.5%)		205	27 (3.7%)	0.006
Tear	Glaucoma	127	2 (1.6%)	52	52	0	0.363
Synovial fluid	Osteoarthrosis	4	0	14	14	0	–

(+): presence of pathology signs; (-): absence of pathology signs.

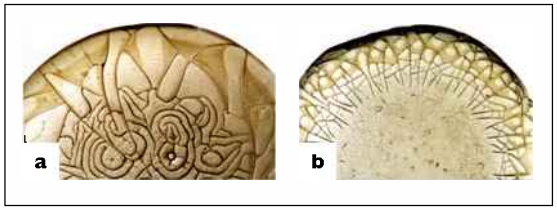

Fig. 4 Blood serum facias of a patient: (a) during a stenocardia attack; (b) 30 minutes after its removal (Magnification × 25).

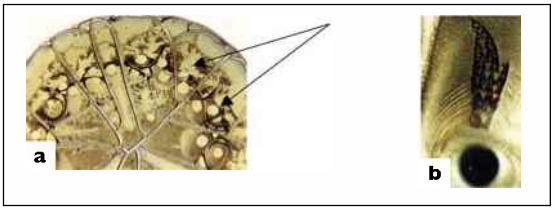

Fig. 5 Facia of blood serum structures: (a) inflammation markers (arrows) (Magnification × 30); (b) separate inflammation marker (arrow) (Magnification × 90).

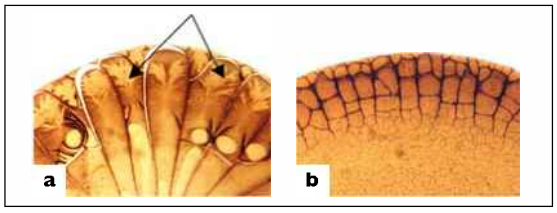

Fig. 6 Facia of the blood serum structures of a child with heavy pneumonia at (a) ten days before death; (b) two days before death (Magnification × 30).

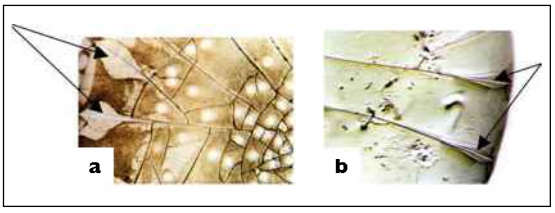

Fig. 7 Fragments of facias: (a) blood serum; (b) urine. Sclerosis markers are the leaf-like structures (arrows) (Magnification × 45).

Quick "response" of biological liquid structures to different outer and inner factors was highly valuable for diagnosing diseases, monitoring the patient and estimating the therapeutic effect of drugs used. Fig. 4 shows dynamic changes in blood serum structures of a patient during a heart attack and 30 minutes after its treatment by medication (Isoket, Schwarz Pharma AG, Monheim, Deutschland; 0.05 ml × 3 receptions of the aerosol under tongue with an interval of 30 s): block cracks (index of acute cardiovascular disorders) changed to radial type of cracks (norm).

A total of 27 patients with heart attacks were investigated. 18 (66.6%) of them responded to medication (Isoket, 1–3 receptions of the aerosol under tongue with an interval of 30 s). Positive clinical effects and normalisation of a morphological picture blood serum facias were observed (similar to what was shown in Fig. 4). Two (7.4%) of them experienced positive clinical effects without normalisation of the morphological picture of the blood serum facias, and seven (25.9%) patients suffered a heart attack which could not be stopped by medication (in the 30 minutes after receiving Isoket) and normalisation of a morphological picture of their blood did not come about.

An inflammatory process marker in blood serum facia was a "tongue"-like structure (Fig. 5). This marker was observed in 205 patients with different inflammatory processes (pneumonia, angina, otitis, cholecystis), as well as in 27 patients with no clinical signs of inflammation.

We believe that this marker showed the level of the organism resistance and disappeared in patients at the terminal stage of the disease. Fig. 6 shows the dynamics in the blood serum structures of a child with pneumonia at a terminal stage. It can be seen that the tongue structures (shown with arrows) are the manifestation of protective inflammatory reactions of the organism. Three-ray cracks appeared as the state of the child was aggravated by prognostic unfavourable structures of congestion phenomena. We think that tongue structures were the result of mutual extermination of auto-wave activity of toxic (inflammatory) proteins and physiological (immune active) proteins.

