# STUDY OF THE PHYSICOCHEMICAL AND CHEMICAL PROPERTIES OF BENCE-JONES PROTEIN FROM MULTIPLE MYELOMA*

by

B. JIRGENSONS, ALTON J. LANDUA, AND JORGE AWAPARA

*The University of Texas, M. D. Anderson Hospital for Cancer Research, Houston, Texas (U.S.A.)*

Patients with multiple myeloma excrete in their urine considerable quantities of Bence-Jones protein. The presence of this abnormal constituent in urine has served as an aid in the diagnosis of multiple myeloma, but the protein itself, although well known for some time, has not been fully characterized. Bence-Jones protein is an albumin-like substance that dissolves in water, precipitates from solution at 50 to 60° C, and goes again into solution at boiling temperature; after cooling, the protein precipitates again. The solubility behavior of the Bence-Jones protein serves as test for its detection in urine but does not represent a consistent and reproducible property. MAGNUS-LEVY[1] has reported that some samples of Bence-Jones protein fail to dissolve when the solution is boiled. Moreover, differences in some properties have been observed for samples excreted by the same patient at different times, indicating that perhaps more than one protein is being excreted. Electrophoretic studies of the Bence-Jones protein indicate that there is a significant difference in mobility between proteins isolated from the urine of different patients[2]. The molecular weight of Bence-Jones protein has been estimated at about 37,000[1]. RUNDLES, COOPER AND WILLETT[3] believe that the Bence-Jones protein represents a low molecular weight protein derived from certain abnormal components of serum. By virtue of its relatively small molecular weight the Bence-Jones protein can pass into the glomeruli and appear in the urine.

The available information on the chemistry of Bence-Jones protein seems to be inconsistent and little agreement exists between values reported by various investigators[4,5,6,7,8]. DENT AND ROSE[7] did not find methionine in the samples investigated by them. They reported that electrophoretic analysis gave only one slightly assymetric peak, but salting out experiments suggested that their sample consisted of two components in the proportion of 9:1. ROBERTS and associates[8] found in their samples 12.6% serine and 8.3% threonine and suggest that such unusually high values of hydroxy amino acids are only found in some virus proteins.

The present study was initiated to establish whether the so-called Bence-Jones protein possesses the same properties and the same chemical composition when isolated from the urine of one patient, but using different methods of isolation. Samples of Bence-Jones proteins were isolated from the urine of one patient at different times and a comparative study of the physicochemical and chemical properties carried out. By so doing it was hoped to clarify some of the existing discrepancies in the literature.

* This work was supported by a grant from the American Cancer Society.

*References p. 632.*

ISOLATION OF BENCE-JONES PROTEIN FROM URINE AND SERUM

Urine of a patient with typical symptoms of multiple myeloma was used as the source of Bence-Jones protein. Urine was collected daily using toluene as preservative. Aliquots of the 24-hour specimens were analyzed by means of the electrophoretic method on filter paper described by DURRUM[9] and DIETRICH and CREMER AND TISELIUS[10]. The urinary excretion of Bence-Jones protein as determined by the electrophoretic method varied from 14.6 to 20.3 grams per 24 hours. The Bence-Jones protein appeared in the filter paper strips as a single homogeneous component, but as it will be shown later its homogeneity is questionable. For the isolation of Bence-Jones protein several methods were used, and the samples thus obtained studied individually.

*Sample D.* 400 ml of filtered urine, from a 24-hour specimen, were dialyzed in a cellophane tube against distilled water until the solution outside the membrane showed negligible change. The operation was carried out at low temperature in the refrigerator. The dialyzed urine was filtered and the protein precipitated with cold acetone at a temperature ranging from $-3^\circ$ C to $-10^\circ$ C. The precipitated protein was filtered through a sintered glass funnel, washed with cold methanol and ether and dried under vacuum in a desiccator. Yield: 3.8 grams.

