gonadotropin system. Hyperthyrotropinemia probably provokes anovulation independently by acting directly on the ovaries.

The study was supported by the Russian Foundation for Basic Research (project code 93-04-06378).

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Effect of Native Venom of Red Cobra (*Naja pallida*) on Morphological and Rheological Properties of Erythrocytes

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 120, № 10, pp. 425-427, October, 1995
Original article submitted March 27, 1995

The effect of native venom of red (spitting) cobra (*Naja pallida*) on human erythrocytes is studied using the methods of electron microscopy and filtration through Millipore filters. Morphological analysis reveals marked transformation of discoid erythrocytes to echinocytes. More prolonged contact of the blood with the venom impairs the deformability of erythrocytes and induces hemolysis and the sludge syndrome. The results indicate a potent cytotoxic effect of native venom of red cobra.

Key Words: *erythrocytes; rheology; cobra venom*

The evolution of zootoxins, and in particular snake venoms, was directed at acquisition of the capacity to selectively damage the vital integrating systems of the organism: the blood, circulatory, and nervous systems. Diffusion of the venom in the organism is facilitated due to its ability to cleave the fibrinogen molecule and considerably reduce its plasma concentration. This results in hypocoagulation, inhibition of erythrocyte aggregation, decreased blood viscosity, and accelerated magistral blood flow [8]. These properties of snake venoms were used to design the drugs arvin, ancrod, and batroxobin for the treatment of peripheral arterial diseases and for the prevention of venous thromboembolism [3]. A study of the effect of snake venoms on the erythrocyte membrane is of special interest, since erythrocytes represent a universal model of the cell membrane [2]. Moreover, erythrocytes are an indispensable component of the rheological system which is responsible for optimal functioning of the micro- and macrocirculation. The most important property of erythrocytes here is their deformability, namely, the properties governing their shape, rigidity, and elasticity.

The above considerations prompted us to study the effect of some rare venoms of elapid snakes on the morphological transformation of human erythrocytes.

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MATERIALS AND METHODS

Naja pallida venom (0.1 μl, 0.028 mg dry substance) was added to 5 ml heparinized blood. The samples were incubated at 37°C for 5, 15, and 20 min. Intact heparinized blood served as the control.

For scanning electron microscopy samples of peripheral blood were fixed in 4% paraformaldehyde on a phosphate buffer (pH 7.4) and treated as de-

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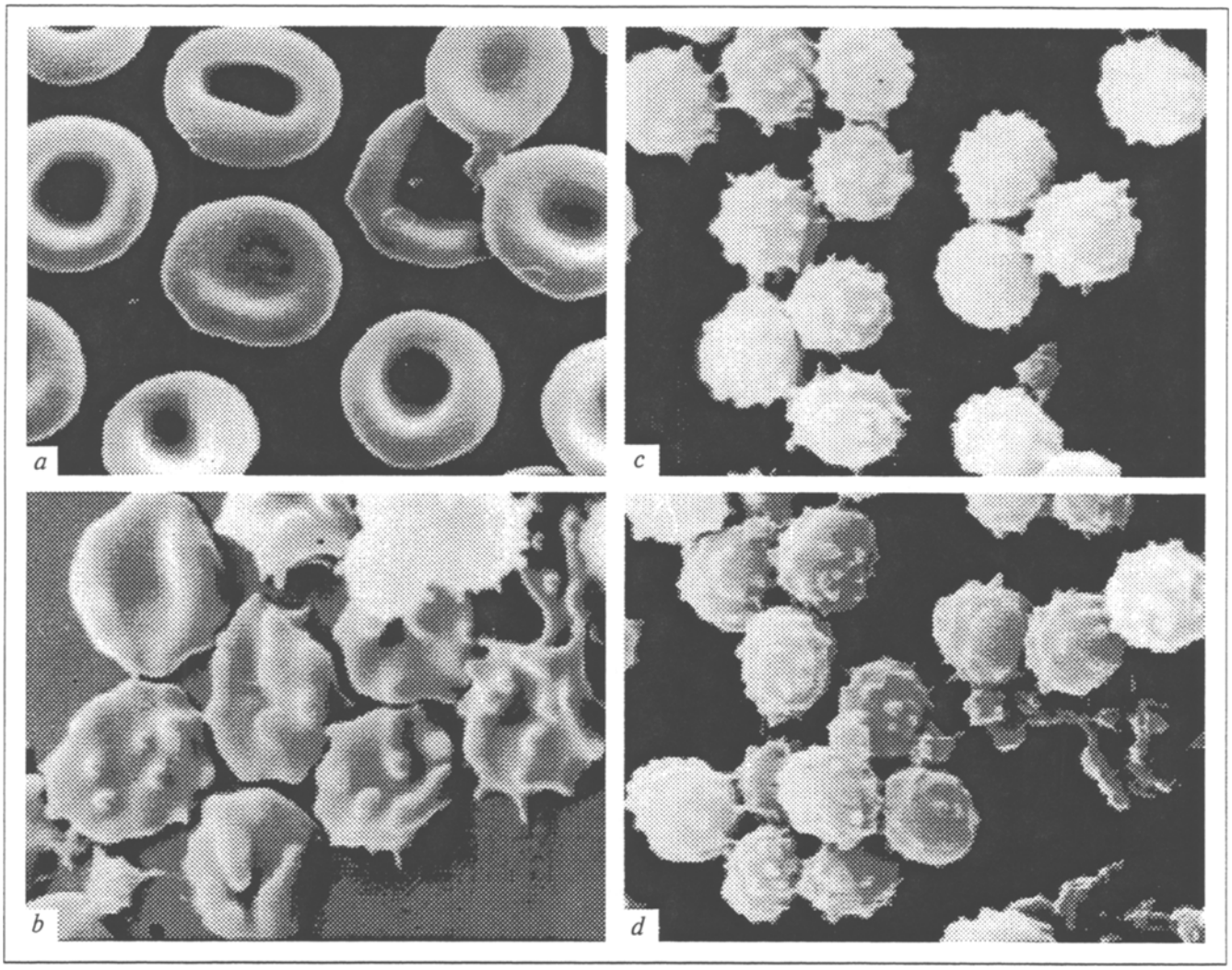

