Data on electrophoretic fractionation of the protein components of fractions II and III, obtained by gel-filtration, in the presence of SDS and dithiothreitol, before and after treatment with 1 mM diamide, are given in Fig. 3. The main components of fraction II are polypeptides with mol.wt. of about 35, 29, and 28 kD. The main components of fraction III are polypeptides with mol.wt. of 29, 28, and 26 kD. On cross-linking with diamide, a relative increase in the content of the polypeptide with mol.wt. of 29 kD was observed in fraction II, and the relative content of this polypeptide was reduced in fraction III. On the basis of these findings it can be postulated that the polypeptide with mol.wt. of 29 kD was most vulnerable to cross-linking by diamide.

It can be concluded from these results that diamide causes the formation of high-molecular-weight protein components as a result of oxidation of SH-groups of crystallins. The experimental use of diamide can be recommended for simulation of the oxidative changes brought about in lens proteins typical for the development of senile cataract.

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CHROMATOFOCUSING OF LOW-DENSITY PLASMA LIPOPROTEINS

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KEY WORDS: low-density lipoproteins; isoelectric point; chromatofocusing; ischemia; tumor.

The heterogeneity of low-density plasma lipoproteins (LDPP) with respect to their charge [15] may be the result of the different protein composition of the lipoprotein particles of this class [10], heterogeneity of apoB — the basic protein of LDPP relative to the isoelectric point pI [11], conformational changes in apoB [13] and also, evidently, its modifications (glycosylation, damage by lipid peroxidation products [8]).

The aim of this investigation was to test chromatofocusing as a method of separating LDPP at the effective isoelectric point as a simpler method than isoelectric focusing, isotachophoresis, or ion-exchange chromatography.

EXPERIMENTAL METHOD

LDPP were isolated by single ultracentrifugation in a stepwise density gradient of NaBr [4]. Plasma was obtained from clinically healthy individuals, patients with coronary heart disease (CHD), documented by coronary arteriography, and patients

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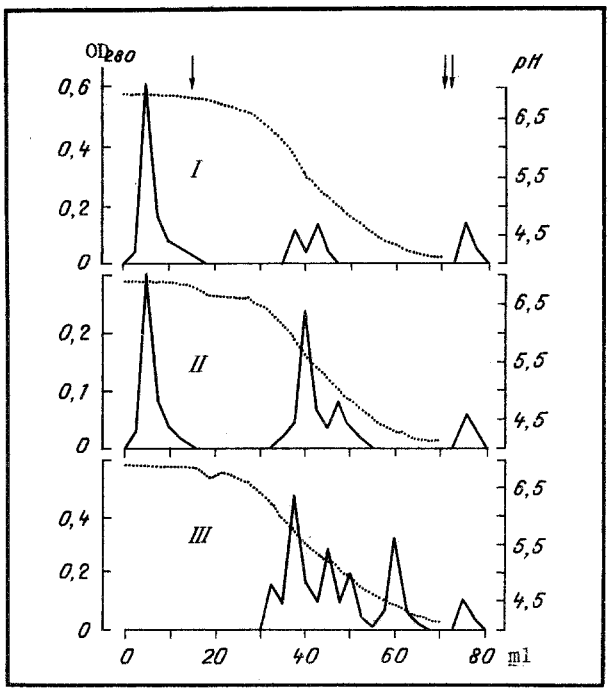

Fig. 1. Three versions of binding and elution of healthy human LDPP on the anion-exchanger PBE-94. Here and in Figs. 2 and 3: single arrow indicates addition of polybuffer, double arrow – addition of 1 M KCl.

with carcinoma of the cervix uteri in stage III-IV.

The lipoproteins (LP) thus obtained were dialyzed against 0.025 M NaCl, pH 7.0. Protein of LDPP was determined by Lowry's method.

Chromatofocusing was carried out on PBE-94 gel (Pharmacia Fine Chemicals, Sweden). The gel was equilibrated with a 0.025 M solution of histidine hydrochloride, pH 6.9, previously passed through a PBE-94 precolumn to remove oxidation products of histidine, absorbing UV light and colored yellow. Analytical chromatofocusing was carried out on a column measuring 1 × 7 cm, and preparative chromatofocusing on a column measuring 1 × 20 cm. The solution of LDPP was applied to the small column in an amount of 1-2 mg as protein, and to the large column in an amount of 16-26 mg. The gel was then washed with the start buffer. Elution was carried out with polybuffer PB-74 (Pharmacia Fine Chemicals), diluted 11 times (pH 4.1), at the rate of 0.2 or 0.5 ml/min. LP firmly bound with the gel were eluted with 1 M KCl. Fractions of 2.5 ml were collected. Optical density was recorded at 280 nm, or in the case of LP stained with Sudan IV, at 650 nm (SF-26, USSR). The pH was measured on a model 262 (USSR) and 701A (Orion, USA) pH-meter.

