Heparin Precipitability of the Macroglobulin in a Patient with Waldenström's Macroglobulinemia

By David Miller

The occurrence of high molecular weight serum globulins as a prominent feature of certain blood dyscrasias was first noted by Waldenström and has been the subject of many reports and of several recent reviews. Although in many of the patients there are features suggesting multiple myeloma, lymphocytic leukemia, or one of the malignant lymphomas, in most instances the picture is not characteristic enough to permit a definite diagnosis. This circumstance, together with the striking biochemical abnormality noted in these cases, has led to the general acceptance of Waldenström's macroglobulinemia as a definite clinical entity. It is now considered to be a neoplasm of the reticuloendothelial system characterized by the proliferation of an abnormal cell type, best described as a lymphoid plasma cell, from which the abnormal macroglobulins probably originate.

With the exception of several studies of the effect of the abnormal protein on blood coagulation, little is known regarding specific reactions of the macroglobulin with substances normally found in the body. Recently, in a patient with macroglobulinemia, precipitation of the abnormal protein with heparin was observed, and a detailed description of the phenomenon is the subject of this report. A full account of the patient's course appears as a Clinicopathologic Conference in the American Journal of Medicine.

Methods

The macroglobulin was isolated by its precipitation following the dilution of serum or plasma in approximately 50 volumes of distilled water. The precipitate was redissolved in a minimal volume of 0.2 M sodium chloride and reprecipitated by dilution in water. After a third precipitation the material was dissolved in 0.2 M sodiun chloride to prepare a solution whose protein concentration was approximately 3 gm. 100 ml. Further dilutions were made just prior to the experiments, and the protein concentration was determined by the method of Lowry et al. using bovine serum albumin as the standard. The preparation was homogeneous by zone electrophoresis, and on ultracentrifugation consisted predominantly of material with a sedimentation constant of 19 Svedberg units, with minor components sedimenting with constants of 7.5 and 27. The apparent heterogeneity of such preparations by ultracentrifugation has been repeatedly noted.

In experiments involving the precipitation of the purified macroglobulin with heparin, the concentration of protein in the supernatant was determined by measuring absorbance at 280 μm with the Beckman DU spectrophotometer. The light absorption of macroglobulin...
solutions was shown to follow Beer’s law at this wavelength. The absorption of heparin was appreciable only at the highest concentrations used, in which case it was corrected for by the use of appropriate blanks.

Heparin in the supernatant was determined as total hexuronic acid by the method of Dische. The precipitates could not be assayed by this method because of interfering substances in the protein.

Sedimentation studies were performed in the Spinco Model E analytical ultracentrifuge at a rotor speed of 42,040 r.p.m. The sedimentation constants have been corrected to 20°C, but not to infinite dilution of protein. The sedimentation of a macroglobulin is especially affected by the presence of lighter components, and the constants obtained in serum or plasma were appreciably lower than those observed with the purified preparation. The importance of this effect in accounting for much of the variability in the sedimentation constants noted in the literature has recently been emphasized. All pH measurements were made with the Beckman pH meter, Model G.

Paper electrophoresis was carried out in barbital buffer, ionic strength 0.1, pH 8.6. Heparin, as the sodium salt, and chondroitin sulfate, derived from hog nasal septum, were obtained from the Nutritional Biochemicals Corporation. Another preparation of heparin, Liquemin Sodium, obtained from Organon, Inc., gave identical results. The potency of the heparin preparations was 100 units/mg. Tris(hydroxy)aminomethane, referred to as Tris, was obtained from the Sigma Chemical Company.

Observations on Blood, Serum and Plasma

The effect of heparin was first observed when it was noticed that blood drawn from the patient showed an increased viscosity when heparin, in a final concentration of 0.1 mg./ml., was used as the anticoagulant. This change was associated with a marked decrease in the erythrocyte sedimentation rate in spite of gross clumping of the red cells. With oxalate, citrate, or versenate as anticoagulants, increased viscosity was not observed and the sedimentation rate was characteristically high. Centrifugation of the heparinized blood at 2000 r.p.m. for one hour resulted in the appearance of three distinct layers: packed red cells at the bottom, clear plasma at the top, and a gelatinous precipitate in the middle.

The addition of heparin in the above concentration to plasma or serum resulted in the formation of a turbid precipitate readily removable by centrifugation. Precipitation occurred equally well in serum and in oxalate or citrate plasma, but was much less marked in versenate plasma. There was no obvious difference at 37°C., at room temperature, or at a C. in the extent of the precipitation. The precipitate could be readily redissolved by the further addition of heparin or by the addition of concentrated salt solutions.

The effect of heparin was further characterized by studies of the supernatant fraction following precipitation. Electrophoresis of an untreated sample of the patient’s serum showed about half of the total protein to consist of a homogeneous component with a mobility similar to that of the slow component of normal gamma globulin. The electrophoretic pattern following heparin precipitation showed almost complete absence of the abnormal protein and no change in the other components. Similarly, ultracentrifuge studies of the serum demonstrated a marked diminution of the major macroglobulin peak and complete absence of the minor macroglobulin peak (corresponding to the
HEPARIN PRECIPITABILITY OF THE MACROGLOBULIN

19 S and 27 S peaks in the purified material) after precipitation of the serum with heparin. No change was noted in the peak representing albumin and normal gamma globulin.

Studies of the Purified Macroglobulin

The addition of heparin to dilute solutions of the purified macroglobulins resulted in immediate turbidity followed by the gradual appearance of a flocculent precipitate. The precipitate could be redissolved by the addition of sodium or potassium chloride or Tris buffer, pH 7.4, to final salt concentrations of approximately 0.5 M, or greater. Precipitation could not be demonstrated in the presence of phosphate ion even at concentrations as low as 0.05 M.

