tion of the erythrocytes is not essential [15]. In the present experiments features of the species difference between rat and mouse were clearly manifested when formaldehyde-treated erythrocytes were used. The success of the attempt to use bone marrow instead of peripheral blood granulocytes offers the prospects of working with small animals, from which it is difficult to obtain a leukocytic concentrate from the peripheral blood.

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MECHANISM OF ACTION OF HEPAIN ON LYMPHOCYTES in vitro

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UDC 615.273.53.015.4:612.112.94

In experiments on CBA mice in which a 2% suspe ·on of red blood cells was used as the antigen, heparin was shown to prevent the migration .. antibodies from antibody-producing cells substantially in vitro. On the addition of substances with the properties of detergents (Triton X-100, deoxycholate) in vitro to a suspension of plaque-forming spleen cells treated with heparin, the ability of the cells to form plaques was partially restored. It is concluded that heparin is able to interact with the outer membrane of immunocompetent cells and to inhibit migration of antibodies synthesized by them into the surrounding medium.

KEY WORDS: heparin; detergents; plaque-forming cells.

Considerable evidence of the ability of heparin to inhibit the development of diseases based on autoimmune conflict has accumulated recently in the literature [1, 2, 8, 11].

The inhibitory action of heparin on plaque formation in vitro has been demonstrated [3, 4]. During the analysis of the results of these investigations it was postulated that the mechanism of action of heparin could be connected with its adsorption of the cell membrane of the lymphocyte, as a result of which obstacles could arise to the migration of antibodies, which will be followed by inhibition of the reaction in progress.

The object of the investigation described below was to test this hypothesis.

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 84, No. 11, pp. 588-590, November, 1977. Original article submitted April 18, 1977.

0007-4888/77/8411-1612$07.50

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TABLE 1. Deblocking Action of Triton X-100 and Deoxycholate on Spleen Cells Treated with Heparin

Treatment of spleen cells	Number of observations	Number of PFC per 10^6 spleen cells	
		$x \pm x$	confidence interval
Control	30	22,4±1,21	24,82—19,98
Heparin	25	3,52±0,32	4,16—2,88
Heparin+ Triton X-100	31	10,02±0,55	11,12—8,92
Heparin+ deoxycholate	26	6,22±0,47	7,16—5,28

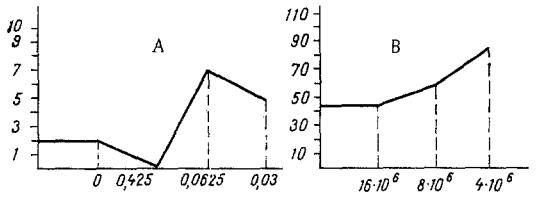

Fig. 1. Action of detergents on spleen cells treated with heparin. A) Various concentrations of detergents; B) various numbers of spleen cells. Abscissa: A) concentration of detergents (in %); B) number of spleen cells; ordinate, number of PFC (per 10^6 spleen cells).

EXPERIMENTAL METHOD

Experiments were carried out on CBA mice obtained from the Stolbovaya Nursery of laboratory animals, Academy of Medical Sciences of the USSR. A 2% suspension of sheep's red blood cells (SRBC) was used as the antigen. Four days after intraperitoneal immunization, the number of plaque-forming cells (PFC) in the spleen of the mice was determined by a modification of Cunningham's method of local zones of hemolysis in semi-liquid medium [9]. The suspension of spleen cells, made up by the usual method, including filtration through a mesh filter, was incubated with heparin in a dose of 70 units to 0.5 ml of the cell suspension at 37°C for 15-20 min. The cell suspension was then washed three times with medium 199, mixed with a 10% suspension of SRBC, and fixed with complement (1 :5). The resulting suspension was poured into specially prepared containers. The containers was incubated at 37°C for 45-60 min. At the end of incubation the number of PFC was counted and the number of antibody-forming cells per 10^6 lymphocytes was calculated.

The substances used for abolishing the heparin blockade of antibody-producing cells were the detergents Triton X-100 and deoxycholate. They were added to the spleen cells immediately after incubation of the cells with heparin. The working dose of the detergent was that which, in the course of 5 min, failed to cause hemolysis of the 10% suspension of SRBC. Cells not treated with heparin or detergent served as the main controls. The effect of abolition of the blockade of plaque formation was assessed as a percentage of the controls. The significance of differences was calculated by the Student–Kolmogorov method.

EXPERIMENTAL RESULTS

The experimental results are summarized in Table 1 and show that the number of PFC in the containers treated with heparin was significantly lower than in the control. The result was not connected with the anti-complementary action of heparin, for the spleen cells, after treatment with heparin, were washed three times to remove the excess of this substance, and complement was added in considerable quantities. The part of the heparin which had such a strong blocking effect on the lymphocytes was evidently in a bound state on the surface of the lymphocyte membrane. This hypothesis is further confirmed by the fact that heparin, as a poly-anion with a surplus negative charge, penetrates with difficulty inside the cell [7] and is able to change the surface ζ potential of the cell membrane [5, 6, 10]. The results of the subsequent experiments with detergents showed that this hypothesis is correct. The final results of these experiments are given in Table 1. Under the influence of detergents the ability of the spleen cells treated with heparin to form plaques was partly restored.

The number of PFC was increased by 2.5-3 times by the action of detergents and was significantly greater than the number of PFC treated with heparin alone.

The specificity of the action of detergents on the lymphocytes was ruled out by an additional series of experiments. Starting from the assumption that, by the laws of action of detergents, their deblocking effect on PFC should strengthen with an increase in their concentration, the following experiment was carried out. Detergents were added in various concentrations to a constant number of PFC treated with heparin. As Fig. 1 shows, an increase in the concentration of surface-active substances led to a stronger deblocking effect.

When surface-active substances belonging to two groups of detergents (deoxycholate) were used, only quantitative differences were found in their effective concentration and the course of the process was in the same direction.

In another series of experiments to rule out specificity in the reaction between detergents and lymphocytes, the hypothesis that the same dose of detergents should have a stronger effect on a small number of PFC than on a large number was tested. The results confirmed the original hypothesis (Fig. 1).

Evidence was thus obtained from the results of these experiments that the process of plaque formation by mouse spleen cells is inhibited by treatment with heparin in vitro. Subsequent treatment with detergents largely abolishes the inhibitory effect of heparin. Presumably heparin has an inhibitory action both on account of obstruction to interaction of T and B lymphocytes and on account of its action on the membrane of the PFC themselves, blocking migration of synthesized antibodies out of the cells.

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