LDPP acetylated and treated with hexobarbital [2, 3] also were used in the work.

Chromatofocusing was used previously to separate LDPP and LP (a) [6], by applying LP to PBE-94 in a solution of 0.025 M imidazole containing 0.2 M NaCl (pH 7.0), i.e., under conditions of inhibition of electrostatic interactions of LDPP with the anion-exchanger PBE-94. However, under these circumstances LP (a) bound with the gel, for their charge is higher than that of LDPP, and they separated into two subfractions with pI of 6.5-6.8 and 4.5-5.0. In the present experiments the start buffer did not contain 0.2 M NaCl. Additionally, after ultracentrifugation of the blood plasma, the zone characteristic of LP (a) and usually located 5-8 mm below the LDPP zone, was absent in all cases.

Three types of binding of healthy human LDPP with PBE-94 were discovered. In the first type (n = 11) most of the LP was not bound with the gel even if applied many times. Minor fractions had pI values of 5.8 and 5.3, and also pI 4.1 (Fig. 1, I). In the first type (n = 3) about half of the LDPP was not bound with the gel, the subfractions had pI values of 5.7 and 5.0, and also pI < 4.1 (Fig. 1, II). In the third type (n = 4) LP were completely bound with the gel and the subfractions had pI values of 6.2, 5.8, 5.2, 4.9, and 4.4 (Fig. 1, III). LDPP of one of the healthy donors, which had a single fraction with pI = 5.8 (not shown in Fig. 1), also may have belonged to this type.

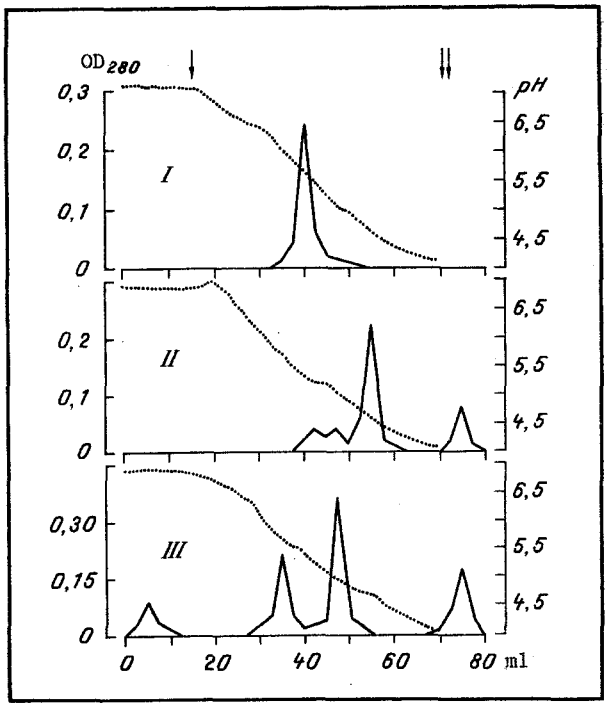

Fig. 2. Chromatofocusing of original LDPP (I), acetylated LDPP (II), and LDPP 2 weeks after isolation (III).

The difference in the character of binding of LDPP from healthy individuals with the anion-exchanger PBE-94 may be due to differences in the character of distribution of the LP-particles by radius [9], leading to a different type of distribution of LDPP by surface charge density and, consequently, to a different type of sorption-desorption of LDPP at the same pI values. Without going into a detailed analysis of the other causes of the existence of several versions of binding and elution of LDPP with PBE-94, we may, however, note that elution of modified LP of this same class was characterized by fundamental differences. Whereas the original LDPP had pI = 5.7 (subfraction, isolated preparatively, chromatofocusing), the acetylated version had pI = 4.6 (Fig. 2, I and II), and that treated with 10 mmoles of hexobarbital had pI = 4.3 (not shown in Fig. 2). These results demonstrate the wide opportunities for chromatofocusing as a method of isolating LDPP strongly modified by charge, or for separating them from unchanged particles.

