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RENAL FUNCTION IN EXPERIMENTAL CADMIUM INTOXICATION

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= Abstract =

Changes in renal functions were studied in rats treated with cadmium. Subcutaneous injections of $CdCl_2$(2 mg Cd/kg·day) for 16 days induced a marked polyuria and a reduction in urine osmolality. These changes were accompanied by increase in urinary excretions of various solutes, such as proteins, glucose, urea, calcium, phosphate, chloride, and potassium. The change in urine flow was proportional to the change in total osmotic solute excretion. Creatinine excretion and $T^c_{H_2O}$ remained unchanged. From these results we suggest that the mechanism of Cd-induced polyuria is an osmotic diuresis initiated by inhibition of proximal tubular reabsorption of various substances.

INTRODUCTION

Chronic exposure to cadmium(Cd) induces various renal functional defects. It has been documented in humans and experimental animals that long-term exposures to Cd result in polyuria,[20,34,48] proteinuria,[2,4,35,39,41] glycosuria,[1,21,36] aminoaciduria[1,3,15,16,21,36], phosphaturia[1,17,20] hypercalciuria,[44] and reduction in urine concentrating ability[20,43]. It has also been reported that Cd-intoxication impairs p-aminohippurate (PAH) secretion and sodium, postassium-activated adenosinetriphosphatase(na-K-ATPase) activity in the kindney[19,32]. The mechanism underlying these changes has not been fully elucidated.

We, therefore, conducted a series of experiments to systematically evaluate alterations in renal tubular transport function in Cd-intoxicated rats. The present paper describes changes in urinary excretion of various substances by Cd-treatment.

The effects of Cd on the Na-K-ATPase and the organic anion transport system in the kidney are reproted elsewhere[22,23]

METERIALS AND METHODS

Sprague-Dawley male rats (250−300 g) were maintained, unless otherwise mandated by experimental protocol, for up to 40 days under standard laboratory conditions with *ad libitum* access to food and water.

After 4 days of baseline period, the experimental group recieved a daily dose of 2 mg Cd ($CdCl_2$ dissolved in saline)/kg (body weight) over 16 days (treatment period), and then followed up for the next 20 days (recovery period). The control group received the same volume of plain saline.

Renal functions were determined at 4 day intervals. Animals were kept in metabolic cages and were denied food and water for 24 hours. Urine was collected under a film of mineral oil in a glass cylinder in order to prevent evaporation. The urine samples were analyzed for creatinine(Wako Technical Bulletin No. 271-10509, Japan), osmolality (Advanced Osmometer, Model 3D2), Na^+ and K^+ (Radiometer Flame Photometer, Model FLM 3), Cl^-(Radiometer Chloride Titrator, Model CMT 10), Ca^{++} (Asan Technical Bulletin No. AM117-K, Korea), phosphate[10], protein[27], urea(Sigma Technical Bulletin No. 640), glucose (Wako Technical Bulletin, No. 270-66509, Japan) and Cd^{++}(Hitachi Atomic Absorption Spectrophotometer, Model 180-30). In some animals blood samples were collected by heart puncture using heparinized syringes, and analyzed for creatinine, osmoality, Na^+ and K^+.

Statistical evaluation of the data was done using the Student's t-test (unpaired comparison) and all results were presented as the mean ±SE.

RESULTS

Figs. 1-4 depict changes in urinary excretion of various substances in the control (saline) and Cd-treated animals during the course of experiment.

Urine flow (V) increased markedly in Cd-treated animals (Fig. 1A). The average V after 16 days of Cd-treatment (experimental day 20) was 71.0±5.8 ml/kg·day, which was more than 3-fold greater than the value observed in matched control animals (22.7± 2.3 ml/kg·day) (Table 1). This polyuria was gradually disappeared after cessation of the Cd-treatment.