*Sample B and E*: 600 ml of urine were mixed with an equal volume of a saturated solution of ammonium sulfate and allowed to stand in the refrigerator for several days. A small quantity of material precipitated and was filtered off. The filtrate was brought to 55% saturation of ammonium sulfate and allowed to stand for several days. The Bence-Jones protein which precipitated was filtered off, dissolved in water and dialyzed against distilled water (Sample B). A second sample of urine was brought to 20% ammonium sulfate saturation, acidified with acetic acid to pH 4.4 and allowed to stand for 2 days in the refrigerator. A small amount of precipitate that formed was filtered off and discarded. The filtrate was brought to 55% saturation with ammonium sulfate and allowed to stand for several days in the refrigerator. The precipitated Bence-Jones protein was filtered off, dissolved in water and dialyzed (Sample E). The dialyzed B and E solutions were mixed and treated with activated charcoal (Norite). The Bence-Jones protein from the decolorized solution was precipitated with acetone at $-5^\circ$ C and the precipitated Bence-Jones protein filtered off. The protein was washed and dried by the same procedure followed with Sample D.

*Sample F.* 800 ml of urine were first treated with activated charcoal (Norite) to remove colored impurities. The urine was mixed, by means of vigorous mechanical stirring, with 1200 ml of saturated ammonium sulfate. The mixture was allowed to stand for several days in the refrigerator. The precipitated protein was filtered, dissolved in water, dialyzed and precipitated with acetone at low temperature following the same procedure used with the previous samples.

*Sample I.* 1000 ml of urine were adjusted to pH 5 with acetic acid, and heated slowly, raising the temperature 0.5° C per minute. During this time the urine was stirred by mechanical means. A faint turbidity appeared when the temperature reached 54° C. This material was removed by filtration, and discarded. Heating was continued and when the temperature reached 57 to 57.5° C the protein precipitated rapidly. After allowing to stand for 30 minutes the protein was filtered, washed with water, acetone and ether consecutively (Sample I). The filtrate was heated until the temperature reached 58°–60° C, at which point a second fraction precipitated from the urine ($I_2$). This fraction was isolated and purified like the others. The filtrate from $I_2$, was again heated, this time to 61.5–65° C, and a third precipitate obtained ($I_3$), which was also filtered and treated like the other samples. Yields: (I): 1.9 grams, ($I_2$): 2.2 grams and ($I_3$): 0.35 grams.

*Sample II.* This sample was obtained by the same method used in the isolation of ($I_2$) but from a different urine specimen, excreted on a different day.

*Sample J.* 2400 ml of urine were adjusted to pH 5.2 with acetic acid and filtered. A saturated solution of ammonium sulfate was added slowly while the mixture was stirred mechanically. At 40% saturation a faint turbidity appeared. As the saturation approached 50% more protein precipitated and nearly complete precipitation occurred when the saturation with ammonium sulfate was brought to 60%. The mixture stood in the refrigerator for 2 days and the precipitated protein was filtered off and treated in the same manner as Sample F, except that precipitation from the dialyzed solution was accomplished using methanol at $-2^\circ$ C.

*Sample K.* 4110 ml of urine, collected at various times, were brought to 60% saturation with ammonium sulfate and allowed to stand in the refrigerator for 4 days. The mixture was centrifugated at 2000 RPM and 5° C for 30 minutes. The precipitate was dissolved in distilled water and filtered. The filtered solution was dialyzed and the solution treated with cold methanol. The precipitated material was treated like the other samples. Yield: 13 grams.

*References p. 632.*

All the samples of Bence-Jones protein obtained by these methods were white powders and readily soluble except those obtained by heat precipitation. The pH of an 0.18% solution of sample D was 5.9 and the solution turned turbid at 56° C. A similar solution of sample B and E has a pH of 4.7 and became turbid at 59.5° C. The precipitated proteins dissolved only partially in boiling water.

