rations are still impure, the important observation is the virtual absence of certain amino acids since some of the amino acids detected must have been derived from impurities. The very low content of tyrosine probably accounts for the low Folin-Lowry color obtained in the area of tyrosinase activity in Fig. 2.

Summary. A procedure for obtaining a 50-fold purification of mammalian tyrosinase on hydroxyapatite has been described. Various highly purified samples of tyrosinase have been studied in the ultracentrifuge. The preparations were not homogeneous. The per cent of copper present in highly purified fractions was shown to be 0.22 to 0.25%, although it cannot be concluded this is an integral part of the enzyme, since prolonged dialysis inactivates the enzyme and the addition of copper did not restore the activity. Qualitative analysis for amino acids by paper chromatography indicated leucine and/or isoleucine, valine, alanine, proline, glutamic and aspartic acid, lysine, glycine, threonine, serine, and cysteic acid (probably from cystine) were present in significant amounts. Traces of phenylalanine, tyrosine, methionine, arginine, and histidine were also present.

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Properties and Structure of Lipoproteins.* (24750)

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A knowledge of properties and structure of serum lipoproteins is important for understanding of their physiological functions. During recent years there have been efforts to relate certain lipoprotein classes to the pathogenesis or prediction of atherosclerosis. So much controversy has arisen that it is imperative that extensive studies of lipid-protein complex be carried out to clarify their structural relationship to their function. Lipid composition and distribution of serum lipoproteins have been studied extensively(1). However, relatively little work has been done on protein moiety of these lipoproteins. The protein portion of lipoproteins may be studied while combined with lipid(2) or following removal of the lipid fraction(3). Extraction of serum lipoproteins with ether removes a high percentage of triglycerides and cholesterol, but considerable phospholipid remains unextracted. Almost complete removal of all lipid materials may be achieved with extraction of lipoproteins with ether-alcohol mixtures(2). In the present work lipoprotein fractions from various sources were extracted with ether, and properties of nonextractable material studied.

Methods and materials. 1. Extraction of low density lipoproteins with ether. Lipoproteins from various sources were prepared by the method of Gofman, Lindgren, and associates(1,4) as follows: Lipoproteins of density less than 1.063 g/ml were obtained by preparative ultracentrifugation in Spinco Model L Ultracentrifuge at 30,000 rpm for 24 hrs. Three volumes of serum were mixed with 6 volumes of sodium chloride solution of density 1.0918 g/ml to final density of 1.063 g/ml. After preparative ultracentrifugation, the top ml of solution was removed as lipoprotein

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fraction. Two more fractions of lower density lipoproteins (density less than 1.006) were collected. Normal fasting serum was centrifuged at 30,000 rpm for 3 and 24 hours, and lipoprotein in top ml of each sample removed. After collection of each of above lipoprotein fractions, they were again centrifuged at 30,000 rpm for 24 hrs to remove any trace of albumin. In addition to fasting serum, lipoproteins of density less than 1.063 g/ml were obtained from essential hyperlipemic human serum, and hypercholesterolemic rabbit serum. The preparations of lipoproteins as obtained above were dialyzed against distilled water for 24 hrs at 4°C. Ascorbic acid (0.5%) was added to both compartments to prevent extensive oxidation of lipoprotein preparations(5). After dialysis, the solutions were added dropwise to 10 volumes of ether at −10°C with stirring. Temperature was increased to −2°C to 3°C, and the solution extracted for 12-16 hrs with constant agitation of the mixture. After extraction, the ether phase and any precipitated material were removed from the aqueous solution, and the latter lyophilized. Analytical ultracentrifugation was carried out on lipoproteins and ether modified lipoprotein preparations in Spinco Model E Analytical Ultracentrifuge. Paper electrophoresis was performed on Spinco Model R apparatus, and paper strips were stained for lipid with oil red O(6) and for protein with bromphenol blue. Cholesterol content and lipid phosphorus were determined on low density lipoproteins before and after ether extraction. Tyrosine-tryptophane ratio was determined for ether modified material. Protein concentrations were determined by biuret method employing bovine albumin as standard. 2. *Incubation of serum with cholesterol-4-C^{14}.* One-tenth ml of normal fasting serum was incubated at 37°C for 24 hrs with 0.5 microcurie of cholesterol-4-C^{14}. The same incubation procedure was carried out with 1/10 ml of serum from patient with essential hyperlipemia. After incubation, 30 μl of incubation mixture were subjected to paper electrophoresis (on Spinco filter paper strips, S and S-2043). The paper strips were stained for protein and lipid and one strip was exposed to X-ray film for 2 weeks and developed to show distribution of radioactivity.

