SERUM POLYSACCHARIDES IN DIABETES MELLITUS

by

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It is generally accepted that the concentration of polysaccharide in the serum of diabetic patients with vascular complications is greater than that in the serum from such patients without vascular complications. GILLILAND, HANNO and STRUDWICK ¹ have also shown that such changes are demonstrable before kidney complications ensue and that an increase in the protein-bound polysaccharide as well as an increase in the $\alpha_2$ and $\beta$ globulins is characteristic of the Kimmelstiel-Wilson syndrome. BERKMAN, RIFKIN and Ross ², however, have doubted whether the Kimmelstiel-Wilson syndrome has a characteristic pattern of serum polysaccharides. The present paper describes the results of an investigation of the serum polysaccharides in a series of 40 diabetic patients, some of whom had renal lesions while others had retinal lesions alone.

Clinical material

The 40 diabetic patients who were either in-patients or out-patients of the Diabetic Department of King's College Hospital were classified in four groups.

Group I. 12 patients who showed no sign of retinal, vascular or renal disease. They were selected as being comparable with Group IV in insulin requirements, age and duration of the diabetes.

Group II. 10 patients with retinopathy of various degrees of severity, usually severe, but showing no evidence of renal damage. Of these patients, only three were free from hypertension (i.e. their blood pressure was lower than 140/90).

Group III. 11 patients without retinopathy but with miscellaneous complications such as myocardial infarction, gangrene of the leg, or clinically obvious arteriosclerosis. Routine investigations showed no evidence of renal damage.

Group IV. 7 patients with hypertension, albuminuria and retinopathy. In 5 of these there was renal failure, two being shown at post-mortem to have the typical lesions of the Kimmelstiel-Wilson syndrome. The diagnosis of this syndrome is notoriously difficult during life but all of the cases in this group showed the clinical picture described by RIFKIN, LEITER and BERKMAN ³.

None of the patients showed evidence of carcinoma, infection or other abnormality known to cause an increase in the concentration of serum polysaccharides. No patient was investigated until at least 20 days after an operation.

Blood samples were collected by venepuncture between 10 and 11 a.m. It was not possible to collect blood from many of the diabetic patients while fasting, and a small series of 8 fasting and 7 non-fasting normal subjects was also investigated.

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Chemical methods

Total protein bound hexose was determined by the anthrone method of Graff, Greenspan, Selman and Holecek 4. The anthrone was employed at a concentration of 0.2% in 95% sulphuric acid as suggested by Dreywood 5. The anthrone reaction with carbohydrate depends on the temperature developed by the reaction of the sulphuric acid in the reagent with the aqueous solution. Many authors have therefore suggested a water or ice bath for the addition of the reagent followed by a heating in a water bath at a constant temperature (Seifter, Dayton, Noric and Muntwyler 4; Roe 7; Kendall and Prudden 8; Mokrash 8. Satisfactory results have been obtained at room temperature by adding the reagent to the solution in Pyrex tubes of 12.5 cm diameter using stirring rods of uniform diameters. The rate of addition of the reagent was controlled by using a 10 ml pipette, the discharge of which took 45 sec. Stirring of the mixture with the rod lasted only while the reagent was added; the rod was then left in the tube. The temperature in the tubes soon after addition of the anthrone reagent rose to 85 or 95°C according to the batches of sulphuric acid used, and regained room temperature in 25 min. Determinations of optical density were carried out half to one hour after mixing. The anthrone reagent was used after 4 hours of preparation and was made up freshly each time. The standard solution of galactose-mannose was prepared in saturated benzoic acid. The final solutions obtained from standards of different concentrations followed Beer's Law, and the various duplicates or triplicates for each determination rarely differed by more than 3%. No better results could be obtained by controlling the rise in temperature with a cold water bath followed by heating in a boiling water bath.

Hexosamine was estimated by the method of West, Clark and Kennedy 10.

Mucoprotein was isolated by the method of Winzler, Devor, Mehl and Smyth 11. The hexose and tyrosine content of the fraction was estimated by the methods of Graff et al. 4 and of Folin and Ciocalteu 12 respectively.

