Electrophoretic and Ultracentrifugal Analysis of Serum Lipoproteins of Normal, Nephrotic and Hypertensive Persons

By Lena A. Lewis, Ph.D., and Irvine H. Page, M.D.

Using a method of ultracentrifugation which brings out clearly the α and β lipoprotein components of serum, studies of normal, hypertensive and nephrotic human beings have been made. There are striking differences in distribution of lipoproteins among the different ages and sexes. During the stages of active atherogenesis in hypertensives and nephrotics, both the α₂ and β lipoproteins and the lighter, higher molecular weight aggregates contribute importantly to the superabundance of plasma lipoproteins. The attribution of an exclusive atherogenesis to any one plasma fraction does not seem to be justified by these or other studies.

MacEboeuf reported in 1929 the preparation from horse serum of a lipoprotein that had a constant lipoprotein composition. The first lipoprotein prepared by ultracentrifugation was by Pedersen from human serum after addition of magnesium sulfate to 0.45 saturation. It had the sedimentation constant of β₁ globulin. Attempts to isolate lipoproteins from serum other than human were at that time unsuccessful. In 1949 Lewis and Page obtained a lipoprotein from dog serum by ultracentrifugation after addition of sodium chloride to 1.5 M concentration. It had the electrophoretic mobility of α₂ globulin. Gofman and coworkers studied lipoprotein concentrates obtained by flotation in the ultracentrifuge of protein solutions made to a density 1.063 by addition of sodium chloride, thus offering a new approach to the characterization of lipoproteins of serum. The number of lipoproteins definable and, correspondingly, the usefulness of this method of analysis was increased by raising the density to 1.21 by addition of potassium bromide as in the modification of Green, Lewis and Page.

1 Electrophoretic analysis of plasma or serum proteins of patients with hypothyroidism, diabetes with retinitis, nephrosis, Cushing's syndrome or malignant hypertension has revealed many abnormalities. But one change common to all was a consistently increased concentration of β globulin. In many, an elevated serum cholesterol was also present. These observations suggested to us that the increase in β globulin was likely due to increase in the lipoprotein fraction and that this increase was in some way associated with vascular disease.

I. Centrifugal Analysis

Methods

A concentrate of lipoproteins was prepared by centrifuging for 13 hours at 30,000 revolutions per minute (centrifugal force 63,000 × g) 5 ml. of serum to which sodium chloride and potassium bromide had been added to bring the density to 1.21. The top 1 ml., which had an oily or milky appearance, was removed and studied in the analytic ultracentrifuge at a density of 1.21 by the method of Gofman using a speed of 52,640 revolutions per minute, equivalent to 250,000 × g. The designation of the unit of measurement, −S₁₃₁₀, represents a negative sedimentation or flotation rate of 1 × 10⁻¹⁵ cm. per second per dyne per gram at a density of 1.21 and a temperature of 26 C. For convenience, 1.21 will be omitted, but is to be understood when "−S" is employed in this paper.

Results

At a density of 1.21 at least three components, based on differences in flotation rate, are resolved in human serum −S(25–40), i.e., β₁ lipoprotein, −S(20–25), i.e., α₂ lipoprotein...
ANALYSIS OF SERUM LIPOPROTEINS

1. Normal Values. The $-S > 70$ concentration of $92\%$ of normal women between 18 and 60 years was less than $50\, \text{mg. per 100}\, \text{ml.}$ (67 persons, 42 being 18 to 34 and 25 being 35 to 60 years of age). The concentration was less than $75\, \text{mg.}$ in $98\%$ of normal men between the ages of 18 and 34 (58 persons), and was greater in $27\%$ per cent in the age group 34 to 60 (99 persons). (See fig. 1.)

