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TRANSFORMATION AND ULTRASTRUCTURAL CHANGES OF ERYTHROCYTES ON SENSITIZATION TO SERUM PROTEIN

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Investigation of transformation of erythrocytes (loss of their usual discoid shape) has revealed many different varieties of these cells in blood diseases, carcinogenesis, and surgical suppurative infection [2, 3, 5]. However, as yet insufficient attention has been paid to morphological changes in erythrocytes in response to injection of a foreign protein, although the role of these cells in immunologic reactions is extremely important [1, 4].

In the investigation described below the shape and ultrastructure of erythrocytes was studied during sensitization of laboratory animals with normal horse serum (NHS).

EXPERIMENTAL METHOD

Experiments were carried out on 40 male Wistar rats (20 experimental and 20 control) weighing 220 ± 21 g. NHS, diluted 1:2 with 0.15 M sodium chloride solution, was injected subcutaneously into the dorsal region in a volume of 1.8-2.4 ml (dose rate 0.6 mg protein/100 g body weight). Control animals were injected with the same volume of sodium chloride solution. Twice the volume of NHS or physiological saline was injected 7 and 22 days later. The use of these doses of serum as sensitizing and reacting doses was chosen because with them the anaphylactic reaction which developed was mild or moderately severe, anaphylactic shock was not observed, and all the animals survived until the end of the experiment. Blood (1 ml) was taken from the tail vessels of the animals 30 min after injection of the reacting dose of NHS and also during the background investigation. As a control of the degree of sensitization of the animals, the leukocyte count and leukocyte formula were determined, with calculation of the morphologic reactivity index (MRI), whereby the intensity of the hemoimmune response of cellular type (HIRCT) could be estimated, the number of cells producing autoantibodies (hemolysins) was studied [4], and the number of circulating immune complexes (CIC) determined [6].

Erythrocytes were sedimented by centrifugation and washed off three times with cold (to 4°C) 0.15 M phosphate buffer, pH 7.2. Next, 0.25 ml of the cell residue was incubated at 4°C for 3 h in 2.5 ml of 0.25% glutaraldehyde solution in phosphate buffer, postfixed for 1.5 h in 1% OsO_4 solution, dehydrated in alcohols of increasing concentration and acetone, and embedded in Araldite. Ultrathin sections were examined in the JEM-100 CX electron microscope. Prepara-

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TABLE 1. Relative Proportions of Different Groups of Erythrocytes in Intact and NHS-Sensitized Rats

Group of animals	Period of investigation	Discocytes	Somatocytes	Echinocytes	Poikilocytes and schizocytes	Spherocytes and erythrocyte "ghosts"
Intact	Background	70,5±5,1	17,2±1,5	2,7±0,2	9,3±0,9	0,3±0,04
	After injection of 0,15 M sodium chloride solution	70,1±6,0	20,1±1,9	1,5±0,2	8,0±0,7	0,3±0,05
Sensitized	Background	72,2±5,6	15,4±1,6	2,0±0,3	10,3±0,8	0,1±0,01
	After injection of reacting dose of NHS	42,7±3,7[a, b]	23,1±2,0[a]	9,4±0,8[a, b]	23,0±1,5[a, b]	1,8±0,15[a, b]

Legend. a) Differences significant compared with background, b) differences significant compared with control at $P < 0.05$ level.

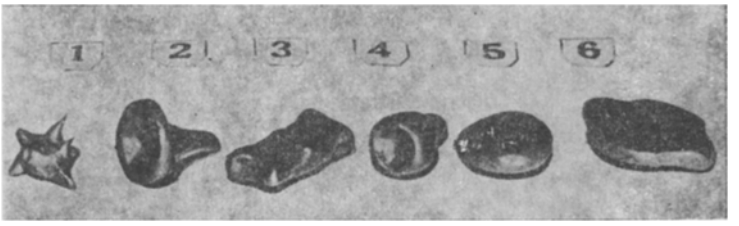

Fig. 1. Transformed erythrocytes of rat sensitized with NHS: 1) Acanthocyte (microform); 2) funnel-shaped poikilocyte; 3) stump-shaped poikilocyte; 4) erythrocyte with newly formed stoma and with comb-like evagination; 5) target cell; 6) bipolarly elongated, flattened erythrocyte. 960 ×. Light microscopy. Diagrammatic, from photographs.

