STUDIES IN SERUM ELECTROLYTES. XV. THE CALCIUM-BINDING PROPERTY OF THE SERUM PROTEINS

(MULTIPLE MYELOMA, LYMPHOGRANULOMA VENEREUM AND SARCOIDOSIS)

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Rona and Takahashi (1) in 1911 first demonstrated by dialysis experiments that serum calcium exists in 2 forms: diffusible, and nondiffusible. Later work by Cushing (2) and by Neuhausen and Pincus (3) employing pressure filtration through collodion membranes demonstrated that approximately half of the total serum calcium is present in either form. Subsequent investigators have confirmed these findings (4) and have shown that in man most of the diffusible calcium is ionized, and most of the nondiffusible calcium is bound to the serum proteins (5).

Previous measurements of the relative amounts of calcium bound by the albumin and globulin fractions of the total serum protein have been unsatisfactory, owing to the difficulty encountered in separating and purifying the proteins without altering their calcium-binding property. As late as 1942 Masket, Chanutin, and Ludewig (6) indicated that the calcium-binding properties of serum albumin and globulin as determined by various investigators were not in agreement presumably owing to differences in the techniques employed. The earlier methods of protein separation were dependent upon salting out procedures which, because of the excess of salts present, required subsequent dialysis in order to purify the proteins. It would seem reasonable that such manipulations might alter the calcium-binding power. In addition, it has since been amply demonstrated that the salting out procedures do not give an albumin-globulin separation comparable to that of electrophoresis (7, 8).

Csapo and Faubl (9) observed that less calcium is carried down by the globulin fraction precipitated with half-saturated ammonium sulfate than by the albumin fraction. Bendien and Snapper (10), likewise, on the basis of protein separations performed by precipitating the globulins with sodium sulfate concluded that most of the nondiffusible calcium is bound to the albumin fraction. This view is also held by Schmidt and Greenberg (11). McLean and Hastings (5) on the basis of measurements made on purified horse serum proteins concluded that the values of the calcium-binding property are 0.716 mgm. of calcium per gram of albumin and 0.744 mgm. of calcium per gram of globulin. Drinker, Green, and Hastings (12), on the basis of measurements on purified fractions of horse serum globulin, concluded that the value of the calcium-binding property varies from 0.20 to 3.44 mgm. of calcium per gram of globulin.

Values for the calcium-binding property of protein have been estimated statistically by plotting the concentrations of the total serum calcium against the concentrations of total serum protein in a scattergram, and applying the formula of Hastings, Murray, and Sendroy (13):

$$[\text{Total Calcium}] = m\,[\text{Total Protein}] + b$$

where "b" represents the diffusible calcium in mgm. per 100 ml. (assuming the latter to be constant) and "m" represents the average binding property of the serum proteins in milligrams per gram of protein. By this method Hastings, Murray, and Sendroy conclude that the value of $m = 0.56$, and that of $b = 5.6$. Peters and Eiserson (14) report the values to be $m = 0.56$, $b = 6.0$; and Greenwald (15) observes that $m = 0.875$, and b varies from 3.7 to 5.0 in the adult and is 6.3 in infants.

This statistical approach by the scattergram method has been applied by Gutman and Gutman (16) to the study of sera obtained from patients suffering from lymphogranuloma venereum and other chronic diseases which present elevated concentrations of serum globulin. Cases of multiple myeloma were not included in this study since the view was held that the increase in serum calcium frequently observed in this condition was

often due to an increase in the diffusible fraction. It was found that when the serum globulin rose above 4.0 grams per 100 ml., the deviation from the Hastings formula was roughly proportional to the increased concentration of globulin. It was calculated that the excess globulin bound only 0.1 to 0.2 mgm. of calcium per gram of excess globulin. As this amount was considered to be negligible, the following values were introduced into the Hastings formula:

$$[\text{Total Calcium}] = 0.83\,[\text{Albumin}] + 7.0.$$

The value of 0.83 represents the calcium-binding property of albumin in mgm. of calcium per gram of albumin. The value of 7.0 is a constant and represents the diffusible calcium plus the calcium bound to globulin.

