

Effect of High Speed Centrifugation on Distribution of Serum Cholesterol. (23284)

J. C. FORBES, C. O. WATLINGTON,* A. J. WASSERMAN,† P. D. CAMP, W. T. TUCKER,
AND O. M. PETERSON

Departments of Biochemistry and Medicine, Medical College of Virginia, Richmond.

The physical state in which cholesterol occurs in the plasma has received a great deal of attention during the last few years, primarily on account of its possible relationship to development of atherosclerosis. We have been interested in a fraction of the total serum cholesterol which is removed when lyophilized serum is extracted for 3 hours with cold anhydrous chloroform[‡] according to a previously described procedure(1). We consider the normal concentration of this "readily extractable cholesterol" (R.E.C.) to be 40 mg or less/100 ml serum since only 5 of 461 male subjects between 17-25 years of age showed values higher than this(2). An elevated concentration was found in 35% of 259 patients who had suffered a myocardial infarction(3). Patients with arteriosclerotic heart disease also tended to have an abnormally high concentration. A very high percentage of the total serum cholesterol of patients with nephrosis was associated with this fraction.

Methods. To obtain information on distribution of this fraction in serum, values for the R.E.C. of serum and of the subnatant portion of the same serum following centrifugation at 20,000 g for 2 hours have been compared. Centrifugation was carried out in cold room about 6°C, using plastic tubes and an angle centrifuge. Following centrifugation, each tube was removed separately, using the utmost care to prevent mixing. The tube was rested on the centrifuge for support and a 0.5 ml pipette, the top closed with a finger, intro-

duced to bottom of tube. After removal of the sample, the tip was wiped with filter paper before adjustment to the mark. The subnatant serum was transferred to a one-half ounce bottle and lyophilized in the usual manner(1). Although no dark field examination of the subnatant fluid was carried out we feel that most, if not all, of the chylomicrons would have risen to the surface during centrifugation but that lipomicrons would not rise. Since the cholesterol present in both chylomicrons and lipomicrons is probably extracted by cold chloroform(4), we assume that the difference between the amount of R.E.C. in the uncentrifuged serum and in the subnatant fluid after centrifugation represents cholesterol which was associated with chylomicrons and rose to the surface during centrifugation. Although the chief purpose of the present study was to determine the relationship, if any, which might exist between the R.E.C. of the serum and amount of cholesterol which rose to the surface during centrifugation, emphasis was laid on selecting subjects diagnosed as having atherosclerosis or as having disease conditions which predispose to atherosclerosis. About two-thirds of the cases had had a myocardial infarction at some previous time or had arteriosclerotic heart disease. The remaining subjects represented a variety of conditions such as diabetes, xanthlasma, xanthomatosis, idiopathic hyperlipemia, and nephrosis, as well as a number of apparently normal individuals.

Results. The general pattern of results is shown in Table I. It will be seen that when the R.E.C. concentration was within normal limits (below 41 mg %) only a small percentage of total serum cholesterol rose to the surface during centrifugation. Sera with elevated R.E.C. values usually showed a greater percentage of the total cholesterol rising to the surface. This was especially marked in sera in which the R.E.C. concentration was

* Medical Student Fellow of National Foundation for Infantile Paralysis during summer of 1956.

† Samuel H. Gellman Resident in Medicine, 1956-57.

‡ To assure uniformity we use "Baker Analyzed" reagent grade chloroform. About 10 g of anhydrous sodium sulfate is added to each 5 lb bottle and the bottles shaken off and on for several days before using.

TABLE I. Readily Extractable Cholesterol Concentration of Serum and of the Subnatant Fluid after Centrifugation at 20,000 g.

No. of tests	Total cholesterol	Readily extractable cholesterol				
	Serum, avg	Serum Range	Serum Avg	Subnatant, avg	Serum minus subnatant, avg	% R.E.C. rising
		mg %				
75	245	<41	29	22	7 ± .5*	24
48	303	41- 70	56	32	24 ± 1.8	43
31	327	71-150	118	40	78 ± 4.6	66
21	324	151-250	204	46	158 ± 5.2	77
27	545	>250	455	107	348 ± 42.0	76

* Stand. error.

above 70 mg %. On the whole the higher the R.E.C. value the greater the proportion of total serum cholesterol which rose to the surface during centrifugation. One case with idiopathic hyperlipemia with a total serum cholesterol of more than 1,000 mg % showed over 90% of total serum cholesterol rising to the surface. The supernatant layer in this case was solid at the end of the centrifugation.

A few subjects with a variety of clinical conditions showed quite a different picture from this general pattern. These showed either no cholesterol or only very little of it rising to the surface during centrifugation in spite of the fact that the R.E.C. was markedly elevated. Five of these subjects had

nephrosis, 3 had diabetes of long duration, 2 had myxedema secondary to radio-iodine administration, one a myocardial infarction superimposed on a previous cerebrovascular accident, and one apparently was a normal subject. These results are shown in Table II.

