Effects of Modifying Urea Hydrolysis in Acute Nephrectomy on Survival, Ammonia in Cecal Contents and Blood Metabolites (37553)

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Germ-free (GF) animals live longer than conventional animals after nephrectomy (1). Uremic colitis of varying severity was observed at death following nephrectomy in most conventional rats but not in rats that were germ-free or had a limited flora (2). These results suggest that gastrointestinal bacteria play a significant role in determining survival times of nephrectomized animals and in the production of gastrointestinal lesions. Bacterial ureases which hydrolyze urea in the intestinal contents to NH_3 and CO_2 have been implicated as an etiologic factor in this syndrome since urea hydrolysis does not occur in germ-free animals (3). The quantities of ammonia liberated in the alimentary tract of conventional animals is equivalent to 25% of the daily urea synthesis and is known to increase as blood urea rises. However, some have suggested that the increase in survival time and the absence of colitis in the germ-free-state are not due to lower gastrointestinal ammonia formation (4).

The studies reported herein were undertaken to evaluate further if the hypothesis that urea hydrolysis in the gastrointestinal tract is a significant factor in the uremic syndrome and to study changes in blood organic acids and metabolites following nephrectomy.

Methods. Experiments were conducted with healthy rats of both sexes weighing 150–250 g and fed a commercial laboratory diet *ad libitum*. Bilateral nephrectomy was performed through a midventral laparotomy under ether–O_2 anesthesia (5). Control animals were sham operated. Feed and water were withheld from both groups following surgery. For collection of portal blood the rats were anesthetized again at 24 hr after surgery and blood was drawn into heparinized syringes. Mixed peripheral blood was obtained following decapitation. To study the effects of modifiers of urea hydrolysis on survival following nephrectomy 30 male rats were divided by weight into 3 treatment groups all of which were nephrectomized. One group served as controls. The other two groups received either neomycin (1.2 mg/ml) and dihydrostreptomycin (0.5 mg/ml) or caprylohydroxamic acid (CHA)[1] (200 μg/ml) in the drinking water for 3 days prior to nephrectomy. CHA was also given orally (250 mg/kg) within 60 min prior to and at 19 and 27 hr following nephrectomy. Colon and cecal contents obtained at death from 6 animals/group were analyzed for NH_3–N and urease activity.

For studies of urea recycling, the animals were placed into individual stainless steel cages for complete urine collection immediately after sc injection of ^{14}C-urea (5.5×10^6 dpm/0.5 ml saline; 9.25 mCi/mmole).

The urine was collected under toluene in bottles containing 0.2 ml of 6 *N* H_2SO_4 and the 24 hr collection was diluted to 50 ml. ^{14}C was assayed in 0.2 ml aliquots of urine added directly to a liquid scintillation cocktail (1, 2). Preliminary investigations showed that a negligible portion of the ^{14}C in the urine was present in forms other than urea. Counting efficiency was determined by the channels ratio method. Total urea flux and urea recycled were calculated by the method of Ford and Milligan (6).

Blood (0.5 ml) for organic acid analysis was mixed with 0.1 ml of 1 *N* NaOH, freeze–

[1] Appreciation is expressed to Eisai Co., Ltd., Tokyo, Japan for supplying the caprylohydroxamic acid.

dried, and stored at −20° until assayed by silica gel partition column chromatography and indicator titration (7). Immediately before application to the silicic acid column each sample was mixed with 0.05 ml of 6 *N* H_2SO_4, 0.2 ml of H_2O and enough dried silicic acid to make a dry, free flowing mixture. Blood ammonia and urea were determined colorimetrically (8). Urease activity in homogenized gastrointestinal contents was assayed by allowing an aliquot of the homogenized material to react with a 3% urea phosphate buffer for 10 min at 37°. Ammonia (free and urea–NH_3) was determined by steam distillation into 20 ml of 2% boric acid. The ammonium borate was tritrated with 0.01 *N* HCl using bromcresol green–methyl red indicator.