By contrast, urine osmolality (U_{osm}) decreased significantly in Cd-treated animals (Fig. 1B). The average U_{osm} after 16 days of Cd-treatment (1151±98 mOsm/kg H_2O) was approximately one half of the corresponding value in control animals (2089±137 mOsm/kg H_2O)(Table 1). Upon cessation of the Cd-treatment, the U_{osm} increased slowly.

In both the control and Cd-treated animals urinary excretion of creatinine (U_{cr}·V) did not change significantly during the whole course of experiment (Fig. 1C), suggesting that the glomerular filtration rate remained unchanged

Urinary excretion of total osmotic substances (U_{osm}·V) rose markedly in Cd-treated animals (Fig. 2A). This change was accompanied by increases in protein (U_{prot}·V), urea (U_{urea}·V), glucose(U_{gl}·V), calcium(U_{ca}·V), and phosphate(U_{PO_4}·V) excretions (Figs. 2B, 2C, 3A, 3B, and 3C). The amount of excretion of these substances after 16 days of Cd-treatment appeared to be 1.5(urea) to 7.7 (glucose) times greater than the correspond-

Table 1. Effect of Cd-treatment on Renal Functions in Rats

		Saline	Cd	P
V	(ml/kg·day)	22.7±2.3	71.0±5.8	<0.05
U_{osm}	(mOsm/kg H_2O)	2089±137	1151±98	<0.05
$U_{cr}\cdot V$	(mg/kg·day)	29.5±1.5	26.3±3.5	NS
$U_{osm}\cdot V$	(m osmoles/kg·day)	45.9±3.0	80.7±7.3	<0.05
$U_{prot}\cdot V$	(mg/kg·day)	171±20	419±56	<0.05
$U_{urea}\cdot V$	(m moles/kg·day)	25.5±1.7	38.9±3.4	<0.05
$U_{gl}\cdot V$	(mg/kg·day)	3.2±0.6	24.7±12.5	<0.05
$U_{ca}\cdot V$	(m moles/kg·day)	0.08±0.02	0.37±0.10	<0.05
$U_{po4}\cdot V$	(m moles/kg·day)	0.69±0.08	1.24±0.14	<0.05
$U_{na}\cdot V$	(mEquiv./kg·day)	1.4±0.3	2.4±0.8	NS
$U_{k}\cdot V$	(mEquiv./kg·day)	4.2±0.7	6.5±1.2	<0.10
$U_{cl}\cdot V$	(mEquiv./kg·day)	3.0±0.4	5.6±0.9	<0.05

Values are mean ± SE of 6~7 animals in each group at the end of 16 days of treatment period.

ing value in the control group (Table 1). Except for phosphate, the change was slowly reverted during the recovery period.

Surprisingly, urinary excretion of Na^+ ($U_{Na}\cdot V$) was drastically reduced in the Cd group, especially during the early phase of Cd-treatment (Fig. 4A). The value of $U_{Na}\cdot V$ after 4 days of Cd-treatment(0.1±0.1 mEquiv./kg·day) was less than 8% of the control value(1.4±0.2). During the later phase of Cd-treatment, however, the $U_{Na}\cdot V$ returned gradually to the control level.

Urinary excretion of K^+($U_K\cdot V$) was increased slightly after 16 days of Cd-treatment, and remained high during the recovery period (Fig. 4B and Table 1). Such a change in K^+ excretion may be attributed in part to the hematuria developed in some animals during this period.

The change of Cl^- excretion ($U_{Cl}\cdot V$) in the Cd group was similar to that of the combined excretion of Na^+ and K^+ (Fig. 4C). However, the absolute amount of Cl^- ex-

