9. F. A. Geneser-Jensen, Z. Zellforsch., 124, 546 (1972).
10. M. Karnovsky and L. J. Roots, J. Histochem. Cytochem., 12, 219 (1964).
11. O. H. Lowry, N. R. Roberts, K. Y. Leiner, et al., J. Biol. Chem., 207, 39 (1954).
12. S. I. Mellgren, W. Markmark, and B. Srebro, Cell Tissue Res., 181, 459 (1977).
13. C. C. D. Shute and P. R. Lewis, Bibl. Anat., 2, 34 (1961).
14. C. C. D. Shute and P. R. Lewis, Z. Zellforsch., 69, 334 (1966).
15. J. Storm-Mathisen, J. Neurochem., 17, 739 (1970).
16. J. Storm-Mathisen and F. Fonnum, Prog. Brain Res., 36, 41 (1972).

MORPHOLOGICAL DIFFERENCES BETWEEN ERYTHROCYTES OF ARTERIAL AND VENOUS RAT BLOOD REVEALED BY SCANNING ELECTRON MICROSCOPY

B. V. Ionov and Academician A. M. Chernukh*

UDC 612.111.014.2-086.3

KEY WORDS: erythrocytes; scanning electron microscopy; arterial and venous blood.

Because the blood shares a common embryogenetic origin with a wide range of tissues derived from the mesenchyme, by using erythrocytes to model various pathological states and processes at the membrane level it is possible to elucidate general principles characteristic of the cell membranes of different organs and tissues.

The most adequate method of studying the shape and surface of erythrocytes at the present time is scanning electron microscopy. Data on normal rat erythrocytes obtained by methods of optical microscopy are inadequate [7] and at times contradictory [8]. Meanwhile morphological characteristics of erythrocytes obtained from arterial and venous blood are not to be found in the literature.

This paper gives a quantitative description of forms of erythrocytes obtained from arterial and venous rat blood studied by scanning electron microscopy.

EXPERIMENTAL METHOD

Fifteen noninbred male rats weighing 200-300 g were used. Under pentobarbital anesthesia blood was taken into a syringe with 2% glutaraldehyde from the bifurcation of the abdominal aorta and from the portal vein. No anticoagulants were used.

The blood was prefixed in 2% glutaraldehyde for 1 h, then centrifuged (1000 rpm) for 5 min, after which the residue was washed twice in 0.1 M phosphate buffer, pH 7.4. The cells were fixed in 1% OsO_4 for 1 h. They were then washed once and dehydrated in acetone of increasing concentration from 40 to 90%, and 3 times in 100% acetone, for 15 min in each case. One drop of fixed and dehydrated erythrocytes was applied to a support previously treated with 0.1 N HCl, acetone, and ether. The preparations were dried in air at room temperature (18-20°C) and sprayed with gold in a vacuum spray. The preparations thus obtained were studied in a Hitachi S-500 (Japan) scanning electron microscope. To obtain quantitative characteristics of the two forms of erythrocytes 400 cells (200 from the artery, 200 from the vein) from each animal were counted and the results subjected to statistical analysis.

EXPERIMENTAL RESULTS

Pictures of erythrocytes from the arterial and venous blood of normal animals obtained in this way gave a sufficiently complete idea of the shapes of the erythrocytes and details of their surface, so that the following classification could be suggested.

*Academy of Medical Sciences of the USSR.

Laboratory of General Pathology and Experimental Therapy, Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 92, No. 12, pp. 749-752, December, 1981. Original article submitted August 21, 1981.

