THE NATURE OF BENCE-JONES PROTEINS

Chemical Similarities to Polypeptide Chains of Myeloma Globulins and Normal γ-Globulins

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The proteins excreted in the urine of patients suffering from multiple myeloma were originally studied by Dr. Henry Bence Jones in 1847 (1, 2) and thus are among the first proteins to have been described. Since that time, a number of efforts have been made to define the nature of Bence-Jones proteins and their relation to myeloma globulins (3). The extensive antigenic cross-reactivity (4–6) among Bence-Jones proteins, myeloma globulins, pathological macroglobulins, and normal γ-globulins would suggest that portions of their molecules are structurally similar or identical. On the other hand, Bence-Jones proteins have the apparently unique and characteristic property of precipitating in the temperature range 45°–60°C, of redispersing upon boiling, and of reprecipitating upon subsequent cooling. Other γ-globulins of higher molecular weight are coagulated irreversibly by heating, usually at temperatures above 60°C. Many speculations on the nature of Bence-Jones proteins have been advanced; none has accounted for their singular properties and for their chemical relation to other γ-globulins.

Recent demonstrations that γ-globulin molecules consist of multiple polypeptide chains linked by disulfide bonds and non-covalent interactions (7, 8) have prompted the hypothesis that Bence-Jones proteins are composed of certain of these polypeptide chains. This hypothesis has been strengthened by the finding (9) that, in certain instances, chains of reduced alkylated myeloma proteins and similarly treated Bence-Jones proteins from the same patient migrate identically upon starch gel electrophoresis.

Two types of chains have been isolated from dissociated γ-globulins. One type consists of chains with molecular weights in the vicinity of 20,000 (L or light chains). The other type consists of chains the molecular weights of which are less precisely determined, but appear to lie in the range of 50,000 to 100,000 (H or heavy chains) (8).

The present communication provides evidence that Bence-Jones proteins are composed of L chains which in some cases appear to be chemically identical to those of the myeloma protein of the same patient. The identity has been estab-

lished in one case by the criteria of starch gel electrophoresis, ion exchange chromatography, nephelometric studies of thermosolubility properties, amino acid analyses, and spectrofluorometric analyses of thermally induced molecular transitions. Related studies on L chains isolated from normal human 7S γ-globulin have shown that they behave as dimers in aqueous solution, having molecular weights in the same range as those of Bence-Jones proteins. Moreover, L chains of normal human γ-globulin have been found to have thermosolubility properties similar to those of Bence-Jones proteins.

Materials and Methods

Proteins.—Bence-Jones proteins were isolated either by dialyzing urine samples of patients with multiple myeloma against running water, or by half saturating the urine with $(NH_4)_2SO_4$ at pH 5.0 and storing at 4°C for 24 hours. Following these procedures the samples were dialyzed against distilled water and lyophilized. Some samples were purified further by zone electrophoresis in starch (10). Myeloma proteins and normal human γ-globulin were isolated from fresh human sera by zone electrophoresis in starch. Human 7S γ-globulin was also obtained as fraction II of Cohn from Lederle Laboratories (Pearl River, New York) Protein concentrations were determined by the modified Folin-Ciocalteu method (11), and also by measuring the optical density of solutions at 280 mμ.

Dissociation, Separation, and Isolation of L and H Chains.—The procedures of reduction and alkylation in the presence and absence of urea, of column chromatography in 6 M urea, and of starch gel electrophoresis in 8 M urea have been described previously (8). To prepare reduced alkylated myeloma protein for the chromatographic isolation of its constituent polypeptide chains the following modified procedure was used: 50 mg of the protein was dissolved in 10 ml of nitrogenated 8 M urea buffered to an apparent pH of 8.0 by addition of 1 M tris-(hydroxymethyl)-aminomethane. The solution was made 0.1 M in β-mercaptoethanol and was allowed to stand for 16 hours at room temperature under prepurified nitrogen. The solution was then made 0.3 M in iodoacetamide, and after standing for 10 minutes at room temperature it was exhaustively dialyzed against distilled water at 4°C and lyophilized. Bence-Jones proteins were treated in an identical fashion for chromatographic comparison. The same procedure was used for normal γ-globulin, except that the reduction was done in 0.15 M NaCl rather than in 8 M urea in order to obtain chains soluble in water (8).

Relevant modifications of the reduction and isolation procedure will be described in the text.

Amino Acid Analyses.—These were performed using ion exchange chromatography as described by Moore *et al.* (12) and Spackman *et al.* (13). In calculating the composition of the myeloma and Bence-Jones proteins, the values were corrected for the moisture and ash content of the lyophilized samples. Samples to be compared were hydrolyzed simultaneously for 30 hours with constant boiling HCl in evacuated sealed glass ampoules immersed in a refluxing bath of toluene at 110°C. Comparisons of the amino acid content of various samples were performed by referring all values to that of alanine normalized to equal one μmole.

Spectrofluorometry and Nephelometry.—The spectrofluorometric procedure has been described in detail elsewhere (14); only essential features are given here. Protein samples were dissolved in the appropriate buffers to a final concentration of 0.2 mg/ml, and the solutions were placed in a thermostatically controlled quartz cuvette. The cuvette contents were excited with light made monochromatic at 280 mμ by passing the output of a xenon lamp (Hanovia 901-C1) through a grating monochromator, (Bausch & Lomb, 33-86-40, slit widths 2 mm). The fluorescent light emitted from the sample at right angles to the incident beam was passed

