Bence Jones Proteins: Nature, Metabolism, Detection and Significance

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ABSTRACT

The nature of Bence Jones proteins is developed by a review of the pertinent literature. This is followed by a discussion of their metabolism and catabolism under normal and abnormal clinical conditions. Various methods for the detection of Bence Jones proteins are critically reviewed and the significance of these proteins in various disorders is assessed.

Nature

In 1845 Henry Bence Jones, a physician and chemical pathologist at St. George's Hospital in London, received a specimen of urine from Dr. Thomas Watson. The urine was from a patient with an illness now known to be multiple myeloma. Accompanying the specimen was a note the text of which is reproduced here:

"The tube contains urine of very high specific gravity. When boiled it becomes highly opaque. On the addition of nitric acid it effervesces, assumes a reddish hue and becomes quite clear; but as it cools assumes the consistence and appearance which you see. Heat reliquefies it. What is it?"

This challenge to Bence Jones resulted in a series of publications in which he clearly described many of the physicochemical features of the urinary protein which later came to bear his name. In his laboratory investigation, Bence Jones placed considerable emphasis on the thermal properties of this urinary substance and these features were used as the basis for defining the presence or absence of this protein in patient's urine for more than a century after the initial description.

The modern era of Bence Jones (BJ) protein analysis began with a report by Edelman and Gally that BJ proteins shared extensive antigenic cross-reactivity with myeloma proteins and normal gamma globulins. Putnam compared peptide maps of pooled human gamma globulin, seven purified pathologic globulins and eight BJ proteins. On the basis of these studies, he concluded that there were two types of BJ protein and that they represented incomplete or aberrant polypeptide chains of normal gamma globulin. Mannik and Kunkel, using Ouchterlony immunodiffusion methods, demonstrated that approximately 60 percent of normal gamma globulin molecules carried one group of antigenic determinants and 30 percent carried a second distinctive group of antigenic characters. Thus, the normal kappa to lambda ratio of two to one for serum immunoglobulins was initially described. Schwartz and Edelman used two-dimensional, high-voltage electrophoresis and compared the tryptic hydrolysates from the
light chain of a myeloma globulin and BJ protein from the same patient. They found that the two were identical. Based on this and other data they suggested that BJ proteins were not aberrant or incomplete polypeptide chains but rather were entire light (L) chains which had not been incorporated into the autologous myeloma protein.

Further evaluation of the tryptic peptide maps of a larger number of BJ proteins from both antigenic groups (kappa and lambda) revealed that a common set of peptides exist for each type of BJ protein, but that the peptides common to the kappa group of BJ proteins are completely different than the common peptides of the lambda group. These observations led Putnam to propose that each BJ protein or L chain was composed of a constant and a variable portion as defined by their amino acid sequence. This proposal has subsequently been confirmed and expanded through elucidation of the primary structure of many BJ proteins by amino acid sequence analysis. These sequence analyses have shown that a BJ protein (or a normal light chain) is composed of approximately 214 amino acid residues. The initial one-half (residues 1 to 107) or amino terminal end represents the variable portion of the molecule (VL). The carboxy terminal portion (residues 108 to 214) corresponds to the constant portion of the polypeptide chain (CL). Thus the CL portion of a particular kappa type BJ protein is essentially identical to the CL region of all other type kappa BJ proteins and normal immunoglobulins containing kappa light chains, but differs considerably from the CL region of all type lambda proteins. An exception to this constancy is the occurrence of the genetic marker Inv at position 191 on the kappa chain. Various amino acids substituted in this position account for the different Inv allotypes. In contrast to the constant portion of the BJ protein or light chain is the variable region where there appears to be a great deal of diversity. In fact, several subgroups of the VL portion of both kappa and lambda chains have been characterized. They undoubtedly play an important role in the antigen binding site of which this portion of the molecule is an integral part.

Metabolism

The intracellular events necessary for the production and secretion of an immunoglobulin molecule are quite similar to those required for other proteins. Heavy (H) chains and light chains are synthesized on separate polyribosomes and are then joined in the cisternae of the endoplasmic reticulum. Under normal conditions (unassociated with malignancy) one H and one L chain form an HL intermediate that combines with a second HL molecule to form a complete immunoglobulin (H₂L₂). Control of some of the intermediate synthetic events appears to be a function (at least for IgG synthesis) of the individual H chain subgroup, each of which has a characteristic number and location of disulfide bonds that are essential to interchain linkage.

