The effect of acidosis on the labelling of urinary ammonia during infusion of [amide-15N]glutamine in human subjects

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In three experiments [amide-15N]glutamine was infused intravenously in male volunteers. After 4–8 h of infusion acidosis was achieved by an oral dose of $CaCl_2$ (1 mmol/kg). In one subject acidosis was maintained for 5 d. The acid load produced an approximately 3-fold increase in urinary NH_3 excretion, with a small (approximately 20%) and transient increase in the isotope abundance of urinary NH_3. Estimates of glutamine production rate (flux) were obtained in two experiments. There was no evidence that it was increased in acidosis. The extra NH_3 production by the kidney represented only a very small part, about 3%, of the total glutamine production rate.

Acidosis: Glutamine metabolism: Urinary ammonia

The principal objective of the work reported here was to examine the effect of acidosis on the labelling of urinary NH_3 during infusion of an amino acid labelled with 15N. In the endproduct method for measuring whole-body protein turnover with a 15N-labelled amino acid, N flux is determined from the isotope abundance in urinary urea or NH_3 or both (Waterlow *et al.* 1978; Fern & Garlick, 1983; Fern *et al.* 1985*a*, *b*). The method has been applied in a number of situations, for example in premature babies (Catzeflis *et al.* 1985), in malnourished children (Jackson *et al.* 1983) and adults (Soares *et al.* 1991), in pregnant women (de Benoist *et al.* 1985) and in the elderly (Golden & Waterlow, 1977).

Being simple and non-invasive, this method is particularly suitable for application to people who are ill. Because many sick patients have some degree of acidosis it is important to know whether and how far acidosis *per se* alters the labelling of urinary NH_3. To the extent that this occurs, it would introduce an error into the calculation of turnover rate.

In this work we have chosen to use as the tracer glutamine (GLN) labelled in the amide (C-5)-N, rather than [15N]glycine, which has been more generally used for measurements of protein turnover, because the amide-N of GLN is the major precursor of urinary NH_3. Further studies on acidosis with [15N]glycine as tracer will be reported later.

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There is a large amount of information in the literature on glutamine metabolism and its relationship to renal NH_3 production in man (Owen & Robinson, 1963; Pitts & Pilkington, 1966; Tizianello *et al.* 1978, 1982), sheep (Heitmann & Bergman, 1978), dog (Pitts *et al.* 1965; Cersosimo *et al.* 1986; Areas *et al.* 1987) and the rat (Squires *et al.* 1976; Schröck & Goldstein, 1981; Squires & Brosnan, 1983; Welbourne, 1986). However, we know of only three studies on man and none on experimental animals in which glutamine labelled with ^{15}N was used (Darmaun *et al.* 1986, 1988; Golden *et al.* 1982), and in none of these was the effect of acidosis examined. Therefore, this work, apart from its immediate objective, provides some further insights into glutamine metabolism in humans in both normal and acidotic states. So far we have concentrated mainly on the effects of acute acidosis, because this is easier to produce experimentally.

We report here the results of two pilot studies, each on a single subject, and results of the main study, in which there were thirteen tests on seven subjects. Pilot 1 was done in London, pilot 2 in Jamaica at the Tropical Metabolism Research Unit and the main study was done in Southampton. In all the tests $[5\text{-}^{15}N]GLN$ was infused intravenously for varying periods. In most of the tests $CaCl_2$ was used to provoke acidosis (Oster *et al.* 1975) since NH_4Cl may alter the rate of NH_3 excretion.

The object of pilot 1 was to examine the effect of an acute acid load on the labelling of NH_3 and urea. In pilot 2 we also looked at the effects of acidosis maintained over 5 d. The purpose of the main study was similar to that of pilot 1, but with a shorter protocol and a larger number of subjects.

METHODS
Subjects

All the subjects were healthy male volunteers. The nature and purpose of the experiments were explained to them and ethical approval was given by the respective ethical committees.

Protocols

The protocols are summarized in Fig 1.

