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Estimation of Renal Secretory Function for Organic Cations by Endogenous N^1-Methylnicotinamide in Rats with Experimental Renal Failure

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To assess whether the secretory clearance of N^1-methylnicotinamide (NMN), an endogenous organic cation, represents renal tubular secretion of the organic cation, the relationship between the secretory clearance of NMN, $CL_{scn(NMN)}$, and that of tetraethylammonium bromide (TEA), $CL_{scn(TEA)}$, was examined in normal and experimental renal failure (ERF) rats. TEA was selected as a representative organic cation secreted by the kidney. ERF was induced by glycerol, folate, salicylate, uranium, and gentamicin, substances which have been demonstrated to produce specific damage to the kidney by pathophysiological studies. Glomerular filtration rate (GFR), $CL_{scn(NMN)}$, and $CL_{scn(TEA)}$ decreased significantly in most of ERF rats, while blood urea nitrogen (BUN) increased significantly in all ERF rats. There was a statistically significant correlation ($r = 0.952$, $p < 0.001$) between the endogenous $CL_{scn(NMN)}$ and $CL_{scn(TEA)}$ in both the normal and ERF rats. Correlation analysis revealed that $CL_{scn(NMN)}$ was superior to GFR in the degree of relationship to $CL_{scn(TEA)}$, but BUN could not be used as an index for the secretion of NMN or TEA. Although the plasma concentration of NMN in most of the ERF rats was much higher than that in the normal rats, it affected neither the urinary clearance of NMN itself nor the excretion of TEA. From these findings, we propose that $CL_{scn(NMN)}$ can be used as an index to assess renal tubular function for the secretion of organic cations that are excreted by both filtration and secretion without reabsorption.

KEY WORDS: renal secretory function; renal clearance; N^1-methylnicotinamide (NMN); tetraethylammonium bromide (TEA); organic cation; experimental renal failure (ERF); folate; glycerol; salicylate; uranium; gentamicin.

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INTRODUCTION

Estimation of the renal clearance of a drug, especially in the patient with renal disease, is clinically important in establishing dosage schedules (1). Usually, the glomerular filtration rate (GFR), determined by creatinine clearance (2), together with blood urea nitrogen (BUN) (3) and *p*-aminohippurate (PAH) clearance (4) in some cases, has been used as the index of renal function. However, in general, only drugs that are filtered and not secreted or reabsorbed in the tubule should have a clearance that is parallel to GFR. The renal clearance of drugs that are secreted or reabsorbed should not be precisely parallel to GFR, since the glomerulo-tubular imbalance has been reported in many patients with various renal diseases (5). Similarly, BUN, which is excreted by filtration and is reabsorbed (3,6,7), may not be parallel to the renal clearance of drugs that are secreted in the renal tubule.

Prediction of the renal clearance of drugs that are secreted by the renal tubules has not been reported. PAH, an organic anion, has been used clinically to examine the secretory capacity of the renal tubule (4) and to estimate renal blood flow (8). However, organic anions have been known to differ from organic cations in their secretory mechanism (9–14). Since there is no appropriate method reported for the prediction of the renal secretory clearance (CL_{scn}) of organic cations, we examined the endogenous organic cation, N^1-methylnicotinamide (NMN), which is secreted by the tubule, as a possible index of renal tubular secretion for organic cations. NMN, which is biosynthesized from exogenous tryptophan or niacin by way of nicotinamide (15,16), was chosen in preference to other endogenous organic cations (9) such as choline and catecholamines because (a) it is excreted by both glomerular filtration and tubular secretion (17–20) without metabolism and tubular reabsorption in the rat (20), (b) it does not bind to plasma proteins (17,20), and (c) it shows renal transport characteristics similar to organic cations such as TEA (12,18,19,22–24). Furthermore, TEA was selected as a model organic cation for the reasons (a) and (b) described above (18,24,25). In the present study, we examined the relationship between $CL_{scn(NMN)}$ and $CL_{scn(TEA)}$ in normal and ERF rats and compared it with that between GFR and $CL_{scn(TEA)}$ or that between BUN and $CL_{scn(TEA)}$, in an attempt to determine if NMN can be used as an index for the renal tubular secretion of organic cation using experimental renal failure rats.

MATERIALS AND METHODS

Materials

NMN, TEA, and folic acid were purchased from Tokyo Kasei Co., Tokyo, Japan. Inulin, mannitol, and glycerol were purchased from Wako

Pure Chemicals Ind. Co., Tokyo. PAH was purchased as 20 v/v % solution from Daiichi Pure Chemicals Co., Tokyo. Sodium salicylate was purchased from Koso Chemicals Co., Tokyo. Gentamicin sulfate (570 μg/mg) was obtained from Shionogi Pharmaceutical Co., Osaka, Japan. ^{14}C-TEA (4.2 mCi/mmol) and ^{3}H-inulin (257.6 mCi/g) were purchased from New England Nuclear Co., Boston, Mass. All other chemicals were reagent grade and were used without further purification.

Experimental Renal Failure (ERF) rats

Male Wistar rats weighing 210–280 g were used in all experiments. Water and commercial chow (CE-2, Clea Japan Inc., Tokyo) were given ad libitum. Glycerol-ERF (26,27) was produced by injection of 10 ml/kg of glycerol solution (50 v/v % in saline) subcutaneously at 24 hr prior to the clearance experiment. Folate-ERF (28–30) was produced by injection of 5 ml/kg of folic acid (0.5 w/v % in 0.3 M sodium bicarbonate) intravenously at 24 hr prior to the experiment. Uranium-ERF (32,33) was produced by injection of 1 ml/kg of uranium nitrate solution (0.5 w/v % in saline) intravenously 120 hr prior to the experiment. Salicylate-ERF (31) was produced by injection of 2.5 ml/kg of sodium salicylate solution (160 w/v % in saline) intravenously at 48 hr prior to the experiment. Gentamicin-ERF (34–36) was induced by injection of 5 ml/kg of gentamicin sulfate solution (0.8 w/v % in saline; stored at 4°C) subcutaneously at 24 hr intervals for 10 days. The clearance study was performed at 24 hr after the final (10th) injection. Normal rats without any treatment were used as controls.

Animal Preparations

Under light ether anesthesia, the femoral vein and artery were cannulated with PE-50 polyethylene tubings for drug administration and blood sampling, respectively. The ureters were cannulated at 2 cm from both kidneys with PE-10 polyethylene tubings. The rats were kept at spine position during the experiment.

Inulin Clearance Study

Inulin infusion was started more than 1 hr after the operation to allow for recovery from the etheral anesthesia. A loading dose of 1 ml/kg of 5.34 w/v % inulin solution (in 3 w/v % mannitol-saline, 0.1 μCi ^{3}H-inulin) was followed by a sustaining infusion of 0.8 w/v % inulin solution (in 3 w/v % mannitol-saline, 13 μCi ^{3}H-inulin/100 ml) into a femoral vein via a PE-50 catheter at the constant rate of 3.0 ml/hr throughout the experiment to attain a steady-state plasma inulin level. After the steady state was

attained, the renal clearance of inulin, NMN, or TEA, was measured as described below.