*Sample L from Serum.* Serum from the same patient was obtained by routine methods. Electrophoretic analyses of the serum showed the following protein distribution:

| | | |
|---|---|---|
| Total: | Protein | 9.10 g per 100 |
| | Albumin | 2.9 g/100 |
| | Globulins | 6.2 g/100 |
| | $\alpha_1$ | 0.22 g/100 |
| | $\alpha_2$ | 0.54 g/100 |
| | $\beta$ | 0.62 g/100 |
| | $\gamma$ | 4.80 g/100 |

The $\gamma$-globulin could not be separated from the abnormal Bence-Jones protein, so that the figure above represents a mixture of the two. When aliquots of serum and urine were mixed, the electrophoretic pattern resulting from the mixture showed an overlap of the abnormal components.

*Isolation of the Bence-Jones protein from blood.*

15 ml of serum were treated with 20 ml of a saturated solution of ammonium sulfate. Most proteins, except the albumin were precipitated. The precipitate was filtered off. The precipitate, (globulins) was taken up in 20 ml of water and dialyzed. A precipitate formed on dialysis. After removal of the precipitate from the dialyzing tube it was discarded. The filtrate contained small amounts of other fractions but 75% was made up by Bence-Jones protein, as determined electrophoretically. The filtrate was brought to 40% saturation with ammonium sulfate and a precipitate formed. The precipitate was removed. Electrophoretic analysis of the solution indicated that this portion was made up of $\gamma$-globulin. To the filtrate, containing ammonium sulfate at 40% saturation was added more ammonium sulfate up to 52% saturation, whereby the Bence-Jones protein precipitated. It was dissolved in water and dialyzed. Again, a small portion of protein precipitated on dialysis, which was discarded. The clear solution contained only the Bence-Jones protein as indicated by electrophoresis.

A sample of this material exhibited all the properties of the Bence-Jones protein, namely reversible appearance of turbidity at 50°–60° C and disappearance on boiling. From the dialyzed solution the protein was precipitated with cold methanol. After precipitation, the moist material was soluble in water and gave a positive Bence-Jones test. However, on drying, its solubility in water decreased considerably. This is comparable to the solubility behavior of a spontaneously crystallizing protein from the serum of a multiple myeloma patient, reported by VON BONSDORFF and associates[11].

## PHYSICAL AND CHEMICAL PROPERTIES OF BENCE-JONES PROTEIN

*Moisture, ash and nitrogen content.* Determinations of moisture, ash and nitrogen were carried out by standard methods. The results are compiled in Table I.

*Amino acid composition.* Amino acids were determined in the hydrolyzed sample following the microbiological assay method of SCHURR and associates[12]. Tryptophan was measured by the method of HOPKINS AND WINKLER as modified by SHAW AND MCFARLANE[13]. Chromatographic analysis on filter paper showed no discernible differ-

*References p. 632.*

TABLE I

NITROGEN, MOISTURE AND ASH CONTENT OF BENCE-JONES PROTEINS

| *Sample* | *Moisture* % | *Ash* % | *Nitrogen** % |
|---|---|---|---|
| B and E | 13.65 | 1.68 | 16.51 |
| D | 10.47 | 0.92 | 14.70 |
| F | 6.00 | 0.29 | 15.81 |
| J | 6.54 | 0.37 | 15.98 |
| K | 9.43 | 0.00 | 16.07 |
| L | 10.11 | 9.73 | 14.25 |
| $I_1$ | 3.53 | 0.56 | 15.43 |
| $I_2$ | 2.21 | 0.60 | 15.17 |
| $I_3$ | 0.59 | 0.59 | 14.39 |
| II | 5.70 | 2.63 | 15.49 |
| Bovine albumin | 1.07 | 0.43 | 15.01 |

* Corrected for moisture and ash content

ences in the amino acid pattern of any of the samples. Tests for phosphate, sulfate and sugars were negative. The hydrolysates were prepared by refluxing the protein with 6 *N* HCl. Samples D and II were hydrolyzed following identical procedures to those reported by ROBERTS and associates[8] whereas samples I and L were hydrolysed for several hours longer. The results are shown in Table II. Sample D was also analyzed for sulfur by the PARR method. The value of 1.04% for this sample was found which is in good agreement with the sum of microbiologically determined sulfur which gives a value of 0.98%.

