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pH AND TEMPERATURE DEPENDENCE OF GLUTAMINE UPTAKE, CARBON DIOXIDE AND AMMONIA PRODUCTION IN KIDNEY SLICES FROM ACIDOTIC RATS

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SUMMARY

1. The effects of medium pH and temperature on glutamine uptake, NH_3 production and CO_2 production were examined using kidney cortex slices from normal and acidotic rats.

2. Uptake of glutamine by kidney slices from normal rats shows a pH optimum of 7·5 at 25 °C and 7·3 at 37 °C. Uptake is optimal, however, at a constant OH^-/H^+ ratio of 10.

3. In slices from acidotic rats greatest uptake was at pH 6·8 at 25 °C and 6·6 at 37 °C. Optimal OH^-/H^+ ratio was 0·4 and constant at both temperatures.

4. CO_2 production from glutamine was greatest at pH 7·0 at 37 °C in slices from control rats. No pH optimum was detected at 25 °C. With slices from acidotic animals, optimal pH for CO_2 production became identical with that for uptake.

5. Both basal and glutamine-stimulated NH_3 production showed no optimal pH but were significantly higher in slices from acidotic rats compared with those from controls.

6. Dependence of glutamine penetration on optimal OH^-/H^+ ratio is considered to reflect a general membrane phenomenon which is produced by either an increase in carrier–substrate complexes or an increase in the number of carriers at this ratio.

7. Cellular penetration of glutamine does not appear to be a limiting factor in production of NH_3 *in vitro*.

INTRODUCTION

In a variety of species, NH_3 is produced by renal cells and is derived principally from glutamine (Van Slyke, Phillips, Hamilton, Archibald, Futcher & Hiller, 1943; Owen & Robinson, 1963; Shalhoub, Webber, Glabman, Canessa-Fischer, Klein, DeHass & Pitts, 1963; Preuss, 1969; Pilkington & O'Donovan, 1971). Glutamine enters the renal cells from both the luminal and anti-luminal borders (Pilkington, Young & Pitts, 1970; Lemieux, Vinay & Cartier, 1974), and is deamidated in a reaction involving a phosphate-dependent glutaminase located in mitochondria (Damian & Pitts, 1970; Curthoys & Weiss, 1974; Kalra & Brosnan, 1974). Deamidation, deamination and subsequent oxidation can provide two molecules of NH_3 and five molecules of CO_2 per molecule of glutamine.

The present studies examined the effect of acid–base balance and medium pH on uptake and metabolism of glutamine by slices prepared from rat kidney cortex. The aim of the study was to determine whether membrane permeability was a limiting factor in ammoniagenesis. In addition to determining the effect of medium acidity on glutamine uptake, NH_3 production and CO_2 production, we related these processes to the OH^-/H^+ ratio in the incubation media. At different temperatures, changes in pH have different effects on the OH^-/H^+ ratio. This is because the pH of neutral water ($OH^- = H^+$) varies inversely with temperature. Effects of medium pH were determined, therefore, at two temperatures: 25 and 37 °C. The results show that glutamine uptake, NH_3 production and CO_2 production by kidney slices are increased by prior acidosis. However, the optimal pH of incubation medium for glutamine uptake and CO_2 production is generally lower in slices from acidotic rats. Furthermore, the optimum pH for glutamine uptake and CO_2 production varies with incubation temperature.

METHODS

Adult male Wistar rats were killed by a blow on the head, and the kidneys were quickly removed, decapsulated and placed in an ice-cold oxygenated incubation medium containing 110 mM-NaCl, 5 mM-KCl, 1·2 mM-$MgSO_4$, 1·0 mM-$CaCl_2$, 5·0 mM-Na acetate and 15 mM-phosphate buffer (12 mM-Na_2HPO_4 and 3 mM-NaH_2PO_4). Phosphate buffer was chosen because of its superiority to other buffers such as Krebs–Ringer bicarbonate, imidazole and Tris for eliciting maximum glutamine uptake at a given pH and temperature (J. George & S. Solomon, unpublished observations). Medium pH was changed by adding HCl and NaOH. Addition of oxidizable substrates such as lactate and others results in a suppression of glutamine uptake and utilization (Preuss, Baird & Eastman, 1978). It has been shown that the addition of such substrates to media used for incubating kidney slices results in glutamine fluxes more closely approximating *in vivo* conditions (Preuss *et al.* 1978). Although acetate *per se* has not been investigated in detail, one would expect that its addition would have effects comparable to lactate. In our laboratory we have used acetate extensively as an added energy source (Park & Solomon, 1977; George & Solomon, 1978). Na acetate (final concentration 5·0 mM) was chosen to be added to the incubation medium in order that glutamine metabolism more closely approximated conditions *in vivo*.