Fig. 1. Effect of native venom of *Naja pallida* on morphology of human erythrocytes. Human erythrocytes in health (*a*), and after 5-min (*b*), 15-min (*c*), and 20-min (*d*) incubation with venom. Scanning electron microscopy. ×4000.

scribed earlier [1]. The erythrocytes were washed free of fixative with distilled water by centrifugation (3 times, 1500 rpm for 10 min). The resultant pellet was resuspended in distilled water. One drop of the suspension was transferred to a cover glass, dried in the incubator for 5-10 min at 45°C, and dehydrated in ascending concentrations of acetone (50, 70, 95, and 100%) three times for 10 min in each solution. The obtained preparations were air-dried, dusted with gold, and examined under a JEM-100 scanning electron microscope.

Erythrocyte deformability was determined by filterability using a Sartorius filtration system and filters (5 μ pore size). The erythrocytes were washed twice with phosphate saline and a 5% erythrocyte suspension in phosphate saline (pH 7.4) was filtered. The results were presented as the percentage ratio of the number of erythrocytes passing through the filter per unit of time to their number in the initial suspension.

RESULTS

A 5-min incubation with *Naja pallida* venom markedly changes the shape of erythrocytes. Practically all erythrocytes were transformed into echinocytelike cells (crenation) with 15-30 randomly scattered conical spines of similar size (Fig. 1, *a, b*).

After a 15-min incubation the mean volume of erythrocytes shrinks and the crenate shape gradually becomes spherical; some erythrocytes approach each other and form cytoplasmic bridges (Fig. 1, *c*).

After a 20-min incubation we observed conglomerates of 7-8 erythrocytes. The number and density of intercellular junctions increase. Some erythrocytes undergo lysis and transform to ghosts (Fig. 1, *d*). The nature of erythrocyte contacts is indicative of the pathological sludge phenomenon rather than of normal cell aggregation.

Changes in erythrocyte deformability are illustrated in Fig. 2. Even the 5-min incubation reduces

the filterability of erythrocytes more than twofold, while after the 15-min incubation filterability is only 20% of the control value. This is evidently due to the spherical transformation of the erythrocytes, which is known to reduce their deformability [6]. After a 20-min incubation only 3-5% of cells pass through the filter, probably because the pores are choked with cell conglomerates.

Thus, morphological analysis showed that *Naja pallida* venom causes a general transformation of human erythrocytes from discocytes to echinocytelike cells. This transformation is probably mediated by the lysolecithin released when the venom comes in contact with the whole blood [5]. The observed changes are probably related to a drop of the ATP content and blocking or inhibition of Ca^{2+},Mg^{2+}-ATPase, which results in a rise of the intracellular Ca^{2+} concentration [4,7]. The morphological state of erythrocytes and their rheological properties progressively deteriorate with the lengthening of incubation, as is evidenced from the decreased filterability, the sludge phenomenon, and hemolysis. The findings suggest that the venom of this species has a potent cytotoxic effect.

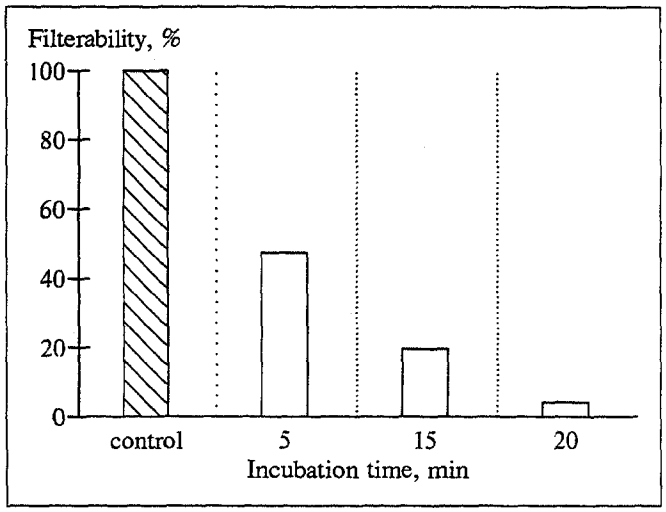

Fig. 2. Changes in filterability of erythrocytes in the course of incubation with venom. Filterability prior to incubation with venom taken as 100%.

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