Serum Content of Macroglobulins (19S Fraction) and Its Variation with Age when Determined by Thin-Layer Gel-Filtration

Lleni Pach de Goldman, Liliana Ballivian, and Ernesto Melgar

Serum proteins were fractionated by thin-layer gel-filtration on Sephadex G-200, and the content of macroglobulins (19S fraction) was determined in 137 samples from apparently healthy subjects of different ages. The relationship between amount of 19S fraction relative to total protein was found to vary widely with age. This variation has to be considered when the method is used for clinical purposes.

Additional Keyphrases normal values, children and adults • Sephadex G-200 • analytical ultracentrifugation compared • umbilical cord blood • lipoproteins, serum • malignant disease

The analytical ultracentrifuge is the most accurate method for separating the serum proteins according to molecular weight. However, ultracentrifugation is possible only in specialized centers, and therefore new methods have been devised to study the composition of the serum proteins for clinical purposes. Thus, Roskes and Thompson (1) introduced gel-filtration as a method to differentiate macroglobulinemic, hyperglobulinemic, and normal sera, and Bergström (2) used a thin-layer gel-filtration technique to estimate quantitatively the macroglobulin component of serum.

During a survey to determine normal values of macroglobulins by use of the thin-layer gel-filtration technique of Bergström, the values one of us found for normal children were strikingly high (3). Therefore, we decided to determine the relationship of macroglobulin concentrations to age in our normal population. The results are presented here.

Materials and Methods

The study was done on 137 sera from apparently healthy subjects, including 70 adults, 54 children of different ages, and 13 sera from umbilical cord blood. The method used to fractionate the serum proteins was that described by Bergström (2), in which one uses thin-layer gel-filtration on Sephadex G-200 that has been poured on grooved glass plates. Two-microliter samples were run in duplicate during 7 h at room temperature, and the proteins were absorbed on Whatman 3MM filter-paper strips. The paper strips were stained with Amido Black; the colored spots were cut out and eluted. The protein concentration of the three serum components (4.5S, 7S, and 19S) was determined by measuring the absorbance at 620 nm with a Model DU Beckman spectrophotometer against a blank eluted from a noncolored piece of the same paper strip. The relative amount of the 19S fraction was calculated from the sum of the absorbancies of the three eluted fractions, and was considered as the macroglobulin component of serum. Total protein concentration of serum was determined by the biuret method (4).

Reagents: Sephadex G-200, superfine, particle size 10 to 40 μ, and Blue Dextran 2000 (used as a marker) were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden.

Results and Discussion

The thin-layer gel-filtration method used in this work permits a fractionation of serum proteins

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into three components, identified according to the order of elution as corresponding to the 19S, 7S, and 4.5S fractions obtained by analytical ultracentrifugation. The quantitative estimates of the protein content in these fractions were reproducible; differences in duplicate samples were never greater than 15% for the 19S fraction. A single serum sample, analyzed 10 times, gave a mean of 7.2%, a standard deviation of ± 0.22%, and a coefficient of variation of 0.03%.

The subjects were separated into five age groups: (1) newborns, (2) children from 0 to 5 years, (3) school children from 6 to 15 years, (4) adults from 16 to 60 years, and (5) adults over 60 years old.

The concentration of total proteins did not differ significantly in the groups 2 to 5, as seen in Table 1, but newborns had lower values of total serum proteins than the other groups, confirming previous observations (5, 6). The mean values for the macroglobulin component of serum from different age groups are also shown. They evidence a wide variation when compared with group 4, but in the age span of this group the values were highly constant, as seen in Table 2. Differences were not significant when the results of the adult group from 16 to 60 years were separated into three subgroups.

The results from group 4 showed no relationship between sex and macroglobulin content of serum. This group consisted of 25 men and 28 women whose serum 19S contents were not significantly different: 5.5% ± 0.39% and 5.9% ± 0.47%, respectively (t = 1.072, p > 0.10). These results do not agree with those in Ericksen's previous report (7), in which higher values of 19S serum proteins were found in women.

There are a few reports on the variation of macroglobulin content of serum with age. Using the ultracentrifuge, Böttinger et al. (8), found that the macroglobulin component of serum remained constant in a group of 46 men, 30 to 71 years old, but they include only four individuals in the 65 to 71 age group. On the other hand, using his thin-layer gel-filtration method, Bergström (2) found higher values in the levels of macroglobulins in the individuals over 64 years. Our results (Table 1) agree with Bergström's observation; the group of adults over 60 years old show a mean value of 8.6%, and, when compared with the group of adults from 16 to 60 years, gave a statistically significant difference in the means (t = 2.500, 0.01 > p > 0.005).

