The Polysaccharide Content of Serum Fractions in Carcinoma, Arthritis, and Infections

M. R. SHETLAR, PH.D., CLARA L. SHETLAR, B.S., VIRGINIA RICHMOND, B.S., AND MARK R. EVERETT, PH.D.

(From the Department of Biochemistry, School of Medicine, University of Oklahoma, Oklahoma City 4, Oklahoma)

A rise of the polysaccharide associated with serum proteins has been noted in malignancy and in several other conditions (7). Seibert, Pfaff, and Seibert (4) determined the carbohydrate content of normal plasma protein fractions isolated by the low temperature ethanolic procedure of Cohn et al. (1) and found considerable carbohydrate to be associated with all globulin fractions. It would be of interest to determine which of the serum protein fractions are responsible for the elevation of serum polysaccharide in malignancy and in other conditions. A study of the application to this problem of the fractionation of serum proteins by sodium sulfate and ammonium sulfate has been made and will be published elsewhere; elevation of polysaccharide associated with the albumin fraction in normal pregnancy has been reported (8). It is the purpose of this paper to describe similar studies on patients with carcinoma, arthritis, and infections.

EXPERIMENTAL

Serum was fractionated into albumin, albumin + α-globulin, and β + γ-globulins by a modified method of Milne (3) with 1.6 per cent and 6.8 per cent sodium sulfate. The γ-globulin was precipitated from serum by the method of Jager and Nickerson (2) using 33 per cent saturated ammonium sulfate. Nonglucosamine polysaccharide (referred to hereafter in this paper simply as “polysaccharide”) was determined by the tryptophan method, as previously described (6), on the albumin and the albumin + α-globulin fractions after precipitation with absolute ethanol. The polysaccharide associated with α-globulin was estimated by the difference between these two determinations. The γ-globulin polysaccharide was determined after dissolving the precipitate from the ammonium sulfate precipitation in 0.9 per cent saline and reprecipitating with absolute ethanol. Estimation of the polysaccharide associated with β-globulin was achieved by subtracting from the total polysaccharide the sum of the polysaccharide associated with albumin, α-globulin, and γ-globulin.

Protein was determined in all fractions by the biuret reaction. The polysaccharide content of each fraction was expressed as a ratio by dividing the polysaccharide by the protein of the fraction in question. For example, albumin polysaccharide + albumin = albumin polysaccharide content.

Patients for study were selected from those admitted to the University hospitals. For the studies on carcinoma, most of the samples were from patients with a tentative diagnosis of malignancy before biopsy samples were taken and before any treatment was initiated. Final diagnosis was established in most cases by biopsy.

RESULTS

A summary of the polysaccharide content of the different serum fractions for 48 patients with carcinoma, 15 with benign tumor, 9 with arthritis, 8 with viral infection, and 9 with bacterial infection, and for 17 normal adults is given in Table 1. The carcinoma group included patients with carcinomas of the skin, 4; lung, 11; stomach, 6; pancreas, 1; rectum, 6; kidney, 1; liver, 1; breast, 5; cervix, 5; prostate, 1, and penis, 1. The group of arthritis patients included 6 cases of rheumatoid, 2 of osteo, and 1 of gouty arthritis. The group with viral infections consisted of 5 cases of poliomyelitis in the convalescent stage; 2 of infectious hepatitis; and 1 of venereal lymphogranuloma. The patients with bacterial infections included 4 with tuberculosis; 1 with bronchopneumonia; 1 with osteomyelitis; 1 with tularemia; 1 with brucellosis; and 1 with an infection of the elbow. These data were compared statistically with those for normal adults by the conventional t test.

The average polysaccharide content of the albumin fraction of carcinoma patients was signifi-
cantly higher than that of normals. A smaller but significant elevation occurred in the benign tumor, arthritis, and bacterial infection groups. However, the average albumin polysaccharide for the carcinoma group was significantly higher than the average for all of the nonmalignant pathologies ($t$ value = 5.6), or that for any single group.

**DISCUSSION**

It appears that elevation of total serum polysaccharide in pathological conditions is related to two factors. First, a carbohydrate-rich fraction or fractions may increase with respect to a carbohydrate-poor fraction—as, for example, an increase of any globulin fraction with respect to albumin. Second, the polysaccharide content of a particular protein fraction may increase. It is obvious that the first factor operated in all pathological conditions reported in this study with the exception of the benign tumor group; the second factor was also present in all pathologies; however, the particular protein fractions in which elevation of polysaccharide occurred differed with the condition. In patients with benign tumors, the polysaccharide content of the albumin fraction was slightly but significantly elevated. The polysaccharide content of the albumin fraction was increased greatly in carcinoma and that of the $\alpha$-globulin was also elevated to a smaller degree, perhaps through increase of the carbohydrate-rich $\alpha_2$-globulin relative to $\alpha_1$-globulin. However, the most striking alteration in the sera of carcinoma patients is the increase in albumin polysaccharide. It is this elevation which apparently accounts for the inability of Seibert, Pfaff, and Seibert (4) to calculate the polysaccharide content of sera from carcinoma patients from electrophoretic analysis and the polysaccharide content of isolated normal human serum fractions. Related to this phenome-

