DISPOSITION OF CREATININE AND UREA IN BILATERALLY NEPHRECTOMIZED RATS*

JUN WATANABE,** JUNJI HIRATE, KIKUO IWAMOTO AND SHOJI OZEKI

Department of Biopharmaceutics, Faculty of Pharmaceutical Sciences, Nagoya City University, 3-1, Tanabedori, Mizuho-ku, Nagoya 467, Japan

(Received February 24, 1981)

Bilaterally nephrectomized rats were used to investigate the disposition of creatinine when renal function is acutely decreased. The percentage of radioactivity recovered in the expired air in 11 hours following intravenous administration of [carbonyl- ^{14}C]creatinine immediately and 24 hours after nephrectomy was 0.54 and 3.24% respectively, and these values were significantly different ($p < 0.05$). As it was considered that expiratory excretion is one of the major elimination routes in nephrectomized rats, mechanism of expiratory excretion was investigated by incubating [carbonyl- ^{14}C]creatinine with the intestinal contents of the rats chronically loaded with creatinine. [carbonyl- ^{14}C]Creatinine was completely metabolized in that intestinal contents, but $^{14}\text{CO}_2$ was not produced. When the mixture of the metabolites was administered orally or intravenously to chronically creatinine loaded rats, however, about 50% of the total radioactivity was excreted into the expired air in 5 hours. Furthermore, biliary excretion of the radioactivity following intravenous administration of [carbonyl- ^{14}C]creatinine in the nephrectomized rats was much greater than that in normal rats. These results indicate that expiratory excretion following intravenous administration of [carbonyl- ^{14}C]creatinine to nephrectomized rats would arise following intestinal absorption of the creatinine metabolites, which seem to be produced by intestinal microflora after biliary excretion of creatinine. The change of disposition for urea by bilaterally nephrectomy was also studied to compare with that for creatinine.

Keywords—creatinine; urea; nephrectomized rat; expiratory excretion; metabolism; biliary excretion; autoradiography

INTRODUCTION

It has been well known that creatinine is metabolically inert and quantitatively excreted into the urine following intravenous administration to man¹⁾ and experimental animals^{2–4)} with normal renal function. In 1974, however, it was shown that 15.9 to 65.7% of endogenous creatinine is metabolized or excreted *via* extra-renal routes in patients with chronically decreased renal function.⁵⁾ Recently, a few papers are also seen which are reporting the disposition of creatinine in patients with chronically decreased renal function.^{6,7)} But the disposition of creatinine

in patients with acute renal disease has not been clarified up to this time.

In our previous study,⁴⁾ it was shown that expiratory excretion of radioactivity following oral administration of [carbonyl- ^{14}C]creatinine to rats was not negligible. This result suggests that expiratory excretion may be one of the major elimination routes of creatinine in acute as well as chronic renal diseases.

However, the disposition of creatinine in acute renal disease is elucidated neither in man nor in experimental animals. It becomes important to clarify the alteration of creatinine disposition in

* This paper constitutes Part VI of the series entitled "Drug Distribution in the Body."

** To whom inquiries should be directed.

such a case as well as chronic renal disease, since the clearance or plasma (serum) concentration of creatinine is clinically used as one of the determinants of dose for many drugs.

In this study, bilaterally nephrectomized rats were used as model animals with acute renal disease, and the experiments were carried out on expiratory excretion, biliary excretion, plasma levels and whole-body autoradiography following intravenous administration of [carbonyl- ^{14}C]-creatinine to the nephrectomized rats. It was considered that expiratory excretion after intestinal metabolism is a major elimination route of the radioactivity in these rats, and the mechanism of $^{14}\text{CO}_2$ formation from [carbonyl- ^{14}C]creatinine was proposed. On the other hand, the change of disposition by nephrectomy for urea, which was excreted into the expired air much more than creatinine following intravenous administration to normal rats,⁸⁾ was also examined and the disposition kinetics of creatinine and urea in nephrectomized rats were compared.

