papers and notes on methodology

Quantitation of apoB-48 and apoB-100 by gel scanning or radio-iodination

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Abstract In this presentation, we have validated two procedures for the separation and quantitation of apoB-48 and apoB-100 by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE): *1*) gamma counting of radio-iodinated lipoproteins and *2*) scanning of stained gels. Total apoB in SDS solutions was determined by absorbance at 220 nm, and validated by amino acid analysis. The absorbance at 220 nm, in contrast to the Lowry procedure, could be used with BSA as a standard without correction factors. At relative apoB-48 concentrations higher than 10% of total apoB, both scanning and radio-iodination gave reliable results. At lower relative apoB-48 concentrations, the radio-iodine method appeared to be superior, but at low total apoB concentrations, the efficiency of radio-iodination was low.—**Zilversmit, D. B., and T. M. Shea.** Quantitation of apoB-48 and apoB-100 by gel scanning or radio-iodination. *J. Lipid Res.* 1989. **30:** 1639–1646.

Supplementary key words apoB-48 quantitation • apoB-100 quantitation • apoB iodination • apoB SDS gel scanning

The quantitation of the principal B apoproteins, apoB-100 and apoB-48, has received only limited attention. In humans and in several species of animals these apoproteins are markers for the origin of the particles in which they occur, and they also mediate, at least in part, the mechanisms of lipoprotein removal from the plasma. Even the quantitative determination of total B protein in various lipoprotein fractions by the procedure of Lowry et al. (1) has given inconsistent results when serum albumin is used as a standard. In their review of 1986, Fisher and Schumaker (2) report a factor of 0.78 to convert apparent apoB concentrations to the correct values, based on findings in four laboratories. Other competent investigators have determined that no correction factor is needed (3), while others simply do not report the use of a correction factor for this spectrophotometric procedure.

In 1987 Poapst, Uffelman, and Steiner (4) reported a method for the quantitation of apoB-48 and apoB-100 by analytical SDS gel electrophoresis, but found differences in the chromogenicities between these proteins as well as a nonlinear relationship of the densitometrically determined values and the quantities of apoB-100 applied to the gels.

The present study was undertaken initially to investigate the possibility that radio-iodination of lipoproteins, followed by SDS gel electrophoresis and coupled with a total apoB determination, might provide a convenient method for the quantitation of the two apoBs. We compared this procedure with quantitation of stained SDS slab gels both by extraction of the Coomassie blue and by scanning.

MATERIALS AND METHODS

Materials

Carrier-free $Na^{125}I$ was obtained from Amersham (Arlington Heights, IL). Iodine monochloride was prepared as described by McFarlane (5). Acrylamide and bisacrylamide were electrophoresis quality reagents obtained from National Diagnostics (Manville, NJ). SDS, Coomassie G-250, and Coomassie R-250 were obtained from Bio-Rad (Richmond, CA) or from Baker (Phillipsburg, NJ). Triton WR-1339 was obtained from Ruger Chemical (Irvington, NJ). Chloramphenicol, gentamicin, ϵ-aminocaproic acid, benzamidine, and aprotinin were obtained from Sigma (St. Louis, MO). Phosphate-buffered saline (PBS) was 50 mM sodium phosphate, pH 7.4, 0.09 M NaCl.

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; PBS, phosphate-buffered saline; BSA, bovine serum albumin; PAGE, polyacrylamide gel electrophoresis.

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Animals

Female New Zealand white rabbits (Becken Farms, Sanborn, NY) were individually caged, kept on a 12-h light/dark cycle, and consumed 100 g/day of Purina Laboratory Rabbit Chow (Ralston Purina, St. Louis, MO) with water ad libitum.

Male Sprague-Dawley rats (Blue Spruce Farms, Altamont, NY) were housed in individual cages and received Prolab 1000 pellets (Agway Country Foods Div., Syracuse, NY) and water ad libitum. All animal procedures conformed to Cornell University guidelines.

The human apolipoprotein B was obtained from LDL from blood bank plasma, by ultracentrifugation as described below for rats and rabbits.

Triton injection

Rats were anesthetized with ether, and Triton WR-1339 (250 mg per kg body wt as a 20% solution in 0.9% NaCl) was injected into the external jugular vein exposed through a small incision in the supraclavicular fossa (through the pectoralis muscle group). Rabbits were injected with the same dose via the marginal ear vein and fed 100 g of chow supplemented with 20% Wesson oil (wt/wt).

