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Effect of Extract from Rhei Rhizoma on Dietary Hyperazotemia in Rats

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Although rats fed on a high-protein diet of 70% casein showed hyperazotemia, administration of the extract from Rhei Rhizoma induced decreases of blood urea nitrogen, ammonia nitrogen in the portal vein, urea concentration in hepatic tissue, and Gln, Lys, Thr, Ala, Gly, Ser, Glu, Pro, Arg, Tyr, Cit, Asp, *etc.* in serum, while significant increases of Glu and Asp in hepatic tissue were observed. The modification of the nitrogen-processing mechanism by rhubarb extract is discussed on the basis of the present results.

Keywords——Rhei Rhizoma; hyperazotemia; blood urea nitrogen; urea; ammonia nitrogen; amino acid; rat

The effects of extracts of various crude drugs on serum constituents of normal rats have been investigated in order to clarify the pharmacological actions of these crude drugs and to find new clinical applications. Blood urea nitrogen-decreasing activity has been observed after administration of extracts of Rhei Rhizoma (*Rheum officinale* BAILLON), Coptidis Rhizoma (*Coptis japonica* MAKINO), Ephedrae Herba (*Ephedra sinica* STAPF), Paeoniae Radix (*Paeonia albiflora* PALLAS var.), Bupleuri Radix (*Bupleurum falcatum* L.), *etc.*[1] Moreover, intraperitoneal administration of extract from Rhei Rhizoma caused decreases of plasma free amino acids, acceleration of serum protein biosynthesis, inhibition of urea production in the liver and kidney, decrease of ammonia nitrogen concentration in the portal vein, increase of glutamine-synthetic activity, inhibition of myoprotein disassimilation, *etc.*, as well as blood urea nitrogen decrease,[2-4] suggesting an acceleration of the nitrogen-reutilizing system. In this paper, further studies were conducted on the modification of the nitrogen-processing mechanism by Rhei Rhizoma extract in hyperazotemic rats.

Materials and Methods

Animals and Treatments——Male rats of the JCL: Wistar strain, initially weighing 90-100 g, were used in this experiment. The animals were fed *ad libitum* either laboratory pellet chow (CLEA Japan Inc., Tokyo; protein 24.0%, lipid 3.5%, carbohydrate 60.5%) (normal group) or 70% casein diet composed of 70% casein, 5.9% α-cornstarch, 15% sucrose, 2% soybean oil, 4% salt mixture,[5] 1% vitamin mixture,[5] 2% cellulose powder, and 0.1% choline chloride. On the 14th day after the start of the synthetic diet, rhubarb extract dissolved in saline (0.5 ml/150 g body weight) was administered intraperitoneally to the rats, while control rats received an equal volume of saline.

Extraction of Rhei Rhizoma——Roots of *Rheum officinale* BAILLON produced in China were finely powdered and extracted at 100°C with water, as described previously.[1] The filtrate was concentrated under reduced pressure to obtain a brown residue.

Analyses——At 6 h after treatment, rats were sacrificed by decapitation. Blood was collected in a conical centrifuge tube for the determination of urea nitrogen and free amino acids. The serum was separated by

centrifugation immediately after collection of the blood. Urea nitrogen was determined by using a commercial reagent (Urea NB-Test Wako obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan) based on the urease-indophenol method.[6] Free amino acids were determined with a Hitachi 835 high-speed amino acid analyzer. Before this determination, the serum was deproteinized by adding 3 volumes of 3% sulfosalicylic acid.

For the determination of ammonia nitrogen, blood was withdrawn through a cannula implanted in the portal vein. Ammonia nitrogen was determined by a modification of the method of Seligson and Seligson,[7] or by using a commercial reagent (Ammonia-Test Wako) based on the indophenol method.[8]

The liver was removed quickly, cooled on ice, and weighed rapidly. A portion of the liver was homogenized with 9 volumes of ice-cold water in a Potter–Elvehjem-type glass homogenizer. The homogenate, diluted about 100-fold with water, was used for the determination of urea by the method of Archibald.[9] The other part of the liver was homogenized with 4 volumes of 3% ice-cold sulfosalicylic acid. The tissue extract obtained by centrifugation was subjected to amino acid analysis.