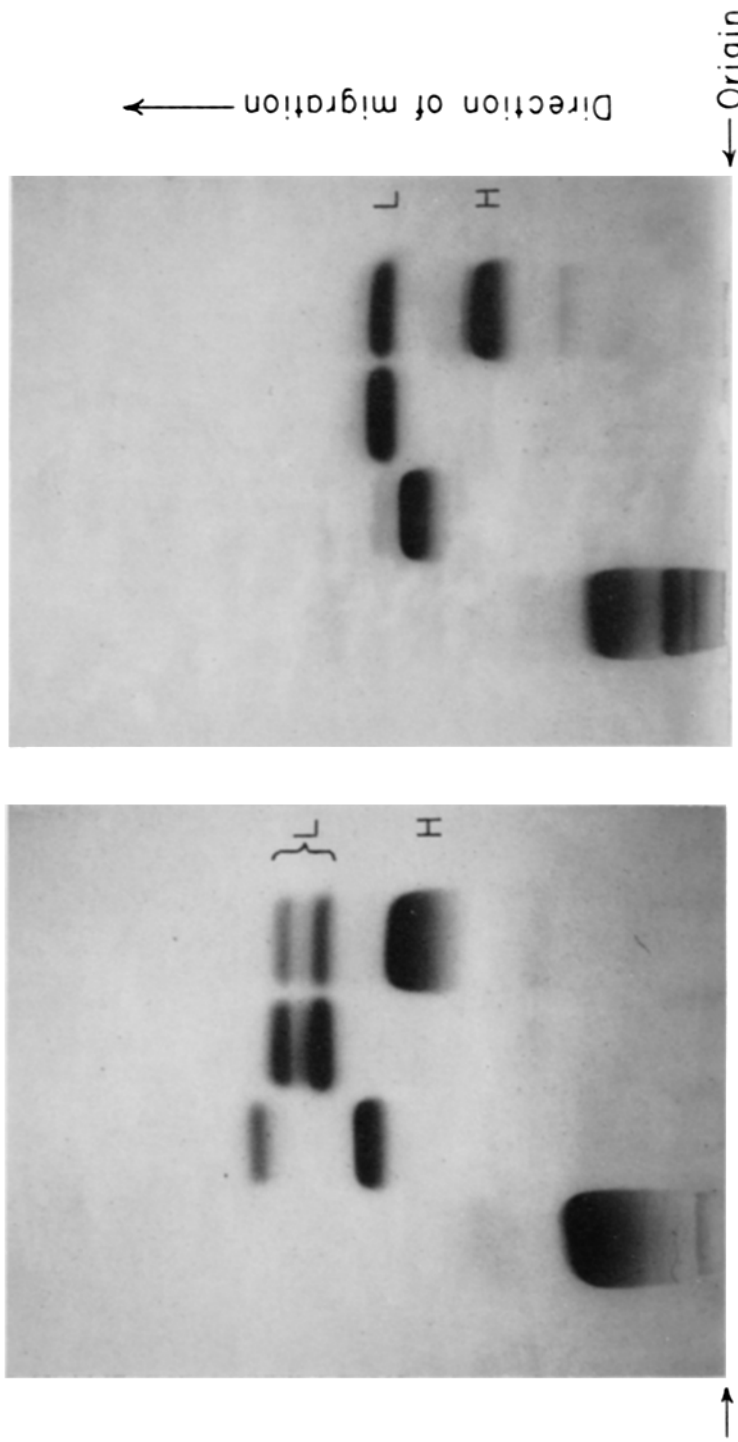

FIG. 1. Starch gel electrophoretic comparison of Bence-Jones proteins and myeloma proteins before and after reduction and alkylation in 8 M urea. (*a*) Patient Haw; (*b*) Patient S, (1) myeloma protein; (2) Bence-Jones protein; (3) Bence-Jones protein, reduced and alkylated; (4) myeloma protein, reduced and alkylated; L—light polypeptide chains, H—heavy polypeptide chains.

through another monochromator (Bausch & Lomb, 33-86-45-49; slit widths 5 mm), to a photomultiplier tube (RCA 1P28), and the resulting signal was amplified and recorded. Temperature measurements were made with a mercury thermometer immersed in the sample, and with a calibrated thermistor thermometer in the cuvette holder.

The temperature of the sample was raised about 1°C per minute while continuously recording the fluorescent signal at the wavelength of maximal fluorescence ($\lambda_{max\ 25°}$). This wavelength was previously determined at 25°C by measuring the emission spectrum of the sample. Wavelengths were measured with a precision of ±1 mμ. Emission spectra were also obtained at temperatures above the transition temperature (T.T.) defined as the temperature at which the increase in fluorescence at $\lambda_{max\ 25°}$ with heating is half-maximal. In certain cases, excitation spectra also were measured above and below the transition temperature. Although the transition temperatures were not measured at thermal equilibrium, they were reproducible to within ±1°C. Moreover, a comparison of transition temperature measurements made on one Bence-Jones protein at thermal equilibrium and made using the procedure outlined above were in agreement.

To obtain qualitative comparisons of the aggregative behavior of the proteins studied, the automatic recording spectrofluorometer was employed as a 90° nephelometer. The mercury line at 546.1 mμ emitted by a mercury-xenon lamp (Hanovia 901-B1) was isolated by the exciting monochromator, and the light scattered by the sample at 90° to the incident beam was passed through the analyzing monochromator set at the same wavelength. By heating the sample at 1°C/minute, the intensity of scattered light was measured as a function of temperature. In view of the complex nature of the scattering phenomena, no effort was made to measure the scattering in reference to a standard. The continuously recorded signal did provide a more detailed picture of changes in aggregation and redispersion than could be obtained by visual inspection, however

Ultracentrifugation.—This was performed in a Spinco model E ultracentrifuge equipped with automatic temperature control and with schlieren and interference optics. Molecular weights were measured by means of equilibrium centrifugation in short columns (15).

RESULTS

Chemical Comparisons of Bence-Jones Proteins and the Chains of Myeloma Proteins.—In Fig. 1 are shown the starch gel electrophoretic patterns of two myeloma proteins from different individuals and of the corresponding Bence-Jones proteins from the same patients. Before dissociation by reduction and alkylation the myeloma protein showed only moderate penetration into the gel. The Bence-Jones proteins penetrated deeply into the gel, (Fig. 1*a* and 1*b*, origins 2). These differences in penetration are consistent with the fact that myeloma proteins have molecular weights of 160,000, whereas the median molecular weight of Bence-Jones proteins is in the vicinity of 44,000 (3).

After reduction and alkylation, the myeloma proteins dissociated into H and L chains, the positions of which are indicated in the photographs (Figs. 1*a* and 1*b*, origin 4). Identically treated Bence-Jones proteins showed exact correspondence of their bands with those of the L chains of the myeloma protein from the corresponding patient. The previously noted individuality of the patterns of different myeloma proteins (8, 9, 16) and Bence-Jones proteins is reflected in the two additional cases examined here.

Of eight sets of myeloma proteins and Bence-Jones proteins obtained from different patients, seven sets showed correspondences of at least one band when examined in this way. Six sets showed complete correspondence of the bands of the reduced alkylated Bence-Jones proteins to those of the L chains of the myeloma protein. In the seventh case, the dissociated myeloma protein showed a band with no counterpart in the Bence-Jones protein, although the remaining