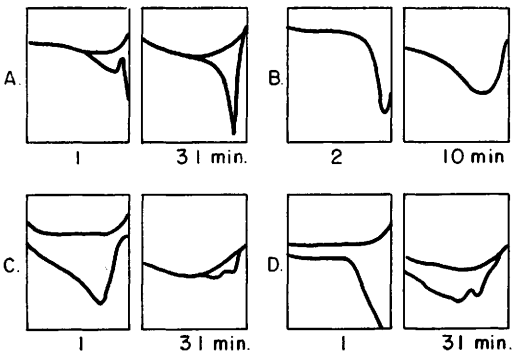

FIG. 1. Ultracentrifugal flotation patterns of various lipoprotein preparations before ether extraction. A. Normal human serum (δ<1.063 g/ml). B. Normal human serum (δ<1.006 g/ml). C. Essential hyperlipemic human serum (δ<1.063 g/ml). D. Rabbit hyperlipemic serum (δ<1.063 g/ml).

Results. Extraction of low density lipoproteins with ether. Ultracentrifuge patterns before and after ether extraction are seen in the following Figures. Fig. 1 shows the pattern of various lipoprotein fractions before extraction procedures. Fig. 2 shows same preparations after ether extraction. Two components appear after extraction of normal serum lipoproteins of density less than 1.063 g/ml, a major faster moving component, followed by a minor component which undergoes only slight sedimentation at 259,700 x g. The extraction product of lipoproteins of density less than 1.006 g/ml prepared at 30,000 rpm for 24 hr shows reduction of faster moving component, and after 3 hr only the component with slight sedimentation remains. The ether extraction product from lipoproteins of density less than 1.063 g/ml for the case of essential hyperlipemia, shows only the slow moving component, and rabbit lipoproteins show predominance of the same component, with only a small amount of the fast moving fraction.

Paper electrophoresis studies were performed on lipoprotein fractions before and after ether extraction. A single electrophoretic component was not seen on paper electrophoresis, regardless of preparation. Before extractions, all human lipoprotein fractions moved toward the anode at pH 8.6 with

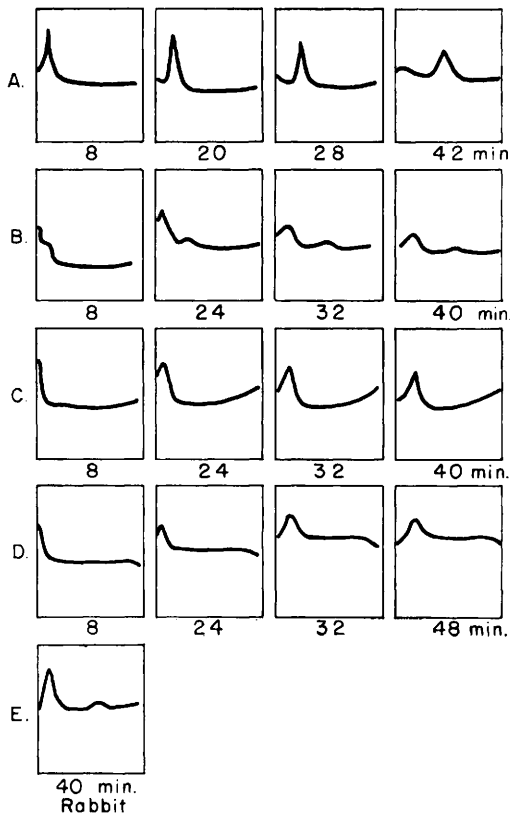

FIG. 2. Ultracentrifugal sedimentation patterns of various patterns after ether extraction. A. Normal human serum (prepared from lipoproteins of $\delta<1.063$). B. Normal human serum (from $\delta<1.006$ after 24 hr). C. Normal human serum (from $\delta<1.006$ after 3 hr). D. Essential hyperlipemia (from $\delta<1.063$). E. Rabbit hyperlipemia (from $\delta<1.063$).

the main component corresponding to beta-lipoprotein of normal serum. A trailing-off of this major component toward the origin was also noted. This trailing phenomenon closely corresponds to the "chylomicron shoulder" noted in normal serum. Intensity of lipid and protein stains on lipoproteins paralleled each other closely. After extraction with ether a similar protein pattern was noted except for small decrease in migration rate toward the anode as compared with normal lipoproteins. Lipid stains were all negative after extraction with ether.