Results. Bilaterally nephrectomized rats showed higher portal blood NH_3–N and urea and higher pH and NH_3–N concentration in cecal contents (Table I). Peripheral blood NH_3–N showed no change in the nephrectomized animals in four experiments (Tables I and II) while portal blood increased, thereby increasing the ratio of portal–peripheral blood NH_3–N concentrations (Table I). At 24 hr following nephrectomy, the male rats had significantly higher concentrations of isocitrate and *trans*-aconitate in portal blood while citrate was significantly lower than in sham-operated controls. In females at 24 hr after nephrectomy, *cis*-aconitate, *trans*-aconitate, citrate and isocitrate were higher relative to corresponding control animals. Peripheral blood glucose decreased following sham operation (Table II), but in nephrectomized animals blood glucose remained constant in females (Table II) and increased in males (Table II). Blood urea increased almost linearly following nephrectomy (Fig. 1) and by 24 hr the concentrations were usually above 100 mg/ml (Table II).

Treating nephrectomized male rats with CHA did not prolong survival, but oral treatment with neomycin and dihydrostreptomycin increased survival times significantly (Table III). CHA or antibiotic treatment did not affect NH_3–N concentration of colon and cecal contents although urease activity was decreased over 50% by CHA treatment and nearly abolished by antibiotic treatment.

Rats treated with antibiotics showed a significant decrease in the amount of urea recycled and hydrolyzed in the gastrointestinal tract and in the total urea flux (Table IV). When 200 μg/ml of CHA were added to the drinking water, followed by an oral dose of 250 mg CHA/kg body weight just before ^{14}C-urea was injected, the amount of urea recycled during the next 24 hr was significantly decreased (Table IV).

TABLE I. Portal Blood NH_3–N and Urea and pH and NH_3–N Concentration in Cecal Contents of Sham-Operated and Nephrectomized Male Rats.

	Treatments		
	Sham-operated	Nephrectomized	N/C[c]
Portal blood (Expt 2)			
NH_3–N[a]			
μg/ml[a]	3.22 ± 0.68	8.11 ± 1.48	2.52
Ratio (portal/peripheral)[a]	4.44 ± 0.49	13.20 ± 3.93	2.98
Urea[a] (mg/100 ml)	20.8 ± 3.2	177.7 ± 10.4	8.54
Cecal contents			
Expt 1			
NH_3–N[a,b]	163 ± 20	436 ± 19	2.67
Expt 2			
pH[a]	7.14 ± 0.07	7.41 ± 0.10	1.04
NH_3–N[a,b]	172 ± 35	248 ± 18	1.44

[a] Treatment means differ ($p < 0.05$).

[b] μg/g wet wt.

[c] N = nephrectomized; C = sham-operated controls.

TABLE II. Metabolites in Portal and Peripheral Blood of Sham-Operated and Nephrectomized Male and Female Rats at 24 hr After Surgery.

	Male[a]			Female[a]		
	Sham-operated	Nephrectomized	N/C	Sham-operated	Nephrectomized	N/C
Portal blood						
cis-Aconitate	—[i]	—[i]	—	—[i]	37 ± 7	—
trans-Aconitate[b,e]	<10[e]	72 ± 11[f]	—	—[i]	10 ± 2[h]	—
Citrate[b]	178 ± 46	96 ± 19	0.54	183 ± 31[g]	1240 ± 360[h]	6.78
Isocitrate[b,e]	—[e,i]	25 ± 2[f]	—	—[g,i]	45 ± 5[h]	—
Glucose[d]	101 ± 6[e]	134 ± 9[f]	1.33	103 ± 5	125 ± 8	1.21
NH_3–N[c]	2.4 ± 0.5[c]	6.1 ± 1.1[f]	2.52	2.1 ± 0.2	3.1 ± 0.2	1.48
Urea[d]	15.7 ± 2.5[e]	134.5 ± 7.9[f]	8.57	11.9 ± 0.6[g]	87.0 ± 0.8[h]	7.31
Peripheral blood						
Glucose[d]	96 ± 5[e]	126 ± 9[f]	1.31	96 ± 2[g]	113 ± 6[h]	1.18
NH_3–N[c]	0.5 ± 0.1	0.8 ± 0.3	1.60	0.7 ± 0.1	0.7 ± 0.1	1.00
Urea[d]	18.3 ± 1.5[e]	132.5 ± 8.1[f]	7.24	10.5 ± 0.7[g]	86.8 ± 2.0[h]	8.27
Peripheral blood (before surgery)						
Glucose[d]	116 ± 5	117 ± 2	—	115 ± 4	113 ± 5	—
Urea[d]	20.1 ± 2.5	19.4 ± 1.4	—	25.5 ± 5.7	23.6 ± 3.9	—