In pilot 1 the subject was a healthy male aged 65 years, weighing 78 kg. After an overnight fast baseline measurements were made. Intravenous infusion of $[5\text{-}^{15}N]$glutamine (95 atom % excess (APE); Prochem, London) dissolved in sterile saline (9 g NaCl/l) at a rate of 178 μmol/h was then started and continued for 16 h. Urine was collected hourly and blood samples every 2 h from an indwelling catheter in the antecubital vein opposite to that of the infusion. From the start of the infusion food was given two-hourly as a sandwich providing one-eighth of a total daily intake of 12·5 MJ and 70 g protein. At 8 h, 10 g $CaCl_2$ (1·15 mmol/kg) dissolved in water was taken orally, and the infusion continued for a further 8 h.

In pilot 2 the subject was a healthy male aged 37 years, weight 64·5 kg. The study was conducted over 5 d. On each day an intravenous infusion of $[5\text{-}^{15}N]GLN$, 97 APE, in normal saline at a rate of 33·3 μmol/h was started in the morning after an overnight fast. Water but no food was given during the infusions. On the first day the infusion was continued for 8 h. $CaCl_2$ (1 mmol/kg) was ingested at 4 h, and the infusion continued over the next 4 h. Baseline samples of blood and urine were taken before the infusion, and at 0·5 h intervals from 2 to 4 h and from 6 to 8 h. Over the next 4 d the subject ingested 2 mmol $CaCl_2$/kg, per d divided into four doses. The protocol was the same as for the first day, except that the infusion was continued for only 4 h.

In the main experiment there were seven subjects, four of whom had one test only, in which acidosis was produced by $CaCl_2$ (1 mmol/kg) given orally. One other subject had a second test with $CaCl_2$, as well as a control infusion with no acid load. Two others had
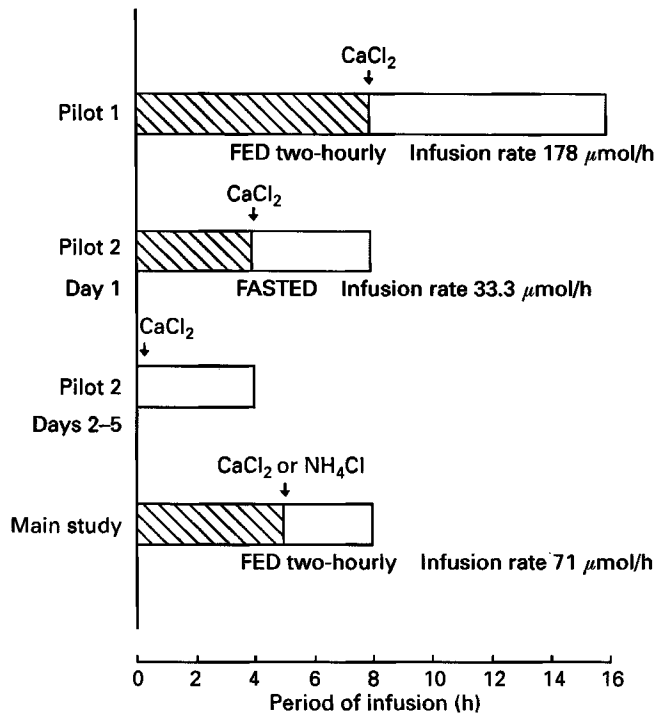
Fig. 1. Summary of protocols for glutamine infusions used for studies with human subjects. (▨), before induction of acidosis; (▢), after induction of acidosis. All infusions started after an overnight fast.

additional tests in which acidosis was provoked by NH_4Cl (2 mmol/kg), followed by control infusions without acidosis. In these three subjects who had repeated tests the average interval between tests was 1 month (range 0·5–3 months).

The protocol was similar to that of pilot 1, but of shorter duration. The subjects were fasted overnight. Baseline samples were taken, and then an intravenous infusion of [5-^{15}N]GLN, 96 APE, at a rate of 71 μmol/h was started. Blood samples were collected hourly from an indwelling catheter in the contralateral antecubital vein, and urine samples were collected every 0·5 h. Every 2 h the subject took a drink of a milk-based mixture (Ensure) which provided 830 kJ and 7 g protein. After 5 h infusion $CaCl_2$ (67 mmol; eight tests) or NH_4Cl (132 mmol; two tests) was ingested and the infusion continued for a further 3 h.

