Morphofunctional Changes in Rat Erythrocytes Caused by Native Poison of Red Cobra (*Naja pallida*)

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The dynamic parameters of the phase-contrast images of rat erythrocytes were analyzed *in vivo* and *in vitro* by the method of contrast-computer microscopy under the effect of lethal dozes of poison from red (spitting) cobra (*Naja pallida*). The results of the analysis provide the information regarding erythrocyte function under normal conditions, the intensity of metabolic processes, and the state of plasma membrane.

Key Words: *phase-computer microscopy; Fourier spectrum; erythrocytes; cobra poison;* LD_{50}

Thorough investigation of damaging effects of zoological poisons on human organs, tissues, and cells, is necessary for the development of adequate detoxification measures.

The methods of automated morphological analysis provide more detail on the structure-function relationships in a biological system. It has been generally agreed that any pathological process and various physico-chemical influences impair the intracellular processes. These delicate impairments are reflected by objective functional manifestations [4].

The present work is an attempt to employ a computer-phase microscope (CPM) for analyzing the reaction of rat erythrocytes to the poison of red cobra. Our goal was to detect the most significant and informative parameters of this reaction by image analysis.

MATERIALS AND METHODS

Erythrocytes were obtained from 40 outbred albino rats of both sexes weighing 160-200 g. The animals were maintained under standard vivarium conditions.

Native poison from red (spitting) cobra (*Naja pallida*) was obtained in the Moscow zoo. The mean lethal dose (LD_{50}) of the poison for albino rats was 0.033 mg/100 g body weight.

The poison was injected intraperitoneally in a 2-fold LD_{50}. In the *in vitro* experiments heparinized blood was used. It was incubated with 0.028 mg (0.06 μl) poison/ml at 37°C for 5, 15 and 30 min. Blood from intact rats served as the control.

Live erythrocytes were studied by the method of phasometry [2] in a Tsitoskan CPM. The microscope was developed at the Moscow State Institute of Radioengineering, Electronics, and Automatics [5].

The microscope is a modified Linnik's interferometer with modulated phase of the reference wave. A helium-neon laser (λ=633 nm) served as a light source. Local values of the phase of the interference signal are measured with a coordinate-sensitive receiver: a dissector and an analog-digital converter. The CFM has the following parameters: objective ×30, the total magnification of the system ×5000, the accuracy of measurement by height and field was 0.5 and 20 nm, respectively, and the image discreteness was 64×64-256×256 pixels. The resolution of the microscope is slightly inferior to that of a raster electron microscope, which allows one to monitor changes in functional processes proceeding in biological objects without fixation and staining.

The CPM, which has identical objectives in the signal and reference shoulders, is based on the

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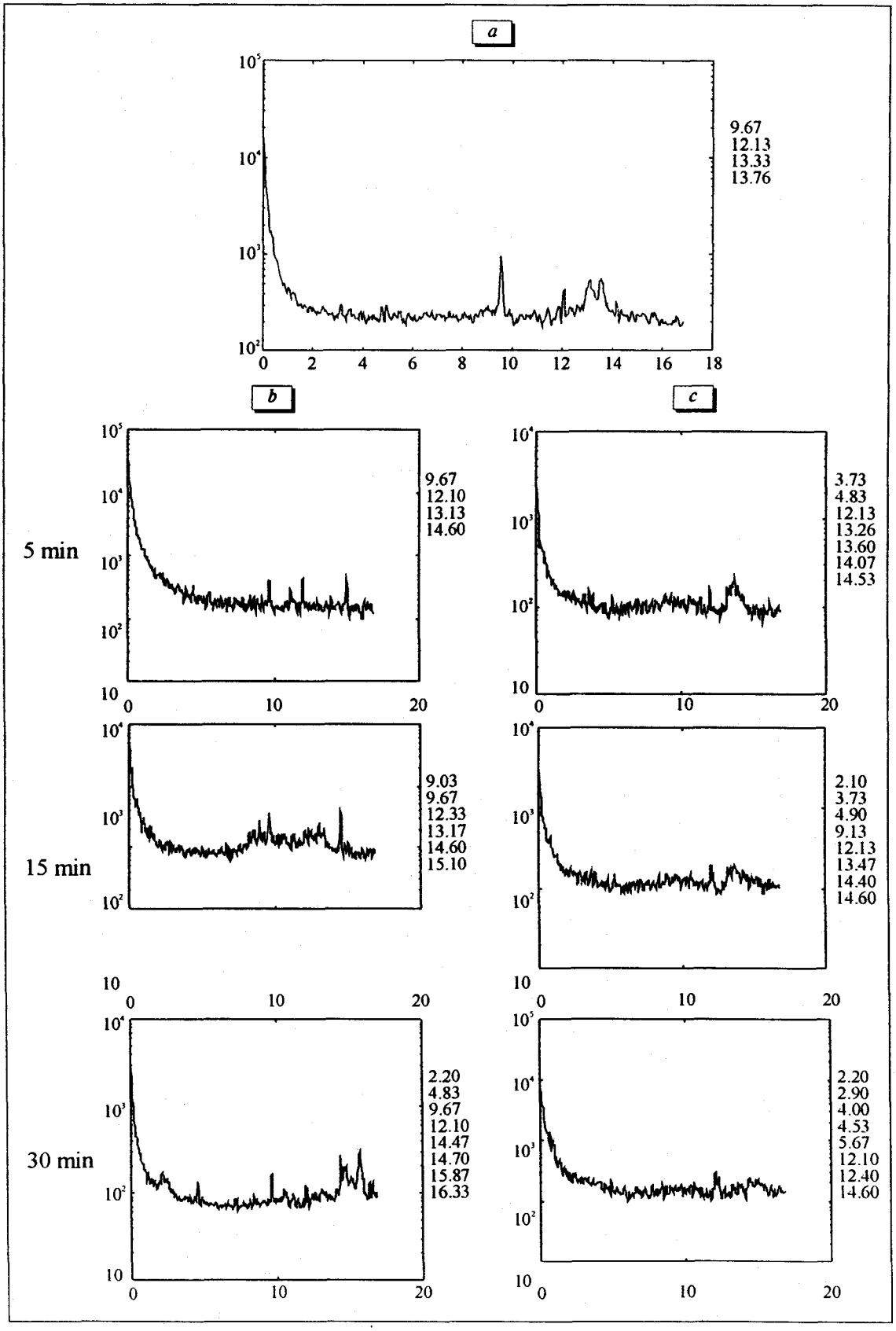

