Some Properties of Human Serum Lipoproteins.* (22178)

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Our concern with the lipides of serum has been principally with respect to their characterization as lipoproteins. In this regard we have been able to demonstrate, by a variety of technics, that carotene, acetal phosphatidyl choline and probably sphingomyelin are *characteristic* of human β_1-lipoprotein(1,2); carotene had already been shown by others (3) to be associated with β_1-lipoprotein. The presence of acetal lipides in serum is not a new observation(4); only recently, however, have studies directed toward demonstrating the protein association of serum acetal phosphatides been reported(1,5). We have been unable to demonstrate a lipide component characteristic of α_1-lipoprotein but do find vit. A to be regularly associated with albumin.

Methods. Preparative Ultracentrifugation. 200 to 400 ml of blood were obtained by veni-puncture from normal, postabsorptive, human subjects and let clot undisturbed at 20°C for an hour. The serum was separated by centrifugation in an International refrigerated centrifuge (15°) and promptly submitted to ultracentrifugation for 8-10 hours at 4-12° using a Spinco E5 rotor at 52640 rpm (ca 200000 g). Appearance of the tubes after centrifugation is diagrammed in Fig. 1. Fractions 1-5 were quantitatively collected by means of a specially constructed aspirating device and examined by one or more of the following procedures: 1) Refractionation in the ultracentrifuge after adjusting density to (a) 1.063 with NaCl or to (b) 1.21 with NaCl and KBr or with D_2O and NaCl. Four to 6 hours at 200000 g with the E5 rotor sufficed to float the desired fraction (β_1-lipoprotein at 1.063 and both α_1 and β_1-lipoproteins at 1.21) into the top ml which

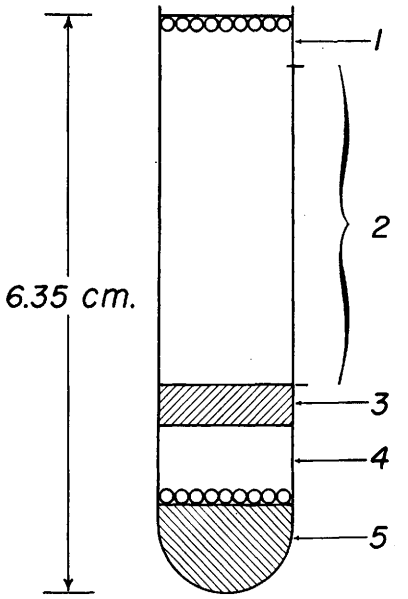

FIG. 1. Serum following 8 hr ultracentrifugation; (1) water-clear to milky; (2) water-clear; (3) bright orange-yellow, turbid; (4) yellow, clear; (5) yellow-brown, clear and includes thin flocculent layer dividing 4 from 5. Typical volume recoveries are 0.6, 3.6, 0.5, 0.7, and 1.0 ml respectively.

was collected by aspiration. 2) Analytical schlieren analyses of all samples were performed after adjusting the density to 1.063 or to 1.21. For these analyses the Spinco An-A rotor operating at 52640 rpm was employed, utilizing both visual inspection of the viewing screen and photographs in the analysis. 3) Chemical analysis on (a) chloroform:methanol (4/1:v/v) extracts and (b) benzene extracts of frozen-dried material. Phosphorus and choline were estimated as described earlier(6); cholesterol(7); total lipide esters (8); carotene from its absorption spectrum (9); and vit. A using both the Carr-Price reaction(9) and the technic of Bessey, *et al.* (10). As here measured choline was an estimate of the lecithin (phosphatidyl choline) and of acetal phosphatidyl choline; sphingomyelin may be estimated by difference from

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TABLE I. Typical Data from Chloroform : Methanol and Benzene (in Parenthesis) Extracts of Frozen-Dried Serum Fractions Obtained by Ultracentrifugation as Described in the Text.

Fraction	Color of extract*	Phosphorus		KOH hydrolyzable choline	Cholesterol		Total carboxylic esters	Carotene	Vit. A	Acetal lipide
		10^{-4} mM/ml fraction								
		Total	KOH hydrolyzable		Total	Non-esterified				
1	W	14.4	12.3	0	10.0	5.1	198	0	0	0
	(W)	(6.3)			(10.0)	(5.0)	(163)	(0)	(0)	(0)
2	W	4.4	4.3	0	3.1	2.0	63	0	0	0
	(W)	(0)			(3.0)	(2.0)	(38)	(0)	(0)	(0)
3	Y	150.0	113.2	85.0	253.1	56.2	735	4+	1+	4+
	(Y)	(4.8)			(171.3)	(55.7)	(200)	(4+)	(0)	(±)
4	Y	64.0	55.1	38.8	54.4	9.0	270	±	2+	+
	(W)	(0)			(1.3)	(1.1)	(0)	(0)	(0)	(±)
5	Y	38.5	33.8	19.4	28.3	3.1	145	0	4+	±
	(W)	(0)			(1.0)	(0.5)	(0)	(0)	(0)	(±)

