Analysis of lipoprotein apoproteins by SDS-gel filtration column chromatography

Charles E. Sparks and Julian B. Marsh

The Medical College of Pennsylvania, Department of Physiology and Biochemistry, Philadelphia, PA 19129

Abstract Rat plasma, containing ^{125}I-labeled triglyceride-rich lipoprotein, was mixed following lipid extraction with 10% SDS buffer and analyzed by gel filtration chromatography on columns using an elution buffer containing 1% SDS. Labeled apoproteins were separated into apo B, apo E, and apo C radioactivity peaks. Labeled peptides, tyrosine, and iodide were also resolved by this method. Isolated lipoprotein fractions were separated into the same components. The method offers the advantages of quantitative radioactivity recovery, large sample volume, and resolution of two apo B proteins.—**Sparks, C. E., and J. B. Marsh.** Analysis of lipoprotein apoproteins by SDS gel filtration column chromatography. *J. Lipid Res.* 181. **22:** 514–518.

Supplementary key words apoprotein B · apo B heterogeneity · triglyceride-rich lipoprotein · VLDL · apoprotein turnover

Lipoprotein turnover studies frequently use ^{125}I-lipoprotein labeling with ICl (1–4), intravenous injection of the labeled lipoprotein, and sequential plasma sampling followed by ultracentrifugation of plasma lipoproteins and subsequent apoprotein analysis (5, 6). When apoprotein-derived peptides occur, trichloroacetic acid solubility may be used to distinguish intact apoproteins from degraded apoproteins (7). When apo B metabolism is studied, the sample may be extracted with tetramethylurea in which apo B is insoluble (8). Uncertainties, however, exist with such experimental designs. The use of ^{125}ICl results in a variable lipid label (4) and residual iodide remains associated with the lipoprotein even after extensive dialysis. During ultracentrifugation, variable quantities of individual apoproteins may be stripped from the lipoprotein and enter higher density fractions (9, 10). Lipoprotein delipidation may extract apoprotein label, especially apo C (11). Tetramethylurea precipitation yields a total apo B fraction (8, 12) revealing little information about the metabolism of heterogeneous apo B subtypes reportedly contained in this fraction (13, 14). Polyacrylamide gel electrophoresis (PAGE) in the presence of SDS (15, 16), ampholine (17–22), or urea (8) effects good resolution of individual apoproteins although some problems may be encountered. Gels stained with Coomassie blue may show variable apoprotein losses and inconstant dye binding of apoproteins hampers calculation of specific activity, and factors have been employed to correct for such differences (23, 24).

Apoproteins have previously been separated using gel filtration column chromatography in the presence of SDS (25–28). The present studies detail the use of SDS columns for separation of apoproteins of triglyceride rich lipoprotein (TRL) in the presence of plasma protein. Improved resolution allows separation of apo B into two component proteins. This method has particular application to the study of apo B metabolism using injected ^{125}I-labeled TRL.

MATERIALS AND METHODS

Preparation of lipoproteins

Lipoproteins were isolated from the sera of male rats of the Fischer 344 strain weighing 200–300 g. The rats were fed a diet containing 68% (w/w) sucrose, 10% (w/w) vegetable oil, and 18% (w/w) casein (ICN Nutritional Biochemicals, Cleveland, OH). The rats were lightly anesthetized by intraperitoneal injection of 30 mg/kg nembutal and blood was obtained by aortic puncture. Lipoproteins were prepared by ultracentrifugation of pooled sera at $d < 1.006$ g/ml (23, 24). The TRL fractions from which chylomicrons had been removed were designated VLDL ($d < 1.006$).

In vitro radioiodinated lipoproteins were prepared using the ICl method as described (1, 2). The ^{125}I-labeled TRL had less than one iodine atom per molecule of apoprotein and specific activities ranged from 1 to 10 dpm/ng protein. Between 6 and 35% of the radioactivity was in lipid, as estimated by the lipid

Abbreviations: PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; TRL, triglyceride-rich lipoprotein ($d < 1.006$ g/ml containing chylomicrons); VLDL, very low density lipoprotein ($d < 1.006$ g/ml); HDL, high density lipoprotein ($1.063 < d < 1.225$ g/ml).

extraction procedure of Lux, John, and Brewer (29) as described previously (23, 24).

