PATTERNS OF TUBULAR PROTEINURIA FROM METALS AND SOLVENTS

R. P. Wedeen
Department of Veterans Affairs, East Orange, New Jersey
University of Medicine and Dentistry of New Jersey and New Jersey Medical School
Newark, New Jersey

I. Udasin and N. Fiedler
University of Medicine and Dentistry of New Jersey, Newark, New Jersey
Environmental and Occupational Health Sciences Institute, Piscataway, New Jersey

P. D'Haese and M. E. DeBroe
University of Antwerp, Edegem, Belgium

E. Gelpi
Institute for Biomedical Research, CSIC, Barcelona, Spain

K. W. Jones
Brookhaven National Laboratory, Upton, New York
Patterns of Tubular Proteinuria from Metals and Solvents

RICHARD P. WEDEEN, IRIS UDASIN, NANCY FIEDLER, PATRICK D’HAESE, MARC E. DEBROE, EMILIO GELPI, AND KEITH W. JONES

Modern concepts of renal disease began with the observation of Richard Bright in 1827 that proteinuria indicates the presence of kidney disease. Bright detected protein by boiling the urine until a white precipitate formed. This precipitate consisted mainly of albumin, which is present in gram-per-liter quantities in urine of patients with glomerulonephritis or Bright’s disease. Whereas glomerular disease is readily detected by measuring albumin in the urine, nonglomerular disease (i.e., tubulointerstitial nephritis), is characterized by the absence of heavy albuminuria in its early phase. Tubulointerstitial nephritis is therefore more difficult to detect until a substantial fraction of kidney function is lost. When the glomerular filtration rate (GFR) is reduced by more than 60%, the blood urea nitrogen (BUN) and serum creatinine concentrations are elevated. The significance of an increased BUN was recognized by Richard Bright.

Normally only a small fraction of circulating albumin and other high-molecular-weight proteins (HMWP) pass through the glomerular capillary filter. Much of the protein that reaches the lower nephron is reabsorbed and catabolized in the proximal tubule so that less than 300 mg of albumin per day appears in the urine. Nevertheless, slightly increased albuminuria (30-300 mg/day), but within normal limits, heralds the future development of kidney disease in patients with diabetes mellitus.

The appearance of minute quantities of low-molecular-weight proteins (LMWP) in the urine has been recognized as the signature of toxic nephropathy since the studies of Friberg in 1948 of urinary excretion of β₂-microglobulin among cadmium workers (Friberg, 1948). LMWP, which are present in only microgram-per-liter quantities, must be distinguished from the heavy albumin-
uria (grams per liter) associated with glomerular disease. In contrast to HMWP, most circulating LMWP pass through the glomerular filter. Following tubular reabsorption and catabolism in the proximal tubule, only microgram quantities of circulating LMWP appear in the urine. Larger quantities of LMWP are excreted in the presence of tubular injury but still in only milligram-per-liter amounts. Lysosomal enzymes may appear in the urine, but these usually originate in damaged tubular cells directly, rather than from the circulation. Other proteins that appear in the urine may be released from the lower urinary tract. Growth factors found in urine are usually assumed to arise from intrarenal structures. Prostanoids are derived from the glomerulous and the interstitium.

The concept of using specific urinary constituents to identify the sites and mechanisms of injury along the nephron has been extended to include structural proteins, prostaglandin metabolites, and growth factors. Obviously, extension of the term “tubular proteinuria” to include biomarkers that are neither protein nor a consequence of tubular dysfunction is imprecise.

With the recent availability of large numbers of highly specific and sensitive assays for proteins it becomes possible to identify the causative agent by the pattern of tubular proteinuria. Tubular proteinuria can suggest the nephrotoxin’s identity when a “fingerprinting” approach is used. The specificity of the fingerprint is, however, limited by the nonspecificity of high- and low-molecular-weight proteinuria in the presence of a leaky glomerulus or whenever renal disease is advanced regardless of etiology. Further oversimplification occurs because of uncertainty about exposure levels and the presence of multiple exposures to nephrotoxins in an occupational setting. The increased tubular proteinuria seen with diabetic nephropathy compared to that in glomerulonephritis at comparable protein excretion rates has been interpreted as indicating diabetes-induced renal tubular damage beyond that caused by proteinuria alone (Yaqoob, 1995). Tubular proteinuria increases with certain diseases such as hypertension (Maruhn et al., 1979), lupus erythematosus (Sesso et al., 1994), and renal transplantation (Jung et al., 1985). It is sensitive to age (Jung et al., 1990), gender (Maruhn et al., 1976), urine flow rate (Jung et al., 1988), drugs (Rossi et al., 1994), alcohol (DeMarchi et al., 1993), and circadian rhythms (Feldmann et al., 1989; Maruhn et al., 1977). The genetic basis for differences in the renal response to toxic injury remains to be determined. Identifying patterns of urinary biomarker excretion can assist both in understanding the pathophysiology of renal disease and in the early detection of toxic environmental exposures. As sensitive indicators of the absorption of toxins, urinary biomarkers can be used to set workplace exposure standards.