Lipoproteins of density less than 1.006 g/ml, prepared for 3 hrs at 30,000 rpm, were examined for cholesterol and phosphorus before and after ether extraction. Before extraction the following amounts of cholesterol and phospholipid were found: 0.91 g cholesterol/g protein and 0.70 g phospholipid/g protein. After ether extraction no cholesterol was found, but 0.35 g phospholipid/g protein remained. The tyrosine-tryptophane ratio was 2.91 moles tyrosine/mole tryptophane.

2. *Incubation of serum with cholesterol-4-C^{14}.* Fig. 3 shows results of incubation of normal serum with cholesterol-4-C^{14}. A certain portion of radioactivity remained at the origin unbound to any protein fraction. The remaining portion of radioactivity appears to be bound specifically with lipoprotein fractions. The highest intensity of radioactivity corresponds with the beta-lipoprotein of the lipid stained strip. Some radioactivity may also be seen in the area of alpha-lipoproteins as well as in the "chylomicron shoulder." The hyperlipemic serum also has a great affinity for cholesterol-4-C^{14} (Fig. 4). The intensity of radioactivity again closely parallels intensity of the lipid stain. Neither radioactivity nor lipid is seen in the usual region of alpha lipoproteins in the case of essential hyperlipemia.

Discussion. Several workers have applied technics of extraction of lipoproteins with organic solvents. Avigan(3) has reported that extraction of lipoproteins of density 1.019-1.063 g/ml with ether produced a single ultracentrifugal and electrophoretic component. This component had a density of 1.137 and $S_{20,w}$ of 12.8. He calculated molecular weight of this ether-modified lipoprotein to be 1.51 x 10^6. Scanu, Lewis, and Bumpus(7) recently carried out ether-alcohol extraction of high density lipoproteins of density 1.063-1.21 g/ml. They found a product with an $S_{20,w}$ of 4.11, and molecular weight of 75,000. This component moved electrophoretically with the alpha lipoprotein in the region of $alpha_1$ globulin. Thus, electrophoretic mobility of neither of above extracted lipoproteins was changed from the original lipoprotein preparation.

Our results indicate that extraction of lipoproteins of density less than 1.063 g/ml with ether produces 2 distinct components as indicated by ultracentrifugal analysis (Fig. 2).

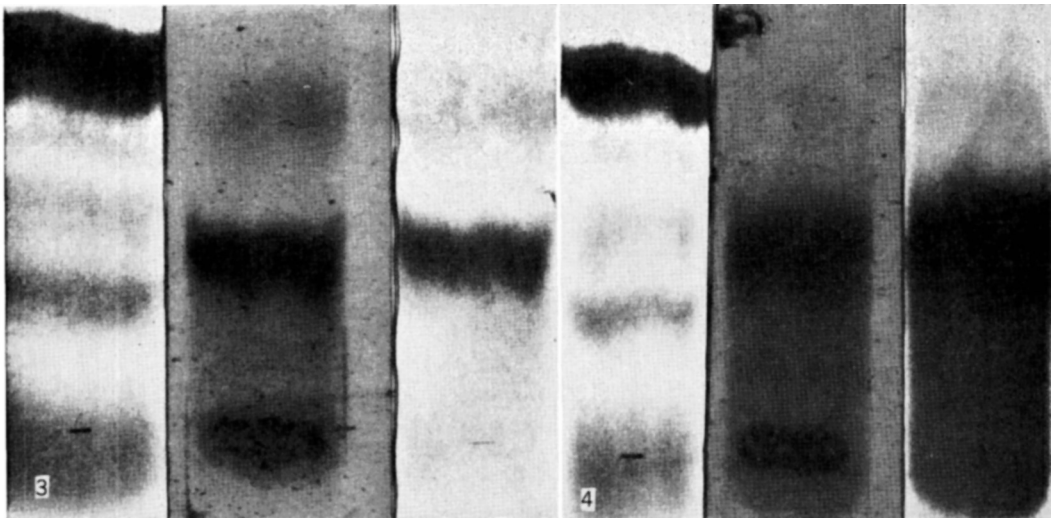

FIG. 3. Incubation of cholesterol-4-C^{14} with normal human serum. Electrophoretic patterns. Left to right: protein stain, radioactivity, lipid stain.

FIG. 4. Incubation of cholesterol-4-C^{14} with hyperlipemic human serum. Electrophoretic patterns. Left to right: protein, radioactivity, lipid.