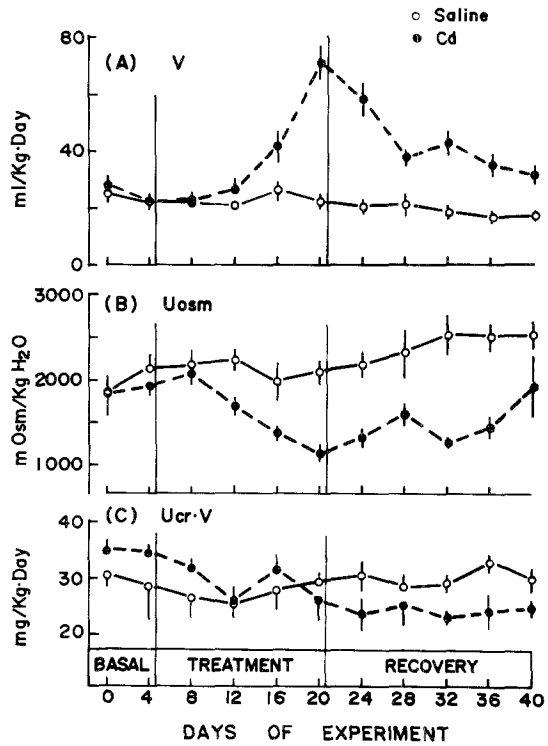

Fig. 1. Changes in urine flow V, urine osmolality (U_{osm}), and endogenous creatinine excretion ($U_{Cr}\cdot V$) in control (saline) and Cd-treated (2 mg/kg·day) rats. Data represent the mean±SE of 7 measurements in each group.

Table 2. Plasma Concentrations of Creatinine, Na^+, K^+ and Osmotic Substances

		Saline	Cd	P
P_{cr}	(㎎/ℓ)	29±5	34±9	>0.5
P_{na}	(mEq/ℓ)	147±3	138±5	>0.1
P_k	(mEq/ℓ)	5.9±0.4	5.0±0.6	>0.2
P_{osm}	(mOsm/kg H_2O)	310±4	316±19	>0.5

Values represent mean ± SE of 6 animals in each group at the 16th day of treatment.

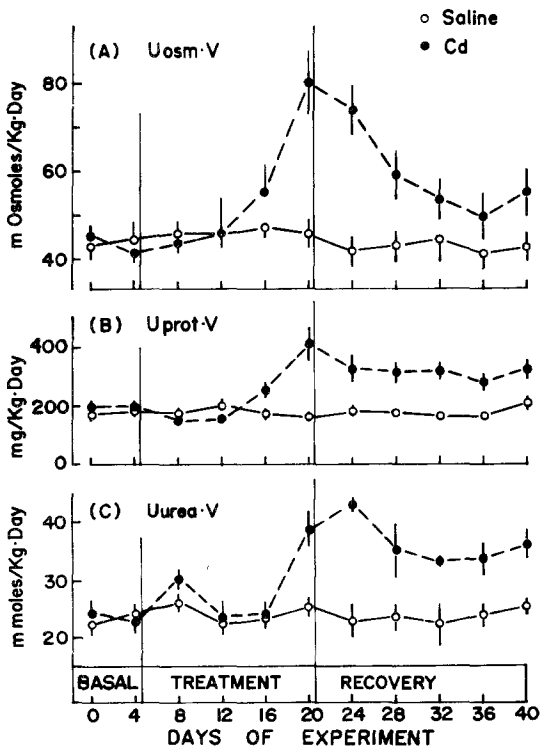

Fig. 2. Changes in urinary excretion of total osmotic substances (U_{osm}·V), protein (U_{prot}·V) and urea (U_{urea}·V) in control (saline) and Cd-treated rats. Data represent the mean±SE of 7 measurements in each group.

creted was always much smaller than the sum of Na^+ and K^+ excreted, indicating that a significant fraction of Na^+ or K^+ was excreted in combination with anions other than Cl^-.

Table 2 compares plasma values of creatinine(P_{Cr}), Na^+ (P_{Na}), K^+(P_K) and osmolality(P_{osm}) in saline-and Cd-treated animals obtained on the 16th day of treatment. In all cases, the value was not significantly different between the two groups. Thus, the Cd-induced changes in solute excretion, as described above, were due to alterations in renal tubular transport processes.

