Effect of Centrifugation at 20,000 × g on Lipid Distribution of Human Sera. (26585)

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Separation of very low-density lipid particles (including the chylomicra) from whole serum by high-speed centrifugation has led to interesting results from several laboratories. Bragdon(1) has shown that hypercholesteremic rabbit serum injected into normal rabbits produced lesions resembling atheroma. However, if the serum was centrifuged at 42,000 rpm for 5 hours and the upper and lower fractions injected separately, lesions were produced by the lower but not by the upper fraction, although both fractions contained appreciable amounts of cholesterol. These findings suggested that cholesterol associated with very low-density lipid particles may play little or no role in production of atherosclerosis. Kellner *et al.*(2) showed that intravenous administration of Triton inhibited development of atherosclerosis in cholesterol-fed rabbits. Forbes and Petterson(3) have reported the effects of centrifugation at 20,000 × g on lipid distribution of sera from rabbits receiving intravenous Triton WR 1339. A large proportion of total serum cholesterol, phospholipids, and neutral fat rose to the surface in contrast to minimal lipid migration in controls. Rabbits rendered markedly hypercholesteremic by cholesterol feeding and given Triton injections showed less cholesterol in the infranatant sera than was present in the infranate from the same animals before the Triton injections, in spite of the augmented hypercholesteremia produced by Triton. The protective effect of Triton in cholesterol-fed rabbits may have resulted from these changes observed in the serum lipid distributions.

Albrink, Man, and Peters(4) have reported that in severe hyperlipemia a considerable amount of the serum cholesterol rose to the surface during high-speed centrifugation. The present report is concerned with the effect of comparable centrifugation on lipid distribution of sera from a diversified group of patients, with particular emphasis on subjects with elevated serum neutral fat or other evidence of disturbed lipid metabolism. The clinical significance of the differences noted will not be discussed here.

Methods. 10 ml of clotted blood were obtained and the serum divided into 2 portions. One portion was centrifuged at 20,000 × g for 2 hours in a Sorval refrigerated centrifuge. A portion of the infranate was carefully removed with a sharp pointed pipet; lipids contained in this fraction are henceforth considered to be in the "soluble" form, consistent with the terminology established by Albrink, Man, and Peters(4). This fraction and the original whole serum were analyzed for cholesterol, neutral fat, and phospholipid phosphorus. A slight modification of a previously described procedure(5) requiring 0.2 ml of serum was used for cholesterol determinations, phospholipid phosphorus was determined by the method of Outhouse and Forbes(6), and neutral fat was determined directly using a modification(7) of the method of Van Handel and Zilversmit(8). In view of the work of many laboratories with similar centrifugation technics, the centrifugation conditions utilized in these studies can be assumed to result in flotation of the chylomicra and some beta-lipoproteins of very low density. Although a few of the determinations in this study were serial ones on the same subjects (particularly in the case of those with familial hyperlipemia), the majority were single determinations on separate subjects.

Results. The results presented in Table I show that among the miscellaneous group of subjects the per cent of cholesterol in the "soluble" form over a wide range of total cholesterol values remained fairly constant at a given neutral fat concentration. Thus, for example, when neutral fat was below 176 mg %, averages over various total cholesterol

TABLE I. Changes in "Soluble" Cholesterol as Related to Total Cholesterol and Neutral Fat Contents of Sera.

	Serum cholesterol ranges							
	<226 mg %		226–300 mg %		301–500 mg %		>500 mg %	
	Avg value (mg %)		Avg value (mg %)		Avg value (mg %)		Avg value (mg %)	
Neutral fat range (mg %)	Serum	Infranate	Serum	Infranate	Serum	Infranate	Serum	Infranate
Miscellaneous group								
<176	186 (37)	179 95*	259 (54)	251 96*	361 (42)	348 96*	532 (2)	519 97*
176– 300	193 (23)	185 95*	264 (23)	246 93*	353 (59)	326 92*	529 (7)	518 97*
301– 500	220 (8)	199 90*	263 (30)	237 90*	339 (44)	332 97*	608 (11)	547 89*
501– 800	203 (1)	160 78*	264 (9)	231 87*	388 (32)	335 86*	637 (16)	525 82*
801–1200	218 (1)	169 77*	249 (1)	107 42*	394 (16)	312 79*	673 (8)	505 73*
1200–2000	—	—	—	—	428 (6)	318 74*	637 (8)	408 64*
>2000	—	—	—	—	427 (3)	301 70*	864 (9)	349 40*
Familial hyperlipemia								
1200–2000	—	—	269 (5)	57 29*	337 (11)	49 14*	884 (5)	100 11*
>2000	—	—	282 (3)	41 14*	—	—	—	—