Paper electrophoresis. Sera were examined by paper-electrophoresis using the apparatus of Flynn and De Mayo 13. The protein fractions were stained on the paper with azocarmine B and the polysaccharides by the method of Kogi and Gronwall 14 with periodic acid-Schiff reagent. Quantitative analyses of the fractions were made within 24 hr of staining using an EEL* scanner using a mixture of 1 bromo-naphthalene and liquid paraffin as clarifier. Each electrophoresis was performed in quadruplicate, thus providing separate duplicates for the staining and subsequent analyses of protein and carbohydrate respectively. Results usually agreed within 5%, but sometimes agreement was less close. Such discrepant results were discarded and a fresh sample of serum was examined. Good proportionality between staining intensity and content of carbohydrate material within the concentrations examined was demonstrated using the technique of Crooke, Harris, Hassan and Warren 15 by staining circles of filter paper upon which equal quantities of solutions containing various amounts of serum or serum mucoprotein had been applied. This mucoprotein was prepared from pooled serum by the method of Winzler et al. 11; after precipitating and washing with phosphotungstic acid, it was washed twice with ethanol and dissolved in 1/8 saturated sodium carbonate.

* Evans Electro-Selenium.
RESULTS

Normal subjects

The results (Table I) are in reasonable agreement with those of others. However, the concentration of serum hexosamine in non-fasting normal subjects is significantly higher than those in fasting subjects. This is in contrast to the findings of Seibert and Atno; however, the difference is not great and the figures for the fasting normals and the normals as a whole are in good agreement with those of others (Berkman, Rifkin and Ross; Seibert and Atno). Protein-bound hexose concentrations are about the same as those of Greenspan, Lehman, Graff and Schoenbach and those of Shetlar, Foster, Kelly and Everett, but are lower than those reported by Berkman et al. and higher than those of Seibert and Atno. These differences may be due to the different techniques and/or to the difference in age of the groups studied. Serum polysaccharide concentrations have been shown to increase with age (Shetlar et al.).

The results with the diabetic patients are summarised in Tables II and III.

Group I. Diabetic subjects without complications

In 10 of the 12 patients in this group the serum hexosamine, total protein-bound hexose and the serum mucoprotein hexose and tyrosine concentrations were all within normal limits. Considering the group as a whole, the results were not significantly different from the values found in normal subjects. In two patients in this group the polysaccharide concentrations were far higher than the others and indeed were as high as those found in any of the groups studied. These 2 patients have shown no sign of complications at the time of writing (20 months after the investigations were performed).

Group II. Diabetics with retinopathy

The subjects in this group showed a highly significant increase in the hexosamine fraction; the increases in the other fractions examined were of doubtful significance. Only three patients in this group were free from hypertension, but the values in each of these three patients were within the same range of the other eight subjects.

