Renal Hemodynamics and Ammoniagenesis

CHARACTERISTICS OF THE ANTILUMINAL SITE FOR GLUTAMINE EXTRACTION

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ABSTRACT Renal production of ammonia by the left kidney was studied in 31 acidotic dogs (NH_4Cl) after acute constriction of the renal artery. Renal ammoniagenesis fell in direct proportion with the reduction in glomerular filtration rate and renal plasma flow. The renal extraction of glutamine by the experimental kidney fell in direct proportion with the reduction in renal hemodynamics. Extracted glutamine remained greater than filtered glutamine indicating that both the luminal and antiluminal transport sites were operative. The relationship between renal extraction of glutamine and ammoniagenesis observed during control was maintained after renal artery constriction (1.7 μmol NH_3 produced for each μmol of glutamine extracted). Systemic venous or renal intra-arterial infusion of glutamine during arterial constriction increased renal production of ammonia to or above control values. These observations indicate that the mechanisms responsible for glutamine extraction and ammonia production were operating normally despite reduced hemodynamics. When measured immediately after arterial clamping, the renal venous pNH_3 was found to rise significantly decreasing progressively thereafter towards control values. The extracted fraction of total glutamine delivered to the kidney (31%) did not change after acute reduction of the glutamine load. Thus, the antiluminal extraction site was incapable of lowering renal venous plasma glutamine concentration below 0.33 μM/ml. In a second series of experiments, the properties of the antiluminal site of transport for glutamine were studied after complete occlusion of the left ureter in acidotic and nonacidotic animals. Under these circumstances, it was demonstrated that the antiluminal site is capable of extracting sufficient glutamine to maintain total ammonia production at 60% or more of control. In acidotic animals, changes in cellular pNH_3 appeared to play a key role on the antiluminal extraction of glutamine since the significant rise in renal blood flow often observed after ureteral occlusion prevented the rise in pNH_3 noted when blood flow remained constant. Thus, when renal blood flow rose glutamine extraction and ammonia production were maintained at control values. In these acidotic animals, glutamine infusion failed to influence ammonia production until luminal transport was restored by release of ureteral clamp and resumption of glomerular filtration. The latter observation establishes that reabsorbed glutamine is utilized at least in part for ammonia production.

INTRODUCTION

Oelert and Nagel have reported that after acute hemorrhage or aortic constriction above the renal arteries in dogs with normal acid-base status, renal ammoniagenesis decreases in proportion with the reduction in renal hemodynamics (1). More recently, Pilkington, Young, and Pitts have shown that in chronically acidotic dogs, ammonia production correlates equally well with glomerular filtration rate and renal blood flow when these parameters are acutely reduced (2). No data on the renal extraction of glutamine were provided in either study. Oelert and Nagel have suggested that reduced ammonia production is dependent mainly on reduction of glomerular filtration since occlusion of the ureter is accompanied by some reduction in ammoniagenesis while renal blood flow actually increases (1). Such conclusion may not be valid during chronic metabolic acidosis where glutamine is extracted from both the luminal and anti-

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luminal sites (2, 3). It is not known whether the same phenomenon occurs after acute reduction of the glutamine load. We have thus examined in detail the relationship between acute reduction in renal hemodynamics, glutamine extraction, and ammonia production in chronically acidotic dogs. Pilkington et al. have calculated that 35% of ammonia produced by intact acidotic dogs is derived from glutamine extracted at the antiluminal site (2). This calculation is based on the assumption that all filtered glutamine is utilized for ammonia production, an event which has never been demonstrated. To define the importance and the respective contribution of the luminal and antiluminal transport sites of glutamine to total ammonia production, we have also studied the extraction of glutamine and ammonia production after complete occlusion of the ureter in acidotic as well as nonacidotic animals.

METHODS

A first group of experiments were performed on 31 female mongrel dogs weighing between 10.9 and 24 kg (mean 17.4±0.57 SE). During the 3 days which preceded the experiment, each animal was given ammonium chloride (0.5 g/kg/day) mixed with soft commercial dog food to induce metabolic acidosis. The animals were also fed dog Purina Chow (Ralston Purina Co., St. Louis, Mo.) and had free access to water. Food was withheld during 12 h before anesthesia but free water intake was allowed. The animals were anesthetized with sodium pentobarbital and intubated. Respiration was controlled by a volume and rate adjustable respiration pump (Harvard Apparatus Co., Inc., Millis, Mass.) to maintain plasma P_{CO_2} near 40 mm Hg while the animals breathed room air. In all experiments, the left kidney was exposed through a midline abdominal incision. The renal artery was carefully dissected and an open Goldblatt clamp was put in place near the aortic origin. In six animals, a no. 25 G needle was introduced in the renal artery between the clamp and the kidney and a 5% dextrose infusion was begun at the rate of 1 ml/min with a Buchler polystaltic pump (Buchler Instruments Div., Nuclear-Chicago Corp., Fort Lee, N. J.). This set-up was used for intraarterial glutamine infusion after renal artery constriction. The left renal vein was catheterized through a femoral vein after ligation of the left ovarian vein. The position of the catheter was checked by palpation. Both ureters were catheterized and urine collected under mineral oil. Heparinized blood samples were drawn anaerobically from the femoral artery and the renal vein. All solutions were delivered through a femoral vein with a Bowman type infusion pump at the rate of 4.7 ml/min. The entire surgical procedure took less than 90 min during which time the animals received approximately 300 ml of isotonic saline. After completion of the surgical procedure, the animals were infused with an isotonic solution made up with equal amounts of 5% mannitol and 0.9% saline and containing appropriate quantities of creatinine and *p*-aminohippurate (PAH). After a 60 min equilibration period following the administration of a priming dose of creatinine, three 10-min collections of urine were taken as controls. The left renal artery was then constricted by tightening the Goldblatt clamp. This resulted in a significant drop in urine flow. The concentration of creatinine and PAH in the sustaining infusion was reduced to minimize variations in plasma concentration. Approximately 90 min elapsed before further collections of urine were taken under conditions of steady albeit reduced urine flow. Three 10-min collections of urine were then obtained along with corresponding femoral arterial and renal venous blood samples. Subsequently, in six animals, L-glutamine (Sigma Chemical Co., St. Louis, Mo.) was infused in a peripheral vein at the rate of 4 μM/kg/min after a priming dose of 200 μM/kg, whereas in six others it was infused directly in the constricted left renal artery at the rate of 7 μM/kg/min. After 15 min, three additional collections of urine were taken. In eight animals, femoral arterial and renal venous blood samples were drawn immediately after constriction of the renal artery and at 5, 10, 15, and 30 min to study early variations in the partial pressure of ammonia (pNH_3). In these eight animals, three additional 10-min urine collections were obtained after the replacement of the mannitol-saline-sustaining solution with isotonic sodium sulfate (Na_2SO_4) to augment acidification of the urine and facilitate cell to lumen diffusion of ammonia (4).