Blood collection and lipoprotein isolation

Rats and rabbits were exsanguinated approximately 5 or 17 h, respectively, after Triton injection in order to maximize the dietary lipid in plasma. Blood was collected in the presence of a preservative mix such that the final concentrations in the collected blood were as follows: Na_2EDTA 1 mg/ml, chloramphenicol 0.02 mg/ml, ϵ-aminocaproic acid 1.3 mg/ml, gentamicin 0.05 mg/ml, benzamidine 0.01 mg/ml, and aprotinin 10 Kallikrein units/ml. Plasma was separated by low speed centrifugation, and $d < 1.006$ g/ml lipoproteins and LDL (d 1.019–1.063 g/ml) were isolated by ultracentrifugation at 4°C according to the method of Hatch and Lees (6).

With the above preservative mix, lipoproteins could be stored for several weeks at 4°C. When degradative products of apoB became apparent on SDS-PAGE, the lipoproteins were discarded in favor of a fresh preparation.

ApoB subspecies separation

The $d < 1.006$ g/ml lipoproteins were delipidated with ethanol–diethyl ether 3:5 (v/v) by the method of Scanu and Edelstein (7) or with butanol–isopropyl ether 45:55 (v/v) by the method of Klein and Zilversmit (8). After resolubilization in 50 mM sodium phosphate, pH 7.2, containing 2% SDS, the apoB-100 and apoB-48 subspecies were separated on a 1.6 × 50 cm Superose 6 column (Pharmacia Inc., Piscataway, NJ) at a flow rate of 0.1 ml/min with an elution buffer of PBS + 0.01% Na_2EDTA and 0.1% SDS. Eluent was monitored at 280 nm for an approximate indication of separation boundaries.

Protein assay

ApoB-100 and apoB-48 fractions from the Superose column were assayed for protein by measuring the absorbance at 220 nm against a crystalline BSA standard (Miles, Elkhart, IN) that had been lyophilized and dried in a vacuum desiccator over P_2O_5. A linear standard curve was obtained for both BSA and apoB to at least an absorbance of 2.0 (representing 200 μg protein/ml). Light scattering was found to be negligible when measured by the Rayleigh light scattering principle (inverse fourth power of the wavelength).

Protein was also measured by the dual wavelength (280/205 nm) method of Scopes (9), by the biuret method of Gornall, Bardawill, and David (10), by the method of Lowry et al. (1), by absorbance at 280 nm, absorbance at 205 nm, and by amino acid analysis.

Electrophoresis

Samples containing 50 to 200 μg protein were delipidated as described above and redissolved in 0.25 to 1.0 ml of 50 mM sodium phosphate, pH 7.2, containing 2% SDS, 5% β-mercaptoethanol, 10% glycerol, and 0.003% bromphenol blue. The mixture was heated to 80°C for 5 min in a 1.5-ml polyethylene micro-centrifuge tube. Four to 40 μg of protein was applied to SDS-slab gels which were bonded to a sheet of Gelbond PAG film (FMC Bio Products, Rockland, ME) during polymerization. Gels containing either 5 or 7.5% acrylamide were run in the Laemmli (11) system at a constant current of 20–40 mamps per gel for 3–4 h. A water-cooled vertical gel apparatus (Hoefer SE600, Hoefer Scientific Instruments, San Francisco, CA) was used to prevent overheating.

Gels were simultaneously fixed and stained for 2 h at 37°C in 0.2% Coomassie blue G-250, 7.5% acetic acid, 46.2% methanol; and destained by diffusion for 15–24 h at room temperature in 7.5% acetic acid, 15% methanol with a Bio-Rad model 556 destaining apparatus (Bio-Rad Laboratories, Richmond, CA). Coomassie blue R-250 did not dissolve at a concentration of 0.2% in the solvent that was used for G-250. We therefore used the method of Fairbanks, Steck, and Wallach (12) for R-250, which uses a stepwise procedure of decreasing dye and alcohol concentrations.

Gels were scanned either on an EC910 densitometer (E-C Apparatus Corp., St. Petersburg, FL) at 605 nm, and areas were determined by planimetry with a K & E digital planimeter (Keuffel & Esser Company, Parsippany, NJ), or on a Bio-Rad model 620 densitometer at 600 nm, and areas were also determined by the 1-D Analyst version 2.01 and 2-D Analyst version 1.01 software from Bio-Rad. The absorption maximum for both apoB-48 and apoB-100 was 600 nm with Coomassie blue G-250.

With the 1-D Analyst software, gel lanes were scanned longitudinally and the band intensity versus band mobility trace was displayed. With the cursor, an appropriate

baseline was drawn and the areas to be integrated were selected. Usually, multiple scans of a given lane at different sites were averaged.