The $-S(25-40)$ (the $\beta_1$ lipoprotein) was less than $150\, \text{mg.}$ in $42\%$ per cent of normal women between 18 and 34 years, contrasting with only $18\%$ per cent of the older 34 to 60 year group. The $-S(25-40)$ was between 150 and

![Fig. 1](http://circ.ahajournals.org/)

![Fig. 2](http://circ.ahajournals.org/)
225 mg. in 62 per cent of the latter. In males the number in the younger group (11 to 34 years of age) having high \( \beta \) lipoprotein was much increased, 72 per cent between 150 and 225 mg. In the group 34 to 60 years of age, males (58 per cent) and females (62 per cent) were alike. Taking the whole group, 20 per cent of males had \( \beta \) lipoproteins greater than 250 mg., and only 9 per cent of females.

Significantly greater in the older women, i.e., 30 per cent with more than 250 mg. per 100 ml. of serum, than in the younger women, i.e., 10 per cent. In contrast, the \( \alpha \) fraction did not change as the men got older. Tables 1 and 2 summarize the normal human serum lipoprotein values.

2. Values in Subjects with Arterial Hypertension. The concentration of serum \( \alpha \) and \( \beta \) lipoproteins of 27 patients with mild essential hypertension was normal. The pattern in severe essential or malignant hypertension (88 cases) showed increased concentrations of \(-S(40-70)\) and \(-S(25-40)\) (\( \beta \) lipoprotein). (See fig. 4.) There was moderate increase in concentration of \( \alpha \) lipoprotein, while \( \beta \) lipoprotein in the women showed a small decrease (fig. 5). The patients with the most severe renal disease exhibited greater increase in \(-S(40-70)\) and the \( > 70 \) components, and the
peak of the $\beta_1$ fraction moved at a somewhat slower rate than normal.

During treatment of malignant hypertensive patients with pyrogens, very marked changes in the distribution of the lipoproteins were observed. There was a large increase in concentration of the $-S > 70$, $-S(40-70)$ and $\beta_1$ lipoprotein. The $\alpha_2$ lipoprotein concentration did not change significantly while the $\alpha_1$ lipoprotein decreases greatly. On stopping treatment, or when the febrile response decreased due to development of tolerance, all lipoproteins tended to revert to pretreatment levels (fig. 6).

**Table 2.** Summary of Differences in Human Lipoprotein Levels, Grouped According to Age and Sex

<table>
<thead>
<tr>
<th></th>
<th>$-S &gt; 70$</th>
<th>40-70</th>
<th>23</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>F vs M</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>18-34 yr</td>
<td>18-34 yr.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F vs M</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>35-60 yr</td>
<td>35-60 yr.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F vs F</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>18-34 yr</td>
<td>35-60 yr.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M vs M</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>18-34 yr</td>
<td>35-60 yr.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$+$ = difference significant at 1% probability level
0 = difference not significant at 1% probability level

**Fig. 4.** $\beta_1$ lipoproteins of serum of patients with severe essential or malignant hypertension, compared with those of normals of the same age (35 to 60 years of age).
The lipoprotein pattern of malignant hypertensives receiving other types of therapy, that is, 1-hydradinophthalazine, hexamethonium, low sodium diet or sodium nitroprusside, showed no changes comparable to those that occurred during pyrogen treatment. The patterns obtained on one patient, while receiving 1-hydradinophthalazine or hexamethonium and hydradinophthalazine, are shown in figure 7. Variations in the concentration of the com-
components seemed to be independent of each other and to have little relation to either the type of treatment or to arterial pressure.

