EDTA-Blood Filtration: A Positive Correlation with Lymphocyte Number

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Blood is filtered through nuclear filters with pore diameter 5 μ under a pressure of 0.1×10^5 dyn/cm^2 and 0.4×10^5 dyn/cm^2. Correlation analysis of the dependence of blood filterability on blood cell count and blood cell adhesiveness is performed. There is a negative correlation between the erythrocyte count and the number of adherent granulocytes. A significant positive correlation is established between blood filterability and the lymphocyte count at a pressure of 0.4×10^5 dyn/cm^2 but not at a pressure of 0.1×10^5 dyn/cm^2.

Key Words: *filterability; lymphocytes; granulocytes; erythrocytes; platelets*

Blood filterability *in vitro* depends on the rheological properties of blood cells and on their count. Erythrocytes, leukocytes, and platelets can hinder blood flow through capillaries; according to published data, none of these cells facilitates blood flow [2]. There is controversy concerning the effects of these cells on blood filterability.

In this study we investigated how blood filterability correlates with blood cell count and adhesive activity of blood cells at various perfusion pressures.

MATERIALS AND METHODS

Blood from 40 donors was studied. It was collected from the cubital vein after an overnight fast. Ethylenediaminetetraacetic acid (EDTA) was used as a preservative. EDTA-blood was filtered through nuclear filters with a pore diameter of 5 μ at constant pressures of 0.1×10^5 and 0.4×10^5 dyn/cm^2. Filterability was assessed by the volume of filtered blood. Adhesiveness of blood cells was studied using a nylon mesh [4,9] and evaluated as the number of adherent cells (the difference between the cell count before and after the blood was passed through the mesh).

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RESULTS

The results of correlation analysis of EDTA-blood filterability as a function of the cellular composition of the blood and the adhesive properties of blood cells are summarized in Table 1. At a pressure of 0.1×10^5 and 0.4×10^5 dyn/cm^2, blood filterability significantly correlated with the number of erythrocytes and adherent leukocytes. The correlation between blood filterability and the number of adherent platelets was statistically insignificant. The negative correlation between blood filterability and erythrocyte counts is not surprising. Although the contribution of the erythrocytes to the obstruction of capillaries is much smaller (750-fold [14]) than that of leukocytes [5,10], the effect of erythrocytes on blood filterability is noticeable due to their very high count. The same holds true for adherent granulocytes. Granulocyte adhesiveness is the most potent decisive factor in the obstruction of filter capillaries upon blood filtration *in vitro* [1] and it markedly increases the ability of granulocytes to obstruct blood capillaries *in vivo*

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TABLE 1. Correlation between EDTA–Blood Filterability, Cell Count, and Adhesiveness of Blood Cells

Pressure, dyn/cm²	Total count						Number of adhesive				
	erythrocytes	leukocytes	lymphocytes	granulocytes	monocytes	platelets	leukocytes	lymphocytes	granulocytes	monocytes	platelets
0.4×10^5	−0.36*	−0.15	0.45**	−0.27	0.14	0.14	−0.24	0.13	−0.42**	0.21	−0.30
0.1×10^5	−0.38*	−0.05	0.12	−0.08	0.03	0.09	−0.22	0.06	−0.33*	0.06	−0.22

Note. Correlation coefficients statistically significant at $p<0.05$ and $p<0.01$ are indicated with one and two asterisks, respectively.

[[6,7]. However, this occurs when the adhesive activity of granulocytes is preserved or induced. In our study this activity was inhibited by EDTA: the adhesion index, i.e., the percent of adherent granulocytes vis-a-vis the total count, was only 7.8±0.4%, whereas in heparinized plasma it was 41.3±6.4% [1]. Obviously, it was this low adhesiveness of granulocytes which diminished their influence on blood filterability, thus allowing other cells to play their role in the process of blood filtration. In studies of blood circulation and filterability special attention is usually paid to platelets due to their strong hemostatic properties such as adhesion and aggregation. However, these properties are suppressed by EDTA. In our study the platelet adhesion index was only 5.4±0.4% *vs.* >50% in heparinized plasma [1]. Since the size of a platelet is much smaller than 5 μ, these cells cannot directly affect blood filterability through filters with a pore diameter of 5 μ by means other than the adhesive-aggregation pathway. Therefore, the absence of a correlation between blood filterability and the number of adherent platelets is logical and predictable.

