Glutamine production rate and its contribution to urinary ammonia in normal man

M. H. N. GOLDEN, P. JAHOOR AND A. A. JACKSON
Tropical Metabolism Research Unit, University of the West Indies, Mona, Kingston, Jamaica

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Summary

1. Glutamine [15N]amide was infused at a steady rate of 33.34 μmol/h into seven male adult volunteers who were in the fed state and normal acid–base status.

2. Plasma glutamine amide N enrichment and urinary ammonia N enrichment rose to a constant value within 3 h.

3. The glutamine production rate was 51.8 ± 7.9 mmol/h.

4. The total ammonia excretion rate was 0.87 mmol/h. Of this excreted ammonia 62.6 ± 9% was derived from the amide N atom of glutamine.

5. The excreted glutamine amide N (0.53 mmol/h) was only 1% of the glutamine production. If half the ammonia formed by the kidney is excreted in urine and half liberated into the renal vein in subjects with normal acid–base status [E. E. Owen & R. R. Robinson (1963) Journal of Clinical Investigation, 42, 263–276], then the kidney accounts for only 2% of glutamine disposal.

6. Whole body protein turnover, measured from the urinary [15N]ammonia enrichment, was 30.3 ± 7.7 g of N/day (2.8 g of protein day⁻¹ kg⁻¹).

Key words: ammonia, ammoniagenesis, glutamine, nitrogen, protein turnover.

Introduction

Although glutamine is the most abundant free amino acid in the body, comprising 69% of the total free amino acid pool of muscle [1], its metabolism has received scant attention. This situation has arisen for two reasons. First, the ease with which glutamine is hydrolysed in vitro to glutamic acid and free ammonia renders analysis by column chromatography unreliable and necessitates particular precautions to be taken in specimen handling and the use of specific enzymatic analysis. Secondly, tracer studies of glutamine metabolism require the amide N moiety to be labelled with 15N. Radioactive carbon is an inappropriate label because the carbon skeleton is common to glutamine, glutamate and α-oxoglutarate; thus the metabolism of these three compounds cannot be confidently separated when this isotope is used.

The only published data on glutamine metabolism in man that we can find are arteriovenous glutamine concentration differences across various organs, which, when combined with measurements of blood flow, give estimates of the net production or utilization of glutamine. These studies consistently show glutamine to be released from limb tissue, presumably muscle [2, 3], and taken up by kidney [4] and the splanchnic bed [2, 3]. This release of glutamine from the periphery and uptake by the viscera is a consistent finding in the rat, dog, monkey and sheep, whether in the fed, postabsorptive, fasted, diabetic or acidotic state. It has been viewed as a non-toxic way of transporting ammonia to the liver and kidney for excretion as urea or free ammonia respectively [5, 6]. That glutamine is the major precursor of urinary ammonia was inferred from the consistent uptake of glutamine by the kidney in quantities sufficient to account for about 60% of renal ammonia production [4]. The actual contribution of glutamine N to urinary
ammonia has only been studied in dogs made chronically acidotic by ammonium chloride ingestion, with renal artery infusions of [15]N-glutamine [7-9]. Under these conditions glutamine was a major precursor of urinary ammonia.

The provision of N for renal ammoniagenesis has long been held to be the major function of glutamine, and various sites such as the liver [11] and muscle [2] have been suggested as the source of glutamine when there is a requirement for increased renal ammoniagenesis. More recently Windmueller & Spaeth [12] have demonstrated that the intestine has a requirement for glutamine: this is the principal respiratory fuel of rat small intestine in both the fed and fasted states. The relative demands of the kidney and intestine for glutamine are unknown. Furthermore, the quantitative relationships between glutamine production and utilization have not been determined for any species. The present study was designed to provide this information for normal man.

Subjects and methods

Subjects

Seven male adult volunteers were studied (Table 1). Each gave informed consent. All were in good health and had never had any disease thought to leave sequelae in muscle, kidney, liver, intestine or brain function.

