

*Kidney International*, Vol. 53 (1998), pp. 416–422

# Renal catabolism of advanced glycation end products: The fate of pentosidine

TOSHIO MIYATA, YASUHIKO UEDA, KATSUNORI HORIE, MASAOMI NANGAKU, SHUICHI TANAKA, CHARLES VAN YPERSELE DE STRIHOU, and KIYOSHI KUROKAWA

*Institute of Medical Science and Department of Medicine, Tokai University School of Medicine, Isehara, and Department of Internal Medicine, University of Tokyo School of Medicine, Tokyo, Japan; and Service de Nephrologie, Universite Catholique De Louvain, Brussels, Belgium*

**Renal catabolism of advanced glycation end products: The fate of pentosidine.** Advanced glycation end products (AGEs) generated through the Maillard reaction significantly alter protein characteristics. Their accumulation has been incriminated in tissue injury associated with aging, diabetes, and renal failure. However, little is known about their clearance from the body. The present study delineates the catabolic pathway of a well-defined AGE product, pentosidine. Synthesized pentosidine given intravenously in rats with normal renal function was rapidly eliminated from the circulation through glomerular filtration, but was undetectable in the urine by chemical analysis. Immunohistochemistry with anti-pentosidine antibody disclosed that pentosidine accumulated transiently in the proximal renal tubule one hour after its administration, but had disappeared from the kidney at 24 hours. After an intravenous load of radiolabeled pentosidine, radioactivity peaked in the kidney at one hour and subsequently decreased, whereas it rose progressively in the urine. Over 80% of the radioactivity was recovered in the 72-hour collected urine. However, only 20% of urine radioactivity was associated with intact pentosidine chemically or immunochemically. In gentamicin-treated rats with tubular dysfunction, up to 30% of the pentosidine load was recovered as intact pentosidine in the urine. The present study suggests that free pentosidine (and possibly other AGEs) is filtered by renal glomeruli, reabsorbed in the proximal tubule where it is degraded or modified, and eventually excreted in the urine. Kidney thus plays a key role in pentosidine disposal.

Advanced glycation and oxidation (glycooxidation) strikingly modifies proteins. This process, the so-called Maillard reaction, links protein amino groups with glucose-derived carbonyl groups and, over the months, yield a variety of advanced glycation end products (AGEs) [1]. Advanced glycation end product modification is instrumental in the catabolism of long-lived matrix proteins and thus contributes to tissue remodeling [1]. It also alters the structure and function of tissue proteins [1], stimulates cellular responses [2–6] through a specific AGE receptor [7–9], and contributes to the generation of reactive oxygen intermediates [10, 11]. Advanced glycation end product levels increase mildly with age, more markedly in diabetes [12, 13], and dramatically in

uremia with or without diabetes [14–16]. The importance of the oxidative stress in AGE genesis has been demonstrated in a large number of studies [10, 17, 18].

To date, little attention has been paid to AGE disposal. The heterogeneity of AGE products ranging from well defined structures, such as protein-linked and free pentosidine [15, 19] and *N*<sup>ε</sup>-(carboxymethyl)lysine (CML) [20], to the ill characterized so-called AGE peptides [21] contributes to the difficulty of this task.

In the present study, we evaluated the catabolic pathway of one AGE structure, pentosidine, present in the serum both in an albumin-linked and a free form [15]. Preliminary studies have suggested that the kidney plays a critical role in the removal of free pentosidine: its half life is directly related to renal function and pentosidine accumulates in renal failure [15]. Our present results demonstrate how the kidney plays a central role in the pentosidine disposal. Free form pentosidine is filtered through the glomeruli and reabsorbed in the proximal tubules where it is modified or degraded to be eventually cleared in the urine.

