4. Reissig, M., Howes, D. W., Melnick, J. L., *J. Exp. Med.*, 1956, v104, 289.

5. Lillie, R. D., *Histopathologic Technic and Practical Histochemistry*, The Blakiston Co., Inc., New York, 1954, 501.

6. von Bertalanffy, L., Masin, M., Masin, F., *Cancer*, 1958, v11, 873.

7. Armstrong, J. A., Niven, J. S. F., *Nature*, 1957, v180, 1335.

8. Melnick, J. L., in *Progress in Medical Virology*, S. Karger, 1958, v1, 59.

9. Drouhet, V., *Ann. Inst. Pasteur*, 1958, v95, 781.

10. Malherbe, H., Harwin, R., *Brit. J. Exp. Path.*, 1957, v38, 539.

11. Sabin, A. B., *Science*, 1959, v130, 1387.

Received January 3, 1961. P.S.E.B.M., 1961, v106.

Binding of Calcium to Bence-Jones Proteins.* (26431)

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Bence-Jones proteins appear in the urine of approximately half of the patients who have multiple myeloma. The fact that these abnormal proteins of small molecular weight are barely detectable in blood signifies that they are efficiently and rapidly transferred from plasma to urine, and in some cases several grams may be excreted per day. Because patients with multiple myeloma frequently have bone lesions, the calcium binding properties of their pathological proteins are of interest. Prior investigations have revealed no special affinity of serum myeloma globulins for calcium(1,2), but the calcium ion binding properties of urinary Bence-Jones proteins have not previously been reported.

Materials and methods. In the present study, Bence-Jones proteins were obtained from the urine of 7 patients† having multiple myeloma by precipitation in ⅔ saturated ammonium sulfate at 4°C. The precipitates were dialyzed exhaustively against distilled water, and the resulting solutions were filtered into flasks and lyophylized. One of the proteins, that from patient C. P., used in these investigations was further purified by column chromatography of a solution of the lyophylized powder on DEAE-SF‡. Just prior to measurements of calcium binding, the lyophylized proteins were dissolved in water, then electrodialyzed in a collodion cell for 24 to 48 hours against flowing distilled water. Standardized calcium hydroxide was then added to effect the desired pH, followed by addition of calcium chloride to reach the desired Ca^{++} concentration. Total calcium values were derived from the measured volumes added. Calcium ion activities were determined by potentiometric measurements using a permselective sulfonated polystyrene-collodion membrane as described by Carr(3). Protein concentrations were determined by weight after drying at 105°C.

Ultracentrifugal analysis of all of the proteins confirmed their classification as Bence-Jones proteins on the basis of sedimentation constants of approximately 3S. Double diffusion in agar gel plates gave precipitin bands in combination with rabbit anti gamma globulin. Electrophoresis by moving boundary and starch gel zone methods indicated varying degrees of heterogeneity with the exception of one homogeneous Bence-Jones protein, C.P., which also was eluted by increasing phosphate, decreasing pH gradient as a

* This investigation was supported in part by a grant from Am. Cancer Soc. and in part by a grant from Nat. Cancer Inst., N.I.H.

† Bone lesions observed by roentgenography: osteolytic lesions, osteoporosis, and vertebral compression in one patient; osteolytic lesions and vertebral compression in one patient; only osteolytic lesions in one patient; only osteoporosis in one patient; and no demonstrable abnormalities in 3 patients.

‡ DEAE-SF: diethylaminoethylcellulose.

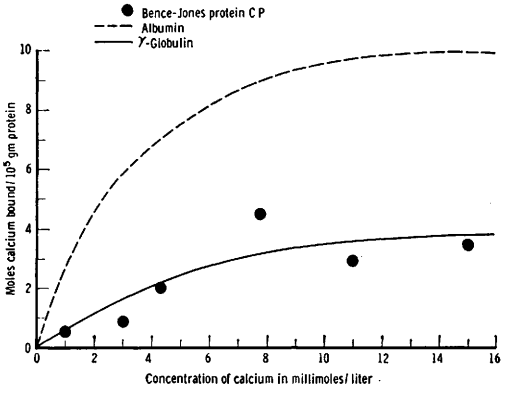

FIG. 1. Binding of calcium to Bence-Jones protein C.P. pH = 7.4. (The drawn curves represent previous results for blood proteins, conc. = 2%; the points are for Bence-Jones protein, conc. = 3.05%.)

homogeneous symmetrical peak from a DEAE-SF column.

Results. Experiments with calcium and Bence-Jones protein C.P. were carried out at pH 7.4 to test the effect of increasing concentrations of calcium ion. The results are summarized in Fig. 1. The solid line represents binding of calcium to gamma globulin previously reported by Carr(4), and the dots illustrate the data for Bence-Jones protein C.P. Binding of calcium with human serum albumin is indicated by the dashed line(4). The Bence-Jones protein, like gamma globulin, shows a comparatively low binding capacity for calcium at pH 7.4. Neither gamma globulins nor Bence-Jones proteins bind calcium to nearly the degree that serum albumin does. In another series of measurements on a different Bence-Jones protein (H.Z.), no detectable binding at pH 7.4 was found, even in a 3% protein solution at a calcium ion concentration of 15 mM/1.

The effect of pH on binding of calcium to the Bence-Jones protein C.P. is shown in Table I. The pH effect appears to be similar to that reported for other proteins(3,4). Calcium binding is higher at pH 10 and decreases progressively until it approaches zero at pH 6.0.