MATERIALS AND METHODS

Materials — [carbonyl- ^{14}C]Creatinine hydrochloride (specific activity, 12.0 mCi/mmol) and ^{14}C -urea (specific activity, 8.6 mCi/mmol) were purchased from The Radiochemical Center, Amersham, England and New England Nuclear, Boston, Mass., U.S.A., respectively. The radiochemical purity of these two chemicals were more than 99%. All other chemicals were of analytical grade and used without further purification.

Animals — Male Wistar rats weighing 250 to 300 g were used. All rats which were used for measuring expiratory excretion, biliary excretion and plasma levels of radioactivity were cannulated into the left external jugular vein with silicone polymer tubing (i.d. 1.0 mm; o.d. 1.5 mm, Dow Corning, Tokyo, Japan) by the method of Upton.⁹⁾ Bilateral nephrectomy of rats was performed by ligating the left and right renal arteries and veins immediately before the administration of those radiochemicals in almost all experiments. Only in the experiment on expiratory excretion following intravenous administration of ^{14}C -

creatinine, nephrectomy was performed immediately or 24 hours before the administration. On the other hand, chronic creatinine loading was performed by allowing rats to drink 8% creatinine solution instead of water for 2 to 3 weeks.

Expiratory Excretion in Nephrectomized Rats following Intravenous Administration of ^{14}C -Creatinine and ^{14}C -Urea — Rats were given 10 $\mu\text{Ci/kg}$ of ^{14}C -creatinine (94.2 $\mu\text{g/kg}$ as creatinine) into the external jugular vein immediately and 24 hours after nephrectomy, or given 10 $\mu\text{Ci/kg}$ of ^{14}C -urea (69.9 $\mu\text{g/kg}$ as urea) immediately after nephrectomy. The rats were then housed individually in the metabolic cage (KN-450, Natsume, Tokyo, Japan), which is equipped to capture $^{14}\text{CO}_2$ in the mixture of ethanolamine-methanol (1:2, v/v).

Biliary Excretion in Nephrectomized Rats following Intravenous Administration of ^{14}C -Creatinine — After bile duct was cannulated with PE-10 tubing (i.d. 0.28 mm; o.d. 0.61 mm, Clay Adams), 10 $\mu\text{Ci/kg}$ of ^{14}C -creatinine (94.2 $\mu\text{g/kg}$ as creatinine) was administered into the external jugular vein immediately after nephrectomy, and bile samples were collected periodically.

Plasma Levels of ^{14}C -Creatinine and ^{14}C -Urea in Nephrectomized Rats following Intravenous Administration — Rats were given 10 $\mu\text{Ci/kg}$ of ^{14}C -creatinine (94.2 $\mu\text{g/kg}$ as creatinine) or ^{14}C -urea (69.9 $\mu\text{g/kg}$ as urea) into the external jugular vein immediately after nephrectomy. Blood samples were withdrawn periodically in a small heparinized tubes and plasma samples were obtained by centrifuging the tubes at 3000 rpm for 15 minutes.

Whole-Body Autoradiography in Nephrectomized Rats following Intravenous Administration of ^{14}C -Creatinine — Rats were given 100 $\mu\text{Ci/kg}$ of ^{14}C -creatinine (942 $\mu\text{g/kg}$ as creatinine) into the tail vein immediately after nephrectomy, and sacrificed at 5 minutes and 24 hours following the administration by soaking them in dry-ice acetone (-78°C) under light anesthesia with ether. The sections (40 μm) were elaborately obtained by a microtome (Yamato 1111, Tokyo, Japan) at about -25°C , and attached to

SALOTAPE (Hisamitsu Seiyaku Co., Ltd., Thosu, Japan). After drying in a freeze-dryer for a few days, the sections were attached on X-ray films (No. 150, Fuji Photo Film Co., Ltd., Tokyo, Japan) in order to be exposed for 20 days at 4°C.

Experiments on in Vitro Metabolism of Creatinine — Intestinal contents (about 5 g in wet weight) were collected from 5 to 10 heads of control or chronically creatinine loaded rats, and 10 μ Ci of 14 C-creatinine (94.2 μ g as creatinine) was added to them. During aerobic incubation of the mixture at 37°C for 24 hours, 14 CO₂ was trapped with ethanolamine-methanol (1:2, v/v). After the incubation the intestinal contents were centrifuged (3000 rpm, 15 min). Metabolites contained in the supernatants were separated by

silica-gel thin layer chromatography (E. Merck, 60F₂₅₄, 0.25 mm thickness).

