The excretion of isotope in urea and ammonia for estimating protein turnover in man with $^{15}\text{N}$ glycine

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Summary

1. Four normal adults were given $^{15}\text{N}$-glycine in a single dose either orally or intravenously. Rates of whole-body protein turnover were estimated from the excretion of $^{15}\text{N}$ in ammonia and in urea during the following 9 h. The rate derived from urea took account of the $[^{15}\text{N}]$urea retained in body water.

2. In postabsorptive subjects the rates of protein synthesis given by ammonia were equal to those from urea, when the isotope was given orally, but lower when an intravenous dose was given.

3. In subjects receiving equal portions of food every 2 h rates of synthesis calculated from ammonia were much lower than those from urea whether an oral or intravenous isotope was given. Comparison of rates obtained during the postabsorptive and absorptive periods indicated regulation by food intake primarily of synthesis when measurements were made on urea, but regulation primarily of breakdown when measurements were made on ammonia.

4. These inconsistencies suggest that changes in protein metabolism might be assessed better by correlating results given by different end-products, and it is suggested that the mean value given by urea and ammonia will be useful for this purpose.

Key words: ammonia, food intake, glycine, nitrogen metabolism, protein biosynthesis, protein metabolism, urea.

Introduction

The stable isotope of nitrogen, $^{15}\text{N}$, is the most frequently used label in studies of whole-body protein turnover in humans. With very few exceptions [1, 2], these methods for measuring protein turnover are based on the transfer of label from an amino acid to an end-product in the urine. All are dependent on four fundamental assumptions. The first presupposes the existence of a single pool of metabolic nitrogen in which the isotope is uniformly distributed: as a consequence the proportion of the isotopic dose excreted in an end-product from this pool should equal the proportion of the nitrogen turnover (flux) excreted as that end-product. The second assumption is that the rate of nitrogen metabolism remains constant during the time of the recycling of isotope from the protein pool. Finally it is also assumed that the $^{15}\text{N}$-labelled amino acid (usually glycine) is a valid tracer for total amino nitrogen.

In the method most commonly used [3] $^{15}\text{N}$ glycine is given by constant infusion and turnover rates are estimated from the excretion of $^{15}\text{N}$ in urinary urea, after this has reached a constant (plateau) value. The major disadvantage of the method is that, because of the large size of the body urea pool and its relatively slow rate of turnover, the plateau may take up to 3 days to be achieved. The technique, therefore, is neither convenient in practice nor capable of detecting short-term changes in protein metabolism.

As a result Waterlow, Golden & Garlick [4] modified the approach to simplify the experimental procedure and to reduce the total time of
measurement. The modified method differs from that of Picou & Taylor-Roberts [3] by the use of a single-pulse dose of isotope ([15N]glycine) and, more significantly, by estimating the rate of nitrogen turnover from the excretion of [15N]-ammonia in urine, over a much shorter period of time. The use of urea as an end-product for measuring nitrogen turnover in their study proved difficult because the amount of [15N]urea could not be assessed simply from its urinary excretion. A substantial amount was still present within the body pool, even 24 h after the dose of [15N]-glycine had been given.

The aim of the present study, therefore, was to modify the single-dose method of Waterlow et al. [4] to obtain, during studies of 9 h, an estimate for nitrogen flux from urea, in addition to an estimate from ammonia. This has been achieved by monitoring the size of the body pool of urea during the experiment and estimating the amount of label in urea retained within this compartment.

The rates of nitrogen turnover derived individually from both end-products have been used to examine two aspects of the experimental protocol, namely the route of isotopic administration [oral and intravenous (i.v.)] and the effect of feeding (absorptive and postabsorptive states).

In addition, the data have been used to investigate the validity of the first fundamental assumption, i.e. the existence of a single homogeneous pool of metabolic nitrogen. To do this the study was designed to fulfil, at least in practical terms, the requirements of the second and third assumptions. To reduce variations in the rate of nitrogen turnover the intake of food was strictly controlled in amount and frequency and, to reduce the possibility of recycling of isotope by protein breakdown, rates of nitrogen flux were estimated over a period of 9 h.

