Pathology of the Kidney in Dysproteinemia
Pathology of the Kidney in Dysproteinemia

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DEDICATION

To three fellow Minnesotans: Elexious Thompson Bell, who awakened my interest in renal pathology, Joel Garrett Brunson, who introduced me to the beauty of experimental pathology and Frank James Dixon, Jr., who combined the two into a meaningful whole.
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PREFACE AND ACKNOWLEDGMENTS

Although a number of excellent textbooks on renal pathology are presently available, monographs, in dealing with a single subject, have several distinct advantages over chapters in traditional textbooks. Most importantly they are “user friendly” since their subject matter is sufficiently circumscribed to permit expanded or detailed visual and textual treatment of specific lesions in a single easy to wield volume. Comparative examples are in the in-depth AFIP fascicles on tumors of single organs or organ systems relative to the much more limited expositions in corresponding chapters in all inclusive textbooks of surgical pathology.

The material presented in this monograph was obtained from 147 cases of renal involvement associated with Dysproteinemia encountered in university and community hospital settings. The described entities account for 4.5% of 3.260 biopsies of native kidneys examined over a period of approximately 25 years. While some of the lesions are quite rare, others are relatively common and seen fairly frequently in a reasonably busy renal biopsy service. Together they constitute a group of extremely interesting lesions in terms of immunology, morphology and pathogenesis.

There is no best or favored classification of renal disease associated with Dysproteinemia. As in any related group of lesions involving single organs, structural, functional and chemical changes, as well as causal and developmental characteristics overlap to a considerable extent. In this monograph the individual renal lesions are classified on the basis of their most distinctive and separate morphologic features.

No mention is made in this volume of the normal anatomy, embryology, or basic mechanisms of injury to the kidney since these subjects are well covered and readily available in numerous texts and it is assumed that the reader is familiar with these fundamental concepts. No effort is made to present recipes for handling, staining and examining renal biopsies as these are also readily available. All of the biopsies were examined by thin section light (2-4 microns), immunofluorescence and transmission electron microscopy by well established techniques. In addition, and of considerable importance, all biopsy specimens were required to be accompanied by detailed clinical histories, serum samples and urine specimens to permit clinicopathologic correlation and additional special laboratory studies to arrive at precise diagnoses.
The individual major sections in this monograph are all organized within the same framework so that easy comparison can be made between similar aspects of different lesions. The individual lesions are discussed in terms of preferred designation, common synonyms, incidence, age, gender, race, significant clinical findings on presentation, important routine and immunology laboratory studies and morphologic findings relative to glomerular, tubular, interstitial and vascular changes. Where appropriate, differential diagnostic features are also included as are discussions concerning known or suspected etiologic factors and pathogenesis. No discussion of treatment of the various lesions is included as long term follow-up of this group of patients was inadequate for this purpose and treatment is well covered in more clinically oriented textbooks on renal disease.

Finally it is important to acknowledge the many nephrologists of northern California and northwestern Nevada who furnished us with the diagnostic renal biopsies, clinical histories, serum and urine samples that made this book possible. The author would also like to thank his friends and coworkers in the laboratories of Sutter Memorial Hospital in Sacramento, CA. These include, but are not limited to, Mary Ann Schendzelos for her remarkable skills in clinical immunology, and Harry Low, Becky LeFavre, Shirley Harner and Heidi Dick for their expertise in electron microscopy, photography and histology. He would also like to remember his fellow pathologists the late doctors John C. Lee and Charles M. (Morrie) Blumenfeld for stimulating discussions concerning many of the lesions described in this text.
INTRODUCTION

Dysproteinemia is defined in the 24th edition of Stedman's Medical Dictionary as “An abnormality in plasma proteins, usually immunoglobulins”. The International Dictionary of Medicine and Biology (1986) sets forth a somewhat broader definition of “Any disease associated with abnormal serum proteins. Qualitative or quantitative abnormalities may involve any serum protein, including lipoproteins”.

Dysproteinemias involving immunoglobulins result from the production and secretion of monoclonal (M) proteins by single transformed clones of B-lymphocytes or plasma cells and the term plasma cell dyscrasia is often used synonymously with Dysproteinemia.

The M-proteins or paraproteins may be produced by aggressively proliferating neoplastic cells as in clinically obvious multiple myeloma, or by less rapidly dividing, more stable, clones of cells in paraproteinemias of undetermined significance on initial discovery. These latter cases may comprise early or indolent myelomas which can be diagnosed with certainty only later in their course, or of small clones which defy precise clinical characterization over extended periods of time.