---

**TABLE 1**

**SUMMARY OF THE PERCENTILE SERUM POLYSACCHARIDE CONTENT OF SERUM PROTEIN FRACTIONS**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Cases</th>
<th>Average Range</th>
<th>CV*</th>
<th>Average Range</th>
<th>CV*</th>
<th>Average Range</th>
<th>CV*</th>
<th>Average Range</th>
<th>CV*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal adults</td>
<td>17</td>
<td>0.61—0.78</td>
<td>0.15</td>
<td>2.93—4.00</td>
<td>0.18</td>
<td>5.65—7.46</td>
<td>0.23</td>
<td>2.39—2.63</td>
<td>0.12</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>48</td>
<td>1.70—5.42</td>
<td>0.58</td>
<td>4.81—8.49</td>
<td>0.37</td>
<td>6.31—9.07</td>
<td>0.64</td>
<td>2.48—3.58</td>
<td>0.22</td>
</tr>
<tr>
<td>Benign tumors</td>
<td>15</td>
<td>0.85—1.8</td>
<td>0.55</td>
<td>2.94—4.41</td>
<td>0.21</td>
<td>5.85—6.40</td>
<td>0.29</td>
<td>2.53—3.34</td>
<td>0.18</td>
</tr>
<tr>
<td>Arthritis</td>
<td>9</td>
<td>1.17—1.58</td>
<td>0.20</td>
<td>4.44—7.41</td>
<td>0.20</td>
<td>7.18—10.13</td>
<td>0.26</td>
<td>2.15—2.63</td>
<td>0.10</td>
</tr>
<tr>
<td>Viral infections</td>
<td>8</td>
<td>0.79—1.94</td>
<td>0.23</td>
<td>2.81—3.45</td>
<td>0.11</td>
<td>7.44—10.39</td>
<td>0.25</td>
<td>2.38—2.50</td>
<td>0.15</td>
</tr>
<tr>
<td>Bacterial infections</td>
<td>9</td>
<td>0.86—1.26</td>
<td>0.24</td>
<td>3.37—4.72</td>
<td>0.24</td>
<td>6.90—8.92</td>
<td>0.29</td>
<td>2.16—2.53</td>
<td>0.11</td>
</tr>
</tbody>
</table>

---

**TABLE 2**

**SUMMARY OF THE PROTEIN DISTRIBUTION AMONG THE SERUM FRACTIONS**

<table>
<thead>
<tr>
<th>Group</th>
<th>Albumin</th>
<th>$\alpha$-Globulin</th>
<th>$\beta$-Globulin</th>
<th>$\gamma$-Globulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal adults</td>
<td>62</td>
<td>11</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>55</td>
<td>12</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>Benign tumors</td>
<td>60</td>
<td>11</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Arthritis</td>
<td>54</td>
<td>11</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Viral infections</td>
<td>59</td>
<td>12</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Bacterial infections</td>
<td>58</td>
<td>12</td>
<td>13</td>
<td>17</td>
</tr>
</tbody>
</table>

* Coefficient of variation.
† Significantly higher than the normal value at the 1 per cent level.
47, rather than 48, cases.

The polysaccharide content of the $\alpha$-globulin fraction was elevated significantly both in patients with carcinoma and in those with arthritis; it was slightly, but not significantly, elevated in patients with bacterial infections. The polysaccharide content of the $\beta$-globulin fraction was elevated significantly in patients with arthritis and in those with viral or bacterial infections. The ratio of polysaccharide to protein for the $\gamma$-globulin fraction was not influenced by any condition studied.

The distribution of total serum protein among the different fractions is summarized in Table 2. The normal values reported are in fair agreement with those given by Milne (3) and by Jager and Nickerson (2). Results for patients with benign tumors were essentially the same as those for normal adults. All other groups exhibited a decrease in albumin and an increase in one or more of the globulin fractions. In contrast to the large increase of $\alpha$-globulin in carcinoma sera reported by Seibert et al. (3), only a slight increase was noted by the method employed. This may indicate that the fractionation method used gives results somewhat different from electrophoresis as employed by Seibert et al. It should be noted that the results of electrophoretic analyses of normal sera by Milne (3) are somewhat different from those of Seibert et al.
non is the finding of Winzler and Smyth (9) with regard to mucoprotein or seromucoid in sera of cancer patients. The albumin fraction, as prepared in our study, should include this seromucoid fraction. In order to determine how much of the elevation of polysaccharide in the albumin fraction is due to mucoprotein, a number of sera from cancer patients were subjected to concurrent albumin polysaccharide and seromucoid determinations. Seromucoid was isolated by the method of Winzler and Smyth (9), with the use of phosphotungstic acid to precipitate the seromucoid after precipitation of other serum proteins by perchloric acid. The carbohydrate moiety was determined by the tryptophan method. A summary of the results is presented in Table 3. Apparently, mucoprotein accounts for some of the elevation of the polysaccharide in the albumin fraction, but, after deducting the mucoprotein polysaccharide, the polysaccharide content of the albumin fraction is still much higher than that in normal sera.

Arthritis is characterized by increases in the polysaccharide content of the albumin, α-globulin, and β-globulin fractions, and a noteworthy elevation of γ-globulin protein resulting in an increase of total γ-globulin polysaccharide. The sera of patients with infections exhibited elevations of polysaccharide content in the β-globulin fraction, with only slight elevations in the albumin fraction.

SUMMARY AND CONCLUSIONS

A study has been made of the distribution of nonglucosamine polysaccharide in the serum protein fractions of sera from patients with carcinoma, benign tumors, arthritis, and infections. A method employing fractionation of the serum proteins with sodium sulfate and ammonium sulfate was used. Sera of carcinoma patients exhibited a greatly elevated content of polysaccharide in the albumin fraction in 45 of 48 cases. Only part of this increase was due to polysaccharide in the mucoprotein fraction. The polysaccharide associated with albumin showed smaller elevations in sera of patients with arthritis, benign tumors, and bacterial infections. The α-globulin polysaccharide was elevated significantly in carcinoma and in arthritis. Polysaccharide of the β-globulin fraction was elevated significantly in arthritis and in viral and bacterial infections. No alterations were noted in the polysaccharide content of the γ-globulin fraction.

REFERENCES


The Polysaccharide Content of Serum Fractions in Carcinoma, Arthritis, and Infections