In 1944 Pillemer and Hutchinson (8) reported a method for the separation of albumin and globulin which gave values that compared favorably with those obtained by electrophoresis. In addition, the method avoided the use of saturated salt solutions and thus appeared to be suitable for the measurement of the calcium bound to each protein fraction. As the normal calcium-binding property of the individual serum proteins, in the opinion of most investigators, has never been determined in a satisfactory manner the following experiments were undertaken to attempt the estimation of these values, and to study their variations in certain diseases.

METHODS

Diffusible calcium was estimated by measuring the concentration of calcium in a protein-free ultrafiltrate of serum. The amount of calcium bound to globulin was estimated from the difference between the concentration of calcium in the original serum, and the concentration of calcium in the same serum after precipitation of the globulins with methanol. The remaining calcium fraction was considered to be bound to albumin.

Samples of blood were withdrawn under oil without stasis and allowed to clot in the refrigerator. The sera were separated approximately 2 hours after withdrawing the blood, and the analyses were made within 48 hours.

Protein-free filtrates were prepared by suction filtration through "Nojax" casing [1] membranes using a Greenberg-Gunther (17) apparatus modified to provide an extra trap and a more reliable method for maintaining water-vapor saturation within the tube containing the filtrate (Figure 1). No protein was demonstrable in the ultrafiltrates by the biuret reaction. The values for specific gravity of the ultrafiltrates ranged from 1.007 to 1.008.

For purposes of comparison, the concentration of calcium and protein have been expressed in terms of serum water. The specific gravity of sera and ultrafiltrates was measured by means of a 2-ml. pyknometer. The percentages of total solids in sera and in ultrafiltrates were obtained by drying weighed samples at 100° to 105° C. to constant weight. The concentration of calcium and protein in relation to the concentration of water was calculated from these measurements in accordance with Sunderman's method (18) for the calculation of such factors.

Measurements of the total serum protein and the protein fractions were made by a modification of the biuret

[1] Made by the Visking Corp., Chicago, Illinois. Diameter 23/32".

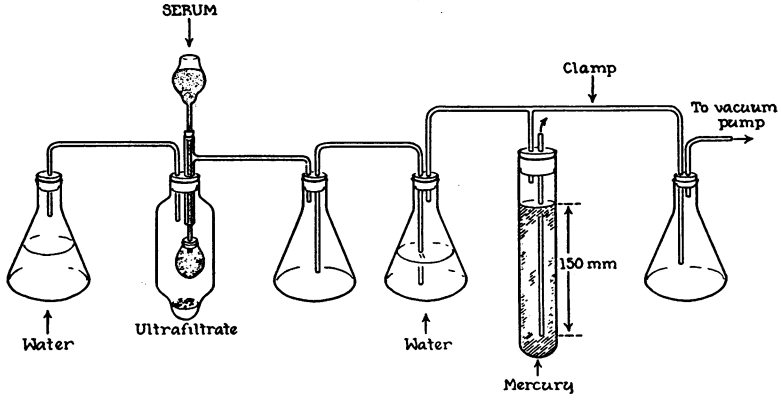

FIG. 1. MODIFIED GREENBERG-GUNTHER APPARATUS FOR THE PREPARATION OF PROTEIN-FREE ULTRAFILTRATES

Serum is contained in semipermeable membrane, and constituents of the ultrafiltrate are drawn through by means of negative pressure. Note water flasks for maintaining constant water vapor pressure within system.

TABLE I

Original data: Measurement of calcium and protein fractions in normals, multiple myeloma, sarcoid, and lymphogranuloma venereum

