REVIEWS

Autogenous Rhythms and Self-Organization of Biological Fluids

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The process by which biological fluids are crystallized makes it possible to identify rhythmic undulatory oscillations occurring in the liquid phase of the medium. Pathological changes in the body impair physiological rhythms at different levels, these impairments being reflected in the biocrystalline structure of a biological fluid during its self-organization. Targeted modifications of the rhythm characteristic of a biofluid may help to evaluate the resistance of the organism to environmental factors.

Key Words: *biological fluids; biorhythm; crystallization; structuralization; spatio-temporal organization*

The theoretical basis of chronobiology is the axiom that there exist spatio-temporal forms of organization of the living matter [2,4,6,7].

Under physiological, highly stressful, or pathological conditions, molecular composition of tissue and chemical interactions between its components responsible for the oscillatory behavior change continuously. Any wave is a signal, and the concentration wave in a chemical medium is not an exception. Activation and inhibition of chemical reactions in a feedback manner provide the basis for rhythmic oscillatory processes in biological systems. These processes, which are best reflected in the structural crystalline organization of biological fluids, determine the functioning of individual elements, systems, and the whole organism.

In terms of synergism, the complex and well coordinated processes occurring in biological fluids can be regarded as self-organizing [5,10-12]. So-called nonequilibrium phase transitions (crystallization of a liquid during freezing or drying) exemplify a self-organization process. This is not a transformation of chaos into order, but rather a phase-transition process from one order of particular quality to another.

We have examined physical parameters of three-dimensional structures arising upon phase transition of various biofluids to solid state during self-organization. Transition of a biological fluid to the solid state is a process whereby a structural order of higher level is achieved.

It is known from general crystallography [8] that crystallization of melted plutonic rocks following volcanic eruptions proceeds in three consecutive stages each of which is characterized by formation of a distinct structural zone: zone of idiomorphic crystals, zone of mixed structures, and amorphous zone. We have observed similar processes in biofluids upon drying.

MATERIALS AND METHODS

Biological fluids (blood serum or urine) were applied as a drop (0.02 ml) onto a slide, allowed to dry at room temperature (18-25°C), and viewed under a microscope in transmitted light at different magnifications.

Urine and serum samples in optical cells were studied under an MBI-15 microscope and a Polivar

