Biosynthesis of Plasma Lipoproteins by Rat Liver Ribosomes*

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SUMMARY

Rat liver ribosomes in vitro incorporated labeled amino acids into proteins which showed the immunological behavior of the plasma lipoproteins. The presence of carrier lipoprotein or carrier lipid was required for the isolation and ultracentrifugal purification of immunologically reactive, labeled low and high density lipoproteins. The identity of the lipoproteins was further characterized by solubility criteria and a peptide-mapping technique. A high correlation between the peptide labeling patterns of ribosome-labeled and slice-labeled preparations was found. It is suggested that rat liver ribosomes in vitro are capable of synthesizing the protein moieties of the plasma lipoproteins. These apo-proteins are then capable of binding lipid.

Rat liver mitochondria and rat kidney ribosomes in vitro were also capable of incorporating radioactive amino acids into lipoproteins, but these proteins were immunologically different from the plasma lipoproteins.

The plasma lipoproteins are known to be synthesized in the liver (1). Haft, Roheim, White, and Eder (2) have shown amino acid incorporation into low and high density plasma lipoproteins in the perfused liver, and Radding, Bragdon, and Steinberg (3) reported similar observations in rat liver slices. The identity of the high density lipoprotein labeled by rat liver slices with high density lipoprotein of plasma was shown (4). The net synthesis of low density plasma lipoproteins has been demonstrated in the perfused rat liver and in rat liver slices (5). Marsh (6) found that whole homogenates of rat liver (devoid of nuclei) and isolated microsomes in the presence of cell sap incorporate labeled amino acids into proteins which, when solubilized by sonic oscillation or French pressure cell treatment, behave, in the presence of carrier, as LDL and HDL, as judged by ultracentrifugal and immunochemical analysis. The HDL labeled in the microsomal-cell sap system was found to consist of the same labeled peptides as HDL labeled in liver slices, as ascertained by peptide-mapping techniques. In order to elucidate further the role of subcellular fractions of rat liver in the synthesis of the protein moieties of the plasma lipoproteins, it was decided to extend the investigations to rat liver ribosomes.

The present work will show that rat liver ribosomes in vitro are capable of incorporating radioactive amino acids into proteins, which, in the presence of carrier plasma, show the ultracentrifugal, immunological, electrophoretic, and solubility behavior of plasma low and high density lipoproteins. The relative specific activities of the LDL and HDL labeled in this system were found to be in accordance with the relative specific activities of LDL and HDL labeled by rat liver slices.

The observation by Marsh (6) that a mitochondrial system in vitro incorporated labeled amino acids into a protein fraction which, after solubilisation, and in the presence of carrier plasma, showed the density centrifugal behavior of lipoproteins was corroborated, but these proteins were shown not to be the protein moieties of the plasma lipoproteins.

MATERIALS AND METHODS

Compounds—14C-Amino acids with a specific activity of 100 to 200 μC per mmole were obtained from New England Nuclear. A mixture of amino acids was prepared containing serine, leucine, isoleucine, valine, and phenylalanine. Standard incubations of mitochondria or ribosomes contained 5 μC of this mixture per ml, unless otherwise mentioned. 1H-Tryptophan with a specific activity of 3.8 C per mmole was obtained from Nuclear-Chicago. ATP was obtained from Schwarz BioResearch as the crystalline disodium salt; GTP as the monosodium salt was obtained from United States Biochemical Corporation. TCA was obtained from Sigma Chemical Company.

The abbreviations used are: LDL, low density lipoprotein; that fraction of the total lipoproteins, after removal of chylomicrons, with a buoyant density between 1.008 and 1.063. The density range of this class of lipoproteins is described as d > 1.008 < 1.063. HDL, high density lipoprotein; that fraction of the total lipoproteins, after removal of chylomicrons, with a buoyant density between 1.063 and 1.21. The density range of this class of lipoproteins is described as d > 1.063 < 1.21. TCA, trichloroacetic acid.

