Our study of 95 serum samples from 37 patients with monoclonal gammopathy revealed distorted irregular monoclonal (M) protein bands after serum protein electrophoresis (SPE) on cellulose acetate membrane. In 71 (75%) of the 95 sera, the M-protein was underestimated and the albumin concentration overestimated. Dilution of the serum sample before SPE eliminated the abnormality of the M-protein bands. By SPE, the mean albumin concentration in these 71 undiluted sera was 45.8 (SD 7.4) g/L vs 37.9 (SD 5.8) g/L for the diluted sera; moreover, this was true of individual samples; measured albumin concentration in each diluted serum sample was always less than in the undiluted serum. As measured by the bromcresol green dye-binding method, the albumin concentration was 32.8 (SD 5.9) g/L. Similarly, the M-protein concentration in SPE was 49.5 (SD 12.3) g/L for the undiluted sera vs 61.8 (SD 15.1) g/L for the diluted sera, and the M-protein concentration in each diluted serum sample always exceeded that in the undiluted serum. Underestimation of M-protein limits the usefulness of M-protein measurement in evaluating the patient's response to therapy and for early detection of disease progression. SPE strips should be carefully inspected visually, and sera with M-protein band abnormalities should be diluted and re-assayed if SPE is to quantify concentrations of M-protein and albumin accurately.

Additional Keyphrases: analytical error · monoclonal gammopathies · plasma cell dyscrasias

Monoclonal proteins (M-proteins) frequently appear in the sera of patients with multiple myeloma and other plasma cell dyscrasias (1–3). Serial measurement of serum M-proteins can reflect both the tumor burden and the clinical course of the disease (3). M-proteins are quantified routinely in many laboratories by serum protein electrophoresis (SPE) after total serum protein in serum has been determined by either refractive-index measurements or colorimetry (4–6). We have observed that on cellulose acetate support medium the presence of distorted or irregular M-protein bands results in underestimation of the M-protein value, with consequent overestimation of the albumin concentration. Underestimation of the M-protein can result in erroneous clinical interpretation of the test results: the test fails to demonstrate the reduction of M-protein values in patients who are responding to therapy and does not show the early increase in M-protein values in patients who develop progressive disease.

In this report, we describe the various types of M-protein band distortions that are observed after SPE on cellulose acetate. We also demonstrate, however, that thorough visual inspection of the electrophoretic pattern and appropriate dilution of the sample before electrophoresis can eliminate this problem and enhance the accuracy of M-protein quantification by SPE.

Materials and Methods

Patients and serum samples. We studied 95 serum samples obtained after venipuncture from 37 patients with monoclonal gammopathies. Of these, 22 patients had IgG-kappa, 11 had IgG-lambda, and one each had IgA-kappa, IgA-lambda, IgM-kappa, and IgM-lambda.

Determination of serum albumin. The concentrations of serum albumin were determined by using the bromcresol green (BCG) dye-binding method reported by Rodkey (7) and modified by Doumas et al. (8) with the aca III analyzer (Clinical and Instrument Systems Division, Du Pont Co., Wilmington, DE). The normal reference interval for serum albumin in our laboratory is 35–47 g/L.

Serum protein electrophoresis. The total protein in serum was determined by refractometry. Serum protein fractions were electrophoretically resolved on cellulose acetate membranes (Beckman Microzone Electrophoresis System; Beckman Instruments, Fullerton, CA) into five protein fractions: albumin, alpha-1, alpha-2, beta, and gamma globulins. These fractions were denatured, stained with Ponceau-S dye, scanned, and quantified with a Model R-112 densitometer (Beckman Instruments).

We electrophoresed, on the same SPE strip, an undiluted serum sample and several dilutions of it (1:2, 1:3, and 1:4) in isotonic saline. We checked the electrophoretic patterns visually to ensure that diluting the serum had eliminated the M-protein band abnormality (see below).

The electrophoretic protein fractions were scanned and quantified densitometrically to obtain values for the serum albumin and M-protein. The dilution that yielded an albumin value closest or equivalent to the albumin value determined by the BCG method and that also eliminated the irregularity of the M-protein bands was considered to be the appropriate dilution. Reference values established in our laboratory by SPE for total serum protein and the five serum protein fractions in healthy individuals were (g/L): total serum protein = 60–82, albumin = 35–47, alpha-1 globulin = 2–3, alpha-2 globulin = 4–7, beta globulin = 5–10, and gamma globulin = 7–14. M-protein bands usually appear on the cellulose acetate strip as an additional discrete and homogeneous band located in the area ranging from the alpha-1 globulin through the gamma globulin region.

