colonies after electrophoretic fractionation, that CFUs with low EPM are in the G_0 stage, and those with high EPM are in the mitotic cycle. Boll [3], who compared EPM of CFUs from normal and regenerating bone marrow, reached a directly opposite conclusion: CFUs in the mitotic cycle possessed lower EPM than CFUs in the G_0 stage.

The nature of CFUs with low and high EPM thus requires further investigation. It can be concluded from data in the literature and the results of the present investigation that fractionation of hematopoietic cells on the basis of differences in their surface charges is a promising method. By means of the electrophoresis method in conjunction with fractionation of cells on the basis of other parameters (volume, density, and so on) it will probably be possible to isolate and identify morphologically and functionally different types of stem cells in hematopoietic tissue.

LITERATURE CITED

1. G. I. Kozinets, Z. G. Shishkanova, V. P. Reshchikov, et al., Probl. Gematol., No. 9, 9 (1979).
2. T. G. Sukiasova, L. L. Eremenko, L. V. Borzova, et al., Lab. Delo, No. 5, 259 (1981).
3. S. Boll, The Recognition of Early Developmental Stages in Haemopoiesis. Thesis, Rijswijk (1980).
4. K. Hannig, Z. Anal. Chem., 181, 244 (1961).
5. M. Meistrich, R. Meyn, and B. Barlogie, Exp. Cell Res., 105, 169 (1977).
6. R. Miller and R. Phillips, J. Cell Physiol., 73, 191 (1969).
7. K. Shortman, Annu. Rev. Biophys. Bioeng., 1, 93 (1972).
8. J. Schubert, F. Walter, and E. Holzberg, Klin. Wschr., 51, 327 (1973).
9. J. Till and E. McCulloch, Radiat. Res., 14, 213 (1961).
10. D. Van Bekhum, M. Van Noord, B. Moat, et al., Blood, 30, 547 (1971).
11. G. Van den Engh and J. Visser, Acta Haemat., 62, 289 (1979).
12. K. Zeiller, J. Schubert, F. Walter, et al., Hoppe-Seyler's Z. physiol. Chem., 353, 95 (1972).

DISTRIBUTION BY RNA CONTENT OF CELLS ISOLATED FROM THE NORMAL AND ATHEROSCLEROTIC HUMAN AORTA

V. A. Kosykh, A. N. Orekhov, and V. S. Repin

UDC 616.132-004.6-008.939.63'915-092.18

KEY WORDS: atherosclerotic changes; cells of human aorta; flow cytofluorometry; RNA content; subpopulations.

Cells of the intima and media of blood vessel walls play a key role in the process of thickening of the intima and formation of the atherosclerotic plaque [3, 5, 6, 8]. So far the cell composition of the human vascular wall has been studied mainly by classicial microscopic methods [3]. Very little is yet known about the functional and metabolic properties of these cells. The writers have developed a method of obtaining human aortic cells in suspension, which has made it possible to study some of the properties of these cells by the use of the method of flow cytofluorometry. Unlike traditional microscopic methods, the method of flow cytofluorometry can be used to analyze rapidly and with great accuracy many cells and to detect differences between them in their physical, biochemical, and functional properties [2, 4]. The object of this investigation was to determine the heterogeneity of a cell population from the intima and media of the normal and atherosclerotic human aorta on the basis of a metabolic parameter such as the RNA content.

Laboratory of Cell and Tissue Culture, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR E. V. Chazov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 5, pp. 104-106, May, 1982. Original article submitted October 23, 1981.