* This research was supported by United States Public Health Service Grant HE-06280, and in part by the Center for Oral Health Research, School of Dental Medicine, University of Pennsylvania. A preliminary report of this work has appeared (Fed. Proc., 25, 581 (1966)).
Preparation of Lipoproteins—Two volumes of rat plasma from heparginized blood, to which 1:10,000 Merthiolate had been added, were carefully layered under 1 volume of 0.15 M NaCl and centrifuged for 1 hour at 15,000 × g in a Sorvall RC-2 centrifuge at 2°. The NaCl layer, containing the chylomicrons, was removed, and the infranatant was adjusted to the appropriate density with 70% NaBr solutions. The lipoproteins were isolated by harvesting the top 1-ml fraction resulting from an 18-hour centrifugation at 100,000 × g in the Spinco No. 40 rotor at 10°. Purification was effected by diluting the top fractions 10-fold with NaBr solutions of the appropriate density and re-centrifugation one or more times under the same conditions. The purity of the lipoproteins was checked in the analytical ultracentrifuge. In the case of LDL, two centrifugations proved sufficient to obtain a protein fraction which showed a single peak. In the case of HDL, three 18-hour centrifugations at 100,000 × g were necessary to obtain a homogeneous fraction.

Preparation of Antibodies against Plasma Lipoproteins—The purified lipoprotein solutions of LDL and of HDL were used to immunize rabbits by the Freund adjuvant technique (14). The purity of the rabbit antibodies was determined with the agar diffusion technique of Ouchterlony as described by Kornfeld (13). The anti-LDL antibodies produced a single line with rat plasma on the Ouchterlony plates. With the anti-HDL antibody or of HDL with anti-LDL antibody was observed.

Extraction of Lipoprotein from Liver Slices and Liver Particulate Fractions—Slices: After incubation, slices were separated from the plasma by centrifugation, and the LDL and HDL were isolated from the medium as described for whole plasma.

Mitochondria: After incubation, mitochondria were subjected to ultrasonic disruption for 16 sec at top speed (17 kHZ) with a Branson model L-75 Sonifier. The temperature was kept below 4° during the process. The resulting solution was centrifuged for 15 min at 15,000 × g to remove the debris, and carrier plasma was added to the supernatant, which was then adjusted to a density of 1.21 with NaBr. The total lipoproteins (d < 1.21)
were harvested and purified twice by ultracentrifugation as previously described.

Ribosomes: After incubation, ribosomes were separated from the medium by centrifugation for 60 min at 150,000 × g. The surface of the pellet was washed once by very gentle swirling of the medium over it. The ribosomes were then treated with 2 M LiCl in 0.005 M potassium phosphate and 0.001 M magnesium sulfate buffer at pH 7.6 as described by Curry and Hersh (16). After overnight incubation at 4°, the mixture was centrifuged for 30 min at 100,000 × g. This method solubilized most of the protein and protein-bound radioactivity. The resulting supernatant was then adjusted to the appropriate density, and the LDL and HDL were harvested by centrifugation with or without the addition of carrier plasma, carrier lipoprotein, or carrier liver lipid.

Antibody-Antigen Reactions—In order to minimize the danger of contamination of the specific antibody-antigen precipitate with coprecipitating, nonrelated protein, it was decided to precede specific antibody antigen reactions with LDL and HDL by treatment of these fractions with unrelated “clearing” antibodies and their antigens, a technique utilized by Peters (17) and by Campbell and Stone (18). Reaction mixtures were routinely treated with anthocyanin plus a rabbit anti-anthocyanin antibody, followed by rabbit anti-bovine γ-globulin and its antigen. Only after these two clearing steps was the specific anti-rat LDL or HDL added. As an added precaution, each specific antibody-antigen reaction was run at the same time with its own internal control tube, which did not receive the antibody but contained an equal amount of labeled antigen mixture. The antibody-antigen precipitates were washed twice with cold 0.15 M NaCl and then dissolved in 0.1 N NaOH for the determination of protein and radioactivity.

Radioactivity Determinations—One milliliter of the final 0.1 N NaOH solutions of the proteins was taken to dryness on a stainless steel planchet and counted in a windowless flow counter for a time sufficient to produce 5% probable error. The results were corrected for self-absorption.

Preparation of Peptides for High Voltage Electrophoresis—Purified LDL was obtained from the incubation of 1 g of rat liver slices in 10 ml of plasma containing 25 μC of labeled amino acid mixture containing aspartic acid, arginine, serine, and valine. Ribosomes from 40 g of rat liver were incubated in the standard incubation system in the presence of 40 μC of the same amino acid mixture. Then 10 ml of rat plasma were added to the 1×10^3 pellet and the LDL was isolated and purified.