Diagnosis	Case	Total calcium serum	Diffusible calcium filtrate	Calcium in globulin-free serum	Total protein serum	Albumin (methanol method) serum	Albumin (salting method) serum	Specific gravity 20/20	Per cent solids serum
		mgm./100 ml.	*mgm./100 ml.*	*mgm./100 ml.*	*grams/100 ml.*	*grams/100 ml.*	*grams/100 ml.*	°C.	*grams/100 grams*
Normals	DL	10.42	4.76	8.09	6.67	4.23	4.87	1.0271	8.64
	AR	10.36	4.16	8.00	6.83	4.00	4.97	1.0261	8.51
	JM	10.20	4.99	7.84	7.13	4.09	4.79	1.0272	8.66
	FS	10.00	4.84	8.09	6.83	4.26	5.00	1.0266	8.52
	AP	10.00	4.99	7.28	6.70	3.40	4.38	1.0274	8.39
Multiple myeloma	JS	16.93	4.12	8.53	10.57	0.79	1.31	1.0452	14.08
	NA	12.20	4.58	7.52	8.05	1.22	2.78	1.0340	11.22
	LP	11.10	4.37	8.73	5.62	3.73	4.22	1.0245	7.63
	LT	11.00	5.72	7.60	5.58	2.15	3.22	1.0253	7.60
	JM	10.80	4.78	8.73	6.21	2.94	4.69	1.0271	8.29
	ES	9.17	4.88	6.32	4.62	1.88	3.14	1.0228	6.65
Sarcoid	JP	10.21	4.65	6.45	7.26	1.68	2.52	1.0289	9.76
Lympho-granuloma venereum	MC	10.50	4.37	9.00	7.16	2.44	3.83		
	TB	9.70	3.54	6.55	7.72	2.80	4.10	1.0318	9.90
	HF	8.88	4.10	5.79	7.72	0.56	0.80	1.0306	9.74
Healed Lympho-gran. ven.	JL	10.21	5.20	7.64	6.76	3.17	3.58	1.0265	8.97

method (19) and the Pregl method for nitrogen (20). The albumin and globulin fractions were separated by the method of Pillemer and Hutchinson (8), in which the globulins are precipitated by methanol and separated by filtration at a temperature below 10° C. The presence of small amounts of methanol was shown not to affect the biuret reaction.

Estimation of the albumin and globulin fractions was also obtained by salting out the globulin with Na_2SO_4 according to the method of Howe (21) as modified by Kingsley (22).

The concentrations of calcium in solution were estimated by the Kramer-Tisdall procedure as modified by Tisdall (23) and by Clark and Collip (24). This procedure was found to yield satisfactory values in serum, in protein-free ultrafiltrates, and in methanol filtrates obtained with the method of Pillemer and Hutchinson. The amount of soluble calcium oxalate, which was found to be negligible in the methanol solutions used, was taken into account in the calculations. In calculating the concentration of calcium in the globulin-free serum filtrate the volume previously occupied by the globulin was neglected since it was shown to amount only to about 7 parts per 1000 parts of filtrate.

All measurements of protein and calcium were performed in duplicate. When the duplicate measurements did not agree within 4 per cent of each other, they were repeated.

RESULTS

The original data from which the calculations have been made are presented in Table I.

In Table II are given the values for the concentrations of the various calcium and protein fractions, calculated in relation to the concentrations of water. The calculated calcium-binding properties of the protein fractions are also shown.

Comparisons of albumin-globulin ratios obtained by methanol precipitation, salting out, and by electrophoresis are given in Table III. It will be seen that our estimations of the albumin-globulin ratios obtained from methanol precipitation of globulin agree reasonably well with those obtained by electrophoresis and thus would confirm the findings of Pillemer and Hutchinson. It has been shown by Dole and Braun (7) that in normal sera the value of the A/G ratio obtained by electrophoresis bears a relation to the value of the A/G ratio obtained by the salting out method of approximately 2:3. A similar relationship between the values of the A/G ratios obtained by methanol precipitation and salting out, respectively, was observed in our normal group.

DISCUSSION

The results obtained in this study are based upon the assumption that globulin does not lose its bound calcium as a result of precipitation of

TABLE II

Calculated data: Calcium and protein partition, and calcium-binding power of the serum proteins in normal and abnormal conditions

