Lipoprotein Patterns by Starch Electrophoresis in Idiopathic Hyperlipemia and Hypercholesteremia

By Fiorenzo Paronetto, M.D., Chun-I Wang, M.D. and David Adlersberg, M.D.

Starch electrophoresis was employed for the study of the lipoprotein patterns in inborn errors of lipid metabolism. Idiopathic hyperlipemia was characterized by marked increases of cholesterol and phospholipid in the alpha-2-lipoprotein fraction of the serum whereas idiopathic hypercholesteremia showed an increase of these lipids in the beta-lipoprotein fraction. The cholesterol and phospholipid contents in the alpha-1-lipoprotein fractions were diminished in both metabolic errors.

In recent years, paper electrophoresis has been extensively used for the study of serum lipoproteins. However, when serum total lipid levels were high considerable adsorption of stainable lipid at the point of application has been observed. Kunkel and Slater were the first to use starch as the supporting medium. They identified two main lipoprotein fractions in human serum. Later, Ackermann and co-workers, Schettler and Kunkel and Trautman reported further observations on lipoprotein patterns in normal persons as well as in patients with abnormalities of lipid metabolism. Previous work from this laboratory was concerned with a comparative analysis of cholesterol and phospholipid in the various serum lipoproteins separated simultaneously by starch and paper electrophoresis. Normal serum and sera from patients with idiopathic hypercholesteremia and with idiopathic hyperlipemia were analyzed for this purpose.

This report presents a detailed study of the lipoprotein distribution by starch electrophoresis in 8 normal persons (controls) and in 7 patients with inborn errors of lipid metabolism. Of these, 4 had idiopathic hyperlipemia and 3 had idiopathic hypercholesteremia.

Material and Methods

Twenty-five milliliters of blood were obtained in the fasting state. The normal group consisted of 2 women, age 26 and 27, and 1 man, age 33.

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Each of the 4 persons with idiopathic hyperlipemia presented lactescence of the serum in the fasting state. The total lipid content of the sera was in repeated determinations consistently 1500 mg. per cent or higher and the cholesterol was over 380 mg. per cent*. Two patients had xanthomata tuberosa; one had mild diabetes, easily controlled with diet alone, subsiding eruptive xanthoma, angina pectoris and intermittent claudication; the fourth subject was asymptomatic.

The fasting sera of the 3 persons with idiopathic hypercholesteremia was persistently clear. The total lipid content was less than 1200 mg. per cent and the cholesterol was over 330 mg. per cent. One of the patients had sustained a myocardial infarction 10 years ago; two others had angina and electrocardiographic evidence of coronary artery disease.

Technic of Starch Electrophoresis

Starch electrophoresis was carried out according to the procedures of Kunkel and Slater with slight modifications. Powdered potato starch "Fisher" (about 450 Cm.) was washed thoroughly with distilled water and then with veronal buffer of pH 8.6 and ionic strength 0.05. The mixture of starch and buffer was then poured into a wax paper mold supported by a glass plate measuring 36 by 17 by 1.5 cm. When the starch appeared solidified the block was divided into equal parts by a narrow longitudinal groove so that duplicate runs of the same serum could be performed simultaneously. Six centimeters from one end of the block, a transverse cut was made in each half of the starch block and extended up to 1.5 cm. from each edge. Two and one-half milliliters of serum diluted with 0.5 ml. buffer was then installed into each cut. A plastic electrophoresis apparatus equipped with a plastic roof and platinum electrodes was used and contact was made with gauze soaked in buffer.

Thirty minutes were allowed for equilibration of the system in a room at 5 C. By applying a constant 450 volts and a current of 18-35 ma., a run 17 cm.

* A table of pertinent data can be obtained from the authors on request.
long could be obtained in a period of 16-18 hours. Albumen was identified as a yellow band moving toward the anode. Two other bands could usually be seen in the regions of about 2-5 cm. and 5-7 cm. from the starting point; the band closer to the starting point appeared brownish and the other one showed a pinkish discoloration. These 2 bands corresponded to the beginning and the end of the α-globulin. After separation each half of the starch block was cut transversely into 1 cm. segments. The segments of one-half were used for lipid analyses and those of the other for protein determinations.