In a second group of 15 animals, experiments were performed on 7 acidotic and 8 nonacidotic female dogs to study ammonia production during complete ureteral occlusion. In these experiments, renal blood flow was measured with an electromagnetic flowmeter (Statham Instruments, Inc., Oxnard Calif., SP-2201). After three 10-min urine collections, the left ureter was completely occluded during 60 min. The concentration of creatinine in the sustaining solution was reduced to avoid significant variations in plasma concentration. The renal extraction of creatinine was measured to ascertain cessation of glomerular filtration during ureteral clamping. Although total cessation of glomerular filtration after complete occlusion of the ureter cannot be fully ascertained by low creatinine extraction ratio, it is most probable that this maneuver results in marked reduction of the glomerular filtration process (5, 6). At least three additional 10-min urine collections were taken 15 min after release of ureteral occlusions. In four of the seven acidotic and three of the eight nonacidotic animals, glutamine was infused during the last 30 min of ureteral clamping via a peripheral vein (4 μM/kg/min) after a priming dose of 200 μM/kg. This infusion was continued after releasing ureteral clamping.

Total ammonia production by the left kidney was estimated as the sum of ammonia added to the renal vein and that excreted in urine per minute. The renal extraction of glutamine was taken as the product of arteriovenous plasma concentration difference and corrected renal plasma flow. During infusion of glutamine in the renal artery, glutamine extraction was calculated as the product of its arterial plasma concentration by the corrected renal plasma flow plus the amount infused per minute in the renal artery minus the amount released in the renal vein. No glutamine could be detected in the urine at any time in significant amount. Exogenous creatinine clearance was used to determine glomerular filtration rate. The corrected PAH clearance was taken as the measure of effective renal plasma flow (except when stated otherwise). Total renal blood flow was calculated using the corresponding hematocrit value. All analytical methods used in this study has been previously reported (7). The partial pressure of ammonia ($_pNH_3$) in arterial and renal venous blood was calculated from total ammonia concentration and pH by the method of Denis, Preuss, and Pitts (8) using the equation of Jacquez, Poppell, and Jeltsch (9). In tables, results are recorded as means ±SE. Statistical difference was determined by a paired *t* test.

TABLE I

Blood, Plasma, and Renal Hemodynamic Values before and after Constriction of the Left Renal Artery

	Control	Clamping, after 90 min	*P*
Blood, *pH*	7.27±0.014	7.25±0.014	NS
Plasma HCO_3^-, *mM/liter*	15.55±0.631	14.89±0.661	NS
Plasma P_{CO_2}, *mm Hg*	35.36±1.842	35.25±1.733	NS
Glomerular filtration rate, *ml/min*	27.15±1.376	14.81±1.277	<0.001
Renal plasma flow, *ml/min*	118.9±6.96	58.0±4.73	<0.001
Renal blood flow, *ml/min*	199.9±12.47	93.1±7.64	<0.001
PAH extraction, %	73.1±1.46	73.4±1.86	NS

Values are means±SE.
n = 31.

RESULTS

In the first group of acidotic animals, the acid-base parameters in blood and plasma did not change after constriction of the renal artery. Glomerular filtration rate averaged 54% of control and ranged from 8 to 93%. Renal plasma flow averaged 49% of control and ranged from 9 to 92%. The renal extraction of PAH did not change after arterial clamping (Table I). There was a proportional relationship between the fall in glomerular filtration rate and that of renal plasma flow. However, the fall in filtration rate at each level was slightly greater than that of renal plasma flow (Fig. 1).

Constriction of the renal artery induced a fall in both urinary and renal vein ammonia so that ammonia production fell in direct proportion with the fall in glomerular filtration rate and renal plasma flow (Figs. 2 and 3). Renal ammoniagenesis averaged 52% of control. In these experiments, arterial and renal venous pNH_3 did not vary significantly after 90 min of clamping. Urinary pH rose from 6.16 to 6.43, titratable acid excretion fell from 8.09 to 2.55 μeq/min and bicarbonate excretion did not change. At the same time, urine flow was reduced by 43%, urinary sodium excretion by 81% and potassium excretion by 51%.

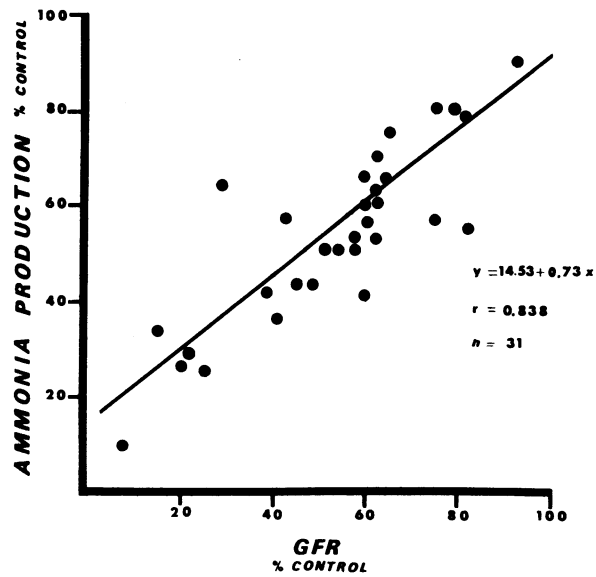

FIGURE 2 Relationship between ammonia production and glomerular filtration rate after acute constriction of the renal artery.

