Quantitation of serum lipoproteins by electrophoresis on agarose gel: standardization in lipoprotein concentration units (mg/100 ml) by comparison with analytical ultracentrifugation

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ABSTRACT Lipoprotein electrophoresis on agarose gel has been modified to allow estimation of the absolute quantity of each fraction. The reproducibility of the method is illustrated by 12 determinations in a single day on serum from one normal subject: mean total dye uptake was 302 ± 9 (SD) "corrected dye units," and the percentages of β-, pre-β, and α-lipoprotein were 56.1 ± 0.9, 29.1 ± 0.4, and 14.8 ± 0.7, respectively. Reproducibility over a period of 8 months was also demonstrated.

Serum lipoproteins of five normal and 15 hyperlipidemic individuals determined by this technique were compared with values obtained by analytical ultracentrifugation. The correlation coefficients were: 0.993 for pre-β-LP vs. VLDL, 0.978 for β-LP vs. LDL, and 0.867 for α-LP vs. HDL. Lipoprotein values obtained by paper electrophoresis were also correlated with those of the analytical ultracentrifuge, but to a lesser degree (r = 0.956, 0.691, and 0.786, respectively). Values for LDL and VLDL which were measured by refractometry after preparative ultracentrifugation were very similar to those obtained from the analytical ultracentrifuge. Serum triglyceride concentration was highly correlated (r = 0.972) with the agarose values for pre-β-LP; serum cholesterol concentration was correlated (r = 0.673) with β-LP. It is proposed that the standard curves of the comparisons with the analytical ultracentrifugal values be used to convert the corrected dye units of electrophoresis on agarose gel to mg/100 ml of specific lipoprotein.

SUPPLEMENTARY KEY WORDS cholesterol · gravimetric total lipids · paper electrophoresis · refractometry · triglyceride

Analysis of serum lipoprotein patterns has become an integral part of the diagnosis and treatment of hyperlipidemic states and other disorders of lipid metabolism (1). As a result, there is a need for a technique for lipoprotein analysis which provides quantitative information and is suitable for widespread use. Two methods are currently available, neither of which fully satisfies both of these criteria.

Analytical ultracentrifugation (2) allows precise quantitation, but it is too laborious and expensive for routine use. The electrophoretic technique (1, 3–8) allows the qualitative identification of lipoprotein fractions, but quantitative determination of these entities has not been fully accomplished.

The chief obstacles to electrophoretic quantitation have been a failure to achieve complete separation of the lipoproteins and variability in the uptake and measurement of the dye which provides a marker for the lipoprotein fractions. An important modification of the electrophoretic technique has recently been developed by Noble (8). His use of a transparent agarose gel in place of paper as the supporting medium has improved

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Abbreviations: LP, lipoprotein; LPE, lipoprotein electrophoresis; CDU, corrected dye units; LDL, low density lipoproteins; VLDL, very low density lipoproteins; HDL, high density lipoproteins; IU, integration units.

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the resolution of the lipoprotein fractions and has permitted easy densitometric estimation of dye uptake. However, the method is not quantitative since lipoprotein concentrations are expressed in arbitrary units which are not corrected for variations in dye uptake.

The objects of this study are: [1] to develop a technique for the quantitative analysis of lipoproteins by electrophoresis on agarose gel; [2] to determine the relationships between the agarose electrophoretic values for serum lipoproteins and values obtained on the same sera by (*a*) analytical ultracentrifugation, (*b*) refractometry after preparative ultracentrifugation, (*c*) paper electrophoresis, and (*d*) chemical and gravimetric lipid analyses; and [3] to provide a method based on these comparisons for converting the dye uptake units of the agarose method into mg/100 ml for each lipoprotein fraction.

METHODS AND MATERIALS

Specimens

Blood samples were obtained after a 12-hr fast from five subjects with normal serum lipid concentrations and 15 individuals with hyperlipidemia of moderate to marked extent. Agarose lipoprotein electrophoresis (LPE) was performed in duplicate on the day of the venipuncture. Paper LPE and ultracentrifugal analysis were initiated within 24 hr on an aliquot which had been stored at 4°C with merthiolate preservative (0.1 mg/ml of serum). Cholesterol, triglyceride, and gravimetric total lipid determinations were performed within 20 days on aliquots which had been stored at −20°C without preservatives.

Agarose LPE

The method for lipoprotein electrophoresis is the same as that developed by Noble (8), with the exception of the modifications described in detail below.

The agarose strips are prepared by delivering 2 ml of the warm agarose solution and spreading quickly to the side edges and to within 25 mm of each end of Cronar polyester film strips measuring 18 × 185 mm. The trough for sample application is formed with a cylindrical steel rod measuring 2 mm in diameter and 10 mm in length.

A volumetric pipette is used to deliver 0.5 ml of the unknown serum into a test tube containing 0.25 ml of the agarose solution at 40–45°C, and the contents of the tube are mixed by inversion. 20 μl of the serum–agarose mixture are delivered into the trough immediately after removing the metal rod, using a disposable micropipette (Scientific Products, Flushing, N.Y.) and a manually operated micropipette control (Kontes of California, Berkeley, Calif.). (The actual delivery of warm serum–agarose mixture, measured with an ^{131}I tracer, was 19.8 ± 0.3 μl [SD].) Electrophoresis is carried out for 2 hr at a constant current of 5 ma per strip supplied by a Beckman Spinco Duostat. Tap water is circulated through the upper and lower cooling plates of the cell and the polarity of the cell is reversed for successive runs. After electrophoresis, the strips are fixed in 5% acetic acid in 70% aqueous ethanol for 40 min at 100–110°C. The strips are stained for 24 hr by rotating at 2 rpm in 6 liters of saturated Oil Red O solution (Beckman Instruments, Spinco Div.).

Densitometry is performed with a Spinco Model RB Analytrol fitted with a B-2 cam, a 520-nm interference filter in the front holder, no interference filter in the rear, and the Microzone scanning attachment. The film strip carrier of the Microzone attachment has been replaced with one constructed to have an aperture slot 118 mm long and 13 mm wide. The motor of the attachment has been replaced with a motor having an output speed of 6 rpm. Strip scanning speed is 48 mm per min. The zero setting is adjusted using base line readings obtained at both ends of the strip.

Standard Strips for Agarose LPE

Variations in dye uptake are corrected using a pool of human sera, previously divided into 5-ml aliquots and stored at −70°C. Aliquots are thawed only once for preparation of the standard strips. Each standard strip is prepared by pipetting 1 ml of a mixture of the pooled serum and warm agarose buffer solution onto the central 60 mm by 18 mm portion of a film strip and spreading this mixture evenly with the pipette (Fig. 1, *A*). Electrophoresis is not carried out on the standard strip, but the procedures for solidification, fixing, drying, and staining are identical to those described above. Four concentrations of standard strips are prepared which contain 0.010, 0.020, 0.029, and 0.038 μl of pooled serum per ml of agarose buffer, respectively. In this range, the relationship of dye uptake to serum concentration is linear. Preparation of the standard strips may be carried out prior to the day of electrophoresis of a given series of unknowns. A set of four standard strips is included every time unknowns are stained.