## METHODS

### Synthesis of pentosidine

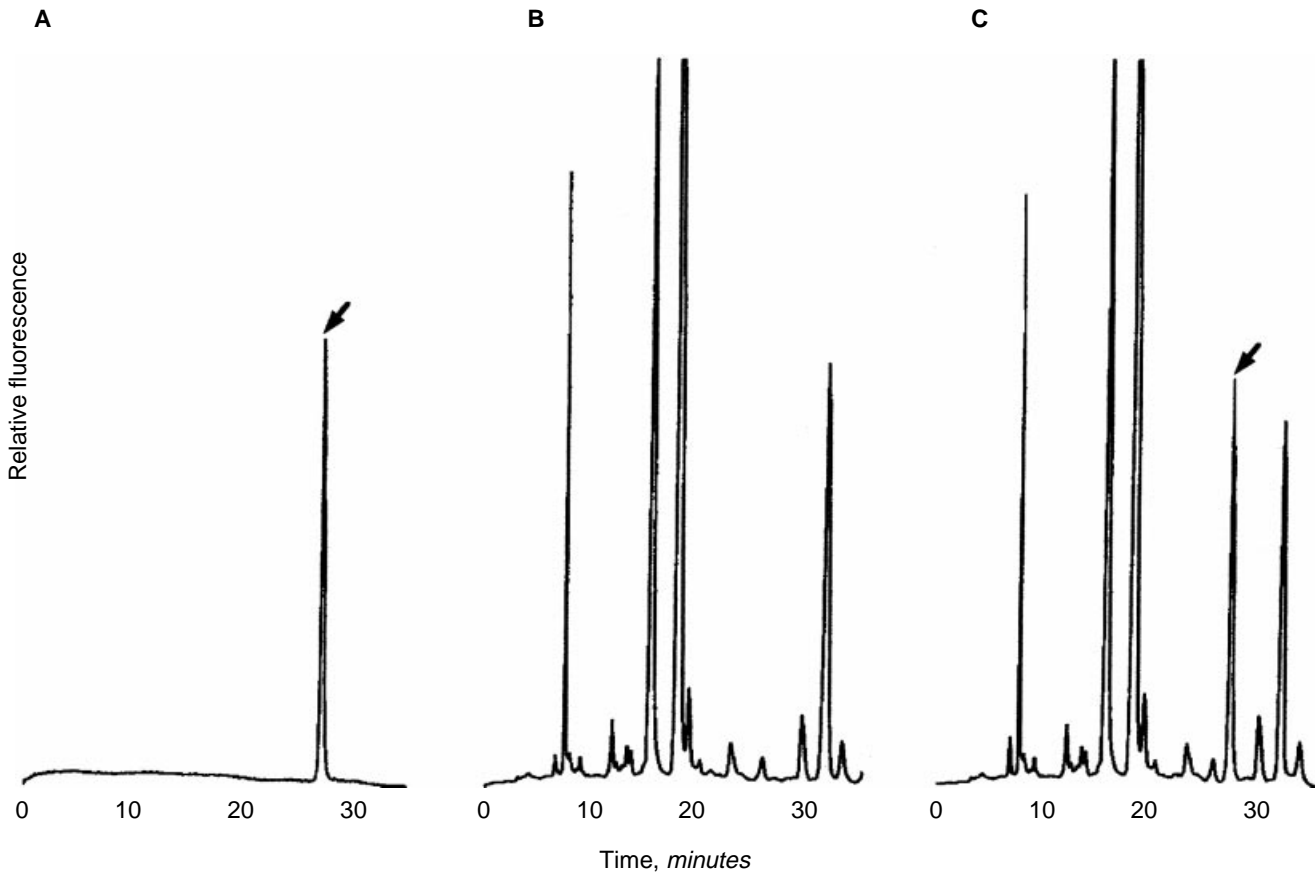
Pentosidine was synthesized according to our previous method [15, 22]. The identity of the final product was confirmed as pentosidine by nuclear magnetic resonance and fast atom bombardment-mass spectrometry.

For synthesis of radiolabeled pentosidine, a suspension of *N*<sup>α</sup>-*t*-butoxycarbonyl-L-lysine and D-ribose in methanol was stirred for three hours at 30°C. Methanol was evaporated under reduced pressure resulting in a dark brown syrup. The residue was purified by column chromatography on Dowex 50 W × 2 (Aldrich, Milwaukee, WI, USA) using a linear gradient from 0.2 M pyridine acetate, pH 3.1, to 2 M pyridine acetate, pH 5.0. The main fraction was collected, concentrated *in vacuo* and lyophilized to yield a partially purified product [*N*<sup>α</sup>-*t*-butoxycarbonyl-*N*<sup>ε</sup>-(1-deoxy-D-ribulos-1-yl)-L-lysine]. The *t*-butoxycarbonyl group was removed by treatment with trifluoroacetic acid for one hour at room temperature. After removal of excess trifluoroacetic acid *in vacuo*, the residue was purified on a reverse-phase high-performance liquid chromatography (HPLC). The thus-obtained product (5 μmol), L-[2, 3-<sup>3</sup>H]-arginine (115 nmol, 185 MBq; Du Pont,

**Key words:** advanced glycation end products, pentosidine, protein, tissue injury.

Received for publication June 27, 1997  
and in revised form August 9, 1997  
Accepted for publication August 10, 1997

© 1998 by the International Society of Nephrology



**Fig. 1. Absence of pentosidine in the urine of normal rats after intravenous pentosidine administration.** Rats with normal renal function were intravenously given pentosidine (10 nmol/rat). The 24-hr collected urine was injected into an HPLC system and separated on a C18 reverse-phase column (B). In some experiments, normal rats urine without pentosidine administration was incubated *in vitro* for 24 hours at room temperature with synthesized pentosidine (100 pmol/ml) and analyzed by HPLC (C). A representative fluorescence profile of TCA supernatant of urine at an excitation-emission of 335/385 nm is shown. (A) Synthesized pentosidine (100 pmol/ml). Arrows indicate the peak for pentosidine.

Wilmington, DE, USA), and L-arginine (5  $\mu$ mol) were incubated in 0.2 ml of 0.3 M phosphate buffer, pH 12.9, for 48 hours at 60°C. After adding 6 N HCl,  $^3$ H-labeled pentosidine was purified on a reverse-phase HPLC. The yield of  $^3$ H-labeled pentosidine was 98 nmol (10 kBq/nmol). More than 95% of the thus-prepared  $^3$ H-labeled pentosidine was immunoprecipitable with anti-pentosidine rabbit IgG, which recognizes free-form pentosidine [22]. The retention time of  $^3$ H-labeled pentosidine on a reverse-phase HPLC was completely identical with that of pentosidine.

