ity for the deuterio-protein will be correspondingly larger. This will permit hindered rotation to take place at a lower temperature in the deuterio-protein than in the hydrogen protein, thus a lower denaturation temperature in deuterio-protein than hydrogen protein. To substantiate this it would be best to attempt a calculation of the temperature difference that would be expected as a result of the differences in moment of inertia.

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ADDED IN PROOF

It should be noted that although the thermal denaturation as observed in these studies is irreversible, Lavorel and Moniot (1962) have observed that with extremely fast heating and cooling the process is apparently reversible. The transition temperature is taken as the onset of the denaturation. Therefore, it is suggested that at the transition temperature we have a reversible process. This would allow the data to be treated by conventional thermodynamic analysis.

REFERENCES

Aranow, R. H., Witten, L., and Andrews, D. H. (1958), *J. Phys. Chem. 62*, 812.
Berns, D. S. (1963), *J. Am. Chem. Soc. 85*, 1676.
Berns, D. S., Crespi, H. L., and Katz, J. J. (1963), *J. Am. Chem. Soc. 85*, 8.
Glasoe, P. K., and Long, F. A. (1960), *J. Phys. Chem. 64*, 188.
Hermans, J., and Scheraga, H. A. (1959), *Biochim. Biophys. Acta 36*, 534.
Kirshenbaum, I. (1951), Physical Properties and Analysis of Heavy Water, New York, McGraw-Hill, p. 1.
Lavorel, J., and Moniot, C. (1962), *J. Chim. Phys. 59*, 1007.
Maybury, R. H., and Katz, J. J. (1956), *Nature 177*, 629.
Mikkelsen, K., and Nielson, S. O. (1960), *J. Phys. Chem. 64*, 632.
Mizushima, S.-I. (1954), *Advan. Protein Chem. 9*, 299.
Ó hEocha, C. (1962) *in* Physiology and Biochemistry of Algae, Lewin, R. A., ed., New York, Academic, p. 421.
Ó hEocha, C., and Ó Carra, P. (1961), *J. Am. Chem. Soc. 83*, 1091.
Tanford, C. (1962), *J. Am. Chem. Soc. 84*, 4240.

The Relationship of Structure to the Thermal Solubility Characteristics of a Bence-Jones Protein*

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A Bence-Jones protein characterized as type A and having a molecular weight of 48,000 was degraded on heating to 100° to a main polypeptide chain and to other unidentified polypeptides of lower molecular weight. Similar degradation products were found after exposure of the Bence-Jones protein to 2 M urea. The main polypeptide chain, isolated by CM-cellulose and DEAE-cellulose chromatography, had a molecular weight of 22,500 and was soluble and stable between 0° and 100°. The peculiar thermal characteristics of the Bence-Jones proteins are explained on the basis that the protein forms an insoluble aggregate between 50° and 70°, which dissolves on heating to 100° owing to deaggregation and degradation to polypeptide chains. On cooling, the various polypeptide chains recombine to form a product resembling the starting material, which may reaggregate at temperatures ranging between 70° and 50°.

Two unusual proteins are elaborated by persons suffering from multiple myeloma. One, the myeloma protein, appears in the serum often in large quantity, and has a molecular weight of approximately 160,000. Although it shares at least one antigenic determinant with the portion of 7 S gamma globulin concerned with antibody function (Olins and Edelman, 1962; Migita and Putnam, 1963), no immunologic activity has as yet been ascribed to it. The other protein occurs mainly in the urine and has a molecular weight of 40,000–60,000. This protein was first described by Henry Bence Jones (1847) and has aroused much curiosity because of its unique thermal characteristics. The Bence-Jones protein precipitates between 45° and 60°, redissolves on heating to 100°, and reprecipitates on subsequent cooling. The nature of the thermal changes have not been explained to date. The relationship of the serum protein to the urinary protein, on the other hand, has been explained recently. As these proteins were found to share polypeptide chains, it has been suggested (Edelman and Gally, 1962) that "Bence-Jones proteins appear to be polypeptide chains . . . that have not been incorporated into myeloma proteins."

One of the difficulties encountered in working with these proteins is the fact that they are not identical in different individuals. As the result of evidence provided by Putnam (1957), it is now commonly accepted that each human with the disease multiple myeloma elaborates individually specific myeloma and Bence-Jones proteins.

Working with a well-characterized Bence-Jones protein from one subject offered an opportunity for intensive study of its structural peculiarities. One of the components of this protein, prepared either by heating to 100° or by urea treatment, was a heat-stable polypeptide chain. At *p*H 5.0 this polypeptide chain remained soluble over a wide temperature range. When associated with lower molecular weight polypeptides also derived from this protein it lost its solubility between 50° and 70°, but not at higher temperatures. This finding suggested that reversible association be-

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tween polypeptides was responsible for the thermal behavior of the Bence-Jones protein. It is hoped that this work will provide a model for studying the thermal characteristics of other Bence-Jones proteins.