Calculations for Agarose LPE

The Analytrol records the absorbance and automatically integrates the areas under the peaks so described. The results may be expressed arbitrarily as "dye units." The absorbance of the Oil Red O solution decreases by about 25% during the two months of utilization. Correction for the changing staining capacity of the dye is made by observing the amount of dye uptake of the

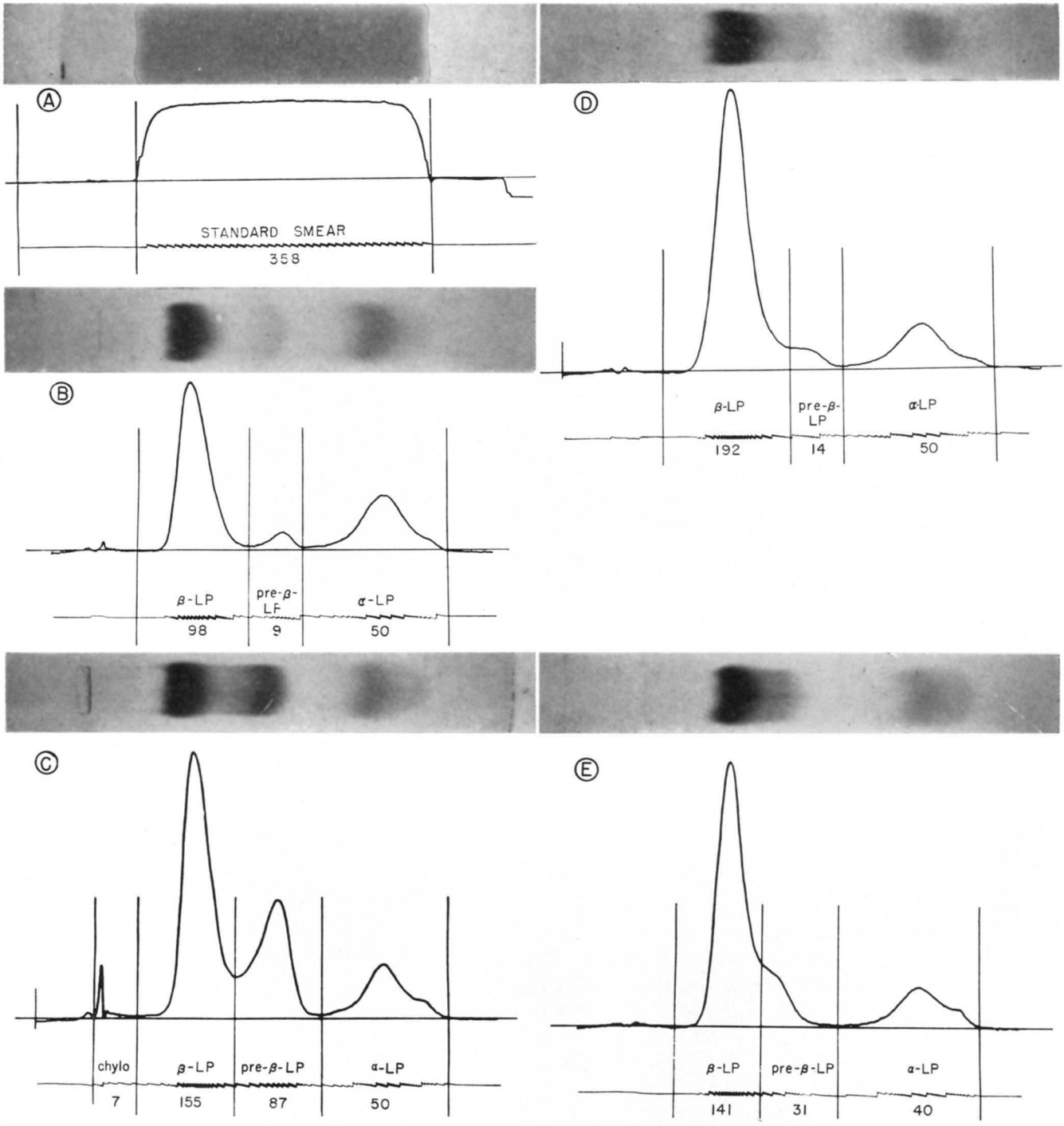

FIG. 1. *A*, a representative standard strip, densitometric scan, and integration tracing. The horizontal plateau of the scan illustrates the homogeneity of the standard application. Dye uptake is 358 integrator units ("dye units"). *B–E*, electrophoretic strips for several different serum types with their densitometric scans and integration tracings. Chylomicrons remain at the origin on the left as seen in *C*. β-LP, pre-β-LP, and α-LP migrate toward the anode on the right. The vertical lines which delineate the lipoprotein zones are drawn according to the rules described in the text. The number of integration units within each zone is expressed in "dye units." Absence of trail and good separation of the β-LP and pre-β-LP zones, shown in *B* and *C*, are characteristic of the agarose method.

four standard strips, measured in "dye units." For each standard strip, a ratio is developed between the dye uptake on an arbitrarily selected reference day of the initial dye lot and that on the day in question. The four ratios are averaged to give the correction factor. The value in "dye units" of each unknown sample is multiplied by the correction factor obtained from standard strips stained on the same day to give the corrected

dye uptake, expressed as "corrected dye units" (CDU). A control specimen from the serum pool is also included in each electrophoretic run as a quality control.

Separation of the lipoprotein fractions is usually sufficiently complete so that chylomicrons, and beta (β-LP), pre-beta (pre-β-LP), and alpha (α-LP) lipoproteins are separated by valleys in the densitometric scans (Fig. 1, *B* and *C*). A perpendicular line is drawn from the middle of the bottom of the valley to the integration tracing of the Analytrol to provide a means for identifying the recorded integrator units associated with each lipoprotein fraction. In unusual instances in which two pre-β-LP peaks are observed, the division of pre-β-LP from β-LP is made at the first valley. Occasionally, sufficient overlap between β-LP and pre-β-LP occurs so that no true valley exists. The following rule was developed for these uncommon situations:

A. If a zero slope is achieved, the vertical line separating β-LP from pre-β-LP is drawn at the onset of zero slope (Fig. 1, *D*).

B. If a zero slope is not achieved and (*1*) if β-LP exceeds pre-β-LP in magnitude, the vertical line is drawn at the onset of the least negative slope that occurs (Fig. 1, *E*), or (*2*) if β-LP is less than pre-β-LP, the vertical line is drawn at the end of the least positive slope that occurs.

The rules outlined above have been developed with the objective of (*a*) representing the lipoproteins accurately, and (*b*) providing a simple and precise procedure for hand calculation.

Other Analyses

The method for performing paper LPE has been described previously (4, 9, 10). A trail of lipid is usually seen from the pre-β-LP region of paper LPE strips back to the origin. This material, which is presumed to be pre-β-LP the migration of which was delayed, falsely raises the measured values for β-LP. Accordingly, the paper LPE values for β-LP are corrected by subtraction of the estimated trail component (10). In the case of agarose LPE, trail is only seen in unusually hyperlipidemic sera of type V pattern. Since this situation is very rare, agarose LPE values for β-LP are not corrected for the trail component.

Analytical ultracentrifugation employing computer analysis of the Schlieren patterns was used to determine the concentrations of high density lipoproteins (HDL), low density lipoproteins S_f 0–20 (LDL), and very low density lipoproteins S_f 20–400 (VLDL) (2). LDL and VLDL were also determined by precision refractometry after preparative ultracentrifugation (11). VLDL determined by this method includes LP's of S_f 20–10^5, and LDL is calculated by subtracting VLDL from total LDL. Chylomicrons were isolated by density gradient ultracentrifugation in a swinging bucket rotor and quantitated by NCH elemental analysis (12).

