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

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THE electrophoretic pattern of the abnormal serum proteins has been well characterized in multiple myeloma, but fewer studies have been reported on the abnormal urinary proteins[1-17] and only one report is available on the protein electrophoretic pattern of a vertebral bone marrow infiltrated by myeloma tissue.[18]

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 millimeter 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.

Urines were tested by heat for Bence Jones proteinuria by the technic of Jacobson and Milner[19] 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.

Urines

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 concentrations of markedly homogeneous discrete globulins of varying mobilities (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 1.—*Urine, Peripheral Venous and Bone Marrow Serum Protein Findings in 35 Cases of Multiple Myeloma*

Case no.	Bence Jones proteinuria		Electrophoretic mobilities of the abnormal urine proteins		Total proteins (Gm. %)	Alb. (Gm. %)	Glob (Gm. %)	Electrophoretic mobility of the abnormal serum globulin	Comparison of the peripheral venous and bone marrow serum protein electrophoresis (components expressed as relative percentages)					
	Heat test	Sulfosalicylic acid test	Dominant homogeneous globulin	Associated proteins					Albumin	Globulins				
										alpha 1	alpha 2	beta 0	gamma	
1	0		Gamma		9.3	3.1	6.2	"M"						
2	Pos.		Beta		6.7	3.8	2.9	Beta	53.1	2.8	12.0	19.4*	12.7	PVS
									57.0	2.1	11.3	18.8*	10.8	BMS
3	Pos.	Pos.	Between alpha 2 & beta		7.3	2.5	4.8	Alpha 2	40.0	7.0	40.0*	6.0	7.0	PVS
									37.3	6.0	41.4*	6.4	8.9	BMS
4	Pos.		"M"		6.2	4.2	2.0	Non-specific	46.2	3.8	12.5	13.8	22.7	PVS
									43.8	7.2	14.6	11.5	22.9	BMS
5	0		Beta		11.6	1.9	9.7	Gamma						
6	Pos.	Pos.	Beta		7.6	2.2	5.4	Gamma						
7	0		Beta, alpha 2		8.5	4.1	4.4	"M"						
8	0	Pos.	"M"	Trace alb.	10.5†	2.4	8.1	Gamma						
9	Pos.		"M"	Trace alb., diffuse glob.	6.3	4.2	2.1	Non-specific	50.8	6.8	13.6	13.7	15.1	PVS
									53.9	5.6	11.3	12.9	16.3	BMS
10	Pos.		"M"	Alb., alpha glob.	13.6†	2.1	11.5	Gamma						
11	0		Beta	Alb., diffuse glob.	7.6	1.8	5.8	Gamma						
12	Pos.		"M"	Trace alb.	8.9	2.8	6.1	"M"						
13	Pos.		Beta	Alb., alpha glob.	12.5	2.9	9.6	Beta	23.6	3.1	2.0	69.3*	2.0	PVS
									23.6	2.0	1.9	70.6*	1.9	BMS
14	0		Gamma, beta	Alb., alpha glob.	13.9	1.8	12.1	Gamma						
15	0		Gamma, beta	Alb., alpha 1	10.9	3.3	7.6	Gamma	27.8	2.0	4.7	4.3	61.2*	PVS
									26.3	0.8	4.5	6.4	62.0*	BMS
16	Pos.	Pos.	Gamma (fast)	Alb., diffuse glob.	15.6	3.2	12.4	Gamma (slow)	29.2	1.6	5.2	8.4	55.6*	PVS
									22.7	1.0	6.4	15.9	54.0*	BMS
17	0		Beta	Trace alb., diffuse glob.	9.8	2.6	7.2	Gamma	42.8	2.5	4.9	6.2	43.5*	PVS
									38.5	3.0	5.2	6.8	46.4*	BMS
18	0		Beta	Alb., trace alpha glob.	7.9	4.8	3.1	"M"						
19	0		Beta	Trace alb., diffuse glob.	6.1	4.1	2.0	Gamma						
20	0		Gamma	Alb., diffuse glob.	6.5	4.6	1.9	Gamma						
21	0		Beta	Alb., diffuse glob.	7.2	2.2	5.0	Beta						
22	0		0	Trace alb., diffuse glob.	6.6	3.0	3.6	Non-specific						
23	0		0	Alb., alpha & beta glob.	7.5†	2.4	5.1	"M"						
24	0		0	Alb., diffuse glob.	9.2	2.8	6.4	Gamma						
25	0	0	0	Alb., diffuse glob.	12.7	3.7	9.0	Gamma						
26	0	0	0	Alb., diffuse glob.	8.8	1.9	6.9	"M"						
27	0	0	0	Trace "M"	7.3	4.4	2.9	"M"						
28	0		0	0	8.1	2.8	5.3	Gamma	22.3	1.4	8.0	8.3	60.0*	PVS
									24.3	2.3	10.0	6.7	56.7*	BMS
29	0		0	0	7.2	2.9	4.3	Gamma						
30	0		0	0	7.7	4.4	3.3	Gamma						
31	0		0	0	6.3	4.5	1.8	Non-specific	53.0	6.2	12.4	13.4	15.0	PVS
									48.5	7.7	11.7	12.7	19.4	BMS