Characterization of M-proteins. The serum M-protein bands detected by SPE were typed by immunoelectrophoresis for at least one sample from each patient. The serum protein fractions were separated by electrophoresis on an agarose membrane ("Immunoelectrofilm"; Kallestad Lab., Inc., Chaska MN) and immunoprecipitated in the gel with anti-IgG, anti-IgA, anti-IgM, anti-kappa, anti-lambda, and anti-human whole serum. The immunoelectrophoretic pattern was interpreted according to the guidelines described by Penn and Batya (9).

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Results

All 95 samples showed distorted or irregular M-protein band abnormalities on the SPE membrane. We grouped these M-protein band abnormalities into three distinct types. Type 1 abnormalities (Figure 1) show marked distortion of the M-protein band: pronounced curvature, fingerlike projections, high-density staining, and a longer vertical dimension than the albumin band. A Type 2 M-protein band abnormality shows only moderate curvature plus high-density staining and a longer vertical dimension than the albumin band (Figure 2).

Figure 3 shows a Type 3 M-protein band abnormality, characterized by a minimal degree of curvature and low-density staining.

Albumin concentrations in samples with Type 1 or Type 2 abnormalities were always overestimated by SPE as compared with the albumin value determined by the BCG method; accordingly, the M-protein was underestimated. Samples with the Type 3 abnormality showed good agreement between albumin values as measured by SPE and BCG. Seventy-one (75%) of the 95 sera showed either Type 1 or Type 2 M-protein abnormalities; these samples were obtained from 26 patients, 17 with IgG-kappa and nine with IgG-lambda monoclonal gammopathies. Five (7%) of the 71 samples had M-protein values of 35 to 39 g/L, those for the remainder (93%) ranging from 40 to 102 g/L. Table 1 shows the mean concentrations of M-protein and albumin as estimated by SPE and of albumin as determined by BCG for this group of patients.

Appropriate dilutions of the sera before SPE eliminated the irregularity of M-protein bands, increased the underestimated M-protein value, and decreased the overestimation of albumin. In every instance, (a) the dilution-corrected value for M-protein obtained by SPE for each diluted serum was always higher than that for the same serum undiluted, (b) the albumin value for each undiluted serum was always higher by SPE than that for the same serum measured by

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**Figure 1.** Type 1 M-protein band abnormality
Position 1 shows the markedly distorted M-protein band from an undiluted serum sample. Positions 2 (1:2 dilution), 3 (1:3 dilution), and 4 (1:4 dilution) show improving correction of the M-protein band abnormality with dilution of the sample. The total serum protein (by refractometry) was 123 g/L; the albumin value (by the BCG method) was 32 g/L. The SPE albumin and M-protein values for the undiluted serum were 60 and 42 g/L; for the 1:2 dilution 47 and 65 g/L; for the 1:3 dilution 45 and 70 g/L; and for the 1:4 dilution 43 and 72 g/L, respectively. Therefore, the appropriate dilution for this sample was 1:4

**Figure 2.** Type 2 M-protein abnormality
Position 1 shows the moderate curvature of the M-protein band plus its high density and length longer than the albumin band. Dilution factors for positions 2–4 are as in Fig. 1. The total serum protein (refractometry) was 144 g/L and the albumin value (BCG method) was 34 g/L. The SPE albumin and M-protein values for the undiluted serum were 49 and 70 g/L; for the 1:2 dilution 41 and 80 g/L; for the 1:3 dilution 39 and 87 g/L; and for the 1:4 dilution 37 and 88 g/L, respectively. Again, the 1:4 dilution was the proper dilution

**Figure 3.** Type 3 M-protein band abnormality is shown in position 1
Dilution factors for positions 2–4 as in Fig. 1. Total serum protein, 78 g/L; albumin, 44 g/L. The SPE albumin and M-protein values for the undiluted serum were 45 and 17 g/L; for the 1:2 dilution 45 and 16 g/L; for the 1:3 dilution 46 and 16 g/L; and for the 1:4 dilution 45 and 16 g/L. Dilution of the serum did not significantly alter the albumin and M-protein values

**Table 1.** Albumin and M-Protein Values (Mean ± SD) for 71 Samples from 26 Patients with Monoclonal Gammopathies

<table>
<thead>
<tr>
<th>Method</th>
<th>Concentration, g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Albumin</td>
</tr>
<tr>
<td>SPE, undiluted serum</td>
<td>45.8 ± 7.4</td>
</tr>
<tr>
<td>SPE, diluted serum</td>
<td>37.9 ± 5.8</td>
</tr>
<tr>
<td>BCG, caa III</td>
<td>32.8 ± 5.9</td>
</tr>
</tbody>
</table>

See Results for dilution factors used.
Table 2. Changes in M-Protein and Albumin Concentrations (g/L) in Sera of Two Patients