Serum cholesterol concentration was analyzed by the standard Technicon Autoanalyzer method (13). Serum triglyceride concentration was measured by an automated fluorometric technique (14).

Total lipids were determined by gravimetric analysis, using a modification of the technique of Folch, Ascoli, Lees, Meath, and LeBaron (15). Lipid material is first extracted from 5 ml of serum with 100 ml of chloroform–methanol 2:1. The mixture is stirred for 20 min, and poured through a fluted filter paper (prewashed with methanol) into a separatory funnel. After two 10-ml washes with the same solvent, 24 ml of 0.73% NaCl is added. The mixture is stored overnight at 4°C; it is then warmed to room temperature and the upper layer is discarded. After adding 1 ml of absolute ethanol, the lower layer is taken to dryness in vacuo on a rotating evaporator. The dried lipid is dissolved in 10 ml of chloroform, and an aliquot is transferred to a tared flask, dried, and weighed.

Gravimetric total lipid determinations were carried out on only 10 of the 20 sera described above. 18 additional fasting samples were obtained from normal and moderately hyperlipidemic individuals. Data from these samples are included in the correlations between agarose LPE and gravimetric total lipid concentration.

RESULTS

Agarose LPE

Fig. 2 shows the effect of the duration of staining on total dye uptake and on the dye uptakes of the three major lipoprotein fractions expressed as percentages of the total. No appreciable changes with staining time were seen in the percentages of dye uptake of β-LP, pre-β-LP, or α-LP. Total dye uptake increased throughout the 72 hr of observation, although most of the uptake took place within the first 24 hr. The 24-hr staining interval was therefore selected for all subsequent experiments.

Correction for variation in staining capacity of the dye solutions was made from the standard strips as described in the Methods and Materials section. This correction factor was used to calculate dye uptake in the 51 independent electrophoretic determinations of the standard serum pool which were carried out over a period of 247 days as a quality control (Fig. 3). The variations in corrected dye unit values appeared to be random; the mean value for this pool was 188 CDU, and the standard deviation was 15 CDU. The mean value without using the correction factor was not significantly different (186 dye units), but a much larger variance

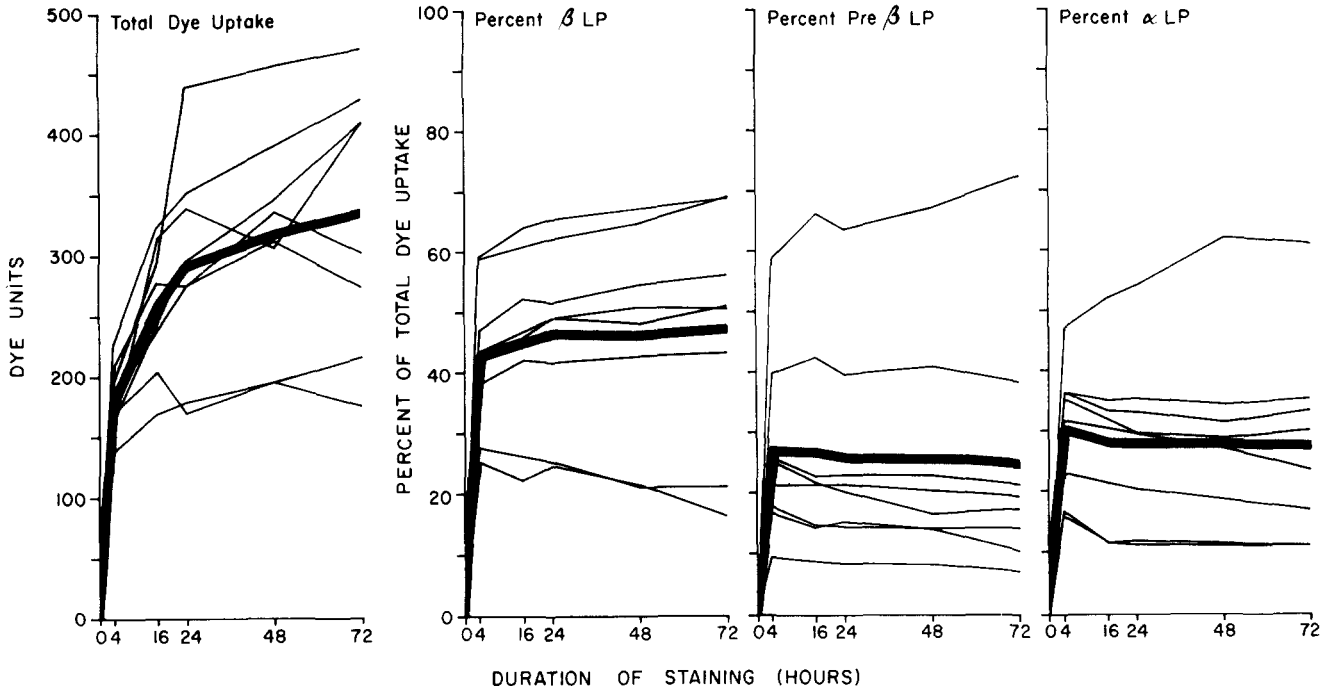

FIG. 2. The effect of duration of staining on dye uptake. For each serum sample, five strips were stained to provide data for the five time intervals. The mean of the eight sera is shown by the heavy dark line.

was observed; the standard deviation of the uncorrected values was 30 dye units. Systematic changes were not apparent in the percentage of dye uptake of each lipoprotein fraction during this period.

The reproducibility of the dye uptake and lipoprotein percentages was studied by performing 12 determinations on a single serum. The total dye uptake was 302 ± 9 CDU (SD), and the percentages of β-LP, pre-β-LP, and α-LP were 56.1 ± 0.9, 29.1 ± 0.4, and 14.8 ± 0.7, respectively.

Comparisons of Values Obtained by Agarose LPE with those of Other Methods

The serum lipid profiles of the study subjects are shown in Table 1. Several patients with unusually high serum lipid concentrations have been included in order to test the full range of the methods.

The close relationships between lipoprotein determinations by analytical ultracentrifugation and agarose LPE values are shown in Figs. 4 and 5. LDL and β-LP are highly correlated for only 16 of the specimens. The four aberrant sera (depicted as solid triangles) are from atypical individuals with marked elevations of pre-β-LP and chylomicrons (type V). These are the only agarose strips that have any trail of lipid between the origin and β regions. It is presumed that β-LP is disproportionately high in these four cases because the measurements include the trail component.

The regression line relating VLDL to pre-β-LP for all 20 sera is shown in Fig. 4 as a dashed line; the correlation coefficient is 0.993. A similar high correlation and a slightly different regression line are seen when the five grossly elevated points are omitted (solid line). The latter relationship, which represents values ranging up to about five times normal, is applicable to normal and moderately hyperlipidemic individuals.

HDL and α-LP values are closely related for all 20 sera (Fig. 5). Chylomicron determinations by the two methods are not highly correlated. The regression line describing this relationship is not presented in Fig. 5; the correlation is statistically significant ($P < 0.01$), but the scatter of the data is too great to allow meaningful interpretation.

