

THE ROLE OF THE KIDNEY IN THE CATABOLISM OF BENCE JONES PROTEINS AND IMMUNOGLOBULIN FRAGMENTS*

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The serum concentrations of the immunoglobulins as with other plasma proteins reflect a balance among their rates of synthesis, rates of catabolism, and patterns of distribution. In recent years, a great deal of knowledge has been obtained concerning the synthesis and distribution of the plasma proteins. However, relatively little is known of the sites and mechanisms of plasma protein catabolism.

Using radioiodinated proteins, it has been shown that each class of immunoglobulins is catabolized at its own individual rate, with survival half times varying from 2.8 days for the newly discovered IgD to 23 days for IgG (1-6). Moreover, a variety of physiological and pathological factors has been shown to affect the survival of one class, leaving the other classes unaffected (2, 7-9). These observations suggest that these proteins are catabolized by processes which are specific for each immunoglobulin class and presumably are related to the portion of the immunoglobulin molecule specific for each class.

The results of such studies indicate that immunoglobulins are destroyed by a random process in a compartment in rapid equilibrium with the intravascular compartment (10, 11). However, these studies provide no information as to the specific organs involved in catabolism. Many other techniques have been utilized to try to obtain such information, including organ perfusion (12), organ extirpation (13, 14), and in vitro incubation of radiolabeled proteins with tissue slices (15) and subcellular fractions (16). However, no consensus has been reached regarding a major role for any individual organ in the catabolism of a specific protein. In general, such studies are hampered because the time period of the study is usually quite short relative to the in vivo life span of the protein, so that it is necessary to extrapolate results to a longer time period. Thus, such methods of study are best applied to proteins with very short survival half times.

In previous work studying the turnover of Bence Jones protein in man, it was shown that the survival of the protein was exceedingly short and also that from 50-90% of its metabolism was accounted for by endogenous catabolism, the remainder being excreted as proteinuria (17). It was also observed that the serum concentrations

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and rates of catabolism of the protein were closely correlated with renal function. Impaired renal function was regularly associated with a marked reduction in Bence Jones protein catabolism. This could be interpreted either as an inhibitory effect of uremia on protein catabolism or as evidence of catabolism of Bence Jones protein by the kidney.

The current studies were undertaken to investigate the role of the kidney in the catabolism of Bence Jones proteins, intact immunoglobulins, and immunoglobulin subfragments. The rates of catabolism of iodinated Bence Jones proteins, whole immunoglobulins, and separated L chains, Fc piece and Fab piece isolated from IgG were measured in nephrectomized, ureter-severed, and unoperated mice. The kidney was shown to play a major role in the catabolism of Bence Jones proteins and isolated L chains.

Materials and Methods

Animals.—NIH strain mice weighing 20–25 g were used in all experiments.

Preparation of Labeled Proteins.—

Human IgG: Human IgG was prepared from fresh normal serum by DEAE-cellulose chromatography (2). Serum was dialyzed against 0.005 M, pH 8.0 phosphate buffer, and applied to a DEAE-cellulose (Selectacel, Brown Company, Berlin, N. H.) column equilibrated and eluted with the same buffer. The protein eluted was shown to be pure IgG as verified by immuno-electrophoresis and the Ouchterlony technique using a specific antiserum directed against IgG H chain and a polyvalent antiserum to whole human serum.

Normal mouse IgG: Normal mouse IgG was kindly supplied by Dr. Stewart Sell. It was prepared by zone electrophoresis of pooled NIS-WS mouse sera. Serial fractions were eluted from the gamma-migrating area of the block and examined by the Ouchterlony technique using a rabbit antiserum against whole mouse serum and an antiserum against the individual mouse immunoglobulins. The fractions thus shown to contain IgG free of contaminating proteins were pooled and concentrated for labeling.

Human Bence Jones proteins: Ammonium sulfate was added to urine from patients with multiple myeloma and λ - or κ -type Bence Jones proteinuria until 80% saturation was obtained. The mixture was allowed to stand at 4°C for 24 hr and the resultant precipitate then redissolved in 0.01 M pH 7.6 phosphate buffer. The redissolved protein was then chromatographed on DEAE-cellulose using the same buffer. The initial peak obtained was shown to consist of pure λ - or κ -Bence Jones protein as verified with the Ouchterlony technique using antisera directed against type-specific L chain determinants, H chain determinants, and whole human serum.

