Effects of Freeze-Thawing and Storage on Ultracentrifugal Properties of Human Serum Lipoproteins. (24831)

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Investigations on the effects of freezing on human serum lipoproteins have appeared in the literature. In one report a comparison of the extractability of lipids with ether was made between liquid and frozen lipoprotein solutions(1). In other studies, extent of lipoprotein change due to freezing and storage was investigated by noting alteration in solubility(2,3). The effects of freeze-thawing and storage on ultracentrifugal properties of lipoproteins have not been studied except for observations relative to one lipoprotein group (4). These effects represent a problem that must be solved before determination of the clinical importance of serum lipoproteins, whenever those conditions are involved. This paper summarizes the results of our investigation into the problem.

Material. Human sera from male adults with elevated concentration of lipoproteins were used. Sera were distributed in samples by pipetting 2 ml of each serum into 10-ml screw-cap vials, which were carefully sealed under nitrogen. *Freezing processes.* For rapid freezing (r.f.), the vials were dipped into dry ice and acetone for 50 sec. For slow freezing (s.f.) vials were set in deep freeze 1 hr. In both processes, final temperature was $-28° \pm 1°C$ as checked with a copper-constantan thermocouple. *Thawing.* For rapid thawing (r.th.), vials were placed in water bath at $+ 37°C$ for 120 sec. For these conditions serum reached a temperature of $24° \pm 1°C$. For slow thawing (s.th.) vials were left at room temperature ($24 \pm 2°C$) for 1 hr, after which temperature equilibrium had usually been reached. *Storage.* Three ranges of storage temperature were studied: (a) between $-30°$ and $-26°C$; (b) between $-5°$ and $0°C$; and (c) between $0°$ and $+4°C$. At temperatures between $-30°$ and $-26°C$ sera appeared constantly and thoroughly frozen in contrast to temperatures between $-5°$ and $0°C$; in this range sera were not always thoroughly frozen. *Ultracentrifugation.* Isolation and analysis of serum lipoproteins were performed by previously described procedures(5). Isolation was carried out in Spinco Model L preparative ultracentrifuge. For isolation of low-density lipoproteins (LDL) serum was diluted with equal volume of NaCl solution ($\rho\ 20°/4° = 1.118$ g/ml). For isolation of total high-density lipoproteins (HDL), serum was diluted with equal volume of $NaNO_3$ in D_2O solution of $\rho\ 20°/4° = 1.390$ g/ml, yielding a background solution of $\rho\ 20°/4° = 1.20$ g/ml. Determinations of flotation rates and concentrations were carried out in Spinco Model-E analytical ultracentrifuge. Lipoproteins were classified as follows: LDL: $S^{\circ}_{f(1.06)}$ 100-400, 20-100, 12-20, 0-12 (flotation rates were corrected for concentration and Johnston-Ogston effects); HDL: lipoproteins with hydrated densities in range between 1.075 g/ml and 1.15 g/ml. Solution densities were determined with 1-ml pycnometer.

Standard errors of measurement are as follows: S°_{f} 0-12: 19; 12-20: 16; 20-100: 22; 100-400: 34; HDL: 10. These standard errors can be expressed as percentage errors by dividing them by their respective means: 7%, 2%, 10%, 12% and 4%.

Results. Table I presents average results of freeze-thawing of 2 sets of sera. Repeated successive cycles (from one to 3 times) of either rapid or slow freeze-thawing of serum produce no significant changes in either concentration or flotation rate of the major classes of lipoproteins.

Once it was established that changes resulting from a single freeze-thawing process were minimal, the effects of storage in deep-frozen

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TABLE I. Freeze-Thawing of Serum. Serum concentration in mg %.

	Avg for 4 sera		Avg for 3 sera	
	Orig.	r.f. r.th. (3×)	Orig.	s.f. s.th. (3×)
$S^{\circ}_{f(1.06)}$ 100-400	239	237	282	281
20-400	216	213	240	235
12- 20	55	55	56	59
0- 12	317	315	325	319
Total LDL	827	820	904	893
HDL	280	291	292	282
Total lipop't'n	1107	1111	1196	1175

state were studied in combination with rapid and with slow freezing and thawing processes. Table II presents average of results for 2 sera for each condition of storage. The discussion of this Table is in line with that of Table III. Table III presents average results of comparative study of storage of 3 sera at temperatures between −30° and −26°C, between −5° and 0°C, and between 0° and +4°C. (Results of analyses intermediate to periods shown in Tables are omitted for brevity.)

At temperatures between −30° and −26°C, lipoproteins with flotation rates above S°_f 20 were more rapidly degraded and showed more extensive degradation over comparable periods of storage than lipoproteins with flotation rates less than $S^{\circ}_{f(1.06)}$ 20. In contrast, $S^{\circ}_{f(1.06)}$ 0-20 as well as HDL lipoprotein classes showed relative stability to storage between these temperatures. After 6 months, the maximum change observed in the HDL class was 30% decrease in concentration.

