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INCORPORATION OF N¹⁵-L-ASPARTIC ACID INTO THE ABNORMAL SERUM
AND URINE PROTEINS OF MULTIPLE MYELOMA
(STUDIES OF THE INTER-RELATIONSHIP OF THESE PROTEINS)¹

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In a consideration of the abnormalities of protein metabolism observed in multiple myeloma, a number of fundamental problems remain unsolved. To state just two of these, it remains to be determined (a) precisely what relationship the abnormal serum globulins of myeloma bear to normal serum gamma globulin, and (b) what is the nature of the possible inter-relationship between the abnormal serum protein and urinary (Bence-Jones) proteins of myeloma when both are present in a particular case. The electrophoretic homogeneity of these abnormal serum and urinary proteins constitutes one of their major physicochemical features. Using this criterion of electrophoretic homogeneity to identify these abnormal proteins, it has been found (1-3) that approximately one-half of a group of one hundred myeloma patients has both a serum and a urine protein abnormality; another one-third of these cases shows only a serum abnormality, with no characteristic proteinuria, and the remaining one-sixth of the cases exhibits a discrete urine protein peak with no abnormal protein peak demonstrable in the serum.

The present study was designed to elucidate the inter-relationship between the serum and urine proteins in the first group of cases, *i.e.*, in those patients with both a serum and a urine abnormality. For this purpose, an isotopically-labelled amino acid was administered to a patient with multiple myeloma and the incorporation and turnover of this label was followed in these two proteins (Ms hereinafter designates the abnormal serum globulin, and Mu the urinary [Bence-Jones] protein). If the time-characteristics of the isotope

curves obtained from Ms and Mu satisfied the criteria for a precursor-product relationship (Ms as precursor, Mu as product), it would be supportive, although not conclusive evidence for such an inter-relationship.

SUBJECT AND METHODS

Case history

The subject (I. A.) was a 50-year-old colored female, admitted to the Delafield Hospital in April, 1954, with a ten-month history of increasingly severe low back pain. Past medical history was non-contributory. In 1951, she was found to have an anemia, the origin of which was obscure. Because of back pain she had been admitted to another hospital in March, 1954, where work-up had revealed: Hgb. 6.2 Gm.; Bence-Jones proteinuria; blood urea nitrogen, 10.7 mg. per cent; total serum protein, 10.8 Gm. per cent; A/G, 4.2/6.6; myeloma cells in bone marrow; and x-ray evidence of a pathological fracture of the third lumbar vertebral body.

When transferred to the Delafield Hospital one month later, laboratory investigation disclosed: Hgb. 4.9 Gm.; wbc 2,800, neutrophils 55 (15-40), lymphocytes 32, eosinophils 3, monocytes 10; platelets 164,000; non-protein nitrogen 23 mg. per cent; ESR 160 mm. per hr. Paper electrophoresis of the serum and urine (Figure 1) confirmed the presence of an electrophoretically homogeneous abnormal protein in both the serum and urine. The serum component (Ms) had the electrophoretic mobility of a γ -globulin; Mu was of beta mobility. Ms, when stained for carbohydrate by the periodic acid-Schiff technique (4), gave a strongly positive reaction, whereas Mu was Schiff-negative (Figure 1). Iliac bone marrow aspiration revealed 50 per cent myeloma cells. Widespread osteoporosis and osteolytic lesions were seen on skeletal survey.

Transient symptomatic benefit was obtained from a course of radiotherapy to the lumbar spine and transfusions in May, 1954, but there was roentgenographic evidence of overall disease progression. A further palliative course of 1,000 r. to the thoracic spine was administered in early September, 1954. No urethane or other chemotherapeutic agent was administered prior to the isotope study. Despite two transfusions in the two weeks pre-

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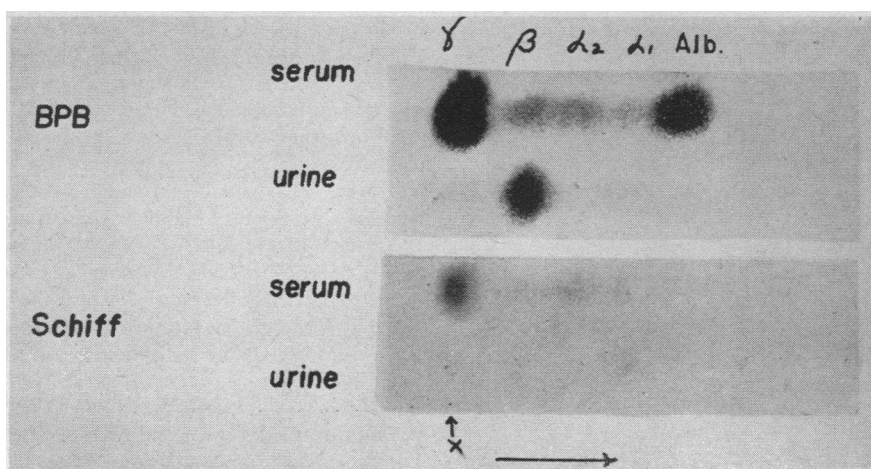


