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Urine Composition in Rats with Adenine-Induced Renal Failure during Treatment with Rhubarb Extract

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The effect of rhubarb extract on the urinary constituents was examined in rats with renal failure induced by adenine. Administration of the rhubarb extract markedly increased the urinary excretion of both urea and creatinine, indicating an improvement of renal clearance in the uremic state. Furthermore, a number of significant differences in the amino acid levels in the urine were observed. Among the essential amino acids, the urinary outputs of threonine, phenylalanine, leucine, and methionine were remarkably lower in the rhubarb extract-treated group than in the control group. Outputs of inessential amino acids (alanine, glycine, glutamic acid, serine, aspartic acid, tyrosine, and cysteine) were also strikingly reduced after the treatment. In addition, the amount of urinary Ca was significantly reduced in the rhubarb extract-treated rats, while urinary inorganic phosphate was significantly elevated. A marked decrease of 2,8-dihydroxyadenine excretion in the urine was noticed. However, no changes were seen in the urinary excretions of protein, glucose, Na, and K throughout the experimental period.

Keywords——Rhei Rhizoma; renal failure; urinary urea; urinary creatinine; urinary amino acid; urinary Ca; urinary P; 2,8-dihydroxyadenine

In the preceding study, the effect of the extract from Rhei Rhizoma was examined in rats with renal failure induced by an adenine diet.[1] On treatment with the rhubarb extract, the levels of urea nitrogen and creatinine in the serum showed a significant decrease, indicating an improvement of renal function. The urea concentrations in the liver and kidney were also decreased after the treatment. In addition, administration of the rhubarb extract to rats markedly decreased the kidney weight and 2,8-dihydroxyadenine content in the kidneys. Accordingly, it appears that the nephrotoxic state caused by tubular obstruction with 2,8-dihydroxyadenine deposits can be mitigated by treatment with rhubarb extract. The present paper describes the effect of rhubarb extract on the urinary constituents.

Materials and Methods

Animals and Diet——Male rats of the JCL: Wistar strain(Hokuriku Labour, Ltd., Toyama, Japan), initially weighing 90—100 g, were placed in a metabolic chamber under a conventional lighting regimen with a dark-night period. The animals were fed on commercial feed (CLEA Japan Inc., Tokyo, type CE-2) for one week after arrival. Then they were fed *ad libitum* on an 18% casein diet containing 0.75% adenine for 30 d. The 18% casein diet had the following composition (in 100 g): casein 18 g, α-cornstarch 57.9 g, sucrose 15 g, soybean oil 2 g, salt mixture[2] 4 g, vitamin mixture[2] 1 g, cellulose powder 2 g, and choline chloride 0.1 g. To this diet, adenine was added at the level of 0.75 g/100 g of diet. During the feeding period, the extract from Rhei Rhizoma (5 mg) was administered intraperitoneally to rats at 10 a.m. every day (rhubarb extract-treated group), while control rats were treated with an equal volume of saline (control group). The 24-h urine samples at the 6th, 12th, 18th, 24th, and 30th d of the feeding period were each collected in a 50 ml Erlenmeyer flask.

Extraction of Rhei Rhizoma——Roots of *Rheum officinale* BAILLON produced in China were finely powdered and extracted at 100 °C with water, as previously described.[3] The filtrate was concentrated under reduced pressure to obtain a brown residue.

Analytical Methods——All reagents were commercial products of the highest grade available. a) Urea was determined by the method of Archibald.[4] b) Creatinine was determined by using a commercial reagent ("Creatinine-Test Wako," obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan) based on the Folin–Wu method.[5] c) Protein was determined by the method of Lowry *et al.*,[6] with bovine serum albumin as a standard. d) Glucose was determined by the method of Momose *et al.*[7] e) Sodium and potassium were determined with an electrolyte measurement apparatus (AHS/Japan Corporation) based on the ion electrode method. f) Calcium was determined by using a commercial reagent ("Calcium C-Test Wako," obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan) based on the orthocresol–phthalein complex compound method.[8] g) Inorganic phosphate was determined by using a commercial reagent ("Phosphor B-Test Wako," obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan) based on the molybdenum blue method.[9] h) Free amino acid was determined with a Hitachi 835 type high-speed amino acid analyzer. Before this determination, the urine was deproteinized by adding 3 volumes of 1% sulfosalicylic acid.

