

## Glutamine Metabolism in the Kidney during Induction of, and Recovery from, Metabolic Acidosis in the Rat

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Experiments were carried out on rats to evaluate the possible regulatory roles of renal glutaminase activity, mitochondrial permeability to glutamine, phosphoenolpyruvate carboxykinase activity and systemic acid-base changes in the control of renal ammonia ( $\text{NH}_3$  plus  $\text{NH}_4^+$ ) production. Acidosis was induced by drinking  $\text{NH}_4\text{Cl}$  solution *ad libitum*. A pronounced metabolic acidosis without respiratory compensation [ $\text{pH} = 7.25$ ;  $\text{HCO}_3^- = 16.9$  mequiv./litre;  $p\text{CO}_2 = 40.7$  mmHg (5.41 kPa)] was evident for the first 2 days, but thereafter acid-base status returned towards normal. This improvement in acid-base status was accompanied by the attainment of maximal rates of ammonia excretion (onset phase) after about 2 days. A steady rate of ammonia excretion was then maintained (plateau phase) until the rats were supplied with tap water in place of the  $\text{NH}_4\text{Cl}$  solution, whereupon  $p\text{CO}_2$  and  $\text{HCO}_3^-$  became elevated [55.4 mmHg (7.37 kPa) and 35.5 mequiv./litre] and renal ammonia excretion returned to control values within 1 day (recovery phase). Renal arteriovenous differences for glutamine always paralleled rates of ammonia excretion. Phosphate-dependent glutaminase and phosphoenolpyruvate carboxykinase activities and the rate of glutamine metabolism ( $\text{NH}_3$  production and  $\text{O}_2$  consumption) by isolated kidney mitochondria all increased during the onset phase. The increases in glutaminase and in mitochondrial metabolism continued into the plateau phase, whereas the increase in the carboxykinase reached a plateau at the same time as did ammonia excretion. During the recovery phase a rapid decrease in carboxykinase activity accompanied the decrease in ammonia excretion, whereas glutaminase and mitochondrial glutamine metabolism *in vitro* remained elevated. The metabolism of glutamine by kidney-cortex slices (ammonia, glutamate and glucose production) paralleled the metabolism of glutamine *in vivo* during recovery, i.e. it returned to control values. The results indicate that the adaptations in mitochondrial glutamine metabolism must be regulated by extra-mitochondrial factors, since glutamine metabolism *in vivo* and in slices returns to control values during recovery, whereas the mitochondrial metabolism of glutamine remains elevated.

The mammalian kidney excretes strong acids largely as their ammonium salts and thus metabolic acidosis in many species is accompanied by increased ammonia\* excretion (Pitts, 1971). Glutamine is the major precursor of urinary ammonia and induction of metabolic acidosis in humans (Owen & Robinson, 1963), dogs (Shalhoub *et al.*, 1963) and rats (Squires *et al.*, 1976) is accompanied by increased renal extraction of plasma glutamine. The regulation of renal glutamine metabolism in rat and in dog is of considerable interest. Metabolic acidosis in the rat is accompanied by increased activities of phosphate-dependent glutaminase (EC 3.5.1.2) (Rector *et al.*, 1955) and of phosphoenolpyruvate carboxykinase (EC 4.1.1.32) (Alleyne & Scullard, 1969). The rates of glutamine metabolism by isolated mitochondria are

increased (Adam & Simpson, 1974; Brosnan & Hall, 1977), which may indicate increased transport of glutamine into mitochondria, and the rates of renal gluconeogenesis are substantially increased (Goodman *et al.*, 1966). All of these effects have been considered to be of regulatory significance. These variables have been investigated with reference to rates of ammonia excretion *in vivo* during acute metabolic acidosis (Alleyne & Scullard, 1969; Alleyne, 1970), and marked agreement was observed between ammonia excretion and phosphoenolpyruvate carboxykinase activity. On the other hand, the idea of an enhanced mitochondrial uptake of glutamine, first suggested by Pitts (1972), has received strong experimental support from the work of Adam & Simpson (1974), Simpson & Adam (1975) and Goldstein (1975). The possibility that the total renal activity of phosphate-dependent glutaminase could be regu-

\* Throughout this paper  $\text{NH}_3$  refers to the dissolved gas and ammonia refers to the sum of  $\text{NH}_3$  and  $\text{NH}_4^+$ .

latory has been generally discounted, since Rector & Orloff (1959) have shown that no increase in activity occurs in the acidotic dog and Goldstein (1965) and Bignal *et al.* (1968) found that administration of actinomycin D to acidotic rats inhibits enzyme induction but does not prevent the increase in ammonia excretion. However, owing to the toxicity of actinomycin D, these experiments were performed for only 1 day, and conclusions drawn are valid only for the first day of acid challenge. In addition Benyajati & Goldstein (1975) indicated that the total activity of glutaminase may be rate-limiting in infant rats.

The present experiments represent an attempt to examine the importance of these various parameters during induction and maintenance of metabolic acidosis in the rat and during recovery from acidosis. These results are compared with ammonia excretion and glutamine renal arteriovenous differences *in vivo* and with blood acid-base parameters.