URINE BIOMARKERS

Table 1 presents a partial list of the urine biomarkers that have frequently been used to detect early renal tubular injury and to localize the site of injury. In addition to albumin, the HMWP frequently identified in urine include transferrin and IgG.
TABLE 1 Urine Biomarkers

Tubular proteinuria

1. Circulating LMWP
   - β2-Microglobulin (B2M)
   - α1-Microglobulin (A1M, HC protein)
   - Retinol binding protein (RBP, α₂-microglobulin)
   - Ribonuclease (RNase)

2. Lysosomal proteins (enzymuria)
   - N-Acetylgalactosaminidase (NAG)
   - Lysozyme (LYS)
   - β-Galactosidase (GAL)
   - α-Glucosidase (GLU)
   - β-Glucuronidase (GRS)
   - Arylsulfatase A (ASA)
   - Glutathione S-transferase (GST, ligandin)
   - Cathespin (CAT)

3. Brush border of proximal tubules
   A. Enzymes
      - Alanine aminopeptidase (AAP)
      - γ-Glutamyltransferase (GGT)
      - Leucine aminopeptidase (LAP)
      - Intestinal alkaline phosphatase (IAP)
      - Total nonspecific alkaline phosphatase (TNAP)
   B. Antigens
      - BB50
      - BBA
      - H5

4. Distal tubule protein
   - Tamm-Horsfall protein (THP)

5. Glomerular structural proteins
   - Glycoaminoglycans (GAG)
   - Fibronectin (FN)
   - Sialic acid

6. Prostanoids
   - Thromboxane B₂ (TXB₂)
   - Prostaglandin F₂₀ (PGF₂₀)
   - 6-ketoprostaglandin F₁₅ (6-keto-PGF₁₅)
   - Prostaglandin E₂ (PGE₂)

7. Kallikrein

FINGERPRINTING

Tubular proteinuria is of particular interest in the study of occupational renal diseases arising from exposure to metals (Pb, Cd, Hg, As, Cr, U, Si, Be) and solvents (e.g., carbon tetrachloride [CCl₄], perchloroethylene [PCE], trichloroethylene [TCE]), because kidney diseases found in a few heavily exposed workers (Table 2) serve as models for understanding toxicity in large populations subjected to continuous but relatively low-level environmental exposures. Tran-
sient tubular proteinuria is regularly seen following excessive exposure to environmental nephrotoxins even when the chronic renal disease resulting from exposure is glomerular (Table 2). Systematic studies by a European Cooperative Study Group of more than 22 parameters including urinary proteins and prostanoids in selected occupations suggest that characteristic patterns of tubular proteinuria can be identified for specific toxin exposures (Table 3).

Lead causes increased N-acetylglucosaminidase (NAG) and thromboxane B2 (TXB2) in the urine (Meyer et al., 1984; Bernard et al., 1989; Cardenas et al. 1993b; Lin et al., 1993; Chia et al., 1994; Pergande et al., 1994; Verberk et al., 1996; Verschoor et al., 1987). Occupational exposure to mercury results in increased IAP (intestinal alkaline phosphatase), NAG, and THP (Tamm-Horsfall protein) excretion (Cardenas et al. 1993a). Cadmium leads to increases in circulating LMWP and lysosomal enzymes in urine (Roels et al., 1993). PCE results in increased urinary 6-keto-PGF1α (6-keto-prostaglandin F1α) (Mutti et al., 1992). A broad spectrum of urine biomarkers similar to those in cadmium nephrotoxicity is seen in Chinese herbs nephropathy (Kabanda et al., 1995). The tubular proteinuria in both cadmium and Chinese herbs nephropathy seem to be directly related to the serum concentration of β2-microglobulin (B2M).