 0007-4888/81/9212-1738$07.50

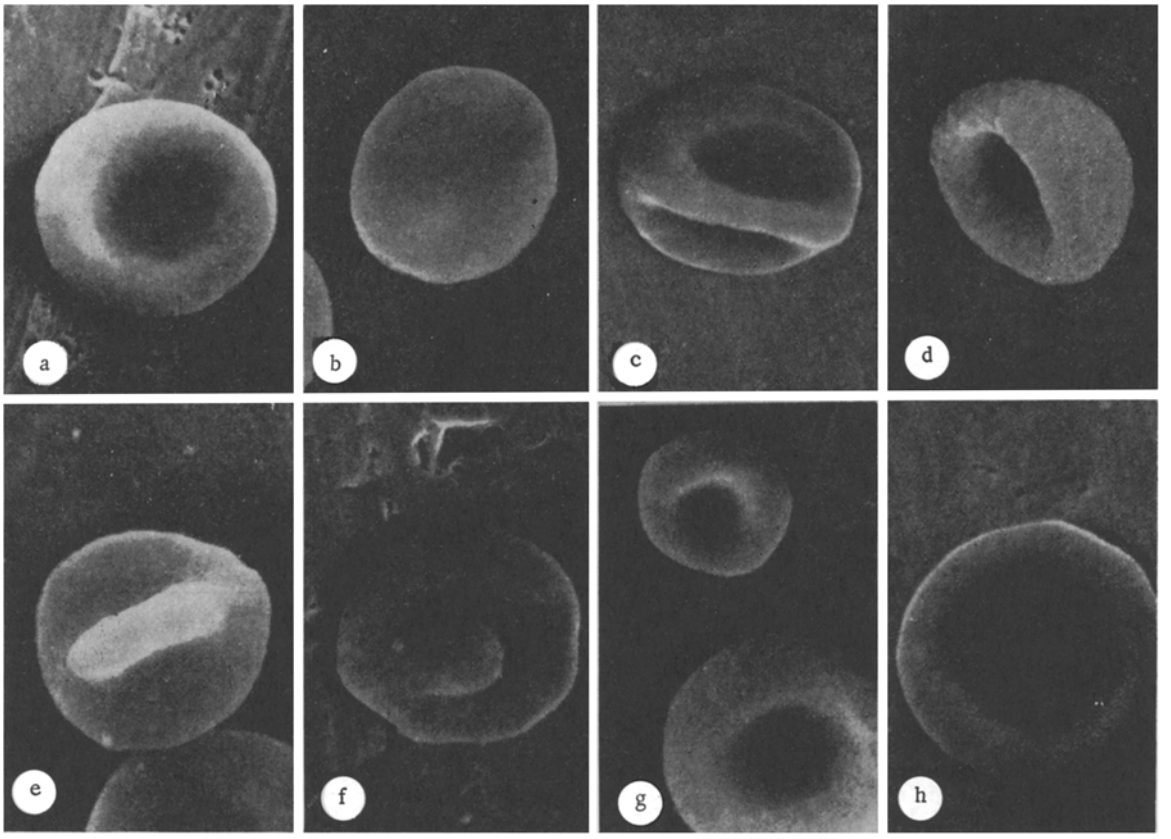

Fig. 1. Morphological characteristics of erythrocytes from arterial and venous rat blood revealed by scanning electron microscopy: a) type 1, biconcave disk, "normocyte"; b) type 2, flattened disk; c) type 3, cell with double indentation resembling a squashed ball; d) type 4, parachute-shaped cell or stomatocyte; e) type 5, cell with crest; f) type 6, cell with conical projection in center; g) type 7, microcyte; h) type 8, macrocyte. Cells with destructive changes, differing in shape (type 9) from those described above, are not illustrated.

Most of the cell population consisted of biconcave cells or flat disks (types 1 and 2). Erythrocytes which differed significantly in shape from normal constituted another group of cells. These included erythrocytes resembling a squashed ball or one with two large indentations (type 3), parachute-shaped cells or, to use the terminology adopted in the Western literature, stomatocytes (type 4), cells with a central crest (type 5), and cells with a conical projection (type 6). Microcytes and macrocytes were classed in types 7 and 8 respectively. Erythrocytes differing in shape from those described above and with traces of destruction of the membranes were distinguished as a separate group (type 9).

Besides the forms described above, which were constantly found, in some animals single spherocytes, echinocytes, or cells with ameboid projections were observed. The rarity of discovery of these forms makes their quantitative evaluation difficult.

Considering the known facts on the behavior of erythrocytes in the blood stream, and on their ability to penetrate into and move along inside capillaries, whose diameter is considerably less than the diameter of the cell itself, i.e., considering data showing the ability of erythrocytes to undergo plastic deformation under physiological conditions [3], the diversity of cell shape observed can be explained by the influence of many different outside forces. First and foremost among them are hydrodynamic factors and the influence of intererythrocytic relationships.