As density of mixture decreased, the proportion of the faster moving component decreased also, and slower moving component increased. At 30,000 rpm for 3 hrs, none of the faster moving fraction was obtained. This faster moving fraction probably corresponds to that prepared by Avigan from lipoproteins of density 1.019-1.063. This lipoprotein fraction would correspond to the Sf 0-12 fraction of Gofman. Our lower density fractions correspond to those with flotation values above Sf 20. These findings suggest that the protein moiety of lipoproteins with lower flotation values (Sf 0-12) may be different from the protein portion of the Sf 20-400 class lipoproteins.

Considerable interest exists as to interrelationships between various lipoprotein classes. Lindgren and co-workers(8) suggested that biological transformation occurs from high Sf lipoproteins to lower Sf lipoproteins, *i.e.*, from chylomicron and lipomicron down to and including lipoproteins of the Sf 0-20 class. They suggest that low density lipoprotein complexes are composed of "cores" of lipid surrounded by high density lipoprotein molecules. As triglycerides are removed from the "core" of the complex, probably through action of the clearing factor, Sf value of lipoprotein molecules is progressively decreased. These workers also suggest that 2 major classes of basic lipoproteins are associated with lipid "cores" to give 2 relatively distinct series of lipoprotein complexes: Sf 20-10^5 and Sf 0-20 class lipoproteins. Conversion of lipoproteins from the class above Sf 20 to the class below Sf 20 would not occur by loss of lipid material alone, but would require participation of another basic component in the chain of transformation. Our findings support the possibility that the basic units of these 2 classes of lipoproteins may be separate distinct entities. The slower moving ultracentrifuge component appears associated with higher Sf classes while the faster component would be associated with lower Sf classes. The almost complete absence of the component associated with Sf 0-20 lipoproteins in serum of patient with essential hyperlipemia suggests the possibility that a block exists at this point in transformation and removal of molecules greater than Sf 20. The same possibility might exist for rabbits that develop markedly hyperlipemic levels so rapidly.

This paper also presents findings concerning binding of a lipid material by lipoprotein complexes. Previous findings have shown that various ionic lipoid substances combine

with lipoproteins *in vitro*(9,10,11). Our work shows that cholesterol is also bound with both alpha and beta lipoproteins *in vitro*. The mechanism of this combination is not clear at the present time, and this could possibly represent an exchange reaction. However, such observations suggest an interesting possibility that blood levels of various lipid substances may be partially determined by binding capacity of serum lipoproteins. A substance similar to that produced after removal of lipids with ether might serve as a basic lipid binding material, and quantity of this substance present might influence amount of lipid bound in the serum. Since little is known concerning mechanisms regulating serum lipid levels, the possible role of this mechanism might deserve further investigation.

Summary. 1. Extraction of serum lipoproteins of density less than 1.063 g ml with ether produces 2 distinct ultracentrifugal components. One is associated with higher density lipoproteins and the other with lower density lipoproteins in the density group less than 1.063. In a case of essential hyperlipemia and with hyperlipemic rabbit serum only the moiety associated with lowest density lipoproteins was present. It would appear that both components are necessary in transformation of lower density lipoproteins to higher ones. Absence of the higher density moiety might result in an accumulation of lipid in lower density lipoproteins as occurred in the case of essential hyperlipemia and lipemic rabbit serum. 2. Also, it was demonstrated that both alpha and beta lipoproteins bind cholesterol *in vitro*. This finding suggests the possibility that serum cholesterol levels may partially depend upon this mechanism of combination.

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Plasma Levels, Excretion and Metabolic Disposition of 4-(p-Dimethylaminostyryl)-quinoline Dihydrochloride in Dogs.*† (24751)

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The efficacy of a series of 4-substituted p-aminostyryl quinoline dyes against experimental carcinomas and lymphomas in rats has been demonstrated by Haddow *et al.*(3) and by Hughes, *et al.*,(4). Bahner(1) has shown that 4-(p-dimethylaminostyryl)quinoline dihydrochloride (4M20) is among the most effective members of the series. The present report presents details of a specific, sensitive, fluorimetric method for estimation of 4M20 in biological materials and results of studies on plasma levels, excretion and metabolic fate of the drug in dogs.

Methods. Estimation of 4M20 in plasma, urine and feces. To 2 ml of plasma, urine, or

* Portions of these data were presented in abstract form in *J. Pharmacol. and Exp. Therap.*, 1958, 122.

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