Changes in Proteins and Lipoproteins in Diabetes and their Relationship to Vascular Degeneration

By J. C. Demanet, M.D., P. E. Gregoire, M.D., and P. A. Bastenie, M.D.

The cause of atherosclerosis and reason for its high incidence in diabetes are not known. In this study proteins and lipoids of serums of patients with diabetes or atherosclerosis are examined by paper electrophoresis to determine whether such changes may be a common factor for both of these conditions.

This paper presents data on lipid and protein changes in the serum of various groups of subjects, with or without diabetes and with or without atherosclerotic lesions. Comparisons of these data might indicate the significance of the common metabolic alterations sometimes held responsible for the development of vascular degeneration.

Methods and Materials

The 97 subjects studied have been grouped in 7 classes (table 1): class 1, 30 normal subjects, 16 to 48 years old, without clinical signs of atherosclerosis; class 2, 27 older subjects (aged 36 to 93) with clinical signs of atherosclerosis; classes 3 to 7, 40 diabetic subjects, 10 being without clinical signs of vascular degeneration, the others presenting various vascular complications. The last class includes 5 cases with albuminuria, hypertension and retinopathy, suggesting the diagnosis of Kimmelstiel-Wilson glomerulosclerosis; 2 cases were verified at autopsy.

Protein electrophoresis was performed by the method of Dustin on Whatman paper no. 1 strips, which were stained with bromophenol blue and read after elution of the chromogenic material.

Electrophoresis of the lipoproteins was effected with the same apparatus and paper, 0.15 ml. of serum being used per test; staining was carried out by the method of Jencks and Durrum for 20 hours in 60 per cent alcohol saturated with oil red 0. The strips were washed with tap water and read after elution with 20 per cent acetic alcohol. With this method electrophoresis for 6½ hours at 300 volts yields well separated α- and β-lipoprotein fractions. In order to estimate absolute quantities of lipids transported by each of the 2 lipoprotein fractions, "total colorable lipids" have been measured by a technic derived from that of Swahn: on equal square pieces of Whatman paper no. 1, increasing quantities were deposited (0.02 to 0.04 to 0.06 ml.) of serum and of a test solution of 1 per cent triolein in absolute alcohol. After stains were applied as described for lipoproteins, photometric reading of the eluate of each specimen yielded figures expressing the total amount of "colorable serum lipids" in milligrams per cent of triolein per 100 ml. serum. Values of α- and β-lipoprotein fractions were then estimated in milligrams of triolein per 100 ml. serum. Sensitivity and reproducibility of these methods have been tested by repeating 10 times the assay on the same serum. The results indicate standard errors between 4.5 and 7.6 per cent for protein fractions (except α-1 globulin), of 4.3 per cent for colorable serum lipids, of 6 per cent for α- and of 2.4 per cent for β-lipoproteins.

Cholesterol has been measured by the method of Schoenheimer and Sperry, and total lipids by the method of Bloor.

Results

Comparison of the 2 series of nondiabetic subjects showed significant alterations in serum proteins and lipids in the group of older atherosclerotic patients. The changes in proteins were characterized by a decrease in the albumin fraction and an increase in the globulins, specially in the α2, but also the β- and γ-globulins. The alteration in lipids consists in an increase of cholesterol, of total lipids, of colorable lipids, and more specifically of the β-lipoproteins.

The findings in 2 series of younger or elder diabetic subjects without signs of im-
<table>
<thead>
<tr>
<th>Classes</th>
<th>Age limits</th>
<th>No. of cases</th>
<th>Serum protein fractions (% of total)</th>
<th>Serum lipids (mg for 100 ml)</th>
<th>Serum lipoproteins</th>
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<td></td>
<td></td>
<td>Alb</td>
<td>α'</td>
<td>α²</td>
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<tr>
<td>1. Normal subjects‡</td>
<td>18-48</td>
<td>30</td>
<td>67.4±4.2</td>
<td>4.3±0.8</td>
<td>7.0±1.3</td>
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<td>2. Atherosclerosis difference from 1</td>
<td>36-93</td>
<td>27</td>
<td>59.9±5.4</td>
<td>4.9±0.9</td>
<td>9.0±2.4</td>
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<td>p&lt;0.001</td>
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<td>3. Diabetes without complication difference from 1</td>
<td>15-55</td>
<td>10</td>
<td>64.7±5.1</td>
<td>4.6±0.6</td>
<td>6.6±1.2</td>
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<td>p&lt;0.02</td>
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<td>4. Diabetes with mild atherosclerosis difference from 1</td>
<td>55-79</td>
<td>10</td>
<td>64.7±5.0</td>
<td>4.1±0.8</td>
<td>7.1±1.0</td>
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<td>p&lt;0.02</td>
<td>p&lt;0.05</td>
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<td>5. Diabetes with severe arteritis difference from 1</td>
<td>50-80</td>
<td>6</td>
<td>59.5±5.8</td>
<td>4.7±0.6</td>
<td>9.7±1.9</td>
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<td>p&lt;0.001</td>
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<tr>
<td>6. Diabetic retinopathy difference from 1</td>
<td>45-70</td>
<td>9</td>
<td>60.7±6.6</td>
<td>4.2±0.8</td>
<td>8.4±1.9</td>
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<td>p&lt;0.001</td>
<td>p&lt;0.02</td>
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<td>7. Kimmelstiel-Wilson disease from 1</td>
<td>42-62</td>
<td>5</td>
<td>48.9±6.2</td>
<td>5.6±1.8</td>
<td>12.0±3.5</td>
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*Normal values after Thannhauser.*
†Normal values after Adlerberget al.*
‡For each group mean values and standard errors (σ) are given. Student-Fischer test has been applied to compare the different groups. Significance is indicated by the p values; nonsignificant results by zero. A dash means that no calculation has been performed, owing to insufficient data.
portant atherosclerotic lesions are not significantly different from each other. Compared to non-diabetic controls, these 2 groups of diabetic subjects show the following changes: increase in β-globulins, increase in cholesterol and total lipids, and increase in colorable lipids, due to marked elevation of the β-lipoproteins.

