ELECTROPHORETIC MOBILITIES OF HIGH DENSITY LIPOPROTEINS OF HUMAN SERUM

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By flotation in solutions of suitable density, the high density lipoproteins of human serum can be separated into two families, one (A*) containing lipoproteins of hydrated density 1.090-1.102 g/ml, and the other (B), those of hydrated density 1.141-1.152 g/ml (Shore, 1957). These high density lipoproteins have been called α_1-lipoproteins, because they presumably migrate in the Tiselius apparatus with the α_1-globulins, though it has been emphasized that this migration was observed with lipoproteins isolated by low-temperature ethanol fractionation (Oncley, 1956). To our knowledge, moving boundary electrophoretic mobilities have not yet been presented on the lipoproteins isolated by density flotation.

The single schlieren peaks of the descending limbs of density isolations of A and B from four sera, measured in the 2 ml cell of the Spinco Model H instrument in pH 8.6 buffer, 0.1μ in Veronal and 10^{-3}M in EDTA, at a potential gradient of 4.95 volts/cm, possessed u's (A, B;) of 6.1, 6.4; 6.25, 6.3; 6.3, 6.4; and 6.3, 6.6. The descending limb patterns were at least as sharp as those of the rising limb. The u's of the rising limb were usually 3-5% higher than those of the descending limb. After about 2 1/2

*Abbreviations: A, lipoproteins of hydrated density 1.090-1.102 g/ml; B, lipoproteins of hydrated density 1.141-1.152 g/ml; u, negative mobility in 10^5 x u cm^2 sec^{-1} $volt^{-1}$; and EDTA, ethylenediamine tetraacetic acid.

hour electrophoresis at 4.95 volts/cm in the Veronal buffer, the rising limbs of three isolations of A resolved into major and minor peaks of u 6.2, 5.4; 6.4, 5.5; and 6.6, 5.6; and the rising limbs of two isolations of B into major and minor peaks of u 6.7, 5.8; and 6.5, 5.7.

Since the lipoprotein u's obtained by us were considerably greater than that (5.2) of α_1-globulin in the same medium, further investigation seemed necessary. The u's of lipoproteins isolated in D_2O-sucrose were not significantly different from those isolated in NaCl and D_2O-$NaNO_3$, arguing against Cl^- or NO_3^- binding as the cause of the high u's. The u's of lipoproteins isolated and electrophoresed in the absence* of EDTA were about 0.1 greater than those of lipoproteins always exposed to 10^{-3}M EDTA. The high u's could not be explained by concentration dependence.

Each mole A and B contained about 12 moles non-esterified fatty acid (probably C-16 and C-18, Dole et al., 1959), with an uncertainty of about 25% in this value. The small amount of lipoprotein analyzed and the uncertainty in lipoprotein molecular weight make it unwise at present to postulate that a difference in u between A and B may reflect a difference in fatty acid content. IRA-400 anion exchange resin that removes the fatty acid from albumin (Dintzis, 1952) has so far not been successful with these lipoproteins. The only B examined, of u 6.6, was able to remove fatty acid from crystalline human serum albumin, resulting in an increase in the lipoprotein u to 7.3. A very preliminary pH-u study indicated an apparently implausible isoelectric point of 3.7 for B. If we assume that the experimentally determined pH of minimum solubility, 5.1-5.3, is that of the isoelectric point, an explanation must be

*In these experiments, O_2 and heavy metals were excluded as much as possible by use of prepurified N_2, redistilled H_2O and D_2O, and recrystallization of the electrophoretic buffer components and salts used to raise the solution density. (EDTA is added to complex heavy metals which catalyze autoxidation of lipoproteins, with a consequent increase in u.)

sought for the 3.7 value. Perhaps both the 3.7 isoelectric point and the high u's at 8.6 of A and B reflect in part the presence of the non-esterified fatty acids, whose ionized carboxyl groups evidently significantly affect the u.

Table I presents the descending limb u's of the proteins of serum alone and serum to which A and B were added. The u's and concentrations of the

Table I

Electrophoretic mobilities of high density lipoproteins, serum proteins, and serum proteins to which high density lipoproteins were added

Solution electrophoresed	Negative mobility[a] x 10^5 cm^2 $volt^{-1}$ sec^{-1}				
	γ-glob.	β-glob.	α_2-glob.	α_1-glob.	alb.
2.2 ml serum[b]	1.1	2.8	4.0	5.2	6.0
lipoprotein A[c]					6.35
lipoprotein B[c]					6.45
2.2 ml serum + 7×10^{-5} mmoles A[c] (about 26 mg)	1.4	3.1	4.1	5.8	6.3
2.2 ml serum + 8×10^{-5} mmoles B[c] (about 15 mg)	1.3	3.0	4.1	5.7	6.2
2.2 ml serum + 10^{-3}M EDTA	1.2	2.9	4.1	5.3	6.2
lipoprotein A[d]					6.3
lipoprotein B[d]					6.4
2.2 ml serum + 7×10^{-5} mmoles A + 10^{-3}M EDTA	1.3	3.1	4.1	5.8	6.3
2.2 ml serum + 8×10^{-5} mmoles B + 10^{-3}M EDTA	1.4	3.1	4.1	5.8	6.4

[a]Measured in pH 8.6, μ 0.1 Veronal, with or without 10^{-3}M EDTA as noted.
[b]All the sera were diluted to 5.1 ml before electrophoresis.
[c]These lipoproteins were isolated and electrophoresed without EDTA.
[d]These lipoproteins were isolated and electrophoresed in 10^{-3}M EDTA.

serum proteins agreed well with the literature values (Dole and Braun, 1944). At least 90% of the added A or B migrated in the region common to the α_1-globulins and trailing edge of the albumin peak. This inter-protein region is included with the α_1-globulins in Table I, and its increased u is evident in the sera with added lipoproteins. Both the concentrations and u's of the inter-

protein region were not significantly changed by conducting the entire experiment, including isolation of A and B, without EDTA. This finding and the observation that the u's of A and B in serum were 5.7-5.8 (not 6.3-6.45), with or without EDTA, suggests that EDTA binding is not the cause of the high u's of A and B in the absence of serum. After A and B were reisolated in D_2O-sucrose from serum to which they had been added, their u's became those, 6.3 and 6.4, before addition.

Other than to state that the u's of the lipoproteins and serum proteins under the various conditions and the multiple moving boundaries of the rising limbs of A and B may be functions of pH, viscosity, and conductance gradients, electrophoretic transport of the ions of the supporting medium, interacting systems, fatty acid binding and exchange, and charge heterogeneity, it does not seem profitable to speculate at this point about the differences in the u's. From our data, we cannot state whether the fatty acids are integral constituents of the lipoprotein molecule, or whether they have been abstracted from other blood proteins during isolation of the lipoprotein.

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