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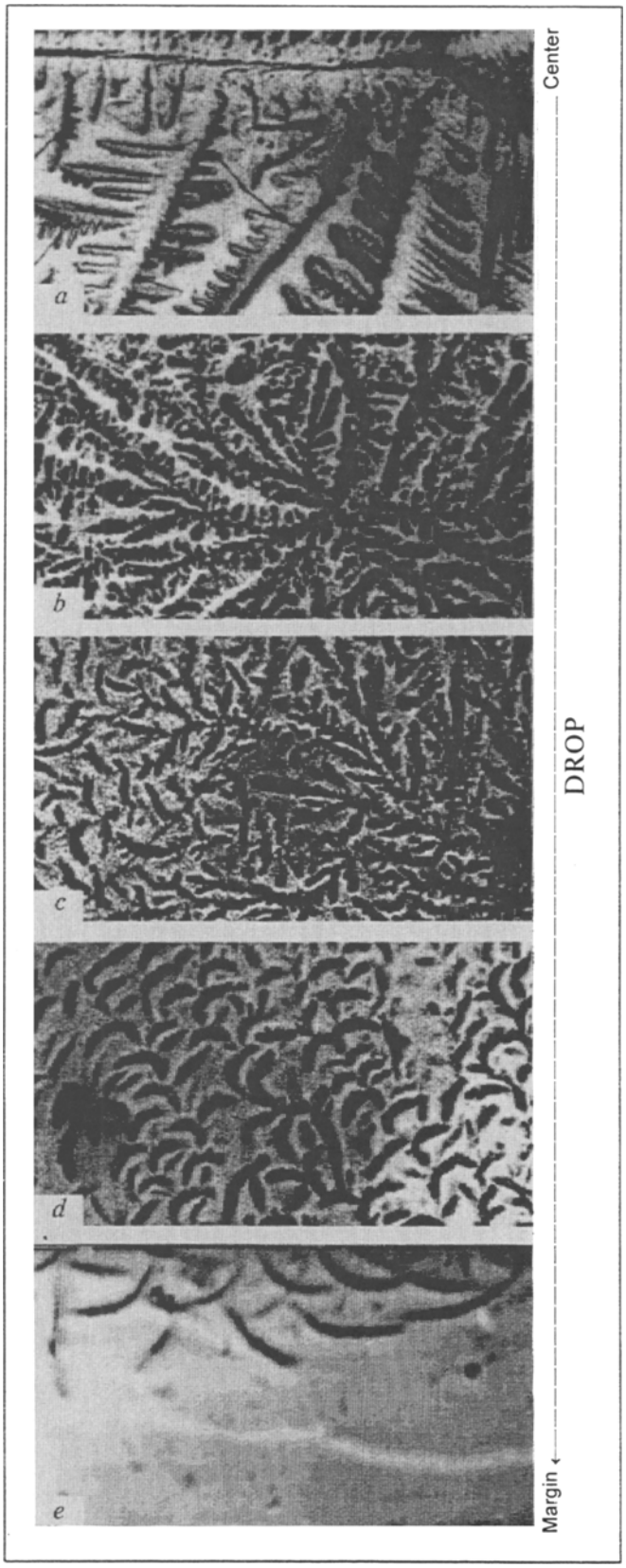

Fig. 1. Crystallization zones (located according to radii) of a serum drop (serum protein content 40 g/liter, serum was diluted 1:1 with physiological saline). ×56. *a*) central (fern-shaped) zone; *b-d*) middle zone between the center and margin; *e*) marginal (amorphous) zone.

polarizing microscope (Reichert-Jung) with the image display. The optical cell was made from two thin glasses (without scratches) freed from fat in alcohol, washed clean with distilled water, and treated with a 0.02% lecithin solution to ensure a weak binding to the support. The sample (0.02 ml) was placed between a glass slide and a coverslip. In order to avoid the influence of uncontrolled factors (nonuniform thickness of the cell, distorted plane of the glasses, etc.), 4-6 identical samples were prepared. The preparations were left at room temperature for at least 48 h before examination. During this period the concentration of organic and mineral components of the biofluid changed due to gradual evaporation of water thought the slit between the slide and the coverslip. These changes induced structural rearrangements of liquid crystals and their transformation into anisotropic double-refracting structures. Anisotropic textures (the patterns of the biofluid structure) were examined at a magnification of 90.

RESULTS

Crystallization of Biofluid as Fixation of its Rhythmic Undulatory Oscillations. We examined more than 700 serum samples air-dried in the form of a drop on a glass slide and/or in the optical cell [9], which enabled us to analyze the basic rhythm of crystallization occurring in this biological fluid.

Figure 1 shows crystallization zones in serum samples diluted 1:1 with physiological saline. The distribution of these zones reflects a particular temporal rhythm of crystallization process. Central zone (Fig. 1, *a*) is occupied mainly by NaCl crystals which form in a relatively free way, without appreciable interference with other structures. Their crystallization begins as the concentration of other salts increases during water evaporation (Fig. 1, *b*). After the concentration of organic substances in the liquid phase had reached the critical level structuralization starts, preventing the growth of salt crystals (Fig. 1, *c* and *d*). Inorganic and organic substances (mainly salts and proteins) compete for free space and free ions, which results in the formation of an intermediate zone between the center and periphery. Finally, structuralization of proteins predominates over other processes, and a marginal amorphous zone is formed (Fig. 1, *e*).

