## Title

THE EFFECT OF CERTAIN LIPID MATERIAL ON THE ULTRA-CENTRIFUGAL PROPERTIES OF SERUM AND EGG LIPOPROTEINS



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## ABSTRACT

Serum and egg lipoproteins interact in vitro with fatty acid and neutral fat emulsion. The resulting lipoprotein in general has a greater flotation rate, suggesting an incorporation of low-density material into the lipoprotein molecules.

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## Introduction

In a previous study of the postirradiation hyperlipoproteinemia[1] it was noted that 30 hours after total body irradiation the class of low-density lipoproteins normally appearing in the rabbit ($S_f$5 to 15) exhibited an increased flotation rate as measured by the ultracentrifugal techniques of Gofman et al.[2] A similar increase in $S_f$ rate was observed in rabbits following the injection of egg lipoprotein.[3] It was possible that the normally occurring lipoprotein had been altered by an interaction in the blood with the abnormal lipids present as a result of irradiation or as a result of the egg lipoprotein injection. It was therefore of interest to determine if the addition in vitro of lipid material could produce similar interactions.

## Methods

Rabbit serum was obtained from the peripheral ear vein of New Zealand White rabbits. The blood was allowed to clot and the serum then separated by clinical centrifugation.

After the various in vitro treatments the serum lipoproteins were analyzed by the following technique:

The serum was subjected to preparative ultracentrifugation, 13 hours at 30,000 rpm, in a NaCl solution of density 1.063 g/ml. Under this centrifugation, all serum lipoproteins of density less than 1.063 g/ml floated to the top of the tube and were pipetted off in the top 1 cc. This top fraction was then spun in an analytic ultracentrifuge (52,640 rpm), and the amount and flotation rates of the various components were determined.

## Results and Discussion

First, it was shown that the effect produced by the injection of egg lipoprotein could be duplicated in vitro. Egg lipoprotein prepared by the procedure of Nichols[4] was added to normal rabbit serum until the concentration of egg lipoprotein in the mixture was about 400 mg%. This mixture was then incubated at 37°C for 24 hr. A volume of saline equal to the egg lipoprotein volume was added to another portion of the rabbit serum and incubated under identical conditions as a control. At the end of 24 hours, the two mixtures were submitted to preparative and analytic ultracentrifugation and the lipoprotein spectrum was determined. It was apparent that the average $S_f$ rate of the native lipoproteins ($S_f$5 to 15) had increased in the mixture containing egg lipoprotein while the lipoprotein spectrum in the saline mixture was unchanged.

Next a lipid emulsion, Lipomul Upjohn*, was substituted for the egg lipoprotein, and a similar effect was observed. A mixture of 8% Lipomul in normal rabbit serum was incubated for 24 hrs at 37° along with a saline control as described above. At the end of 24 hours, the two samples were spun in a clinical centrifuge at 1,500 rpm for 15 minutes and the excess Lipomul was removed by pipetting off the top layer. The lipoproteins remain uniformly distributed throughout the tube during this low-speed centrifugation. The Lipomul-free serum was then analyzed by the usual ultracentrifugal procedures. Figure 1a shows the lipoprotein pattern of the rabbit serum that had been mixed with the Lipomul. Figure 1b shows the pattern of the saline control. It is obvious that the Lipomul has interacted with the native lipoproteins to produce molecules of a higher average $S_f$ rate. The use of Lipomul rather than egg lipoprotein to produce this effect has the advantage that the Lipomul can be easily separated from the lipoproteins after the mixture has been incubated. This, of course, is due to a large difference in flotation rate between the lipid emulsion and the native lipoproteins.

From Fig. 1 it is seen that the concentration of the native lipoprotein is unchanged but that the average $S_f$ rate has increased. This is also often the case following irradiation. This would suggest that the density of the native lipoproteins has been decreased by the incorporation of a small amount of

* Lipomul-Oral, Upjohn: Vegetable oil 40%, dextrose, anhydrous, 10%, preserved with sodium benzoate, 0.1%.