Diagnosis	Case	Calcium partition					Protein partition *			Binding power		Increased globulin fraction
		Total	Diffusible	Bound	Bound to albumin	Bound to globulin	Total	Albumin	Globulin	Albumin	Globulin	
		mgm. per 100 grams H_2O	*mgm. per 100 grams H_2O*	*mgm. per 100 grams H_2O*	*mgm. per 100 grams H_2O*	*mgm. per 100 grams H_2O*	*grams per 100 grams H_2O*	*grams per 100 grams H_2O*	*grams per 100 grams H_2O*	*mgm. per gram*	*mgm. per gram*	
Normals	AR	11.03	4.18	6.85	4.34	2.51	7.27	4.26	3.01	1.02	0.83	
	DL	11.11	4.78	6.33	3.84	2.49	7.11	4.51	2.60	0.85	0.96	
	FS	10.65	4.86	5.79	3.75	2.04	7.27	4.54	2.73	0.83	0.75	
	JM	10.87	5.01	5.86	3.34	2.52	7.60	4.36	3.24	0.77	0.78	
	AP	10.62	5.01	5.61	2.71	2.90	7.12	3.61	3.51	0.77	0.83	
Average										0.85 (s.e. = 0.045)	0.83 (s.e. = 0.040)	
Multiple myeloma	JS	18.86	4.14	14.72	5.38	9.34	11.77	0.88	10.89	6.11	0.86	β
	NA	13.29	4.60	8.69	3.59	5.10	8.77	1.33	7.44	2.70	0.68	γ
	JM	11.47	4.80	6.67	4.47	2.20	6.60	3.12	3.48	1.43	0.63	γ
	LP	11.73	4.39	7.34	4.83	2.51	5.94	3.94	2.00	1.23	1.26	α
	LT	11.62	5.74	5.88	2.29	3.59	5.89	2.27	3.62	1.01	0.99	α
	ES	9.60	4.90	4.70	1.73	2.97	4.84	1.97	2.87	0.88	1.03	α
Sarcoid	JP	11.00	4.67	6.33	2.28	4.05	7.82	1.81	6.01	1.26	0.67	γ
Lympho-granuloma venereum	HF	9.55	4.12	5.43	2.11	3.32	8.30	0.60	7.70	3.52	0.43	γ
	MC	11.19	4.39	6.80	5.20	1.60	7.63	2.60	5.03	2.00	0.32	γ
	TB	10.44	3.56	6.88	3.48	3.40	8.31	3.01	5.30	1.16	0.64	γ
Healed lymphogran. venereum	JL	10.92	5.22	5.70	2.96	2.74	7.23	3.39	3.84	0.87	0.71	

* By the method of Pillemer and Hutchinson (8).

TABLE III

Comparison of methanol, Na_2SO_4,† and electrophoretic separation of albumin and globulin*

Diagnosis	Case	Albumin		Globulin		A/G ratio		
		Methanol	Na_2SO_4	Methanol	Na_2SO_4	Methanol	Na_2SO_4	Electrophoresis
		grams/100 grams H_2O	*grams/100 grams H_2O*	*grams/100 grams H_2O*	*grams/100 grams H_2O*			
Normals	AR	4.26	5.29	3.01	1.98	1.42	2.67	
	JM	4.36	5.11	3.24	2.49	1.34	2.05	
	FS	4.54	5.32	2.73	1.95	1.66	2.73	
	DL	4.51	5.19	2.60	1.92	1.73	2.70	
	AP	3.61	4.65	3.51	2.47	1.03	1.88	
Multiple myeloma	ES	1.97	3.29	2.87	1.55	0.69	2.12	0.62
	LT	2.27	3.40	3.62	2.49	0.63	1.36	0.46
	LP	3.94	4.46	2.00	1.48	1.97	3.01	1.30
	JS	0.88	1.46	10.89	10.31	0.08	0.14	0.23
	NA	1.33	3.03	7.44	5.74	0.18	0.53	0.26
	JM	3.12	4.98	3.48	1.62	0.90	3.07	1.00
Sarcoid	JP	1.81	2.71	6.01	5.11	0.30	0.53	
Lympho-granuloma venereum	HF	0.60	0.86	7.70	7.44	0.07	0.11	
	MC	2.60	4.08	5.03	3.55	0.52	1.15	
	TB	3.01	4.41	5.30	3.90	0.57	1.13	
Healed lym-phogran. venereum	JL	3.39	3.83	3.84	3.40	0.88	1.13	

* By the method of Pillemer and Hutchinson (8).

