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EXPERIMENTAL MORPHOMETRIC STUDIES DURING THE ERYTHROCYTE FILTERABILITY TEST

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KEY WORDS: erythrocyte morphology; filterability; membranes; morphometric characteristics.

Several pathological states are linked with disturbances of the rheologic properties of the blood, which are largely dependent on erythrocyte deformability [6]. Consequently, assessment of erythrocyte deformability by the use of the filterability test on blood samples is becoming increasingly important. As a rule what is measured is the number of erythrocytes in suspension, passing without undergoing hemolysis through a microfiltration membrane with pores of known diameter in the course of a known time interval [1]. The microfiltration membranes used are made from various polymer materials and have pores of widely different shape – from straight cylinders in track membranes to twisted channels in membranes obtained from polymer solutions (Fig. 1). As was shown previously, the shape of the pores in the membrane has a significant effect on the degree and multiplicity of change in shape of the cells, which determines the pattern of movement of erythrocytes in the membrane [3].

A more precise and correct interpretation of results of the filterability test on blood samples could help with the development of a technique which would allow the shape of the cells to be investigated actually in the membrane. This would facilitate the transition from the phenomenologic level of examination of the filterability test to the analysis of the mechanisms of interaction of two discrete systems: the cell assembly and the porosity of the membrane-films, which constitute a periodic colloidal system [4].

In the investigation described below deformability of the blood cells was studied by the use of a combined method of morphometric analysis of filtration membranes and erythrocytes in them.

EXPERIMENTAL METHOD

Blood samples from healthy donors (men) and groups of rabbits kept on a high cholesterol diet served as the test objects. The hematocrit index varied from 0.003 to 0.2. Membranes used were obtained from the firms of Millipore and Nucleopore Pall, and were used under filtration conditions and also during statistical contact between the blood sample and membranes, soaked beforehand with buffer solution. Membranes with cellular material immobilized on them were fixed in 0.25% glutaraldehyde, after which the membrane was examined in a scanning electron microscope (Hitachi, Japan). (The photomicrographs were prepared by head of laboratory Dr. S. A. Gusev, to whom the authors are grateful.) Morphometric analysis of the cells and free regions of the membranes was carried out by means of our own program on the IBAS-2 system (Opton, West Germany). During morphometry of the membranes, image inversion was used. The program of morphometry of the cells, like that developed previously for evaluating the state of erythrocytes in patients with familial hyperlipoproteinemia [5], contained assessment of

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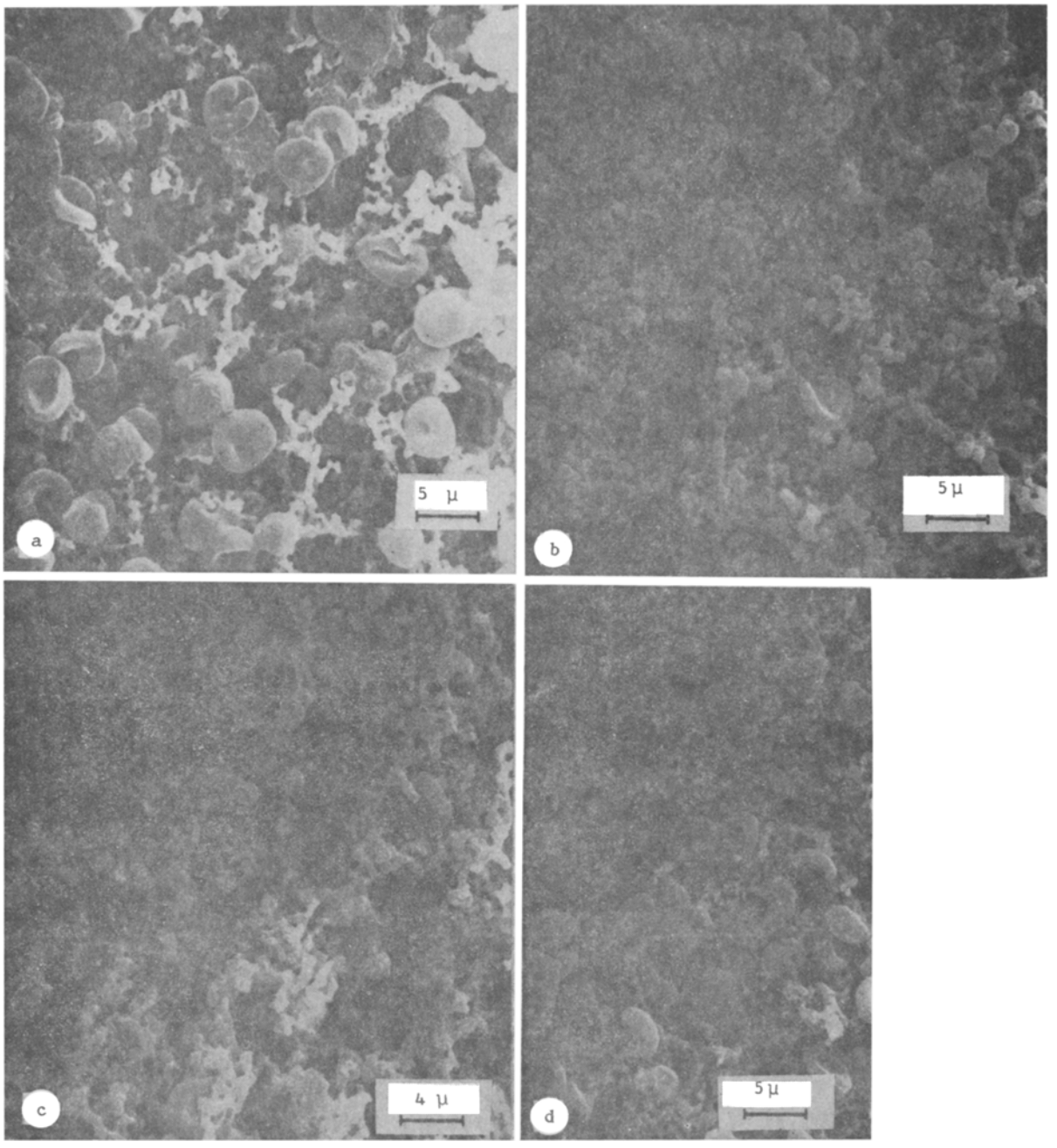