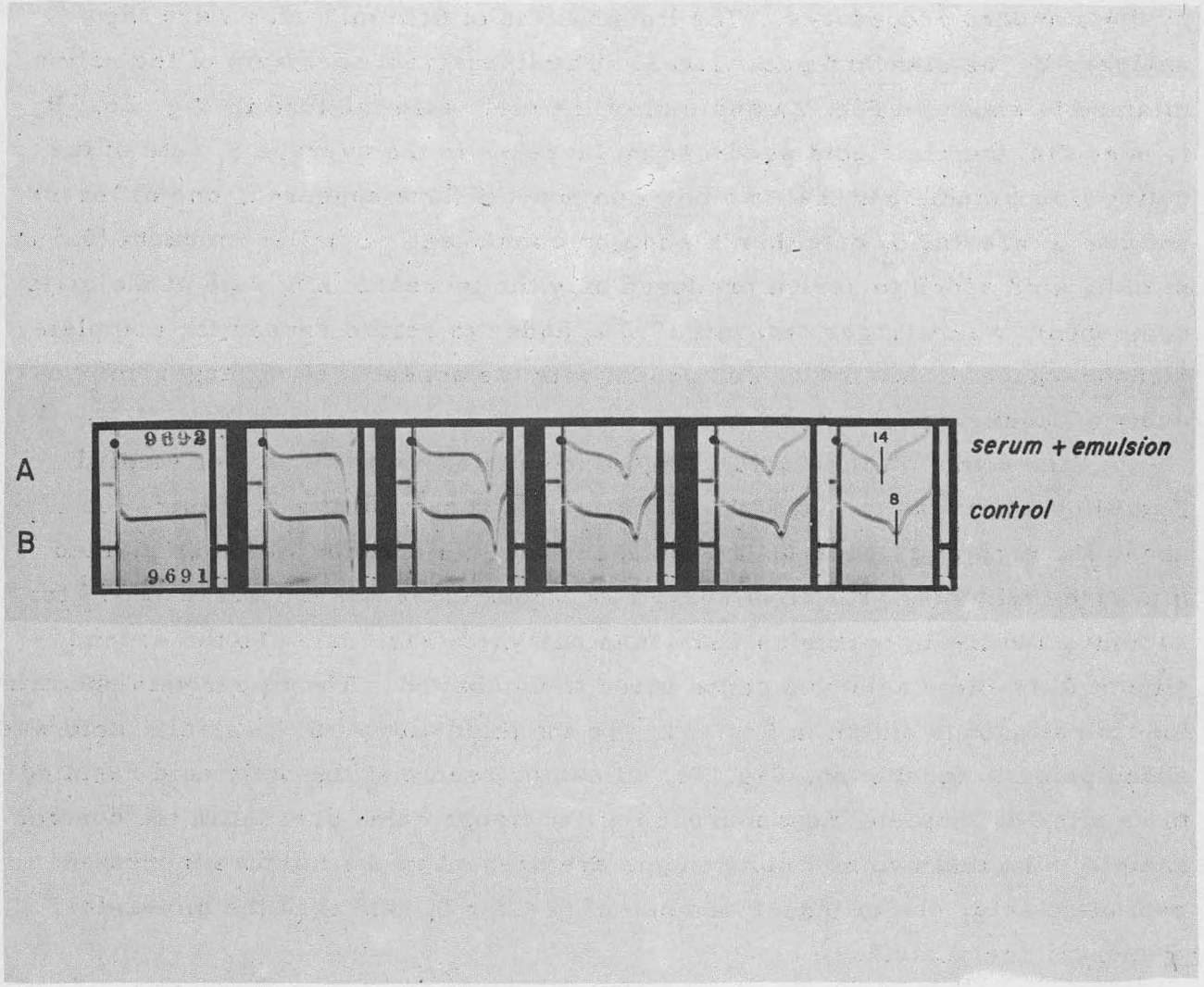


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Fig. 1. Serum lipoprotein patterns (rabbit) following incubation with an emulsion (A) and saline (B). From left to right, successive frames are at 0, 6, 12, 22, 30 and 38 minutes after the rotor has reached 52,640 rpm. The flotation rates of the major component are shown in frame No. 6.

low-density material or by the exchange of material already present for material of lower density.

