METHODS

Use of Morphostructural Reaction of Blood Serum for Toxicological Evaluation of Drugs

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We prorposed a simple and economic method for determination of general toxic effects of drugs consisting in evaluation of serum morphology by polarization light microscopy.

Key Words: *blood serum; morphotypes; drugs; toxicological evaluation; polarization microscopy*

An important task of toxicological studies is detection of side effects of the studied substances. These studies provide the data for calculating the ratio of therapeutic and toxic doses and for determining the target organ. This latter task cannot always be fulfilled, because some substances possess a wide spectrum of harmful effects on many vital organs and systems. An objective toxicological evaluation of drugs can be made using a complex of hematological, biochemical, and pathomorphological studies [4]. Electron microscopy, histochemical, autoradiographic, immunological, cytochemical, cytogenetic, and other methods were used for this purpose, but all these methods are time-consuming and expensive. These methods can be used in toxicology after preliminary screening of drug tolerance on animals, which can be performed using simple informative methods for evaluation of the effects of these compounds on the body. Therefore the search for less involved and less expensive methods for evaluation of the effects of chemical compounds remains an important problem.

We analyzed the possibility of using morphological analysis of the blood serum by polarization microscopy [8] in toxicological studies of substances with potential antileprous activity.

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MATERIALS AND METHODS

Chronic toxicity of hydrobunide and furasonal, which was previous demonstrated *in vitro* [1], and their antibacterial activity [2] (using Shepard's model [10]) were studied on 100 CBA mice of similar weights and sex kept under similar conditions. Dapsone (diaminodiphenylsulfone; DDS), the main antileprosy drug, served as the reference drug. The animals were divided into 4 groups: group 1 received DDS (25 mg/kg), group 2 hydrobunide (40 mg/kg), group 3 furasonal (75 mg/kg), and group 4 animals (control) received distilled water. The doses of the test drugs were selected in preliminary experiments. The drugs were dissolved in distilled water and administered through a gastric tube (0.5 ml) twice a week for 6 months. The drug effects were evaluated by pathomorphological analysis of the viscera (liver, kidneys), measurements of serum ALT, AST, total bilirubin, creatinine, and urea on a Mitsubishi Super Z-818 biochemical analyzer using ECO-MED-POLL reagents.

In parallel, the structure of dry blood sera from experimental animals was studied in optic cells [8]. To this end, 0.02 ml serum was applied onto a standard

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TABLE 1. Results of Biochemical Tests (*M*±*m*)

Group	AST, U/liter	ALT, U/liter	Bilirubin, μmol/liter	Urea, mmol/liter	Creatinine, μmol/liter
1	263.57±4.33	79.00±3.79	8.48±0.53	9.54±0.51	7.82±0.54
2	265.60±7.67	88.80±5.46*	8.97±1.19	11.32±0.84*	10.50±0.75*
3	279.20±3.76	90.20±4.64**	9.34±0.83**	11.53±1.73*	9.40±1.68
4	259.86±6.27	71.74±4.00	7.06±0.67	9.22±0.56	8.07±0.52

Note. *$p<0.05$, **$p<0.01$ compared to group 4.

slide (75×25 mm), covered with a 18×18 mm slide, and dried at 18-25°C and 50-70% humidity. The samples were examined in polarized light in a MZ-12 stereomicroscope (Leica) equipped with a polarization device and Pixera TV camcorder.

The results were processed using Excel software. The significance of differences was evaluated using Student's *t* test.

RESULTS

Serum biochemical parameters (Table 1), results of morphological analysis of the viscera, and data of polarization microscopy of serum aggregation samples from control animals were considered as the normal values. Thread-like textures and small spherulites predominated in the control group (Fig. 1, *a*), with solitary needle-shaped and polymorphic crystal morphological types (Table 2).

Quantitative parameters of serum biochemistry in animals treated with dapsone were close to the control.

Microscopic structure of the liver in group 1 mice little differed from the normal. Hepatocytes with hypertrophic basophil nuclei were observed in just few cases, the number of binucleated cells increased compared to the control. Histological study of renal tissue from these animals showed no pathological changes.

Serum morphotype composition in animals treated with DDS was similar to that in the control.

Serum bilirubin content and AST activity in animals treated with hydrobunide were somewhat higher than in the control. ALT activity and the levels of creatinine and urea increased significantly ($p<0.05$).