FIG. 1. PAPER ELECTROPHORETIC PATTERNS OF THE SERUM AND URINE OF PATIENT I. A., STAINED FOR PROTEIN WITH BROMPHENOL BLUE (BPB) AND FOR CARBOHYDRATE WITH THE SCHIFF TECHNIQUE

"X" indicates the site of sample application, and the arrow designates the direction of migration.

ceding the study, the blood count on the day of isotope administration was: Hgb., 7.0 Gm.; rbc, 2.47 million; wbc, 2,500; platelets 88,000. Further data at this time (9/24/54): Non-protein nitrogen, 43 mg. per cent; uric acid, 4.1 mg. per cent; calcium, 12.8 mg. per cent.

The isotope study was well tolerated, and, throughout the 17-day observation period, appetite and food intake were excellent. An intercurrent cystitis secondary to the presence of an indwelling catheter responded satisfactorily to antibiotics. The effect of this cystitis on the qualitative nature of the proteinuria, and the procedural changes necessary for urine protein separations, are described below. Three transfusions of packed red blood cells from 500 cc. of whole blood were given during the 17-day period.

Following the isotope study, a therapeutic trial of cortisone and subsequently a course of urethane were administered with slight subjective (but no objective) evidence of benefit. Urethane was discontinued after 6 weeks (total dose 105 Gm.) because of pancytopenia. Two weeks prior to death, the patient developed nitrogen retention, and despite supportive measures she expired on 28 December 1954.

Autopsy confirmed the widespread osseous destruction with myeloma tissue. The kidneys showed the tubular epithelial degenerative changes and the proteinaceous casts considered typical for Bence-Jones protein damage. Fine vacuolization of the tubular epithelial cells was also seen. The immediate cause of death appeared to have been an extensive bilateral lobular pneumonia.

Physico-chemical characteristics of the subject's myeloma serum (Ms) and urinary (Mu) proteins

Figure 2 shows the moving boundary electrophoretic patterns of the whole serum, and Figure 3, the electro-

phoretic and ultracentrifugal diagrams of the isolated serum (Ms) and urine (Mu) proteins. Throughout the experimental period, the total serum protein concentration remained at 14 Gm. per cent with the following percentage distribution of components: albumin 15.9, alpha-1 globulin 2.4, alpha-2 globulin 4.9, beta-globulin 6.1, gamma-globulin (Ms) 70.7 per cent. Thus, the Ms component was present at a concentration of approximately 10 Gm. per cent. The electrophoretic mobility of Ms in Veronal buffer, pH 8.6, ionic strength 0.1, was 1.3×10^{-5} cm.² sec.⁻¹ volt⁻¹. The sedimentation constant $S_{20,w}$ of Ms = 6.0 S. Mu had a mobility of 2.7×10^{-5} cm.² sec.⁻¹ volt⁻¹, and a sedimentation constant $S_{20,w}$ of 3.5 S. The mono-dispersity of these proteins is apparent from the

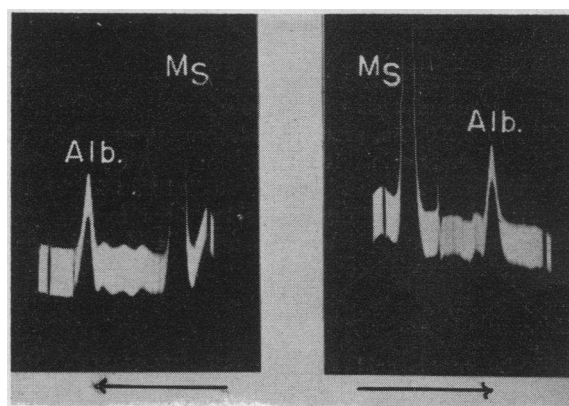


FIG. 2. ELECTROPHORETIC PATTERNS, ASCENDING (LEFT) AND DESCENDING (RIGHT) BOUNDARIES OF WHOLE SERUM OF SUBJECT I. A., OBTAINED IN VERONAL BUFFER, pH 8.6

The arrows indicate the direction of migration.

