Metabolic Acidosis and Urinary Acidification Defect during the Course of Hemorrhagic Fever with Renal Syndrome

To evaluate urinary acidification defect and its contribution to metabolic acidosis (MA) during hemorrhagic fever with renal syndrome (HFRS), we serially analyzed acid-base balance and urinary acidification indices in 10 HFRS patients. Data of the patients were compared with those of 8 normal volunteers (NC). MA was observed in 6 of 8 patients in the oliguric phase, 5 of 7 in the early diuretic phase, 8 of 10 in the late diuretic phase and 2 of 9 in the convalescent phase. HFRS patients with MA had a higher plasma anion gap in the oliguric and early diuretic phases than NC and a higher plasma Cl/Na ratio in the late diuretic phase than NC. As compared with acid-loaded NC, HFRS patients had a higher urine pH in the oliguric, early diuretic and late diuretic phases, a higher urine anion gap (UAG) in the oliguric and early diuretic phases and a lower urinary NH_4^+ excretory rate in the oliguric, early diuretic and late diuretic phases. Overt distal acidification defect was observed in 6 of 8 patients in the oliguric phase, 3 of 7 in the early diuretic phase, 5 of 10 in the late diuretic phase and none of 9 in the convalescent phase. None of the convalescent patients had latent acidification defect. In conclusion, urinary acidification defect is marked in the oliguric and diuretic phases of severe HFRS and may play a role in the development of a high anion gap (AG) metabolic acidosis in the earlier phase and hyperchloremic MA in the later phase, but rapidly recovers in the convalescent phase.

Key Words : *Hemorrhagic fever with renal syndrome; Metabolic acidosis; Urinary acidification defect*

Jin Suk Han, Gheun-Ho Kim,* Jaeho Earm,†
Kwon Wook Joo, Wooseong Huh,‡ Un Sil Jeon,
Curie Ahn, Suhnggwon Kim, Jung Sang Lee

Department of Internal Medicine, Seoul National
University College of Medicine, Seoul, Korea
* Department of Internal Medicine, Hallym
University College of Medicine, Seoul, Korea
† Department of Internal Medicine, Chungbuk
National University College of Medicine, Chongju,
Korea
‡ Department of Internal Medicine, Samsung
Medical Center, Seoul, Korea

Address for correspondence
Jin Suk Han, M.D., Ph.D.
Associate Professor of Internal Medicine, Seoul
National University College of Medicine,
28 Yongon-dong, Chongno-gu, Seoul 110-744,
Korea
Tel: +82.2-760-2392, Fax: +82.2-3673-2392
E-mail : jshan@plaza.snu.ac.kr

INTRODUCTION

The clinical characteristics of hemorrhagic fever with renal syndrome (HFRS) are fever, hemorrhagic manifestations and variable degrees of renal insufficiency (1). The etiologic agents of HFRS are Hantaan, Seoul and Puumala viruses of the genus *Hantavirus*, family Bunyaviridae (2) and recently other hantaviruses, still poorly characterized, have been isolated from patients in the United States (3) and Europe (4). In Korea, Hantaan and Seoul viruses cause HFRS (5), and more than 500 serologically confirmed cases occur annually (6).

HFRS has a complex course during the illness with five recognizable phases: febrile, hypotensive, oliguric, diuretic and convalescent (7). Acute renal failure is one of the important clinical presentations in HFRS (8), and patients from oliguric to the convalescent phase follow a course similar to that of acute tubular necrosis (5). Impaired tubular function is reflected in a loss of urinary concen-

trating ability and decreased sodium reabsorption together with the increased excretion of urinary β_2-microglobulin (4).

Metabolic acidosis in HFRS has been described as only a minor biochemical abnormality accompanying acute renal failure for decades (9). On the other hand, residual tubular defects of urinary acidification and ammonia production had been reported in Korean hemorrhagic fever (10) and nephropathia epidemica (11). The present study was undertaken to evaluate urinary acidification defect and its contribution to the development of metabolic acidosis during the course of HFRS.

PATIENTS AND METHODS

Ten serologically confirmed HFRS patients were studied during the admission to Seoul National University Hospital, a tertiary referral hospital in Korea. The pa-

tients showed a typical course of acute renal failure; eight out of the ten patients presented with oliguria, and the others were nonoliguric but azotemic at admission. All of the oliguric patients and one of the nonoliguric patients were managed with intermittent hemodialysis during their initial hospital stay, and predialysis samples were obtained. None of the patients received oral or intravenous alkali replacement.