Experimental

Materials.—The Bence-Jones protein was precipitated from the urine of patient (Pa)[1] with multiple myeloma by half saturation with $(NH_4)_2SO_4$. The precipitate was dialyzed and stored as a lyophilate. It was purified by DEAE-cellulose[1] chromatography with gradient elution by a potassium phosphate buffer of *p*H 8.0. The fractions comprising the major peak (approx 95% of all the protein eluted) were dialyzed against distilled water and stored as a lyophilate. A single peak appeared on rechromatography. The protein obtained from urines collected over a number of months appeared to be identical when tested by starch gel electrophoresis as well as by amino acid analyses.

Methods.—Starch gel electrophoresis (Smithies, 1955) was carried out in sodium borate buffer (0.05 M, *p*H 9.1). Preparative column electrophoresis was carried out in a Pyrex cylinder (14 cm length, 5 cm diameter) with polyacrylamide (Cyanogum 41, American Cyanamid Co.) as the supporting medium (Raymond and Weintraub, 1959). The column was cut into 0.25-cm sections, which were homogenized in water and centrifuged. The protein concentration of the fractions was measured by ninhydrin. Free-boundary electrophoresis was carried out in various buffers, in a Spinco Electrophoresis-Diffusion apparatus, Model H.

Ultracentrifuge analyses were carried out in the Spinco Ultracentrifuge, Model E. Sedimentation constants were determined at varying concentrations at 20° (± 0.1°) in sodium acetate buffer (0.1 M, *p*H 5.0). In calculations of these constants the usual corrections were applied for the viscosity of the buffer. Molecular weight determinations were made by the approach to equilibrium method. The samples had a protein concentration of 3–5 mg/ml. The protein concentration at the meniscus and the amount of protein sedimenting away from the mensicus were determined with a Nikon microcomparator (Stangert Corporation, Far Rockaway, New York). The data were analyzed according to Erlander and Foster (1959). The molecular weights obtained by this method were compared to those obtained by sedimentation velocity. The partial molar volume of protein was calculated from the amino acid composition.

Diffusion analyses were carried out in acetate buffer, (0.1 M, *p*H 5.0) in a Spinco Electrophoresis-Diffusion apparatus, Model H.

CM-cellulose (Brown Company) columns were eluted with acetate buffer (0.02 M, *p*H 4.5) and a gradient of 0–0.3 M KCl. DEAE-cellulose (Brown Company) columns were eluted with phosphate buffer (0.01 M $HPO_4^=$, *p*H 8) and a gradient of 0–0.3 M $H_2PO_4^-$. The conductivity of the effluent was measured in a Wheatstone bridge at 4°. The protein concentration of the effluent was evaluated by absorption at 280 mμ in a Beckman spectrophotometer, Model DU.

Protein nitrogen was determined after micro-Kjeldahl digestion by means of Nessler's reagent.

Amino acid analyses were performed by the method of Spackman *et al.* using an automatic analyzer (Woods and Engle, 1960). The samples were hydrolyzed in evacuated sealed ampoules with constant-boiling HCl for 20 hours at 110°.

The agar gel double-diffusion method of Ouchterlony was used with rabbit antisera against fraction II human gamma globulin.

All heat experiments were carried out under atmospheric conditions.

Preparations were dialyzed under atmospheric conditions against buffered solutions containing the desired concentration of reagent grade urea.

[1] Abbreviations used in this work: CM-cellulose, carboxymethyl-cellulose; DEAE-cellulose, diethylaminoethyl-cellulose.

Table I

Relative Amino Acid Content of Bence-Jones Protein, and L Chains Prepared by Heating or Urea[a]

Amino Acids	B-J Protein	L-Chain (heat)[b]	L-Chain (urea)[b]
Lysine	0.88	—	—
Histidine	0.18	—	—
Arginine	0.37	—	—
Aspartic acid	1.00	1.00	1.00
Threonine	1.39	0.88	0.87
Serine	2.60	2.04	1.88
Glutamic acid	1.65	1.40	1.36
Proline	1.29	1.16	1.17
Glycine	1.35	1.83	2.19
Alanine	1.41	0.99	0.84
Half-cystine	0.29	0.21	0.23
Valine	1.31	1.07	1.13
Methionine	Trace	0.00	0.00
Isoleucine	0.40	0.52	0.49
Leucine	1.04	0.87	0.86
Tyrosine	0.65	Trace	0.00
Phenylalanine	0.41	0.31	0.28

[a] The values are given as moles of amino acid/mole of aspartic acid. [b] The short column was not run for the L chains.

Results

Characterization of the Bence-Jones Protein.—The Bence-Jones protein was of the "α" type, as reversible heat precipitation occurred over a relatively wide range of *p*H (Putnam *et al.*, 1959). On immunologic assay, the protein was found to possess "A" type antigenic determinants (Korngold and Lipari, 1956) when analyzed in agar gel double diffusion with an appropriate typing rabbit antiserum. In confirmation of the typing, the protein lacked terminal amino groups (Putnam, 1957). The protein was not attacked by carboxypeptidase (Worthington).