Extraction of 2,8-Dihydroxyadenine——2,8-Dihydroxyadenine deposits was extracted according to the method of Bendich *et al.*[10] The preparation had ultraviolet (UV), infrared (IR) (KBr), and mass spectra (MS) [*m/z*: 167 (M^+, $C_5H_5N_5O_2$)] similar to those of 2,8-dihydroxyadenine reported previously.[11]

Statistics——Significance of differences between the control and rhubarb extract-treated groups was tested by the use of Student's *t*-test.

Results

Urine Volume

Table I shows the urine volume of the rhubarb extract-treated and control rats fed on a 0.75% adenine diet. The feeding of adenine diet produced a significant increase to 23.0—27.4 ml/d, while the urine volume in rats fed on a 18% casein diet was about 14.0 ml/d during the feeding period (data not shown). Adenine administered orally caused polyuria. Under these dietary conditions, the urine volume of the rhubarb extract-treated group was decreased by about 5—13% at the 6—18th d as compared with the control value, but there was no statistically significant difference between the control and rhubarb extract-treated groups during the test period.

Specific Gravity

The specific gravity of urine from each group was almost the same throughout the experimental period (Table I).

TABLE I. Effect of Rhubarb Extract on Urine Volume and Urinary Specific Gravity

	Feeding period (d)	Control	Rhubarb extract
Urine volume (ml/d)	6	23.0±2.8 (100)	20.1±0.9 (87)
	12	26.5±2.1 (100)	24.0±1.3 (91)
	18	27.4±2.5 (100)	26.0±1.8 (95)
	24	26.5±1.7 (100)	27.2±2.4 (103)
	30	24.0±3.4 (100)	24.3±2.0 (101)
Specific gravity	6	1.01±0.01 (100)	1.01±0.01 (100)
	12	1.01±0.01 (100)	1.01±0.01 (100)
	18	1.01±0.01 (100)	1.01±0.01 (100)
	24	1.01±0.01 (100)	1.01±0.01 (100)
	30	1.01±0.01 (100)	1.01±0.01 (100)

Values are means±S.E. of 6 rats.
Figures in parentheses are percentages of the control value.

TABLE II. Effect of Rhubarb Extract on Urea, Creatinine, Protein, and Glucose Levels in the Urine

	Feeding period (d)	Control	Rhubarb extract
Urea (mg/d)	6	134.0±17.7 (100)	142.5±18.1 (106)
	12	150.5± 8.2 (100)	206.9±19.0[b] (137)
	18	157.6±22.7 (100)	216.9±26.6[a] (138)
	24	149.3±18.3 (100)	183.6±24.3 (123)
	30	143.1±29.1 (100)	144.6± 6.0 (101)
Creatinine (mg/d)	6	3.02±0.05 (100)	3.16±0.22 (105)
	12	3.42±0.10 (100)	3.75±0.11[b] (110)
	18	3.65±0.26 (100)	4.48±0.34[b] (123)
	24	3.71±0.16 (100)	4.00±0.15 (108)
	30	4.13±0.27 (100)	4.18±0.30 (101)
Protein (mg/d)	6	26.1±1.7 (100)	23.9±2.3 (92)
	12	37.6±2.3 (100)	33.2±2.0 (88)
	18	38.0±5.0 (100)	39.6±2.8 (104)
	24	45.5±3.4 (100)	47.9±2.1 (105)
	30	37.0±3.2 (100)	34.3±4.4 (93)
Glucose (mg/d)	6	2.6±0.2 (100)	2.7±0.5 (104)
	12	2.8±0.3 (100)	2.6±0.2 (93)
	18	3.1±0.2 (100)	2.7±0.2 (87)
	24	2.5±0.2 (100)	3.0±0.2 (120)
	30	3.1±0.7 (100)	3.0±0.5 (97)

Values are means±S.E. of 6 rats.
Figures in parentheses are percentages of the control value.
a) Significantly different from the control value, $p<0.1$.
b) $p<0.05$.