Expiratory Excretion following Intravenous or Oral Administration of the Mixture of Creatinine Metabolites — The supernatant separated from the intestinal contents which were collected from chronically creatinine loaded rats and incubated with 14 C-creatinine was administered intravenously or orally to other groups of chronically creatinine loaded rats. Collection procedure of the expired air was the same as described above.

Radioactivity Measurement — The radioactivity was determined in a Mark II liquid scintillation spectrometer (Nuclear-Chicago Corporation, Des Plaines, Ill., U.S.A.). All samples were directly determined with 10 ml of toluene-Triton X-100

TABLE I. *Expiratory Excretion in Nephrectomized Rats following Intravenous Administration of [Carbonyl- 14 C]Creatinine (94.2 μ g/kg) or 14 C-Urea (69.9 μ g/kg) immediately (A) and 24 Hours (B) after Nephrectomy*

(A) Immediately after nephrectomy

Experiment No.	Creatinine			Urea	
	0–2 h	0–11 h	0–24 h	0–2 h	0–24 h
1	0.15 ^{a)}	0.47	1.12	7.63	37.8
2	0.20	0.58	1.28	4.55	31.9
3	0.22	0.60	1.39	6.39	32.5
4	0.20	0.52	11.7	—	—
Mean $\pm$ S.D. ^{b)}	0.19 $\pm$ 0.03	0.54 $\pm$ 0.06	3.89 $\pm$ 5.22	6.19** $\pm$ 1.55	34.1** $\pm$ 3.2

(B) Twenty-four hours after nephrectomy

Experiment No.	Creatinine		
	0–2 h	0–11 h	0–24 h
1	0.20	2.18	death ^{c)}
2	0.23	2.57	7.33
3	0.18	1.40	death
4	—	1.34	death
5	—	6.81	death
6	0.20	5.15	death
Mean $\pm$ S.D.	0.20 $\pm$ 0.02	3.24* $\pm$ 2.23	—

a) Values represent % of dose,

b) Standard deviation,

c) Died in 11 to 24 h following administration,

* Significantly different from the case of "(A) immediately after nephrectomy" at $p < 0.05$,

** Significantly different from the corresponding values with creatinine at $p < 0.001$.

liquid scintillator (PPO 5 g, POPOP 300 mg, toluene 700 ml, Triton X-100 300 ml). The counting efficiencies were automatically determined by ^{133}Ba external standardization method and cpm was converted to dpm.

RESULTS

1. Expiratory Excretion in Nephrectomized Rats following Intravenous Administration of ^{14}C -Creatinine and ^{14}C -Urea

The results of expiratory excretion in nephrectomized rats following intravenous administration of ^{14}C -creatinine immediately and 24 hours after nephrectomy are shown in Table I. When the administration was performed at 24 hours after nephrectomy, most of rats died at 11 to 24 hours after the administration. However, the cumulative expiratory excretion in 11 hours after the administration to 24 h-nephrectomized rats was significantly larger than the case of immediate administration after nephrectomy ($p < 0.05$).

The results with ^{14}C -urea, which was excreted into the expired air much more (about 12%)⁸⁾ than creatinine (about 1%)⁴⁾ following in-

travenous administration to normal rats, are also shown in Table I. Considerably high radioactivity in expired air was observed immediately after the administration in contrast to creatinine, and cumulative expiratory excretion in 24 hours following the administration of ^{14}C -urea was also significantly larger than that of ^{14}C -creatinine ($p < 0.001$).

2. Plasma Levels of ^{14}C -Creatinine and ^{14}C -Urea in Nephrectomized Rats following Intravenous Administration

Plasma elimination curves of ^{14}C -creatinine and ^{14}C -urea in nephrectomized rats following intravenous administration immediately after nephrectomy are shown in Fig. 1 and Fig. 2, respectively. Estimated pharmacokinetic parameters for these two chemicals are summarized in Table II.