Methods

Experimental subjects

Four healthy volunteer subjects, one female and three males, were used in all studies. Each was fully informed of the experimental procedure and all gave their consent. The study was approved by the Ethical Committee of University College Hospital, London. Details of each subject were as follows (no., sex, age, height, weight): 1, female, 32 years, 178 cm, 57 kg; 2, male, 34 years, 178 cm, 69 kg; 3, male, 34 years, 178 cm, 71 kg; 4, male, 31 years, 169 cm, 67 kg.

Experimental procedure

Rates of protein synthesis and breakdown were determined over a 9 h period after a dose of [15N]glycine had been given under two conditions: postabsorptive (fasting), measured between midnight and 09.00 hours when there was no food intake, and absorptive (fed), measured between 10.00 and 19.00 hours while the subjects were repeatedly feeding. Movement of the subjects was unrestricted. During the fasting periods they were sleeping and during the fed periods they were predominantly sedentary.

On the day preceding studies on fasting subjects a total of six meals was eaten at 2 h intervals starting at 08.00 hours and ending at 18.00 hours. Between 22.00 and 24.00 hours urine was collected to determine the baseline enrichment of ammonia and urea. At 24.00 hours a venous blood sample was taken just before administering the dose of [15N]glycine. Urine was collected until 09.00 hours the next morning and subsequently for 15 h. Blood samples were taken at the end of each urine collection. During studies on fed subjects the protocol was very similar except that the meals were consumed during the experiment. The first meal was eaten at 08.00 hours, 2 h before the dose was administered. Four urine collections were made from 0 to 3, 3 to 6, 6 to 9 and 9 to 24 h after the dose was given. In addition a baseline urine collection was made before the dose was given (08.00 to 10.00 hours) and blood samples were taken at the time of dose and at 9 and 24 h after it.

[15N]Glycine (Prochem, BOC Ltd, London; 200 mg; 96.6 atom %) was given either orally as a solution in water or by slow intravenous injection dissolved in NaCl solution (150 mmol/l: saline) and sterilized by Millipore filtration. A slow injection over a period of 1 h was adopted to minimize the possibility of abnormal metabolism of glycine: a dose of 200 mg (i.v.) of glycine in 1 min would be approximately 12 times the amount of glycine incorporated into whole-body protein over this time. The effect of an overload of glycine was investigated in one oral study in which a total of 1000 mg of glycine (200 mg of [15N]glycine) was given. In addition in one study 100 mg of [15N]urea (Prochem; 32.1 atom %) was given as a pulse i.v. injection.

The meals consisted of two standardized diets, eaten alternately. The nitrogen and energy content of the two diets was designed to be the same and the total consumption from all six meals approximated normal daily requirements (10.1 MJ and 77.2 g of protein for males and 8.3 MJ and 68.2 g of protein for the female subject). Dietary nitrogen was supplied in the form of milk protein (74%) and wheat protein (25%). Water intake was unrestricted except during the hour preceding a blood sampling when it was not permitted at all.
Urine collections were made directly into storage vessels containing 20 ml of HCl (6 mol/l) and 8 mg of chlorohexidine gluconate (ICI Ltd, Macclesfield, Cheshire, U.K.). Venous blood samples (20 ml) were taken into heparin and the plasma was stored at \(-20^\circ\text{C}\).

**Estimation of urinary ammonia, urinary and plasma urea and total urinary nitrogen**

Urinary ammonia was measured by both acid titration after aeration into saturated boric acid and directly by the Berthelot reaction [5]. Values given by the Berthelot reaction were 96.6% (SEM 0.7) of those by acid titration. Results from both methods were averaged for each urine sample. Urinary and plasma urea were also measured by the Berthelot reaction before and after treatment with urease (Sigma type VII; Sigma Chem. Co., Poole, Dorset, U.K.). Assays were calibrated with stabilized urea standards (Preciset; Boehringer Corp., Lewes, Sussex, U.K.). Total nitrogen in urine was measured by the micro-Kjeldahl method [6]. Distilled ammonia was titrated with HCl (0.01 mol/l) with methyl red as the indicator.

**Determination of \(^{15}\)N-enrichment of urinary ammonia and urea and of plasma urea**

Urinary ammonia was isolated in HCl (1 mol/l) after aeration of strongly alkaline urine for 2 h. The pH of the urine was then adjusted to 6.5 and the urea nitrogen converted into ammonia by incubation with urease (2 units; Sigma type VII) for 1 h at 37\(^\circ\)C in sodium phosphate buffer (200 mmol/l). Ammonia was collected as before. For one subject, subject no. 3, enrichment of urinary ammonia, urinary and plasma urea after precipitation of plasma protein with an equal volume of trichloroacetic acid (200 g/l).

