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Assessment of Urinary Retinol-Binding Protein as an Index of Proximal Tubular Injury

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The urinary excretion of retinol-binding protein (RBP), β_2-microglobulin (β_2-m), and β-*N*-acetyl-D-glucosaminidase was monitored in patients with renal tubular damage secondary to multiple injuries, rhabdomyolysis, antibiotic treatment, or poisoning by various chemicals such as solvents, heavy metals, or pesticides. In almost all cases, RBP proved to be a more sensitive index of renal tubular damage than was β-*N*-acetyl-D-glucosaminidase and, being more stable in acid urine, a more practical analyte to measure than was β_2-m. We corroborated this finding by studying the relationships between these three analytes in more than 150 patients. On the average, an increase in the urinary excretion of β-*N*-acetyl-D-glucosaminidase becomes detectable when urinary RBP already exceeds the normal value by 50- to 100-fold. In urines with pH >6, RBP and β_2-m concentrations are well correlated (r = 0.93, n = 150), β_2-m tending to be more frequently positive (i.e., >311 μg/L). But in urines with pH <6 (about 30–40% of the samples), the RBP/β_2-m concentration ratio increases as pH decreases, up to 500 in some patients with massive tubular injury. Because the renal uptake of proteins involves a saturable process, the urinary excretion of RBP, like that of β_2-m, specifically reflects the reabsorption capacity of proximal tubules only when the glomerular filtration rate is normal or slightly impaired (i.e., serum creatinine <20 mg/L). Under these conditions the determination of RBP protein in urine appears the most appropriate test when early detection of tubular injury is desirable.

Additional Keyphrases: *retinol-binding protein · β_2-microglobulin · β-N-acetyl-D-glucosaminidase · enzymuria · tubular proteinuria · renal-function toxicology*

In clinical practice renal dysfunction is usually detected by estimating the glomerular filtration rate from results for serum creatinine, creatinine clearance, or blood urea nitrogen. Concentrations of urinary albumin or total protein are also frequently used as an index of glomerular damage, because in most cases a clinically significant proteinuria (i.e., >0.5 g/L) can be ascribed to an increased glomerular permeability. Early injury to the proximal tubule, on the contrary, is less frequently looked for because slight damage to that part of the nephron is difficult to detect and seems to have little impact on the overall function of the kidneys (*1*).

During the last decade, however, sensitive and precise methods have become available to detect proximal tubular damage. These tests rely either on the ability of proximal tubular cells to reabsorb low-molecular-mass proteins (M_r <40 000; e.g., β_2-microglobulin, β_2-m) or on the release into urine of high-molecular-mass enzymes of tubular origin such as β-*N*-acetyl-D-glucosaminidase (NAG) (*1*).[1] Currently the most widely used test for this is the determination of urinary β_2-m, for which several immunoassays are commercially available. Because of its instability in acid urines, however, β_2-m is not the most suitable analyte to measure in screening for tubular damage (*2–5*). Measurement of retinol-binding protein (RBP), a low-M_r protein more stable in acid urine, might be more appropriate (*2, 3, 6*). Sufficiently sensitive assays for determining RBP in urine have only recently been developed (*6–9*); so far, none is yet commercially available. Little information is thus available on the value of this analyte for detecting early damage to the proximal tubule. In the present study we have compared the behavior of urinary RBP, β_2-m, and NAG in various clinical situations, to better define the respective values of these markers of renal tubular injury.

Materials and Methods

The population examined included patients with drug-induced renal damage (e.g., from analgesics, antibiotics), chronic intoxication by heavy metals (cadmium, lead, mercury), or acute tubular necrosis due to rhabdomyolysis or to poisoning by various chemicals such as pesticides or solvents. We were able to collect 24-h urines from 57 patients; for the others, we analyzed untimed urine samples. No correction for urine dilution was made except when graphically representing results from two patients. These two patients had a normal creatinine clearance, so we could correct their urinary outputs according to the creatinine concentration.

