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MICROVISCOSITY OF SUBFRACTIONS OF HIGH-DENSITY LIPOPROTEINS OF HUMAN BLOOD

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The results of many epidemiologic investigations have shown that a high plasma concentration of cholesterol of high-density lipoproteins (HDLP) is an antirisk factor of ischemic heart disease (IHD) [12]. This fact accounts for the great interest shown by research workers in HDLP and, in particular, in the two subfractions $HDLP_2$ and $HDLP_3$, which differ in composition and properties and which probably perform different functions in the body [6].

The metabolism of the protein–lipid particles of plasma is currently receiving intensive study. One of the essential characteristics of lipoproteins, which determines their role in reactions of this type, is the microviscosity of the lipid regions [7, 10]. Evidence has been obtained of a change in the microviscosity of individual classes of liproproteins in dyslipoproteinemias in man [13], and also in experimental atherosclerosis [3, 5, 9]. Meanwhile the microviscosity of HDLP subfractions in persons with different HDLP cholesterol levels has hardly been studied at all. It has been claimed their antiatherogenic properties are due not only to the high concentration of HDLP, but also to their physicochemical features [4].

The object of this investigation was to study the microviscosity of HDLP subfractions in subjects with different plasma HDLP concentrations. The fluorescent probe pyrene, which has already proved its value in the study of microviscosity of lipoproteins in previous investigations [3, 5], was used to study this problem.

EXPERIMENTAL METHOD

The microviscosity of the principal subfractions of HDLP was studied in 20 subjects aged 30–59 years with different HDLP cholesterol levels (from 33 to 80 mg%). $HDLP_2$ (1.063 g/ml < d < 1.125 g/ml) and $HDLP_3$ (1.125 g/ml < d < 1.210 g/ml) were obtained by consecutive ultracentrifugation [2]. HDLP cholesterol was determined on a Technicon AAII automatic analyzer after preliminary sedimentation of very low- and low-density lipoproteins [8]; phospholipids were determined by Svannborg's method [15].

The microviscosity of the lipoproteins was estimated as the ratio between the intensities of fluorescence at the maximum of the spectrum for two forms of pyrene — the monomer (F_m, 395 nm) and the eximer (F_e, 470 nm) [5]. The fluorescence measurements were made on a

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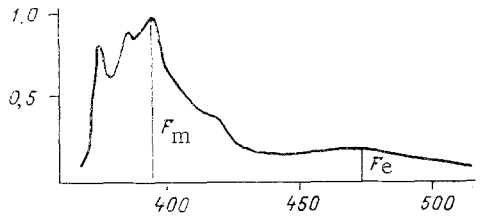

Fig. 1. Fluorescence of pyrene in $HDLP_3$ of a person with a HDLP cholesterol concentration of 72 mg%. Concentration of $HDLP_3$ phospholipids 0.02 mg/ml, of pyrene 4 μM. F_m) Intensity of fluorescence of monomers; F_e) the same of eximers of pyrene. Abscissa, wavelength of fluorescence (in nm); ordinate, intensity of fluorescence (in relative units).

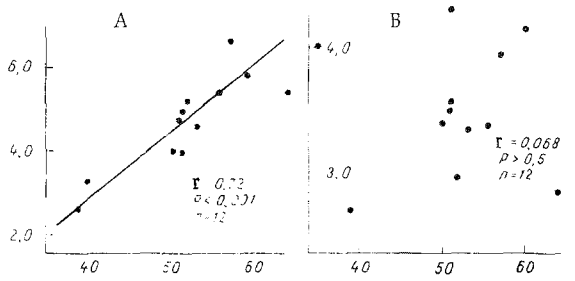

Fig. 2. Changes in F_m/F_e parameter of pyrene depending on concentrations of HDLP cholesterol in $HDLP_3$ (A) and $HDLP_2$ (B) isolated from blood plasma of subjects with normal HDLP cholesterol level. Concentration of probe and of lipoprotein phospholipids the same as in Fig. 1. Abscissa, concentration of HDLP cholesterol (in mg%); ordinate, ratio F_m/F_e.

Hitachi MPF-2a spectrofluorometer in cylindrical cuvettes 5.5 mm in diameter, with excitation of fluorescence at 334 nm.

EXPERIMENTAL RESULTS

The typical fluorescence spectrum of pyrene in human $HDLP_3$ with a high content of HDLP cholesterol (72 mg%) is illustrated in Fig. 1, which also shows the parameters measured (F_m/F_e). Statistical analysis (Fig. 2A) revealed high correlation ($r = 0.72$, $P < 0.001$) between the F_m/F_e parameter of pyrene in $HDLP_3$ and the concentration of total HDLP cholesterol for the group of subjects with a normal HDLP cholesterol concentration (i.e., according to the criteria adopted in [1], from 34 to 70 mg%). However, the points corresponding to the F_m/F_e parameter in $HDLP_3$ for subjects with hyper-α-liproproteinemia (HDLP cholesterol over 71 mg%) did not obey this relationship and the mean values of this parameter for those subjects were indistinguishable from normal (Table 1).

An increase in the microviscosity of $HDLP_3$, the substrate for lecithin cholesterol acyltransferase (LCAT) [11], with an increase in the HDLP cholesterol level is bound to have some effect on the course of this reaction. We know [14] that LCAT acts better on a more liquid substrate.

The microviscosity of $HDLP_3$, estimated relative to eximerization of pyrene, was below that for $HDLP_3$ of subjects with a normal HDLP cholesterol concentration. The reason probably is that $HDLP_2$ has a smaller protein fraction than $HDLP_3$ [6]. In the case of hyper-α-lipoproteinemia differences in the microviscosity of $HDLP_2$ and $HDLP_3$, however, disappear (Table 1).

No correlation was found for $HDLP_2$, unlike $HDLP_3$, between the F_m/F_e parameter and the HDLP cholesterol concentration (Fig. 2B). The reason may perhaps be that environmental fac-

TABLE 1. Fluorescence Parameter F_m/F_e of Pyrene in Human $HDLP_2$ and $HDLP_3$ (M ± m)

Group of subjects	$HDLP_2$	$HDLP_3$	P
Normal, HDLP cholesterol 51 ± 3 mg% (n = 12)	3,5±0,1	4,7±0,3	<0,02
Hyper-α-lipoproteinemia, HDLP cholesterol 76 ± 2 mg% (n = 4)	4,2±0,6	4,6±0,3	>0,5

tors such as the character of the diet, the mode of life, and so on [1], have a pronounced effect on the $HDLP_2$ concentration and also, perhaps, on its physical properties.

Under normal conditions positive correlation thus exists between the pyrene fluorescence parameter F_m/F_e in $HDLP_3$ and the HDLP cholesterol concentration. The microviscosity of $HDLP_2$ of subjects with a normal HDLP cholesterol level is less than that for $HDLP_3$. In hyper-α-lipoproteinemia no differences in the microviscosity of $HDLP_3$ and $HDLP_2$ are found compared with the normal state.

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