Lipoproteins Quantitated by Paper Electrophoresis as an Index of Atherosclerosis

By Moses Wurm, M.S., Robert Kositchek, M.D., and Reuben Straus, M.D.

Numerous studies have been devoted to the diagnosis of coronary atherosclerosis in the absence of classical clinical signs and symptoms. These studies have emphasized the relationship of blood lipids to atherogenesis. Serum cholesterol, phospholipids, and cholesterol/phospholipid ratios have been employed for diagnostic purposes with questionable success. A more reliable index for distinguishing atherosclerotic patients from normal individuals has been claimed for the lipoproteins per se, or their cholesterol content, separated by chemical fractionation, ultracentrifugal flotation, and starch and moving boundary electrophoresis.

For routine clinical use the lipoprotein procedures are almost, if not entirely, unavailable. A practical solution for this problem has been the development of the paper electrophoretic procedure for such separation of serum lipoproteins by Swahn and Jencks et al. Heretofore, these methods have not been found to be sufficiently reliable.

A new procedure for separating 5 lipid-containing fractions of serum by paper electrophoresis and a method of quantitation have been reported from our laboratory. It is the purpose of this report to present the results of an initial study correlating lipoprotein distributions in "normal" individuals and in atherosclerotic patients.

Material and Method

The present series consists of 40 consecutive, unselected patients classified as abnormal on the basis of unequivocal evidence of one or more myocardial infarctions or arteriosclerotic cerebrovascular accidents. In addition, many of these patients presented ancillary clinical symptoms of cardiovascular disease such as angina, hypertension, nephrosclerosis, retinopathy, arteriosclerotic gangrene, and positive familial histories of coronary disease. Three patients in this group also exhibited xanthomata with familial and refractory hypercholesterolemia. In this group there were 32 males, ranging in age from 38 to 74 years, with a mean age of 55 years, and 8 females, ranging in age from 31 to 70 years, with a mean age of 54 years.

Another group of 40 individuals selected in the same manner, represent the controls (normal). These were so classified by the exclusion of atherosclerotic cardiovascular disease on the basis of negative findings on routine history and physical examination and by electrocardiography. While this group of "normals" consisted chiefly of healthy individuals, some patients with nonrelated disease were included and admittedly some degree of asymptomatic atherosclerosis may be presumed to be present. This group is represented by 25 males ranging in age from 10 to 80 years, with a mean age of 48 years, and 15 females ranging from 11 to 70 years, with a mean age of 34 years. Since this experiment was set up on a blind basis, initially 2 children, one of each sex, were included. If these are omitted, the age distribution for males is 33 to 80, with a mean age of 49 years, and for females 23 to 70, with a mean age of 47 years.

The usual clinical evaluation also included laboratory examinations such as complete blood counts, urinalysis, serum protein-bound iodine, liver-function tests, blood glucose, serum urea nitrogen and uric acid, blood serologic tests, and Papanicolaou tests of vaginal exfoliated cells. The results of these tests were found to be noncontributory and are not further considered.

Fasting blood specimens were drawn in the early morning and all analytical procedures were started within 24 hours. The lipid spectrum for each individual was determined as follows: the concentration of cholesterol in the serum was measured by the method of Abell et al.; lipid phosphorus was assayed by Bloor extract of serum by a modification of the method of Fiske and Subbarow and
ELECTROPHORESIS OF LIPOPROTEINS

Figure 1
Frequency distribution of cholesterol values in patients with manifest coronary artery disease and controls. Line graph represents the per cent of normal individuals at each concentration level.

Figure 2
Frequency distribution of phospholipid values in patients with manifest coronary artery disease and controls. Line graph represents the per cent of normal individuals at each concentration level.

Figure 3
Frequency distribution of total lipid values in patients with manifest coronary artery disease and controls. Line graph represents the per cent of normal individuals at each concentration level.

