THE α$_2$ LIPOPROTEINS OF HUMAN SERUM. CORRELATION OF ULTRACENTRIFUGAL AND ELECTROPHORETIC PROPERTIES

Henry G. Kunkel, Rodes Trautman

Research Article

THE α_2 LIPOPROTEINS OF HUMAN SERUM. CORRELATION OF ULTRACENTRIFUGAL AND ELECTROPHORETIC PROPERTIES

By HENRY G. KUNKEL AND RODES TRAUTMAN

(*From The Rockefeller Institute for Medical Research, New York, N. Y.*)

Two main groups of lipoproteins have been characterized in the serum of normal adults. These have been termed the α_1 and the β_1 lipoproteins on the basis of their electrophoretic mobility (1–5). The broad spectrum of low density lipoproteins that have been observed particularly in patients with arteriosclerosis by means of the analytical ultracentrifuge (6) have not been classified in terms of electrophoretic mobility because of the difficulties in characterization by classical electrophoretic procedures (7–9). Nevertheless, these lipoproteins have usually been considered as β lipoproteins.

Electrophoresis on filter paper, obviating solution density problems and permitting lipid analyses of the more stable whole serum has revealed the two main "alpha" and "beta" lipoprotein components. Another fraction observed at the site of origin is considered to represent adsorbed lipoproteins (4, 5) particularly the chylomicrons (10).

This fraction is not observed by zone electrophoresis in a starch supporting medium, but an additional lipoprotein peak in the α_2 region has been found in the sera of fasting normal (4, 11) and arteriosclerotic (12) individuals. Relatively little information is available regarding this component except that it was shown to rise after meals, particularly fatty meals, as indicated by phospholipid determinations (11).

The present report represents an attempt to define the α_2 lipoproteins further in terms of both zone electrophoretic and density ultracentrifugal properties. This fraction was examined in the sera of a limited group of fasting normal and arteriosclerotic individuals and was found to consist primarily of lipoproteins less dense than d_4^{20} 1.018.

METHODS

Preparative ultracentrifugation of the human sera was carried out in accordance with the basic principles of differential density centrifugation of the lipoproteins as described by Lindgren, Elliott, and Gofman (13) and de Lalla and Gofman (14) with very minor modifications. The Spinco swinging bucket rotor SW39L was used in the Spinco Model E ultracentrifuge instead of the angle preparative rotors. Various low molecular weight solutes were used to effect density increments for isolation of low density fractions and initially layered solutions were routinely employed.

The purposes of this study were met by a gross fractionation with no density gradient of solvent but rather one due to the distribution of the serum albumin after the centrifugation to effect sufficient compartmentization to quantitatively maintain the separation of the serum into top and bottom fractions. Very slow acceleration and deceleration were employed, approximately 1000 rpm per min. In the Model LH preparative machine the insertion of a drive current meter enabled acceleration and braking at a given constant current similar to the operation of the Model E. This required constant manual slow movement of the speed dial. In general the Model L machine proved less satisfactory for accurate work with the swinging bucket rotor because of disturbance just prior to stopping.

In order to be able to carry out electrophoresis of the ultracentrifugal fractions without dialysis, the density increments were made with D_2O or sucrose solutions or barbital buffer pH 8.6, ionic strength 0.05, with rutin added to give fractions containing 1 to 10 μg. per ml. rutin (15). For preparation of lipoproteins less dense than 1.20 g per ml., a saturated stock solution of KBr and $NaNO_3$ (1.58 μg. per ml.) was used to effect the density increase and the angle rotor 40.3 was used. This enabled 1.5 ml. of 1.20 solvent to be layered initially on the mixture of 3.0 ml. serum and 1.5 ml. of the 1.58 μg. per ml. stock solution. Divided sector center pieces were used in the analytical ultracentrifugation.

The supporting media employed for the zone electrophoretic separations were thoroughly washed with at least 4 volumes of buffer over a filter prior to pouring the starch block. This was found to be important particularly with the 0.05 ionic strength buffer to prevent pH changes during the course of the experiments due to the ionic exchange at the surface of the granules. The procedures used for preparing the self supporting block were similar to those described previously (11, 16). The entire system was encased in polyethylene sheeting and rested on a glass plate. No upper glass plate was employed.