For the determination of urea and creatinine in the urine, 6-h urine was collected in a 50 ml Erlenmeyer flask. Urea was determined by the method of Archibald.[9] Creatinine was determined by using a commercial reagent (Creatinine-Test Wako) based on the Folin–Wu method.[10]

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

Results

Effect of Rhubarb Extract on Urea Nitrogen Level in the Serum

Table I shows the effect of rhubarb extract on urea nitrogen level in the serum after intraperitoneal administration. The urea nitrogen-decreasing effect of rhubarb extract was confirmed by treatment with graded doses of this extract. Six hours after administration, the level of urea nitrogen in the serum depended on the amount of rhubarb extract administered to rats, and the administration of 10 mg decreased it by 32% from the control level. However, administration of 1 mg of rhubarb extract caused only a 9% decrease in the urea nitrogen level.

Effect of Rhubarb Extract on Urea Concentration in the Liver

Intraperitoneal administration of the extract from Rhei Rhizoma to rats caused a significant decrease of the urea concentration in the liver. As shown in Table II, the value for hepatic urea per g of tissue was about 24—32% lower at the dosage level of 2.5, 5, and 10 mg/rat in the rhubarb extract-treated group as compared with the control group. In particular, rhubarb extract significantly reduced the urea concentration by 32% as compared with the control upon intraperitoneal administration of 5 and 10 mg/rat. The effect of the rhubarb extract was the same whether calculated per g of tissue or per tissue.

Effect of Rhubarb Extract on Free Amino Acids Concentrations in the Serum and Liver

As shown in Fig. 1, the concentrations of amino acids in serum were significantly

Table I. Effect of Graded Doses of Rhubarb Extract on Urea Nitrogen in the Serum

Treatment	Dose (mg/rat)	Urea nitrogen (mg/dl)
Normal	—	15.1±0.5
Control (saline)	—	29.0±1.5 (100)
Rhubarb extract	1	26.3±1.6 (91)
Rhubarb extract	2.5	22.3±0.8[a] (77)
Rhubarb extract	5	21.0±1.3[a] (72)
Rhubarb extract	10	19.8±0.8[b] (68)

Values are means±S.E. of 6 rats. Figures in parentheses are percentages of the control value. Significantly different from the control value, *a*) $p<0.01$, *b*) $p<0.001$.

Table II. Effect of Graded Doses of Rhubarb Extract on Urea in the Liver

Treatment	Dose (mg/rat)	Urea (mg/g tissue)
Normal	—	0.11±0.01
Control (saline)	—	0.34±0.02 (100)
Rhubarb extract	1	0.30±0.01 (88)
Rhubarb extract	2.5	0.26±0.02[a] (76)
Rhubarb extract	5	0.23±0.02[a] (68)
Rhubarb extract	10	0.23±0.01[b] (68)

Values are means±S.E. of 6 rats. Figures in parentheses are percentages of the control value. Significantly different from the control value, *a*) $p<0.01$, *b*) $p<0.001$.