**Lipid Analyses.** After the starch segments were dried overnight at 40°C the lipid content of each segment was extracted with a boiling mixture of chloroform-methanol (2:1) and filtered. Purification of the filtrate was carried out according to the method of Folch. The purified extract was brought to 25 ml. with chloroform-methanol (2:1) in a volumetric flask. Five milliliters were taken for phospholipid analysis. The remaining 20 ml. were evaporated to dryness and re-extracted with acetone-alcohol (1:1) for the determination of total and free cholesterol. The phospholipid and cholesterol contents of each segment were plotted on graph paper. Lipoprotein fractions were determined planimetrically and expressed both in percentage of the total and in milligrams per fraction.

The standard lipid partition was performed in all sera employing methods used in this laboratory.

**Protein Analyses.** The protein content of each segment was determined by the Biuret method. Hyperlipemic sera were usually turbid and produced abnormally high results. However, the procedure was adequate for the localization of the serum protein components.

**RESULTS**

With the aid of the protein analysis the lipoprotein fractions of the "control" sera, could be identified into 2 definite peaks corresponding to alpha-1- and beta-globulin (fig. 1). In these sera the region of the α-2-globulin was completely flat.

The cholesterol content of α-1-lipoprotein varied from 25 to 38 per cent of the total, that of α-2-lipoprotein from 3.4 to 6 per cent, and that of β-lipoprotein from 58.6 to 70 per cent. The esterified cholesterol was 70 per cent in α-1-lipoprotein fraction and 59 per cent in β-lipoprotein. The phospholipid content of α-1-lipoprotein varied from 12.2 to 60.0 per cent of the total, that of α-2-lipoprotein from 1.0 to 5.6 per cent, and that of β-lipoprotein from 39 to 55 per cent. The cholesterol-phospholipid ratio averaged 0.58 in α-1-lipoprotein and 1.35 in β-lipoprotein.

Compared with direct chemical analysis of the serum the recovery of cholesterol from the starch segments amounted to 88 per cent and that of phospholipid to 72 per cent. The recovery of esterified cholesterol (75.5 per cent) was slightly lower than that of total cholesterol indicating a better recovery of free cholesterol.

In idiopathic hyperlipemia, the cholesterol and phospholipid distribution differed decidedly from that observed in "healthy" controls (fig. 2). A high spike of the cholesterol curve in the α-2-region was the prominent feature. The amount of cholesterol decreased in α-1-lipoprotein and increased markedly in β-lipoprotein. Nevertheless, the relative proportion of cholesterol in the β-lipoprotein fraction was moderately decreased when compared with that seen in the control sera; it ranged in the α-1-lipoprotein 2.5 to 14.7 per cent of the total, in the α-2 28.0 to 36.5 per cent and in β-lipoprotein 49.1 to 69.5 per cent. The percentage of esterified cholesterol in α-1-lipoprotein, studied in one instance, was considerably lower than that of the controls (55 per cent vs. 70 per cent), whereas that in β-lipoprotein was slightly higher (61 per cent vs. 59 per cent).

The phospholipids were increased in both the α-2- and β-lipoprotein fractions; a well-defined peak in the α-2-lipoprotein was seen.
only in 2 of the 4 patients studied. Percentage-wise, the phospholipid varied in the \( \alpha-1 \)-lipoprotein from 11.4 to 29.6 per cent of the total, in \( \alpha-2 \)-lipoprotein from 22 to 28 per cent and in \( \beta \)-lipoprotein from 42.4 to 68.6 per cent. The increase of phospholipid in \( \alpha-2 \)- was thus associated with a marked decrease in \( \alpha-1 \)-lipoprotein fraction. The cholesterol-phospholipid ratio in the lipoprotein fractions showed in comparison with the controls a considerable decrease in \( \alpha-1 \)-lipoprotein (0.32 vs. 0.58) as well as in \( \beta \)-lipoprotein (0.93 vs. 1.35).

The recovery of cholesterol came close to the values seen in the controls (83 vs. 88 per cent) whereas the recovery of phospholipid was higher (86 vs. 72 per cent).