The sera or lipoprotein fractions were usually diluted

with ⅓ volume of buffer but were not dialyzed except where the high density of 1.2 was used because of the adverse effects of this procedure on the state of the lipoproteins. Lipid free human albumin was always added to the isolated lipoprotein fractions to bind fatty acids which would otherwise increase lipoprotein mobilities (17). Initial experiments without albumin demonstrated that such mobility increases commonly occurred. The separation was carried out employing a field strength of approximately 8 volts per cm. and special care was taken to avoid heating. The temperature in the starch block was kept at $10 \pm 5°$ C. Absolute mobilities were not calculated; instead the standard Greek letter identification relative to albumin was used.

Following the separation, starch segments approximately $6 \times 1.2 \times 1.2$ cms. in size were dried slightly for 5 minutes in front of a fan and stirred with 20 ml. of organic solvent. This mixture was poured over a coarse grade ground glass filter and filtered under suction. An additional 10 ml. of organic solvent was then added to wash and displace the material left in the starch and on the filter. Alcohol-ether (3–1) was frequently employed as the solvent and recoveries of phospholipid of the order of 95 per cent could usually be obtained. Occasional recoveries were as low as 80 per cent but these could be improved if extraction was carried out immediately after separation. Alcohol-ether-chloroform (3-1-1) was also used as solvent in a number of experiments and good recoveries were obtained. This solvent gave a slightly higher phosphorus base line with the starch alone but in general proved quite suitable. The phospholipid content was measured and converted to milligrams as previously described (4). Starch presents a number of hazards in regard to the extraction of lipids and a wide literature is available on the affinity of certain lipids for the starch granule particularly in the presence of polar solvents (18). However, with certain precautions good recoveries at least of cholesterol and phospholipid were achieved. Those experiments where poor recoveries were obtained are not included in this report although they did furnish some information particularly relating to the mobility of isolated fractions. The polyvinyl resin (Geon 400×65)[1] was used as the supporting medium in a number of experiments. This is a non-swelling medium in contrast to starch. The separation of the serum proteins was slightly inferior to that obtained with the starch medium and dilution of the serum with buffer was particularly important. No problems were encountered in extraction and recoveries of approximately 95 per cent were found. The protein distribution was obtained by analyses of a separate serum sample fractionated on the same starch or polyvinyl block and partitioned into identical size segments as for the lipid analyses.

RESULTS

Chemical analyses on the segments obtained by starch electrophoresis of whole serum at pH 8.6
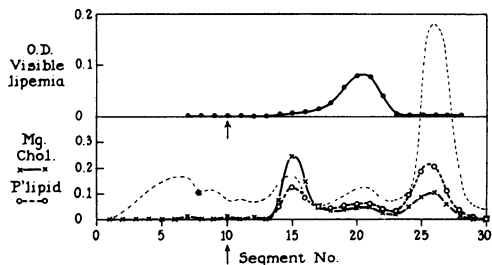
FIG. 1. ZONE ELECTROPHORESIS IN A STARCH SUPPORTING MEDIUM OF POST-PRANDIAL SERUM

The upper curve represents the visible lipemia in eluates of different fractions. The lower curves show the distribution of cholesterol and phospholipid in the different lipoproteins. The protein distribution is also illustrated by the finely dashed curve. Segment 15 contains the peak of the β_1 lipoprotein; segment 21, the peak of the α_2; segment 26, the peak of the α_1. The arrow indicates the origin; the anode is at the right.