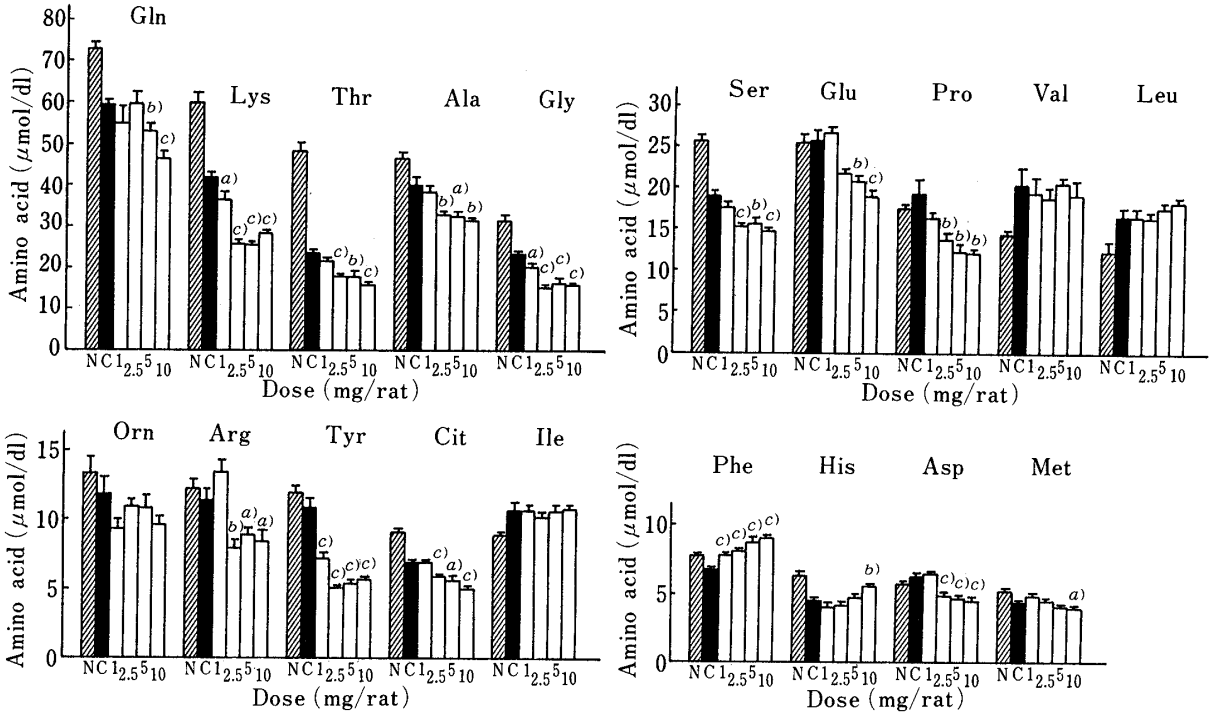

Fig. 1. Effect of Graded Doses of Rhubarb Extract on Free Amino Acids in the Serum

Values are means±S.E. of 6 rats. Significantly different from the control value, *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$. N, normal; C, control (saline).