In idiopathic hypercholesteremia, the distribution of cholesterol in the lipoprotein fractions was characterized by a marked increase in the \( \beta \)-lipoprotein fraction (fig. 3). The \( \alpha-2 \)-fraction remained flat, as in the controls. The relative proportion of cholesterol ranged in \( \alpha-1 \)-lipoprotein from 10.1 to 12.2 per cent of the total, in \( \alpha-2 \)-lipoprotein from 2.3 to 9.7 per cent and in \( \beta \)-lipoprotein from 80.0 to 87.1 per cent. The percentage of esterified cholesterol in \( \alpha-1 \)- (70 per cent) and \( \beta \)-lipoprotein

Fig. 2. Lipoprotein pattern of a person (C. Ne) with idiopathic hyperlipemia and xanthoma tuberosa. Note large \( \alpha-2 \) and \( \beta \) peaks. Elevation of cholesterol more pronounced than that of phospholipids. Cholesterol and phospholipid contents of the \( \alpha-1 \)-lipoprotein reduced. The cholesterol:phospholipid ratio reduced in all 3 fractions, especially in \( \alpha-1 \)-lipoprotein. Notations as in figure 1.

Fig. 3. Lipoprotein pattern of a person (M. Kl) with idiopathic hypercholesteremia and preceding myocardial infarction. Note peak of \( \beta \)-lipoprotein with marked increase of both cholesterol and phospholipid. In contrast with idiopathic hyperlipemia, there is a slight elevation of phospholipid in \( \alpha-2 \) region without a concomitant increase in cholesterol. Correspondingly there is a marked reduction of cholesterol:phospholipid ratio in \( \alpha-2 \)-lipoprotein without any significant changes of the ratio in \( \alpha-1 \) and \( \beta \)-lipoprotein. Notations as in figure 1.

(66 per cent) in idiopathic hypercholesteremia was somewhat similar to that observed in the controls.

The phospholipid distribution was characterized by a marked increase in \( \beta \)-lipoprotein (62.4–66.3 per cent) and by a small but definite increase in \( \alpha-2 \)-lipoprotein (7.3–18.6 per cent); there was a marked decrease of phospholipid in \( \alpha-1 \)-lipoprotein fraction (16.0–30.3 per cent). These changes resulted in a marked decrease in cholesterol:phospholipid ratio in the \( \alpha-2 \)-fraction (0.45 vs. 1.3) in the controls. Similarly, the recovery of cholesterol and phospholipid was close to that of the controls.

As mentioned previously, 3 colored bands could be seen in the starch block after the separation of serum components by electrophoresis. The brown band, seen 2–5 cm. from the starting point, occupied a wider area in the two disorders of lipid metabolism and was more pronounced in idiopathic hyperlipemia than in idiopathic hypercholesteremia. One may speculate that the intensified brownish band was caused by the higher serum carotene concentration usually seen in conditions associated with marked elevation of serum lipids.

DISCUSSION

The distribution of cholesterol and phospholipid in the lipoprotein fractions, in the control
Table 1.—Cholesterol and Phospholipid Content and Cholesterol Phospholipid Ratio in Lipoprotein Fractions of Control Sera Separated by Starch Electrophoresis

<table>
<thead>
<tr>
<th>Lipoprotein fractions</th>
<th>Author</th>
<th>No. of Cases</th>
<th>( \alpha-1 )</th>
<th>( \alpha-2 )</th>
<th>( \beta )</th>
<th>( \text{Cholesterol/Phospholipid} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol Total</td>
<td>Ackermann and co-workers(^4)</td>
<td>1</td>
<td>32.0</td>
<td>10.0</td>
<td>58.0</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>Schettler(^3)</td>
<td>1</td>
<td>38.0</td>
<td>8.0</td>
<td>53.5</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>Present study</td>
<td>3</td>
<td>30.5</td>
<td>4.8</td>
<td>64.7</td>
<td>0.57</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>Ackermann and co-workers(^4)</td>
<td>1</td>
<td>42.0</td>
<td>11.0</td>
<td>37.0</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>Schettler</td>
<td>1</td>
<td>54.5</td>
<td>11.2</td>
<td>33.3</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>Present study</td>
<td>3</td>
<td>50.0</td>
<td>3.1</td>
<td>46.3</td>
<td>0.57</td>
</tr>
<tr>
<td>Cholesterol/Phospholipid</td>
<td>Kunkel and Slater</td>
<td>5</td>
<td>1.17-1.31</td>
<td>58.0</td>
<td>64.7</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>Ackermann and co-workers(^4)</td>
<td>1</td>
<td>0.72</td>
<td></td>
<td></td>
<td>64.7</td>
</tr>
<tr>
<td></td>
<td>Present study</td>
<td>3</td>
<td>1.20</td>
<td>78.7</td>
<td>77.5</td>
<td>0.57</td>
</tr>
</tbody>
</table>