Renal Clearance of Exogenous NMN, Exogenous ^{14}C-TEA, and Inulin: Experiment I

NMN was injected into a femoral vein through a PE-50 catheter at a dose of 8 mg/kg. Blood samples (0.2 ml) were taken at 5, 20, 40, 60, and 80 min after the injection from a femoral artery via a PE-50 catheter. Urine in each blood-sampling period was collected through the two ureters via PE-10 catheters. Plasma and urine samples were stored at −20°C until assay for inulin and NMN. After samples for NMN were taken, ^{14}C-TEA was injected into a femoral vein at 8 mg/kg (12 μCi/kg) through a PE-50 catheter. Blood samples (0.1 ml) taken at 20, 40, 60, and 80 min after the injection, together with urine samples collected in each blood-sampling period, were assayed for TEA and inulin. Inulin clearance was used as GFR, and the secretory clearance of NMN, $CL_{scn(NMN)}$, or TEA, $CL_{scn(TEA)}$, was calculated by $CL_{scn} = CL_r - GFR$.

Renal Clearance of Endogenous NMN, Exogenous ^{14}C-TEA, and Inulin: Experiment II

After the steady-state plasma inulin concentration was attained, four successive blood samples (0.2 ml) at 20 min intervals and three successive urine samples during each blood-sampling period were taken for the assay of inulin and endogenous NMN. After the fourth blood sample was taken, ^{14}C-TEA (8 mg/kg, 12 μCi/kg) was injected into a femoral vein, and blood samples (0.1 ml) were taken at 20 min intervals for 4 hr after the injection. Blood and urine samples collected were assayed for inulin and TEA, respectively. GFR, $CL_{r(NMN)}$, and $CL_{r(TEA)}$ were calculated in the same manner as described above.

Effect of NMN on the Excretion of TEA

At 80 min postintravenous injection of 8 mg/kg of ^{14}C-TEA, 100 mg/kg of NMN was injected intravenously, and then the changes of CL_{scn}, GFR, and urine volume were examined.

Plasma Level of Endogenous NMN and Its Stability

The plasma NMN concentration was determined in two groups of rats. One group was given only water and commercial chow, and the other was given commercial chow and 0.05 or 0.5 w/v % nicotinic acid solution overnight ad libitum. At 30 min after initiation of infusion of inulin-mannitol

solution, blood samples were obtained three times at 20 min intervals in the same manner described above, and the constancy of the plasma NMN concentration was examined.

Determination of Inulin, TEA, and NMN

Radioactivity of ^{3}H-inulin and ^{14}C-TEA in plasma or in urine was determined in a Tri-Carb liquid scintillation spectrophotometer (Packard Instruments Corp., model 3255, Downers Grove, Ill.). Fifty μl of plasma or urine sample was added to a scintillation vial containing 10 ml of counting solution (toluene 2 L, Triton-X 1 L, PPO 8 g, POPOP 0.2 g). An appropriate crossover correction was made to separate the radioactivities of ^{3}H and ^{14}C. NMN was determined by the method of Clark *et al.* (37) with slight modification, i.e., 0.3 ml of 17 w/v % trichloroacetic acid was added to 50 μl of plasma and was stirred. The fluorescence was measured at 430 nm (excited at 370 nm) in a Hitachi MPF-4 spectrofluorometer (Hitachi Ltd., Tokyo) within 48 hr after the addition of formic acid. A standard curve was prepared by adding acetophenone, 3 M KOH, and formic acid as described above to each tube containing a different concentration (0.04–0.16 μg/ml) of NMN in 1 ml of 80 v/v % ethyl alcohol. Over this concentration range, the intensities were linearly related to the concentration of NMN with a correlation coefficient of at least 0.980. The limitation of the sensitivity of the assay was 20 ng/ml. The TCA extract from serum containing exogenous authentic NMN yields more than 89 % of the fluorescence intensity of similarly treated TCA extracts from 10^{-4} M HCl containing the same amount of NMN.

Assay of BUN and Transaminase Activities

The arterial plasma obtained at the end of the clearance experiment was assayed for BUN by the indophenol method (38, 39) using a commercial kit (UN kit; Daiichi Pure Chemicals Co. Ltd., Tokyo). Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GTP) were assayed by the modified Reitman-Frenkel method (40) using a commercial kit (Hepatest A; Daiichi Pure Chemical Co. Ltd., Tokyo).

Light Microscopy of Kidney Specimens

After the clearance study, the rats were killed by injection of 2 ml air through the femoral artery cannula, and both kidneys were excised and washed with distilled water. The tissue fragments were fixed in 4% neutral buffered formaldehyde solution, and paraplast sections were stained conventionally with hematoxylin and eoxin.

Measurement of Hematocrit Value

The hematocrit of arterial blood was measured prior to the other measurements using a Hematocrit tube (VC-HO75H, Termo Co., Tokyo) by centrifugation for 4 min at 3000 rpm.

Statistical Analysis

The regression lines of the two groups of data were obtained by the linear least squares method. The difference between the two groups of data and the difference between the two relation coefficients were examined for their significance with the Student's *t*-test. All clearance values in this study were normalized for the body weight in kg.

RESULTS

Changes in Renal Physiology by ERF

Parameters of pathophysiological changes by experimental renal failure (ERF) in rats are shown in Table I. BUN, a simple index of the renal dysfunction, increased significantly in all ERF rats. Kidney weight increased significantly in the folate-, glycerol-, and uranium-ERF rats. Urine volume decreased significantly in the uranium-ERF rats. The *p*H of urine was not changed by any ERF. Hematocrit values decreased significantly in the folate- and glycerol-ERF rats. Liver wet weight, GOT, and GPT were not changed by ERF, and were 34.2–39.3 g/kg, 43.4–55.7 Karmen's unit, and 15.0–34.0 Karmen's unit, respectively.