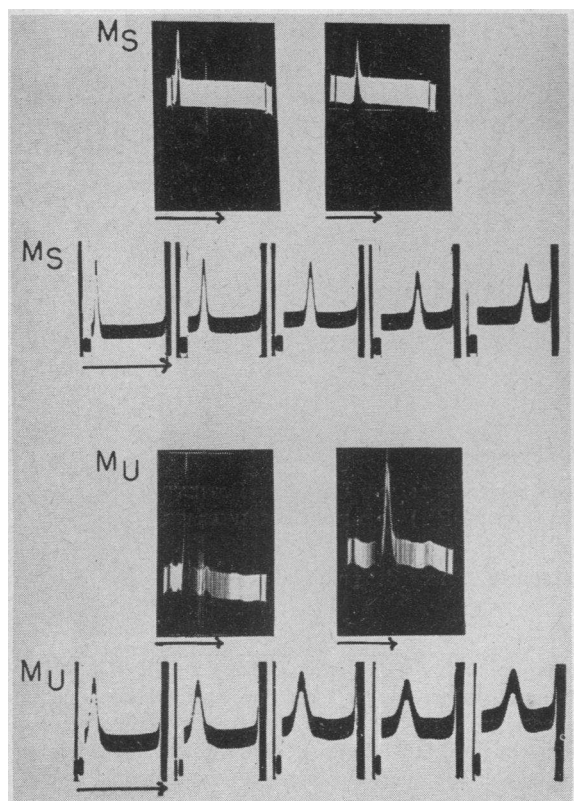


FIG. 3. ELECTROPHORETIC AND ULTRACENTRIFUGAL PATTERNS OF MS AND MU

The electrophoretic patterns were obtained in Veronal buffer, pH 8.6, and descending boundaries are shown. For Ms, the electrophoretic patterns were photographed at 15 and 85-minute intervals; for Mu, at 38 and 80 minutes. The arrows indicate the direction of migration.

In the ultracentrifugal patterns, sedimentation proceeds to the right as indicated by the arrows, and all photographs were taken at 16-minute intervals.

electrophoretic and ultracentrifugal diagrams. End amino group analysis² of Ms disclosed: glutamic acid, 1.61 M/160,000 Gm. protein; and phenylalanine, 1.40 M/160,000 Gm. protein. It is noteworthy that this is the only myeloma protein of over 20 studied thus far by Dr. Putnam in which phenylalanine has been found as an amino end group. N-terminal amino acid analysis of Mu was not carried out.

Experimental protocol

N^{15} -L-aspartic acid ($\text{COOH}\cdot\text{CH}_2\cdot\text{CH}\cdot N^{15}\text{H}_2\text{COOH}$) was prepared from fumaric acid and ammonia containing

² This N-terminal amino group analysis was kindly performed by Dr. Frank W. Putnam, formerly of the Department of Biochemistry, University of Chicago, and now Professor of Biochemistry, University of Florida College of Medicine, Gainesville, Florida.

N^{15} in the presence of *E. coli* according to the method of Wu and Rittenberg (5). A single dose of 10.6 Gm. of N^{15} -L-aspartic acid, containing 31 per cent excess N^{15} was administered along with 2 Gm. of NaHCO_3 immediately preceding a breakfast of eggs, toast, milk and coffee. No untoward gastrointestinal side-effects were experienced. Food intake throughout the experimental period was satisfactory. Blood samples were obtained at 1, 2.5, 4, 8, 12, 16, 20, 24, 30, 36 and 48 hours, twice daily on the third, fourth and fifth days, and daily thereafter to the termination of the experiment on the seventeenth day. An indwelling bladder catheter permitted complete and precisely timed urine collections. All stools were collected for 6 days.

Serum proteins were fractionated by dilution with 3 volumes of 0.1 M NaCl and dialysis at 2 to 3°C against 1.64 M ammonium sulfate buffered to pH 7.2 as outlined by Grisolia and Cohen (6). The abnormal myeloma globulin (Ms) precipitates were washed twice with 1.64 M ammonium sulfate dissolved in 0.15 M NaCl and re-precipitated with 5 per cent trichloroacetic acid (TCA). The proteins in the supernate from the ammonium sulfate dialysis, comprising principally albumin, with approximately 15 per cent alpha and beta globulins, were precipitated with 5 per cent TCA. This fraction is hereinafter designated Alb. (α , β) in recognition of its residual contamination with these alpha and beta globulin constituents. All TCA-precipitated proteins were washed a minimum of five times with 5 per cent TCA to remove non-protein nitrogenous constituents.

