Proceedings of the Iowa Academy of Science

Volume 69 | Annual Issue Article 34

1962

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A Study of the Electrophoretic Mobilities of Multiple Myeloma Proteins

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Over a period of several years our laboratory has been carrying out electrophoretic analysis of plasma, serum and body fluids from individuals with various diseases. One disease in which we have been interested is that of multiple myeloma.

Many workers have investigated the electrophoretic distribution of abnormal serum proteins in multiple myeloma. They based their reports on the findings of from one to 282 cases (Hardy and Putnam, 1955; Gutman, et al., 1936). The majority of investigators have used barbiturate buffer at pH 8.6 for electrophoretic analysis (Putnam, 1953; Osserman, 1955). A few have employed different buffers at varying pH's (Kekwick, et al., 1940; Gutman, et al., 1941). Bence-Jones protein in the urine and its relation to the myeloma protein in the serum has been the subject of several reports. These studies were based on the techniques of electrophoresis, ultra-centrifugation, immunoelectrophoresis and chemical separation. Until the advent of immunochemical procedures, especially immunoelectrophoresis in agar, the presence of Bence-Jones protein in the serum was not substantiated. There is convincing evidence that the Bence-Jones proteins are, in fact, present in minute amounts in the serum of many patients with multiple myeloma (Osserman, 1961).

During electrophoresis the abnormal protein migrated at speeds corresponding to the range from β-globulin to γ-globulin (Adams, et al., 1949; Putnam and Udin, 1953; Rundles, et al., 1951; Conn and Klatskin, 1954; Reiner and Stern, 1953). Conn (1954) and Griffith, et al. (1953) reported a multiple myeloma peak with the mobility of α_2-globulin and called it the α_2 globulin type of multiple myeloma, though it is possible that other investigators overlooked the α_2 globulin type because the peak tended to fuse with the α_2 globulin. Reiner and Stern (1953) found that in 91 cases none of the sera yielded a completely normal pattern. Myeloma globulins of the β type were shown to contain two different components by ultracentrifugation (Putnam, 1955). Osserman and others have classified the abnormal proteins as the slow γ-globulin, mid γ-globulin, fast γ-globulin, "M" type, and β-globulin type. Multiple myeloma serum proteins and Bence-Jones proteins have been shown to share anti-

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genic determinants in common with the normal γ-globulins. The development and application of immunoelectrophoresis has revealed further information concerning the two major types of myeloma protein: γ and β globulin types (Osserman and Lawler, 1961).

This paper presents results of electrophoretic analysis of serum specimens from proven multiple myeloma cases in whom the diagnosis was confirmed by either bone marrow aspiration, the presence of Bence-Jones protein in the urine, roentgenograms, or autopsy. The distribution of the patterns into the various types of myeloma is based on a practical application of electrophoretic mobility ratios.

Experimental

The serum or plasma samples were diluted with 3 volumes of barbital buffer pH 8.6 and ionic strength of 0.1. The resulting solutions were dialyzed against the buffer at 5°C for three days with daily change of buffer in preparation for electrophoresis. The Tiselius apparatus was used for electrophoretic analysis during which 25 milliamperes of current were passed for 120 minutes under the standardized conditions established in our laboratory. The analytical results were obtained from descending patterns. The relative mobility of the myeloma protein was obtained by measuring the distance from its peak to the peak of anomaly and dividing this by the distance of the albumin peak to the peak of the anomaly. The clinical confirmation of the diagnosis was obtained from the medical records of each patient.

Results

Tables 1 to 6 illustrate typical results obtained from an electrophoretic analysis of serum specimens from 86 patients with multiple myeloma. In every table the average percentage composition of the albumin and globulin components is compared to an average of the serum proteins from a group of normal individuals. Also included are average mobility ratios of the globulin peaks versus the albumin peak from a large series of normal serum patterns. The value for the mobility ratio of the predominate abnormal peak is located next to the percentage composition value for that component to facilitate comparison of the various types of multiple myeloma. The data included under clinical confirmation was obtained from a careful study of the medical records of each patient and consultation with the patient's physician when possible. These clinical findings included bone marrow examination (BM), the presence of Bence-Jones protein (BJ), positive x-ray findings (x-ray) and confirmation of multiple myeloma at autopsy (autopsy).

Table I. Serum Proteins in Multiple Myeloma. The Alpha-2 Type.