† By the method of Howe (21).

the protein. To our knowledge, no evidence bearing upon this assumption is available.

McLean and Hastings (5) and others (6, 25) have indicated that in solutions containing calcium and protein, the following mass law relationship holds as a first approximation:

$$\text{I.} \qquad \frac{[\text{Calcium Proteinate}]}{[Ca^{++}] \times [\text{Protein}^{=}]} = K.$$

From this equation the amount of calcium bound to protein would be partially dependent upon the concentration of calcium ion. Comparisons are, therefore, made only upon those cases having a diffusible calcium concentration within a relatively narrow range (4.18 to 5.22 mgm. per 100 grams H_2O). One case of lymphogranuloma (TB) had a diffusible calcium concentration below this range, and 1 case of multiple myeloma (LT) had a concentration above it. The calculated binding power of the proteins in these cases is not considered to be comparable with others of the series.

The average value for the calcium-binding property of total serum protein in our normal subjects was 0.84 (s.e. = 0.029) mgm. of calcium per gram of protein. This value compares favorably with that of 0.87 derived statistically by Greenwald (15). It is, however, higher than that of 0.56 by Hasting *et al.* (13) and by Peters and Eiserson (14). The average value for the calcium-binding property of serum globulin in our series was 0.83 (s.e. = 0.040) mgm. of calcium per gram of total globulin which is fairly close to the value of 0.74 obtained by McLean and Hastings (5) for horse serum globulin. The average calcium-binding property of albumin from normal subjects was 0.85 (s.e. = 0.045) mgm. per gram, as compared to the value of 0.716 obtained by McLean and Hastings (5). These authors indicate that owing to the tendency of serum albumin to lose its calcium-binding power during purification, it would seem likely that their value did not represent the full calcium-binding power of serum albumin under natural conditions.

It has long been known that the concentration of serum albumin shows a strong tendency to decrease in diseases characterized by hyperglobulinemia. Heretofore, studies of the properties of albumin in hyperglobulinemic sera by various precipitation procedures (26, 27) and by electrophoresis (28 to 32) have shown no indication of any qualitative change in the albumin fraction. In the experimental results presented in this paper, it will be noted that most of the patients studied show an increase in the calcium-binding property of their serum albumin, some to a marked degree. This phenomenon is seen in all 3 diseases.

If it be assumed that

$$[\text{Ca Proteinate}] = [\text{Total Calcium}] - [Ca^{++}]$$

following the relationships of Marrack and Thacker (33) and McLean and Hastings (5), then from Equation I:

$$\text{II.} \qquad \frac{[\text{Total Calcium}] - [Ca^{++}]}{[Ca^{++}]} = K\,[\text{Total Protein}].$$

McLean and Hastings (5) have shown that in human serum practically all the diffusible calcium is ionized, and the non-diffusible calcium is practically all bound to protein. If, therefore, on the left-hand side of the Equation II, the expression [Calcium bound to Protein] is substituted for the numerator, and the expression [Diffusible Calcium] is substituted for the denominater, then:

$$\text{III.} \qquad \frac{[\text{Calcium bound to Protein}]}{[\text{Total Protein}]} = K\,[\text{Diffusible Calcium}].$$

If we now consider the specific application of Equation III to albumin:

$$\text{IV.} \qquad \frac{[\text{Calcium bound to Albumin}]}{[\text{Albumin}]} = K\,[\text{Diffusible Calcium}].$$

Equation IV is plotted in Figure 2, "K" having been determined from the data for normal sera. The points obtained from normal sera are all close to the curve. The points derived from the sera obtained from patients, however, are for the most part well outside the range of dispersion of the normals. This suggests either that an appreciable amount of non-diffusible calcium is bound to substance other than protein, or else that a protein different from normal serum albumin makes its appearance in the albumin fraction in significant quantity in the diseases studied.