The delipidated protein was treated at 0° with performic acid according to the oxidation method of Hirs (19). It was then precipitated with 40 volumes of ether at 0° and washed twice with ether to remove residual formic acid. After drying, the proteins were mixed thoroughly with 0.2 N NH₄HCO₃ at pH 8.2 and digested with 10 μg of trypsin per mg of protein at 37° for 8 hours. This was followed by digestion with 10 μg of chymotrypsin per mg of protein under the same conditions. Remaining high molecular weight material was precipitated with 5% TCA, and the supernatant was extracted with butyl acetate by the method of Young (20) to remove TCA. The samples were lyophilized twice. They then were dissolved in a minimal volume of buffer and streaked onto 6-cm-wide strips of Whatman No. 3MM paper in thin streaks 3 cm in length. A one-dimensional separation was obtained by high voltage electrophoresis in a Savant instrument as described by Katz, Dreyer, and Anfinsen (21). A current of 57 volts per cm was applied for 50 min. The buffer was pyridine-acetic acid-water, 1:10:289, at pH 3.6. This separation technique resulted in more than 20 ninhydrin-positive bands for both LDL and HDL protein. Peptides from slice-labeled LDL, ribosomal-labeled LDL, and unlabeled plasma LDL were subjected to high voltage electrophoresis in one run.

In another experiment, 1-H-tryptophan was used as the single labeling agent. The LDL from a slice incubation (1 g of slices, 50 μC of 1-H-tryptophan) and the HDL as well as the albumin from a ribosomal incubation (20 mg of protein, 50 μC of 1-H-tryptophan) were prepared. The albumin was extracted from the d < 1.21 infranatant by the acid-alcohol method of Debro, Tarver, and Korner (22). The S-S links were oxidized and the resolubilized proteins were digested according to the method of Margolis and Langdon (23) with 20 μg of trypsin per mg of protein for 22 hours at 37°. Remaining high molecular weight material was precipitated by adjusting the pH of the incubation mixture to 3.6 with 1 N acetic acid, followed by heating at 90° for 10 min. The lyophilized peptides were subjected to high voltage electrophoresis at 50 volts per cm for 80 min. This enzymatic degradation and separation technique resulted in 14 ninhydrin-positive bands for HDL protein. The peptides from slice-labeled HDL, ribosomal-labeled HDL, carrier plasma HDL, and ribosome-labeled albumin were subjected to high voltage electrophoresis in the same run. After sectioning, the paper strips were cut into 1-cm sections, and the peptides were eluted with water and counted with Bray’s solution (24) in a liquid scintillation spectrometer. The samples were counted until an accuracy of ±10% had been reached.

RESULTS

Mitochondrial Lipoprotein Synthesis—Rat liver mitochondria incubated in vitro in the presence of 20 μg of RNase incorporated radioactive amino acids into total protein with a specific activity of about 10% of a whole homogenate. Sonic disruption solubilized about 45% of the protein and 35% of the protein-bound radioactivity. To the sonic extracts of mitochondrial incubations (containing 20 mg of mitochondrial protein), 2 ml of carrier plasma were added, and the total lipoproteins (d < 1.21) were harvested and purified. Treatment of these solutions with antibodies against plasma lipoproteins produced immune precipitates which did not contain measurable radioactivity (Table I). Mitochondria were harvested from 2 g of liver slices which had been incubated in 10 ml of medium with 9 μC of the labeled amino acid mixture, and were washed five times. The immune precipitates of the total labeled lipoproteins (d < 1.21) derived from these mitochondria again did not contain measurable radioactivity (Table I).

Ribosomal Lipoprotein Synthesis—When labeled ribosomal protein was solubilized by LiCl treatment and carrier plasma was added, the low and high density plasma lipoproteins were labeled (Fig. 1), and the labeling of the plasma lipoproteins followed that of the total ribosomal protein. Puromycin (10^-4 M) inhibited both total incorporation and the labeling of lipoproteins by 95%. Controls incubated at 0° showed less than 2% of the incorporation into both ribosomal protein and total lipoprotein seen at 37°. The incorporation of radioactivity into the LDL and HDL followed that into the total ribosomal protein, depending on the concentration of labeled amino acids in the incubation medium. The specific activity of LDL was higher than that of HDL (Fig.
1). The specific activities measured for the lipoproteins are not true specific activities, since whole plasma was used as a carrier.

**Isolation and Purification of Lipoproteins**—The labeled lipoproteins did not lose activity after successive recentrifugation (Table II). When LDL and HDL were isolated separately from the LiCl supernatant, 2% and 4%, respectively, of the counts present floated to the top during the initial centrifugation, and the radioactivity persisted on successive centrifugations.