Two or three slices of cortex approximately 0·4–0·5 mm thick were cut from each kidney using a Stadie Riggs microtome, and stored in the same incubation medium until used. For the measurement of O_2 consumption, glutamine uptake and CO_2 production, slices (approximately 200 mg) were placed in Warburg incubation flasks equipped with two side arms and a central well. The reaction chamber of the flasks contained 3·0 ml incubation medium and 0·1 ml [^{3}H]inulin (New England Nuclear). Only one side arm was used and it contained both 0·1 ml L-[U-^{14}C]glutamine (Amersham/Searle) and 0·1 ml stock L-glutamine (Sigma Chemicals). The concentration of glutamine in the medium was 1 mM. At the start of the incubation period the medium contained [^{3}H]inulin and [^{14}C]glutamine to give specific activities of approximately 0·1 and 0·01 μCi ml^{-1} medium, respectively. Small pieces of accordion-type, folded filter paper moistened with 0·2 ml of 20% KOH were placed in the central well in order to trap the evolved $^{14}CO_2$. The flasks were attached to manometers and equilibrated at 25 °C or 37 °C in the water-bath of a Warburg apparatus, and measurements of O_2 consumption (Q_{O_2}) made using standard manometric techniques (Umbreit, Burris & Stauffer, 1972).

At time zero, glutamine was tipped into the reaction chamber to begin the experiment and then the kidney slices were incubated for 30 min. In previous experiments we had found that the uptake of glutamine was maximal at 40 min and increased linearly up to that time (George & Solomon, 1978). After incubation, slices were quickly removed from the medium, rinsed in glutamine-free medium for about 2 s, and blotted on filter paper. A small portion of each slice was removed, weighed and dried overnight at 100 °C to determine the total water content of slices. The remainder of the tissue was weighed and transferred to a small tube containing 2 ml distilled water and kept overnight in the refrigerator at 4 °C to leach out [^{3}H]inulin and [^{14}C]glutamine from the tissue according to the method described by Law (1975). More than 98% of the radioactivity was recovered

in this manner. The filter paper and remaining KOH from the central well of the flasks were transferred to counting vials containing Phase Combining System (Amersham/Searle), and activity due to $^{14}CO_2$ was determined using a liquid scintillation spectrometer (Packard Tri-Carb). Thus, knowing the total counts min^{-1} of $^{14}CO_2$ at the end of incubation, total counts min^{-1} of [^{14}C]glutamine at the start of the incubation, total quantity of glutamine in medium and wet weight of tissue, the amount of glutamine oxidized to CO_2 could be calculated. It is important to point out that the measurement of evolved CO_2 is only a partial measurement of glutamine metabolism since all the permeating glutamine may not be completely oxidized to CO_2. The next day the tubes containing tissue were centrifuged for 10 min at 3000 *g* and portions of supernatant fluid were pipetted into a counting vial for measurement of radioactivity. Radioactivity of the incubation medium at the end of the incubation period was also determined. After correction for cross-over between ^{14}C and ^{3}H, the activities of ^{14}C and ^{3}H in the tissue and medium were computed. From these values the extracellular ([^{3}H]inulin) space and ^{14}C activity in the cellular space were calculated.

The metabolism of glutamine may result in a spectrum of products (glucose, glutamate, aspartate, and α-ketoglutarate among others: Goodman, Fuisz & Cahill, 1966; Vinay, Lemieux & Gougoux, 1979). It was not the concern of this study to determine what the end products were, but rather to attempt to evaluate the effects of medium pH, temperature and acidosis on influx of glutamine into the tissue. Since the only source of ^{14}C was the added glutamine, the radioactivity in the intracellular fluid plus that trapped as CO_2 approximated the uptake of glutamine. Accordingly, glutamine taken up and left in the cells was calculated by multiplying cell:medium ratio of counts (counts min^{-1} (ml intracellular fluid)$^{-1}$:counts min^{-1} (ml medium)$^{-1}$) by the concentration of glutamine in the medium. The fraction of glutamine metabolized to CO_2 was added to the equivalent amount remaining in the cells to obtain total influx.

It should be pointed out that some metabolites of glutamine probably leak back into the medium. However, it has been shown that the leak for glutamate is small (Vinay *et al.* 1979), and since glutamate is probably the main metabolite in these studies, the error introduced by neglecting the leak is small.

For measurements of NH_3, slices were placed in 25 ml Erlenmeyer flasks containing 6·4 ml incubation medium and 0·2 ml of glutamine equilibrated at 25 or 37 °C. The final concentration of glutamine in the medium was 1 mM. The flasks were sealed with rubber stoppers and incubated for 30 min. At the end of incubation, 50 μl of concentrated HCl was injected through the rubber stoppers into the flasks to trap all of the NH_3 as NH_4^+ ions. Concentration of NH_3 in the incubation medium was determined using an NH_3 electrode (Orion Research Inc., Mass.) after the samples were alkalinized by the addition of 50 μl saturated NaOH.