The variants of erythrocyte morphology in rats described above agree on the whole with pictures of human erythrocytes published by various workers [2, 6]. The best classification giving a quantitative indication of the relative percentages of each type of cells is that suggested by Kozinets et al. [1].

TABLE 1. Number of Different Forms of Erythrocytes (in %) in Arterial and Venous Blood (M ± m)

Type of cells	Arterial blood	Venous blood
1	69,06±2,08	61,83±2,13*
2	17,43±1,17	24,63±1,47*
3	4,60±0,50	3,26±0,60
4	1,76±0,18	3,33±0,65*
5	1,70±0,46	1,66±0,40
6	3,53±0,61	2,23±0,39
7	0,63±0,16	0,63±0,15
8	0,20±0,36	0,20±0,36
9	1,33±0,19	1,90±0,35

*P < 0.05.

TABLE 2. Coefficient of Variation (C) of Different Forms of Erythrocytes in Arterial and Venous Blood

Parameter studied	Blood	Type of cells								
		1	2	3	4	5	6	7	8	9
C	Arterial	11,68	26,05	42,39	39,77	—	67,42	—	—	57,89
	Venous	13,39	23,21	71,52	75,97	—	69,05	—	—	71,789

The effectiveness of quantitative assessment of dispersion of the forms of erythrocytes was demonstrated in the present investigation during a comparative analysis of the morphological and physiological state of the membranes of erythrocytes from venous and arterial blood. No difference could be found in the shape or surface of the erythrocytes, but there were significant differences in the quantitative proportions in the two cases (Table 1). It will be clear from Table 1 that the number of three types of cells differed significantly. For instance, there were more biconcave erythrocytes (type 1) in arterial blood and more flat disks (type 2) and stomatocytes (type 4) in venous blood.

These data suggest that erythrocytes in venous blood are more able to change their shape than those in arterial blood. This is confirmed by the significantly larger number of cells with the classical shape and, consequently, the smaller number of forms of abnormal erythrocytes in arterial blood. Calculations of the coefficient of variation (C) for each form of cells provide further indirect confirmation of this conclusion. These calculations were not carried out for cases when the standard error was equal to or greater than the arithmetic mean ($\sigma \geqslant M$). The data in Table 2 show that in every case but one (type 2 — flat disk) the coefficient of variation was higher for several values representing the relative percentages of those cells in venous blood.

Attention is drawn to the fact that there were significantly more stomatocytes (type 4) — cells shaped like a parachute or cup) in venous blood. The writers showed previously [4] that this shape is characteristic of erythrocytes forming aggregates of a certain type. At the same time, we know that disturbances of the aggregative state of blood are more marked in the venous compartment [3]. It can accordingly be suggested that in this case we have indirect evidence of differences in the degree of physiological aggregation taking place in the venous and arterial compartments of the circulatory system respectively.

An independent investigation is necessary to elucidate the mechanisms lying at the basis of differences in the relative proportions of the different forms of erythrocytes in venous and arterial blood. On the basis of existing data it can be postulated that the facts observed are connected either with the state of the hemoglobin (oxidized or reduced form) or with the rheologic and physicochemical properties which are different in the corresponding parts of the vascular system, and also with the influence of biologically active substances [5].

LITERATURE CITED

1. G. I. Kozinets, I. V. Ryapolova, Z. G. Shishkanova, et al., Probl. Gematol., No. 7, 10 (1977).
2. L. D. Krymskii, G. V. Neistaiko, and A. G. Rybalov, Scanning Electron Microscopy of Vessels and Blood [in Russian], Moscow (1976).
3. A. M. Chernukh, P. N. Aleksandrov, and O. V. Alekseev, The Microcirculation [in Russian], Moscow (1975).
4. A. M. Chernukh, O. V. Alekseev, and B. V. Ionov, Byull. Éksp. Biol. Med., No. 2, 226 (1981).
5. A. M. Chernukh, Inflammation [in Russian], Moscow (1979).
6. M. Bessis, R. J. Weed, and P. F. Leblond, Red Cell Shape, New York (1973).
7. J. Kiczak, Acta Physiol. Pol., 27, 183 (1976).
8. R. Tanaka and K. Nakai, Jpn. J. Pharmacol., 27, 413 (1977).