The concentrations of albumin, β_2-m, and RBP in urine or serum were determined by an automated latex agglutination test (*10*). The detection limit of this immunoassay in urine is 10 μg/L and its precision (CV) lies between 5 and 10% (*10*). We measured the activity of NAG (EC 3.2.1.30) in urine by the fluorimetric method of Tucker et al. (*11*). The upper limit of normal, defined as the mean + 2SD of the urinary concentrations observed in a group of 100 healthy subjects, was for albumin 31.8 mg/L; β_2-m, 311 μg/L; RBP, 308 μg/L; and NAG, 2.56 U/L. Because these urinary parameters were not normally distributed, these upper limits of normal were calculated from log-transformed data. The reference intervals for serum β_2-m and RBP were 1–2.4 mg/L and 20–50 mg/L, respectively. The concentrations of creatinine in urine and serum were determined by the Jaffé method as described by Henry (*12*) and Heinegård and Tiderström (*13*). The reference interval for serum creatinine was 8–13 mg/L. The activity of creatine kinase (CK; EC 2.7.3.2) in serum was assayed by using the "CK-NAG activated test kit" (Boehringer Mannheim, Mannheim, F.R.G.) for which normal values range from 24 to 195 U/L.

Results

Case Reports

Patients with multiple injuries. Figure 1 shows the course of urinary concentrations of RBP, β_2-m, albumin, and NAG in a 16-year-old patient with multiple injuries and undergo-

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[1] Nonstandard abbreviations: RBP, retinol-binding protein; β_2-m, β_2-microglobulin; NAG, β-*N*-acetyl-D-glucosaminidase; CK, creatine kinase.

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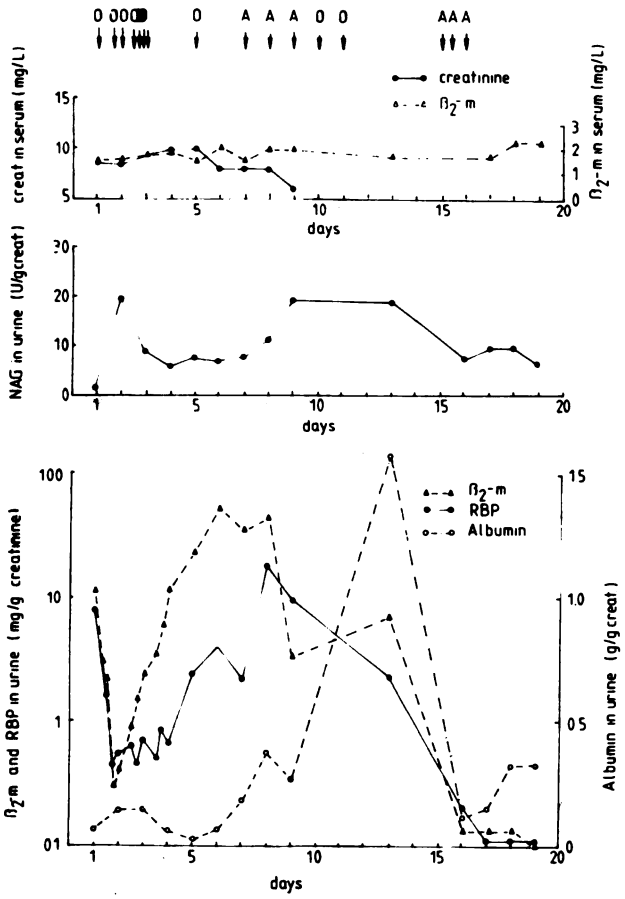

Fig. 1. Time course of (*upper*) the serum concentration of creatinine (—) and β_2-m (------) and of the urinary excretion of (*middle*) NAG, (*lower*) β_2-m (△), RBP (●—●), and albumin (○) in a patient with multiple injuries treated with oxacilline (*O*) and amykacin (*A*)

The *arrows* indicate the times when the antibiotics (dose = 1 g) were administered in the infusate

ing antibiotics therapy. At the day of admission, the patient presented with high concentrations of β_2-m and RBP in urine. But the concentrations of creatinine and β_2-m in serum and the endogenous creatinine clearance (Ccr, 128 mL/min) were normal, and the urine contained only traces of protein. On the next day, the urinary excretion of both β_2-m and RBP declined to nearly normal values, then drastically increased just after the intravenous administration of high doses of oxacilline antibiotic. Approximately 10 days were required for apparent complete recovery of the proximal tubules. The diuresis of this patient varied from 3.9 to 1.7 L/24 h. These changes in proximal tubular function were not detectable by the determination of NAG and albumin in urine nor by that of creatinine or β_2-m in serum. A massive increase in the urinary excretion of RBP and β_2-m was observed in two other cases of multiple injuries, indicating that tubular damage not necessarily leading to tubular necrosis is probably a common finding in this situation.