In vivo labeled lipoproteins were prepared by intraperitoneal injection of either 0.5 mCi ^{3}H-labeled L-amino acids (New England Nuclear, Boston, MA, NET 250), or intravenous injection of 0.25 mCi of ^{14}C-labeled L-amino acids (New England Nuclear, Boston, MA NEC 445). Labeled TRL was isolated 2 hr after injection of radioactive amino acids.

Preparation of samples for column analysis

Plasma containing ^{125}I-labeled lipoproteins was delipidated using the method of Lux, et al. (29) as described previously (23). Briefly, the method involves adding 1 mg dextran T-500 (Pharmacia Fine Chemicals Co., Piscataway, NJ) per ml plasma followed by delipidation. After collection of the precipitate by centrifugation, the protein pellet was washed in pure diethyl ether. The air-dried precipitate from up to 0.2 ml plasma was dissolved in 0.5 ml of 10% SDS buffer composed of 0.1 M Tris (hydroxymethyl) aminomethane, adjusted to pH 7.4, containing 10% (w/v) SDS, 10% (v/v) glycerol, 10% (v/v) 2-mercaptoethanol. The tubes were sealed and incubated at room temperature for 18 hr. The sample was then heated at 100°C for 10 min with vigorous mixing. More than 99% of the ^{125}I radioactivity was recovered in the combined lipid extract and the solubilized plasma. Whole plasma, containing the ^{125}I-labeled lipoproteins, was prepared for column analysis by dilution with an equal volume of 10% SDS buffer, heating for 3–5 min at 100°C, and cooling to room temperature. The radioactivity in lipid was determined by direct measurement or by the difference between delipidated plasma and whole plasma radioactivity. Labeled lipoprotein fractions in 0.15 M NaCl/2 mM EDTA, pH 7.4, were prepared for column analysis by dilution with an equal volume of 10% SDS buffer, heating at 100°C for 3–5 min and cooling to room temperature. Delipidated plasma, whole plasma, or lipoprotein fractions were stable for up to 6 months at −20°C after dilution with the 10% SDS buffer.

Apoprotein separation and analysis

Labeled apoproteins were separated on 165 × 1.5 cm glass columns (Bio-Rad Laboratories, Richmond, CA) containing Sepharose CL-6B (Pharmacia Fine Chemicals, Piscataway, NJ) equilibrated in a column buffer containing 0.1 M sodium phosphate, pH 7.4, and 1% SDS. Up to 2.0 ml of sample, prepared as described above, was applied under a layer of buffer and apoproteins were eluted at an average flow rate of 7–8 ml/hr. Fractions were collected at 30-min intervals and radioassayed. Columns remained useful for up to 6 months without significant alterations of apoprotein exclusion volumes. Between 95 and 100% of applied radioactivity was recovered in the eluted fractions.

SDS-polyacrylamide gel electrophoresis

Following lipid extraction of the lipoproteins, apoproteins were dissolved in 1% SDS in 0.1 M sodium phosphate, pH 7.4, and heated for 2 min at 100°C in the presence of 1% 2-mercaptoethanol. SDS-polyacrylamide gel electrophoresis (PAGE) was carried out in 5 or 10% gels and the apoprotein bands were identified by staining with Coomassie blue as previously described (23, 24) or by gel slicing and radioassay in the case of ^{125}I-labeled apoproteins.

Radioactivity measurements

Radioassays of ^{125}I-labeled lipoproteins and column fractions of ^{125}I-labeled apoproteins were made in a Searle 1144 gamma scintillation spectrometer with a counting efficiency of 82%. Radioassays of ^{3}H- or ^{14}C-labeled lipoproteins were made in a Packard Tri-Carb liquid scintillation spectrometer with an internal standard used for quench corrections. Counting errors in all cases were less than 5%. All results are expressed as ±S.E.M.

