2. M. L. R. Angulo, B. F. Sanchez, and E. L. Rodriguez-Echandia, Cell Tissue Res., 192, 67 (1978).
3. G. Donegani and G. Filogamo, Boll. Soc. Ital. Biol. Sper., 47, 156 (1971).
4. A. I. Farbman, Dev. Biol., 11, 110 (1965).
5. A. I. Farbman, in: Handbook of Sensory Physiology, Berlin (1971), p. 51.
6. L. Guth, in: Handbook of Sensory Physiology, Berlin (1971), p. 63.
7. M. Jacobson, Developmental Neurobiology, New York (1978).
8. T. Lomo and C. R. Slater, J. Physiol. (London), 275, 391 (1978).
9. R. G. Murray, in: Handbook of Sensory Physiology, Berlin (1971), p. 31.
10. S. Salmons and F. A. Sreter, Nature, 263, 30 (1976).
11. F. A. Sreter, M. Elzinge, S. Salmons, et al., FEBS Lett., 57, 107 (1975).
12. A. A. Zalewski, Exp. Neurol., 23, 18 (1969).
13. A. A. Zalewski, Exp. Neurol., 35, 519 (1972).

A NEW APPROACH TO THE STUDY OF ERYTHROCYTE AGGREGATION

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The study of the mechanism of interaction (aggregation) of cells is an important problem in modern biology and medicine. Aggregation of blood cells (erythrocytes), a fundamental stage in microcirculatory disturbances in many pathological states [7], plays a special role. To study the mechanism of erythrocyte aggregation in model experiments *in vitro*, investigators have used dextran, a neutral polymer of glucose, and high-molecular-weight blood plasma proteins, namely fibrinogen and γ-globulin, as inducers of aggregation [1, 8]. According to some workers [5], erythrocyte aggregation stimulated by dextran is similar in its general features to aggregation taking place in various pathological states, and it provides a convenient model with which to study cellular interaction. The mechanism of the aggregating action of dextran is the formation of "bridges" of polymer macromolecules adsorbed on their membrane between neighboring cells [4]. However, no attention is paid in the mechanism of erythrocyte aggregation suggested by the authors cited to changes in cell shape (discoid—spherical transformation), which take place in many diseases in which intravascular erythrocyte aggregation is observed [11].

It was accordingly decided to study the possibility of inducing aggregation of erythrocytes by substances causing changes in their shape.

EXPERIMENTAL METHOD

Human erythrocytes, washed and resuspended (1:200) twice in buffered physiological saline (20 mM Tris-HCl and 146 mM NaCl, pH 7.4) were used. Erythrocyte aggregation was studied in a highly sensitive aggregometer of our own design, by a photometric method [3]. The viscosity of the erythrocyte suspension (77%) was studied by means of a VIR-75M rotatory viscosimeter (designed by A. N. Sundukov) with shearing velocities of 2.2 to 85.0 sec^{-1}. Erythrocyte morphology was studied in a phase-contrast microscope. The erythrocytes were first fixed in 1% glutaraldehyde solution. The known crenating agents 2,4,6-trinitrophenol (TNP) and 2,4-dinitrophenol (DNP) were used.

EXPERIMENTAL RESULTS

It was found that TNP (1 mM) induced sharp changes in shape of the erythrocytes: total disappearance of the discoid forms and the appearance of crenocytes and spherocrenocytes (Fig. 1b). It was also found that TNP, in the above dose, stimulated marked erythrocyte aggrega-