TABLE II

AMINO ACID CONTENT OF BENCE-JONES PROTEINS

(Grams of amino acid per 100 grams protein)*

| *Amino Acid* | *Sample* | | | | | |
|---|---|---|---|---|---|---|
| | *D* | *II* | $I_1$ | $I_2$ | $I_3$ | *L* |
| Alanine | 8.11 | 6.96 | 7.92 | 7.96 | 7.31 | 6.79 |
| Arginine | 6.66 | 5.59 | 5.27 | 4.99 | 5.31 | 3.74 |
| Aspartic acid | 6.95 | 6.71 | 8.77 | 9.05 | 8.86 | 7.68 |
| Cystine | 3.74 | 3.56 | 4.36 | 3.29 | 3.48 | 2.69 |
| Glutamic acid | 10.50 | 9.08 | 6.22 | 9.92 | 5.91 | 8.21 |
| Glycine | 7.07 | 7.84 | 9.65 | 9.64 | 7.76 | 7.42 |
| Histidine | 1.61 | 1.55 | 1.72 | 1.82 | 1.65 | 1.95 |
| *Iso*leucine | 2.62 | 2.86 | 3.24 | 2.77 | 2.75 | 1.51 |
| Leucine | 8.30 | 7.55 | 9.61 | 8.82 | 8.39 | 9.54 |
| Lysine | 7.05 | 8.25 | 6.83 | 6.99 | 6.52 | 5.91 |
| Methionine | 0.42 | 0.45 | 0.34 | 0.25 | 0.24 | 0.54 |
| Phenylalanine | 6.24 | 4.48 | 11.45 | 9.46 | 8.01 | 6.19 |
| Proline | 7.25 | 5.96 | 6.04 | 5.57 | 5.14 | 7.18 |
| Serine | 6.85 | 6.30 | 5.90 | 7.56 | 7.07 | 4.18 |
| Threonine | 14.24 | 14.17 | 13.13 | 10.90 | 8.43 | 9.35 |
| Tryptophan | 2.04 | 3.26 | 3.79 | 2.62 | 2.20 | 3.79 |
| Tyrosine | 4.62 | 4.79 | 8.35 | 8.76 | 9.23 | 9.39 |
| Valine | 9.28 | 8.86 | 7.25 | 7.97 | 7.99 | 7.46 |
| Total | 113.55 | 108.22 | 119.84 | 118.34 | 106.25 | 103.52 |

* Corrected for moisture and ash content.

*References p. 632.*

*Isoelectric point.* The isoelectric point of the various samples was determined by several methods: (1) by precipitation with sodium lauryl sulfate according to NEURATH AND PUTNAM[14], (2) by precipitation with methanol at different pH values, (3) by the electrometric method, and (4) by microelectrophoresis on filter paper[9,10]. The results obtained by method (1) are shown in Table III in which bovine serum albumin has been included for comparison. Agreement of these values was observed by the other methods of determination.

TABLE III

ISOELECTRIC POINT BY PRECIPITATION WITH DETERGENT

| | |
|---|---|
| Sample D | 4.91 |
| Sample F | 5.22 |
| Sample J | 4.91 |
| Sample K | 5.13 |
| Bovine albumin | 4.70 |

*Acid-base binding capacity.* Varying amounts of standard acid (HCl) or base (NaOH) were added to separate 1 ml samples of a 4.86% solution of sample K in water. The total volume was maintained constant at 10 ml, the temperature at 23° C and the pH was measured after samples had equilibrated for 30 minutes. The results are presented in Table IV. Between pH 2 and 12 Bence-Jones protein binds much less $H^+$ and $OH^-$ than serum albumin. Below pH 2 and above pH 12 the acid base binding capacity increases very rapidly, indicating that at such pH the protein is strongly hydrolyzed.