In normal rats, β and k_{10} , which are the parameters relating to elimination, for creatinine were significantly larger than those for urea ($p < 0.01$), and $(V_d')_{\text{extrap}}$ for creatinine was about three times that of urea.⁴⁾ In case of nephrectomized rats, however, β and k_{10} for creatinine were significantly smaller than those for urea ($p < 0.01$), and $(V_d')_{\text{extrap}}$ for these two chemicals were much the same.

FIG. 1. Plasma Levels of ^{14}C -Creatinine following Intravenous Administration to Nephrectomized Rats Administration was performed immediately after nephrectomy. Each point represents the mean $\pm$ S.D. for three animals. (Only the point in 24 hours is for one animal.) Dose: $10 \mu\text{Ci/kg}$ ($94.2 \mu\text{g/kg}$ as creatinine).

FIG. 2. Plasma Levels of ^{14}C -Urea following Intravenous Administration to Nephrectomized Rats Administration was performed immediately after nephrectomy. Each point represents the mean $\pm$ S.D. for three animals. Dose: $10 \mu\text{Ci/kg}$ ($69.9 \mu\text{g/kg}$ as urea).

TABLE II. Pharmacokinetic Parameters for ^{14}C -Creatinine and ^{14}C -Urea following Intravenous Administration to Nephrectomized Rats (Value for Parameter $\pm$ Standard Error^{a)})

Parameters	Creatinine ($n=31$)		Urea ($n=30$)	
A, dpm/ml	4.23×10^4	$\pm 3.55 \times 10^3$	4.10×10^4	$\pm 5.05 \times 10^3$
B, dpm/ml	3.10×10^4	$\pm 9.25 \times 10^2$	$3.36 \times 10^4^{**}$	$\pm 4.70 \times 10^2$
α , min^{-1}	3.46×10^{-2}	$\pm 4.94 \times 10^{-3}$	$3.55 \times 10^{-1}^{**}$	$\pm 4.75 \times 10^{-2}$
β , min^{-1}	7.22×10^{-5}	$\pm 6.38 \times 10^{-5}$	$5.09 \times 10^{-4}^{**}$	$\pm 1.52 \times 10^{-4}$
k_{10} , min^{-1}	1.70×10^{-4}	$\pm 1.48 \times 10^{-4}$	$1.13 \times 10^{-3}^{**}$	$\pm 3.50 \times 10^{-4}$
k_{12} , min^{-1}	1.98×10^{-2}	$\pm 3.19 \times 10^{-3}$	$1.94 \times 10^{-1}^{**}$	$\pm 3.52 \times 10^{-2}$
k_{21} , min^{-1}	1.47×10^{-2}	$\pm 1.94 \times 10^{-3}$	$1.60 \times 10^{-1}^{**}$	$\pm 1.40 \times 10^{-2}$
V_1 , ml/kg	3.03×10^2	$\pm 1.52 \times 10^1$	2.98×10^2	$\pm 2.07 \times 10^1$
V_2 , ml/kg	4.09×10^2	$\pm 8.76 \times 10^1$	$3.61 \times 10^2^*$	$\pm 7.71 \times 10^1$
AUC, dpm $\cdot$ min/ml	4.30×10^8		6.61×10^7	
$(V_d')_{\text{extrap}}$, ml/kg ^{b)}	7.16×10^2		6.61×10^2	
$(V_d')_{\beta}$, ml/kg ^{c)}	7.13×10^2		6.60×10^2	
$t_{1/2\beta}$, h	160		23	

a) W.E.Deming, "Statistical Adjustment of Data," Jhon Wiley and Sons, Inc., New York, 1946.

b) $(V_d')_{\text{extrap}} = (\text{Dose})/B$, c) $(V_d')_{\beta} = V_1 \cdot k_{10}/\beta$,

* Significantly different from creatinine at $p < 0.05$, ** Significantly different from creatinine at $p < 0.01$.

FIG. 3. Cumulative Biliary Excretion of Radioactivity in Normal (●) and Nephrectomized (○) Rats following Intravenous Administration of ^{14}C -Creatinine

In experiment for nephrectomized rats, administration was performed immediately after nephrectomy. Each point represents the mean $\pm$ S.D. for three animals. Dose: $10 \mu\text{Ci/kg}$ ($94.2 \mu\text{g/kg}$ as creatinine).

