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## Metabolism of Bence Jones Proteins \*

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Bence Jones proteins are molecules of about 40,000 mol wt present in the urine of many patients with plasma cell malignancy. These microglobulins, which are synthesized in plasma-cell tumors (1, 2), share many immunochemical and physicochemical features with the larger 6.6 S  $\gamma$ -globulins. That Bence Jones proteins are similar to or identical with components of the larger  $\gamma$ -globulins has been suggested (3-5). Evidence has been recently obtained that microglobulin components similar to Bence Jones proteins may be present in normal urine (6-8) and serum (9).

The rate of synthesis and metabolic fate of these proteins are poorly understood, both in malignant plasma cell diseases and in the normal state. Putnam and Hardy (10) and Osserman and associates (11) observed that newly synthesized Bence Jones protein was excreted rapidly by patients with multiple myeloma; they suggested that massive proteinuria (as high as 50 g per day) may be present and that Bence Jones proteins are excretory products of protein metabolism. It was not clear in these studies whether all the newly synthesized Bence Jones protein was excreted or whether only a part was excreted and the remainder catabolized. Evidence that some Bence Jones protein is catabolized was noted by Meyer and Putnam (12), who reported that only a fraction of injected  $C^{14}$ -labeled Bence Jones protein appeared in the urine of a single patient with multiple myeloma when he was reinjected with his own labeled Bence Jones protein. Rabbits given the same protein also excreted only a part of the injected protein intact. Perkins, Doherty, and Towbin (13) reported after two studies with intravenously administered  $I^{131}$ -labeled Bence Jones protein that only 20% of the injected  $I^{131}$  was excreted bound to protein, the remaining 80% being excreted as iodide, presumably representing

catabolism of the protein. In studies with Bence Jones protein formed by the transplantable mouse plasma-cell tumor MPC-2, Humphrey and Fahey (14), injecting  $I^{131}$ -labeled Bence Jones protein into normal and tumor-bearing mice, found that 4 to 48% of the injected  $I^{131}$  was excreted bound to protein, the remainder appearing as iodide. These observations indicate that Bence Jones protein is catabolized as well as excreted in the urine. In addition, observations in ten patients with multiple myeloma and detectable levels of Bence Jones protein in the serum (15) indicate that Bence Jones protein might not be immediately cleared from the circulation and that other factors, such as renal function, might play a part in their metabolic behavior.

The present studies were undertaken to investigate the metabolic behavior of Bence Jones proteins. After the iv injection of  $I^{131}$ -labeled Bence Jones protein, the relative contribution of catabolism and urinary loss to the over-all metabolism of Bence Jones proteins was determined. The absolute turnover (synthetic rate) of Bence Jones protein was estimated in patients who were in a steady state of Bence Jones protein production and excretion. Bence Jones protein (BJ) turnover studies differ from studies of albumin and  $\gamma$ -globulin turnover in normal subjects in several important features. Urinary protein loss, impaired renal excretion of iodide, and rapid rates of protein catabolism (i.e., very rapid relative to the rates of distribution of the protein and of the excretion of the radioiodide released from the protein) result in an accumulation of radioiodide in the body water. Therefore, the usual parameters of the protein turnover investigation were supplemented by determinations of protein-bound radioactivity and nonprotein-bound (predominantly free iodide) radioactivity in the plasma and urine at intervals of 1 day or less throughout the study.

Bence Jones protein metabolism was studied in patients with massive Bence Jones proteinuria, smaller amounts of urinary Bence Jones protein,

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or no detectable urinary Bence Jones protein. The subjects included patients with normal and abnormal renal function. The metabolic behavior of  $I^{131}$ -labeled Bence Jones protein from three patients was studied. In addition, because of the possibilities that urinary Bence Jones protein had been altered on passing through the kidneys and that studies employing labeled urinary Bence Jones protein might not reflect the metabolic behavior of newly synthesized protein, the metabolic behavior of serum Bence Jones protein was studied in two patients and compared with that of the urinary protein. Fifteen studies in ten patients form the basis of this report.

### Materials and Methods

*Clinical material.* Ten subjects, 50 to 65 years old, were hospitalized at the Clinical Center of the National Institutes of Health. Thirteen of the fifteen studies were conducted at the Metabolism Service of the National Cancer Institute. Urinary analyses included microscopic examination of the sediment, cell counts, and total 24-hour protein excretion. The urine was tested for Bence Jones protein by the techniques of Putnam and co-workers (16). Blood urea nitrogen (BUN), serum creatinine and uric acid levels, phenolsulfonphthalein (PSP) excretion, and creatinine clearance were also measured. Serum proteins were characterized by immunoelectrophoresis and paper electrophoresis in all cases and by starch gel electrophoresis and ultracentrifugation (17) in cases having multiple myeloma or macroglobulinemia. In each case with Bence Jones proteinuria, the protein was isolated from the urine and from the serum where present (15) and characterized by immunochemical and ultracentrifugal techniques.

Four patients (ZO, DR, SE, and HP) had multiple myeloma and were excreting 0.3 to 30.0 g of Bence Jones protein per day; the first three also had advanced renal insufficiency. Four patients with macroglobulinemia (MT, JB, FC, and JG) were studied, one (MT) also with Bence Jones proteinuria and renal insufficiency. Bence Jones proteinuria was not detected in the other three. Patient JG had malignant hypertension with renal insufficiency and was under treatment with antihypertensive agents. Patients FC and JB had normal renal function. Two patients without malignant disease of the plasma-cell system were studied, one (FL) with azotemia and no proteinuria and the second (NMc) with proteinuria but no azotemia. None of the patients evidenced excessive fluid retention.

*Preparation of radioiodine-labeled Bence Jones proteins.* Midstream or catheterized urine was collected aseptically from three patients (DR, ZO, and SE) excreting large quantities of Bence Jones proteins. The urine was passed immediately through a 0.22 Millipore

filter. It was dialyzed<sup>1</sup> for two successive 2-hour periods at 4° C against a hundredfold volume of sterile 0.01 M potassium phosphate buffer, pH 8. Separation of Bence Jones protein from other urinary proteins was achieved by anion exchange chromatography on diethylaminoethyl (DEAE) A-50 Sephadex.<sup>2</sup> Both the adsorbent and the buffer solutions had been autoclaved and were sterile and nonpyrogenic. Approximately 1 g of adsorbent was used per 100 mg of protein. The adsorbent was equilibrated with the 0.01 M phosphate buffer at pH 8. The dialyzed urine samples were applied to the column under air pressure of 10 pounds per square inch, and the protein was eluted by stepwise application of pH 8 potassium phosphate buffers of increasing molarity from 0.01 M to 0.10 M. The appearance of protein in samples of the effluent was determined by turbidity on addition of 10% trichloroacetic acid. The Bence Jones proteins of these three patients were eluted with 0.05 to 0.08 M potassium phosphate buffers, pH 8. The effluents were collected in 8-ml vol and were immediately filtered through a 0.22 Millipore filter into sterile vials.