A similar increase in average $S_f$ rate is observed upon incubation of serum lipoproteins with oleic acid. Some 0.5% normal saline was added to an aliquot of human serum and this mixture incubated for 24 hours. To another aliquot of the same serum 0.5% oleic acid was added and this mixture also was incubated for 24 hours. The lipoproteins of both mixtures were then analyzed by the standard procedures. The lipoproteins of both mixtures were then analyzed by the standard procedures. The lipoprotein spectrum of the saline mixture is shown in Fig. 2a and that of the oleic acid mixture in Fig. 2b. It is seen that the oleic acid produced an increase in the average $S_f$ rate of the native lipoproteins and that two new components have appeared, one of lesser and one of greater $S_f$ rate than the major component. Smaller amounts (0.1%) of oleic acid added to serum produced only the increase in $S_f$ rate of the major component, while larger amounts (0.7%) added to serum caused the complete disappearance of this major component with the appearance of larger components of lesser and greater $S_f$ rate.

The effect of oleic acid on egg lipoprotein in vitro was also studied. Egg lipoprotein was mixed with normal rabbit serum until a concentration of about 200 mg% egg lipoprotein was reached. This solution was then divided into three aliquots. The first served as a control; it was incubated at $37^{\circ}$ for 24 hours and the lipoproteins were then analyzed (Fig. 3a). To the second aliquot 0.7% oleic acid was added prior to incubation. The lipoprotein spectrum for this aliquot is shown in Fig. 3b. To the third aliquot 0.7% stearic acid was added prior to incubation, Fig. 3c. It can be seen that the oleic acid resulted in an almost complete disappearance of the lipoproteins present in the control sample; also that two new components are present in the mixture incubated with oleic acid, one of lesser and one of greater $S_f$ rate than the molecules present in the control.

The addition of stearic acid had essentially no effect on the lipoprotein spectrum. Whether this is because the stearic acid was added in solid form while the oleic was a liquid, or because of some fundamental difference between saturated and unsaturated fatty acids, is not known.

Shore et al.[5] have shown that fatty acid is liberated when lipoproteins are incubated with postheparin plasma, and Nichols et al.[4] have shown that sodium oleate produces changes in the solubility of egg lipoprotein similar to