For measurement of \(^{15}\)N, samples containing approximately 0.1 mg of nitrogen, as NH\(_4\)Cl, were treated with sodium hypobromite to liberate nitrogen [8]. \(^{15}\)N-abundance was determined in a single-collector mass spectrometer (AEI, Manchester, U.K.) with a precision of 0.001 atom % excess. With the dosages used the abundances ranged from about 0.3 to 0.5 atom % excess in ammonia in the first 3 h collection to about 0.05 atom % excess in plasma and urinary urea at 9 h.

**Calculation of total-body water**

Total-body water for each subject was estimated from anthropometric data by the equations of Watson, Watson & Batt [9].

**Calculation of the rate of protein turnover**

The rate of nitrogen flux was calculated from the equation given by Waterlow, Garlick & Millward [10]: i.e., \(Q = E_x \cdot d/e_x\), where \(Q\) is the rate of nitrogen flux (g of nitrogen/9 h), \(E_x\) is the excretion of ammonia or urea (g of nitrogen/9 h), \(d\) is the dose of isotopic nitrogen (g of \(^{15}\)N) and \(e_x\) is the amount of isotope excreted in the urine as ammonia in 9 h or, in the case of urea, is the sum of the amount excreted in 9 h and the amount retained in the urea pool of the body at the end of 9 h (g of \(^{15}\)N/9 h).

The rate of ammonia excretion was taken to equal the actual amount of ammonia nitrogen excreted in the urine during the experimental period. The rate of urea excretion was taken to equal the amount of urea nitrogen excreted in the urine after adjusting for changes in the body urea pool. The size of this pool was calculated on the basis that the urea concentration was represented by that of plasma water and its volume of distribution by the total-body water. The water content of plasma was assumed to be 92% of the volume.

Rates of protein synthesis and breakdown in the whole body were derived from the expression \(Q = E_t \cdot d/e_t\), where \(E_t\) is the corrected rate of excretion of total nitrogen in urine, \(Z\) is the rate of whole-body protein synthesis, \(I\) is the rate of intake of nitrogen from the diet and \(B\) is the rate of whole-body protein breakdown. All units are expressed as g of nitrogen/9 h. The corrected excretion of total nitrogen in urine \((E_t)\) has been obtained from the actual excretion after allowing for changes in the body pool of urea nitrogen. A factor of 6.25 was used to convert g of nitrogen into g of protein.

**Results**

**General aspects of nitrogen metabolism**

Table 1 shows the variation in the rate of urinary excretion of total nitrogen, in the concentration of urea nitrogen in plasma water and in the rate of excretion of ammonia and of urea under postabsorptive (fasted) and absorptive (fed) conditions. The actual rate of excretion of total nitrogen in the fasted state was 59% of that in the fed state (see also Table 4). However, this does not include an adjustment for fluctuations in the
size of the urea pool of the body, which fell during the postabsorptive period and increased during food absorption. When these changes were taken into account the corrected excretion of total nitrogen in the fasted state was only 44% of that in the fed state. The decrease resulted from a substantial reduction in the excretion of urea and a smaller reduction in the excretion of ammonia.

The absolute amount of nitrogen in the urea space of the body amounted to 4.5–6.0 g. It is therefore quantitatively important because it delays the excretion of labelled urea in the urine. Table 2 shows the estimated distribution of labelled urea between the urine and the body pool 9 h after the dose was given. The proportion had decreased to 10% after 24 h. Again intravenous and oral dosage gave similar distributions.

**Rate of flux of whole-body nitrogen**

The values for nitrogen flux (Q) for both the absorptive and postabsorptive studies are given in Table 3. In each case the flux is presented as individual estimations based on the excretion of either ammonia or urea. The results of the absorptive study show two important features. First, there was a difference in the magnitude of the flux given by each end-product. In almost every case the value derived from ammonia was lower than that from urea. Second, the results for each end-product showed considerable variation within individual subjects: the variance for the rates given by ammonia in the absorptive study ranged from 4.1 for subject no. 3 to 18.8 for subject no. 2. However, it is noticeable that when values from urea and ammonia were averaged (end-product average) there was less variation within and between subjects. The variance of the end-product average for each subject was smaller than would be expected for that of a mean of two independent variables. In this instance the variance of the end-product average should have been a quarter of the sum of the variances of urea and ammonia. The observed reduction in the

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**Table 1. General aspects of nitrogen metabolism**

Values are means ± SEM of eight postabsorptive and 20 absorptive observations. *Values for total nitrogen excretion and urea excretion have been adjusted for changes in the size of the urea pool of the body during the 9 h experimental period.

