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FILTERABILITY OF LEUKOCYTES IN WHOLE BLOOD

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Although the number of leukocytes in the blood is very much less than that of erythrocytes of platelets, they play the key role in the capillary blood flow [11]. The number of leukocytes in the blood is inversely proportional to its microcirculatory properties [1, 7]. However, the role of the filtration properties of the leukocytes themselves in the capillary blood flow has been studied completely inadequately.

The aim of this investigation was to study dependence of filterability of leukocytes in the filtration properties of whole blood.

EXPERIMENTAL METHOD

Experiments were carried out on blood from 25 healthy male donors. The blood was taken from the cubital vein of the fasting subjects. Heparin (Richter) in a concentration of 10 IU/ml blood was used as anticoagulant, because heparin disturbs leukocyte functions by a lesser degree than other anticoagulants [4]. Blood samples containing different numbers of erythrocytes and leukocytes were produced by combining different volumes of packed erythrocytes and leukocyte-enriched or leukocyte-deprived plasma. Fractionation of the blood into packed erythrocytes and leukocyte-enriched plasma wa carried out by free sedimentation of erythrocytes. Plasma free from leukocytes was prepared by centrifugation of leukocyte-enriched plasma at 400*g*. The blood was filtered through filters from the firm of Nucleopore (USA), 13 mm in diameter, with an internal pore diameter of 5 μm, and under constant pressure of 100 cm water, for 30 sec. The filtered blood was collected in a test tube containing EDTA (blocking cell adhesion to the walls of the tube [5]), the volume was made up to 10 ml with Hanks' medium, and the number of erythrocytes and leukocytes per unit volume was counted. Each blood sample was filtered 3 times, and the mean value was taken as the result. Cells were counted on a "Coulter-S-Unior" automatic analyzer (France).

In the experiments of series I blood from 15 donors was tested. Four or five blood samples from each of them, differing in their concentration of leukocytes (2-8 · 10^9/liter), containing the same number of erythrocytes (2-2.5 · 10^{12}/liter) were filtered. The efficiency of filtration of the leukocytes was estimated by the leukocyte filtration index (LFI), which is the ratio of the leukocyte concentration in the filtrate to that in the blood before filtration. The filtration capacity of the whole blood was determined as the number of erythrocytes passing through the filter. The effect of leukocytes on the filtration capacity of the blood was judged by the index of leukocyte-dependent reduction of blood filtration (LDRBF), the percentage decrease in the number of electrolytes passing through the filter in response to an increase in the blood leukocyte concentration from 2-8 · 10^9/liter.

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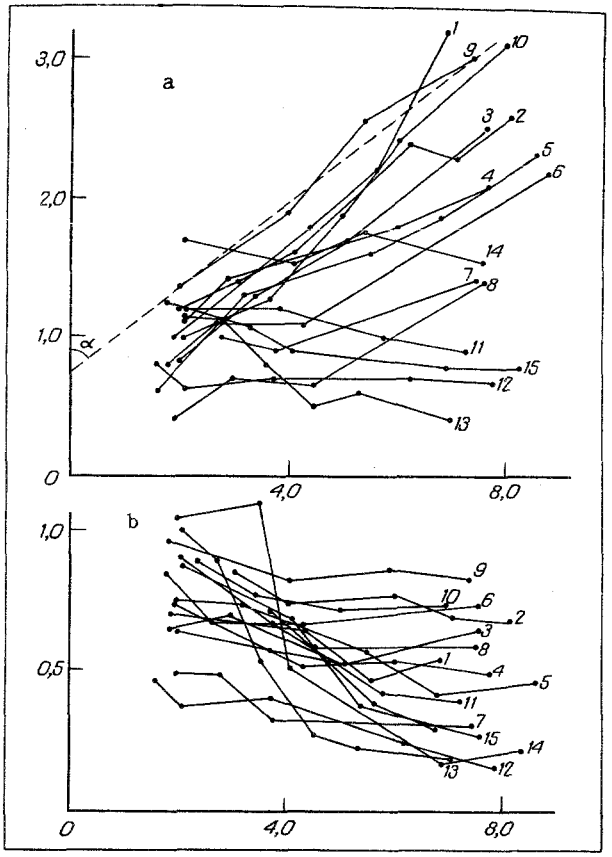

Fig. 1. Filtration of leukocytes (a) and erythrocytes (b) in whole blood. a: Abscissa, number of leukocytes in blood undergoing filtration ($\cdot 10^9$/liter); ordinate, number of leukocytes in filtrate ($\cdot 10^9$/liter); numbers indicate serial numbers of donors. In Fig. 1a, broken line shows angle α for blood of donor No. 9. b: Abscissa, number of leukocytes in blood undergoing filtration ($\cdot 10^9$/liter); ordinate, number of erythrocytes in filtrate ($\cdot 10^{12}$/liter).

In the experiments of series II, three blood samples each of 10 donors, differing in erythrocyte concentration ($0\text{-}5 \cdot 10^{12}$/liter), but containing equal numbers of leukocytes ($4\text{-}5 \cdot 10^9$/liter) were filtered. The leukocyte filtration index was estimated.

EXPERIMENTAL RESULTS

The results of the investigations of series I (curves showing the time course of the number of filtered leukocytes depending on their number in the blood before filtration) are given in Fig. 1a, which shows that this dependence differed in different cases. The number of filter-passing leukocytes of donors 1-10 increased in proportion to their number in the blood and the filtration index either remained or fell moderately (Table 1). The reason for this is evidently that with an increase in the concentration of leukocytes in the blood, these cells utilize the pores of the filter more effectively (they either occupy a larger number of pores if a reserve is present, or more than one leukocyte begins to pass simultaneously through some of the pores [3]). However, movement of the leukocytes along capillaries is preserved and a syndrome of capillary occlusion by leukocytes does not develop. Filtration of the particles is directly proportional to their number in the suspension before filtration, provided that the conditions of filtration are appropriate for the particles, and it is this which determines its success [1]. This appropriateness reflects the good filtration properties of the leukocytes.