Initial investigations suggested that the formation of immunoglobulin molecules was characterized by the production of an excess of light chains and this was considered the source of the small amount of L chains present in normal serum and urine. Other workers, however, have shown that balanced synthesis of H and L chains is most characteristic of nonmalignant immunoglobulin production. These investigators also demonstrated the existence of a small, rapidly utilized intracellular pool of free light chains that may be important in controlling the assembly of immunoglobulins. Their results did not exclude the possibility that a small amount of light chain might appear in the circulation as a result of cell death, cytoplasmic shedding or unbalanced synthesis by a limited number of cells.
In malignant plasma cells of human and animal origin, a wide spectrum of synthetic events has been shown to occur. Zolla and coworkers\textsuperscript{55} demonstrated three basic patterns of immunoglobulin synthesis by malignant plasma cells: (1) production of only L chains, (2) balanced synthesis where H and L chains are made in equal amounts and (3) an unbalanced synthesis in which intact immunoglobulin molecules and excess L chains are made and secreted. It was also demonstrated that the formation of an HL intermediate was common in the synthesis of a complete IgG myeloma protein and that dimerization of L chains occurred intracellularly.\textsuperscript{55} Excretion of the monoclonal L chain (i.e., BJ protein) into the urine as a monomer or a dimer was usually the same as the state of polymerization when secreted by the malignant plasma cell. However, in a number of cases where free light chains were clearly demonstrated to be secreted by the cells, they were not detected in the urine. This suggests that the presence or absence of BJ protein in the urine is dependent on the rate and amount of free light chain synthesized and the patient's renal status (\textit{vida infra}).

The investigations of Solomon,\textsuperscript{42} Wochner\textsuperscript{51} and Miettinen\textsuperscript{29} and their collaborators have all contributed to establishing the kidney as the major organ responsible for the catabolism of normal light chains and BJ proteins. In a normal individual, the glomerular filtration apparatus effectively controls the amount and type of protein that enters the tubular lumen usually allowing only trace amounts of albumin to be excreted while significantly larger amounts of proteins with molecular weights less than 50,000 daltons are presented to the tubular epithelium. The proximal tubular epithelium actively takes up and catabolizes a large number of these smaller proteins and, thus, only a limited amount of the filtered protein is ultimately excreted in the urine. The importance of this tubular reabsorptive process has been shown by an analysis of the type of proteinuria that results when the proximal tubules are selectively poisoned with sodium maleate or in clinical disorders such as the Fanconi syndrome, Wilson's disease and chronic cadmium poisoning.\textsuperscript{30} In these conditions the processes of protein uptake and catabolism diminish and increasing amounts of small molecular weight proteins appear in the urine. In conditions characterized by a selective proximal tubular disease, there is usually little or no decrease in the glomerular filtration rate. However, when renal damage is more severe and involves the glomeruli and tubules, there is a decrease of the glomerular filtration rate. This results in less protein being filtered and presented to the tubular epithelium; therefore, catabolism and excretion are diminished. In addition, many small proteins (e.g., L chains) that would have undergone catabolism or been excreted by the kidneys remain in the circulation and have a significantly prolonged half-life.

Individuals with normal renal function remove labelled BJ proteins at a rate of 10 to 42 percent of the intravascular pool each hour; the half-life of a BJ protein or L in the circulation is approximately four hours.\textsuperscript{29,42} Miettinen and Kekki\textsuperscript{29} injected \textsuperscript{131}I-labelled BJ protein into normal subjects and found that only 3.6 percent of the labelled protein was recovered intact in the urine. The bulk of the injected BJ protein was degraded within the body. The kidney was clearly demonstrated to be the site of this degradative process in the studies of Wochner and others.\textsuperscript{51} They showed that the survival time of human BJ protein was quite short in both unoperated and ureterligated mice; but in nephrectomized animals it was markedly prolonged. The location of this active catabolism within the kidney was the epithelial lining of the convoluted tubular cells. Tan and Epstein\textsuperscript{48} studied homogenates of normal human kidney tissue and found that the highest catabolic activity directed at L chains was in the lysosomal rich fraction. This process was most active at pH 5.0 and in the presence of a reducing agent. Interestingly, the first step in the degradative process resulted in the formation of in-
soluble polymers. This was followed by the appearance of half molecules with either constant or variable antigenic features upon continued incubation. Morphologic confirmation of this work has demonstrated that 80 percent of injected kappa chains are reabsorbed by the proximal tubular cells where they form crystal-like structures within phagolysosomes.  