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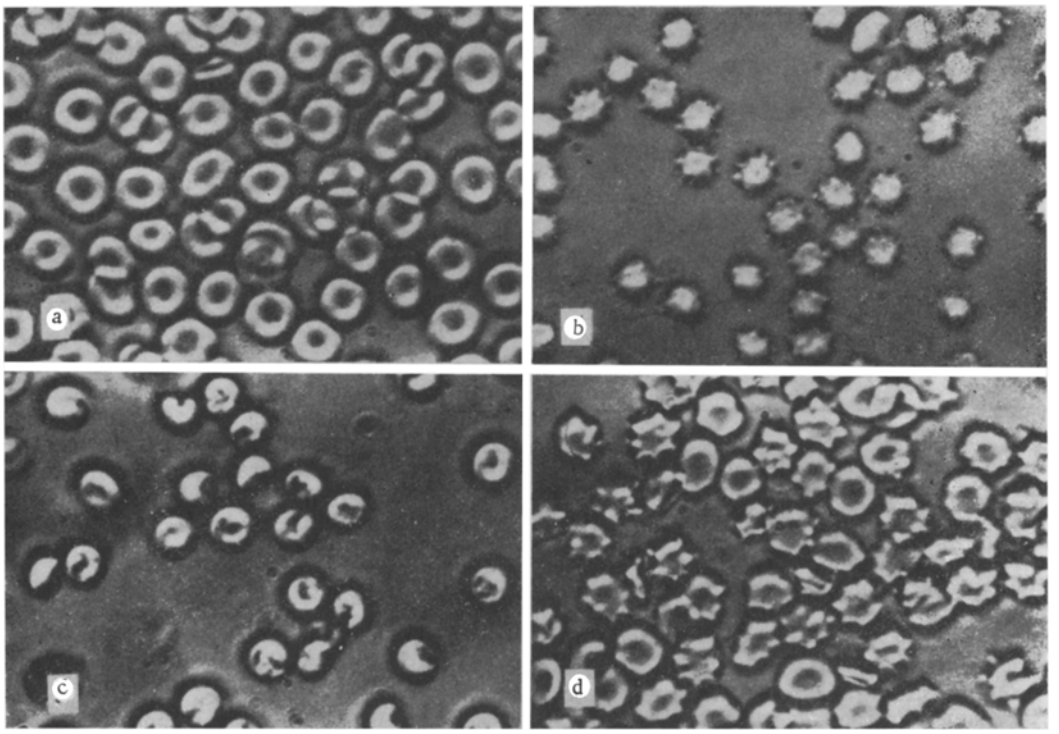

Fig. 1. Effect of crenating agents and chloropromazine on erythrocyte morphology: a) intact erythrocytes, b) erythrocytes after addition of TNP, c) erythrocytes after addition of chloropromazine, d) erythrocytes after addition of TNP preceded by chloropromazine. 900 ×.

tion, which was completely absent in the control experiments (Fig. 2). The viscosity of the blood at a low shearing velocity is known to be determined mainly by aggregation of erythrocytes. The appearance of aggregation is due to an increase in viscosity of the erythrocyte suspension treated with TNP, which is mainly at a low shearing velocity.

Addition of TNP to glutaraldehyde-fixed intact discoid erythrocytes did not change their shape or cause their aggregation.

The clear correlation between the degree of change in shape of the erythrocytes and the intensity of their aggregation was confirmed by experiments with another crenating agent, DNP. In the same dose (1 mM) DNP caused much less transformation of shape of the erythrocytes than TNP. This was the reason for the significantly weaker aggregation of erythrocytes treated with DNP (Fig. 2).

The change in shape of the erythrocytes thus played a leading role in their aggregation. Yet another important piece of evidence in support of our hypothesis is the results of the experiments with chlorpromazine. This agent also substantially modifies the shape of erythrocytes, causing the formation of cup-shaped forms or stomatocytes [6]. According to a recent hypothesis on changes in erythrocyte shape, based on a bilayer model of their membrane structure [12], crenating agents act mainly on the outer surface of the bilayer, stretching it and causing the formation of crenated forms. Stomatocyte agents, on the other hand, act on the inner surface of the lipid membrane bilayer. In certain concentrations the action of these substances may be balanced, in which case the shape of the erythrocyte remains practically unchanged [6].