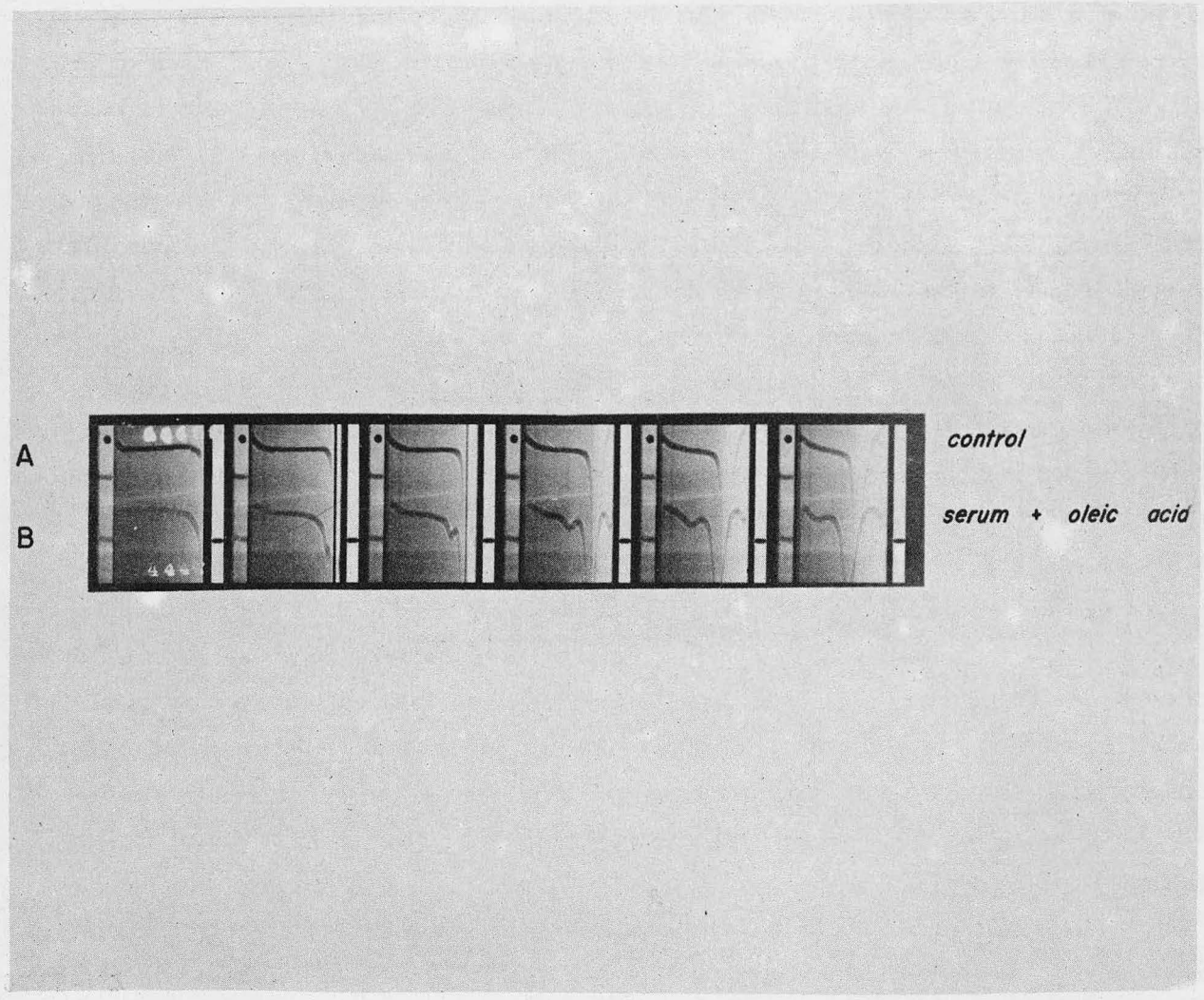


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Fig. 2. Serum lipoprotein patterns (human) following incubation with saline (A) and oleic acid (B).