Histopathology of ERF

In histopathology by light microscopy, we found the following results in each ERF rat. In the glycerol-ERF rats, renal tubules were more damaged than glomeruli, and this finding coincided with those reported by Preuss (41) and Westenfelder *et al.* (42). In the gentamicin-ERF rats, extensive necrosis was observed in renal tubules of the cortical area; similar findings were reported by Luft *et al.* (43), Kosek *et al.* (44), and Houghton *et al.* (45). In the folate-ERF rats, in spite of the decrease in glomerular filtration function, necrosis was not so severe as that observed in the glycerol-ERF rats. Schmidt *et al.* (46) and Schubert (47) reported similar results. The increase in kidney weight by folate treatment was also observed, and this finding coincided with that reported by Preuss *et al.* (48). In the uranium-ERF rats, both glomeruli and renal tubules were extensively damaged as reported by Carone and Spector (49) and Blantz and Konnen (50). In the salicylate-ERF rats, no remarkable change except expansion of renal tubules was

Table I. Pathophysiological Changes of Experimental Renal Failure (ERF) in Rats[a]

	Normal	Folate	Glycerol	Salicylate	Uranium	Gentamicin
Body weight (g)	234.2 ± 6.3	240.3 ± 7.4	240.8 ± 4.9	225.7 ± 9.9	245.7 ± 3.5	259.7 ± 8.4
Kidney weight (g/kg)	7.7 ± 0.1	13.5 ± 0.9[c]	10.1 ± 0.6[d]	9.5 ± 1.0	10.7 ± 0.4[c]	8.8 ± 0.7
BUN (mg/dl)[b]	19.1 ± 2.6	90.2 ± 10.4[e]	58.3 ± 11.6[e]	30.3 ± 1.1[c]	136.2 ± 3.5[e]	53.3 ± 4.6[e]
Urine volume (ml/hr/kg)	5.44 ± 1.36	7.26 ± 1.53	4.52 ± 1.07	4.01 ± 0.32	1.24 ± 1.07[c]	3.32 ± 0.53
*p*H of urine	6.24 ± 0.18	5.58 ± 0.07	5.71 ± 0.11	6.13 ± 0.12	6.41 ± 0.62	5.87 ± 0.08
Hematocrit value	0.46 ± 0.01	0.42 ± 0.01[c]	0.35 ± 0.01[e]	0.44 ± 0.01	0.48 ± 0.02	0.46 ± 0.00

[a] Values are means ±SE of four to six rats.
[b] BUN: blood urea nitrogen.
[c] Significantly different from the normal rats at $p = 0.01$.
[d] Significantly different from the normal rats at $p = 0.05$.
[e] Significantly different from the normal rats at $p = 0.001$.

observed; this result was slightly different from that reported by Calder *et al.* (51).

Excretion of TEA and NMN in ERF-rats

Figure 1 shows the plasma decline of TEA (a) and NMN (b) in normal and ERF rats following intravenous injection of 8 ml/kg of each, respectively. The plasma concentration of TEA or NMN showed a slower decline in the ERF rats than in the normal rats. The solid lines in Fig. 1a were drawn by fitting the observed values as described above. The plasma decline curves of NMN were not calculated, since the observed plasma concentration of NMN included those of endogenous NMN. Figure 2 shows the relationship between renal excretion rates and mean plasma concentrations during the urine-collecting periods in each representative normal and ERF rat following 8 mg/kg intravenous injection of TEA (a) and NMN (b), respectively, since a large interindividual variation was observed in the folate-, gentamicin-, and glycerol-ERF rats. Linear relationships between the renal excretion rates and the mean plasma concentrations were observed in all cases.

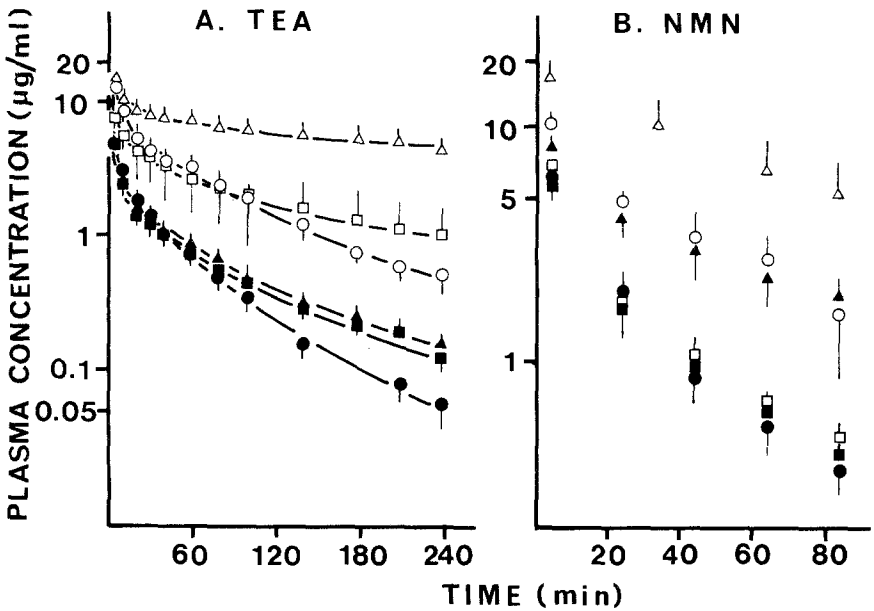

Fig. 1. Plasma decline of TEA (a) and NMN (b) following respective intravenous administration of 8 mg/kg in normal and ERF rats. Each set of points represents means ±SE of three to six rats, as follows: ●, normal rats. ERF rats: □, folate; ○, glycerol; ■, salicylate; △, uranium; and ▲, gentamicin.

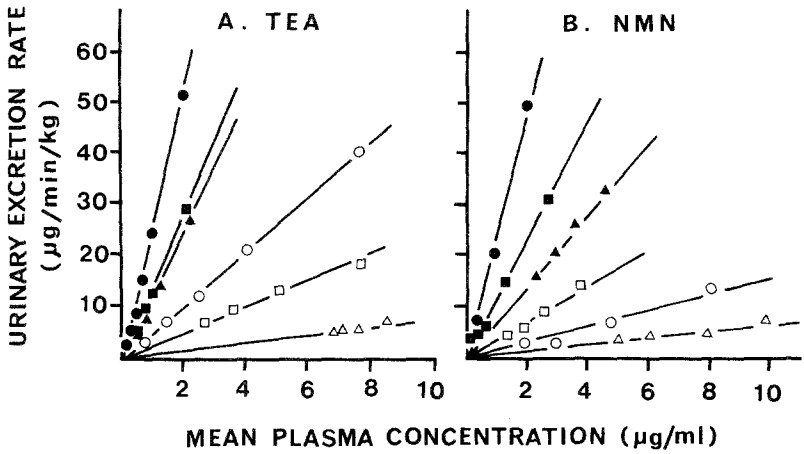

Fig. 2. Relationship between the urinary excretion rates and mean plasma concentrations of TEA (a) and of NMN (b) following respective intravenous administration of 8 mg/kg in each representative normal and ERF rat. Slope of each solid line represents the mean renal clearance of a rat. For symbols, see Fig. 1.

Renal Clearances of TEA and NMN in Normal and ERF rats

The changes in renal clearances for TEA and NMN produced by ERF after 8 mg/kg intravenous administration, respectively, are listed in Tables II and III. $CL_{r(TEA)}$, $CL_{scn(TEA)}$, $CL_{r(NMN)}$, and $CL_{scn(NMN)}$ decreased significantly in all ERF rats. Since the excretion of TEA or NMN did not show saturation over the concentration ranges in Fig. 2, $CL_{r(TEA)}$ or $CL_{r(NMN)}$ of each rat was calculated as the mean of all measurements. The GFR decreased significantly in all ERF rats except the salicylate-ERF rats. The ratio of $CL_{r(TEA)}$ or $CL_{r(NMN)}$ to GFR was always above unit in all ERF rats.