The relationship between lipoprotein determinations by analytical ultracentrifugation and paper LPE values are shown in Fig. 6.

The relationship between serum cholesterol concentrations and agarose β-LP values is shown in Fig. 7. The correlation is relatively low, even after omitting the four atypical sera. A high correlation is noted between serum triglyceride concentration and pre-β-LP. The correlation coefficient between gravimetric determinations of total lipid concentration and total lipoproteins measured by agarose LPE is 0.992 (Fig. 8).

The correlation coefficients for all comparisons among the five methods are summarized in Table 2. All correla-

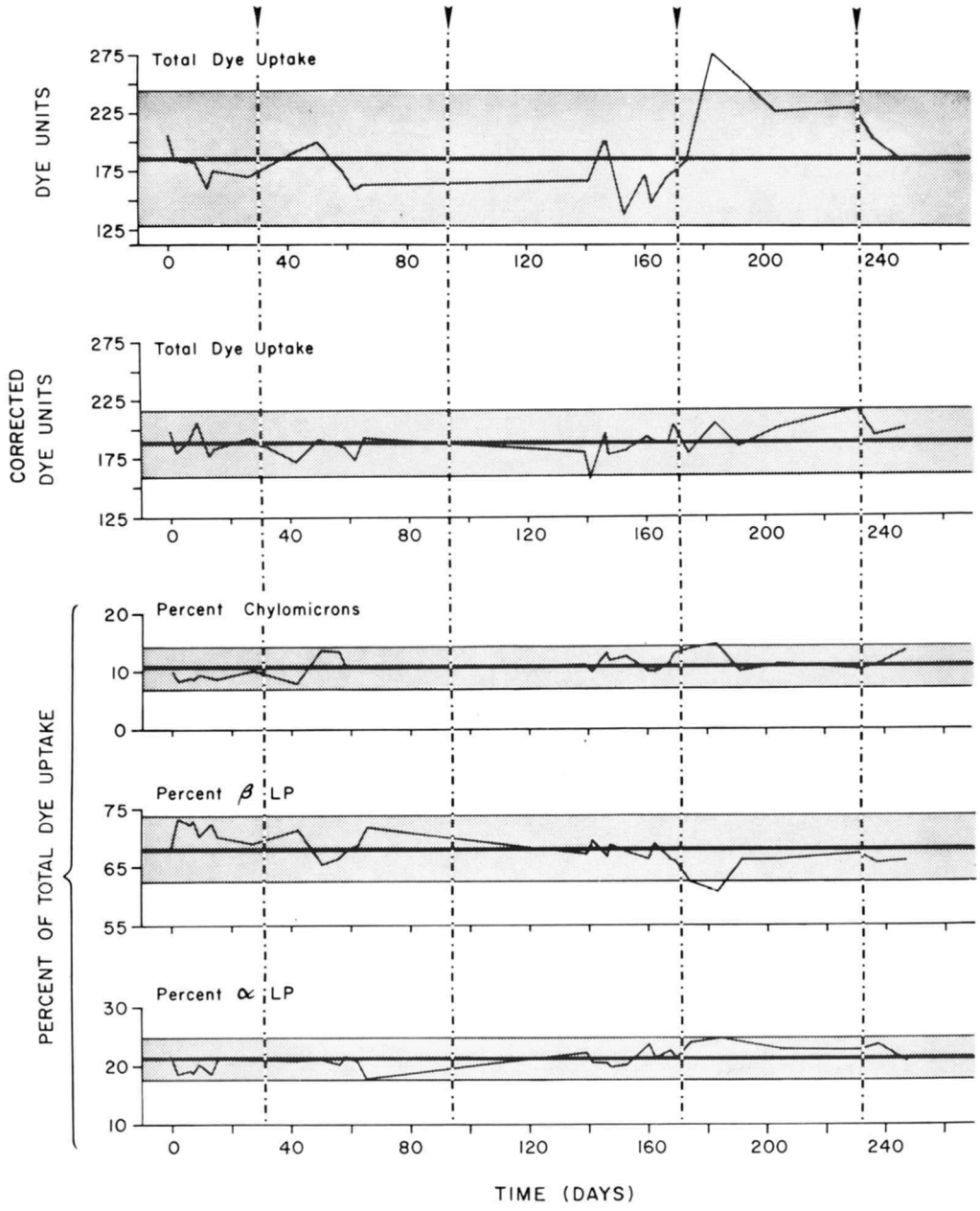

FIG. 3. Electrophoretic determinations of the control serum pool. The 95% confidence limits are shown by the stippled area above and below the mean (heavy horizontal line). The Oil Red O solution was replaced on the four days indicated by arrows. Total dye uptake before and after correction for staining variation is shown.

tions that include β-LP, LDL, or cholesterol have been calculated after exclusion of the four unusually lipemic type V sera. The remaining values represent all 20 specimens.

The standard errors of estimating lipoprotein concentrations by the several methods are shown in Table 3. These data are based on the assumption that the lipoprotein values determined by analytical ultracentrifugation are correct. The standard errors for the agarose LPE method are less than one-half of those for paper LPE or chemical lipid determinations. The standard error of estimating individual lipoprotein fractions by agarose LPE ranges from 8 to 14% of the mean lipoprotein concentration.

DISCUSSION

The central role of serum lipoproteins in the diagnosis and treatment of disorders of lipid metabolism (1) has led to a search for improved analytical techniques.

TABLE 1 SERUM LIPID AND LIPOPROTEIN CONCENTRATION

Subject and Age	Cholesterol	Triglyceride	Total Lipids		Lipoprotein Analysis: By Electrophoresis, Agarose	By Electrophoresis, Paper	By Analytical Ultracentrifugation*	By Refractometry after Ultracentrifugation
	mg/100 ml	*mg/100 ml*	*mg/100 ml*		*CDU*	*IU*	*mg/100 ml*	*mg/100 ml*
G.H. 23	152	70	490	Chylo or $S_f > 400$	0	16.0	1.8	
				β-LP or LDL	73	78.5	228.6	222.1
				Pre-β-LP or VLDL	30	22.0	79.3	78.3
				α-LP or HDL	45	32.0	310.1	
S.H. 29	170	67	480	Chylo or $S_f > 400$	0	27.0	0.5	
				β-LP or LDL	80	75.0	221.2	250.2
				Pre-β-LP or VLDL	23	11.0	22.8	66.0
				α-LP or HDL	55	33.0	376.1	
S.R. 27	155	64		Chylo or $S_f > 400$	1	12.0	0	
				β-LP or LDL	109	78.0	317.1	315.5
				Pre-β-LP or VLDL	26	23.0	49.8	61.1
				α-LP or HDL	37	20.0	202.0	
D.H. 28	132	81	486	Chylo or $S_f > 400$	0	19.0	0.5	
				β-LP or LDL	80	96.5	219.5	243.7
				Pre-β-LP or VLDL	24	22.0	35.0	69.7
				α-LP or HDL	34	23.5	197.2	
A.T. 27	126	29	404	Chylo or $S_f > 400$	0	16.0	0.1	
				β-LP or LDL	61	74.5	191.0	181.2
				Pre-β-LP or VLDL	10	7.0	4.1	12.4
				α-LP or HDL	43	33.5	265.8	
E.T. 45	261	44	710	Chylo or $S_f > 400$	0	17.0	0.5	
				β-LP or LDL	169	107.0	565.9	621.4
				Pre-β-LP or VLDL	12	13.0	5.4	27.8
				α-LP or HDL	49	38.0	302.9	
D.W. 66	260	133		Chylo or $S_f > 400$	0	13.0	0.5	
				β-LP or LDL	180	100.0	587.2	573.7
				Pre-β-LP or VLDL	39	20.0	89.7	109.7
				α-LP or HDL	63	27.0	347.7	
H.H. 65	278	73	874	Chylo or $S_f > 400$	1	36.0	1.8	
				β-LP or LDL	173	112.0	551.0	550.7
				Pre-β-LP or VLDL	19	13.0	40.4	57.6
				α-LP or HDL	55	39.5	333.7	
R.F. 51	198	203		Chylo or $S_f > 400$	5	26.0	16.6	
				β-LP or LDL	131	80.5	440.5	405.2
				Pre-β-LP or VLDL	70	41.0	174.2	226.1
				α-LP or HDL	40	15.0	231.0	
R.P. 56	218	255		Chylo or $S_f > 400$	2	19.0	6.2	
				β-LP or LDL	144	71.0	453.3	467.4
				Pre-β-LP or VLDL	101	40.0	248.7	295.4
				α-LP or HDL	36	27.0	224.2	