We divided the clinical course of acute renal failure in HFRS into oliguric, early diuretic, late diuretic and convalescent phases. The oliguric phase was characterized by a daily urine volume less than 500 mL. After the diuretic phase had started, azotemia usually continued to increase for the first few days of diuresis (early diuretic phase) and then decreased (late diuretic phase). The convalescent phase was defined as the time when azotemia is resolved (serum creatinine ≤ 1.4 mg/dL). Arterial blood gas analysis, serum electrolytes and creatinine, urine pH and urinary electrolytes and ammonium were measured serially in the oliguric, early or late diuretic and convalescent phases in all the patients except for one who was discharged in the late diuretic phase. During the convalescent phase, the rapid NH_4Cl loading test was done to verify any residual latent defect in the distal urinary acidification (12). An oral load of 0.2 g/kg of NH_4Cl was made to ensure arterial bicarbonate concentration below 18 mmol/L, and urine pH was measured hourly for eight hours.

For comparison with the patients in the acute stage, eight normal volunteers were also studied with acid loading using ammonium chloride (NH_4Cl) in a daily oral dose of 0.1 g per kilogram of body weight for 3 successive days. On the fourth day arterial blood and urine samples were obtained for determination of arterial blood gas analysis, urine pH, urinary electrolytes and ammonium excretion.

Arterial blood gas analysis was performed with a blood gas analyzer (NOVA, Waltham, MA), and urine pH was measured by a pH meter (Beckman, Fullerton, CA). Serum and urine electrolytes and creatinine were measured on an autoanalyzer (Beckman, Fullerton, CA), and urinary ammonium concentration was determined by Conway's microdiffusion method (13). When urine samples were obtained for pH determination, they were collected under mineral oil and all subjects were instructed to void in the upright position (14). The plasma anion gap was equal to the difference between sodium and the sum of chloride and bicarbonate in the plasma samples (15), and the urine anion gap was calculated from sodium plus potassium minus chloride values (in mmol/L) in the first morning urine samples (16).

Data are described as means ± SE. Statistical comparisons among the phases and between patients and normal controls were performed with Mann-Whitney U test. Correlations between variables of interest were analyzed by linear regression. Differences were considered significant at p<0.05.

RESULTS

Characteristics of metabolic acidosis in HFRS

In HFRS patients, serum creatinine concentrations were 10.3 ± 1.3, 12.6 ± 2.1, 4.3 ± 0.9 and 1.3 ± 0.1 mg/dL in the oliguric, early diuretic, late diuretic and convalescent phases, respectively. The data of arterial blood gas in each phase of HFRS patients are shown in Table 1. Arterial blood pH in the oliguric, early diuretic, late diuretic and convalescent phases were 7.35 ± 0.02, 7.36 ± 0.04, 7.38 ± 0.02 and 7.41 ± 0.02, respectively. Plasma bicarbonate concentrations were 15.1 ± 1.0, 16.1 ± 1.4, 18.9 ± 1.1 and 24.4 ± 0.9 mmol/L in the oliguric, early diuretic, late diuretic and convalescent phases, respectively.

The individual data of arterial blood gas were analyzed, and metabolic acidosis was defined as arterial blood pH <7.4 and plasma bicarbonate concentration <24 mmol/L. Metabolic acidosis was observed in six out of eight patients in the oliguric phase, five out of seven in the early diuretic phase, eight out of ten in the late diuretic phase and two out of nine patients in the convalescent phase. Plasma anion gaps in the patients with metabolic acidosis were 14.0 ± 1.7, 16.6 ± 1.9, 8.4 ± 0.9 and 7.5 ± 2.5 mmol/L in the oliguric, early diuretic, late diuretic and convalescent phases, respectively. As compared with the baseline values of eight normal volunteers (6.9 ± 1.3 mmol/L), HFRS patients had a high plasma anion gap in the oliguric and early diuretic phases (p<0.05). Plasma Cl/Na ratios in the patients with metabolic acidosis were 0.778 ± 0.011, 0.760 ± 0.012, 0.798 ± 0.008 and 0.769 ± 0.003 in the oliguric, early diuretic, late diuretic and convalescent phases, respectively. As compared with the baseline values of normal controls (0.763 ± 0.006), HFRS patients had higher plasma Cl/Na ratio in the late diuretic phase (p<0.05). Fig. 1 shows the changing pattern of plasma