* W = White; Y = Yellow.

the total and KOH phosphorus values(6). The desirability of using frozen-dried preparations in a study of this kind has been elaborated elsewhere(11). 4) Paper chromatographic analysis of both chloroform: methanol and benzene extracts(12). 5) Paper electrophoresis (ionophoresis) both a Kunkel-type apparatus and a Spinco Model R were used at pH 8.6 (0.05 *M* sodium barbital) usually operating at 250 volts, 2 m.a. for 20 hours. Whole serum was submitted to the techniques described above to aid in interpretation of the fraction data.

Results. With respect to their lipoprotein content, fractions 1 and 2 were normally quantitatively insignificant, together accounting for only 7% of the serum cholesterol and 14% of the phosphatide although occupying 66% of the volume. Fraction 1 consisted of chylomicrons which were principally triglyceride and cholesterol(2), all of which was readily extractable from frozen-dried samples by benzene, while only 50% of their phosphatide was so extracted (Table I). Fraction 2 was characterized by its low lipide content; it should be recognized that this fraction is adjacent, above and below, to fractions of high lipide content (Fig. 1).

Fraction 3 usually contained all of the β_1-lipoprotein and perhaps 50% of the α_1-lipoprotein as judged by the schlieren tracings (Fig. 2). The β_1-lipoprotein was easily separable from α_1-lipoprotein by ultracentrifugation at density 1.21. This separation was accomplished in the An-A rotor; centrifugation continued until, by inspection of the viewing screen, the yellow β_1-lipoprotein peak disappeared to the left; the α_1-peak was then nearly halfway across the field. Removal of the analytical cell from the rotor permitted aspiration of the subnatant solution which

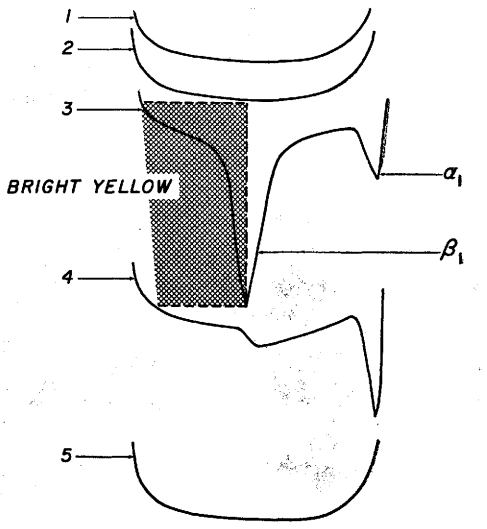

FIG. 2. Flotation schlieren patterns (22 min. pictures) from fractions 1-5 after adjusting density to 1.21 with D_2O • NaCl. Occasionally an α_1-peak was observed in 5. When adjusted to density 1.063 the β_1-peak had an S_f of 6. The yellow color of the β_1-peak in curve 3 was readily observed on the viewing screen during the run.

TABLE II. Representative Cholesterol-Phosphatide, Free-Total Cholesterol Mole-Ratios of Various Human Blood Lipoprotein Fractions: (a) This Paper, (b) Others.

Source	Cholesterol / Phosphatide	Free cholesterol / Total cholesterol
a. Fraction 1	.69	.51
2	.68	.51
3	1.9	.27
4	1.0	.20
5	.75	.14
D_2O • NaCl β_1	2.8	.28
α_1*	1.6	.25
Diabetic β_1†	1.8	.49
b. Cohn fractionation(3)		
β_1	2.1	.27
α_1	1.4	.27
Starch ionophoresis (13)		
β_1	2.1	.27
α_1	.58	.22
Ultracentrifugation(3)		
$\beta_1(S_f4)$	2.4	.25

* Shown not to be free of β_1.

† Obtained by preparative ultracentrifugation of serum from patient in diabetic acidosis. This material collected at the top of the tube as an orange-yellow waxy layer similar to Hunter's(2) cream-layer β_1.

contained the α_1-lipoprotein and some β_1. The bulk of the β_1-lipoprotein remained on the upper face of the cell as an orange wax, easily lifted off; these materials were then examined by ionophoresis, paper chromatography, etc. (Table II). As in the case of ionophoresis of fraction 3, these β_1 samples migrated more rapidly than the β_1-lipoprotein in whole serum (Fig. 3), probably due to the absence of *plain* β_1-globulin. In all cases β_1-lipoprotein was characterized by intense osmiophilia, evidently due to the carotene, and by a strong reaction for acetal lipide. By means of various spot-tests and paper chromatography(12) the major acetal lipide component of β_1-lipoprotein was identified as acetal phosphatidyl choline(2,12). The α_1-lipoprotein was identified through its paper electrophoretic mobility on staining with bromophenol blue; it was not osmiophilic and did not stain for acetal lipide. Albumin and non-lipide globulins were absent from the D_2O • NaCl preparations.

