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Characterization of Cadmium Proteinuria in Man and Rat

by Robert R. Lauwerys,*† Alfred Bernard,‡ Harry A. Roels,* Jean-Pierre Buchet* and Claude Viau**

In workers chronically exposed to cadmium and without signs of renal insufficiency, plasma proteins with molecular weight ranging from 11,800 to 450,000 are excreted in greater amount in urine. Increased urinary excretion of low and high molecular weight proteins can occur independently. Because of its greater stability in urine and provided a sensitive immunological technique is used, the determination of retinol-binding protein is a more practical and reliable test of proximal tubular function than β_2-microglobulin.

The evaluation of renal function of workers removed from cadmium exposure indicates that cadmium-induced renal lesions, albeit of slow progression, are not reversible when exposures ceases. In workers chronically exposed to cadmium or removed from cadmium exposure, metallothionein in urine is directly correlated with cadmium in urine but not with cadmium in blood or years of cadmium exposure. The association between cadmium in urine and metallothionein in urine is independent of the status of renal function and the intensity of current exposure to cadmium.

Whereas the repeated IP injection of high doses of cadmium to rat gives rise to a mixed or tubular type proteinuria, the prolonged oral administration of cadmium results mainly in the development of a glomerular type proteinuria. The former is usually reversible after cessation of treatment whereas the latter is not. Circulating antiglomerular basement membrane antibodies have been found in man and in rat chronically exposed to cadmium. The pathogenic significance of this finding deserves further investigation.

In man, an increased urinary excretion of plasma proteins is usually the earliest detectable biological sign of chronic effect of cadmium on the kidney (*1*). It is well known that the tubular dysfunction induced by cadmium results in a marked urinary excretion of low molecular weight proteins among which β_2-microglobulin has been the most investigated. We summarize in this paper several personal observations related to cadmium proteinuria in man and rat.

*Unité de Toxicologie Industrielle et Médicale, Université Catholique de Louvain, 30.54 Clos Chapelle-aux-Champs, 1200 Brussels, Belgium.

†Author to whom reprint requests should be sent.

‡Chargé de recherches du Fonds National Belge de la Recherche Scientifique.

**Research Fellow from the Institut de Recherches en Santé et Sécurité du Travail du Québec, Canada

Observations in Man

Excretion of Plasma Proteins in Workers Chronically Exposed to Cadmium

In early 1970, when we started to investigate groups of workers exposed to cadmium in smelters and in a cadmium-nickel battery factory, we used to measure the total amount of proteins excreted in urine by a biuret method and to characterize the urinary proteins by gel electrophoresis (on agarose or on sodium dodecyl sulfate polyacrylamide) after about 100-fold concentration of urine. On electrophoresis, we noticed that some proteinuria had a gomerular-type pattern (i.e., proteinuria composed mainly of high molecular weight proteins as found in glomerular dysfunction); some had a tubular-type pattern (i.e., with electrophoretic bands corresponding to low molecular weight proteins) and some had a mixed-type pattern (*2*).

The quantitative determination of specific pro-

Table 1. Urinary excretion of proteins in control and cadmium-exposed workers.

	Control ($n = 27$)		Exposed ($n = 31$)		
	Mean[a]	Range	Mean[a]	Range	p
Age, yr	57	42–73	59.3	47–70	NS
β_2-microglobulin in urine, μg/g creatinine	80.7	7.9–362	721	7.4–91900	<0.001
Retinol-binding protein in urine, μg/g creatinine	105	38–547	665	79–62900	<0.001
Albumin in urine, mg/g creatinine	7.4	2.21–164	14.6	2.3–135	<0.05
Ferritin in urine, μg/g creatinine	11.5	2.2–93.5	25.4	5.0–142	<0.005
Ferritin in urine, μg/g creatinine[b]	4.5	0.9–55	18.6	5.0–135	<0.001

[a]Geometric mean, except for age.
[b]Standardized for a serum ferritin concentration of 100 μg/L.

teins in urine (β_2-microglobulin by radioimmunoassay and albumin, transferrin, orosomucoid and IgG by nephelometry) tended to confirm our qualitative observations by electrophoresis: high molecular weight proteins such as albumin (MW 69,000) were frequently excreted in greater amount in workers chronically exposed to cadmium and sometimes without concomitant increased β_2-microglobulinuria (*3–5*).

