Effects of Loop Diuretic on Ammonium Excretion after 24 hours Unilateral Ureteral Obstruction in Rats

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Abstract

To characterize the ammonium transport defect in obstructive nephropathy, the inhibition of ammonium reabsorption at the thick ascending limb of Henle's loop by furosemide was studied in 24 hrs. unilateral ureteral obstructed (UUO) rats. After the release of obstruction, the kidney was unable to lower urine pH. The fractional bicarbonate excretion of the damaged kidney was 10 times the value of the normal kidney.The rate of ammonium excretion ($U_{[NH4^+]}V$) and ammonium index ($U_{[NH4^+]}V/GFR$) were both markedly reduced. Thus, the impaired urine acidification was due to the reduction in hydrogen ion secretion, bicarbonate reabsorption and ammonium excretion. The blockage of Na^+-K^+ (NH_4^+) - $2Cl^-$ cotransporter at the thick ascending limb by loop diuretic, furosemide, resulted in increasing ammonium index in both the contralateral control kidney (CCK) and the post-obstructed kidney (POK). This indicated that the loop of Henle of POK still functioned. However, the Henle's loop of the POK may be defective since the degree of the increase was smaller than that observed in the CCK.

The improvement of functioning nephron activity was induced by increased RBF and GFR from right nephrectomy (RNx). That method caused a marked increase in GFR and also in the ammonium index. It was suggested that the ammonium index of the POK is in part dependant on GFR, which may vary with the activity of functioning nephron. Moreover, the ammonium index of the POK in the right nephrectomy peroid is insignificantly different from the control value of the CCK. This data indicated indirectly that ammonium excretion defect after release of 24 hrs. UUO is mediated by impair ammoniagenesis and / or proximal tubule ammonium secretion. More information is still required.

1. Introduction

Unlike bicarbonate and phosphate buffer, ammonium excretion is important for urine acidification since it is generated within the renal tubular cell itself from available amino acid. Observation carried out in human subjects receiving acid and alkali added to their diet indicated that change in renal net acid excretion occurs mainly as a result of varying ammonium excretion [1,2].

The kidney forms ammonia from nitrogen precursors extracted from arterial blood especially glutamine. The production takes place in mitochondria of proximal tubule, the chief site of renal ammonium production [3,4].Micropuncture and microcatheterization studies [5-11] showed the pathway of ammonium transfer from early nephron to the final urine as demonstrated in Figure 1. The glomerular filtrate contain a substantial fraction (10-30%) of excreted ammonium. Produced ammonium is secreted from proximal tubular cell and a small amount of secreted ammonium is back diffused to the interstitium. About 70% of excreted ammonium was delivered to the loop of Henle. Both lumen positive

transepithelial drive and the facilitated cotransport system, Na^+-K^+-(NH_4^+)-2Cl^-, cause NH_4^+ reabsorbtion from the thick ascending limb. A "single effect" of countercurrent multiplication provided by the later generates the highest concentration of ammonium, 160% of excreted ammonium, at the bend of Henle's loop. Ammonia is also secreted along the collecting duct by transepithelial NH_3 concentration and will be trapped as NH_4^+ to be finally excreted into urine.

Urinary tract obstruction is an important clinical problem since it may lead to chronic renal failure. Understanding of pathophysiology of urinary tract obstruction is essential for clinical diagnosis and rational management. Renal function after the release of complete acute unilateral ureteral obstruction (24 hrs.UUO) in rats was investigated in this study. Not only the glomerular filtrate, reabsorptive and secretory function but also the renal acid-base regulation is reduced after the release of 24 hrs.UUO. Impaired urine acidification has been observed in patients [12,13] and experimental animals [14-16]. After the release of 24 hrs. UUO, the post-obstructed kidney (POK) excreted more bicarbonate than the contralateral control kidney (CCK). Wall et al. [15] demonstrated that acidification defect after the release of UUO was associated with decreased excretion of ammonium. Bloudon et. al. [17] demostrated that the production of ammonium from glutamine decreased by 30% of the normal kidney after release of 24 hrs. UUO. Besides, Buerkert and associates [18] found a decrease in ammonium excretion as a result of the reduced number of functioning nephron in the remnant kidney. However the possible mechanism for decreased excretion of ammonium after release of UUO is still undetermined. Therefore, the present study was undertaken in a rat model permitting more information concerning urinary ammonium excretion after release of 24 hrs. UUO. Since the thick ascending limb of Henle 's loop is the major concentrated ammonium region, Na^+-K^+-2 Cl^- cotransporter blocker, furosemide, was used indirectly in an investigation of ammonium excretion defect of the damaged kidney after 24 hrs. UUO.