Patient	Albumin %	α_1 %	α_2 %	β Mob. Ratio	β %	γ' %	γ %	Clinical Confirmation BM	BJ	X-ray	Autopsy
V. L.	47.9	8.0	23.3	0.667	16.2	1.4	3.2		X	X	
M. M.	39.0	13.0	19.5	0.673	15.1	1.7	11.7	X	X	X	
G. S.	46.2	7.3	23.6	0.674	11.8	1.0	10.1	X	X		
R. D.	45.8	7.1	19.3	0.686	14.2	2.7	10.9		X		
	47.9	10.4	23.2	0.635	11.7	3.9	3.1				
F.C.	29.3	18.4	23.6	0.653	16.1	2.5	10.1	X		X	
A. R.	38.6	12.7	21.5	0.630	9.2	2.7	15.3	X		X	
Average 6 patients 7 serum	42.1	11.0	22.0	0.660	13.5	2.3	9.2				
Average Normal Mobility Ratio		0.847		0.678	0.493	0.385	0.223				
Average Normal Serum	61.1	5.1	8.8		12.4	2.0	10.6				

Table II. Serum Proteins in Multiple Myeloma. The Fast Beta Type.

Patient	Albumin %	α_1 %	α_2 %	β %	β Mob. Ratio	γ' %	γ %	Clinical Confirmation BM	BJ	X-ray	Autopsy
L. C.	23.9	4.2	7.4	62.8	0.556	0.2	1.5	X			
H. C.	19.5	3.8	2.7	70.4	0.529	0.4	3.2	X			
M. C.	30.3	4.8	12.8	48.0	0.525	0.4	3.7	X			
E. F.	53.3	4.4	9.9	27.4	0.509	1.6	3.4	X	X	X	
	51.7	4.5	10.6	28.1	0.500	1.4	3.7				
L. McG.	31.3	4.8	6.5	55.2	0.540	0.5	1.7	X	X	X	
	29.8	2.9	4.6	61.4	0.560	0.4	0.9				
Average 5 patients 7 serums	34.3	4.2	7.8	50.5	0.531	0.7	2.6				
Average Normal Mobility Ratio		0.847	0.678		0.493	0.385	0.223				
Average Normal Serum	61.1	5.1	8.8	12.4		2.0	10.6				

Table III. Serum Proteins in Multiple Myeloma. The Intermediate Beta Type.

Patient	Albumin %	α_1 %	α_2 %	β %	β Mob. Ratio	γ' %	γ %	Clinical Confirmation BM	BJ	X-ray	Autopsy
L. C.	21.0	3.7	8.1	61.6	0.467	..	5.6			X	
	46.6	7.5	10.7	25.8	0.457	1.2	8.6				
A. B.	37.1	9.2	11.0	32.9	0.479	2.0	9.6	X			
O.	55.3	8.0	15.0	15.2	0.490	1.6	4.9	X	X		
V. O.	60.1	3.4	11.8	19.3	0.460	0.9	4.5	X			
A. P.	41.0	6.6	17.2	18.0	0.466	1.9	15.3				X
W. T.	52.5	5.6	8.7	15.3	0.471	9.7	8.2				
K. G.	14.4	4.1	7.4	72.2	0.446	..	1.9		X	X	
H. S.	26.9	8.0	10.5	52.1	0.417	..	2.5				
Average 8 patients 9 serums	39.4	6.2	11.1	34.7	0.461	1.5	6.8				
Average Normal Mobility Ratio		0.847	0.678		0.493	0.385	0.223				
Average Normal Serum	61.1	5.1	8.8	12.4		2.0	10.6				

Table IV. Serum Proteins in Multiple Myeloma. The Fast Gamma Type.

Patient	Albumin %	α_1 %	α_2 %	β %	%	γ' Mob. Ratio	γ %	Clinical Confirmation BM	BJ	X-ray	Autopsy
L. A.	40.1	4.7	10.2	15.8	27.6	0.333	1.6	X		X	
P. C.	26.7	4.7	8.5	7.9	52.2	0.331	..	X			
H. E.	33.4	5.7	8.4	7.6	44.9	0.326	..	X		X	X
D. G.	13.3	4.2	7.0	11.5	27.1	0.330	36.9	X			
W. G.	22.1	5.0	7.2	5.1	59.2	0.382	1.4	X			X
M. J.	30.2	3.4	4.1	8.8	52.9	0.361	0.6		X		
R. K.	32.0	2.2	8.1	15.8	32.3	0.302	9.6	X			
C. L.	24.6	5.3	7.0	4.6	58.5	0.372	..	X			
A. P.	30.9	3.6	8.6	8.0	47.5	0.339	1.4			X	
A. T.	40.2	6.8	14.4	14.4	22.5	0.351	1.7	X		X	
Average 19 patients 23 serums	33.6	5.4	10.3	10.0	35.4	0.340	7.3				
Above Normal Mobility Ratio		0.847	0.678	0.493		0.385	0.223				
Average Normal Serum	61.1	5.1	8.8	12.4	2.0		10.6				