Effect of NMN on the Excretion of TEA

The effects of coadministered NMN (100 mg/kg) on $CL_{r(TEA)}$, GFR, and urine volume in the normal rats are summarized in Table IV. The $CL_{r(TEA)}$, GFR, and urine volumes were not changed by the coinjection of 100 mg/kg of NMN in the normal rats.

Plasma Level of Endogenous NMN and Its Stability

The plasma levels of endogenous NMN were summarized in Table V. The plasma levels of endogenous NMN were high enough to determine in the normal rats, but they were too low to determine in some ERF rats in spite of their general tendency to ascend in the ERF rats. Nicotinamide

Table II. Clearance Parameters for TEA in Normal and ERF Rats[a]

Parameter	Normal	Folate	Glycerol	Salicylate	Uranium	Gentamicin
$CL_{r(TEA)}$ (ml/min/kg)	39.90 ± 3.72	20.35 ± 6.05	8.92 ± 2.70[C]	26.44 ± 2.42[d]	0.10 ± 0.08[e]	15.02 ± 3.95[e]
GFR (ml/min/kg)	5.48 ± 0.63	1.33 ± 0.40[c]	1.70 ± 0.51[d]	4.83 ± 0.26	0.06 ± 0.05[c]	2.58 ± 0.63[e]
$CL_{scn(TEA)}$ (ml/min/kg)[b]	34.42 ± 3.6	19.02 ± 5.65[e]	7.21 ± 2.20[c]	23.02 ± 1.95[e]	0.05 ± 0.04[c]	12.44 ± 3.40[e]

[a] Values are means ±SE of three to six rats. Dose, 8 mg/kg i.v.
[b] $CL_{scn(TEA)}$ was calculated by $CL_{scn(TEA)} = CL_{r(TEA)} - GFR$.
[c] Significantly different from the normal rats at $p = 0.001$.
[d] Significantly different from the normal rats at $p = 0.01$.
[e] Significantly different from the normal rats at $p = 0.05$.

Table III. *Renal Clearance Parameters for Exogenous NMN in Normal and ERF Rats*[a]

Parameter	Normal	Folate	Glycerol	Salicylate	Uranium	Gentamicin
$CL_{r(NMN)}$ (ml/min/kg)	33.72 ± 2.91	16.44 ± 4.30[c]	8.95 ± 2.91[e]	23.15 ± 1.44[d]	0.17 ± 0.15[e]	14.74 ± 2.82[c]
GFR (ml/min/kg)	6.33 ± 0.65	1.00 ± 0.27[e]	1.97 ± 0.51[c]	5.42 ± 0.61	0.07 ± 0.06[e]	3.00 ± 0.66[c]
$CL_{scn(NMN)}$ (ml/min/kg)[b]	27.43 ± 2.74	15.44 ± 4.06[d]	6.98 ± 1.35[e]	17.74 ± 1.35[d]	0.10 ± 0.07[e]	11.74 ± 2.20[c]

[a] Values are means ±SE of three to six rats. Dose, 8 mg/kg i.v.
[b] $CL_{scn(NMN)}$ was calculated by $CL_{scn(NMN)} = CL_{r(NMN)} - GFR$.
[c] Significantly different from the normal rats at $p = 0.05$.
[d] Significantly different from the normal rats at $p = 0.01$.
[e] Significantly different from the normal rats at $p = 0.001$.

Table IV. Effect of NMN on the Renal Clearance of TEA, GFR, and Urine Volume in Normal Rats

	Control[a] ($n=6$)	NMN[b] ($n=3$)
$CL_{r(TEA)}$ (ml/min/kg)	39.90 ± 3.72	38.50 ± 3.10
GFR (ml/min/kg)	5.48 ± 0.63	5.26 ± 0.55
Urine volume (ml/min/kg)	5.51 ± 1.33	5.73 ± 1.37

[a] TEA was injected at a dose of 8 mg/kg without NMN.
[b] TEA was injected at 8 mg/kg simultaneously with NMN of 100 mg/kg.

solution given as above produced plasma NMN levels high enough to determine, and these levels were higher in the ERF rats than in the normal rats. The plasma NMN levels were almost constant over the experimental period (80 min) in all rats studied.

Renal Clearance of Endogenous NMN

The $CL_{r(NMN)}$ and $CL_{scn(NMN)}$ of the endogenous NMN together with GFR in the normal and ERF rats are listed in Table VI. All values, except GFR in the salicylate-ERF, were decreased significantly by ERF, but they did not show a significant difference from the corresponding values listed in Table III, where the data were obtained from the exogenous NMN and inulin studies.

Relationship Between GFR and Secretory Clearance

Figure 3 shows the relationships between GFR and $CL_{scn(TEA)}$ (a), and that between GFR and $CL_{scn(NMN)}$ (b) in experiment I for every normal and EFR rat. The correlation coefficients were 0.812 ($p<0.001$), and 0.722

Table V. Endogenous Plasma NMN Levels and NMN Levels Following Free Access to Various Concentrations of Nicotinamide Solution for 12 to 24 h[a]

	Concentration of nicotinamide solution		
	None	0.05%	0.5%
Normal	0.19–0.45 (7)[b]	0.31–0.85 (6)[b]	1.78–3.59 (2)[b]
Folate	0.38–1.45 (4)	1.41–3.21 (2)	3.89–15.52 (2)
Glycerol	3.73–8.80 (4)	1.70–3.93 (4)	5.66–6.13 (2)
Salicylate	0.00–1.24 (3)	0.40–1.15 (2)	3.63–4.92 (2)
Uranium	0.00–2.78 (4)	n.d.	15.79–20.02 (2)
Gentamicin	0.00–2.93 (4)	n.d.	2.20–3.06 (2)

[a] Plasma level of NMN was presented as μg/ml, n.d., no experiment performed.
[b] Number of experiments was shown in the parenthesis.

Table VI. Renal Clearance of Endogenous NMN in Normal and ERF Rats[a]

Parameter	Normal	Folate	Glycerol	Salicylate	Uranium	Gentamicin
$CL_{r(NMN)}$ (ml/min/kg)	25.09 ± 2.06	5.57 ± 1.65[c]	5.76 ± 1.15[c]	17.42 ± 4.41[d]	1.63 ± 0.86[c]	14.54 ± 2.67[e]
GFR (ml/min/kg)	6.27 ± 0.51	0.45 ± 0.16[c]	2.20 ± 1.05[d]	4.28 ± 0.91	0.29 ± 0.26[c]	3.24 ± 0.94[d]
$CL_{scn(NMN)}$ (ml/min/kg)[b]	18.82 ± 1.91	5.12 ± 1.53[c]	3.52 ± 0.54[c]	13.14 ± 3.62[d]	0.80 ± 0.55[c]	11.30 ± 3.62[d]

[a] Values are means ±SE of three to nine rats.
[b] $CL_{scn(NMN)}$ was calculated by $CL_{scn(NMN)} = CL_{r(NMN)} - GFR$.
[c] Significantly different from the normal rats at $p = 0.001$.
[d] Significantly different from the normal rats at $p = 0.05$.
[e] Singificantly different from the normal rats at $p = 0.01$.