## Experimental

### Animals

Male Sprague-Dawley rats (250–400 g), obtained from Canadian Breeding Farms and Laboratories, St. Constant, La Prairie, Que., Canada, were used in all experiments. They were fed on Purina rat chow (Ralston Purina of Canada, Don Mills, Ont., Canada) and, depending on the experiment, were allowed free access to either tap water or to 1.5% (w/v)  $\text{NH}_4\text{Cl}$ . Recovery simply involved replacing the  $\text{NH}_4\text{Cl}$  drinking solution with tap water. For the measurement of urinary ammonia excretion, rats were placed in individual metabolic cages (Acme Metal Products, Chicago, IL, U.S.A.) and urine was collected under mineral oil. For the measurement of renal arteriovenous differences of glutamine, rats were anaesthetized with sodium pentobarbital (6.5 mg/100 g body weight, intraperitoneally,) blood samples were taken, plasma was deproteinized and glutamine was measured as described by Squires *et al.* (1976). Blood  $p\text{CO}_2$  and blood pH were measured on conscious rats with an IL-213 blood-pH gas analyser (Instrumentation Laboratories, Lexington, MA, U.S.A.). Rats were placed into a restraining cage and blood samples were taken into 100  $\mu\text{l}$  heparinized capillary tubes after the tail had been pricked with a sharp scalpel. This procedure obtains capillary blood.  $\text{HCO}_3^-$  concentration was calculated from the  $p\text{CO}_2$  and pH by using the Henderson-Hasselbach equation:

$$\text{pH} = 6.10 + \log \left( \frac{[\text{HCO}_3^-]}{0.03 p\text{CO}_2} \right)$$

### Use of metabolic cages

The use of metabolic cages entails certain errors. In the measurement of urinary ammonia excretion the

principal potential errors are (a) spillage of the  $\text{NH}_4\text{Cl}$  drinking solution into the urine, (b) spillage of food and faeces into the urine, (c) evaporation of urine on the collecting funnel, (d) evaporation of the collected urine and (e) bacterial degradation of urea and other nitrogenous substances in urine to yield  $\text{NH}_3$ . These potential errors were minimized, and where possible the extent to which they occurred was estimated.

The spout of the water bottle was placed outside the cage, about 2.5 cm from the back wall, and the rats had access to it through an opening in the back wall. Thus any spillage could not contaminate the urine. There was considerable spillage of food into the urine when cubed chow was used, since the rats withdrew cubes from the feeding cup into the body of the cage and broken pieces readily fell through the wire mesh floor into the urine. This problem was minimized by grinding the food to a powder. However, inevitably, some food (about 1 g/day) found its way into the urine. That this could not be a significant source of ammonia in the urine was shown by experiments in which 1 g of food was soaked in 15 ml of water for 24 h. The quantity of  $\text{NH}_3$  subsequently found in the water was less than 1% of the normal daily excretion. Contamination from faeces also presents a problem. A similar experiment showed that such contamination was not appreciable.

The collecting funnels were treated with a silicone solution (Siliclad: Clay Adams, Parisippany, NJ, U.S.A.) every other day to improve urine drainage. The extent of evaporation from the funnels was estimated as follows. Urine was collected for 24 h and the collecting funnels were then washed thoroughly with water. The collected urine and the water washes were then analysed for creatinine (Di Giorgio, 1974). On average, the washes contained 6% of the total excreted creatinine. Corrections were made for this error.

Urine was collected under mineral oil to prevent evaporation. No other protection against bacterial contamination and action was provided, but experiments were carried out to determine whether freshly voided urine stored in this fashion for 24 h resulted in increased ammonia concentrations. No increase was found.

In general, fluid intake did not vary during the induction of acidosis, except for the first day when intake was about a half of that of control rats. Urine volume was constant during the acidotic regimen. However, during recovery, fluid intake and urine volume was increased by 50–100%.

### Urine analyses

Urinary ammonia and phosphate were measured on suitably diluted samples by the methods of Kirsten *et al.* (1963) and Fiske & Subbarow (1925) respectively. Titratable acidity was measured by titrating

samples of urine, diluted 1:10 with 0.167M-NaCl, with 2mM-NaOH in 0.167M-NaCl. Titrations were carried out to blood pH. The ionic strength of plasma is equivalent to that of 0.167M-NaCl (Van Slyke *et al.*, 1925) and therefore errors in the determination of titratable acid due to the effect of ionic strength on the  $pK'$  values of urinary acids (principally the  $pK'_2$  of phosphoric acid; Nutbourne, 1961) will be minimized.

#### *Enzyme assays*

The entire right kidney was removed, freed from fat and connective tissue, weighed, cut into small pieces with scissors and suspended in 50vol. of an ice-cold homogenization medium consisting of 0.25M-sucrose, 0.15M-NaF, 3mM-Tris and 1mM-EGTA, adjusted to pH7.4 with HCl. Homogenization was carried out in a smooth-glass Potter-Elvehjem homogenizer with a motor-driven Teflon pestle (clearance 0.15mm). One part of the homogenate was centrifuged for 30min at 30000g and 0°C and 0.1ml samples of the supernatant were used for the measurement of phosphoenolpyruvate carboxykinase activity. The assay used was that of Seubert & Huth (1965), except that NaF was omitted from the incubation medium, since it was present in the homogenization medium. In our standard assay 0.1ml samples of homogenate were incubated for 15min. Phosphate-dependent glutaminase was assayed on uncentrifuged samples of the homogenate by the spectrophotometric method of Curthoys & Lowry (1973). The inclusion of NaF in the homogenate was shown to be without effect on the activity of this enzyme.