Mouse Bence Jones proteins: Urine from mice bearing the plasmacytoma MPC-2 of Dr. M. Potter was dialyzed against 0.005 M pH 8.0 phosphate buffer and then chromatographed on a DEAE-cellulose column, using a linear gradient from 0.005–0.3 M phosphate (pH 8.0). The initial protein peak was shown on agar double diffusion analysis to be pure mouse Bence Jones protein using antisera directed against mouse L chain, intact mouse immunoglobulins, and whole mouse serum.

Human L chains: L chains were prepared by reduction and alkylation (18). Human Cohn fraction II, IgG, was subjected to reduction with 0.1 M β -mercaptoethanol at pH 8.0 (tris-HCl buffer). Alkylation was initiated after 1 hr with the addition of cold 0.1 M iodoacetamide. The mixture was then dialyzed against cold distilled water (4 hr) and, finally, against 1.0 M acetic acid. L chains were isolated by Sephadex G-130 (mixture of Sephadex G-200 and G-100) chromatography using 1.0 M acetic acid buffer. The second of two peaks was shown to contain

pure L chains free of H chains with specific anti- κ - and anti- λ -L chain antisera as well as specific anti-H chain antiserum in Ouchterlony analysis.

Rabbit Fc piece: Rabbit Cohn fraction II was purified by passage through a DEAE-cellulose column equilibrated with pH 6.3, 0.01 M phosphate buffer. Papain digestion was then carried out on the purified material according to the method of Porter (19). Digestion was performed at pH 7.5 (0.1 M phosphate) in the presence of papain (1 mg/100 mg protein), cysteine (0.02 M), and EDTA (0.02 M); digestion was carried out for $\frac{1}{2}$ hr at 37°C, and the reaction was terminated by dialysis in the cold against 0.03 M borate pH 8.4 buffer containing 0.001 M *p*-chloromercuribenzoate. Fc fragment crystals formed in the dialysis bag and were separated and redissolved in 0.1 M acetate pH 5.0. A second crystallization was then accomplished against the borate buffer and the crystallized material again dissolved in pH 5.0 acetate. The Fc fragment thus obtained was tested with specific anti-H chain and anti-L chain antisera in Ouchterlony analysis and shown to be uncontaminated with L chain determinants.

Rabbit Fab fragment: The supernatant solution obtained after the above described papain digestion and precipitation of Fc fragment was dialyzed against 0.01 M pH 5.8 acetate buffer and chromatographed on carboxymethyl (CM) cellulose using the same buffer. The initial peak was obtained and shown to contain only Fab fragment using antisera to the H or L chains or to the Fc piece of IgG.

Iodination of the proteins: Each of the purified proteins was labeled with ^{131}I by the iodine monochloride technique of McFarlane (20). There was from 0.3 to 1.0 moles of iodine per mole of protein in each of the final products. Over 98% of the radioactivity of all preparations was precipitable by 20% phosphotungstic acid.

Method of measuring protein catabolism: Protein catabolic rates are usually determined from the rate of decline of radioactivity in the whole animal or in the serum following an intravenous dose of radioiodinated protein. The basic assumptions of these techniques as usually performed are that radioiodine released on protein catabolism be quantitatively excreted from the body, and that the rate of protein catabolism be slow relative to the rates of protein distribution between body compartments and to the rates of excretion of the radioactive catabolic products from the body. With very rapidly catabolized proteins such as Bence Jones proteins or with any protein in nephrectomized or ureter-severed animals these requirements are not fulfilled, and so different techniques must be used.

The technique used in the present study was chosen to circumvent the problems imposed by delayed or absent excretion of released radioiodide from the body. Catabolism was assessed by determining the rate of decline in protein-bound (acid-precipitable) radioactivity of the whole body with time. The protein-bound radioactivity of the body was estimated by measuring the protein-bound activity of aliquots of whole homogenized animals killed at appropriate intervals after injection of the labeled proteins. The fraction of the radioactivity remaining protein bound is the fraction of the injected protein not yet catabolized.

Experimental protocol: All mice were maintained on drinking water containing potassium iodide (0.6 g/liter) for at least 2 days prior to their use to block thyroidal uptake of released radioiodide. In each experiment 60–80 mice were placed in three groups: nephrectomized, ureter-ligated or ureter-severed, and unoperated controls. Operated animals were anesthetized with chloral hydrate and ether. Bilateral nephrectomies were performed through flank incisions, and the ureters were severed or ligated bilaterally using a midabdominal incision. The animals were injected with 1–2 μc of the ^{131}I -labeled protein within an hour after surgery unless otherwise noted. At timed intervals, 3–6 animals from each group were killed and the fraction of the injected labeled protein that had been catabolized was determined as follows:

For nephrectomized and ureter-operated animals, no excretion of radioactivity to the outside was possible. After these animals were killed, their carcasses were homogenized in a Waring Blendor along with 200 ml of water. Duplicate 3-ml aliquots of the homogenate were

removed. The protein of one aliquot was precipitated by the addition of an equal volume of 20% phosphotungstic acid. The precipitated preparations were filtered and the clear supernatants were counted at the same time as the untreated homogenate aliquots in an automatic well-type gamma counter. The fraction of the total radioactivity which was protein bound was thus determined. For these animals, this fraction is equivalent to the fraction of injected protein which was not yet catabolized.