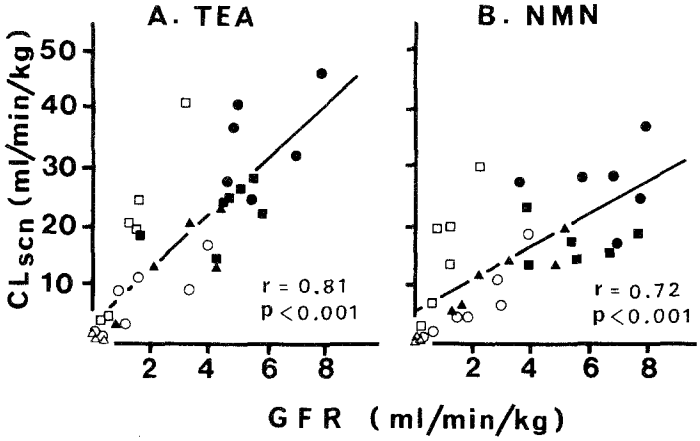

Fig. 3. Correlation between GFR and $CL_{scn(TEA)}$ (a), and between GFR and $CL_{scn(NMN)}$ (b) following intravenous administration of 8 mg/kg of TEA and NMN, respectively. Panel A: $CL_{scn(TEA)} = 4.91GFR + 3.11$ ($n = 34$), $r = 0.81$ ($p < 0.001$). Panel B: $CL_{scn(NMN)} = 2.88GFR + 5.02$ ($n = 34$), $r = 0.72$ ($p < 0.001$). For symbols, see Fig. 1.

($p < 0.001$), respectively. A similar relationship was observed between GFR and the endogenous $CL_{scn(NMN)}$ in experiment II ($r = 0.820$, $p < 0.001$). In the folate-ERF rats, the extent of decrease in GFR, $CL_{scn(TEA)}$, and $CL_{scn(NMN)}$ showed a large difference from those of the other ERF rats.

Relationship Between 1/BUN and GFR, 1/BUN and CL_{scn}

Figure 4 shows the relationships between 1/BUN and $CL_{scn(TEA)}$ (a) ($r = 0.715$, $p < 0.001$), and between 1/BUN and GFR (b) ($r = 0.874$, $p < 0.001$) in the normal and ERF rats. In the folate-ERF rats 1/BUN greatly decreased; the values are nearly equal to 1, and a large interindividual variation was observed in $CL_{scn(TEA)}$ (Fig. 4a) but not observed in GFR (Fig. 4b).

Relationship Between $CL_{scn(NMN)}$ and $CL_{scn(TEA)}$

The relationships between exogenous $CL_{scn(NMN)}$ and $CL_{scn(TEA)}$ (a) and between endogenous $CL_{scn(NMN)}$ and $CL_{scn(TEA)}$ (b) in the normal and ERF rats are shown in Fig. 5. The correlation coefficients were 0.959 ($p < 0.001$) and 0.952 ($p < 0.001$), respectively. They were significantly higher than that between GFR and $CL_{scn(TEA)}$ ($p < 0.05$) or that between BUN and $CL_{scn(TEA)}$ ($p < 0.001$). The two regression lines of Fig. 5 coincided in their slopes and passed nearly through each origin.

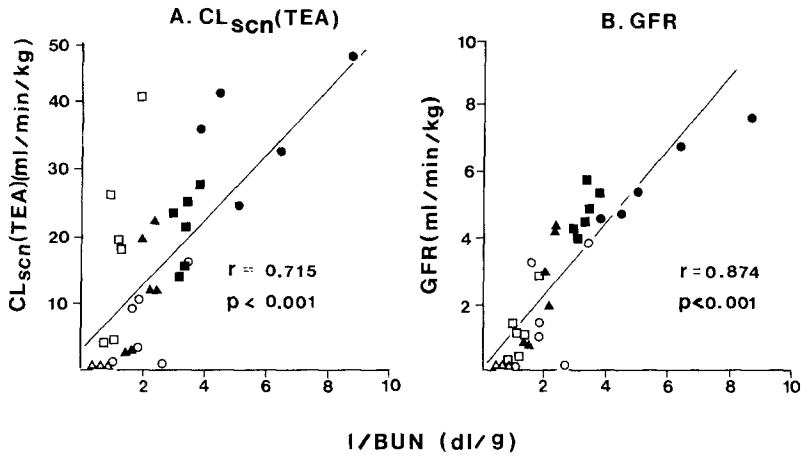

Fig. 4. Correlation between (1/BUN) and $Cl_{scn(TEA)}$ (a), and between (1/BUN) and GFR (b) in normal and ERF rats. Panel A: $CL_{scn(TEA)} = 5.34(1/BUN) + 3.20$ ($n = 32$), $r = 0.715$ ($p < 0.001$). Panel B: $GFR = 1.10(1/BUN) + 0.0748$ ($n = 32$), $r = 0.874$ ($p < 0.001$). For symbols, see Fig. 1.

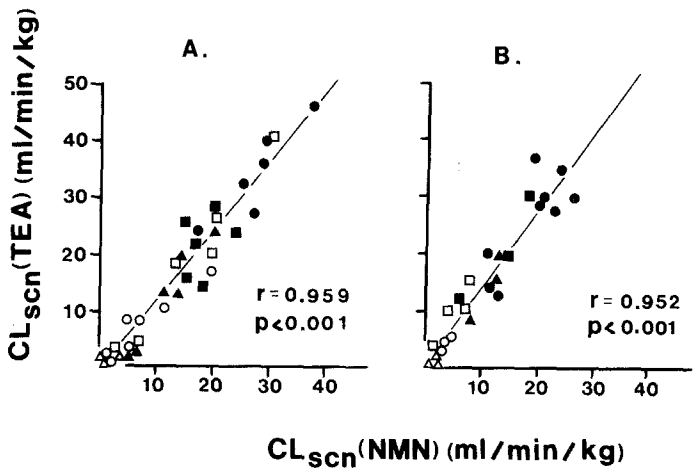

Fig. 5. Correlation between $CL_{scn(NMN)}$ and $CL_{scn(TEA)}$ in normal and ERF rats. Panel A: NMN and TEA were injected at 8 mg/kg as described in experiment I. $CL_{scn(TEA)} = 1.26CL_{scn(NMN)} + 0.70$ ($n = 34$), $r = 0.959$ ($p < 0.001$). Panel B: $CL_{scn(NMN)}$ was determined from endogenous NMN. TEA was injected at 8 mg/kg as described in experiment II. $CL_{scn(TEA)} = 1.39\ CL_{scn(NMN)} + 0.39$ ($n = 25$), $r = 0.952$ ($p < 0.001$). For symbols, see Fig. 1.