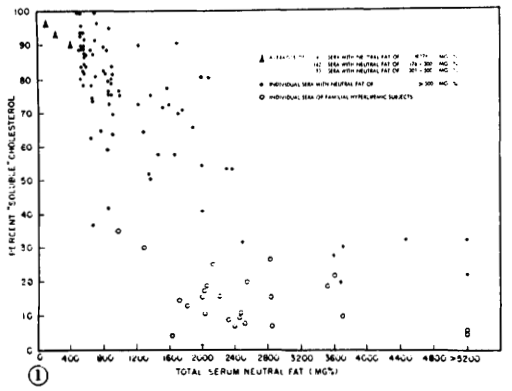
( ) = No. of determinations. * % total serum cholesterol.

ranges revealed that 95 to 97% of serum cholesterol was "soluble," even in sera with total cholesterols over 500 mg %. This relationship between "soluble" and total cholesterol was however subject to considerable individual variability and became less and less uniform as both neutral fat and total cholesterol concentrations increased. Sera from 3 subjects with familial hyperlipemia consistently showed exceptionally small amounts of "soluble" cholesterol.

As neutral fat concentrations increased, progressively more cholesterol rose to the surface with resultant steady decreases in per cent of total cholesterol in the "soluble" state. This inverse relationship between neutral fat and per cent "soluble" cholesterol is further illustrated in Fig. 1. The data suggested the possibility that familial hyperlipemics had somewhat lower amounts of "soluble" cholesterol at a given neutral fat concentration than subjects with other forms of hyperlipemia.

The relationship between total and "soluble" neutral fat is presented in Fig. 2. The general linearity illustrated by the vast majority of subjects indicated that "soluble" neutral fat increased proportionally as total neutral fat increased, at least up to a whole serum concentration of 2000 mg %. However, it was striking to observe the departure from this general linearity exhibited by subjects with familial hyperlipemia, even at whole serum concentrations well below 2000 mg % (2 subjects with acute pancreatitis showed cholesterol and neutral fat concentrations and distributions similar to that of the familial hyperlipemics; however, the lipid determinations approached normal with clinical improvement). Concerning those few hyperlipemic subjects from the "miscellaneous" group which fell among the familial hyperlipemic subjects in Fig. 1 and 2, the possibility exists that they also had the familial disorder without verification of the familial aspect, or that they had idiopathic hyperlipemia closely related to the familial type.

Phospholipid phosphorus changes were followed in over 200 sera (Table II). In general,



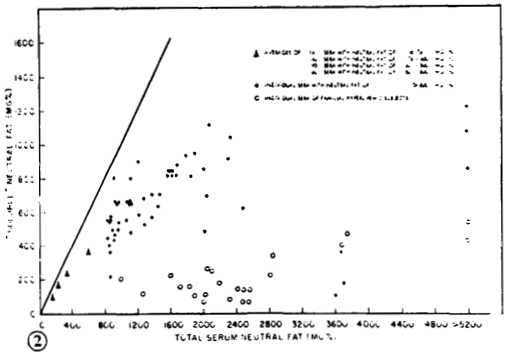


FIG. 1. Relationship between total neutral fat concentration and per cent "soluble" cholesterol in human sera (aggregates of points which were very close together have been averaged to avoid crowding of figure).

FIG. 2. Relationship between total and "soluble" neutral fat concentrations in human sera (aggregates of points which were very close together have been averaged to avoid crowding of figure).

when neutral fat was below 176 mg %, very little, if any phospholipid or cholesterol rose to the surface. Although there was considerable individual variation, the average percentage change in cholesterol and phospholipid following centrifugation showed good agreement except in the case of the familial hyperlipemics where a much smaller percentage of cholesterol than of phospholipid was "soluble."

