METHODS

Crystallography of Biological Fluid as a Method for Evaluating Its Physicochemical Characteristics

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Using an integral qualitative and quantitative approach to the studies of initiation of the biological material crystallogenesis, we showed in experiments with normal human saliva that the external characteristics of biological fluid (pH, osmolality, and environmental temperature) determine the results of crystallization (tesigraphic facies). The main external (macroenvironment) and inner (microenvironment) factors of biological fluid crystal formation, determining specific features of the tesigraphic facies, were distinguished and classified. The informative value of differential analysis of biomaterial properties by means of modulating the environmental conditions is established.

Key Words: *dehydration; crystallization; biological fluids; facies; factors*

Crystallographic methods of investigation attract much attention of biologists and medics [1,2,4,5,7-10]. The diagnostic role of crystallography in medicine was established [1,7-9], but many other aspects of the biocrystallization phenomenon (indicator and prognostic significance, monitoring of body status, *etc.*) remain virtually not studied [3].

Evaluation of the significance of studies of crystal forming and initiating characteristics of biological substrates under different dehydration conditions is a difficult problem, which is explained by very low number of methods adequately evaluating the result of crystallization [1,4,5,10].

We interpreted the tesigraphic facies of biological media with consideration for the micro- and macroenvironment as exemplified by the saliva.

MATERIALS AND METHODS

A total of 125 micropreparations of dry biological media from 95 healthy subjects (saliva) and 30 patients with alveococcosis of the liver (saliva, urine) were studied.

The method of differential tesigraphy (detection of the initiator potential of biological medium towards the basic substances modulating the physicochemical characteristics and forming the micro- and macroenvironment) [3,6] was used.

The temperature of dehydration, osmotic characteristics of the medium (hypo-, iso-, and hyperosmolality), pH of the biosystem (neutral or shifted to the acid or alkaline values), and co-crystallization with chemically and bioactive basic substances (0.1, 0.9, and 10% NaCl, 0.1 N HCl solution, 0.01 N KOH solution, 2% procaine solution, 0.1% epinephrine solution, and 40% ethanol) were evaluated.

In order to evaluate the impact of various factors of the dried biological substrate crystal formation, the effects of osmotic characteristics and pH of the medium were studied using facies prepared by the method of differential tesigraphy with 5 basic substances (0.1, 0.9, and 10% NaCl solutions, 0.1 N HCl, and 0.01 N KOH). The basic tesigraphic coefficient (Q), characterizing the initiator potential

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of biological material, and the zonal coefficient (*P*), reflecting the heterogeneity of the fluid composition, were considered as the most significant parameters of the micropreparation.

RESULTS

Multifactorial analysis of dispersions (ANOVA) showed that the osmotic and pH values were essential for the studied tesigraphic parameters (Tables 1, 2). For *Q* and *P* the level of significance for the combination of factors was $p=0.023$ and $p=0.048$, respectively. Classical statistical analysis of tesigraphy data was supplemented and confirmed by the results of analysis of dispersions. For example, the increase in osmolality of the medium in which crystallogenesis was realized was paralleled by increase in the initiator capacity of the biosubstrate (for *Q*) and evenness of the facies elements distribution (*R*). The minimum level of the sample fragmentation (for *I*) was observed only in the presence of optimal osmolality of the medium, while any shifts in this parameter augmented the disorders in the facies integrity ($p<0.05$).

These trends were confirmed for the impact of the medium acidity. For example, the increase of pH caused a significant ($p<0.05$) increase in *Q* and *R*. As pH is essential for the status of the salivary protein component, deviations from the neutral level caused a decrease in the marginal zone diameter ($p<0.05$).

Study of variations in the tesigraphic facies with consideration for the thermal regimen of crystallization showed significant ($p<0.01$) differences only between the samples dried by heating (45-55°C) and cooling (0-4°C), but not between dehydration at 20-25°C and in a flow of warm air.

Hence, the main factors of dynamics and crystal formation of biological media, in addition to their qualitative and quantitative composition, are the micro- and macroenvironment of crystallizing substances (Fig. 1).

The manifestation of the initiator capacity of normal human saliva varies greatly, depending on the microenvironment of forming crystals. Due to this fact it is possible to carry out a multiparametrical analysis of the initiator potential of the biological fluid with compounds, simulating different microenvironment for the biological medium, serving as the basic substances for the tesigraphic test. Coordinated analysis of the basic tesigraphic parameters (*Q* and *P*) using initiated series of 5 substances with different characteristics showed that a stringent tesigraphy pattern (sum of values of parameters for all basic substances) corresponded to alveococcosis of the liver (Table 3). The initiator potential of biological substrates with composition differring because of the parasite (alveococcus) presence) was identical.