The abnormal serum immunoglobulins may consist of monoclonal light chains alone, intact monoclonal immunoglobulin molecules or rarely of monoclonal heavy chains alone. Large amounts of these proteins are typically found in classical myeloma and are easily detected by routine serum paper or cellulose acetate electrophoresis and identified by immuno-electrophoresis or immunofixation. If light chains are secreted in excess of intact immunoglobulins or are the only chains produced by the neoplastic cells, they are excreted in the urine and readily demonstrated there. In paraproteinemias of undetermined significance, however, detection and identification of smaller amounts of monoclonal protein may require studies on concentrated specimens, especially concentrated urine samples.
Monoclonal proteins in serum or urine may be present in excess but not express detectable qualitative abnormalities. Some, however, may exhibit amino acid deletions, additions or substitutions; abnormally short or long light or heavy chains; hydrophobic chain segments and excessive glycosylation or polymerization. It has been postulated that any one of these molecular aberrations may be important in the pathogenesis of the injury produced by the deposition of these proteins in the kidney or other organs.

Polyclonal, as well as monoclonal immunoglobulins, may also exhibit qualitative abnormalities resulting in the deposition of fibrils and microtubules in presumed immune complex induced fibrillary glomerulonephritis and immunotactoid glomerulonephritis.

Dysproteinemias of renal significance also include non-immunoglobulin proteins which may be presumed to be of normal structure and present in the serum in excess, or may be qualitatively abnormal. In renal amyloidosis precursor proteins include serum amyloid A-related protein (SAA), transthyretins in certain ethnic groups, apolipoprotein A-1, fibrinogen A-alpha chain and lysozyme. With the exception of SAA, amyloidoses associated with these other proteins are relatively rare autosomal dominant diseases related to single amino acid substitutions.

Renal involvement in Dysproteinemia is usually due to the deposition of protein in specific anatomic sites within the kidney. It is the site as well as the type of deposit which dictate the morphologic and functional changes that characterize the various lesions encountered. Renal functional abnormalities are frequently the first indication of a Dysproteinemia. Conditions due to the rapid proliferation of neoplastic clones of cells may also secondarily involve the kidney. Nephrocalcinosis, uric acid deposits and pyelonephritis may occur. Functionally significant parenchymal infiltration by malignant clonal cells may also occasionally take place.
PART I

LESIONS ASSOCIATED WITH MONOCLONAL IMMUNOGLOBULIN PRODUCTION AND NONFIBRILLAR PROTEIN DEPOSITS
Thirty patients with clinically symptomatic and progressive disease satisfied at least 1 major and 1 minor criteria or 3 minor criteria for the diagnosis of multiple myeloma as presented in Table 1-1.

**TABLE 1-1  DIAGNOSTIC CRITERIA FOR MULTIPLE MYELOMA***

| Major: 1) | 30% or > marrow plasma (myeloma) cells or solitary plasmacytoma on tissue biopsy.  
| 2) Serum M-protein > 3.5gm/dl for IgG, > 2.0 gm/dl for IgA or monoclonal light chain urine excretion of > 1.0gm/24hrs.  
| Minor: 1) | 10-30% marrow plasma (myeloma) cells.  
| 2) Serum M-protein present but < major criteria.  
| 3) Lytic bone lesions.  
| 4) Decrease of normal immunoglobulins to < 600 mg/dl IgG, < 100mg/dl IgA and < 50mg/dl IgM. |

* Modified from Barlogie, B. (1)

Of the 30 patients, 27 or 90% exhibited light chain cast nephropathy (LCCN) on biopsy. In addition 3 of these 27 patients (11%) also displayed early amyloid deposits and 2 of the 27 (7%) characteristic early deposits of light chain deposition disease (LCDD). These numbers compare favorably with those observed in other renal biopsy and necropsy series of patients with myeloma where up to 86% have obstructive cast nephropathy (2), 11% AL-amyloidosis and 5% LCDD(3).

Many of the biopsies of LCCN also revealed prominent tubular epithelial cell changes consistent with direct tubular toxicity. Of the 3 myeloma patients not showing LCCN, 1 patient with lambda light chain proteinuria also exhibited tubular proteinuria and early histologic evidence of tubulotoxicity in the absence of light chain casts. The 2 additional patients showed chemical and morphologic evidence of Fanconi’s syndrome in association with kappa light chain proteinuria without significant cast formation.

Biopsies from 2 patients with LCCN also revealed histologic evidence of calcium deposition in association with hypercalcemia. One additional patient demonstrated uric acid deposits due to hyperuricemia and 2 patients presented with significant clinical and histologic evidence of pyelonephritis.
LIGHT CHAIN CAST NEPHROPATHY (LCCN)

COMMON SYNONYMS:
1) Bence-Jones cast nephropathy
2) Myeloma cast nephropathy
3) Myeloma kidney

INCIDENCE:
The overall incidence of multiple myeloma in the U.S. population is approximately 4/100,000 (4). It accounts for 1% of all malignancies and slightly greater than 10% of hematopoietic malignancies (5).