TABLE IV

ACID-BASE BINDING CAPACITY OF BENCE-JONES PROTEIN (K) AND HUMAN SERUM ALBUMIN

| *pH* | *$H^+$ Bound ($\cdot 10^5$) per gram protein* | | *$OH^-$ Bound ($\cdot 10^5$) per gram protein* | |
|---|---|---|---|---|
| | *Albumin* | *Bence-Jones (K)* | *Albumin* | *Bence-Jones (K)* |
| 1 | 160 | 200 | | |
| 2 | 145 | 20 | | |
| 3 | 130 | 5 | | |
| 4 | 20 | 2 | | |
| 5 | 0 | 1 | | |
| 6 | | | 20 | 1 |
| 7 | | | 30 | 1 |
| 8 | | | 40 | 2 |
| 9 | | | 55 | 2 |
| 10 | | | 65 | 6 |
| 11 | | | 95 | 14 |
| 12 | | | 140 | 56 |
| 13 | | | 160 | 1588 |

*Viscosity.* Viscosity was measured in dilute solutions (0.2–1.0%) with an Ostwald capillary viscometer at 37 and 34.8° C, 2 ml of solution were introduced. The flowing time of 2 ml water in the first viscometer at 37° C was 166.8 seconds, for another, at 34.8° C was 117.9 sec. The reduced viscosity $\eta_{sp}/c$ was calculated in the usual fashion, the intrinsic viscosity was obtained by extrapolation of the reduced viscosity values to zero concentration ($c = 0$). Intrinsic viscosity of sample D was found to be 0.033,

*References p. 632.*

of sample B and E 0.045 and of sample F 0.044. Viscosity changes little with changes in pH. Thus, the reduced viscosity of a 0.85% solution of B and E at pH 3.6 was found to be 0.063, at pH 4.2, 0.049 and at pH 4.72, 0.050. Denaturing agents affect the viscosity of the solutions, as they do with many other proteins. In 0.5% Aerosol solution, the intrinsic viscosity of all samples was found to be 0.080. Exactly the same values were obtained in 0.5% solutions of sodium lauryl sulfate. Sample J rendered the same figures in detergent solutions but in 5 *M* guanidine hydrochloride its intrinsic viscosity was 0.170.

*Optical rotation.* Measurements were made with a BELLINGHAM-STANLEY instrument in 10 or 20 cm tubes using sodium light. Accuracy of the readings is ± 0.01 degree. The results of these measurements are recorded in Table V. All denaturing reagents like guanidine hydrochloride or detergents increase the negative rotation considerably, as it was found for legumin by JIRGENSONS[15] and for β-lactoglobulin by GROVES and associates[16] and CHRISTENSEN[17]. Different samples of protein dried under the same conditions show significant differences in optical rotation. The dependence of rotation on pH particularly on the acid side is reminiscent of other proteins[18], but on the alkaline side the rotation changes are negligible.

TABLE V

SPECIFIC ROTATION OF BENCE-JONES PROTEIN DETERMINED IN ONE PER CENT. SOLUTIONS

| *Sample* | *In Water* | *In Guanidine Hydrochloride 5 M* |
|---|---|---|
| B and E | − 44.0° | − 90.5° |
| J | − 38.6° | − 92.5° |
| D | − 41.1° | − 97.0° |

SPECIFIC ROTATION OF SAMPLE B AND E AT VARIOUS pH VALUES

| *pH* | *Specific Rotation* |
|---|---|
| 1.9 | − 64.2 |
| 2.6 | − 57.2 |
| 5.0 | − 44.0 |
| 9.7 | − 38.8 |
| 11.3 | − 38.4 |