#### *Experiments with isolated mitochondria*

Kidney-cortex mitochondria were prepared as previously described (Kalra & Brosnan, 1974). Oxygen consumption was measured at 30°C with a Clark-type oxygen electrode as described by Brosnan & Hall (1977). Glutamine, glutamate, 2-oxoglutarate and succinate were used as substrates, all at a final concentration of 2mM. Mitochondrial ammonia production was measured by a modification of the incubation procedure of Hird & Marginson (1968). The incubation medium contained 0.14M-KCl, 15mM-KH<sub>2</sub>PO<sub>4</sub>, 1mM-MgCl<sub>2</sub>, 2mM-EDTA, 23mM-glucose, 2mM-ADP, 10mM-sucrose (from the mitochondrial suspension), 20mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] (pH7.2), bovine serum albumin (fatty acid-free) (1mg/ml) and dialysed hexokinase [at least 8 units ( $\mu$ mol/min)]. Glutamine (2mM) was used as substrate. Incubations were carried out at 30°C in unstoppered 25ml Erlenmeyer flasks, shaken at 100 strokes/min. After 15min, the incubations were terminated by the addition of 0.2ml of 70% (w/v) HClO<sub>4</sub>. Denatured protein was removed by centrifugation (2000g for 10min),

and the supernatant was used for the assay of ammonia. Ammonia was measured after removal of glutamine by an adaptation of the method of Sherrard & Simpson (1969). NH<sub>4</sub><sup>+</sup>, present in 0.5ml samples of the supernatants, was adsorbed on batches (about 0.3g) of the cation-exchange resin AG 50W-X8 (50-100 mesh; Na<sup>+</sup> form; Bio-Rad Laboratories, Richmond, CA, U.S.A.). Glutamine was removed by washing several times with NH<sub>3</sub>-free water and the adsorbed NH<sub>4</sub><sup>+</sup> was then eluted with 1ml of 2M-CsCl and measured colorimetrically by the indophenol reaction (Di Giorgio, 1974).

#### *Experiments with kidney-cortex slices*

Slices (approx. 20-30mg) were cut free-hand and were incubated as described by Krebs *et al.* (1963) for 90min, except that the concentration of Ca<sup>2+</sup> was 1.25mM. After termination of the reaction by addition of 0.45ml of 70% (w/v) HClO<sub>4</sub> the contents of each flask were homogenized in a Potter-Elvehjem homogenizer, the precipitated proteins removed by centrifugation (2000g for 10min), the supernatants neutralized with K<sub>3</sub>PO<sub>4</sub>, and glucose (Bergmeyer & Bernt, 1974), glutamate (Bernt & Bergmeyer, 1974) and ammonia (see above) were measured.

#### *Materials*

Enzymes, cofactors and substrates were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. CsCl (Biological grade) was purchased from Schwarz/Mann, Orangeburg, NY, U.S.A.

#### *Presentation of data and statistical treatment*

Data are presented as means  $\pm$  s.d. with the numbers of experiments in parentheses. Enzyme activities are expressed as total activity per 24h, to facilitate comparison with daily ammonia excretion. Statistical significance was determined by Student's *t* test. *P* values of less than 0.05 were taken to indicate statistical significance.

#### **Results**

##### *Urinary acid excretion and renal arteriovenous differences for glutamine*

The renal excretion of acid by rats during and after NH<sub>4</sub>Cl-induced acidosis is shown in Fig. 1. Urinary pH fell immediately, reaching its lowest point after 2 days' acidosis. Thereafter the pH increased, returning to values close to control after 7 days. Urinary pH increased during recovery. Titratable acidity also increased 2-3-fold during NH<sub>4</sub>Cl-induced acidosis and returned to control values after 1 day of recovery. There was little titratable acid in the urine after 2 days

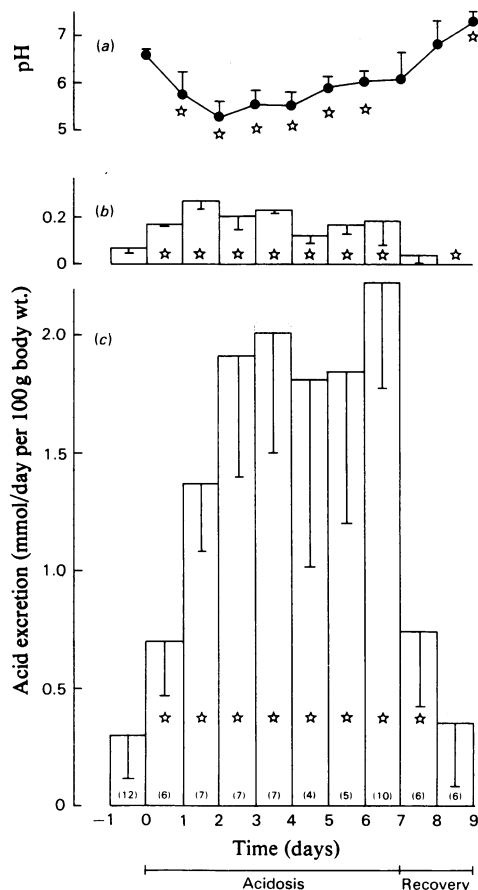


Fig. 1. Excretion of ammonia (c) and titratable acid (b) and urine pH (a) during and after metabolic acidosis. The vertical bars indicate 1 s.d.; numbers of experiments are given in parentheses. The stars indicate a significant difference from the control ( $P < 0.05$ ).

of recovery. Although substantial quantities of phosphate (Table 1) are excreted during recovery, the virtual absence of titratable acid is due to the relatively elevated urinary pH. Urinary phosphate excretion increased 2-fold on day 2 of acidosis, but was not significantly increased on other days. Titratable acid is essentially attributable to phosphate and the increased titratable acidity evident during  $\text{NH}_4\text{Cl}$ -induced acidosis is generally attributable to increased acid excretion (lower urinary pH) in the face of constant urinary phosphate. However, on the second day of acidosis the peak of titratable acid excretion is accomplished by a low urinary pH and an elevated excretion of phosphate (Table 1).