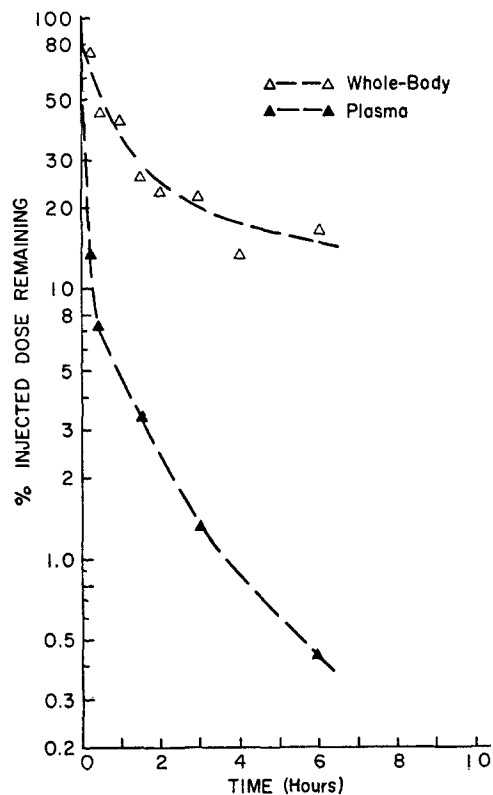


FIG. 1. Plasma and whole-body survival of human Bence Jones protein in the mouse. At 6 hr, when 17% of the injected radioactivity remained protein bound in the body, less than 0.5% of the injected activity was found within the plasma.

Urinary excretion of radioactivity could occur in the unoperated animals. The radioactivity in these animals was measured immediately after injection of the radioiodinated protein and again after the animals were killed using a small animal bulk counter. The fraction of the initial radioactivity remaining in the carcass was determined from these measurements. The carcasses were then processed as described above. It was thus possible to determine the fraction of the initial injected radioactivity which remained protein bound within the body at the time of death of the animals. This figure was calculated as the product of the fraction of the initial radioactivity remaining in the carcass and the fraction of this radioactivity which was protein bound.

The fraction of injected protein remaining protein bound (uncatabolized) was thus obtained for each mouse. The values for from three to six animals killed at each time point were averaged and plotted on semilogarithmic graph paper against time (Figs. 1-7).

In unoperated animals the relative contributions of endogenous catabolism and urinary loss of intact protein were determined according to the method of Gitlin et al. (21). This tech-

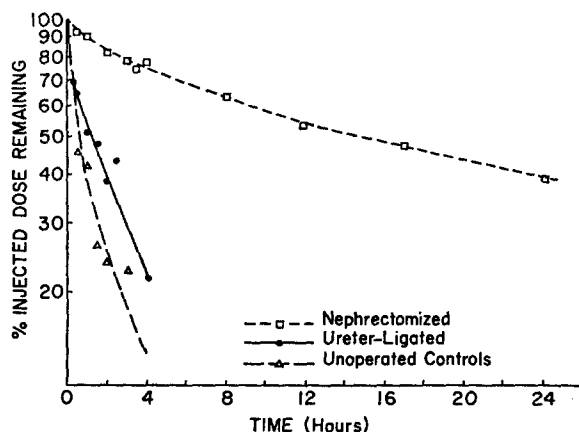


FIG. 2. Survival of human type κ -Bence Jones protein in the mouse. The catabolism of Bence Jones protein was found to be markedly impaired in nephrectomized animals.