Recently, we have again determined urinary proteins with molecular weight ranging from 11,800 up to 450,000 in 27 control and 31 cadmium-exposed workers. Their age ranged from 47 to 70 years (cadmium workers) and from 42 to 73 (control workers), respectively. A statistically significant increased urinary excretion of the four proteins measured was observed in cadmium workers (Table 1). Chromatography of urine on Sephadex G-200 or G-75 confirmed that the intact protein, and not fragments, was detected by the immunoassay technique used for their determination.

Comparison of Retinol-Binding Protein and β_2-Microglobulin Determination in Urine for the Early Detection of Tubular Proteinuria

It is generally accepted that proteins with molecular weight below 40,000, such as β_2-microglobulin, retinol-binding protein or lysozyme, are reabsorbed by a similar mechanism in the proximal tubule. Since their reabsorption is normally almost complete, impairment of proximal tubular function is usually associated with a high relative increased urinary excretion of these proteins.

Hitherto, one of the most widely used tests for the early detection of tubular proteinuria induced by cadmium is the determination of urinary β_2-microglobulin. Retinol-binding protein can also be used for the early detection of tubular dysfunction (*6*). In urine samples immediately neutralized after collection, there is an excellent correla-

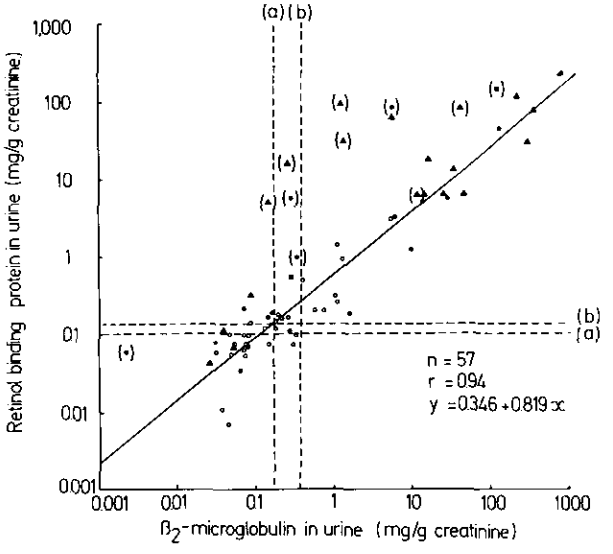

FIGURE 1. Correlation between β_2-microglobulin and retinol-binding protein in urine from patients with various renal diseases. The values from urines with a pH < 5.6 (in brackets) were not used in calculating the correlation coefficient. The dashed lines represent the 95th percentile (*a*) or the geometric mean + 2 geometric SD (*b*) of the values found in a group of 150 healthy subjects (*6*).

tion between β_2-microglobulin and retinol-binding protein concentration in urine (Fig. 1). The β_2-microglobulin test presents, however, a major pitfall resulting from the instability of the protein in acid urine. When urinary pH drops below about 5.5, a time- and temperature-dependent degradation of the protein occurs. As this degradation is very rapid and may start in the bladder, the neutralization of pH immediately after urine collection does not necessarily suffice to avoid misleading results.

This is clearly illustrated by Figures 2 and 3. Urinary β_2-microglobulin and retinol-binding protein were follwed up in a patient hospitalized for acute tubular necrosis (Fig. 2). The increased urinary excretion of β_2-microglobulin was proportionally less marked than that of retinol-binding

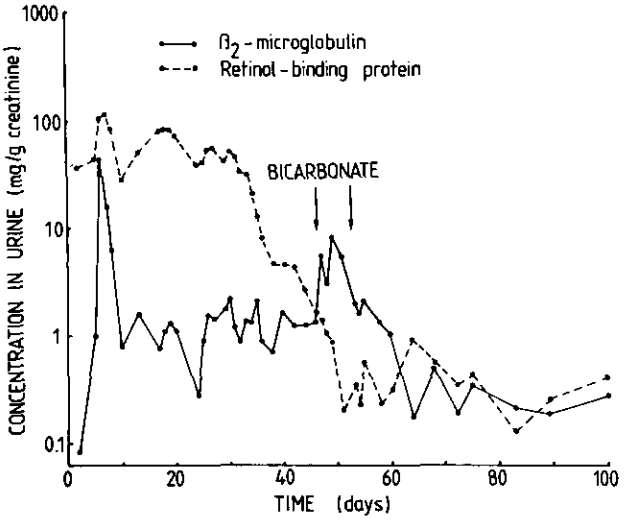

FIGURE 2. Follow up of urinary β_2-microglobulin and retinol-binding protein in a patient with acute tubular necrosis. The arrows indicate the period during which bicarbonate was administered.