Urea

Table II compares the amount of urinary urea of the rhubarb extract-treated and control rats fed on a 0.75% adenine diet. In the control group, there were no remarkable changes in urinary urea excretion throughout the experimental period. Repeated intraperitoneal administration of the extract from Rhei Rhizoma to rats fed the adenine diet caused a significant increase of the urea output in the urine from 206.9 mg/d at the 12th d to 216.9 mg/d at the 18th d and around 145—184 mg/d thereafter.

Creatinine

As shown in Table II, administration of the rhubarb extract to rats resulted in an increase of creatinine excretion in the urine; the excretion was about 8—23% higher at the 12—24th d in the rhubarb extract-treated group as compared with the control group. In particular, the urinary output of creatinine at the 18th d was significantly elevated.

Protein

Table II compares the protein excretion in the urine of the rhubarb extract-treated and control rats fed on a 0.75% adenine diet. In the control group, the feeding of adenine diet caused an increase of protein output until the 24th d, followed by a gradual decrease from the 24th to the 30th d. Although the renal histological changes were characterized by degeneration of tubular epithelia with dilatation of the tubular lumina, deposits of amorphous birefringent crystals in the proximal and distal tubuli, and the formation of foreign body granuloma,[11-15] the present data appeared to suggest that rats fed an adenine diet may develop glomerular

TABLE III. Effect of Rhubarb Extract on Electrolytes in the Urine

	Feeding period (d)	Control	Rhubarb extract
Na (mM/d)	6	1.16±0.10 (100)	1.06±0.12 (91)
	12	1.15±0.07 (100)	1.10±0.09 (96)
	18	1.04±0.13 (100)	0.82±0.04 (79)
	24	0.89±0.09 (100)	0.79±0.08 (89)
	30	0.76±0.11 (100)	0.83±0.10 (109)
K (mM/d)	6	0.65±0.02 (100)	0.71±0.04 (109)
	12	0.74±0.01 (100)	0.73±0.10 (99)
	18	0.71±0.06 (100)	0.63±0.04 (89)
	24	0.63±0.04 (100)	0.63±0.04 (100)
	30	0.57±0.01 (100)	0.57±0.02 (100)
Ca (mg/d)	6	5.4±0.3 (100)	5.6±0.4 (104)
	12	7.4±0.6 (100)	5.5±0.4[a] (74)
	18	6.0±0.4 (100)	5.9±0.4 (98)
	24	5.0±0.3 (100)	5.1±0.4 (102)
	30	4.7±0.5 (100)	4.8±0.5 (102)
P (mg/d)	6	9.0±0.4 (100)	10.1±0.6 (112)
	12	8.8±0.2 (100)	11.8±1.1[a] (134)
	18	8.4±0.8 (100)	12.0±1.4[a] (143)
	24	9.7±0.9 (100)	9.7±1.0 (100)
	30	8.7±0.5 (100)	8.3±0.2 (95)

Values are means±S.E. of 6 rats.
Figures in parentheses are percentages of the control value.
a) Significantly different from the control value, $p<0.05$.

disease. However, there were no appreciable differences between the control and rhubarb extract-treated groups throughout the feeding of adenine diet.

Glucose

As shown in Table II, there were no significant changes in the amount of urinary glucose of rats of the rhubarb extract-treated and control groups.

Electrolytes

Though the data are not shown, adenine-fed rats exhibited a significant increase in the amounts of urinary Na, K, and Ca, while the urinary excretion of P showed a significant decrease during the feeding period.[16] As shown in Table III, the value for urinary Ca was about 26% lower at the 12th d in the rhubarb extract-treated group as compared with the control group. However, there was no difference between the control and rhubarb extract-treated groups at the 6th, 18th, 24th, and 30th d. In addition, the amount of urinary P increased to 12.0 mg/d on average at the 18th d. On the other hand, no changes were seen in the urinary excretion of Na and K throughout the experimental period.