Obstructive light chain cast nephropathy (LCCN) is the most common and serious renal complication of myeloma. It occurs in 50% of myeloma patients in some autopsy series (6) and up to 86% in renal biopsy series where selection occurs because of clinical renal disease. LCCN accounts for 18% of the total number of cases of renal involvement due to Dysproteinemia described in this monograph.

AGE, SEX AND RACE:
LCCN as a complication of myeloma is a disease of older individuals with a reported median age of 61 years (5). It is uncommon in the young with an incidence of only 2.2% in patients under 40 years and 0.3% in patients under 30 (7). In the present series, as seen in Figure 1-1, the majority of cases occurred in the 5th through 8th decades with a median age of 58.5 years and a range of 24 to 84 years. Interestingly 11% of the patients in this group were under age 40.

Twice as many males as females demonstrated LCCN. This 2:1 ratio is somewhat higher than that of 3:2 usually given in the literature (8).

Although African-Americans are reported to have twice the incidence of multiple myeloma than Whites (8), in this study 70% of the patients were Caucasian. This percentage is probably a reflection of the rural and semi-rural character of the population in this biopsy area similar to that found by Kyle, et al (9) in Olmstead County, Minnesota.

CLINICAL AND LABORATORY FINDINGS:
Figure 1-2 presents the significant clinical findings on presentation of patients with LCCN and Table 1-2 their pertinent laboratory results.

As is evident in Figure 1-2, 81% of the patients with LCCN presented with signs and symptoms of renal failure. These consisted of prominent fatigue, weakness, anorexia and variable weight loss. In some patients a history of very recent onset was associated with pure light chain myeloma which is often responsible for acute renal failure in LCCN (10). In several patients the acute onset of renal failure was also associated with additional factors of dehydration, urinary tract infection and hypercalcemia (11). In other cases slowly developing symptoms without an acute episode were indicative of progressive renal failure which is the most common presentation of LCCN.

As presented in Table 1-2 the mean serum creatinine level for all patients was 8.8 mg/dl with a range of 1.4 mg/dl in a patient without renal failure to 18.6 mg/dl in a patient with advanced disease.

Serum potassium levels varied with the severity of renal failure reaching a value of 6.8 meq/l in the most severely azotemic patient.
Approximately one third of the patients exhibited elevation of their blood pressure. This in part was probably related to their renal failure but was also felt to be due to age related vascular disease as some of the renal biopsies exhibited significant arterio- and arteriolosclerosis. This preexisting renal vascular involvement was probably a significant secondary factor in the development of renal failure in some of the patients with LCCN.

Almost one third of the patients were also noted to exhibit generalized or conjunctival and mucous membrane pallor and the majority a normochromic, normocytic anemia with a mean hematocrit of 24.8%. The anemia associated with multiple myeloma is secondary to marrow replacement by myeloma cells. The degree of anemia is related to the total tumor mass. The patient with the lowest hematocrit of 13.1% in this study also exhibited multiple lytic lesions of the pelvis, proximal femurs and lumbar vertebrae on bone-scan and a serum k-IgA peak of 5.5 gm/dl. He satisfied the clinical criteria for a myeloma with high tumor mass estimated to be > 1.2 X 10^12 myeloma cells/M^2 (12). In addition it has been reported that serum erythropoietin levels are low for this degree of anemia present (13) possibly due to excessive production and activity of interleukin-1 and tumor necrosis factor beta (14). This is probably also a contributing factor to the anemia of myeloma.

Seven or 26% of the patients complained of bone pain which in 5 patients was associated with lytic bone lesions. Of the remaining 2 patients, one had a compression fracture of the 3rd lumbar vertebra in association with spinal osteopenia and the other patient no discernable skeletal lesions on scan. Of the patients with lytic bone lesions 3 with the most extensive involvement demonstrated hypercalcemia with serum calcium levels as high as 14.4 mg/dl.

The majority of patients with LCCN had elevated serum uric acid levels with a mean value of 13.3 mg/dl and a range of 6.8 to 19.3 mg/dl.

Five or 19% of the patients presented with fever, neutrophilia and localized signs of infection. Three of these patients had clinical and radiographic evidence of pneumonia and 2 patients clinical and laboratory evidence of a urinary tract infection.

Only 4 or 15% of the LCCN patients on initial presentation had a prior diagnosis of multiple myeloma emphasizing the repeated observation that renal involvement is frequently the first indication of the disease (11,15).

Importantly, none of the patients exhibited edema, marked hypoalbuminemia or significant hypercholesterolemia even though the majority had very significant proteinuria with a mean quantitative urine protein of 413 mg/dl. This indicated the non-nephrotic character of their proteinuria and in association with their age and renal failure strongly suggested the diagnosis of LCCN.