The urinary protein preceding the experiment and during the first two days of the study consisted almost exclusively of the abnormal, electrophoretically homogeneous, Mu component. Accordingly, the urine samples during this initial period were half-saturated with ammonium sulfate, and the precipitated protein re-dissolved in water, precipitated with 5 per cent TCA, dissolved again in 0.75 M NaOH, re-precipitated with TCA, and washed five times with 5 per cent TCA. With the development of the intercurrent cystitis on the second day of the study, there was gross hematuria and contamination of Mu with blood protein components. It thus became necessary to carry out an extensive fractionation procedure on the urine samples subsequent to the second day, as follows: (a) urine acidified to pH 5.5 and half-saturated with ammonium sulfate; (b) the resultant precipitate dissolved in water, followed by a 48-hour water dialysis (at 10°C) which precipitated hemoglobin and small amounts of other globulins, leaving Ms, Mu and albumin in solution; (c) precipitation and separation of Ms globulin by dialysis against 1.64 M ammonium sulfate at pH 7.2; (d) removal of ammonium sulfate in the supernate by water dialysis; (e) concentration by osmotic dialysis against 25 per cent polyvinylpyrrolidone; (f) precipitation of Mu and albumin with 5 per cent TCA; and (g) washing five times in 5 per cent TCA. Electrophoretic analysis of these samples after step (e), *i.e.*, after separation of Ms and prior to TCA precipitation, demonstrated them to be comprised of 85 per cent of Mu, and 15 per cent

albumin and alpha globulins, the latter representing the extent of residual contamination with serum protein constituents. Further purification of these urine protein samples could not be carried out due to the limitations of sample size. It will be seen that this complication did not appreciably alter the form of the Mu isotope concentration curve.

Glutamic and aspartic acids were isolated from the TCA-precipitated proteins after hydrolysis with 6 N HCl using the basic polyamine, formaldehyde resin (Amberlite IR-4) according to the technique described by Cannan (7). Urea (both serum and urine) was isolated as the di-xanthydryl-urea derivative. Urinary ammonia was liberated with saturated potassium carbonate and transferred by aeration into boric acid. The Kjeldahl method was employed for all nitrogen determinations. N^{15} abundance was determined with the mass spectrometer.

RESULTS

Over 70 per cent of the administered N^{15} was excreted in the urine as urea nitrogen within the