Bence Jones protein was isolated from the sera of two patients (DR and ZO) by anion exchange chromatography on DEAE-sephadex. Eight ml of plasma from each patient was dialyzed against a hundredfold volume of 0.01 M potassium phosphate buffer, pH 8, as described for the urine samples. The bulk of the 6.6 S  $\gamma$ -globulins was eluted from the column in the first chromatogram fraction, and the Bence Jones proteins were eluted subsequently with 0.06 to 0.08 M potassium phosphate buffer, pH 8.

Preparation of the purified urinary or serum Bence Jones proteins required about 4 hours. The proteins were promptly labeled with reducing agent-free  $I^{131}$  by the iodine monochloride method of McFarlane (18). Free radioiodine was removed by dialysis against a sterile solution of 0.14 M NaCl, and the iodinated protein was filtered through a 0.22 Millipore filter. There was less than one mole of iodine per mole of protein (mol wt, 40,000) in the product. Sterile human albumin was added subsequently, providing a protein concentration of 3.5 g per 100 ml to prevent damage to the Bence Jones protein by selfradiation. Before administration, the iodinated proteins were found to be sterile and nonpyrogenic.

Each Bence Jones protein preparation was characterized by ultracentrifugation, starch gel electrophoresis, immunoelectrophoresis and Ouchterlony agar double diffusion tests with polyvalent antigamma globulin antiserum and antiwhole human serum antiserum (15, 17). The results are summarized in Table I and Figure 1. The proteins were seen as a single peak with a sedimentation coefficient ( $S_{20,w}$ ) of 3.4 to 3.8 S in the ultracentrifuge. They exhibited the characteristic reversible thermal insolubility and gave pH-heat precipitation curves of the beta type described by Putnam and associ-

<sup>1</sup> Cellulose casings, Visking Corp., Chicago, Ill.

<sup>2</sup> Lot no. TO-7874N, Pharmacia, Uppsala, Sweden.

TABLE I  
Properties of Bence Jones protein\* labeled with  $I^{131}$

BJ protein	Electrophoretic analysis† Mobility on paper	Ultra-centrifugation
	mm from origin	$S_{20,w}$
ZO urinary BJ	+15	3.8 S
ZO serum BJ	+15	3.7 S
DR urinary BJ	+ 2	3.8 S
DR serum BJ	+ 2	3.7 S
SE urinary BJ	+12	3.4 S

\* The ZO, DR, and SE Bence Jones proteins were immunochemical Type II proteins.

† Starch gel electrophoresis revealed that each of the Bence Jones proteins consisted largely of 1 component.

ates (16). The urinary Bence Jones protein preparations were found to be free of other proteins, but the serum preparations also contained some normal  $\gamma$ -globulin. The isolated serum Bence Jones protein had antigenic, electrophoretic, ultracentrifugal, and thermal behavior similar to that of the urinary protein from the same patient (15). The association of radioiodine with Bence Jones protein was established by quantitative measurement of radioactivity distribution after starch gel electrophoresis.

**Experimental protocol.** Five patients received a single preparation of  $I^{131}$ -urinary Bence Jones from patient SE, and another five subjects received a preparation of the labeled urinary Bence Jones from patient ZO. Two patients (ZO and DR) received preparations of their own serum and urinary Bence Jones proteins and the urinary protein from another patient in separate studies. Patients were given 0.5 ml of Lugol's solution every 8 hours to inhibit the thyroidal uptake of radioiodine. Approximately 5 mg of the iodinated protein containing 50  $\mu$ c of  $I^{131}$

was administered intravenously from a calibrated syringe. Plasma samples were obtained after 10 to 20 minutes, 3, 6, 9, and 12 hours, and then twice daily for the remainder of the study. Urine collections were made in 3-hour lots for the first 12 hours and then daily for the remaining 6 to 10 days of the study. The urines were collected and refrigerated with toluene added to each bottle. In addition, two 48-hour stool collections were obtained from each patient. Stool collections were then discontinued if no significant radioactivity was detected.

**Measurement of radioactivity.** Total and nonprotein-bound  $I^{131}$  radioactivities were determined in plasma and urine samples, the difference in the two giving protein-bound values. For determination of nonprotein-bound  $I^{131}$  in plasma, 3 ml water and 1 ml 60% perchloric acid solution were added to 2 ml plasma. The mixture was centrifuged, and 3 ml of clear supernatant fluid was placed in counting vials. To determine the nonprotein-bound  $I^{131}$  in urine, 1 ml 10% albumin solution and 2 ml 60% perchloric acid were added to 8 ml of the patient's urine. The material was centrifuged, and 4 ml of the clear supernatant fluid was placed in counting vials. Appropriate correction factors were applied so that the counts in the supernatant fluids were equivalent to the free iodide counts in 1 ml of whole urine or untreated plasma.

The plasma and urine samples were counted in an automatic gamma ray well-type scintillation counter employing a sodium iodide crystal. Standards made from dilution of the iodinated material given to each patient were counted concurrently with the plasma and urine samples. Stool samples, collected in paint cans, were brought up to a constant volume and were shaken on a paint can shaker. The homogenized samples were counted in a gamma ray scintillation counter with an appropriate standard to a counting accuracy of  $\pm 3\%$ .

**Data analysis.** The calculations used to determine rates of BJ catabolism and excretion required meas-

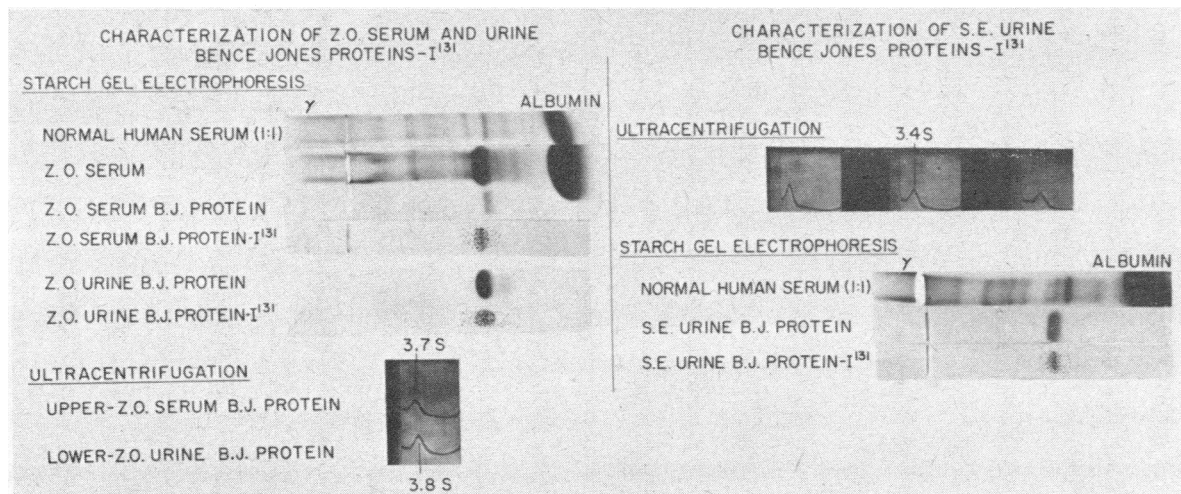


FIG. 1. CHARACTERISTICS OF ZO URINARY BENCE JONES PROTEIN, ZO SERUM BENCE JONES PROTEIN, AND SE URINARY BENCE JONES PROTEIN.