The calcium-binding property of globulin in the sera from patients with multiple myeloma was increased above the normal range in 3

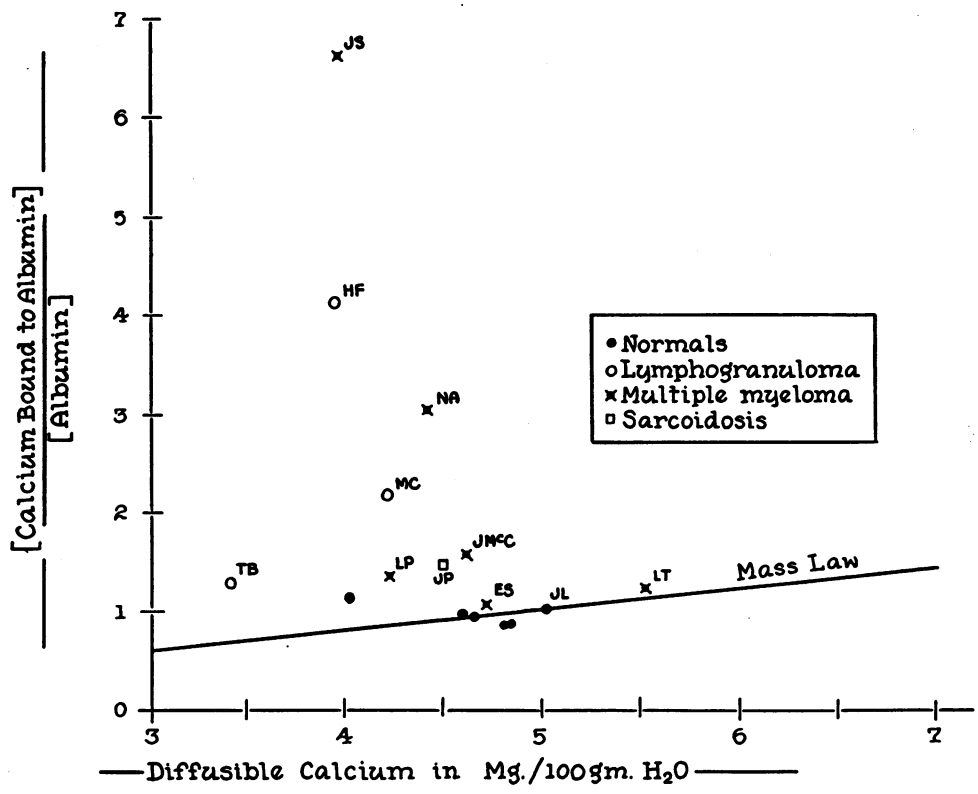

FIG. 2. EQUATION IV PLOTTED FOR ALBUMIN OF NORMALS AND OF CASES OF MULTIPLE MYELOMA, SARCOIDOSIS, AND LYMPHOGRANULOMA VENEREUM

sera, normal in 1, and below normal in 2. Variation might be expected on the basis of electrophoretic patterns (34 to 37) and of studies of the base-binding properties of globulin (16, 38, 39). In the cases characterized by a predominance of the alpha globulin fraction, the average calcium-binding property of globulin is elevated. Where the beta fraction predominates, the average calcium-binding property is normal. As surmised by Gutman and Gutman (16), gamma globulin appears to have a low calcium-combining power. The sera from patients with lymphogranuloma venereum, sarcoidosis, and tuberculosis have characteristically an increase in serum gamma globulin (29, 30, 32, 40, 41). In the 2 long-standing cases of lymphogranuloma venereum in our series, HF and MC, a marked decrease was apparent in the calcium-binding property of serum globulin. Values at the lower end of the normal range were found in the early cases of lymphogranuloma venereum and in the case of sarcoidosis. According to the data of West and Jefferson (42), the total calcium tends to decrease in tuberculosis, and it is likely that the relative increase in gamma globulin found in this disease may be the cause of this.

The considerable differences in the calcium-binding power of the alpha, beta, and gamma globulin fractions suggested by our data are in harmony with the findings of Drinker, Green, and Hastings (12). It should be noted in this connection, however, that the actual proteins in the various globulin fractions separated by electrophoresis, may differ in disease from those found in the normal, for as shown by Cohn (43), each fraction probably consists of a mixture of proteins of similar physical characteristics.