The renal excretion of ammonia by rats during and after  $\text{NH}_4\text{Cl}$ -induced acidosis is also shown in Fig. 1. The excretion of ammonia in the urine was

Table 1. Urinary phosphate excretion during and after  $\text{NH}_4\text{Cl}$ -induced acidosis

Results are expressed as means  $\pm$  s.d. for four experiments. \*Significantly different ( $P < 0.05$ ) from control.

Experimental procedure	Urinary phosphate (mmol/24 h per 100 g body wt.)
Controls	$0.163 \pm 0.032$
1 day of acidosis	$0.207 \pm 0.054$
2 days of acidosis	$0.341 \pm 0.083^*$
3 days of acidosis	$0.236 \pm 0.065$
4 days of acidosis	$0.214 \pm 0.024$
5 days of acidosis	$0.144 \pm 0.022$
6 days of acidosis	$0.185 \pm 0.084$
7 days of acidosis	$0.174 \pm 0.111$
7 days of acidosis	$0.193 \pm 0.022$
+1 day of recovery	
7 days of acidosis	$0.161 \pm 0.025$
+2 days of recovery	

elevated in rats given 1.5%  $\text{NH}_4\text{Cl}$  to drink. This increase is statistically significant after 1 day, but further increases occurred until a plateau was attained by 48 h. No further change in ammonia excretion was observed until the  $\text{NH}_4\text{Cl}$  was replaced by water, when an abrupt fall occurred.

Measurements of renal arteriovenous differences for glutamine (Fig. 2) indicated that in general the renal extraction of glutamine paralleled ammonia excretion. The arterial plasma concentration of glutamine fell immediately, reaching a new steady state after about 2 days. It returned to the control value immediately on recovery.

The response of the kidney in these experiments can therefore be divided into three phases: an onset phase which lasts for about 2 days, during which there is a progressive increase in glutamine extraction and ammonia excretion, a plateau phase beginning at about 48 h and a recovery phase which is quite rapid.

#### Blood acid-base parameters

During the onset phase there was a large increase in  $\text{H}^+$  concentration (the pH fell from 7.40 to 7.25 in the first day) and a large fall in  $[\text{HCO}_3^-]$  (Fig. 3). These parameters returned to control values during the plateau phase and an overshoot was evident during recovery in which the  $\text{HCO}_3^-$  concentration rose above the control values. The  $\text{pCO}_2$  remained constant during the  $\text{NH}_4\text{Cl}$  regimen, but there was a striking increase in  $\text{pCO}_2$  after 1 day of recovery. However, after 2 days recovery the  $\text{pCO}_2$  was back to control values.

#### Glutaminase activity and mitochondrial ammoniogenesis

Phosphate-dependent glutaminase activity increased during the onset phase; a plateau was reached

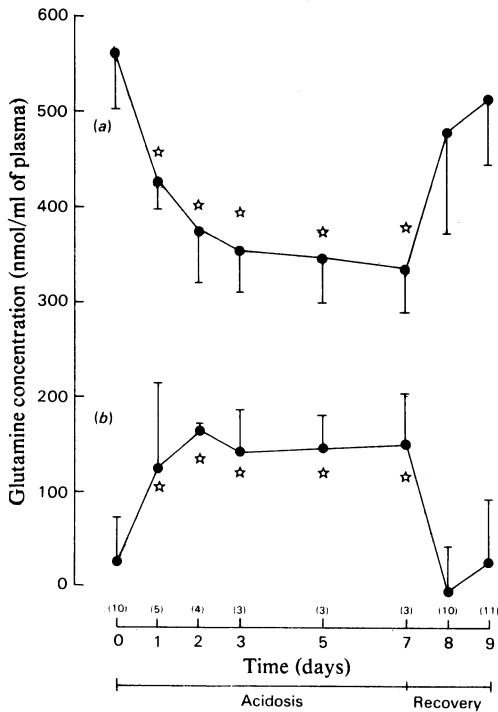


Fig. 2. Arterial glutamine concentrations (a) and renal arteriovenous difference for plasma glutamine (b) during and after metabolic acidosis

The vertical bars indicate 1 s.d.; numbers of experiments are given in parentheses. The stars indicate a significant difference from the control ( $P < 0.05$ ).

after 3 days and no further change in activity was observed even during the recovery phase, when ammonia excretion was returning to normal (Fig. 4). The pattern of ammoniagenesis by isolated mitochondria paralleled the activity of glutaminase (Fig. 4).