Rhabdomyolysis. A 23-year-old woman developed severe rhabdomyolysis after a suicide attempt with benzodiazepines (Figure 2). The serum activity of CK in serum increased to 170 000 U/L. At admission, serum creatinine was 22 mg/L (C_{cr}, 20 mL/min) but slowly returned to normal during hospitalization (44 mL/min at day 23). The diuresis ranged from 2.1 to 4 L/24 h. The urinary excretion of NAG was increased during the whole observation period but showed little variation except for a very high value on day 2.

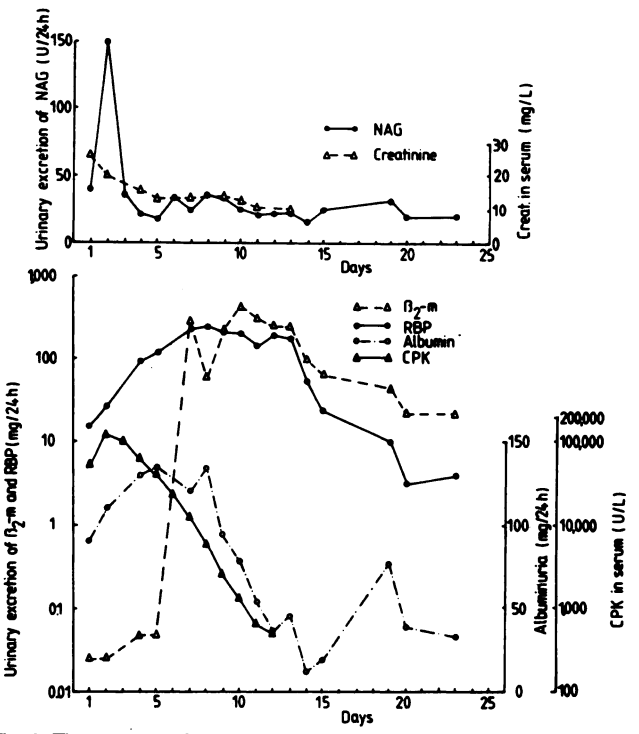

Fig. 2. Time course of concentrations of creatinine and creatine kinase (CPK) in serum and of the urinary excretion of NAG, β_2-m, RBP, and albumin in a patient with rhabdomyolysis

Despite the improvement of renal function, however, the urinary excretion of RBP still increased by 10-fold after admission, exceeding 200 mg/24 h between days 6 and 11 (i.e., >1000-fold normal values). During the first days, β_2-m was completely degraded in urine, the pH of which was below 6, but from day 6, its behavior paralleled that of RBP, with maximal values exceeding 300 mg/24 h. Because the amount of β_2-m filtered in normal human kidneys is around 300 mg/24 h, we concluded that the tubular uptake of β_2-m and presumably also that of RBP was completely abolished in this patient between days 6 and 13. At this stage, however, the concentration of β_2-m in the patient's serum was about 6 mg/L, i.e., slightly above the estimated renal threshold for that protein (see below). Thus the massive excretion of β_2-m and RBP in this patient were probably due to both the increasing concentrations of these proteins in serum and the decreased endocytotic activity of damaged tubular cells.

Acute poisonings. Several cases of acute poisonings were examined for the presence of tubular damage. Figure 3 shows the changes in the concentrations of β_2-m and RBP in a 33-year-old patient who ingested approximately 60 g of Diquat (a bipyridylium herbicide). The concentration of creatinine and β_2-m in plasma and the creatinine clearance (between 120 and 160 mL/min) remained within normal limits. This patient was maintained on an osmotic diuresis of 10 to 18 L/24 h till day 11, then 4 to 6 L/24 h from day 12 to 17. This high diuresis, however, did not prevent the occurrence of a marked tubular impairment that nearly completely inhibited the tubular reabsorption of β_2-m and RBP. The urinary excretion of these proteins returned to normal after 18 days, paralleling the disappearance of the Diquat. In that patient, however, renal damage was easily detectable because the urinary excretion of albumin increased to 15 g/24 h.