PCE is commonly used in dry cleaning operations. It is representative of a group of hydrocarbons often referred to as “solvents,” which have been implicated as causes of glomerulonephritis (Wedeen, 1997). Absorption of solvents regularly induces tubular proteinuria for a variety of circulating LMWP and lysosomal enzymes. This tubular effect is not clearly related to the solvent nephropathy that presents clinically as immunologically mediated glomerulonephritis. However, the possibility that solvents modify the antigenicity of normal tissue proteins or alter the immune response itself remains to be examined. In contrast to cadmium, tubular proteinuria associated with lead, mercury, chromium and PCE has not been shown to predict the later development of kidney failure.

Urine obtained from residential areas heavily contaminated with chromium in Hudson County, New Jersey, showed that environmental exposure to chro-

### TABLE 2 Agent-Related Occupational and Environmental Renal Diseases

<table>
<thead>
<tr>
<th></th>
<th>Tubulointerstitial nephritis</th>
<th>Glomerulonephritis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Cd</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Hg</td>
<td>(+++)</td>
<td>+</td>
</tr>
<tr>
<td>As. Cr. U</td>
<td>(+++)</td>
<td>+</td>
</tr>
<tr>
<td>Solvents</td>
<td>(+++)</td>
<td>++</td>
</tr>
<tr>
<td>Si</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Be</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

NOTE: (+++) = tubular proteinuria usually without clinically important interstitial nephritis.
mrium did not cause increased excretion of NAG, mAlb or IAP in 55 subjects whose mean urine chromium concentration was low: 0.3 ± SEM (standard error of the mean) 0.8 μg/g creatinine (Wedeen et al., 1996). Similarly, environmental exposure to mercury in contaminated condominiums in Hoboken, New Jersey, did not result in tubular proteinuria despite urine Hg concentrations averaging 25 ± 4.4 μg/g creatinine (N = 29). The mean urine NAG concentration was 2.1 ± 1.3 units per gram (U/g) creatinine in these subjects (upper limit of normal, 4.64 U/g) (Taylor et al., 1997). Urine EGF excretion correlated weakly but significantly with NAG, mAlb, and THP corrected for urine creatinine in those exposed to mercury in their homes. The two prostanoids measured in the urine (TXB₂ and 6-keto-PGF₁₀) also showed significant correlations with one another when corrected for creatinine. Subjects in this study were 14 females and 15 males, including 3 children between 3 and 8 years of age. Two subjects whose urine mercury concentrations were reported to be higher than 200 μg/L were excluded from the analyses.

Subjects with exposure to mercury excreted significantly more IAP and less TXB₂ and 6-keto-PGF₁₀ in their urine than did the lead-solvent workers described below. There was no difference in NAG, THP, mAlb, or EGF excretion between these two groups of subjects (Table 4).

In a study of workers exposed to lead and/or industrial solvents in New Jersey, we found that urine NAG correlated positively with current blood lead concentration but not with bone lead determined by in vivo tibial K X-ray fluorescence. In vivo tibial X-ray fluorescence (XRF) is a new, noninvasive method for measuring the lead content of bone. It uses a 50 mCi ¹⁰⁰Cd radioactive source to excite characteristic K X-rays from lead atoms in the mid tibia, which are detected with a 50 mm diameter high-purity, germanium detector. The K X-ray

### Table 3: Fingerprint Patterns of Urine Biomarkers from European Cooperative Study

<table>
<thead>
<tr>
<th>Protein</th>
<th>Pb (48 μg/dl)</th>
<th>Cd (5 μg/g cr)</th>
<th>Hg (22 μg/L)</th>
<th>PCE (15 ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAP</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>TNAP</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>NAG</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>RBP</td>
<td>+</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>THG</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B2M</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>mAlb</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TXB₂</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6KPGF</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
</tbody>
</table>

**NOTE:** Refer to Table 1 for full names of proteins.

**SOURCES:** Adapted from Cardenas et al. 1993a, b; Mutti et al., 1992; Roeis et al., 1993.
technique records bone calcium (coherent elastic scatter) so that the lead content is normalized to calcium (i.e., the lead:calcium ratio is measured). Conversion of the Pb:Ca ratio to lead concentration depends on the estimated bone composition. Solvent and lead exposures were relatively low in these workers; mean blood lead was $8.2 \pm 0.9 \mu g/dl$ ($N = 67$), and mean bone lead was $3.9 \pm 0.7$ ppm (parts per million) wet weight ($N = 67$). There was no significant correlation between blood and bone lead concentrations (Figure 1). No significant correla-