*Oxygen consumption of mitochondria with glutamine and related substrates*

The mitochondrial adaptation that occurred during acidosis is also evident as an increased capacity to oxidize glutamine (Brosnan & Hall, 1977). The increased oxidation of glutamine was evident even after 1 day, and rose to plateau values after 3 days (Table 2). However, in agreement with the observations on renal mitochondrial ammoniagenesis (Fig. 4), the mitochondrial oxidation of glutamine did not decrease during the recovery phase, when ammonia excretion *in vivo* returned to normal. The same pattern of mitochondrial glutamine metabolism was

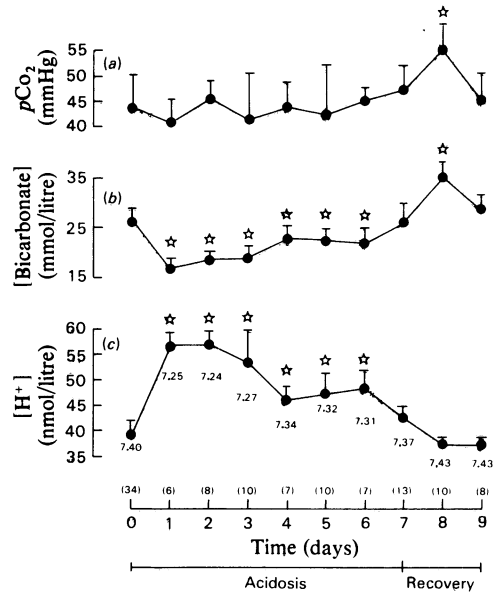


Fig. 3.  $H^+$  (c) and  $HCO_3^-$  (b) concentrations and partial pressure of  $CO_2$  (a) during and after metabolic acidosis. The vertical lines indicate 1 s.d.; numbers of experiments are given in parentheses. The numbers under the  $H^+$  concentrations indicate the blood pH that corresponds to each concentration. The stars indicate a significant difference from the control ( $P < 0.05$ ).

also evident when the phosphate concentration in the incubation medium was lowered to 4mM (results not shown). Table 2 also shows oxygen consumption by isolated mitochondria with glutamate, 2-oxoglutarate and succinate as substrates. No adaptive changes in the metabolism of these substrates occurred.

*Phosphoenolpyruvate carboxykinase activity*

The activity of phosphoenolpyruvate carboxykinase increased in the kidneys of acidotic rats (Fig. 5). Plateau values for this enzyme activity were attained between 1 and 2 days of acidosis, but fell immediately when the rats were returned to tap water.

*Studies with kidney-cortex slices*

Slices from chronically acidotic rats produced more ammonia, glutamate and glucose from glutamine than did slices from control animals (Table 3). When, after 7 days, the acidotic rats were returned to tap water the rate of glutamine metabolism by slices decreased immediately. Ammonia formation and

glutamate formation returned to control values within 1 day, whereas the rate of gluconeogenesis from glutamine was significantly decreased after 1 day and had returned to the control level after 2 days of recovery. The rate of gluconeogenesis from malate, lactate and pyruvate was also measured (Table 4) and similar patterns were observed.

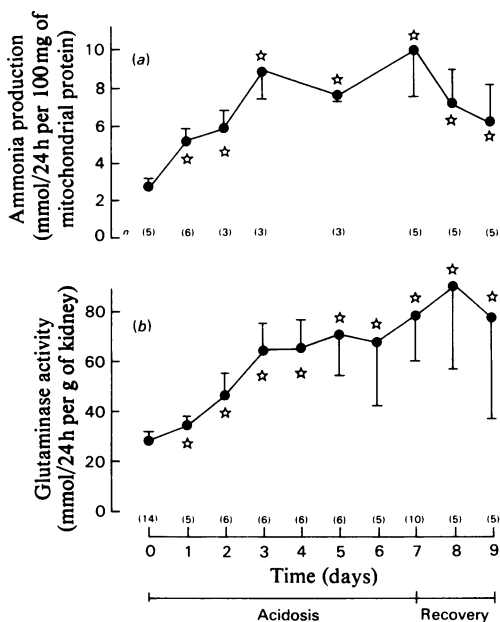


Fig. 4. Renal mitochondrial ammoniogenesis from glutamine (a) and renal phosphate-dependent glutaminase activity (b) during and after metabolic acidosis

The vertical lines indicate 1 s.d.; numbers of experiments are given in parentheses. The stars indicate a significant difference from the control ( $P < 0.05$ ).

## Discussion

There are three principal means by which strong metabolic acids may be excreted by the kidney. A trivial amount may be excreted as free  $H^+$  ions. A portion may be excreted as titratable acid owing to the conversion of  $Na_2HPO_4$  into  $NaH_2PO_4$  in the renal tubules. The bulk of the acid is excreted as ammonium salts. This pattern of excretion is borne out in the data in Fig. 1. Adult animals eating a defined diet will generally be in a state of phosphate balance. However, during severe acidosis,  $Ca_3(PO_4)_2$  may be mobilized from bone (owing to its increased solubility at lower pH) and the phosphate may serve to excrete  $H^+$  ions (White *et al.*, 1973). In fact, 1 mol of  $Ca_3(PO_4)_2$  permits the excretion of 4 equiv. of  $H^+$ .