Endogenous or basal NH_3 was measured as the NH_3 produced in control slices in the absence of added glutamine. Total NH_3 is the NH_3 produced in the presence of added 1 mM-glutamine. The difference between endogenous and total NH_3 was taken to be the NH_3 produced from the added glutamine, and was referred to as the glutamine-stimulated NH_3.

Metabolic acidosis was induced in rats by adding 0·28 M-NH_4Cl to their drinking water for 1 week (Goldstein, 1975; Tannen & Kunin, 1976) while maintaining the animals on standard laboratory chow. Normal controls were pair-fed a similar diet and allowed access to tap water. To control for any possible renal effects of NH_3 *per se*, a group of rats received 0·28 M-NH_4HCO_3 in their drinking water for 1 week. Determinations of pH of blood and urine were made in controls and acidotic animals using a pH meter (Radiometer 27, Copenhagen) and the results are shown in Table 1.

A two-sample, two-tailed, Student's *t* test was used to test significance levels, and *P* value less than 0·55 were regarded as indicating significance.

RESULTS

Glutamine uptake was determined in kidney slices isolated from adult male rats as a function of medium pH and temperature. The kidney slices were incubated at 25 or 37 °C in a medium whose pH was varied between 6·5 and 8·0. Even though the total glutamine uptake determined at each medium pH had relatively large standard errors, uptake was maximum at a certain medium pH for the two temperatures studied. The optimum pH for maximum glutamine uptake is 7·5 at 25 °C and 7·3 at

37 °C (Table 2). However, when the values for glutamine uptake were evaluated as a function of medium OH^-/H^+ ratio, it was found that optimal uptake occurs at an OH^-/H^+ ratio of 10 regardless of the temperature of incubation (Fig. 1).

In order to test whether alterations in acid–base balance change glutamine transport characteristics, kidney slices prepared from acidotic rats were incubated in media with pH values ranging from 6·4 to 7·8. As in the case of normal rats, accumulation of glutamine in slices from acidotic rats was pH-dependent and optimal pH values were lower for acidotic rats, being 6·8 at 25 °C and about 6·6 at 37 °C. As

TABLE 1. Measurements of blood and urine pH of normal and acidotic rats

	Blood pH	Urine pH
Controls, non-treated (7)	7·39 ± 0·01	6·80 ± 0·01
Normal, NH_4HCO_3-treated (6)	7·40 ± 0·02	6·62 ± 0·3
Acidotic, NH_4Cl-treated (6)	7·20 ± 0·03*	5·50 ± 0·09*

Values are means ± S.E. The number of animals used in each group is given in parentheses.
* Significantly different from non-treated and NH_4HCO_3-treated controls ($P < 0·05$).

TABLE 2. pH and temperature dependency of glutamine uptake in kidney slices from normal and acidotic rats

Group	Incubation temperature (°C)	Maximum glutamine uptake	P value	Optimal pH	Optimal OH^-/H^+
Normal ($n = 7$)	25	63·1 ± 6·3	n.s.	7·5	10·08
Normal ($n = 8$)	37	68·9 ± 3·4		7·3	9·50
Acidotic ($n = 6$)	25	66·6 ± 2·0*	< 0·02	6·8	0·40
Acidotic	37	94·9 ± 9·5**		6·6	0·38

Total glutamine uptake values are expressed in μmol (kg wet tissue)$^{-1}$ min^{-1}, mean ± S.E.
* No significant difference between acidotic and normal groups at 25 °C ($P > 0·60$).
** Significant difference between acidotic and normal groups at 37 °C ($P < 0·02$).

shown in Table 2, at 25 °C, maximum rates of glutamine uptake in slices from normal and acidotic rats are not significantly different from each other. However, at 37 °C, glutamine uptake in slices from acidotic rats is significantly higher than uptake in slices from normal rats. Moreover, when the glutamine uptake values for slices from acidotic rats were plotted as a function of medium OH^-/H^+ ratio, maximum glutamine uptake was found to occur at an OH^-/H^+ ratio of 0·4 regardless of incubation temperature (Fig. 2).

