LEUKOCYTOLYSIS AND PHAGOCYTOSIS UNDER HYPEROSMOTIC CONDITIONS

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Cytolysis, as a method of clinical immunologic diagnosis [1, 3], including the specific leukocytolysis test, the neutrophil alteration test, and analysis of intracellular criteria of damage to these cells [1, 7, 8], has definite shortcomings: the considerable variability of the data, the small difference in the degree of cytolysis of cells of sensitized patients and control individuals. The problem of the initial phagocytic activity of neutrophils (PAN) and their ameboid reaction, determined by the neutrophil damage index (NDI) test, likewise remains unsolved [7, 8].

The aim of this investigation was to study the effect of nonspecific factors such as the osmotic activity of the medium [4] and the physicochemical properties of the substances, with particular reference to low-molecular-weight nonelectrolytes (glycerol and glucose), on leukocytolysis and PAN. These factors have a hemolytic (erythrolytic) action, and in the case of leukocytes, it has not received adequate attention in the literature.

EXPERIMENTAL METHOD

The leukocytolytic action was evaluated by the method in [5], which we have modified, and PAN against *Staphylococcus aureus* was determined by the method in [6]. Leukocytes in heparinized blood (0.1 ml heparin, "G. Richter," Hungary, to 5.0 ml blood) were counted in a counting chamber and the morphology of the blood cells was studied, with calculation of the differential leukocyte formula (control). A solution of the nonelectrolytes in increasing concentration from 0.04 to 4.0 M was introduced into seven test tubes, each in a volume of 0.1 ml. To each tube was then added 0.1 ml of blood, the contents were mixed, and the tubes allowed to stand for 1 h at room temperature. After careful mixing, 0.2 ml of 3-5% acetic acid was added to 0.2 ml of diluted (1:10) blood from each tube, and after mixing the number of leukocytes in each sample was counted. The supernatant was poured off, a film prepared from the residue, and the leukocyte formula counted in it.

Altogether six series of experiments were carried out with each preparation.

EXPERIMENTAL RESULTS

Analysis of the data in Table 1 shows that both nonelectrolytes induced marked leukocytolysis. Under the microscope, some of the destroyed cells and preserved leukocytes were visible in the films. This result was confirmed by a decrease in the number of cells in the samples: the number of leukocytes in the control was $(12.36 \pm 1.645) \cdot 10^9$/liter in the experiments with glycerol and $(14.24 \pm 0.745) \cdot 10^9$/liter in those with glucose. Dependence of the leukocytolytic action on equimolar concentrations of nonelectrolytes was found to be a parabolic function: it was more marked in hypotonic than in hypertonic solutions; optimal concentrations were 2.0 M of glycerol and 0.5 M

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TABLE 1. Effect of Nonelectrolytes on Blood Leukocyte Count ($\cdot 10^9$/liter) (M ± m)

Concentration of solutions, M						
0,04	0,15	0,5	1,0	2,0	3,0	4,0
2,52±0,25*	3,08±0,47*	4,00±0,42*	3,72±0,65*	7,26±1,15*	5,04±0,35*	5,28±0,40*
6,92±0,58*	10,96±0,99	13,90±0,71	12,18±1,34	11,42±1,01	11,40±2,12	8,68±2,02*

Legend. Asterisk indicates values for which $p < 0.01$ compared with control.