The present experiments showed for the first time that if chloropromazine (0.5 mM) is used to restore the shape of erythrocytes when modified by TNP (disappearance of spheroechinocytes, the appearance of discoid forms and of echinocytes 1 in Fig. 1d), aggregation of the blood cells can be sharply reduced (Table 1). When the normal shape of the erythrocytes is restored the viscosity of their suspension also falls (Fig. 2), in agreement with the most recent data [9, 10]. In the above dose chlorpromazine itself causes slight aggregation of erythrocytes (Table 1) and the formation of stomatocytes (Fig. 1c).

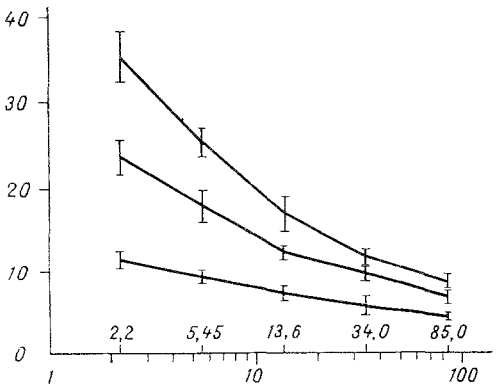

Fig. 2. Effect of TNP and chlorpromazine on viscosity of erythrocyte suspension (n = 20). Abscissa, shearing velocity (sec^{-1}); ordinate, viscosity of erythrocyte suspension (in cP). 1) Control, 2) chlorpromazine on TNP, 3) TNP.

TABLE 1. Effect of Crenating Agents and Chlorpromazine on Erythrocyte Aggregation (n = 20; M ± m)

Agents	Maximal amplitude of aggregation curve, mm
Control	0 (no aggregation)
2, 4, 6-TNP	215,2±39,4
2, 4, 6 TNP + chlorpromazine	108,4±16,3
2, 4-DNP	59,6±7,70
Chlorpromazine	27,9±4,20

Discoid—spherical transformation of erythrocytes, not only when stimulated chemically but also taking place in the course of time in stored erythrocytes without the addition of any substances of any kind, also is accompanied, as we showed, by aggregation. For instance, in a suspension of washed erythrocytes (either for 24 h at 37°C or for 48 h at 4°C) kept for 24 h at room temperature marked spontaneous erythrocyte aggregation took place. In the phase-contrast microscope changes in the shape of all cells were observed in this suspension — echinocytosis and spheroechinocytosis.

These experiments thus provide a new insight into the problem of intercellular interaction and they confirm the previous hypothesis [2] that changes in the shape of the cells play a definite role in the mechanism of their aggregation. The use of agents with differential action on the outer and inner surface of the lipid membrane bilayer could prove to be a useful tool for the study of the mechanism of cellular reactions in various branches of medicine and biology.

LITERATURE CITED

1. K. M. Lakin, V. A. Makarov, and M. S. Ovnatanova, Farmakol. Toksikol., No. 2, 188 (1975).
2. G. Ya. Levin, Yu. A. Sheremet'ev, and S. V. Petrov, Byull. Eksp. Biol. Med., No. 7, 13 (1977).
3. G. V. R. Born, Nature, 194, 927 (1962).
4. S. Chien and K.-M. Jan, Microvasc. Res., 5, 155 (1973).
5. S. Chien, S. Simchon, R. E. Abbott, et al., J. Colloid Interface Sci., 64, 461 (1977).
6. B. Deuticke, Biochim. Biophys. Acta, 163, 494 (1968).
7. L. Dintenfass, Cardiovasc. Res., 2, 337 (1977).
8. K.-M. Jan and S. Chien, J. Gen. Physiol., 61, 655 (1973).
9. H. J. Meiselman, Biorheology, 15, 225 (1978).
10. J. Palek, A. Liu, D. Liu, et al., Blood, 50, 155 (1977).
11. H. Schmid-Schonbein, Wein. Klin. Wochenschr., 90, 245 (1978).
12. M. P. Sheetz and S. J. Singer, Proc. Natl. Acad. Sci. USA, 71, 4457 (1974).
13. M. P. Sheetz, R. G. Painter, and S. J. Singer, J. Cell Biol., 70, 193 (1976).