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Opsonic Characteristics of Chylomicron-Bound Protein (38782)

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The Kupffer cells of the reticuloendothelial system have been implicated in the removal of cholesterol-containing chylomicrons from the circulation and the parenchymal cells clear native (natural) chylomicrons (1, 2). However, Stein and Stein (3) have shown that Kupffer cells acquired triolein chylomicrons; this chylomicron ingestion was not evident in the parenchymal cells.

Opsonin has never been shown to be an intermediary in the reaction, even though combination with opsonin has been demonstrated to be a prerequisite to the clearance of some colloids and particles (4–6).

The quantity of protein associated with the chylomicrons is significantly increased when the lipid particles enter the blood (7, 8). Roheim *et al.* (9) showed that the apoprotein that combines with the lipid particles is plasma protein, and Salpeter and Zilversmit (10) demonstrated by electron microscopy the protein coat on the chylomicrons.

Murray (4) showed that natural opsonins combine with gelatin-coated radiogold colloid and this agglutination of the radiogold particles forms the basis for an assay for opsonin. This report demonstrates the complexation *in vivo* of an opsonic-like material with blood-borne, postingestion fat particles which presumably are chylomicrons and, in addition, shows the multivalency of this material; i.e., that the material can combine with another reactive particle even though it is already in combination with one reactive particle.

Materials and Methods. Sixty ml of blood was withdrawn, using a heparinized syringe, from the cubital vein of a volunteer who had had a steak dinner 9 hr earlier. The blood was centrifuged at 200 *g* for 15 min at 5° in an International PR2 refrigerated centrifuge. The supernatant was recentrifuged at 1200 *g* for 20 min at 5° to remove platelets; and the plasma supernatant, which was found to be lipemic, was filtered through a 0.45 μm filter to remove any residual formed elements. In order to define the composition of the lipid fraction, a lipid analysis was performed.

The plasma (4 ml) was combined with 150 units of heparin, 0.5 mCi of radiogold-198 (Aureotope—E. R. Squibb and Sons, lot No. 55LT003), and a concentrated aliquot of the lipid particles contaminated with platelets (0.2 ml out of 1 ml). The platelets were found to play no part in the subsequent reactions. Controls were without plasma in one and without gold in another. The mixtures were incubated at 37° for 1 hr in a water bath with intermittent mixing, after which they were centrifuged at 1200 *g* for 20 min at 5°. The supernatants were aspirated and the precipitates washed once with a 5 ml aliquot of saline. The washed precipitates were suspended in 3% glutaraldehyde solution for fixation. The samples were counted and prepared for electron microscopy as previously described (11).

Results. Duplicate results of the cholesterol determination on a 2-channel analyzer gave 252 and 261 mg%. The triglyceride results were 365 and 385 mg/100 ml, when run in duplicate. The lipemia was classed as a type IV, as the β- and pre β-levels were elevated as seen by electrophoretic analysis.

The radiogold colloid binding by the plasma sample and the control without plasma was so high that eight half-lives passed before the samples were able to be counted. The ratio of the sample with plasma to the sample without added plasma was 7.68.

Figure 1 shows the appearance of radiogold colloid when agglutinated by human plasma. The black dots are the gold particles and the granular material is the protein in this micrograph.