Protocol

The infusion of subject no. 1 was used as a pilot study to determine the most appropriate sampling times and feeding regimens for the subsequent studies [13]. This subject ate one egg sandwich (52 g of white bread, 5 g of butter and 60 g of egg) immediately before the infusion, and one-quarter sandwich at hourly intervals during the study. L-Glutamine [15]N-amide (97 atoms excess, Prochem), dissolved in sodium chloride solution (150 mmol/l), was infused at a rate of 33.34 μmol of [15]N/h into a peripheral arm vein. Urine was collected immediately before the infusion and at 0.5 h intervals for the succeeding 6 h. Blood samples (20 ml) were drawn from an indwelling cannula in the contralateral forearm at hourly intervals.

The subsequent six subjects ate their habitual breakfast between 07.30 and 08.00 hours. At 08.30 hours they each consumed 24 g of white bread. At 08.55 hours they emptied their bladders, gave a baseline blood sample and ate 12 g of bread; the infusion of L-glutamine [15]N-amide, at the same dose rate as in subject no. 1, was started at 09.00 hours. The subjects drank 100 ml of iced water, ate 12 g of bread and emptied their bladders at 0.5 h intervals for 4 h. Five subsequent blood samples were taken at 0.5 h intervals from 2 to 4 h after the start of the infusion. Subject no. 1 sat throughout the study: except when voiding the other subjects were lying at rest in bed throughout the study.

Analytical procedures

Urine pH was measured on a portion of urine within 3 min of voiding, with a Radiometer pH electrode; the remaining urine was kept in sealed containers, on ice, until free ammonia was assayed and extracted. This was always within 2 h of the end of the infusion. Urine ammonia was assayed by the indophenol reaction [14]. A portion of urine containing 130 μmol of free

Table 1. Details of the subjects and enrichments (atoms % excess) of urinary ammonia and plasma glutamine amide N, at isotopic equilibrium, during constant infusion of glutamine [15]N-amide

All enrichments are relative to preinfusion [15]N abundance. Numbers in parentheses give the coefficient of variation of the samples at plateau. Diet A was hourly egg sandwich and diet B was half-hourly bread and water.

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>Diet</th>
<th>Mean urine pH</th>
<th>Time to plateau (h)</th>
<th>Enrichment (atoms % excess)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Urine ammonia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Plasma glutamine amide N</td>
</tr>
<tr>
<td>1</td>
<td>36</td>
<td>67.3</td>
<td>A</td>
<td>7.08</td>
<td>1.0</td>
<td>0.0556 (17.7)</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>68.6</td>
<td>B</td>
<td>7.08</td>
<td>1.0</td>
<td>0.0457 (5.5)</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>66.4</td>
<td>B</td>
<td>7.32</td>
<td>1.0</td>
<td>0.0428 (5.2)</td>
</tr>
<tr>
<td>4</td>
<td>29</td>
<td>66.4</td>
<td>B</td>
<td>6.70</td>
<td>0.5</td>
<td>0.0301 (4.4)</td>
</tr>
<tr>
<td>5</td>
<td>35</td>
<td>69.1</td>
<td>B</td>
<td>7.27</td>
<td>2.5</td>
<td>0.0463 (5.7)</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>76.8</td>
<td>B</td>
<td>6.94</td>
<td>2.5</td>
<td>0.0426 (4.2)</td>
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<td>7</td>
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<td>77.3</td>
<td>B</td>
<td>7.16</td>
<td>3.0</td>
<td>0.0283 (10.0)</td>
</tr>
<tr>
<td>Mean</td>
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<td>7.08</td>
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<td>0.0416</td>
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<tr>
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<td></td>
<td>0.23</td>
<td></td>
<td>0.0096</td>
</tr>
</tbody>
</table>
ammonia was adjusted to pH 12 with 30% sodium hydroxide and the ammonia collected by entrainment in acid-washed air and trapping in HCl (0.1 mol/l) in a Leurquin apparatus.