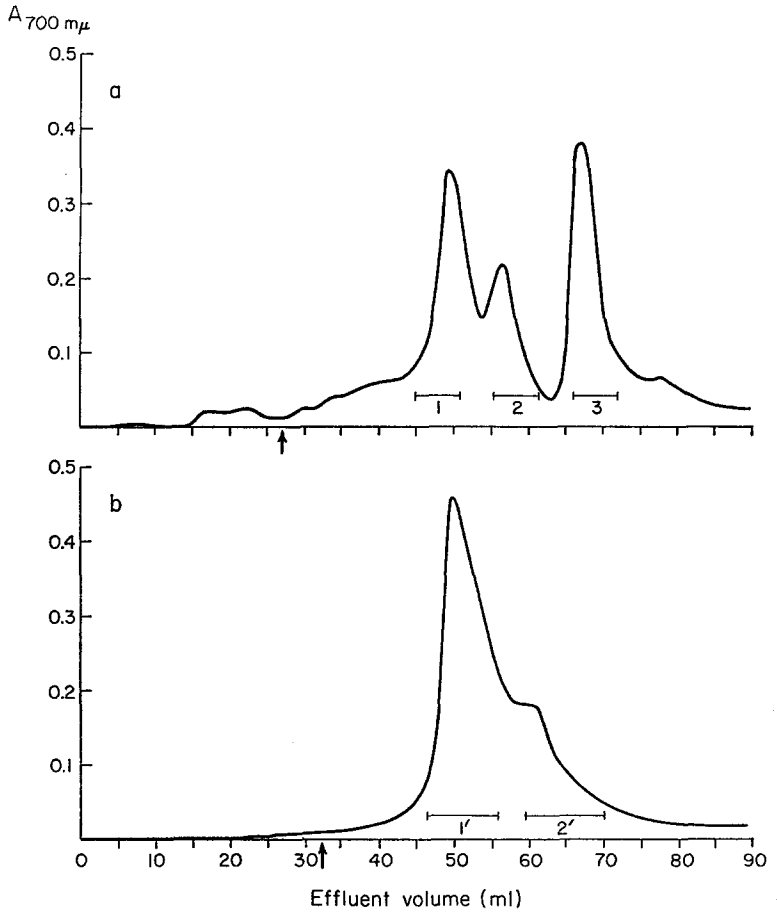

FIG. 2. Chromatography on carboxymethylcellulose of reduced alkylated myeloma protein and Bence-Jones protein (patient Haw).

(*a*) Reduced alkylated myeloma protein. Column dimensions: 25 × 0.9 cm; gradient: 0.01 M to 1.0 M sodium acetate buffer in 6 M urea, apparent pH 5.9; mixing chamber volume: 550 ml; column load: 50 mg protein, recovery 67 per cent.

(*b*) Reduced alkylated Bence-Jones protein under same conditions. Column load: 35 mg; recovery 60 per cent; ↑ —start of gradient; 1, 2, 3; 1′, 2′—fractions taken for further analysis; $A_{700\ m\mu}$—optical density of Folin reaction at 700 mμ.

bands did correspond. In the eighth case, there was no correspondence in the mobilities of the bands of the dissociated myeloma protein and Bence-Jones protein.

The reduced alkylated myeloma protein and Bence-Jones protein from patient Haw (Fig. 1*a*) were chromatographed separately on carboxymethylcellulose in 6 M urea under similar conditions, (Fig. 2). The derivatives of the Bence-Jones protein emerged in a position identical to that of the first peak of the chromatogram of the myeloma protein. Starch gel electrophoresis of these fractions corroborated this chromatographic correspondence (Fig. 3). The two bands corresponding to L chains seen in Fig. 1 were present but were not completely resolved for this material, which had been reduced and alkylated under different conditions. (See Materials and Methods.) Fraction 1 obtained from the chromatogram of the treated myeloma protein (Fig. 2) and fraction 1′ of the chromatographed Bence-Jones protein migrated identically in the starch gel (Fig. 3) and appeared to represent the slower of the bands corresponding to L chains. Fractions 2 and 2′ appeared to contain both types of L chains. Fraction 3 of the reduced alkylated myeloma protein consisted of H chains, (Fig. 3).

Amino acid analyses were performed on the myeloma protein and the Bence-Jones protein of patient Haw and also on the chains isolated by chromatography. The compositions of the myeloma protein and Bence-Jones protein are given in Table I. Distinct differences are apparent in the amino acid content of the two proteins.

In Table II the amino acid analyses of the myeloma protein, the Bence-Jones protein, and the isolated chains of the myeloma protein are compared. To afford an accurate comparison of the ratios of the amino acids stable to acid hydrolysis, the values are all referred to that of alanine normalized to 1 μmole. The reduced alkylated Bence-Jones protein, the chromatographic fraction 1′ of the Bence-Jones proteins, and the chromatographic fraction 1 of the myeloma protein all had compositions that were identical within the error of the method. As expected, the analysis of the myeloma protein yielded values intermediate between those obtained for the constituent L and H chains.

After the reduction and alkylation procedure, no half-cystine was found for either the Bence-Jones protein or the myeloma protein, thus indicating that reduction and alkylation were complete (Table II). The half-cystine and S-carboxymethylcysteine contents of the two proteins are summarized in Table III.

Isolation of L Chains of Normal Human 7S γ-Globulin.—The L chains ot normal 7S γ-globulin were isolated for the purpose of comparing their properties with those of Bence-Jones proteins. In order to obtain the L chains in soluble form, the γ-globulin was reduced in the absence of urea (8). The products of partial reduction were dissociated and chromatographically isolated in 6 M urea (Fig. 4). Verification of the separation was obtained by means of starch gel electrophoresis of the material from each peak. These patterns indicated that