The fifth group of 6 diabetic patients with severe peripheral atherosclerosis displays the same changes in serum proteins as the group of atherosclerotic patients without diabetes: decrease in albumin, increase in globulins, specially α2- and β-globulins. In this group the values of cholesterol, of lipids and of β-lipoproteins are very high.

In the group with diabetic retinopathy the same changes in proteins and in the lipids except cholesterol were observed. Changes are most marked both for proteins and lipids in the class of diabetic patients with retinopathy and nephropathy. The albumin fraction is very depressed and the increase in globulins concerns all fractions. Cholesterol and total lipids reach extremely high values and the marked increase in colorable lipids is due to the very high augmentation of the β-lipoproteins.

Although the diabetic condition is characterized by a definite increase in total serum lipids, in most cases the respective fractions transported with α- and β-proteins are not markedly altered. However the partition of the lipids between α and β fractions is profoundly altered in cases with clinical evidence of specific abnormalities of the vessels, and most so in patients with diabetic glomerulosclerosis.

**Discussion**

The methods used for paper electrophoresis of serum proteins are satisfactory for clinical research. The results obtained in this investigation are closely comparable to those reported by Jencks et al.6 It is true that paper electrophoresis used in the determination of lipoproteins yield only semiquantitative results. As discussed by Eder,7 however this method has definite advantages over more intricate procedures.

With these limitations it appears that the changes in serum proteins and lipids, which are observed in diabetes and in atherosclerosis, are very similar.

In the present study, atherosclerosis is characterized by a reduction in serum albumin and and increase in α2-, β- and γ-globulins and by a definite increase of the serum cholesterol, total lipids and β-lipoproteins. These observations are in keeping with numerous previous reports on proteins,6,8,9 on cholesterol,10,11 on lipids, and on electrophoretically separated lipoproteins.7,13-16

Diabetes, even when under good control and without vascular degeneration, differs from the normal state by a definite increase of β-globulins and the same alterations in lipid metabolism, that are found in non diabetic atherosclerosis. With development of vascular lesions, the anomalies do not undergo qualitative changes but α2-globulins increase as in atherosclerosis; also the cholesterol and other lipids may reach very high values.

Similar observations have been made by other authors for proteins,17-20 cholesterol and lipids21-27 lipoproteins separated by ultracentrifugal techniques,23,27,28 and by paper electrophoretic methods.3,15,29

While some of these changes, in particular the increase of α2-globulins, only become apparent with the development of severe atherosclerotic changes, it is difficult to establish to what extent the metabolic changes are primary or secondary to the vascular lesions. Schertenlieb and Tuller,31 in a recent paper report "a tendency for those protein changes to be present before there is clinical evidence of vascular damage." It is clear that in the Kimmelstiel-Wilson syndrome many of the changes may be due to some functional alteration similar to those of the nephrotic syndrome. However, the observations made in uncomplicated diabetes suggest that increase in the β-lipoproteins and in the total lipids together with elevation of the β-globulins play a definite role in the development of the vascular degeneration, so common in the course of diabetes.
SUMMARY

Paper electrophoresis of serum proteins and lipids has been performed in 97 subjects viz. 30 normal subjects, 27 nondiabetic patients with atherosclerosis, 40 diabetic patients, some with vascular degeneration.

Certain alterations in serum proteins and lipids are of constant occurrence in atherosclerosis: decrease in albumin, increase in $\alpha_2$, $\beta$- and $\gamma$-globulins, increase in serum cholesterol, total lipids and $\beta$-lipoproteins. Some of these changes, especially increased $\beta$-globulins and lipids, are present in diabetes even without detectable signs of vascular degeneration.

SUMMARIO IN INTERLINGUA

Electrophorese a papiro esseva effectuate pro le proteinas e lipidos seral de 97 subjectos, i.e. 30 normales, 27 atheroscleroticos non diabetic, e 40 diabeticos, certes sin e alteres con degeneration vascular.


REFERENCES


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Subsequently, in the celebrated Commentaries upon which our grandfathers in the profession were educated, Heberden gave a fuller account of his experience with the disease. The name which he adopted can not be regarded as altogether satisfactory, since it was already in use in designating affections of the throat, with which its literal meaning—a strangling—is much more in harmony. In one sense, however, the term is fairly appropriate, since, as noted by Gairdner, the words anxiety and anguish, expressive of two of the most prominent features of the disease, have a derivation from the same Greek word as angina.—WILLIAM OSLER. Lectures on Angina Pectoris and Allied States, 1897.
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Circulation. 1959;19:863-867
doi: 10.1161/01.CIR.19.6.863

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