TABLE I. Binding of Calcium by Bence-Jones Protein C.P. at Several pH Values. Protein concentration = 1.69%.

pH	Added Ca	Measured Ca	Bound Ca	Bound Ca, moles/10^5 g protein
	mmoles/l			
10	4.64	3.23	1.41	8.34
8.9	4.64	3.38	1.26	7.50
7.9	4.64	3.71	.93	5.50
7.0	4.64	4.20	.44	1.63
6.0	4.64	4.62	.02	.12

Bence-Jones protein from patient H.Z. which was slightly heterogeneous electrophoretically possessed a very low calcium binding capacity. There was a slight though significant degree of binding at pH 8.8 (3.8 moles/10^5 g), but at pH 8 binding was only half as great, and at pH 7.4 there was no detectable binding. Three additional Bence-Jones proteins studied at pH 7.4 gave results similar to protein H.Z., whereas 2 additional Bence-Jones proteins indicated slight degrees of calcium binding similar to protein C.P. illustrated in Table I. There was no correlation between the severity of bone lesions in the patients studied and the extent to which calcium was bound by their Bence-Jones proteins.

Discussion. All of the data indicate that calcium is not very strongly bound by Bence-Jones proteins. The proteins with the highest affinities for calcium among those studied had binding capacities no greater than those reported for gamma globulin, a protein which has a low capacity for binding divalent cations. These proteins bind calcium to a much smaller extent than does serum albumin.

Carr and Woods(5) have shown that binding of magnesium to a variety of proteins was quantitatively similar in each case to binding of calcium, and further studies demonstrating competitive binding of these 2 ions to serum albumin indicated that binding sites on the protein common for both ions are involved(6). It is very probable that magnesium, as well as calcium, is only slightly bound by Bence-Jones proteins.

It is evident from these results that bone destruction frequently associated with plasma cell proliferation in patients who have multiple myeloma cannot be due to a direct chemical interaction of bone calcium with the Bence-Jones proteins which are believed to arise *de novo* in malignant plasma cells. The "punched out lesions" evident on roentgenographic examination of the bones of

these patients are probably due to lytic factors created by proximate proliferative activity of tumor tissue. It is possible that the generalized osteoporosis occurring in other patients may be due to these local factors operating in a more uniformly disseminated form of the disease, while the lack of bone lesions in still other patients with multiple myeloma may signify a less advanced stage of tumor infiltration.

Summary. Determinations of calcium ion activities by potentiometric measurements using permselective sulfonated polystyrene-collodion membranes indicate that calcium is not very strongly bound by Bence-Jones protein. It is concluded that bone destruction frequently associated with plasma cell proliferation in patients who have multiple myeloma cannot be due to a direct chemical interaction of bone calcium with the Bence-Jones proteins.

1. Gutman, A. B., Gutman, E. B., *J. Clin. Invest.*, 1937, v16, 903.

2. Prasad, A. S., *A.M.A. Arch. Int. Med.*, 1960, v105, 560.

3. Carr, C. W., in *Electrochemistry in Biology and Medicine,* edited by T. Shedlovsky, John Wiley and Sons, Inc., New York, 1955, 266.

4. ———, *Arch. Biochem. Biophys.*, 1953, v46, 424.

5. Carr, C. W., Woods, K. R., *ibid.*, 1955, v55, 1.

6. Carr, C. W., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 546.

Received January 3, 1961. P.S.E.B.M., 1961, v106.

Antibody Response in Hematologic Patients. (26432)

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Previous studies from this laboratory(1) demonstrated that antibody response to subcutaneous injection of phenolized tularemia vaccine in 92 of 105 splenectomized patients was similar to that observed in normal controls. Failure of the remaining 13 patients to exhibit antibodies was attributed to underlying disease rather than absence of spleen. As a correlative study, antibody response of patients with hematologic diseases and intact spleens was studied. Killed tularemia vaccine (Foshay) was employed because the incidence of naturally-occurring tularemia in this area is low, and the vaccine is not generally available. Thus it could be assumed that any response would be due to a primary antigenic stimulus.

Materials and methods. The subjects used in the study were selected at random from patients visiting the hematology clinics at University Hospital. "Normal" controls were selected from presumably healthy volunteers (medical students and employees). All received a single 0.5 ml subcutaneous injection of Foshay killed tularemia vaccine. Blood for serologic studies was obtained before immunization and 3-6 weeks after. Antibody titers were determined by the bacterial agglutination test described earlier(1).

Results. The response of 162 hematologic patients and 48 control subjects to a single injection of tularemia vaccine is presented in Table I. Antibody titers varying from 1:20 to 1:640 were detected in 45 of 48 (93.7%) controls. In contrast (Table I) only 67 of 162 (41.4%) patients with hematologic disorders showed agglutination titers varying from 1:10 to 1:640. The lowest incidence of antibody response was seen in chronic lymphatic leukemia where only 2 of 25 (8.0%) had titers of 1:10 and 1:640, respectively. Similarly only 5 of 20 (25.0%) lymphosarcoma patients showed titers varying from 1:10 to 1:320. A total of 15 of 37 (40.5%) Hodgkin's patients exhibited titers from 1:10 to 1:640. In the remaining smaller groups (Table I) antibodies were detected in 13 of 19 (68.4%) pernicious anemia and 5 of 12 (41.7%) chronic myeloid