[a] Means of 5 or 6 animals per treatment ± SEM; C = sham-operated; N = nephrectomized.
[b] nequiv/ml blood.
[c] μg/ml blood.
[d] mg/100 ml blood.
[e,f] Treatment means for male animals differ significantly ($p < 0.05$).
[g,h] Treatment means for female animals differ significantly ($p < 0.05$).
[i] Metabolite not detected in portal blood in these animals.

Discussion. The results show that nephrectomy causes a marked alteration in the metabolism of citrate and related organic acids and that there is a sex difference in this response in rats. Elliot and Freeman (9) reported that plasma citrate increased to a maximum at 4 hr after nephrectomy and then declined to concentrations below control at 24 hr. The sex of the animals was not stated, however, the reported results agree with our data in males (Table II). The blood citrate concentration remained elevated longer in females than in males. At 24 hr postnephrectomy citrate was elevated 6.8-fold in females over their sham-operated controls. The reasons for the transient hypercitricemia following nephrectomy are not clear. Parathyroidectomy 4 days prior to nephrectomy abolished hypercitricemia (9). Simpson (10) concluded that nephrectomy caused no decrease in the rate of oxidation of plasma citrate and that the temporary increase in plasma citrate is due to an increase in the rate of production of plasma citrate rather than a decrease in the rate of its oxidation. The reasons for the apparent difference between males and females in this respect remains to be determined.

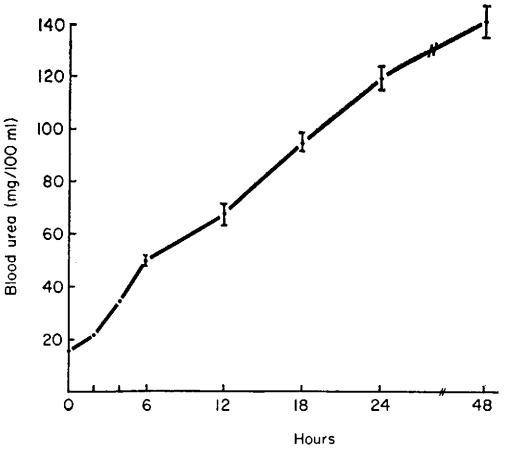

FIG. 1. Blood urea concentrations as a function of time following bilateral nephrectomy of rats.

TABLE III. Effects of Antibiotics[a] and Caprylohydroxamic Acid (CHA) Treatment of Nephrectomized Male Rats on Survival Time, and Ammonia Concentration and Urease Activity of Gastrointestinal Contents (Expt 5).

	Nephrectomized	Nephrectomized + CHA[b]	Nephrectomized + antibiotic
Survival time[c] (hr)	39.6 ± 3.5[f]	41.4 ± 2.9[f]	60.8 ± 6.9[g]
NH_3–N (mg/g dry wt)	3.4 ± 0.6	3.0 ± 0.4	2.3 ± 0.3
Urease[d,e]	11.7 ± 1.9[f]	5.2 ± 0.8[g]	0.6 ± 0.1[h]

[a] Neomycin (1.2 mg/ml) and dihydrostreptomycin (0.5 mg/ml) were given in the drinking water for 3 days prior to nephrectomy.

[b] CHA (200 ppm) was given in drinking water for 3 days prior to nephrectomy and an oral dose of 250 mg/kg was given immediately preceding and at 19 and 27 hr following nephrectomy.

[c] Means of 10 animals/treatment ± SEM.

[d] Means of 6 animals/treatment ± SEM.