Free Amino Acid

The amounts of free amino acids in the urine are shown in Fig. 1. Though the urinary level of free amino acids was too low to be quantified in rats fed on 18% casein diet, the adenine-fed rats had a total amino acid level of around 26.7 μmol/24 h at the 6th d of the feeding period. The animals that continued on the adenine diet showed a significant rise in the urinary excretion of amino acids during the test period. In particular, the excretions of alanine, glycine, lysine, glutamic acid, threonine, and histidine in the urine of rats which had in-

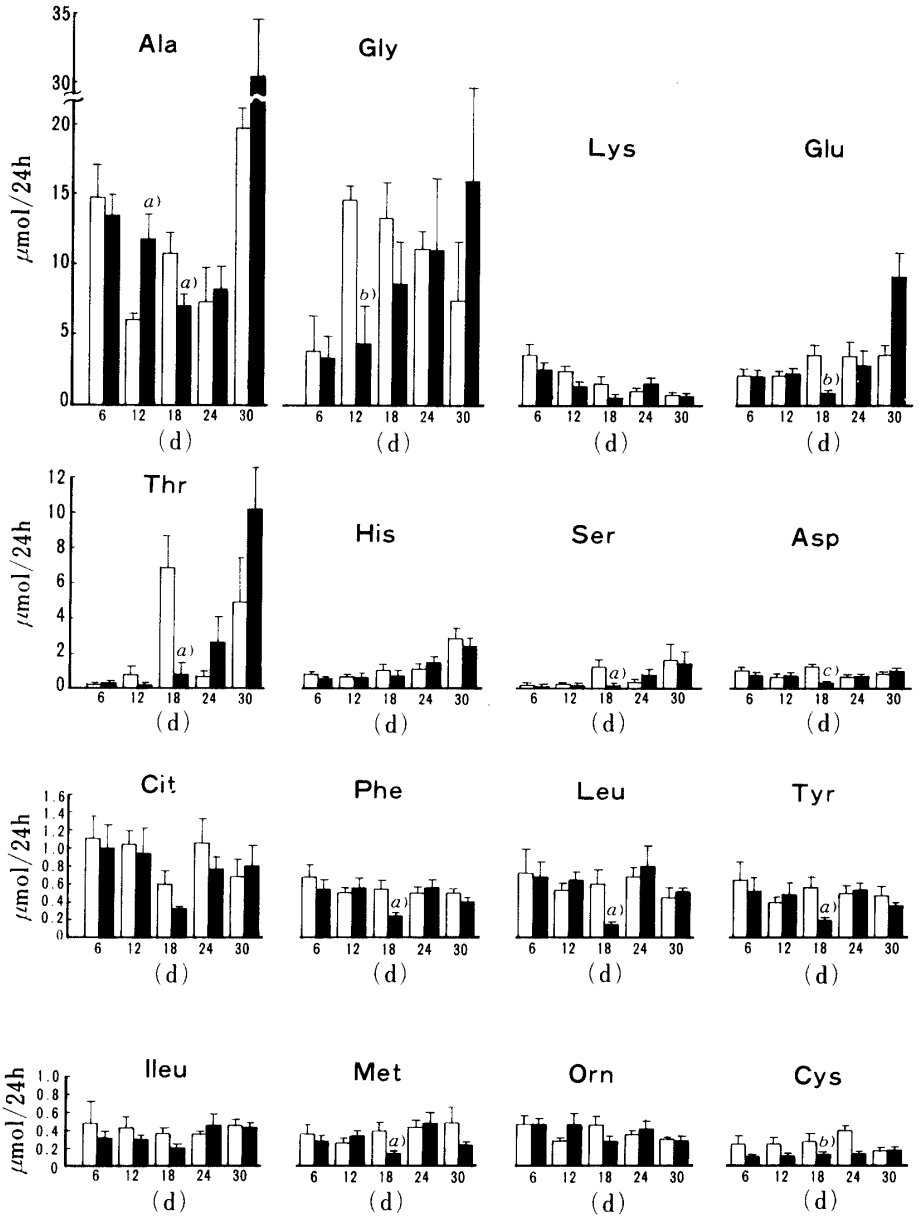

Fig. 1. Free Amino Acids Excretion in the Urine of Rats of the Control and Rhubarb Extract-treated Groups