2. Material and Methods

Fifteen male Wistar rats weighing 170-250 g. were operated on, ureteral obstruction was carried out by double ligation of the left ureter at a distance of about one third from the bladder with silk thread. They were then returned to a metabolic cage where only water was allowed ad libitum for 24 hours prior to the clearance study.

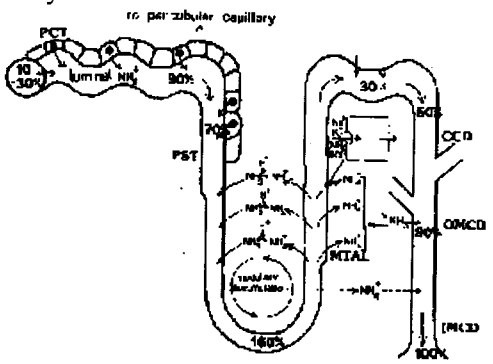

Figure1 The pathway of ammonium transfer from early nephron to the urine. PCT = proximal convoluted tubule, PST = proximal striaght tubule, MTAL = medullary thick ascending limb of Henle 's loop, CCD = cortical collecting duct, OMCD = outer medullary collecting duct and IMCD = inner medullary collecting duct.

For clearance study, the rat was anasthetized by intraperitoneal injection of sodium thiobarbital (inactin) 100 mg./kg. BW. The rat was placed on a heating table and its body temperature was regulated by a yellow spring temperature controller in order to maintain the body temperature at 37°C. Tracheostomy was performed to allow aspiration of any secretion which may block the airway under anesthetic condition. The right femoral artery was cannulated for measuring the arterial blood pressure (ABP) and periodic arterial blood sampling during the course of the experiment. The ABP was monitored with a pressure transducer (Statham P23 DE) and recorded on a Grass polygraph recorder. The right femoral

vein was cannulated for intravenous infusion, using a Harvard infusion pump. The right carotid artery was cannulated for additional fluid infusion by another infusion pump. The infused fluid composition and the rate of infusion are illustrated in Table 1.

Table 1 The composition of infusate in normal saline and rate of infusion during clearance study.

Period	Femoral vein		Carotid artery	
	Composition	rate (µl/min)	Composition	rate (µl/min)
Control (C)	10 g %inulin	20	0.8 g %inulin	20
Furosemide (Fu)	10 g %inulin & Furosemide*	20	0.8 g %inulin	20
Furosemide after right nephrectomy (Fu + RNx)	10 g %inulin & Furosemide*	20	normal saline	20

*Furosemide from Lasix, Hoechst Pharmaceutical Limited

Thirty minutes after the release of 24 hours of left ureteral obstruction, the normal and obstructed kidney function were quantitated using clearance method. The complete experimental protocol is shown in Figure 2. For each experiment, urine from both kidneys was collected for 3 periods, namely, control (C), furosemide (FU) and furosemide after right nephrectomy (FU + RNx). During each period, 3 urine samples each lasting 30 minutes were collected.

To prevent severe volume depletion during diuresis, normal saline was administered to replace urine loss. The difference between volume of urine and total fluid infusion was determined and the same volume of normal saline was gradually injected intravenously during 30 minutes of subsequent urine collection. At the end of the FU period, the right normal kidney was nephrectomized by ligating right renal vessels with cotton thread to increase blood flow to left kidney and increase GFR of the obstructed kidney.

Blood samples were collected for blood gas and chemical analysis at about one hour intervals as depicted in Figure 2. An equal volume of 6% bovine serum albumin in normal saline was then administered intra-arterially to replace the volume of blood sample. At the end of each experiment, both kidneys were decapsulated and weighed.

All urine samples were collected under light mineral oil to preserve total CO_2. To prevent loss of ammonium, each volume of urine sample was immediately diluted with 10% perchloric acid (PCA) for chemical analysis.