Group III. Diabetics with miscellaneous complications

The serum hexosamine and also the tyrosine of the mucoprotein of this series of
<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
<th>Mean Age</th>
<th>Duration</th>
<th>Insulin (units/day)</th>
<th>Hexosamine (mg/100ml) (a)</th>
<th>Total protein bound hexoses (mg/100ml) (b)</th>
<th>Ratio</th>
<th>Mucoproteins Hexose (mg/100ml) (c)</th>
<th>Tyrosine (mg/100ml) (d)</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Diabetics with no apparent complications (12 subjects)</td>
<td>46</td>
<td>17</td>
<td>47</td>
<td>112.0 ± 13.6</td>
<td>132.0 ± 42.2</td>
<td>0.94 ± 0.15</td>
<td>12.1 ± 4.7</td>
<td>4.12 ± 1.04</td>
<td>2.98 ± 0.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(24–70)</td>
<td>(7–35)</td>
<td>(32–64)</td>
<td>(99–133)</td>
<td>(87–249)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td>Diabetics with retinopathy (11 subjects)</td>
<td>60.3</td>
<td>11.1</td>
<td>26</td>
<td>140.2 ± 17.4</td>
<td>155.8 ± 33.1</td>
<td>0.92 ± 0.11</td>
<td>16.9 ± 5.1</td>
<td>4.8 ± 0.93</td>
<td>3.50 ± 0.65</td>
</tr>
<tr>
<td>Group III</td>
<td>Diabetics with miscellaneous complications (11 subjects)</td>
<td>62.4</td>
<td>18</td>
<td>37</td>
<td>134.9 ± 19.7</td>
<td>158.8 ± 28.8</td>
<td>0.89 ± 0.11</td>
<td>18.4 ± 7.7</td>
<td>5.05 ± 1.1</td>
<td>3.59 ± 1.20</td>
</tr>
<tr>
<td>Group IV</td>
<td>Diabetics with K.W. syndrome (7 subjects)</td>
<td>44.5</td>
<td>17.7</td>
<td>47</td>
<td>145.2 ± 23.0</td>
<td>174.8 ± 24.7</td>
<td>0.83 ± 0.06</td>
<td>21.0 ± 6.7</td>
<td>5.92 ± 1.2</td>
<td>2.95 ± 0.64</td>
</tr>
</tbody>
</table>

Figures in parenthesis give ranges. Other results given as mean ± standard deviation.
**TABLE III**

**STATISTICAL ANALYSIS**

Probability that observed differences are due to chance.

<table>
<thead>
<tr>
<th>Groups compared</th>
<th>Hexosamine (a)</th>
<th>Total protein bound hexoses (b)</th>
<th>Ratio a/b</th>
<th>Mucoprotein hexose (c)</th>
<th>Mucoprotein tyrosine (d)</th>
<th>Ratio c/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal and diabetics without complications</td>
<td>&lt;0.2, &gt;0.1</td>
<td>&lt;0.3, &gt;0.2</td>
<td></td>
<td>0.5</td>
<td>&lt;0.2, &gt;0.1</td>
<td></td>
</tr>
<tr>
<td>Diabetics without complications</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- and diabetics with retinopathy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- and diabetics with miscellaneous complications</td>
<td>&lt;0.001</td>
<td>&gt;0.001</td>
<td>0.7</td>
<td>&lt;0.5, &gt;0.2</td>
<td>&lt;0.2, &gt;0.1</td>
<td>&lt;0.05, &gt;0.02</td>
</tr>
<tr>
<td>- and diabetics with Kimmelstiel-Wilson syndrome</td>
<td>&lt;0.001</td>
<td>&lt;0.05, &gt;0.02</td>
<td>&lt;0.001</td>
<td>&lt;0.001, &gt;0.01</td>
<td>&lt;0.001</td>
<td>0.9</td>
</tr>
<tr>
<td>Diabetics with retinopathy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- and diabetics with miscellaneous complications</td>
<td>0.5</td>
<td>0.8</td>
<td>0.5</td>
<td>0.6</td>
<td>0.5</td>
<td>0.8</td>
</tr>
<tr>
<td>- and diabetics with Kimmelstiel-Wilson syndrome</td>
<td>0.6</td>
<td>&lt;0.3, &gt;0.2</td>
<td>&lt;0.1, &gt;0.05</td>
<td>&lt;0.1, &gt;0.05</td>
<td>&lt;0.1, &gt;0.05</td>
<td>&lt;0.1, &gt;0.05</td>
</tr>
<tr>
<td>Diabetics with miscellaneous complications and</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>diabetics with Kimmelstiel-Wilson syndrome</td>
<td>0.3</td>
<td>&lt;0.3, &gt;0.2</td>
<td>0.7</td>
<td>0.5</td>
<td>&lt;0.2, &gt;0.1</td>
<td>0.2</td>
</tr>
</tbody>
</table>
patients were significantly in excess of normal but the average age of this group is greater than that of any other group. These changes in polysaccharide concentration might therefore be due to the arteriosclerosis which may have occurred with increasing age and independently of the diabetes.