Figure 2 shows chylomicrons in combination with a proteinaceous material that is

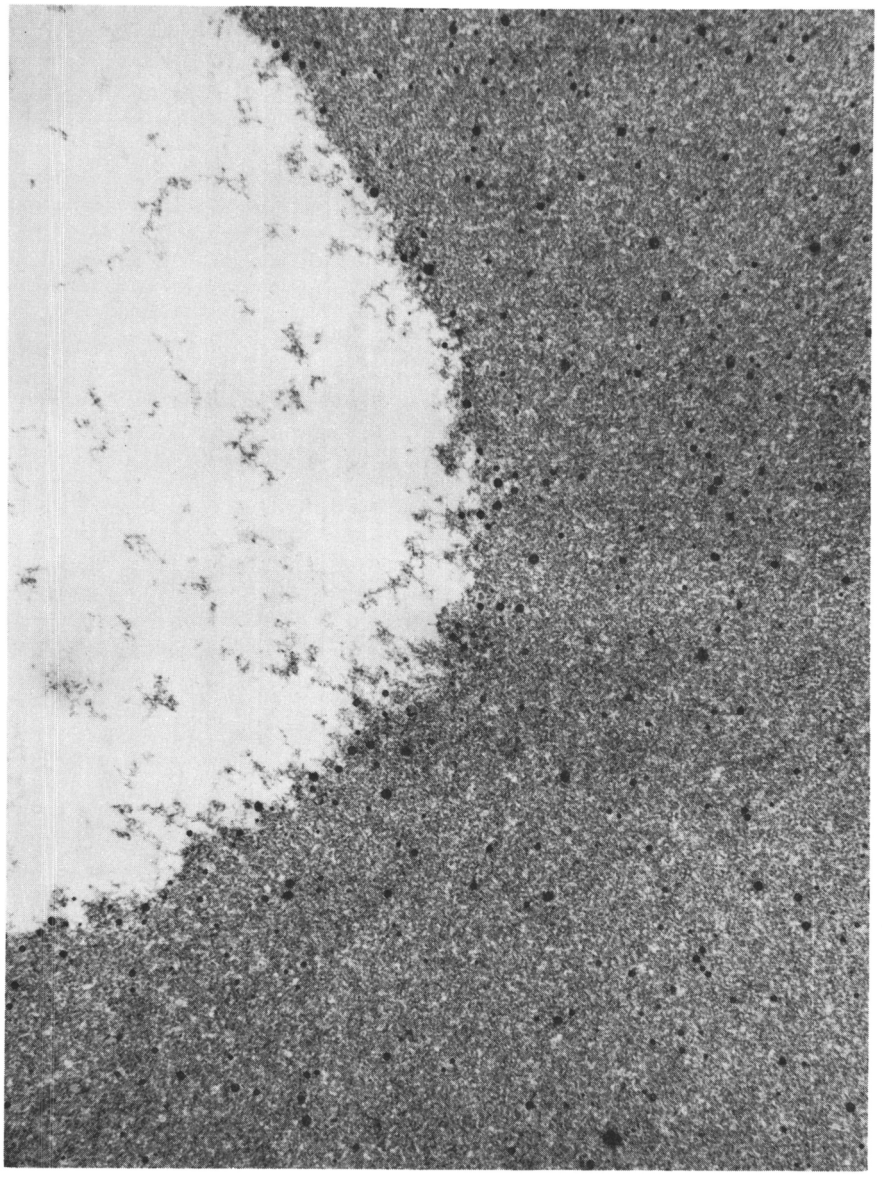

FIG. 1. The agglutination of radiogold colloid by human plasma, followed by centrifugation, results in the formation of a cohesive, membrane-like mass, a section of which is shown here. The black dots are the gold particles and the granular material is the protein.

mainly fibrillar in form. This sample was obtained from the lipid particles in the plasma but without adding additional plasma to the mixture. Thus, it is likely that the protein–lipid combination took place either *in vivo* or, if *in vitro*, then before the fractionation of the blood took place.

There is no indication of the nature of the protein attached to the chylomicrons in Fig. 2, but the possibility exists that it is opsonin. In order to test this possibility, radiogold colloid was incubated with this material. It may be seen in Fig. 3 that the radiogold particles adhered to this protein material, thus fulfilling a previously suggested characteristic of opsonic protein; i.e., its ability to bind to radiogold colloid stabilized with gelatin (4, 12, 13).