Figure 4
Frequency distribution of cholesterol/phospholipid ratio in patients with manifest coronary artery disease and controls. Line graph represents the per cent of normal individuals at each concentration level.

converted to phospholipids by a factor of 25; and total lipids were estimated gravimetrically by a modification of the method of Sperry. The apparatus and technic of paper electrophoresis routinely employed in this laboratory during the last 4 years, together with our method for visualizing lipoproteins with Fat Red 7 B and direct scanning of the electrophoretic pattern for purposes of quantitation, have been previously reported. With this technic 5 lipoprotein fractions can be demonstrated, namely; lipalbumin, alpha-1 lipoprotein, alpha-2 lipoprotein, beta lipoprotein, and gamma lipoprotein plus neutral fat fractions, which are evaluated in terms of relative concentration. Beta/alpha ratios were calculated by dividing the beta lipoprotein value, exclusive of the gamma plus neutral fat fraction, by the combined values of alpha-2, alpha-1, and lipalbumin fractions. Beta/lipalbumin ratios were derived similarly from the values of these individual fractions. Statistical constants were calculated according to standard methods. Significance of differences between groups was determined by the Fisher t test, for which a value of 3.0 or more was considered by us to be acceptable.

Each type of datum also was examined for its significance in predicting the atherosclerotic status of the individual. For this purpose, the percentage of normal individuals was determined at each level of the variable being examined. Two levels of confidence are considered, namely, the 100 per cent level, at which all individuals without exception can be classified as either "normal" or abnormal, and the 80 per cent level for such individual
Table 1
Lipid and Lipoprotein Values in Coronary Atherosclerotic Patients and Symptomless Controls

<table>
<thead>
<tr>
<th>Blood fraction</th>
<th>No. of subjects</th>
<th>Mean ± standard deviation</th>
<th>No. of subjects</th>
<th>Mean ± standard deviation</th>
<th>Fisher t test</th>
<th>Significant</th>
<th>Per cent of population</th>
<th>Less than</th>
<th>More than</th>
<th>Per cent of population</th>
<th>Less than</th>
<th>More than</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol*</td>
<td>38</td>
<td>248 ± 53.6</td>
<td>35</td>
<td>273 ± 60.4</td>
<td>1.85</td>
<td>no</td>
<td>7</td>
<td>139</td>
<td>432</td>
<td>14</td>
<td>178</td>
<td>354</td>
</tr>
<tr>
<td>Phospholipid*</td>
<td>38</td>
<td>292 ± 49.7</td>
<td>35</td>
<td>296 ± 50.7</td>
<td>0.337</td>
<td>no</td>
<td>10</td>
<td>225</td>
<td>420</td>
<td>0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>Total lipid*</td>
<td>38</td>
<td>946 ± 255</td>
<td>35</td>
<td>1066 ± 384</td>
<td>1.54</td>
<td>no</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cholesterol/</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>phospholipid</td>
<td>38</td>
<td>.85 ± .12</td>
<td>35</td>
<td>.92 ± .09</td>
<td>2.32</td>
<td>no</td>
<td>8</td>
<td>65</td>
<td>—</td>
<td>33</td>
<td>0.80</td>
<td>1.00</td>
</tr>
<tr>
<td>Lipalbumin†</td>
<td>40</td>
<td>19.8 ± 5.86</td>
<td>40</td>
<td>13.2 ± 3.96</td>
<td>5.78</td>
<td>very, .001</td>
<td>32</td>
<td>10.4</td>
<td>21.5</td>
<td>56</td>
<td>12.7</td>
<td>17.7</td>
</tr>
<tr>
<td>Alpha-1 lipo-</td>
<td>40</td>
<td>4.67 ± 2.07</td>
<td>40</td>
<td>3.80 ± 1.43</td>
<td>2.17</td>
<td>no</td>
<td>12</td>
<td>2.2</td>
<td>7.0</td>
<td>22</td>
<td>2.3</td>
<td>6.5</td>
</tr>
<tr>
<td>protein†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha-2 lipo-</td>
<td>40</td>
<td>7.06 ± 2.35</td>
<td>40</td>
<td>5.07 ± 1.86</td>
<td>4.14</td>
<td>very, .001</td>
<td>11</td>
<td>2.7</td>
<td>9.6</td>
<td>55</td>
<td>4.8</td>
<td>7.4</td>
</tr>
<tr>
<td>protein†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta/lipo-</td>
<td>40</td>
<td>52.5 ± 9.20</td>
<td>40</td>
<td>62.5 ± 8.84</td>
<td>4.88</td>
<td>very, .001</td>
<td>15</td>
<td>46.8</td>
<td>76.5</td>
<td>40</td>
<td>51.1</td>
<td>70.7</td>
</tr>
<tr>
<td>protein†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gamma plus neutral fat†</td>
<td>40</td>
<td>15.5 ± 4.56</td>
<td>40</td>
<td>15.4 ± 6.78</td>
<td>0.023</td>
<td>no</td>
<td>3</td>
<td>7.2</td>
<td>14</td>
<td>9.5</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Beta/alpha</td>
<td>40</td>
<td>1.81 ± 0.68</td>
<td>40</td>
<td>3.22 ± 1.48</td>
<td>5.41</td>
<td>very, .001</td>
<td>22</td>
<td>1.54</td>
<td>4.72</td>
<td>65</td>
<td>2.00</td>
<td>2.62</td>
</tr>
<tr>
<td>Beta/lip-</td>
<td>40</td>
<td>3.02 ± 1.36</td>
<td>40</td>
<td>5.55 ± 3.11</td>
<td>4.65</td>
<td>very, .001</td>
<td>32</td>
<td>2.56</td>
<td>6.82</td>
<td>51</td>
<td>2.97</td>
<td>5.12</td>
</tr>
</tbody>
</table>