With the 2-D software, the entire gel was digitized, with contour lines representing the stain density. From the printout of this whole gel scan, a given sample lane was selected by means of the axes on the printout. The digitized data for that sample lane were then "extracted" from the whole image and displayed as a trace on the monitor. The difference between this trace and a normal 1-D trace is that, instead of trying to select "representative" slices to scan, the entire lane is displayed, thus averaging out anomalies in stain density across a given band. In lane to lane comparisons, peak areas were normalized to the same lane width. The software documentation omitted any mention of this necessary adjustment.

Amino acid analysis

Amino acid analyses were carried out by the Biotechnology Facility of the Cornell University Chemistry Department. Protein (0.5 to 5.0 μg) was lyophilized, then hydrolyzed for 2 h at 150°C with 6 N HCl vapor in vacuo with the addition of norleucine as an internal standard. Amino acid residues were determined by high performance liquid chromatography on a Waters "Pico-tag" 2.0 mm × 15 cm column, and amino acyl mass was calculated from the sum of the residue weights, after subtracting the water of hydrolysis (13). The amino acid composition is in good agreement with previously published values (**Table 1**).

TABLE 1. ApoB-100 amino acid composition: residues/1000

					Present Study	
	Ref. 14	Ref. 15	Ref. 16	Ref. 4	Mean (n = 5)	SD
Asp	97.4	106.6	131.0	110.7	99.0	4.7
Thr	58.4	66.4	66.5	63.7	61.0	4.9
Ser	74.6	86.4	114.0	92.2	88.8	5.4
Glu	114.0	116.2	132.0	125	115.8	5.6
Pro	34.7	38.5	39.4	38	47.0 (n = 2)	
Gly	44.2	47.3	57.0	50	51.6	6.5
Ala	56.1	60.5	72.2	62	62.8	2.3
Cys	6.2	4.5	6.6	4.6	N.D.	
Val	56.1	55.6	37.1	48.5	56.6	1.1
Met	16.6	16.0	13.3	17.1	20.2	0.8
Ile	56.1	60.3	37.5	52.9	59.4	2.6
Leu	108.0	118.5	110.0	111.2	131.2	8.6
Tyr	27.1	33.6	29.5	34.8	42.4	4.0
Phe	48.4	49.8	55.1	41.7	50.0	2.2
His	23.3	25.7	27.1	28.1	26.4	1.1
Lys	61.7	79.9	65.1	82.9	74.8	1.8
Arg	29.4	33.8	28.5	33.2	39.6	1.1
Trp	5.2	N.D.	6.2	N.D.	N.D.	

Ref. 14, Margolis and Langdon (1966); Ref. 15, Kane et al. (1980); Ref. 16, Cardin et al. (1982); Ref. 4, Poapst et al. (1987).

Iodination

Delipidated lipoproteins, redissolved in 2% SDS, 50 mM sodium phosphate, pH 7.4, were iodinated by the method of McFarlane (17). Approximately 1 mg (2 nmol) protein in 0.3 ml was iodinated at room temperature by the addition of 0.1 ml of a premixture of 10 μCi ^{125}I, 60 nmol ICl, and 1 M glycine, pH 8.5. After 30 sec the reaction was stopped with 350 μmol of β-mercapto-ethanol. At an average efficiency of 33%, one atom of iodine would be bound per 50,000 daltons of apoB. Unbound iodine was removed on a Superose 6 column.

Intact lipoproteins were iodinated by the method of Bilheimer, Eisenberg, and Levy (18) in order to reduce lipid labeling. One to 5 ml of lipoprotein solution containing 0.3–1 mg/ml protein in 0.2 M glycine, pH 10, was iodinated with 10 to 100 μCi of ^{125}I by the addition of ICl such that at an average efficiency of 5%, one atom of iodine would be bound per 50,000 daltons of protein. Unbound iodine was removed during delipidation and on a Superose 6 column in the presence of 0.1% SDS. After both iodination procedures, the iodinated apoB was indistinguishable from noniodinated apoB by SDS-PAGE.

Individual bands from a dried gel were cut out, and ^{125}I was measured in plastic vials in a Beckman Gamma 8000 counting system. Equal counting efficiencies were observed for all samples (approximately 70%).

RESULTS AND DISCUSSION

The intent of the present investigations was to determine the suitability of radio-iodination as a method for quantitating the relative amounts of apoB in plasma lipoprotein components. This was motivated by reports that scanning of stained electrophoretograms was not suitable for reasonably accurate determinations of proteins, because of their inherently different staining characteristics (19), even in the case of two proteins that are structurally very similar (4). The stoichiometry for the binding of Coomassie blue to protein is not completely understood; however, it appears to be not only a function of amino acid composition and macromolecular structure (20), but also a function of dye concentration and acid/alcohol concentration (19).