3. Values in Patients with the Nephrotic Syndrome. The serum lipoprotein pattern of patients in the nephrotic phase of nephritis was bizarre. The concentration of the rapidly rising components $-S > 70$, and $-S(40-70)$ was increased, and $-S 30 (\beta_1)$ and $-S 23$ (\(\alpha_2\)) which normally are well resolved, separated poorly and were much increased in concentration (table 3). The first analysis on patient 7 was taken March 15, 1951, during the stage of acute nephritis. Before treatment was started the $-S > 70$ was elevated to 130 mg., the $-S(40-70)$ to 90 mg. and the $\beta_1$ and $\alpha_2$ lipoprotein components showed no separation. The wide spread of the peak indicated its heterogenous nature. The two, $\beta_1$ and $\alpha_2$, were present in a concentration of over 260 mg. per 100 ml. The $\alpha_1$ lipoprotein was within normal limits throughout the study. The electrophoretic pattern was typical of that observed in the nephrotic syndrome, albumin 0.92, $\gamma$ globulin 0.37 Gm. per 100 ml. with very large $\alpha_2$ and $\beta$ globulin components. Following a course of nitrogen mustard, 0.1 mg. per kilogram intravenously daily for four days, progressive clinical improvement, especially in renal function, was observed. The lipoprotein pattern and electrophoretic pattern reverted toward normal finally becoming so three months after the nitrogen mustard was initiated. The serum cholesterol concentration had decreased from an initial value of 1380 mg. to 257 mg. per 100 ml.

In contrast with patient 7, patient 9 showed no clinical improvement during the period of study, and the serum lipoprotein and electrophoretic patterns remained abnormal.

**FIG. 7.** Serum lipoprotein patterns of patient with malignant hypertension during treatment with 1-hydrizinophthalazine or hexamethonium and hydrizinophthalazine.

- - - - - - blood pressure; - - - - - $\beta$ lipoprotein; - - - - $\alpha_1$ lipoprotein; - - - - - $\alpha_2$ lipoprotein.

II. Electrophoretic Analysis

*Method*

Electrophoretic analysis of serum, plasma or serum lipoprotein concentrates was carried out by Longsworth's modification of the Tiselius method. Total protein concentration of serum or plasma was determined by Pregl modification of the Kjeldahl method. When phosphate buffer, pH 7.8, is employed, electrophoretic analysis resolves the following components in order of decreasing mobility: albumin plus $\alpha_1$, $\beta$, $\phi$, $\gamma$ globulin. The $\beta$-globulin complex is usually resolved as a double peak, the more rapidly migrating $\beta_1$, the slower $\beta_2$ globulin.

The electrophoretic mobility of the lipoproteins seems to be dependent on the relative amounts of amino acids in the molecule, the components with the higher nitrogen to lipid ratio exhibiting faster migration rates.

The pattern of the lipoprotein concentrates reported here obtained in the standard electrophoresis cell are similar to those obtained by paper electrophoresis of serum by Swahn and by zone electrophoresis by Kunkel and Slater.
Results

As had been observed previously, the electrophoretic serum protein pattern of patients with mild essential hypertension was normal. In patients with severe essential or malignant hypertension (76 cases) the β globulin was elevated (fig. 8) and the α₁ globulin showed a wider range of concentration than normal. Albumin was frequently decreased.

The electrophoretic pattern of the lipoproteins of serum of severe essential and malignant hypertensives concentrated by ultracentrifugation showed large amounts of α₂ and β globulin. The lipoproteins accounted for approximately 40 to 60 per cent of the total area of the β globulin and 30 to 40 per cent of the α₂ globulin fraction in the serum of nephrotics, and for 20 to 50 per cent of the β globulin and 15 to 40 per cent of the α₁ globulin in severe essential
or malignant hypertensives. In normal sera
the lipoproteins were only 15 to 30 per cent
of the \( \beta \) globulin area and 7 to 15 per cent of the
\( \alpha_2 \) globulin area. The increase in \( \alpha_2 \) and \( \beta \)
globulin concentration in severe essential and
malignant hypertensive sera and in nephrotic
sera is, therefore, largely accounted for by
increased concentrations of lipoproteins.

![Graph showing \( \alpha_2 \) globulin concentrations in normal and hypertensive sera.]

Fig. 8. The \( \alpha_2 \) and \( \beta \) globulin concentrations in
patients with severe essential and malignant hyperten-
sion, compared with normal human beings.