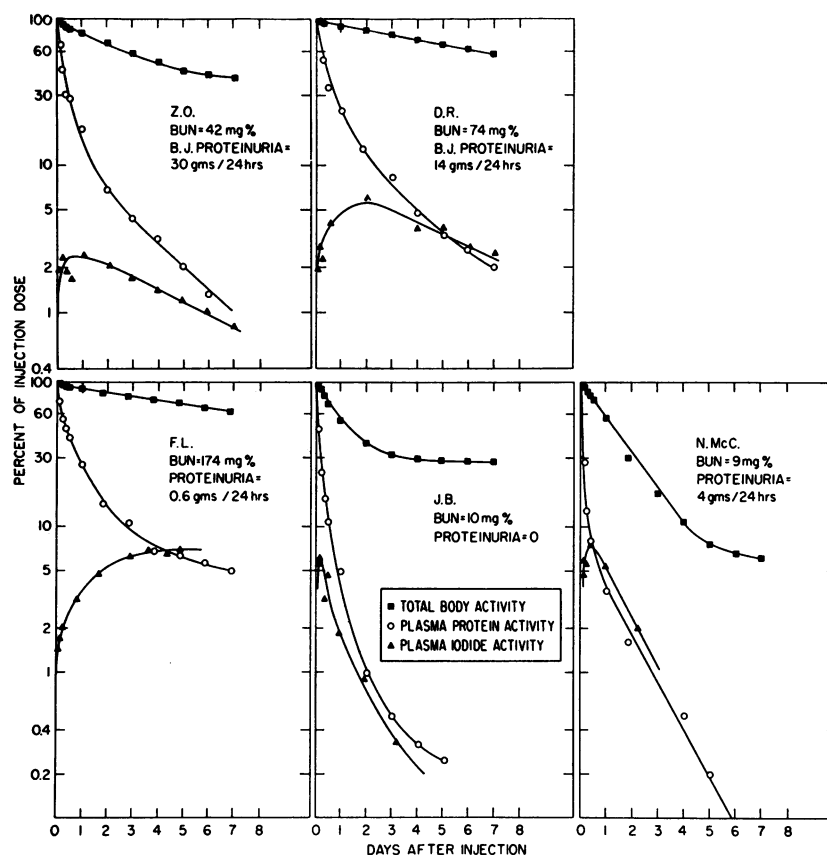


FIG. 2. METABOLISM OF  $I^{131}$ -LABELED URINARY BENCE JONES PROTEIN FROM DONOR ZO. The recipient's initials, blood urea nitrogen (BUN), and 24-hour urinary protein content are given in each box with the metabolic data.

urement of the plasma volume, intravascular BJ pool, intravascular and total body content of radioiodide, and urinary excretion of labeled BJ protein and radioiodide.

The plasma volume was determined by isotope dilution techniques. 1) Plasma volume = injected dose of  $I^{131}$ -labeled BJ/radioactivity per ml of plasma at zero time (determined by extrapolation of the semilogarithmic plot of the 10- and 20-minute serum values to the zero time intercept).

2) Total circulating BJ protein = plasma volume  $\times$  serum BJ concentration.

3) Total body radioactivity = the injected dose less cumulative excretion of radioactivity in the urine and stools. Daily excretion of radioactivity in the stools was always less than 0.2% of the dose.

4) The total circulating BJ activity and total circulating nonprecipitable activity = the respective serum activities per ml  $\times$  the plasma volume. The total body radioactivity, the total circulating Bence Jones radioactivity, and the total circulating nonprecipitable radioactivity were plotted as a percentage of the administered dose against time on semilogarithmic paper in Figures 2 to 4.

5) Total body nonprecipitable radioactivity = nonpre-

cipitable  $I^{131}$  per ml of plasma  $\times$  the plasma volume  $\times$  f, where f = the ratio of the total body iodide pool to the plasma iodide pool. The ratio f was not directly measured but was taken to be 8 (see Discussion).

The protein-bound radioactivity lost in the urine and the radioiodide released from the protein after catabolism were expressed as functions of the mean circulating  $I^{131}$  BJ activity. This method of analysis requires the assumption that catabolism occurs in the plasma compartment (or a compartment in rapid equilibrium with the plasma) but does not require that the total body pool of Bence Jones protein be known, that the pools be in equilibrium, or that the patient be producing Bence Jones protein.

6) Proteinuric rate, as percentage per hour = urinary protein-bound  $I^{131}$  excreted in a period  $\times$  100 / (integrated mean circulating protein-bound  $I^{131}$  in the period  $\times$  hours in the period). For the patients with Bence Jones proteinemia and proteinuria, the grams of Bence Jones lost in the urine per day may be calculated from the product of the daily proteinuric rate (Equation 6) and the total circulating Bence Jones protein (Equation 2). The values for the grams of BJ protein lost as proteinuria thus calculated were compared to the experimentally deter-

mined values for the grams of BJ proteins lost in the urine of these patients, thus providing a check on the accuracy of the estimate of the proteinuric rate.

7) Catabolic rate, as percentage per hour = nonprecipitable  $I^{131}$  released from BJ in a period  $\times 100 / (\text{integrated mean circulating protein-bound } I^{131} \text{ in the period} \times \text{hours in the period})$ . In this calculation the nonprecipitable  $I^{131}$  released from protein breakdown in a urine collection period equals the urinary excretion of radioiodide plus the change in the total body nonprecipitable radioactivity in that period, as determined from Equation 5.

The proteinuric and catabolic rates were determined from each urinary collection period; the values presented in tabulation results represent the mean value for all the periods for a given patient.

8) The ratio of proteinuric (P) to catabolic (C) rates was determined *a*) from the above values (ratio of Equation 6 to 7), or, alternatively *b*) by a modification of the cumulative excretion method used by Gitlin, Janeway, and Farr (19) for the analysis of the protein turnover in patients with nephrosis.  $P:C = \text{cumulative urinary excretion of precipitable } I^{131} \text{ over the whole period of the study} / \text{cumulative urinary excretion of nonprecipitable } I^{131} \text{ over the whole period of the study}$ . This method assumes that only a negligible quantity of nonprecipitable  $I^{131}$  remains in the body water at the completion of the

study. This was true in all cases except patient FL, who had significant retention of iodide in the body water. The determination of the P:C ratio from the cumulative excretion of radioactivity does not require that an estimate of the body iodide pool be made, nor that the curve of serum radioactivity be determined.