Glutamine metabolized to CO_2 was studied for slices isolated from normal and acidotic rats. In slices from normal rats, maximum CO_2 production occurred at pH 7·0 and 37 °C and it was approximately 30 μmol (kg wet tissue)$^{-1}$ min^{-1}. Slices incubated at 25 °C failed to show an optimum pH and CO_2 production remained at a level of about 6 μmol (kg wet tissue)$^{-1}$ min^{-1}. In slices from acidotic rats, CO_2 production

increased almost two-fold compared with slices from normal rats at both 25 and 37 °C. The optimum pH values for maximum CO_2 production in acidotic rats were found to be about 6·8 at 25 °C and about 6·6 at 37 °C, the same optimum values observed also for total glutamine uptake for this experimental group. Since this is the case, when the CO_2 production data for acidotic rats are plotted as a function of medium

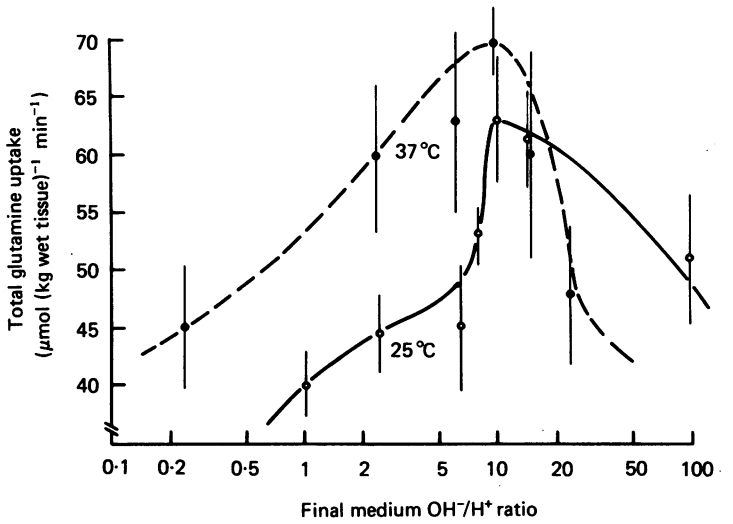

Fig. 1. Total glutamine uptake as a function of medium OH^-/H^+ ratio at 25 and 37 °C in kidney slices from normal rats. The incubation period was 30 min. Each point represents the mean of six or seven (25 °C) and seven or eight (37 °C) experiments. Vertical bars represent ±1 S.E. Curves are drawn freehand in all Figures.

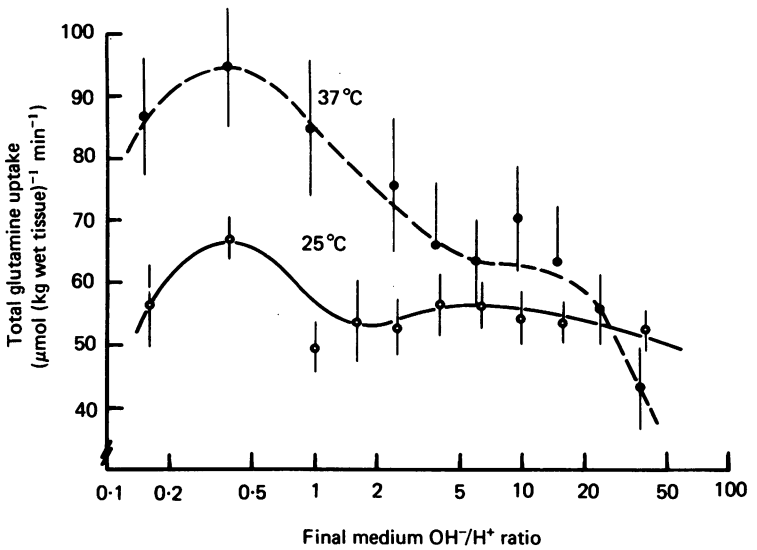

Fig. 2. Total glutamine uptake in slices from acidotic rats as a function of medium OH^-/H^+ ratio at 25 and 37 °C. The incubation period was 30 min. Each point represents the mean of six experiments. Vertical bars represent ±1 S.E.

OH^-/H^+ ratio, maximum CO_2 production is seen to occur at about the same OH^-/H^+ ratio of 0·4 regardless of the incubation temperature (Fig. 3).

Measurements of NH_3 expressed as μmol (kg wet tissue)$^{-1}$ min^{-1} in kidney slices from normal and acidotic rats as a function of incubation medium pH at 37 °C are shown in Table 3. Basal NH_3 production in slices was not affected by the pH of incubation medium; however, it was higher in slices from acidotic rats. Glutamine-stimulated NH_3 production was also higher in slices from acidotic rats and was increased in slices incubated at higher medium pH. These observations are consistent with previous reports (Alleyne & Roobol, 1974; Relman & Narins, 1975).