Plasma and urine samples were analyzed for polyfructosan by an anthorne method [19], sodium-potassium concentration by atomic absorption spectophotometer (Model AA575 Varian Tectron), ammonium concentration by microdiffusion and colorimetric determination using indophenol reaction [20], pH-PCO_2 by blood gas analyser, Radiometer, Copenhagen. Since the urine pH was beyond the range of its capacity, the pH of some of these urine samples was determined by a pH meter (Backman PHASAR-I digital pH meter). These data were used for calculation of GFR, bicarbonate concentration, excretion rate (UxV), fractional excretion (FEx) of Na^+,K^+ and ammonium index ($U_{[NH4^+]}$ V/GFR). The mean values for each period were calculated by averaging individual data from all three urine samples during that period. Thes data were statistically compared using an unpaired t-test among the group after a general test for homogeneity of variance or by pair t-test within each group. They were considered to be statistically different when P-values were less than 0.05.

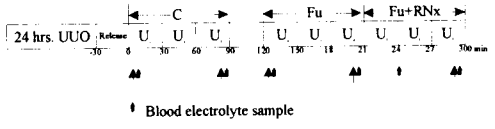

Figure 2 The experimental protocol

3. Results

Arterial blood composition of the 24 hrs. UUO rats in this study are shown in Table 2. The arterial blood pressure (ABP), hematocrit (Hct), plasma pH, PCO_2, HCO_3^-, $Na^+$and K^+ are within the normal range.

As shown in Table 3, a mild diuretic state during control period was induced by intravenous administration of normal saline to increase urine volume from the POK for chemical analysis sufficiency. However, GFR 546±17 ml/min. 100gBW. is within the normal range. Na^+ and K^+ excretion and fractional excretion rate of CCK indicate that the CCK had a normal reabsorptive function. While the data demonstrates the marked impaired function as the urine flow rate of the POK is only 20% and the GFR is approximate 15% of the CCK. However, Na^+ and K^+ reabsorption is insignificantly lower than the CCK.

The CCK could excrete acidic urine with a mean UpH of 6.11. The mean U-PCO_2 of 20.7 mmHg. is lower than the corresponding plasma value of 35.9 mmHg. and the urinary bicarbonate concentration ($U_{[HCO3]}$ was 2.5 mM.) These results indicate that the CCK can reabsorb HCO_3^- and secrete H^+. Moreover, the low fractional excretion of bicarbonate indicates that about 99% of filtrated bicarbonate was reabsorbed. In contrast, the POK was unable to acidify urine and had a defective bicarbonate reabsorption. The ammo
nium excretion of the POK is depressed to only 5% and the ammonium index is only 30% of the compared value in CCK. These results indicated defective ammonium excretion after release of 24 hrs. UUO.

Furosemide led to highly elevated urine flow rate by the CCK to 92 ml/min. 100 g BW. but decreased GFR by about 10% from the pretreatment value. This reduction indicated volume depletion due possibly to insufficient fluid replacement. The GFR of the POK was also reduced. Volume depletion may result in an increased adrenergic activity and angiotension II level. Both changes play an important role in increasing the renal vascular resistance. The significant reduction in renal plasma flow after furosemide infusion may consequently reduce SNGFR [21].

Period / Blood composition	C	Fu	Fu+RNx
ABP (mmHg)	126±3	114±3	113±3
Hct (%)	49±1	46±1	42±1
pH	7.44±0.02	7.49±0.02	7.48±0.02
pCO_2 (mmHg)	35.5±2.2	31.3±1.7	31.5±2.2
HCO_3^- (mM)	23.4±1.0	22.9±1.4	22.9±1.7
Na^+ (mEq/L)	137±1	136±2	138±1
K^+ (mEq/L)	5.0±0.6	4.5±0.5	4.6±0.4

Table 2 Arterial blood composition of rat during control (C), Furosemide (Fu), and Furosemide after right nephretomy (Fu+RNx) period.

Excretion and fractional excretion rate of $Na^+$and K^+ are significantly elevated. UpH of the CCK is insignificantly increased to 6.5 after furosemide infusion. This is partly due to an increase in excretion and fractional excretion rate of bicarbonate to approximate 5 and 6 times of the control value, respectively. The increased distal delivery of bicarbonate resulted in the augmentation of U-PCO_2. Ammonium excretion and ammonium index increased by about 30% ($P<0.01$) and 50% ($P<0.01$) above pretreatment value, respectively as depicted in Figures 3 and 4. This result may indicate an increased ammonium secretion or marked reduction in ammonium reabsorption by the thick ascending limb of Henle's loop after furosemide administration if plasma ammonium concentration was assumed to be relatively constant throughout the course of the experiment.