Initially the delipidated lipoproteins were iodinated in a medium of 2% SDS by the method of McFarlane (17). The iodination in that environment was chosen in order to expose the tyrosine groups of the two proteins to an equal extent. The specific activities of the two proteins were calculated from the amount of ^{125}I and the absorbance of the two apoBs at 220 nm in the eluates of the Superose 6 columns. The specific activities of the apoB-100 were 25 ± 1.5% (mean ± SE, n = 29) lower than that of apoB-48. Since it is known that some large pro-

teins, especially those with hydrophobic regions, do not completely unfold in the presence of 1.4% SDS (21), we increased the SDS concentration to 10% for the iodination, but the difference in specific activities remained the same. Oddly enough, when samples of d < 1.006 g/ml, isolated from rats or rabbits fed a high-fat meal and injected with Triton WR-1339, were iodinated in their native form, the specific activities of the apoB-100 and apoB-48 were essentially the same.

The above findings raised the possibility that the determination of protein by the 220 nm absorbance method might be affected by differences in tyrosine or tryptophan content or possibly by the presence of carbohydrate in the protein chain. It has, for example, been recognized that the Lowry method overestimates apoB by about 30% when serum albumin was used as a standard (2). We therefore compared the 220 nm absorbance method with the biuret determination and the dual wavelength method