Simultaneously with measurements of glutamine uptake and CO_2 production, O_2

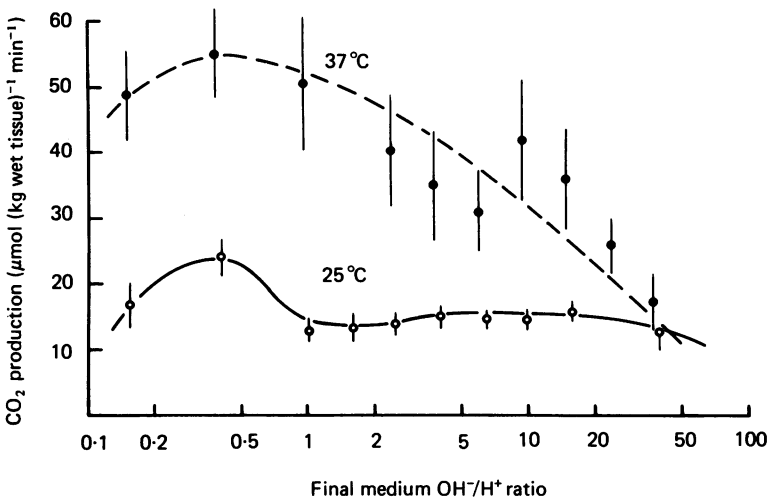

Fig. 3. CO_2 production in kidney slices from acidotic rats as a function of final medium pH at 25 and 37 °C. The results are from six experiments. Vertical bars represent ±1 S.E.

TABLE 3. Effect of pH on NH_3 production in kidney slices from normal and acidotic rats

	Total NH_3			Basal NH_3		
pH ...	6·8	7·2	7·6	6·8	7·2	7·6
Controls, non-treated	33·8±0·71	39·8±1·95	47·4±2·24	19·2±0·96	19·8±0·77	18·3±0·89
Acidotic, NH_4Cl-treated	61·3±5·54	61·4±3·32	70·9±4·27	24·1±0·77*	24·7±0·49	24·8±0·29
	Glutamine-stimulated NH_3					
pH ...	6·8	7·2	7·6			
Controls, non-treated	14·5±1·19	20·0±2·09	29·1±2·41			
Acidotic, NH_4Cl-treated	37·1±5·59	36·7±3·84	46·1±4·27			

Values are expressed in μmol (kg wet tissue)$^{-1}$ min^{-1}, mean±S.E. All incubations were carried out at 37 °C.

There was a significant difference in NH_3 production between slices from normal and acidotic rats ($P < 0·001$, except for * where $P < 0·02$).

consumption by the slices was determined as a function of pH and temperature. At 25 °C, Q_{O_2} (μl (g wet tissue)$^{-1}$ min^{-1}) in ten experiments at pH 7·0 was 11·0±0·64 in controls and 8·9±0·49 in slices from acidotic rats. However, at 37 °C, Q_{O_2} in eight experiments at pH 7·0 was 15·9±2 in controls and 16·4±1·2 in slices from acidotic rats. Variations of incubation medium pH in both control and acidotic slices did not produce any significant change in Q_{O_2}. Thus, even though Q_{O_2} increases with higher incubation temperature of the slices, incubation medium pH and acidosis do not increase Q_{O_2}.

In order to determine whether the increase in total glutamine uptake, CO_2 production and NH_3 production observed in slices from acidotic animals were related to the prior acidosis or to increased intake of NH_3, slices from NH_4HCO_3-treated animals were studied. The results are shown in Table 4. There are no differences in glutamine uptake, CO_2 production and NH_3 production between controls and NH_4HCO_3-treated animals.

TABLE 4. Effects of NH_4HCO_3 and HN_4Cl treatment on glutamine uptake, CO_2 production and NH_3 production in rat kidney slices

	Glutamine uptake		CO_2 production		Total NH_3 production	
pH ...	7·0	7·4	7·0	7·4	7·2	7·6
Controls, non-treated	50·1±3·18	50·9±4·75	7·2±1·71	5·0±0·75	39·8±1·95	47·4±2·24
NH_4HCO_3-treated	45·2±2·92	52·6±3·09	6·5±0·29	5·0±0·27	37·8±1·13	43·3±1·59
NH_4Cl-treated	59·1±3·36	61·0±3·89	18·9±3·05	18·8±2·86	61·4±3·82	70·9±4·27

Values are expressed in μmol (kg wet tissue)$^{-1}$ min^{-1}, mean±S.E. The incubations were done at 25 °C.

There was no significant difference in glutamine uptake, CO_2 production and NH_3 production between controls and NH_4HCO_3-treated animals. NH_4Cl-treated animals show significant increase in glutamine uptake, CO_2 production and NH_3 production compared with controls and NH_4HCO_3-treated animals ($P < 0·01$).