On starch gel electrophoresis the protein migrated somewhat more slowly than serum albumin, but there were traces of slightly faster and slower components. The preparation also was slightly heterogeneous on free-boundary electrophoresis in different buffers. In borate buffer (0.05 M, *p*H 9.0) the single, slightly skewed boundary had a mobility of 4.4×10^{-5} cm^2 volt^{-1} sec^{-1}. The isoelectric point was 4.9. The preparation gave a symmetrical boundary in the ultracentrifuge ($s^0_{20,w}$ = 3.7 S). The diffusion constant was 6.6×10^{-7} cm^2 sec^{-1}. The partial molar volume of the protein, estimated from its amino acid composition, was 0.716. The molecular weight calculated from S and *D* (sedimentation velocity) was 48,000. The approach to sedimentation equilibrium gave a molecular weight of 47,900. The molecular weight was 44,200 when calculated from the amino acid composition (exclusive of tryptophan and carbohydrate). The relative amino acid composition of the protein is given in Table I. The protein contained only traces of hexose and hexosamine and therefore was free of

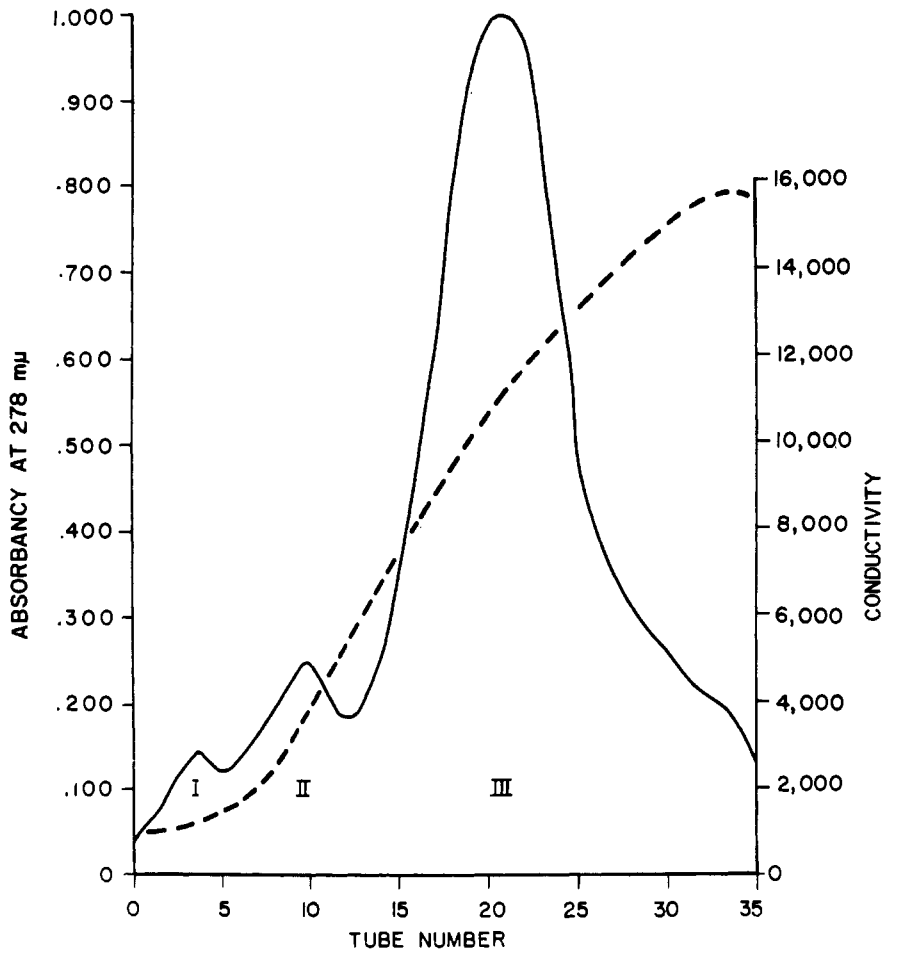

FIG. 1.—Elution diagram of 100 mg Bence-Jones protein from a 30 × 2—cm column of CM-cellulose. Ten-ml fractions were collected. The protein concentration (solid line) is estimated from the optical density measured at 278 mμ, the concentration of the eluent (interrupted line) is given as conductivity.

urinary glycolipoprotein (Weicker and Huhnstock, 1962).