The concentration of L chains in normal urine is three to four micrograms per ml and the kappa to lambda ratio of 1.9 closely simulates that of serum. The urinary L chains may exist as monomers, dimers and tetramers with a greater tendency for polymer formation as the concentration increases. Fragments of light chains which retain the antigenic features of the constant or variable portion of the molecule have also been detected in urine.

Individuals with a compromised renal status clear BJ proteins and L chains at a rate of 1.8 to 3.9 percent of the intravascular pool per hour. This is considerably lower than their normal counterparts. The half-life of a labelled BJ protein is also much longer (8 to 32 hours vs. 4 hours in the normal subject) in these individuals. Patients with multiple myeloma and normal renal function have a great capacity to catabolize BJ proteins in spite of the large amounts of monoclonal L chains that may be synthesized. However, declining renal status from any cause sets a vicious cycle in motion with rising serum levels of BJ protein, additional cast formation and further decline of renal function. The insoluble polymers formed during the normal sequence of catabolic events take on added significance in this context and may contribute to the renal injury seen so often in patients with multiple myeloma and collectively termed "myeloma kidney," a lesion whose etiology continues to defy clarification.

Detection

Procedures for the identification of BJ proteins include (1) heat precipitation, precipitation with acids and/or salts, and (3) electrophoresis and immunoelectrophoresis. Methods for accurate quantitation of the amount of BJ protein have also been described.

For many years, BJ proteins were defined by their ability to precipitate when heated to 45 to 60° followed by their redissolution on boiling. Although the mechanism of this phenomenon was not appreciated, these unusual thermal properties provided laboratories with a relatively simple technique for their identification. Putnam and his collaborators demonstrated that the extent of aggregation and precipitation by these proteins was dependent on the pH, the ionic strength and the electrolyte composition of the reaction mixture. The pH was the most important variable, with an optimum precipitation of all proteins occurring in the range 4.6 to 5.4. Edelman and Gally used spectrofluorometric analysis to demonstrate that BJ proteins and L chains underwent characteristic reversible molecular transitions when heated and that pH changes markedly influenced these events. Solomon and McLaughlin analyzed the thermal solubility properties of a BJ protein and its isolated C_L and V_L fragments. They found that the whole molecule and its V_L portion had identical heat precipitating properties while the C_L portion had none. Differences in the primary structure of the V_L portion of the molecule were thought to be responsible for the variable thermal solubility properties of individual BJ proteins. Ghose also found that the V_L portion of the molecule was the determinant of a BJ protein's heat precipitating qualities. This suggested that the formation of a precipitate is the result of thermally induced conformational changes with exposure of hydrophobic bonds leading to increased aggregation and precipitation.

In spite of our increased understanding of the mechanism(s) responsible for the heat precipitation of BJ proteins, the use of this test is justifiably declining. The reasons for this include (1) the necessity for rigid pH control, the inability of the test to detect BJ protein at less than 145 mg per dl,
(3) the recognition that other proteins (e.g., transferrin) found in the urine may closely simulate the thermal solubility properties of BJ protein\(^7\) and (4) the recognition that not all BJ proteins, as defined immunohimically, possess the characteristic heat precipitating qualities.\(^3\)\(^,\)\(^4\) Lindstrom et al\(^26\) reviewed the ability of a standard heat test to detect BJ proteins in urines from patients with multiple myeloma. They found the heat precipitation test positive in only 13.6 percent of patients and recorded several instances of BJ protein being present by immunologic methods when screening tests were repeatedly negative. This phenomenon has been confirmed in our laboratory including one instance of a patient with L chain disease who was losing 10 to 14 g of protein every 24 hours in his urine and had several negative heat tests. Urine electrophoresis and immunoelectrophoresis revealed that virtually all of his urinary protein was a lambda type monoclonal (BJ) protein. Recent publications confirm the inadequacy of heat tests to screen urines effectively for BJ protein. Stone and Frenkel\(^46\) noted that only six of the 18 urines obtained from patients with L chain myeloma had a positive heat precipitation test. This is particularly undesirable in a disorder like L chain disease where BJ proteinuria may be the only laboratory manifestation of a monoclonal protein. The heat precipitation test for BJ protein cannot be recommended for the routine screening of urine. In the future, it will play a less important role in the laboratory evaluation of patients with suspected immunoproliferative disorders.