All subjects were male except D.W. and U.D.
* Chylomicrons were determined by NCH analysis.
† Determination of LDL by this method is invalid in the presence of a large excess of VLDL.

Semiquantitative electrophoretic methods have been developed (3, 5, 16–18), but these have permitted determination only of relative quantities of lipoproteins; each fraction was expressed as a percentage of total lipid-staining material. Estimates of lipoprotein concentration have been made from the product of the total serum lipid concentration and the percentage of each electrophoretic fraction, but the method for determining total lipids was tedious (19) or relatively imprecise (20). Moreover, the results were not verified by comparison with data from the analytical ultracentrifuge. Correlations between values obtained by electrophoresis on agarose and those of the ultracentrifuge have been reported (21), but the agarose LPE technique was not truly quantitative because the units of dye uptake were not corrected for variations in staining capacity.

A quantitative technique for the electrophoresis of serum lipoproteins on agarose gel is described in this report. The method is simple and inexpensive, and provides satisfactory reproducibility. Comparison of values obtained by this method with those of the analytical ultracentrifuge shows high correlations between (*a*) β-LP and LDL, (*b*) pre-β-LP and VLDL, and (*c*) α-LP and HDL. If analytical ultracentrifugation is considered to be the primary standard technique, then the standard error of estimating LDL and VLDL by

TABLE 1 (*continued*)

Subject and Age	Cholesterol	Triglyceride	Total Lipids		Lipoprotein Analysis: By Electrophoresis, Agarose	By Electrophoresis, Paper	By Analytical Ultracentrifugation*	By Refractometry after Ultracentrifugation
	mg/100 ml	*mg/100 ml*	*mg/100 ml*		*CDU*	*IU*	*mg/100 ml*	*mg/100 ml*
C.V. 57	212	247		Chylo or $S_f > 400$	1	13.0	1.0	
				β-LP or LDL	122	73.0	348.4	328.1
				Pre-β-LP or VLDL	110	46.0	280.4	330.2
				α-LP or HDL	52	26.5	354.3	
W.T. 42	221	490	1080	Chylo or $S_f > 400$	0	39.0	13.9	
				β-LP or LDL	105	73.0	330.9	290.2
				Pre-β-LP or VLDL	183	73.0	513.2	625.1
				α-LP or HDL	24	13.0	185.0	
B.S. 50	273	241		Chylo or $S_f > 400$	0	37.0	3.7	
				β-LP or LDL	175	105.5	536.6	519.3
				Pre-β-LP or VLDL	78	33.0	185.0	259.1
				α-LP or HDL	59	42.5	390.7	
J.V. 34	310	342		Chylo or $S_f > 400$	1	17.0	6.1	
				β-LP or LDL	161	92.0	593.5	536.6
				Pre-β-LP or VLDL	104	51.0	324.5	382.3
				α-LP or HDL	55	25.0	405.0	
J.H. 62	256	766	1525	Chylo or $S_f > 400$	0	50.0	44.8	
				β-LP or LDL	100	80.5	359.4	258.5
				Pre-β-LP or VLDL	273	97.0	817.0	946.3
				α-LP or HDL	35	18.5	303.5	
B.H. 37	254	957		Chylo or $S_f > 400$	6	46.0	280.5	
				β-LP or LDL			277.7	†
				Pre-β-LP or VLDL	348	84.0	927.4	1262.2
				α-LP or HDL	34	26.0	204.8	
C.K. 47	304	1293		Chylo or $S_f > 400$	2	41.0	209.3	
				β-LP or LDL	83	50.0	210.5	287.8
				Pre-β-LP or VLDL	486	127.0	1238.9	1411.8
				α-LP or HDL	25	11.0	153.8	
F.R. 36	282	1305	2246	Chylo or $S_f > 400$	41	150.0	566.5	
				β-LP or LDL			349.5	†
				Pre-β-LP or VLDL	416	129.0	820.0	981.3
				α-LP or HDL	43	20.0	210.9	
F.M. 55	282	1981		Chylo or $S_f > 400$	172	67.0	1006.0	
				β-LP or LDL			457.3	†
				Pre-β-LP or VLDL	372	84.0	726.5	1341.6
				α-LP or HDL	48	44.0	271.8	
U.D. 43	409	3758	5892	Chylo or $S_f > 400$	24	114.0	869.0	
				β-LP or LDL			324.2	†
				Pre-β-LP or VLDL	1349	249.0	3810.4	4036.5
				α-LP or HDL	28	12.0	247.3	

agarose LPE is 8 and 9% of the lipoprotein concentrations, respectively. These figures apply to a heterogeneous group of normal and moderately hyperlipidemic samples, i.e., triglyceride concentration ranging from 29 to 766 mg/100 ml and cholesterol concentration ranging from 126 to 310 mg/100 ml. It should be noted, however, that no attempt has been made to study the effects of changing fatty acid composition or the abnormal lipoprotein of biliary obstruction.

The standard errors for estimating LDL or VLDL by paper LPE are more than double those for the agarose technique. The superiority of agarose over paper stems from (*a*) the greater resolution of the lipoprotein classes, (*b*) the absence of trail from all sera except a few of type V pattern, and (*c*) the advantage of a transparent medium for more precise densitometry and for greater sensitivity.