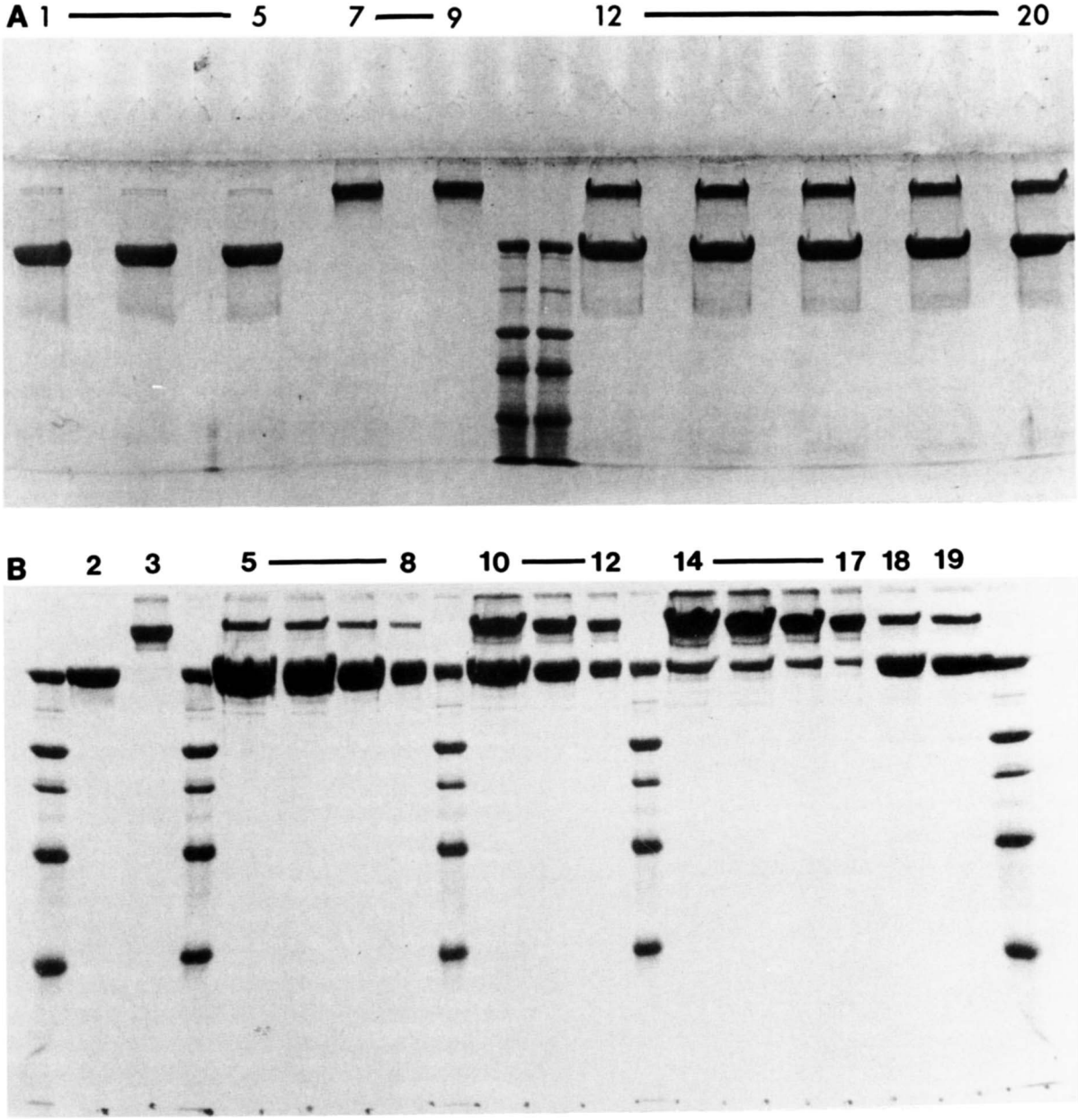

Fig. 1. A: 5% acrylamide SDS-PAGE slab gel; lanes 12–20, d < 1.006 g/ml apoB from Tritonized rat; lanes 1–5, apoB-48 from Superose 6 column; lanes 7–9, apoB-100 from Superose 6 column; lanes 10, 11, molecular weight standards: myosin (200 k), β-galactosidase (116 k), phosphorylase B (97 k), BSA (66 k), and ovalbumin (43 k) at dye front. B: 7.5% acrylamide SDS-PAGE slab gel; lanes 18, 19, d < 1.006 g/ml apoB from Tritonized rat; lanes 1, 4, 9, 13, 20, molecular weight standards as in Fig. 1A; lanes 2 and 3, apoB-48 and apoB-100, respectively, for preparation of known mixtures; remainder of lanes, from left to right: serial dilutions of apoB-48/apoB-100 mixtures shown in Table 3.

of Scopes (9), both of which primarily measure peptide bonds. The results were also compared to the method of Lowry et al. (1), to absorption at 280 nm, and to amino acid analysis. Comparisons showed close agreement between the results of these various methods for apoB quantitation, except for the Lowry procedure, which gave values about 13% higher, and absorption at 280 nm, which overestimated apoB by 22%.

There are, however, certain disadvantages to some of the procedures for protein determination. The biuret determination requires mg amounts of protein. The absorbance at 205 nm, in the presence of most buffer components, exhibits a very high blank reading. The amino acid analysis is rather slow if one only wants a quantitation of the protein. We observed that the hydrolysis of apoBs with 6 N HCl at 150°C required at least 2 h. On the basis of sensitivity and simplicity, we prefer to measure absorbance at 220 nm for the determination of proteins dissolved in 0.1% SDS.

In order to assess the adequacy of gel staining and scanning procedures, purified fractions of apoB-100 and apoB-48 obtained from Superose 6 columns, equilibrated with 0.1% SDS, were prepared. These fractions were then mixed in different proportions, and aliquots were applied to vertical 5% or 7.5% acrylamide slab gels (**Fig. 1**). The gels were then stained, destained, and scanned as described in the Methods section. At first, each lane was scanned across three different sites, and the peak areas, determined by planimetry, were averaged. Not only was this procedure quite laborious, but in cases in which the stained bands looked nonuniform and showed the presence of "lobes", the results from the three-site scans were rather variable. Even though averaging three scans compensated for these deficiencies, we subsequently investigated the use of the Bio-Rad 620 scanner, utilizing a two-dimensional summation technique whereby the entire stained area is integrated.

TABLE 2. Comparison of % apoB-48 from gel scan, dye elution, and ^{125}I-labeled apoB

	Gel Scan	Dye Elution[a]	^{125}I[b]
	% apoB-48 (mean ± SD)		
VLDL (4)	61.6 ± 0.6[c]	64.9 ± 1.6	61.1 ± 1.2
Known mixtures			
30.6% ApoB-48 (3)[d]	30.3 ± 0.8	33.0 ± 1.4[e]	29.4 ± 1.6
51.9% ApoB-48 (3)	54.4 ± 3.4	55.7 ± 2.3	53.6 ± 0.8
67.5% ApoB-48 (3)	72.8 ± 2.8	79.0 ± 3.5	68.4 ± 0.9

Iodination of intact VLDL (d < 1.006 g/ml) from Triton WR 1339-injected rats, the composition of which is shown on the top line, mean of four lanes. Superose 6 fractions from this VLDL were used to prepare the known mixtures (sp act of apoB-100 and apoB-48 were 938 cpm/μg and 934 cpm/μg, respectively).

[a]Coomassie blue elution with 25% pyridine in water.

[b]Gamma counting of gel strips.

[c]Duplicate EC 910 densitometer scans per lane (all others were single scans per lane).

[d]Number of replicate lanes.

[e]Duplicate samples, n = 2.