Fig. 1. Erythrocytes on a membrane of reticular structure: a) above, b) below, c) shear section, d) erythrocytes on entering the pores of a membrane.

the parameters of minimal and maximal diameters, shape factors, and projection areas of the cells. The structure of the membranes was assessed on the basis of radii of correlation and linear dimensions of morphological details [2].

EXPERIMENTAL RESULTS

Photomicrographs of membranes made from cellulose esters (Fig. 1a, b, c, d) and polyamide (Fig. 2a, b, c), and of polycarbonate track membranes (Fig. 2d), through which the erythrocyte suspensions were passed, are shown in Figs. 1 and 2. Analysis of the photomicrographs shows that the type of membrane structure influences the shape of the cells, depending on the shape of the pores and in shear sections (Figs. 1c and 2c) the erythrocytes are in the form of spicules and discocytes, alternately deformed and regaining their initial shape.

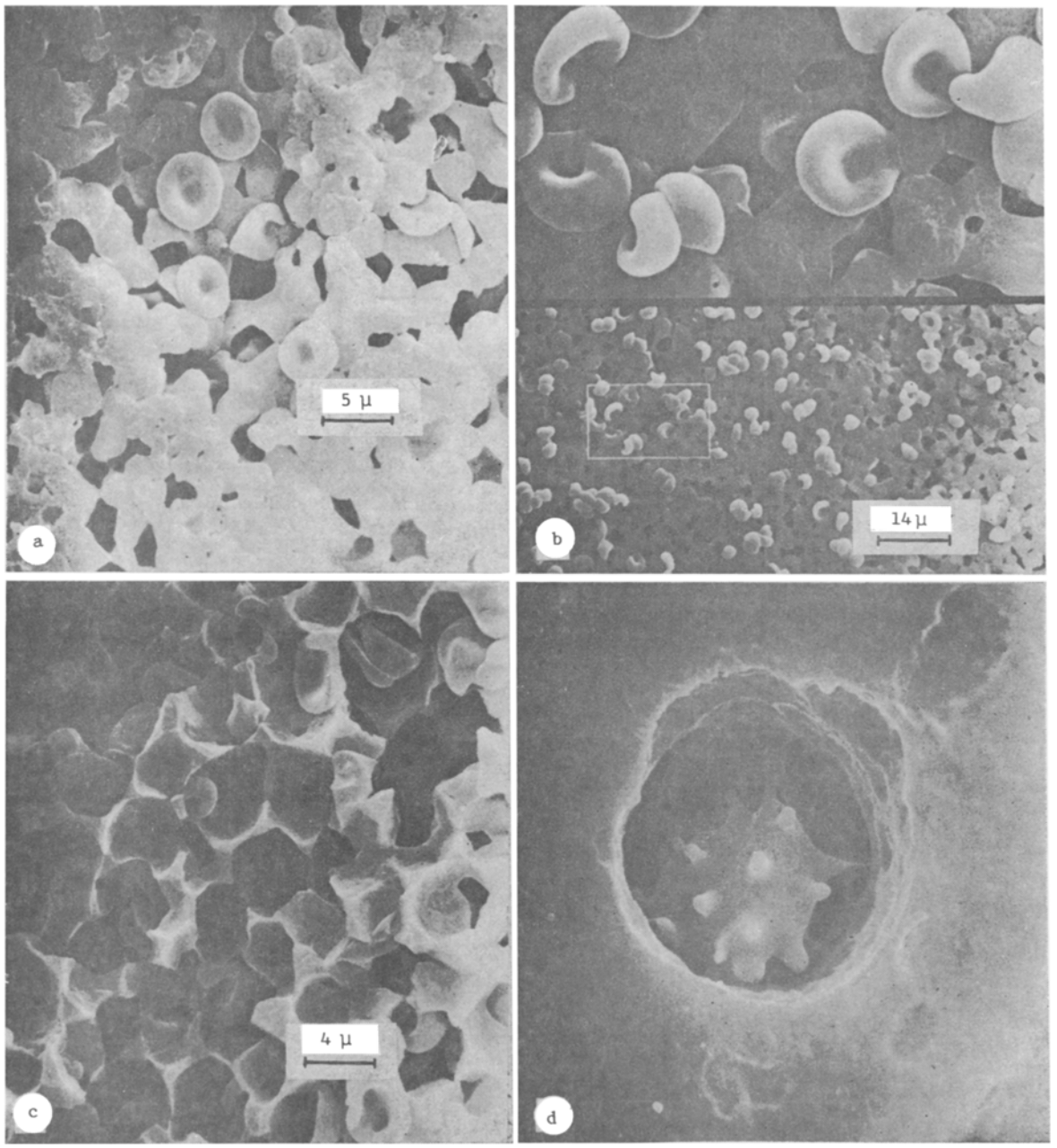

Fig. 2. Erythrocytes on a membrane of cellular structure. a) Above, b) below, c) shear section, d) erythrocyte in the cylindrical pore of a track membrane.

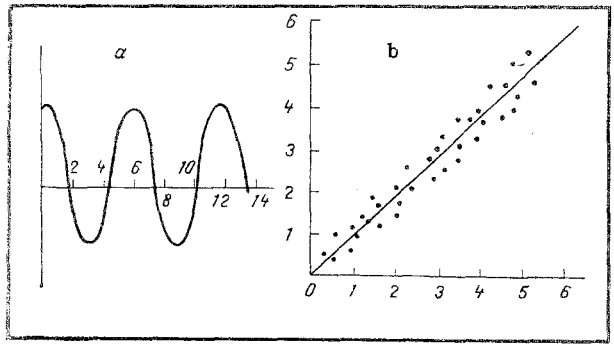

Fig. 3. Autocorrelation function describing periodic nature of membrane structure (a) and connection between dimensions of cells and pores in membrane (b).