Blood, anticoagulated with one unit of ammonia-free heparin (Weddel Pharmaceuticals Ltd), was immediately placed in a crushed-ice bath. All subsequent steps were carried out at 0–4°C unless indicated. The blood was centrifuged for 10 min and the plasma proteins were precipitated from the plasma (approx. 12 ml) with 10 ml of ice-cold 10% (w/v) perchloric acid and rapidly centrifuged. The perchloric acid was removed by adjustment to pH 7 with 30% potassium hydroxide. After centrifugation to remove the precipitated potassium perchlorate, the samples were brought to pH 10.5 with 30% sodium hydroxide and free ammonia was removed by aeration for 3 h. It is essential to maintain the samples at 0°C during this procedure to prevent glutamine hydrolysis. Under these conditions approximately 1.4% of the glutamine is hydrolysed [115]. The samples were then adjusted to pH 4.9 with hydrochloric acid (1 mol/l) and 1 ml of sodium acetate buffer (1.5 mol/l), pH 4.9, was added to give a final buffer concentration of 75 mmol/l. One unit of glutaminase I (Grade V, Sigma Chemical Co.) was added and the sample incubated in a water bath at 37°C for 4 h. The samples were then cooled to 0°C, made alkaline (pH 10.5) with 30% sodium hydroxide and the ammonia liberated from glutamine amide was removed in the Leurquin apparatus by acid-washed air entrainment for 3 h. The resulting samples contained about 6 μmol of ammonium chloride.

Nitrogen gas was evolved by the method of Sprinson & Rittenberg [16], in miniaturized Rittenberg tubes. The enrichment was measured in a dual collector mass spectrometer (Micromass 602C), with the capillaries uncrimped.

Calculations and theory

The steady-state dilution of the 15N in plasma was used to estimate the glutamine production rate from the formula

\[ \frac{d}{c_{GN}} = \dot{p}_{Gln} \]

where \( \dot{p}_{Gln} \) = glutamine production rate (mmol/h), \( d \) = dose of 15N in mmol/h and \( c_{Gln} \) = the enrichment of glutamine amide N over base line in atoms excess [117]. The production rate thus measured, with infusion into and sampling from the plasma, gives an estimate of the amount of glutamine entering the plasma compartment which is new to the plasma. It does not include glutamine which leaves the plasma and subsequently returns to the plasma; the production rate may thus be less than the total turnover of plasma glutamine, and in the steady state is equal to the irreversible disposal rate of glutamine. The total disposal rate will be underestimated by the amount of label lost from glutamine, which is subsequently reincorporated into glutamine and enters the plasma compartment. The pool in which the labelled glutamine is distributed is thus not equivalent to the plasma pool, but will include the interstitial fluid and a theoretical volume of intracellular fluid corresponding to the volume that would be occupied by the labelled intracellular glutamine if it had the same enrichment as the plasma glutamine.

With regard to experiments with other labelled amino acids used to determine protein synthesis rates we have, in the past, used the term flux to denote the same quantity as production rate [18]. We have avoided use of this term in this paper because it is also used to denote a different quantity, the unidirectional transport from one compartment to another, by investigators studying transport kinetics.

The proportion of urinary ammonia derived from glutamine amide N was calculated from the ratio of the enrichment of urinary ammonia to the enrichment of plasma glutamine amide N, on the assumption that glutamine amide N was the only enriched precursor of urinary ammonia.

The proportion of glutamine amide produced that was dispensed of by excretion as urinary ammonia (\( E_{Gln(NH_3)} / \dot{p}_{Gln} \)) was calculated on the assumption that labelled and unlabelled glutamine were metabolized in the same way; thus this proportion should equal the proportion of the 15N dose excreted as urinary ammonia:

\[ E_{Gln(NH_3)} / \dot{p}_{Gln} = \epsilon_{NH_3} \dot{E}_{NH_3} / d \]

where \( E_{Gln(NH_3)} \) = that proportion of total urinary ammonia that is derived from glutamine amide N, \( \dot{E}_{NH_3} \) = the urinary ammonia excretion rate and \( \epsilon_{NH_3} \) = the enrichment of urinary ammonia (atoms excess, atoms % excess \( \times 10^{-2} \)). The estimate of this proportion of glutamine disposal by the kidney is not dependent upon the measurement of plasma glutamine amide N enrichment, as seen from the right-hand term of this equation.