0007-4888/82/9305-0675$07.50

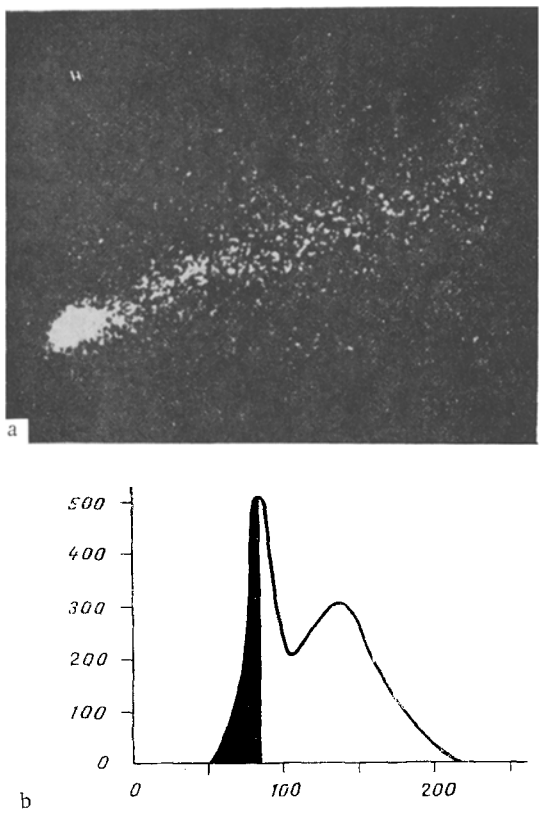

Fig. 1

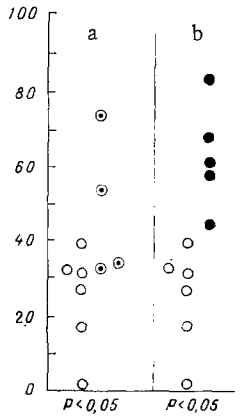

Fig. 2

Fig. 1. Distribution of cells from intima of normal aorta. a: Abscissa, scattering of light; ordinate, intensity of red fluorescence; b: abscissa, intensity of red fluorescence (channel No.), ordinate, number of cells per channel.

Fig. 2. Relative number of cells of subpopulation A (in %) in cell population from intima of normal and atherosclerotic vessels. a: Empty circles — normal (vessels of group 1), circled dots — pathology (vessels of group 2); b: empty circles — normal, filled circles — pathology (vessels of group 3).

EXPERIMENTAL METHOD

Experiments were carried out on a segment of thoracic aorta taken from 15 men aged 4-53 years 1.5-3 h after death. The segment of aorta was cut longitudinally into sections, the adventitia with part of the adjacent media was removed, and the inner surface was assessed visually. The segment was classed in one of three groups depending on the type of the atherosclerotic changes on its inner surface: 1) an outwardly normal surface with no signs of atherosclerotic changes; 2) the surface of the vessel with predominance of lipid streaks on it; 3) the surface of the vessel with predominance of lipofibrous plaques.

In each case the intima was separated from the media. Examination with the light microscope confirmed that separation of the antima from the media had taken place at the inner elastic membrane, the anatomical boundary between these layers of the vessel wall. The intima and media were separated into thin fibers, which were dispersed with collagenase and elastase. Solutions of collagenase and elastase were made up in isotonic phosphate buffer, pH 7.4, containing 25 mM HEPES, 10% embryonic calf serum, and 0.3% glucose. Fragments of media were incubated in a 0.2% solution of elastase (type I, from Sigma, USA) for 2 h, and then dispersed in a 0.3% solution of collagenase (type II, from Worthington, USA) for 4 h at 37°C. Fragments of intima were dispersed by a mixture containing 0.15% collagenase and 0.01% elastase (type III, from Sigma, USA) for 5-6 h at 37°C. To disperse 1 g wet weight of tissue, 10 ml of the enzyme solution was used. The isolated cells in suspension were stained with the fluorochrome acridine orange by method 1 [1] and the intensity of red fluorescence (wavelength over 600 nm), corresponding to the RNA content in each cell, was determined by flow