*Mg. per cent concentration.
†Relative per cent concentration.

classification, which allows for a possible error of 1 in 5.

Results

Cholesterol

The data for serum cholesterol values in 73 individuals of the combined sample population are compiled in the histogram (fig. 1). It will be noted that the frequency distribution of this serum component shows a relatively poor normal distribution for either the group of controls or the abnormal individuals, and is also true for the combined population. The reason for this deviation is not apparent because there are many factors, not considered in the present study, that may contribute to the concentration of blood cholesterol. When the 2 groups, "normal" and atherosclerotic, are compared statistically it is found that they are different only on a 5 per cent confidence level (t = 1.85) (table 1). This is not considered sufficiently significant by our criteria.

The continuous graph also shown in figure 1 depicts the per cent of "normal" individuals found at each level of serum cholesterol. It will be noted that at the extreme low value of 199 mg. per cent, the chances are 3 to 1 that the subject is normal. With increasing concentration of serum cholesterol, the chance of finding a normal individual progressively decreases, so that one who displays a serum cholesterol level in the vicinity of 399 mg. per cent is favored to be an atherosclerotic by a 2 to 1 ratio. By extrapolation, it becomes apparent also that the confidence with which normal and atherosclerotic subjects can be identified increases rapidly as serum cholesterol levels continue to decrease or increase. At approximately 135 and 475 mg. per cent only "normal" and abnormal, individuals respectively, may be encountered. Actually our data show that an absolute determination of "normal" can be made at cholesterol values below 159 mg. per cent and a diagnosis of atherosclerosis at values above 432 mg. per cent. These limits however apply
to only 5 individuals, or 7 per cent of the sample tested. Below a cholesterol level of 178 mg. per cent and above 354 mg. per cent an individual can be correctly classified 80 per cent of the time. This level of confidence applies to 10 individuals only, or 13 per cent of the population tested.

**Phospholipids**

Figure 2 illustrates the frequency distribution of phospholipid values. It will be noted that both "normal" and abnormal patients are almost equally distributed at every level of phospholipid concentration. The high degree of overlapping values for both groups is reflected also by the low $t$ value of 0.34, (table 1), indicating no significant difference. It is apparent that the predictive value of serum phospholipid concentration for the individual is extremely low, since only 7 patients, or 10 per cent of the sample population, can be distinguished absolutely as normal or atherosclerotic. This occurrence, furthermore, is considered to be purely fortuitous, since no concentration ranges can be found in which a portion of the population can be classified on an 80 per cent confidence level, and no significant trend in the distribution of values can be identified.

**Total Lipids**

Serum total lipid concentrations appear to be the least meaningful of all determinations. From figure 3 and its statistical data (table 1) it is evident that these values for the 2 groups of patients are not significantly different and that no diagnostic inference for the individual can be found. This is emphasized particularly by the observation that both the highest and the lowest values of serum lipids were found in abnormal patients.

**Cholesterol/Phospholipid Ratio**

Comparison of figures 1 and 4 reveals a striking similarity in the frequency distribu-

---

*Figure 5*

*Representative electrophoretic pattern of serum lipoproteins with density distribution curve.*

---

_Circulation, Volume XXI, April 1960_
tion of cholesterol values and cholesterol/phospholipid ratios. From the calculated Fisher t value it will be noted that the difference in this variable between normal and abnormal groups (table 1) is of a low order of significance. Only 2 individuals, or 3 per cent of the entire sample, with cholesterol/phospholipid ratios below 0.65 can be differentiated with absolute confidence. If one accepts an 80 per cent chance of classifying an individual, then 24 persons, or 33 per cent of the population with cholesterol/phospholipid values less than 0.80 and greater than 1.00 can be differentiated correctly.

