2. M. S. Petrova, M. E. Sukhareva, S. A. Azarova, and N. M. Brodova, *Vopr. Okhr. Mat.*, № 5, 17-20 (1986).
3. P. V. Sergeev, A. S. Dukhanin, S. I. Ogurtsov, *et al.*, *Byull. Eksp. Biol. Med.*, **111**, № 1, 44-46 (1991).
4. E. M. Barry, G. Clofent, W. Crist, *et al.*, *J. Bact.*, **17**, 720-726 (1991).
5. L. Bonasera, *Boll. Soc. Ital. Biol. Sper.*, **61**, 811-817 (1985).
6. S. Durant, D. Duval, and F. Homo, *J. Steroid Biochem.*, **17**, 61 (1982).
7. A. Munck, P. M. Guyre, and N. J. Holbrook, *Endocr. Rev.*, **5**, 25-44 (1984).
8. T. Murayama, H. Hashimoto, M. Kubota, *et al.*, *J. Neurochem.*, **55**, 1631-1638 (1990).
9. A. Takalts, S. Grinstein, D. Goets, *et al.*, *Acta Biochim. Biophys. Hung.*, **24**, 191-202 (1989).

Nonspecific Leukocytolysis *In Vitro* as a Test of Comparative Cytotoxicity of Low-Molecular Nonelectrolytes

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In experiments with hypo-, iso-, and hypertonic solutions taken at equimolar concentrations for equilibrium of osmotic conditions the leukocytolytic action of each of three nonelectrolytes was shown to be a parabolic function of osmotic concentrations. The comparative degree of leukocytolysis for urea is superior to that for glycerin and glucose and in inverse proportion to their molecular weight. As this difference depends on the chemical component of action, nonspecific leukocytolysis *in vitro* is considered as a test of comparative cytotoxicity in contrast to the specific (immunologic) cytotoxicity.

Key Words: *leukolysis; nonelectrolytes; osmosis; cytotoxicity*

At present two methods are generally used for the study of leukocytes as a biological object. The first approach, involving cell count in counting chambers and morphological examination of blood cells [7], serves a diagnostic purpose and is used for assessment of reactivity (for example, the leukocytic index of intoxication) [9] as well as of adaptive reactions of the organism [5]. The second includes a group of techniques among which immunologic assays are used [1,3,8,11] to study the leukocytic response to *the in vitro* action of various infectious and noninfectious agents, as well as to determine specific cytotoxicity [8,11]. The reaction of leukolysis is specific and has been recommended as a tool for comparative study in cases of chemical and drug standardization with regard for their allergenic action [3]. However, these recommendations are hardly implemented due to procedural difficulties, which have been described as follows: "so far, purely osmotic phenomena of irritation are almost impossible to distinguish from purely chemical phenomena" [10]. Nevertheless, this problem (of comparing the nonspecific cytotoxicity of chemical substances) may be partially solved by drawing on a classic proposition of chemistry, namely, that equimolar solutions of nonelectrolytes (NE) of low molecular weight have equal osmotic pressure [6].

The aim of the present investigation was to analyze the quantitative changes and qualitative indexes of nonspecific leukolysis *in vitro* and to validate the leukocytolysis phenomenon as a test of

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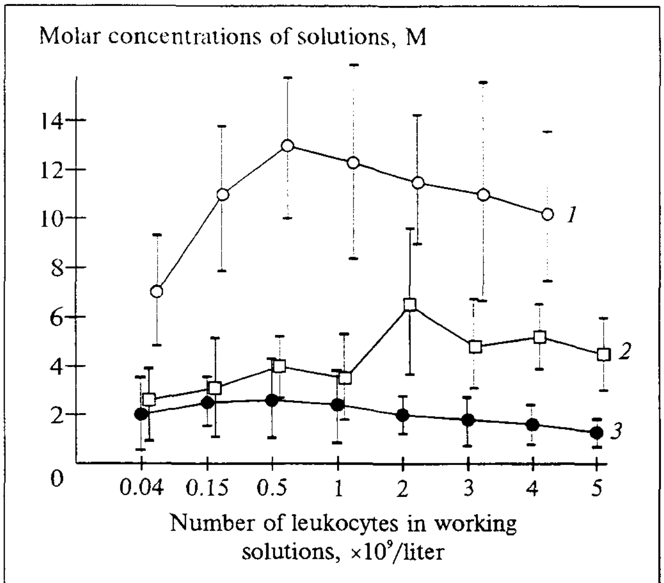

Fig. 1. Resistance of blood leukocytes to osmotic and chemical effects of equimolar NE solutions. 1) glucose; 2) glycerin; 3) urea.

nonspecific comparative cytotoxicity on the example of hypo-, iso-, and hyperosmotic solutions of low-molecular NE of various molecular weight.

MATERIALS AND METHODS

The leukocytolytic effect of urea, glycerin, and glucose on the blood of intact animals (dogs) was estimated by a procedure described previously [4]. Morphological study of blood cells including differential leukocyte counting was performed as described elsewhere [7]. Each NE was tested in 6 experimental series. Comparative calculations of the osmotic activity of the solutions were performed according to the van't Hoff equation [6].