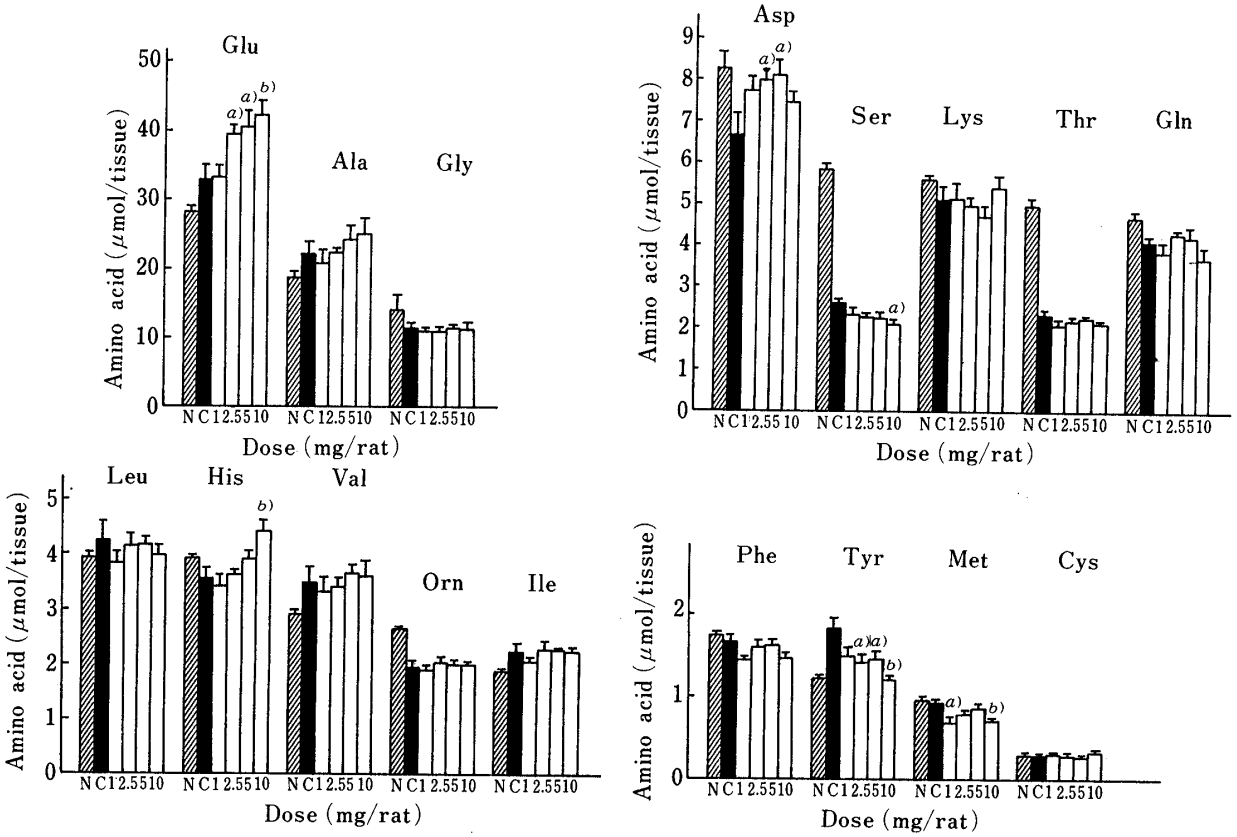

Fig. 2. Effect of Graded Doses of Rhubarb Extract on Free Amino Acids in the Liver

Values are means±S.E. of 6 rats. Significantly different from the control value, *a*) $p<0.05$, *b*) $p<0.01$. N, normal; C, control (saline).

TABLE III. Effect of Rhubarb Extract on Ammonia Nitrogen in the Portal Vein

Treatment	Dose (mg/rat)	NH_3–N (μg/dl)
Normal	—	305.9±31.0
Control (saline)	—	397.8±43.2 (100)
Rhubarb extract	5	284.4±29.6[a] (71)

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

TABLE IV. Effect of Rhubarb Extract on Urea and Creatinine Excretions in the Urine

Treatment	Dose (mg/rat)	Urea (mg/6 h)	Creatinine (mg/6 h)
Normal	—	37.7±9.0	1.57±0.20
Control (saline)	—	56.2±6.9 (100)	1.70±0.05 (100)
Rhubarb extract	5	41.8±3.7 (74)	1.60±0.06 (94)

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

decreased at 6 h after administration as compared with the control values, as follows: Gln (11—22% at 5, 10 mg/rat); Lys (13—39% at 1, 2.5, 5, 10 mg); Thr (25—33% at 2.5, 5, 10 mg); Ala (16—20% at 2.5, 5, 10 mg); Gly (14—34% at 1, 2.5, 5, 10 mg); Ser (18—23% at 2.5, 5, 10 mg); Glu (19—27% at 5, 10 mg); Pro (29—38% at 2.5, 5, 10 mg); Arg (21—30% at 2.5, 5, 10 mg); Tyr (33—53% at 1, 2.5, 5, 10 mg); Cit (15—27% at 2.5, 5, 10 mg); Asp (22—28% at 2.5, 5, 10 mg); and Met (10% at 10 mg). The concentrations of Phe and His were increased by 14—33% (1, 2.5, 5, 10 mg) and 24% (10 mg), respectively. Thus, the total amino acids level in the serum of the rhubarb extract-treated group was sharply decreased at dosage levels of 2.5, 5, and 10 mg/rat. Figure 2 shows the effect of rhubarb extract on free amino acids in the liver. The concentrations of Glu, Asp, and His were increased by 20—29% (at 2.5, 5, 10 mg), 21—22% (at 2.5, 5 mg), and 25% (at 10 mg), respectively. The concentrations of Ser, Tyr, and Met were decreased by 20% (at 10 mg), 19—33% (at 2.5, 5, 10 mg), and 25—26% (1, 10 mg), respectively.