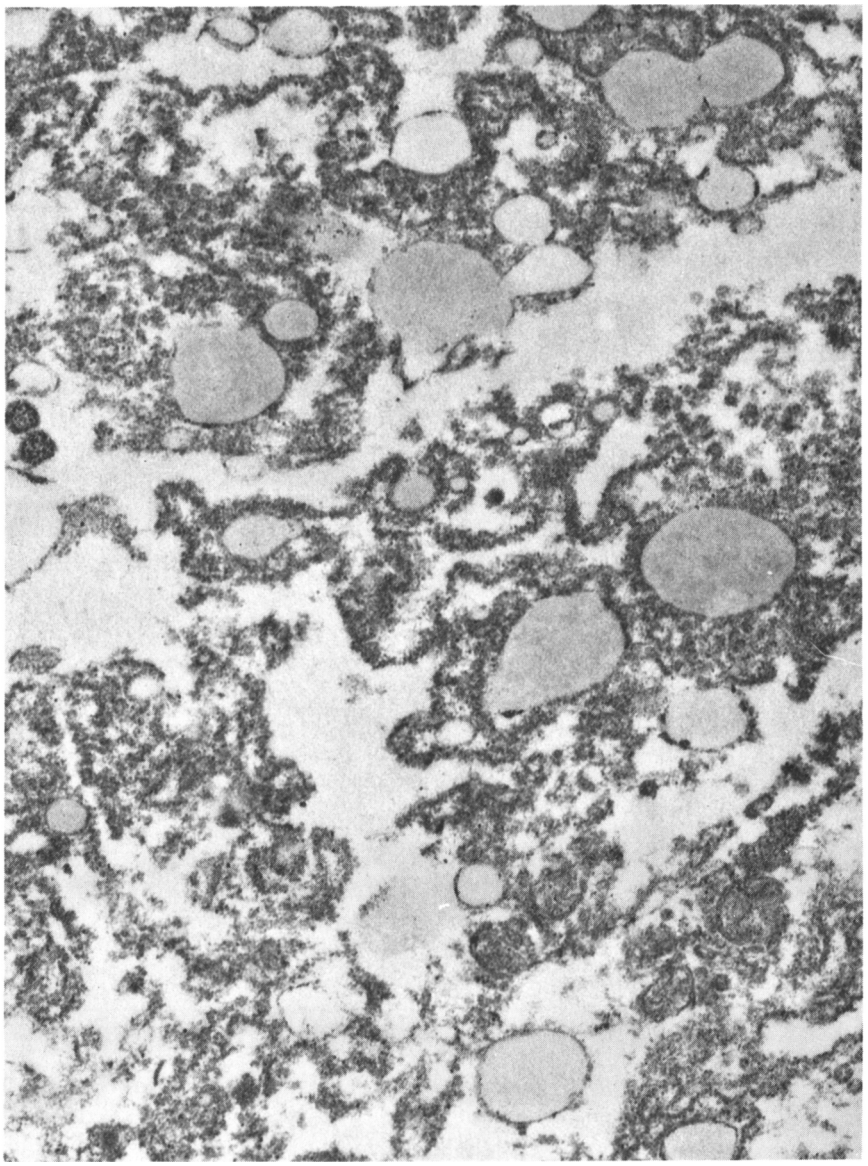

FIG. 2. Chylomicrons in combination with protein-like material. This sample was not treated with radiogold colloid. Magnification = 37,000 ×.

Discussion. The clearance of cholesterol-containing chylomicrons (1, 2) and of neutral fat (3, 14) is a function of the reticuloendothelial system. Chylomicrons are cleared via liver parenchymal cells and adipose tissue and thus are not delayed by loading the RES with colloids (1, 2, 14, 15).