TABLE 3. Comparison of % apoB-48 from gel scans and ^{125}I-labeled apoB

	ApoB Applied per Lane	Gel Scans		^{125}I[c]
		1D[a]	2D-1D[b]	
	μg	*%*		*%*
VLDL		68.8	69.4	66.6
Known mixtures				
9.58% ApoB-48	3.37	17.0	13.1	10.5
9.58% ApoB-48	6.61	17.3	15.8	10.5
9.58% ApoB-48	9.99	16.6	17.1	10.5
9.58% ApoB-48	12.8	18.0	16.5	10.5
49.9% ApoB-48	3.25	51.3	51.5	47.0
49.9% ApoB-48	6.37	50.4	52.7	46.8
49.9% ApoB-48	12.7	52.2	53.4	46.8
90.5% ApoB-48	3.40	85.2	87.5	86.5
90.5% ApoB-48	6.66	84.8	86.4	86.6
90.5% ApoB-48	10.1	84.0	87.1	86.4
90.5% ApoB-48	13.4	81.3	86.0	86.3

Rat VLDL (d < 1.006 g/ml) from Triton WR-1339-injected rats, delipidated and iodinated in SDS, the composition of which is shown on the top line (mean of two lanes). Specific activities of apoB-100 and apoB-48 in Superose 6 fractions were 3180 and 4295 cpm/μg, respectively. These fractions served as sources for known mixtures.

[a]Bio-Rad 620 densitometer one-dimensional.

[b]Bio-Rad 620 densitometer two-dimensional scan converted to one-dimensional before integration.

[c]Gamma counting of gel strips.

Table 2 shows the results of experiments in which gel scanning data were compared to those obtained by dye elution and by counting of the radioiodine in the stained bands. The top line of data in Table 2 shows the apoB composition of the rat VLDL harvested after injecting Triton WR-1339 into chow-fed rats. By all three methods the composition was between 61 and 65% apoB-48. It should be noted that the rat VLDL was iodinated as intact VLDL and that the specific activities of the two apoBs were nearly identical. When this VLDL was delipidated and then subjected to chromatography on Superose 6 in an SDS-containing buffer, apoB-100 and apoB-48 fractions were collected and protein contents were determined by absorbance at 220 nm. Known mixtures (30.6, 51.9, 67.5% B-48) of the two apoBs were then applied to three replicate SDS slab gel lanes. The two apoB bands were scanned after which the dye was extracted with 25% pyridine (22). Subsequently the bands were counted for ^{125}I-labeled apoB. The results show agreement among the three procedures with the known composition of the three mixtures.

In **Table 3**, mixtures of the apoBs over a wider concentration range were compared by two scanning procedures and the measurement of ^{125}I in the apoB bands. A photograph of this gel is shown in Fig. 1B. In this instance the iodination was performed after delipidation of the VLDL

and resolubilization of the apoBs in the SDS buffer. In contrast with the results observed with the iodination of the intact lipoproteins, the specific activity of the apoB-48 was 35% greater than that of apoB-100. The VLDL (top line, Table 3) was obtained from rats as in the previous experiment (Table 2), and the apoB mixtures were prepared in the same manner, based on absorbance at 220 nm. The scanning in the experiment was performed both by triplicate one-dimensional scans of the same lane (Table 3, 1D) and by two-dimensional scans that were subsequently displayed as a single set of peaks prior to integration (Table 3, 2D-1D). The ^{125}I column in Table 3 was calculated by taking into account the difference in specific activities of the two apoBs. In most instances, the agreement among the procedures for the rat VLDL and the mixtures of known compositions was satisfactory. However, in the case of relatively low apoB-48 percentages (9.58% apoB-48), the scanning procedures were less reliable. In a different experiment (data not shown), with a set of 10 replications on each of two gels, a known mixture of 4.3% apoB-48 showed 5.7% apoB-48 by the iodination method and 5.02% apoB-48 by the 2D-1D gel scans. In Table 3, the results (10.5%) obtained by radioiodination were remarkably reproducible and much closer to the known value (9.58%) than the scanning results (13.1–18.0%). It should also be noted that the calculated % apoB-48 was reproducible for varied amounts applied to the gels.

In **Table 4**, the scanning of a 6.9% apoB-48 mixture gave low values: 3.9 and 5.4%, whereas the iodination results agreed with the known composition. The results of scanning, after staining with Coomassie blue, showed good agreement with the iodination method and with the known values for the mixtures with higher proportions of apoB-48. At low concentrations of apoB-48, the ^{125}I procedure was more accurate and convenient, particularly in those cases in which we iodinated the lipoproteins directly, so that no corrections for differences in apoB specific activities had to be made.