TABLE 1. Morphometric Data for Membranes and Erythrocytes

Detail of structure	Pore			Erythrocyte		
	dimensions	Ff**	Fell***	dimensions	F **	Fell***
Outlet of mouth on surface	3,0±0,2	0,67±0,07	0,60±0,06	2,8±0,3*	0,55±0,02	0,53±0,02
Pore space	7,0±0,5	0,8±0,1	0,7±0,1	6,5±0,5	0,84±0,03	0,88±0,05

Legend. *) Width of spicule of erythrocyte at base, **) Ff = area/(perimeter)^2 × 4πFf – the shape factor [5], ***) Fell = a/b, where a and b are hemiaxes of an ellipse, and Fell denotes the ellipticity factor [5].

The ideal membrane for erythrocyte morphometry is a track membrane (Fig. 2d), for the precise shape of the orifices of the cylindrical pore means that the details of morphology of an echinocyte can be observed. It is important to emphasize that a combined quantitative study of cells and of details of membrane morphology enables relations between the dimensions of the pores in the membranes and the dimensions of the cells to be assessed, so that the results can be correctly interpreted. For instance, the mouths of the pores of track membranes measure 6.0-7.0 μ, and disparity with the values indicated by the manufacturer (8.0 μ according to the specification) can be explained by the use of hydrodynamic methods of testing filtration membranes during their manufacture and preliminary evaluation.