By contrast, in another patient who ingested approxi-

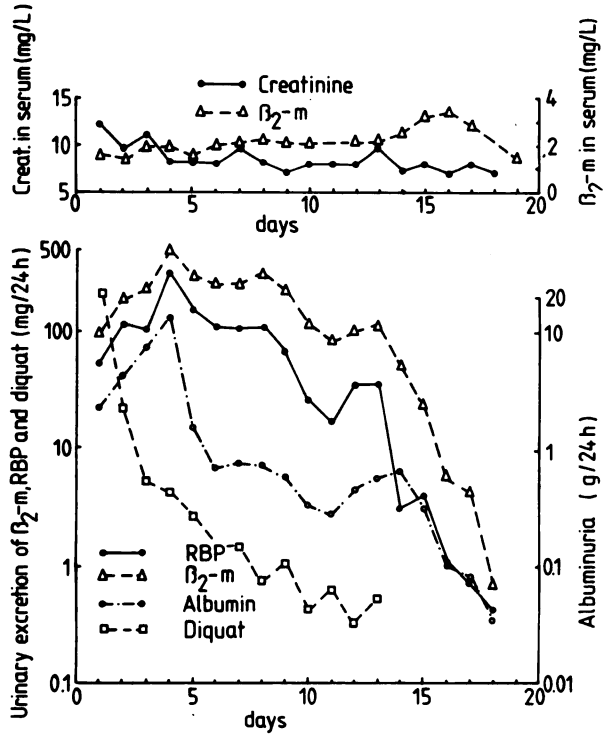

Fig. 3. Serum concentrations of creatinine and β_2-m (*upper*) and (*lower*) urinary excretion of RBP, β_2-m, albumin, and Diquat in a case of acute intoxication by Diquat

Diquat, □; other symbols as in Fig. 1

mately 75 g of tetrachloroethylene (Figure 4), the only evidence of mild tubular damage was the decrease in urinary excretion of RBP. The albuminuria, the concentrations of creatinine and β_2-m in serum, and the creatinine clearance (101 mL/min at day 1) were normal; the urinary excretion of NAG showed an isolated increase within 6 h after the admission, but β_2-m could not be reliably estimated because of the acidity of urine. The diuresis was 1.2 and 1.95 L/24 h on day 1 and 2, respectively.

Relationship between Urinary RBP or β_2-m and Renal Insufficiency

Like creatinine, low-M_r proteins in serum such as β_2-m and RBP are eliminated by glomerular filtration; their serum concentration is thus inversely correlated to the glomerular filtration rate (*14*). The urinary excretion of β_2-m invariably increases when its concentration in serum exceeds the threshold of 5 mg/L (*14*). Here we have examined whether the same phenomenon occurs with urinary RBP. The urinary excretion of RBP cannot be directly related to the serum concentrations of RBP, the major part of which forms a high-M_r complex with transthyretin, which cannot be filtered through the renal glomerulus and thus behaves quite differently from unbound RBP. However, the existence of a renal threshold for RBP can be demonstrated indirectly from the relationships between urinary RBP and the serum concentrations of β_2-m and creatinine. Table 1 shows that the urinary excretion of RBP, like that of β_2-m, suddenly increases when the concentration of the latter exceeds 4–6 mg/L in serum; on the average, this corresponds to a creatinine concentration of 19 mg/L in serum. More than 80% of the patients with values for β_2-m and creatinine above these thresholds in serum exhibit an urinary excretion of β_2-m and RBP that is more than 100-fold higher than normal (i.e., >10 mg/L).