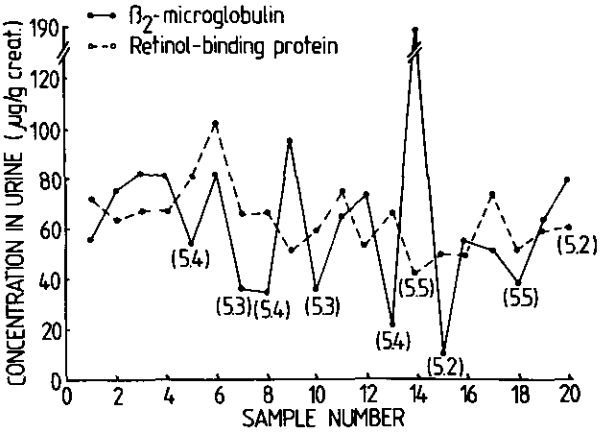

FIGURE 3. Concentration of β_2-microglobulin and retinol-binding protein in spot urine samples collected from a control worker over a 20-day period. The values of urinary pH below 5.6 are indicated within brackets.

protein mainly during the time interval when the patient was in metabolic acidosis. Since during that period, the concentration of β_2-microglobulin in serum (> 20 mg/L) was consistently much higher than the T_m for that protein (around 5 mg/L), one would expect much higher urinary β_2-microglobulin concentration. The degradation of β_2-microglobulin in acid urine is confirmed by the increased β_2-microglobulin excretion following the administration of bicarbonate. Spot urine samples were also collected from a control worker over a 20-day period and analyzed for β_2-microglobulin and retinol-binding protein (Fig. 3). Immediately after collection, the urine pH was brought to 7 with phosphate buffer. It is evident that despite the rapid neutralization of the collected urine, the variability of β_2-microglobulin concentration (CV = 58%) is much greater than that of retinol-binding protein (CV = 21.7%). A variable degradation of β_2-microglobulin in the bladder may partly explain this phenomenon. Because of its greater stability in urine and provided a sensitive immunological technique is used, the determination of retinol-binding protein is a more practical and reliable test of proximal tubular function than β_2-microglobulin (*6*).

Reversibility of Cadmium-Induced Proteinuria

We have had the opportunity to examine 19 workers while they were exposed to cadmium and after their removal from cadmium exposure. Eighteen of them had at least one renal biological parameter abnormal before their removal from exposure. Their last examination took place from 0.3 to 7.9 years after the date of removal from cadmium exposure.

Comparison of the renal function parameters (serum creatinine, total proteinuria, aminoaciduria, albuminuria, β_2-microglobulin and the urinary excretion of retinol-binding protein) before and after the cessation of exposure indicated that cadmium-induced renal lesions, albeit of slow progression, are not reversible when exposure ceases (*7*).

Metallothionein in Urine of Cadmium-Exposed Workers

In collaboration with Professor Garvey, we have measured metallothionein level in urine of 94 cadmium workers (*8*). Seventy-three were still exposed to cadmium and 21 were removed from exposure or retired. Sixty-six had no biological signs of renal dysfunction, whereas 28 had an increased β_2-microglobulin. The interrelationship between metallothionein in urine, cadmium in urine, cadmium in blood and years of cadmium exposure were examined in the various subgroups. The study of the correlations between these variables demonstrates that metallothionein in urine is directly correlated with cadmium in urine but not with cadmium in blood or years of cadmium exposure. The association between cadmium in urine and metallothionein in urine is independent of the status of renal function and the intensity of current exposure to cadmium (Fig. 4).

Observation in Rats

The nature of cadmium proteinuria induced in animals has frequently been investigated after administration of relatively high doses of cadmium by the SC or IP routes. These experiments cannot be considered as representative of the occupational or environmental exposure of man to cadmium.