The protein was further purified by CM-cellulose chromatography. The elution pattern obtained is shown in Figure 1. The two minor components lacked the thermal characteristics of Bence-Jones proteins. Component I was dialyzable. Component II had a sedimentation constant of 2.2 S. On starch gel electrophoresis it migrated to the same region as the starting material. Most of the protein eluted in component III. On starch gel electrophoresis, component III appeared to be slightly heterogeneous, resembling the starting material. When the isolated component III was again developed by CM-cellulose chromatography, it again exhibited the lower molecular weight components. It is likely that even on gentle manipulation the Bence-Jones protein undergoes degradation to lower molecular weight components.

Heat Experiments.—The initial temperature of precipitation of the Bence-Jones protein in acetate buffer (0.1 M, *p*H 5.0) was 52° and precipitation continued until approximately 70°. At 97° the precipitate went into solution. Reprecipitation occurred on subsequent cooling with only 10% of the original material remaining in solution. CM-cellulose chromatography of this solution yielded two fractions (Figure 2). One fraction eluted at 2,000 mho and was a mixture of dialyzable low molecular weight material, henceforth called L* polypeptides, and a material with a sedimentation coefficient of 2.2 S. The latter migrated as a single band on starch gel electrophoresis. This material, henceforth called L-chain, lacked the thermal as well as the antigenic properties of the parent compound. The second fraction eluted at 10,000 mho and appeared to be unchanged Bence-Jones protein, possessing thermal, immunologic, electrophoretic, and sedimentation characteristics of the starting material.

A three-step procedure was devised for a more detailed analysis of the changes occurring during heating (see Figure 3). Step 1: Five hundred mg of the Bence-Jones protein (in acetate buffer, 0.1 M, *p*H 5.0) was heated to 70° and the resulting precipitate was separated from the solution. An aliquot of the solution was fractionated on CM-cellulose chromatography. Step 2: The precipitate was resuspended in buffer and heated to 100° and allowed to cool. The material remaining in solution was analyzed by CM-cellulose chromatography. Step 3: An aliquot of the supernatant obtained in step 1 was heated to 100°, cooled, and then fractionated on CM-cellulose. Figure 3 shows the CM-cellulose chromatography elution patterns of solutions obtained in each step. Four different products were recovered. The L-chain was the major product and appeared mainly in step 2. Traces of the L-chain were also found in solutions of step 3. Step 1 gave rise to a fraction containing small amounts of a material sedimentating at 2.5 S which was strongly bound to CM-cellulose. Unchanged Bence-Jones protein and L* polypeptides were found in all solutions.

A minimum concentration of the Bence-Jones proteins is required for manifestation of their thermal characteristics. The minimum concentration for the

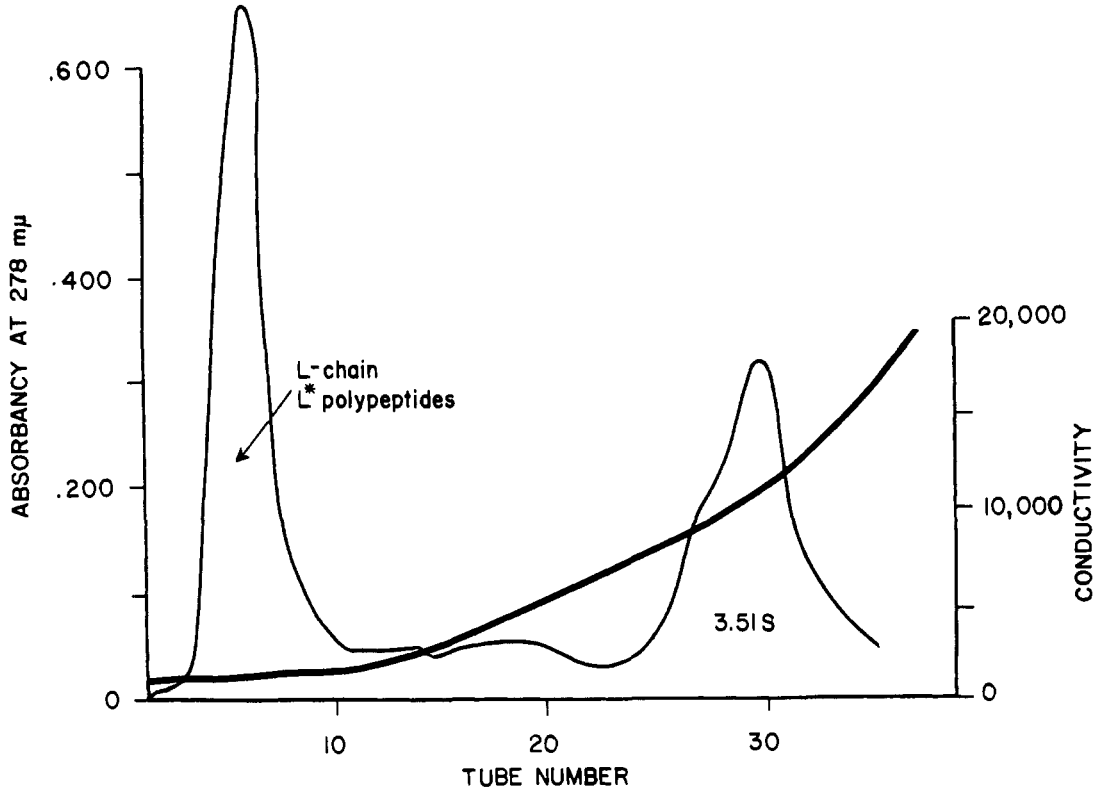

FIG. 2.—Elution diagram from CM-cellulose of solution obtained after heating 200 mg Bence-Jones protein to 100°.