#### Animal experiments

Eight-week-old Wistar rats (Charles River Japan Inc., Yokohama, Japan) were fed for one week a casein-based diet (AIN-76; Clea Japan Inc., Tokyo, Japan) with low pentosidine content as estimated by HPLC assay (0.3 nmol/g). Rats given an intravenous load of  $^3$ H-pentosidine (270 kBq/26.8 nmol/rat), were housed in a metabolic cage with a trap for expired air. Whole blood and plasma samples, kidney and liver homogenates in distilled water, were dissolved in tissue solubilizer (Soluene-350; Packard Instruments Company, Meriden, CT, USA). Specimen radioactivity was counted on a scintillation counter. Radioactivity was also measured in the collected urine, in feces homogenized in distilled water and dissolved in tissue solubilizer, in expired air trapped in

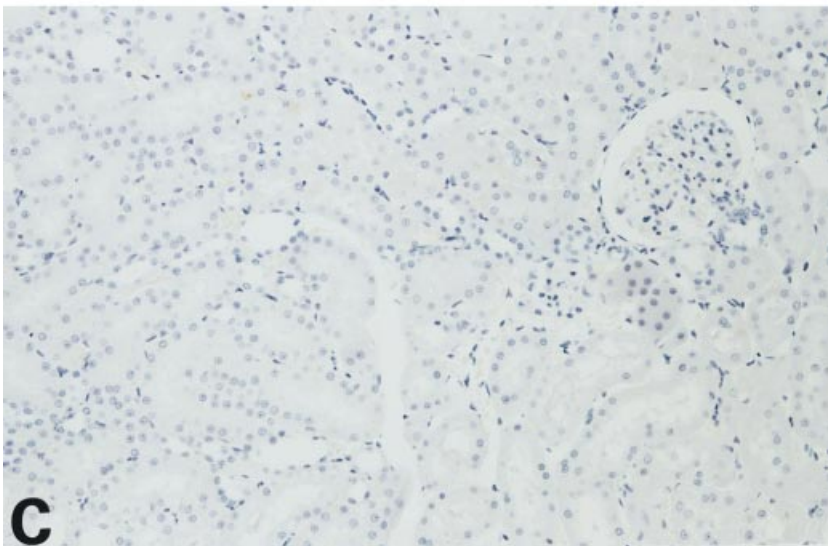
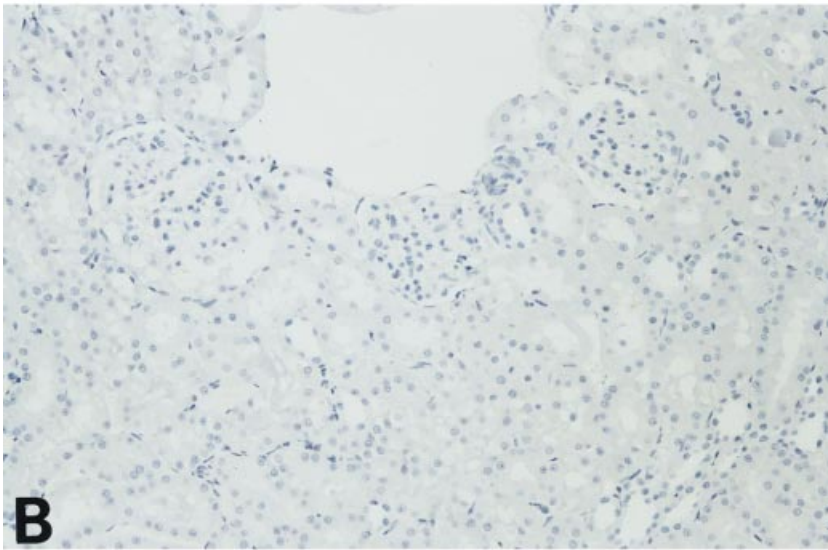
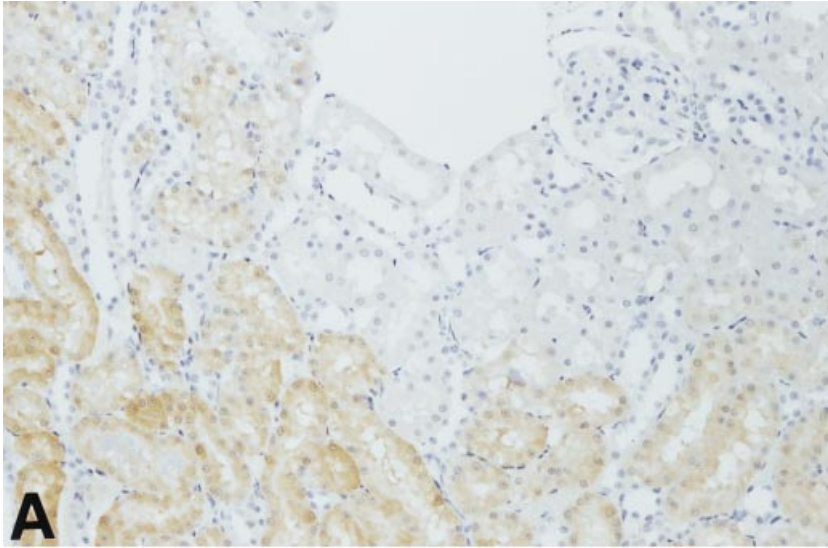
distilled water and, finally, in the final carcass dissolved in 5 N sodium hydroxide.

To evaluate the role of tubular cells in the handling of pentosidine, normal rats were given a daily intravenous injection of gentamicin (80 mg/kg) for nine days. Both gentamicin-treated and normal control rats then received an intravenous load of synthesized pentosidine by intravenous injection (10 or 100 nmol/rat) and were subsequently kept in a metabolic cage. Pentosidine levels were determined by HPLC assay.

Creatinine clearance, blood urea nitrogen, and excreted fraction of filtered sodium were measured in all rats. All studies were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and protocols were approved by the institutional Animal Care and Use Subcommittee.