RESULTS AND DISCUSSION

Analysis of TRL apoproteins by SDS-PAGE resolved apo C, apo E, and two apo B bands. Calibration of SDS-PAGE using molecular weight standards including thyroglobulin (330,000) and apoferritin (220,000), showed the two apo B proteins to be between 200,000 and 400,000 daltons in size corresponding to the rat apo B components described by Krishnaiah, et al. (14). Co-electrophoresed ^{125}I-labeled apoproteins were eluted from corresponding unstained gels and specific ^{125}I-labeled apoproteins were analyzed by the column procedure which allowed identification of apoprotein bands of TRL run by SDS-columns. In **Fig. 1**, SDS-column analysis of ^{125}I-labeled TRL apoproteins present in plasma is shown. The apoprotein peaks are identified. The calculated values for the distribution coefficients (K_d) for the ^{125}I-labeled apoproteins is presented in the legend. A molecular weight calibration curve was constructed using the calculated K_d values for standard proteins by plotting the logarithm of the molecular weight versus K_d. From the calibration curve, the approximate molecular size of the TRL apoproteins was calculated to be: apo C = 9600, apo E = 37,000, and apo B = 250,000 and 500,000.

We have designated the apo B proteins of higher and lower molecular weight apo B_h and apo B_l. Be-

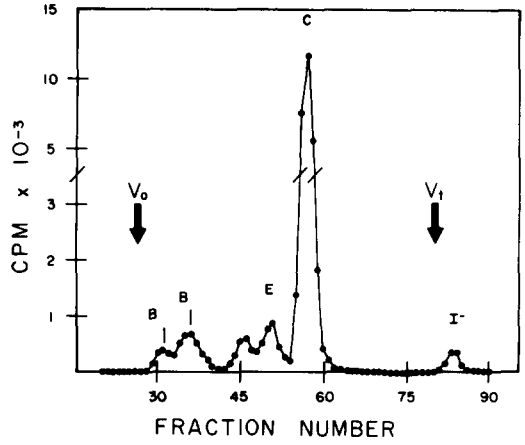

Fig. 1. Radioactivity distribution of ^{125}I-labeled TRL analyzed by SDS columns where radioactivity per fraction was plotted against fraction number. A 20-μl sample of ^{125}I-labeled TRL was added to 1 ml of rat plasma. A 0.2-ml aliquot was then delipidated and dissolved in 0.5 ml of 10% SDS buffer as in Methods and analyzed on a SDS column. Eluted fractions of 3.5 ml were collected every 30 min and radioassayed. The peaks are labeled apo B (B), apo E (E), apo C (C), and I^- for radioactive iodide. The first apo B peak of higher molecular weight represents apo B_h and the second apo B peak of lower molecular weight represents apo B_l. The K_d was calculated by the formula $K_d = V_e - V_o/V_t - V_o$. The effluent volume ($V_e$) of marker substances was determined using four different columns and are as follows; blue dextran = 105.6 ± 2.4, apo B_h = 113.9 ± 2.3, apo B_l = 131.2 ± 2.1, apo E = 191.1 ± 1.5, apo C = 217.0 ± 1.2, ^{14}C-amino acids = 283.1 ± 0.8, 2-mercaptoethanol = 292.0 ± 1.8, ^{125}iodide = 321.7 ± 1.6, bromophenol blue = 358.1 ± 2.0. The calculated K_d of these marker substances was determined using blue dextran as the V_o and 2-mercaptoethanol as the V_t and are as follows; apo B_h = 0.054 ± 0.007, apo B_l = 0.146 ± 0.005, apo E = 0.469 ± 0.005, apo C = 0.601 ± 0.008, ^{14}C-amino acids = 0.946 ± 0.004, ^{125}iodide = 1.158 ± 0.015, bromophenol blue = 1.351 ± 0.007.