The mechanism of hypercalcemia in multiple myeloma, has long been a controversial point. Cantarow (44) pointed out that, although plausible, the hypothesis that hypercalcemia in multiple myeloma is dependent upon hyperproteinemia, is far from being well-established. The fact that there often was no correlation between the con-

centration of total serum calcium and total serum protein in this disease convinced some authors that the mechanism was not one primarily dependent upon the calcium-protein relationship (26, 27, 45). It has been shown by Gutman and Gutman (26) that in some cases the hypercalcemia is caused by an increase in the diffusible calcium fraction, presumably owing to breakdown of bone. Case LT in our series illustrates this condition. The other cases in the present series, however, indicate that in some instances, at least, the hypercalcemia is directly dependent upon alteration of the calcium-binding property of serum protein. The combination of increased binding property of the serum albumin and increased concentration of serum globulin having normal or elevated binding power, may lead to an increase in bound calcium. Hypercalcemia is not observed in such diseases as lymphogranuloma venereum presumably owing to the low calcium-binding power of the gamma globulin formed in this disease.

SUMMARY

The calcium-binding property of serum albumin and globulin obtained from normal subjects has been estimated. It was found that approximately the same amount of calcium is bound by both albumin and globulin, the average being 0.84 mgm. of calcium per gram of protein.

In diseases characterized by abnormality of the serum proteins, the calcium-binding property of albumin may increase, in some cases to as much as 6 times the normal value. The calcium-binding property of globulin in such diseases varies from the normal, and the direction of variation appears to depend upon the predominating electrophoretic globulin fraction present.

One or more of several mechanisms appear to be responsible for the hypercalcemia frequently observed in multiple myeloma. These include increased calcium-binding property of the serum albumin; increased concentration of serum globulin; and increased concentration of diffusible calcium.

Dr. Florence B. Seibert made the electrophoretic measurements which the authors gratefully acknowledge.

CLINICAL ABSTRACTS

1. JS (Lankenau Hospital No. A47636). Patient was a 56 year old married negro male who had bone pains, weakness and weight loss for 8 months, and recurrent epistaxis for 1 month. Physical examination was essentially unrevealing except for emaciation. X-ray films showed numerous areas of bone destruction in the skull, ribs, pelvis, and the lumbar vertebrae. A mass projected from a destroyed rib into the thoracic cage. Examination of the sternal marrow revealed typical myeloma cells. Urine contained Bence-Jones protein, and a 24-hour specimen contained 3.2 grams of albumin per liter. The erythrocyte count was 3.5 million per cmm. The leucocyte count was 14,100 per cmm., with neutrophils 72 per cent, and lymphocytes 28 per cent. Serum inorganic phosphorus was 5.0 mgm. per 100 ml. The blood clotted rather slowly, but once clotted formed a firm mass from which serum was not expressed.

2. LT (Pennsylvania Hospital No. 77922), a 67 year old white male admitted with a fracture of the right humerus. Patient had a plasmacytoma removed from the right nostril 8 years ago. Numerous recurrences were treated with further surgery and with irradiation. Two years ago he developed collapse of the fourth and fifth lumbar vertebrae, and 1 year ago he developed a fracture of the right clavicle. A course of stilbamidine was given. Temperature on admission was 102° F. There was crusting and pungent odor in the right nostril. There was a mass in the right clavicle, and deformity of the right humerus. X-rays revealed multiple lesions in the right humerus, radius and scapula, in the ribs, skull, and the left femur.

3. LP (U. of P. Hospital No. 4781183), a 71 year old colored man, who in August, 1946, noted a lump over the sternal region. There was slow enlargement of the lump with pain in the region. There was some cough and wheezy respiration. In December, 1946, patient developed swollen abdomen and edema of the legs. On physical examination there was some mottled pigmentation of the legs. A hard rounded walnut-sized tumor was present over the left fourth costochondral junction. Rhonchi were heard throughout the chest. There was a systolic murmur, but no cardiac enlargement. The liver was felt 2 fingers below the costal margin. Pitting edema of the legs was present. Biopsy of the sternal mass revealed plasma cell myeloma. X-ray studies revealed multiple bony defects in the skull, humeri, scapulae, clavicles, sternum, and thoracic cage. There seemed to be a mass behind the heart. Leucocyte count was 12,350 per cmm. with 20 per cent plasmocytes in the peripheral blood. Urine showed moderate albumin, and contained Bence-Jones protein. There was a progressively increasing anemia.