Analytical methods

Blood pH was measured in the Intensive Care Unit with an accuracy of at least 0·05 pH units. Urinary pH, titratable activity and concentrations of NH_3 and urea were measured by standard methods (Kaplan, 1965). For determination of isotope abundance in the urinary endproducts a sample of urine was made alkaline and the NH_3 removed by aeration and collected in dilute acid. After removal of NH_3 the sample was brought to pH 5 and incubated with urease (EC 3.5.1.5; Sigma) for 30 min. After alkalinization the NH_3 liberated was collected as before.

In pilot 1 the isotope abundance of the NH_3 collected was measured in a modified single-collector mass spectrometer (MS 20; AEI, Manchester). In pilot 2 it was measured in a dual-collector mass spectrometer (VG Micromass 602C; VG Isogas, Winsford, Ches.), and in the main study a triple collector Sira 10 (VG Isogas) was used.

Plasma GLN concentration was measured in pilot 1 only, by standard ion-exchange chromatography.

The isotope abundance of plasma GLN was measured after liberation of the amide-N by glutaminase (EC 3.5.1.2) as previously described by Golden et $al.$ (1982), but only in pilot 2 were these measurements satisfactory. In pilot 1 the samples had to be stored deep-frozen at $-20°$ for 3 years because the MS 20 mass spectrometer, the only instrument available at that time, was not sensitive enough for these measurements. During this time glutamine was evidently hydrolysed. When calculating abundance it was assumed that all the ^{15}N in the sample of plasma was derived from the amide-N of glutamine. Abundance was then estimated as μmol ^{15}N per ml plasma divided by μmol GLN per ml plasma, as previously determined by chromatography. However, it was evident that some of the NH_3 was lost during the prolonged period of storage, since the calculated APE of the precursor glutamine was lower than that of the product NH_3, which is impossible. Nevertheless, the results should be comparable within the experiment.

RESULTS
Acidosis

The urinary data are summarized in Table 1. The values before acidosis relate to the last 2–2·5 h before the acid load; 1 h was then allowed for adjustment and the data for that 1 h are not included in Table 1.

Table 1 shows that a single dose of $CaCl_2$ produced an acidosis which, however, was compensated since there were no significant falls in blood pH or pCO_2 (data not shown). The acidosis with NH_4Cl was slightly more intense than that with $CaCl_2$ because the dose was larger. In the main experiment there was a wide variation between subjects in excretion of acid and NH_3 but each subject was fairly consistent from one period to another. In chronic acidosis blood pCO_2 fell from a baseline value of 41 mmHg to a mean value of 29 mmHg on days 3–5. There was no fall in blood pH.

Labelling of urinary ammonia

Fig. 2 shows the time-course of the labelling of urinary NH_3 in the main experiment. The rise towards a plateau was slower than that reported in the studies of Golden et $al.$ (1982) in which plateau was achieved in 2–3 h. After the acid load there was an increase, of the order of 20–25 %, in the APE of NH_3, with a time-lag of about 2 h. This increase implies that either the precursor glutamine was more highly labelled or a smaller proportion of urinary NH_3 was being derived from unlabelled precursors (see p. 89).

The proportion of the dose of ^{15}N excreted in NH_3 represents the proportion of the flux of GLN-amide-N excreted in this endproduct. It is evident from Table 2 that even at the peak of acidosis renal NH_3 production accounted for only a small proportion of the GLN flux. In pilot 1 we have no explanation for the higher rate of excretion of ^{15}N expressed as a proportion of the dose, unless it was that this subject was almost twice as old as any of the others.

Urea

Urea excretion and labelling were measured in pilot 1 and the main study but not in pilot 2. In pilot 1 excretion decreased significantly after the establishment of acidosis, from a mean of 19·1 mmol/h before acidosis to 11·2 mmol/h after acidosis. This was in keeping with the findings of others (e.g. Häussinger et $al.$ 1984; Monson et $al.$ 1984) that acidosis suppresses urea formation. However, this result was not confirmed in the main experiment,