The amount of protein-bound radioactivity in the LDL and HDL fractions was quite dependent on the presence of carrier plasma or carrier lipoprotein. When the fractions floating at $d < 1.063$ and at $d > 1.063 < 1.21$ were harvested without the prior addition of carrier, only 40 to 60% of the counts which would have floated up in the presence of the added carrier were found in the top 2 ml of the initial centrifugation (Table II).

**Reaction of Labeled Lipoproteins with Antilipoprotein Antibodies**—The labeled protein present in the fractions isolated in the presence of carrier and purified by ultracentrifugation were treated with two clearing antibody-antigen reactions followed by treatment with specific antibodies against LDL and HDL. The results in Table III show clearly that 60 to 100% of the radioactivity remaining in solution after the two unrelated immune reactions reacts with the specific anti-LDL and anti-HDL antibodies.

A further study of the specificity of the immunochemical reaction was carried out with rat kidney ribosomes, since rat kidney does not synthesize plasma lipoproteins (1). When kidney ribosomes were incubated in the standard system, their efficiency in the incorporation of the radioactive amino acids into total proteins was found to be 30% of that of rat liver ribosomes. When these radioactive proteins were solubilized by 2 M LiCl and carrier plasma was added, the twice purified radioactive

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**Table I**

<table>
<thead>
<tr>
<th>Labeling conditions</th>
<th>Total radioactivity in extracted protein</th>
<th>Radioactivity in isolated lipoproteins*</th>
<th>Radioactivity in immune precipitate of lipoproteins</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In vitro</strong></td>
<td>1,112 cpm</td>
<td>20 cpm</td>
<td>&lt;2 cpm</td>
</tr>
<tr>
<td></td>
<td>2,573 cpm</td>
<td>38 cpm</td>
<td>&lt;2 cpm</td>
</tr>
<tr>
<td><strong>In vivo</strong></td>
<td>24,000 cpm</td>
<td>127 cpm</td>
<td>&lt;2 cpm</td>
</tr>
<tr>
<td></td>
<td>78,300 cpm</td>
<td>253 cpm</td>
<td>2 cpm</td>
</tr>
</tbody>
</table>

* After sonic disruption, the extracted protein was mixed with 2 ml of rat plasma, and the total lipoproteins ($d < 1.21$) were harvested, purified, and treated with specific antibodies to LDL and HDL as described in the text.

The lipoproteins were treated with two unrelated clearing antibodies before the treatment with the specific antibodies to LDL and HDL.

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**Table II**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Total radioactivity in protein of LiCl supernatant</th>
<th>Added carrier</th>
<th>Lipoprotein class isolated</th>
<th>Radioactivity on initial isolation</th>
<th>Specific radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cpm</td>
<td></td>
<td></td>
<td>Total cpm</td>
<td>Specific cpm/mg</td>
</tr>
<tr>
<td>1</td>
<td>90,000 cpm</td>
<td>4 ml plasma</td>
<td>LDL + HDL</td>
<td>7,020 cpm</td>
<td>1,910 cpm/mg</td>
</tr>
<tr>
<td>2</td>
<td>50,000 cpm</td>
<td>2 ml plasma</td>
<td>LDL + HDL</td>
<td>3,210 cpm</td>
<td>2,000 cpm/mg</td>
</tr>
<tr>
<td>3</td>
<td>119,000 cpm</td>
<td>4 ml plasma</td>
<td>LDL + HDL</td>
<td>14,300 cpm</td>
<td>2,485 cpm/mg</td>
</tr>
<tr>
<td>4</td>
<td>21,000 cpm</td>
<td>1 ml plasma</td>
<td>LDL + HDL</td>
<td>1,529 cpm</td>
<td>3,240 cpm/mg</td>
</tr>
<tr>
<td>5a</td>
<td>129,000 cpm</td>
<td>2 ml plasma + 600 µg LDL</td>
<td>LDL + HDL</td>
<td>1,765 cpm</td>
<td>2,300 cpm/mg</td>
</tr>
<tr>
<td>5b</td>
<td>129,000 cpm</td>
<td>None</td>
<td>LDL</td>
<td>60 µg</td>
<td></td>
</tr>
<tr>
<td>5c</td>
<td>129,000 cpm</td>
<td>2 ml plasma + 600 µg LDL</td>
<td>HDL</td>
<td>5,150 cpm</td>
<td>2,180 cpm/mg</td>
</tr>
<tr>
<td>5d</td>
<td>193,000 cpm</td>
<td>None</td>
<td>HDL</td>
<td>2,360 cpm</td>
<td></td>
</tr>
<tr>
<td>6a</td>
<td>112,000 cpm</td>
<td>4 ml plasma + 1 mg LDL</td>
<td>LDL</td>
<td>2,809 cpm</td>
<td>1,461 cpm/mg</td>
</tr>
<tr>
<td>6b</td>
<td>112,000 cpm</td>
<td>None</td>
<td>LDL</td>
<td>987 µg</td>
<td></td>
</tr>
<tr>
<td>6c</td>
<td>112,000 cpm</td>
<td>4 ml plasma + 1 mg LDL</td>
<td>HDL</td>
<td>3,881 cpm</td>
<td>870 cpm/mg</td>
</tr>
<tr>
<td>6d</td>
<td>112,000 cpm</td>
<td>None</td>
<td>HDL</td>
<td>1,251 µg</td>
<td></td>
</tr>
</tbody>
</table>