#### High-performance liquid chromatographic assay

For quantitation of free form pentosidine, urine samples were mixed with an equal volume of 10% trichloroacetic acid (TCA) and centrifuged at  $5,000 \times g$  for 10 minutes. The supernatant was filtered through a 0.5  $\mu$ m-pore filter and diluted with distilled water. Pentosidine was determined on a reverse-phase HPLC according to our previous method [15, 22]. The effluent was monitored using a fluorescence detector (RF-10A; Shimadzu) and



**Fig. 2. Immunohistochemical detection of pentosidine in renal proximal tubules of rats administered with pentosidine.** Renal tissue sections from normal rats intravenously given pentosidine (10 nmol/rat) were immunostained with either anti-pentosidine rabbit IgG (A and C) or anti-pentosidine rabbit IgG preincubated with an excess of synthesized pentosidine (B). A and B show the tissue sections one hour after administration of pentosidine; C is 24 hours after administration. The nuclei were counterstained with Meyer's hematoxylin (A-C  $\times 200$ ). Note that proximal renal tubules are stained positive for pentosidine at one hour after administration (A), but pentosidine staining is undetectable at 24 hours after administration (C).

**Table 1.** Radioactivity concentration in tissue after intravenous administration of  $^3\text{H}$ -pentosidine to rats

Time hours	Radioactivity concentration			
	nmol Eq of pentosidine/ml		nmol Eq of pentosidine/g	
	Blood	Plasma	Kidney	Liver
1	0.03 ± 0.01	0.04 ± 0.01	4.58 ± 0.55	0.03 ± 0.01
3	0.02 ± 0.01	0.03 ± 0.01	2.70 ± 0.37	0.03 ± 0.01
8	0.02 ± 0.00	0.03 ± 0.00	0.93 ± 0.03	0.03 ± 0.01

Data represent the mean value ± SD ( $N = 3$ ).

**Table 2.** Cumulative excretion of radioactivity in urine, feces and expired air after intravenous administration of  $^3\text{H}$ -pentosidine to rats

Time hours	Cumulative excretion of radioactivity % of dose			
	Urine	Feces	Expired air	Total
1	27.42 ± 6.26	—	—	—
3	43.53 ± 7.64	—	—	—
8	64.56 ± 6.43	—	—	—
24	77.40 ± 14.92	2.19 ± 2.07	1.56 ± 1.02	81.15 ± 15.67
48	80.10 ± 14.10	3.16 ± 2.04	2.99 ± 2.46	56.83 ± 13.33
72	82.99 ± 13.37	3.44 ± 2.09	4.00 ± 2.86	90.42 ± 12.53
Carcass 72 hours				10.33 ± 1.32

Radioactivity was converted to % of dose. Data represent the mean value ± SD ( $N = 3$ ).

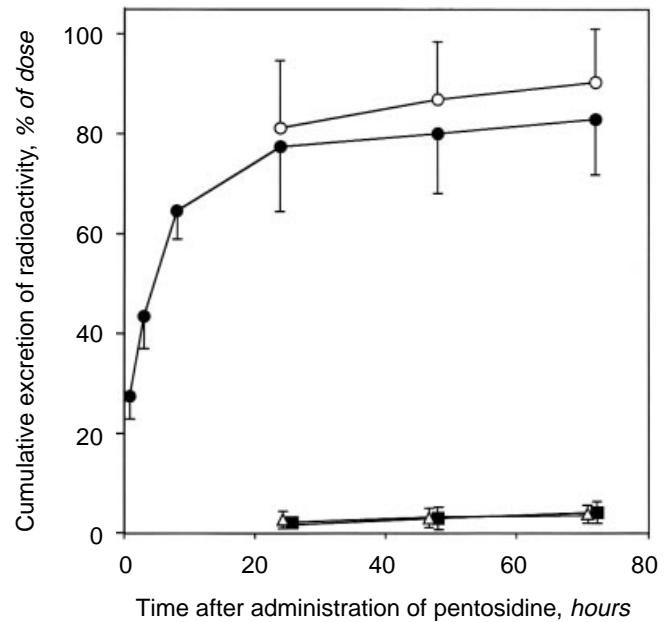
an excitation-emission wavelength of 335/385 nm. Synthesized pentosidine was used to obtain a standard curve. The limit of the detection was 10 pmol of pentosidine per ml of urine.

### Immunohistochemistry

Rat renal tissues were obtained from three animals given an intravenous load of synthesized pentosidine (10 nmol/rat). Specimens were fixed in 10% formalin and embedded in paraffin. Sections cut at 2  $\mu\text{m}$  were mounted on slides coated with 3-aminopropyltriethoxy silane (Sigma, St. Louis, MO, USA), deparaffined, rehydrated in distilled water, and then incubated with 0.5 mg/ml of Pronase (Dako, Glostrup, Denmark) in a buffer solution containing 0.05 M Tris-HCl, pH 7.2, and 0.1 M NaCl for 15 minutes at room temperature. The sections were washed with PBS containing 0.05% Tween and blocked in 4% skim milk for two hours. They were subsequently incubated with 10  $\mu\text{g}/\text{ml}$  anti-pentosidine rabbit IgG [22] overnight in humid chambers at room temperature, followed by the detection with 3,3'-diaminobenzidine solution containing 0.003%  $\text{H}_2\text{O}_2$ . Competition experiments was performed with the anti-pentosidine antibody preincubated at 37°C for five hours with an excess of synthesized pentosidine. Non-immune rabbit IgG was used as a negative control.

### Statistical analysis

Data are expressed as means ± SD. Student's *t*-test was used for a statistical evaluation of significant difference between the two groups.