Table 1. Data of arterial blood gas analysis in each phase of HFRS

Phase	Arterial pH	$PaCO_2$ (mmHg)	$[HCO_3^-]$ (mmol/L)
Oliguric (n=8)	7.35 ± 0.02	27.5 ± 1.1	15.1 ± 1.0
Early diuretic (n=7)	7.36 ± 0.04	28.7 ± 1.1	16.1 ± 1.4
Late diuretic (n=10)	7.38 ± 0.02	32.9 ± 1.1	18.9 ± 1.1
Convalescent (n=9)	7.41 ± 0.02	39.8 ± 1.5	24.4 ± 0.9

Values are means ± SEM.
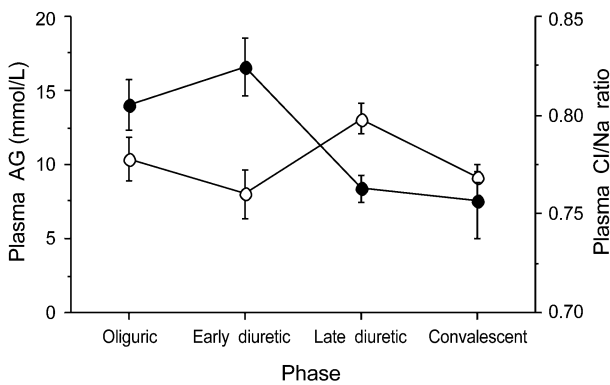HFRS, hemorrhagic fever with renal syndrome.

Fig. 1. Changing pattern of plasma anion gap (AG) and chloride-to-sodium (Cl/Na) ratio in the patients with metabolic acidosis during the course of hemorrhagic fever with renal syndrome (closed circle, plasma AG; open circle, plasma Cl/Na ratio).

anion gap and Cl/Na ratio in the patients with metabolic acidosis during the course of HFRS.

Throughout the course of HFRS, creatinine clearance correlated with arterial blood pH and with plasma bicarbonate concentration (Fig. 2). The lower creatinine clearance was, the lower arterial blood pH ($r=0.37$, $p<0.05$) and plasma bicarbonate concentration were ($r=0.71$, $p<0.01$).

Measures of urinary acidification during the course of HFRS

Table 2 shows the indices of urinary acidification in HFRS patients and NH_4Cl-loaded normal controls. Urine pH in the HFRS patients appeared to have no changes throughout the course: 6.52 ± 0.32, 5.96 ± 0.20, 5.95 ± 0.27 and 6.11 ± 0.18 in the oliguric, early diuretic, late diuretic and convalescent phases, respectively. However,

Table 2. Measures of urinary acidification in HFRS patients

Phase	Urine pH	Urine NH_4^+ (mmol/day)	Urine AG (mmol/L)
Oliguric (n=8)	$6.52\pm0.32^*$	$2.9\pm1.3^\dagger$	$46.7\pm5.9^\dagger$
Early diuretic (n=7)	$5.96\pm0.20^*$	$8.6\pm1.6^\dagger$	$28.3\pm2.6^\dagger$
Late diuretic (n=10)	$5.95\pm0.27^*$	$22.9\pm2.5^\dagger$	6.9 ± 2.3
Convalescent (n=9)	6.11 ± 0.18	18.1 ± 2.9	22.2 ± 5.5
Acid-loaded NC (n=8)	5.09 ± 0.07	52.6 ± 3.7	-16.2 ± 5.5

Values are means $\pm$ SEM.
HFRS. hemorrhagic fever with renal syndrome; Urine AG, urine anion gap; Acid-loaded NC, NH_4Cl-loaded normal controls.
$*p<0.05$ compared to acid-loaded NC by Mann-Whitney U test.
$^\dagger p<0.01$ compared to acid-loaded NC by Mann-Whitney U test. The values of convalescent phase are not compared to those of acid-loaded NC, because acidemia recovers in the convalescent phase of HFRS (Table 1).

when only the patients with metabolic acidosis were considered, urine pH showed a tendency to decrease ($r=-0.71$, $p<0.01$) as the phases progressed (Fig. 3). Urinary ammonium excretory rates in the oliguric, early diuretic, late diuretic and convalescent phases were 2.9 ± 1.3, 8.6 ± 1.6, 22.9 ± 2.5 and 18.1 ± 2.9 mmol/day, respectively. Urine anion gaps in the oliguric, early diuretic, late diuretic and convalescent phases were 46.7 ± 5.9, 28.3 ± 2.6, 6.9 ± 2.3 and 22.2 ± 5.5 mmol/L, respectively. The urinary ammonium excretory rate in the oliguric phase was lower ($p<0.05$) than in the late diuretic phase, while the urine anion gap in the oliguric phase was higher ($p<0.05$) than in the late diuretic phase. When the patients in each phase were considered altogether, the urine anion gap correlated inversely ($r=-0.57$, $p<0.01$) with urinary ammonium excretory rate (Fig. 4).