Acetylation, incidentally, usually blocks about 40% of amino groups of apoB, and this theoretically ought to lead to a shift of the isoelectric point of only 0.1 pH unit. The actual shift in the value of pI was 1.1 pH unit. These data may be evidence of conformational changes of apoB in the composition of acetylated LDPP, which are not detected by the circular dichroism method [2]. A similar conclusion regarding conformational changes was drawn from a study of isoelectric points of apocytochrome C and homocytochrome C. Methylation of one lysine group in position 75 in the first case led to a shift of pI of 1 pH unit, but in the second case it did not affect the isoelectric point [12].

It will be noted that 2 weeks after isolation of the LDPP and their keeping at 4°C in 0.025 M NaCl, pH 7.0, there was a change in the electrical properties of the LP-particles. Additional subfractions were discovered with isoelectric points of 5.0 and pI less than 4.1 (Fig. 2, III). This shows that the subfraction with pI = 4.1 is formed during keeping of LDPP and also, probably, during their isolation.

The possibilities of chromatofocusing for the discovery of LP with anomalous isoelectric point were utilized during analysis of LDPP of patients with CHD and tumors. In the first case, enrichment of LDPP is possible, either with the positively [1] or the negatively [5] charged subfraction, in the second case enrichment with the negatively charged subfraction on account of an increase in the degree of sialization of apoB in patients with cancer of the breast and cervix uteri [14]. In three patients with CHD and normolipidemia, all LDPP bound with the anion-exchanger. The contribution of the subfraction with pI < 4.1 was considerable (up to 20%). In one case the subfraction with pI = 6.3 predominated, in another case that with pI = 5.4, and in a third case, with pI = 4.4 (Fig. 3A, B, C). In other words, the results do not give grounds for the conclusion that there is a

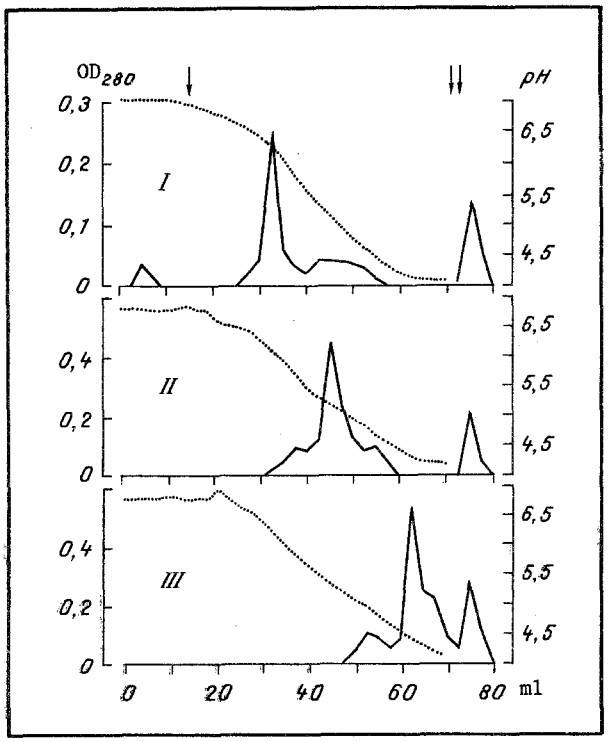

Fig. 3. Chromatofocusing of LDPP of three patients with CHD.

significant shift of the effective isoelectric point of LDPP in patients with CHD, in the direction of an increase. A decrease in the value of the isoelectric point of LDPP on account of the appearance of a subfraction with pI < 4.1 can be mentioned. An increase in the contribution of this subfraction to some degree may be used as a diagnostic sign of CHD. Meanwhile, its existence in the bloodstream is doubtful. As was pointed out above, and its presence in isolated LDPP is due to peroxide modification of the LP-particles. In this case the liability of the LDPP of patients with CHD of this modification is higher than LP of the same class from healthy individuals.

LDPP of the patients with tumors bound with PBE-94 to the extent of 50%. In one case the subfractions had pI values of 6.2 and 5.0, and in a second case 5.8 and 4.8, and in a third case 6.3, 5.9, and 4.3 (not shown in Fig. 3). Consequently, it cannot be concluded from the results that LDPP was enriched by the negatively charged subfraction in patients with cancer of the cervix uteri.

On the whole the facts obtained suggest that chromatofocusing is a convenient way of fractionating LDPP by effective isoelectric point. Meanwhile, for the complete characterization of the electrical properties of LP this method alone is insufficient, for LDPP in the tissue fluid of the skin have a higher negative charge (pre-β-mobility) than plasma LDPP (β-mobility), but they are virtually indistinguishable in their isoelectric point from plasma LP [7].

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FILTERABILITY OF LEUKOCYTES IN WHOLE BLOOD

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KEY WORDS: filtration; leukocytes; erythrocytes.

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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