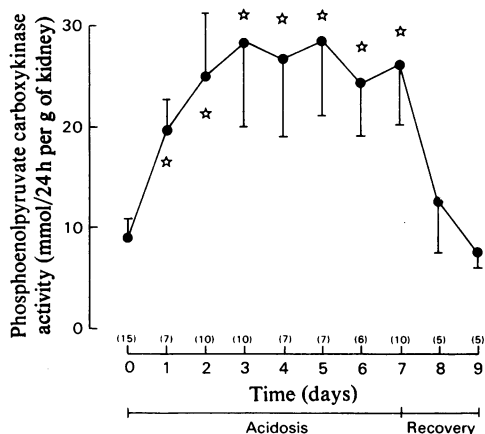


Fig. 5. Renal phosphoenolpyruvate carboxykinase activity during and after  $NH_4Cl$ -induced acidosis

The vertical lines indicate 1 s.d.; numbers of experiments are given in parentheses. The stars indicate a significant difference from the control ( $P < 0.05$ ).

Table 2. Oxygen consumption of kidney-cortex mitochondria from rats during and after  $NH_4Cl$ -induced acidosis in the presence of glutamine and related substrates

All substrates were added at 2 mM. Results are expressed as means  $\pm$  s.d. for the numbers of experiments in parentheses. \* Significantly different ( $P < 0.05$ ) from control.

Experimental procedure	Oxygen consumption (ng-atoms of O/min per mg of mitochondrial protein)			
	Glutamine	Glutamate	2-Oxoglutarate	Succinate
Controls	18.2 $\pm$ 9.6 (8)	12.5 $\pm$ 7.9 (8)	61.9 $\pm$ 18.3 (8)	61.7 $\pm$ 16.8 (8)
1 day of acidosis	34.6 $\pm$ 13.4 (12)*	12.9 $\pm$ 6.3 (12)	71.1 $\pm$ 14.0 (12)	46.3 $\pm$ 10.4 (12)
3 days of acidosis	45.0 $\pm$ 11.1 (9)*	14.8 $\pm$ 10.5 (9)	78.2 $\pm$ 8.6 (9)	54.4 $\pm$ 15.9 (9)
5 days of acidosis	34.1 $\pm$ 7.3 (9)*	10.6 $\pm$ 5.8 (9)	57.2 $\pm$ 28.8 (9)	51.7 $\pm$ 13.4 (9)
7 days of acidosis	38.5 $\pm$ 14.1 (8)*	10.1 $\pm$ 6.3 (8)	63.0 $\pm$ 25.6 (8)	46.9 $\pm$ 8.3 (8)
7 days of acidosis	43.8 $\pm$ 8.4 (7)*	12.6 $\pm$ 5.1 (8)	6.81 $\pm$ 11.2 (8)	59.6 $\pm$ 17.8 (8)
+1 day of recovery				
7 days of acidosis	42.4 $\pm$ 5.6 (8)*	12.9 $\pm$ 5.5 (8)	73.7 $\pm$ 28.8 (8)	62.8 $\pm$ 10.0 (8)
+2 days of recovery				

Table 3. *Metabolism of glutamine (2mM) by kidney-cortex slices from rats during and after NH<sub>4</sub>Cl-induced acidosis*  
Results are expressed as means  $\pm$  s.d. for the numbers of experiments in parentheses. \*Significantly different ( $P < 0.05$ ) from control.

Experimental procedure	Formation of product ( $\mu\text{mol}/90\text{ min per g wet wt.}$ )		
	Ammonia	Glutamate	Glucose
Control	106 $\pm$ 15 (5)	13.6 $\pm$ 6.1 (5)	9.4 $\pm$ 0.7 (5)
7 days of acidosis	196 $\pm$ 41 (4)*	28.2 $\pm$ 4.3 (3)*	39.7 $\pm$ 4.6 (4)*
7 days of acidosis +1 day of recovery	113 $\pm$ 19 (5)	13.8 $\pm$ 3.1 (5)	19.6 $\pm$ 1.0 (3)*
7 days of acidosis +2 days of recovery	95 $\pm$ 22 (4)	14.0 $\pm$ 2.6 (4)	8.9 $\pm$ 1.9 (4)

Table 4. *Gluconeogenesis from various substrates by kidney-cortex slices from rats during and after NH<sub>4</sub>Cl-induced acidosis*  
All substrates were added at 10mM. Results are expressed as means  $\pm$  s.d. for the numbers of experiments in parentheses.  
\*Significantly different ( $P < 0.05$ ) from control.

Experimental procedure	Glucose production ( $\mu\text{mol}/90\text{ min per g wet wt.}$ ) from:		
	Malate	Lactate	Pyruvate
Control	42.2 $\pm$ 5.2 (4)	12.2 $\pm$ 2.5 (5)	44.8 $\pm$ 8.3 (5)
7 days of acidosis	51.8 $\pm$ 11.0 (4)	37.9 $\pm$ 8.4 (4)*	120.5 $\pm$ 27.9 (4)*
7 days of acidosis +1 day of recovery	35.4 $\pm$ 6.1 (4)	24.9 $\pm$ 3.8 (5)*	63.8 $\pm$ 19.0 (5)
7 days of acidosis +2 days of recovery	28.1 $\pm$ 9.5 (3)	16.2 $\pm$ 3.2 (3)	49.0 $\pm$ 15.2 (4)