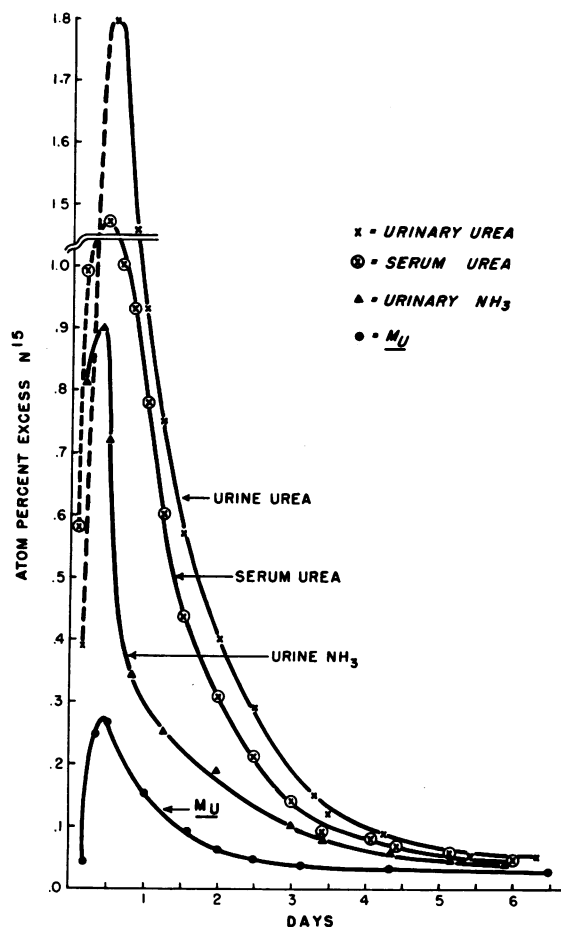


FIG. 4. N^{15} ABUNDANCE IN SERUM AND URINARY UREA NITROGEN, URINARY AMMONIA AND THE MYELOMA URINARY (MU) PROTEIN

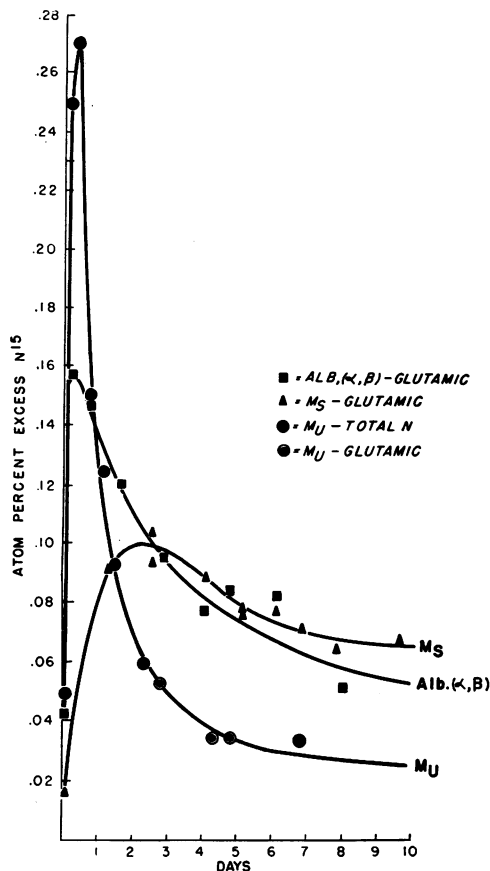


FIG. 5. N^{15} ABUNDANCE IN ▲ MS-GLUTAMIC ACID, ■ ALB. (α , β)-GLUTAMIC ACID, ● MU TOTAL NITROGEN AND ○ MU GLUTAMIC ACID

first three days. Another 6 per cent appeared as urinary urea nitrogen between the third and the eighth days. Urinary ammonia nitrogen accounted for only one per cent of the N^{15} loss, and total stool nitrogen for another 4.8 per cent. Thus, over 80 per cent of the administered label was excreted as non-protein nitrogen.

The isotope abundance curves for serum and urinary urea, urinary ammonia and for the Mu protein are shown in Figure 4. The Mu curve is seen to be essentially similar in form and time-characteristics to the urea and ammonia curves, indicating that the metabolic turnover of this abnormal protein proceeds at a rate comparable to that of the non-protein nitrogenous substances.

The isotope curves for Ms, Alb. (α , β), and Mu are shown in Figure 5. By contrast with the rapid incorporation and decline of isotope in Mu, the maximal isotope concentration in Ms was not

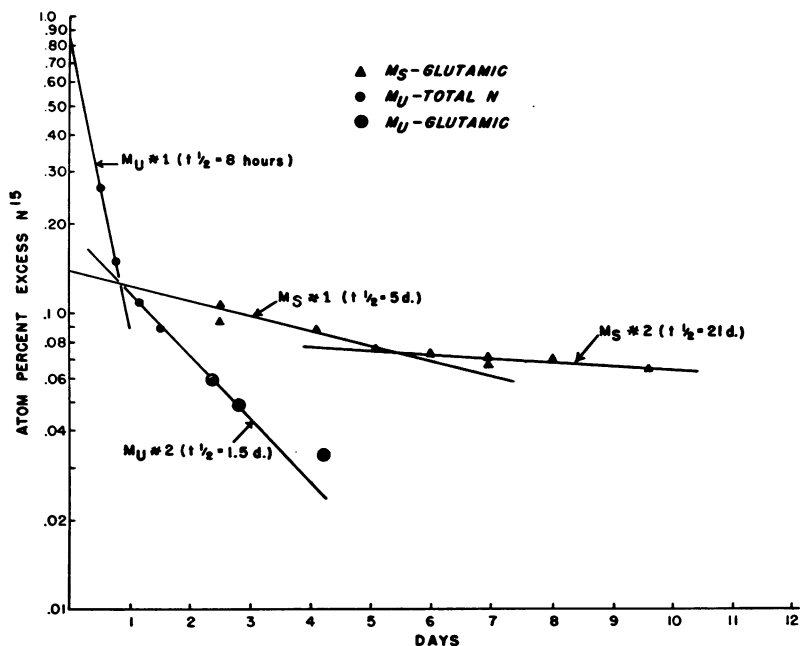


FIG. 6. SEMI-LOGARITHMIC PLOT OF N^{15} ABUNDANCE DATA FOR ▲ MS-GLUTAMIC ACID, ● MU-TOTAL NITROGEN AND ○ MU-GLUTAMIC ACID

reached until the second day, and the subsequent decline proceeded at a much slower pace than for Mu. The isotope curve for Alb. (α , β) was intermediate in its time-characteristics between these two myeloma protein curves, showing faster rate of decline than Ms, but considerably slower than Mu.

Semi-logarithmic plots of the declining portions of the Ms and Mu isotope curves are shown in Figure 6. In both instances, the data appear to show the best fit to two separate exponential functions. For Ms, the first exponential (E^1) has a $t_{1/2}$ of 5 days; the second (E^2), a $t_{1/2}$ of 21 days. For Mu, (E^1) has a $t_{1/2}$ of 8 hours, and (E^2), of 1.5 days. The Alb. (α , β) isotope curve (not shown in Figure 6) follows a similar double exponential decline pattern, with the $t_{1/2}$ of (E^1) equal to 3.5 days; $t_{1/2}$ of (E^2) equal to 12.5 days.