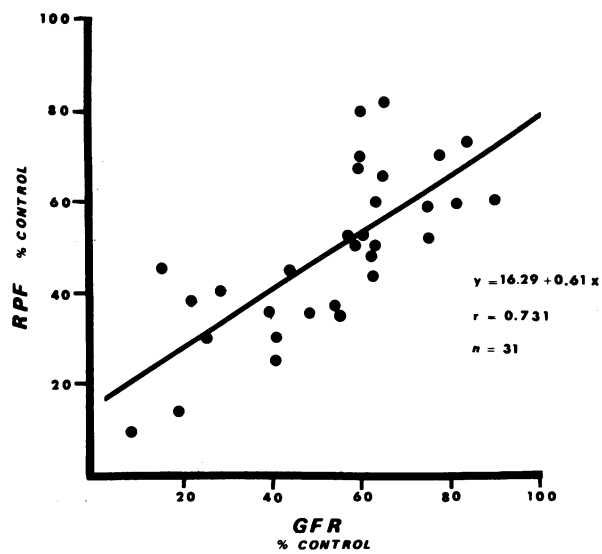

FIGURE 1 Relationship between renal plasma flow and glomerular filtration rate after acute clamping of the renal artery.

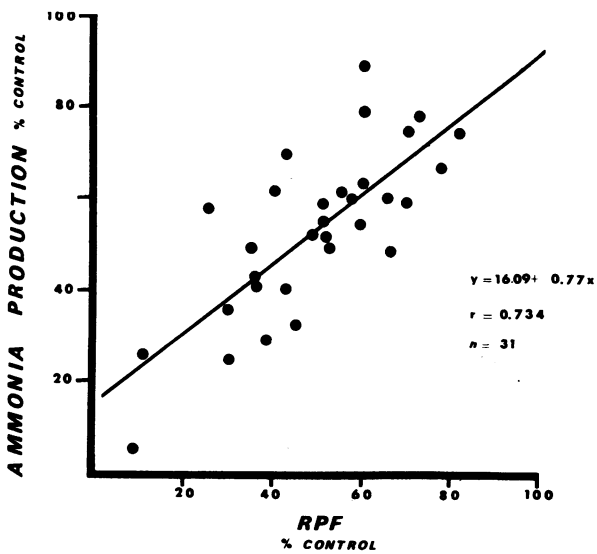

FIGURE 3 Relationship between ammonia production and renal plasma flow after acute constriction of the renal artery.

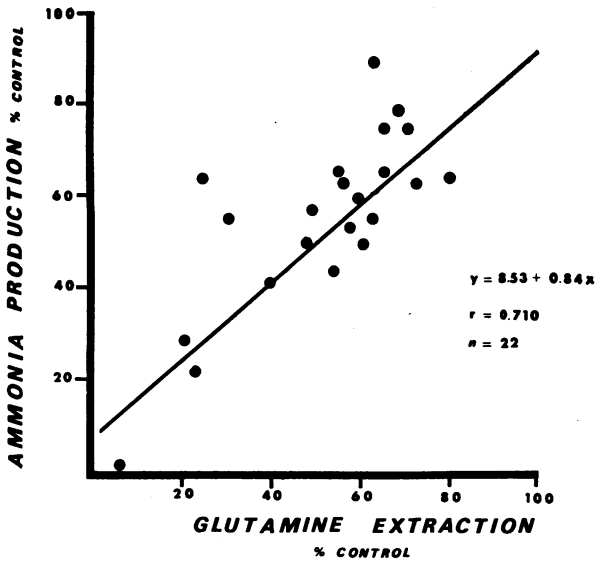

FIGURE 4 Relationship between ammonia production and renal extraction of glutamine after constriction of the renal artery.

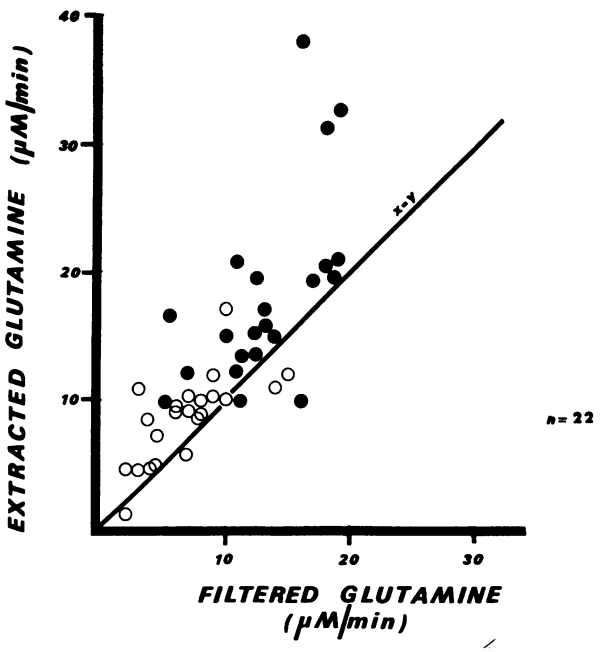

FIGURE 5 Relationship between extracted and filtered glutamine before and after constriction of renal artery.

In the 22 experiments where the renal extraction of glutamine was measured, it fell in direct proportion with the fall in ammoniagenesis (Fig. 4). The fall in glutamine extraction correlated equally well with the reduction of either glomerular filtration rate or renal plasma flow. In these experiments, extracted glutamine exceeded filtered glutamine slightly during both the control period and after constriction of the renal artery (Fig. 5). Filtered glutamine averaged 74% of extraction during control and 84% after constriction. Arterial and renal venous plasma glutamine did not change significantly after constriction of the renal artery so that the arteriovenous difference remained nearly identical before and after clamping. In addition, extraction of the total glutamine load delivered to the kidney did not exceed 31% during both control and renal artery constriction (Table II).