In normal controls, three days of NH_4Cl administration produced overt metabolic acidosis; arterial bicarbonate concentrations decreased from 25.8 ± 0.9 to $19.6\pm$
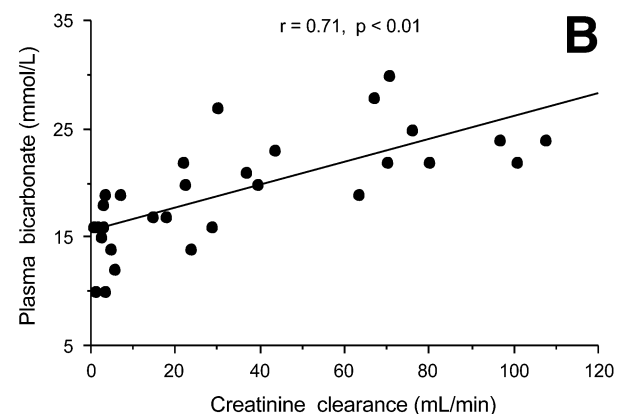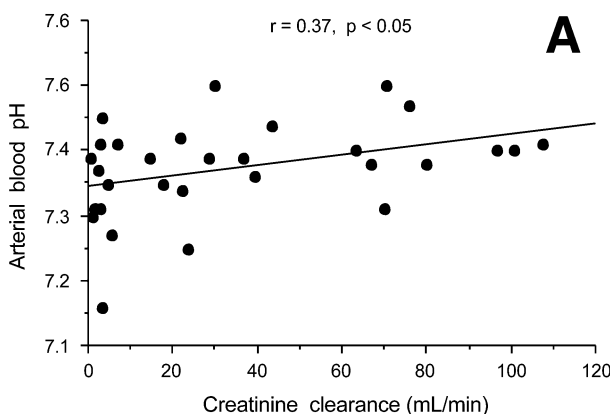

Fig. 2. Correlations between creatinine clearance and arterial blood pH (A) and between creatinine clearance and plasma bicarbonate concentration (B) in patients with HFRS.
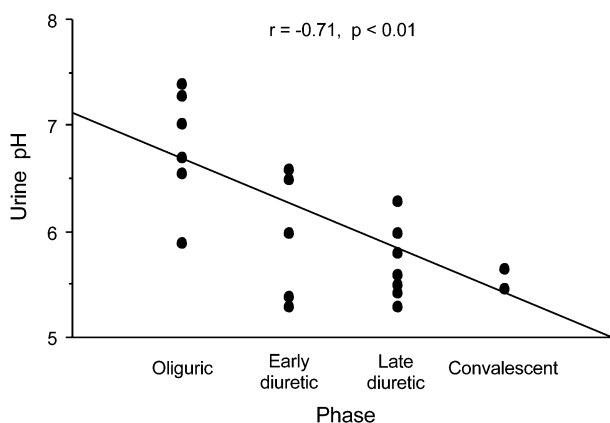
Fig. 3. Decreasing tendency of urine pH in relation to the phases of HFRS in the patients with metabolic acidosis (The association is analyzed by linear regression).
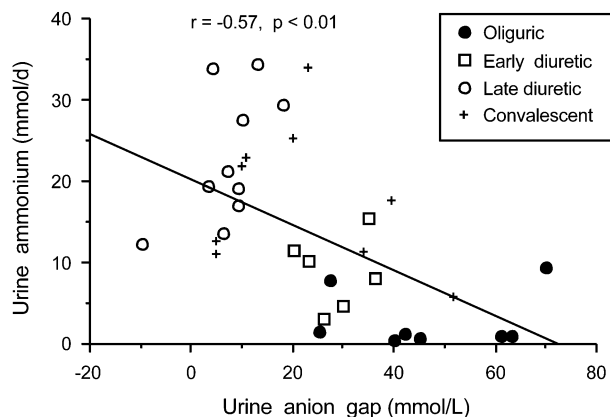

Fig. 5. The number of patients with and without overt distal acidification defect during the course of hemorrhagic fever with renal syndrome (open bars, without overt distal acidification defect; filled bars, with overt distal acidification defect).