The initial rapid rates of decline in isotope concentration in these three proteins (the E^1 s) undoubtedly represent the net resultant of several functions of isotope distribution and dilution into amino acid and protein pools of different magnitudes proceeding concomitantly with protein degradation and excretion (Mu). The fact that single exponential functions approximate the data during these early periods is probably fortuitous. The

second decay functions should more nearly reflect the respective rates of protein degradation, with the probable implicit error of isotope recycling. In the case of Mu, there exists the added factor of excretion. The extent to which the isotopic label is liberated by protein breakdown back into the metabolic nitrogen pool and reincorporated into these same proteins is impossible to estimate.

Figure 7 shows the isotope curves for Ms glutamic, aspartic and total protein nitrogen. The time-characteristics of these three curves are essentially similar, supporting the postulate of uniformity of turnover of the formed protein molecule, and, as expected, the isotope abundance in the individual amino acids exceeds the N^{15} concentration in total protein nitrogen. It is particularly noteworthy that glutamic N^{15} concentration was uniformly higher than aspartic N^{15} , although aspartic had been administered.³ This concentration

³ Wu and Rittenberg (5) have noted the same concentration differential, *i.e.*, plasma and tissue protein glutamic N^{15} exceeding aspartic N^{15} after N^{15} -aspartic administration. This is consistent with the recognized rapid rate at which the amino nitrogen of aspartic acid exchanges with the nitrogen of the metabolic pool. Aspartic acid is so rapidly deaminated that its amino group may be considered to behave metabolically like ammonia.

difference for glutamic, aspartic and total protein nitrogen was also found in the Alb. (α , β) samples (Figure 8).

The Mu samples during the first 48 hours were too small to permit isolation of glutamic and aspartic acids, and only total protein N^{15} could be measured. When the pooled Mu samples of the third and subsequent days were available for glutamic and aspartic isolations, these were performed. As indicated in Figures 4, 5, and 6, the glutamic N^{15} values appeared to follow the curve of the earlier Mu-total N points. In these later Mu samples, the glutamic N^{15} values and the total Mu protein N^{15} values were not significantly different within the limits of accuracy of the analytic methods.

DISCUSSION

A number of physico-chemical studies of myeloma serum and urinary proteins (8-10) have documented a consistent difference in molecular weights of these two constituents. Although the serum globulins vary considerably in molecular weight in individual cases, they most frequently have been found to have sedimentation and diffusion constants indicative of molecular weights in the range of 160,000. The urinary Bence-Jones proteins, by contrast, are usually of much smaller size, with mean molecular weights in the range of 35,000 to 40,000. The sedimentation constants of Ms and Mu of the patient in this present study are consistent with molecular weights in these respective ranges.

A study (4) of the carbohydrate content of myeloma serum and urine proteins in our laboratory indicated that, whereas the myeloma serum

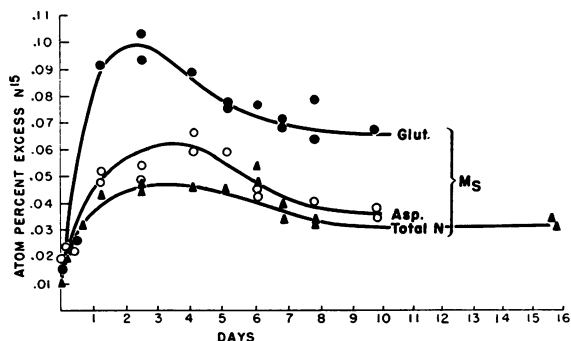


FIG. 7. N^{15} ABUNDANCE IN THE ● GLUTAMIC, ○ ASPARTIC AND TOTAL PROTEIN NITROGEN ▲ FRACTIONS OF MS

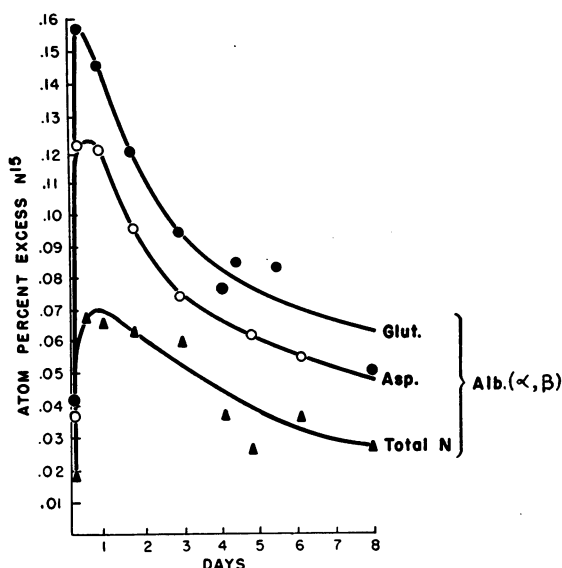


FIG. 8. N^{15} ABUNDANCE IN THE ● GLUTAMIC, ○ ASPARTIC AND TOTAL PROTEIN NITROGEN ▲ FRACTIONS OF ALB. (α , β)