Fig. 6 shows the relationship between ammonia production and glutamine extraction in absolute amounts before and after constriction of the renal artery. The ratio of 1.81 found after constriction is not significantly different from that of 1.63 observed during control (Table II). For all experimental phases (control, clamping, clamping and glutamine infusion) ammonia production averaged 1.70 μM/min per μmol of glutamine extracted (Fig. 6). Systemic venous or renal arterial infusion of glutamine during clamping of the renal artery restored both ammonia production and renal extraction of glutamine near or above control values (Fig. 7). During systemic infusion of glutamine, the plasma arterial glutamine concentration almost doubled increasing from 0.499 to 0.893 μM/ml. When glutamine was infused directly in the renal artery, arterial (femoral) glutamine concentration did not change significantly. The values for pNH_3 in the renal venous blood rose from

TABLE II

Renal Handling of Glutamine after Renal Artery Constriction (Left Kidney)

	Control	Clamping, after 90 min	P
Arterial glutamine, μM/ml	0.513±0.025	0.483±0.027	NS
Renal venous glutamine, μM/ml	0.351±0.023	0.332±0.023	NS
(A-V) Glutamine, μM/ml	0.162±0.011	0.151±0.008	NS
Extracted glutamine, μM/min	17.89±1.641	8.56±0.790	<0.001
Filtered glutamine, μM/min	13.28±0.872	7.24±0.855	<0.001
$\frac{\text{Extracted}}{\text{Filtered}}$ Glutamine	1.35±0.109	1.18±0.140	NS
Total arterial glutamine load to the kidney, μM/min	56.65±5.097	27.38±2.641	<0.001
% of the total glutamine load extracted	31.6±2.10	31.3±1.79	NS
Total NH_3 production, μM/min	29.25±1.988	15.48±1.325	<0.001
$\frac{\text{Total } NH_3 \text{ production}}{\text{Extracted glutamine}}$	1.63±0.104	1.81±0.114	NS

Values are means±SE.
$n = 22$.

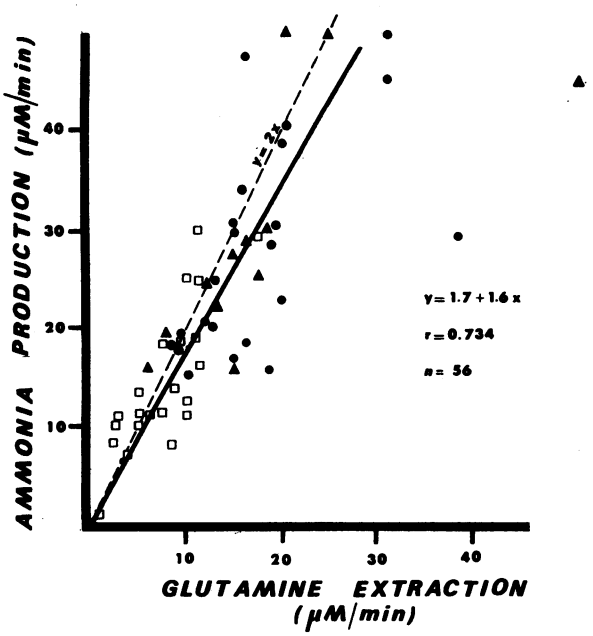

FIGURE 6 Relationship between ammonia production and glutamine extraction in absolute amounts after constriction of the renal artery and glutamine infusion. ●, control; □, clamping; ▲, glutamine infusion after clamping.

52 to 69 mm Hg during systemic glutamine infusion and from 61 to 133 mm Hg when glutamine was infused directly in the renal artery. The reduction in renal

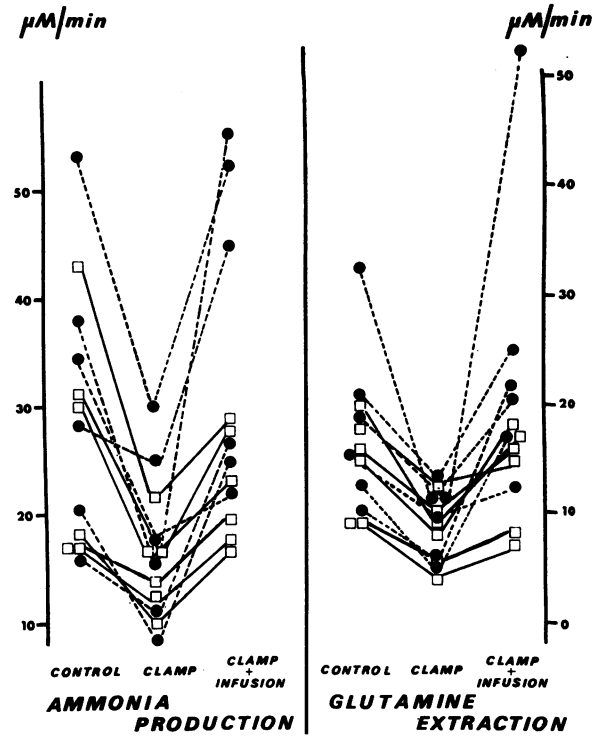

FIGURE 7 Effect of systemic or renal intra-arterial infusion of glutamine on ammonia production and extraction of glutamine after constriction of the renal artery. □, systemic infusion; ●, renal artery infusion.