sera, is in good agreement with the observations of Kunkel and Slater, Ackermann and co-workers, and Schettler (table 1). Kunkel and Slater\(^3\) identified only the \( \alpha \) and \( \beta \) component. Ackermann and associates\(^4\) suggested the importance of an "intermediate fraction" in the region of \( \alpha-2 \) \( \beta \)-1-globulin in addition to the two main lipoprotein fractions. The "intermediate fraction" correlates well with the \( \alpha-2 \) fraction seen by Schettler,\(^3\) Kunkel and Trautman\(^6\) and by us in this study.

In idiopathic hyperlipemia the lipoprotein pattern was characterized by a huge \( \alpha-2 \) fraction as well as a marked elevation of the \( \beta \)-lipoprotein component. In a single case of idiopathic hyperlipemia studied by Schettler\(^3\) a separate \( \alpha-2 \)-lipoprotein peak was not present. Nevertheless, the figures of the \( \alpha-2 \)- and \( \beta \)-lipoprotein of his case are in good accord with the sum of \( \alpha-2 \)- and \( \beta \)-lipoprotein seen in our patients.

In idiopathic hypercholesteremia both the cholesterol and phospholipid contents of the \( \beta \)-lipoprotein were markedly increased. Moderate increase in the phospholipid content of the \( \alpha-2 \)-lipoprotein was seen while the cholesterol content remained unchanged. In a single case of idiopathic hypercholesteremia studied by Kunkel and Slater\(^3\) no elevation of cholesterol or phospholipid was found in the \( \alpha-2 \) component. In a subsequent publication Kunkel and Trautman\(^6\) found in a limited group of "normal and atherosclerotic individuals" the phospholipid content of the \( \alpha-2 \) fraction ranging from 4–34 per cent of the total. Ackermann's group\(^4\) observed in one case of coronary artery disease associated with hypercholesteremia an increase of both cholesterol and phospholipid in the intermediate fraction to 21 and 30 per cent of the total, respectively. Since neither of these investigators separated idiopathic hyperlipemia from idiopathic hypercholesteremia a strict comparison of the results is impossible.

It was of interest to compare the sum of cholesterol, as well as that of phospholipid, in \( \alpha-2 \)-plus \( \beta \)-lipoprotein in the two errors of lipid metabolism. The absolute content of cholesterol in \( \alpha-2 \)-plus \( \beta \)-lipoprotein was considerably higher in idiopathic hyperlipemia than in idiopathic hypercholesteremia (363 vs. 266.6 mg.). Similarly, the phospholipid content for \( \alpha-2 \)-plus \( \beta \) was considerably higher in idiopathic hyperlipemia (367 vs. 248.4 mg.). Despite these differences in the absolute figures, the percentage distribution of cholesterol and phospholipid in \( \alpha-2 \)-plus \( \beta \)-lipoprotein was approximately identical in both conditions. The relative cholesterol content in \( \alpha-2 \)-plus \( \beta \)-lipoprotein amounted to 91.8 per cent in idiopathic hyperlipemia and 89.5 per cent in idiopathic hypercholesteremia; the corresponding figures for phospholipid was 78.7 per cent and 77.5 per cent, respectively.
The consistent α-2- peak in idiopathic hyperlipemia suggested a causal relationship with the high concentration of serum triglycerides seen in this condition. It appeared profitable to study the effect of fat loading on the lipoprotein pattern of "normal" persons. Data in the postprandial lipemia to be published elsewhere showed that certain cholesterol and phospholipid molecules migrated with the mobility of the α-2-lipoprotein.