DISCUSSION

The present study was designed to assess whether changes in the renal secretory function for the organic cation can be predicted by use of $CL_{scn(NMN)}$ in renal disease. NMN and TEA were selected as representative of endogenous and exogenous cations, which are filtered and secreted in the kidney without reabsorption, respectively. The BUN of ERF rats was significantly higher than that of the normal rats in decreasing order of uranium-, folate-, glycerol-, gentamicin-, and salicylate-ERF. The kidney weight was increased significantly by folate-ERF as reported by Preuss *et al.* (28), and also by uranium-ERF (Table I). The significant decrease in hematocrit values by folate- and glycerol-ERF, and that in urine volume by uranium-ERF, suggested a possible difference in pathogenesis among the employed renal failure models.

The plasma concentration of either TEA or NMN showed a slower decline in the ERF rats than that in the normal rats (Fig. 1). Accordingly, the plasma concentration of each compound was at higher levels in the ERF rats than in the normal rats, suggesting an injured excretory function of kidney for TEA or NMN by ERF. However, the linear relationship between the urinary excretion rate and mean plasma concentration of NMN (Fig. 2) suggested that the renal excretion of TEA or NMN following respective injection of 8 mg/kg was not saturated even in the ERF rats. Assuming tubular secretion of TEA or NMN by a carrier-mediated transport, as proposed by many workers (12,14,52,53), the half-saturation concentration of the carrier for the secretion ($K_{m(scn)}$) of TEA or NMN must be much larger than the plasma level (0.17–120 μM) of each compound in this study. Ross *et al.* (20) reported the $K_{m(scn)}$ value of NMN to be approximately 0.3 mM.

The values of $CL_{r(TEA)}$, $CL_{r(NMN)}$, and GFR in the normal rats (Table II) were comparable with those reported previously (20,25). They were decreased significantly by ERF, except GFR in the salicylate-ERF, but the ratio of $CL_{r(TEA)}$ or $CL_{r(NMN)}$ to GFR was always much greater than unity, suggesting the primary role of tubular secretion in the excretion of TEA or NMN even in the ERF rats. Since it has been reported that the reabsorption of TEA or NMN is absent in normal rats (20,54), and in the present study the urinary *p*H, an important factor affecting renal reabsorption, was not changed by in ERF rat studied (Table I), the tubular reabsorption of TEA or NMN might be negligible when compared with $CL_{r(TEA)}$ or $CL_{r(NMN)}$ even in the ERF rats. In the normal rats, both $CL_{r(TEA)}$ and $CL_{r(NMN)}$ were close to the renal plasma flow, suggesting a blood flow limited process (55). However, in all groups of ERF rats studied, no change in the renal plasma flow was observed, while CL_{scn} showed a decrease, suggesting a secretion limit (55).

The excretion of TEA was unaffected by endogenous NMN in all rats studied, since the linear relationship between the excretion rate of TEA and its mean plasma concentration was maintained in all ERF rats (Fig. 2), and the coadministered NMN (100 mg/kg) did not affect $CL_{r(TEA)}$ or $CL_{scn(TEA)}$ in the normal rats (Table IV). This was further supported by observations that the bolus injection of NMN (100 mg/kg) at the midpoint of the experimental period for TEA clearance resulted in no change of $CL_{r(TEA)}$ or GFR (data was not shown). Furthermore, the large value of $K_{m(scn)}$ for NMN when compared with its concentration in the TEA-secretory system, which may be common with NMN (12,18,19,22–24), also supports the above consideration. The constancy of the plasma level of endogenous NMN during the experimental period suggested that the balance was kept between NMN biosynthesis and its elimination from the body. The fluctuation of the plasma level of endogenous NMN in the ERF rats may be due to the variance in $CL_{r(NMN)}$. As described above, some ERF rats without cinotinamide administration showed relatively low plasma levels of NMN, and this may be due to the small amounts of chow taken, since the origin of NMN is nicotinamide in the chow. Furthermore, other possibilities such as a decrease in the intestinal absorption of nicotinamide and/or its metabolism in the liver, still remain.

GFR is one of the important clinical indices of renal disease and was determined by inulin clearance in the present study (Table I). As shown

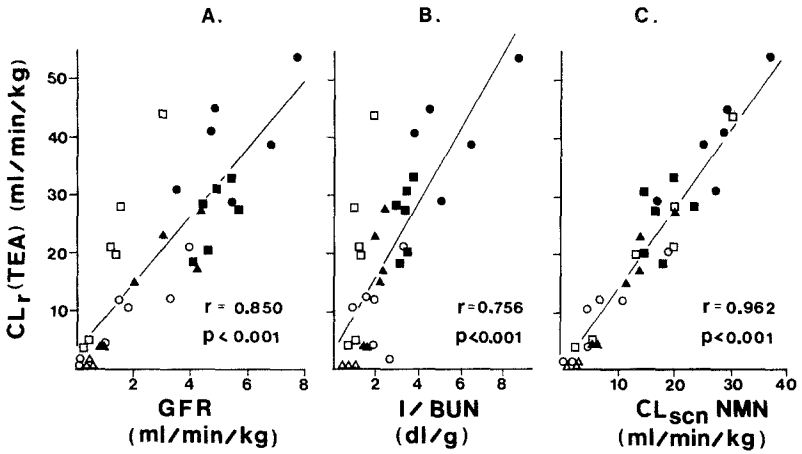

Fig. 6. Correlation between $CL_{r(TEA)}$ and GFR, $CL_{r(TEA)}$ and 1/BUN, and $CL_{r(TEA)}$ and $CL_{scn(NMN)}$, in normal and ERF rats. Panel A: $CL_{r(TEA)} = 5.82(GFR) + 3.13$ ($n = 34$), $r = 0.850$ ($p < 0.001$). Panel B: $CL_{r(TEA)} = 6.44(1/BUN) + 3.28$ ($n = 32$), $r = 0.756$ ($p < 0.001$). Panel C: $CL_{r(TEA)} = 1.43\ CL_{scn(NMN)} + 0.511$ ($n = 34$), $r = 0.962$ ($p < 0.001$). For symbols, see Fig. 1.