Histological study of liver tissue from group 2 mice showed focal granular degeneration and necrosis of solitary hepatocytes, paralleled by lymphomacrophagal infiltration; moderate degeneration of the convoluted tubular epithelium was observed in the renal tissue of experimental mice.

Serum anisotropic structures of group 2 animals presented mainly as dendritic and polymorphic forms (Fig. 1, *b*), with few small and medium-sized spherulites.

Biochemical analysis of the serum from animals treated with furasonal showed a significant (*vs.* the control) increase of ALT and AST activities and bilirubin and urea concentrations ($p<0.05$). Histological study revealed pronounced structural changes in the visceral organs. Diffuse degeneration and necrosis of groups of hepatocytes in the central and peripheral compartments of the hepatic lobules were seen in the liver, necrosis of convoluted tubular epithelium was detected in the kidneys.

Serum aggregation samples from animals of this group contained mainly colored needle-shaped textures and spherulites, which were either defective, or contained foreign incorporations in the main structure (Fig. 1, *c*, *d*).

Biological fluid is a highly dynamic most sensitive tissue, as it is a complex system with characteristic patterns of fluctuations in physicochemical, biochemical, and morphological parameters caused by autowave processes of atoms and molecules of dissolved substances and the solvent.

Rhythmological characteristics of biological fluids can be imaged during transfer of the system from one phasic state into another, *e. g.* drying of a droplet. Any biological medium undergoes the following phase transitions during drying: molecular solution — micellar solution — liquid crystal — solid crystal [5]. Molecular complexes with similar autowave characteristics form the concentration wave or crystal structures, depending on the dehydration conditions. Any deviations in the qualitative or quanti-

TABLE 2. Characteristics of Serum Texture Distribution (*M*±*m*)

Group	Morphotypes		
	basic, %	secondary, %	atypical
1	81.57±3.78	18.43±3.78	0
2	37.00±4.18*	63.00±4.18*	0
3	8.80±1.39*	34.60±4.56*	56.60±5.37*
4	89.71±3.47	10.29±3.47	0

Note. *$p<0.001$ compared to group 4.