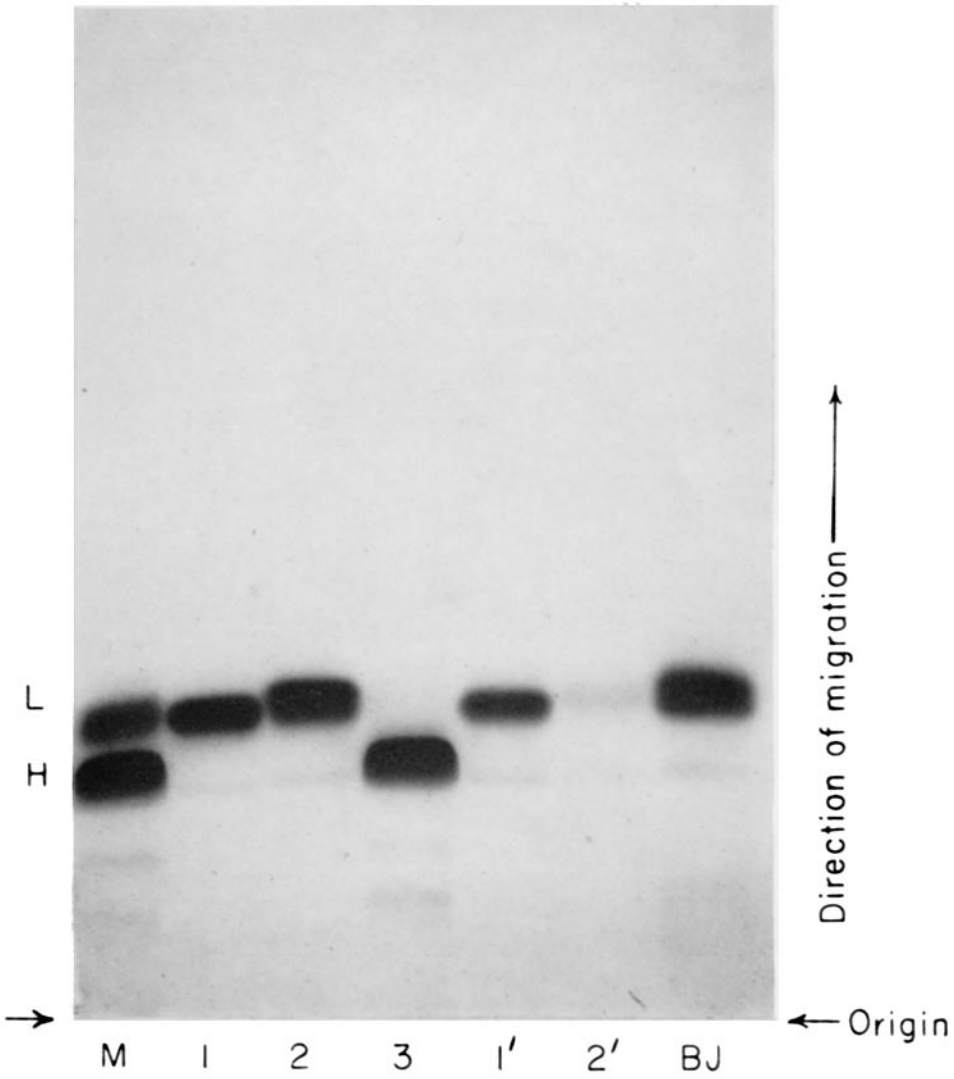

FIG. 3. Starch gel electrophoretic comparison of chromatographically isolated fractions of fully reduced alkylated Bence-Jones protein and myeloma protein (patient Haw). M—reduced alkylated myeloma protein; BJ—reduced alkylated Bence-Jones protein; 1, 2, 3—successive fractions of chromatographed dissociated myeloma protein (see Fig. 2*a*); 1′, 2′—successive fractions of chromatographed dissociated Bence-Jones protein (see Fig. 2*b*). (Fraction 2′ was applied to the origin in low concentration. The sample of Bence-Jones protein contained small amounts of myeloma protein as evidenced by the lightly staining slow bands.)

the L chains were isolated free of H chains in the first peak. The second peak contained the H chains but also contained a small amount of L chains and of material that appeared to correspond to unreduced γ-globulin.

A sedimentation velocity analysis of the isolated L chains in 0.02 M sodium phosphate buffer, pH 7.0, made 0.2 M in KCl, showed a single peak with an s_{20} of 3.8 (Fig. 5). In the same buffer, approximately 84 per cent of this material had a molecular weight of 41,000 ± 1500. The remainder had a molecular weight of 200,000. In 6 M urea made 0.2 M in KCl, 60 per cent of the L chain preparation

TABLE I

*Amino Acid Composition of a Myeloma Protein and Bence-Jones Protein from the same Patient**

Amino acid‡	Myeloma protein	Bence-Jones protein
Lysine	7.77	6.75
Histidine	2.87	1.70
Arginine	4.55	4.86
Aspartic acid	8.82	8.82
Threonine	7.71	8.24
Serine	9.65	10.83
Glutamic acid	11.98	14.86
Proline	6.72	5.14
Glycine	4.47	4.37
Alanine	3.84	4.34
Half-cystine	2.27	2.34
Valine	8.91	6.70
Methionine	1.09	0.74
Isoleucine	2.79	3.75
Leucine	8.08	7.78
Tyrosine	5.69	6.79
Phenylalanine	4.15	5.14

* Patient Haw.

‡ All values expressed as grams amino acid per 100 gm protein, calculated from the results of a single analysis of each sample.

had a molecular weight of 20,000 ± 1,000, suggesting that dissociation of a dimer had occurred in this solvent. The remainder of this material had molecular weights in the range of 40,000 to 60,000. In the absence of urea, the molecular weight of the L chains is comparable to those of most Bence-Jones proteins (3). The dissociation by 6 M urea is similar to that observed by Heimer (17) for a Bence-Jones protein in 4 M urea.

Thermosolubility Properties of Bence-Jones Proteins, L chains of Myeloma Proteins, and L Chains of Normal γ-Globulin.—The L chains of completely reduced alkylated myeloma proteins or of completely reduced alkylated normal 7S γ-globulin were insoluble in aqueous buffers at room temperature. Suspensions of these materials dissolved completely upon boiling, and reprecipitated upon cool-

ing to temperatures below 60°C. Similar behavior was observed for Bence-Jones proteins reduced and alkylated either in 0.15 N NaCl or in 8 M urea.

The soluble L chains from partially reduced myeloma protein (patient Haw) had thermosolubility properties similar to those of the Bence-Jones protein from the same patient. Dissolved to a concentration of 0.5 mg/ml in sodium phos-

TABLE II

Relative Amino Acid Content of a Myeloma Protein Compared to That of Its L and H Polypeptide Chains, and to That of the Bence-Jones Protein from the Same Patient

Amino acid*	Myeloma protein (Haw)	Reduced alkylated myeloma protein	Bence-Jones protein (Haw)	Reduced alkylated Bence-Jones protein	Reduced alkylated Bence-Jones protein fraction 1'‡	Reduced alkylated myeloma protein fraction 1§	Reduced alkylated myeloma protein fraction 3§
Lysine	1.23	1.20	0.95	0.91	0.96	0.93	1.30
Histidine	0.32	0.28	0.17	0.14	0.15	0.16	0.38
Arginine	0.61	0.59	0.57	0.57	0.58	0.57	0.61
Aspartic Acid	1.54	1.54	1.36	1.39	1.40	1.39	1.58
S-carboxymethylcysteine	0.00	0.42	0.00	0.40	0.31	0.37	0.44
Threonine	1.50	1.52	1.42	1.43	1.46	1.43	1.52
Serine	2.13	2.14	2.12	2.10	2.24	2.20	2.07
Glutamic Acid	1.88	1.88	2.07	2.02	2.10	2.06	1.78
Proline	1.35	1.37	0.92	0.91	0.95	0.92	1.60
Glycine	1.38	1.39	1.20	1.19	1.22	1.17	1.50
Alanine	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Half-cystine	0.44	0.00	0.40	0.00	0.08	0.00	0.00
Valine	1.77	1.80	1.18	1.19	1.20	1.20	2.04
Methionine	0.17	0.16	0.10	trace	0.11	0.10	0.21
Isoleucine	0.49	0.50	0.59	0.59	0.60	0.58	0.45
Leucine	1.47	1.44	1.22	1.23	1.25	1.21	1.53
Tyrosine	0.73	0.74	0.77	0.76	0.77	0.79	0.71
Phenylalanine	0.58	0.59	0.64	0.65	0.66	0.64	0.56

* Values listed are moles of amino acid per mole of alanine in the sample.