*Synthetic rate.* The absolute turnover of Bence Jones protein, which in a steady state equals the grams synthesized, may be determined from the product of the total circulating Bence Jones protein and the fraction of the intravascular BJ turning over per unit time. This technique requires an accurate determination of the proteinuric and catabolic rates, the plasma volume, and the serum BJ concentration. Alternatively, the absolute turnover of Bence Jones protein may be determined from the following formula:

9) *a*) BJ turnover = grams lost as proteinuria + grams catabolized. *b*) Grams lost as proteinuria/grams catabolized = P:C ratio. *c*) Grams catabolized = grams lost as proteinuria/P:C ratio. *d*) Substituting *c* in *a*, BJ turnover = grams lost as proteinuria + (grams lost as proteinuria/P:C ratio). This second method requires only that the P:C ratio and the grams of Bence Jones excreted per day be determined. The two methods of calculating the absolute turnover rate require different determinations and give slightly different results. The second method

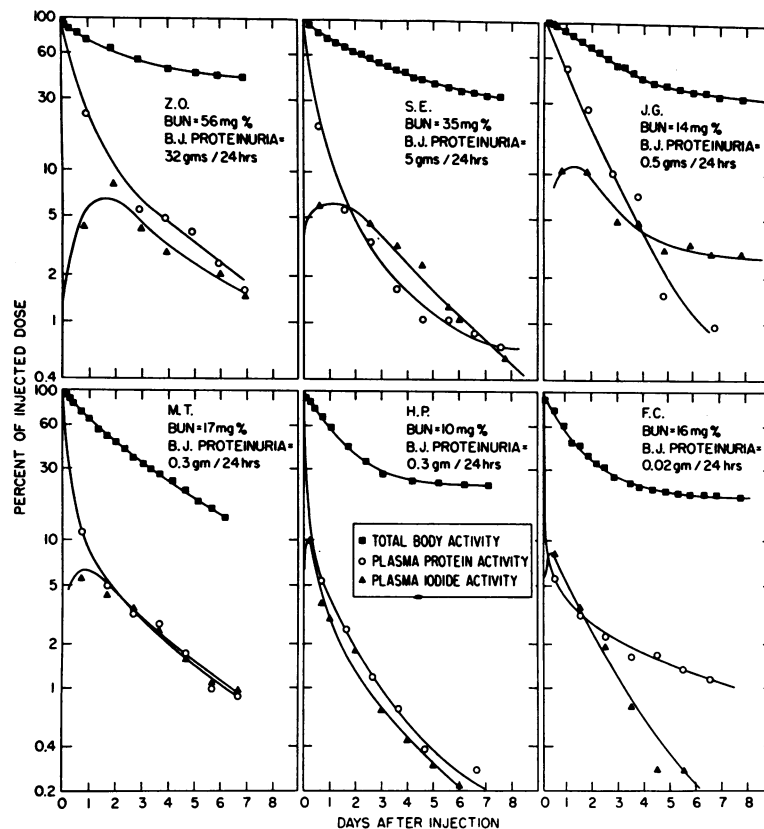


FIG. 3. METABOLISM OF  $I^{131}$ -LABELED URINARY BENCE JONES PROTEIN FROM DONOR SE.

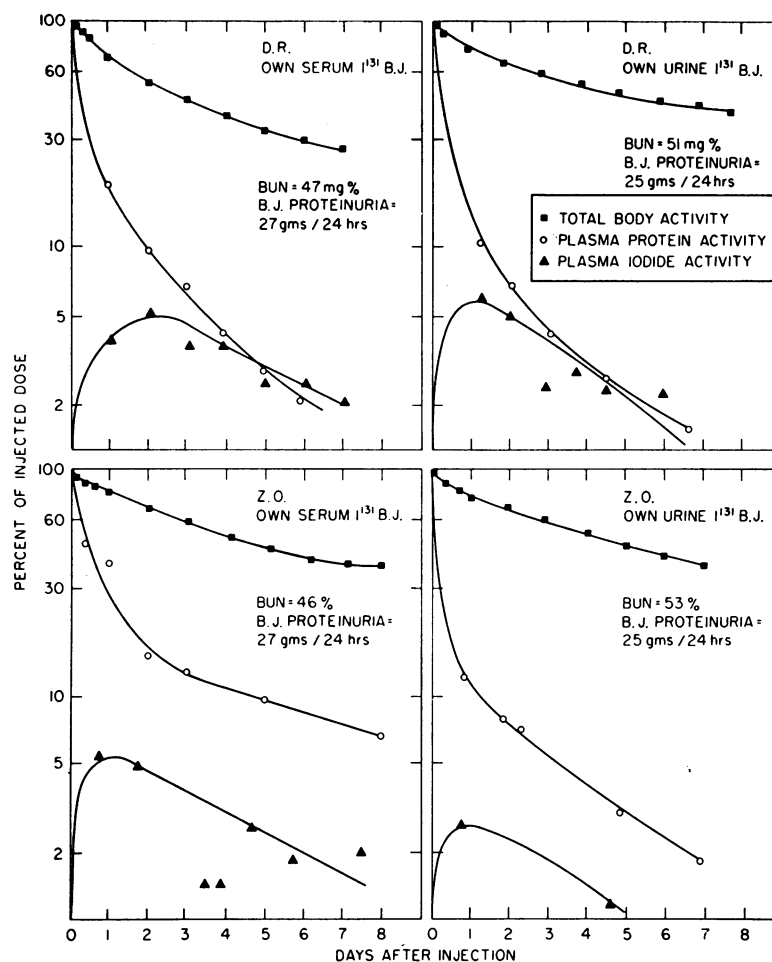


FIG. 4. METABOLIC STUDY OF  $I^{131}$ -LABELED SERUM AND URINARY BENCE JONES PROTEINS FROM DR AND ZO.

was used in the present study, since the P:C ratio (Equation 8b) and the grams of BJ excreted in the urine (experimentally determined) can be quite accurately obtained.

### Results

Total body radioactivity, total plasma protein-bound radioactivity, and free iodide radioactivity are plotted for each of the studies in Figures 2 to 4. The calculated turnover data are shown in Table II. Since the results appeared to be related to the rate of Bence Jones protein synthesis and to the renal status of the patient, the observations were divided into four groups: those made in patients *a*) with Bence Jones proteinuria and marked renal impairment, *b*) with Bence Jones proteinuria and normal renal function, *c*) without Bence Jones protein but with normal renal function, and *d*) without Bence Jones protein but with renal ab-

normalities. The course of a typical study is presented in the first case considered below.

*Patients with marked Bence Jones proteinuria and renal impairment: Patient ZO.* ZO had multiple myeloma with marked Bence Jones proteinuria (25 to 32 g excreted per day), Bence Jones proteinemia (1.05 to 1.5 g per 100 ml), and renal functional impairment (BUN, 42 to 56 mg per 100 ml). She was studied with four preparations of  $I^{131}$ -labeled Bence Jones protein: two separate studies with her own urinary Bence Jones protein, one with her serum Bence Jones protein, and one with the urinary Bence Jones protein of patient SE.