Sclerosis markers (leaf structures) were observed in different types of biological liquids. Fig. 7 shows leaf-like structures in the peripheral zones of blood serum facia of a patient with a heavy system atherosclerosis and urine facia of a patient with a chronic kidney insufficiency.

We observed leaf-like structures in the blood serum of all patients with clinical displays of atherosclerosis in blood vessels. This was observed in 875 patients. Plaque-formed structures (Fig. 8) indicated the excessive content of toxins in the organism, which may be of either endogenous or exogenous origin. Markers of intoxication were observed in 163 (91.5%) of 178 patients at late stages of chronic ischaemia of brain and in 251 (93.7%) of 268 patients with ischaemic illness of the heart.

During research of the structural organisation of the tear fluid of glaucomatous patients, we took into consideration its specifics depending on the stage of the disease. The facia at the early stage (17 patients) had a wide peripheral protein zone (Fig. 9a). The facia of patients at the second (developed) stage (21 patients) possessed a narrower peripheral protein zone (Fig. 9b). The feature typical of both stages was the formation of the

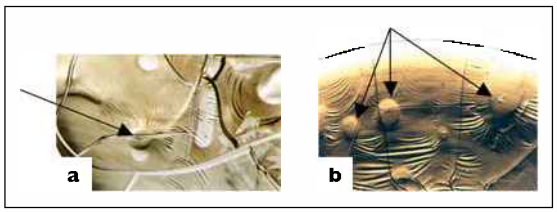

Fig. 8 Fragments of blood serum facias. Plaque-formed structures (arrows) in patients with (a) food intoxication; (b) chronic ischaemia of the brain (Magnification × 45).

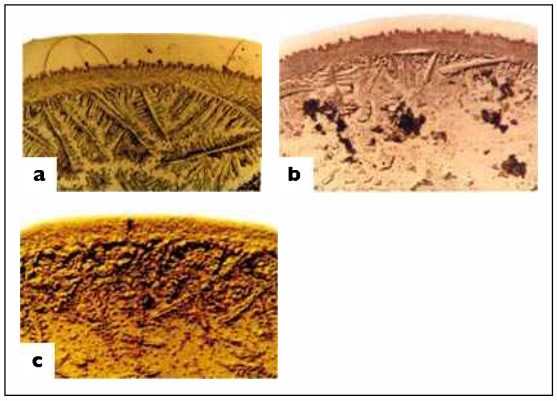

Fig. 9 Fragments of tear facias of patients at (a) early; (b) developed; and (c) terminal stages of glaucoma (Magnification × 45).

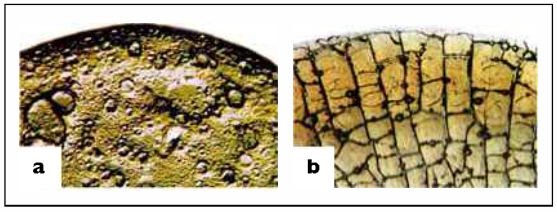

Fig. 10 Fragments of synovial fluid facias of patients at (a) initial stages; (b) advanced stages of osteoarthrosis (Magnification × 45).

intermediate zone. Such an area appeared in the facia of the biological fluids at the pathological states, connected with high intra-organ pressure in different organs (eye, skull, uterus). We concluded that the intermediate zone was the elements of facia, formed by specific proteins, which blocked the outflow from closed cavities of the organism. The main feature in the structure organisation of tear in the developed stage was the complete absence of the peripheral protein zone. Crystals of salt are massive structures with wide branches, oriented on the centre of the facia and covering the whole surface. The facia of tear of the patients in the terminal stage of glaucoma (14 patients) was characterised by a peripheral organo-mineral zone (Fig. 9c).

The morphology of the synovial fluid in the norm (four patients with a trauma of a knee joint) was characterised by a small pore structure (Fig. 3c). During early stages of osteoarthrosis (three patients), a synovial fluid facia was characterised by local growth of the pore structures (Fig. 10a). As osteoarthrosis progressed, the system organisation (11 patients) of the synovial fluid became abnormal (Fig. 10b).