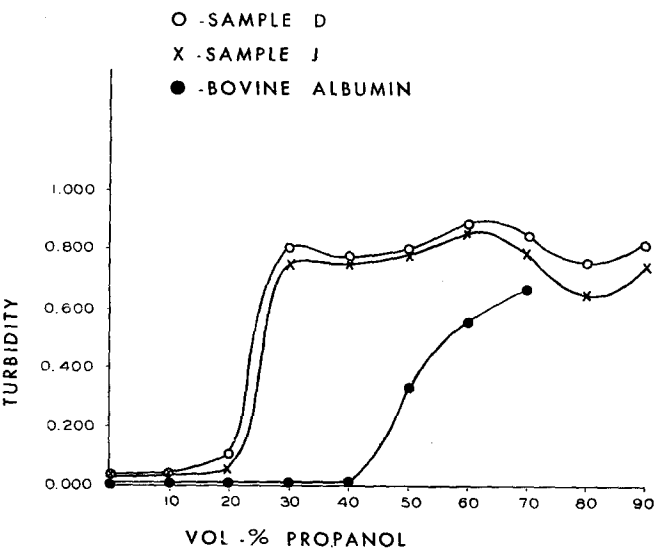


Fig. 1. Flocculation of Bence-Jones protein at various concentrations of *n*-propanol. The concentration of protein is 0.1 % and the temperature 23–24° C. Readings taken after 20 hours.

*Homogeneity.* The criterion used to ascertain the degree of homogeneity in various samples was based on flocculation with propanol. JIRGENSONS[19] observed in coagulation series with increasing amounts of propanol (at a constant pH and constant protein concentration) mixtures of proteins showed in the series two or more turbidity maxima at different propanol concentrations. The method, as applied to some of the samples of Bence-Jones protein revealed that neither sample was homogeneous. For comparison, crystalline bovine albumin was also studied and the results are shown in Fig. 1.

*References p. 632.*

## DISCUSSION

The discrepancies in properties of Bence-Jones protein as reported by different investigators may be ascribed to the multiplicity of procedures used for isolation. The results of this study points clearly to significant differences in properties of various samples isolated from the urine of one patient but collected at different times and by using different methods for the isolation of the Bence-Jones protein. Moreover, the isolated protein which appears as a homogeneous component in paper electrophoresis behaves as a mixture in flocculation tests. The presence of methionine in our samples is in agreement with other reports. The amino acid composition of the Bence-Jones protein resembles the composition reported by other investigators. However, some discrepancies exist, among which the content of serine and threonine are conspicuous. We find much lower values for serine and higher for threonine than those reported by ROBERTS and associates[8]. A similar discrepancy exists in the concentration of glutamic acid and phenylalanine. Some significant variations in amino acid composition were observed in the three samples obtained by heat coagulation at different temperatures (I, $I_2$, and $I_3$ in Table II). This variation seems to indicate that partial fractionation of Bence-Jones protein has been accomplished. Other differences in properties were found as shown in the experimental section, but they could be ascribed to denaturation that may have occurred during the isolation procedure. However, denaturation may be ruled out on the basis of viscosity measurements. The intrinsic viscosity values of 0.03 to 0.04 indicate that we have a non-denatured spheroprotein. Furthermore, the conditions used in the isolation from urine were certainly very mild.

## ACKNOWLEDGEMENT

The authors are grateful to Mr. ROBERT FUERST for carrying out the microbiological assays.

## SUMMARY

1. Several samples of Bence-Jones protein were isolated from urine of a patient with multiple myeloma. The isolation was carried out by different methods and from different urine specimens of the same patient. A sample of the same protein was also isolated from serum of the patient.

2. The isoelectric point, acid-base binding capacity, viscosity, optical rotation, and amino acid composition of the various samples were determined. Significant variations in properties and chemical composition were found for several samples.

3. The homogenity of the isolated Bence-Jones protein was determined by paper electrophoresis and by flocculation with propanol. The samples tested were found to give one single component by paper electrophoresis, but behaved like a mixture of proteins in the flocculation test.

## RÉSUMÉ

1. Plusieurs échantillons de protéines de Bence-Jones ont été isolées de l'urine d'un malade ayant un myelome. L'isolement a été fait par des méthodes différentes, utilisant différents échantillons d'urine du même malade. Un échantillon de la même protéine a été isolé du serum du malade.