When studying the electrophoretic and
ultracentrifuge patterns of lipoprotein concen-
trates, striking similarities were observed,
which were emphasized by plotting the ultra-
centrifuge pattern on a flat base line (fig. 9).
The electrophoretic pattern of nephrotic
serum lipoprotein concentrate exhibited clear
\( \alpha_1 \) and \( \alpha_2 \) globulin peaks; the \( \beta_1 \) peak skewed
to the slow side and additional slower migrating
components corresponding to \( \alpha_1 \), \( \alpha_2 \), \( \beta \) and
\(-S(40-70)\) components, respectively, of the
ultracentrifuge pattern. The pattern of pa-
tients with essential hypertension showed
\( \alpha_1 \), \( \alpha_2 \) and \( \beta_1 \) peaks, and in some a very small
component trailing behind the \( \beta_1 \) lipoprotein,
similar to those of normal sera. Malignant
hypertensives usually had a component or
components with migration rates less than that
of \( \beta_1 \) lipoprotein and corresponding to com-
paratively large \(-S(40-70)\) and >70 components in the ultracentrifuge pattern.

![Graph showing \( \beta \) globulin concentrations in normal and hypertensive sera.]

![Graph showing ultracentrifuge and electrophoresis patterns of lipoprotein concentrates in essential hypertensive and nephritic patients.]

Fig. 9. Electrophoretic and ultracentrifugal pat-
terns of lipoprotein concentrates of serum of an
essential hypertensive patient and a nephritic (ne-
phrotic phase) patient.

The sera of about 10 per cent of severe
essential or malignant hypertensives were
characterized by very poor resolution of the
\( \alpha_2 \) from the \( \beta \) globulin. After removal of the
lipoproteins separation was sharp. The elec-
trophoretic pattern of the lipoprotein concen-
trates was often poorly resolved, in part
due to the fact that the mobility of the \( \beta \)
lipoprotein was greater in both hypertensives
and nephritics than in normal lipoprotein
concentrates. In the ultracentrifuge negative
sedimentation of the \( \beta \) lipoprotein fraction was
often slower than normal, approaching that of
\( \alpha_2 \) lipoprotein, being \(-S\) 25 to 27 instead of
\(-S\) 30. \( \alpha_2 \) globulin of normal sera has a nega-
tive sedimentation of about \(-S\) 23.
DISCUSSION

The combined use of electrophoretic and ultracentrifugal analysis of serum has advantages over either one alone in that the information derived from both in many cases is additive rather than substitutive.

There are certain characteristics of the lipoproteins of normal serum which deserve comment. The $-S > 70$ component was usually greater in men than in women. This lipoprotein, if that is what it really is, which is very light, contains much chylomicron-like material, along with other lipids. Its neutral fat concentration is high. It probably represents fat in the early stages of its metabolism. The fact that it is greater in amount in plasma of men than of women has suggested that the preponderance of atherosclerosis in men might be in part due to this fact. As we have pointed out on numerous occasions before, we do not believe any one factor alone is responsible for the multifaceted mechanism of the genesis of atherosclerosis.

The earlier results of Lewis and Page\(^6\) suggested a close relationship between the increased quantities of $\beta$ globulin and the occurrence of vascular disease. This is not to say that vascular disease need always result when $\beta$ globulin is elevated. Again, the contrast in the concentration of $\beta$ lipoprotein ($-S 25-40$) between male and female is important. Even in the younger males the concentrations were much increased over the concentration in the corresponding age group of females. In the older group the concentration in women and men became more and more alike. Taken as a whole, about twice as many males have $\beta_1$ lipoproteins greater than 250 mg. than females. The increased $\beta$ lipoproteins plus increased $-S > 70$ both point to males as victims of future vascular disease.

As Gofman and co-workers\(^6\) first suggested, the $-S(40-70)$ (i.e., $S_1 10-20$) may be a highly important fraction of plasma in relation to atherogenesis. They found a correlation between its elevation and coronary atherosclerosis in human beings. The ultracentrifugal analysis using the higher density potassium bromide medium also shows an increased number of males with this component greater than in females.