TABLE 1. Relationship between Medium Osmolality and Initiated Crystallogenesis of Normal Human Saliva (*M*±*m*)

Parameter	Hypotonic solution	Isotonic solution	Hypertonic solution
Basic tesigraphic coefficient	1.72±0.21*	2.12±0.23	2.67±0.24*
Zonal coefficient	1.80±0.12	1.86±0.20	1.92±0.19
Coefficient of evenness of facies elements distribution	1.76±0.16*	2.50±0.18	2.81±0.24*
Coefficient of porosity degree	2.47±0.22*	1.75±0.16	2.55±0.21*
Facies destruction degree	2.28±0.19	2.09±0.17	1.34±0.11*
Coefficient of marginal zone manifestation	2.09±0.20	2.06±0.18	2.13±0.23

Note. *$p<0.05$ compared to isotonic solution.

TABLE 2. Relationship between the Medium pH Shift and Tesigraphic Values in Normal Subjects (*M*±*m*)

Parameter	pH<7	pH=7	pH>7
Basic tesigraphic coefficient	1.73±0.16*	2.22±0.24	3.15±0.28*
Zonal coefficient	1.84±0.19	1.86±0.17	2.26±0.20*
Coefficient of evenness of facies elements distribution	1.94±0.15*	2.50±0.19	3.09±0.22*
Coefficient of porosity degree	2.19±0.14	2.31±0.16	2.44±0.21
Facies destruction degree	1.84±0.16	1.91±0.20	1.78±0.15
Coefficient of marginal zone manifestation	1.44±0.13*	2.06±0.18	1.22±0.10*

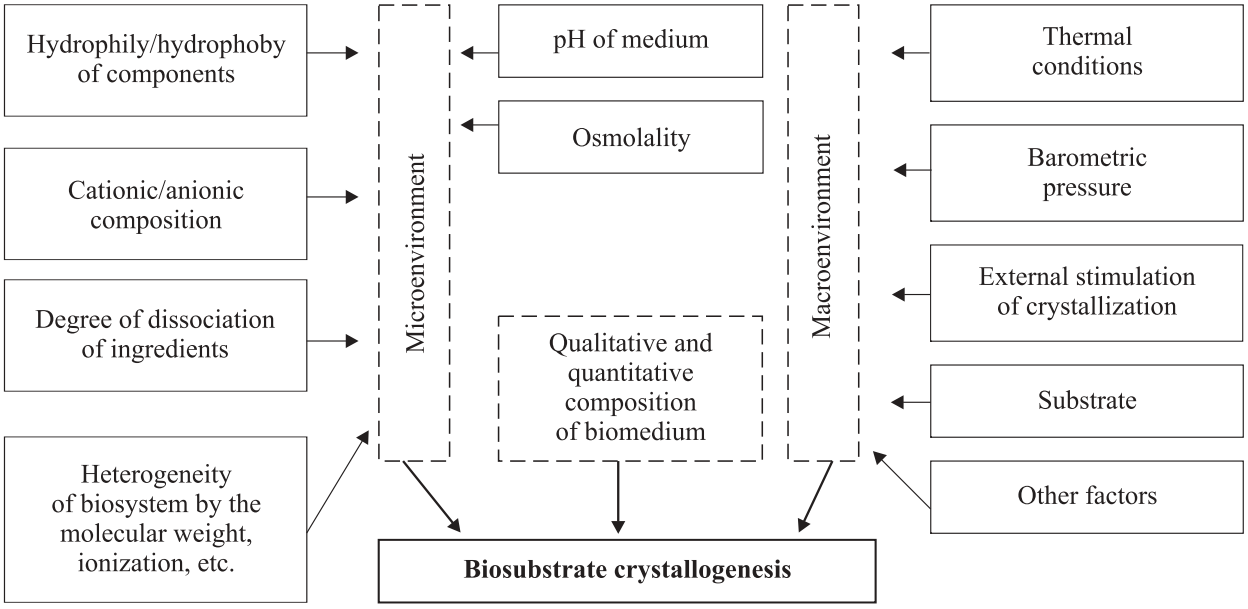

Fig. 1. Factors limiting the biological substrate crystal formation.

TABLE 3. Coordination Analysis of Initiator Profile of Biological Fluids as Exemplified by the Saliva and Urine of Patients with Hepatic Alveococcosis

Coefficient	Biological medium				
	NaCl		0.1% epinephrine	2% procaine	40% ethanol
	0.9%	10%			
Basic tesigraphic coefficient					
Saliva	+	+	—	— (pronounced transformation of elements)	—
Urine	+	+	+	—	—
Zonal coefficient					
Saliva	*	<2	≥2	>2	>2
Urine	<2	≥2	<2	≥2	≈2

Note. +: activation; —: ingibition; *uncertain parameter.

Hence, crystallogenesis of biological substrates is a dynamic process of removal of the liquid part of the biological medium, determined by the composition of dehydrating mixture and the micro- and macroenvironment of crystallizing substances. The most significant factors of macro- and microenvironment are pH, osmolality, and thermal conditions of crystallization. The heterogeneity of the initiator potential of the biological substrate (its different behavior under different environmental conditions) in the presence of different basic substances permits a sufficiently complete evaluation of the information capacity of the biological medium.

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