This mechanism may have been operative on the second day of metabolic acidosis, when a doubling of phosphate excretion occurred. In this connection it is probably significant that the most severe acidosis was evident on the second day, as demonstrated by the nadir in urinary and blood pH. Maximal rates of ammonia excretion were not achieved until about the beginning of the third day of the NH<sub>4</sub>Cl regimen. Previous studies have shown that 2–4 days are required for maximal rates of ammonia excretion to be attained (Rector *et al.*, 1955; Welbourne, 1974). The immediate fall in ammonia excretion during recovery (Table 1; Dies & Lotspeich, 1967) is quite dissimilar from the human studies of Pitts (1948) and of Welbourne *et al.* (1972). In these studies, high rates of ammonia excretion persisted for days after the subjects had ceased to ingest NH<sub>4</sub>Cl. This excretion of ammonia was considered necessary to restore acid–base balance by the excretion of excess acid and the retention of Na<sup>+</sup>. Ammonia excretion does not persist in the rat during recovery and this may be due to a restoration of acid–base balance on the seventh day of acidosis (Fig. 3).

The excretion of ammonia (Fig. 1) was paralleled by renal arteriovenous differences for glutamine (Fig. 2), which reached its maximum after 2 days of acidosis. Ammonia excretion also reached maximum after 2 days. This was evidenced by the attainment of plateau rates of ammonia excretion on day 3 of acidosis (i.e. urine collected from 48 to 72h). There-

fore we can conclude that renal glutamine metabolism had fully adapted after approx. 2 days. The rapid fall in ammonia excretion during recovery is accompanied by a similar decrease in renal glutamine arteriovenous difference. In fact, the latter was not significantly different from zero after 1 day of recovery. Hence, the decreased urinary ammonia excretion is not due to a continued increased glutamine metabolism with a diversion of most of the NH<sub>3</sub> into the venous blood (owing to decreased NH<sub>3</sub> trapping in the alkaline urine) but rather, represents decreased renal glutamine hydrolysis. NH<sub>3</sub> excretion was slightly elevated over the first day of recovery, but was completely back to control values on the second day of recovery (i.e. urine collected from 24 to 48 h of recovery). Thus the rate of renal glutamine metabolism had returned to normal after about 24h of recovery.

The decrease in arterial glutamine concentration during acidosis confirms our previous observations (Squires *et al.*, 1976). The decrease was evident after 1 day and was maximal after 2 days. Glutamine concentrations returned to normal during recovery, and hence the blood glutamine concentration was inversely proportional to the rate of renal glutamine extraction. The significance of these observations is not clear.

A very remarkable observation was the improvement (i.e. return towards normal) of the acid–base parameters during chronic metabolic acidosis. The

explanation for this phenomenon probably lies in the renal adaptation in glutamine metabolism. Thus during the first few days there was an appreciable acidification of the blood and a sharp decrease in blood  $[\text{HCO}_3^-]$ . This clearly indicates that during this period more acid was ingested than was excreted and that the accumulation and buffering of excess  $\text{H}^+$  ions in the body caused the observed changes in pH and  $[\text{HCO}_3^-]$ . However, as the renal adaptation in glutamine metabolism becomes manifest, increased quantities of acid are excreted as their ammonium salts and the quantity of acid excreted can approximate to the quantity ingested. Hence the blood acid-base parameters returned to normal. There was no simple correlation between the rate of ammonia excretion and the blood acid-base parameters. Maximal rates of ammonia excretion were associated with normal acid-base status, and submaximal ammonia excretion with pronounced acidosis. Thus direct regulation of renal ammonia production by changes in systemic acid-base status is not the mechanism by which renal glutamine metabolism is controlled.

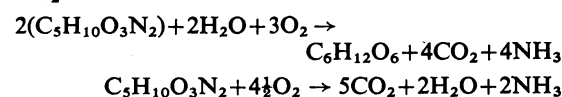
Since the  $p\text{CO}_2$  did not alter significantly during the acidosis, no respiratory compensation was evident. A similar lack of respiratory compensation in rats with  $\text{NH}_4\text{Cl}$ -induced acidosis has been observed by Levine & Nash (1974). Large increases in  $p\text{CO}_2$  and  $[\text{HCO}_3^-]$  were observed during recovery, although blood pH was essentially normal. These changes probably represent a metabolic alkalosis with a substantial respiratory compensation. The reasons for these changes are not apparent. The pattern of glutaminase induction is essentially similar to that reported previously (Rector *et al.*, 1955). It is noteworthy that glutaminase activity continued to increase between 48 and 72 h of acidosis, although the maximal rates of ammonia excretion were achieved by 48 h. Thus there is some dissociation between glutaminase activity and ammonia excretion during the onset and plateau phases. A more marked dissociation may be deduced from the work of Curthoys & Lowry (1973). Although these authors did not measure ammonia excretion, their protocol for inducing acidosis was identical with that used in the present work and thus the excretion pattern should be similar to that in Fig. 1. Curthoys & Lowry (1973) found that phosphate-dependent glutaminase activity measured in micro-dissected proximal convoluted tubules had increased about 2-, 5-, 12- and 25-fold after 1, 2, 4 and 7 days respectively of  $\text{NH}_4\text{Cl}$ -induced acidosis. A very striking dissociation between glutaminase activity and ammonia excretion was evident during the recovery phase (Figs. 1 and 4). The activity of glutaminase remained elevated, although the rate of ammonia excretion returned to normal. The mitochondrial parameters (glutaminase activity, mitochondrial ammoniogenesis and oxygen consump-

tion) did not return to control values until after about 10–14 days of recovery (results not shown).