**Fig. 3.** Cumulative excretion of radioactivity in the urine, feces, and expired air after intravenous administration of  $^3\text{H}$ -pentosidine into rats. Rats with normal renal function ( $N = 3$ ) were administered intravenously with  $^3\text{H}$ -pentosidine (270 kBq/26.8 nmol/rat) and kept in the metabolic cage. The radioactivity in the collected urine, feces, expired air were counted on a scintillation counter. Radioactivity was converted to % of dose initially administered. Data are expressed as the means ± SD. Symbols are: (○) total; (●) urine; (△) feces; (■) expired air.

## RESULTS

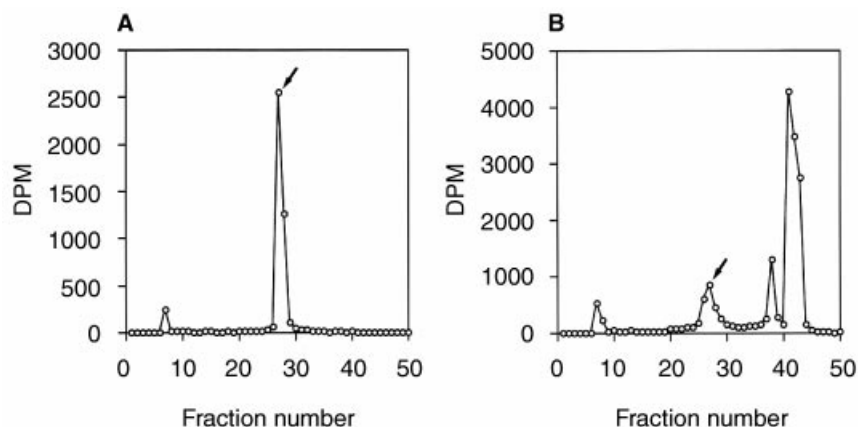
### Normal rat studies

No intact pentosidine was detectable by reverse-phase HPLC in the 24-hour urine collected in normal rats after an intravenous load of synthetic pentosidine (10 nmol/rat; Fig. 1B). By contrast, synthetic pentosidine incubated in rat urine for 24 hours at room temperature yielded an HPLC fluorescent peak (indicated by an arrow in Fig. 1C) with a retention time identical with that of pentosidine (Fig. 1A).

Pentosidine accumulation was detected by immunohistochemistry in the proximal renal tubule one hour after the intravenous injection of synthetic pentosidine (Fig. 2A). No immunostaining was further demonstrable after 24 hours (Fig. 2C). In the absence of a pentosidine load, no immunostaining was visible in normal rats (data not shown). Preabsorption of the antibody with pentosidine (Fig. 2B) or non-immune rabbit IgG abolished immunostaining, demonstrating the specificity of the immunoreaction.

Taken together with our previous demonstration that intravenous pentosidine was rapidly cleared from the plasma of normal rats [15], these results suggest that synthetic pentosidine was filtered through the renal glomeruli and reabsorbed by the proximal renal tubule within 24 hours. Reabsorbed pentosidine disappeared rapidly from the tubules, its ultimate fate remaining undefined.

The renal catabolic pathway of synthetic pentosidine was further evaluated in normal rats given an intravenous load of radiolabeled pentosidine. One hour after the injection, up to 35% of the radioactive load accumulated in the kidneys versus only less than 2% in blood, plasma, and liver (Table 1). At eight hours, less



**Fig. 4. Absence of intact pentosidine in the urine of normal rats administered intravenously with radiolabeled pentosidine.** Rats with normal renal function were intravenously given  $^3\text{H}$ -labeled pentosidine (270 KBq/26.8 nmol/rat). The 72-hour collected urine was injected into an HPLC system and separated on a C18 reverse-phase column. The eluent was monitored for radioactivity by liquid scintillation counting. A representative elution profile is shown. (A) Radiolabeled pentosidine; (B) the 72-hour collected urine. An arrow indicates the peak for  $^3\text{H}$ -labeled pentosidine.

**Table 3.** Urinary excretion of pentosidine in normal rats and gentamicin treated rats with tubular dysfunction

	Normal rats Pentosidine nmol/rat			Gentamicin treated rats 80 mg/kg/day $\times$ 9 Pentosidine nmol/rat		
	0	10	100	0	10	100
CCr $\mu\text{l}/\text{min}$	1022.32 $\pm$ 110.08	913.85 $\pm$ 204.86	1057.88 $\pm$ 192.97	210.55 $\pm$ 109.25	185.85 $\pm$ 48.47	258.17 $\pm$ 146.80
FENa %	0.43 $\pm$ 0.10	0.31 $\pm$ 0.12	0.21 $\pm$ 0.09	6.00 $\pm$ 1.57	7.12 $\pm$ 1.85	6.54 $\pm$ 3.34
Excretion of pentosidine in the 17.5 hours collected urine nmol	ND	ND	0.45 $\pm$ 0.17	ND	3.20 $\pm$ 1.65	21.17 $\pm$ 11.56

Data represent the mean value  $\pm$  SD ( $N = 5$ ).

than 7% of the radioactive load was still detectable in the kidney. At that time, 21.5%, 20.1% and 14.1% of the radioactivity were found in the TCA precipitable fractions of plasma, liver, and kidney, respectively, suggesting that a fraction of radiolabeled pentosidine was associated with tissue proteins.

The cumulative excretion of radioactivity in urine, feces and expired air is shown in Table 2. At 72 hours, 83% of the initial dose was eliminated in the urine and only 7% in the feces and expired air, the remaining 10% being recovered in the carcass (Fig. 3).