tions of "squeezed drop" were obtained from the rest of the washed erythrocytes, and 500 cells were counted with a light microscope under a magnification of 900×. For convenience of statistical analysis all forms of erythrocytes were grouped as follows: cells of unchanged shape (discocytes), transformed erythrocytes (stomatocytes, echinocytes, poikilocytes, and schizocytes, and hemolyzed forms (spherocytes and erythrocyte "ghosts"). To obtain an integral assessment of the process of cell transformation in individual cases the transformation index (TI) was calculated, i.e., the ratio of the total number of transformed erythrocytes to the number of discocytes. The experimented results were subjected to statistical analysis by the t test [8].

EXPERIMENTAL RESULTS

Sensitization and injection of the reacting dose of protein caused a significant ($P < 0.05$) increase in the number of leukocytes [$(14.7 \pm 1.95) \cdot 10^9$/liter in the experiment, $(11 \pm 2.1) \cdot 10^9$/liter in the control , eosinophils (9.3 ± 1.3 and $3.4 \pm 0.6\%$, respectively), and cells forming hemolysins ($2.8 \pm 1.1\%$ in the experiment, $0.45 \pm 0.08\%$ in the control) in the rats. CIC also appeared in the blood of the sensitized animals (up to 0.36 ± 0.05 conventional extinction unit), whereas in the control group none were detected. These results indicated the development of sensitization of the rats to the foreign protein. MRI in all the experimental animals exceed 18 points at the last determination, evidence of the development of a positive HIRCT, and confirming the presence of sensitization [1, 9].

According to the results of light microscopy, the number of transformed erythrocytes in the control animals remained low (29.5-29.9%) throughout the experiment, whereas the increase in this parameter at the last investigation, compared with the background level, led to a threefold increase in TI. In the sensitized animals, among cells with modified shape, poikilocytes and stomatocytes were most frequently observed (Table 1), followed by echinoovalocytes and echinostomatocytes (mixed type of transformation) and schizocytes (Fig. 1). The appearance of large lanceolate or of bipolarly elongated erythrocytes, annulocytes, and spherocytes, and forms with a pinched off cell membrane, resembling thread-like myelin figures with spherical swellings at their end, possibly as a result of clasmatosis [7], also was noted.

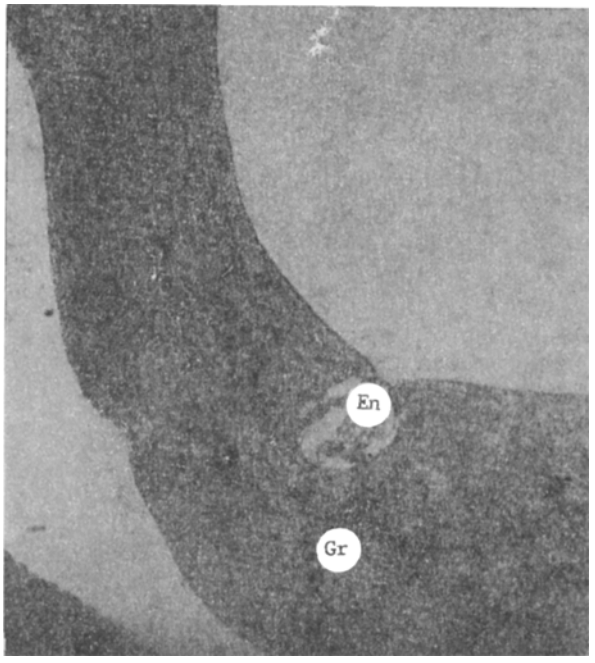

Fig. 2. Ultrastructure of poikilocyte of rat sensitized with NHS. En) Endovesicle; Gr) concentration of hemoglobin-containing granules in central part of cell. 13,000 ×.

