An Electrophoretic Study of the Abnormal Proteins
in Urine, Peripheral Venous and Bone Marrow Sera
in Multiple Myeloma

By ROBERT T. S. JIM

The electrophoretic pattern of the abnormal serum proteins has been well
categorized in multiple myeloma, but fewer studies have been reported
on the abnormal urinary proteins and only one report is available on the
protein electrophoretic pattern of a vertebral bone marrow infiltrated by myeloma
tissue.

In a paper electrophoretic study of concentrated whole urines, peripheral
venous blood and bone marrow sera of 35 patients with multiple myeloma,
distinct and abnormal proteins of marked homogeneity were found in many of
the urines while the electrophoretic pattern of the bone marrow sera did not
appear to differ significantly from the corresponding peripheral venous sera.

Methods

Fresh urine centrifuged free of sediment were placed in cellulose casings with thymol
added as preservative, and concentrated approximately 100-150X by dialysis against 25 per
cent polyvinylpyrrolidone (PVP) at 6 C, or in a few cases by fan evaporation after 24 hour
dialysis against water at 4 C. Serum was obtained from clotted venous blood and from
approximately 0.5 ml of bone marrow material aspirated from the sternum or iliac crest
through an Unger needle. Slight hemolysis was noted in some of the bone marrow sera. The
bone marrow aspirates were all grossly and microscopically cellular. Twenty cubic milli-
meter quantities of the concentration whole urine and sera were subjected to electrophoresis
on Whatman #3 filter paper (supported between siliconized glass plates) in Veronal buffer
pH 8.6, ionic concentration 0.1, at 320 volts for 8 hours at room temperature and the paper
subsequently stained with 1 per cent bromphenol blue dye. Quantitation of the stained
bands was accomplished with the Spinco Analytrol Scanner.

Urine samples were tested by heat for Bence Jones proteinuria by the technic of Jacobson and
Milner in cases 3, 6, 9, 11, 13, 16-23, 25-28 and 32-35 while results on the other cases were
obtained from hospital charts where exact conditions of the testing were not recorded.

Results

Results of electrophoresis of the urine, peripheral venous and bone marrow
sera are summarized in table 1.

Urine

Twenty-seven out of 35 urines studied contained protein material on electrophoresis. Twenty-one out of the 35 urines or 60 per cent demonstrated large
congections of markedly homogeneous discrete globulins of varying mobilites
(cases 1-21, see figure 1 for illustrative examples). Of these 21 urines, 7 demonstrated
single discrete globulins alone (cases 1-7) while the other 14 were also
associated with smaller amounts of albumin and more diffuse globulin (cases

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<table>
<thead>
<tr>
<th>Case no.</th>
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<th>Electrophoretic mobility of the abnormal serum globulin</th>
<th>Comparison of the peripheral venous and bone marrow serum protein electrophoresis (components expressed as relative percentages)</th>
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Table 1.—Urine, Peripheral Venous and Bone Marrow Serum Protein Findings in 35 Cases of Multiple Myeloma

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**Table 1.**—Continued

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<tr>
<th>Case no.</th>
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* Indicates the abnormal homogeneous globulin component.
† Cryoglobulin present.
PVS denotes peripheral venous serum, BMS denotes bone marrow serum.

**Fig. 1.**—Illustrative examples of the abnormal serum (upper) and corresponding urine (lower) proteins on electrophoresis in multiple myeloma.
8–21). In 3 urines two separate but equally distinct homogeneous globulins were found in each urine (cases 7, 14–15). The mobilities of the discrete globulins varied from that of gamma to alpha 2 globulins, being slightly slower (cases 1, 3), the same as (cases 2, 12–13, 20–21) or greater than (cases 5–6, 8, 10–11, 16–19) their corresponding abnormal serum components. In 2 out of the 3 urines with two abnormal globulin components (cases 7, 15) one migrated with the mobility of the abnormal serum globulin while the other migrated slightly faster; in the third urine one component moved slower and the other component moved faster than the abnormal serum globulin (case 14). All urines positive for the abnormal discrete globulins also demonstrated corresponding abnormal serum globulins with the exception of 2 sera showing nonspecific protein patterns (cases 4, 9). Six urines exhibited only albumin and diffuse globulins of the nonspecific type (cases 22–27) although the majority of sera from these patients contained abnormal serum globulins. None of the 27 urines containing protein showed albumin alone. Eight urines failed to show any protein material on electrophoresis (cases 28–35).

With one exception, a patient with Waldenström's macroglobulinemia and Bence Jones proteinuria, electrophoresis of 83 non-myelomatous urines with proteinuria due to other etiologies failed to reveal the distinctly predominant homogeneous globulin pattern seen in the myeloma urines. These included urines from patients with a variety of renal diseases, infections, lymphomas, leukemias, malignancies, hypertensive and arteriosclerotic cardiovascular diseases, collagen diseases, granulomas and metabolic diseases. Nonspecific protein patterns were found consisting (a) mainly of albumin with smaller amounts of various globulin components (see fig. 2), (b) diffuse but occasionally more distinct globu-

![Fig. 2.](image) Electrophoretic appearance of proteinurias observed in a variety of non-myelomatous diseases. Note the discrete homogeneous globulin in the urine of a patient with Waldenström's macroglobulinemia resembling the abnormal myeloma urine globulin.
ABNORMAL URINARY PROTEINS IN MULTIPLE MYELOMA

![Image of protein electrophoresis patterns]

Fig. 3. Comparative appearance of peripheral venous and corresponding bone marrow serum protein electrophoretic patterns (case 15).

In components in association with small amounts of albumin in a few urines and (c) occasionally small amounts of diffuse globulin alone.