We think that formation of these zones reflects the first-level rhythm of biological fluid crystallization and regard this rhythm as physiological.

When serum protein content is as high as 90 g/liter and the relative protein concentration is similar to that of salts, structures in the form of second-level concentric waves are formed (Fig. 2). X-ray

Fig. 2. Crystallization pattern of a serum drop (serum protein content 90 g/liter). The arrow indicates second-level concentric waves. ×56.

spectral analysis of chemical elements comprising concentric waves revealed alternation of salts and proteins in these zones. Such a distribution of waves results from the virtually equal conditions under which organic and inorganic components of blood serum have to compete for free space. This leads to the formation of waves of other (lower) amplitudes and higher frequency, which reflects changes in the concentration of chemical substances in the biological fluid. We regard this rhythm as the second level of rhythmic oscillations. Autonomous closed zones of the rhythm emerge, referred to as sectoral rhythms. Figure 2 shows radial waves characteristic of amorphous structures in the solid phase of biological fluids and looking like "cracks". In hyperproteinemia (protein concentration 120 g/liter), the number of autonomous rhythmologic zones is increased. The second-level rhythms may be regarded as physiological in general; however, they dominate when the serum protein content is abnormally high.

Thus, organizational rhythms in a biofluid can be registered owing to the presence of biocrystalline structures, i.e., the crystallization process makes it possible to register rhythmic undulatory oscillations in the liquid phase of the medium which cannot be detected by other (for example, biochemical) methods.

A chemical reaction in which differently colored waves of oxidation-reduction processes alternate has been described [1-3]. This process was discovered in Russia in the 1950s and is known as the Belousov–Zhabotinsky's reaction. Using special indicators, we observed the emergence and alternation in a protein medium of circular waves, each having a particular color. We believe that the detected shapes of bio-

TABLE 1. Effects of Laser Therapy on the Composition of Serum Texture in Patient with Bronchial Asthma

Day of therapy	Composition of serum texture, %											
	before exposure to laser radiation				5 min after exposure				60 min after exposure			
	B	S	SA	A	B	S	SA	A	B	S	SA	A
1	20	60	0	20	40	50	0	10	20	60	10	10
2	20	60	5	15	50	40	10	0	40	40	10	10
3	30	60	10	0	100	0	0	0	60	40	0	0
4	50	40	10	0	100	0	0	0	90	10	0	0
5	90	10	0	0	100	0	0	0	100	0	0	0

Note. Here and in Table 2: B = basic structures; S = secondary structures; SA = secondary structures with atypical signs; A = atypical structures.