In contrast, women usually had greater concentrations of $-S(2-8)$, i.e., $\alpha_1$ globulin, than men and they tend to be significantly greater in older women. The $\alpha_1$ fraction, on the contrary, did not change as men got older.

The $\alpha_2$ lipoprotein (i.e., $-S 20-25$) was the most constant of all the fractions, being less than 20 mg. in most people regardless of age or sex.

To get some notion of the biochemical significance of these fractions, comparison during disease states has been made. Hypertensive patients are important in this relationship chiefly because atherosclerosis and hyperplastic arteriosclerosis are an integral part of the disease. It is usually from one or both of these that the patients die. Early in the disease the lipoproteins are normal, but as it progresses, a steady increase in $-S(40-70)$ and $-S(25-40)$ components occurs. The $\alpha_2$ lipoproteins increased but to a lesser degree while $\alpha_1$, in women at least, decreased.

Using alcohol fractionation, Barr observed\(^6\) an increased $\alpha_1$ lipoprotein in serum of patients following treatment with estrogens. Similar increases in the $\alpha_1$ globulin were obtained by ultracentrifugation of some of these same sera in our laboratory. The difference in $\alpha_1$ lipoprotein concentration observed in the serum of the two sexes may be due to hormonal action. There is also the possibility that the ratio of the $\alpha_1$ lipoprotein to $\beta$ lipoprotein concentration provides additional information on the state of dispersal of the plasma lipids not provided by concentrations of each alone.

Treatment of patients with malignant hypertension with pyrogens has proved useful in a number of patients, as pointed out several years ago.\(^1\) Especially striking is the betterment of the necrotizing hemorrhagic arteriolitis. During treatment when fever occurs twice daily, the larger molecular lipoprotein aggregates increase greatly while the more dense $\alpha_1$ fraction decreases. The $\alpha_2$ fraction showed no significant change. When treatment was stopped, or the febrile reaction decreased due to development of tolerance, and clinical
improvement had occurred, all lipoprotein fractions tended to revert to pretreatment levels. We suppose that the increase in the less dense or larger molecular lipoproteins means either that the increased metabolism due to fever has demanded an increased turnover of lipid or, less likely, that the fever has slowed the passage of fats down the metabolic ladder to the lower molecular weight species. In any case, there seems to be no obligate relationship between this phenomenon and the resolution of the vascular disease except that it indicates a very active metabolism of tissue including blood vessels. That it is a metabolic event and not due to lowering of blood pressure is attested to by the failure of change when hypotensive drugs such as 1-hydrizinopthalalazine or sodium nitroprusside are given.

The nephrotic syndrome has been associated in the minds of most clinicians with severe lipemia, indeed it has been called on occasion a lipid diabetes. Vascular disease of the atherosclerotic type is hastened by appearance of the syndrome. The utilization of fat appears to be normal; some abnormality in transport seems to be at fault. We suggested some time ago that the loss of protein in the urine without equivalent loss of lipid might account in part for the accumulation of lipid in the blood. The urine of these patients contains large amounts of protein with almost no lipid.

As would be expected, the lighter, larger molecular weight -S > 70 and 40-70 components were increased. The β₁ lipoproteins were abnormal in their response to resolution and were much increased in concentration as found originally by Longsworth and MacInnes. This fraction, as we have suggested, is often associated with developing vascular disease. The α₂ fraction is also greatly augmented in concentration. Thus all but the α₁ lipoproteins are sharply increased while the albumin and γ globulin are decreased. It is the albumin which makes up most of the protein in the urine of nephrotics. Failure of glomerular filtration or complete tubular reabsorption could account for the accumulation of lipoproteins with concurrent loss of albumin.

Thus, both in severe hypertensives and in nephrotics during periods when atherosclerosis tends to develop rapidly, lipoproteins are present in superabundance.