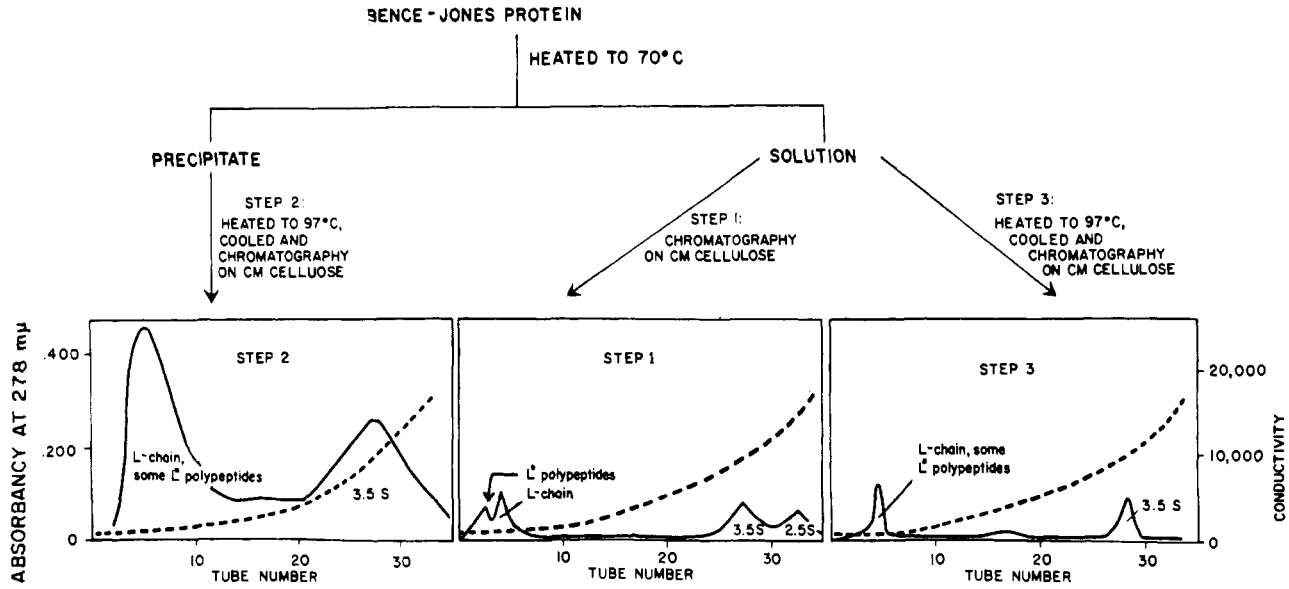

FIG. 3.—Flow chart for procedure used to obtain products of the heat degradation of 500 mg Bence-Jones protein. Elution diagrams of the degradation products from 24 × 1.5-cm columns of CM-cellulose, collected in 10-ml fractions.

protein under study was 0.36 mg/ml (8 μM). Below this concentration there was no visible precipitation either upon heating to 70° or after heating to 100°. Moreover, by diluting on heating to 100° it was possible to prevent reprecipitation of a concentrated solution which had coagulated at 70° and redissolved at 97°. In one experiment 50 mg of the protein, dissolved in 5 ml of *p*H 5.0, 0.1 M buffer was heated to 100° and diluted with 200 ml of boiling water. On cooling a clear solution was obtained which was lyophilized. The lyophilized material failed to precipitate when heated above 52°. This material was dialyzed and analyzed by CM-cellulose chromatography. The only product found eluted at 2,000 mho. It had the characteristics of the L-chain. The yield was approximately 30%. In another experiment, although similarly executed, there remained some undissolved protein following heating to 100° and dilution. After clearing of the solution by centrifugation and lyophilization a concentrated solution gave a positive heat test. On CM-cellulose chromatography considerable amounts of material with the characteristics of the original Bence-Jones protein were recovered at the expected conductivity. The L* polypeptides not bound to the

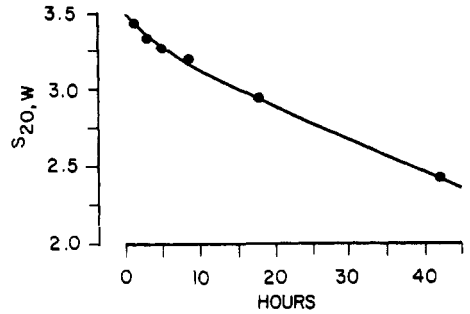

FIG. 4.—The dependence of the sedimentation coefficient of the Bence-Jones protein upon length of time of exposure to 2 M urea in 0.1 M, *p*H 5, acetate buffer.

ion exchanger, and L-chain binding only slightly to the ion exchanger were also recovered. The variables which govern the amount of recovery of unchanged starting material have not been elucidated so far, but a number of additional experiments suggest that these are the duration of heating at 100°, the concentration of the sample, and *p*H.