Whole body protein turnover (in previous publications termed flux) was derived from the dose of 15N as glutamine amide N and the steady-state enrichment of urinary ammonia as previously described [119].

All enrichments were taken as the increment in 15N abundance over the same N moiety measured.
in the baseline sample. Results are expressed as means ± SD.

Results

The urinary pH for the six subjects was 7.08 ± 0.23 with a range of 6.7 to 7.3 (Table 1). We presume that these subjects were all in a normal acid–base state.

In the pilot study (subject no. 1) the enrichment of plasma glutamine amide N reached a steady state within 1 h and remained at plateau, within the limits of precision of the measurement, for 6 h. The measured enrichments from the other studies confirmed that from the hours 2 to 4 glutamine amide is in isotopic equilibrium. The high coefficient of variation (28%) seen in the pilot study was reduced to 6.7 ± 2.0% in subsequent studies, largely because of more careful control of the physiological state: in particular the dietary intake, fluid intake and activity were closely controlled.

The coefficient of variation of the plateau values for urinary ammonia enrichment also showed a high value for the pilot study (17%), falling to 5.8 ± 2.1% in subsequent studies. The urinary ammonia enrichments rose to a plateau value, confirming that an isotopic steady state had been achieved within the time course of these experiments. However, the rate of rise to plateau enrichment in the different subjects was quite variable. In subjects nos. 1–4 the plateau in urinary ammonia was achieved by 1 h; in contrast in subjects nos. 5–7 there was a slower increase in the enrichment so that isotopic equilibrium was not achieved for 2.5–3 h. No clinical, racial, antecedent dietary habit, or measured characteristic, could be identified that varied with these differing time courses to plateau. The slow rise to plateau could not be associated with incomplete bladder emptying.

The different rates of rise to plateau are shown for subjects nos. 2 and 5 in Fig. 1. The glutamine production rate (disposal rate) was 52 ± 8 mmol/h (Table 2). The production rate was lowest in the pilot study. There was no clear relationship between either the urinary pH or the ammonia excretion rate and the glutamine production rate; however, this study was designed to produce a homogeneous group of subjects, and thus any relationship between the small variations in acid–base status and glutamine production could be expected to be overshadowed by other variables.

![Fig. 1. Enrichment in plasma glutamine amide N (○) and urinary ammonia N (■) with time, during an intravenous infusion of glutamine [15N]amide at 33.34 μmol of [15N]/h, in subjects nos. 2 and 5. The points for urinary ammonia enrichment are plotted at the middle of the urine collection period.](image-url)

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Urinary NH₃ (mmol/h)</th>
<th>Glutamine production rate (mmol/h)</th>
<th>Glutamine amide N derived urinary NH₃ (mmol/h)</th>
<th>NH₃ derived from glutamine amide N (%)</th>
<th>Glutamine amide N produced excreted as NH₃ (%)</th>
<th>Whole body turnover of N (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.88</td>
<td>41.6</td>
<td>0.61</td>
<td>69.4</td>
<td>1.5</td>
<td>21.6</td>
</tr>
<tr>
<td>2</td>
<td>0.85</td>
<td>46.3</td>
<td>0.54</td>
<td>63.3</td>
<td>1.2</td>
<td>26.3</td>
</tr>
<tr>
<td>3</td>
<td>0.41</td>
<td>53.7</td>
<td>0.28</td>
<td>68.9</td>
<td>0.5</td>
<td>28.0</td>
</tr>
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<td>4</td>
<td>1.37</td>
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<td>0.78</td>
<td>56.7</td>
<td>1.2</td>
<td>39.9</td>
</tr>
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<td>0.75</td>
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<td>25.9</td>
</tr>
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<td>6</td>
<td>0.93</td>
<td>44.8</td>
<td>0.50</td>
<td>54.2</td>
<td>1.1</td>
<td>28.2</td>
</tr>
<tr>
<td>7</td>
<td>0.91</td>
<td>59.7</td>
<td>0.46</td>
<td>50.7</td>
<td>0.8</td>
<td>42.4</td>
</tr>
<tr>
<td>Mean</td>
<td>0.87</td>
<td>51.8</td>
<td>0.53</td>
<td>62.6</td>
<td>1.0</td>
<td>30.3</td>
</tr>
<tr>
<td>SD</td>
<td>0.29</td>
<td>7.9</td>
<td>0.15</td>
<td>9.0</td>
<td>0.3</td>
<td>7.7</td>
</tr>
</tbody>
</table>
Glutamine metabolism in normal man

The administered dose of $[^{15}N]$glutamine represented less than 0.1% of the total entry of new glutamine to the plasma, and is thus unlikely of itself to have given rise to any perturbation of glutamine metabolism.