<table>
<thead>
<tr>
<th></th>
<th>Postabsorptive</th>
<th>Absorptive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total nitrogen excretion (mg of nitrogen/9 h)</td>
<td>3227 ± 175</td>
<td>5435 ± 192</td>
</tr>
<tr>
<td>Corrected*</td>
<td>2543 ± 160</td>
<td>5837 ± 133</td>
</tr>
<tr>
<td>Plasma urea nitrogen (mg of urea nitrogen/l of plasma water)</td>
<td>152 ± 5</td>
<td>139 ± 4</td>
</tr>
<tr>
<td>Experimental time (h)</td>
<td>9</td>
<td>134 ± 4</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>152 ± 7</td>
</tr>
<tr>
<td>Change in the size of the urea pool of the body between 0 and 9 h (mg of urea nitrogen)</td>
<td>-684 ± 81</td>
<td>+402 ± 111</td>
</tr>
<tr>
<td>Urea excretion rate (mg of urea nitrogen/9 h)</td>
<td>2696 ± 165</td>
<td>4561 ± 183</td>
</tr>
<tr>
<td>Corrected*</td>
<td>2012 ± 189</td>
<td>4963 ± 125</td>
</tr>
<tr>
<td>Ammonia excretion rate (mg of ammonia nitrogen/9 h)</td>
<td>175 ± 11</td>
<td>240 ± 11</td>
</tr>
</tbody>
</table>

**Table 2. Percentage distribution of $^{15}$N urea between the urea pool of the body and the urine 9 and 24 h after a dose of $^{15}$N glycine or $^{15}$N urea**

Values are means ± SEM. Figures in parentheses indicate the number of observations. *Total $^{15}$N urea (urine + body pool) accounted for 92.4% (SEM 3.4; n = 4) of the injected dose at 9 h and 92.7% (SEM 1.7; n = 4) at 24 h.

<table>
<thead>
<tr>
<th>$^{15}$N dose</th>
<th>Dietary condition</th>
<th>$^{15}$N urea in body pool (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine (200 mg, i.v.)</td>
<td>Postabsorptive</td>
<td>65-4 ± 2.1 27.1 ± 1.0 (4)</td>
</tr>
<tr>
<td>Glycine (200 mg, oral)</td>
<td>Postabsorptive</td>
<td>59-1 ± 2.3 27.1 ± 3.0 (4)</td>
</tr>
<tr>
<td>Glycine (200 mg, i.v.)</td>
<td>Absorptive</td>
<td>48-4 ± 1.9 19.7 ± 3.2 (4)</td>
</tr>
<tr>
<td>Glycine (200 mg, oral)</td>
<td>Absorptive</td>
<td>50-3 ± 1.8 20.8 ± 2.0 (8)</td>
</tr>
<tr>
<td>Glycine (1000 mg, oral)</td>
<td>Absorptive</td>
<td>47-5 ± 1.3 17.8 ± 2.9 (4)</td>
</tr>
<tr>
<td>Urea (100 mg, i.v.)*</td>
<td>Absorptive</td>
<td>37-7 ± 2.3 10.0 ± 0.2 (4)</td>
</tr>
</tbody>
</table>
Protein turnover in man

TABLE 3. Rate of nitrogen turnover (flux) in individuals measured between 0 and 9 h after a dose of [15N]glycine (g of nitrogen/9 h)