**Bone Marrow Sera**

A comparison of the electrophoretic protein pattern of the peripheral venous and bone marrow sera is summarized in table 1 (see figure 3 for illustrative example). In 12 myeloma patients studied, electrophoresis of the sera obtained from aspirated bone marrow material exhibited essentially no striking differences from the peripheral blood sera (cases 2–4, 9, 13, 15–17, 28, 31–32, 34). The bone marrow sera demonstrated essentially the same protein concentration as the peripheral sera although in 8 cases the bone marrow albumin level appeared slightly lower than that in the peripheral sera. Sufficient hemolysis of the bone marrow serum occurred in case 16 to produce a higher beta globulin concentration than the peripheral serum beta globulin and in case 32 was marked enough to make it impossible to separate the alpha 2 from beta globulin. Whenever the abnormal serum globulin was found in the peripheral sera it was also present in the corresponding bone marrow sera. Likewise, with nonspecific peripheral serum protein electrophoretic patterns similar nonspecific patterns were found in the corresponding bone marrow sera (cases 4, 9, 31).

**Discussion**

In multiple myeloma a high incidence of an abnormal globulin of marked homogeneity may be found in the urine on electrophoretic study, occurring either as an isolated discrete globulin or as the predominant globulin in association with smaller amounts of albumin and more diffuse globulin. The occurrence of this characteristic globulin pattern is rare in diseases other than multiple myeloma. Although strongly suggestive, this urinary homogeneous globulin is not specific nor pathognomonic for multiple myeloma.

The incidence of the abnormal homogeneous globulin in the urines studied could not be correlated with the age, sex, stage, course or duration of the disease. The abnormal urine globulin was found early or late in the course of the disease. Of the 21 urines positive for the typical abnormal globulin (cases 1–21), 16
were from patients with normal plasma non-protein nitrogens while the other 5 patients showed slight azotemia. Whether the association of albumin with the typical abnormal globulin denoted more serious renal impairment could not be determined due to inadequate renal functional studies. Azotemia appeared to be more common when albumin was found in addition to the typical homogeneous urine globulin. Of the 6 patients who came to autopsy in whom urine electrophoretic studies were performed (5 urines with the typical globulin pattern and 1 with the nonspecific pattern), microscopic examination of the kidneys of these patients revealed only nonspecific findings of tubular degeneration, occasional tubular casts, slight pyelonephritis and no massive infiltration by myeloma cells.

Approximately half of the myeloma urines with the characteristic homogeneous globulin exhibited the classical heat reaction for Bence Jones proteinuria. Failure to elicit the latter reaction may have been due to an improperly performed test, a protein concentration insufficient for heat coagulation or in view of the electrophoretic findings, the occurrence of this unique heat physicochemical reaction in only certain of the numerous abnormal urinary proteins found in multiple myeloma.

While the exact site of formation of Bence Jones protein still remains unknown, more recent studies with isotopic labeling of Bence Jones protein with $^{14}$C glutamic acid and lysine have indicated the de novo synthesis of Bence Jones protein directly from the free amino acids of the body pool rather than the conversion from plasma or tissue proteins or renal cleavage of the abnormal protein.

The slight differences between the electrophoretic protein pattern of the peripheral venous and bone marrow sera do not appear to be significant. The bone marrow serum protein electrophoretic pattern was probably that of venous serum and not the true protein content of the myeloma cell.

**Summary**

1. Abnormal and distinct homogeneous globulins of different electrophoretic mobilities have been found in a high percentage of concentrated whole urines from patients with multiple myeloma, occurring either as a single isolated globulin or as the dominant globulin in combination with smaller amounts of albumin and other globulin components. In contrast, the electrophoretic pattern of non-Bence Jones protein observed in a wide variety of diseases is dominated by albumin in association with smaller amounts of various globulin components usually more heterogeneous in nature.

2. The abnormal dominant globulins were found in 21 out of 35 or 60 per cent of the myeloma urines studies, while only 28.5 per cent were positive for Bence Jones protein by the conventional heat test.

3. Electrophoresis of concentrated whole urines in multiple myeloma appears to be a more sensitive technic for the detection of the abnormal urinary protein than the conventional heat test and may be a useful additional diagnostic procedure when the latter reactions cannot be elicited.

4. In patients with multiple myeloma the electrophoretic pattern of the protein in bone marrow serum does not appear to differ significantly from the corresponding protein pattern of peripheral venous serum.
ABNORMAL URINARY PROTEINS IN MULTIPLE MYELOMA

SUMMARIO IN INTERLINGUA

1. Anormal e distincte globulinas homogenee de differente mobilitate electrophoretic esseva trovate in un alte procentaeg de specimens de urina concentrate obtenite ab patientes con myeloma multiple. Illos occurreva in certe casos como isolate globulina unice in altere casos como le globulina dominante in combination con minor quantitates de albumina e altere componentes globulinic. In contrasto con isto, le configuration electrophoretic de proteina de typos non Bence Jones que es observe in un grande varietate de morbos es dominante per albumina in association con minor quantitates de varie componentes globulinic, e istos es usualmente de natura plus heterogenee.

2. Le anormal globulinas dominante esseva trovate in 21 cx 35 urinas a myeloma studiate. Isto amonta a 60 pro cento, duramite que solmente 28,5 pro cento del specimens esseva positive pro proteina Bence Jones secundno le essayo conventional a calor.

3. Le electrophorese de specimens de concentrate urina integre in casos de myeloma multiple pare esser un plus sensibile technica pro le detection de anormal proteina urinari que le essayo conventional a calor. Illo representas forsan un manovra diagnostic supplementari pro uso in cases in que le essayo a calor remane negative.

4. In patientes con myeloma multiple, le configuration electrophoretic del proteina in sero de medulla ossee non pare differer significafivensenste ab le corespontente configuration proteinic de sero venose peripheric.

REFERENCES

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