Experiments with Urea.—On analytical ultracentrifugation Bence-Jones proteins, dissolved in buffered urea solutions, exhibited a single homogeneous peak. The sedimentation constants of the solute showed a time-dependent decrease (Figure 4). Moreover, the sedimentation constants were nearly independent of urea concentrations ranging from 2 M to 8 M and of hydrogen ion concentrations between *p*H 4 and *p*H 8. CM-cellulose chromatography of the Bence-Jones protein exposed to 2 M urea at *p*H 5.0 resulted in recovery of three distinct fractions. As indicated in Figure 5, the respective yield of these fractions was a function of the time of exposure to urea. Urea slowly degraded the Bence-Jones protein to two fragments, with eventual disappearance of the starting material. The newly formed fragments resembled the ones which were obtained on heating. The material with the least affinity for CM-cellulose was dialyzable through Visking membranes (L* polypeptides), and the material with somewhat more affinity for CM-cellulose had a sedimentation constant of 2.2 S. When material containing more than 0.02 mole of NaCl was developed by CM-cellulose chromatography the two fractions tended to elute as a single peak. The mixture had an observed sedimentation constant of 1.2 S. Prolonged dialysis of this mixture, however, produced a shift of the sedimentation constant to values approaching 2.2 S. The homogeneous boundaries seen on ultracentrifugation of these mixtures reflect the inability of the ultracentrifuge to resolve slowly sedimenting components. The sedimentation constants obtained therefore reflected the variable amount of different molecular species in a mixture.

The nondialyzable degradation product was an L-chain. It sedimented at 2.2 S and lacked the thermal characteristics of the parent compound. It failed to react with rabbit anti-human gamma globulin. On starch gel electrophoresis it had nearly the same mobility as the parent compound.

Comparison of L-Chains Obtained on Exposure to Urea and on Heating.—The L-chain obtained on exposure to urea and the L-chain obtained on heating to 100° were indistinguishable on CM-cellulose as well as on DEAE-cellulose chromatography. The L-chains were also indistinguishable by starch gel as well as by polyacrylamide-column electrophoresis. Both preparations failed to coagulate on heating to 97°, and they lacked the antigenic determinants which characterized the parent compound. Analyses of the amino acid content of L-chains isolated by polyacrylamide column electrophoresis (Table I) are suggestive of the identity of the two preparations. The L-chain obtained on exposure to urea was heated to 100° for 30 minutes and then analyzed by CM-cellulose chromatography. Ninety-five per cent of the material eluted at approximately 2,000 mho and the remaining material appeared at 12,000 mho. The major fraction appeared to be unchanged L-chain. The minor fraction was not further characterized.

The L-chain obtained on heating to 100° was made 2 M with respect to urea. The solution was analyzed in the ultracentrifuge. When kept in the centrifuge cell a gradual shift to lower sedimentation constants was observed over an 18-day period. It would appear that L-chains exposed to urea for prolonged periods undergo further degradation.

L* *Polypeptides.*—Mixtures of L-chain and L* polypeptides obtained after CM-cellulose chromatography exhibited two bands on starch gel electrophoresis. The faster moving band was associated with the L* polypeptides, as prolonged dialysis resulted in the disappearance of this band.

In a yield study following a heating experiment, the recovery of L-chain and L* polypeptides was measured by CM-cellulose chromatography using slow gradient elution, starting with a sodium acetate buffer of *p*H 4.5 which had a conductivity of 250 mho. Two distinct peaks were found, one comprising L* polypeptides and a second peak, eluting somewhat later, having the characteristics of L-chain. The material in these distinct fractions was analyzed for nitrogen content by Kjeldahl digestion. The ratio of L* polypeptides to L-chain was 37:43.

DEAE-cellulose chromatography also afforded some resolution of mixtures of L* polypeptides and L-chain. The major peak, eluting at low ionic strength, corresponded to the L-chain. The minor peaks, appearing later, were dialyzable and hence L* polypeptides (Figure 6).

Resolution of L-chains and L* polypeptides was also accomplished by gel filtration with Sephadex G-25. In such an experiment, a mixture of original starting material, L-chain, and L* polypeptides was resolved by obtaining the former two species at column volume, and the L* polypeptides as at least six distinct fractions retarded by the gel. After determination of the L-chain concentration in the effluent, the ratio of L-chain to L* polypeptides was estimated to be unity.