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>End-product</th>
<th>Postabsorptive Dose of [15N]glycine (mg)</th>
<th>200 i.v.</th>
<th>200 oral</th>
<th>Absorptive 2000 oral</th>
<th>(mean)</th>
<th>(variance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ammonia</td>
<td>9.3</td>
<td>13-6</td>
<td>12-8</td>
<td>18.1</td>
<td>13-5</td>
<td>14-9</td>
</tr>
<tr>
<td></td>
<td>Urea</td>
<td>18-2</td>
<td>13-5</td>
<td>32-1</td>
<td>22-2</td>
<td>26-7</td>
<td>27-3</td>
</tr>
<tr>
<td></td>
<td>End-product average</td>
<td>13-7</td>
<td>13-5</td>
<td>22-4</td>
<td>21-7</td>
<td>20-1</td>
<td>20-1</td>
</tr>
<tr>
<td>2</td>
<td>Ammonia</td>
<td>10.5</td>
<td>10-6</td>
<td>13-1</td>
<td>16-8</td>
<td>17-2</td>
<td>19-5</td>
</tr>
<tr>
<td></td>
<td>Urea</td>
<td>13-6</td>
<td>15-5</td>
<td>32-8</td>
<td>23-0</td>
<td>24-7</td>
<td>26-9</td>
</tr>
<tr>
<td></td>
<td>End-product average</td>
<td>12-0</td>
<td>13-0</td>
<td>23-0</td>
<td>19-9</td>
<td>21-0</td>
<td>21-8</td>
</tr>
<tr>
<td>3</td>
<td>Ammonia</td>
<td>13-4</td>
<td>13-0</td>
<td>20-0</td>
<td>24-3</td>
<td>20-8</td>
<td>23-3</td>
</tr>
<tr>
<td></td>
<td>Urea</td>
<td>17-2</td>
<td>12-8</td>
<td>28-4</td>
<td>24-6</td>
<td>25-0</td>
<td>20-8</td>
</tr>
<tr>
<td></td>
<td>End-product average</td>
<td>15-3</td>
<td>12-9</td>
<td>24-2</td>
<td>24-5</td>
<td>22-9</td>
<td>22-1</td>
</tr>
<tr>
<td>4</td>
<td>Ammonia</td>
<td>10-9</td>
<td>11-6</td>
<td>12-9</td>
<td>16-6</td>
<td>22-9</td>
<td>15-0</td>
</tr>
<tr>
<td></td>
<td>Urea</td>
<td>13-4</td>
<td>9-4</td>
<td>27-3</td>
<td>23-6</td>
<td>22-7</td>
<td>20-6</td>
</tr>
<tr>
<td></td>
<td>End-product average</td>
<td>12-2</td>
<td>10-5</td>
<td>20-1</td>
<td>20-1</td>
<td>22-8</td>
<td>17-8</td>
</tr>
</tbody>
</table>

The variance of the end-product average indicates that, in the fed state, the rate of flux estimated from ammonia is inversely related to the rate of flux estimated from urea [11]. The relationship is shown in Fig. 1. A similar connection could not be shown in the postabsorptive study, but the number of observations was limited and there was less difference between the rates given by each end-product.

Table 3 also shows that increasing the dose of glycine from 200 to 1000 mg did not appear to affect the estimate of flux from ammonia, urea or the end-product average. Only in subject no. 4 was there a slight indication that the larger dose was producing different, in this case lower, values for flux. Waterlow et al. [4] reported that the calculated rate of protein synthesis in two obese subjects was affected by the amount of glycine given, the larger the dose the lower the apparent rate of synthesis. Their doses were between 150 and 1600 mg of glycine and the rate was calculated from the urinary excretion of ammonia. We observed very little, if any, effect of the amount of glycine in the dose. An important difference between these two studies was the feeding schedule which, in the present study, involved smaller and more frequent intakes of food.

Although 200 mg of glycine cannot strictly be regarded as a tracer dose, it is unlikely to create errors in estimating the rate of nitrogen flux by inducing abnormalities in glycine metabolism.

Rates of whole-body protein synthesis and breakdown

The calculated rates of protein synthesis (Z) and breakdown (B) in the whole body (g of protein/9 h) are presented in Fig. 2. These have been derived from the flux rates (Table 3) by subtracting the corrected excretion of total nitrogen in the urine (Table 4) for protein synthesis or, the rate of intake of dietary nitrogen over the experimental period, for protein breakdown. When [15N]glycine was given orally under postabsorptive conditions there was no significant difference between the rates given by ammonia or urea for either synthesis (60.5 and 63.9 g of protein/9 h respectively) or breakdown (76.3 and 80.0). This agreement did not extend to when the oral dose was given with food. Here the rates given by ammonia were significantly lower than those from urea, both for synthesis (78.5 and 113.2) and breakdown (60.1 and 94.3). With
intravenous administration of the dose there was never agreement between the end-products, in either the fed or fasted condition. In the post-absorptive situation rates for synthesis indicated by ammonia and urea were 52.9 and 81.6, and for breakdown were 68.8 and 97.5. When the i.v. dose was given while the subjects were feeding, the two end-products gave very large discrepancies in the rate of synthesis (55.9 and 151.5) and in the rate of breakdown (35.7 and 132.2).
Table 5. Relative changes in the rate of protein synthesis and breakdown in the whole body between the absorptive and postabsorptive condition