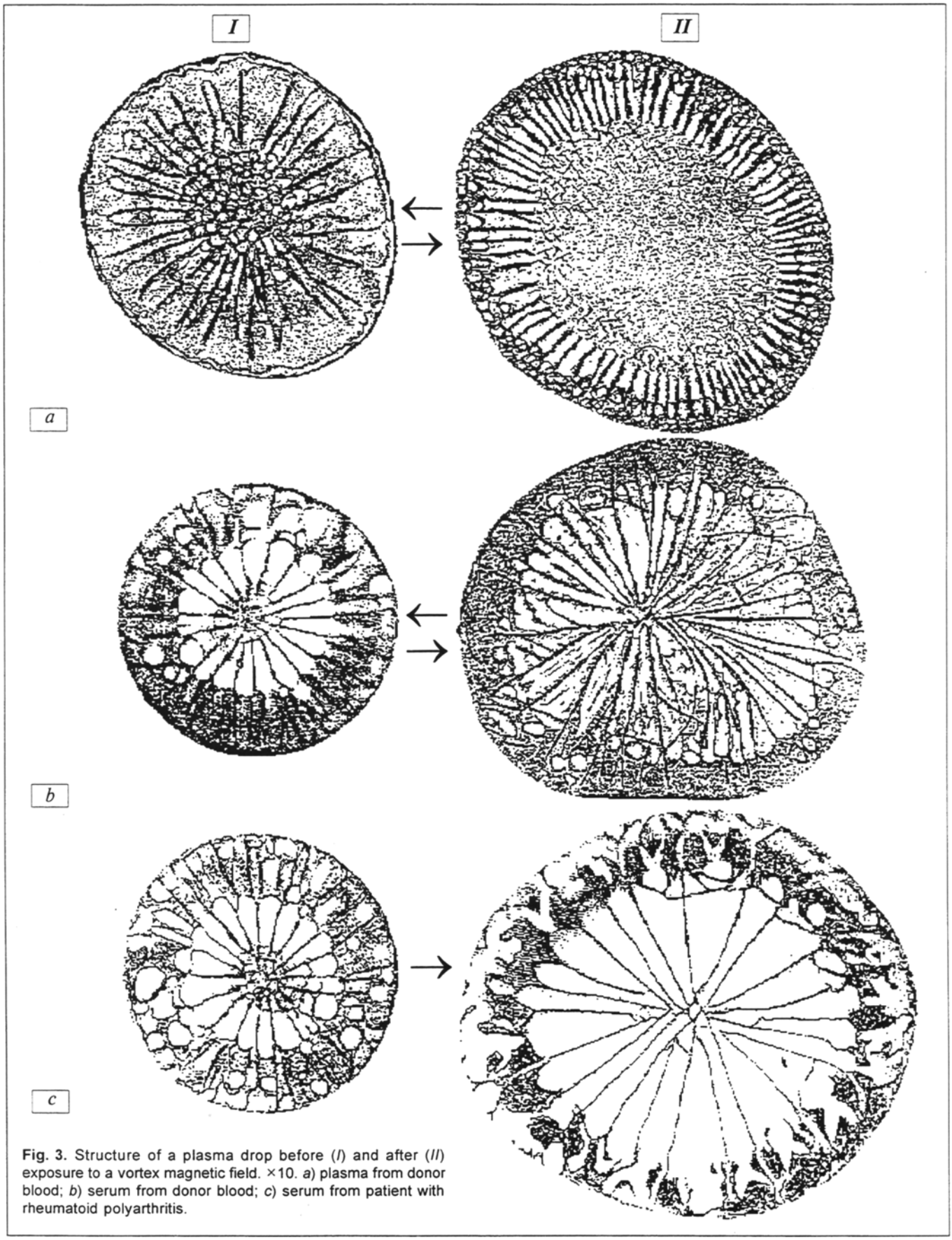

Fig. 3. Structure of a plasma drop before (*I*) and after (*II*) exposure to a vortex magnetic field. ×10. *a*) plasma from donor blood; *b*) serum from donor blood; *c*) serum from patient with rheumatoid polyarthritis.

crystalline structures and the Belousov—Zhabotinsky's waves have the same basis, namely, rhythmic undulatory processes by which the concentrations of chemical substances are altered in the studied object. Autowaves are self-sustaining waves in so-called active media, i.e., those containing an energy source maintaining the parameters of autowaves at a constant level.

Effect of External Undulatory Influences on Autogenous Rhythms of Biological Fluids. We have found that the rhythms of biofluids change under the action of external undulatory influences such as a vortex magnetic field. In the relevant experiments, biological fluids were exposed for 3 min to a vortex magnetic field generated by two (5 and 6 kHz) sources. Drops of the biofluids were allowed to dry before and 1, 2, 3, and 4 h after exposure to the magnetic field.

Induced rhythm altered the autogenous rhythm of biofluids during transition to the solid phase. Figure 3, *a* shows the structure of a dried drop of plasma from a healthy subject before and after exposure to a vortex magnetic field. This exposure increased the diameter of the drop, changed the wave amplitude, caused zonal transposition of the first-level waves (central wave moved to periphery), and increased 2.5- to 3-fold the frequency of the radial rhythm waves.