‡ Fraction obtained from chromatographic separation, see Fig. 2*b*.

§ Fractions obtained from chromatographic separation, see Fig. 2*a*. Fraction 1 consisted of L chains. Fraction 3 consisted of H chains (Fig. 3).

phate buffer, pH 7.0, $\Gamma/2 = 0.2$, both proteins showed the development of turbidity at 58°–60°C, which cleared upon boiling and reappeared upon cooling.

The L chains of partially reduced human 7S γ-globulin were soluble in aqueous buffers at room temperature. In sodium acetate buffers, pH 5.0, $\Gamma/2 = 0.2$, and at a protein concentration of 0.5 mg/ml, solutions of normal L chains showed visible clouding at 50°C, with clearing upon boiling, and reprecipitation upon cooling. Material from the second peak of the chromatogram shown in Fig. 4 flocculated irreversibly at 70°C in the same buffer.

A nephelometric comparison (Fig. 6) illustrates in more detail the behavior

described above. Human 7S γ-globulin, its partially reduced alkylated derivative, and materials from the first and second peak of the chromatogram in Fig. 4 were compared with a Bence-Jones protein. Both the Bence-Jones protein and the L chains of the human γ-globulin showed increasing light scattering as the temperature was raised in the region of 50°–60°C. Above 60°C the scattering diminished and reached the original values at 90°C. The scattering increased again after cooling. All of the other preparations began to scatter at 65°C and undewent irreversible flocculation between 75° and 85°C.

Spectrofluorometric Comparisons of Bence-Jones Proteins and L Chains.—The thermosolubility properties of Bence-Jones proteins suggest the possibility that changes in their molecular conformation occur between 45° and 60°C. Spectrofluorometry has been found to provide a sensitive measure of the conformational changes of ribonuclease brought about by heating (14). Spectrofluorometric

TABLE III

*Comparison of the Half-Cystine Content of a Myeloma Protein with That of a Bence-Jones Protein from the Same Patient**

	Myeloma protein	Reduced alkylated myeloma protein	Bence-Jones protein	Reduced alkylated Bence-Jones protein
Moles half-cystine per 100,000 gm sample...	18.9	—	19.5	—
Moles S-carboxymethylcysteine per 100,000 gm sample.....	—	17.7	—	18.3

* Patient Haw.

techniques therefore were used to study Bence-Jones proteins, the L chains of a myeloma protein, and L chains of normal human γ-globulin.

Changes in the fluorescence intensity and shifts in the wavelengths of maximal emission were found upon heating these proteins. In Fig. 7 are shown the fluorescence emission spectra of a Bence-Jones protein measured at 25°C and at a temperature above the transition temperature. The $\lambda_{max,\ 25°}$ was 337 mμ; at all temperatures above the transition temperature the wavelength of maximal emission shifted to 340 mμ. Upon cooling to 25°C, the wavelength of maximal emission returned to 337 mμ and thus the shift was reversible.

A typical curve obtained by recording the fluorescence at $\lambda_{max,\ 25°}$ as a function of temperature is given in Fig. 8. As the temperature was raised the fluorescence intensity declined. At 52°C a sharp increase in the intensity was noted, and above 60°C the intensity again declined. The transition temperature was 56°C. In general, the shapes of these transition curves were similar for different Bence-Jones proteins, the ascending slope extending over a range of 10°C.

The transition temperatures and λ_{max} values determined for seven Bence-

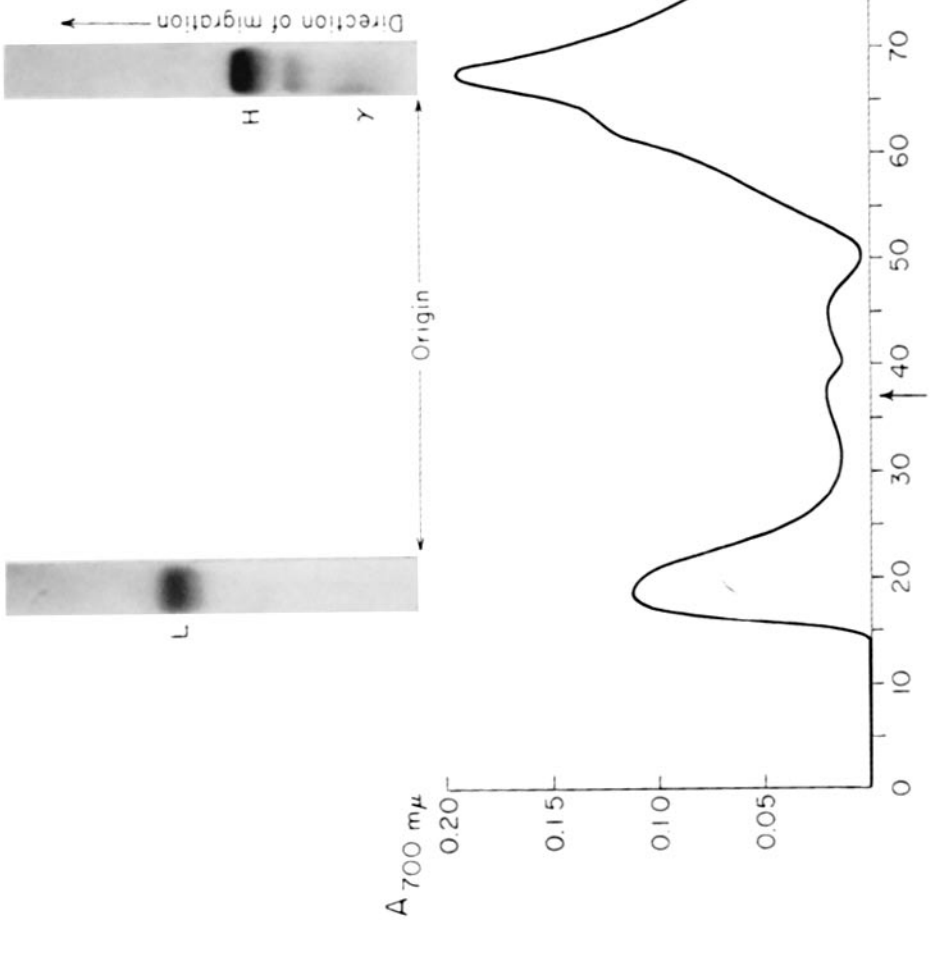

FIG. 4. Chromatography on carboxymethylcellulose of partially reduced and alkylated human fraction II γ-globulin. Column dimensions: 35 × 0.9 cm; gradient: 0.01 M to 1.0 M sodium acetate buffer in 6 M urea, apparent pH 6.0; mixing chamber volume: 550 ml; ↑—start of gradient; $A_{700\ m\mu}$—optical density of Folin reaction at 700 mμ. The starch gel electrophoretic patterns of the separated components are shown above the peaks of the chromatogram to which they correspond. L — light polypeptide chains; H — heavy polypeptide chains; γ — apparently undissociated γ-globulin.