After administration of her urinary Bence Jones protein labeled with  $I^{131}$  (Figure 2, ZO; Table II, ZO no. 2), there was a rapid decline in the serum protein-bound radioactivity and a rapid

rise of the free iodide in the plasma to 2% of the injected dose, indicating a buildup of iodide in the body water to 16% of the injected dose following protein catabolism. After reaching a peak, the free iodide in the body water fell slowly, indicating moderate impairment of renal excretion of iodide. This was confirmed by iv administration of  $\text{NaI}^{131}$ , which indicated that the  $t_{1/2}$  of iodide excretion was 55 hours, compared to the normal 8 to 12 hours (20).

There was a gradual decrease in the whole body radioactivity as radioactivity was excreted in the urine, both protein-bound and as iodide. At the end of 6 days, 28.7% of the administered radioactivity was lost in the urine as  $\text{I}^{131}$ -labeled Bence Jones protein, and 31.7% was excreted as free iodide. The fraction of the plasma protein radioactivity lost in the urine per hour, the "proteinuric rate," was 2.5% per hour in ZO (Table II). The fraction of her plasma protein-bound radioactivity converted to iodide per hour by catabolism of the iodinated protein, termed "catabolic rate," was 2.9%. The higher catabolic rate indicates that catabolism played a slightly greater role than excretion in the loss of Bence Jones protein in this patient.

The relative contributions of excretion and catabolism to protein loss can be expressed as the proteinuric:catabolic (P:C) ratio. In patient ZO, the preceding data indicate a P:C ratio of 0.86. This ratio can be checked from the cumulative excretion of iodide and protein-bound radioactivity in the urine throughout the study (Equation 8b). In ZO the P:C ratio calculated in this way is 0.91, in sufficiently good agreement with the figure of 0.86 obtained above to confirm that catabolism contributed more than excretion to protein loss and that the estimate of the iodide pool used for the determination of the catabolic rate was reasonable.

At the time of study, ZO had a serum Bence Jones protein concentration of 1.3 g per 100 ml and a plasma volume of 2,665 ml. Thus the total circulating Bence Jones protein was 35 g. The product of the daily proteinuric rate, determined by isotopic data, and the total circulating Bence Jones protein should equal the quantity of Bence Jones protein excreted in the urine. The calculated quantity of urinary Bence Jones protein was 23 g per day (Table II), somewhat less than the

30 g per day observed. The estimated rate of Bence Jones protein synthesis was 68 g per day (Equation 9).

In this study 39.6% of the injected dose of  $\text{I}^{131}$  was still present in the body at the end of day 6, as estimated from the cumulative excretion of radioactivity, yet only 0.9% of the injected dose was present in the serum bound to protein and only 0.4% as free iodide. The latter corresponds to approximately 3.2% of the injected dose as iodide and 1.8 to 2.7% as protein in all body pools under normal distribution. Thus, approximately 34% of the administered radioactivity was unaccounted for. In 6 of the 7 studies that were carried to the point that less than 2% of the administered radioactivity remained in the plasma at the completion of the experiment, from 6 to 34% of the dose could not be accounted for. This will be discussed further later in this report.

The other Bence Jones turnover studies performed on Patient ZO, using another preparation of her urinary Bence Jones protein, her serum Bence Jones protein (Figure 4), and a urinary Bence Jones protein from patient SE (Figure 3), gave data of the same order as that discussed above (Table II). The mean proteinuric rates in the individual studies ranged from 1.4 to 3.0% per hour; the catabolic rates, from 1.8 to 2.9% per hour. Approximately equal quantities of the labeled Bence Jones protein were catabolized and excreted as protein. The mean calculated proteinuric rate was 15.2 g per day as estimated from the fractional proteinuric rate and the circulating Bence Jones Pool, compared to the 28.5 g per day experimentally observed. The estimates of protein synthesis ranged from 41 to 81 g per day. Five per cent of the labeled protein was still in the serum at the sixth day after administration of the  $\text{I}^{131}$  serum Bence Jones protein, compared to 1% in 3 studies with urinary BJ protein. This discrepancy probably indicates persistence of labeled 6.6 S  $\gamma$ -globulin contaminant from the serum Bence Jones protein preparation. The turnovers of labeled urinary and serum Bence Jones protein were otherwise quite comparable.

*Patient DR* (Figures 2, 4) had multiple myeloma, marked Bence Jones proteinuria (14 to 27 g per day excreted), Bence Jones proteinemia (0.85 to 2.0 g per 100 ml), and renal functional impairment (BUN, 47 to 74 mg per 100 ml serum).



TABLE II  
*Observations on Bence*

Recipients	Donors of BJ	Date	BUN	BJ prote- inuria	Serum BJ con- centration	Plasma volume
			mg/100 ml	g/day	g/100 ml	ml
Bence Jones proteinuria, poor renal function						
ZO	ZO U* no. 1	10/61	53	25	1.1	3,400
ZO	ZO U no. 2	2/62	42	30	1.3	2,665
ZO	SE U	10/61	56	32	1.1	3,020
ZO	ZO S	11/61	46	27	1.1	3,230
DR	DR U	10/61	51	25	0.85	3,500
DR	ZO U	2/62	74	14	2.0	2,890
DR	DR S	10/61	47	27	1.1	2,600
SE	SE U	10/61	31	5	0.3	3,800
Bence Jones proteinuria, good renal function						
HP	SE U	10/61	10	0.3	0	2,600
MT	SE U	10/61	17	0.3	0	4,750
No Bence Jones protein, good renal function						
JB	ZO U	2/62	10	0	0	4,625
FC	SE U	10/61	16	0.02	0	4,980
NMc	ZO U	2/62	9	4.0†	0	4,575
No Bence Jones protein, altered renal function						
JG	SE U	10/61	14‡	0.5	0	1,190
FL	ZO U	2/62	174	00.6	0	2,150

\* U = urinary Bence Jones protein; S = serum Bence Jones protein.

† Proteinuria but not with Bence Jones protein.

‡ Tubular function impaired (see text).

§ Value for FL correlated for nonprecipitable radioactivity in the body water.

He was studied with preparations of labeled Bence Jones proteins from his own serum and urine and with a labeled Bence Jones protein from ZO. The results of studying him were similar to those of ZO (Table II). The proteinuric rates were 0.9 to 2.5% per hour, and catabolic rates were 2.7 to 3.1% per hour. The proteinuric to catabolic ratios ranged from 0.33 to 0.93. Fifty-two to 75% of the Bence Jones protein lost from the body was catabolized, and the remainder appeared as protein in the urine. The mean calculated proteinuric rate from the three studies was 17 g per day compared to the 22 g per day as determined from analysis of the urine. As with ZO, the estimated synthetic rate was 58 to 81 g per day in the three studies. DR's labeled urinary Bence Jones protein was handled comparably to his serum Bence Jones protein (Figure 4).

*Patient SE* (Figure 3), who also had multiple

myeloma with Bence Jones proteinuria, proteinemia, and renal impairment, metabolized the protein in a fashion comparable to ZO and DR. The proteinuric rate was 2.2% per hour, and the catabolic rate, 3.9% per hour; the estimated synthetic rate for Bence Jones protein was 13 g per day. Both calculated and observed values for proteinuria were 5 g per hour.