The structure of a dried drop of serum from a healthy subject before and after exposure to the same vortex magnetic field is shown in Fig. 3, *b*. It can be seen that this drop underwent similar but less pronounced changes than those depicted in Fig. 3, *a*. The original rhythmologic parameters of both plasma and serum were restored 4 h after exposure to magnetic field.

By contrast, serum samples from patients with a severe disease (rheumatoid polyarthritis or renal amyloidosis) retained the rhythm induced by the magnetic field and were unable to resume their own rhythm. We designated this phenomenon as rhythmologic instability. Figure 3, *c* shows the structure of a dried drop of serum from a patient with rheumatoid polyarthritis after exposure to the vortex magnetic field, which increased the diameter of the drop, caused a well-defined change in the rhythm of

Fig. 4. Microstructure of the marginal sector in a drop of serum from the same patient with rheumatoid polyarthritis as in Fig. 3 after exposure to vortex magnetic field. The arrow indicates Arnold's tongues. ×500.

circular oscillations (the central wave disappeared), and slightly altered the rhythm of radial waves.

In addition, specific sectoral rhythms appeared within the circular wave of the first-level rhythm, and microscopic examination of their structure revealed peculiar zones (shown by arrow in Fig. 4). Such zones, which are referred to as Arnold's tongues in rhythmology [2,13], result from superposition of alternative waves of particular amplitudes and frequencies. These waves are then annihilated and an arrhythmic space is formed, whereas some areas of Arnold's tongues retain the undulatory rhythm. Such areas appear as variously shaped triangles.

We believe that targeted modification of the autogenous rhythm of a biological fluid system can be used to evaluate its resistance to endogenous and exogenous agents. The information thus obtained characterizes the potential of this system and is suitable for predicting the course and outcome of a pathological process.

Significance of Autogenous Rhythms for Crystal Formation in Biofluids. Our studies showed that a biofluid exhibits a set of biorhythms whose specific

TABLE 2. Composition of Serum Textures Before and After Drug Therapy

Patients	Number of patients	Composition of serum texture, %									
		before treatment					after treatment				
		B	S	SA	A	AM	B	S	SA	A	AM
Chronic respiratory and gastrointestinal diseases	192	12	72	2	8	6	42	58	-	-	-
Cancer (stage III or IV)	212	6	5	31	48	10	-	9	40	44	7

Note. AM = amorphous structures.

features determine not only the undulatory organization of the biofluid as a whole (*en masse*), but also the characteristics of crystal formation upon transition to the solid phase. This can be clearly seen when the shape of serum crystals is examined.

We studied anisotropic structure of sera from 210 healthy individuals, 94 patients with chronic respiratory disease, 98 patients with gastrointestinal diseases, and 212 patients with stage III or IV cancer. Analysis of the results enabled us to classify serum crystals according to their shapes in health and disease [7].

Sera from healthy individuals contained medium-sized and large spherulites which we regard as the basic form of texture (Fig. 5, *a*). Textures with secondary growth at the periphery, either fan-like or needle-like, dominated in sera from chronically ill patients (Fig. 5, *b*). Textures with defects and variously colored foreign inserts in the structure of the main crystal (mixed textures) formed in sera from cancer patients (Fig. 5, *c*). These textures were regarded as atypical. A secondary structure with atypical signs was also identified.

Inhibited structuralization is an abnormality of self-organization of a biological fluid. It manifests itself as a considerable delay (6 or more days) in crystal formation in serum in the optical cell. We have designated such a state as amorphous.

The detection of different textures in healthy subjects and patients may be explained by rhythmologic variability which manifests itself in the process of self-organization. We believe that the emergence of colored inserts or foreign inclusions in the texture is due to the loss of their own rhythm by some organic and mineral components of biological fluid and acquisition of a foreign rhythm induced by rhythmologically stable neighboring structures. The components of biological fluid are included into unique crystalline structure because their rhythms are almost identical.