* When the lipoprotein fractions were isolated in the absence of carrier plasma, the amount of protein present was too small to allow determination of the specific activity. Unlabeled carrier was therefore added after isolation.
The presence of carrier lipoprotein or carrier lipid was required for the isolation by density centrifugation of immunologically reactive lipoprotein from the LiCl supernatants of incubated rat liver ribosomes. The radioactive lipoprotein which floated at d < 1.063 and cl < 1.21 in the absence of carrier lipoprotein (see Table II) was further purified in the presence of carrier lipo-

protein and allowed to react with specific antibodies. Only a very small percentage (1 to 4% for LDL; 9 to 12% for HDL) of immune precipitates, in comparison with the 60 to 80% found in the specific precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate precipitate 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necessary to investigate further the identity of these lipoproteins, especially since the results of the antibody experiments (see Table III) did not always show a total precipitation by the specific antibodies. The peptide-labeling patterns of the ribosome-labeled LDL and HDL were compared with those from LDL and HDL labeled in liver slices. The results for the LDL, shown in Fig. 2, indicate a striking similarity between the tryptic and chymotryptic peptide patterns. Comparison of the labeling patterns of the tryptic peptides of ribosome-labeled HDL and Table VII

<table>
<thead>
<tr>
<th>Stage</th>
<th>Total protein</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before urea treatment</td>
<td>0.221</td>
<td>220</td>
</tr>
<tr>
<td>After urea treatment</td>
<td>0.167</td>
<td>232</td>
</tr>
<tr>
<td>Urea-insoluble fraction</td>
<td>0.054*</td>
<td>184</td>
</tr>
</tbody>
</table>

* Determined by difference.

**DISCUSSION**

In previous work from this laboratory (6), rat liver microsomes were shown to incorporate labeled amino acids into proteins, which, after the addition of carrier, displayed the ultracentrifugal and immunochemical characteristics of low and high density plasma lipoproteins. While isolated mitochondria were shown to
label lipoproteins, these were not identified as plasma lipoproteins. The present work has shown clearly that the labeled lipoproteins are not plasma lipoproteins, since they did not react with specific antisera. It would be very surprising if mitochondria synthesized any of the plasma proteins, particularly since most of the soluble proteins they contain are not labeled in vitro (30). The early observations probably represent a partial solubilization of the mitochondrial membrane lipoprotein, which Roodyn (31) has shown to be the most likely kind of protein labeled in vitro.

Since the microsomal fraction previously used to study lipoprotein biosynthesis contains much lipid, it became interesting to determine whether isolated ribosomes, almost devoid of lipid, were capable of synthesizing the polypeptide chains of LDL and HDL which, in the presence of carrier, could be identified in such. The extraction of labeled protein from ribosomes in vitro by the use of 2 M LiCl proved to be a simpler and gentler procedure than the sonic disruption or pressure cell treatment previously used to liberate labeled lipoprotein from microsomes. We attribute the fact that LDL labeling was superior to HDL labeling in ribosomes—in agreement with data in vivo (32) but contrary to the work with microsomes (6)—to the method of protein extraction. Since the extraction of protein from ribosomes is more complete, however, the possibility of contamination is increased, and many of the experiments in the present investigation were designed to strengthen the conclusion that the labeled proteins concerned are the protein moieties of plasma LDL and HDL.