Table 1. *Urine pH, titratable acid and ammonia excretion before and after induction of acidosis in human subjects**

	pH		Titratable acid (mmol/h)		NH$_3$ (mmol/h)	
	Before	After	Before	After	Before	After
Acute acidosis†						
Pilot 1	6·72	4·93	0·28	0·90	1·46	2·32
Pilot 2						
First day	7·06	4·93	0·36	1·69	0·45	1·53
Main experiment						
CaCl$_2$: Mean	7·02	5·22	−0·08	1·75	0·94	2·36
SD	0·29	0·42	0·39	0·74	0·28	0·82
NH$_4$Cl	6·96	5·14	−0·36	2·54	1·30	3·15
Controls	7·07	7·045	0·08	0·02	0·85	0·99
Chronic acidosis						
Pilot 2, days 2–5	4·92		1·36		1·95	

* For details of procedures, see pp. 84–86.

† For pilots 1 and 2 and main experiment respectively measurements were made 4, 2 and 2·5 h before and 4, 2 and 2 h after acidosis, with a gap of 1, 2 and 1 h. For the controls measurements were made at times equivalent to those in experiments in which acid was given.
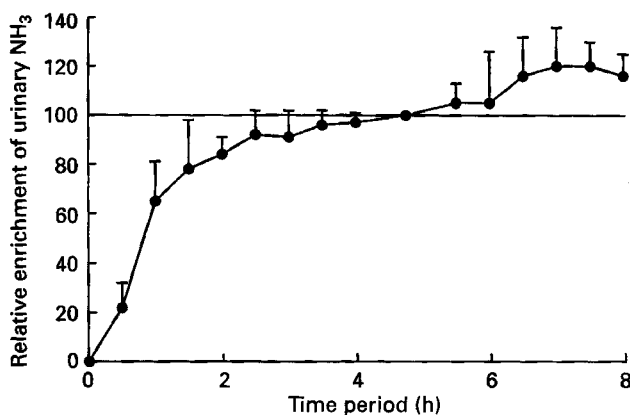

Fig. 2. Main experiment. Isotope enrichment of urinary ammonia during 8 h infusion of [2-^{15}N]glutamine. Each period was 30 min. Because there was much variation between subjects, for each subject the mean enrichment in samples 9 (4·5 h) and 10 (5·0 h) was taken as 100, and the enrichment in samples at earlier and later times expressed as a percentage of this value. Values are means and between-subject standard deviations represented by vertical bars. For details of procedures, see pp. 84–86.

in which the acid load was not followed by any significant change in the urea output. There was much variation between subjects, but no rank order correlation between the rate of urea excretion and the extent of acidosis, as judged by urinary pH, titratable acid or NH$_3$ output.

The time-course of the labelling of urea fits that which would be expected if the urea pool is turning over with a half-life of approximately 10 h.

Table 2. *Percentage of the dose of ^{15}N excreted/h in urinary ammonia before and after acute acidosis and during chronic acidosis in human subjects**

	Before acidosis		After acidosis	
	Period†(h)	Percentage of dose (/h)	Period†(h)	Percentage of dose (/h)
Acute acidosis				
Pilot 1	6–8	2·30	9–12	5·54
			12–15	7·53
Pilot 2, day 1	2–4	0·83	6–8	3·30
Main experiment‡	4–5	0·53	6–7	1·29
			7–8	1·62
Chronic acidosis				
days 2–5 (data for day 4 missing)			2–4	3·1

* For details of procedures, see pp. 84–86.

† From $t = 0$ at start of the infusion.

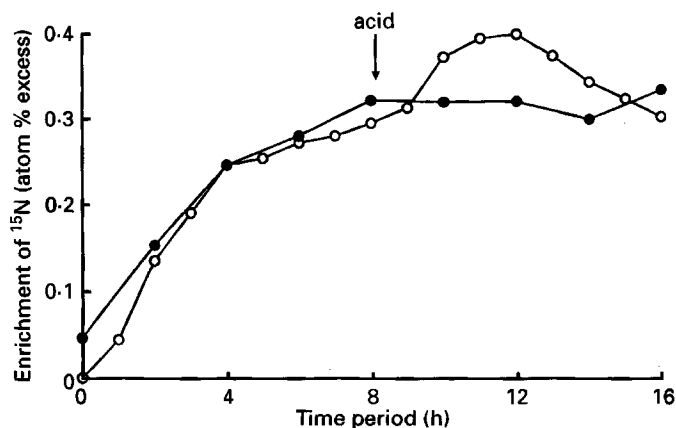‡ Results with $CaCl_2$ and NH_4Cl combined.