2. Le point iso-électrique, la capacité d'attachement acide-base, la viscosité, la rotation optique et la composition en amino acides des échantillons différents ont été déterminés. Des différences sensibles dans la propriété et la composition chimique ont été trouvés pour des échantillons différents.

3. L'homogénéité des protéines de Bence-Jones isolées a été déterminée par l'électrophorèse sur papier et par la flocculation au propanol. Les échantillons examinés se sont comportés comme une protéine seule à l'électrophorèse sur papier et comme un mélange de protéine à l'examen par la flocculation.

*References p. 632.*

## ZUSAMMENFASSUNG

1. Mehrere Proben von Bence-Jones Protein wurden vom Harn eines Patienten mit Multiple Myeloma isoliert. Die Isolierung wurde nach verschiedenen Methoden von verschiedenen Harnproben des Patienten ausgeführt. Eine Probe von demselben Protein wurde auch vom Blutserum des Patienten hergestellt.

2. Der isoelektrische Punkt, Bindungsfähigkeit von Säure und Alkali, die Viskosität, und der Gehalt von Aminosäuren wurden an verschiedenen Proben des Proteins bestimmt. Beträchtliche Unterschiede wurden in den genannten Eigenschaften und in der chemischen Zusammensetzung gefunden.

3. Die Einheitlichkeit der Proben wurde durch mikroelektrophoretische Versuche und durch Ausflockung mit Propanol bestimmt. Es wurde gefunden dass das Protein elektrophoretisch einheitlich ist, sich dagegen in den Flockungsversuchen wie eine uneinheitliche Substanz benimmt.


## REFERENCES


1 A. MAGNUS-LEVY, *Z. Physiol. Chem.*, 243 (1936) 173.
2 A. B. GUTMAN, *Advances in Protein Chem.*, 4 (1948) 155.
3 R. W. RUNDLESS, G. R. COOPER, AND R. W. WILLET, *J. Clin. Invest.*, 30 (1951) 669.
4 F. G. HOPKINS AND H. SAVORY, *J. Physiol.*, 42 (1911) 189.
5 H. O. CALVERY AND R. H. FREYBERG, *J. Biol. Chem.*, 109 (1935) 739.
6 J. DEVINE, *Biochem. J.*, 35 (1941) 433.
7 C. E. DENT AND G. A. ROSE, *Biochem. J.*, 44 (1949) 410.
8 E. ROBERTS, G. B. RASAMARA, AND H. B. LEWIS, *Proc. Soc. Exptl. Biol. Med.*, 74 (1950) 237.
9 E. L. DURRUM, *J. Am. Chem. Soc.*, 72 (1950) 2943.
10 H. D. CREMER AND A. TISELIUS, *Biochem. Z.*, 320 (1950) 273.
11 B. VON BONSDORFF, H. GROTHER, AND T. PACKALEN, *Folia Haematologica*, 59 (1938) 191.
12 P. E. SCHURR, H. T. THOMPSON, L. M. HENDERSON, J. N. WILLIAMS, AND C. A. ELVEHJEM, *J. Biol. Chem.*, 182 (1950) 39.
13 J. L. D. SHAW AND W. D. MCFARLANE, *J. Biol. Chem.*, 132 (1940) 387.
14 H. NEURATH AND F. W. PUTNAM, *J. Am. Chem. Soc.*, 66 (1944) 692.
15 B. JIRGENSONS, *J. Polymer Sci.*, 5 (1950) 179.
16 M. L. GROVES, N. J. HIPP, AND T. L. MCMEEKIN, *J. Am. Chem. Soc.*, 73 (1951) 2790.
17 L. K. CHRISTENSEN, *Nature*, 163 (1949) 1003.
18 A. J. ALMQUIST AND D. M. GREENBERG, *J. Biol. Chem.*, 105 (1934) 519.
19 B. JIRGENSONS, *Biochem. Z.*, 268 (1934) 404.


Received April 22nd, 1952