The injection of carbon particles competitively interfered with the clearance of RE test lipid emulsion, which is a combination of glycerol:triglyceride:phospholipid: 10:10:1 in 5% dextrose-in-water (16). However, the injection of carbon particles did not interfere with the clearance of natural chylomicrons (17, 18). In other comparisons, the clearance of chylomicrons of cholesterol was hampered by carbon injection, whereas the clearance of natural chylomicrons was not (2, 19). Furthermore, the Kupffer cells of the liver are important to the clearance of cholesterol chylomicrons (19–22), whereas the parenchymal cells remove normal chylo-

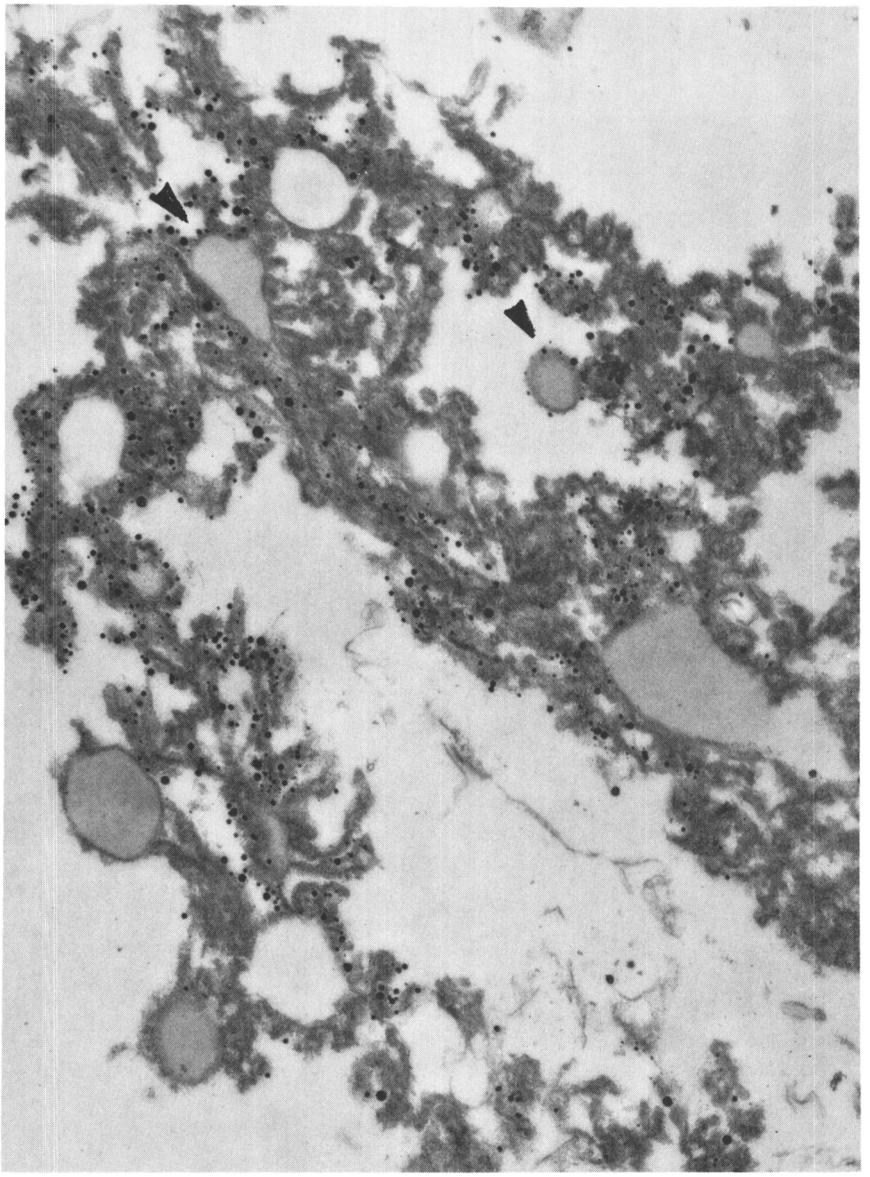

FIG. 3. Chylomicrons in combination with protein-like material; the complex has been treated with radiogold colloid. Magnification = 33,900 ×.

microns with a high triglyceride content (21, 23, 24) although Stein and Stein (3) reported a role of Kupffer cells in the removal of triolein chylomicrons. Thus, RE test lipid emulsion and cholesterol chylomicrons are treated as foreign particles by the RES, whereas native chylomicrons are handled differently.