In order to determine the dependence of the precipitation on the concentration of heparin, varying amounts of heparin were added to dilute solutions of the purified macroglobulin. The additions were made so as to maintain constant the pH, ionic strength, and protein content. After two hours at room temperature, the tubes were centrifuged at 2000 r.p.m. for 30 minutes, and the protein concentration of the supernatant was determined and compared with that of a control to which no heparin was added. The results (table 1) indicate that maximal precipitation occurs over a 50 to 100 fold range of heparin concentration. It should be noted that, even at concentrations of heparin which inhibit precipitation, the contribution of heparin to the total ionic strength is relatively small, and the inhibition of precipitation by excess heparin would not appear due to its effect on the ionic strength. This is further borne out by the fact that, over most of the range of heparin concentration, there is only a small decrease in the amount of protein precipitated when the buffer concentration is increased by 0.05M. Such a change in buffer concentration represents a greater increase in ionic strength than would result from increments of heparin adequate to completely inhibit precipitation.

No decrease in the heparin concentration of the supernatant could be demonstrated under conditions of maximal precipitation (e.g., tubes 4 to 6.

Table 1.—The Precipitation of Macroglobulin at Varying Concentrations of Heparin

<table>
<thead>
<tr>
<th>Tube</th>
<th>Heparin μg/ml.</th>
<th>Per cent protein precipitated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>1</td>
<td>5000</td>
<td>1.4</td>
</tr>
<tr>
<td>2</td>
<td>2500</td>
<td>.9</td>
</tr>
<tr>
<td>3</td>
<td>1000</td>
<td>28</td>
</tr>
<tr>
<td>4</td>
<td>500</td>
<td>68</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>86</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>86</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>85</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>72</td>
</tr>
<tr>
<td>9</td>
<td>2.5</td>
<td>—</td>
</tr>
<tr>
<td>10</td>
<td>1.0</td>
<td>18</td>
</tr>
<tr>
<td>11</td>
<td>.5</td>
<td>14</td>
</tr>
<tr>
<td>12</td>
<td>.25</td>
<td>—</td>
</tr>
</tbody>
</table>

Initial protein concentration: 800 μg/ml. Heparin concentration: as shown. A: 0.2 M Tris buffer, pH 7.4. B: 0.15 M Tris buffer, pH 7.4.
table 1). As the limit of sensitivity of the assay used was about 10 μg./ml., it would appear that heparin, if it does complex with protein, does not combine in a mole ratio of much over one.

**Studies of the Macroglobulin Subunits**

Deutsch and Morton demonstrated the dissociation of abnormal macroglobulins into subunits or "monomers" following the reductive cleavage of disulfide bonds by mercaptoethanol. Removal of the sulfhydryl reagent by dialysis results in reaggregation unless the sulfhydryl groups of the monomer are blocked, e.g., with iodoacetate. In the present case, the monomer (sedimentation constant, 6 S) which resulted from the dialysis with iodoacetate did not precipitate with heparin in contrast to both the reaggregated material and the untreated macroglobulin.

**Specificity of the Heparin-Macroglobulin Interaction**

Chondroitin sulfate, when substituted for heparin over a wide range of concentration, did not precipitate the macroglobulin. Other protein preparations tested with heparin with negative results included human gamma globulin (American Red Cross Immune Globulin) and the isolated euglobulins from two other patients with Waldenström's macroglobulinemia.

**Discussion**

The precise nature of the heparin-macroglobulin interaction which has been described is not understood. The precipitation appears to be relatively specific with respect to both the acid mucopolysaccharide and the protein. Whether it is simply due to the formation of a stable and insoluble heparin-protein complex, or whether it is due to a disruption of the protein fine structure without the formation of a stable complex, cannot be decided on the basis of the data presented. The solubility of the precipitates at higher concentrations of heparin superficially resembles the solubility of antigen-antibody precipitates in the presence of excess antigen. However, the very broad range of heparin concentration over which maximal precipitation occurs (approximately 50 to 100 fold) is different from the case in most antigen-antibody systems and weighs against this interpretation.

The heparin precipitable fraction of plasma studied by Smith and von Korff differs from the macroglobulin described in the present report in that it was present in plasma but not in serum, was precipitable only in the cold, and was precipitable by other sulfated polysaccharides, including chondroitin sulfate.

The occurrence of heparin precipitability in macroglobulinemia is probably not common, and no statement regarding its significance can be made. Whether such a heparin-protein interaction is in some way related to the profusion of tissue basophils noted in many cases of macroglobulinemia is an intriguing question. A more practical consideration is the possibility of the intravascular precipitation of macroglobulin following the therapeutic administration of heparin. It might therefore be advisable to exclude heparin precipi-
Tability of the macroglobulin by in vitro testing before administering heparin to patients with Waldenström’s macroglobulinemia.

**Summary**

The macroglobulin isolated from a patient with macroglobulinemia was shown to be specifically precipitable by heparin. Maximal precipitation occurred at relatively low (by weight) concentrations of heparin and persisted over a broad range of heparin concentration. The possible clinical significance of the finding has been briefly discussed.

**Sommario in Interlingua**

Esseva monstrate que le macroglobulina isolate ab un patiente con macro-globulinemia esseva specificamente precipitable con heparina. Le maximo del precipitation occurreva a relativamente basse concentrationes (per peso) de heparina e persisteva a transverso un extense gamma de concentrationes del heparina. Le possibile signification clinic del observation es discutite brevemente.

**Acknowledgment**

I wish to thank Miss Carmelita Lowry of the Department of Biological Chemistry of the Washington University School of Medicine for very kindly performing the ultracentrifuge studies. I am also indebted to Dr. Herman N. Eisen of the Department of Medicine for many helpful discussions.

**References**