TABLE 4. Comparison of % apoB-48 for ^{125}I-labeled apoB, Coomassie Blue G-250, and R-250 methods[a]

	Calculated from		
	G-250	R-250	^{125}I[b]
VLDL	64.6 ± 3.0[c]	65.3 ± 2.6[c]	
Known mixtures			
6.9% ApoB-48	5.4 ± 0.3[c]	3.9 ± 0.2[d]	6.9 ± 0.2
50.2% ApoB-48	48.7 ± 0.3[c]	48.2 ± 1.3[d]	50.7 ± 0.4
90.0% ApoB-48	88.0 ± 1.3[c]	89.4 ± 1.9[d]	87.9 ± 0.5

Rat VLDL (d < 1.006 g/ml) from Triton WR-1339-injected rats, delipidated and iodinated in SDS, the composition of which is shown on the top line. Specific activities of apoB-100 and apoB-48 in Superose 6 fractions were 6170 and 8090 cpm/μg, respectively. These fractions served as sources for known mixtures.

[a]All measurements with EC 910 densitometer (mean ± SD).
[b]Gamma counting of gel strips.
[c]Averages of 16 gel lanes, 1 scan per lane.
[d]Average of 3 lanes (3 scans per lane).
[e]Average of 2 lanes (3 scans per lane).

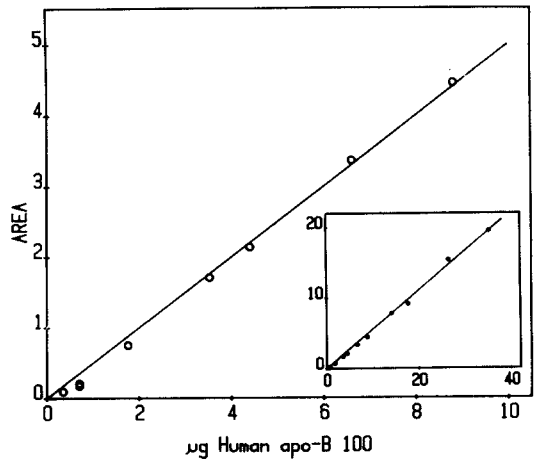

Fig. 2. Total stain (area) as a function of human LDL apoB-100 mass (μg) for two ranges of mass: 0–10 μg and 0–40 μg (inset). Numbers on X-axis are the amounts applied per lane. Mass was determined by absorbance at 220 nm, and area was determined with a Bio-Rad 620 densitometer using the 2-D and 1-D software. The slopes were determined by the method of least squares analysis for the equation $y = bx$. (For 0–10 μg, $b = 0.499$, $r^2 = 0.998$; for 0–40 μg, $b = 0.553$, $r^2 = 0.998$.)

Fig. 2 and **Fig. 3** show standard curves for gel scans of human and rabbit apoB-100. The inset in Fig. 2 shows the linear regression for the same data between 0 and 35 μg. The slopes for the two curves are almost identical. **Fig. 4** shows a comparison of standard curves for rat apoBs determined in different aliquots of a known mixture. In six gels with Coomassie blue G-250 obtained from Bio-Rad or Baker, the mean ± SD for the ratio of apoB-48 slope/apoB-100 slope was 1.03 ± 0.11.

In a recent publication by Poapst el al. (4), several calibration curves of apoB staining were shown in which a pronounced deviation from linearity was present in the standard curves. This contrasts with our data (Fig. 2) which show good linearity over two ranges of aliquots. However, Poapst et al. (4) quantitated apoB by tube gel electrophoresis, and they used Coomassie blue R-250 instead of the G-250 used in our studies. Table 4 shows that, when 16 aliquots of a rat VLDL apoB were applied to the same gel, the percentage of apoB-48 calculated from the two stains was 65% with less than a 5% coefficient of variation. The table also shows that when different mixtures of the apoBs were analyzed, there was no significant effect of the type of Coomassie blue employed. In addition, the agreement of the values determined by averaging three scans per lane gave very good agreement with the known amounts of the two apoBs in the mixture and with the iodination procedure. We cannot account for the pronounced nonlinearity of staining observed by Poapst el al.

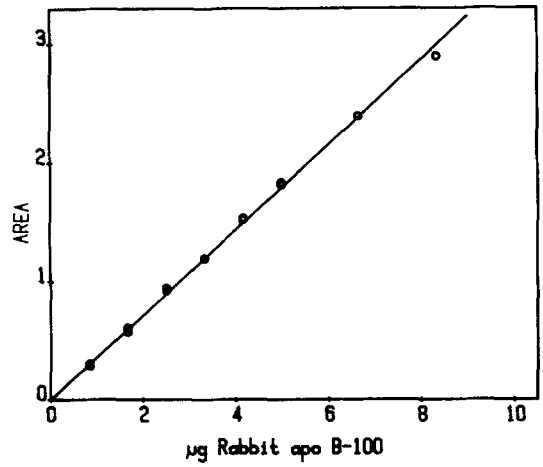

Fig. 3. Total stain (area) as a function of rabbit LDL apoB-100 mass (µg). Numbers on X-axis are the amounts applied per lane. Mass was determined by absorbance at 220 nm, and area was determined with a Bio-Rad 620 densitometer using the 2-D and 1-D software. The slope was determined by the method of least squares analysis for the equation $y = bx$. ($b = 0.359$, $r^2 = 0.999$.)