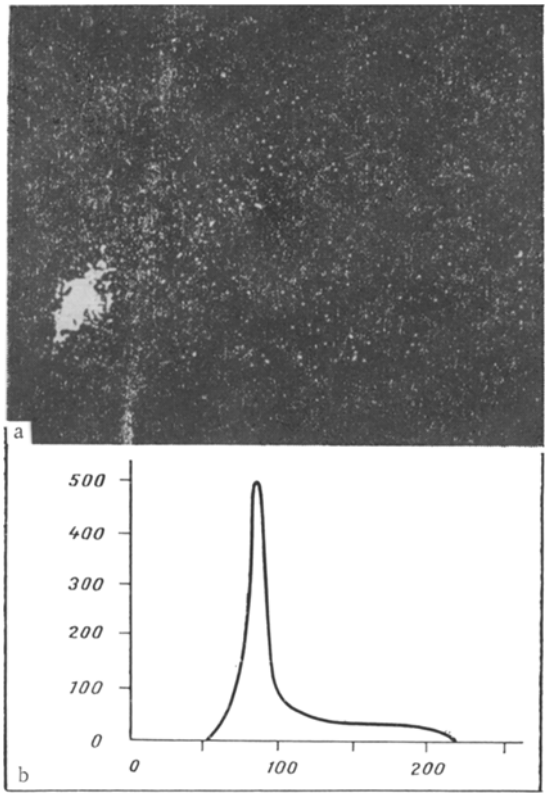

Fig. 3. Distribution of cells from intima of blood vessel with lipofibrous plaques. Legend as to Fig. 1.

cytofluorometry. The analysis was carried out on the FACS II flow cytofluorometer (from Becton-Dickinson, USA), exciting fluorescence at a wavelength of 488 nm. The distribution of cells by RNA content was obtained by analysis of 50,000 cells from each specimen. The significance of differences was estimated by the Wilcoxon—Mann—Whitney nonparametric criterion.

EXPERIMENTAL RESULTS

The distribution of cells from the intima of thenormal human aorta is shown in Fig. 1a for two parameters: intensity of red fluorescence corresponding to the RNA content in the cell, and scattering of light, which gives information about the size of the cell, for one increases parallel with the other, although the relationship between them is not described by a simple function [7]. Two regions of this distribution correspond to two cell subpopulations. Cells with a low RNA content (subpopulation A) are smaller in size (scattering of light) than cells with a high RNA content (subpopulation B). The frequency distribution of these same cells for red fluorescence is shown in Fig. 1b. It is bimodal in character: The narrow peak on the left corresponds to cells of subpopulation A, the wide peak on the right to subpopulation B. To determine the relative number of cells in each peak the frequency distirbution was determined planimetrically. Assuming that the distribution in the left peak was close to normal, the number of cells in the shaded region will correspond to half the total number of cells in subpopulation A. Using this method of estimation, the cell populations from the intima and media of normal vessels and of vessels with atherosclerotic changes were analyzed.

Analysis showed that the cell population from the intima of vessels with atherosclerotic changes also consisted of cells of two subpopulations. However, the relative number of cells of subpopulation A was appreciably greater in this population (Fig. 2). In the cell population from the intima of vessels with lipofibrous plaques (group 3) the contribution of cells of subpopulation A was greater than that of cells of subpopulation B, and in some cases cells of the subpopulation A became the principal cell type (Fig. 3a, b).

The cell population from the media also consisted of two cell subpopulations, but the relative number of cells of subpopulation A in the cell population from the media of vessels with atherosclerotic changes was identical to normal (data not shown).

The results of this investigation thus indicate that cell populations from the intima and media of the human aorta consist of two subpopulations which differ in their intracellular RNA content. Analysis of the cell populations from the intima of vessels with atherosclerotic changes showed an increase in the contribution of cells of subpopulation A (cells with a low RNA content) compared with normal. It can be postulated that cells of subpopulation A are metabolically less active than cells of subpopulation B.

LITERATURE CITED

1. Z. Darzynkiewicz, in: Flow Cytometry and Sorting, New York (1979), p. 285.
2. M. J. Fulwyler, Blood Cells, 6, 173 (1980).
3. J. C. Geer and M. D. Haust, Smooth Muscle Cells in Atherosclerosis, Basel (1972).
4. R. Ross and J. A. Glomset, New Engl. J. Med., 295, 369 (1976).
5. R. Ross and J. A. Glomset, New Engl. J. Med., 295, 420 (1976).
6. G. C. Salzman, P. F. Mullaney, and B. J. Price, in: Flow Cytometry and Sorting, New York (1979), p. 105.
7. H. C. Stary, Adv. Exp. Med. Biol., 43, 59 (1973).