Analysis of photomicrographs of objects made with the use of different methods of washing the erythrocytes also led to clarification of some technical details. For instance, centrifugation, as described in a number of publications, is evidently not a satisfactory way of preparing the erythrocytes, for it causes considerable changes in the morphology of the cells. Accordingly, in our view it is essential to make sure that the morphology of the original erythrocytes is unchanged by direct methods, in any investigation of blood by the filterability test. With verification in this way, the choice of filtration membrane can be made, with consideration paid to the hygienic and chemical properties of the polymer matrix.

It will be clear from some of the photomicrographs that before they assume the shape of the capillary of the filtration membrane, which measures about 1.2 μ, cells of healthy animals become bispicular in shape, so that they are able to enter the mouth of the pore and to penetrate into the threshold space of the membrane (Fig. 1d). The results of combined morphometry are given in Table 1. Comparison of the morphometric data for pores and erythrocytes shows that their morphometric parameters, as might be expected, coincide under these circumstances ($p \geq 0.95$). Since the pores widen immediately below the surface of the membrane, after assuming the spicular shape, the cells restore their discocyte shape, which is characterized by the previous morphometric parameters.

Next, the cells again become spicular at the point of narrowing of the pore, in the substance of the membrane, and movement of the cells during the filterability test becomes a sequence of reversible transitions from discocytes into spicular cells. The mean statistical parameters of shape (Ff) of the cells, periodically and in accordance with changes in the structure of the pore are 0.85 for the discocyte and 0.55 for the bispicular form respectively, with transition through a form that is morphologically completely congruent with the capillaries of the porous membrane.

It is clear from Fig. 3 that membranes are organized structures. Photomicrographs of cellulose nitrate membranes in static contact with blood suspensions show that absence of the compulsory factor (filtration pressure) does not prevent the cells from penetrating into the pores of the film and virtually to fill the entire porous space of the membranes, as was observed previously [3].

No such phenomenon was observed in photomicrographs of nuclear filters with pores measuring 6.0-7.0 μ in diameter.

This suggests that the phenomenon observed, namely spontaneous movement of cells into a porous membrane with structure of reticular and cellular types, determined by congruence of the cells and the membrane pores, is confirmed by data of concurrent morphometry given in Table 1. Congruence is not a characteristic feature of the track membranes which have been studied. We consider that further development of the test of spontaneous movement of cells into the porous space of a membrane, soaked with phosphate buffer solution is worthwhile.

In all cases the filterability test must include analysis of the type of structure of the membranes used and of the shape and size of the pores in the chosen microfilters. An essential condition for a correct interpretation of the results obtained during development of the filterability test and of the change from the phenomenologic level of analysis to the study of its mechanisms is the concurrent study of erythrocytes and polymer filters. Introduction of the concept of morphometric correlation between parameters of cell structure and porosity of the polymer film during passage of cells through it provides a basis for the methodology of membrane screening for the filterability test and also enables parameters of the periodic structure of membranes to be estimated from data of the joint morphometry of the membrane and cells immobilized by it.

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ULTRASTRUCTURAL BASIS OF INTRAVASCULAR THROMBOSIS IN THE LIVER IN OBSTRUCTIVE JAUNDICE

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Numerous investigations have shown that a syndrome of disseminated intravascular clotting (DIC) develops in a number of pathological states, and is frequently regarded as a component of terminal states [3, 5, 7]. The liver is known to synthesize several blood clotting factors, and development of the DIC syndrome accordingly assumes particular importance. On the basis of the study of the coagulation properties of blood in vitro in liver diseases, development of a DIC syndrome has been postulated [1, 6, 8, 11], and confirmed at autopsy [5]. However, the morphological features of intravascular blood clotting in the vascular bed of the liver have not been fully explained.

It was decided to study the vascular bed of the liver and particular features of the ultrastructure of platelet thrombi in experimental cholestasis.

EXPERIMENTAL METHOD

Liver tissue from 28 rats with a model of obstructive jaundice was investigated by the method described previously [4]. The experimental animals were decapitated on the 10th, 15th, and 20th days of the experiment (five rats acted as the control, eight died from complications of secondary biliary cirrhosis on the 18th and 20th days). Pieces of liver tissue were pro-

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