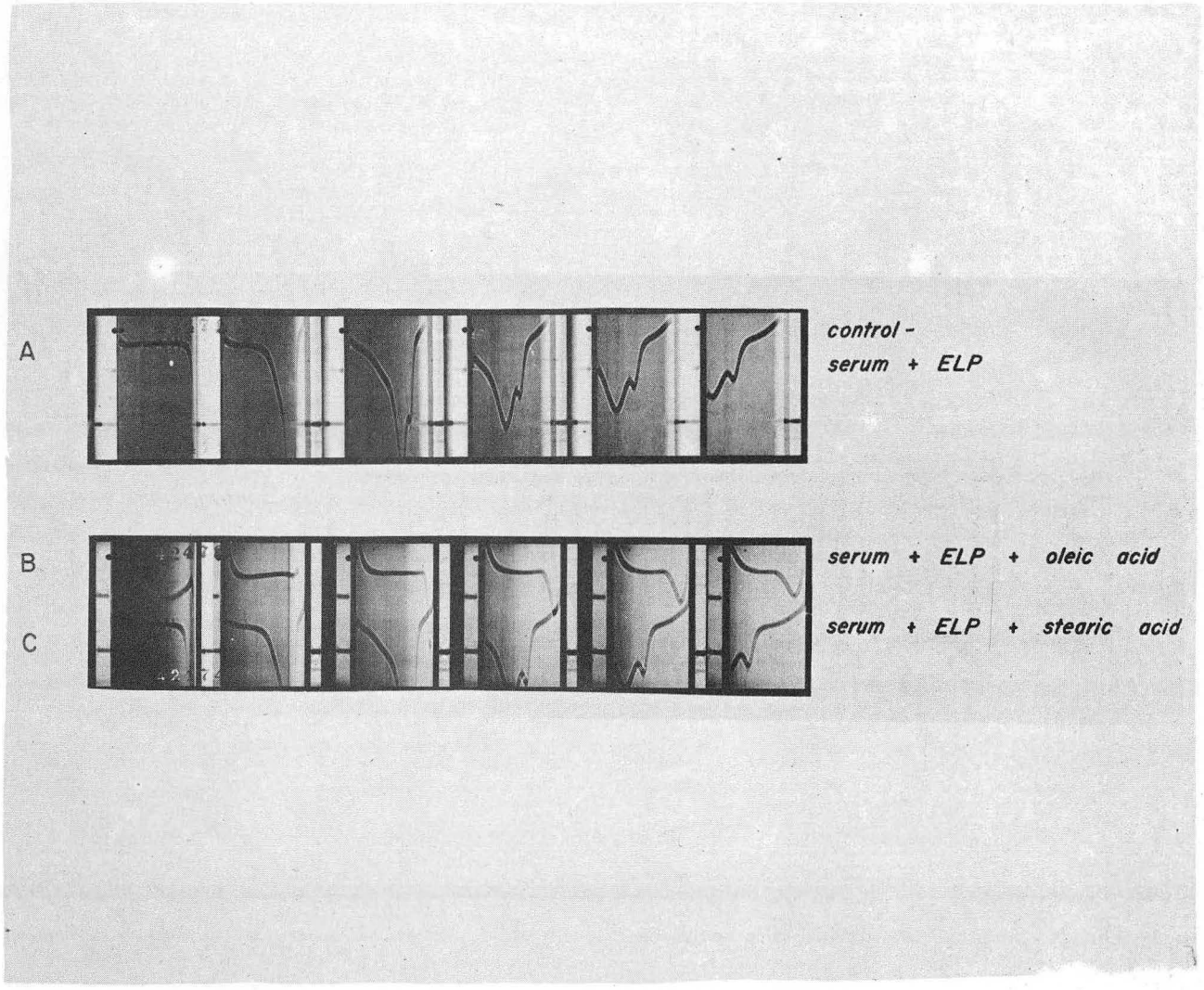


Fig. 3. Egg lipoprotein patterns following incubation with saline (A), oleic acid (B), and stearic acid (C).

those produced by postheparin plasma. When to these two facts are added the changes in the lipoprotein spectrum produced by the incubation with fatty acid, the possibility suggests itself that the action of heparin on lipoproteins in vivo is brought about by fatty acids released into the blood by the action of heparin on sources high in neutral fat.

The in vitro effect of oleic acid added to serum lipoproteins may be summarized as follows:

(1) With small amounts added (0.1%) there is an increase of the average $S_f$ rate of the lipoproteins present.

(2) With larger amounts added (0.5%) the increase in $S_f$ rate is observed, and in addition two new components appear, one of greater and one of lesser $S_f$ rate than the component originally present.

(3) With still larger amounts added (0.7%) the original component disappears and one of the two components remaining is of greater, the other of lesser $S_f$ rate.

## Summary

(1) Certain lipid materials have been shown to interact, in vitro, with serum and egg lipoproteins.

(2) In general this interaction produces molecules of higher average flotation rates.

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