After the addition of carrier plasma or carrier lipoprotein, the specific activity of the isolated HDL and LDL remained fairly constant after three ultracentrifugal isolations, although in some experiments (Table II) there was a decline in specific activity on the second isolation, followed by a rise in the third purification. In no case was the final specific activity less than that observed after the first isolation. We conclude that the labeled protein cannot be separated from the carrier lipoprotein by centrifugal means. In general, we found that the addition of purified lipoproteins as carrier was superior to the addition of whole rat plasma.

The purified lipoproteins reacted with antibodies which were specific for LDL or HDL (Table III). In the case of LDL about two-thirds of the label was removed from solution by this means, while in the case of HDL more than three-quarters specifically reacted, and in one experiment (Table III, Experiment 4) 100% of the label reacted with specific antibodies. As noted under "Materials and Methods," care was taken to avoid nonspecific adsorption of the label onto an immune precipitate. Proof of the specificity of the antibody reactions, in addition to the experiments with mitochondria, was obtained in the experiments with kidney ribosomes (Table IV), in which labeled protein was found in the ultracentrifugally isolated lipoprotein fractions. However, these did not react at all with the specific antibodies to plasma LDL or HDL. Kidney tissue might be expected to synthesize membrane or other tissue lipoprotein, but not plasma protein.

With the isolated LDL fraction, two further tests—lack of solubility in 6 M urea and precipitation with heparin—again indicated that the labeled protein behaved in the same manner as the carrier LDL (Table VII). Finally, the peptide map of the ribosome-labeled peptides from LDL were compared with the patterns obtained from the same lipoproteins isolated from the medium in which liver slices had been incubated. The results (Fig. 2) indicate a very close concordance of the labeling patterns.

The peptide maps from the ribosome-labeled HDL in which 3H tryptophan was used as the labeling agent showed good agreement with the corresponding liver slice-labeled peptides (Fig. 3) except for two peaks of radioactivity, at 7 and 10 cm from the origin, which appeared only in the latter. Since the ultracentrifugal purification of HDL is more difficult than LDL, and since not all of the label was precipitable with specific antibody, we are inclined to ascribe this difference to the presence of an impurity, although it is possible that differences in the degree of chain initiation and chain lengthening between the ribosomal system and the slice system may have contributed to this result. As a control, 3H-labeled albumin, isolated in the same experiment from the ribosomes with the aid of carrier albumin, was also subjected to the peptide-mapping procedure, and a quite different pattern was obtained (Fig. 4). Unfortunately, we did not obtain sufficient labeling in these experiments to use this technique on antibody-precipitated HDL protein.

The requirement for the addition of carrier to reveal the presence of labeled specific lipoproteins indicates that complete lipoproteins are not made by the ribosome-pH 5 enzyme system. The labeled protein isolated at d = 1.21 in the absence of carrier did not react appreciably with specific antisera. The question whether the labeled apoproteins were combining with the lipid or the protein moieties of the carrier appears to be answered by the experiments shown in Table VI, in which it was found that equilibration of the LiCl extract of the ribosomes with petroleum ether solutions of lipids greatly increased the label recovered after centrifugation at d = 1.21 and treatment with specific antibodies. Scanu (33) has shown that delipidized HDL can recombine with phospholipid emulsions, and Sodhi and Gould (25) have carried out similar binding experiments. It also does not seem likely that extensive protein-protein interactions would occur at the high salt concentrations (about 3 M) used in the preparation of lipoproteins. In the case of LDL, the labeled protein was not very soluble in 6 M urea.

The finding that liver ribosomes are capable of synthesizing the polypeptides of the plasma lipoproteins does not indicate whether a lipid-poor apoprotein is actually secreted as such by the liver. Roheim, Miller, and Eder (34) have recently provided evidence for the presence of apoproteins in rat plasma, capable of binding lipid when perfused through fatty livers. The identity of this apoprotein with any of the usual LDL or HDL apoproteins has not been established. It is also not clear whether the carbohydrate or covalently linked fatty acids which have been found (35-37) in plasma lipoproteins are required for lipid binding. The present experiments provide no information on whether carbohydrate or lipid is attached to the polypeptides being synthesized on the polyribosomes, although Lawford and Schachter (38) recently have shown that some, but not all, of the glucosamine can be introduced into the peptides of glycoproteins while they are still attached to ribosomes. Experiments designed to investigate these and related problems are currently in progress.

Acknowledgment—We wish to thank Dr. Fred Karush for his gift of clearing antibodies and antigens.

REFERENCES
Biosynthesis of Plasma Lipoproteins by Rat Liver Ribosomes
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