**Lipoproteins**

Electrophoretic strips stained with Fat Red 7B reveal the presence of 5 lipid fractions,\(^5\) which are illustrated in figure 5. It is of interest to examine these fractions individually and in combination for the degree to which they correlate with manifest coronary artery disease and, conversely, the extent to which they can be used as a diagnostic index.

**Gamma Lipoprotein Plus Neutral Fat Fraction**

The least significant of all the variables measured in this portion of the study appears to be the gamma lipoprotein and neutral fat fraction. Figure 6 and the related statistical data (table 1) reveal a marked overlapping in the frequency distribution of the "normal" and atherosclerotic groups. The skewness of the distribution observed in the entire sample population probably is associated with the age and diet of these patients. Since the percentage of "normals" at each concentration level of this fraction approaches 50 per cent as shown by the line graph, it is concluded that this has a low predictive value for classifying the individual. Actually, only 2 (3 per cent) and 11 (14 per cent) persons in our sample population can be classified on an absolute and on an 80 per cent confidence level, respectively. This result is not unexpected, since it probably reflects the low order of diagnostic significance found for the serum total lipids described above.

**Alpha-1 Lipoprotein**

The analysis of our data dealing with alpha-1 lipoprotein concentration is summarized in figure 7. Although the difference in mean concentration between the "normal" and abnormal groups is more significant \((t = 2.17)\) than that found for any of the chemically determined variables, the data still do not satisfy our criteria for acceptance. Twelve per cent of the population can be identified with absolute certainty as "normal" or atherosclerotic when alpha-1 lipoprotein values are below 2.2 and above 7.0 per cent. If confidence limits of 4 to 1 are acceptable, then relative con-
Electrophoresis of Lipoproteins

Frequency distribution of alpha-2 lipoprotein values in patients with manifest coronary artery disease and controls. Line graph represents the per cent of normal individuals at each concentration level.

Concentration of this lipoprotein fraction below 2.3 and above 6.5 per cent will include 18 individuals or 22 per cent of the population.

**Alpha-2 Lipoprotein**

The distribution of the relative concentrations of alpha-2 lipoproteins is shown in figure 8. It will be noted that each group, as well as the total population, displays a well-normalized frequency distribution. Although a considerable degree of overlapping is noted, comparison of the mean values for the 2 groups is found to be significantly different \( t = 4.14 \). In addition, the possibility of differentiating “normal” from abnormal on an individual basis is considerably increased since 55 per cent of the total population can be classified with 80 per cent reliability, whereas only 11 per cent of the sample can be classified with absolute assurance.

**Beta Lipoproteins**

Our findings with respect to the relative concentrations of beta lipoproteins are shown in figure 9. The range of values for the entire population as well as for each group of patients closely assumes a normal distribution curve. On a group basis, normal individuals can be satisfactorily differentiated from atherosclerotic patients, since the difference in the mean values for each is highly significant \( t = 4.88 \). For the purpose of classifying individuals, beta lipoprotein values are approximately as satisfactory as alpha-2 lipoproteins.

**Lipalbumin**

The relative concentrations of lipalbumin are graphically presented in figure 10, from which it can be seen that overlapping values for the “normal” and abnormal subjects occur in a narrow portion of the entire range and include a relatively small fraction of the entire population. It appears possible to identify the atherosclerotic status of an individual with absolute certainty if the lipalbumin concentration is below 10.4 or above 21.5 per cent. The tail-end of the distribution beginning with these values include 26 individuals, or 32 per cent of the sample. If one accepts a 4 to 1 probability for correct diagnosis, then 56 per cent of the population can be satisfactorily classified when the concentration is below 12.7 and above 17.7 per cent. The calculated Fisher’s \( t \) of 5.78 also indicates that the difference in the means for the 2 groups is highly significant.