**Group IV. Diabetics with Kimmelstiel-Wilson syndrome**

There is a marked and highly significant increase in the concentration of all the fractions analysed with the exception of the total protein-bound hexose, the increase in which was of doubtful significance.

**Paper electrophoresis studies**

Because of the generally acknowledged difficulties in the accurate quantitative estimation by staining of serum proteins after electrophoresis on filter paper, the results will not be presented in detail. Adequate information may be obtained by simple visual assessment of the paper strip. In general the quantity of polysaccharide bound to protein and detected by staining by the periodic acid Schiff reaction agreed with the quantities determined chemically. In the normal person the periodic acid staining material was mainly bound to the \( \beta \)-globulin and to a less extent to the \( \alpha_2 \)-globulin. The \( \alpha_1 \) and \( \gamma \) globulins and the albumin bound very little, if any, periodic acid staining material.

In the patients of Group I (uncomplicated diabetes), of Group II (diabetes with retinopathy) and of Group III (diabetes with miscellaneous complications) the electrophoretic patterns of the serum protein and of the periodic acid staining material were not obviously different from the patterns found in normal people. In agreement with the results of direct chemical analysis, increased periodic acid staining material was found in the miscellaneous group of patients, and this material was mainly found to be associated with the \( \alpha_2 \) globulin.

The results obtained with the patients of Group IV (diabetes with renal complications) were very striking. In about two-thirds of the cases a higher than normal peak of periodic acid staining material was present in the \( \alpha_2 \)-globulin fraction, lower peaks being found in the \( \beta \) and \( \alpha_1 \) globulin and sometimes also present in the albumin and \( \gamma \) globulin. In the remaining third, however, periodic acid staining material had also migrated with the albumin fraction, and in these cases it appeared that albumin was an important carrier of this material. In three cases of group IV, the albumin concentration appeared lower and the \( \alpha_2 \) globulin concentration higher than normal. In the other four cases the \( \alpha_2 \) globulin fraction was not obviously raised even though the chemical estimation and the periodic acid staining had shown a high concentration of protein-bound polysaccharide. The periodic acid stain is not specific for saccharides, and lipides and lipoprotein complexes may well be partially responsible for the periodic acid staining of the protein fraction on the paper.

**DISCUSSION**

The present results are in agreement with those of others in showing that the serum polysaccharides are in greater concentration in diabetic patients with renal and retinal lesions than in diabetic patients without such complications. The greater increase in those patients with the Kimmelstiel-Wilson syndrome (i.e., with hyper-
tension, retinopathy and albuminuria) compared with that in patients with retinopathy alone supports the views of Gilliland, Hanno and Strudwick that there is a correlation between the severity of the lesions and the concentration of polysaccharides in the serum. This increase is not specific for the Kimmelstiel-Wilson syndrome or for retinopathy as is shown clearly by the increase in serum polysaccharides in two of the patients free from complications. In these two the increase was even greater than that in the patients with the renal complications. These observations together with the increase in protein-bound polysaccharides known to occur in a variety of diseases including cancer, collagen disease, tubercle and sarcoid as well as in pregnancy and in fever, show without doubt that the protein-bound polysaccharide concentration in serum would be a very unreliable indication of the presence of the Kimmelstiel-Wilson syndrome.

The ratio of hexosamine to protein-bound hexose approximated closely to unity in all groups except in Group IV in which the ratio was significantly lower than was found in the other groups. The ratio of carbohydrate to tyrosine in the mucoprotein fraction showed minor differences in the various fractions but statistical analysis revealed that these differences were not significant. This is in agreement with the findings of Winzler et al. who also found the composition of their mucoprotein fraction was fairly constant in various pathological conditions.

The statistical analysis of the results shows clearly that it is the hexosamine content of the serum which appears to be the most sensitive indication of the onset of complications in diabetes. The statistical significance of the alteration in this fraction in Group IV is greater than that in the other fractions, and in Group II with retinopathy it is the only fraction which shows a significant increase. It would appear, therefore, that the relative simplicity of estimation of this fraction in contrast to the rigid standardization necessary for the estimation of the protein-bound hexose fraction makes the former the investigation of choice in the investigation of diabetes for the onset of complications. As has clearly been shown this estimation must not be regarded as specific in the detection of these complications.