At temperatures between −5° and 0°C all lipoprotein classes appeared to be stable for at least 28 days. Thereafter, S°_f 0-20 and HDL lipoprotein classes appeared to be more resistant to degradation than the other lipoprotein classes. After 21 to 28 days of storage, the HDL lipoprotein class showed some qualitative changes not evident in Table III. These changes were a broadening of lipoprotein distribution accompanied by increase in flotation rate of the major peak.

At temperatures between 0° and +4°C, all lipoprotein classes appear to be stable for at least 14 days. Thereafter, there is no consistent pattern of degradation. Qualitative changes frequently appear as concentration increases and decreases within any broad lipoprotein class, without any net concentration change of that class. After 7 to 14 days of storage, the HDL lipoprotein class showed a broadening of lipoprotein distribution and increase in flotation rate of the major peak.

Conclusions. 1) Storage of serum at a temperature between −5° and 0°C yielded stable values for human serum lipoproteins for at least 4 weeks. 2) Storage of serum between 0° and +4°C for as long as 2 weeks also revealed no quantitative change in lipoprotein pattern. 3) Storage for even a few days at −28°C was associated with alterations in relative proportions of lipoprotein fractions. It is interesting to mention that under similar treatment, lipoproteins in salt solutions undergo faster degradation than those in serum.

Summary. The effects of freeze-thawing and of storage on ultracentrifugal characteristics of human serum lipoproteins have been studied. Two different rates of freezing and thawing have been used. Storage has been carried out at 3 different temperatures: be-

TABLE II. Storage of Serum at Temperature between −30° and −26°C. Serum concentration in mg %.

	Avg for 2 sera							
		r.f.—storage—r.th.				s.f.—storage—s.th.		
Days of storage→	Orig.	7	180	360	Orig.	7	180	360
$S^{\circ}_{f(1.06)}$ 100-400	274	212	162	51	137	140	72	Traces
20-100	230	152	129	45	195	160	68	Traces
12- 20	44	37	45	33	67	51	47	31
0- 12	220	198	217	220	314	274	252	224
Total LDL	768	599	553	349	713	625	439	235
HDL	258	255	264	203	247	270	203	189
Total lipoprotein	1026	854	817	552	960	895	642	444

TABLE III. Storage of Serum at Temperatures between −30° and −26°C, −5° and 0°C, and 0° and +4°C. Serum concentration in mg %.

		Avg for 3 sera s.f.—storage—s.th.								
		−30° and −26°C			−5° and 0°C			0° and +4°C		
Days of storage→	Orig.	7	28	128	7	28	178	7	78	178
$S^{\circ}_{f\,(1.06)}$ 100-400	250	235	187	98	258	267	151	253	230	108
20-100	356	137	124	87	363	350	140	348	301	208
12- 20	41	24	25	24	39	41	32	42	44	40
0- 12	270	232	247	227	272	284	230	278	272	233
Total LDL	917	628	583	436	932	942	553	921	847	589
HDL	232	236	237	199	227	224	166	257	216	195
Total lipoprotein	1149	864	820	635	1159	1166	719	1178	1063	784

tween −30° and −26°C, between −5° and 0°C, and between 0° and +4°C. Adequate preservation of lipoproteins stored as serum at a temperature between −5° and 0°C was maintained for 4 weeks, between 0°C and + 4°C for 2 weeks, and between −30° and −26°C for a few days.

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Changes in Renal Tissue Composition Induced in Rabbits by Various Intravenous Doses of Mercaptomerin Sodium.* (24832)

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Previous studies(1,2) have shown that the organic mercurial diuretic agent, mercaptomerin sodium, localizes in kidneys when administered parenterally to animals or human subjects. The renal histopathologic effects of the organic mercurials are well known, but the nature of the changes in tissue composition is still obscure. The purpose of the present study in normal rabbits was to determine what changes in water, sodium and potassium contents of renal tissue were produced by intravenous injection of various doses of mercaptomerin sodium.

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Material and methods. Forty-four normal albino male rabbits were kept in individual metabolism cages and fed a stock diet of compressed pellets. Tap water was given without restriction. Six control animals were not injected. Six animals received intravenous injection of mercury of 0.5 mg/kg of body weight; 9 animals were given 1 mg/kg; 5 were given 5 mg/kg and 6 each were given 10, 15 and 20 mg/kg. The mercury was given as commercial lyophilized mercaptomerin sodium (Thiomerin), dissolved in 1 or 2 ml of water immediately prior to injection. All test animals were killed by air embolism 48 hours after administration of the mercurial. Both kidneys were removed and weighed, and the cortices were then separated from the medul-