Table V. Serum Proteins in Multiple Myeloma. The Intermediate Gamma Type.

Patient	Albumin %	α_1 %	α_2 %	β %	γ' %	γ %	γ Mob. Ratio	Clinical BM	Clinical BJ	Confirmation X-ray	Confirmation Autopsy
F. B.	34.4	14.1	16.1	14.1		21.3	0.299	X			
C. B.	19.0	5.9	12.1	7.1	1.0	54.9	0.248	X	X		
B. B.	18.0	6.7	10.0	5.3	2.5	57.5	0.217	X		X	
C. E.	29.2	7.7	10.9	6.8	1.3	44.1	0.254	X	X	X	
H. M.	27.3	4.2	6.0	11.5		51.0	0.271	X		X	
J. P.	15.3	4.4	6.7	4.8	1.0	67.8	0.283	X			
K. S.	39.6	6.8	11.7	20.4	4.2	17.3	0.279			X	
F. S.	21.2	3.7	7.5	6.1		61.4	0.283			X	
J. T.	51.2	6.0	11.5	8.1	1.0	22.2	2.250			X	
U. W.	15.2	4.3	6.4	4.7	0.7	68.7	0.234	X		X	
Average 24 patients 29 serums	33.6	6.1	10.2	10.9	3.6	35.2	0.251				
Average Normal Mobility Ratio		0.847	0.678	0.493	0.385		0.223				
Average Normal Serum	61.1	5.1	8.8	12.4	2.0	10.6					

Table VI. Serum Proteins in Multiple Myeloma. The Slow Gamma Type.

Patient	Albumin %	α_1 %	α_2 %	β %	γ' %	γ %	γ Mob. Ratio	Clinical BM	Clinical BJ	Confirmation X-ray	Confirmation Autopsy
Z. B.	21.5	6.1	11.8	7.5	0.8	52.3	0.182				X
F. B.	18.0	4.7	3.5	2.9	0.6	70.3	0.190	X			
I. C.	18.7	1.4	3.4	6.5	0.5	69.6	0.144	X		X	
H. E.	17.2	2.8	6.2	7.4	1.9	64.5	0.177	X			
E. F.	23.3	4.2	5.0	7.1	2.1	58.3	0.201	X		X	
E. M.	19.5	3.7	6.9	6.6	1.1	62.2	0.191	X			
O. M.	28.3	5.6	11.2	6.2		48.7	0.136	X			
P. P.	17.4	4.1	8.6	3.6	2.1	64.2	0.141				X
N. P.	24.0	4.0	10.5	7.1	0.9	54.4	0.000	X	X	X	
D. U.	31.1	8.2	15.5	11.7	1.0	32.5	0.147	X			
Average 24 patients 32 serums	28.3	5.0	10.2	8.5	45.2	45.2	0.166				
Average Normal Mobility Ratio		0.847	0.678	0.493	0.385		0.223				
Average Normal Serum	61.1	5.1	8.8	12.4	2.0	10.6					

Discussion

We have used the relative mobility of the abnormal proteins versus albumin instead of their individual mobilities because of many factors: the patterns have been collected in a span of over fifteen years during which period many electrophoresis cells have been replaced and the analysis have been carried out by different personnel.

By employing the relative mobilities we found that the patterns of six patients who had a higher than normal α_2-globulin concentration averaging 22.0% had the highest mobility ratio with an average of 0.660. Reiner and Stern (1953) reported six cases which they classified as α_2-globulin type myeloma protein with a relative concentration of 9.5 to 82.7% and mobility which varied from 3.6-4.5 x 10^{-5}. Conn and Klatskin (1954) reported only one patient with an abnormal α_2-globulin protein. In Table I the serum of every patient had an α_2-globulin concentration that was two to three times normal.