Before right nephrectomy, furosemide infusion led to a reduction in GFR of POK by approximately 40% ($P<0.01$). The FE_{Na+} is insignificantly increased but the FE_{H20} increased significantly by 70% of pretreatment value ($P<0.05$) albiet the unchanged urine flow rate. These results indicated a tendency to reduce tubular water reabsorption during furosemide infusion in POK. During this same period, potassium excretion was insignificantly increased by 40% whereas the fractional potassium excretion was 3 times the control value ($P<0.01$). Furosemide induced a slight fall in UpH to 7.07 without changing U-PCO_2. The bicarbonate excretion rate was insignificantly decreased in proportion to the reduction in GFR. The ammonium excretion remains relatively

constant and is not reduced albeit a reduction GFR. The ammonium index is,therefore, markedly elevated by 50% (<0.01).

Furosemide with contralateral nephrectomy results in increased GFR and massive diuresis, natriuresis and kaliuresis by the POK. However urine flow rate, GFR , absolute and fractional excretion of water, sodium and potassium are lower than those values during diuresis peroid of the normal kidney. The data indicate a loss of bicarbonate reabsorption efficiency of the POK. However, furosemide does not markedly decrease bicarbonate reabsorption in POK as in CCK. Ammonium excretion is raised more than 4 times of control period which is only 20% of that of the control kidney in diuresis period. The ammonium index is also elevated by about 140% from the value in the control period which is 50% of the value in the CCK. Thus, furosemide infusion after the release of UUO and contralateral nephrectomy results in increased ammonium secretion and excretion whereas UpH fell.

4. Discussion

The renal impairment after 24 hrs. UUO in rats involved severe suppression of GFR. A decrease in SNGFR and preglomerular vasoconstriction may be responsible for this change [22-24]. The impairment of tubular reabsorptions of water and sodium are also observed in the present study. Several factors may contribute including the heterogeneity of individual nephron, decreased sodium and water reabsorption by deep nephron [25] and loop of Henle [26] especially medullary thick ascending limb [27] and unresponsiveness of the collecting duct to ADH. Our results are in agreement with the concept that is flow dependent of potassium secretory along the distal tubule and collecting duct [28-30] since mild diuretic induction in the present experiment causes higher excretion rate and higher fractional excretion of potassium of the POK than the other [31]. Furthermore, the undifferent fractional excretion of potassium of the POK and the CCK implicate that potassium secretion by distal nephron may not be severely depressed as its secretion may increase with tubular fluid flow.

The acidification defects of the damaged kidney include high urine pH and low tubular bicarbonate reabsorption. It is well documented that hydrogen ion secretion in the distal tubule and collecting duct was defective after UUO [15,32-34]. Micropuncture data demonstrated that the defect of bicarbonate reabsorption occured beyond the proximal tubule probably at the level of late distal tubule of the surface nephron or the collecting duct [15] despite the fact that proximal tubule is the major site for bicarbonate reabsorption [35-37]. Moreover, ammonium excretion was markedly decreased as reported by others[14,15,25]. A marked reduction in ammonium index, 30% of the normal kidney, implicate both lower ammonium generation and secretion by the POK. In a model of chronic renal failure using a remnant kidney in the rats, a defect in renal ammonium excretion is also observed to be associated with an impairment of medullary transfer of ammonium from the loop of Henle to collecting duct [18]. Thus a reduction in ammonium secretion at collecting duct may in part contribute to the reduction of ammonium excretion in the POK. However, there has been no report the showed defective transport of ammonium at other tubular segment which may also contribute to the reduction in ammonium excretion after UUO.

Furosemide administration in the presence of the normal kidney induces a reduction in GFR of both CCK and POK. This result may in part be due to the volume depletion. The effect of furosemide itself may partly be responsible for the suppressed GFR since furosemide can produce a temporary decrease in GFR due to an increase in the hydrostatic pressure especially at the proximal tubule [38]. The damaged nephron seems to be responsive to furosemide mainly by reduction of salt and water reabsorption in the loop of Henle. However, the magnitude and degree of these reductions are smaller than those observed in the CCK. Several factors may possibly be involved. The reduced RBF would decrease the transport of drug to the obstructed kidney.