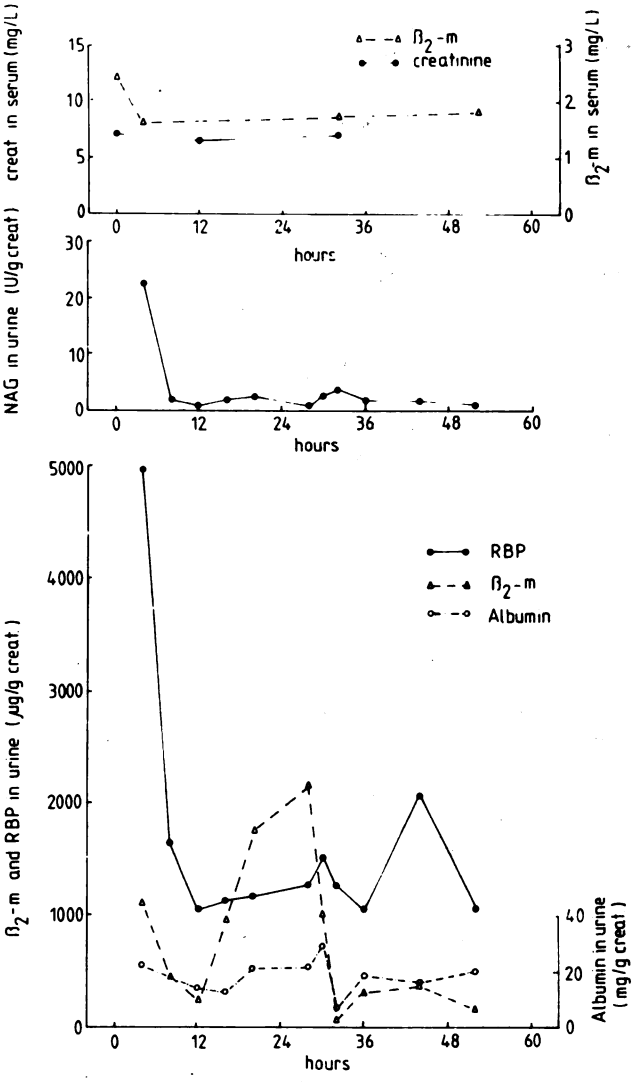

Fig. 4. Serum concentration of creatinine and β_2-m and urinary excretion of NAG, RBP, β_2-m, and albumin in a patient with acute tetrachloroethylene poisoning

Symbols as in Fig. 1

Relationships between RBP, β_2-m, and NAG Concentrations in Urine

We examined the relationships between RBP, β_2-m, and NAG in urine for more than 150 patients presenting with various types of renal damage. The relationship between RBP and β_2-m in urine is strongly dependent on the urinary pH. In urine with pH <6, the RBP/β_2-m ratio increases as pH decreases, because proteolytic enzymes degrade β_2-m but not RBP (Figure 5). This RBP/β_2-m ratio can increase up to 500 in urines from patients with severe tubular injury (see Figure 5) because, in that situation, impairment of tubular function can be associated with a massive release of proteolytic enzymes from damaged tubular cells. By contrast, when only urines with pH >6 are considered, the concentrations of β_2-m and RBP are well correlated (r = 0.93) with a slope close to 1 (Figure 6). Under these conditions, the β_2-m test tends to be more frequently positive (i.e., >311 μg/L) than the RBP test. Seventeen patients exhibited an increased urinary excretion of β_2-m alone vs

Table 1. Relationships between Concentrations (mg/L) of β_2-m and RBP in Urine and β_2-m and Creatinine in Serum

β_2-m (serum), range	β_2-m (urine[a]) n	Geometric mean (and range)	% pos.[b]	RBP (urine) n	Geometric mean (and range)	% pos.[b]	Creatinine (serum), mean ± SD[c]
<2	34	1 (0.038–32)	14.7	39	0.89 (0.066–60)	12.8	9.8 ± 0.31
2–<4	43	5.1 (0.019–50)	13.7	62	0.69 (0.021–62)	19.4	12.2 ± 0.37
4–<6	13	2.2 (0.040–160)	38	21	1.8 (0.037–83)	43	18.6 ± 0.27
6–<10	5	38.7 (5–50)	80	9	12.2 (0.11 –65)	78	22.9 ± 0.55
>10	7	85 (48–150)	100	14	45 (13–112)	100	56.2 ± 3.5

[a] Urines with pH > 6. [b] Percent with concentration >10 mg/L. [c] n is the same as for RBP (urine).

only three with increased RBP (marginal $\chi^2 = 9.8$, $P < 0.005$).

RBP in urine appears to be a more sensitive index of tubular injury than is NAG, the relative increase in the urinary concentration of RBP averaging 10-fold more than that of NAG (Figure 7). Of 152 patients, 38 had increased urinary excretion of RBP alone vs only seven with increased excretion of NAG (marginal $\chi^2 = 21.4$, $P < 0.001$). In four of these patients, the concentrations of β_2-m and creatinine in serum exceeded 6 and 20 mg/L, respectively, so that the discrepancy between NAG and RBP observed in these subjects might result from a saturation of the tubular uptake of RBP. But for the other patients, who had normal or slightly increased concentrations of β_2-m ($\bar{x}$ ± SD, 1.6 ± 0.77 mg/L) or creatinine (8.2 ± 3.1 mg/L) in serum, the assay of urinary NAG obviously failed to detect the tubular injury. Comparing results for β_2-m and NAG in urine with pH >6 supported the same conclusion (results not shown).