*Patients with Bence Jones protein and normal renal function.* Patients HP and MT had multiple myeloma and macroglobulinemia, respectively. Both excreted small amounts of Bence Jones protein in the urine but had no detectable renal functional impairment. These patients had proteinuric rates of 3.4 and 1.4% per hour, respectively, i.e., of the same order as those found in ZO, DR, and SE. HP and MT, however, had catabolic rates of 31 and 10% per hour, which are markedly greater than those observed (1.8 to

TABLE II  
*Jones protein turnover*

Fate of I <sup>131</sup> -BJ at 6 days								
Remain- ing in plasma	Cumulative urinary excretion		Protein- uric rate	Cata- bolic rate	Proteinuric catabolic ratio (Equa- tion 8b)	Proteinuric catabolic ratio (Equa- tions 6, 7)	Calcu- lated BJ protein- uria	Synthetic rate
	Precip- itable	Nonpre- cipitable						
%	%	%	%/hour	%/hour			g/day	g/day
2.0	35.2	20.2	3.0	1.8	1.74	1.66	27	41
1.3	28.7	31.7	2.5	2.9	0.91	0.86	23	68
1.6	35.2	27.4	2.4	2.3	1.28	1.04	19	67
5.8	24.0	35.0	1.4	2.0	0.69	0.70	12	81
2.0	29.3	30.0	2.5	2.7	0.97	0.93	18	58
2.5	13.1	30.7	0.9	2.7	0.43	0.33	15	62
1.9	29.6	45.0	2.2	3.1	0.66	0.70	17	81
1.0	24.7	34.3	2.1	3.9	0.72	0.56	5	13.0
0.3	9.3	67	3.4	31.3	0.13	0.11	0	3.0
2.3	5.8	91.0	1.4	10.4	0.064	0.13	0	2.7
<0.1	0	71	0	16.3	0	0	0	
0.2	2.8	76.5	1.1	41.6	0.037	0.026	0	
<0.1	1	90.9	0.54	25.0	0.032	0.022	0	
0.9	7.8	60	0.25	2.4	0.13	0.10	0	
5.6	17.7	77.2	0.92	1.8	0.23§	0.33	0	

3.9% per hour) in the three patients discussed above. Approximately 90% of the labeled Bence Jones protein lost to the body was catabolized, whereas only 10% was lost as such in the urine. During the first day, when the majority of the administered protein was catabolized, radioiodide accumulated rapidly in the serum and body water (Figure 3) and was quickly cleared in subsequent days consistent with normal renal function. These two patients synthesized 2.7 to 3.0 g of Bence Jones protein per day, or about 5% of that produced by ZO and DR.

*Patients without Bence Jones proteinuria and with normal renal function:* JB (Figure 2), FC (Figure 3), and NMc (Figure 2). Patients JB and FC had macroglobulinemia. FC excreted 1.1% of the serum protein-bound I<sup>131</sup> into the urine per hour, but JB excreted none in this form. Both had exceedingly rapid Bence Jones catabolic

rates, with 16 and 42%, respectively, of the plasma protein-bound radioactivity being catabolized per hour. Thus 96 to 100% of the Bence Jones protein lost to the body was catabolized, and 0 to 4% was excreted as urinary protein.

Although Patient NMc excreted 4 g of non-Bence Jones protein in the urine per day, in every other respect he had relatively normal renal function with normal BUN and rapid clearance of free iodide from the body water. He catabolized Bence Jones protein in a fashion comparable to JB and FC, who also had normal renal function, and showed a proteinuric rate of only 0.54% per hour and a catabolic rate of 25% per hour.

*Patients without Bence Jones protein production but with renal abnormalities:* JG (Figure 3) and FL (Figure 2). Patient JG had macroglobulinemia. Despite normal BUN, he had evidence of renal tubular damage, including a PSP excre-

tion of 4% in 15 minutes and 22% in 2 hours, and a very slow clearance of iodide from the body water. This patient had a proteinuric rate of only 0.25% per hour and a low catabolic rate of 2.4% per hour.

Patient FL had chronic nephritis with extreme renal functional impairment (BUN, 174 mg per 100 ml serum). Although this patient catabolized the labeled protein at a rate of 2.8% of the plasma pool per hour, less than 1% of the dose was excreted as iodide in the first 24 hours, and only 18% was excreted in 6 days because of impaired renal function.

### Discussion

*Methodological.* The metabolism of slowly catabolized serum proteins has been successfully studied with proteins labeled *in vivo* with  $C^{14}$  or  $N^{15}$  amino acids or *in vitro* with  $I^{131}$ . Certain assumptions inherent in some techniques of analyzing protein turnover data are not fulfilled when proteins with a very short survival are studied, and new methods of conducting the studies and analyzing the data must be used. Studies using labeled precursor amino acids give spuriously long protein survival values because of persistence of labeled precursor. This represents a relatively minor error in the study of albumin and  $\gamma$ -globulin, which have survival  $t_{1/2}$  of more than 20 days, but makes accurate studies of proteins with survivals of a few hours impossible. Because the  $I^{131}$  label is not reutilized in patients receiving iodide solutions, this isotope was used in our studies of Bence Jones protein metabolism.

In the majority of metabolic studies using  $I^{131}$ -labeled proteins, results have been expressed either in terms of the  $t_{1/2}$  of protein survival, derived from a semilogarithmic plot of the plasma or whole body radioactivity curves (21, 22), or as daily urinary activity expressed as a percentage of the retained body radioactivity. These methods of analyzing the data normally require the following assumptions. *a)* The labeled protein behaves in the body in the same way as unlabeled protein. *b)* Catabolism and loss of protein occur from both the intravascular and extravascular compartments in proportion to the mass of proteins in these pools. *c)* The proteins in the various pools are in rapid exchange, and after an initial distribution phase, the specific activity of labeled proteins is

the same in all pools. *d)* The rate of excretion of iodide liberated by a breakdown of the labeled protein should be very rapid compared to the rate of protein catabolism. Campbell, Cuthbertson, Matthews, and McFarlane (23), McFarlane (24), Lewallen, Berman, and Rall (25), and Veall and Vetter (26), however, have presented evidence that catabolism of the serum protein occurs in the vascular compartment or a pool that equilibrates very rapidly with the intravascular pool. Proteinuria also clearly represents loss from the intravascular pool. Gitlin and associates (19) have shown in patients with significant proteinuria that specific activities of proteins are seldom uniform in all compartments and that at sites in close temporal proximity with the intravascular pool, where protein loss and catabolism occurs, there may be a much lower specific activity than in the extravascular pools. Thus assumptions *b)* and *c)* are not fulfilled, but when the rate of catabolism is low relative to rates of distribution between compartments, the error in calculation of the rate of protein catabolism from whole body radioactivity curves is small. When, as with Bence Jones proteins, rates of catabolism and loss are very rapid, meaningful data cannot be obtained from whole body radioactivity curves alone or from urinary data expressed in terms of whole body radioactivities. For this reason, in our study, both the daily excretion of protein-bound radioactivity (proteinuric rate) and the release of iodine from the catabolized Bence Jones protein (catabolic rate) are expressed in terms of the mean plasma protein-bound radioactivity for that collection period. This assumes that catabolism occurs in a pool closely associated with the plasma but does not require that the size of the extravascular pool be known or that the various pools be in equilibrium.