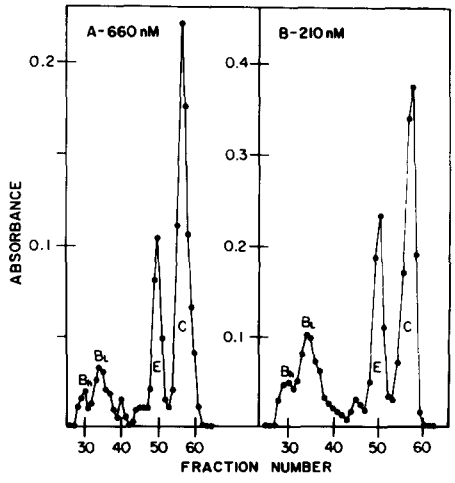

Fig. 2. SDS-Gel filtration column chromatography of solubilized TRL apoproteins. Protein concentration was measured by a modification of the Lowry method (35) and is plotted as absorbance at 660 nM using 1 ml of each fraction in the assay in column A. Peptide bond absorbance at 210 nM is plotted in column B. Both columns are plotted against fraction number at a flow rate of 3.9 ml per 30-min fraction. The apoprotein peaks are marked apo B_h (B_h), apo B_l, (B_l), apo E (E), and apo C (C). A sample containing 2 mg of apo-TRL was applied to each column in 10% SDS-buffer as described in Methods.

TABLE 1. Amino acid composition of Apo B_h and Apo B_l from rat VLDL[a]

Amino Acid	Apo B_h	Apo B_l
	Residues per 1,000	
Cysteic acid	6.5	5.6
Methionine (sulfoxide)	4.8	5.1
Aspartic acid	78.3	75.8
Threonine	63.3	61.8
Serine	72.2	71.5
Glutamic acid	123.0	121.7
Proline	34.1	34.8
Glycine	57.7	62.4
Alanine	90.6	97.5
Valine	60.0	60.7
Isoleucine	59.8	56.0
Leucine	121.1	119.7
Tyrosine	27.5	29.2
Phenylalanine	54.4	56.7
Lysine	81.1	79.9
Histidine	24.9	24.2
Arginine	40.5	40.6

[a] The values shown are the averages of a duplicate analysis of a single preparation from the pooled serum of 40 rats. Amino acid analysis was carried out after hydrolysis in 6 N HCl for 24 hr at 110°C.

cause of the known difficulties of analyzing the molecular weight of high molecular weight hydrophobic proteins in the presence of SDS, we have chosen not to designate the proteins by molecular weight until studies of the type carried out by Steele and Reynolds (30, 31) have been performed. Columns were run on 2 mg of apo TRL obtained from the pooled plasma of 50 rats fed ad lib and the apoprotein separation by SDS-columns is presented in **Fig. 2.** Each of the apoproteins from the columns was analyzed by SDS-5% PAGE and found to migrate as a single band corresponding in mobility to the same apoprotein of the original apo TRL. The pure apo B_h and apo B_l corresponded to the mobility of the two apo B proteins described by Krishnaiah, et al. (14). Amino acid analysis was performed on apo B_h and apo B_l and the results are presented in **Table 1.** The apo B_h and apo B_l had very similar amino acid compositions. The amino acid composition was also very similar to rat apo LDL ($1.006 < d < 1.04$ g/ml) (32) and rat apo B (14). There was no tendency for apo B aggregation in the delipidation of whole plasma as shown in **Table 2.** When apo TRL was separated on columns where the apo B_h and apo B_l had K_d's of 0.4–0.7 again there was no tendency to aggregate as evidenced by the absence of higher molecular weight components. Using the method of Holmquist and Carlson (33, 34), the apo B of ^{125}I-labeled TRL quantitatively precipitated with isopropanol and the precipitated apo B had the same column mobility as in the apo TRL when analyzed on SDS-columns. Both apo B components were present in in vivo labeled

TABLE 2. Distribution of labeled TRL apoprotein radioactivity in plasma

Preparation	Apo B_h	Apo B_l	Apo E	Apo C	$\frac{\text{Apo } B_l}{\text{Apo } B_h}$
			Percent		
^{125}I-TRL[a] (n = 4)	3.7 ± 0.3	8.4 ± 0.6	7.3 ± 0.4	75.7 ± 1.5	2.37 ± 0.28
^{125}I-TRL delipidated (n = 4)	4.1 ± 0.6	8.9 ± 0.7	7.6 ± 0.6	73.9 ± 2.4	2.24 ± 0.12
^{3}H-VLDL (n = 3)	19.0 ± 11.3	33.9 ± 8.2	8.0 ± 3.4	38.6 ± 10.8	1.80 ± 0.17