**Beta/Alpha Ratio**

The distribution of the beta/alpha ratios for “normal” and patients with manifest coronary artery disease is shown in figure 11. To conform with the beta/alpha ratios published by others\(^{17,18}\), the lipalbumin, alpha-1,
and alpha-2 lipoprotein fractions are included in the "alpha" portion of this ratio. It will be noted that this value in our group of normal subjects shows a typical Gaussian distribution, except for a marked tailing toward the higher values. This undoubtedly reflects the uncertainty in the classification of "normal" individuals on clinical evidence alone. The group of abnormal individuals shows a similar tailing toward higher values. Nevertheless, these 2 groups display significantly different beta/alpha ratios (t value of 5.41). It is also apparent that 22 per cent of the total sample population can be correctly classified with absolute confidence when beta/alpha ratios fall below 1.54 (normal persons) and above 4.72 (abnormal persons). On an 80 per cent confidence level, 65 per cent of the entire population can be satisfactorily classified, particularly when the observed beta/alpha ratio is below 2.00 or greater than 2.62. Within this confidence limit, therefore, only a relatively small portion of any random population may be expected to fall within the doubtful range.

**Beta/Lipalbumin Ratio**

The qualitative character of the lipoprotein pattern prepared by our method of electrophoresis reveals that the alpha lipoproteins represent a minor portion of the lipoprotein pattern and, therefore, can be expected to make a small contribution to the foregoing beta/alpha ratio. The lipalbumin fraction, on the other hand, represents a larger physical share of the pattern and plays a greater determinant role in the calculation of this ratio. We have examined, therefore, our data with respect to the usefulness of the beta/lipalbumin ratio in our sample population (figure 12). This index displays a high degree of reliability (t = 4.65) for the separation of "normal" and abnormal groups. In addition it will be noted that the atherosclerotic status of one third of our population can be correctly classified without chance of error, when this variable is below 2.56 or greater than 6.82. On the other hand, if an 80 per cent level of confidence is acceptable, more than half of the population can be diagnosed when values are less than 2.97 or more than 5.12 (table 1).

**Discussion**

It is commonly accepted that an abnormality in lipid metabolism is intimately associated with the pathogenesis of atherosclerosis.\(^{19, 20}\) Knowledge of the particular lipids involved or of a precise mechanism is still lacking. In fact, it would appear that this and even the entire subject of atherogenesis and the significance of various proposed laboratory diagnostic procedures for its detection have arrived at a contentious level.\(^{21, 22}\)

Numerous measurements of serum lipids as
ELECTROPHORESIS OF LIPOPROTEINS

Figure 12
Frequency distribution of beta/lipalbumin ratios in patients with manifest coronary artery disease and controls. Line graph represents the per cent of normal individuals at each level of values.

diagnostic of atherosclerosis have been suggested, namely; cholesterol,23-26 phospholipids,20, 23, 25, 27 cholesterol/phospholipid ratio,25 cholesterol-uric acid/phospholipid ratio,28 total lipids,24 neutral fats,29 saturated and unsaturated fatty acids,29 as well as lipoproteins determined by chemical fractionation,30 ultracentrifugation,8 and electrophoresis.17, 27, 31

Unfortunately, the information gained by simple chemical analysis has not been deemed sufficiently significant.18, 24, 27 Current adherence to cholesterol determinations as an index of atherosclerosis appears to be a compromise decision according to Keys, "... partly because there is more information about this than any other relevant item of analysis and partly because we still insist there is no evidence that other recommended analytical items have really significantly different or greater diagnostic or prognostic value.32, 33 This opinion is shared also by Adlersberg and Sobotka,20 Clough,21 and Lawry et al.34

In essence, we are in agreement with the foregoing. Our observations, however, indicate that serum cholesterol concentrations can have only an extremely limited diagnostic value. By extrapolation of the curve (figure 1) depicting the proportion of normal individuals at each concentration level, it is apparent that only at the extreme values, below 150 or over 400 mg. per cent does this variable reflect the atherosclerotic status of the individual. These findings are in agreement with those of Wagner and Poin dexter26 as well as Sperry,35 who stated, "unless the total cholesterol content of serum of a patient is extremely low or high, we can not be certain that the amount found is abnormal for that person." Therefore, a manifest weakness of this determination is revealed by the fact that an overwhelming majority of a random population may be expected to fall within the nonsignificant range.

Although phospholipids may play an important role in the solubility of cholesterol and other lipids, from our data at least, their serum concentration per se has no significance in revealing the atherosclerotic status of either the individual or groups. Contrariwise, it may be concluded that the extremely low Fisher t value which we have observed tends to establish an identity between normal and atherosclerotic subjects with respect to this lipid component. In a sense this is not surprising, since the concentration of phospholipids roughly parallels that of total lipids in the blood, an observation supported by a high coefficient of correlation (r = 0.91).