Another assumption in the use of whole body radioactivity curves or curves of urinary radioactivity alone is that labeled iodide be promptly excreted after catabolism of the protein. This requirement was not met in the present studies. In ZO and DR, in whom 2.91% of the intravascular Bence Jones protein was catabolized per hour, only 1.1% of the  $I^{131}$  body iodide was excreted per hour ( $t_{1/2} = 55$  to 66 hours as determined with  $NaI^{131}$  given intravenously before the protein turnover study). In the extreme case of

patient FL, with marked renal functional impairment, there was significant catabolism of Bence Jones protein but hardly any detectable excretion of radioiodide for the first few days of the study, the majority of the radioiodide released from protein catabolism being retained in the body water. Since a similar buildup of radioiodide in the body water occurs to some extent in all patients, one must consider not only radioiodide excreted in the urine but also the alteration in radioiodide in the body water in calculating the rate of catabolism of Bence Jones protein. The significance of relative delay in iodide excretion is not limited to the study of Bence Jones proteins but applies equally to all protein turnover studies where the rate of catabolism is high, as it is in exudative enteropathy, fibrinogen turnover (27), and such, or where the fractional excretion rate of iodide is low, as it may be in renal disease.

The problem posed by delayed iodide excretion may be handled, using different radioisotopes of iodine, by studying the metabolism of iodide simultaneously with that of the iodinated proteins. Although this was not done in this study, the proportion of radioiodide in the plasma was determined throughout, and by assuming a factor of eight for the ratio of total body to plasma iodide, the radioiodide in the body water could be estimated. Several observations indicate that a factor of eight was appropriate to the present work. Factors greater than eight are precluded since they would result in values for total body radioactivity greater than the administered dose. If lower factors are used, a sudden, and highly improbable apparent increase in the extravascular protein-bound radioactivity occurs. The iodide pool size, using the factor of eight, agrees well with previously determined values (20, 28). The proteinuric:catabolic ratios obtained by use of the iodide factor (Equation 8a) can be checked by a measure of the proteinuric:catabolic ratios from the cumulative urinary excretion of radioiodine throughout the study (Equation 8b), which does not require an estimate of the iodide pool size. Calculations of proteinuric:catabolic ratios by these techniques generally were in good agreement (Table II).

Seven studies were carried to the point that only negligible radioactivity was present in the plasma and urine. In one study, all of the injected radioactivity was demonstrated in the urine, but in six

of these studies only 66 to 94% of the injected radioactivity was apparently excreted in the urine and feces. The apparent retention of radioactivity could result from incomplete collection of radioactivity lost from the body or from radioactivity retained in metabolically inactive sites. Loss of urine appears unlikely, since the patients were kept on metabolic balance conditions in a nursing unit where complete urine collection is routinely performed. None of the patients excreted significant radioactivity in the stools collected during the study, but loss of activity through the skin was not estimated. Uptake of radioiodide by the thyroid was restricted by the use of Lugol's solution, and no significant localization of iodine in the thyroid was found by surface scanning or, in one patient, by post-mortem for radioactivity in tissue. Although protein-bound radioactivity may be stored inside phagocytic cells, much in the way that thorotrast or other colloidal foreign materials are stored, further study using whole body counting techniques is needed to explain this apparent retention of radioactivity.

*Biological.* Bence Jones protein was rapidly lost from the plasma into the urine or by catabolism in all patients. The sum of proteinuric and catabolic rates ranged from 2.6% per hour of the circulating pool of labeled BJ protein to over 42% per hour. This may be compared to a turnover of 0.4% per hour of the circulating pool for 6.6 S  $\gamma$ -globulin in five of these patients (29). Catabolism was a significant factor in the fate of Bence Jones protein in all studies, with 50 to 100% of the total daily loss of Bence Jones protein because of catabolism.

In assessing the factors that determined the fate of injected Bence Jones protein, we observed that Bence Jones proteins of different origins were treated in the same manner by one patient, whereas a single Bence Jones protein behaved quite differently in a variety of patients. Two factors relevant to this phenomenon are the rate of Bence Jones protein production and the renal status of the recipient.

The quantity of Bence Jones protein presented to the kidney appeared to influence the proteinuric rate, as seen in Figure 5. In all patients without Bence Jones protein, the proteinuric rate was low (0 to 1.1% per hour, mean value of 0.58% per hour). In nine of the ten studies in patients with

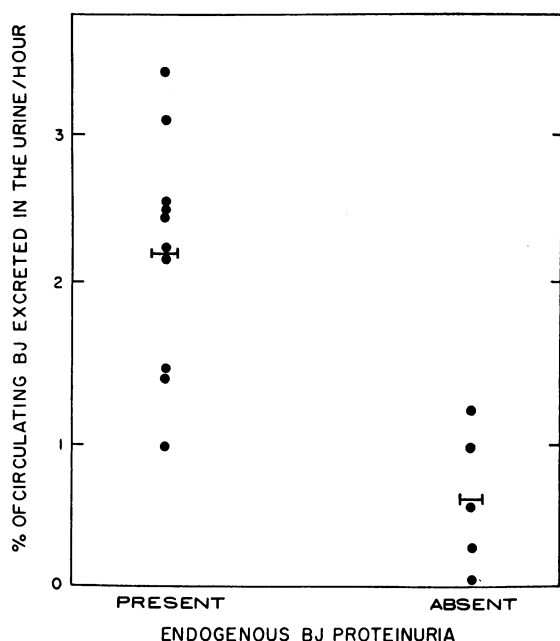


FIG. 5. THE RELATIONSHIP OF THE PROTEINURIC RATE OF  $I^{131}$ -LABELED BENCE JONES PROTEIN TO ENDOGENOUS BJ PROTEINURIA.

Bence Jones proteinuria, however, the proteinuric rate was high (1.4 to 3.4% per hour; mean value, 2.3% per hour). There was no constant relationship between the proteinuric rate and renal functional status. The possibility that Bence Jones protein may normally pass through the glomerular membrane and be reabsorbed by the renal tubules is supported by the demonstration of a greater fractional rate of excretion of radioactive Bence Jones protein in patients who were producing Bence Jones protein. In patients with endogenous Bence Jones protein production, the tubular transfer mechanisms may be partially saturated by the unlabeled Bence Jones protein. Similar filtration and reabsorption by the kidney has been postulated for albumin (30).