globulins contained a significant quantity of conjugated Schiff-positive material, presumably protein-bound hexose and hexosamine, the urinary myeloma proteins were apparently devoid of these substances. These observations were subject to at least three possible interpretations, namely (I) that Ms and Mu are two distinct protein moieties, independently elaborated, and independently metabolized, (II) that the urinary protein (Mu) represents a fragment of the serum globulin (Ms) after removal of the conjugated carbohydrate moiety, or (III) that the protein excreted in the urine represents a portion of a larger body pool of this material which is functioning as a precursor of the serum globulin, *i.e.*, prior to conjugation with its carbohydrate moiety.

If the second postulate were correct, *i.e.*, Ms is the precursor of Mu, the isotope concentration maximum in Ms should have preceded and exceeded the isotope maximum in Mu. Since just the opposite relationships were observed between these two turnover curves, postulate II would appear to be invalid, leaving the choice between I and III. As will be seen shortly, after consideration of other available data, this choice cannot, as yet, be clearly and conclusively made.

Essentially similar results to those herein reported have been obtained by Putnam, Hardy,

TABLE I

	Labelled compound	Serum globulin		Urinary (Bence-Jones) protein		Albumin
		S ₂₀	T _½	S ₂₀	T _½	T _½
Putnam and Hardy (11)	NH ₂ ·CH ₂ ·C ¹³ OOH	6.2 : 8.8	17-20 d.	3.08	18 hours	No data
Hardy and Putnam (12)	N ¹⁵ H ₂ ·CH ₂ ·COOH	6.6 (Cryo-)	? about 20 d.	2.14	1.8 d.	No data
This paper	HOOC·CH ₂ ·CH·N ¹⁵ H ₂ COOH	6.0	E ¹ = 5 d. E ² = 21 d.	3.5	E ¹ = 8 hrs. E ² = 1.5 d.	E ¹ = 3.5 d. E ² = 12.5 d.

Meyer, and Miyake (11-13) in studies using C¹³-glycine in one patient, N¹⁵-glycine in a second, and DL glutamic acid-1C¹⁴ in a third. A comparison of their results, from studies (11) and (12) with the data from the present study, is outlined in Table I. Several significant differences in the protocols of these three studies are notable, *viz.*, Putnam and Hardy's first subject (11) was receiving urethane throughout the experimental period; the subject of the second study had evidence of advanced renal damage and nitrogen retention, and had also received urethane just prior to the isotope experiment. This patient's serum protein was a cryoglobulin, with only aspartic acid in the N-terminal position. The subject of the study herein reported was selected because of the relatively typical nature of the abnormal serum and urine proteins exhibited, and the apparent freedom from the possible effect of prior therapy or renal damage. Despite these differences, and the fact that different amino acids were employed, the observed results in these three studies are in essential agreement with regard to the turnover rates of the myeloma proteins. It must be recognized that the biosynthetic labelling procedure employed in this study and in the studies of Putnam and his co-workers has, implicit within it, a serious source of error due to reincorporation of the isotope. This factor of recycling is primarily operative in the later time periods. Accordingly, the calculated half-lives must be considered as only very gross approximations to the true biological life-spans of these protein constituents. Within these clearly recognized limitations, however, one may speculate on the possible interpretations of the observed isotope turnover curves of Ms and Mu.

The extremely rapid turnover of Mu may in part be explained by the rapid sequestration of

this protein in the urine, and, hence, its non-availability for later reincorporation of "recycling isotope." The approximately 15 per cent residual contamination of the later Mu samples with albumin and minimal amounts of alpha globulins due to the intercurrent cystitis might be expected to have introduced an error in the direction of prolonging the observed half-life of the urinary protein, since the serum Alb. (α , β) fraction displayed a much slower turnover rate than that found for Mu.

An isotope turnover study of the albumin in the urine of a patient with nephrosis, comparing the isotope half-life in urinary albumin with serum albumin of the same subject, would probably represent the best reference with which to evaluate the function of urinary sequestration on the form of the isotope turnover curve. Although turnover studies of serum proteins in nephrosis have been reported, the urinary proteins have not been examined.