The ammonia excretion rate, 0.87 mmol/h, correlated with the urinary pH ($r = 0.93, P < 0.01$). The ratios of enrichments of the urinary ammonia and glutamine amide N showed that 63 ± 9% of the urinary ammonia was derived directly from plasma glutamine amide N. The range of values was from 50 to 75%.

We attempted to measure the enrichment of the free ammonia in plasma; however, the quantities of ammonia recovered were extremely small (approx 0.5 μmol) and below the limits of reliable measurement with our mass spectrometer (0.8 pmol). The results are consequently open to considerable error; six samples gave an average enrichment of 13% of the plasma glutamine amide N enrichment. As the glutamine pool is many times larger than the free ammonia pool, and there is some hydrolysis of the glutamine (1-4%), it is unlikely that free ammonia contributed more than negligible amounts of $[^{15}N]$ to urinary ammonia. If there were a contribution of $[^{15}N]$ from arterial free ammonia the contribution from glutamine amide N would be proportionately less.

One per cent of administered $[^{15}N]$ was excreted in urinary ammonia per unit time; thus only 1% of total plasma glutamine was disposed of by way of urinary ammonia. Owen & Robinson [4] showed that half the ammonia produced by the kidney of man in acid–base equilibrium was excreted in the urine, the other half being released into the renal vein. Thus our figures suggest that 2% of glutamine disposal is by the kidney in normal man and 98% is by other routes.

Whole body protein turnover, calculated from the plateau enrichment of urinary ammonia, was 30.3 g of N/day; when expressed in terms of protein ($\times 6.25$) this represents 2.8 ± 0.6 g of protein day$^{-1}$ kg$^{-1}$ body wt.

Discussion
As far as we are aware, this is the first estimate of glutamine production rate; therefore, the only data we can use to put our results in context are estimates of net glutamine exchange across organs. Marliss et al. [2] measured the plasma arteriovenous glutamine concentration difference, across the forearm of normal postabortive man, as 0.175 mmol/l. If we assume a total carcass blood flow of 1 l/min [20], then the net addition of glutamine to the blood would be 10.5 mmol/h (about one-fifth of the total production rate in our subjects). However, this direct comparison assumes: 1, that glutamine metabolism is similar in postabsorptive and fed man; 2, that the estimate of carcass blood flow is accurate; 3, that forearm is representative of whole carcass. All of these assumptions are liable to error. In rats the net hindquarter glutamine output increases fourfold after a meal [21]. If this relationship holds for man our data and those of Marliss et al. [2] would be in close agreement.

It is likely that muscle is the major source of glutamine in man; certainly it comprises by far the largest glutamine pool in the body [11], and in animals substantial efflux from muscle continues under all physiological conditions so far studied [5, 22].

The two tissues which consistently consume glutamine are the kidney and intestine. What are the relative demands of these two tissues for glutamine? Our data show that only about 2% of glutamine is disposed of by the kidney. Could the intestine account for the utilization of the balance? In the fasted rat net intestinal glutamine uptake is 191 nmol min$^{-1}$ g$^{-1}$ [11], which rises to 484 nmol min$^{-1}$ g$^{-1}$ when the intestine is perfused with four amino acids [12]. If man's intestine weighs 1 kg and it has the same consumption as in the rat, then intestinal glutamine consumption would be 29 mmol/h. Alternatively, if splanchnic blood flow is 1.5 l/min [20], and the difference between the fed and fasted states seen in rats pertains in man, then the measured glutamine concentration difference across the splanchnic bed of 0.11 mmol/l of plasma [2] gives a net utilization of 25 mmol/h by the intestine and liver.