The use of paper electrophoresis followed by periodic acid-Shiff staining of the protein fraction thus separated requires much further investigation before it can be recommended for the investigation of diabetic patients. Rigid standardization of technique and equipment is needed before the results of one laboratory may be readily compared with those in another.

ACKNOWLEDGEMENTS

We wish to acknowledge our gratitude to Drs. R. D. Lawrence and W. G. Oakley for their permission to allow us to investigate their patients. We thank also Drs. K. J. Gurling and J. A. Robertson who were kind enough to assist in the selection of suitable cases. One of us (D.V.A.) thanks the British Council for a grant which enabled him to visit this country for the period during which the work was carried out. We are grateful to Dr. Ian Gilliland for helpful discussion.

SUMMARY

Hexosamine, total protein-bound hexose, and the hexose and tyrosine content of the mucoproteins in serum have been estimated in fifteen normal subjects and
forty diabetic patients. Polysaccharides and proteins were also estimated by paper electrophoresis. The diabetic patients were divided into four groups, those without complications, those with retinopathy, those with miscellaneous complications and those with the Kimmelstiel-Wilson syndrome.

The concentrations of the serum polysaccharides were considerably elevated in the group of patients with the Kimmelstiel-Wilson syndrome; the patients with retinopathy showed an increase in the concentration of serum hexosamine. Although there was some evidence that the concentration of these substances increased with the severity of the complications, this was not a regular phenomenon because two patients without apparent complications showed very high concentrations of serum polysaccharides.

Changes in serum hexosamine seem to be the most sensitive indication of the onset of complications in diabetes, but cannot be regarded as specific.

Résumé


Les concentrations des sérum-polysaccharides étaient considérablement plus élevées dans la groupe des sujets à syndrome Kimmelstiel-Wilson; ceux souffrant de rétinopathie montraient un accroissement de la concentration en hexosamine du sérum. Certains faits indiquent que la concentration de ces substances augmente avec la gravité des complications; pourtant, il ne s'agit pas là d'un phénomène régulier, puisque deux patients sans complications apparentes montraient des concentrations très élevées de sérum-protéines.

Des changements de la teneur en hexosamine du sérum semble être l'indication la plus sensible du commencement de complications du diabète; on ne peut cependant pas considérer cette indication comme spécifique.

Zusammenfassung


denn zwei Patienten ohne sichtbare Komplikationen zeigten sehr hohe Serum-
polysaccharidkonzentrationen.

Änderungen des Serumhexosamin-Gehaltes scheinen das empfindlichste An-
zeigen für das Eintreten von Komplikationen bei Diabetes zu sein, können aber
nicht als spezifisch angesehen werden.

**Résumé**

Гексозамина, общая гексоза связанная с протеином и гексоза и тирозин, содержащимся в мукопротеинах сыворотки были оценены на 15 нормальных субъектах и на 40 больных
диабетом. Полисахариды и белки были также оценены электрофорезом на бумаге.
Больные диабетом были разделены на 4 группы: на группу без осложнений, на группу с болезнью сетчатой оболочки, на группу с различными осложнениями и на группу больных межкапиллярным склерозом почечного клубочка.

Концентрации полисахаридов в сыворотке были значительно повышенны в группе с межкапиллярным склерозом почечного клубочка. Пациенты с болезнью сетчатой оболочки показали увеличение сывороточного гексозамина. Хотя и были признаки, что концентрация этих веществ увеличивалась в зависимости от серьезности осложнений, это не было постоянным явлением потому что два пациента без явных осложнений показали очень высокие концентрации сывороточных полисахаридов.

Изменения в сывороточном гексозамине кажутся самым чувствительным показателем начала осложнений в диабете, но не могут быть рассматриваемы как специфические.

**REFERENCES**


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