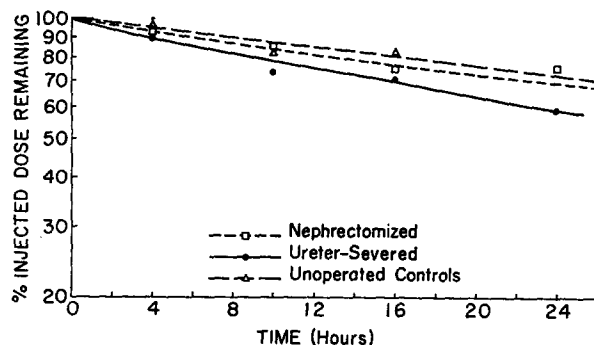


FIG. 3. Survival of human IgG in the mouse. The rate of catabolism of IgG was comparable in nephrectomized, ureter-severed, and control mice.

nique depends on the fact that all of the radiolabel excreted as a result of endogenous catabolism ultimately appears in the urine as iodide or iodinated tyrosine and is not precipitable with phosphotungstic acid, while radiolabel excreted as proteinuria is precipitable with acid. Accordingly, at a time when essentially all of the injected radioactivity has been excreted, the cumulative excretion of acid-soluble radioactivity compared to that of acid-precipitable radioactivity provides a reliable estimate of the relative contributions of endogenous catabolism and proteinuria. Thus, unoperated animals were placed in metabolic cages after injection, and all urine was collected until excretion of radioactivity was for practical purposes

complete. The cages were rinsed with saline and aliquots of the combined urine and washings, along with added albumin as a carrier protein, were precipitated with 20% phosphotungstic acid, and counted as described above for mouse homogenates. The ratios of nonprecipitable to total and precipitable to total radioactivity in the urine were taken as estimates of the relative contributions of endogenous catabolism and proteinuria, respectively, to the overall metabolism of the protein. The whole-body metabolic rate (catabolic plus proteinuric rate)

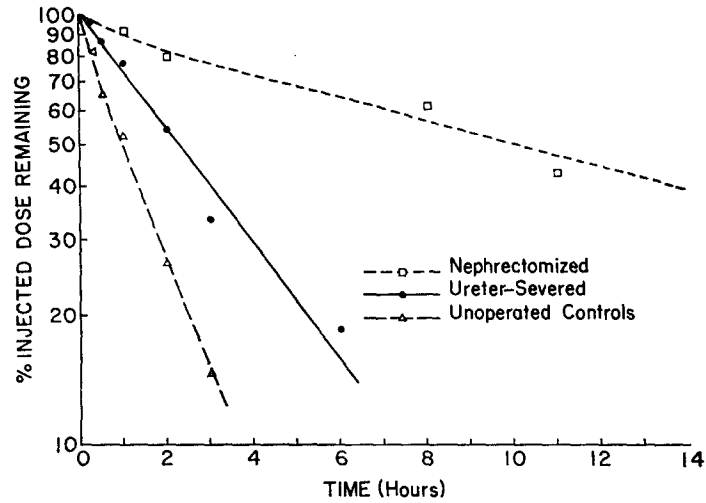


FIG. 4. Survival of human L chains in the mouse. The catabolism of L chains was found to be markedly impaired in nephrectomized animals.

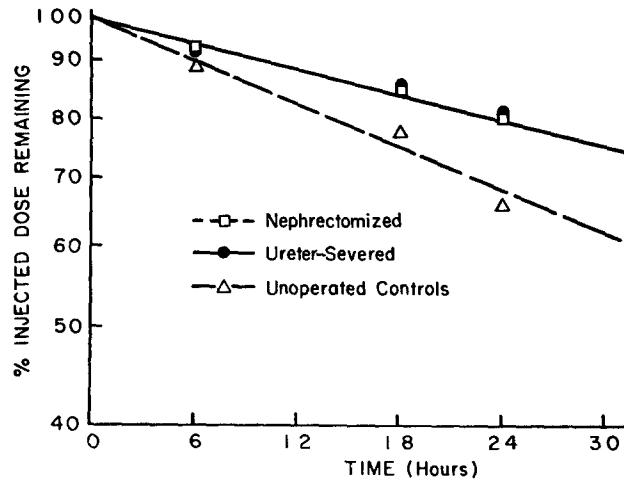


FIG. 5. Survival of rabbit Fc fragment in mouse. The Fc portion of the H chain of rabbit IgG was catabolized at the same rate in ureter-severed and nephrectomized groups.

was estimated for control animals by dividing the \log_2 (0.693) by the survival half time, estimated graphically from the protein-bound radioactivity curves. The proteinuric rate and the catabolic rate were calculated by multiplying this whole-body metabolic rate by the fraction of the overall metabolism accounted for by proteinuria and endogenous catabolism respectively.