[a] Expressed as percent of apoprotein radioactivity. The ^{125}I-lipid radioactivity was measured independently and subtracted from the apo C and total radioactivity prior to calculation. The lipid label in ^{125}I-TRL comigrated with apo C and was almost entirely present in phospholipids. In in vivo labeled lipoproteins, some label appears in neutral lipids that were extracted by diethyl ether prior to dissolving the sample in SDS buffer.

TRL and the results are presented in Table 2 and **Fig. 3.** It is unlikely that the smaller apo B_h is a result of proteolytic activity since no peptides were seen in the column analysis of labeled TRL and both peaks were present in in vivo labeled TRL isolated in the presence of protease inhibitor (toluene sulfonyl fluoride) and azide.

In addition to the analysis of apoproteins, the present method allows for the measurement of labeled peptides and amino acids which separate from ^{125}-iodide in study of degradation of ^{125}I-labeled apoproteins. Both ^{125}iodide and bromophenol blue had elution volumes greater than total volume indicating interaction with the agarose gel as shown in Fig. 1. There was consistent contamination of all preparations of ^{125}I-labeled TRL in spite of extensive dialysis with ^{125}iodide radioactivity (2–5% of the total radioactivity) and this iodide could be measured reproducibly by SDS-columns.

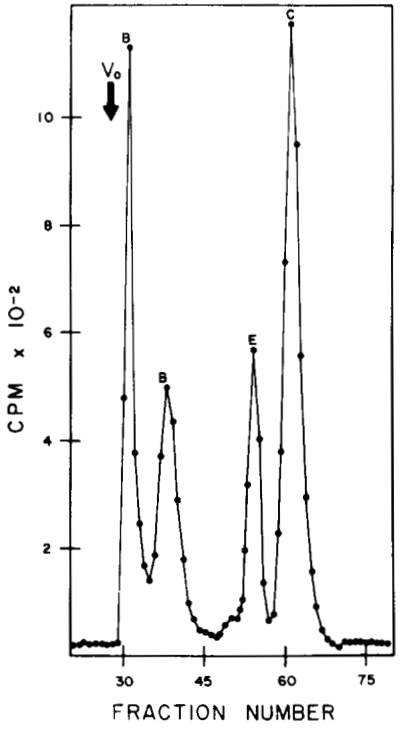

Fig. 3. Radioactivity distribution of ^{14}C-labeled TRL analyzed by SDS columns. Radioactive ^{14}C-labeled TRL was prepared from sucrose-fed rats fed ad lib injected with ^{14}C-labeled amino acids, as discussed in Methods. The ultracentrifugally isolated ^{14}C-labeled TRL fraction was added to an equal volume of 10% SDS buffer and applied to the SDS column. Fractions of 3.5 ml were collected every 30 min, radioassayed, and the radioactivity per fraction was plotted against fraction number. The apoprotein bands are indicated above each peak as in Fig. 1.

The SDS-columns offer the advantage of complete radioactivity recovery even in the presence of plasma proteins. The disadvantage of whole plasma analysis is the inability to determine individual apoprotein specific activity in each lipoprotein fraction. In addition, complete resolution of apo E from apo A-I and apo A-IV is not possible in samples containing these apoproteins. The low molecular weight apo C proteins separate as a group. The individual plasma lipoprotein density classes as isolated by ultracentrifugation can be readily fractionated by SDS-columns. Separation of unlabeled SDS-solubilized TRL apoproteins by SDS-columns is presented in Fig. 2. The protein measurement is made by a modification of the method of Lowry, et al. (35) or by absorbance at 210 nM. In analysis of labeled proteins, the radioactivity is measured and apoprotein specific activity is calculated. In samples containing apo A-I, apo A-IV, and apo E, the column fractions containing these apoproteins can be pooled and the individual apoprotein specific activities determined by SDS-PAGE analysis. Individual apo C protein specific activity measurement requires analytical isoelectric focusing.