Fig. 1. Fourier spectrum and table of frequencies recorded in native erythrocytes and erythrocytes treated with the poison of Naja pallida *in vitro* and *in vivo*. *a*) control; *b) in vivo* experiments; *c)* erythrocytes incubated with the poison *in vitro*. Abscissa: frequency of periodic components of the spectrum, Hz; ordinate: power, arb. units.

comparison of the wave front reflected from the object surface with the reference wave front reflected from a high-quality mirror. The final signal is subjected to quantization with subsequent record of phase distribution is the form of a digital code.

The data are displayed as a topogram: distribution of the absolute values of phase heights in pseudocolor with 16 color coding. The digital matrix of the obtained phase profile is focused in the memory of the computer.

Cell dynamics was studied by analyzing temporary processes. For this purpose a profile was selected on the topogram and local values of the phase of the scattered wave were measured in 32 points of the profile structure (the real geometrical coordinate was identified with the number of the point). The number of consecutive records of a scanning was 1024 at observation time of 32.77 sec. The results of measurements were arranged in a 32×1024 matrix, in which the lines represent the space variable (phase height in the consecutive points) and the columns represent the temporary variable (consecutive measurements in one point), and organized in a databank for subsequent study of the records.

The activity of dynamic processes of the cells was assessed by Fourier analysis with automatic determination and formation of a table of the maximum power frequencies.

RESULTS

Analysis of the dynamic parameters of phase images of intact rat erythrocytes revealed "zones of silence" in the 1-9 Hz range and high-intensify processes occurring at frequencies of 9.67, 12.13, 13.33 and 13.76 Hz (Fig. 1).

The following changes in the Fourier spectra were observed in the *in vivo* experiments: the power of the reference frequencies recorded in the control decreased and an activity appeared in the 14.33-14.78 Hz range. A time-dependent increase in the spectrum intensity was observed in 15.56-16.33 Hz range.

The effect of native poison on whole blood *in vitro* manifested itself in a sharp reduction (by 8 fold) of spectral power in high-frequency range and in practically complete suppression of the activity of structural and metabolic processes occurring in at 9.67 Hz. These changes were time-dependent. A 2-fold increase in spectral power was observed in the 2.20-5.67 Hz range.

We believe that the spectrum of characteristic frequencies and their intensity reflect the intensity of intracellular metabolism. The contribution of mechanical, electrical, and diffuse processes associated with the cell membranes as well as that of interaction, distribution and redistribution of receptors, aggregation state of the cytoplasm, and conformation of macromolecules to the spectra cannot be ruled out. So far, there is no definite interpretation of these data. However, analysis of spectral parameters may be helpful in the establishment of a certain quantitative standard reflecting the normal state of cells for the use in further investigations.

A comparison of dynamic parameters of intact and poison-treated erythrocytes *in vitro* and *in vivo* revealed common constant frequencies: 9.67 and 12.10 Hz and "zones of silence" in the 4.8-9.6 Hz range.

The 14.1-14.7 Hz range frequencies with identical power were recorded in poison-treated erythrocytes both *in vivo* and *in vitro* and were not observed in the control. This range may reflect the binding of the poison components to the erythrocyte plasma membrane.

It is noteworthy that the time-dependent increase in spectral power in the 15.5-16.3 Hz range was recorded *in vivo* but not *in vitro*. This range may characterize the compensatory reaction of the organism to the poison.

The *in vitro* decrease in the intensity of the high-frequency component of the spectrum, suppression of the constant frequency 9.67 Hz to the background level, and intensification of slow-rate cell processes, which are reflected by the low-frequency components may be associated with degradation of the plasma membrane with subsequent hemolysis. This assumption is consistent with our previous findings confirming high cytotoxicity of the poison [3] which is determined by the presence of cardiotoxin and phospholipase A_2 [6,7]. The different dynamics of morphofunctional state of erythrocytes observed *in vivo* may be related to the fact that the poison causes no intravascular hemolysis [1].

Thus, we have demonstrated different responses of rat erythrocytes to the red cobra poison *in vivo* and *in vitro*.

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