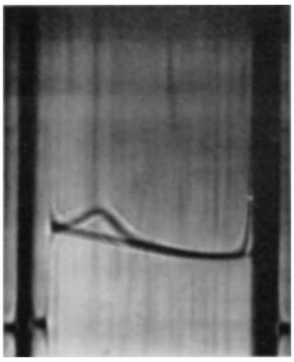

FIG. 5. Ultracentrifugal pattern of L chains of normal human 7 S γ-globulin. Solvent: 0.02 M sodium phosphate buffer, pH 7.0, + 0.2 M KCl; speed: 50,740 RPM; time of photograph: 96 minutes; phase plate angle: 40°.

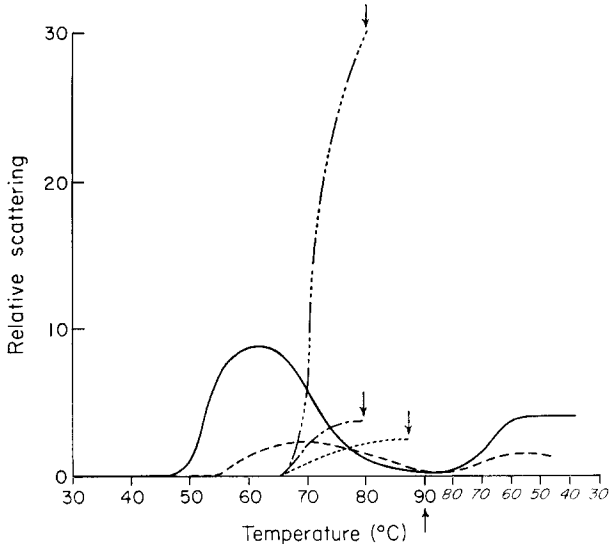

FIG. 6. Nephelometric comparison of the thermosolubility properties of a Bence-Jones protein, normal human 7 S γ-globulin, and the polypeptide chains of normal human γ-globulin. —— Bence-Jones protein (patient P); ----- L chains of normal human 7 S γ-globulin; ···· H chain fraction of normal human 7 S γ-globulin; —···— normal human 7 S γ-globulin; —·— partially reduced alkylated normal human 7 S γ-globulin; ↓—Irreversible flocculation; ↑—Cessation of heating at rate of 1°C/minute. Samples were allowed to cool at a much slower rate.

Jones proteins are collected in Table IV. All of the samples were heated in phosphate buffer, pH 7.0, $\Gamma/2 = 0.2$; under these conditions the samples used did not become turbid upon heating to temperatures above the transition temperature. Sample P also was examined at different ionic strengths. Changing the

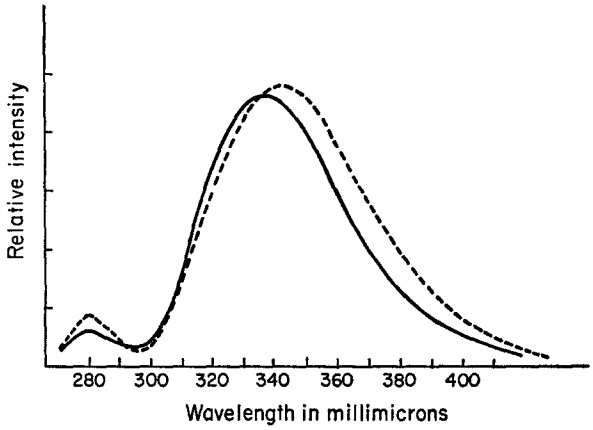

FIG. 7. Fluorescence emission spectra of Bence-Jones protein P. —— emission spectrum measured at 25°C; —— emission spectrum measured at 60°C; transition temperature of sample: 55°C; exciting wavelength: 280 mμ. The small peak at this wavelength represents scattered exciting light. Spectra are uncorrected for spectral dependence of the monochromator and detector system. Solvent: phosphate buffer, pH 7.0, ionic strength 0.2.

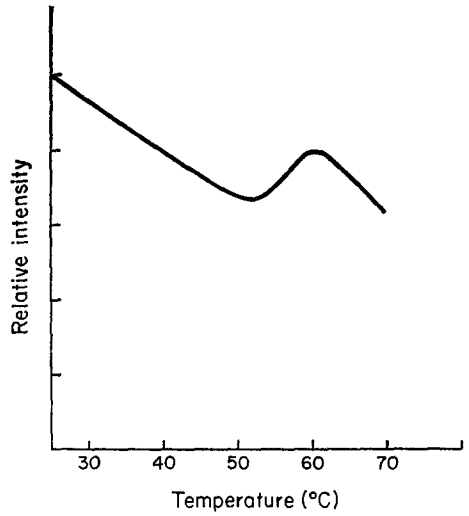

FIG. 8. Dependence on temperature of the fluorescence intensity of Bence-Jones protein Z. Transition temperature: 56°C; exciting wavelength: 280 mμ. Intensity was measured at the wavelength of the maximum of the emission spectrum at 25°C. (343 mμ). Solvent: phosphate buffer, pH 7.0, ionic strength 0.2.

ionic strength of the phosphate buffer at pH 7.0 from 0.05 to 0.2 did not affect the transition temperature which was 55°C (Table IV). In distilled water, however, the transition temperature rose to 58°C and aggregation occurred, as indicated by an increase in the peak of scattered exciting light and by the observa-

tion of visible turbidity. Sample T which showed a marked shift in the wavelength of maximal emission was also examined above and below its transition temperature for changes in the excitation spectrum. There was no detectable shift in the wavelength of maximal excitation.

Each Bence-Jones protein had a unique combination of transition temperature and shift in the wavelength of maximal emission. In the case of samples Har and R, the wavelength shifts were only slowly reversible, occurring over

TABLE IV

Transition Temperatures and Shifts of the Wavelengths of Maximal Emission of Bence-Jones Proteins and L Chains

Protein* samples	Transition temperature	$\lambda_{max, 25°}$ ‡	$\lambda_{max, T.T.}$ §	Reversibility of transition
	°C	λmμ	λmμ	
B	63	335	340	Reversible
Haw	60	345	345	
L chains (Haw myeloma)	59	345	345	
Har	58	331	335	Slowly reversible
Z	56	343	339	Reversible
R	56	330	339	Slowly reversible
P	55	336	340	Reversible
L chains (normal γ-globulin)	51	335	340	Reversible
T	50	344	335	Irreversible

No corrections were made for the spectral dependence of the monochromator and detector system.

* Dissolved in sodium phosphate buffer pH 7.0, $\Gamma/2 = 0.2$.

‡ $\lambda_{max, 25°}$—wavelength in mμ of maximum of the fluorescence emission spectrum measured at 25°C.