We think that the positive correlation between the filterability of EDTA-blood and the lymphocyte count at a perfusion pressure of 0.4×10^5 dyn/cm² (Table 1) is the most interesting result of this study. Such a correlation was absent at a pressure of 0.1×10^5 dyn/cm². This is not consistent with the literature. A lymphocyte is quite a large cell [11] with a pronounced rigidity [8] and it can hinder capillary flow by itself [3]. Our results show that lymphocytes facilitate blood filterability. Presumably, when whole blood (but not a lymphocyte suspension [3]) is filtered, some indirect mechanism responsible for the influence of lymphocytes on blood flow is triggered, which not only abundantly compensates for lymphocyte resistance in the capillary bed, but also greatly surpasses it, since the resultant effect of lymphocytes on blood filterability proves to be positive. The magnitude of this indirect influence depends on the perfusion pressure: it starts operating after the pressure has been elevated from 0.1×10^5 to 0.4×10^5 dyn/cm². This implies a mechanical genesis of the positive effect of lymphocytes on the filterability of EDTA-blood. In addition, certain morphological and functional properties of lymphocytes may promote their stimulatory effect when blood passes though a 5-μ capillary. The adhesive activity of lymphocytes is not high [13]. The cell size (6.5 μ [11]) is slightly larger than the capillary diameter, and the cell membrane can shrink the cell diameter almost to the nuclear diameter due to the numerous folds on its surface. The nucleus, on the other hand, is practically undeformable [12] and round, having a diameter of 4.5 μ [11], i.e., it is slightly smaller then the capillary diameter. Therefore, the lymphocyte can readily enter a 5-μ capillary, but moves along it with its membrane "rubbing against" the capillary wall, thought without adhering to it. Since the lymphocyte has a large, rigid nucleus, it cannot overcome any obstacle in the capillary such as an adherent granulocyte or platelet aggregate. There are two possibilities for a lymphocyte: it can overcome the obstacle by dislodging the adherent granulocyte from the capillary wall or by destroying the platelet aggregate, or it can fail to overcome the obstacle and completely block the capillary. The behavior of lymphocytes in the capillary blood flow *in vitro* is probably determined by the ratio between the perfusion pressure and the firmness of the obstacle. A perfusion pressure of 0.4×10^5 dyn/cm² proved to be sufficient for a lymphocyte to overcome weakly adherent (due to the presence of EDTA) granulocytes and platelets, while a pressure of 0.1×10^5 dyn/cm² was insufficient. If experiments had been performed with heparinized blood, where adhesiveness of granulocytes and platelets is incomparably greater than in EDTA-blood, even a pressure of 0.4×10^5 dyn/cm² would surely have been insufficient. But this is just a hypothesis not corroborated by facts. At the present time, we can speak only of the phenotype of a positive effect of lymphocytes on EDTA-blood filtration through 5-μ filters. The genesis of this phenomenon is unclear and requires further investigation.

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Role of Estrogens in the Regulation of Prolactin Receptors in Liver Cells of Female Rats

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Strong expression of prolactin receptors in sinusoidal domains and cytoplasmic granules of hepatocytes, which is independent of cell location in the hepatic lobule and is positively regulated by estrogens, is revealed in pubertal female rats. In estrogen-treated animals, Prolactin receptors are also exposed in the perinuclear space of some hepatocytes surrounding the central veins. Estrogens regulate the intensity of prolactin receptors expression in hepatocytes, but not the number of cells containing these receptors.

Key Words: *immunohistochemistry; prolactin receptors; rat liver; estrogens*

Prolactin produces a broad spectrum of metabolic effects on the liver [2,4]. Prolactin receptors (PR) are present in the liver of humans and various animal species. The receptor protein has been isolated in pure form, and monoclonal antibodies to it have been obtained [2,6,11,12].

With the aid of radioligand techniques it has been demonstrated that estrogens positively regulate the PR content in rat liver [2]. It is unclear however, whether estrogens control the PR level in each liver cell or regulate the number of prolactin-sensitive cells in this organ. Immunohistochemical identification of liver cells containing PR in female rats with different estrogen status was the aim of this study.

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MATERIALS AND METHODS

Experiments were performed on pubertal female albino rats of a mixed population either intact or 25-30 days after ovariectomy. Estradiol-17β (E_2) was administered to ovariectomized females in a dose of 10 μg in 0.4 ml of propylene glycol during a 14-day period. The animals were sacrificed 24 h after the last injection of the hormone.

Prolactin receptors were visualized by the indirect immunoperoxidase technique using murine anti-PR monoclonal antibodies (U6) as the immunoglobulin G fraction isolated from ascitic fluid. Monoclonal antibody U6 is specific for a

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