Fig. 3. Pilot 1. ^{15}N (atom % excess) in plasma glutamine amide-N (●——●) and in urinary ammonia (○——○) before and after the acid load in a male volunteer. For details of procedures, see pp. 84–86.

Glutamine turnover

Plasma GLN concentration was measured by chromatography in pilot 1. It did not change with the induction of acidosis. As mentioned on p. 86, it is likely that hydrolysis and loss during prolonged storage resulted in an underestimate of the labelling of GLN, but there is no reason why the values should not be compared within the experiment. The apparent APE of GLN reached a plateau in 8 h (Fig. 3) compared with 2–3 h in the studies of Golden *et al.* (1982) and Darmaun *et al.* (1986). The interesting point is that the acidosis had no effect on the APE of GLN, but, as in the main study, there was a sharp rise in the APE of NH_3.

Results from pilot 2 are shown in Table 3. In this experiment the more prolonged acidosis led to a fall in GLN production rate, most evident on day 2. Nevertheless, although there was a several-fold increase in the amount of urinary NH_3 derived from GLN, it still represented only 4 % of the glutamine flux.

Table 3. *Pilot 1. The effect of chronic acidosis in one subject over 4 d**

Day of study...	1 (baseline)	2	3	4	5
NH$_3$ excretion (mmol/h)	0·45	2·64	1·00	2·60	1·55
Glutamine production (B; mmol/h)	42·2	28·8	35·5	—	38·0
Percentage of glutamine produced excreted as NH$_3$(C)†	0·83	4·20	1·90	—	3·23
Amount of urinary NH$_3$ derived from glutamine amide-N (mmol/h)‡	0·35	1·21	0·67	—	1·23
Percentage of urinary NH$_3$ derived from glutamine amide-N§	78	46	67	—	79

* For details of procedures, see pp. 84–86.

† Calculated as $\dfrac{\text{rate of }^{15}\text{N excretion in urinary NH}_3}{\text{rate of infusion of }^{15}\text{N}}$.

‡ Calculated as B × C.

§ Calculated as $\dfrac{\text{APE of urinary NH}_3}{\text{APE of plasma glutamine}} \times 100$, where APE is atoms % excess.

DISCUSSION

The initial objective of these experiments was to examine the effect of acidosis on the labelling of urinary NH$_3$ when the label was derived from GLN amide-N, the main precursor of NH$_3$. In acute acidosis a 3-fold increase in the rate of NH$_3$ output was accompanied by a transient increase in isotope abundance, which at its peak was 20% above the level before acidosis (Fig. 3). If acidosis altered the pattern of precursors of NH$_3$, so that less was derived from GLN and more from other amino acids such as glycine, one would expect the isotope abundance to decrease. The literature on this point is conflicting. Owen & Robinson (1963) found no change in acidosis in the proportion of NH$_3$ derived from GLN, whereas according to Tizianello *et al.* (1982) 'in man, at an early stage of metabolic acidosis, glutamine fails to sustain its role as the nearly exclusive precursor of ammonia'. Our results tend to support those of Owen & Robinson (1963).

The question has been posed: what are the sources of the extra urinary NH$_3$ in acidosis (Goldstein *et al.* 1980)? If the precursors remain essentially unchanged, a 3-fold increase in NH$_3$ output will represent a 3-fold increase in the rate of GLN extraction by the kidney. Thus, in the dog, Areas *et al.* (1987) found a linear relationship between renal GLN extraction and urinary NH$_3$ production. Similar results have been recorded by others; in man by Owen & Robinson (1963) and Tizianello *et al.* (1978), in sheep by Heitmann & Bergman (1978), in the dog by Lotspeich (1967) and Cersosimo *et al.* (1986) and in the rat by Goldstein *et al.* (1980) and Welbourne (1988).