The number of erythrocytes from these donors passing through the filter decreases with an increase in the number of leukocytes in the blood (Fig. 1b). The reason is evidently that with an increase in the number of leukocytes in the blood they occupy an ever-increasing number of pores, and the velocity of movement of the leukocytes along the 5-micron capillaries is

TABLE 1. Filtration Properties of Leukocytes in Whole Blood

Donor	Angle α*	LDRBF, %	LFI		
			a	b	c
1	48	22,5	0,48	0,40	0,48
2	54	18,6	0,60	0,39	0,33
3	53	0	0,40	0,34	0,33
4	68	34,3	0,55	0,34	0,30
5	62	38,2	0,40	0,31	0,28
6	64	22,1	0,42	0,26	0,26
7	74	34,7	0,30	0,22	0,17
8	65	14,3	0,20	0,16	0,19
9	52	14,4	0,66	0,49	0,41
10	53	19,2	0,50	0,38	0,39
11	101	56,0	0,60	0,22	0,13
12	90	46,8	0,50	0,14	0,10
13	113	80,0	0,57	0,11	0,05
14	103	85,5	0,60	0,22	0,09
15	95	69,3	0,85	0,34	0,21

Legend. Concentration of leukocytes in blood before filtration: a) $2 \cdot 10^9$/liter, b) $5 \cdot 10^9$/liter, c) $8 \cdot 10^9$/liter.

*See Fig. 1a.

TABLE 2. Filtration of Leukocytes in Whole Blood in the Presence of Different Concentrations of Erythrocytes ($M \pm m$)

Concentration of erythocytes in blood before filtration ($\times 10^{12}$/liter)	LFI
0	1,72±0,22
2,5	1,76±0,26
5	1,64±0,28
	$p>0,05$

significantly less than that of the erythrocytes, and movement of the erythrocytes is inhibited [2, 9]. However, filtration of whole blood in this case is not very greatly reduced: by 0 to 38.2% of its level in the presence of a small number of leukocytes (Table 1).

The pattern was different in the case of donors 11-15: FLI was sharply reduced in these donors, but in donors 11, 13, and 15, the absolute number of leukocytes passing through the filter also fell sharply when their number in the blood was increased (Fig. 1, Table 1). This was evidently due to arrest of the leukocytes in the capillaries and their occlusion. Under those conditions an increase in the number of leukocytes in the blood does not necessarily lead to an increase in their number in the filtrate, for the total channel of the filter is reduced more rapidly, and it ceases to function. If firm occlusion can take place as a result of the presence of two or more leukocytes simultaneously in a capillary, the leukocytic capillary occlusion syndrome can be expected if the number of leukocytes in the blood is high (above a certain level).

However, the question arises: with what number of leukocytes in the blood does their filtration index reflect their true filtration properties? Whereas in donors 1-10 the filtration index was relatively constant, in donors 11-15 it differed sharply with different concentrations of leukocytes in the blood. In our view, the filtration properties of leukocytes (and their ability to occlude the capillaries) can be characterized more adequately by the direction of the curve on the filtration graph. This direction is reflected by the angle formed by intersection of the curve and the ordinate of the graph (Fig. 1a). The efficiency of filtration of whole blood was very closely connected with this parameter (the coefficient of correlation between values of the angle α and the index of the leukocyte-dependent reduction of whole blood filtration was +0.91). This dependence is directed from leukocytes to erythrocytes, i.e., filtration of the erythrocytes in the whole blood depends on leukocytes, but not vice versa. This is shown by the results of the experiments of series II, in which the level of leukocyte filtration remained constant in the absence or in the presence of erythrocytes, or of different concentrations of them (Table 2). It can accordingly be concluded that filtration of leukocytes in the composition of whole blood is independent of erythrocytes. This result is in agreement with known data on the morphologic [8] and rheologic properties of the leukocytes [7] and their behavior in the microcirculatory system [2].

Thus the filterability of leukocytes from different donors differs sharply, and together with the number of leukocytes, it determines the filtration properties of whole blood. Meanwhile, erythrocytes had no effect on filtration of leukocytes in whole blood.

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IDENTIFICATION OF A PROTEIN WITH THE PROPERTIES OF TENASCIN IN EMBRYONIC CARTILAGE TISSUE MATRIX AND IN CULTURES OF HUMAN EMBRYONIC FIBROBLASTS

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A leading role of macromolecular components of the extracellular matrix (ECM) in processes of cell differentiation, regulation of cell movement, morphogenesis of organs, and embryonic development, growth, and aging, has been demonstrated during the last 20 years [3, 6]. Although the structure of the main micromolecular components of the ECM of cartilage tissue, namely collagen and proteoglycan aggregates (PGA), has now been characterized, much still remains unexplained in the interaction between these components during organization of the matrix. Participation of a whole range of molecular structures of varied nature has been suggested in these interactions (collagens IX, X, and XI, proteoglycans, chondronectin, and other glycoproteins) [8, 9, 11]. Recently a new protein, tenascin, with high molecular weight (>100 kD) has been described in the ECM of chick embryos and hamsters. It may occupy in the cartilage matrix a position similar to that occupied by laminin in the matrix of basement membranes [11]. Tenascin has also been found in certain human tumors [5].

In the course of a study of structural changes in the proteoglycan component of the cartilage matrix at different stages of embryonic development, we found a protein, and the investigation described below was devoted to a study of its properties.

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