### Table 1-2

<table>
<thead>
<tr>
<th>Lab Test</th>
<th>Mean Value</th>
<th>Patient Range</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCRT %</td>
<td>24.8</td>
<td>13.1 to 38.0</td>
<td>35.0 to 49.0</td>
</tr>
<tr>
<td>Cr mg/dl</td>
<td>8.8</td>
<td>1.4 to 18.6</td>
<td>0.5 to 1.4</td>
</tr>
<tr>
<td>K meq/l</td>
<td>5.6</td>
<td>3.5 to 6.8</td>
<td>3.5 to 5.3</td>
</tr>
<tr>
<td>Ca mg/dl</td>
<td>10.2</td>
<td>8.1 to 14.4</td>
<td>8.5 to 10.3</td>
</tr>
<tr>
<td>U-ACID mg/dl</td>
<td>13.2</td>
<td>6.8 to 19.3</td>
<td>4.0 to 8.5</td>
</tr>
<tr>
<td>CHOL mg/dl</td>
<td>209</td>
<td>133 to 273</td>
<td>&lt;200</td>
</tr>
<tr>
<td>TSP gm/dl</td>
<td>7.5</td>
<td>4.7 to 14.4</td>
<td>6.0 to 8.0</td>
</tr>
<tr>
<td>ALB gm/dl</td>
<td>3.6</td>
<td>2.4 to 5.9</td>
<td>3.8 to 5.5</td>
</tr>
<tr>
<td>QUP mg/dl</td>
<td>413</td>
<td>25.0 to 1,380</td>
<td>12.5 to 20.0</td>
</tr>
</tbody>
</table>

**HCRT:** hematocrit, **Cr:** serum creatinine, **K:** serum potassium, **Ca:** serum calcium, **U-ACID:** serum uric acid, **CHOL:** total serum cholesterol, **TSP:** total serum protein, **ALB:** serum albumin, **QUP:** quantitative urine protein.

### Monoclonal Protein:

The monoclonal immunoglobulin isotype produced and secreted by neoplastic plasma cells in large series of myeloma patients are: 58 to 61% IgG, 20 to 23% IgA, 17 to 20% kappa or lambda light chains alone and 1 to 2% IgD (1,16,17). Monoclonal IgE, IgM and biclonal immunoglobulins are only rarely encountered (1). The kappa/lambda light chain ratio mirrors that of immunoglobulins produced by normal plasma cells with about 67% kappa and 33% lambda light chain assembled (1). In IgD myeloma, however, lambda light chains are much more frequently associated with delta heavy chains and an increase in free light chains is often observed (17).

Light chain proteinuria occurs in 100% of patients with light chain myeloma and is stated to be present in 92% of patients with IgD, 70% with IgA...
and 65% with IgG myeloma (10). Of the patients with LCCN documented in this monograph, 12 of 27 or 44% exhibited only kappa or lambda light chains in their urine as depicted graphically in Figure 1-3 and illustrated in Figure 1-4. As seen in Figure 1-3, 56% of the LCCN patients demonstrated intact monoclonal immunoglobulins which consisted of IgG in 37%, IgA in 11% and IgD in 7.4% of the total of 27 cases.

Since light chain cast formation in distal renal tubules is directly related to the amount of light chains excreted in the urine (18) it is not surprising that slightly greater than ½ of the LCCN patients had light chain myeloma or IgD myeloma. Similar findings were noted by Ronco, et al. where 37% of LCCN patients had myeloma cell clones producing only light chains (19).

The light chain ratio for all 27 patients described in this monograph was 63% kappa and 37% lambda or very close to the ratio for normal immunoglobulins. Comparable findings have been noted in many other studies. In several, however, a reversed ratio has been observed. Ronco, et al. found a kappa:lambda ratio of 37%:63% in 30 biopsy patients (19). In an additional 13 patients in the same study, however, a more normal ratio of 62%:38% was noted. Bernstein and Humes also reported an increased incidence of lambda light chains in their series of patients with multiple myeloma and renal failure (20).

MORPHOLOGIC FINDINGS:

GROSS PATHOLOGY:

Because all of the renal tissue in this study was obtained by biopsy, the only gross feature available for correlation was that of kidney size in those patients in which abdominal x-rays were obtained. Bilateral renal length was either normal or commensurate with the age and vascular status in the oldest patients. The demonstration of normal sized kidneys in elderly patients with renal failure should raise the suspicion of myeloma with LCCN (21).