The correlations between the values obtained by agarose LPE and analytical ultracentrifugation are considerably higher than those previously reported (21) for the standard Noble agarose LPE technique (8). Three major modifications of the Noble technique are responsible:

1. The use of a narrow agarose strip allows the entire width of the stained area to be scanned by the Analytrol. This eliminates the large variances due to uneven sample

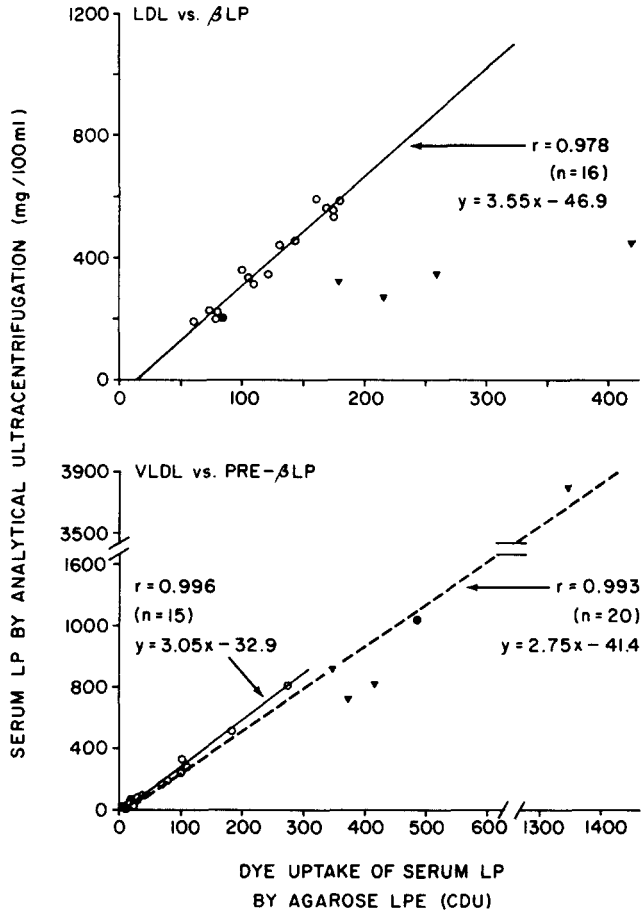

FIG. 4. The relationship between lipoprotein determinations by analytical ultracentrifugation and agarose LPE values. Analyses of LDL are compared to those of β-LP in the upper portion of the figure. The four sera from type V individuals with striking elevations of pre-β-LP associated with chylomicrons and trail are depicted as solid triangles. The regression line and correlation coefficients represent the remaining 16 samples. The values for VLDL and pre-β-LP are shown in the lower portion of the figure. Regression lines for the entire group (dashed line) and for the 15 individuals whose serum triglyceride concentrations were 766 mg/100 ml or less (solid line) are shown. The solid triangles represent the type V individuals, mentioned above, and the solid circle depicts an additional type V serum which contained chylomicrons and high pre-β-LP, but no trail.

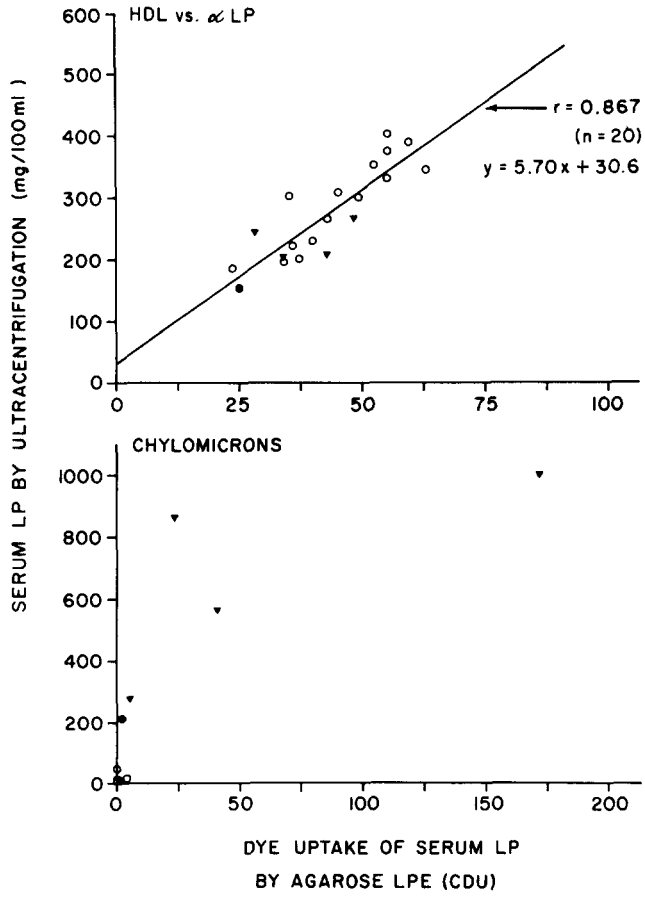

FIG. 5. The relationship between lipoprotein determinations by ultracentrifugation and agarose LPE values. The symbols used are the same as those in Fig. 4.

application which were observed in preliminary experiments in this laboratory using the wide strips specified by Noble (8).

2. Staining with Oil Red O at 40°C for 24 hr provides more consistent and complete uptake of dye. Lower temperatures and shorter time intervals proved to be less satisfactory. Although dye uptake increased for at least 72 hr, 88% of the 72-hr uptake occurred during the first 24 hr. This interval was chosen for this quantitative method because it consistently provides near-maximal uptake. Shorter intervals are satisfactory for qualitative electrophoretic techniques because dye uptake occurred at about the same rate in each lipoprotein fraction.

3. The use of a standard serum preparation allows correction for variations in staining capacity of the dye solution. Use of this correction factor reduced the variance of dye uptake by 50% in an 8-month series of analyses of aliquots from the frozen serum pool. It is assumed, though as yet unproven, that once the standard serum has been frozen, no further alterations occur during continuous storage at −70°C. The development of an artificial standard in place of the serum pool might prove superior in this regard, and it would provide a better means for standardization of results from different laboratories.

In the current report, one aliquot of the pooled standard serum was used to correct for variations in staining (the standard strips), and a second aliquot was used as an internal standard for quality control (the 51 electrophoretic determinations). Inclusion of such an internal standard is recommended for general use of the method; preferably the two types of standards should be aliquots from different serum pools.

Total corrected dye uptake measured by the current agarose LPE technique is highly correlated with the

TABLE 2 CORRELATION COEFFICIENTS FOR THE FIVE METHODS

	Lipoprotein or Lipid		
	Pre-β-LP or VLDL or TG	β-LP or LDL or Chol	α-LP or HDL
Number of subjects	20	16*	20
Correlation coefficients			
Agarose LPE			
vs. analytical ultracentrifugation	0.993	0.978	0.867
vs. paper LPE	0.971	0.685	0.708
vs. Chol or TG	0.972	0.673	
vs. refractometry	0.994	0.972	
Paper LPE			
vs. analytical ultracentrifugation	0.956	0.691	0.786
vs. Chol or TG	0.942	0.220	
vs. refractometry	0.961	0.675	
Analytical ultracentrifugation			
vs. Chol or TG	0.947	0.680	
vs. refractometry	0.990	0.958	
Chol or TG			
vs. refractometry	0.977	0.677	

LPE, lipoprotein electrophoresis; Chol, serum cholesterol concentration; TG, serum triglyceride concentration; refractometry, determined by refractive index after preparative ultracentrifugation. All correlations are statistically significant ($P < 0.01$) except for that between β-LP determined by paper LPE and serum cholesterol concentration (NS).

* The four sera exhibiting type V pattern and trail on agarose LPE have been omitted. The correlation coefficients do not apply to such individuals.