The cholesterol/phospholipid ratio appears to be a function of the change in the serum cholesterol level,20 which is demonstrated by the similarity in the continuous curves shown
in figures 1 and 3. Except for an occasional reference to the contrary, the cholesterol/phospholipid ratio, according to most investigators, bears no relationship to the presence or absence of atherosclerosis. Our own findings show a low level of significance for separating groups and for predicting the status of an individual. By the same reasoning as in the case of cholesterol, the cholesterol/phospholipid ratios may be expected to provide a useful index only at extremely high or low values. Such extreme values, however, are not likely to appear, especially since our data show these variables to have a high coefficient of correlation ($r = 0.84$). This interrelationship has been reported also by others.

It will be noted that total lipid measurements provide a poor index for establishing differences both between normal and atherosclerotic groups and with respect to identifying individuals, since this variable shows zero reliability at every level of concentration. This presumably is related closely to the dietary habits of the individual.

Lipoprotein analyses by paper electrophoresis have yielded considerable variation in results reported by different laboratories. This variation is confirmed by our experiences with the technique used by those investigators. With our method of electrophoresis but using Durrum's staining procedure, we can routinely demonstrate up to 4 lipid zones. From approximately 200 such Oil Red O-stained patterns, quantitated by our procedure previously described, we have found normal, doubtful, and abnormal beta/alpha lipoprotein ratios ranging from 1.0 to 4.0, 4.0 to 6.0, and 6.0 to 12.0, respectively. This observation has been confirmed more recently by 32 normal and 27 atherosclerotic patients in the present series and 20 tuberculous patients (part of another study), in which Oil Red O and Fat Red 7B lipoprotein patterns were run simultaneously. The relatively greater scatter and poorer reproducibility of the Oil Red O values appear to be the result of the deep coloration of the paper background, which, in turn, produces even lower density readings for those lipoproteins present in low concentration, namely, the alpha and lipalbumin fractions. Undoubtedly other factors such as variations in color hue of stained patterns, even when using a single batch of Oil Red O, also contribute to the variability that we find when using this dye.

By our present technic of lipoprotein staining, 5 lipid zones are consistently demonstrated that are found to coincide with their respective protein fractions. The beta lipoprotein and the gamma plus neutral fat fraction, the "O" zone of Adlersberg, have been described repeatedly in the literature. Our demonstration of an alpha-2 lipoprotein is confirmed by the previous observations of Kunkel and Trautman, Moinat et al., and Ackerman et al. The presence of an alpha-1, as well as an alpha-2 lipoprotein and lipalbumin such as we find, also has been described by DeGennes and Polonovski. As early as 1941, Blix et al. have claimed that each of the protein fractions contains some lipids. More recently Eiber et al. have shown that ultracentrifugal pretreatment of serum produces a significant reduction in peaks of beta, alpha-2, and gamma globulin as observed in moving boundary electrophoresis. The conclusion that lipids exist in association with all the protein fractions of the serum is inescapable. The alpha lipoproteins generally referred to in the literature, however, represent a combination of all lipoproteins with mobility greater than that of the beta fraction.

All lipoproteins fractionated by paper electrophoresis, except the gamma lipoprotein plus neutral fat fraction, reveal considerably more significant information relative to atherosclerosis than any of the chemical studies. Of these, the beta lipoproteins, lipalbumin, the beta/alpha ratio and the beta/lipalbumin ratio appear to offer indices that make it possible to classify correctly a large percentage of individuals in our sample. For example, our data dealing with the lipalbumin fraction alone enable one to distinguish the normal from the abnormal group with an exceedingly high degree of confidence ($t = 5.8$) and it is also possible to diagnose one third of the pop-
ELECTROPHORESIS OF LIPOPROTEINS

ulation individually with absolute certainty. Better than one half of the population can be classified individually if an 80 per cent level of confidence is acceptable.

In view of the low relative concentrations of the alpha-1 and alpha-2 lipoproteins we think that they play a rather minor role in transport and metabolism of lipids. On the other hand, our data would support the idea that lipalbumin is considerably more important in the handling of blood lipids than either of these. This view is also indirectly borne out by the fact that the hyperlipemia associated with nephrosis essentially is the result of a hypoalbuminemia. It may be inferred thereby that the lipalbumin fraction may have greater significance in reflecting lipid metabolism than was previously appreciated.

