In vitro Effect of Surface Active Agents on Human Serum Lipoprotein and Protein Patterns.* (24028)

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Several authors have described changes in electrophoretic migration rates of serum lipoproteins under various conditions. Sachs and Danielson(1) recently reported that incubation of soy bean phosphatides with human serum produced increased migration velocity of serum lipoproteins on paper electrophoresis. Laurell(2) and Gordon(3) previously reported that incubation of serum with fatty acids produced a similar effect on migration of serum lipoproteins. Injection of heparin into hyperlipemic subjects also causes increased velocity of migration of lipoproteins when analyzed by electrophoresis technic(4). However, this effect occurs only *in vivo*. In a study of mechanisms involved in producing these changes several surface active agents of different characteristics have been investigated to determine their effects on serum lipoprotein and protein patterns.

Materials and methods. The following surface active agents were incubated with normal and lipemic human serum: a) sodium lauryl sulfate; b) commercial aryl-alkyl sulfonated anionic detergent, Lakeseal;‡ c) non-ionic polyoxyethylene sorbitan detergent, Tween 80; d) cationic detergent, benzalkonium chloride; and e) equal mixture of Tween 80 and benzalkonium chloride. Corn oil phosphatide preparation,§ containing lecithin, cephalin, and inositol phosphatides was also incubated with serum. Five to 20 mg of each of above agents were added to 2 ml serum aliquots, and equal aliquots without added agents served as controls. Sera were incubated at 37°C for 18 hours. After incubation samples were subjected to paper electrophoresis and to ultracentrifugal studies. Electrophoresis of samples was performed on Spinco Model R electrophoresis system in barbital buffer of pH 8.6 and 0.75 ionic strength. The apparatus was operated at 5 milliamperes for 16 hr. Serum aliquots of .01 ml were used for protein staining, and .02 ml aliquots were used for lipid staining. Protein was stained with bromphenol blue, and lipid with oil red 0 according to modified method of Durrum(5). Ultracentrifugation studies were performed on Spinco Model E analytical ultracentrifuge by the method of Gofman and associates(6,7). The above agents were incubated with normal serum and with serum lipoproteins obtained by preparative ultracentrifugation of serum at 30,000 rpm for 13 hr at specific gravity 1.0635. In the latter case serum lipoproteins were concentrated 3-fold before incubation with the agents.

Results. Table I shows effects of various surface active agents on migration rates of serum lipid components. Anionic surface active agents, sodium lauryl sulfate and Lakeseal, when incubated with serum increased migration velocity towards the anode of both alpha and beta lipid fractions at pH 8.6 when compared to control patterns. Incubation of

TABLE I. Effects of Various Surface Active Agents on Migration Rates of Serum Lipoproteins *In Vitro*.

Sample	Mean distance moved from origin at 5 milliamperes in 16 hr in mm	
	β-Lipoprotein	α-Lipoprotein
Sodium lauryl sulfate	+53 (+31)	+85 (+61)
Lakeseal	+49 (+30)	+84 (+61)
Tween 80	0 (+29)	0 (+61)
Benzalkonium chloride	+12 (+30)	+52 (+61)
Tween 80 + benzalkonium chloride	−36 (+31)	−25 (+75)
Corn phosphatide	+56 (+33)	+90 (+64)

Control values shown in parentheses.

(+) Plus sign designates movement from origin toward anode.

(−) Minus sign designates movement from origin toward cathode.

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‡ Supplied by Finger Lakes Chemical Co.

§ Avitol supplied by Mr. M. Mattikow of Refining Unincorp., N. Y.

TABLE II. Effects of Surface Active Agents on Ultracentrifuge Patterns of Serum and Concentrated Lipoproteins.

Sample	Lipoprotein conc. S_f 0-12 (mg %)	S_f 12-400 (mg %)
Serum lipoproteins		
Lakeseal	175 (200)	138 (135)
Corn phosphatides	281 (287)	227 (77)
Sodium lauryl sulfate	8 (200)	95 (125)
Concentrated lipoproteins		
Lakeseal	244 (266)	243 (128)
Corn phosphatide	55 (155)	216 (128)
Tween 80*	218 (287)	55 (77)
Tween 80†	48 (287)	12 (77)
Benzalkonium chloride	71 (266)	236 (128)

Control values shown in parentheses.
* Incubation for 6 hr. † Incubation for 18 hr.

serum with non-ionic detergent, Tween 80, resulted in complete loss of mobility of all serum lipids toward either electrode. The cationic detergent, benzalkonium chloride, when incubated with serum decreased migration velocity of both fractions towards the anode. No differences were noted between effects of any of these agents on normal and hyperlipemic serum.

Incubation of serum with anionic agents resulted in formation of prealbumin protein component on the electrophoretic pattern. This effect was especially pronounced by sodium lauryl sulfate, which was accompanied by diminution of alpha$_1$, alpha$_2$ globulins and albumin components. None of the other surface active agents produced changes in protein distribution as detected on paper electrophoresis.

