Simplified Method for Estimation of Serum and Plasma Viscosity in Multiple Myeloma and Related Disorders

By DORIS J. WRIGHT and DAVID E. JENKINS, JR.

In the present study, the red blood cell pipette was tested and found to be a suitable viscometer for detection of the hyperviscosity syndrome in multiple myeloma and macroglobulinemia. Additional studies demonstrated that in normal subjects and in most patients there was little difference in relative viscosity values whether serum or plasma was used and whether the test was performed at room temperature or 37°C. Based on these observations, a rapid screening test for the measurement of serum or plasma viscosities was described.

PROMPT RECOGNITION of the potentially fatal hyperviscosity syndrome in Waldenström's macroglobulinemia and multiple myeloma is of great clinical importance since dramatic relief in symptoms may result from the lowering of viscosity by plasmapheresis. The usual technique for measuring viscosity is relatively simple but requires equipment frequently not available in clinical laboratories. Moreover, it is common practice to perform this test using serum and a 37°C waterbath. The present study was undertaken to develop a more practical clinical method for this procedure. The results of this study show that the readily available red blood cell pipette can serve as an adequate viscometer. In addition, by using plasma in place of serum and by carrying out the test at room temperature rather than at 37°C, a rapid and accurate screening test, adaptable even to the hospital ward, can be readily performed.

DEFINITIONS AND METHODS

The viscosity of a fluid represents the property of that fluid to resist flow. For protein solutions this resistance to flow is influenced by both concentration and intrinsic viscosity of individual proteins in solution. The intrinsic viscosity, a physicochemical property of the protein molecule, is in turn influenced by the molecular size and shape of that protein.

Figure 1 compares the standard Ostwald viscometer and the “red cell pipette viscometer.”
SERUM AND PLASMA VISCOSITY IN MULTIPLE MYELOMA

517

Oxalate, citrate, and EDTA used as anticoagulants. Heparin was not used because of its known capacity to precipitate plasma proteins in certain disease states.7

Fig. 1.—Comparison of the Ostwald viscometer and the red blood cell pipette viscometer. Aside from availability, the red cell pipette has several additional advantages including ease of filling and cleaning, requirements for only 1 ml. of sample, and rapid flow time (90 seconds for water, 2-3 minutes for normal serum or plasma). In extremely viscous samples, the white blood cell pipette can be used in place of the red cell pipette.

used to measure viscosity in this study. Each instrument contains a bulb reservoir and a narrow gauge outflow tube. In the figure, the red cell pipette is supported by a cork placed in a Coplin jar. A second hole in the cork maintains an open system. It is important that the pipette tip does not touch the side of the receptacle tube, thereby impeding flow of the sample. Other methods of support can also be used but the pipette must be maintained in a vertical position during use. For routine testing at room temperature we have found it most convenient to use a clamp and ring stand to support the cork containing the red cell pipette.

The Ostwald viscometer was filled by placing approximately 5 ml. of serum or plasma into the larger reservoir column. The instrument was then tilted until the smaller reservoir was filled. The red cell pipette was filled by pulling the sample into the pipette from below, with suction applied through the standard piece of tubing supplied with red cell pipettes. For each instrument, the time of flow between the lines immediately above and below the bulb reservoir was recorded. The flow time of the test solution in seconds divided by the flow time for water in seconds gave the relative viscosity of that solution. Determinations were carried out either at room temperature (23°C) or at 37°C in an incubator with a glass door. Following each determination, the instruments were thoroughly rinsed with saline, then distilled water, and finally dried with acetone. Thorough cleansing is imperative since dirt or dried proteins will impede flow and give falsely high values. The saline rinse avoids the precipitation of serum or plasma proteins which will occur with direct water rinsing.

Several different red cell pipettes were tested in these studies. Pipettes with star-shaped beads (Yankee, Clay-Adams; Propper Trophy), as shown in Fig. 1, gave highly reproducible results. Twenty successive viscosity determinations were performed on samples of pooled serum and plasma with a Clay-Adams pipette. With pooled serum the coefficient of variation was 4.0 per cent and 0.1 per cent at 20°C and 37°C, respectively. For pooled plasma the coefficient of variation at 23°C was 0.1 per cent and at 37°C, 2.3 per cent. Pipettes with cylindrical beads were somewhat less satisfactory, since occasionally the cylindrical beads would partially occlude the lower opening of the pipette bulb producing a slight increase in flow time.

RESULTS

Serum (or plasma*) viscosities were performed on 20 control subjects, 29

*Oxalate, citrate, and EDTA used as anticoagulants. Heparin was not used because of its known capacity to precipitate plasma proteins in certain disease states.7
Fig. 2.—Comparison of serum viscosities at 37°C using the Ostwald viscometer (V) and the red cell pipette (R).