Fig. 4. Inverse correlation between urinary ammonium excretion and urine anion gap in patients with HFRS.

Fig. 6. The result of rapid NH_4Cl loading test in HFRS patients during the convalescent phase.

0.6 mmol/L after acid loading ($p<0.01$). Urine pH, urinary ammonium excretory rate and urine anion gap in eight NH_4Cl-loaded normal controls were 5.09 ± 0.07, 52.6 ± 3.7 mmol/day and -16.2 ± 5.5 mmol/L, respectively. As compared with the NH_4Cl-loaded normal controls, urine pH of HFRS patients was higher ($p<0.05$) in the oliguric, early diuretic and late diuretic phases, urinary ammonium excretion was lower ($p<0.01$) in the oliguric, early diuretic and late diuretic phases and urine anion gap was higher ($p<0.01$) in the oliguric and early diuretic phases (Table 2).

We defined the overt distal acidification defect as a urine $pH>5.5$ in the face of metabolic acidosis and serum $[HCO_3^-] <20$ mmol/L. Six out of eight patients in the oliguric phase, three out of seven in the early diuretic phase, five out of ten in the late diuretic phase and none out of nine in the convalescent phase revealed the
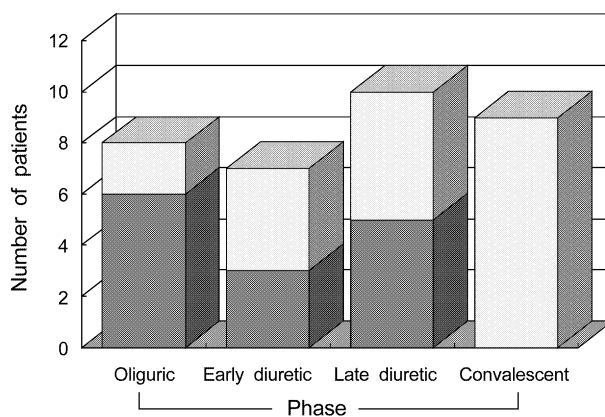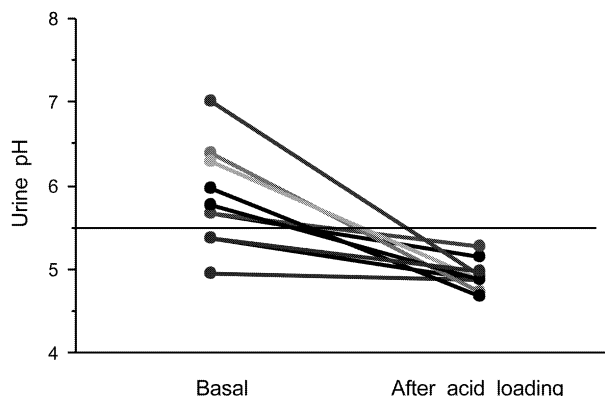
overt distal acidification defect (Fig. 5). After the NH_4Cl loading, urine pH values of all the convalescent patients were below 5.5 (Fig. 6.).

DISCUSSION

The metabolism of dietary protein yields 50-100 mmol/day of fixed non-volatile acids, which must be excreted by the kidneys to maintain acid-base homeostasis (17). Predictably, acute renal failure is often complicated by metabolic acidosis with widening of the serum anion gap (18), and acute renal failure is one of the important presenting manifestations of HFRS (8). Therefore, it is expected that metabolic acidosis frequently accompanies the clinical course of HFRS. Surprisingly, however, there have been only a few reports describing or studying meta-

bolic acidosis in HFRS (9, 19-22).

We found that metabolic acidosis was prevailing from oliguric to the diuretic phase in HFRS. Most of the patients in the early phases had metabolic acidosis, and the incidence of the patients with metabolic acidosis showed a tendency to decrease from oliguric to the convalescent phase. The mean arterial blood pH in the oliguric and late diuretic phases were 7.35 and 7.38, respectively, and the mean arterial plasma bicarbonate concentration in the oliguric and late diuretic phases were 15.1 and 18.9, respectively. These data are contrary to a previous comprehensive study reported by Hunter et al.(21), in which, although most patients in the oliguric phase had marked renal failure and retention of anions such as inorganic phosphate, sulfate and organic acids, carbon dioxide combining power decreased only slightly, with minimum values ranging between 20.6 and 24.0 mmol/L. The explanation for this was not clear(9).