Fraction 4 usually contained the rest of the α_1-lipoprotein as demonstrated by ionophoresis and by ultracentrifugation at density 1.21 in D_2O • NaCl. Similarly fraction 5 was shown to contain most of the vitamin A (osmiophilia in the albumin region, *cf.* Fig. 3) and occasionally some α_1-lipoprotein. In the D_2O • NaCl refractionation the vitamin A was found to be below the top milliliter.

Ionophoretically, fractions 1 and 2 were negative except for traces of albumin. Fraction 3 showed a trace of γ-globulin, all the β_1-lipoprotein, some α_1-globulin (lipoprotein?) and a moderate amount of albumin. Fraction 4 showed a moderate amount of γ-globulin, no

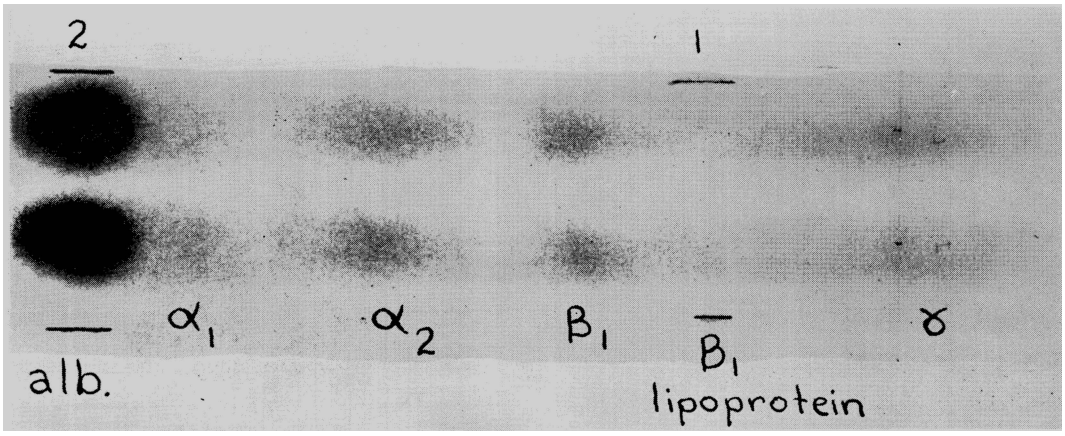

FIG. 3. Ionophoresis of 10 lambda aliquots of 2 normal human sera. The paper was dried promptly at room temperature by means of a fan and exposed to OsO_4 vapors for 15 min.(12) resulting in a browning in the zones delineated by the pencil lines 1 and 2. The paper was then stained for protein using bromophenol blue. On duplicate specimens it was demonstrated that usually only the β_1 osmophilic region was positive for acetal lipide.

β_1-lipoprotein, considerable β_1, a_2 and a_1 globulins and albumin. Fraction 5 was similar to 4 except for markedly diminished a_1 and a marked increase in the other globulins and albumin.

Discussion. The behavior of frozen-dried β_1-lipoprotein in chloroform: methanol and in benzene as here described (Table I) renders less useful the notion that *all* protein-bound lipides can be estimated by the solvent partition method(6,12). In this case carotene and cholesterol, for example, seem to be loosely bound, *i.e.*, extractable by benzene. On the other hand it is apparent that the other blood lipoproteins (excepting chylomicrons) are more resistant to benzene extraction.

Summary. Normal, postabsorptive, human serum was fractionated by means of the ultracentrifuge and the lipoproteins studied with the aid of paper chromatography, ionophoresis and by chemical procedures. The characteristic features of β_1-lipoprotein and methods for its isolation are described.

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Development of Arthritis, Periarthritis and Periostitis in Rats Given Adjuvants.* (22179)

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In this investigation skeletal muscle was incorporated into a Freund-type water-in-oil emulsion and the mixture administered by various routes to rats. Within a short time, ranging from 14 to 45 days, a high percentage of the animals which received intracutaneous inoculations began to develop tender swellings of the ankles, the paws, the tail and the digits. Single or multiple joints were involved. The experiment was repeated on two further occasions. In the third trial a different strain of rats was used. The original results were readily reproducible.

Materials and methods. Originally both male and female rats of the Wistar strain were used. They ranged in weight from 230 to 280 g. The final series contained female rats of the Long-Evans strain, weighing 180 to 220 g. All animals were fed a standard pellet diet of Purina chow and water *ad libitum*. They were weighed frequently and inspected almost daily throughout the investigation period. The inoculum was prepared as follows: a healthy rat of the same species to be subsequently used in the study was killed

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