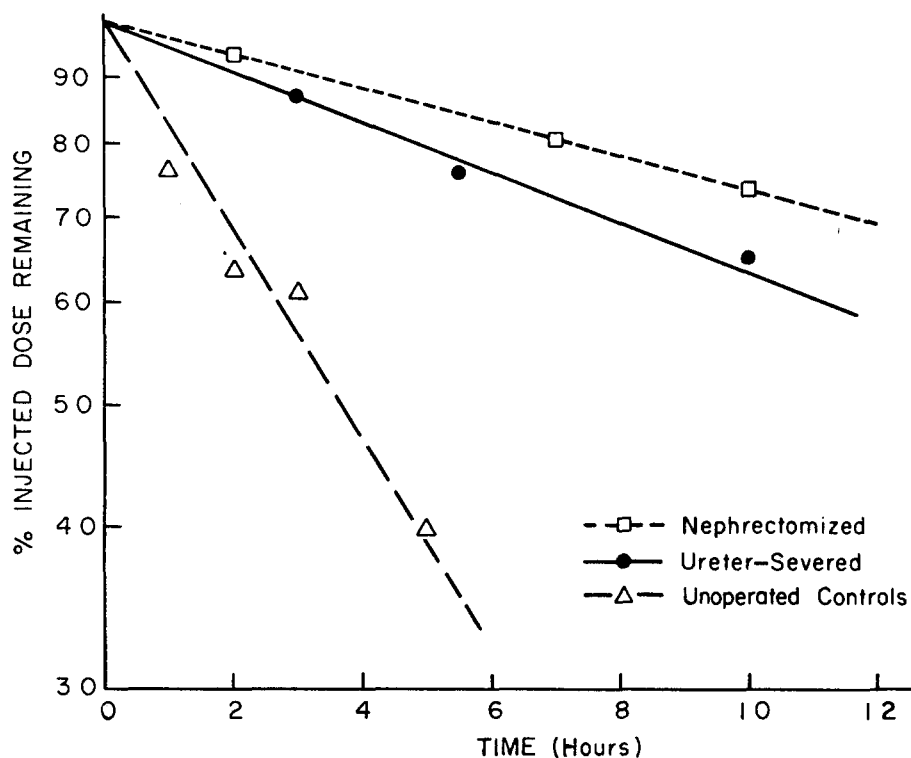


FIG. 6. Survival of rabbit Fab fragment in mouse. The rate of catabolism of Fab piece was only slightly less in nephrectomized animals compared to ureter-severed animals, but was much less in these groups than in non-uremic control animals.

Validity of methodology: The release of iodine from iodinated protein in vivo has been shown to provide a valid measure of protein catabolism (10). Using the present technique it is also required that there be no coprecipitation of iodine with protein on treatment with phosphotungstic acid, nor should iodide be reincorporated into protein once released. Similarly, it is required that no deiodination of the protein occur during the homogenization and precipitation procedures. These requirements were fulfilled as shown by the following studies:

1. Six ureter-severed and six control mice who had received potassium iodide in their drinking water for 48 hr were given sodium- ^{131}I intravenously. 18 hr later, they were killed, homogenized, and the precipitability of the radioactivity determined as discussed above. In each case 98–100% of the radioactivity was nonprecipitable, indicating that no significant coprecipitation or reincorporation of the radiolabel took place.

2. To evaluate the effect of homogenization and subsequent handling of the samples on protein binding of iodine, four to six animals were injected with each preparation, immediately killed, and then processed in the same manner as the other experimental animals. For each preparation 98–100% of the radioactivity was precipitable. Thus, no radiolabel was released from the protein during handling of the sample.

It will be seen that the decline of protein-bound activity of the whole-body after injection of Bence Jones protein was not linear with time but tended to have a progressive decrease in slope. A similar decrease in slope was seen in a previous study of Bence Jones metabolism in man (17). Three possible explanations for this phenomenon were considered, including (a) reincorporation of radioiodine into proteins, (b) contamination of the protein injected with a slowly catabolized radiolabeled protein, or (c) transfer of radiolabeled protein from the cata-

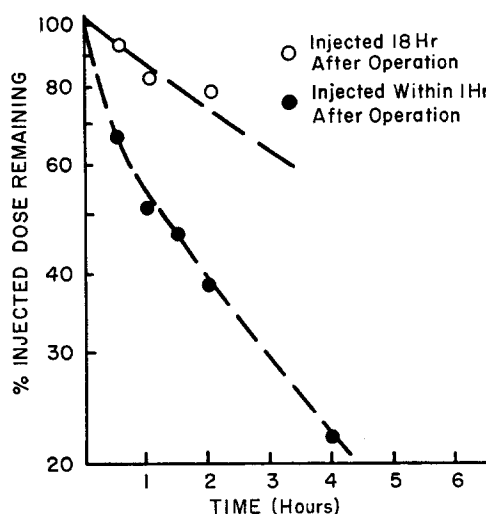


FIG. 7. Survival of human Bence Jones protein in groups of ureter-severed mice injected within 1 hr or at 18 hr after operation. The uremic mice injected 18 hr after the surgical procedure catabolized Bence Jones protein at a much slower rate than animals injected within 1 hr of the procedure.