Table 3 summarizes change in Cd excretion in Cd-treated animals. The Cd excretion was not significantly elevated after 4 days of Cd-treatment, but it increased drastically after 16 days of treatment. During 20 days of recovery period, the excretion was significantly declined.

DISCUSSION

Although chronic exposure to cadmium induces abnormalities in various organs in the body, the kidney is believed to be the most critical organ[37]. The events between Cd-ingestion and development of renal dysfunction can be summarized as follows[13,24] : The Cd ingested is bound to metallothionein, a

Table 3. Urinary Excretion of Cadmium in Cd-treated Animals

	$U_{cd} \cdot V$(μg/kg · day)
Pretreatment	0.23±0.14
Cd, 4 days	0.58±0.22
Cd, 16 days	163.63±41.98
Recovery, 20 days	59.30±20.95

Values are mean ± SE of 7 animals.

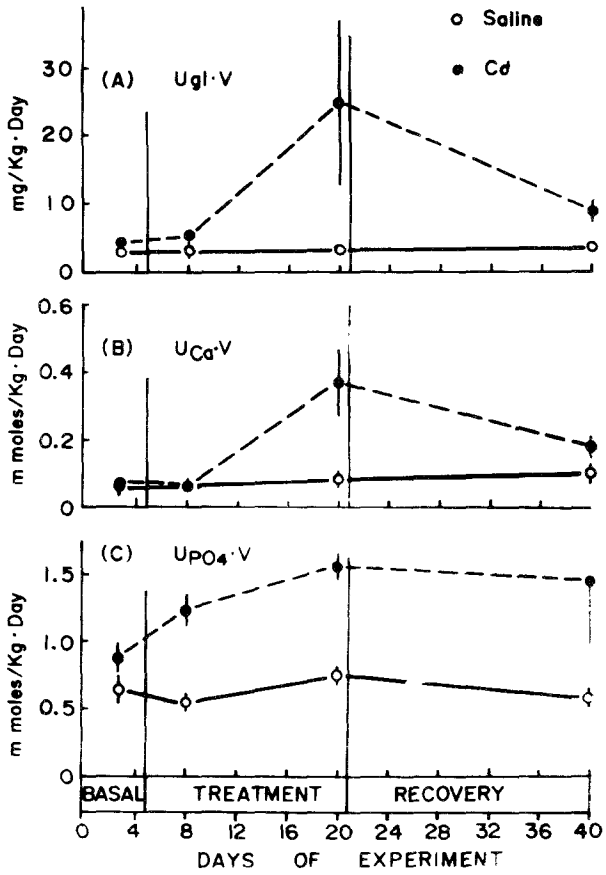

Fig. 3. Changes in urinary excretions of glucose ($U_{gl} \cdot V$), calcium($U_{Ca} \cdot V$), and phosphate ($U_{PO4} \cdot V$) in control saline and Cd-treated rats. Data represent the mean±SE of 6-7 measurements in each group.

Cd-binding low molecular weight protein[9,11]. The Cd-metallothionein complex is transported via blood to the kidney, filtered through glomeruli, and absorbed into proximal tubular cells by endocytosis[12,45]. After entering lysosomes, the complex is catabolized and the free Cd released is bound to newly synthesized metallothionein in the tubular cell. It is proposed that kidney damage is prevented until a stage is reached at which the kidney can no longer produce enough metallothionein, and at this time the non-metallothionein-bound Cd ions become very toxic[33,38]. The time to reach this point appears to depend on the form of Cd given and dosage schedule.