3. Biliary Excretion in Nephrectomized Rats following Intravenous Administration of ^{14}C -Creatinine

The time course of cumulative biliary excretion of radioactivity in nephrectomized rats following intravenous administration of ^{14}C -creatinine immediately after nephrectomy is shown in Fig. 3 along with that in normal rats. It is obvious that biliary excretion increases remarkably by nephrectomy.

4. Whole-Body Autoradiography in Nephrectomized Rats following Intravenous Administration of ^{14}C -Creatinine

Whole-body autoradiograms in nephrectomized rats following intravenous administration of ^{14}C -creatinine immediately after nephrectomy are shown in Fig. 4. In 5 minutes, the autoradiogram had the same appearance as that in normal rat,⁴⁾ except that creatinine did not distribute at all in the kidney of nephrectomized rat. In 24 hours, however, relatively high radioactivity existed in the intestinal tract. Enhanced biliary excretion may be responsible for this distribution pattern of the radioactivity in the nephrectomized

(A) 5 minutes

(B) 24 hours

FIG. 4. Autoradiograms (5 minutes (A) and 24 hours (B)) showing the Distribution of Radioactivity (Dark Area) in Nephrectomized Rats following Intravenous Administration of ^{14}C -Creatinine. Administration was performed immediately after nephrectomy. Dose: $100\ \mu\text{Ci/kg}$ ($942\ \mu\text{g/kg}$ as creatinine).

TABLE III. *In Vitro* Formation of $^{14}\text{CO}_2$ from [Carbonyl- ^{14}C]Creatinine in the Intestinal Contents from Two Groups of Rats

Groups	Average recovery (% of dose) with range	
	0–11 h	0–24 h
Control ($n=2$)	0.08 (0.06–0.10)	0.11 (0.08–0.13)
Chronically loaded with creatinine ($n=2$)	0.10 (0.08–0.11)	0.68 (0.64–0.71)

rat.

5. Metabolism of ^{14}C -Creatinine in Vitro and Expiratory Excretion following Intravenous or Oral Administration of the Mixture of Creatinine Metabolites

In vitro production of $^{14}\text{CO}_2$ was very little in both experiments with intestinal contents from control and chronically creatinine loaded rats (Table III). As shown in Fig. 5, however, ^{14}C -creatinine was almost completely converted to three major metabolites (M-1, M-2, M-3) in the intestinal contents of chronically creatinine loaded rats, in contrast to the metabolic profile in control rats.

The current concept on the metabolic pathway

of creatinine in the intestinal tract has been mentioned.¹⁰⁾ According to this metabolic pathway, the radioactive chemicals which can be formed from [carbonyl- ^{14}C]creatinine are 1-methylhydantoin, creatine, sarcosine, glyoxylate and glycolate. By TLC method, M-1 and M-3 were identified with the standards of sarcosine and 1-methylhydantoin, respectively. But M-2 could not be identified yet with any standards.

When the mixture of the creatinine metabolites was administered intravenously or orally to chronically creatinine loaded rats, about 50% of the administered radioactivity was excreted into the expired air in 5 to 7 hours in both routes of administration. These results are shown in Fig. 6.

DISCUSSION

Most studies on the disposition of creatinine in renal failure have been performed with severe chronic renal failure, and the disposition of creatinine with acute renal failure has yet been clarified neither in man nor in experimental animals. In this study, bilaterally nephrectomized

FIG. 5. Typical Radio-thin-layer Chromatograms for the Supernatants of *in Vitro* Incubation Mixture from Control (A) and Chronically Creatinine Loaded Rats (B)

Solvent system: Ethyl Acetate : Methanol : Ammonia Water = 5:2:1.

FIG. 6. Expiratory Excretion in Chronically Creatinine Loaded Rats following Intravenous (○) or Oral (●) Administration of the Mixture of Creatinine Metabolites

Each point represents the mean $\pm$ S.D. for three animals.

rats were used as model animals of acute renal failure.