The results in Table 2 indicate the proportion of the glutamine flux extracted by the kidney for NH$_3$ formation, but they do not answer the key question: whether the increased extraction is accompanied by an increase in flux. In the main experiment, admittedly flawed by technical problems, no evidence of an effect of acidosis on glutamine labelling and, hence, on production rate was found (Fig. 3). In pilot 2 the production rate actually fell with acidosis (Table 3). We conclude that the flux is so large in relation to NH$_3$ production that no increase is necessary.

It is true that our values for GLN production rate are twice as high as those found by Darmaun *et al.* (1986), Dutra *et al.* (1992) and Matthews *et al.* (1993). Darmaun *et al.* (1986) suggested as a possible reason that in their studies arterialized blood was used for

Table 4. *Effect of acidosis on inter-organ flux rates (mmol/h per whole animal; 50 kg) in the sheep (from Heitmann & Bergman, 1978)*

	Uptake	Release	Net
Viscera and liver			
Normal	V_{VC} 9·4	V_{CV} 6·4	+3·0 Metabolism
Acidotic	V_{VC} 10·8	V_{CV} 8·1	+2·7 Metabolism
Muscle and peripheral tissues			
Normal	V_{MC} 2·4	V_{CM} 4·7	−2·3 Synthesis
Acidotic	V_{MC} 0·9	V_{CM} 4·7	−3·8 Synthesis
Kidney			
Normal	V_{KC} 0·5	V_{CK} 1·2	−0·7 Urine
Acidotic	V_{KC} 1·6	V_{CK} 0·3	+2·3 Urine
Production rate = $V_{CM} + V_{CV} + V_{CK}$			
Normal = 12·3			
Acidotic = 13·1			

V_{CV}, V_{CM}, V_{CK}, flux from viscera, muscle and kidney respectively to central pool; V_{VC}, V_{MC}, V_{KC}, flux from central pool to viscera, muscle and kidney respectively.

sampling, but recorded arterio–venous differences in GLN concentration in peripheral blood (e.g. Tizianello *et al.* 1978) are not enough to explain the discrepancy, which clearly needs further investigation. However, even if our values for GLN flux are too high by a factor of 2, still only a small proportion of the flux would be used for NH_3 production.

The absence of a change with acute acidosis in whole-body GLN flux does not mean that there is no change in GLN kinetics. Darmaun *et al.* (1986) took blood samples every 15 min, and achieved plateau labelling in about 2 h. Assuming that the rise to plateau could be represented by a single exponential, they calculated a rate-constant of 0·025 min. From this and the production rate they estimated an exchangeable pool size for GLN of 207 μmol/kg. About two-thirds of this pool could be accounted for by extracellular GLN. Darmaun *et al.* (1986) pointed out that the intracellular pool was some fifty times greater than the calculated 'exchangeable' pool and, therefore, postulated that there was a very large 'immiscible' pool, probably mostly in muscle. In reality, however, this pool need not be regarded either as immiscible, or as a sink from which there is no return. The pool is simply so large and turning over so slowly that the effect of it does not show up in a short-term isotope-abundance curve; it behaves like muscle in measurements of whole-body protein turnover.

In man we do not have enough data to quantify the absolute exchanges between tissues. Arterio–venous differences only give the net exchanges. The only studies we know of in which isotopic measurements were combined with arterio–venous differences to allow estimates of absolute rates of uptake and release are those of Heitman & Bergmann (1978) in the sheep. Their findings are summarized in Table 4. If a steady-state is assumed in each pool, the net values of influx–efflux, if negative, must be balanced by *de novo* synthesis; if positive, by net disposal or metabolism. It seems that the main effects of acidosis are: in the peripheral tissues a decrease in uptake balanced by an increase in net synthesis; in the viscera an increase in GLN release, with a small decrease in disposal; and in the kidney a substantial switch from net release to net uptake.

In man the information summarized by Darmaun *et al.* (1986) indicates that GLN uptake by the kidney and release by muscle are of the same order (30 μmol/kg per h). This is the reason for the question posed earlier about the sources of extra NH_3 in acidosis.

However, the high rate of GLN flux allows flexibility in the system, and if the results from sheep apply to man, the increase in GLN uptake by the kidney is achieved by a series of redistributions of components of the flux, each quite small in itself. We do not propose to discuss possible biochemical mechanisms for achieving these adjustments, because we have no new observations that bear on this subject.

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