In the present study, subcutaneous injections of $CdCl_2$ to rats at a daily dose of 2 mg Cd/kg for 12-16 days resulted in a marked increase in urinary Cd-excretion, and produced various renal functional changes, characteristic of chronic Cd-intoxication. The animal developed a marked polyuria (Fig. 1A), proteinuria (Fig. 2B), glycosuria (Fig. 3A), phophaturia (Fig 3C), hypercalciuria (Fig. 3B), and reduced urinary concentrating ability (Fig, 1B) as observed in other Cd-intoxicated animals[1,2,3,4,15,16,17,20,21,34,35,36,39,41,43,44,48]. Glomerular filtration rate, as judged by creatinine excretion, was normal (Fig. 1C) as were plasma concentrations of creatinine, Na^+,K^+,

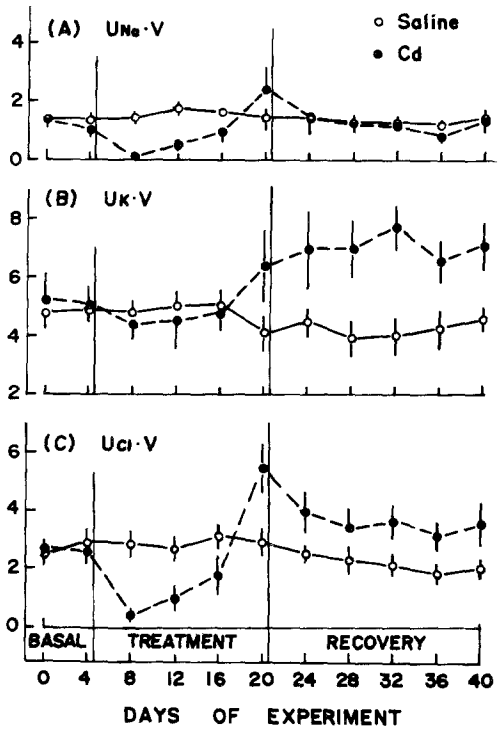

Fig. 4. Changes in urinary excretions of sodium (U_{Na}·V), potassium (U_K·V), and chloride (U_{Cl}·V) in control (saline) and Cd-treated rats. Data represent the mean±SE of 7 measurements in each group.

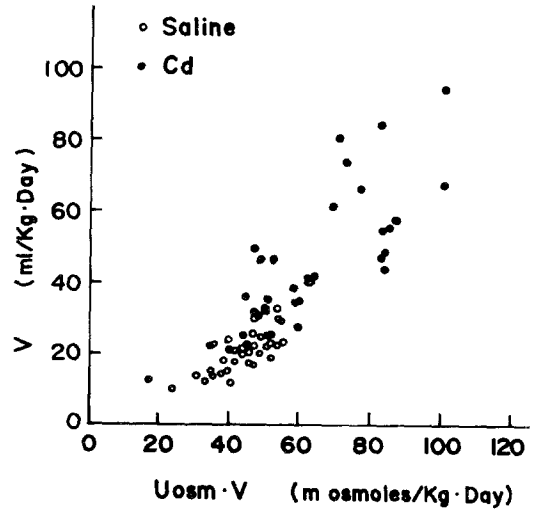

Fig. 5. Variation of urine flow (V) as a function of urinary excretion of total osmotic substances (U_{osm}·V) in control (saline) and Cd-treated rats.

and osmolality (Table 2). Injections of a higher dose of $CdCl_2$ resulted in a rather high mortality. We, therefore, concluded that subcutaneous administration of 2 mg Cd/kg·day for about two weeks induces typical renal functional defects and consequently produces a model suitable for study of renal tubular functions in chronic Cd-intoxication.

The polyuria developed in Cd-treated animals was accompanied by a marked increase in excretion of total osmotic substances (Fig. 2A), suggesting that the nature of the polyuria was an osmotic diuresis. Indeed, as depicted in Fig. 5 which relates individual urine flow and osmotic excretion at various stage of Cd treatment, the increase in urine output appeared to be directly proportional to the increase in osmotic excretion. The value of $T^c_{H_2O}$ calculated for the animals sacrificed on the 16th day of Cd treatment (−119±21 ml/kg·day, N=5) was exactly comparable to the control value(−119±19, N=4). It is, therefore, apparent that the increase in urine flow and the decrease in urine osmolality in Cd-intoxicated animals did not involve alterations of ADH-dependent water reabsorption, but was associated with an osmotic diuresis. Major increase in solute excretion was observed for protein, urea, glucose, phosphate, and Ca^{++} (Figs. 2 and 3). Excretion of K^+ and Cl^- were also increased, but the extent was small (Fig. 4). Although we have not determined amino acid excretion, it is believed to be also increased, as observed in numerous other studies[1,3,15,16,21,36].