	CCK		POK		
	C	Fu	C	Fu	Fu+RNx
V (ml/min.100g.BW.)	$24_{\pm}4$	$92_{\pm}8^{***}$	$5_{\pm}1$	$5_{\pm}1$	$46_{\pm}5^{***,\#\#\#}$
GFR (ml/min.100g.BW.)	$546_{\pm}17$	$486_{\pm}8$	$90_{\pm}8$	$54_{\pm}7^{**}$	$162_{\pm}13^{***,\#\#\#}$
FE_{H_2O} (%)	$4.3_{\pm}0.7$	$19.4_{\pm}1.3^{***}$	$5.4_{\pm}0.7$	$9.0_{\pm}1.2^{*}$	$28.0_{\pm}2.7^{***,\#\#\#}$
$U_{[Na+]}$(mEq/L)	$110_{\pm}11$	$131_{\pm}3$	$108_{\pm}4$	$108_{\pm}5$	$122_{\pm}4^{\#}$
$U_{[Na+]}V$(nEq/min.100g.BW.)	$2,714_{\pm}608$	$11,096_{\pm}1,394^{***}$	$540_{\pm}87.2$	$576_{\pm}131$	$5,667_{\pm}775^{***,\#\#\#}$
$FE_{[Na+]}$ (%)	$3.8_{\pm}0.8$	$19.3_{\pm}1.9^{***}$	$4.8_{\pm}0.7$	$8.5_{\pm}1.1$	$27.1_{\pm}2.8^{*,\#\#}$
$U_{[K+]}$(mEq/L)	$43.0_{\pm}8.5$	$14.3_{\pm}1.1^{**}$	$26.9_{\pm}2.3$	$33.4_{\pm}3.1$	$18.8_{\pm}2.4^{*,\#\#}$
$U_{[K+]}V$(nEq/min.100g.BW.)	$630_{\pm}55$	$1,205_{\pm}80^{***}$	$108_{\pm}13$	$150_{\pm}24$	$681_{\pm}4.2^{***,\#\#\#}$
$FE_{[K+]}$ (%)	$24.6_{\pm}2.6$	$63.0_{\pm}6.1^{***}$	$25.5_{\pm}2.3$	$67.4_{\pm}9.5^{*}$	$103.4_{\pm}7.4^{***,\#\#}$
U_{pH}	$6.24_{\pm}0.18$	$6.50_{\pm}0.07$	$7.26_{\pm}0.10$	$7.07_{\pm}0.12$	$6.62_{\pm}0.15^{**}$
U-PCO_2 (mmHg)	$20.7_{\pm}1.2$	$25.5_{\pm}1.2$	$15.7_{\pm}1.8$	$15.2_{\pm}1.5$	$21.0_{\pm}1.2^{*,\#\#}$
$U_{[HCO_3-]}$(mM)	$2.5_{\pm}1.5$	$2.3_{\pm}0.5$	$8.1_{\pm}1.2$	$5.1_{\pm}1.3$	$3.2_{\pm}0.6^{**}$
$U_{[HCO_3-]}V$(nmol/min.100.BW.)	$40_{\pm}17$	$207_{\pm}55^{*}$	$58_{\pm}12$	$30_{\pm}7$	$145_{\pm}26^{*,\#\#}$
$FE_{[HCO_3-]}$(%)	$0.35_{\pm}0.19$	$1.99_{\pm}0.61^{*}$	$2.24_{\pm}0.49$	$2.25_{\pm}0.53$	$3.35_{\pm}0.62$
$U_{[NH4+]}$(mM)	$17.2_{\pm}5.4$	$3.8_{\pm}0.4^{*}$	$3.9_{\pm}0.8$	$3.0_{\pm}0.4^{*}$	$1.5_{\pm}0.2^{*,\#\#}$
$U_{[NH4+]}V$(nmol/min.100.BW.)	$252_{\pm}26$	$327_{\pm}24^{**}$	$12_{\pm}1$	$11_{\pm}1$	$57_{\pm}5^{***,\#\#\#}$
$U_{[NH4+]}V$/GFR(mM)	$467_{\pm}51$	$7144_{\pm}64^{**}$	$152_{\pm}15$	$233_{\pm}22^{**}$	$372_{\pm}29^{***,\#\#\#}$

* p< 0.05, ** p< 0.01,*** p< 0.001 from C period

# p< 0.05, ## p< 0.01, ###p< 0.001 from Fu period

Table 3 Effect of furosemide and / or right nephrectony on renal function of contralateral control kidney (CCK) and post obstructed kidney (POK).