MICROSCOPIC PATHOLOGY:

Glomeruli:

The glomeruli in LCCN frequently appear normal except in those cases associated with AL-amyloidosis and LCDD. Occasionally subtle glomerular changes associated with hyperviscosity occur as illustrated in
Figure 1-5. Increased serum viscosity in myeloma is most commonly associated with monoclonal IgA (22).

Figure 1-5: Glomerulus of a patient with lambda-IgA myeloma and a serum viscosity of 2.48 centipoise. Note the rouleaux of intracapillary erythrocytes (arrows) in association with several small “intraluminal thrombi” (arrow heads) H&E stain X 370.

In some instances the protein in Bowman’s space is very dense, prominently eosinophilic and more compressive of glomerular structures as shown in Figure 1-8. In these cases because of the large amount of protein present, its monoclonal composition can be easily demonstrated by the less sensitive immunoperoxidase technique applied to sections of paraffin embedded tissue and examined by conventional light microscopy as seen in Figure 1-9.

When examined by electron microscopy, as shown in Figure 1-10, the dense, suggestively laminated appearance of the protein in Bowman’s space is seen to be due to the presence of polymerized kappa light chains which have a propensity to self-aggregate when highly concentrated.

In rare cases crystals of monoclonal light chains are noted in Bowman’s space as a consequence of the crystallization of refluxed tubular protein as shown in Figure 1-11. These crystals typically invoke a cellular response similar to that noted about light chain casts within more distal tubular lumens.
Figure 1-8: Glomerulus of another patient with kappa light chain myeloma and LCCN exhibiting very dense, suggestively laminated protein filling Bowman’s space. H&E stain X 475.

Figure 1-9: Glomeruli of the same patient illustrated in Figure 1-8. A) Positive staining of dense protein for kappa light chains. B) Negative staining for lambda light chains in a similarly involved Glomerulus. Immuno-peroxidase stains for human kappa and lambda light chains X380.

Figure 1-10: Electron micrograph of a portion of a Glomerulus from the same patient illustrated in Figures 1-8 and 1-9 showing parallel fibrils of polymerized kappa light chains (arrows) in Bowman’s space X 33,480.

Figure 1-11: Glomerulus from a patient with lambda light chain myeloma and LCCN showing rhomboid shaped crystals of light chains within Bowman’s space and adjacent tubular lumens. The crystals are surrounded by multinuclear giant cells and mononuclear cells in both locations. H&E stain X 475.

TUBULES:

As indicated by its descriptive designation the most striking changes in Light Chain Cast Nephropathy involve the renal tubules and secondarily the adjacent interstitium. The tubules contain very distinctive casts which are often most notable within distal and collecting tubular lumens. In cases with marked light chain proteinuria and extensive cast formation, numerous casts within proximal convoluted tubules are also present.

The cast containing tubules are often distended and exhibit epithelial cell atrophy. Typically the casts are brightly eosinophilic, dense and frequently appear brittle and fractured in both paraffin embedded light microscopic sections as seen in Figure 1-12A and in frozen sections as illustrated in Figure 1-12B.

By electron microscopy the casts are very electron dense and encroach upon and compress the tubular epithelial cells as seen in Figure 1-13.

Figure 1-12: A) Eosinophilic, dense, fractured light chain casts within the lumens of distended tubules exhibiting marked epithelial cell compression and atrophy. H&E stain X 370. B) Fractured intensely staining kappa light chain casts. FITC anti-kappa light chain serum X 370.
Electron micrograph of a portion of a dense light chain tubular cast (*) encroaching upon and compressing tubular epithelial cells. A segment of tubular basement membrane (arrow heads) is present in the left upper corner X 12,110

With periodic acid Schiff (PAS) stains the light chain containing casts within distal tubules are PAS-positive due to the associated presence of Tamm-Horsfall glycoprotein. In more proximal tubular lumens the casts are either PAS-negative or only weakly PAS-positive as noted in Figure 1-14A. These latter casts are composed almost entirely of light chains which usually do not contain carbohydrate and are not glycosylated and consequently do not have 1-2 glycol groups available to react with the Schiff reagent (23). This negative or only weakly PAS-positive staining differs strikingly from the intensely PAS-positive staining of usual tubular casts as depicted in Figure 1-14B and should immediately suggest the presence of casts composed predominantly of immunoglobulin light chains. In addition while the light chain casts may be multilamellar they do not exhibit the concentric or circumferential lamellation as is so typical of IgA containing casts in other types of tubular atrophy as illustrated in Figure 1-14B.

The obstructing light chain casts in LCCN which are composed primarily of light chains can be easily identified by either immunofluorescent microscopy, as illustrated in Figure 1-15, or by immunoperoxidase staining as seen in Figure 1-16.

Some of the casts may also contain small amounts of immunoglobulin, albumin and fibrinogen as well as Tamm-Horsfall protein (2,24). Typically the staining for the former components is much less intense than that for the light chains excreted in the patients urine and the casts containing them much less numerous.