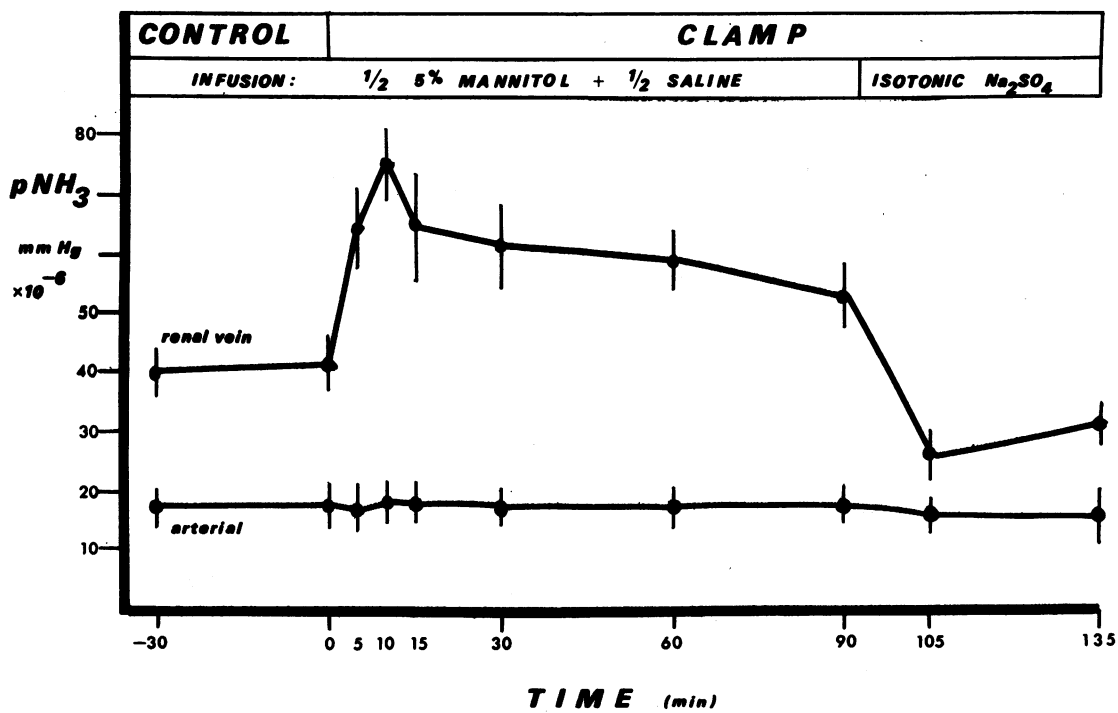

FIGURE 8 The effect of renal artery constriction on renal venous pNH_3 during infusion of either mannitol and saline or isotonic sodium sulfate.

hemodynamics induced by arterial constriction was unchanged during glutamine infusion.

When sodium sulfate was substituted for the mannitol-saline mixture during arterial clamping, urinary excretion of ammonia rose near control values (from 6.6 to 13.6 μeq/min). At the same time, urinary pH fell significantly (from 6.47 to 5.27), titratable acid excretion increased to control levels (from 3.1 to 9.6 μeq/min), and bicarbonate almost disappeared from the urine. However, as ammonia excretion rose, release of ammonia in the renal vein decreased in a proportional fashion so that total production of ammonia by the clamped kidney remained unchanged. The values for arterial and renal venous pNH_3 after clamping of the renal artery and infusion of sodium sulfate are shown on Fig. 8. When measured immediately after clamping the renal artery renal venous pNH_3 almost doubled during the first 15 min. It fell progressively thereafter remaining slightly above control values at 90 min. When sodium sulfate was substituted for the saline and mannitol mixture, renal

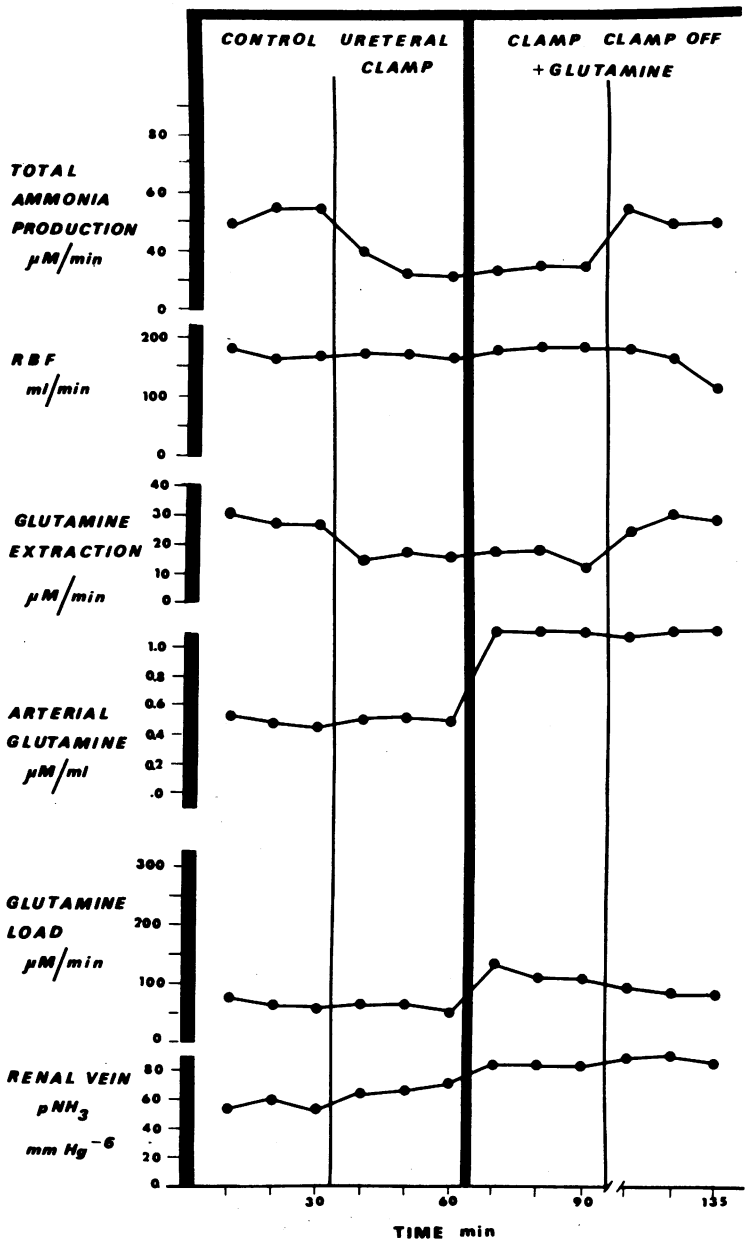

FIGURE 9 The effect of ureteral occlusion and subsequent glutamine infusion on ammonia production and glutamine extraction in an acidotic dog (no. 14) with constant renal blood flow.