TABLE 3 STANDARD ERRORS OF ESTIMATING SERUM LP CONCENTRATION

	Lipoprotein or Lipid		
	Pre-β-LP or VLDL or TG	β-LP or LDL or Chol	α-LP or HDL
Number of subjects	15*	16†	20
Mean lipoprotein concentration by analytical ultracentrifugation (mg/100 ml)	191	385	276
Standard error of estimating lipoprotein concentration (mg/100 ml)‡			
by agarose LPE	18	32	38
by paper LPE	41	111	48
by Chol or TG	24	113	
by refractometry	14	44	

LPE, lipoprotein electrophoresis; TG, serum triglyceride concentration; Chol, serum cholesterol concentration; refractometry, determined by refractive index after preparative ultracentrifugation.

* The five sera with TG above 766 mg/100 ml have been omitted. The standard error values do not apply to individuals with higher TG values.

† The four sera exhibiting type V pattern and trail on agarose LPE have been omitted. The standard error values do not apply to such individuals.

‡ Calculation assumes the values obtained by analytical ultracentrifugation to be correct.

total serum lipid concentrations measured gravimetrically, in accord with previous observations (22). The correlation is present despite the widely varying lipoprotein distributions of the sera studied and despite the facts that the affinity for Oil Red O varies with the lipid under study (3, 22) and that different lipoproteins have dissimilar lipid compositions (23). In this study, the average dye uptakes of the lipid portions of the three major lipoproteins were similar: dye uptake in CDU per mg of lipid was calculated to be 2230 for pre-β-LP, 2030 for β-LP, and 1560 for α-LP.[1]

The very high correlation between serum triglyceride concentration and pre-β-LP is a reflection of the fact that this class of lipoproteins contains 50–60% triglyceride by weight, whereas HDL and LDL contain 12% or less (23). The correlation coefficient of 0.972 is similar to that previously reported (11). Serum cholesterol concentration and β-LP were also correlated ($r = 0.673$), reflecting the fact that LDL contains 46% cholesterol (23). The correlation is much lower than that between triglyceride and pre-β-LP because HDL and VLDL also contain relatively large proportions of cholesterol (22% and 13–18%, respectively) (23).

The correlations between the agarose LPE values and those of the analytical ultracentrifuge are sufficiently high to provide a means for converting CDU to mg/100 ml. The preferred approach is the analysis of a series of about 20 sera by agarose LPE and analytical ultracentrifugation. Quantitation of subsequent samples is accomplished directly from the relationship of the two sets of values. Precision refractometry after preparative ultracentrifugation can be substituted for analytical ultracentrifugation if the latter technique is unavailable. If precision refractometry is also not practicable, then the regressions determined in this study can be used to provide quantitation. This indirect method must be validated by determining the relationship between the corrected dye units in the new laboratory and those presented in this paper. In practice, this is best accomplished by a comparison between the values obtained by agarose LPE and gravimetric analysis. The Appendix contains a detailed description of this procedure. These standardization methods are valid for sera of widely varying lipid content and provide a practical means for determination of lipoprotein concentrations by serum electrophoresis.

[1] Calculated by using the mean values for dye uptake and ultracentrifugal lipoprotein concentration for the 20 sera, and the assumption that pre-β-LP is 88% lipid, β-LP is 78% lipid, and α-LP is 50% lipid (23).

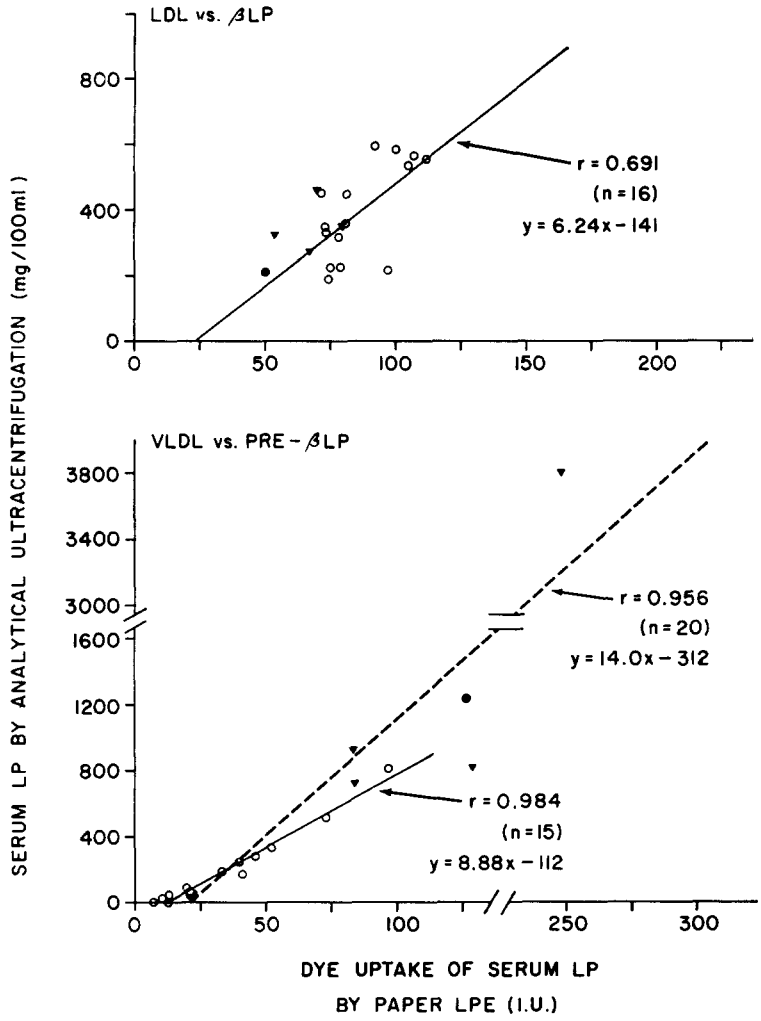

FIG. 6. The relationship between lipoprotein determinations by analytical ultracentrifugation and paper LPE values.

APPENDIX

Use of Gravimetric Total Lipid Analyses for Standardization of Quantitative Agarose LPE

For technical reasons, the units of dye uptake reported in this paper (CDU) will not be identical to the units in other laboratories (CDU*). If analytical ultracentrifugation is unavilable, CDU* can be converted to CDU by performing gravimetric total lipid analyses as described below.

Two large serum pools are stored at −70°C in 5-ml aliquots; each aliquot will be thawed only once and discarded after use. One is used to prepare the "standard strips," which are stained each day to allow the raw units of dye uptake (DU*) to be converted into units corrected for staining variance (CDU*). This correction normalizes all data to an arbitrarily selected reference day (day 0). The second serum pool is electrophoresed each day as an internal standard; total CDU* for this pool must fall within the acceptable range established in the first 20 analyses (mean ± 2 SD).