bolic pool (intravascular pool or a pool in rapid equilibrium with it) to a slowly equilibrating extravascular noncatabolic pool. Reincorporation of iodide into protein was excluded by the administration of $\text{Na } ^{131}\text{I}$ in the studies discussed above. To test the other possibilities, a group of control mice was injected with ^{131}I Bence Jones protein and handled in the usual manner except that protein-bound plasma radioactivity was also determined at the time of sacrifice for all animals. The specific activity (cpm/ml) of plasma was obtained and plotted against time as a percentage of the plasma activity present 5 min after injection. No decrease in slope of the serum curves was observed as would have been seen if a slowly catabolized protein contaminant had been injected (Fig. 1). At 6 hr, when 17% of the injected radioactivity remained protein bound in the body, less than 0.5% of the injected activity was found within the plasma. Thus, more than 97% of protein-bound activity was located in the extravascular space at that time. The protein-bound radioactivity in various organs (spleen, kidney, liver, lung, thyroid, gastrointestinal tract, and carcass with viscera removed) was also determined at 6 hr after injection of the iodinated Bence Jones protein. Over 80% of the precipitable radioactivity

was in the carcass with viscera removed. These studies suggest that the progressive decrease in slope of the whole-body radioactivity curves was secondary to distribution of the labeled protein into extravascular (skin, muscle) noncatabolic pools.

RESULTS

The half times of protein survival ($T_{1/2}$) and the relative roles of proteinuria and catabolism in the overall metabolism of IgG, type κ - and λ -human Bence Jones proteins, mouse Bence Jones protein, isolated L chains, Fc piece, and Fab piece are shown in Table I. The major factor in the overall metabolism of each of the proteins studied was found to be endogenous catabolism, with proteinuria a less significant factor. This was true even for Bence Jones proteins, with catabolism accounting for 75-82% of the overall metabolism.

TABLE I
The Proteinuric and Catabolic Rates of Immunoglobulin and Subfragment Molecules

Protein	Half time of survival	Proteinuric rate	Catabolic rate	Overall metabolism due to catabolism
	<i>hr</i>	<i>% body pool/hr</i>	<i>% body pool/hr</i>	<i>%</i>
IgG (human)	88	0	0.8	100
Bence Jones protein				
Human (type κ)	1.4	9	40	82
Human (type λ)	0.8	22	65	75
Mouse (MPC-2)	1.0	14	54	79
L chain (human IgG)	1.0	Not done	Not done	Not done
Fc piece (rabbit)	39	0.3	1.5	83
Fab piece (rabbit)	3.6	7	12	63

The catabolism of Bence Jones protein was found to be markedly impaired in nephrectomized animals. Control animals catabolized 50% of the κ -human Bence Jones protein in 1.4 hrs after intravenous injection, while nephrectomized animals did not do so until 17 hr after injection (Fig. 2, Table II). Ureter-ligated and ureter-severed animals catabolized 50% of the administered Bence Jones protein by 1.6 hr; thus the operative procedure, early uremia, and the prevention of loss of Bence Jones protein in the urine did not significantly affect Bence Jones metabolism. It should be noted that ureter-severed and ureter-ligated animals behaved identically in their metabolism of Bence Jones proteins. Similar results were obtained using type λ human Bence Jones protein and Bence Jones protein isolated from the urine of mice bearing the MPC-2 plasma cell tumor. In each case the control and ureter-severed animals catabolized the Bence Jones protein at 5-10 times the rate observed in nephrectomized animals (Table II). In contrast, there was no significant difference between groups of control, ureter-severed, and nephrectomized mice in the rate of catabolism of

intact IgG from either human or mouse origin (Fig. 3, Table II). These results indicate that the kidney plays a major role in the catabolism of Bence Jones proteins but not of intact IgG.