The use of SDS-columns has improved the ability to account for all radioactivity in samples obtained from metabolic experiments, but must be supplemented by other methods, as indicated, to obtain specific activities of all of the apoproteins.

We wish to thank Arline Ritz, Bruce LaMont, Sarah Massey, and Oleh Hnatiuk for their excellent technical assistance. We are particularly indebted to Dr. Joel Rosenbloom for

the amino acid analyses. This work was supported in part by N.I.H. Grant No. PO1-HL-22633.

Manuscript received 8 August 1980 and in revised form 14 November 1980.

REFERENCES

1. McFarlane, A. S. 1958. Efficient trace-labeling of proteins with iodine. *Nature.* **182:** 53.
2. Bilheimer, D. W., S. Eisenberg, and R. I. Levy. 1972. The metabolism of very low density lipoproteins. 1. Preliminary in vitro and in vivo observations. *Biochim. Biophys. Acta.* **260:** 212–221.
3. Fidge, N. H. 1974. A review of methods and metabolic studies associated with the radioiodination of lipoproteins. *Clin. Chim. Acta.* **52:** 5–13.
4. Fidge, N. H. 1974. Studies on the radioiodination of very low density lipoproteins obtained from different mammalian species. *Clin. Chim. Acta.* **52:** 15–26.
5. Eisenberg, S., and R. I. Levy. 1975. Lipoprotein metabolism. *Adv. Lipid Res.* **13:** 1–89.
6. Schaefer, E. J., S. Eisenberg, and R. I. Levy. 1978. Lipoprotein apoprotein metabolism. *J. Lipid Res.* **19:** 667–687.
7. Bierman, E. L., O. Stein, and Y. Stein. 1974. Lipoprotein uptake and metabolism by aortic smooth muscle cells in culture. *Circ. Res.* **35:** 136–150.
8. Kane, J. P. 1973. A rapid electrophoretic technique for identification of subunit species of apoproteins in serum lipoproteins. *Anal. Biochem.* **53:** 350–364.
9. Mahley, R. W., and K. S. Holcombe. 1977. Alterations of the plasma lipoproteins following cholesterol feeding in the rat. *J. Lipid Res.* **18:** 314–324.
10. Fainaru, M., R. J. Havel, and K. Imaizumi. 1977. Apoprotein content of plasma lipoproteins of the rat separated by gel chromatography or ultracentrifugation. *Biochem. Med.* **17:** 347–355.
11. Scanu, A. M., and C. Edelstein. 1971. Solubility in aqueous solutions of ethanol of the small molecular weight peptides of the serum very low density and high density lipoproteins. *Anal. Biochem.* **44:** 576–588.
12. Faergeman, O., T. Sata, J. P. Kane, and R. J. Havel. 1975. Metabolism of apoprotein B of plasma very low density lipoproteins in the rat. *J. Clin. Invest.* **56:** 1396–1403.
13. Kane, J. P., D. A. Hardman, and H. E. Paulus. 1980. Heterogeneity of apolipoprotein B: isolation of a new species from human chylomicrons. *Proc. Natl. Acad. Sci. USA.* **77:** 2465–2469.
14. Krishnaiah, K. V., L. F. Walker, J. Borensztain, G. Schonfeld, and G. S. Getz. 1980. Apolipoprotein B variant derived from rat intestine. *Proc. Natl. Acad. Sci. USA.* **77:** 3806–3810.
15. Weber, K., J. R. Pringle, and M. Osborn. 1969. Measurement of molecular weights by electrophoresis on SDS-acrylamide gel. *In* Methods in Enzymology. Vol. X, XVI. C. H. W. Hirs and S. N. Timashesi, editors. Academic Press, New York. 3–27.
16. Swaney, J. B., H. Reese, and H. A. Eder. 1974. Polypeptide composition of rat high density lipoprotein: characterization by SDS-gel electrophoresis. *Biochem. Biophys. Res. Commun.* **59:** 513–519.