We have no satisfactory explanation for the difference in serum cholesterol distribution of these subjects when compared with the others who showed markedly elevated R.E.C. concentrations. The fact that the sera of 5 of the 6 nephrotic patients showed very little cholesterol rising to the surface during the centrifugation is difficult to explain. Albrink, Mann, and Peters(5) found a large percentage of the total serum cholesterol of a 3-year-

TABLE II. Difference between the Readily Extractable Cholesterol Concentration of the Serum and Subnatant Fluid in Sera Which Did Not Conform to the Usual Pattern, in 13 Patients.

Age, yr	Sex	Total serum cholesterol, mg %	Readily extractable cholesterol, mg %			Diagnosis and remarks
			Serum	Subnatant	Serum minus subnatant	
21	♂	1138	1130	986	144	Nephrosis with edema
		1166	1030	1041	nil	4 days later
40	♂	509	465	463	nil	Nephrosis with edema
		618	589	589	nil	25 days later
27	♀	652	571	573	nil	Nephrosis, 17 days postpartum
3	♂	478	443	440	nil	Nephrosis
4	♀	852	822	500	322	"
40	♀	467	411	327	84	Diabetes for 15 yr
		465	413	315	98	6 days later
53	♀	509	456	456	nil	Diabetes for 30 yr
69	♀	350	257	204	53	" " 26 yr
37	♂	545	495	500	nil	Mitral stenosis with edema at intervals for 2 yr
56	♀	521	448	404	44	Myocardial infarction superimposed on previous cerebrovascular accident
50	♂	691	583	530	53	Myxedema from radio-iodine
72	♀	618	488	500	nil	<i>Idem</i>
54	♀	386	282	224	58	Apparently normal subject

old nephrotic child, rising to the surface when the serum was centrifuged at 20,000 g for 1 hour. All patients studied by us were under some form of treatment when blood samples were collected, but since the kind of treatment varied it is doubtful whether this can explain why the cholesterol distribution in these subjects was so different from that seen in the majority of the cases. Reference should be made to the fact that in sera of nephrotic subjects who showed a small amount of cholesterol rising to the surface during centrifugation, the degree of lipemia was definitely less than we are accustomed to see in nephrotic patients. Serum phospholipid phosphorus was determined in a number of cases but we were unable to find any definite correlation between these values and the R.E.C. of either the serum or subnatant fluid.

It is, of course, premature to suggest any diagnostic value to the fractionation procedure, but it is interesting that the sera of many patients with myocardial infarction contained significant amounts of cholesterol associated with chylomicrons and rose to the surface during centrifugation. This may be considered as supportive evidence for the colloidal-macromolecular theory of the pathogenesis of atherosclerosis, especially as stated by Heuper(6) and Moreton(7). The results on the nephrotic sera, however, would seem to throw some doubt on this but it is possible that a satisfactory explanation for the apparent discrepancy can be obtained.

Summary. As a general rule a fairly large percentage of total serum cholesterol rose to the surface when sera containing markedly elevated amounts of "readily extractable cholesterol" were centrifuged at 20,000 g for

2 hours. Sera having a normal "readily extractable cholesterol" concentration showed very little of the cholesterol rising to the surface under the same conditions. A few subjects, including most nephrotic patients studied, differed markedly from this pattern. In these cases, only a small amount of cholesterol rose to the surface during centrifugation in spite of the fact that the sera had markedly elevated "readily extractable cholesterol" concentrations. It is suggested that the cholesterol which rose to the surface is cholesterol which is associated with chylomicrons, while that which did not rise but which is a part of the "readily extractable cholesterol" is cholesterol present in lipomicrons.

We wish to acknowledge our indebtedness to Drs. R. Bates, W. R. Jordan, and R. H. Kirkland for their cooperation in obtaining blood samples on some of their patients.

1. Forbes, J. C., Dillard, G. H. L., Porter, W. B., and Petterson, O., *PROC. SOC. EXP. BIOL. AND MED.*, 1948, v68, 240.
2. Forbes, J. C., Camp, P. D., Jordan, W. R., Petterson, O. M., and Korn, C., *South. Med. J.*, 1954, v47, 226.
3. Forbes, J. C., *Am. Pract. and Dig. of Treat.*, 1955, v6, 907.
4. Taylor, P. C., Forbes, J. C., and Petterson, O. M., *PROC. SOC. EXP. BIOL. AND MED.*, 1956, v91, 562.
5. Albrink, M. J., Mann, E. B., and Peters, J. P., *J. Clin. Invest.*, 1955, v34, 147.
6. Hueper, W. C., *Am. J. Clin. Path.*, 1956, v26, 559.
7. Moreton, J. R., *J. Lab. and Clin. Med.*, 1950, v35, 373.

Received May 23, 1957. P.S.E.B.M., 1957, v95.