In occasional cases of LCCN the casts are composed of distinct crystals associated with mononuclear and giant cells as shown in Figure 1-17.

By electron microscopy the crystals may be large, overlapping and rhomboid in shape or small and needle or plate-like in appearance. Almost always the electron dense crystalline material is associated with finely granular less electron dense protein as seen in Figure 1-18.

Figure 1-14: A) PAS negative staining light chain casts in a patient with kappa light chain myeloma and LCCN. B) Intense PAS staining tubular casts in a patient with renal failure associated with nephrosclerosis. By immunofluorescent microscopy these casts contained abundant IgA and presumable Tamm-Horsfall protein. Note the concentric lamellated appearance of the casts. AB-PAS stains X 370.

Figure 1-15: Serial sections from a patient with Kappa LCCN. A) Intense staining for kappa light chains. B) Trace to negative staining of the same casts for lambda light chains. FITC anti-kappa and lambda light chain serum X 370.

Figure 1-16: Biopsy from a patient with kappa LCCN. A) Positive staining of tubular casts for kappa light chains. B) Negative staining of the casts for lambda light chains. Immunoperoxidase anti-kappa and lambda light chain serum X 370.
A very conspicuous, almost pathognomonic morphologic feature of LCCN, is the cellular reaction incited by the light chain casts within the tubular lumens. Only infrequently in Waldenstrom’s macroglobulinemia (25), and rarely in other conditions unrelated to multiple myeloma (26), are similar appearing casts noted. Characteristically the light chain casts in LCCN are surrounded by mononuclear cells and multinucleated giant cells as illustrated in Figure 1-19.

Many years ago, Bell correctly suggested that the cells were macrophages (27). Allen among others, however, considered the cells to be of epithelial cell origin and the giant cells to be "a syncytium of tubular epithelial cells rather than foreign body giant cells" (28). The controversy as to the lineage of these cells continued until recently when antibodies against cytochemical markers established their monocyte/macrophage origin (32-34) as illustrated in Figure 1-20.

In some cases neutrophiles as well as macrophages and giant cells are present about the light chain casts. In these instances the cells appear actively phagocytic by both light microscopy as noted in Figure 1-21 and electron microscopy as seen in Figure 1-22. The periphery of the casts have a scalloped or moth-eaten appearance and the cells appear to be ingesting cast material.

The inflammatory cells apparently gain access to the intratubular casts from the interstitium (peritubular capillaries) through discontinuities or breaks in the tubular basement membranes (18).
Prominent degenerative changes of the tubular epithelium is noted in association with the casts as shown in Figures 1-19 and 1-21. These tubular epithelial cell changes, however, are not confined just to the cast containing tubules but are widespread and involve non-cast bearing tubules as well. This probably explains the lack of uniform correlation between the extent of cast formation and impairment of renal function that has been reported (14,35).

Figure 1-21: Light chain casts surrounded and invaded by monocytes, macrophages and scattered neutrophiles. A scalloped and moth-eaten appearance of the casts (•) is seen as is the presence of apparent cast material within the cytoplasm of occasional phagocytes (arrow). H&E X 370.

Figure 1-22: Electron micrograph showing endocytic phagocytosis (arrow) of light chain cast material (•) by an activated mononuclear phagocyte with cytoplasmic pseudopods. X 13,460.

INTERSTITIUM
Continuous accretion of light chains by tubular casts over time is believed to result in tubular rupture with extrusion of casts and tubular contents into the adjacent interstitium (18,36). Very likely digestion of tubular basement membranes by hydrolytic enzymes of phagocytic origin contributes to this process as well by creating discontinuities of breaks in the basement membranes. The presence of light chain cast material within the peritubular interstitium provokes an inflammatory reaction which may be very intense and characterized by the presence of neutrophiles as well as mononuclear cells resulting in a severe interstitial nephritis as depicted in Figure 1-23.

Figure 1-23: Early acute interstitial nephritis associated with destruction of a large segment of the basement membrane of a cast containing tubule (arrows) by inflammatory cells. H&E X 370.

As inflammation proceeds there is an associated proliferation of fibroblasts, as noted in Figure 1-24, eventuating in interstitial fibrosis and tubular atrophy as seen in Figure 1-25.

Figure 1-24: Advanced interstitial nephritis with extensive destruction of parenchymal structures and proliferation of fibroblasts (arrows). H&E X 370.
Figure 1-25: Interstitial fibrosis and tubular atrophy in end-stage nephritis of LCCN. H&E X 115.