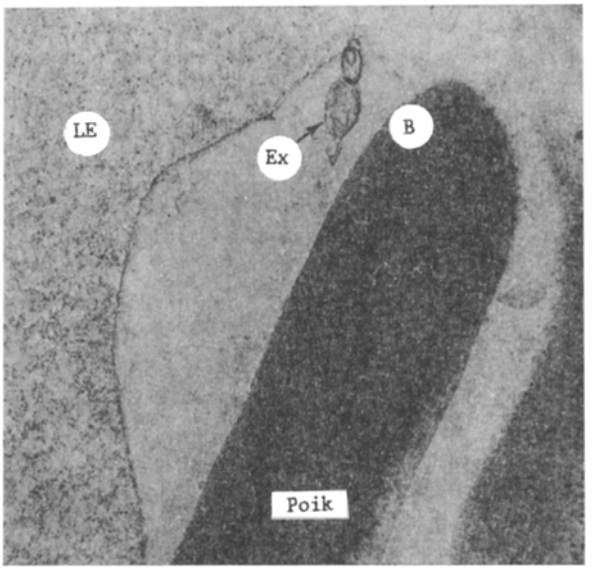

Fig. 3. Ultrastructure of areas of lysed erythrocyte (LE) and poikilocyte (Poik). Ex) Exovesicle; B) concentration of hemoglobin-containing granules in the form of a band. 13,000 ×.

The electron-microscopic investigation showed that the cytolemma of the discocytes of intact animals was undamaged, and the hemoglobin-containing granules of high electron density were uniformly arranged. The cell membrane of the somatocytes, like that of the discocytes, remained double-layered, but it was thickened in some areas. Local loosening of the structure of the cytolemma was found in some echinocytes and poikilocytes, or even fusion of its outer and inner layers. However, their number, like the number of areas with exovesicle formation, did not exceed three to six (with respect to each feature) per 100 cells studied. Fusion and enlargement of the hemoglobin-containing granules took place in some erythrocytes, and they were concentrated in the form of narrow bands in the inner juxtamembranous zone. Injection of NHS appreciably increased the heterogeneity of the ultrastructural organization of the erythrocytes. The number of exo- and endovesicles exceeded 10-15 (with respect to each

feature) per 100 erythrocytes. Exovesiculation affected not only the outer layer, as in the control, but also the inner layer of the cytolemma. These changes were observed particularly often in poikilocytes and schizocytes, but they were also commonly found in the stomatocytes and echinocytes, although they were hardly ever observed in the control animals. In all transformed erythrocytes fusion of hemoglobin granules was observed mainly in the central zones; the width of these areas was increased to 1.5-3 times that in the control (Fig. 2). The number of erythrocytes with signs of hemolysis (erythrocyte "ghosts"; Fig. 3) was doubled.

Sensitization of albino rats with NHS is thus reflected in the state not only of leukocytic, but also of erythrocytic hematopoiesis. Changes in the shape and ultrastructure of the erythrocytes under these circumstances, amounting in some cases to injury and destruction, are evidence that they participate in the immune response of the body to antigenic stimulation by foreign protein as target cells [1, 7].

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ADVANTAGES OF ULTRASONIC SURGICAL INSTRUMENTS FOR USE IN EXPERIMENTAL NEUROPHYSIOLOGY

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The use of ultrasonic instruments (USI) in neurosurgery, ophthalmology, otorhinolaryngology, traumatology, and other branches of medicine is increasing at the present time. This is because of some important advantages of the method, and in particular, the reduction of tissue trauma and the hemostatic effect. The wide use of this type of energy to divide biological tissues has become possible with the introduction of resonance rods of variable section, allowing concentration of oscillatory movements (from 23,000 to 60,000/sec, with an amplitude of up to 100 μ or more) on the cutting edge of the surgical instrument. Physiological tolerance of the brain to the action of USI has been determined experimentally [1, 4-6]. In this connection existing types of instruments are being improved and new ones created [2, 3], and the scope for their use in neurosurgery is being widened.

The aim of this investigation was an experimental study of the effect of ultrasonic techniques on the functional state of the brain and its individual structure in order to determine the most appropriate and least traumatic method of removal of certain brain structures chosen for study.

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