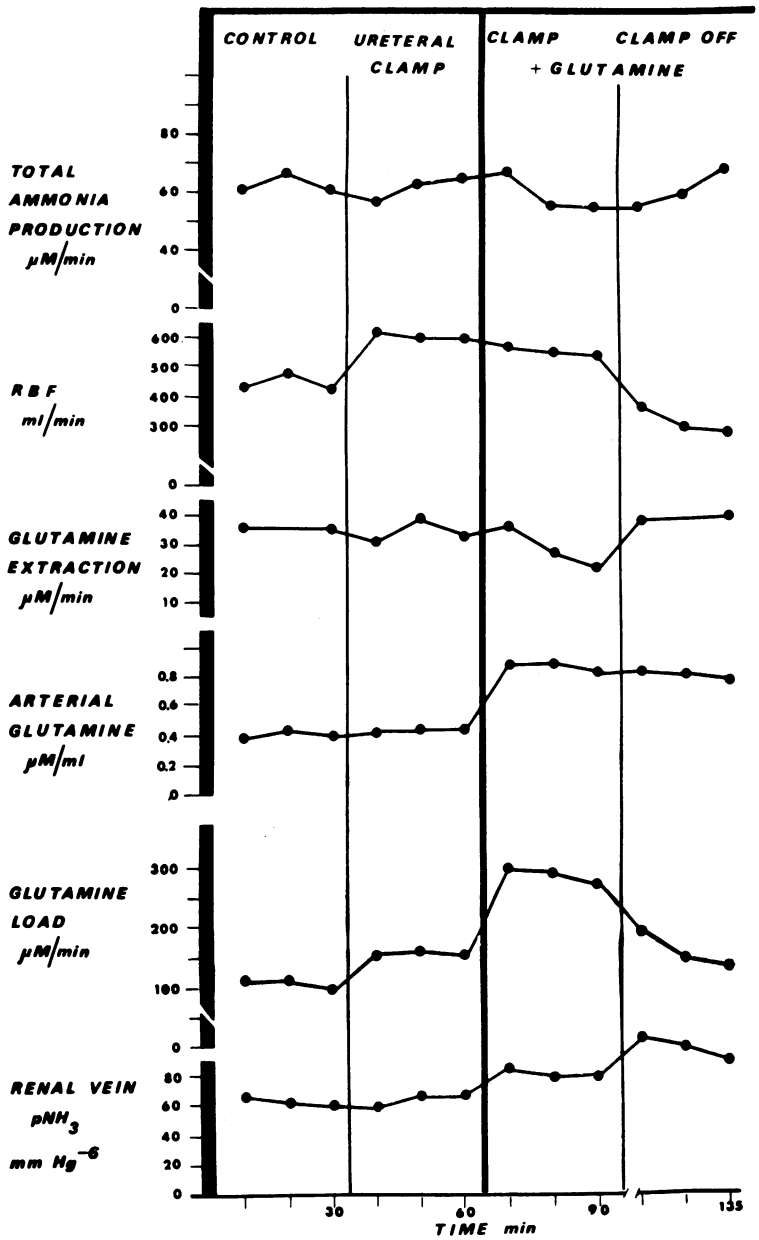

FIGURE 10 The effect of ureteral occlusion and subsequent glutamine infusion on ammonia production and glutamine extraction in an acidotic dog (no. 18) with rising renal blood flow.

venous pNH_3 fell rapidly within 15 min to reach values below that observed in the control period. In these studies, arterial blood pNH_3 did not change (Fig. 8).

Complete ureteral occlusion resulted in two types of renal hemodynamic response in both acidotic and non-acidotic dogs. Renal blood flow either rose by 45% or did not vary. The acidotic animals without changes in renal blood flow showed a significant decrease in ammonia production and glutamine extraction after ureteral occlusion (Fig. 9). In these experiments, arterial plasma glutamine did not vary while the percentage of the total glutamine load extracted by the kidney fell by some 25%. Renal vein pNH_3 showed a net tendency to rise (Fig. 9) but these variations were not significant at the 0.05 level. In contrast, total ammonia production and glutamine extraction did not change after ureteral occlusion in acidotic animals presenting a significant rise in renal blood flow (Fig. 10). In this situation, the total load of glutamine delivered to the kidney rose significantly while arterial glutamine concentration did not change. The percentage

of the total glutamine load extracted by the kidney was therefore decreased by 40%. Renal venous pNH_3 remained constant (Fig. 10). When the changes in ammonia production are corrected to eliminate variations in renal blood flow, it is obvious (as shown in Fig. 11) that in all animals total ammonia production will fall by 40% after complete ureteral occlusion.

When L-glutamine is infused in acidotic animals during continued ureteral occlusion, no change in renal extraction of glutamine or total ammonia production is observed irrespective of variations in renal blood flow (Figs. 9–11). However, after releasing the ureteral clamp, renal ammonia production and glutamine extraction rise in spite of a fall in renal blood flow (Figs. 9 and 10). This response is more clearly illustrated in Fig. 11 where variations in renal blood flow are artificially eliminated.

In the nonacidotic animals, the already low total ammonia production fell by some 30% after ureteral occlusion irrespective of the changes in renal blood flow. The fall in ammonia production averaged 40% when corrected for variations in renal blood flow. (Fig. 11). Renal venous pNH_3 did not change in these experiments. When L-glutamine was infused during ureteral clamping, ammonia production and glutamine extraction rose near control values (Fig. 11). However, when the ureteral clamp was released, no additional effect was observed with continued glutamine infusion.

In all animals, the renal extraction of creatinine fell from more than 20% to less than 4% within 20 min to reach values of less than 1% after 60 min of ureteral occlusion. Upon release of the ureteral clamp, creatinine extraction returned to near control values.

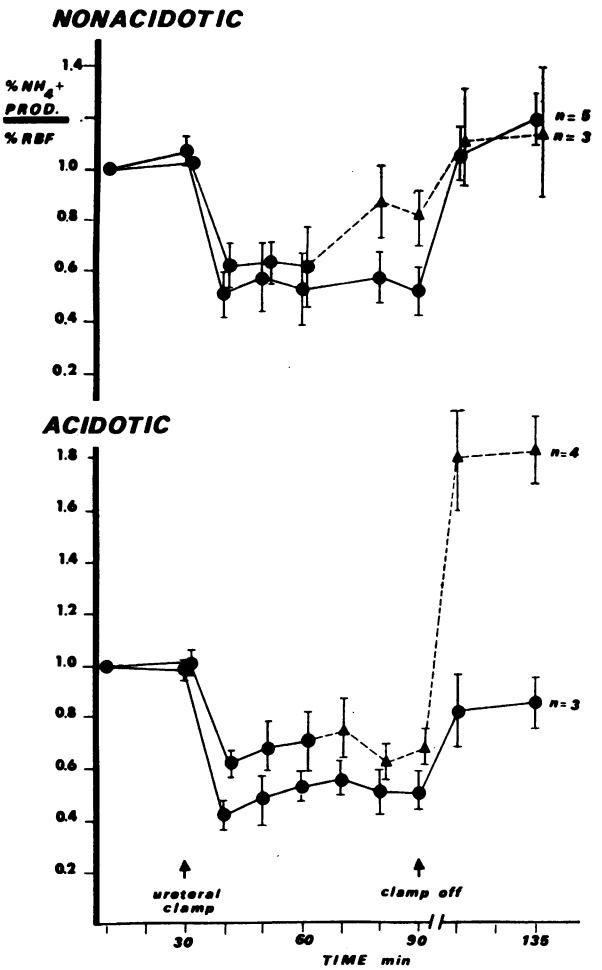

FIGURE 11 The effect of ureteral occlusion and glutamine infusion on ammonia production corrected for changes in renal blood flow in acidotic and nonacidotic dogs. ●, no glutamine; ▲, glutamine infusion.