in Fig. 3, GFR did not show a high correlation with $CL_{scn(TEA)}$ ($r = 0.81$, $p < 0.001$) or $CL_{scn(NMN)}$ ($r = 0.72$, $p < 0.001$), probably due to the mechanical difference between glomerular filtration and tubular secretion. In the folate-ERF rats, although GFR greatly decreased, little interindividual variation was observed. On the contrary, a large interindividual variation was observed in CL_{scn}, suggesting a glomerulo-tubular imbalance. BUN, another clinical index of renal disease, did not show a good correlation with $CL_{scn(TEA)}$ (Fig. 4a; $r = -0.689$, $p < 0.001$) or with $CL_{scn(NMN)}$ ($r = -0.689$, $p < 0.001$) in the normal and ERF rats, though it showed a good correlation with GFR to some extent (Fig. 4b; $r = -0.828$, $p < 0.001$). McNay *et al.* (56) reported that there was no correlation between BUN and the renal extraction ratio of TEA in the dog with azotemia by bilateral ureteral venous anastomosis. This finding did not agree with the result of this study, which may be due to the difference of ERF. Especially in the folate-ERF rats the disagreement is remarkable, the reason is unclear at the present time. It has been reported that BUN is excreted in urine mainly by glomular filtration and is reabsorbed partly by a passive transport (3,6,7). From these findings, it was suggested that the excretory mechanism of BUN may be different from that of TEA or NMN. In contrast to GFR or BUN, both $CL_{scn(NMN)}$ obtained from exogenous and endogenous NMN were highly correlated with $CL_{scn(TEA)}$ ($r = 0.959$, $p < 0.001$, and $r = 0.952$, $p < 0.001$, respectively) in the normal and all ERF rats (Fig. 5). Both correlation coefficients were significantly higher than those from GFR vs. $CL_{scn(TEA)}$ ($p < 0.05$) and from BUN vs. $CL_{scn(TEA)}$ ($p < 0.001$). These results may support the fact that TEA and NMN share the same secretory system as suggested by many investigators (12,18,19,22–24). Accordingly, the renal secretory function for an organic cation like TEA in ERF can be estimated more accurately by $CL_{scn(NMN)}$ than by GFR or by BUN (Fig. 6). In conclusion, it is suggested that the renal secretory clearance of endogenous NMN($CL_{scn(NMN)}$) may be a useful index of the renal secretory clearance of organic cations that are excreted from the kidney by glomerular filtration and tubular secretion without reabsorption.

REFERENCES

1. L. Dettli. Drug dosage in renal disease. *Clin. Pharmacokin.* **1**:126–134 (1976).
2. W. L. Chiou and F. H. Hsu. Pharmacokinetics of creatinine in man and its implications in the monitoring of renal function in dosage regimen modifications in patients with renal insufficiency. *J. Clin. Invest.* **15**:427–434 (1975).
3. H. Halkin, L. B. Sheiner, C. C. Peck, and K. L. Melmon. Determination of the renal clearance of digoxin. *Clin. Pharmacol. Ther.* **17**:385–394 (1975).
4. H. W. Smith, N. Finkelstein, L. Aliminosa, B. Crawford, and M. Graber. The renal clearances of substituted hippuric acid derivatives and other aromatic acids in dog and man. *J. Clin. Invest.* **24**:388–404 (1945).

5. S. Miwa, S. Tojo, H. Obata, M. Narita, T. Tsuchiya, and K. Hirose. Renal failure and its clinical aspects. *Saishin Igaku* **14:**1797–1807 (1959).
6. J. P. Kassirer. Clinical evaluation of kidney function: glomerular function. *N. Engl. J. Med.* **285:**385–389 (1971).
7. D. B. Morgan, M. E. Carver, and R. B. Payne. Plasma creatinine and urea: creatinine ratio in patients with raised plasma urea. *Br. Med. J.* **2:**929–932 (1977).
8. W. H. Cargill. The measurement of glomerular and tubular plasma flow in the normal and diseased human kidney. *J. Clin. Invest.* **28:**533–538 (1949).
9. A. Farah and B. Rennick. Studies on the renal tubular transport of tetraethylammonium ion in renal slices of the dog. *J. Pharmacol. Exp. Ther.* **117:**478–487 (1956).
10. B. R. Rennick, D. M. Calhoon, H. Gandia, and G. K. Moe. Renal tubular secretion of tetraethylammonium in the dog and the chicken. *J. Pharmacol. Exp. Ther.* **110:**309–314 (1954).
11. B. R. Rennick and A. Farah. Studies on the renal tubule transport of TEA ion in the dog. *J. Pharmacol. Exp. Ther.* **116:**287–295 (1956).
12. B. R. Rennick. Renal tubule transport of organic cations. *Am. J. Physiol.* **240:**F83–F89 (1981).
13. C. R. Ross and A. Farah. *p*-Aminohippurate and N^1-methylnicotinamide transport hypothesis. *J. Pharmacol. Exp. Ther.* **151:**159–167 (1966).
14. C. R. Ross, N. I. Pessah, and A. Farah. Studies on the uptake and runout of *p*-aminohippurate and N^1-methylnicotinamide in dog renal slices. *J. Pharmacol. Exp. Ther.* **160:**381–386 (1968).
15. J. W. Huff and W. A. Perzwig. N^1-methylnicotinamide, a metabolite of nicotinic acid in the urine. *J. Biol. Chem.* **150:**395–400 (1943).
16. B. C. Johnson, T. S. Hamilton, and H. H. Mitchel. The excretion of nicotinic acid, nicotinamide, nicotinuric acid, and N^1-methylnicotinamide by normal individuals. *J. Biol. Chem.* **159:**231–236 (1945).
17. K. H. Beyer, H. F. Russo, S. R. Gass, K. M. Wilhoyte, and A. A. Pitt. Renal tubular elimination of NMN. *Am. J. Physiol.* **160:**311–320 (1950).
18. R. E. Green, W. E. Ricker, W. L. Attwood, Y. S. Koh, and L. Peters. Studies of the renal tubular transport characteristics of N^1-methylnicotinamide and tetraethylammonium compounds in the avian kidney. *J. Pharmacol. Exp. Ther.* **126:**195–201 (1959).
19. B. R. Rennick, A. Kandel, and L. Peters. Inhibition of the renal tubular excretion of tetraethylammonium and N^1-methylnicotinamide by basic cyanine dyes. *J. Pharmacol. Exp. Ther.* **118:**204–219 (1956).
20. C. R. Ross, F. Diezi-Chomety, and F. Roch-Ramel. Renal excretion of N^1-methylnicotinamide in the rat. *Am. J. Physiol.* **228:**1641–1645 (1975).
21. K. Besseghir, B. Pearce, and B. Rennick. Renal tubular transport and metabolism of organic cations by rabbits. *Am. J. Physiol.* **240:**F308–F314 (1981).
22. A. Kandel and L. Peters. Observations concerning the renal tubular transport characteristics of three quarternary bases in dogs. *J. Pharmacol. Exp. Ther.* **119:**550–558 (1957).
23. L. Peters, K. J. Fenton, M. L. Wolf, and A. Kandel. Inhibition of the renal tubular excretion of N^1-methylnicotinamide (NMN) by small doses of a basic cyanine dye. *J. Pharmacol. Exp. Ther.* **113:**148–159 (1955).
24. L. Peters. Renal tubular excretion of organic bases. *Pharmacol. Rev.* **12:**1–35 (1960).
25. M. Mintum, K. H. Himmelstein, R. L. Schroder, M. Gibaldi, and D. D. Shen. Tissue distribution kinetics of tetraethylammonium ion in the rat. *J. Pharmacokin. Biopharm.* **8:**373–409 (1980).
26. H. G. Preuss. Tubular function in experimental acute tubular necrosis in rats. *Kid. Int.* **10:**S51–S57 (1976).
27. C. Westenfelder, G. J. Arevalo, P. W. Crawford, P. Zerwer, R. L. Baranowski, F. M. Birch, W. R. Earnest, R. K. Hamburger, R. D. Colemen, and N. A. Kurtzman. Renal tubular function in glycerol-induced acute renal failure. *Kid. Int.* **18:**432–444 (1980).
28. H. G. Preuss, F. R. Weiss, R. H. Janicki, and H. Goldin. Studies on the mechanism of folate-induced growth in rat kidneys. *J. Pharmacol. Exp. Ther.* **180:**754–758 (1972).