Dialysis experiments with a sample containing L-chain and L* polypeptides indicated that 40% of the nitrogenous material was dialyzable through Visking membranes.

DISCUSSION

As Bence-Jones proteins differ from one another, the conclusions drawn from an analysis of a single specimen need not necessarily hold for the entire class of compounds. Nevertheless, the findings of this study appear to complement work done by others on different specimens.

In previous work (Edelman and Gally, 1962) two essential conditions, namely, unfolding of the protein structure followed by cleavage of disulfide bonds, appeared to be involved in the recovery of L-chains. In the study reported here the dissociation of the protein was accomplished either by urea alone or by heating to 97°. It is unlikely that splitting of disulfide bonds played a role in the degradation of the protein

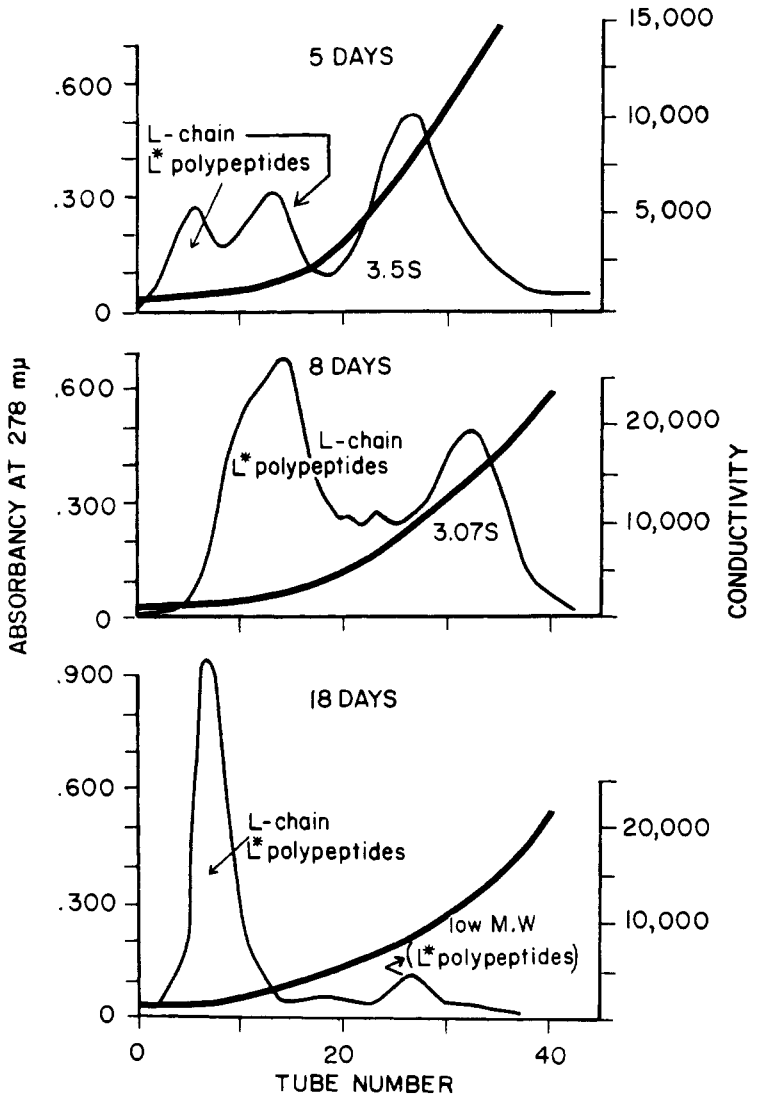

FIG. 5.—CM-cellulose chromatography elution patterns of the Bence-Jones protein exposed to 2 M urea in 0.1 M, *p*H 5.0, acetate buffer for 5 days, 8 days, and 18 days, respectively.

and the separation of the L-chain from the L* polypeptides.

The L-chains prepared in this study had a molecular weight of 22,500. This value is in good agreement with molecular weights determined for L-chains of other Bence-Jones proteins and their congeners when prepared by methods which also involved reduction of disulfide bonds (Edelman and Gally, 1962). The preparations which were subjected to molecular weight determinations were homogeneous when tested by Trautman plots. As the L-chains had a molecular weight of nearly half the parent compound, the possibility had to be considered that the Bence-Jones protein was a dimer of identical L-chains. In view of the significant differences in amino acid composition of the L-chains and the parent compound, this appears to be a remote possibility.

The data presented indicate that the Bence-Jones protein is composed of a heat-stable L-chain linked to an unknown number of L* polypeptides by noncovalent bonds such as hydrogen bonds, hydrophobic bonds, and Van der Waals' forces. The L* polypeptides comprise approximately one-half the parent compound, but no information is available as yet concerning their size, number, or identity. Studies designed to characterize these polypeptides are now in progress.