[e] 1 unit = 1 mg NH_3–N released in 10 min at 37° in a urea phosphate buffer.

[f–h] Treatment means without a common superscript differ ($p < 0.05$).

The survival of nephrectomized rats treated orally with CHA was not increased in these studies. Swales, Tange and Evans (4) administered acetohydroxamic acid (AHA) subcutaneously after nephrectomy and observed a decreased survival time. However, the total AHA administered approached the LD_{50} dose. Both AHA and CHA are urease inhibitors but CHA has a K_i for urease of 9.3×10^{-8} *M vs* 1.2×10^{-6} *M* for AHA (11). On this basis, CHA should be the more effective inhibitor. In the present studies, CHA was administered orally rather than by injection to avoid the need for its diffusion into the lumen of the intestine which is the site of urease activity. This treatment was effective in lowering urease activity of gastrointestinal contents (Table III). Total urea recycled into the gastrointestinal tract and hydrolyzed in other intact animals treated similarly with CHA decreased by 36% (Table IV). However, only when antibiotics were given was survival increased (Table III), urease activity markedly decreased (Table III), and urea hydrolyzed in the gastrointestinal tract decreased appreciably. Be-

TABLE IV. Urea Recycling in Rats Treated with Antibiotics or Caprylohydroxamic Acid (CHA) (Expt 6).

	Treatments[a]		
	Control	Antibiotic[b]	CHA[c]
	(5)	(6)	(5)
Body wt (g)	172 ± 6.6	173 ± 7.9	182 ± 8.9
Urinary urea excretion[d]	564 ± 27	531 ± 24	537 ± 49
Urea recycled[d]	172 ± 18	56 ± 20	111 ± 11
Urea flux[d]	736 ± 44	586 ± 34	648 ± 52
Blood urea[e]	32 ± 2.1	25.9 ± 1.9	—

[a] Data expresses as means ± standard deviation. Number of animals per treatment in parentheses.

[b] Neomycin (1.2 mg/ml) and dihydrostreptomycin (0.5 mg/ml) were given in the drinking water for 5 days prior to administering ^{14}C-urea.

[c] CHA (200 ppm) was given in drinking water for 5 days and on day of experiment, an oral dose of 250 mg CHA/kg was given.

[d] mg/24 hr.

[e] mg/100 ml blood.

cause bacterial urease activity exceeds that required to hydrolyze urea which enters the bowel normally, it appears that more than 50% of the urease activity has to be inhibited before urea recycling and hydrolysis is affected. To date antibiotics have been the only means which consistently reduce urea hydrolysis and thus the only treatment expected to have therapeutic value if urea hydrolysis is a significant factor in the survival of uremic animals. The present data or the data of Swales, Tange and Evans (4) do not refute the hypothesis that the toxic effects of ammonia are important in uremia. Ammonia concentrations in cecal contents of nephrectomized rats were elevated (Table I) and the pH of the contents was about 0.3 units higher than control (Table I). This increase in pH would approximately double molecular NH_3 concentrations. Molecular NH_3 is believed to readily transgress cell membranes and is believed principally responsible for mammalian toxicity (12). It is apparent that determining the role of urea hydrolysis and ammonia release in the uremic syndrome will require more effective and specific approaches for decreasing gastrointestinal urea hydrolysis.

Summary. Ammonia concentrations were increased in portal blood and in cecal contents of nephrectomized compared to sham-operated control rats at 24 hr after surgery. Portal blood urea was increased 7- to 8-fold in both male and female nephrectomized rats compared to sham-operated controls. Portal concentrations of *trans*-aconitate, isocitrate and glucose were increased in male nephrectomized rats at 24 hr after surgery while in female rats, *cis*- and *trans*-aconitate, citrate and isocitrate were elevated in portal blood at 24 hr after surgery. The increase in citrate in female rats was quite marked at 24 hr while in male rats citrate was lower than control. Oral treatment of nephrectomized rats with caprylohydroxamic acid did not affect the survival time following nephrectomy, while treatment with neomycin in the drinking water for 3 days prior to nephrectomy significantly prolonged survival following nephrectomy.

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