Feasibility of Correlating Autogenous Biorhythm in Clinical Practice. From the viewpoint of rhythmology, physiotherapy or drug therapy is an intervention in the basic biorhythm of the organism (in rhythms involving the entire body, its anatomical parts, or specific chemical structures). The benefits of such an intervention result from adequate alterations of biorhythm, i.e., restoration and stabilization of the intrinsic rhythms of body structures.

This is supported by changes induced by laser therapy in the crystalline texture of serum from a patient with asthma (Table 1). Before laser therapy (acupuncture), the textures consisted predominantly of basic and atypical structures. After the first session of laser therapy, the texture composition changed: the percent of basic structures increased, whereas that of secondary and atypical structures decreased. However, 1 h after the first session, there was a tendency toward restoration of the original rhythm. It was only after the fifth (last) session that the serum texture was entirely composed of stable basic structures. Throughout the treatment, normalization of the texture composition coincided with improvements in the general condition of the patient and its breathing.

Normalization of serum rhythm coincided with improved condition of patients with other diseases. Table 2 illustrates restoration of physiological rhythms of serum crystallization in 192 patients with chronic respiratory and gastrointestinal diseases after effective drug therapy. In 212 cancer patients receiving close range radiation therapy, the composition of textures remained practically unchanged, indicating stability of pathological rhythms.

Biorhythms in the Diagnosis and Monitoring of Urolithiasis. Our studies showed that pathological changes occurring in the body can disrupt the first-level physiological rhythms of biofluid self-organization. For example, in the urine of patients with urolithiasis these changes manifested themselves as the loss of the boundary between salt and protein waves during the formation of zones. As a result, salt crystals were formed in the protein zone (Fig. 6).

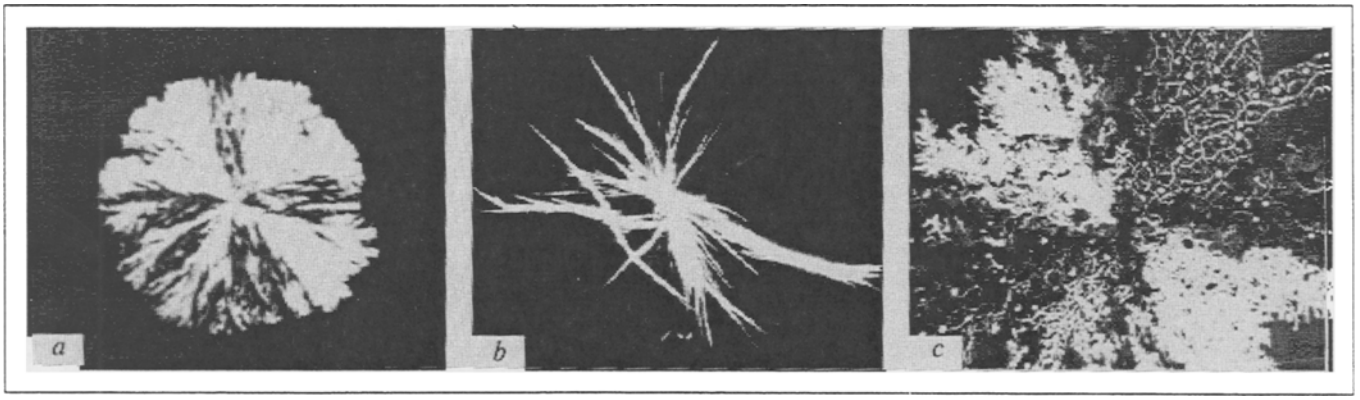

Fig. 5. Serum textures under a polarizing microscope. ×90. *a*) large spherulite (basic form); *b*) needle-like (secondary form); *c*) mixed (atypical form).