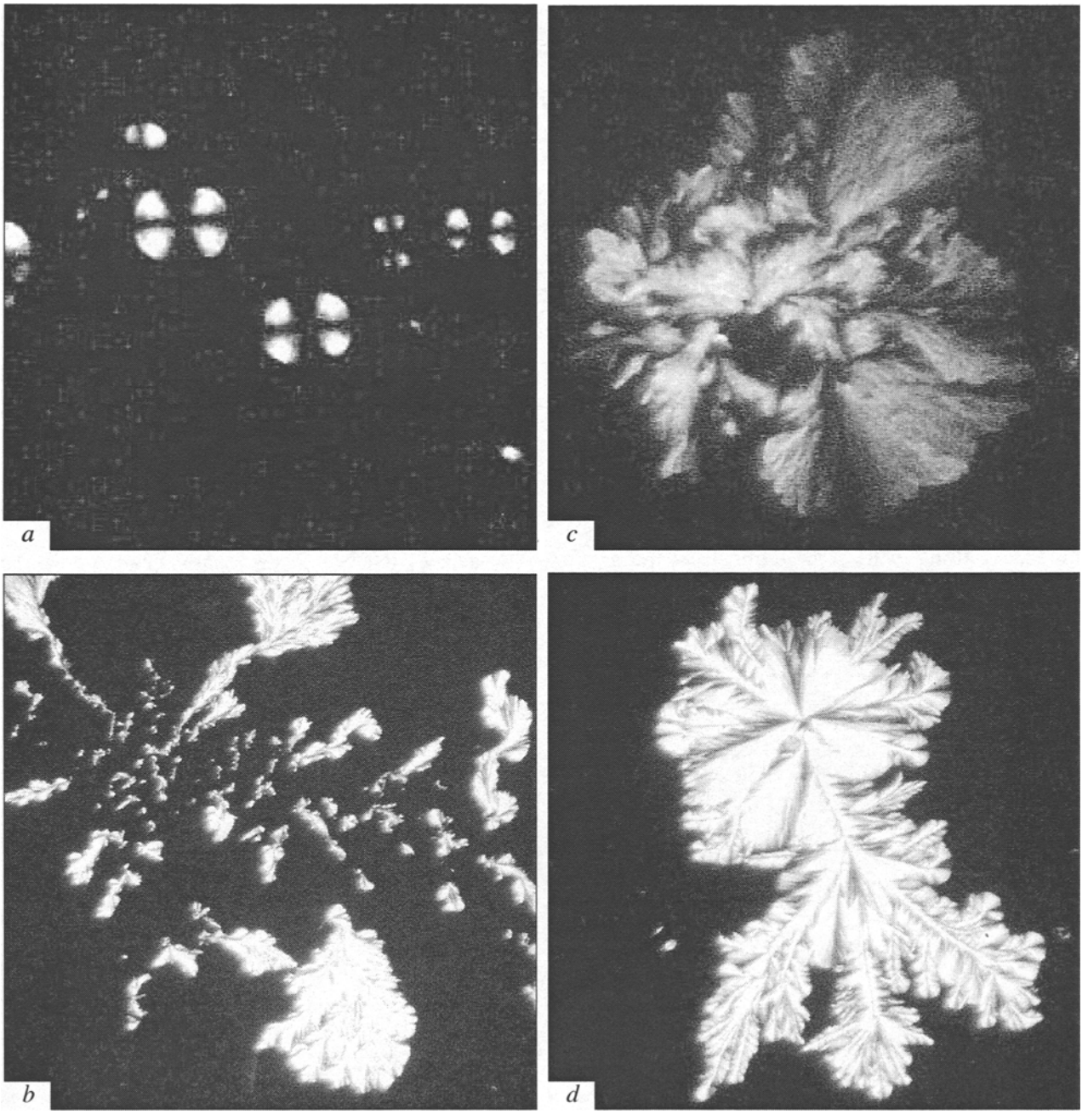

Fig. 1. Morphological types of experimental animal sera. *a*) basic (small spherulites); *b*) secondary (polymorphic, dendritic); *c*, *d*) atypical. Polarization microscopy: *a*) ·100; *b*) ·20; *c*) ·70; *d*) ·40.

tative composition of the environment modify the intermolecular interactions and lead to the formation of a new wave (vibration) gradient, involving alteration of structural and optic characteristics of biological fluids.

The crystal shape is a natural system for indication of physiological and pathological organic structures whose composition completely reflects the results of metabolic processes in the body [8]. Three main morphological types of anisotropic structures forming during drying are distinguished: basic, secondary, and atypical. The first of these are textures of compensated homeostasis (small and medium-sized spherulites and thread-like morphotypes). Secondary structures including fan-like, dendritic, polymorphic, needle-shaped, *etc.* morphotypes characterize the adapted status of homeostasis. The third group unites atypical morphotypes or textures of decompensated homeostasis with defective shape, incorporations of other structures in the main crystal shape, and differing from monochromatic secondary and basic forms by color range. Crystallization of liquid media of the body is described for some diseases [3,6,7,9].

Polarization microscopy of serum samples from control animals showed the predominant content of basic crystal forms with negligible content of secondary textures.

Quantitative and qualitative composition of serum morphotypes of animals treated with DDS little differed from the control. The data correlated with the results of biochemical and pathoanatomical studies, indicating the absence of toxic effect of the drug upon long treatment.

Biochemical and histological findings in animals treated with hydrobunide indicated an unfavorable effect of this drug on the hepatobiliary and urinary systems. Serum content of secondary morphotypes increased in group 2 animals, which was paralleled by decreased content of basic morphotypes, indicating tension of the adaptation mechanisms.

Furasonal treatment led to the appearance of atypical crystals in the sera, which is in line with the data of pathomorphological studies of the viscera and appreciable deviations in the biochemical parameters, indicating toxic effects of the drug.

Hence, our study demonstrated the possibility of using polarization optic method for examination of animal sera for tentative toxicological evaluation of drugs. The data of this method are in line with the results of more labor- and time-consuming pathoanatomical and biochemical methods, which recommends polarization optic microscopy for evaluation of qualitative and quantitative composition of morphological types of serum anisotropic structures as a preliminary method for evaluating drug tolerance by animals. The predominance of basic textures in the serum in the presence of negligible content of secondary and absence of atypical anisotropic structures indicates good prospects of the studied drug and prompts its further investigation. Changed ratio of basic to secondary textures towards accumulation of secondary textures is a sign of unfavorable effect of the drug on the host, while the appearance of atypical textures is a sign of toxicity. The advantages of the proposed method are obvious: small volume of material can be analyzed, the method is simple and rapid.

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