§ $\lambda_{max, T.T.}$—wavelength in mμ of maximum of the fluorescence emission spectrum measured at temperatures above the transition temperature.

several hours after cooling to room temperature. The transition temperatures of the L chains and the Bence-Jones protein of patient Haw agreed within 1°C.

The L chains of human γ-globulin had a transition temperature of 51°C at pH 7.0 and also showed a reversible wavelength shift (Table IV). Partially purified H chains and partially reduced alkylated normal γ-globulin showed no evidence of a fluorescence transition until heated to 70°C, at which point both the fluorescence and the scattering increased. It was not possible to decide whether the increase in fluorescence in these instances resulted from a molecular transition or merely from a change in light path or re-emission resulting from the presence of large aggregates.

The closely similar behavior of L chains of human γ-globulin and of Bence-Jones proteins was emphasized in a study of the pH dependence of the transition

temperature (Fig. 9). Above pH 10 and below pH 5 the transition temperature declined, and at pH 2 the transition was complete even at room temperature. In addition, the change in intensity of fluorescence resulting from the transition was smaller at acid pH values. In all instances the transition temperature values went through shallow maxima around pH 6 and pH 9. At pH 5 all of the sam-

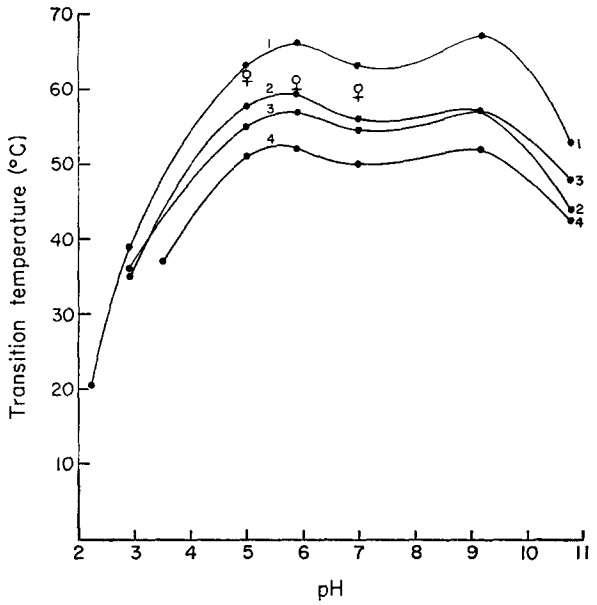

FIG. 9. Dependence on pH of the transition temperatures of Bence-Jones proteins and L chains. 1—Bence-Jones protein, patient B; 2—Bence-Jones protein, patient Z; 3— Bence-Jones protein, patient P; 4—L chains of normal human 7 S γ-globulin; O—Bence-Jones protein, patient Haw; +—L chains of myeloma protein, patient Haw.

Buffers: pH 2.2, pH 2.9 } glycine HCl
pH 3.5—sodium formate
pH 5.0, pH 5.9, pH 7.0 } sodium phosphate
pH 9.2, pH 10.8 } glycine—NaOH

The ionic strength of all buffers was 0.2.

ples showed an increase in the intensity of the scattered exciting light (280 mμ) when the temperature was raised above the transition temperature. This appeared to be the result of aggregation, as verified in most cases by visible clouding of the solutions.

The comparison of the transition temperatures of Bence-Jones protein (Haw) and the L chains of the myeloma protein from this patient is given in Fig. 9 for three different pH values. The transition temperatures agreed within 1°C. Ex-

amination of these proteins at other pH values was made difficult by the fact that the slope of the transition was shallow, and therefore a precise determination of the transition temperature was not possible.

DISCUSSION

The structural studies described here suggest that Bence-Jones proteins are composed of polypeptide chains of the L type. In one instance, a Bence-Jones protein and the L chains of the myeloma protein from the same patient were studied using a variety of methods. No differences could be found in their starch gel electrophoretic, and chromatographic behavior, in their amino acid composition, and in their thermosolubility properties. Investigation of the thermosolubility properties and spectrofluorometric behavior of L chains derived from normal γ-globulin as well as from myeloma globulin indicated that their behavior resembled that of Bence-Jones proteins. These findings suggest that reversible temperature dependent structural changes are a general property of the L chains of both normal and pathological human γ-globulins.

Some Bence-Jones proteins appear to be composed of L chains that are the same as those present in the myeloma proteins of the same patient. Although this has been shown by extensive criteria in only one instance, it has been strongly suggested in other instances by correspondences in the starch gel electrophoretic patterns of L chains and Bence-Jones proteins. The starch gel electrophoretic patterns of the different myeloma proteins and Bence-Jones proteins so far examined (8, 9, 16) have been individual and unique. It is unlikely, therefore, that the correspondences described above are adventitious.

In the case of the abnormal proteins obtained from patient Haw, the amino acid analyses of chromatographically isolated L chains and Bence-Jones proteins were similar or identical. Until exhaustive studies are done on the amino acid sequence of L chains and Bence-Jones proteins in a similar instance, their complete identity cannot be considered proven. Consideration of the physicochemical similarities of these proteins, however, would favor the notion that they are identical.

In addition to providing evidence on the identity of L chains and Bence-Jones protein, the amino acid analyses have yielded information on the number of disulfide bonds in the myeloma protein and its chains. Assuming that its molecular weight is 160,000, the whole myeloma protein would contain 15 disulfide bonds (Table III). From the data in Table I and Table II a minimal molecular weight of 20,000 was calculated for the Bence-Jones protein and the L chains of the myeloma protein. On the basis of this value, which is in good agreement with the ultracentrifugal value obtained for the L chains of normal γ-globulin, the units of the Bence-Jones protein and the L chains of the myeloma protein, each contained four half-cystine residues.

The exact number of polypeptide chains in individual myeloma proteins is

not known. The data on the amino acid content of the myeloma protein (Haw) and its chains appear to be consistent with a model comprised of two L chains of molecular weight 20,000 and two H chains of molecular weight 55,000 to 70,000. Agreement also was obtained assuming a model consisting of three L chains of molecular weight 20,000 and two H chains of molecular weight 60,000. Even in this instance a complete balance of amino acid residues is not achieved, and additional evidence is required before this problem is resolved.

One case has been found in which there was no starch gel electrophoretic correspondence between the L chains of the myeloma protein and the Bence-Jones protein. Immunologic studies have indicated that the Bence-Jones protein possessed antigenic determinants not present in the myeloma protein. In contrast, in those cases where correspondence was found in the starch gel, the myeloma protein appeared to contain all of the antigenic determinants possessed by the Bence-Jones protein (18). Extension of these immunologic and structural correlations to other examples, and to an analysis of various types of Bence-Jones proteins and β-myeloma globulins is in progress.