□, control group; ■, rhubarb extract-treated group. Values are mean ± S.E. of 6 rats. Significantly different from the control group, *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$.

gested adenine were significantly higher than in the control group (fed 18% casein diet). In patients with chronic renal failure, certain deviations from the normal free amino acid pattern have been observed.[17] Similar changes have been noted following the experimental induction of chronic uremia in animals.[18] Many amino acids and peptides are excreted in the urine, possibly because of decreased ability of renal tubular cells to catabolize the filtered protein. Thus, our animal model system may also be suitable for studies of amino acid reabsorption by the renal tubules. Therefore, it seemed of interest to determine whether or not the intraperitoneal administration of rhubarb extract modified the amino acid output in the urine. As illustrated in Fig. 1, the urinary excretions of alanine, glutamic acid, threonine, serine, aspartic acid, phenylalanine, leucine, tyrosine, and methionine were significantly lower at the 18th d in rats of the rhubarb extract-treated group, but there was no statistically

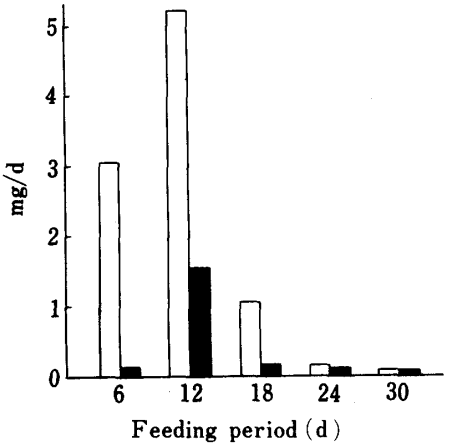

Fig. 2. 2,8-Dihydroxyadenine Content in the Urine of Rats of the Control and Rhubarb Extract-treated Groups

□, control group; ■, rhubarb extract-treated group. Six rats were used in each group.

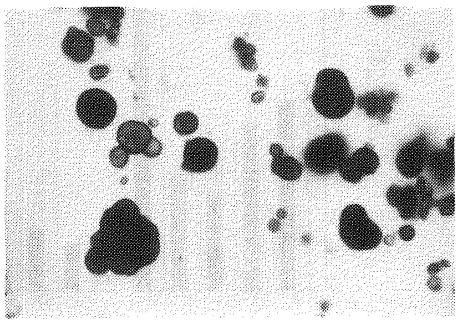

Fig. 3. 2,8-Dihydroxyadenine Deposits in the Urine of Rats of the Rhubarb Extract-treated Group at the 12th d (×100)

significant difference between the control and rhubarb extract-treated groups at the 6th, 12th, 24th, and 30th d except in the case of alanine. A decrease was also observed in the amount of urinary glycine at the 12th d and cysteine at the 24th d. Lysine, histidine, citrulline, isoleucine, and ornithine in the rhubarb extract-treated group showed no appreciable changes as compared with the control group.

2,8-Dihydroxyadenine

The rats of the rhubarb extract-treated group showed a significant decrease in the urinary excretion of 2,8-dihydroxyadenine at the 6th, 12th, and 18th d (Fig. 2). The urinary sediment (2,8-dihydroxyadenine) was brown, round particles similar to 2,8-dihydroxyadenine stones due to the adenine phosphoribosyltransferase defect in man (Fig. 3).[19] However, this is thought to be the first report of 2,8-dihydroxyadenine deposits in an experimental animal.

Discussion

It has recently been shown by us that animals given large oral doses of adenine have renal damage attributable to 2,8-dihydroxyadenine.[11-15] Disturbance of renal function induced by tubular obstruction by deposits of this adenine metabolite was thought to explain several biochemical observations.[20-22] From this point of view, we previously examined the effect of rhubarb extract in rats with chronic renal failure induced by adenine feeding,[1] and found that the nephrotoxic state caused by tubular obstruction with 2,8-dihydroxyadenine deposits can be mitigated by treatment with rhubarb extract. In the present experiment, the effect on the urinary constituents was further studied in order to evaluate the possible therapeutic significance of the rhubarb extract.