Lipoproteins in both diseases are greatly increased in concentration, but so are the lighter high molecular weight aggregates. It can only be said that chronic elevation of the β lipoproteins is a more unusual event, that elevation of the chylomicron-like fraction occurs temporarily with each fatty meal. The attribution of an exclusive atherogenesis to any one serum fraction is not justified from these or other studies.

**SUMMARY**

1. Ultracentrifugal analysis of sera of normal, hypertensive and nephrotic human subjects was made, using a density of 1.21. Normal males had greater concentration of the -S > 70 fraction than females at all ages. The -S(25-40) (i.e., β lipoproteins) was low in younger females, tending to rise with increasing age. In younger males this fraction was increased and almost equal to the concentration in females of the older age (34 to 60 years) group. The -S(40-70) fraction (i.e., S₁ 10–20 class of Gofman) was present in some instances in both sexes but much less in young females and slightly less in young males than in men and women of older groups. The -S(20–25) (i.e., α₂) fraction was small and about equal in quantity in most normal people. The -S (2–8) (i.e., α₁) fraction was more concentrated in women than in men; especially in the older women. In contrast this fraction did not change as men got older.

2. The concentration of α and β lipoproteins in early hypertension is normal for the corresponding age and sex. As hypertension becomes severe or malignant the -S(40–70) (S₁ 10–20), -S(25–40) (β₁ lipoprotein) and -S(20–25) (α₂ lipoprotein) increased. Patients with the most severe renal disease exhibited especially great increase in the -S(40–70) and > 70 fractions.

3. During treatment of malignant hypertension with pyrogens a large increase occurs in the -S > 70, -S(40–70) and β lipoproteins. A marked decrease in α₁ lipoprotein, with no change in the α₂ fraction characterized the period of febrile reaction to the pyrogen.
The lipoprotein pattern reverted back to pretreatment patterns on discontinuing treatment. Other hypotensive drugs such as Apresoline and sodium nitroprusside did not produce similar lipoprotein changes.

4. The nephrotic phase of glomerulonephritis is characterized by great increase in the concentration of the $-S > 70$, 40–70 and $\alpha_2$ lipoproteins, as resolved by ultracentrifugation. The electrophoretic pattern of serum lipoprotein concentrate as compared with normal serum concentrates showed large $\alpha_2$ and $\beta$ globulin components and additional components with electrophoretic mobility less than that of $\beta_1$ lipoprotein.

5. Lipoproteins are in great abundance during the periods when atherogenesis is rapid in patients with severe essential and malignant hypertension and the nephrotic phase of glomerulonephritis. Both the $\alpha_2$ and $\beta$ lipoproteins and the lighter higher molecular weight aggregates contribute importantly to this abundance.

Acknowledgment

We wish to thank Mr. Richard Wetzel for help with the statistical treatment of the data.

Sumario Español

Se han hecho estudios en individuos normales, hipertensos y nefróticos usando un método de ultracentrifugación que demuestra claramente los componentes lipoprotéicos $\alpha$ y $\beta$ del suero. Hay diferencias notables en la distribución de las lipoproteínas entre diferentes edades y sexos. Durante las etapas de aterogenesia en hipertensos y nefróticos las lipoproteínas $\alpha_2$ y $\beta$ y los agregados livianos de peso molecular alto contribuyen significativamente a la superabundancia de lipoproteínas del plasma. La atribución de una aterogenesia exclusiva a cualquier fracción del plasma no nos parece a nosotros justificada de acuerdo a estos y otros estudios.

REFERENCES

10 BARK, D. P.: Personal communication.
Electrophoretic and Ultracentrifugal Analysis of Serum Lipoproteins of Normal, Nephrotic and Hypertensive Persons
LENA A. LEWIS and IRVINE H. PAGE

Circulation. 1953;7:707-717
doi: 10.1161/01.CIR.7.5.707

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1953 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/7/5/707

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/