DISCUSSION

The present experiments show that the uptake of glutamine by kidney slices from both normal and acidotic rats varies with medium pH and that the optimal pH changes with temperature. Knowing the pH and temperature of incubation the ratio between hydroxyl ion and hydrogen ion was calculated (OH^-/H^+ = antilog 2 (pH − pN), where pN is the pH of water: Howell, Baumgardner, Bondi & Rahn, 1970; Rahn, 1974). The optimum pH for maximum glutamine uptake in slices from non-acidotic rats was about 7·5 at 25 °C and 7·3 at 37 °C. Evaluation of the data showed that the glutamine uptake in slices from non-acidotic rats is at its maximum at an OH^-/H^+ ratio of 10 (Fig. 1, Table 2). The optimum pH for maximum uptake of glutamine was lower in slices from acidotic rats than from normals. The optimum pH in slices from acidotic rats was about 6·8 at 25 °C and 6·6 at 37 °C. Optimal uptake was again found to occur at a fixed OH^-/H^+ ratio, in this case of 0·4 (Fig. 2, Table 2). Thus, glutamine permeability in slices from non-acidotic and acidotic rats is optimum at a fixed OH^-/H^+ ratio independent of incubation temperature.

Our studies also shown that the oxidative metabolism of glutamine is dependent upon medium pH and temperature. In slices from both normal and acidotic rats

metabolism of glutamine to CO_2 was elevated as the temperature of the incubation medium was increased. In slices from normal rats, maximum CO_2 production occurred around pH 7·0 at 37 °C. At 25 °C, no optimum was seen for CO_2 production. Slices from acidotic rats metabolized twice the amount of glutamine to CO_2 compared with slices from non-acidotic rats and showed dependency on both pH and temperature. In these slices, maximum CO_2 production occurred at an OH^-/H^+ ratio of 0·4 (Fig. 3), a value observed for optimal uptake of glutamine also.

Little is known about the permeation of glutamine across plasma membranes of kidney cells. Glutamine enters the renal cells through both tubular and peritubular membranes (Pilkington *et al.* 1970), and serves as the major substrate for the production of NH_3. From data on the apparent concentration gradient of glutamine between tissue and blood, it has been suggested by several investigators that an active 'pump' exists at the peritubular face of proximal tubule cells for transport of glutamine (Shalhoub *et al.* 1963; Pitts, 1964; Balagura-Baruch, Shurland & Welbourne, 1970). Lemieux *et al.* (1974) showed that the transport of glutamine across the anti-luminal membrane of dog renal cell is gradient-limited; the concentration of glutamine in the renal venous plasma could not be lowered below 0·33 μmol ml^{-1} despite prolonged contact of the plasma with the renal cells following clamping of the renal artery. These observations suggest a passive peritubular transport mechanism for glutamine.

In present experiments, in which the uptake of glutamine was studied using rat kidney slices, glutamine may enter the cells through both luminal and anti-luminal membranes (Kamm & Asher, 1970; Irias & Greenberg, 1972; Preuss, Vavatsi-Manos & Vertuno, 1973). It has been reported previously that glutamine transport is increased in metabolic acidosis (Van Slyke *et al.* 1943; Shalhoub *et al.* 1963; Owen & Robinson, 1962; Pitts, 1964, 1973). The present results also show that prior acidosis enhances glutamine uptake in slices incubated at 37 °C. When incubated at 25 °C, slices took up less glutamine and no difference was seen in the levels of maximum glutamine uptake between slices from normal and acidotic rats.

The present observations, indicating an apparent dependence of glutamine permeation on a certain OH^-/H^+ ratio, are not unique to the transport of glutamine. Park & Hong (1976) studied toad skin ATPase and found that the enzyme is in the most active catalytic state at an OH^-/H^+ ratio of 16, regardless of temperature. Recently, Park & Solomon (1977) studied the uptake of PAH and phenol red in rat kidney cortex slices and found the optimal pH for uptake of PAH and phenol red to be different at different incubation temperatures. When the uptake of PAH and phenol red was evaluated as a function of the OH^-/H^+ ratio in the medium, it was found that the maximum uptake is constant at an OH^-/H^+ ratio of about 10, regardless of temperature.

In the present study it was observed that the maximum glutamine uptake in slices from normal rats also occurred at an OH^-/H^+ ratio of about 10, independent of temperature. In slices from acidotic rats, maximum glutamine transport was at an OH^-/H^+ ratio of about 0·4, a value far below that observed in slices from normal rats. Thus, in slices from normal kidneys, maximum uptake of glutamine occurs when acidity of the medium is near that of normal blood. The lower optimum pH for glutamine transport seen in acidotic rats tends to enhance uptake and, thereby, has adaptive value.