In our considerations of the beta/alpha ratio, we have followed the established precedent in calculation. For example, Kanabrocki et al. and Adlersberg et al. calculate beta/alpha ratios by excluding the gamma and neutral fat fraction and dividing the beta lipoprotein fraction by the balance of the lipidophilic material. With this method of calculation the difference in mean values between normal and abnormal groups in our study is highly significant (t = 5.41). We have found the beta/alpha ratio to be highly correlative with the atherosclerotic status of the individual, since 65 per cent of the total sample population can be classified on an 80 per cent confidence level with values falling below 2.00 and above 2.62. Similar conclusions can be drawn from our data in terms of beta/lipalbumin ratios.

Of the 5 lipoprotein fractions that we have examined, only 2, the beta lipoprotein and the lipalbumin, provide the most useful data, which is not surprising, since these fractions are present in largest concentration and may be presumed to play dominant roles in lipid metabolism. Our data would appear to be considerably more useful than those reported by Jencks et al. who, while claiming that lipoprotein electrophoretic patterns distinguish groups of normal controls from patients with myocardial infarcts, show scatter diagrams in which the degree of overlap between these 2 groups reveals that it is impossible to distinguish more than a very small fraction of the population on an individual basis.

In order to compare Gofman's atherogenic index with ours we have replotted Gofman's data concerned with Sf 12-20 lipoproteins (fig. 13). Remarkably similar trends are noted between Gofman's index and our beta/alpha ratios (fig. 11) but more detailed analysis is not possible without the original data.

Unquestionably an area of overlapping values exists in all such determinations. This can be accounted for by our inability to separate adequately the symptomless atherosclerotic individual from the true normal. In addition, this area of doubt is also contributed to by a considerable number of individuals who, having displayed evidence of coronary artery disease, spontaneously or through medical management, may have adjusted their lipid metabolism so as to interrupt the phenomenon of atherogenesis. This type of course would suggest, therefore, that the pathogenesis of the disease may not be a continuous but rather an intermittent process, and in a sense holds promise for its eventual management. The myocardial infarction, the coronary insufficiency, and the thrombosis are the result of the coronary atherosclerosis, which itself is a secondary manifestation of an underlying metabolic disease. An abnormal atherogenic index in terms of lipoproteins, therefore, must be construed to imply the developing phase of the disease of the arterial wall per se, and not necessarily the extent to which it exists at the time of sampling.

The data thus far discussed appear to warrant further investigation to include a larger population, which is currently in process.

Summary

Data dealing with cholesterol, phospholipid, their ratios, and total lipids in a group of 40 "normal" and 40 individuals with manifest coronary artery disease have been evaluated and have been found to have a low order of significance in determining the atherosclerotic
status for both individuals and for groups.

Serum lipoproteins separated by paper electrophoresis and visualized with Fat Red 7B have been shown to occur in 5 fractions. Of these, lipalbumin and beta lipoproteins as well as beta/alpha and beta/lipalbumin ratios reveal a high level of significance for distinguishing normal from abnormal individuals and groups.

Acknowledgment

The authors wish to acknowledge the technical assistance of Mrs. Marylin Kattan and Miss Shirilyn Shulman.

Summario in Interlingua

Datos relative a cholesterol, phospholipido, le proportion inter le duo, e lipidó total, eseva evalutata in 40 subjectos normal e in 40 con manifesto morbo de arteria coronari. Esseva trovate que ille datos es paucó significative in determinar le stato atherosclerotic tanto de individuos como etiam de gruppos. Esseva mostrate que le lipoproteinas del sero, quando separate per electrophoresa a papiro e visualisate con rubio grasse 7B, occurre in 5 fractiones. Inter istos, le valores pro lipalbumina e le lipoproteinas beta e pro le proportiones beta a alpha e beta a lipalbumina revela un alte grado de signification in le differentiation inter individuos e gruppos normal e anormal.

References

8. JENCKS, W. P., DURRUM, E. L., AND JETTON, M.

WURM, KOSITCHEK, STRAUS

22. GOPMAN, J. W., HONIG, M., JONES, H. B., LAUFER,
ELECTROPHORESIS OF LIPOPROTEINS


Lipoproteins Quantitated by Paper Electrophoresis as an Index of Atherosclerosis
MOSES WURM, ROBERT KOSITCHEK and REUBEN STRAUS

Circulation. 1960;21:526-537
doi: 10.1161/01.CIR.21.4.526

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1960 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/21/4/526

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/