4. NA (U. of P. Hospital No. 4467961). Patient was a 55 year old white female who complained of low back pain, left knee pain, and increasing fatigue for 1 year. Physical examination was essentially negative. X-ray films revealed punched-out areas of bone destruction in the skull, ribs and long bones. Pathologic fractures were sustained above the left knee, and subsequently in the other femur and a humerus. Sternal marrow and rib biopsy both showed typical myeloma cells. Urea clearance was 47 per cent of normal. Urine never contained Bence-Jones protein, but showed 1+ to 2+ albumin.

Leucocyte cell count ranged from 2300 to 5500 cells per cmm. Erythrocyte count ranged from 2.4 to 2.9 million per cmm. Treatment with radioactive phosphorus, X-rays, and intravenous stilbamidine was attempted without noteworthy result.

5. JM (Temple U. Hospital No. 122318). Patient was a 52 year old white male who complained of weakness and tiredness for 1 year, following an attack of "pleurisy." There was a 50-pound weight loss. Physical examination revealed a large liver and spleen. X-ray films of the bones were negative. Chest X-ray films revealed some rounded soft densities, the nature of which was uncertain, in the right lung, as well as some pneumonitis. Examination of the sternal marrow showed typical myeloma cells. Urine contained no Bence-Jones protein. Leucocyte count ranged from 3400 to 5400 per cmm., neutrophils 66 to 73 per cent, lymphocytes 24 to 25 per cent, monocytes 3 to 8 per cent. Autopsy showed widespread myelomatosis.

6. ES (Temple U. Hospital No. 122883). Patient was a 58 year old white male who complained of weakness of the legs for 2 months, and inability to stand for 3 weeks. Physical examination suggested a tumor at the fourth thoracic vertebra with signs of pressure on the cord at this level. X-ray films showed complete destruction of the body of this vertebra with kyphosis. The tumor was removed and proved to be plasma cell myeloma. There was no evidence of other lesions. Urine contained no Bence-Jones protein.

7. HF (Philadelphia General Hospital No. 170882). Patient was a 48 year old negro female, who was found to have a rectal stricture 1 year before performance of the present studies. The lygranum test was moderately positive at the time the stricture was first noted. A colostomy was performed because of progressive narrowing of the rectal lumen.

8. MC (U. of P., O.P.D. No. 43176). Patient was a 31 year old married negro female, who gave a history of rectal stricture and bleeding of 3 years' duration. Physical examination was unrevealing except for the presence of a stricture 1 inch above rectal sphincter. The lygranum test, done shortly before the present studies, was strongly positive.

9. TB (U. of P., O.P.D. No. 42189). Patient was a 25 year old married negro male, who noted bilateral inguinal tenderness and swelling 3 weeks prior to the present studies. The inguinal lesions progressed to typical buboes. Aspiration of these revealed frank pus. Oral temperature was 100.0° F. A lygranum test was applied, but the patient did not return for follow-up.

10. JL (U. of P. Hospital No. 4680289). Patient was a 28 year old married negro male, who noted 3 shallow penile ulcers, and right inguinal tenderness. The latter progressed to a typical bubo, and yielded frank pus on aspiration. The lygranum test was positive. Two weeks after the onset of his illness, serum protein was 6.8 per cent, albumin 2.8, globulin 4.0 (by method of Howe). Sulfadiazine therapy was started at this time, and continued for 1 month at which time the present studies were performed. At this time, symptoms and signs of the disease had completely subsided.

11. JP (U. of P. Hospital No. 4677177). Patient was a 13 year old negro male, who over an 8-month period had had enlarged tender parotids, swelling of the upper lids, night sweats, dyspnea, tonsilitis, and cervical adenitis. X-ray films of the chest were reported as showing "numerous areas suggesting interstitial infiltration almost approaching nodulation." Tuberculin tests were consistently negative. Biopsy of a cervical lymph node showed tubercles composed of macrophages and multinucleated giant cells without caseation. The histologic appearance was considered to be typical of sarcoid.

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