Table II shows the results from seven patterns whose largest protein concentration was found in the β-globulin fraction ranging from 27.4% to 62.8% with an average of 50.5%. The abnormal peaks migrated with the leading edge of the β-globulin peak with mobility ratios from 0.500 to 0.560 with an average of 0.531. Table III on the other hand presents data from eight patients whose serum contained abnormal proteins that migrated with the medium speed of the β-globulin fraction. The concentration varied from 15.2% to 72.2% with an average of 34.7% while their relative mobilities ranged from 0.417 to 0.490, averaging 0.461. Again Reiner and Stern (1953) list fourteen cases with abnormal peaks near the mobility of the β-globulin peak (2.5-3.6 x 10^{-5}) and a relative concentration of 31.8-8.95.3% Putnam and Udin (1953) list three cases as β-globulin type myeloma protein. Gutman, et al. (1941), using phosphate buffer with pH of 7.4 and ionic strength of 0.2 obtained six patterns of the β type protein.

Many investigators have reported another myeloma protein called the "M" component which migrated at the same speed as the γ'-globulin. Ossermann (1955) reported 3 cases, and Gutman et al. (1941), one case. We have found as Table IV illustrates that the relative mobility of the abnormal protein in twenty three serums was from 0.262 to 0.400 with an average of 0.340. Only three patterns had a relative mobility below 0.300 and only one as high at 0.400. The abnormal protein was found following the β-globulin peak very closely and the concentration percentage ranged from 15.5 to 68.0 in serum and 8.3 to 13.7 in plasma.

Table V was prepared from patterns that had a γ-globulin

type peak and whose relative mobilities varied from 0.202 to 0.299 averaging 0.251. Of the 29 serum specimens the percentage concentration of the abnormal protein ranged from 6.5 to 69.2 with an average of 35.2. These patterns would compare with those of Putnam and Udin (1953) numbers 8a to 13 which have a relative mobility ratio of 0.200 to 0.250.

Table VI contains 32 patterns from 24 patients whose abnormal protein component migrated at the speed of a slow γ-globulin. The mobility ratios varied from 0.000 to 0.223 averaging 0.166 while abnormal protein concentrations ranged from 10.6% to 70.3% with an average of 45.2%. Putnam and Udin (1953) reported eight patterns with similar relative mobility ratios (0.091 to 0.183).

Summary

1. A series of 107 serum specimens from 86 patients with proven muliple myeloma have been subjected to electrophoretic analysis.
2. When the mobility of the abnormal protein peak was compared to that of albumin in the same electrophoretic pattern a relative mobility was obtained for the myeloma protein. On the basis of the relative mobilities the serum specimens were classified as alpha-2, fast beta, intermediate beta, fast gamma, intermediate gamma or slow gamma type of multiple myeloma. The majority of the patients exhibited abnormal proteins migrating in the general range of the gamma globulin with the following distribution: alpha-2, 7%; fast beta, 6%; intermediate beta, 9%; fast gamma, 22%; intermediate gamma, 28%; and slow gamma 28%.

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Identification of Basic Concepts of Chemistry Appropriate for Grade Seven

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Abstract. One of the greatest results of the increased popular interests in science has been the development of advanced high school science courses. These courses have necessitated the reexamination of junior high school science curricula. In line with this reconsideration the University High School of the State University of Iowa has developed a special junior high school science sequence to aid in this preparation.

The seventh grade science course is entitled Matter and serves as the foundation where basic concepts are learned. Those chemical concepts most effectively learned by this age group are: (1) the nature of matter (2) atomic theory and structure (3) periodicity of elements and the periodic chart (4) molecular theory (5) chemical bonding (6) valence and formula writing (7) ionization in solutions (8) concentrations of solutions (9) nature of acids, bases, and salts (10) elementary organic chemistry concepts.

Preliminary findings portend the success of this program.

Since the Russian achievements in space in October of 1957, educators in this country have begun serious reconsiderations of the adequacy of science programs which are to prepare students for participation in a society which is becoming more and more technologically and scientifically oriented. May Brodbeck has pointed out that the growing prestige of the scientist and his increasing role in social affairs as well as the use of the threat of new weapons by all sides in world diplomacy make science of special concern to both the reflective citizen and scientist. the layman, feeling the impact of science on his life far beyond the convenience of new gadgets and inventions begins to wonder just what man has wrought(1). This concern has manifested itself in serious reexaminations in education of the science curriculum itself.

The reexamination, in turn has resulted in positive action;

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