TABLE 1.—*Continued*

Case no.	Bence Joces proteinuria		Electrophoretic mobilities of the abnormal urine proteins		Total proteins (Gm. %)	(Alb. Gm. %)	Glob. (Gm. %)	Electrophoretic mobility of the abmornal serum globulin	Comparison of the peripheral venous and bone marrow serum protein electrophoresis (components expressed as relative percentages)					
	Heat test	Sulfosalicylic acid test	Dominant homogeneous globulin	Associated proteins					Albumin	Globulins				
										alpha 1	alpha 2	beta "M"	gamma	
32	0		0	0	8.9	4.5	4.4	Beta	43.7	3.7	8.8	28.6*	17.2	PVS
									37.0	3.1		42.5*	17.4	BMS
33	0		0	0	9.5	3.7	5.8	Gamma						
34	0		0	0	9.1†	3.1	6.0	Gamma	34.2	2.4	8.5	9.3	45.5*	PVS
									33.3	2.4	11.5	10.9	41.8*	BMS
35	0		0	0	7.4	3.0	4.4	Gamma						

* Indicates the abnormal homogeneous globulin component.

† Cryoglobulin present.

PVS denotes peripheral venous serum, BMS denotes bone marrow serum.

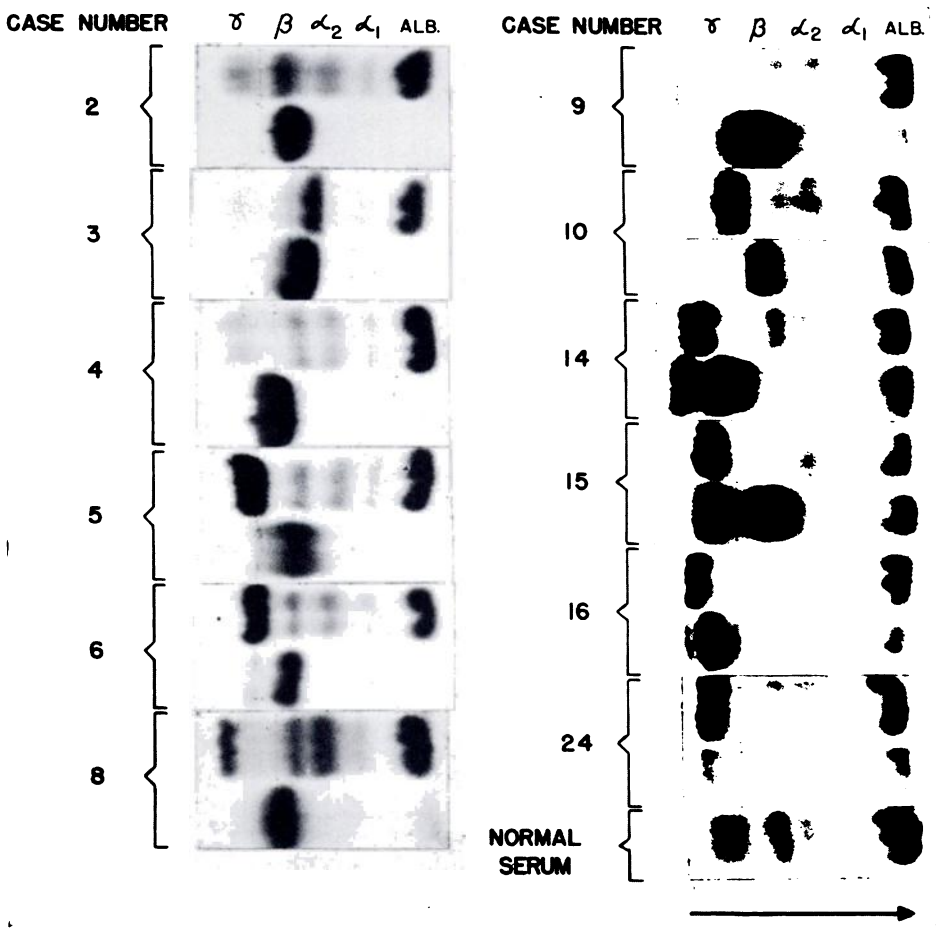

FIG. 1.—Illustrative examples of the abnormal serum (upper) and corresponding urine (lower) proteins on electrophoresis in multiple myeloma.