Summary

Lipoprotein patterns by starch electrophoresis were studied in 3 "healthy" controls, 4 persons with idiopathic hyperlipemia and 3 persons with idiopathic hypercholesteremia.

In the control sera, 2 well-defined lipoprotein fractions were identified: α-1- and β-lipoprotein. The relative cholesterol content of these two fractions was 30.5 and 64.7 per cent, respectively; 4.8 per cent cholesterol was found in the intermediate zone corresponding to the α-2- area. The phospholipid content was 50.0, 46.9 and 3.1 per cent for the α-1-, β- and α-2-lipoprotein, respectively.

In idiopathic hyperlipemia, the lipoprotein patterns were characterized by a marked increase in cholesterol and phospholipid content in the α-2-lipoprotein fraction amounting to 32 per cent for the cholesterol and 25.1 per cent for the phospholipid. There was an associated decrease of cholesterol and phospholipid in α-1-lipoprotein.

In idiopathic hypercholesteremia, the lipoprotein pattern was characterized by decided increases in the β-lipoprotein fraction of cholesterol and phospholipid content to 84 and 64.7 per cent, respectively. Again, there was a concomitant decrease of cholesterol and phospholipid in α-1-lipoprotein fraction. The α-2-lipoprotein showed an elevated phospholipid content (12.8 per cent) with a practically normal cholesterol content (5.5 per cent).

In le seros de controlo, 2 ben-definite fraciones lipoproteinicas esseva ideificate: lipoproteina alpha-1 e beta. Le contento relative de cholesterol in iste fraciones esseva 30,5 e 64,7 pro cento, respectivamente. In le zona intermediate, correspondent al area de alpha-2, 4,8 pro cento de cholesterol esseva trovate. Le contento de phospholipido esseva 50,0, 46,9, e 3,1 pro cento in le lipoproteinas alpha-1, beta, e alpha-2, respectivemente.

In hyperlipemia idiopathic, le configuration lipoproteinica esseva caracterisate per marcate augmentos del contento de cholesterol e phospholipido in le fraction lipoproteinica alpha-2. Iste augmentos amontava a 32 pro cento pro cholesterol e a 25,1 pro cento pro phospholipido. Esseva constatate un reduction concomitante del contento de cholesterol e phospholipido in le lipoproteina alpha-1.

In hypercholesteremia idiopathic, le configuration lipoproteinica esseva caracterisate per pronunciate augmentos del contento de cholesterol e phospholipido in le fraction lipoproteinica beta. Iste augmentos amontava a 84 e 64,7 pro cento, respectivamente. Etiam in iste casos, un reduction concomitante del contento de cholesterol e phospholipido esseva notate in le fraction lipoproteinica alpha-1. Le fraction alpha-2 monstrava un augmento de 12,8 pro cento in le contento de phospholipido e un nivello praticamente normal (+5,5 pro cento) in le contento de cholesterol.

Summario in Interlingua

Le configurationes lipoproteinica esseva studiate per amyleoelectrophoresis in 3 normal subjectos de controlo, 4 individuos con idiopathic hyperlipemia, e 3 individuos con idiopathic hypercholesteremia.

REFERENCES

7 Paronetto, F., Wang, C. I. and Adlersberg,
Pressoreceptor Reflexes after Hemorrhage

It has previously been shown that the recovery of arterial pressure after hemorrhage depends on the intactness of pressor reflex mechanisms (Heymann and co-workers, 1933). It is also known that under normal conditions reduction of sinuscarotid pressure induces arterial hypertension by increase in both peripheral resistance and cardiac output.

Prof. Bouckaert and his associates have recently shown, by experiments in which pressure in the isolated carotid sinuses could be varied independently of arterial pressure of the animal before and after bleeding, that during hypovolemia induced by bleeding, reflex increase in total peripheral resistance plays a much greater part in restoring arterial pressure than augmentation of cardiac output.

It should be observed, however, that the technical procedures employed included vagotomy, hence perhaps obliterated an increase in cardiac output through cardiac acceleration, such as takes place normally. Since heart rate changes were probably negligible, the obvious corollary would seem to me that pressor reflexes from the carotid sinuses did not aid venous return by venomotor action.

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