17. Gidez, L. I., J. B. Swaney, and S. Murnane. 1976. Analysis of rat serum apolipoproteins by isoelectric focusing. I. Studies on the middle molecular weight subunits of HDL and VLDL. *J. Lipid Res.* **18:** 59–68.
18. Swaney, J. B., and L. I. Gidez. 1976. Analysis of rat serum apolipoproteins by isoelectric focusing. II. Studies on the low molecular weight subunits. *J. Lipid Res.* **18:** 69–76.
19. Haglund, H. 1971. Isoelectric focusing in pH gradients. A technique for fractionation and characterization of ampholytes. *Methods Biochem. Anal.* **19:** 1–104.
20. Albers, J. J., and A. M. Scanu. 1971. Isoelectric fractionation and characterization of polypeptides from human serum very low density lipoproteins. *Biochim. Biophys. Acta.* **236:** 29–37.
21. Albers, J. J., V. Albers, and F. Aladjem. 1971. Isoelectric heterogeneity of the major polypeptide of human serum high density lipoproteins. *Biochem. Med.* **5:** 48–55.
22. Edelstein, C., C. T. Lim, and A. M. Scanu. 1973. The serum high density lipoproteins of *Macacus rhesus*. II. Isolation, purification, and characterization of their two major polypeptides. *J. Biol. Chem.* **248:** 7653–7660.
23. Marsh, J. B., and C. E. Sparks. 1979. Hepatic secretion of lipoproteins in the rat and the effect of experimental nephrosis. *J. Clin. Invest.* **64:** 1229–1237.
24. Marsh, J. B., and C. E. Sparks. 1979. Lipoproteins in experimental nephrosis: plasma levels and composition. *Metabolism.* **28:** 1040–1045.
25. Day, C. E., and R. S. Levy. 1968. Determination of the molecular weight of apoprotein subunits from low density lipoproteins by gel filtration. *J. Lipid Res.* **9:** 789–793.
26. Brown, W. V., R. I. Levy, and D. S. Fredrickson. 1969. Studies of the proteins in human plasma very low density lipoproteins. *J. Biol. Chem.* **244:** 5687–5694.
27. Marsh, J. 1976. Apoproteins of the lipoproteins in a nonrecirculating perfusate of rat liver. *J. Lipid Res.* **17:** 85–90.
28. Capuzzi, D. M., C. E. Sparks, and J. L. DeHoff. 1979. Effect of residual enzymes on degradation of radioiodinated VLDL by collagenase-dispersed hepatocytes. *Biochem. Biophys. Res. Commun.* **90:** 587–595.
29. Lux, S. E., K. M. John, and H. B. Brewer. 1972. Isolation and characterization of apo Lp-Gln ii (Apo A-II), a plasma high density apolipoprotein containing two identical polypeptide chains. *J. Biol. Chem.* **247:** 7510–7518.
30. Steele, J. C. H., Jr., and J. A. Reynolds. 1979. Characterization of the apolipoprotein B polypeptide of human plasma low density lipoprotein in detergent and denaturant solutions. *J. Biol. Chem.* **254:** 1633–1638.
31. Steele, J. C. H. Jr., and J. A. Reynolds. 1979. Molecular weight and hydrodynamic properties of apolipoprotein B in guanidine hydrochloride and sodium dodecyl sulfate solutions. *J. Biol. Chem.* **254:** 1639–1643.
32. Koga, S., D. L. Horwitz, and A. M. Scanu. 1969. Isolation and properties of lipoproteins from normal rat serum. *J. Lipid Res.* **10:** 577–588.
33. Holmquist, L., and K. Carlson. 1977. Selective extraction of human serum very low density apolipoproteins with organic solvents. *Biochem. Biophys. Acta.* **493:** 400–409.
34. Holmquist, L., K. Carlson, and L. A. Carlson. 1978. Comparison between the use of isopropanol and tetramethylurea for the solubilization and quantitation of human serum very low density apolipoproteins. *Anal. Biochem.* **88:** 457–460.
35. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.