Only ~30% of the 72-hour urine radioactivity was immunoprecipitated with the anti-pentosidine antibody. On a reverse-phase HPLC (Fig. 4B), approximately 20% of urine radioactivity coincided with the retention time of pure radiolabeled pentosidine (indicated by an arrow in Fig. 4A), the remaining radioactivity coinciding with fractions with different retention times. These results demonstrate that most of the urinary radioactivity is associated with substances chemically and immunochemically distinct from intact pentosidine, and suggest that, after glomerular filtration, pentosidine undergoes a degradation or modification process. Urine *per se* is not thought to be the cause of pentosidine modification, because synthetic pentosidine remained completely immunoprecipitable after a 24-hour incubation with rat or human urine at room temperature. Furthermore, as shown in Figure 1C, by reverse-phase HPLC its retention time remained identical with that of pentosidine.

#### Gentamicin-treated rat studies

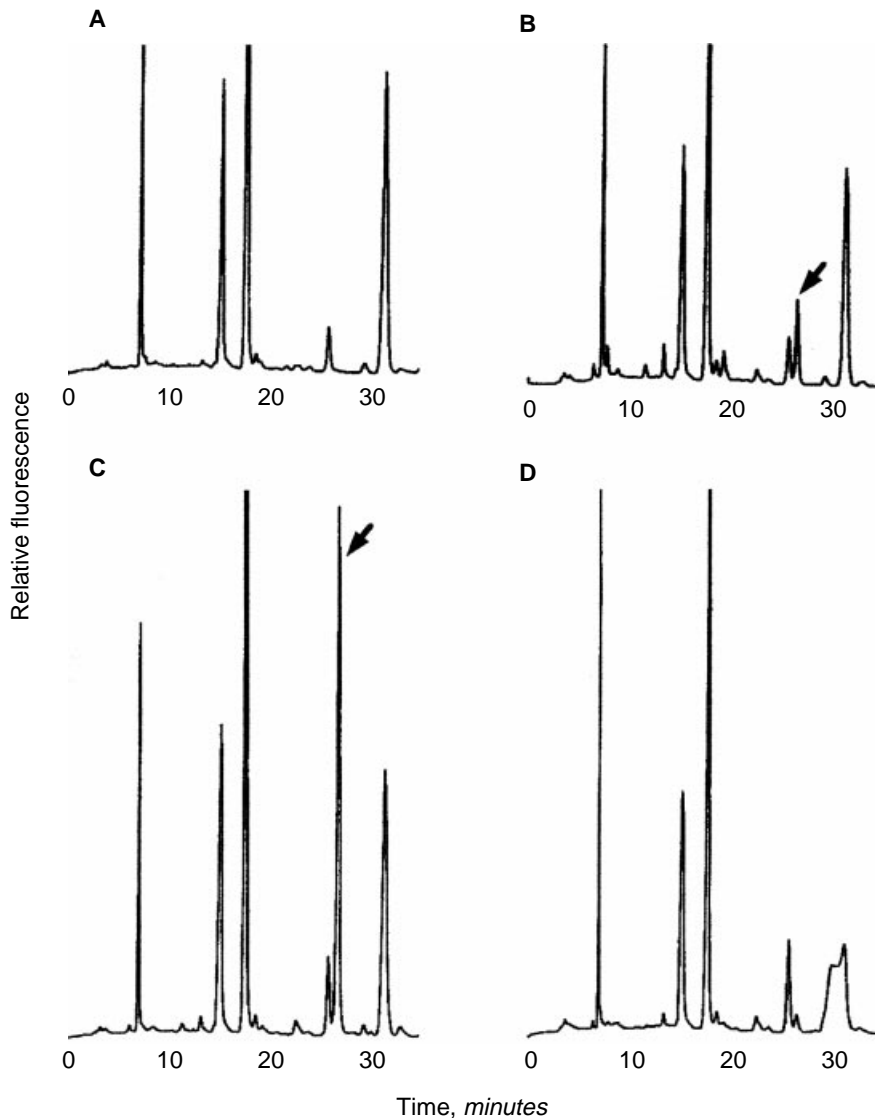
Gentamicin treatment lowered glomerular filtration to 20% of control (Table 3). The amount of pentosidine recovered in the

urine within 17.5 hours after various intravenous pentosidine loads is given in Table 3 for both control and gentamicin-treated rats. In control rats, no intact pentosidine was detected after a 10 nmol pentosidine load (Fig. 1B) and only minimal amounts after a 100 nmol load. By contrast, in gentamicin-treated rats, 30% and 20% of the load were recovered after a 10 and 100 nmol/rat load, respectively. Intact pentosidine is recovered in the urine of gentamicin-treated rats as demonstrated by the presence, on a HPLC profile, of peaks (Fig. 5 B, C) with the same retention time as synthetic pentosidine. The pentosidine peaks were immunoprecipitated with anti-pentosidine antibody (Fig. 5D). No pentosidine was detected in the urine of gentamicin-treated rats in the absence of a pentosidine load (Fig. 5A).

#### DISCUSSION

The present study demonstrates the key role of the kidney in the disposal of an AGE product, pentosidine, and provides new insights in the renal catabolic pathway for AGEs. We have previously shown that the half-life of synthetic pentosidine given to rats is prolonged by renal failure, suggesting its removal by glomerular filtration [15]. Interestingly, however, we now report that, for the same load, no pentosidine appears in the urine, a finding compatible with extensive tubular reabsorption. This hypothesis is further supported by the demonstration by immunohistochemistry of the transient accumulation of pentosidine in proximal tubules.