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-

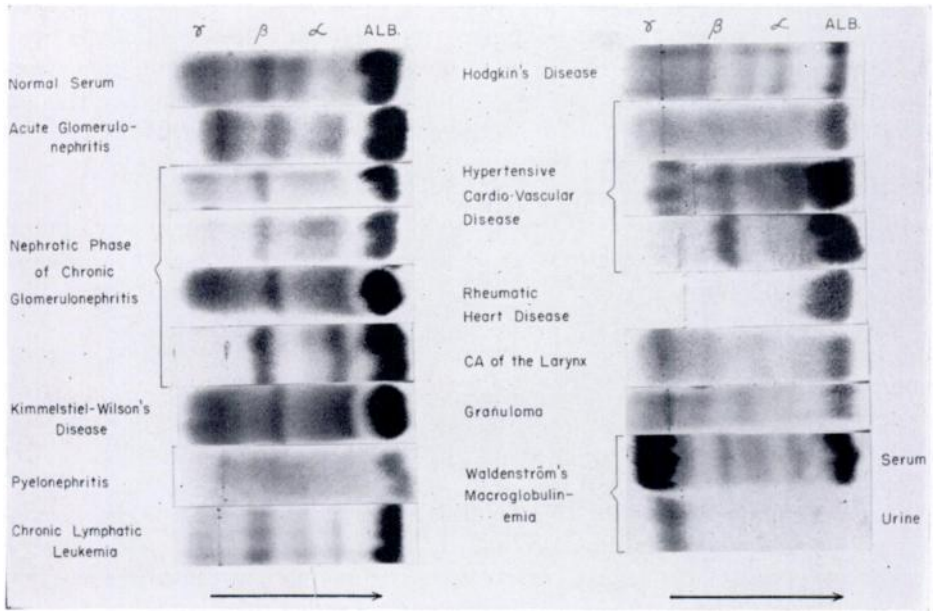

FIG. 2.—Electrophoretic appearance of proteinurias observed in a variety of non-myelomatous diseases. Note the discrete homogeneous globulin in the urine of a patient with Waldenström's macroglobulinemia resembling the abnormal myeloma urine globulin.

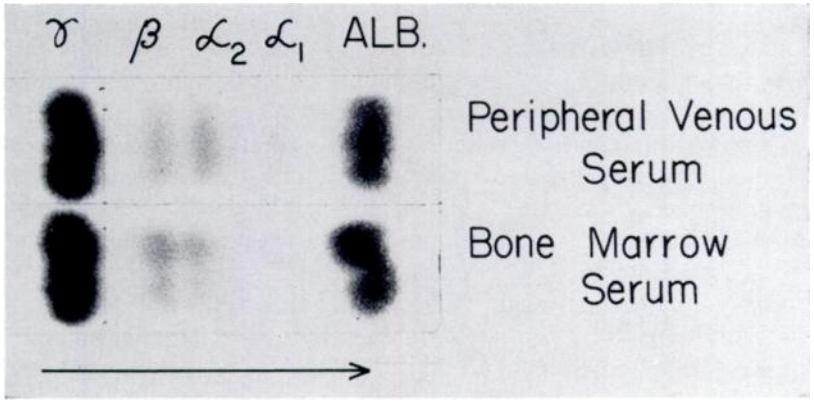

FIG. 3. Comparative appearance of peripheral venous and corresponding bone marrow serum protein electrophoretic patterns (case 15).

lin 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 C^{14} 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.[21]

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.

SUMMARIO IN INTERLINGUA

1. Anormal e distincte globulinas homogenee de differente mobilitate electrophoretic esseva trovate in un alte procentage 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 observate in un grande varietate de morbos es dominate per albumina in association con minor quantiates de varie componentes globulinic, e istos es usualmente de natura plus heterogenee.

2. Le anormal globulinas dominante esseva trovate in 21 ex 35 urinas a myeloma studiate. Isto amonta a 60 pro cento, durante que solmente 28,5 pro cento del specimens esseva positive pro proteina Bence Jones secundo 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 representa forsan un manovra diagnostic supplementari pro uso in casos 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 significativemente ab le correspondente configuration proteinic de sero venose peripheric.

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