The contribution that maintenance of a fixed OH^-/H^+ ratio makes to determining glutamine uptake is not clear. Kinetic analysis of PAH uptake in rat kidney slices showed that the maximum velocity (V_{max}) for influx of PAH occurred at the same OH^-/H^+ ratio at 25 and 37 °C, whereas the carrier substrate affinity (apparent K_m) was independent of such a ratio (Park & Solomon, 1977). In the same study, efflux rates for PAH also showed pH optima, which were identical to those uptake. Therefore, the preservation of a fixed OH^-/H^+ ratio may be important in determining the mobility of a carrier–substrate complex and not necessarily the affinity of carrier–substrate complex. Alternatively, an optimal OH^-/H^+ratio could provide conditions that maximized the number of transport sites. Whether glutamine uptake is similarly changed by changing the OH^-/H^+ratio will have to be determined in additional studies. It is of interest, however, that both glutamine and the organic acid transport system share an optimal OH^-/H^+ ratio of 10. Such an observation is consistent with both glutamine and these organic acids being transported by a common pathway, or alternatively with the change in OH^-/H^+ ratio altering some general property of the plasma membrane that affects transport velocity of such substances. If the latter is the case, change in the OH^-/H^+ ratio in acidosis would imply that chronic acidosis somehow affects membrane properties.

TABLE 5. Comparison of increased glutamine uptake and CO_2 production induced by acidosis

pH	Glutamine uptake	CO_2 production
7·0	9	11
7·4	10	13·5

All values are expressed as μmol (kg wet tissue)$^{-1}$ min^{-1}. Incubations were carried out at 25 °C. Data were calculated from results in Table 4.

Since permeation of mitochondria by glutamine can be a limiting factor in NH_3 production, the issue arises as to whether cellular permeation could also be a limiting factor. Some insight can be obtained by comparing the effect of acidosis in increasing glutamine uptake, CO_2 production and NH_3 production. An approximation of these relationships are shown in Table 5. Nearly all of the glutamine entering the cell is metabolized and both nitrogen atoms appear as NH_3. If increased metabolism of glutamine were to increase penetration by increasing the gradient between cell and medium, one would expect that the added glutamine metabolized would exceed the acidosis-induced increment in glutamine uptake. We would then get an increased production of NH_3 and the added amount of NH_3 produced would be greater than could be accounted for by the increment in uptake. If, however, metabolism were limited and cellular penetration was not a limiting factor, then one would further expect that the added increment of glutamine metabolized would be less than the increment in glutamine uptake. Such is not seen at 25 °C. If the data obtained at 37 °C are examined, however, it is found that added uptake of glutamine exceeds the increment in NH_3 production brought about by acidosis. It would, therefore, seem that at least *in vitro* at 37 °C, cellular permeation is not a limiting factor in glutamine metabolism or, presumably, in NH_3 production.

The functional significance of these changes in membrane characteristics described above is that they may prevent the penetration of glutamine from becoming a limiting factor in ammoniagenesis. The shift in the optimal OH^-/H^+ ratio to a lower value during acidosis allows for an increased penetration of glutamine at the lower plasma pH. Although the optimum OH^-/H^+ ratio is lower than would be encountered in any physiological situation, the change is adequate, however, to account for an increased glutamine uptake. As a result, it would be appropriate to suggest that membrane changes may underlie, in part, the increased ammoniagenesis found in chronic acidosis.

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REFERENCES

ALLEYNE, G. A. O. & ROOBOL, A. (1974). Regulation of renal cortex ammoniagenesis. I. Stimulation of renal cortex ammoniagenesis by plasma isolated from acutely acidotic rats. *J. clin. Invest.* **53**, 117–121.

BALAGURA-BARUCH, S., SHURLAND, L. & WELBOURNE, T. C. (1970). Effects of α-keto-glutarate on renal ammonia release in the intact dog. *Am. J. Physiol.* **218**, 1070–1075.

CURTHOYS, N. P. & WEISS, R. F. (1974). Regulation of renal ammoniagenesis. *J. biol. Chem.* **249**, 3261–3266.

DAMIAN, A. C. & PITTS, R. F. (1970). Rates of glutaminase 1 and glutamine synthetase reactions in rat kidney *in vivo*. *Am. J. Physiol.* **218**, 1249–1255.

GEORGE, J. P. & SOLOMON, S. (1978). Dependence of glutamine metabolism and ammonia synthesis on sex in rat kidney slices. *J. Endocr.* **79**, 271–276.

GOLDSTEIN, L. (1975). Glutamine transport by mitochondria isolated from normal and acidotic rats. *Am. J. Physiol.* **229**, 1027–1033.

GOODMAN, A. D., FUISZ, R. E. & CAHILL, G. F. (1966). Renal gluconeogenesis in acidosis, alkalosis and potassium deficiency: its possible role in regulation of renal ammonia production. *J. clin. Invest.* **45**, 612–618.