Discussion

Of the three markers of tubular damage we compared, urinary RBP seems the most suitable for the early detection of proximal tubular injury. This low-M_r protein is much more sensitive than urinary NAG for the early detection of tubular impairment. Unlike β_2-m, RBP is stable in acid urines, which makes it more suitable for detecting renal tubular change in most clinical situations. This practical advantage of RBP over β_2-m, demonstrated previously in vitro and in urines from healthy subjects (*3*), is now clearly demonstrated in clinical situations. When acid degradation of β_2-m can be excluded, there is a close association (r = 0.93) between β_2-m and RBP, suggesting that both proteins respond to tubular impairment with approximately the same sensitivity.

A more careful examination of the results reveals that a

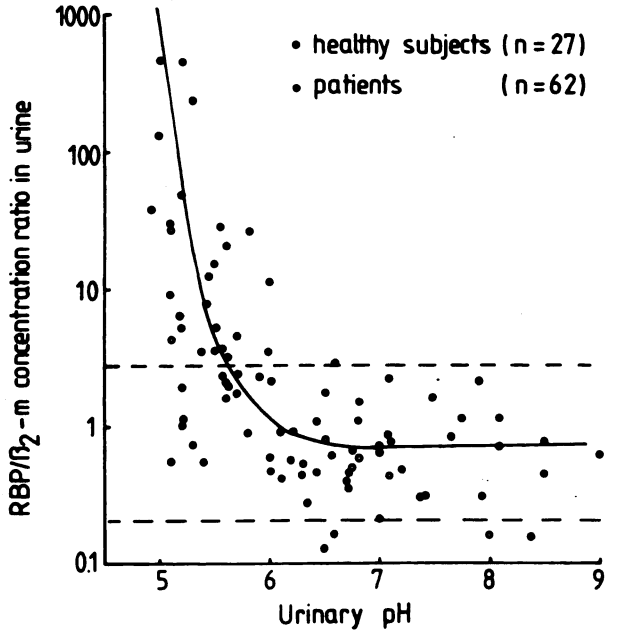

Fig. 5. Effect of urinary pH on the RBP/β_2-m concentration ratio in urines from healthy subjects (○) or from patients (●) with various renal diseases

The *dashed lines* represent the mean ± 2SD of the values observed in the urine from 31 healthy subjects (urinary pH >6)

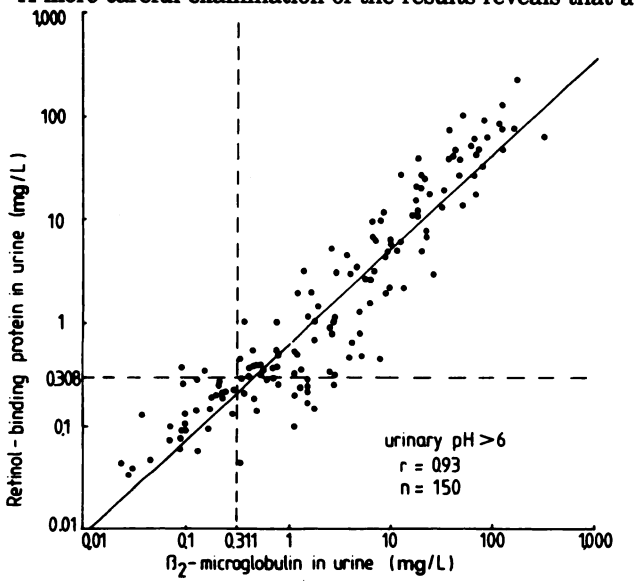

Fig. 6. Correlation between RBP and β_2-m in urine with pH >6 from 150 different patients

The equation of the *solid line* is $y = 0.011 + 0.929x$. The standard deviation of the slope is 0.03. *Broken lines* represent the upper limits of normal