DISCUSSION

Constant changes in the molecular composition of organism tissues and characteristics of chemical interactions between the different components occur in physiological, extreme and pathological states. The molecular forms are also constantly changing. All these create a complicated specific picture of oscillating behaviour of the system that can be determined at any moment by cuneiform dehydration. Wave structures of the facia of a biological liquid reflect functional behaviour of its components and of the system in general. These changes are markers of homeostasis at the molecular level and can serve as the basis for diagnosing different diseases at their early stages. Transition of a biological liquid from the hydrated to the dehydrated state brings into order elements of uncoordinated information that it contains in the liquid state, creating a structural ensemble. It possesses complex information on the state of the organism, such as physiological norm, age peculiarities, different pathological states, and results of therapeutic action. On the basis of the cuneiform dehydration, we have developed 19 diagnostic methods patented in different countries. According to the modern scientific ideas, all living beings acquire forms and functions with the help of self-organisation. The process of organisation and disorganisation of the matter is one of the basic forms of its movement. Living matter is always in a state of physical and chemical non-equilibrium. Parameters of the non-equilibrium state herewith always change within critical limits. Such dynamic but stable non-equilibrium is the basis of the living matter function.

Self-organisation is characterised by the appearance of special forms of order far from the state of equilibrium given the accordant external and internal conditions and the destruction of structures close to the state of equilibrium given arbitrary conditions.[1] Like in ontogenesis, phylogenesis and evolution of the living matter in aggregate, simple structures are formed first, while more complicated are formed from simpler ones.[2] Deviations from the physical and chemical equilibrium are a non-specific factor of the appearance of new structures. The condition that determines the specifics of their composition is the complementary relation among the structure-building elements and the concordance of their behaviour. Specifically, within a certain space and time period of the chaotic disordered medium, there appears a group of elements acting in concordance and thus distinguishing themselves from the medium by

forming an appropriate structure. Therefore, structuring is a method of individualisation of a complementary group of chemical elements in the medium.

Any structure of a biological element has its space and time characteristics since it is in constant movement determined by space and time parameters. Appearance of an ordered-in-time-and-space collective behaviour of the system means that it has some specific regulatory abilities. The space-time structure is a global and fundamental characteristic feature of a substance. There is no scientific branch not using, in some form or other, an idea of structure. The desire to find a universal explanation for structural and energetic laws of the material world in the second half of the 20th century lead to the appearance of synergetic (from the Greek synergy – a cooperative action) – a self organisation theory that explains the processes of appearance, stability decay and renaissance of various structures of the living and the non-living matter. Synergetics treats the self-organisation as the basic mechanism for forming space-time structures of substance and in fact considers it similar to the notion of "being".

Irrespective of how the theoretical ideas concerning the character of interconnection in the material world transform, one fact is unchanged – any physical and chemical connections are elements of the global energetic field and provide for the process of self-organisation of the matter. At the same time, self-organisation is determined by internal features of a system appearing spontaneously as a result of the interaction of a large number of subsystems and is their coordinated cooperative effect. A system is a group of elements acting in cooperation, and reacts as a unique whole to the changes of internal or external conditions to its existence for the purpose of maintaining its basic qualities. A system is called self-organising if it acquires some space-time structure without any specific external influence. A specific external influence is one that imposes its structure upon the system. In the case of a self-organising system, it does not experiences a specific external influence.[3]

Formation of structures out of a homogeneous non-differentiated medium is one of the fundamental problems in physics, chemistry and biology. In open systems, which constantly get energy and substances from their surroundings, fixed non-balanced states with a high degree of order can appear. The precondition is the coordinated (cooperative) system behaviour, which can appear in case of deviations from the equilibrium in separate parts of the system. The stability and reliability in functioning of the organism as a whole are also based on the self-organisation principle: the function of an element forms its structure, and the structure in turn defines its function. In line with modern physics data, auto-waves of biological structures are formed by auto-waves of their elements. The rhythms of these elements define the auto-wave characteristics of the whole biological object by calibering synchronisation when interacting cooperatively. If we examine deeper levels of the substance organisation, we will see that they are represented by positrons, neutrons, electrons, quarks, and quantum. Quantum possesses the characteristics of both: a particle and a wave. When quantum forms a more complex structure, its dual properties coexist. The properties of the particles define the structure of a material object, whereas wave properties define its function. The higher the organisation level of a material object is, the more complex its structure and function.