HOWELL, B. J., BAUMGARDNER, F. W., BONDI, K. & RAHN, H. (1970). Acid–base balance in cold-blooded vertebrates as a function of body temperature. *Am. J. Physiol.* **218**, 600–605.

IRIAS, J. J. & GREENBERG, R. E. (1972). Relationship of renal gluconeogenesis to control of ammonia formation. *Am. J. Physiol.* **223**, 750–755.

KALRA, J. & BROSNAN, J. T. (1974). The subcellular localization of glutaminase isoenzymes in rat kidney cortex. *J. biol. Chem.* **249**, 3255–3260.

KAMM, D. E. & ASHER, R. R. (1970). Relation between glucose and ammonia production in renal cortical slices. *Am. J. Physiol.* **218**, 1161–1165.

LAW, R. O. (1975). The inulin space, solute concentrations, and weight changes in rat renal medullary slices incubated in iso-osmolal media, and their modification during anoxia and hypothermia. *J. Physiol.* **247**, 37–54.

LEMIEUX, G., VINAY, P. & CARTIER, P. (1974). Renal hemodynamics and ammoniagenesis. Characteristics of the antiluminal site for glutamine extraction. *J. clin. Invest.* **53**, 884–894.

OWEN, E. E. & ROBINSON, R. R. (1963). Amino acid extraction and ammonia metabolism by the human kidney during the prolonged administration of ammonium chloride. *J. clin. Invest.* **42**. 263–276.

PARK, Y. S. & HONG, S. K. (1976). Properties of toad skin Na–K-ATPase with special reference to effect of temperature. *Am. J. Physiol.* **231**, 1356–1363.

PARK, Y. S. & SOLOMON, S. (1977). pH–temperature dependence of organic acid transport in rat kidney slices. *Am. J. Physiol.* **233**, 382–387.

PILKINGTON, L. A. & O'DONOVAN, D. J. (1971). Metabolism of glutamine in cortex slices from dog kidney during acid–base alterations. *Am. J. Physiol.* **220**, 1634–1639.

PILKINGTON, L. A., YOUNG, T. K. & PITTS, R. F. (1970). Properties of renal luminal and antiluminal transport of plasma glutamine. *Nephron* **7**, 51–60.

PITTS, R. F. (1964). Renal production and excretion of ammonia. *Am. J. Med.* **36**, 720–742.

PITTS, R. F. (1973). Production and excretion of ammonia in relation to acid–base regulation. In *Handbook of Physiology*, sect. 8, *Renal Physiology*, ed. ORLOFF, J. & BERLINER, R. W., pp. 455–496. Washington, D.C.: American Physiological Society.

PREUSS, H. G. (1969). Renal glutamate metabolism in acute metabolic acidosis. *Nephron* **6**, 235–246.

PREUSS, H. G., BAIRD, K. & EASTMAN, S. T. (1978). The regulation of renal ammoniagenesis in the rat by extracellular factors. II. Ammoniagenesis by rat kidney slices incubating in normal acidotic sera. *Metabolism* **27**, 1639–1647.

PREUSS, H. G., VAVATSI-MANOS, O. & VERTUNO, L. L. (1973). Effects of glutamine deamination on glutamine deamidation in rat kidney slices. *J. clin. Invest.* **52**, 755–764.

RAHN, H. (1974). Body temperature and acid–base regulation. *Pneumonologie* **151**, 87–94.

RELMAN, A. S. & NARINS, R. G. (1975). The control of ammonia production in the rat. *Med. Clin. North Am.* **59**, 583–593.

SHALHOUB, R., WEBBER, W., GLABMAN, S., CANESSA-FISCHER, M., KLEIN, J., DEHASS, J. & PITTS, R. F. (1963). Extraction of aminoacids from and their addition to blood plasma. *Am. J. Physiol.* **204**, 181–186.

TANNEN, R. L. & KUNIN, A. S. (1976). Effect of potassium on ammoniagenesis by renal mitochondria. *Am. J. Physiol.* **231**, 44–51.

UMBREIT, W. W., BURRIS, R. H. & STAUFFER, J. H. (1972). In *Manometric and Biochemical Techniques*, 5th edn. Minneapolis: Burgess Publishing Co.

VAN SLYKE, D. E., PHILLIPS, R. A., HAMILTON, P. B., ARCHIBALD, R. M., FUTCHER, P. H. & HILLER, A. (1943). Glutamine as source material of urinary ammonia. *J. biol. Chem.* **150**, 481–482.

VINAY, P., LEMIEUX, G. & GOUGOUX, A. (1979). Characteristics of glutamine metabolism by rat kidney tubules: a carbon and nitrogen balance. *Can. J. Biochem.* **57**, 346–356.