$\Gamma/2$ 0.05 are shown in the lower part of Figure 1. The protein curve serves to define the mobility of the lipid distribution shown as both total cholesterol and phospholipid. The albumin is in segments, 23–29, gamma globulin is in 2–11. This figure shows that the lipoproteins are distributed among three major groups. In this buffer the fastest lipoprotein peak falls more directly under the albumin than in buffer at 0.1 ionic strength. The α_1 protein peak is also invisible at this buffer concentration (11). The next lipid peak falls on the α_2 globulin protein peak. However, the maximum of this intermediate peak is not always half way between the other two and does show some variation with age of the serum and conditions of electrophoresis. The slowest lipoprotein can have its maximum anywhere in the space occupied by the β-globulin. Despite the variation in position it is termed here the β_1 lipoprotein region. The first experiment on the nature of the lipoproteins in the α_2 region is shown in the upper part of Figure 1. The optical density (O.D.) was measured on saline eluates of the segments. This shows that the visible lipemia in the post-prandial serum migrates in the α_2 region. The slight elevation in the β region can be larger and variations in the lipemia pattern were obtained in different experiments even with the same serum. The lipemia sometimes migrated differently in freshly drawn serum than in serum where the lipemic particles had been resuspended after settling at the surface. These observations as well as others (19) constitute evi-
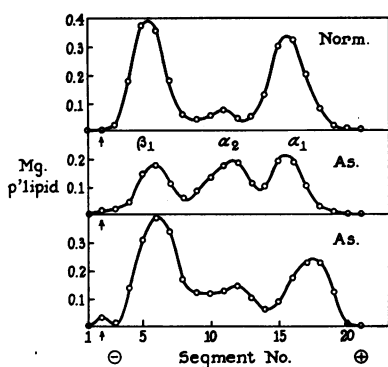
FIG. 2. LIPOPROTEIN CURVES OBTAINED BY PHOSPHO-
LIPID ANALYSIS OF ELECTROPHORETIC FRACTIONS FROM A
NORMAL SERUM AND FROM THE SERUM OF TWO PATIENTS
WITH ARTERIOSCLEROSIS ILLUSTRATING VARIATIONS IN
THE RELATIVE SIZE OF THE α_2 COMPONENT

FIG. 4. PHOSPHOLIPID CURVES OF STARCH ELECTRO-
PHORESIS FRACTIONS FROM WHOLE SERUM AND THE TOP
AND BOTTOM FRACTIONS OF THE SAME SERUM SEPARATED
IN THE ULTRACENTRIFUGE AT d_4^{20} 1.006

The top fraction migrates in the α_2 position and the α_2
component disappeared in the bottom fractions.

dence that adsorption is playing only a minor role
in this medium even with these large particles since
the turbidity boundary in free electrophoresis also
usually migrates with the α_2 globulin (20).

Three fasting sera have been chosen in Figure 2
to illustrate the range in concentration of the α_2
lipoprotein. In the upper curve a serum from a
normal young male is shown with about 4 per cent
of the phospholipid in the α_2 region. The middle
and lower curves are from arteriosclerotic individu-
als with 38 per cent and 21 per cent, respectively,
in the α_2 region. The recovery, calculated in the
upper and lower separation, was 95 and 91 per
cent, respectively.

That the α_2 peak is not characteristic of starch
alone is shown in Figure 3. The upper curve is
a pattern from a fasting serum from another pa-
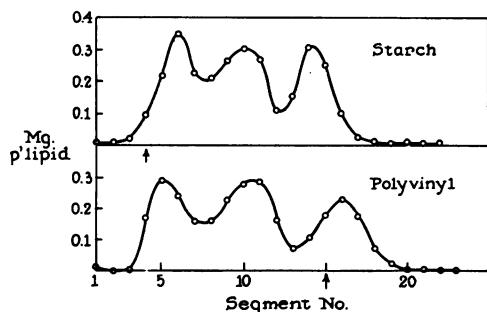tient with arteriosclerosis separated in starch and

FIG. 3. COMPARISON OF THE LIPOPROTEIN CURVES OB-
TAINED BY ELECTROPHORETIC SEPARATION OF THE SAME
ARTERIOSCLEROTIC SERUM IN TWO DIFFERENT SUPPORTING
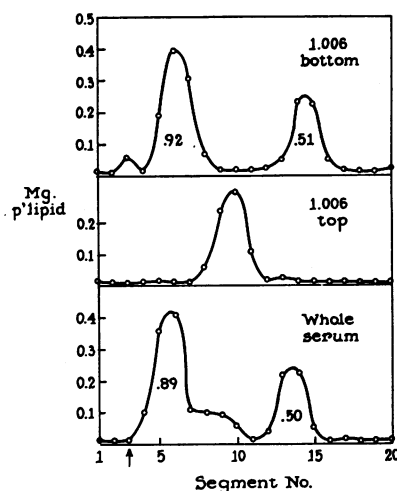MEDIA, STARCH AND POLYVINYL—1.5 ML. OF SERUM USED
IN EACH CASE

the lower curve in polyvinyl. The general shape
and amount of the various peaks are quite com-
parable despite a marked difference in electro-
osmotic flow as indicated by the different locations
of the point of origin in the two patterns of Fig-
ure 3. Evidence was obtained for some retarda-
tion of the β_1 lipoprotein in traversing these porous
media. In the starch the maxima usually appeared
on the slower side of the β-globulin protein peak
but in the polyvinyl in which the migration rela-
tive to the medium actually was in the opposite di-
rection, the maximum was usually found on the
faster mobility side of this β-peak.