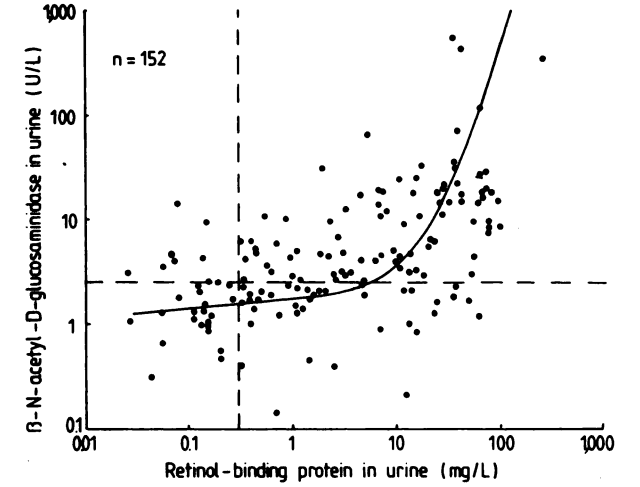

Fig. 7. Relationship between the urinary activity of NAG and the concentration of RBP in urine from 152 different patients

Broken lines represent the upper limits of normal: RBP, 308 μg/L; NAG, 2.56 U/L

small fraction (about 10%) of the patients with urinary pH >6 exhibit a slight increase in the urinary excretion of β_2-m without change in that of RBP, whereas the opposite is rarely observed. The reason for this discrepancy is unknown. These patients did not present with abnormal concentrations of β_2-m or RBP in serum. Perhaps in the development of tubular dysfunction, the tubular transport mechanism of β_2-m is impaired earlier than that of RBP, but the experimental evidence that human β_2-m and RBP compete for presumably a common transport system in rat kidneys does not favor this hypothesis (Bernard et al., ms. in preparation).

Contrary to RBP, which is synthetized by the liver only, β_2-m is present on the membrane of virtually every cell as a constituent of the Class I molecules of the major histocompatibility complex. An isolated increase of β_2-m in urine could thus represent an increased cell membrane turnover as a result of renal tissue destruction and cell regeneration. Whatever its origin, this slightly lower sensitivity of urinary RBP is, for most clinical applications, a minor drawback as compared with the instability of β_2-m in acid urine. However, when a very early detection of tubular injury is desirable, e.g., in occupational or environmental medicine, it may be useful to measure both proteins.

The urinary excretion of RBP, like that of β_2-m, is probably diagnostically significant as a specific index of proximal tubular impairment only when the glomerular filtration rate is only slightly or not at all decreased (i.e., serum creatinine <20 mg/L). The urinary excretion of RBP and β_2-m is indeed increased in virtually all patients with advanced renal insufficiency because of the existence of a renal threshold (*14–16*). Presumably, this threshold results from the tubular uptake of proteins being saturable, but we cannot exclude the possibility that the endocytotic transport of proteins in the proximal tubule is invariably disturbed by a certain degree of renal insufficiency—e.g., because of uremia (*14*). The renal threshold observed for β_2-m in the present study was between 4 and 6 mg/L, which agrees with the value of 5 mg/L reported by Wibell and Evrin (*14*). Elsewhere, the renal threshold for free RBP, estimated by Sephadex G-75 chromatography of serum samples from patients with various degrees of renal insufficiency (Bernard et al., ms. submitted for publication) was 25 mg/L, corresponding to average serum concentrations of β_2-m and creatinine of 5 and 20 mg/L, respectively.

As shown in this study, abnormalities in the urinary excretion of RBP or β_2-m are frequently observed in the absence of a decrease in glomerular filtration rate and even of a detectable proteinuria. In several clinical situations (e.g., multiple injuries, short treatment with antibiotics), these changes are probably not clinically relevant and represent no more than a transient subclinical deterioration of the tubular function. In some cases (e.g., rhabdomyolysis), monitoring of the urinary excretion of RBP could be recommended to assess the risk of occurrence of tubular necrosis. However, when the proximal tubules are repeatedly or chronically injured (e.g., multiple courses of antibiotics, long-term occupation or environmental exposure to nephrotoxic chemicals), an increase in the urinary excretion of low-M_r proteins might indicate a progressive loss of tubules and subsequently of nephrons, which, because of the large reserve capacity of the human kidney, would remain clinically silent until a considerable amount of kidney parenchyma was irreparably damaged (*17*). For instance, in the case of chronic exposure to cadmium, an increase in the urinary excretion of RBP or β_2-m might predict, if exposure is maintained, irreversible renal damage (*18*).

A growing number of drugs and chemicals in our environment are potentially nephrotoxic. In most cases, the primary target is the renal tubule. The definition of safe levels of exposure to these chemicals or the development of new drugs causing no or little damage to the proximal tubules requires simple and reliable methods capable of ascertaining renal injury in the absence of changes in the glomerular filtration rate. The determination of urinary RBP meets this objective.

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