Fig. 3.—Serum viscosity at 23°C and 37°C using the red cell pipette. Subjects studied: 20 controls, 29 myeloma-macroglobulinemia patients, and 20 polyclonal hyperglobulinemia subjects.
SERUM AND PLASMA VISCOSITY IN MULTIPLE MYELOMA

Fig. 4.—Comparison of serum (S) and plasma (PL) viscosities in 20 controls, 10 myeloma-macroglobulinemia patients, and eight polyclonal hyperglobulinemia subjects.

patients with myeloma or macroglobulinemia, and 20 patients with polyclonal hyperglobulinemia. Fresh serum and plasma were used in these studies to avoid alteration of viscosity through freezing and thawing. The 20 control subjects were hospitalized patients with normal serum globulin levels. Five of the 29 myeloma-macroglobulinemia patients exhibited hypogammaglobulinemia by paper electrophoresis. In the remaining 24, hyperglobulinemia was present in association with a narrow-based monoclonal peak on serum electrophoresis. In each of these patients, the immunoglobulin responsible for the monoclonal peak was determined using commercial immunoglobulin assay kits. (Immunoplates, Hyland Laboratories, Los Angeles, Calif.) The 20 patients with polyclonal hyperglobulinemia all had serum globulin levels of more than 4.0 Gm./100 ml. in association with a broad-based increase in gamma globulin on serum electrophoresis.
Figure 2 establishes that the red cell pipette can be used as a viscometer. Comparable serum viscosity values were obtained with the Ostwald viscometer and the RBC pipette at 37°C in 20 control subjects, 10 myeloma-macroglobulinemia patients, and 16 subjects with polyclonal hyperglobulinemia. The normal serum viscosity range reported in the literature is 1.4–1.8. Our controls gave comparable values.

Figure 3 compares serum viscosity at room temperature and 37°C using the red cell pipette. Good correlation was observed in all three groups of subjects studied.

Figure 4 shows the comparison of serum and plasma viscosities. At room temperature and at 37°C, individual subjects in all three groups showed similar results using either serum or plasma.

Serum viscosity according to Ig types is shown in Fig. 5. Of the 20 myeloma-macroglobulinemia patients, the five with hypogammaglobulinemia had serum viscosity values similar to those of our control group. The 16 γG myeloma patients had values from 1.4 to 12.4. The five γA myeloma patients ranged from 1.9 to 13.0, and the three γM subjects from 2.2 to 4.8. Again, comparable serum viscosity values were observed at either room temperature or 37°C.

**DISCUSSION**

This report describes a simplified technique for determining serum and plasma viscosities with the RBC pipette. As a screening test, the procedure can be performed at room temperature using plasma. This eliminates the need for an incubator and obviates the need for clot retraction which often is impaired in myeloma and macroglobulinemia. If increased viscosity is observed...
at room temperature with plasma, the test should be repeated at 37°C with plasma or preferably serum. If no difference is demonstrated between 37°C and room temperature, then serial studies can be carried out at the latter temperature with plasma. Increased viscosity at room temperature, not present at 37°C, would suggest the presence of a cryoglobulin or cryogel. In patients with extremely high viscosities and slow flow time, the test may be performed more rapidly with the white blood cell pipette instead of the red cell pipette. Flow time with the white cell pipette is approximately 10 times more rapid than that observed with the red cell pipette.

The hyperviscosity syndrome has been seen most frequently in patients with Waldenström’s macroglobulinemia. Its occurrence might be expected in this disorder because of the relatively high intrinsic viscosity of γM globulin. Hyperviscosity has also been described in myeloma. Whether the hyperviscosity in myeloma is due to aggregation of myeloma proteins or to the presence of myeloma proteins with high intrinsic viscosity has not been established in all instances. In three previously reported cases of hyperviscosity associated with γG myeloma, aggregation has been demonstrated in two patients but not in third. Analytical ultracentrifugal studies of serum from one of our patients with γG myeloma and hyperviscosity failed to show evidence for aggregation. This patient had a 6.4S peak which did not change with reduction in relative viscosity from 12.4 to 5.6 with plasmapheresis. Analytical ultracentrifugation was also performed on the serum of one of our patients with γA myeloma and hyperviscosity. Both the hyperviscous sample (relative viscosity 13.0) and the specimen with normal relative viscosity (2.6) contained 8.9S peaks. These findings indicate that this patient’s γA myeloma protein was present as a dimer but that no additional polymerization could be detected in the hyperviscous specimen.

The hyperviscosity syndrome presents variable symptoms in different patients. A bleeding diathesis separate from any thrombocytopenia, a distinctive retinopathy with “sausage-link” or “box car” venous distention and hemorrhages, varying neurological symptoms, and less frequently congestive heart failure have all been reported to occur. Symptoms are commonplace with a relative viscosity of 10.0 and uncommon with values below 3. The viscosity threshold for any one symptom varies from patient to patient, but remains constant in the individual patient. In our group of 29 patients with myeloma and macroglobulinemia four had symptoms of hyperviscosity, including two patients with γA myeloma and two patients with γG myeloma. Each of these four patients had initial viscosity values greater than 6.0. Vigorous plasmapheresis plus alkylating agents were employed in three of the four, resulting in clinical improvement and reduction in serum viscosity in all three. The fourth patient, with γA myeloma, was terminally ill when the hyperviscosity was detected and he received no therapy directed specifically at this complication.

REFERENCES
Simplified Method for Estimation of Serum and Plasma Viscosity in Multiple Myeloma and Related Disorders

DORIS J. WRIGHT and DAVID E. JENKINS, JR.