Because of the structural relationships between IgG and Bence Jones proteins, it was of interest to study the metabolism of IgG fragments, including L chains, Fc piece, and Fab piece. The catabolism of L chains isolated from pooled human IgG was found to be comparable to that of Bence Jones proteins. While ureter-severed and unoperated controls catabolized 50% of the injected L chains in 1.9 and 1.0 hr, respectively, nephrectomized animals did not do so for 9.5 hr (Table II, Fig. 4). In contrast, the Fc portion of the H chain of rabbit

TABLE II
Effect of Nephrectomy and Ureter Severing on the Catabolism of Immunoglobulins and Subfragments

Protein	Time for catabolism of 50% of the injected dose		
	Unoperated control	Ureter-severed	Nephrectomized
	<i>hr</i>	<i>hr</i>	<i>hr</i>
Bence Jones protein			
Human (type κ)	1.4	1.6	17
Human (type λ)	0.8	1.4	9
Mouse MPC-2	1.0	1.5	10
IgG (human)	88	86	88
IgG (mouse)	60	58	60
L chain (human IgG)	1.0	1.9	9.5
Fc piece (rabbit)	39	76	76
Fab piece (rabbit)	3.6	11.3	13.5

IgG was metabolized at the same rate in ureter-severed and nephrectomized groups (Fig. 5). This rate was somewhat slower than that observed in control animals, in part due to the elimination of direct urinary loss of intact Fc piece (Table II) and in part possibly due to the effects of uremia. The rate of catabolism of the rabbit Fab piece (consisting of the Fd fragment of the H chain attached to an L chain) was slightly less in nephrectomized animals compared to ureter-severed animals (Fig. 6).

These results indicate that the kidney is required for normal catabolism of L chains and Bence Jones proteins, which are dimers of L chains, but that it does not appear to be required for catabolism of intact IgG, Fc piece, or probably of Fab piece.

The presence of advanced uremia was associated with impairment of Bence Jones protein catabolism. All of the foregoing studies were performed using animals injected with labeled proteins within an hour after operation. However,

when ureter-severed or ureter-ligated animals were injected 18 hr after the surgical procedure, they catabolized Bence Jones protein at a much slower rate than animals injected within 1 hr of the procedure (Fig. 7). It should also be noted that both ureter-severed and nephrectomized animals catabolized Fab piece (Fig. 6) and to a lesser degree Fc piece (Fig. 5), at a much slower rate than control animals or sham-operated animals, suggesting that even "early" uremia may inhibit the catabolism of these molecules.

DISCUSSION

Bence Jones protein and isolated L chains are broken down rapidly by an endogenous catabolic process which is quantitatively of much more importance in their overall metabolism than loss in the urine as intact proteins. This catabolic process is markedly impaired in nephrectomized mice, but not in those with bilateral ureteral interruption, indicating that catabolism is dependent upon the presence of renal tissue, and probably takes place within the kidney. Whole IgG molecules, the Fc immunoglobulin fragment, and to a lesser extent the Fab fragment, do not participate in this process since these molecules have the same survival in nephrectomized and ureter-severed animals. The catabolic process is impaired in the presence of advanced uremia as indicated by prolonged survival of Bence Jones proteins in ureter-severed animals that were not injected until 18 hr after surgery.

Previous work had indicated that the kidneys do not play a significant role in the catabolism of albumin except possibly in the presence of severe nephrosis (13, 23). In some animals with experimental nephrosis, nephrectomy decreases the fractional catabolic rate for albumin. In these animals there is increased filtration of albumin through the glomerulus exposing the protein to the renal tubular cells. It has been suggested that the filtered albumin may be absorbed and catabolized by the tubular cells (23). It should be noted that large quantities of albumin must be present in the filtrate before renal catabolism of albumin can be demonstrated. In the current study, it might be speculated that Bence Jones proteins and L chains are also filtered and then catabolized by the tubular cells since these are small molecules (mol wt < 45,000) and their appearance in the urine is proof that they are exposed to the tubular epithelium. However, the rabbit Fc and Fab immunoglobulin fragments are also small and appear in the urine and so must be exposed to the tubule. Yet these proteins are not catabolized significantly by the kidney. Similarly, no renal catabolism was observed for another immunoglobulin fragment appearing in the urine of mice with MOPC-20-plasma cell tumors. This molecule contains parts of both L and H chains of mouse IgA. Thus, the mere presence of protein in the renal tubule is not sufficient for significant protein catabolism to take place. Furthermore, ureter-severed and ureter-ligated mice were identical in their catabolism of Bence Jones proteins, and ureteral ligation is known to stop

glomerular filtration abruptly. Thus, although the mechanism by which renal catabolism takes place in the intact animal may involve filtration of the protein and reabsorption by the tubular cells, glomerular filtration does not appear to be an absolute requirement for this catabolism to take place.