In Figure 4 is shown the phospholipid distribu-
tion for still another whole serum from a patient
with arteriosclerosis along with the top and bottom
ultracentrifugal fractions separated at the serum's
own protein-free density d_4^{20} 1.006. The top frac-
tion was from 2.5 ml. of serum compared to the
whole and bottom fractions from 1 ml. of serum.
When corrections were made for these volume dif-
ferences 0.26 mg. phospholipid was found in the
top fraction and 0.25 mg. disappeared from the α_2
component of the whole serum. The three pat-
terns in Figure 4 were obtained on the same starch
block. This shows that in this serum at least 95
per cent of the α_2 region is accounted for by lipo-
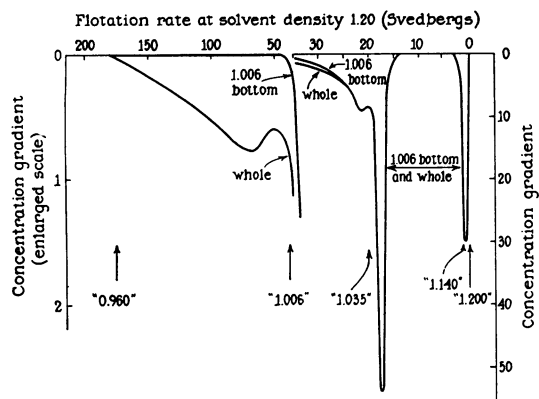proteins less dense than 1.006. The top and bot-

FIG. 5. ANALYTICAL ULTRACENTRIFUGE SCHLIEREN PATTERN OF THE LIPOPROTEINS FROM THE WHOLE SERUM AND THE 1.006 BOTTOM FRACTION ILLUSTRATED IN FIGURE 4

Lipoprotein molecular density is indicated in quotation marks; concentration gradient in arbitrary units. Note the reduction to zero of the light material with flotation rate greater than 50, thus indicating the point of separation in the preparative ultracentrifuge.

tom fractions together thus serve as a check on the electrophoretic procedure. The total phospholipid in the β_1 and α_1 components is indicated in Figure 4 and shows that there was little change in these following centrifugation.

The analytical ultracentrifuge was used as a control for this quantitative preparative fractionation. Figure 5 shows the analytical ultracentrifugal patterns of the lipoproteins from the top and bottom fractions of Figure 4 isolated and centrifuged at a density d_4^{20} 1.20. The actual photograph from
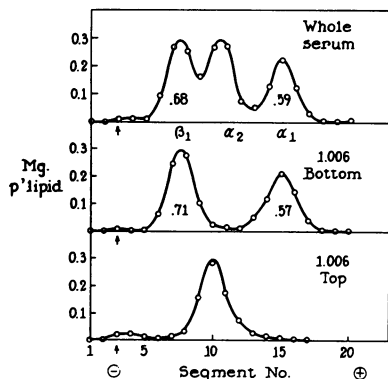

FIG. 6. LIPOPROTEIN CURVES SIMILAR TO THOSE OF FIGURE 4 BUT FOR A DIFFERENT ARTERIOSCLEROTIC SERUM WITH A LARGER α_2 COMPONENT

The top fraction illustrated is from another serum.

this experiment is not shown, but rather the measurements made on a two-dimensional comparator. Several frames were analyzed, and the concentration gradient distributions with respect to radius were converted to a distribution with respect to sedimentation rate (13). The pattern drawn in Figure 5 is idealized to the extent that some of the diffusion has been eliminated by extrapolation to the latest time picture.