As shown in Table I, the expiratory excretion in nephrectomized rats following intravenous administration of ^{14}C -creatinine increased significantly when administered 24 hours after nephrectomy as compared with the case of immediate administration after nephrectomy ($p < 0.05$). This result may be brought about by the increment of metabolic capacity of intestinal microflora which would be able to metabolize creatinine. Such an increment of the metabolic capacity or population of the microflora may originate from the consequence that creatinine is transferred to a considerable extent into the intestinal tract by nephrectomy, as it is suggested from the results of the experiments on biliary excretion (Fig. 3) and whole-body autoradiography (Fig. 4).

As it was considered that major elimination route of radioactivity in nephrectomized rats following intravenous administration of ^{14}C -creatinine was expiratory excretion (fecal excretion was negligible in nephrectomized rats as well as normal rats⁴⁾), the authors had great interest in the mechanism of expiratory excretion. Therefore, metabolism experiments were carried out *in vitro* by incubating ^{14}C -creatinine with the intestinal contents of control or chronically creatinine loaded rats, but $^{14}\text{CO}_2$ was hardly produced in both intestinal contents (Table III). In the intestinal contents of chronically creatinine loaded rats, however, ^{14}C -creatinine was almost completely converted into the three main metabolites (Fig. 5). When the mixture of these metabolites was administered intravenously or orally to chronically creatinine loaded rats, about 50% of administered radioactivity was excreted into the expired air in 5 to 7 hours in either administration route (Fig. 6). These results indicate that expiratory excretion of $^{14}\text{CO}_2$ in nephrectomized rats following intravenous administration of [carbonyl- ^{14}C]creatinine arises following intestinal absorption of creatinine metabolites, which seem to be produced by intestinal

microflora.

In normal rats, β and k_{10} for creatinine were significantly larger than those for urea ($p < 0.01$).⁴⁾ As shown in Table II, however, these parameters for creatinine have changed to be significantly smaller than those for urea ($p < 0.01$) by nephrectomy, since expiratory excretion, which is the main elimination route of radioactivity in both creatinine and urea in nephrectomized rats, was much larger in urea than creatinine. It is interesting to note that approximately three times difference in $(V_d')_{\text{extrap}}$ (for creatinine $>$ urea) in normal rats disappeared by nephrectomy (Table II). This results may indicate that mechanism and/or extent of renal transfer of these two chemicals are considerably different.

REFERENCES

- 1) R.Dominguez and E.Pomerene: Recovery of creatinine after ingestion and after intravenous injection in man, *Pro. Soc. Exp. Biol. Med.*, **58**, 26–28 (1945).
- 2) E.Katoh and S.Abe: The daily tests of renal function, *Nippon Rinsho*, **34**, 2817–2824 (1976).
- 3) J.Greenberg, I.L.Schwartz, M.Spinner, L.Silver and N.Starr: Apparent volumes of distribution of *p*-aminohippurate and creatinine in the dog, *Am. J. Physiol.*, **168**, 86–92 (1952).
- 4) J.Watanabe, J.Hirate, K.Iwamoto and S.Ozeki: Distribution of creatinine following intravenous and oral administration to rats, *J. Pharm. Dyn.*, **4**, 329–335 (1981).
- 5) J.D.Jones and P.C.Burnett: Creatinine metabolism in humans with decreased renal function: creatinine deficit, *Clin. Chem.*, **20**, 1204–1212 (1974).
- 6) W.E.Mitch and M.Walser: A proposed mechanism for reduced creatinine excretion in severe chronic renal failure, *Nephron*, **21**, 248–254 (1978).
- 7) W.E.Mitch, V.U.Collier and M.Walser: Creatinine metabolism in chronic renal failure, *Clin. Sci.*, **58**, 327–335 (1980).
- 8) M.Shibata, K.Iwamoto, J.Hirate, K.Hori and J.Watanabe: Excretion of ^{14}C -urea in rats: "Abstracts of the 99th Meeting of Pharmaceutical Society of Japan," 1979, p. 562.
- 9) R.A.Upton: Simple and reliable method for serial sampling of blood from rats, *J. Pharm. Sci.*, **64**, 112–114 (1975).
- 10) J.D.Jones and P.C.Burnett: Creatinine metabolism and toxicity, *Kid. Internat.*, **7**, S294–S298 (1975).