Measurement of catabolic rates higher than 10% per hour cannot be made with any degree of accuracy. There can be no doubt, however, about the high rate of Bence Jones protein catabolism, since in some patients (EG and HP) more than half the injected protein-bound activity appeared as iodide in the urine in a few hours. In others, with poor renal function, much of the liberated labeled iodide was retained in the body water for a few days. Such high rates have been found when

denatured proteins are injected, these being phagocytosed and promptly digested (31). In the present experiments, denaturation can be excluded because in other patients the same labeled Bence Jones protein preparation was catabolized at a low rate. For example, the highest recorded catabolic rate (42% per hour in FC) was given by the urinary Bence Jones protein from SE. The same preparation in ZO gave one of the lowest catabolic rates (2.3% per hour, Table II).

The renal status of the patients appeared to be an important factor in determining the catabolic rate of the Bence Jones protein. As seen in Figure 6, the Bence Jones protein catabolic rates were relatively low (1.8 to 3.9%) in the nine patients with blood urea nitrogen levels above 30 mg per 100 ml. In five of the six patients with normal blood urea levels, however, Bence Jones catabolic rates ranged from 10 to 41%. The sixth patient (JG) with a low blood urea level and a low catabolic rate had evidence of severe renal tubular damage with slow renal iodide clearance and

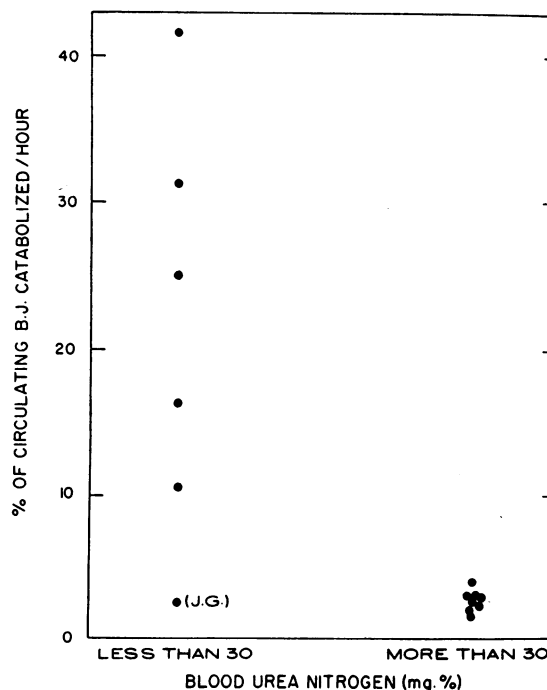


FIG. 6. RELATIONSHIP OF THE CATABOLIC RATE OF  $I^{131}$ -LABELED BENCE JONES PROTEIN TO RENAL FUNCTION (BUN). Patient JG had a normal blood urea level but had evidence of severe renal tubular damage with slow renal iodide clearance and a PSP excretion of 4% in 15 minutes and 22% in 2 hours.

a PSP excretion of 4% in 15 minutes and 22% in 2 hours. These data indicate that the kidney may play a role in Bence Jones catabolism. Although the gastrointestinal tract and the liver have been suggested as major sites of catabolism of albumin and  $\gamma$ -globulin (32, 33), there is some evidence that the kidney plays a significant role in the catabolism of these proteins (34), especially when proteinuria and protein reabsorption are prominent.

In two patients (ZO and DR) Bence Jones proteins were readily detected in the serum. An unusually high synthetic rate and low catabolic rate combined to raise the serum Bence Jones protein to levels of 1 to 2 g per 100 ml in each patient. Renal damage's reduction of the Bence Jones catabolic rate indicates one of the means by which Bence Jones proteins may increase to detectable levels in the serum. In a study of ten patients with Bence Jones proteinemia (15), all were found to have advanced renal disease.

The Bence Jones proteins used in these turnover studies were of the same immunochemical type (Type II). Two types of Bence Jones proteins occur in human disease and differ in their immunochemical and physicochemical properties (35-38). Since ZO, DR, and SE Bence Jones proteins used in these turnover studies were of Type II, the information we obtained may not be applicable to Bence Jones proteins of Type I. Also, Bence Jones proteins have been found in two sizes (39, 40). Some have a molecular weight of about 20,000, equivalent to one L polypeptide chain. Most Bence Jones proteins, however, have a molecular weight of about 40,000 and are composed of two L chains (41). The labeled Bence Jones proteins used in our study belonged to the latter form, as did the Bence Jones proteins of all recipients with multiple myeloma. Further studies would be needed to determine the metabolic properties of the smaller Bence Jones proteins and of immunochemical Type I Bence Jones proteins.

Patients with multiple myeloma apparently have a much greater rate of Bence Jones protein synthesis than is reflected by the quantity of BJ in the urine. As little as 10% of the Bence Jones protein was excreted by patients forming about 3 g per day. Even in patients having 25 to 30 g excreted Bence Jones protein, less than half of

the Bence Jones synthesized per day appeared in the urine. These rates of protein synthesis indicate a vigorous synthetic mechanism. Studies of the turnover rate of relatively small numbers of myeloma proteins in man indicate that 35 g of myeloma protein may be synthesized daily (42).

The patients with minimal or no Bence Jones proteinuria and normal renal functions, who exhibited a low rate of excretion but a high rate of Bence Jones protein catabolism, may provide some indication of the manner of metabolism of  $\gamma$ -microglobulins in normal man. Such components, similar to Bence Jones proteins, have been detected in small amounts in the urine (6-8) and serum (9) of normal subjects. Normal urine contains approximately 5 mg per day (8). If one assumes that this represents 10% of the amount available for excretion per day, that the catabolic rate was 20% per hour (estimates based on observations in JB, FC, NMc, and MT), and that the normal microglobulins behaved metabolically in the same manner as the Bence Jones proteins studied, then the present turnover studies indicate that about  $\frac{1}{4}$  g of  $\gamma$ -microglobulins may be formed daily.

### Summary

The metabolic fate of Bence Jones proteins was investigated in fifteen studies in ten patients. Three different Bence Jones proteins were labeled with  $I^{131}$ , and the amount and rate of Bence Jones protein lost in the urine and catabolized in the body were determined.

Both catabolism and urinary loss contributed to the removal of Bence Jones protein from the blood. The relative contributions of catabolism and of urinary loss depended on the quantity of Bence Jones protein formed and on the renal status of the subject.

The proteinuric rate ranged from 0 to 3.4% of the intravascular pool per hour. The proteinuric rate was highest in patients synthesizing Bence Jones protein. The possibility that Bence Jones proteins or similar  $\gamma$ -microglobulins are filtered by the glomerulus and reabsorbed by normal renal tubular cells is considered.

Labeled Bence Jones protein was catabolized at rates of 1.8 to 42% of the intravascular pool per hour. The catabolic rate correlated with renal functional capacity. In subjects with normal

renal function, daily catabolic rates of 10 to 42% per hour were observed. Lower catabolic rates (1.8 to 3.9% per hour) were observed in patients with poor renal function. These observations indicate that the kidneys may play a role in the catabolism of Bence Jones proteins.

The demonstration that catabolism contributes significantly to the metabolic fate of Bence Jones protein indicates that the urinary Bence Jones protein is only a portion of the total Bence Jones protein synthesized per day. Factors determining the urinary losses of Bence Jones protein are discussed.

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