RESULTS

The results presented in Table 1 clearly demonstrate the comparative values of osmotic pressure for NE solutions in relation to the total osmotic blood pressure (7.6 atm), which is mainly dictated by NaCl and other electrolytes [10]. Analysis of the tabulated data, first, confirms the correlation between the osmotic pressure and the concentration of the particular NE solution and, second, attests to the different osmotic pressures in solutions prepared from equal-weight samples of different NE. The higher the molecular weight of NE taken in equal-weight ratios, the lower the osmotic pressure of their solutions in comparison to NE of lower molecular weight.

The data depicted (Fig. 1) show that all NE both in hypo- and in hypertonic concentrations exert a marked lytic effect on leukocytes. The number of leukocytes decreases parabolically. Consequently, there is a range of low (0.04 M) and high (3-5 M) concentrations of preparation solutions at which lysis of leukocytes is significant, whereas within concentration limits of 0.5-1 M for urea and glucose and 2 M for glycerin this action is less pronounced (the peak of the parabolic curve on Fig. 1).

Furthermore, the comparative number of leukocytes in equimolar solutions of different NE is the lowest for urea and the highest for glucose. In other words, the degree of leukocytolytic action of urea is greater *in vitro* than that of glycerin and glucose (all other conditions being equal). The lytic effect of glycerin on blood leukocytes is higher than that of glucose but lower than that of urea. The confidence intervals of the mean numbers of leukocytes (Fig. 1) show that the difference in the extent of the leukocytolytic action between urea and glucose (at all concentrations) and between each of the three NE pairs (in the range of hypertonic concentrations) is reliable.

It is noteworthy that the sequence of three NE (urea - glycerin - glucose) arrayed in order of diminishing leukocytolytic activity exhibits an inverse dependence on the molecular weight: the

TABLE 1. Comparative Data on Osmotic Activity of Aqueous Solutions of Low-Molecular NE at 20°C in Equal-Weight Concentrations (Calculations by van't Hoff Equation)

Concentration of solutions, %	Osmotic pressure of solutions, atm			
	urea (60.06)	dimethylsulfoxide (78.13)	glycerin (92.1)	glucose (180.16)
3.003	12.03	9.25	7.84	4.01
6.006	24.05	18.49	15.69	8.02
12.012	48.11	36.98	31.37	16.04
18.018	72.17	55.47	47.06	24.06
24.024	96.22	73.96	62.74	32.08
30.030	120.28	92.46	78.44	40.09

Note. Figures in parentheses are molecular weights.

lower the molecular weight of an NE, the higher its leukocytolytic action, and vice versa.

Morphological investigations of formed elements of the blood have confirmed the above pattern of leukocytolysis. Here, the resistance of granulocytes to the impact of hypo- and hypertonic NE solutions is lower as compared to mononuclears.

Thus, the results of our study make it possible to distinguish two components in the phenomenon of nonspecific leukocytolysis: osmotic and chemical. The osmotic component is plotted as a parabolic curve (Fig. 1), which represents lysis as a function of osmotic concentrations. The similar character of this correlation for the three NE is due to the fact that osmotic concentrations are probably determined only by the total number of molecules dissolved and do not depend on the chemical nature of the substance [6]. The correlation itself reflects the different resistance of mononuclears and granulocytes to osmotic effects of different extent.

Since the conditions of our experiments (osmotic, pH, temperature, blood dilution, and so on) were similar, the discrepancy in the leukocytolytic action of NE is explained by their individual chemical properties (the chemical component). This component is graphically expressed (Fig. 1) by the various levels at which the "dose-effect" curves are situated on the ordinate axis. Each level is typical for each NE and reflects the extent of its leukocytolytic action.

The data obtained enable us to consider nonspecific leukocytolysis *in vitro* as a test of the comparative cytotoxicity of low-molecular NE as distinct from the specific (immunologic) cytotoxicity. The correlation between the cytotoxicity of NE and their molecular weight is substantial, a fact which is of fundamental importance in the context of evaluating cell damage [2,10] as an aspect of general pathology.

REFERENCES

1. A. D. Ado, *General Allergology* [in Russian], Moscow (1978), p. 274.
2. A. D. Ado, *Problems of General Nosology* [in Russian], Moscow (1985), p. 62.
3. O. G. Alekseeva and L. A. Dueva, *Industrial Compound Allergy* [in Russian], Moscow (1978), pp. 207-210.
4. M. P. Vavilov, L. A. Apollonova, and E. K. Nazarova, *Klin. Lab. Diagnost.*, No. 6, 40-43 (1993).
5. L. Kh. Garkavi, E. B. Kvakina, and M. A. Ukolova, *Adaptive Reactions and Resistance of the Organism* [in Russian], Rostov-on-Don (1979).
6. A. J. Gordon and R. A. Ford, The Chemist's Companion, Wiley (1972)
7. *Laboratory Methods of Clinical Trials. A Handbook Book,* Ed. V. V. Men'shikov [in Russian], Moscow (1987), pp. 123-124.
8. *International Register of Potentially Toxic Chemicals. United Nations Program on the Environment* [Russian translation], Geneva (1985), Pt. A, pp. 406-410.
9. *Wounds and Wound Infection. A Handbook for Physicians.* Eds. M. I. Kuzin and B. M. Kostyuchenok [in Russian], Moscow (1990), p. 216.
10. M. Verworn, *Allgemeine Physiologie. Ein Grundriss der Lehre von Leben,* Jena (1909) [Russian translation] (1911), Vol. 2, pp. 241-268.
11. *Immunologische Arbeismethoden*, Jena (1984), Ed. H. Friemel [Russian translation], Moscow (1987), pp. 226-246.