29. U. Schmidt, J. Torhorst, M. Huguenin, and U. C. Dubach. Acute renal failure after folate: Na^+, K^+-ATPase in isolated rat renal tubule. Ultramicrochemical and clinical studies. *Eur. J. Clin. Invest.* **3:**169–178 (1973).
30. G. E. Schubert. Folic acid induced acute renal failure in the rat: morphologic studies. *Kid. Int.* **10:**S46–S50 (1976).
31. I. C. Calder, C. C. Funder, C. R. Green, K. N. Ham, and J. D. Tange. Comparative nephrotoxicity of aspirin and phenacetin derivatives. *Br. Med. J.* **4:**518–521 (1971).
32. R. C. Blantz and K. Konnen. The mechanism of acute renal failure after uranyl nitrate. *J. Clin. Invest.* **55:**621–635 (1975).
33. F. A. Carone and W. G. Spector. The suppression of experimental protein-uria in the rat by compounds that inhibit increased capillary permeability. *J. Pathol. Bacteriol.* **80:**55–62 (1960).
34. D. G. Houghton, M. Hartnett, M. C. Boswell, G. Porter, and W. Bennett. A light microscopic analysis of gentamicin nephrotoxicity in rats. *Am. J. Pathol.* **82:**589–599 (1976).
35. U. C. Kosek, R. I. Mazze, and M. J. Cousins. Nephrotoxicity of gentamicin. *Lab. Invest.* **30:**48–57 (1974).
36. F. C. Luft, G. R. Aronoff, A. P. Evan, B. A. Connors, M. H. Weinberger, and S. A. Kleit. The renin-angiotensin system in aminoglycoside-induced acute renal failure. *J. Pharmacol. Exp. Ther.* **220:**433–439 (1982).
37. B. R. Clark, R. M. Halpern, and R. A. Smith. A fluorimetric method for quantitation in the picomole range of N^1-methylnicotinamide and nicotinamide in serum. *Anal. Biochem.* **68:**54–61 (1975).
38. J. K. Fawcett and J. E. Scott. A rapid and precise method for the determination of urea. *J. Clin. Pathol.* **13:**156–159 (1960).
39. R. L. Searcy and F. M. Cox. A modified technique for ultramicro estimations of urea nitrogen. *Clin. Chim. Acta* **8:**810–812 (1963).
40. S. Reitman and S. Frankel. A colorimetric method for the determination of serum glutamic pyruvic transaminases. *Am. J. Clin. Pathol.* **28:**56–63 (1957).
41. H. G. Preuss. Tubular function in experimental acute tubular necrosis in rats. *Kid. Int.* **10:**S51–S57 (1976).
42. C. Westenfelder, G. J. Arevalo, P. W. Crawford, P. Zerwer, R. L. Baranowski, F. M. Birch, W. R. Earnest, R. K. Hamburger, R. D. Coleman, and N. A. Kurtzman. Renal tubular function in glycerol-induced acute renal failure. *Kid. Int.* **18:**432–444 (1980).
43. F. C. Luft, G. R. Aronoff, A. P. Evan, B. A. Connors, M. H. Weinberger, and S. A. Kleit. The renin-antigiotensin system in aminoglycoside-induced acute renal failure. *J. Pharmacol. Exp. Ther.* **220:**433–439 (1982).
44. J. C. Kosek, R. I. Mazze, and M. J. Cousins. Nephrotoxicity of gentamicin. *Lab. Invest.* **30:**48–57 (1974).
45. D. G. Houghton, M. Hartnett, M. C. Boswell, G. Porter, and W. Bennett. A light microscopic analysis of gentamicin nephrotoxicity in rats. *Am.. J. Pathol.* **82:**589–599 (1976).
46. U. Schmidt, J. Torhorst, M. Huguenin, and U. C. Dubach. Acute renal failure after folate: Na^+, K^+-ATPase in isolated rat renal tubule. Ultramicrochemical and clinical studies. *Eur. J. Clin. Invest.* **3:**169–178 (1973).
47. G. E. Schubert. Folic acid-induced acute renal failure in the rat: Morphologic studies. *Kid. Int.* **10:**S46–S50 (1976).
48. H. G. Peuss, F. R. Weiss, R. H. Janicki, and H. Goldin. Studies on the mechanism of folate-induced growth in rat kidneys. *J. Pharmacol. Exp. Ther.* **180:**754–758 (1972).
49. F. A. Carone and W. G. Spector. The suppression of experimental protein–uria in the rat by compounds that inhibit increased capillary permeability. *J. Pathol. Bacteriol.* **80:**55–62 (1960).
50. R. C. Blantz and K. Konnen. The mechanism of acute renal failure after uranyl nitrate. *J. Clin. Invest.* **55:**621–635 (1975).
51. I. C. Calder, C. C. Funder, C. R. Green, K. N. Ham and J. D. Tange. Comparative nephrotoxicity of aspirin and phenacetin derivatives. *Br. Med. J.* **4:**518–521 (1971).
52. A. Farah, B. R. Rennick, and M. Frazer. The influence of some basic substances on the transport of tetraethylammonium ion. *J. Pharmacol. Exp. Ther.* **119:**123–127 (1957).

53. P. D. Holohan and C. R. Ross. Mechanisms of organic cation transport in kidney plasma membrane vesicles: 2. *p*H studies. *J. Pharmacol. Exp. Ther.* **216:**294–298 (1981).
54. B. Rennick, G. K. Moe, R. H. Lyons, S. W. Hoobler, and R. J. Neligh. Absorption and renal excretion of the tetraethylammonium ion. *J. Pharmacol. Exp. Ther.* **91:**210–217 (1947).
55. C. K. Shim, Y. Sawada, T. Iga, and M. Hanano. Effect of experimental acute renal failure on intrinsic renal tubular secretory clearance of organic cations in rats. *J. Pharm. Dyn.* **6:**787–789 (1983).
56. J. L. McNay, S, Rosello, and P. G. Dayton. Effects of azotemia on renal extraction and clearance of PAH and TEA. *Am. J. Physiol.* **230:**901–906 (1976).