The half-life of serum Alb. (α , β) as measured in our myeloma subject was approximately 12.5 days. This is considerably shorter than the values for albumin of normal subjects reported from other studies (14, 15) which utilized biosynthetic labelling techniques.⁴ Thus, London (14) obtained a half-life for albumin of 20 days in normal subjects after feeding N¹⁵-glycine. Masouredis and Beeckmans (15) found comparable albumin half-

⁴ These data are more closely comparable to the experimental situation of this study than are the more extensive reports (15, 17, 18) of albumin half-life as measured by *in vitro* labelled I¹³¹-albumin. The values for half-life of I¹³¹-albumin are about one-half to one-fifth as long as the values for the biosynthetically labelled protein. This shorter life-span of the I¹³¹-albumin may reflect a significant alteration (? partial denaturation) of the albumin molecule in the *in vitro* labelling process, resulting in a shortened life-span.

lives of 27.6 days in a patient with polycythemia vera, and 39.4 days in a subject with inactive rheumatic heart disease when they employed orally administered C^{14} -glycine. Volwiler, Goldsworthy, MacMartin, Wood, Mackay, and Fremont-Smith (16) report a range of 23 to 44 days in half-life for albumin in normal subjects when they used orally administered S^{35} -cystine. Whereas the rapid turnover of the Alb. (α , β) fraction in our myeloma subject may partly be due to the contamination of albumin with alpha and beta globulins of more rapid turnover rates than albumin itself, it is suggestive of a true augmentation in the rate of albumin degradation in this derangement of protein metabolism. The lack of data on patients with other neoplastic diseases and associated hypoalbuminemia, however, prevents any speculation regarding the specificity or significance of this phenomenon.

An extensive range of values for the half-life of normal gamma globulin has been reported from the several laboratories engaged in these studies. As in the case of other plasma protein fraction life-span studies, differences in labelling techniques and methods of protein fractionation appear to exert a profound influence on final calculated values of turnover rates. Thus, Volwiler and his co-workers (16) using orally administered S^{35} -cystine obtained gamma globulin half-lives of 48 to 85 days in normal subjects, and 31 days in a patient with cirrhosis. A half-life range from 19 to 60 days for this fraction has been reported by Armstrong and his associates (19, 20). The half-life value of approximately 21 days for the Ms protein herein reported, and the comparable values for the myeloma serum globulins reported by Putnam and Hardy (11, 12) are not clearly divergent from the available isotope turnover data for normal gamma globulin fractions, but again it must be stressed that the differences in labelling and fractionation techniques preclude any reliable comparisons.

In conclusion, it may be stated that the *relative* rates of turnover of the abnormal serum and urine proteins in a case of multiple myeloma have been studied after the oral administration of N^{15} -L-aspartic acid, in an effort to ascertain whether the urinary protein could be a product or a fragment of the larger molecular-sized serum constituent.

Because the isotope concentration maximum of the urine protein (Mu) preceded and exceeded the isotope maximum in the serum globulin (Ms), the latter (Ms) cannot be construed to have been the precursor of the former (Mu). Having observed similar results in their isotope studies of those abnormal myeloma proteins, Putnam, Hardy, Meyer, and Miyake (11-13) have suggested that the opposite inter-relationship may exist, *i.e.*, that the Bence-Jones urinary proteins may represent precursors or abortive products of serum globulin synthesis. This alternative hypothesis is surely worthy of further consideration, but the present authors do not believe that the currently available experimental data justify this interpretation. It would appear that Mu is rapidly synthesized and rapidly excreted, whereas Ms is more slowly elaborated and degraded. The experimental evidence fails to establish that either protein is the precursor or product of the other.

SUMMARY

1. The rates of incorporation and degradation of serum albumin, the abnormal serum (Ms) and urine (Mu) proteins in a patient with multiple myeloma have been studied employing orally administered N^{15} -L-aspartic acid as the biosynthetic label.
2. The turnover rate of Mu was found to be extremely rapid, with a half-life of 1.5 days, whereas the myeloma serum globulin, Ms, was found to have a much slower turnover rate (half-life = 21 days).
3. The isotope concentration maximum in Mu preceded and exceeded the isotope maximum in Ms.
4. These data are interpreted as being incompatible with the hypothesis that the larger molecular-sized serum globulins of myeloma are precursors of the smaller urinary (Bence-Jones) proteins. They also fail to establish the opposite inter-relationship, *i.e.*, that the Bence-Jones proteins are "abortive precursors" of serum globulin synthesis.
5. Until further data prove to the contrary, the thesis that Ms and Mu are separate constituents, independently elaborated, would seem most readily acceptable.

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