Because of great individual variation of lipoprotein distribution in whole serum, fourteen sera were examined in this same manner, and all were found to yield top fractions of α_2 mobility. Figure 6 illustrates the whole serum pattern compared to that of the bottom fraction from a patient with arteriosclerosis having a relatively large α_2 lipoprotein component. The top fraction from another serum similarly high in α_2 is also shown.
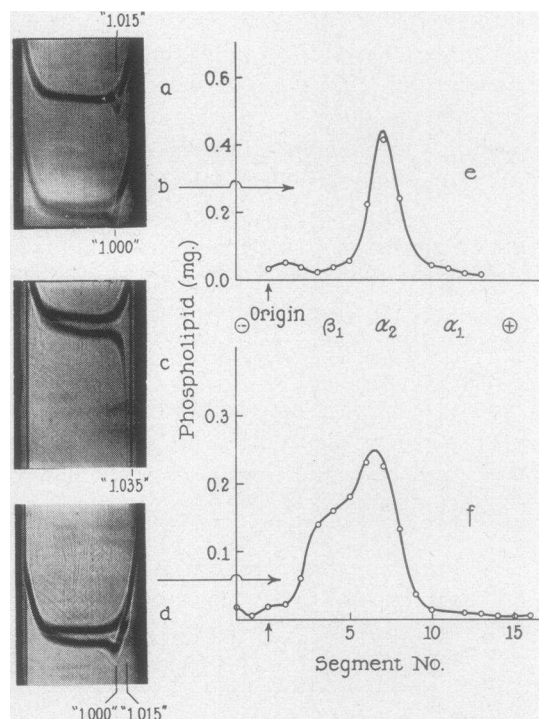

FIG. 7. CORRELATION BETWEEN ANALYTICAL ULTRACENTRIFUGE PATTERNS AND ELECTROPHORETIC DISTRIBUTION OF FRACTIONS ISOLATED BY PREPARATIVE ULTRACENTRIFUGATION

a, b, Second and top fractions from d_4^{20} 1.018,
c, d_4^{20} 1.07 top fraction,
d, Mixture of a and b,
e, Starch electrophoretic pattern of b showing only α_2,
f, Starch electrophoretic pattern of d showing α_2 and β_1 lipoproteins.

Studies were carried out to determine at what density the division between α_2 and β_1 occurred. Figure 7 shows an experiment at an increased density over the protein-free density of d_4^{20} 1.018. Solvent of the same density was layered at the top prior to centrifugation so that those molecules of about the same density as the solvent, 1.018, would not contaminate the top fraction. This top fraction is shown in Figure 7b, and as a control the second fraction containing the 1.015 to 1.018 molecules is shown in 7a. It has a slower flotation rate as expected. The relationship of this division to the lipoprotein spectrum of the whole serum can be seen by comparing with 7c. The electrophoresis of the top fraction is shown in 7e, being almost entirely α_2. In a separate preparation, the top and second fraction (above the albumin boundary) were mixed. This yielded the centrifugal pattern of Figure 7d, and the electrophoretic pattern of Figure 7f, which contains β_1 lipoprotein as well as the α_2. Thus the density 1.018 in this serum splits the α_2 and β_1 region. A d_4^{20}, 1.018 corresponds to Gofman $S_f 12$ provided the published flotation rate dependence on solvent density is universal (13).

Whereas no light lipoproteins (d_4^{20} less than 1.018) were found with β_1 mobility, it was observed that in three sera out of fourteen studied, removal of these lipoproteins still did not eliminate all of the α_2 region of the whole serum. An example is shown in Figure 8, which is the whole serum and the d_4^{20} 1.018 bottom fraction of the same serum which was shown in Figure 7. Here it can be seen that less than half of the α_2 region was removed at a preparative density of 1.018.
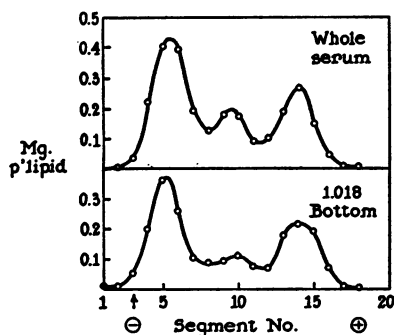
FIG. 8. LIPOPROTEIN PATTERNS OF A SERUM SHOWING ONLY PARTIAL REMOVAL OF THE α_2 COMPONENT AFTER SEPARATION AT d_4^{20} 1.018

Further results obtained by increasing the preparative density revealed that this remaining α_2 lipoprotein has a density between 1.02 and 1.07. This α_2 could be due to the d_4^{20} 1.05 ($S_{1.21}$ 20–25) component suggested by Lewis and Page (9). However, other sera which showed a distinct 1.05 component in the analytical ultracentrifuge did not show α_2 material of this density.