Other reports stated variable results on the incidence and degree of metabolic acidosis in HFRS. Powell reported a moderate decrease of blood carbon dioxide combining power between 30 and 40 vol. % in about 25% of the patients (19). Barbero et al. reported a low carbon dioxide combining power in 68% of the patients between the 5th and 12th days of the disease (20). Kang et al. observed metabolic acidosis in twelve of twenty-eight patients during the oliguric phase (22). These discrepancies could be attributed to the differences in the characteristics of the subjects such as the severity of illness, presence of other associated complications including vomiting and the laboratory methods used in the studies. The patients enrolled in this study might have been more seriously ill than those included in previous reports, and during the period of admission our patients seldom exhibited nausea and vomiting.

Consistent with the new reference range for the plasma anion gap which shifted downward, primarily because of an upward shift in chloride values using modern electrode technology (23), the baseline plasma anion gap and Cl/Na ratio in our normal controls were 6.9 ± 1.3 mmol/L and 0.763 ± 0.006, respectively. Our HFRS patients with metabolic acidosis had a higher plasma anion gap in the oliguric and early diuretic phases, and higher plasma Cl/Na ratio in the late diuretic phase than NH_4Cl-loaded normal controls. Thus, the patients seemed to have high anion gap metabolic acidosis in the early phases and normal anion gap hyperchloremic metabolic acidosis in the late phases.

Throughout the course of HFRS, contrary to the result of Kang et al. (22), creatinine clearance correlated with arterial blood pH and with plasma bicarbonate concentration. The lower creatinine clearance was, the lower arterial blood pH and plasma bicarbonate concentration

were. This suggests that the metabolic acidosis in HFRS may be similar to that frequently associated with acute renal failure due to various causes. As the pattern of serum electrolytes and acid-base composition depends on the stages of chronic renal failure (24), a high anion gap metabolic acidosis may be present predominantly in the oliguric and early diuretic phases of HFRS and an element of hyperchloremia may be prevailing in the late diuretic phase. Thus, it is suggested that the frequently observed metabolic acidosis during the acute stage of HFRS may be originated mainly from the retention of anions associated with azotemia in the early phases and from the impaired urinary acidification associated with residual renal tubular damage in the late phases.

While urine pH in HFRS patients with metabolic acidosis showed a tendency to decrease as the phases progressed, distal acidification defect could be inferred to be present during the acute stage of HFRS. From oliguric to the diuretic phase the mean urine pH was much higher than 5.5 in the face of low arterial blood pH and bicarbonate concentration, and urinary ammonium excretory rates were lower compared with those of NH_4Cl-loaded normal controls. We also observed the decreasing pattern of urine anion gap, a useful index of distal urinary acidification in hyperchloremic metabolic acidosis (25), from oliguric to the diuretic phase. As in the cases of chronic renal failure (26, 27), an inverse correlation between urinary ammonium excretion and urine anion gap was demonstrated throughout the course of HFRS.

Recovery from acute renal failure is often incomplete, as evidenced by the presence of residual defects in both renal structure and function (28). A case of residual tubular defect in urinary acidification and ammonia synthesis, presenting with mild hyperchloremic metabolic acidosis without impairment of glomerular filtration, was found two years after the acute phase of severe HFRS (10). The nephropathy induced by Puumala virus is much more benign than that caused by Hantaan virus and Seoul virus, the prototype of which is Korean hemorrhagic fever (29). Therefore, clinical recovery would be more complete in patients with nephropathia epidemica than in those with more severe HFRS. Mild renal tubular dysfunctions, however, were reported in several cases of nephropathia epidemica, and renal biopsy revealed nonspecific mild changes (11). From our individual data, we observed the overt distal acidification defect in about half of the patients from oliguric to the diuretic phase. In convalescent phase, however, none of our patients had latent distal acidification defect.

In summary, the urinary acidification defect in HFRS was marked in the oliguric and diuretic phases. It might play a part in the development of a high anion gap metabolic acidosis associated with severe azotemia in the early

phases and hyperchloremic metabolic acidosis associated with mild azotemia in the late phase. The urinary acidification defect was improved rapidly in all the subjects, and we could not find any residual urinary acidification defect in patients in their convalescent phase of HFRS. The residual urinary acidification defect seems to be very rare after the acute stage of HFRS.

Acknowledgment

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