Biology abounds in complex systems. The cell consists of a membrane, a nucleus and cytoplasm, which in turn include a variety of different structural level components. An organism does not contain a finite quantity of cells, which interact quite cooperatively to perform specific functions. A biological system can pass into different states; therefore it needs information to choose the optimal one. This choice is genetically determined. However, the surrounding environment is also important. So, the peculiarity of the state is determined by the interior parameters of the system, as well as the "context". New information is formed as the result of the system-environment interaction, together with the genetic data of the system. One of the most striking peculiarities of any biological system is the high degree of coordination between its separate parts. Thousands of local metabolic processes can occur in a cell simultaneously and cooperatively. Clearly, all these coordinated and coherent processes take place due to the information exchange. Information should be formed and delivered to the address. Therefore, information is the decisive element in the existence of life itself.[3,4]

The morphology of biological systems is based on the step principle: from simple structures, more complex ones are built. The space orientation of elements defines the structure. However, it is not the elements, but the bonds between them that are of importance. Auto-waves are derivatives of material structures, and they are formed on the same principle. Weak auto-waves of some atoms would be corrected by waves of atoms with more active auto-wave rhythm in the process of synchronisation when forming a molecule or a crystal. As a result, there appears the auto-wave structure of the second level, which in turn shapes the next rhythmic level, pending the main auto-wave rhythm of the whole biological object. The leading rhythms define the main function of the object, whereas other waves define specific (individual) traits of this very function. Hence, the auto-waves of elements shape the auto-waves of systems and the latter are coordinated by their auto-wave rhythms of a higher level.

All cells are built of molecules and that is why the role of the link-up mechanisms is enormous. It is known that a

coupling of small molecules with big ones is the basis for many biological processes, for example the metabolism of nutrients and hormonal influence. The interaction of macromolecules is a part of such phenomena as muscle contraction, antibiotic effect, nervous impulse deliverance and many others. It has been established that the basic cell macromolecules, proteins, nucleic acids and polysaccharides, are responsible for building of cell components, chemical transformation catalysis, cell movements, and cell heredity. The information supply of biological macromolecules provides this vital functioning. Information which defines the space configuration of the surface lies in the sequence of macromolecule subunits and the attending elements. This configuration governs the processes of mutual recognition between various molecules or various parts within the same molecule with the help of weak non-covalent interactions.[4]

The problem of the interrelation between the substance and motion is solved by means of wave mechanisms (quantum theory) in physics while the problem of interrelation between the structure and function is solved by means of auto-wave processes in biological systems in medicine and biology. Millions of auto rhythms differ in their character, intensity, wave length and vector orientation function in the human organism constantly or temporarily. In the normal state, these rhythms are synchronised; in pathology, desynchronisation develops. The rhythmic state of the organism is a special physiological sphere, in which all metabolic processes take place.

Metabolic and wave processes of the organism are closely bound and mutually determine the character of each other. On the basis of obtained data,[5,6] we believe that in live systems, molecule and cell contacts are enabled by the effect of microbiolocation. Molecules "talk" to each other by means of wave processes. With the help of these processes, they learn from each other, exchange information, determine the need for entering into closer contacts and uniting into functional blocks, for building complex biological structures. Molecules, by means of the wave processes, destroy chemical structures which are not essential for the normal activity of an organism. For example, effector-molecule does not need to "examine" the whole structure of the target-object, it recognises it with all its peculiarities by emitted auto-waves. The process of recognition happens in no time and may take place at a significant distance as the result of auto-wave biolocation of molecules and molecular complexes. An active molecule connects with the target-molecule by definite parts. As a result, we observe auto-wave specificity of these parts that also makes possible a more delicate detection and provides the joining of molecules with optimum space orientation.

Facia of a biological liquid is a complex of stabilised and structured information on the functional processes that go on in the organism. The method of cuneiform dehydration of biological liquids developed by the autors enables visualisation at the molecular (biochemical) level of organisation of biological systems by way of its transformation into the macro level. Structures of the macro level become applicable for the study by routine analytical methods. Further decoding of the versatile structures of the facia of biological liquids (i.e. detection of their connection with a certain pathological or physiological state of a man) gives broad possibilities for monitoring the whole range of metabolic processes, which go on in the human organism with extreme complexity and dynamism.

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