The mechanisms whereby solute excretion is increased in the Cd-treated animal are not entirely identified. It is known that filtered glucose, amino acids, and phosphate are mostly reabsorbed in the proximal tubule via co-transport system with Na^+ [5,8,50]. Thus, increased urinary excretion of these substances would indicate that Na^+ transport in the proximal tubule is inhibited. However, urinary excretion of Na^+ was not significantly elevated even after 16 days of Cd treatment (Fig. 4A). Whether these indicate that Cd treatment altered the Na^+-independent tansport of the above substances or alternatively, Na^+ transport was actually reduced at the proxiaml tubule, but it increased at the distal tubule, is difficult to ascertain. There are, however, some indirect lines of evidence which favour the latter possibility. In the present study, Na-K-ATPase activity of renal cortical microsomes was significantly reduced in rats treated with Cd for 16 days[22] Although renal cortex contains distal tubules as well as proximal tubules, total mass of the proximal segements will be much greater than that of the distal segments. Thus, a marked reduction in the enzyme activity, as observed in the present study, would imply that active sodium pump in the proximal tubules is inhibited. Nishiyama and Nakamura[31] have observed that chronic treatments of rats with Cd(2 mg/kg·day) resulted in a marked increase in aldosterone secretion. If a similar change occurred in the Cd-treated rats in the present study, Na^+ reabsorption in their distal tubules could be increased. Direct analysis of Na^+ transport in various segments of renal tubule would verify this point.

The Cd-induced proteinuria, as observed in the present study, has been repeatedly documented in human patients[41,49] and in animals[4,35,39]. Quantitative protein analysis by several investigators[35,41] showed a high excretion of low molecular weight proteins, indicating that main cause of the proteinuria is decrease in tubular reabsorption. The mechanism of this change has not been fully elucidated. It is known that the process of tubular reabsorption of filtered low molecular weight proteins, such as enzymes, immunoproteins, and peptide hormones, is mainly confined to the proximal tubule and that the process is saturable, revealing a high Tm[28,29]. Studies using stop-flow[26], microperfusion[7], isolated tubule[6], or isolated perfused kidney[28] technics indicated that protein molecules are accumulated in the proximal tubular cell and the accumulation depends entirely on the transport from the luminal side and not from the peritubular side[28]. The process of luminal uptake appears to be unaffected by a change in Na^+ reabsorption, but it is markedly inhibited by cytochalasin B (which impairs endocytosis in other cells) at doses not affecting Na^+, K^+, goucose, and water reabsorption[18]. A number of studies[28,29] indicated that small proteins absorbed into renal cells are catabolized and released to peritubular side as amino acids. These facts may suggest that under normal conditions, low molecular weight proteins filtered through glomeruli are first accumulated into proximal tubular cells by endocytotic process in the brush-border membrane, then catabolized within the cell, and the catabolic products are transferred to the circulation across the basolateral membrane. Thus, impairments of endocytosis would lead to massive loss of proteins to the urine. We, therefore, speculate that the Cd-induced proteinuria is a consequence of alterations in en-

docytotic mechanisms in the proximal tubular brush-border membrane.