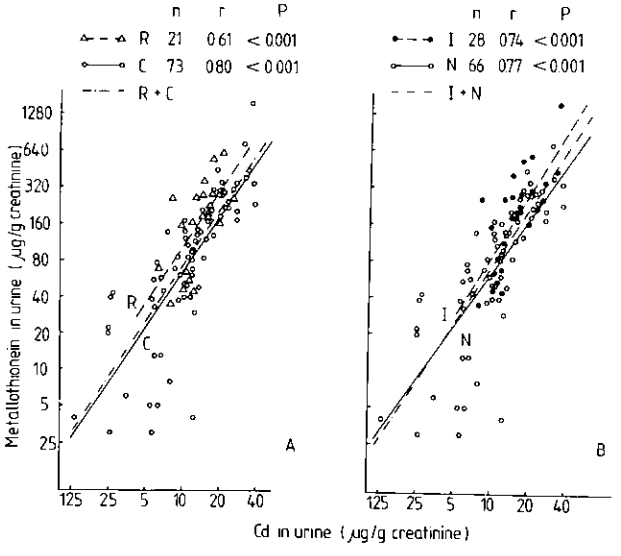

FIGURE 4. Relationship between urinary metallothionein and urinary cadmium: (*A*) workers currently exposed to cadmium (n = 73, group C) and workers removed from cadmium exposure (n = 21, group R); (*B*) workers with normal (n = 66, group N) and increased (n = 28, group 1) β_2-microglobulinuria; (–·–) the regression line for the total population is also shown (*8*).

Experiments were undertaken to test whether the mode of cadmium administration had any influence on the cadmium-induced proteinuria. Two different experimental models of cadmium proteinuria were developed. In one group of rats, cadium was given IP at the dose of 1 mg Cd/kg, 5 times/week for 2 months; in another group, cadmium was given orally (200 ppm in drinking water for 11 months). The concentration in urine of total protein was measured by the biuret method and the patterns of urinary proteins were studied by gel filtration on Sephadex G-75 and by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Whereas the repeated IP injection of high doses of cadmium gave rise to a mixed (or in a few cases a tubular) type proteinuria, the prolonged oral administration of cadmium resulted mainly in the development of a glomerular type proteinuria (Fig. 5). Furthermore, the proteinuria induced by repeated injection of high doses of cadmium was reversible following the cessation of treatment. On the contrary, the proteinuria induced by oral administration of 200 ppm cadmium for 11 months was not reversible (*7*). During the 11 months of the PO experiment, there is an evident age-related decline of the renal function of the rats. This decline which has been