Table II records the effects of surface active agents on lipoprotein ultracentrifugal patterns. No constant relationship was found between effects of these agents on electrophoretic and ultracentrifugal patterns. Sodium lauryl sulfate and Tween 80 appear to increase the density of lipoproteins present, while the cationic agent, benzalkonium chloride and corn oil phosphatide decrease the lipoprotein densities. The anionic agent, Lakeseal, shows less pronounced changes on lipoprotein densities. Differences between control values represent usual variations found in lipoprotein concentrations of different sera.

Discussion. Results obtained after incubation of serum with surface active agents of different ionic charges offer a possible explanation for the mechanism of effects produced on lipoproteins by soy bean phosphatides, fatty acids and heparin injection. The results indicate that direction and rate of migration of serum lipids depend upon the charge present on ionic group of the active agent. This phenomenon might be readily explained by assuming that the surface active agent is bound by the lipoprotein molecule producing a net change in the charge of the molecule. Anionic agents would thus increase rate of migration of the lipid toward the anode as observed in this experiment. Depending upon degree of binding by lipoproteins the cationic surface agent would either decrease rate of migration towards the anode or cause direction of migration to be towards the cathode. Surrounding of lipoprotein molecules by the bound non-ionic agent, Tween 80, would decrease their migration in an electric field as evidenced experimentally by complete lack of mobility of serum lipid after incubation with Tween 80.

Friedman and associates(8) demonstrated that serum lipoproteins rapidly bind Tween 80 after intravenous injection. Injection of Tween 80 produces a marked hyperlipemic response by enhancing ability of lipoproteins to take up lipid from the liver. The results of our experiment indicate that Tween 80 enhances the binding of the cationic surface active agent, benzalkonium chloride, by lipoproteins. This increased binding of the cationic agent produces a marked reversal in migration direction of serum lipoproteins.

Binding of fatty acids by lipoprotein would result in an increased negative charge of the lipoprotein molecule and an increased migration rate of the molecule towards the anode. This mechanism was suggested by Gordon(3) to explain electrophoretic changes produced by interaction of sodium oleate and human serum. Similar effects were noted with soy bean phosphatides(1), and with a corn oil phosphatide used in this study. Sachs and Danielson(1) found the active component of soy bean phosphatide to reside in the alcohol-insoluble lipositol fraction. Although the complete chemical structure of lipositol is un-

known, this phosphatide contains phosphoric acid esterified with inositol suggesting it to be an anionic agent. The effects of heparin on electrophoretic mobility of lipoproteins *in vivo* remain unexplained. However, the effects on both alpha and beta lipoprotein are qualitatively the same as those seen after incubation with anionic agents *in vitro*(4).

Lipoprotein densities studied ultracentrifugally are unrelated to ionic charges of surface active agents; however, density changes suggest binding of agents by lipoproteins.

The prealbumin fraction observed after incubation with anionic agents has also been reported after injection of heparin(9) and after incubation of serum with sodium oleate(3). Reasons for the marked difference between the effects of these agents on lipid and protein patterns are not apparent. If all lipids are bound to only a small portion of total serum, protein changes in the migration of this small portion might not be detected on paper electrophoresis.

Summary. The effects of incubation of serum with various surface active agents on serum lipids and protein patterns have been studied on electrophoresis and in the ultracentrifuge. Direction of migration of lipid in electrophoresis depends upon the charge of surface active agents. Anionic surface active agents increase migration rate of both alpha and beta lipoproteins toward the anode. Cationic agents either decrease migration rate of lipids toward the anode or cause them to move toward the cathode. Non-ionic agents cause complete loss of mobility of all lipids toward either electrode. These effects could be explained by assuming that surface active agents are bound to lipoproteins and contribute their charge in the migration of these lipoproteins. Effects of surface active agents on protein patterns are less pronounced. Surface active agents either increase or decrease density of serum lipoproteins as studied in the ultracentrifuge suggesting that these agents are bound to lipoproteins. However, prediction of effects of these agents on lipoprotein densities cannot be made from knowledge of the charge present on the agent.

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Measurement of Compounds of Vit. B_6 Group in Blood.* (24029)

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Using a spectrophotofluorometric technic (1), Duggan and Udenfriend have shown that the Vit. B_6 group could be detected in concentrations as low as 0.001 μg/ml(2). We confirmed these observations, and extended the use of this technic to demonstrate effects of hydrogen peroxide and ultraviolet irradiation on these fluorescent curves. Initial studies established optimal conditions for fluorescence of these compounds to be in 0.1 molar phosphate buffer of a pH 6.75. The ideal concentration for studying activity was found in the range of a 1 micromole/l. Table I outlines the fluorescence of a 1 micromolar stand-

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