In some cases of LCCN, as seen in Figure 1-26, the phlogogenic response to extruded cast material is less intense and consists of lymphocytes, macrophages and multinucleated giant cells surrounding cast fragments in which the light chain components are apparently relatively resistant to digestion by macrophage proteases (37).

Figure 1-26: Chronic granulomatous interstitial nephritis with lymphocytes, macrophages and multinucleated giant cells surrounding extruded cast material (*). One giant cell contains an apparent cast fragment within its syncytial cytoplasm (arrow). H&E X 475.

Although microscopic infiltrates of neoplastic plasma cells are reported in 10% of autopsied myeloma cases, infiltration of the interstitium is rarely seen in needle biopsy specimens, for when they do occur they are small and widely scattered.

VESSELS

In the absence of associated amyloidosis or LCDD no specific vascular changes are present in biopsies of patients with LCCN. Because LCCN usually occurs in older individuals, significant arterio- and arteriolosclerosis is frequently present as illustrated in Figure 1-27.

The presence of significant pre-existing vascular disease in many patients with LCCN is undoubtedly important in the development of their renal failure. This is especially true in patients with acute renal failure associated with dehydration and hypovolemia in which acute tubular necrosis (ATN) due to decreased renal blood flow is an additive factor in the development of their tubular damage. Microscopic assessment of arterial disease and ATN of distal tubules is of considerable value in predicting the reversibility of renal failure in patients with LCCN.

Figure 1-27: Biopsy from a 59-year-old female with lambda-IgA myeloma and LCCN. Prominent fibrous intimal and elastic lamellar thickening of the wall of an interlobular artery branch have resulted in significant luminal attenuation. Hyaline arteriolosclerosis of an adjacent afferent arteriole is also present (arrow). AB-PAS stain X 370.

DIFFERENTIAL DIAGNOSIS:

The major microscopic changes in LCCN are so distinctive that the diagnosis is almost always readily apparent in adequate biopsy specimens. Often the diagnosis can be made on H&E stained frozen sections of that portion of the biopsy obtained for immunofluorescent microscopy and rapid reports can be conveyed to the attending nephrologists concerned as to the cause of their patients' renal failure.

ETIOLOGY AND PATHOGENESIS:

The etiology or cause of the neoplastic transformation and proliferation of plasma cells in multiple myeloma is unknown. The identification of immunoglobulin light chains as the major component
of obstructing tubular casts in myeloma patients with renal failure has established them as the primary pathogenic agents of LCCN.

Under suitable conditions of tubular fluid flow rate, pH, NaCl and calcium concentration cast forming monoclonal light chains (LC) bind to a specific peptide portion of Tamm-Horsfall protein (THP) (39-41). This glycoprotein is anchored to the luminal surface of epithelial cells of the thick ascending limb of Henle’s loop and early distal convoluted tubules. A portion of the THP is released as a soluble protein by the action of proteases (42), and aggregation of this portion with light chains results in the formation of insoluble LC-THP precipitates and cast formation within the distal tubules (40). Due to continuous local accretion of the precipitate the casts enlarge and distend the tubules which may rupture with extrusion of cast material and tubular contents into the peritubular interstitium (18). In this site the particulate THP is bound to a single class of sialic acid specific receptors on the surface of neutrophiles (43) resulting in their activation, release of reactive oxygen metabolites (44) and the induction of an acute inflammatory response. As inflammation proceeds activation of macrophages by particulate THP also results in the release of superoxide radicals as well as glucosaminidase and gelatinase (45), resulting in further tissue damage and eventual fibrosis.

Not all light chains produced by myeloma cells form tubular casts with THP. Occasional myeloma patients excrete large amounts of light chains in their urine for many years without measurable adverse effects on renal function (16,46). The specific physicochemical properties of light chains responsible for their aggregation with THP are unknown. It has been proposed that ionic interaction between light chains with high isoelectric points (pl) and THP with a pl of 3.5 in acidic tubular fluid is responsible for their aggregation leading to cast formation (47,48). More recent studies, however, have failed to support this contention (2,49,50) and the importance of light chain pl is still uncertain.

**LIGHT CHAIN TUBULOTOXICITY**

Although renal tubular obstruction by light chain casts is felt to be the most important factor in producing severe tubular damage in multiple myeloma, there is considerable evidence that soluble light chains are also directly toxic to tubular epithelium. As mentioned earlier, morphologic evidence of tubular epithelial cell damage is not confined just to cast containing tubules but is widespread, involving cast free proximal as well as distal convoluted tubules. Prominent reduction in the height of proximal tubular epithelial cells, vacuolization of their thinned cytoplasm and nuclear pyknosis in association with interstitial edema is frequently striking as seen in Figure 1-28.

In Alcian Blue-PAS stained sections the interstitial edema is characterized by an increase in blue staining glycosaminoglycans as depicted in Figure 1-29A, and in immunofluorescent stained frozen sections for fibrin(ogen) as seen in Figure 1-29B.