The loop of Henle may also be defective and may reduce its responsiveness to furosemide. However, ureteral obstruc tion has been shown to be associated with a reduction in the amount of luminal Na^+-K^+-2Cl^- cotransport in medullary thick ascending limb [27]. Therefore, furosemide should block the intact thick ascending limb of obstructed nephron and excrete total K^+ from ascending limb to the urine. This expectation is confirmed by furosemide infusion which causes increased fractional excretion of potassium from control period and comparable with the control kidney. However, furosemide block Na^+-K^+ [NH_4^+]-2 Cl^- cotransporter induces lower ammonium index in the POK than in the CCK. Thus, decreased NH_4^+ excretion defect after release of 24 hrs. UUO is partly occured at thick ascending limb of Henle loop.

In the normal kidney, furosemide induces a moderate increase in ammonium excretion and ammonium index. It directly inhibits Na^+ - K^+(NH_4^+)-2Cl^- cotransporter at thick ascending limb of Henle's loop and therefore the unreabsorbed $NH4^+$ is flushed out . Besides an increase in urine flow rate by furosemide would favour an elevation of ammonium excretion by stimulating ammonium secretion along the distal nephron. The increase in luminal fluid flow rate enhances the gradient for NH_3 concentration [41]. Since this loop diuretic also leads to an increase in UpH of CCK, thus stimulation of distal hydrogen secretion may not be the major factor responsible for an increase in ammonium excretion by furosemide.

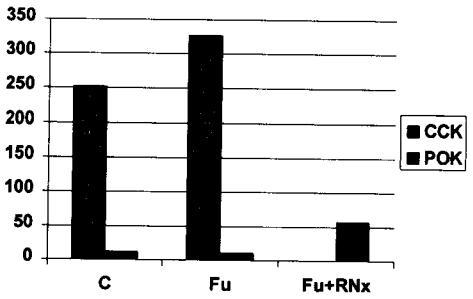

Figure 3 Effect of furosemide on ammonium excretion. Value are mean + SE.*,**,*** Significantly different at p<0.05,0.01 and 0.001, respectively.

In the POK, furosemide infusion causes a significant elevation in ammonium index. This pattern of change is similar to that observed in the CCK. The result may indirectly indicate that thick ascending limb of Henle's loop of the damaged nephron is still able to reabsorp Na^+, K^+,Cl^- and NH_4^+. Moreover, inhibition of NH_4^+reabsorption at this site may contribute to the elevation of ammonium index if GFR was not extremely decreased. Under this condition, tubular fluid flow rate as well as ammonium secretion along the nephron especially at proximal tubule may likely be reduced. However, the ammonium index was still markedly depressed compared to the CCK (50% of that value of the CCK in the control period). This severe depression of the ammonium index after UUO, even though inhibited NH_4^+ reabsorption at thick ascending limb of Henle's loop may be due to the generation and/or secretion of ammonium at proximal tubule, was markedly reduced.

After right nephrectomy, GFR of obstructed kidney increased to 180% of control period. The ammonium index of the POK in this period is approximate 280% of control period. This data indicates the effect of the increment of RBF and GFR on ammonium excretion. Elevation of GFR under this condition may also increase ammonium generation and secretion at the proximal tubule. Increasing blood flow as well as SNGFR would favour an increased delivery of substrate for ammoniagenesis and proximal secretion of ammonium. It is not known at present if changes in physical parameters alone are the major contributing factors in increasing ammonium index as the role of neural influence on renal ammoniagenesis after UUO can not be excluded. Moreover, ammonium index of the POK after right nephrectomy is insignificantly different from the control value of the CCK (data do not show) while GFR of the POK is only 30% of the CCK in control period. Thus, the major site of defect NH_4^+ excretion after released of 24 hrs. UUO should be proximal tubule.

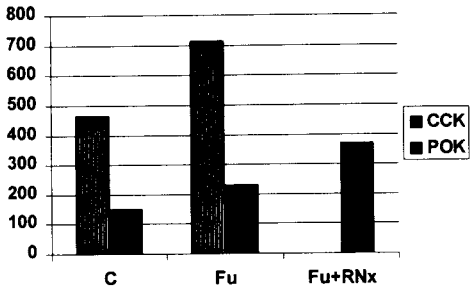

Figure 4 Effect of furosemide on ammonium index. Value are mean + SE.*,**,*** Significantly different at $p<0.05$,0.01 and 0.001, respectively.

5.Acknowledgement

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