Comparisons of the starch gel electrophoretic patterns of dissociated normal γ-globulins and myeloma proteins have emphasized their generic similarity in structure as well as their differences in chemical homogeneity (8, 9). The heterogeneity of normal γ-globulins appears to be related mainly to the heterogeneity of L chains on different molecules, as indicated by the diffuse spreading of the zones corresponding to L chains. Dissociated myeloma proteins yield different patterns containing sharp bands corresponding to L chains, with mobilities comparable to those of the L chains of normal γ-globulin. The physicochemical studies reported here extend and emphasize the similarities between the properties of L chains of myeloma proteins and normal γ-globulins.

The spectrofluorometric techniques employed in these studies allow a detailed comparison of certain physical properties of Bence-Jones proteins and their congeners. Using these methods it has been found that Bence-Jones proteins and L chains undergo characteristic molecular transitions when heated. These conformational changes may be studied under a wider variety of conditions than are possible with analysis of precipitation alone, since thermally induced precipitation of Bence-Jones proteins occurs only within a narrow range of pH (19).

Previous studies have shown that the quantum yield of fluorescence (14) of free tryptophan and of tryptophanyl residues in proteins decreases with increasing temperature. The two descending portions of the curve shown in Fig. 8 are probably due to a temperature-dependent quenching process of the type found for free tryptophan. The sharply ascending portion appears to result from a change in molecular structure influencing the environment of some or all of the tryptophanyl residues. Similar spectrofluorometric changes have been found in studying the effect of temperature on the tyrosine fluorescence of ribonuclease,

and these changes correlated well with the molecular transition known to occur in that protein (14). The shifts in the wavelength of maximal tryptophan emission of heated Bence-Jones proteins resemble those that occur when proteins are treated with various denaturing agents (20). It is possible that these shifts result from alteration of the vibrational levels and electronic configuration of tryptophan following changes in the hydrophobic interactions of this amino acid.

The nature of the conformational changes brought about by heating Bence-Jones proteins and L chains is unknown. The ultracentrifugal studies and amino acid analyses are compatible with the notion that most Bence-Jones proteins and isolated L chains are dimers in aqueous solutions at temperatures below the transition temperature. Unfolding and dissociation of these dimers may be responsible for the thermally induced changes in the fluorescence of these proteins (14). Similarly, the decrease of the transition temperature at acid pH may be related to the reported dissociation of Bence-Jones proteins in this pH region (21). It is also possible, however, that changes in tertiary structure alone are responsible for the changes in fluorescence. Whatever the process, net charge plays an important role, as indicated by the lowering of the transition temperature at extremes of pH. The studies of Putnam *et al.* (19) on the thermal aggregation of Bence-Jones proteins under various conditions also emphasize the importance of electrostatic interactions in these phenomena.

Bence-Jones proteins from different patients probably differ in their tertiary structure, since they differ both in transition temperatures and in λ_{max} values. Notwithstanding these differences, there are generic similarities in their structure, as indicated by the foregoing analyses of transition temperatures as a function of pH (Fig. 9). These findings and the evidence that these proteins undergo reversible changes in intramolecular and intermolecular interactions make them excellent models for the study of factors influencing protein structure.

The structural features of L chains responsible for their thermal behavior are likely to be of significance in determining the properties of specific antibodies. Antigenic analyses have shown that Bence-Jones proteins cross-react (22, 23) with the proteolytically produced S fragments (24) of the γ-globulin molecule which are known to contain the antibody-combining site. The L chains derived from normal γ-globulin also cross-react with this fragment (25), thus suggesting that the S fragment contains L chains or portions of L chains. Moreover, starch gel electrophoresis of dissociated guinea pig antibodies of different specificities has shown differences in the patterns of their L chains (26). It is therefore possible that L chains of different types are involved in the acquisition of immunologic specificity, and that the structural features that they share with Bence-Jones proteins play a role in this process (27).

Isotopic tracer studies (28, 29) have yielded results consistent with the inter-

pretation that Bence-Jones proteins are by-products or precursors of myeloma protein synthesis. In those instances in which the myeloma protein contains L chains of the same type as those of the Bence-Jones proteins, a likely mechanism for the excessive production of Bence-Jones protein would be asynchronous synthesis of L and H chains by the same plasma cell line. In those cases in which the Bence-Jones protein and the L chains of the myeloma protein are not related, it may be that the myeloma protein is produced by one cell line and that the Bence-Jones protein is produced by other cells incapable of forming H chains. The study of model systems such as the transplantable plasmacytoma of mice (30, 31) in conjunction with the structural approaches outlined here should permit a detailed analysis of the kinetics of chain synthesis in multiple myeloma. Such studies may have a bearing upon the related question of whether L chains are formed asynchronously in normal animals. A comparison of L chains and the recently described (32) γ-related urinary proteins of low molecular weight might provide information to support this possibility.

A urine sample containing reversibly coagulable protein was sent to Dr. Jones in 1845 by a practitioner, Dr. Watson, who also sent a note describing its behavior. The description terminated with the question "What is it?" Dr. Jones concluded that it was the "hydrated deutoxide of albumin." (2) The above studies would supply the question with another qualified answer: Bence-Jones proteins appear to be polypeptide chains of the L type that have not been incorporated into myeloma proteins.

SUMMARY

The chemical relations among Bence-Jones proteins, myeloma proteins, and normal γ-globulins have been investigated by a variety of means. Starch gel electrophoresis in 8 M urea of reduced alkylated Bence-Jones proteins yielded patterns of bands corresponding to those of the light (L) polypeptide chains of the dissociated myeloma protein from the same patient.

One instance in which this correspondence was found was chosen for extensive study. Chromatography on carboxymethylcellulose in 6 M urea was employed to isolate the light (L) polypeptide chains and heavy (H) polypeptide chains of the completely reduced and alkylated myeloma protein. Isolation of similarly treated Bence-Jones protein from the same patient corroborated the correspondence to the L chains of the myeloma protein. Amino acid analyses indicated that the compositions of the Bence-Jones protein and the L chains of the myeloma protein were identical. Moreover, the thermosolubility properties and spectrofluorometric behavior of the isolated L chains and Bence-Jones protein were similar.

Ultracentrifugal analyses of the L chains of normal human 7S γ-globulin showed that their molecular weight in 6 M urea was 20,000. In aqueous solution their molecular weight was 41,000, suggesting that they exist as dimers under

these conditions. The L chains of normal human γ-globulin were found to have reversible thermosolubility properties similar to those of Bence-Jones proteins. The H chains of normal human γ-globulin did not share these properties.

Using spectrofluorometric methods, characteristic molecular transitions were found upon heating Bence-Jones proteins and L chains. These transitions were indicated by an increase in the intensity of fluorescence at well defined temperatures as well as by reversible shifts in the wavelength of maximal emission.

The findings suggest that Bence-Jones proteins are composed of L chains of the type found in normal and pathological γ-globulins.

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