As shown in Table II, the administration of rhubarb extract to adenine-fed rats caused a significant increase in the urinary excretion of both urea and creatinine, especially at the 12th and 18th d. These experimental results support the view that the extract from Rhei Rhizoma improves the renal clearance in the uremic state induced by adenine feeding, and are consistent with the previous observations[1] that the extract from Rhei Rhizoma caused a significant decrease in the levels of both urea nitrogen and creatinine in the serum after repeated intraperitoneal administration to rats fed the adenine diet. The urea concentrations in the liver and kidney were also decreased after the treatment.

Accordingly, these results confirm that this rat model can be used for the investigation of alterations in nitrogenous products associated with uremia. On the other hand, previous reports from our laboratory have shown that adenine administration caused certain abnormalities in the blood amino acid pattern,[22] particularly in the concentration of urea cycle intermediates; the arginine concentration was significantly increased, whereas the amount of ornithine was significantly decreased in the adenine-fed rats compared with the control animals (18% casein-fed rats). Abnormalities in the metabolism of the urea cycle intermediates have been observed. Further, arginase activity was shown to be inhibited by adenine feeding.[21] The enzymatic reactions which lead to urea breakdown may be inhibited, with the consequent accumulation of glycine, arginine, and aspartic acid. Furthermore, the intake of adenine greatly increased the levels of guanidinosuccinic acid and methylguanidine in the serum; these are prime candidates for the role of uremic toxins.[22] Thus, dietary adenine may influence the concentration of various metabolites, *e.g.*, ammonia and guanidino compounds, as a result of urea retention or the toxic effect of urea itself. It would be interesting to investigate the effect of rhubarb extract on the levels of ammonia, guanidino compounds, and so forth.

Another important observation was that the extract from Rhei Rhizoma caused a number of significant differences in amino acid levels in the urine. As shown in Fig. 1, the urinary outputs of threonine, phenylalanine, leucine, methionine, and some inessential amino acids (alanine, glycine, glutamic acid, serine, aspartic acid, tyrosine, and cysteine) were significantly lower in the rhubarb extract-treated group as compared with the control group at the 12th, 18th, or 24th d of the test period. In addition, a greater effect on the urinary excretion of Ca was noted at the 12th d in rats of the rhubarb extract-treated group, while the amount of urinary P was increased by 43% at the 18th d as compared with the control group (Table III). From these results, it seems likely that the extract from Rhei Rhizoma improves the proximal or distal function in rats with impairment of renal function due to tubular obstruction with 2,8-dihydroxyadenine deposits. These effects may be regarded as reflecting a decrease in the 2,8-dihydroxyadenine content in the kidneys reported previously;[1] a significant decrease of 2,8-dihydroxyadenine content in the kidneys was observed at the 6th, 18th, and 24th d. Furthermore, a drastic decrease of 2,8-dihydroxyadenine excretion in the urine was noted at the 6th, 12th, and 18th d (Fig. 2). Thus, prevention of 2,8-dihydroxyadenine production may be an important mechanism of action of rhubarb extract.

Especially when present in a supernormal concentration, adenine is converted by xanthine oxidase to 2,8-dihydroxyadenine, a urate analogue characterized by extremely low solubility.[10] Although 2,8-dihydroxyadenine is considered to be a minor metabolic product in the breakdown of adenine, Philips *et al.*,[23] Shields *et al.*,[24] and Lindblad *et al.*[25] reported that its relative insolubility makes it a potential source of renal damage. However, it is of great interest that an anomaly such as impairment of renal function and disturbance can be improved by treatment with rhubarb extract.

On the other hand, there were no remarkable changes in the amount of urinary glucose of rats of the rhubarb extract-treated and control groups throughout the experimental period (Table II). Glucose is reabsorbed in the proximal tubules, chiefly in the convoluted portion, at normal plasma glucose concentration. As the maximal capacity of the tubules for reabsorbing glucose is reached, much more glucose is excreted in the urine. Thus, further information concerning the effect of rhubarb extract on the blood glucose seems necessary. The urinary excretion of protein could not be improved by the rhubarb extract (Table II). However, further studies on the effect of rhubarb extract in rats with chronic renal failure, seem desirable.

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