The reabsorbed pentosidine is rapidly cleared as it disappears from the tubules within 24 hours. Its fate is elucidated by an analysis of the kinetics of labeled pentosidine injected to normal



**Fig. 5. Presence of intact pentosidine in the urine of rats with tubular dysfunction.** Rats with tubular dysfunction due to administration with gentamicin (80 mg/kg/day) for nine days were intravenously given pentosidine (10 or 100 nmol/rat). The 17.5-hour collected urine was injected into an HPLC system and separated on a C18 reverse-phase column. Representative fluorescence profiles of TCA supernatant of urine at an excitation-emission of 335/385 nm are shown. (A) The urine of rats without pentosidine administration; (B) the urine of rats with 10 nmol/rat of pentosidine administration; (C) the urine of rats with 100 nmol/rat of pentosidine administration; and (D) the urine of rats with 100 nmol/rat of pentosidine administration, which were incubated with anti-pentosidine rabbit IgG. Arrows indicate the peak for pentosidine.

rat. During the first hour, radioactivity accumulates mainly in the kidney. After 72 hours, more than 80% of the radioactivity is recovered in the urine. However, less than a third of the urinary radioactivity is immunoprecipitable with anti-pentosidine antibody, and only 20% migrates with intact pentosidine on HPLC. These findings suggest that the majority of pentosidine has been catabolized during the tubular reabsorptive process.

The claim that filtered pentosidine is reabsorbed to a major extent is supported by our observations in gentamicin-treated rats. This model is characterized by a primary lesion of tubular cells, an early step of which is a disturbance of lysosomal function [23] with a subsequent degradation of glomerular filtration. In this experimental group, up to 20 to 30% of the injected pentosidine are recovered intact in the urine. This observation further raises the hypothesis that pentosidine handling by tubular cells is linked with lysosomal function.

Is the present model of pentosidine disposal, that is, filtration and catabolism during the reabsorptive process, applicable to other AGE epitopes? Bendayan et al [24] has demonstrated, in

the cellular compartment of mice proximal tubular cells, the presence of tagged glycosylated bovine serum as well as native bovine serum albumin in their cellular compartment. Of greater interest, the same group [25] followed the fate of intact as well as proteolysed AGE-BSA, the so-called AGE peptides injected in rats. On immunoelectron microscopy with a polyclonal anti-AGE antibody, this group demonstrated the presence of AGE peptide, but not of AGE-BSA, in proximal tubular cells, first in early then in late endosomes and lysosomes. The recognized AGE epitopes were not identified. Nevertheless, these observations fit very well with our hypothesis that AGEs are destroyed in the kidney by a process of filtration and tubular reabsorption, reminiscent of many other substances such as low molecular weight proteins, insulin, and various other hormones.

Our present observations demonstrate that exogenous, synthetic pentosidine binds rapidly to tissue proteins as evidenced by the radioactivity present at eight hours in TCA-precipitable fractions of plasma, liver, and kidney. This finding raises the question of a possibly deleterious role of pentosidine contained in

various prepared foods. If a protein binding exogenous pentosidine develops the same characteristics as proteins transformed into AGE proteins by the glycoxidative stress, prevention of AGE-related disorders such as atherosclerosis, diabetic complications, and dialysis-related amyloidosis might benefit from dietary adaptations.

The relevance of our results for humans remains to be established. Preliminary immunohistochemical studies in normal human kidney suggest that it might be the case. Indeed, pentosidine is demonstrable in renal proximal tubules (unpublished observations).

#### ACKNOWLEDGMENTS

This study was supported by grants from Research for the Future Program of the Japan Society for the Promotion of Science (96L00303).

Reprints requests to Toshio Miyata, M.D., Ph.D., Institute of Medical Sciences and Department of Medicine, Tokai University School of Medicine, Bohseidai, Isehara, Kanagawa 259-11, Japan.

#### APPENDIX

Abbreviations used in this article are: AGEs, advanced glycation end products; CML, N<sup>ε</sup>-(carboxymethyl)lysine; HPLC, high-performance liquid chromatography; TCA, trichloroacetic acid.