Effect of Rhubarb Extract on Ammonia Nitrogen in the Portal Vein

Table III shows the effect of rhubarb extract on ammonia nitrogen concentration in the portal vein. The administration of rhubarb extract to rats produced a significant decrease to 284.4 μg/dl. Ammonia nitrogen concentration in the portal vein was decreased by 29% at a dose of 5 mg/rat, as compared with the control value.

Effect of Rhubarb Extract on Urea and Creatinine Levels in the Urine

As shown in Table IV, administration of the rhubarb extract to rats resulted in a decrease of urea excretion in the urine; the excretion was 26% lower in the rhubarb extract-treated group as compared with the control group, but the difference was not statistically significant. Furthermore, no change was seen in the urinary excretion of creatinine, and there was no appreciable change in the urine volume.

Discussion

Rats fed on a high protein diet have been reported to show hyperazotemia,[11-13] and such alterations were confirmed in this experiment. The administration of rhubarb extract to

these rats was found to result in a normal or nearly normal level of blood urea nitrogen. The level of blood urea nitrogen was significantly lower at doses of 1, 2.5, 5, and 10 mg/rat in rats of the rhubarb extract-treated group as compared with the control. This urea nitrogen-decreasing effect with rhubarb extract was not considered to be caused by the increase of urea excretion into urine but to be a reflection of the depression of ureapoiesis in the liver. A factor in the depression of ureapoiesis may be assumed to be the decrease of ammonia, which is the nitrogen source of ureapoiesis. Detailed examination revealed a significant decrease of ammonia nitrogen concentration in the portal vein after the administration of rhubarb extract. Decrease of the nitrogen source may, therefore, be a factor of the depression of ureapoiesis after the intraperitoneal administration of the extract from Rhei Rhizoma. The mechanism of the blood urea nitrogen-decreasing activity of the rhubarb extract was very similar to those observed in normal rats.[2-4]

On the other hand, the concentrations of Gln, Lys, Thr, Ala, Gly, Ser, Glu, Pro, Arg, Tyr, Cit, Asp, *etc.* in serum were decreased in the rhubarb extract-treated group. Among these amino acids, the decrease of Cit or Asp, which is the substrate of argininosuccinate synthetase, a rate-limiting enzyme of the urea cycle, is thought to be one of the factors which induced the depression of ureapoiesis. However, decrease of Tyr and increases of Glu and Asp were observed in hepatic tissue, and the increase of Glu was especially marked. Thus, it was supposed that in the liver the metabolic pattern is shifted toward glutamic acid production. Further studies seem necessary to establish the effects of rhubarb extract on glutamate dehydrogenase, aspartate aminotransferase, and transaminases, which are enzymes concerned in the urea cycle, amino acid metabolism, *etc.*

Purgative, antibacterial, astringent, stomachic, and cholagogic effects of Rhei Rhizoma are anticipated. So far, a series of studies in the laboratories of the present authors, that is, experimental results using rats fed on a laboratory pellet chow,[2,3] fasted rats,[4] rats with chronic renal failure,[14-18] and rats fed on a high protein diet (this work), have confirmed that the extract can modify the metabolism of nitrogen in the body.

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