DISCUSSION

The present study demonstrates that renal ammoniagenesis is directly affected by acute reduction in renal hemodynamics. The observed fall in ammonia production correlated equally well with the reduction in glomerular filtration rate and renal plasma flow. Such correlation was still apparent even at very low values for both parameters achieved by severe constriction of the renal artery. These results confirm those obtained in a small number of dogs by Oelert and Nagel during normal acid-base balance (1) and Pilkington et al. during metabolic acidosis (2) as well as those of Aber, Morris, Housley, and Harris in human subjects with emphysema breathing oxygen (10). It is now clear from the present work that the fall in ammonia production is associated with a proportional decrease in the renal extraction of glutamine. The present study also establishes the following new findings: (*a*) both the luminal and antiluminal transport sites for glutamine extraction are operative in metabolic acidosis at all levels of glomerular filtration and renal plasma flow; (*b*) the mechanisms responsible for glutamine extraction and ammonia production are functioning normally after acute constriction of the renal artery; (*c*) failure of the antiluminal transport site to lower renal venous glutamine concentration below 0.33 μM/ml suggests the existence of another rate-limiting step at this site in addition to cellular pNH_3 changes, (*d*) in the absence of significant glomerular filtration, antiluminal glutamine transport is capable of sustaining ammoniagenesis at 60% and more of control; and (*e*) at least part of filtered glutamine is utilized for ammonia production.

It has been demonstrated in the dog that the capacity of the renal tubular cell to reabsorb glutamine in both metabolic alkalosis and acidosis is so high that only traces of this aminoacid appear in the urine even during conditions of exogenous glutamine loading (2, 11). Under normal acid-base status, filtered glutamine may exceed the amount extracted by the kidney (2, 3). It

is thus evident that in this situation an undetermined fraction of filtered glutamine must return to the renal venous circulation. It is also quite possible that significant extraction of glutamine normally takes place across the antiluminal membrane of the renal tubular cell (2). It has been shown that in chronic metabolic acidosis, glutamine enters the renal tubular cell at both luminal and antiluminal sites since filtered glutamine is significantly lower than the total extraction of this aminoacid by the kidney (2, 3). Such observations indicate that the antiluminal membrane can be the site of a bidirectional flux of glutamine. In the present study, extracted glutamine exceeded filtered glutamine in most instances even after renal artery constriction. We can therefore conclude that after acute reduction of renal blood flow and glomerular filtration during acidosis, glutamine extraction although significantly depressed still occurs both at the luminal and antiluminal sites of the tubular cell, the latter site accounting for at least 15% of the total glutamine extracted by the kidney. Although we cannot define the relative contribution of each transport site to glutamine extraction and ammonia production under these circumstances, it is obvious that the extraction of glutamine was affected at both sites of transport following renal artery constriction.

Theoretically, each molecule of glutamine could yield two molecules of ammonia after extraction and utilization by the renal tubular cell. The contribution of glutamine to intrarenal synthesis of other aminoacids such as alanine and serine (3, 12, 13) may lower this ratio to values somewhat less than the theoretical ratio. The observed value of 1.6–1.8 μmol of ammonia produced by the kidney per min for each micromole of glutamine extracted is not different from that reported by others during acidosis alone (2, 14) and acidosis with glutamine loading (11). It is of interest that this relationship did not change after acute constriction of the renal artery. This suggests that the mechanisms responsible for the utilization of glutamine by the renal tubular cell were operating normally after arterial clamping. That extracted glutamine continues to be utilized normally after constriction of the renal artery is further suggested by the fact that either systemic venous or renal intra-arterial infusion of glutamine restores both ammonia production and renal extraction of glutamine near or above control values. These observations emphasize the importance of substrate availability in renal ammoniagenesis during acute reduction of renal hemodynamics. It is also significant that other tubular functions such as extraction of PAH and acidification of the urine were not affected after renal artery constriction.

The extraction of glutamine by the kidney did not exceed 31% of the total load during both control and after constriction of the renal artery. Whereas net extraction of glutamine fell by more than 50%, the renal arteriovenous glutamine difference did not change after constriction. Since we are dealing with a situation whereby renal ammoniagenesis is stimulated by a state of chronic metabolic acidosis, one wonders why the renal tubular cell seems incapable of extracting more than 30% of the glutamine load when the latter is acutely reduced. Even if all filtered glutamine is utilized, it is obvious that the antiluminal site is incapable of extracting the same amount of glutamine as before constriction. Otherwise lower concentrations of glutamine in the renal venous blood would have been observed and no direct relationship between reduced hemodynamics and glutamine extraction would have been noted. Since this did not occur, one must envisage a limitation for the renal tubular cell to establish a higher concentration gradient for glutamine with the peritubular blood. It is of interest that the concentration of glutamine in the renal venous blood remained around 0.35 μmol/ml after constriction. This value is essentially the same as that found in whole renal tissue of the dog during metabolic acidosis (15). This observation could suggest that the transport of glutamine at the antiluminal site is a passive phenomenon. However, an active transport could also have been modified after arterial clamping by intracellular changes.