![Graph](image_url)

**FIGURE 1** Correlation between blood lead and bone lead in lead-solvent workers (determined by in vivo tibial X-ray fluorescence) was not statistically significant.
Correlation was found between blood or bone lead and total nonspecific alkaline phosphatase (TNAP), IAP, THP, retinol binding protein (RBP), mAIB TXB₂, 6-keto-PGF₁₀, or EGF (Wedeen et al. 1997). A weak but significant positive linear correlation was found between NAG and blood lead \( r = 0.3, p < .01 \) but not between NAG and bone lead (Figure 2). This finding is consistent with the observation of a correlation between NAG excretion and blood lead concentration by dos Santos et al. (1994). It is similarly consistent with the report by Lin et al. (1993) that increased NAG excretion was more prevalent in lead-exposed subjects than in controls but that there was no correlation between NAG excretion and body lead burden assessed by the EDTA (ethylenediaminetetraacetic acid) lead-mobilization test. The effect of blood lead on NAG excretion is consistent with Chia et al. (1994), who found that urinary NAG correlates best with renal lead content (Chia et al., 1994). Mean values for the urinary markers did not exceed the upper limits of normal established by the European Cooperative Study Group except for 6-keto-PGF₁₀. Although there was no significant correlation between urine TXB₂ or 6-keto-PGF₁₀ and blood or bone lead, the mean urine 6-keto-PGF₁₀ of 345.6 ng/g exceeded the upper limit of normal of 151 ng/g (Taylor et al., 1997). The absence of correlation between NAG excretion and body lead stores (Figure 3) suggests that the renal tubular effects of lead are related to the circulating blood lead concentration rather than to lead stores. The New Jersey lead-solvent worker cohort demonstrates the effect of blood lead on renal function at lower blood lead levels than has previously been reported.
FIGURE 3 Correlation between NAG and blood lead in lead-solvent workers was statistically significant.

**Site Specificity**

It is possible to identify the site along the nephron that is responsible for the appearance of certain LMWP in urine because they either originate in specific tubular cells or normally are reabsorbed and catabolized in specific renal tubule segments. Nephron site specificity is illustrated for a few of the urinary compounds under consideration in Figure 4: mAlb appears in urine after a breakdown of the glomerular filtration barrier; RBP and B2M appear when proximal tubular reabsorptive and catabolic processes are impaired. The lysosomal enzyme NAG appears in the urine when proximal tubule intracellular lysosomes are damaged, thus releasing enzymes. The enzymes IAP and TNAP are released into the urine when the proximal tubule brush border is injured. Finally, Figure 4 shows that THP can enter the urine in increased quantities when there is injury to the distal tubule.

Not indicated in Figure 4 is the potential for specific proteins to be added to the urine in the lower urinary tract. It has been reported, for example, that fibronectin and β-glucuronidase are added to urine in the bladder in the presence of bladder cancer (Ho and Kuo, 1995; Malmstrom et al., 1993).

**CONCLUSIONS**

Using modern technology, minute quantities of LMWP, prostanoids, growth factors, and intrarenal and extrarenal enzymes can be measured in urine. Excre-
FIGURE 4 Association of urinary biomarkers with specific nephron sites.

ory patterns that are characteristic for the site and mechanism of renal injury often can be found. It is possible to recognize urinary biomarker patterns that suggest the putative environmental nephrotoxin. This fingerprinting approach has become an effective tool in recent years as urine from cohorts with known occupational nephrotoxin exposures has been analyzed for patterns of specific constituents in European cooperative studies. Our own studies performed in subjects with occupational and environmental exposures in New Jersey confirm the pattern specificity and threshold effects for chromium, mercury and lead. In addition, we have been able to show that increased N-acetylglucosaminidase excretion following lead exposure correlates with current (blood lead) but not with cumulative (bone lead) exposure.

The success of recent cooperative efforts has been in part due to the absence of clinical renal failure in study subjects. Urinary biomarkers indicate early renal injury. As renal failure progresses, excretory patterns become nonspecific. Moreover, renal injury that results in tubular proteinuria may not progress to renal failure. Nevertheless, biomarkers of renal injury can help establish
acceptable exposure levels and identify the need for long-term surveillance to ascertain when clinical renal disease may result. Research supported in part by US Department of Energy, Contract No. DE-AC02-98CH10886 (KWJ).

REFERENCES


TUBULAR PROTEINURIA FROM METALS AND SOLVENTS