TABLE 2. Effect of Glycerol (Gly) and Glucose (Glu) on Phagocytic Activity of Neutrophils (M ± m)

Parameter	Preparation	Concentration of solutions, M						
		0,04	0,15	0,5	1,0	2,0	3,0	4,0
Number of phagocytic neutrophils, per cent	Gly	46±5,5	48±5,8	41±11,2	33±10,2	28±14,0	34±7,0	22±5,0*
	Glu	56±8,7	39±7,9	18±7,0	21±4,5	15±2,0	14±2,4*	14±4,8*
Phagolytic index of ingestion	Gly	2,5±0,24	3,1±0,27	3,0±0,51	2,2±0,33	1,2±0,39*	2,0±0,18	1,5±0,20
	Glu	2,1±0,94	1,12±0,22	0,94±0,37	0,38±0,10	0,28±0,05*	0,28±0,06*	0,34±0,11
Index of ingestion	Gly	0,53±0,10	0,97±0,26	0,73±0,03	0,57±0,05	0,44±0,15	1,24±0,52*	0,93±0,29
	Glu	0,61±0,06	0,59±0,25	1,28±0,62	0,76±0,18	1,26±0,39	0,88±0,22	2,99±0,72*

Legend. Asterisk indicates data for which $p < 0.01$ compared with values at 0.15 M concentration.

of glucose, when despite the existence of leukocytolysis, the relatively largest number of leukocytes still remained. Comparison of the quantitative characteristics of leukocytolysis in equimolar solutions of the nonelectrolytes shows that glycerol has a leukocytolytic action 2-3 times stronger than glucose.

The morphologic investigation showed that in hypotonic and hypertonic solutions (3.0-4.0 M) of nonelectrolytes the mononuclears have greater osmotic resistance than polymorphonuclear leukocytes (PML). Within the concentration range 0.5-2.0 M a relative and absolute increase in the number of PML was observed: in 0.5 and 2.0 M glycerol solutions the relative and absolute numbers of neutrophils were 5.5 and 12.0% or $0.373 \cdot 10^9$/liter and $1.098 \cdot 10^9$/liter, whereas in the same concentrations of glucose, it was 82 and 91% or $10.25 \cdot 10^9$/liter and $14.74 \cdot 10^9$/liter respectively. Meanwhile it was found that glucose has a milder cytolytic action than glycerol: a lower degree of cytolysis of the cells, less severe intracellular degenerative changes in the leukocytes. The qualitative parameters of leukocytolysis thus confirm the trend of changes in the numerical parameters.

It follows from Table 2 that the parameters of PAN in the control were the number of phagocytic neutrophils (35 ± 8.7%), the phagocytic index (2.3 ± 0.46), and the index of digestion (0.69 ± 0.05). Comparison of these results shows that PAN was maintained in the solution of nonelectrolytes. This indicates sufficient resistance of the functions of PNL. In hypotonic solutions PAN rose by 11-16% compared with the control, remained at the optimal level in isoosmotic (0.15 M) medium, and fell (with respect to the first two parameters) in hypertonic solutions. In this case hypertonic solutions of glycerol reduced PAN less than did glucose, which stimulated their digestive power more strongly.

Thus parallel determination of PAN and investigation of leukocytolysis, differing from the method in [5] in that it was carried out in a hypertonic medium also, thus yielded important facts. Hypertonic solutions of glycerol and glucose, like hypotonic, in vitro induced nonspecific cytolysis of leukocytes, some of which remained viable and, in particular, preserved their PAN. The results indicate that the degree of leukocytolysis depends on PAN, confirming that PAN can be stimulated by products of leukocytolysis [2].

The great resistance of mononuclears to strong osmotic influences of the two nonelectrolytes agrees with data showing the faster rate of migration and the shorter life span of granulocytes than of mononuclears [2] and also with information indicating the greater osmotic resistance of the latter [5].

The parabolic character of dependence of leukocytolysis on concentrations of the glycerol and glucose solutions is linked, in our opinion, with the osmotic action of the nonelectrolytes, for the difference in the leukocytolytic action of equimolar solutions of these nonelectrolytes, which have equal osmotic pressure, indicates a nonosmotic component of this action. This state of affairs in principle can serve as the basis for the use of equimolar

solutions in reactions of specific leukocytolysis, and not of concentrations of therapeutic agents containing equal weights. In this way the accuracy and reproducibility of the results of laboratory diagnosis of drug allergy can be increased.

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