In the present study, the partial pressure of ammonia in the renal vein rose immediately and significantly during the first 15 min after clamping of the renal artery. It fell however progressively thereafter but remained slightly above control at 90 min. This observation is different from that of Oelert and Nagel who reported no change in renal venous pNH_3 during suprarenal aortic clamping (1). Such discordance may be attributed to the fact that in the latter studies pNH_3 was not measured before 20 min after clamping whereas most of the animals studied were not acidotic and showed low ammonia production (1). It is of interest that renal venous ammonia has been found to rise markedly upon the release of total renal artery clamping with return to control levels within 5 min (16). If as suggested by Denis et al. (8) renal venous pNH_3 reflects the pNH_3 in the renal tubular cell, one could postulate that the diminished antiluminal extraction of glutamine observed after renal artery constriction is secondary to the rise in cellular pNH_3. Available evidence indicates than an increase in pNH_3 of tubular cells lowers renal extraction of glutamine and production of ammonia (17) by inhibiting the glutaminase I reaction (18, 19) and inducing intracellular pH changes (18). It is quite possible that the immediate and transient rise in renal venous pNH_3 which follows renal artery constriction sets a new rate of antiluminal glutamine extraction and ammonia production with subsequent restoration of

pNH_3 to control values. Infusion of sodium sulfate led to a rapid decrease in pNH_3 whereas total production of ammonia remained unchanged during continued constriction of the renal artery. In fact, the only effect of sodium sulfate was to induce a shift of ammonia from the renal vein to the tubular fluid after the rapid drop in urinary pH induced by the unreabsorbable anion (4). The same phenomenon has been shown to occur in acidotic dogs with normal renal hemodynamics (5). The fact that renal ammoniagenesis does not change when cellular pNH_3 decreases further suggests the existence of an additional limit for antiluminal glutamine extraction which is independent of pNH_3 changes and characterized as previously mentioned by the failure to establish lower renal vein glutamine concentration.

Total or near total reduction of luminal transport by complete ureteral clamping discloses that the antiluminal site is capable of extracting all the glutamine necessary to maintain renal ammoniagenesis in acidotic dogs at 60% of control when renal blood flow is constant and even at 100% when blood flow rises. Such values are much higher than the derived value of 35% calculated by Pilkington et al. in intact acidotic dogs (2). It is also obvious that the observed rise in renal blood flow does not affect glutamine extraction and ammonia production by simply increasing the glutamine load delivered to the kidney since infusion of glutamine sufficient to double arterial concentration of this aminoacid has no effect on the antiluminal extraction of glutamine regardless of blood flow changes. Severe reduction of ammonia diffusion in the tubular lumen after ureteral occlusion should result in intracellular accumulation of ammonia and a rapid rise in pNH_3. Such an effect is supported by the rise in renal venous pNH_3 observed in the acidotic animals showing no change in renal blood flow. It is likely that the elevation of renal blood flow which often takes place following occlusion of the ureter (20, 21) prevents a rise in cellular pNH_3 through a washout effect which allows the maintenance of constant ammonia production. It would thus appear that the antiluminal site of glutamine extraction is markedly affected by a rise in pNH_3. The latter could either modify the cellular utilization of glutamine or inhibit the antiluminal transport of this aminoacid. It is unlikely that intracellular accumulation of ammonia free-base affects the transport of glutamine directly at the peritubular border since intravenous infusion of ammonium chloride induces a rise in pNH_3 and decreased glutamine extraction from the renal blood while the concentration of free glutamine in the renal cortical cells actually increases (18). On the other hand, ammonia could modify the transport of glutamine across the mitochondrial membrane within the renal tubular cell since such a transport appears to be a rate-limiting factor in the utilization of glutamine and production of ammonia in chronic acidosis (22).

The present study does not define the mechanism whereby glutamine is transported across the peritubular border of the renal cell. Failure of the latter to establish an overt concentration gradient for glutamine with the peritubular blood after acute constriction of the renal artery has already been mentioned as an indication that the transport of glutamine at the antiluminal site may be a passive phenomenon. In this respect, doubling the arterial concentration of glutamine during complete ureteral occlusion should enhance the intracellular diffusion of glutamine at the antiluminal border and stimulate ammonia production. However, the fact that glutamine extraction and ammonia production did not rise in acidotic animals during glutamine infusion regardless of whether renal blood flow increased or remained constant may be taken as evidence favoring already saturated active transport. The observed rise of pNH_3 in dogs with constant renal blood flow could be viewed as the major rate-limiting factor in the extraction of glutamine and production of ammonia. Already saturated transport could explain the lack of influence of glutamine infusion in animals with rising renal blood flow in which pNH_3 did not change. It is possible however that a greater exogenous load of glutamine might have resulted in increased glutamine extraction and ammonia production. It has been shown that glutamine loading increases ammonia production in acidotic dogs (10). The present study also demonstrates such an effect after acute constriction of the renal artery. Under these circumstances however, both the luminal and antiluminal sites for glutamine transport are available. It is noteworthy that when the ureteral clamp is released and the luminal site restored, ammonia production rises significantly during continued glutamine infusion when corrected for changes in renal blood flow. It would thus appear that the presence of the active luminal site of transport (2) is necessary to observe stimulation of ammoniagenesis by glutamine infusion. This observation as well as the fact that ammonia production corrected for blood flow changes drops by 40% when the ureter is completely occluded constitutes strong evidence that at least part of the reabsorbed glutamine is utilized for ammonia production.

It is of interest that our findings in nonacidotic antimals after ureteral occlusion are in perfect agreement with those of Oelert and Nagel (1). Ammonia production fell by 30% regardless of blood flow changes whereas pNH_3 remained essentially unchanged. With regard to the interpretation of the data, we must disagree with these authors when they state that renal ammonia production is predominantly dependent on the supply of substrate to the luminal membrane of the tubular cells (1). This cannot be so if according to their own data

and ours the antiluminal site is able to extract sufficient glutamine to maintain ammonia production at 70% of the control value after constriction of the ureter. It is quite difficult to explain why glutamine infusion, in contrast to its lack of effect in the acidotic animals, was able to restore glutamine extraction and ammonia production near control values during ureteral occlusion. It should be pointed out that in these animals, total ammonia production is quite low when compared with that of acidotic animals. Thus, a slight influence of glutamine on ammonia production may be sufficient to restore ammonia production. It is clear however that upon release of ureteral clamping, glutamine infusion had no additional effect in these animals.

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