A marked increase of Ca^{++} excretion in Cd-treated rats in the present study (Fig. 3B) and hypercalciuria observed in Cd-exposed humans[44] indicate that proximal tubular transport of Ca^{++} is significantly impaired by Cd. Micropuncture studies in rats[25] have shown that reabsorption of the bulk of filtered Ca^{++} closely parallels that of Na^+, occurring predominantly in the proximal tubule. The isolated tubule study by Ng et al.[30] has suggested that the mechanism of Ca^{++} transport in the proximal convoluted tubule, which accounts for more that 60% of the total kidney Ca^{++} reabsorption under normal conditions[25], is mainly passive. Thus, one may expect that this portion of Ca^{++} transport would also be reduced when tranport of other solutes, such as proteins, glucose, amino acids phosphate, and possibly Na^+, is decreased by Cd exposure. Whether the active Ca^{++} transport mechanism in the proximal straight tubule[42] and PTH- and calcitonin-dependent Ca^{++} transport mechanisms in the thick ascending limb of Henle and distal convoluted tubule[46,47] are also affected by Cd treatment is not certain. However, in view of a marked increase in Ca^{++} excretion(Fig. 3B), we presume that the main site of reduced Ca^{++} transport in the Cd-treated animal is the proximal convoluted tubule.

Elevation of urea (Fig. 2C) and K^+(Fig. 4B) excretions in Cd-treated animals may be a consequence of impaired solute transport in the proximal tubule. Rejection of solutes in the proximal tubule would retard water reabsorption, and hence increase fluid delivery to the distal nephron. This would increase the driving force for K^+ secretion in the distal tubule[14] and decrease the driving force for urea reabsorption in the collecting duct[51]; consequently, urinary excretion of these substances would be increased. For K^+ excretion, there may be additional reasons to increase after Cd exposure. These include an enhanced excretion of impermeable anions, such as phosphate (Fig. 3C), and an increased aldosterone secretion, as observed by others[31], both of which facilitate K^+ secretion in the distal tubule[14].

One final point deserves comments is a drastic reduction in Na^+ excretion during the early phase of Cd-exposure. As depicted in Fig. 4A, Na^+ excretion became almost negligible after 4 days of Cd-treatment; then it returned to the control level during the subsequent period of Cd-treatment. Again, the mechanism of this response is not precisely understood, but we presume that the pattern represents difference in time course between the Cd-induced alterations of proximal and distal tubular Na^+ transort processes. In other words, a short-term exposure to Cd resulted in an increased Na^+ reabsorption from the distal nephron without altering proximal tubular Na^+ transport, but a long-term exposure impaired the proximal tubular transport as well; thus, the urinary excretion of Na^+ remained low until the proximal tubular impairment progressed to such and extent that the effect on the distal tubular transport process was completely masked. The early reduction of Na^+ excretion after Cd-exposure has also been obserbed in other experiments. Perry et al.[40] reported that the amount of urinary Na^+ in rats was significantly decreased 1~2 days after Cd-injection. Similar observation was made by Nishiyama and Nakamura[31], and they suggested that this may be due to in-

creased secretion of aldosterone. In the latter study, plasma aldosterone level of rats was markedly elevated during 7 days of Cd-treatment. As described elsewhere[22], the Na-K-ATPase activity of renal cortical microsomes in the present experiment appeared to be not significantly altered during the early phase of Cd treatment. For instance, the enzyme activity (V_{max}, 81.9 mmoles Pi/g protein per hr; Km, 0.71 mM) measured after 4 days of Cd-treatment, at which time urinary Na^+ excretion declined to the lowest level, was exactly comparable to that observed in control animals(V_{max}, 82.0; Km, 0.76). These data may suggest that the antinatriuretic effect of Cd treatment does not involve activation of proximal tubular sodium pump, but is associated with an increase in Na^+ reabsorption in the distal tubule, perhaps by the aldosterone activation. If this were true, then antinatriuresis will persist as long as proximal tubular transport mechanisms remain unaltered. However, as discussed above, Na^+ reabsorption in the proximal tubule seems to be inhibited in long-term Cd exposure (more than 2 weeks of Cd treatment in the present study). Thus, it seems that the urinary excretion of Na^+ in chronic Cd exposure is determined by relative changes in proximal and distal tubular Na^+ transport functions. Certainly, much more investigations are needed to prove this notion with confidence.

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