The 19S component of serum, obtained by ultracentrifugation, is a heterogeneous fraction composed of approximately the same amounts of α-globulins and γ-globulins (9). Flodin and Killander (10) reported that, in addition to the α-globulin and γ-globulin components, almost 90% of the lipoprotein in serum that (in the analytical ultracentrifuge) accompanies the 19S fraction migrates on gel-filtration with the 19S fraction. This method-re-

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Subject's age, yr.</th>
<th>No. samples</th>
<th>Total protein, g/100 ml</th>
<th>Macroglobulin, %*</th>
<th>Mean ± the standard error of the mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Newborn</td>
<td>13</td>
<td>6.63</td>
<td>2.7 ± 0.54</td>
<td>0.0-6.3</td>
</tr>
<tr>
<td>2</td>
<td>0-5</td>
<td>27</td>
<td>7.19</td>
<td>9.9 ± 0.67</td>
<td>4.4-18.2</td>
</tr>
<tr>
<td>3</td>
<td>6-15</td>
<td>27</td>
<td>7.29</td>
<td>7.3 ± 0.48</td>
<td>2.1-11.5</td>
</tr>
<tr>
<td>4</td>
<td>16-60</td>
<td>53</td>
<td>7.25</td>
<td>5.6 ± 0.30</td>
<td>0.5-12.5</td>
</tr>
<tr>
<td>5</td>
<td>Over 60</td>
<td>17</td>
<td>7.06</td>
<td>8.6 ± 1.17</td>
<td>2.9-15.1</td>
</tr>
</tbody>
</table>

* Mean ± the standard error of the mean.

<table>
<thead>
<tr>
<th>Group</th>
<th>Age, yr.</th>
<th>No. samples</th>
<th>Proteins, g/100 ml</th>
<th>Macroglobulin, %*</th>
<th>Mean ± the standard error of the mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>4A</td>
<td>16-30</td>
<td>27</td>
<td>7.25</td>
<td>5.5 ± 0.40</td>
<td>4A/4B:t = 0.714; 0.35 &gt; p &gt; 0.20</td>
</tr>
<tr>
<td>4B</td>
<td>31-45</td>
<td>16</td>
<td>7.28</td>
<td>6.1 ± 0.74</td>
<td>4A/4C:t = 0.286; 0.50 &gt; p &gt; 0.35</td>
</tr>
<tr>
<td>4C</td>
<td>46-60</td>
<td>10</td>
<td>7.21</td>
<td>5.3 ± 0.58</td>
<td>4B/4C:t = 0.851; 0.35 &gt; p &gt; 0.20</td>
</tr>
</tbody>
</table>

* Mean ± the standard error of the mean.

related behavior of the lipoproteins can be responsible for the differences observed in the results, since there is an increased lipoprotein content of serum from old men (11).

The macroglobulin levels in umbilical cord blood were very low in our series (Table 1), which contradicts Ericksen's observation (7). He found, with the ultracentrifuge, that the 19S proteins are greater in serum of newborns than in that of adults, but, as pointed out by Bergström (2), the results of the gel-filtration method cannot be directly compared with the ultracentrifuge analysis. Low values for macroglobulins in newborns may result from decreased synthesis of immunoglobulins during fetal life (12) and the fact that at birth the concentrations the 19S immunoglobulins are about 10% as great as in normal adults (13); moreover, the serum lipoprotein concentration at birth is also lower than in adulthood (14). In two samples from this group we were not able to see the 19S fraction, not because they were lacking but because of the decreased sensitivity of the method, as was proved by diluting samples of normal sera.

The amount of macroglobulins found in both groups of children was considerably higher than in adults, mainly in the younger group, whose values could be considered, sometimes, as pathologic (Table 1). Bergström in his series (2), considered
as normal a 19S component corresponding to less than 10% of the total protein. In this work, 15 of 27 samples from children up to 5 years old had values higher than 10%. Ericksen (7) found in sera from a group of 11 children, up to 12 years of age, a macroglobulin content about one and a half times that of serum from adults. An explanation of this increase can be found in the work of Ganrot and Scherstén (15), who report higher values of the α1-macroglobulin in infants than in adults. They thought this fact suggested a role of the α1-macroglobulins in regulating growth processes, since the growth hormone binds to these proteins (16). γ-Macroglobulins (13) and lipoproteins (17) increase after birth, but the concentrations are always smaller than or near to those of adults, so the possibilities are limited that the noteworthy enhancement of the 19S fraction in small children could be related to the increase of those proteins.

Our study of the macroglobulin content of serum in various immunoproliferative diseases gave values under 12% in multiple myeloma (four cases), lymphosarcoma (four cases), reticulum cell sarcoma (four cases), lymphatic leukemia (three cases), and Hodgkin’s disease (four cases), but in two cases of Waldenström’s macroglobulinemia the values were 24.7 and 26.8%, respectively. Normal values were also found in myelocytic leukemia (four cases), cryoglobulinemia (one case), aplastic anemia (three cases), hemolytic anemia (two cases), erythematous systemic lupus (three cases), and tuberculosis of the lymphatic glands (three cases).

Finally, we can establish that the thin-layer gel-filtration method is useful for quantitative estimation of the macroglobulin fraction of serum, but there are some factors, related to the age of the patient, that we have to consider before concluding that a sample is abnormal. For this, additional information should be obtained through paper- and immunoelectrophoresis.

References