The oxygen consumption of isolated kidney-cortex mitochondria with glutamine and related substrates (Table 2) essentially confirmed the mitochondrial ammonia-production experiments in that glutamine oxidation adapted readily during acidosis, but did not return to control values during the recovery phase. In addition, since there was no enhanced oxidation of glutamate, 2-oxoglutarate or succinate, the adaptation in glutamine metabolism in isolated mitochondria must be quite specific.

Rather good agreement is evident between the activity of the carboxykinase and ammonia excretion during the entire experimental protocol. The activity measured probably reflects that in the proximal tubule (Guder & Schmidt, 1974; Schmidt & Guder, 1976). Especially striking was the rapid return of both parameters to control values within 1 day of recovery, in marked contrast with the stability of glutaminase activity. Our present data do not permit us to determine the mechanism of this decrease in activity. However, the half-life of this enzyme is quite short [3.4 h according to Longshaw *et al.* (1972) and 13 h according to Iynedjian *et al.* (1975)], so that a 50% decrease in activity over 24 h is not surprising.

Phosphoenolpyruvate carboxykinase is important in the disposal of the carbon skeleton of glutamine. It is now appreciated that the first two steps of glutamine metabolism (glutaminase and glutamate dehydrogenase) do not produce  $\text{NH}_3$  but rather produce  $\text{NH}_4^+$ , which, of course, cannot buffer protons. For  $\text{NH}_3$  to be produced as the nitrogenous end product, it is necessary that the glutamine carbon be metabolized to a neutral compound. The most likely neutral compounds are glucose and  $\text{CO}_2$ :



Phosphoenolpyruvate carboxykinase will be involved in both of these pathways (Cahill, 1975) and thus alterations in its activity cannot be interpreted to represent alterations in the rate of renal gluconeogenesis *in vivo*.

Kidney slices were used in the study of recovery, because slices represent an intact-cell preparation in which the enhanced metabolism of glutamine, evident during acidosis *in vivo*, is maintained *in vitro*. In agreement with many workers, we found that the production of all three of the major products of glutamine metabolism (ammonia, glutamate and glucose) was increased during acidosis. Production of ammonia and glutamate returned to control values within 1 day, although the rate of gluconeogenesis decreased by 50% after 1 day and returned to control values after 2 days.



The pathways of gluconeogenesis from glutamine, lactate, pyruvate, and malate differ considerably from each other in the mitochondrial steps involved (Rognstad & Katz, 1970). Despite these differences it is remarkable that the rates of gluconeogenesis from all four substrates vary qualitatively in the same manner (Tables 3 and 4). Gluconeogenesis from all four substrates is increased during acidosis, in essential agreement with Goodman *et al.* (1966). During recovery the rates of gluconeogenesis from all four substrates rapidly return to control values, the return being complete after 2 days. This strongly suggests that the regulation of gluconeogenesis is occurring in that portion of the gluconeogenic pathways that is common to all four substrates, the conversion of cytoplasmic oxaloacetate into glucose. These changes in gluconeogenic rate are similar to the changes in phosphoenolpyruvate carboxykinase activity (Fig. 5). However, the agreement between enzyme activity and gluconeogenic rate is not precise, since gluconeogenesis was still elevated after 1 day of recovery, whereas the carboxykinase activity had returned to control levels.

The experiments reported here have important implications for the hypotheses that ammonia excretion *in vivo* is regulated by adaptive increases in either the mitochondrial transport of glutamine or glutaminase activity. Although initial rates of glutamine uptake by isolated mitochondria are not yet amenable to measurement, it is well established in both rat (Adam & Simpson, 1974) and dog (Simpson & Adam, 1975) that kidney mitochondria from acidotic animals accumulate greater amounts of radioactive glutamate when incubated with [<sup>14</sup>C]glutamine than do mitochondria from normal animals. Kidney mitochondria from acidotic rats and dogs also produce more ammonia when incubated with glutamine than do mitochondria from normal animals. Of course these observations, at least in the rat, may alternatively be attributed to the increased activity of mitochondrial glutaminase. The rate of ammoniogenesis and of oxygen consumption by isolated mitochondria with glutamine as substrate is determined by the rate-limiting step in glutamine hydrolysis, whether transport or glutaminase. Our data unequivocally demonstrate that the mitochondrial capacity to metabolize glutamine remains elevated during recovery, although glutamine metabolism *in vivo* returns to normal. Thus regulation of ammonia production in the rat is not brought about solely by alterations in the mitochondrial capacity to metabolize glutamine. Of course, the present data do not permit extrapolation of this conclusion to other species. Glutamine metabolism in slices, however, did return to control values during recovery. Thus, during recovery, some regulatory factor is operative in slices that is not present in isolated mitochondria. Furthermore this regulatory factor is capable of suppressing

the expression in slices of an increased mitochondrial capacity to metabolize glutamine. Presumably the same regulatory factor is operative *in vivo*. The importance of phosphoenolpyruvate carboxykinase for renal ammoniogenesis has been emphasized by two other studies. Specific inhibition of this enzyme in perfused kidneys (Ross, 1976) and kidney slices (Bennett & Alleyne, 1976) from chronically acidotic rats resulted in decreased ammonia production from glutamine. Whether or not phosphoenolpyruvate carboxykinase activity is the factor that controls ammoniogenesis during recovery remains to be determined.

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