Di Luzio (1) fed one group of dogs olive oil and another group lard to induce lipemia. The clearance rate of colloidal gold in the two lipemic groups were then compared to the rate in a normal group of dogs. The olive oil-fed group showed an intravascular half-life that was 80.8% of normal and the lard-fed group showed an intravascular half-life of 70.6% of normal. In other words, the results indicate that both lipemic groups cleared colloidal gold faster than normal.

Saba and Di Luzio (5) and Saba *et al.* (6) demonstrated the dependence of Kupffer cell phagocytosis of a variety of foreign particles,

including RE test lipid emulsion, on the presence and participation of a system of α_2-globulin proteins called opsonins. Murray (4) has shown that the agglutination of gelatin-stabilized radiogold is due to these opsonins.

The presence of protein on chylomicrons has been previously noted (8, 10). Although chylomicrons in lymph contain some protein, there is an increase in the quantity of protein associated with the chylomicrons when the lipid particles enter the blood (7–9).

The identification of the chylomicron-associated protein reported here is based upon its additional reactivity with gelatin-stabilized radiogold colloid. Although it is likely that the observed protein is contaminated with other plasma proteins, Simon (25), using electrophoresis, reported that gold bound to plasma traveled with the alpha and beta globulins. Murray and Katz (13) confirmed the enhancement of gelatin-stabilized radiogold clearance by alpha and beta globulin fractions of plasma. Allen *et al.* (26) identified purified opsonin as an alpha$_2$-globulin, based upon a phagocytosis assay as well as radiogold agglutination. Our attempts to confirm the work of Simon (25) showed the primary localization of the radiogold in the alpha$_2$-globulin area (27).

Tonaki *et al.* (11) has shown that the incubation of gelatin-stabilized RE test lipid emulsion with plasma resulted in the coating of the lipid particles with a protein-like material, which appears as a granular substance on electron micrographs. This was also true of human serum albumin microspheres when they were incubated with plasma and then examined by electron microscopy. The appearance of this protein-like substance on microspheres in rarified areas of protein is that of short fibers attached at one end.

In the present study, the granular material is seen as a matrix around the colloidal gold particles (Fig. 1). When the plasma and colloidal gold are interacted and the mixture centrifuged, a pseudomembrane can be observed which demonstrated the agglutination reaction. Thus, we assume that the granular material is opsonin.

Figure 2 shows a ribbonlike material surrounding and interconnecting the chylomicrons. This material stains like protein. The interaction of the chylomicrons and the material presumably took place *in vivo*. If colloidal gold is incubated with this material, the gold is adsorbed to it, as shown in Fig. 3. Thus, this material also demonstrates an "opsonic" property. Note that the colloidal gold adsorbs not only to the dark material which stains like protein, but it also adsorbs to the material which immediately surrounds the chylomicrons (arrows in Fig. 3).

On the basis of these results, it must be concluded that native chylomicrons react with opsonin-like protein in the blood and that this may contribute to the clearance of lipid from the blood. However, from the quoted work above, it would appear that there are different opsonin systems, as evidenced by the differential handling of native chylomicrons and artificial lipid emulsions, either cholesterol or triglyceride in nature, even though both systems appear to act with colloidal gold.

It is possible that opsonin represents a multifaceted molecule that is capable of reacting with a variety of foreign particles; thus, the portion of a reacted molecule of opsonin that is still exposed is capable of reacting with another specificity of molecule, as has been shown in this study. The findings suggest divalency of opsonin and open up the possibility of multivalency.

Summary. A blood protein in complex with native chylomicrons was labeled with radiogold colloid, thus indirectly identifying the protein material in the complex as opsonin and suggesting the possibility that native chylomicrons may utilize the opsonin system for its clearance from the circulation. The protein in question has been shown to be at least divalent, in that the protein in combinaton with one type of particle could still react with another. It is felt that these studies provide further insight into the complex surface events that result during the process of opsonization.

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