Numerous methods that rely on the ability of various acids and/or salts to precipitate the BJ protein in urine have been proposed. They are reviewed by Naumann,\(^31\) Hobbs\(^21\) evaluated six of the more commonly used methods and compared the results with two variations of the heat precipitation test and immunoelectrophoresis. He reported failure rates ranging from five to 52 percent, with the best results (5 percent failure rate) obtained by layering the urine over concentrated hydrochloric acid and noting the formation of a precipitin ring. Interestingly, Hobbs reported a 52 percent failure rate when using the toluene-sulfonic acid method of Cohen and Raducha.\(^13\) Stone and Frenkel\(^46\) using the same method obtained a positive result in 30 of 35 patients with L chain myeloma. The failures all occurred in patients with less than 400 mg of protein per 24 hours. In general, these tests are not sensitive to low levels of BJ protein, or they fail to discriminate BJ protein from other urinary proteins. Attention should also be called to the misleading results obtained with the Dipstix\(^*\) test in patients with BJ proteinuria. This procedure is notable for its inconsistent and frequent negative results in the presence of significant BJ proteinuria.\(^20\)\(^,\)\(^46\) In fact, a negative Dipstix test and a positive sulfosalicylic acid test have been proposed as a presumptive test for BJ protein in the urine.\(^17\) Laboratories using any of the above techniques should be aware of the shortcomings inherent in most if not all of these procedures.

Immunoechemical analyses have clearly demonstrated the presence of gamma globulin and gamma globulin subfragments in urine from normal individuals.\(^49\) The latter include intact IgG, IgG Fc fragment, free L chains and fragments and IgA molecules of the secretory type. The IgG Fc fragment may account for 15 percent of the gamma globulin present in normal urine. It is the product of catabolic events involving the complete IgG molecule. The L chains present in normal urine account for more than half the globulin fraction. Their kappa to lambda ratio remains approximately two to one and their concentration may vary from 2.5 to 4.0 mg per liter.\(^5\)\(^,\)\(^6\) Immunoelectrophoretic examination of the urinary L chains reveals a diffuse electrophoretic mobility comparable to that seen with normal serum immunoglobulins.\(^21\) The L chains in

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normal urine may be monomers, dimers or trimers.

Electrophoresis and immunoelectrophoresis of a concentrated urine specimen is the most sensitive method currently available for the detection of BJ proteinuria. The use of high quality anti-kappa and anti-lambda antisera in immunoelectrophoretic techniques provides a dimension of specificity that is unavailable with any other method. Materials and technical methods are readily available and will not be considered. However, some remarks on anti-light chain antisera appear warranted.

Kappa and lambda chains are a heterogeneous protein population. Antigenic differences have allowed the division of both light chain types into several subgroups. Antisera prepared against certain BJ proteins have been found to contain antibody populations unique to antigenic sites on the VL portion, the CL portion and the hinge region between CL and VL. Not all BJ proteins are capable of generating these various specificities. In addition, L chain antisera prepared using whole immunoglobulin or Fab fragments that contain bound kappa or lambda chains will have different properties than antisera prepared with BJ protein as the immunogen. In particular, antisera against monoclonal L chains may react very well with other BJ proteins of the same type but poorly or not at all with a similar L chain bound to an intact immunoglobulin. This antiserum may be useful in determining the type of BJ protein present in serum or urine but may fail to detect monoclonal proteins (especially IgA) present in serum and should not be used to evaluate the kappa/lambda ratio in routine sera. Therefore, any laboratory involved in the detection and analysis of BJ proteins by immunoelectrophoresis should require complete information regarding the preparation of antisera purchased from commercial sources. In addition, panels of serum and urine known to contain certain monoclonal proteins should be frozen (−70°) and used to evaluate any new antiserum before it is put into routine use.

The incidence of BJ proteinuria associated with multiple myeloma and other disorders is dependent on the method used for detection. In large series, the heat test is positive in only 50 percent of the tested patients. The use of a concentrated urine specimen, electrophoresis and immunoelectrophoresis increases the number of positives to 70 or 80 percent.

**Significance**

A search for BJ protein is most often associated with the laboratory evaluation of a patient suspected of having multiple myeloma. However, monoclonal L chains may be found in the urine of patients with other immunoproliferative disorders (Waldenström's macroglobulinemia, chronic lymphocytic leukemia), amyloidosis, carcinoma and perhaps in a few patients with no underlying disease. As previously noted, concentrated urine electrophoresis and immunoelectrophoresis leads to the detection of BJ protein in approximately 70 percent of patients with myeloma. The absence of BJ proteins in the other 30 percent of patients may reflect a lack of sensitivity in the method or a balanced synthesis by the malignant plasma cells so that no free monoclonal L chain is available for excretion.