These calculations serve to demonstrate that muscle could be the major source of glutamine and intestine the major tissue for glutamine disposal. Furthermore, as glutamine is the principal respiratory fuel of the intestine, at least in the rat [11, 12], changes in intestinal glutamine utilization are likely to overshadow changes in consumption by other organs. The kidney seems to play a quantitatively minor role in glutamine disposal. Even in chronically acidic man, net renal glutamine uptake reaches only 6.1 mmol/h [4], or about 12% of our measured glutamine production rate. It seems therefore unnecessary to postulate a specific requirement for increased muscle production of glutamine in chronic acidosis, as minor changes in metabolism by other organs could easily accommodate the increased renal demand for ammoniagenic substrate [10, 23].
If there is, by and large, efflux of glutamine from muscle and influx into intestine, we can quantify the components of glutamine metabolism in normal feeding man. This is illustrated in Fig. 2. With a muscle glutamine pool of 382 mmol and production rate of 52 mmol/h, the fractional turnover rate for muscle glutamine will be $0.14 \text{ h}^{-1}$ ($T_{0.5} = 5.1 \text{ h}$). A similar calculation for the extracellular glutamine pool gives a fractional turnover rate of $8.0 \text{ h}^{-1}$ ($T_{0.5} = 5.0 \text{ min}$). The total extracellular glutamine is less than 2% of the muscle free pool and is turning over very rapidly. Acute changes in glutamine demand could easily be accommodated by relatively small changes in muscle glutamine fractional turnover.

Because of the slow fractional turnover of the muscle glutamine pool, relative to the time course of our experiments, it is unlikely that $^{15}$N released from labelled glutamine amide would be reincorporated into glutamine by muscle and released in significant amounts. The free muscle glutamine pool, by virtue of its relative enormity, may act as a $^{15}$N sink in these experiments [17]. It should be emphasized that the steady-state dilution of the infused $^{15}$N-glutamine is caused by unlabelled glutamine entering the plasma pool: it is not affected by $^{15}$N loss from this pool. There is no information available to assess the magnitude of the unidirectional influx of glutamine into muscle tissue.

The extremely rapid turnover of plasma glutamine indicates that all subjects should have achieved $^{15}$N equilibrium in this pool in 0.25 h. Unfortunately we did not take early blood samples to follow the rise to plateau. However, it is unlikely that a delay in achieving plateau enrichment in plasma explains the observed delay in reaching equilibrium in urinary ammonia enrichment. As there is no evidence for a renal delay pool, we suggest that there may have been subtle changes in metabolic state during the experiment. The high coefficient of variation of enrichments in subject no. 1, where strict control conditions were not applied, suggests that glutamine production rate is labile and responsive to minor metabolic changes.

Our direct measurements of the contribution of glutamine amide N to urinary ammonia confirm the inference drawn by Owen & Robinson [4] from the uptake of glutamine by the kidney, that glutamine amide is the major source of urinary ammonia in man. Their estimate of the proportion of ammonia derived from glutamine is identical with ours (63%). Forty-four per cent of urinary ammonia was found to be derived from glutamine amide in eleven chronically acidicotic dogs by Pitts' group [7-9]. It remains to be seen...
whether this difference is due to the acidotic state, a species difference or to the production of acidosis with ammonium chloride.

The estimates of whole body protein turnover derived from urinary ammonia enrichment are only slightly lower than those obtained with leucine, glycine [25] or lysine [26]. It is to be expected that amide N will give a lower estimate because it lies on the excretory pathway for N [27]. The $^{15}$N method assumes that the particular compound used as a tracer should be incorporated into the end product in the same proportion as total N is incorporated into that end product. The surprisingly close approximation of glutamine amide N to this assumption (assessed by comparison with the other amino acids) indicates that a substantial proportion of total amino N may pass through the amide N of glutamine on its way to excretion.

The technique described for measuring glutamine metabolism in vivo is relatively quick, simple and atraumatic. Under these conditions physiological perturbations which affect glutamine metabolism can be usefully studied.

Acknowledgments

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References