#### REFERENCES

- BROWNLEE M, CERAMI A, VLASSARA H: Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. *N Engl J Med* 318:1315–1321, 1988
- VLASSARA H, BROWNLEE M, MANOGUE KR, DINARELLO CA, PASAGIAN A: Cachectin/TNF and IL-1 induced by glucose-modified proteins: Role in normal tissue remodeling. *Science* 240:1546–1548, 1988
- MIYATA T, INAGI R, IIDA Y, SATO M, YAMADA N, OSA O, MAEDA K, SEO H: Involvement of  $\beta$ 2-microglobulin modified with advanced glycation end products in the pathogenesis of hemodialysis-associated amyloidosis: Induction of human monocyte chemotaxis and macrophage secretion of tumor necrosis factor- $\alpha$  and interleukin 1. *J Clin Invest* 93:521–528, 1994
- IIDA Y, MIYATA T, INAGI R, SUGIYAMA S, MAEDA K:  $\beta$ 2-Microglobulin modified with advanced glycation end products induces interleukin-6 from human macrophages: Role in the pathogenesis of hemodialysis-associated amyloidosis. *Biochem Biophys Res Commun* 201:1235–1241, 1994
- MIYATA T, IIDA Y, UEDA Y, SHINZATO T, SEO H, MONNIER VM, MAEDA K: Monocyte/macrophage response to  $\beta$ 2-microglobulin modified with the advanced glycation end products. *Kidney Int* 49:538–550, 1996
- MIYATA T, NOTOYA K, YOSHIDA K, HORIE K, KUROKAWA K, MAEDA K, TAKETOMI S: Advanced glycation end products enhance osteoclast-induced bone resorption in cultured mouse unfractionated bone cells and in rats implanted subcutaneously with devitalized bone particles. *J Am Soc Nephrol* 8:260–270, 1997
- NEEPEER M, SCHMIDT AM, BRETT J, YAN SD, WANG F, PAN YC, ELLISTON K, STERN D, SHAW A: Cloning and expression of a cell surface receptor for advanced glycosylation end products of proteins. *J Biol Chem* 267:14998–15004, 1992
- SCHMIDT AM, YON SD, BRETT J, MORA R, NOWYGRAD R, STERN D: Regulation of human mononuclear phagocyte migration by cell surface-binding proteins for advanced glycation end products. *J Clin Invest* 92:2155–2168, 1993
- MIYATA T, HORI O, ZHANG JH, YAN SD, FERRAN L, IIDA Y, SCHMIDT AM: The receptor for advanced glycation endproducts mediates the interaction of AGE- $\beta$ 2-microglobulin with human mononuclear phagocytes via an oxidant-sensitive pathway: Implication for the pathogenesis of dialysis-related amyloidosis. *J Clin Invest* 98:1088–1094, 1996
- BAYNES JW: Perspectives in diabetes. Role of oxidative stress in development of complications in diabetes. *Diabetes* 40:405–412, 1991
- YAN SD, SCHMIDT AM, ANDERSON GM, ZHANG J, BRETT J, ZOU YS, PINSKY D, STERN D: Enhanced cellular oxidant stress by the interaction of advanced glycation end products with their receptors/binding proteins. *J Biol Chem* 269:9889–9897, 1994
- DYER DG, DUNN JA, THORPE SR, BAILIE KE, LYONS TJ, MCCANCE DR, BAYNES JW: Accumulation of Maillard reaction products in skin collagen in diabetes and aging. *J Clin Invest* 91:2463–2469, 1993
- BEISSWENGER PJ, MOORE LL, BRINK-JOHNSON T: Increased collagen-linked pentosidine levels and advanced glycosylation end products in early diabetic nephropathy. *J Clin Invest* 92:212–217, 1993
- ODETTI P, FORGARTY J, SELL DR, MONNIER VM: Chromatographic quantitation of plasma and erythrocyte pentosidine in diabetic and uremic subjects. *Diabetes* 41:153–159, 1992
- MIYATA T, UEDA Y, SHINZATO T, IIDA Y, TANAKA S, KUROKAWA K, VAN YPERSELE DE STRIHOUC, MAEDA K: Accumulation of albumin-linked and free-form pentosidine in the circulation of uremic patients with end-stage renal failure: Renal implications in the pathophysiology of pentosidine. *J Am Soc Nephrol* 7:1198–1206, 1996
- MIYATA T, UEDA Y, YOSHIDA A, SUGIYAMA S, IIDA Y, JODAU M, MAEDA K, KUROKAWA K, VAN YPERSELE DE STRIHOUC: Clearance of pentosidine, an advanced glycation end product, by different modalities of renal replacement therapy. *Kidney Int* 51:880–887, 1997
- AHMED MU, DUNN JA, WALLA MD, THORPE SR, BAYNES JW: Oxidative degradation of glucose adducts to protein: Formation of 3-(N<sup>ε</sup>-lysino)-lactic acid from model compounds and glycated proteins. *J Biol Chem* 263:8816–8821, 1988
- WELLS-KNECHT KJ, ZYZAK DV, LITCHFIELD JE, THORPE SR, BAYNES JW: Mechanism of autoxidative glycosylation: Identification of glyoxal and arabinose as intermediates in the autoxidative modification of protein by glucose. *Biochemistry* 34:3702–3709, 1995
- SELL DR, MONNIER VM: Structure elucidation of a senescence cross-link from human extracellular matrix. *J Biol Chem* 264:21597–21602, 1989
- AHMED MU, THORPE SR, BAYNES JW: Identification of N<sup>ε</sup>-carboxymethyllysine as a degradation product of fructoselysine in glycated protein. *J Biol Chem* 261:4889–4894, 1986
- MAKITA Z, RADOFF S, RAYFIELD EJ, YANG Z, SKOLNIK E, DELANEY V, FRIEDMAN EA: Advanced glycosylation end products in patients with diabetic nephropathy. *N Engl J Med* 325:836–842, 1991
- MIYATA T, TANEDA S, KAWAI R, UEDA Y, HORIUCHI S, HARA M, MAEDA K, MONNIER VM: Identification of pentosidine as a native structure for advanced glycation end products in  $\beta$ 2-microglobulin-containing amyloid fibrils in patients with dialysis-related amyloidosis. *Proc Natl Acad Sci USA* 93:2353–2358, 1996
- OLBRICHT CJ, FINK M, GUTJAHRE E: Alterations in lysosomal enzymes of the proximal tubule in gentamicin nephrotoxicity. *Kidney Int* 39:639–646, 1991
- BENDAYAN M, LONDONO I: Reabsorption of native and glycated albumin by renal proximal tubular epithelial cells. *Am J Physiol* 40:F261–F268, 1996
- GUGLIUCCI A, BANDAYAN M: Renal fate of circulating advanced glycated end products (AGE): Evidence for reabsorption and catabolism of AGE-peptides by renal proximal tubular cells. *Diabetologia* 39:149–160, 1996