The phosphopeptide described by Hack (21) and Havel, Eder, and Bragdon (22) which accounts for 5 to 10 per cent of the serum phospholipid is not specifically separated from the α_1 lipoproteins in this procedure of electrophoresis of whole serum. Several sera were freed of lipids and lipoproteins less dense than 1.20 in a layered preparative method and pipetted deep to remove all top fractions. The bottom fractions were concentrated by ultrafiltration and analyzed. They showed a ratio of less than $\frac{1}{15}$ cholesterol to phospholipid. On electrophoresis, the amount in the α_2 region equivalent to the 1.5 ml. serum usually used was within the experimental error. Most, if not all, was in the α_1-albumin region, and hence the α_1 lipoprotein peak of whole serum combines this material with the high density lipoproteins.

DISCUSSION

Although the series studied was quite limited, it did serve to indicate that the procedure of zone electrophoresis employed here does not provide a preparative technique superior to density fractionation. It does appear that the very light lipoproteins ($d_4^{20} < 1.018$, $S_f > 12$) migrate as a distinct fraction in the α_2 region rather than as part of the β_1 lipoproteins. These lipoproteins that are so heterogeneous in respect to size appear relatively homogeneous electrophoretically.

The relationship of the electrophoretic zones observed in this study to lipoprotein components obtained by ultracentrifugal methods is shown in summary in Figure 9. The vertical axis is the relative concentration distribution in the absence of diffusion. The density fractionation distribution is shown inverted along the diagonal axis because of similarity to analytical flotation patterns at a high solvent density (d_4^{20} 1.2). It should be emphasized that Figure 9 is a somewhat idealized illustration and that the distribution of lipoproteins will differ considerably depending on the type of
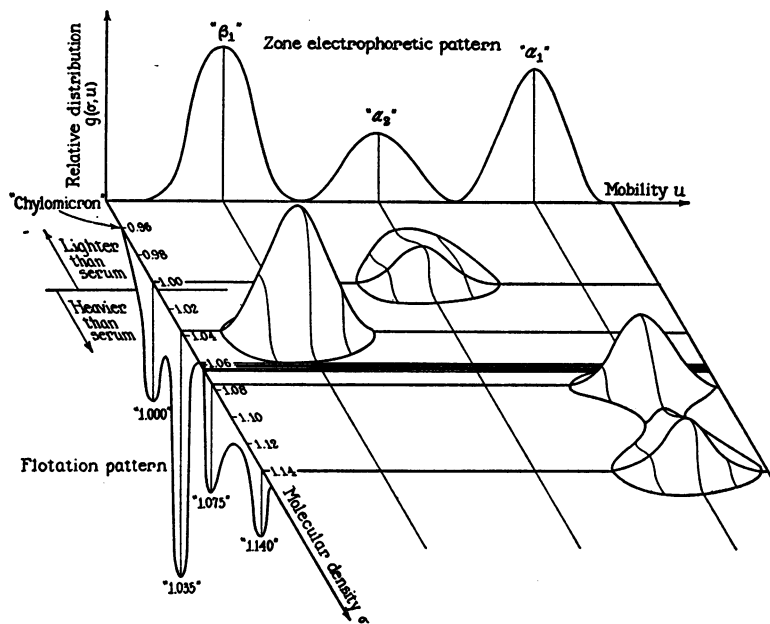
FIG. 9. THREE DIMENSIONAL CHART OF THE IDEALIZED DISTRIBUTION OF
LIPOPROTEINS IN SERUM

The vertical axis is the assumed relative concentration in the absence of
diffusion. The horizontal axis is the electrophoretic distribution at pH 8.6
$\Gamma/2$ 0.05. The diagonal axis is from preparative ultracentrifuge results in
a solvent density gradient from d_4^{20} 1.01 to 1.20.

measure which is used for the relative quantita-
tion of the lipoproteins; here phospholipid is im-
plied for both the mobility dimension and for
zones successively floated out of serum by in-
creasing the solvent density. Further subdivision
of the lipoproteins will also, undoubtedly, be ob-
tained with more refined technique and different
conditions of electrophoresis or centrifugation.
It also seems highly probable that lipoproteins in
pathological as well as some normal sera will not
always appear in the groups indicated in the three
dimensional diagram shown above. The yellow
lipoprotein described by Hunter in certain normal
sera (23) and the lipoproteins appearing post
heparin (13, 24) may be examples.