The reaction sequence of the thermal decomposition of the Bence-Jones protein, L–L*, may be written as follows:

$$L - L^* \xrightarrow[52\text{-}70^\circ]{k_1} (L - L^*)_n \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} L - L^* \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} L + L^* \quad \text{(97° / at 97°)}$$

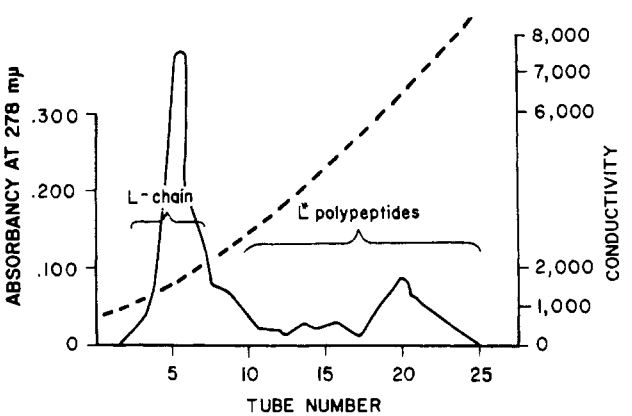

FIG. 6.—Resolution of L-chain and L* polypeptides by chromatography on a DEAE-cellulose column (24 × 1.5 cm).

Heating of L–L* results in formation of an insoluble polymer $(L–L^*)_n$. This is a first-order reaction, and is irreversible. It may require an activation step involving loss of some amino acids or low molecular weight polypeptides (see Figure 3). On further heating to 97° $(L–L^*)_n$ depolymerizes. This reaction is reversible. At this temperature L–L* also undergoes degradation to L and L*. The L does not form L–L or $(L–L)_n$, and L* does not form L*-L* or $(L^*-L^*)_n$. The failure to encounter $(L-L^*)_n$ or L-L* on heating a highly dilute solution might be attributed to the concentration dependence of k_{-3}. Failure to recover increasing amounts of L and L* on prolonged heating of high concentrations of L-L* is explained by $k_3 < k_{-3}$. The proposed mechanism also explains in part the heat-precipitation sequence of the Bence-Jones protein. The key to the unique behavior of the Bence-Jones proteins is the remarkable resistance of L-L* and L-chain to heat denaturation. Although L-chain and L* polypeptides do not form $(L-L^*)_n$ on heating, it appears likely that they combine with intact L-L* to form insoluble polymers on cooling.

The mechanism described above may also explain the reactions of L-L* on prolonged exposure to urea. The chief difference between the heat degradation and the degradation by urea is that the latter does not involve a $(L-L^*)_n$ polymer and also that the L-chain eventually degrades slightly on exposure to urea.

Many details of the proposed scheme need further elucidation. Particularly baffling is the recombination of the L-chain with the numerous L* polypeptides. The recombination as well as the polymerization of L-L* apparently does not involve disulfide interaction, as 0.025 M iodoacetate (in 0.1 M acetate, *p*H 5.0) failed to interfere with the heat test. Putnam *et al.* (1959) previously reported that iodoacetamide failed to influence the amount of protein precipitated on heating solutions to 56°. Further work also needs to be done on other Bence-Jones proteins to ascertain whether they all conform to an L-L* structure. This phase of the work assumes great importance in view of the finding of Edelman and Gally (1962) that the amino acid composition of the L-chain of Bence-Jones protein (Haw), obtained with urea and mercaptoethanol, was identical with the parent compound. Their protein might have been of type L-L, i.e., a dimer of L-chains. A comparison of the amino acid contents of the L-chains produced by the methods described herein and by the method of Edelman and Poulik (1961) is now being made and will be reported on separately.

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References

Edelman, G. M., and Gally, J. A. (1962), *J. Exptl. Med. 116*, 207.

Edelman, G. M., and Poulik, M. D. (1961), *J. Exptl. Med. 113*, 861.

Erlander, S. R., and Foster, J. F. (1959), *J. Polymer Sci. 37*, 103.

Jones, Henry Bence (1847), *Lancet II*, 88.

Korngold, L., and Lipari, R. (1956), *Cancer 9*, 262.

Migita, S., and Putnam, F. W. (1963), *J. Exptl. Med. 117*, 81.

Olins, D. E., and Edelman, G. M. (1962), *J. Exptl. Med. 116*, 635.

Putnam, F. W. (1957), *Physiol. Rev. 37*, 512.

Putnam, F. W., Easley, C. W., Lynn, L. T., Ritchie, A. E., and Phelps, R. A. (1959), *Arch. Biochem. Biophys. 83*, 115.

Raymond, S., and Weintraub, L. (1959), *Science 130*, 711.

Smithies, O. (1955), *Biochem. J. 61*, 629.

Weicker, H., and Huhnstock, K. (1962), *Nature 196*, 480.

Woods, K. R., and Engle, R. L. (1960), *Ann. N. Y. Acad. Sci. 87*, 754.