Light chain disease is a myelomatous disorder in which BJ protein in the serum and/or urine is the only monoclonal protein detected. This entity comprises approximately 20 percent of the cases in large series of myeloma. It is often a rapidly progressive disease and is frequently associated with renal disease and amyloidosis. In other patients with multiple myeloma a phenomenon known as "BJ escape" may occur. These are usually patients with an IgG or IgA myeloma and are initially responsive to chemotherapy but later become refractory. This increased re-
sistance to therapy is heralded by a rapid increase in the amount of monoclonal L chain excreted but no corresponding increase of the monoclonal IgG or IgA. "BJ escape" may occur as a result of clonal selection by the therapeautic agent. Experimental support for this concept is available.\textsuperscript{32}

Waldenström's macroglobulinemia is another disorder in which the reported incidence of BJ proteinuria varies from zero to 60 percent as a result of different methods of testing.\textsuperscript{14,15,28} Urine protein electrophoresis and immunoelectrophoresis will detect BJ proteins in fifty percent or more of these patients. The urinary monoclonal L chain and the L chain portion of the monoclonal IgM found in the serum are the same in most instances. BJ proteinuria may also be found in patients with lymphocytic lymphomas and chronic lymphocytic leukemia.\textsuperscript{28,33} This is not surprising in light of the probable B cell nature of most of these tumors.\textsuperscript{38}

Monoclonal immunoglobulins, primarily IgG and IgA, are often present in patients with nonmyelomatous diseases\textsuperscript{53} or perhaps with no illness (benign monoclonal gammopathy). The reasons for this remain unknown. However, the presence or absence of BJ proteins in these patients has been used as a criterion for distinguishing the benign gammopathy from those that are associated with a malignant immunoproliferative disease. Dammacco and Waldenström\textsuperscript{15} used concentrated urine electrophoresis and immunoelectrophoresis and found that 23.8 percent of 42 patients with a benign monoclonal gammopathy had a monoclonal L chain in their urine. The physicochemical properties of the BJ proteins in these patients did not differ from those in the urine of myeloma patients. However, the amount of BJ proteinuria never exceeded 60 mg per liter in the patients with a benign monoclonal gammopathy. Concentrations varied widely in the myeloma patients and most of them had significantly greater levels with some patients excreting 10 g per liter of monoclonal L chain. Longterm followup on the patients with benign monoclonal gammopathy will be necessary to assure that they were not in a premelomatous state. Thus one should be cautious in using BJ proteinuria to discriminate a benign from a malignant monoclonal gammopathy.

Amyloidosis and a plasma cell dyscrasia occur more often in the same patient than coincidence would allow.\textsuperscript{4,23} In most if not all of these patients there is a monoclonal protein—frequently a BJ protein. Until very recently, this relationship was unexplained. However, in the past few years Glenner and his colleagues\textsuperscript{19} have demonstrated, by chemical methods, that the amyloid fibrils in a group of cases are complete immunoglobulin L chains or their variable (amino-terminal) fragments. This observation would explain the frequent occurrence of amyloidosis in patients with L chain disease.\textsuperscript{46} Isobe and Osserman\textsuperscript{23} have detected a monoclonal protein in one hundred percent of their patients with primary amyloidosis. Forty-six of their 50 patients had a BJ protein, most often of lambda type. Stone and Frenkel's\textsuperscript{46} findings in their patients with amyloidosis and L chain disease are similar. They observed that the patients who secrete monoclonal L chains with "amyloidogenic" properties usually have a disease course dominated by their amyloidosis rather than their myeloma. Similar observations have been made in our patients.

**Summary**

Bence Jones proteins are monoclonal light chain proteins. They are made and secreted by plasma cells usually in association with a malignant immunoproliferative disorder. They circulate in the plasma, are catabolized by the kidney and excreted into the urine. The heat tests traditionally used for the detection of Bence Jones proteins have been shown to lack specificity and sensitivity and are being replaced by electrophoretic and immunologic methods. Although BJ proteins are most often found in patients with mul-
multiple myeloma, they may be present in a variety of other disorders.

References

35. Putnam, F. W.: Structural relationships among normal human gamma globulin, myeloma globu-