Variations in the gross chemical composition of
certain molecular density bands has been pointed
out (13, 22). Consideration of this further reso-
lution and the finding (4, 25) that the Cohn IV +
V + VI fraction in biliary cirrhosis contains ma-
terial of β_1 mobility and not the usual α_1, together
with the present observation (not shown in Fig-
ure 9) that the α_2 band can occasionally contain
lipids more dense than 1.018 point out the diffi-

culties involved in assessing the results of one
procedure in terms of another. The error involved
in using the electrophoretic term, "β lipoproteins,"
for the low density lipoproteins on the basis of
their solubility properties is apparent from the
present study.

The fasting sera in the present series which
showed a large α_2 fraction by zone electrophoresis
were all obtained from patients with arteriosclero-
sis and contained increased amounts of $S_f > 12$
lipoproteins. The number studied, however, was
too small to draw any conclusions regarding this
condition. The problem is made more difficult by
the distinct relation of this component to particu-
lar diets and variations regarding the time of the
fasting period. The high "β" to α_1 ratio reported
in the sera of patients with arteriosclerosis exam-
ined by paper electrophoresis (26, 27) and by
Cohn fractionation methods (28) is accounted for
in part by the inclusion of the α_2 group of lipo-
proteins in the "β" fraction obtained by these
procedures.

Determination of the neutral fat content of the
α_2 fraction presented a number of difficulties

mainly because of interference from the buffers used for the electrophoretic separation. However, a limited number of observations indicated that it was high in neutral fat relative to the α_1 and β_1 zones. This was to be expected from the known composition of the low density lipoproteins (22, 29). There also appeared to be a parallelism between the neutral fat content of the whole serum and the height of the α_2 peak. More extensive analyses of neutral fat and other special lipids were not carried out because it became apparent that these could be carried out more satisfactorily on fractions obtained directly by preparative ultracentrifugation.

SUMMARY

1. A separate lipoprotein component migrating in the α_2 region was observed following separation by zone electrophoresis in a starch or polyvinyl supporting medium. When expressed in terms of phospholipid this ranged in concentration from 4 to 40 per cent of the total lipoproteins of the fasting serum in a limited series of normal and arteriosclerotic individuals.

2. Isolation of top and bottom fractions by ultracentrifugation at different densities followed by electrophoresis indicated that the lipoproteins less dense than d_4^{20} 1.018 or with $S_f > 12$ migrated as α_2 proteins. Disappearance of the α_2 lipoprotein component from whole serum when these low density lipoproteins were removed substantiated the results with the isolated lipoproteins. Three out of 14 sera examined by combined ultracentrifugation and electrophoresis showed high density lipoproteins in the α_2 fraction in addition to those of the lighter group.

3. A three dimensional map of the serum lipoproteins based on the results of combined electrophoresis and ultracentrifugation is presented. The difficulties involved in correlating one isolation procedure with another in the case of lipoproteins are discussed.