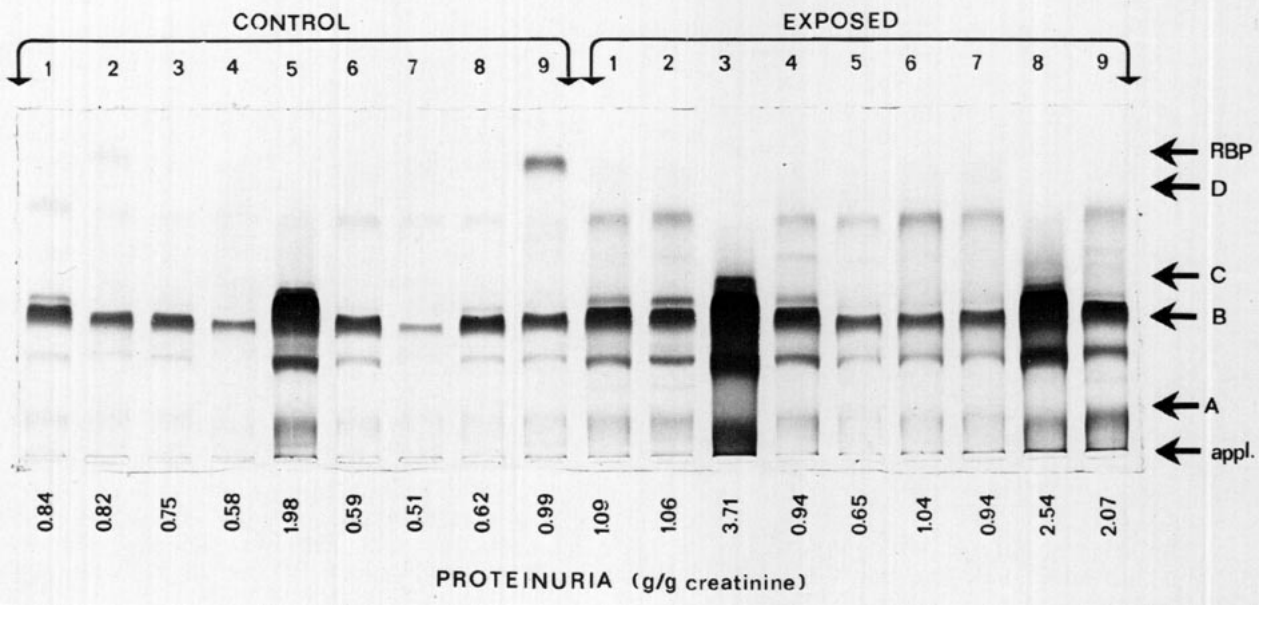

FIGURE 5. Electrophoresis on sodium dodecyl sulfate-polyacrylamide gel of urinary proteins from rats given 200 ppm Cd in drinking water for 11 months and trom control rats. The letters refer to calibration proteins: (*A*) IgG; (*B*) bovine serum albumin; (*C*) egg albumin; (*D*) chymotrypsinogen; (RBP) retinol-binding protein (*9*).

called chronic progressive nephrosis usually leads to the development of a glomerular proteinuria (*10*). It is possible that in our PO experiment, the cadmium treatment has exacerbated the age-related decline of renal function, favoring the development of a glomerular proteinuria.

Origin of Cadmium Proteinuria

Increased urinary excretion of β_2-microglobulin and retinol-binding protein (free form, i.e., not bound to prealbumin) which passes freely through the glomerulus indicates generally a defect in reabsorption by the proximal tubule. In some cadmium workers, the plasma level of β_2-microglobulin may be significantly increased, probably due to a glomerular defect and in this case, the urinary excretion of β_2-microglobulin may not be related to the intensity of tubular dysfunction.

The glomerular type proteinuria can have different origins.

(1) In the advanced stage of cadmium poisoning, glomerular proteinuria may result from the loss of nephrons due to tubular-interstitial nephritis. We have observed, however, that high molecular weight proteinuria may be an early manifestation of the toxic effects of cadmium on the kidney.

(2) High molecular weight proteins present in tubular cells might be released if the cells are damaged by cadmium. Among the various high molecular weight proteins (albumin, transferrin, IgC, ferritin) that we have detected in excessive amount in urine of cadmium-exposed workers, we considered that this possibility only applies to ferritin. The quantity found in urine of some workers is quite high and, therefore, is unlikely to result from an increased glomerular permeability only.

(3) An incomplete reabsorption by the proximal tubule might also be responsible for an increased excretion of high molecular weight proteins. Our previous results indicating that in cadmium-exposed workers tubular and glomerular proteinuria can occur independently is not an argument against this hypothesis since it is known that different mechanisms are involved in the tubular reabsorption of low and high molecular weight proteins.

(4) Chronic cadmium exposure might also lead to an increased glomerular permeability. Cadmium could bind to the negatively charged components of the glomerular capillary wall and reduce the electrostatic restriction to polyanionic proteins, such as albumin and ferritin. An immunological reaction as it has been described for inorganic mercury might also be involved. The recent finding of circulating anti-laminin antibodies in a few workers and in rats chronically exposed to cadmium offers some support to the latter hypothesis (Bernard et al., in preparation).

Conclusion

The studies summarized above suggest that long-term moderate exposure to cadmium may induce an increased urinary excretion of plasma proteins by two different mechanisms: a decreased tubular reabsorption and an increased glomerular permeability. Both mechanisms may, of course, be active concomitantly. The relative importance of both mechanisms may depend on the rate of cadmium absorption, the age of the exposed person and possibly his immunological background. Whatever the underlying mechanism of cadmium-induced proteinuria, our results clearly demonstrate the usefulness of evaluating the urinary excretion of both low and high molecular weight proteins in order to detect as early as possible any adverse effect of cadmium on the kidney.

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