Many previous observations have defined reactions of transport and metabolism of immunoglobulins which appear to be related most closely to the Fc portion of the immunoglobulin molecule (1-9). In the current study, it is the L chain moiety which appears to be susceptible to the renal catabolic process. The combination of H chains and L chains in the form of an intact immunoglobulin molecule was not susceptible to renal catabolism and only partial susceptibility could be demonstrated for the rabbit Fab piece containing the L chain and just the Fd portion of the H chain. The remainder of the H chain, as the Fc piece, was unaffected by the catabolic process. Thus, another metabolic reaction is demonstrated which is related to a specific portion of the immunoglobulin molecule, the L chain moiety. Whether attachment of the H chain inhibits access to a catabolic site, inhibits the catabolic process itself, or possibly acts by some other mechanism will require further study.

Proteinuria is a frequent concomitant of renal disease and may be of two major types. The most common is typified in its severe form by the nephrotic syndrome. Here the urinary proteins represent a filtrate of the plasma, with smaller proteins such as albumin appearing in greater quantities than the larger ones (24). This type of protein loss appears to be a result of glomerular damage, with leakage of excess protein into the tubule resulting in proteinuria. The other major type of proteinuria occurs in subjects with disease predominantly affecting the renal tubules such as renal tubular acidosis or the Fanconi syndrome (25). In this situation, the proteins excreted are not simply a filtrate of the plasma. Instead, the urine contains relatively little albumin, but a variety of other proteins are observed. Most of these are as yet unidentified, but they are of low molecular weight and migrate predominantly in the alpha and beta regions on electrophoresis. The pathogenesis of this type of proteinuria is not yet clear. It may be that these proteins appear in the urine because of failure of the tubules to reabsorb and catabolize proteins that are filtered. The current observations demonstrate that at least some proteins (Bence Jones proteins and isolated L chains) may be catabolized by the kidney, thus lending indirect support to this hypothesis.

The recovery of Bence Jones proteins from the urine has now been recognized as a major sign of disease for more than a century (26). In recent years, L chains have also been recovered in urine of normal individuals, but their significance is still unclear (27-29). Isotopic studies suggest that they are products of *de novo* synthesis and not breakdown products of immunoglobulins (29, 30). It is clear from the current and previous studies (17) that the quantity of either L chains or Bence Jones proteins recovered from the urine cannot be taken as an

indication of the rate of production of these substances. Rather, they are synthesized at rates considerably greater than the rate of urinary loss would indicate, the remainder being catabolized within the kidney. Furthermore, the presence of renal disease and uremia impairs this catabolic process. Thus, patients with multiple myeloma who have renal disease may have a significantly reduced rate of catabolism of Bence Jones protein that contributes to the development of detectable serum levels of Bence Jones protein. It may be predicted that other diseases associated with renal dysfunction will be found in which altered metabolism of L chains occurs. Indeed, one recent observation of increased serum concentrations of L chains in systemic lupus erythematosus (31) may be such an instance, although no correlation could be made between the increase in L chain concentration and the presence of azotemia in these patients. Other diseases, especially those with renal tubular dysfunction, will bear further investigation in this regard.

SUMMARY

The role of the kidney in the catabolism of Bence Jones proteins, intact IgG molecules, and isolated L chains, Fab and Fc fragments of IgG, was studied. The proteins were purified, radioiodinated, and their survival times measured in nephrectomized, ureter-severed, and control mice.

Active endogenous catabolism was the major factor in overall Bence Jones metabolism since excretion as proteinuria accounted for less than 25% of the total metabolism. The survival times of λ - and κ -type human Bence Jones proteins and the Bence Jones protein produced by mice with MPC-2 plasma cell tumor were exceedingly short in both unoperated and ureter-severed mice, with 50% of the injected protein catabolized in from 0.8-1.6 hr. In contrast, the survival of Bence Jones protein was markedly prolonged in nephrectomized animals, with 50% of the injected dose catabolized in from 9 to 17 hr. This tenfold decrease in catabolic rate indicates that the kidneys are the major site of breakdown of Bence Jones proteins. Similar studies with other proteins indicated that the kidneys are also the major site for catabolism of isolated L chains but not of intact IgG molecules. The Fc immunoglobulin fragment was not catabolized and the Fab fragment only partially catabolized by the kidney. When ureter-severed animals were allowed to develop advanced uremia before being studied, the survival of Bence Jones protein was greatly prolonged, indicating that the catabolic process is impaired in the presence of uremia. The nature of this renal catabolism remains unknown.

These observations suggest that the Bence Jones proteins and L chains observed in the urine of patients may reflect only a small fraction of such molecules synthesized by these patients. Furthermore, they provide an explanation for the prolongation of Bence Jones protein survival and the development of Bence

Jones proteinemia observed in subjects with multiple myeloma and impaired renal function.

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