REFERENCES

1. Oncley, J. L., Gurd, F. R. N., and Melin, M., Preparation and properties of serum and plasma proteins. XXV. Composition and properties of human β-lipoprotein. J. Am. Chem. Soc., 1950, 72, 458.
2. Gurd, F. R. N., Oncley, J. L., Edsall, J. T., and Cohn, E. J., The lipoproteins of human plasma. Disc. Far. Soc., 1949, 6, 70.
3. Fasoli, A., Le lipoproteine del plasma sanguigno; Published by Prenico Ganassini, Milan, Italy, 1951.
4. Kunkel, H. G., and Slater, R. J., Lipoprotein patterns of serum obtained by zone electrophoresis. J. Clin. Invest., 1952, 31, 677.
5. Nikkilä, E., Studies on the lipid-protein relationships in normal and pathological sera and the effect of heparin on serum lipoproteins. Scandinav. J. Clin. & Lab. Invest., 1953, 5, Suppl. 8.
6. Gofman, J. W., Jones, H. B., Lindgren, F. T., Lyon, T. P., Elliott, H. A., and Strisower, B., Blood lipids and human atherosclerosis. Circulation, 1950, 2, 161.
7. Trautman, R., and Gofman, J. W., Hydrodynamic factors in moving-boundary electrophoresis. An introduction to the electrophoretic study of lipoproteins. J. Phys. Chem., 1952, 56, 464.
8. Trautman, R., Clinical moving-boundary electrophoresis: Nonplanar difficulties due to the presence of lipoproteins in normal and pathological serum. Arch. Biochem. & Biophys., 1954, 53, 85.
9. Lewis, L. A., and Page, I. H., Electrophoretic and ultracentrifugal analysis of serum lipoproteins of normal, nephrotic and hypertensive persons. Circulation, 1953, 7, 707.
10. Swahn, B., Studies on blood lipids. Scandinav. J. Clin. & Lab. Invest., 1953, 5, Suppl. 9.
11. Kunkel, H. G., and Bearn, A. G., Phospholipid studies of different serum lipoproteins employing P$_{32}$. Proc. Soc. Exper. Biol. & Med., 1954, 86, 887.
12. Ackermann, P. G., Toro, G., and Kountz, W. B., Zone electrophoresis in the study of serum lipoproteins. J. Lab. & Clin. Med., 1954, 44, 517.
13. Lindgren, F. T., Elliott, H. A., and Gofman, J. W., The ultracentrifugal characterization and isolation of human blood lipids and lipoproteins, with applications to the study of atherosclerosis. J. Phys. & Coll. Chem., 1955, 55, 80.
14. de Lalla, O. F., and Gofman, J. W., Ultracentrifugal analysis of serum lipoproteins in Methods of Biochemical Analysis, 1954, Vol. 1, p. 459.
15. Ray, B. R., Davisson, E. O., and Crespi, H. L., Experiments on the degradation of lipoproteins from serum. J. Phys. Chem., 1954, 58, 841.
16. Kunkel, H. G., Zone electrophoresis in Methods of Biochemical Analysis, 1954, Vol. 1, p. 141.
17. Gordon, R. S., Jr., Interaction between oleate and the lipoproteins of human serum. J. Clin. Invest., 1955, 34, 477.
18. Schoch, T. J., The fractionation of starch. Advances Carb. Chem., 1945, 1, 247.
19. Carlson, L. A., Electrophoretic studies of serum lipoproteins. I. A description of apparatus and technique for their separation in starch medium. Acta chem. Scandinav., 1954, 8, 510.
20. Laurell, C. B., Preliminary data on the composition and certain properties of human chylomicrons. Scandinav. J. Clin. & Lab. Med., 1954, 6, 22.
21. Hack, M. H., A serine phosphate in human serum. Federation Proc., 1955, 14, 222.

22. Havel, R. J., Eder, H. A., and Bragdon, J. H., The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. J. Clin. Invest., 1955, 34, 1345.

23. Hunter, F. M., Description of a β_1 lipoprotein found in chylomicron layer obtained by ultracentrifugation of serum. Proc. Soc. Exper. Biol. & Med., 1955, 88, 538.

24. Herbst, F. S. M., Lever, W. F., Lyons, M. E., and Hurley, N. A., Effects of heparin on the lipoproteins in hyperlipemia. An electrophoretic study of the serum alpha and beta lipoproteins after their separation by fractionation of the plasma proteins or ultracentrifugal flotation. J. Clin. Invest., 1955, 34, 581.

25. Eder, H. A., Russ, E. M., Pritchett, R. A. R., Wilber, M. M., and Barr, D. P., Protein-lipid relationships in human plasma: In biliary cirrhosis,

obstructive jaundice, and acute hepatitis. J. Clin. Invest., 1955, 34, 1147.

26. Rosenberg, I. N., Young, E., and Proger, S., Serum lipoproteins of normal and atherosclerotic persons studied by paper electrophoresis. Am. J. Med., 1954, 16, 818.

27. Gottfried, S. P., Pope, R. H., Friedman, N. H., and Di Mauro, S., A simple method for the quantitative determination of alpha and beta lipoproteins in serum by paper electrophoresis. J. Lab. & Clin. Med., 1954, 44, 651.

28. Barr, D. P., Russ, E. M., and Eder, H. A., Protein-lipid relationships in human plasma. II. In atherosclerosis and related conditions. Am. J. Med., 1951, 11, 480.

29. Lindgren, F. T., Nichols, A. V., and Freeman, N. K., Physical and chemical composition studies on the lipoproteins of fasting and heparinized human sera. J. Phys. Chem., 1955, 59, 930.