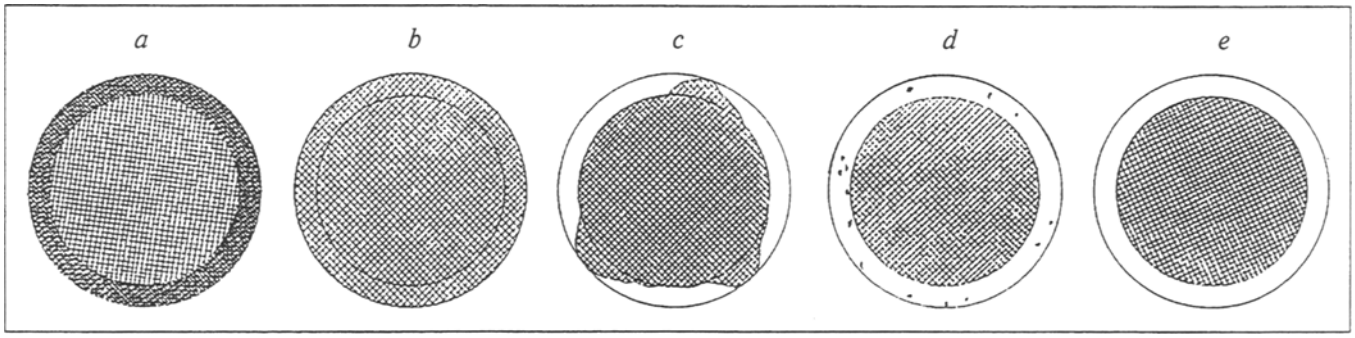

Fig. 6. Urine samples from patients with urolithiasis and different rates of stone formation in the kidneys: *a*) very high rate; *b*) high rate; *c*) moderate rate; *d*) low rate; *e*) no stone formation (shown for comparison).

We think that this phenomenon is based on the above-mentioned mechanism, i.e., the loss of their own rhythm by some salts. Subsequently, they acquire the rhythm of proteins and are structurized in the protein zone (or, more precisely, in the protein rhythmic wave) which is amorphous. The loss by salts of their own rhythm may account for their instability in the liquid phase; as this instability increases stones are formed.

When the treatment of urolithiasis was effective, a decrease in the stone formation could be traced over time: massive crystallization of salts in the protein zone was seen during the period of intense stone formation, individual conglomerates of salt crystals were associated with the periods of moderate formation, and the emergence of a salt-free marginal zone indicated that stone formation had ceased completely. During the period of stone self-destruction, which is usually accompanied by renal colics, excessive "amorphization" of urinary salts was observed, so that salt crystals could not form even in the center of a dried drop of urine.

Biorhythms as the Basis of Functioning of Material Objects. Our studies have shown that biological fluids display characteristic rhythmic oscillations differing in physical nature and spatial orientation and ranging widely from the macrolevel (extending throughout the biofluid) to the molecular level.

With this in mind, we hypothesized that rhythmic oscillatory processes provide the basis for all functions performed by their material carrier. Therefore, the functions of matter in general are, in the final analysis, rhythmic oscillations of its elements, complexes, and systems. Due to complementarily of rhythmic oscillations, identical as well as different molecules and atoms interact with each other. The term "complementarily" is used here to describe a complete (or almost complete) coincidence of the frequencies and amplitudes of oscillations of material particles. The rhythm of these oscillations may change depending on environmental parameters (ambient temperature, pressure, presence of catalysts, etc.) with the corresponding change in the nature of the forming chemical compounds. Environmental factors may destroy chemical compounds by altering individual rhythms of their elements. For example, dissolution of a solid substance in a liquid is determined by complementarily of their oscillatory rhythms.

Thus, examination of specific rhythmologic parameters of the solid-phase states of biological fluids opens new prospects for understanding the fundamentals of vital processes as well as in developing qualitatively new diagnostic and therapeutic technologies, by which the functions of living matter can be controlled at different levels of organization, and theoretical concepts of how functions of the matter are organized and established.

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