Discussion. The data presented do not permit evaluation of the physico-chemical association of the cholesterol undergoing flotation because the supernate was not further divided into chylomicra and very low-density lipoproteins. However, the work of a number of investigators suggested the possibility that some of the cholesterol undergoing flotation was associated as an integral part of chylomicra. Bragdon *et al.*(9,10) demonstrated that chylomicra, washed free of unassociated or loosely bound lipoproteins, contained small amounts of cholesterol, phospholipids, and protein, and that cholesterol content could be increased in experimental animals by cholesterol feeding. Lindgren *et al.*(11) suggested that chylomicra have a triglyceride "core" containing cholesterol and phospholipids surrounded by high density lipoproteins. The possibility of cholesterol-lipoprotein comlexes within chylomicra was suggested by the work of Rodbell and Fredrickson(12) demonstrating the association of alpha-lipoproteins with chylomicra and the work of Korn(13) showing that alpha-lipoproteins were required for lipoprotein lipase hydrolysis of a coconut oil emulsion.

The difference in flotation of cholesterol and neutral fat between the miscellaneous group and familial hyperlipemics with comparable neutral fat concentrations may be due to differences in the protein component of some of the lipoprotein complexes. Rodbell(14) found that the protein component of lipoproteins in the range of Sf 100-400 from patients with idiopathic hyperlipemia was characterized by peptides with N-terminal aspartic acid, serine and threonine. The N-terminal residue of this class in normal subjects was apparently

TABLE II. Changes in "Soluble" Cholesterol and Phospholipids as Related to Neutral Fat Contents of Sera.

Neutral fat range (mg %)	No. of determinations	Cholesterol			Phospholipid phosphorus		
		Serum (mg %)	Infranate (mg %)	% "soluble"	Serum (mg %)	Infranate (mg %)	% "soluble"
<176	85	265 ± 5.8*	256 ± 6.1*	96	8.5 ± .18*	8.2 ±.14*	96
176- 300	53	318 ± 16	303 ± 13	95	9.3 ± .28	9.0 ±.32	96
301- 500	37	354 ± 13	327 ± 13	92	11.3 ± .33	10.4 ±.31	92
501-1000	19	434 ± 30	346 ± 29	79	13.6 ± .78	11.1 ±.79	81
>1000	33†	458 ± 41	94 ± 16	20	13.5 ±1.0	6.4 ±.49	49

* Stand. error of mean.

† Familial hyperlipemic subjects.

glutamic acid(15). In idiopathic hyperlipemics, Furman(16) has shown that lipoproteins with the characteristics of alpha$_1$-lipoproteins can be dissociated by sonic vibration from the low-density beta-lipoprotein fraction separated at D 1.006.

Since there is evidence to suggest that chylomicron cholesterol plays little or no role in atherogenesis(17), further studies are in progress to determine if the presence of clinical atherosclerosis correlates better with "soluble" than with total serum cholesterol.

Summary. The effects of centrifugation at 20,000 × g for 2 hours on lipid distribution of various human sera have been studied. In general, when neutral fat was below 176 mg %, very little, if any, cholesterol or phospholipids rose to the surface. When neutral fat content was markedly elevated, as much as 90% of both cholesterol and neutral fat and 50% of phospholipids underwent flotation. However, subjects with similar degrees of marked hyperlipemia appeared to separate into 2 major groups represented by moderate and marked degrees of flotation of neutral fat, the latter group being composed primarily of subjects with familial hyperlipemia. An inverse correlation was found between whole serum neutral fat content and percentage of total cholesterol remaining in the subnatant serum. Variations from these general patterns and possible clinical interpretations are being studied.

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On the Hemolytic Activity of Mouse Complement.* (26586)

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As judged by the usual hemolytic tests, mouse serum appears to lack complement (C′). By use of a reaction system containing a very low concentration of sensitized cells, McGhee(1) demonstrated one unit of hemolytic C′ activity per ml in 7 of 12 mouse sera, and 2 units per ml in the serum of a single animal, but even with his sensitive test system, 4 out of 12 sera had no hemolytic activity. According to Ritz(2), the first component, C′1, is present since EA treated with mouse serum can be hemolyzed by subsequent incubation with guinea pig endpiece. Brown

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