(4). The use of tube gels by Poapst et al., instead of vertical slabs used in our experiments, may not be relevant, since Nestel, Billington, and Fidge (23) have reported linearity for apoB-48 and apoB-100 with dye eluted from tube gels. A linear standard curve that consistently passes through the origin is of great advantage, because far fewer standard samples would have to be included in any one set of determinations, thus allowing a larger number of unknowns.

We have been unable to obtain an adequate supply of human lipoprotein fractions so as to address the question

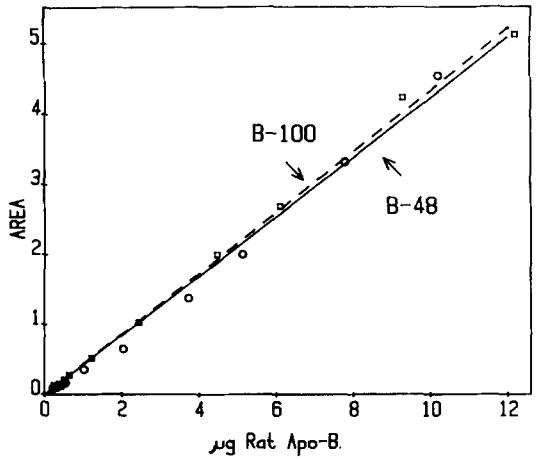

Fig. 4. Comparison of chromogenicities of d < 1.006 g/ml rat apoB-100 (○) and rat apoB-48 (□). Numbers on X-axis are the amounts applied per lane. Mass was determined by absorbance at 220 nm, and area was determined with a Bio-Rad 620 densitometer using the 2-D and 1-D software. The slopes were determined by the method of least squares analysis for the equation $y = bx$. (For apoB-100, $b = 0.433$, $r^2 = 0.999$; for apoB-48, $b = 0.423$, $r^2 = 0.995$.)

TABLE 5. Chromogenicities of apoBs relative to BSA[a]

	Gel Scan[b]
BSA	1.00
Rabbit LDL apoB-100	1.04 ± 0.14
Rat VLDL apoB-100	0.916 ± 0.12
Human LDL apoB-100	0.975 ± 0.16
Rat VLDL apoB-48	1.03 ± 0.11

VLDL (d < 1.006 g/ml) from Triton WR-1339-injected rats.
[a]A comparison of the slope of each standard curve (four points per slope, 1–10 µg) to the slope of BSA. Slopes were determined by linear regression. Stained with Coomassie blue G-250.
[b]Scanned by Bio-Rad 620 2D to 1D conversion before integration (area/µg protein). Mean ± SD (number of gels = 3).

of whether human apoB-48 and apoB-100, derived from different density fractions, show different chromogenicities (4). However, in three additional tests of rat, human, and rabbit apoB-100 as well as with rat apoB-48 and BSA, respective chromogenicities with Coomassie blue G-250 were compared. **Table 5** shows relatively close agreement between the staining of the four apoB fractions and that of BSA. One could, therefore, use BSA as an internal standard for the quantitation of these apoproteins.

The following general conclusions are warranted by the current data. *1*) For the measurement of total apoB in a medium containing SDS, the measurement of absorbance at 220 nm gives reliable results when BSA is used as a standard. The measurement at 220 nm is, however, affected by the presence of Tris buffer or azide. The useful range with a 1-cm light path is 2–200 µg protein/ml. *2*) Iodination of intact lipoprotein fractions containing apoB-100 and apoB-48 resulted in equal specific activities for the two apoBs. Iodination in 2% SDS solutions results in a 25–30% higher specific activity for apoB-48 than for apoB-100. *3*) Quantitation of B-100/B-48 by iodination and counting of gel strips gives reliable information down to very low relative concentrations of apoB-48. This is an advantage, because most lipoprotein fractions obtained under physiological conditions contain relatively small amounts of apoB-48. One disadvantage of iodination is the requirement for increasing amounts of radio-iodine when the concentration of total apoB is less than 1 mg/ml. *4*) Staining of apoB fractions with Coomassie blue shows good linearity for one-dimensional and two-dimensional scans over a range of 0.35–35 µg of apoB. The two-dimensional scans gave better accuracy than the one-dimensional results, particularly when the stained bands showed gross deviations from a rectangular, evenly stained profile.

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