<table>
<thead>
<tr>
<th>Date of sampling</th>
<th>Total Albumin, BCG</th>
<th>Albumin, M-Protein</th>
<th>Albumin, M-Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Undiluted serum</td>
<td>Diluted serum</td>
<td>Diluted serum</td>
</tr>
<tr>
<td>Patient with progressive disease*</td>
<td>6-19-82</td>
<td>103</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>6-24-82</td>
<td>114</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>7-19-82</td>
<td>115</td>
<td>39</td>
</tr>
<tr>
<td>Patient responding to therapy</td>
<td>3-29-83</td>
<td>125</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>4-27-83</td>
<td>116</td>
<td>33</td>
</tr>
</tbody>
</table>

*The appropriate dilutions for the samples on all these dates were 1:4. The M-protein was identified as IgG kappa.

The BCG method, and (c) the albumin value for each undiluted serum was always higher than that for the same serum after dilution when both were assayed by SPE. Because there was such a consistent bias for both the albumin and M-protein values for the diluted and undiluted specimens, we assumed (but did not compute) statistical significance.

Appropriate dilutions of the sera before SPE always lowered the overestimated albumin values on SPE of every sample; however, only eight (11%) of the 71 samples that had overestimated SPE albumin values for the undiluted sera had the SPE albumin concentrations in the diluted sera equivalent to their BCG albumin values; the SPE albumin concentrations in the diluted sera of the remaining 63 (89%) samples were 6% to 26% greater than those determined by the BCG method.

The "appropriate" dilutions that provided the SPE albumin values closest or equivalent to the corresponding BCG albumin values for 15 of the 71 samples were 1:2. Greater dilutions (1:3 and 1:4) did not improve the agreement of the albumin values. For three of these 15 samples, the SPE values for albumin in the diluted sera were equivalent to the values for albumin in the undiluted serum by BCG.

The 1:3 dilutions were the most appropriate dilutions for 19 of the 71 samples. Use of 1:4 dilutions of these 19 sera did not improve the SPE albumin value. For only one of the 19 sera was the SPE albumin concentration for the diluted serum equivalent to its undiluted BCG albumin value.

The remaining 37 samples required 1:4 dilutions for the SPE albumin values to be close or equivalent to their corresponding BCG albumin values. For four of these 37 samples, the SPE albumin values for the diluted sera were equivalent to their undiluted BCG albumin values.

All of the remaining 24 sera (25%) from 18 patients (12 IgG-kappa, two with IgM-lambda, and one each with IgA-kappa, IgA-lambda, IgM-kappa, and IgM-lambda) showed the Type 3 M-protein band abnormality. For 15 (63%), M-protein values ranged from 14 to 34 g/L, three (12%) from 35 to 39 g/L, and six (25%) from 40 to 50 g/L. Dilution of these sera eliminated the M-protein band abnormalities but did not alter the M-protein or albumin values.

Table 2 presents data for two patients whose diluted sera clearly demonstrated results by SPE that were consistent with the clinical findings. However, SPE of the undiluted sera failed to reflect the clinical findings.

Discussion

Serum M-protein and albumin values are clinically signif-

icant prognostic factors for patients with multiple myeloma (10), and serial measurements of serum M-protein during the course of the disease are useful monitors for the clinical treatment of patients with monoclonal gammopathies (1-3, 10). SPE provides a simple method for simultaneously assaying both M-protein and albumin concentrations (4-6, 11), but precautions must be taken to assure its accuracy. In SPE, when any protein fraction exceeds a limiting concentration, dye binding is not linearly related to protein concentration (6). This type of error causes the M-protein to be underestimated and also indicates falsely increased amounts of the other protein fractions. The albumin concentration is affected most, being the major fraction of the serum proteins (6). In addition, because of the shape of the narrow aperture of the densitometer, each protein fraction must migrate in a regular rectangular shape, similar vertical length, and homogeneous staining. Proper dilution of the serum before applying it to the SPE strip should correct the band distortion and diminish the degree of the nonlinear dye binding by lowering the M-protein concentration. Although the pre-electrophoretic dilution treatment of the sera provided an albumin value equivalent to that determined by the BCG method only in eight (11%) of the 71 samples we studied, it improved the results for all the other samples so they were closer to the more nearly accurate albumin value.

Densitometric scanning of electrophoretic patterns is considered to permit an objective quantification of serum albumin and M-protein concentrations (4, 11). However, the electrophoretic patterns should be thoroughly inspected visually before quantification, to check for small M-protein bands on the SPE membrane.

Finally, we conclude that, to provide clinically reliable information for patient care, serum samples displaying M-protein band abnormalities on the SPE strip must be diluted and re-assayed.

References