10 sera are obtained on day 0, ranging in total lipid content from about 400 to 1600 mg/100 ml. Total lipid levels are determined gravimetrically and agarose LPE is carried out. The data are described by the regression equation

$$y^* = m^*x^* + b^*$$

where

y^* = gravimetric lipid content (in mg/100 ml)

x^* = total LP by agarose LPE (in CDU*)

m^* = slope

b^* = intercept

The intercept should be close enough to the origin to allow its omission without an important error. Then,

$$m^* = \frac{y^*}{x^*}$$

In Fig. 8 the total lipid concentration is related to the total LP content by the equation

$$y = mx + b$$

where

y = gravimetric lipid content (in mg/100 ml)

x = total LP by agarose LPE (in CDU)

m = slope = 3.42

b = intercept = −6.6

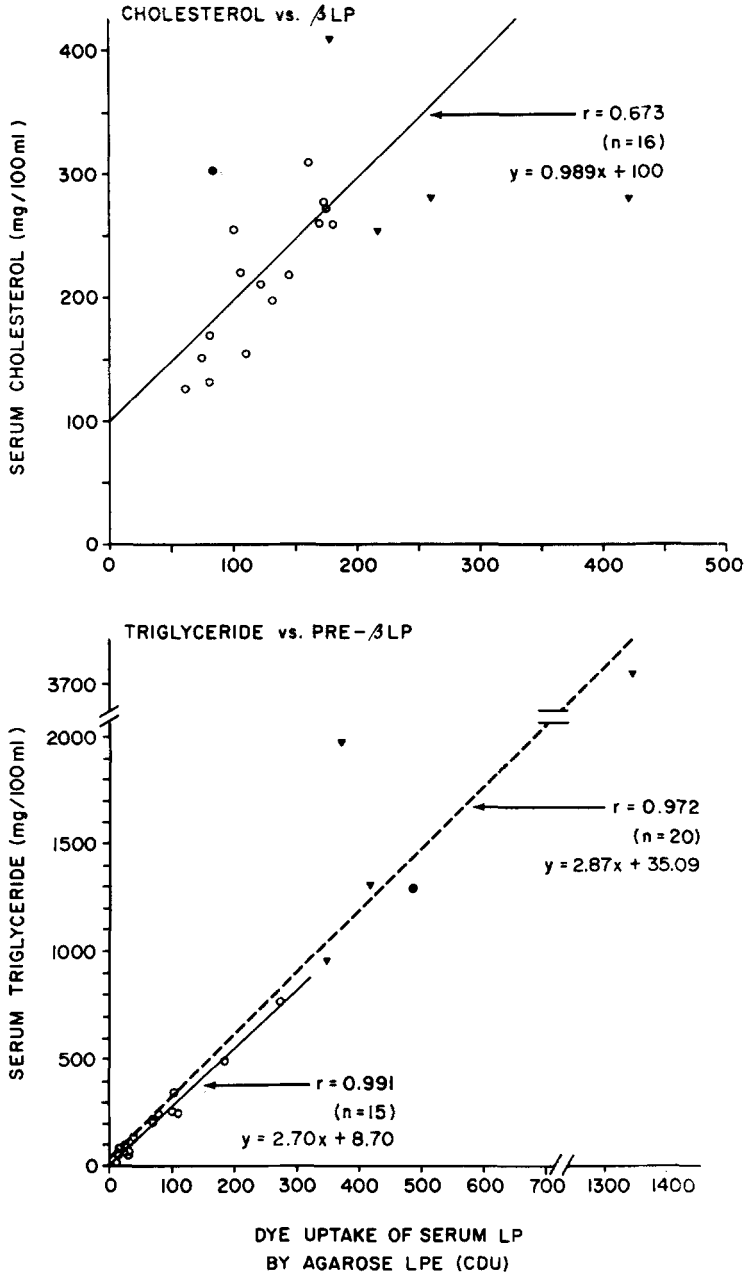

FIG. 7. The relationship between chemical lipid determinations and agarose LPE values.

The intercept of −6.6 is sufficiently small to be neglected without serious error. Then,

$$m = 3.42 = \frac{y}{x}$$

The relationship between the mean total lipid concentration and the mean total LP level is assumed to be the same for any randomly selected 10 or more sera. This assumption is supported by our experience with four groups of sera analyzed over a 16-month period, three of which are included in Fig. 8. It follows from this assumption that any difference between m^* and m must be due to the difference between CDU* and CDU, or

$$\frac{m^*}{m} = \frac{\text{CDU}}{\text{CDU}^*}$$

thus,

$$\text{CDU} = k \times \text{CDU}^*$$

where

$$k = \frac{m^*}{m} = \frac{m^*}{3.42}$$

and should remain constant indefinitely. CDU are transformed to mg/100 ml using the regression equations given in Figs. 4 and 5.

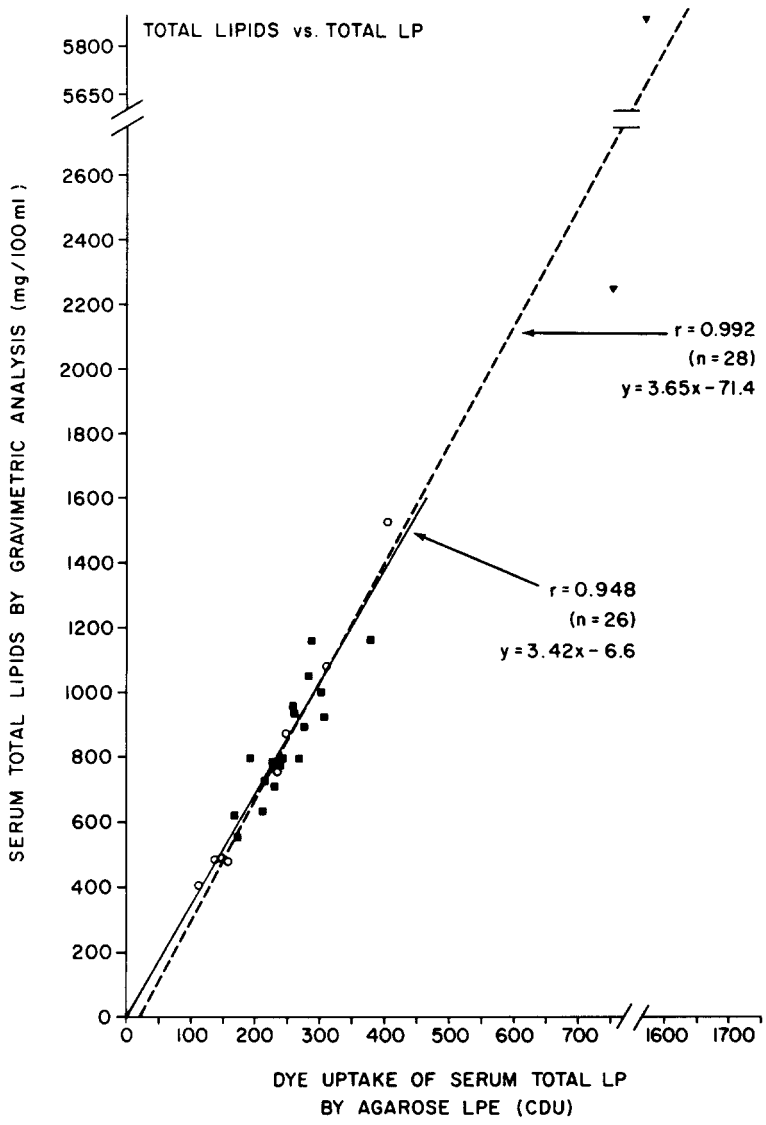

FIG. 8. The relationship between total lipid content determined gravimetrically and total lipoprotein measured by agarose LPE. Only 10 of the 20 sera in Table 1 were analyzed gravimetrically (open circles and solid triangles). Data from 18 additional subjects are shown as solid squares. The regression line for all 28 sera is shown as a dashed line, and that for the 26 individuals after exclusion of the two markedly hyperlipidemic sera is shown as a solid line.

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