The small amount of free kappa and lambda light chains filtered into the urine in normal individuals is almost completely reabsorbed and catabolized by
the proximal convoluted tubules (51,52). In myeloma patients with prominent light chain proteinuria, the cytoplasm of the less damaged proximal convoluted tubular epithelial cells typically contain numerous protein resorption droplets. By immunofluorescent microscopy these stain intensely for the light chain detected in the patient’s urine as seen in Figure 1-30.

If tubulotoxic, the intracytoplasmic light chains would be expected to cause observable cell damage. One of the patients with lambda-IgM myeloma in this study exhibited refractory tubular proteinuria and early microscopic changes of tubulotoxicity in the absence of light chain casts. The histologic changes consisted of prominent apical blebs associated with focal loss of brush borders and cytoplasmic clearing or vacuolization of proximal tubular cells packed with protein resorption droplets as illustrated in Figure 1-31. These findings are similar to those described by Sanders, et al. in kidney specimens from patients with light chain related disease without evidence of intraluminal light chain precipitation or cast formation (53).

Direct tubulotoxicity of myeloma light chains has been well documented in experimental studies. A single class of low affinity, high capacity binding sites with relative selectivity for light chains has been identified on the brush border membranes of human and rat proximal tubule cells (54). These receptors mediate endocytosis and delivery of the light chains to degradative sites within acidified vesicles (55). This process interferes with the uptake of alanine, glucose and phosphate by the brush borders and their intracellular transport (56,57). Lysosomal injury by light chains during their catabolism is evidenced by an increase in both intracellular and urinary N-acetyl-beta-D-glucosaminidase in association with an increase in the number and size, as well as autophagic vacuolation, of the lysosomes containing light chains (58,59). Perfusion of isolated rat nephrons by light chains also results in cytoplasmic vacuolation, focal loss of brush borders and fragmentation and desquamation of proximal tubule cells containing light chains within endosomes and activated lysosomes (60). Defective Lysosomal acidification (61) as well as inhibition of Na-K-ATPase activity and gene expression by light chains within proximal tubule cells has also been reported (62). These experimental studies lend strong support for the role of direct toxic injury to renal tubules by at least some light chains in humans with multiple myeloma. As is the case with LCCN, the physicochemical properties of these tubulotoxic light chains have yet to be determined (63).

LIGHT CHAIN INDUCED FANCONI’S SYNDROME

Further evidence for the direct tubulotoxicity of immunoglobulin light chains is found in cases of adult Fanconi’s syndrome associated with multiple myeloma (64). Two cases in this study revealed clinical and chemical features of this entity in association with kappa-light chain proteinuria without significant cast formation.

One of these cases was a 61-year-old male admitted to the hospital for evaluation of back
pain due to a destructive lesion of the body of his 5th lumbar vertebra. Routine urinalysis revealed proteinuria and glucosuria. Twenty-four-hour urine protein was > 5gm. Paper electrophoresis of his concentrated urine disclosed the presence of an anomalous protein with alpha-2 mobility combined with a pattern consistent with tubular proteinuria. Serum paper electrophoresis failed to demonstrate any detectable M-protein as shown in Figure 1-32A. Immunoelectrophoresis identified the anomalous urine protein as free kappa-light chains which were also present in trace amount in the patient’s serum as seen in Figure 1-32B.

A sternal bone marrow aspirate contained 22% immature plasma cells. Quantitative serum immunoglobulin determinations revealed a reduced IgG of 700mg/dl (normal 770-1130), IgA of 62mg/dl (80-200) and IgM of 77 mg/dl (90-170). Additional special studies showed widespread impairment of proximal tubular transport function with glucosuria, generalized aminoaciduria, hypouricemia, hypophosphatemia and hypokalemia. Urine glucose was 8gm/24hrs, with a fasting blood sugar of 93mg/dl (normal 70-125). Eight of 8 measured urine amino acid levels were elevated. Serum uric acid was 2.0mg/dl (normal 4.0-8.5), PO4 1.4mg/dl (2.1-4.3) and K 2.6meq/l (3.5-5.3). Radiography revealed bone demineralization consistent with hypophosphatemic osteomalacia (65).

Light microscopic sections of the patient’s renal biopsy demonstrated numerous needle shaped crystals within the cytoplasm of the proximal tubular epithelial cells as illustrated in Figure 1-33A. The crystals exhibited positive staining for kappa light chains by the immunoperoxidase technique as seen in Figure 1-33B.

By electron microscopy the crystals were needle shaped, very electron dense and bound by distinct membranes as shown in Figure 1-34. They were similar in appearance to those described previously in the literature (66).