Values are means ± SEM of four observations in each group. Statistical significance of differences between absorptive and postabsorptive rates was assessed by Student's paired t-test: *P < 0.05; **P < 0.02; ***P < 0.01. Results were calculated from comparison of columns 1 and 3 shown in Table 3 for i.v. dosage and columns 2 and 4 for oral dosage. These were designed to be paired studies with only 7 days between measurements.

<table>
<thead>
<tr>
<th>End-product</th>
<th>Postabsorptive rate/absorptive rate (%)</th>
<th>Protein synthesis</th>
<th></th>
<th>Protein breakdown</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>i.v.</td>
<td>oral</td>
<td>i.v.</td>
<td>oral</td>
</tr>
<tr>
<td>Ammonia</td>
<td>97.9 ± 9.2</td>
<td>83.7 ± 9.8</td>
<td>223.4 ± 39.3***</td>
<td>143.7 ± 21.9</td>
</tr>
<tr>
<td>Urea</td>
<td>54.4 ± 4.9***</td>
<td>54.9 ± 8.6**</td>
<td>75.0 ± 6.6*</td>
<td>89.2 ± 9.9</td>
</tr>
<tr>
<td>Average</td>
<td>65.0 ± 2.8***</td>
<td>64.8 ± 5.0***</td>
<td>100.1 ± 5.0</td>
<td>100.8 ± 7.9</td>
</tr>
</tbody>
</table>

Table 6. Percentage of isotope excreted in ammonia or contained in urea (excreted and retained) in the first 9 h relative to that excreted in 24 h

Values are means ± SEM of seven observations in the postabsorptive group and 16 in the absorptive group.

<table>
<thead>
<tr>
<th></th>
<th>[15N]Ammonia</th>
<th>[15N]Urea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postabsorptive</td>
<td>80.7 ± 1.5</td>
<td>72.5 ± 1.2</td>
</tr>
<tr>
<td>Absorptive</td>
<td>82.1 ± 1.4</td>
<td>83.4 ± 1.6</td>
</tr>
</tbody>
</table>

As with the measurement of flux, this variation could be improved by using the end-product average. In Fig. 2 the rates of synthesis and breakdown were very similar for either oral or i.v. administration of isotope. In the postabsorptive study the end-product average indicated rates for synthesis of 62.2 (oral) and 67.2 (i.v.). Under absorptive conditions the rates were 95.9 and 103.7 respectively. The agreement was similar for breakdown; postabsorptive rates were 78.0 (oral) and 83.1 (i.v.) and absorptive rates were 77.1 and 84.0.

Table 5 compares the rates of synthesis and breakdown of protein in the fed and fasted states. When the rates of synthesis derived from ammonia were compared, no statistically significant differences between the absorptive and postabsorptive condition were seen with either route of isotope administration. In contrast, rates derived from urea showed a large decrease in synthesis with fasting. This was apparent for both i.v. and oral doses. A greater discrepancy was seen for breakdown. With both i.v. and oral studies ammonia indicated an increase in the rate, whereas urea indicated a decrease. Comparison of the rates given by the end-product average showed good consistency between both routes and indicated a decrease in the rate of synthesis with no change in the rate of breakdown.

As all the above studies the rate of protein metabolism was based on the excretion of isotope over a 9 h experimental period. The method of calculation assumes that by this time the excretion of isotope is essentially complete. The data in Table 6, however, indicate that more isotope is excreted in both ammonia and urea between 9 and 24 h. This phenomenon has been discussed previously by Waterlow et al. [10], who concluded that the best period of collection of urine was a compromise between the time taken to clear all the isotope from the metabolic pool and the need to make short measurements to prevent recycling of label from the breakdown of rapidly turning-over proteins. It is not possible from the present study to distinguish between these two alternative explanations for the continued excretion of isotope. It is, nevertheless, interesting to note that the proportionate increase in excretion between these times is very similar for urea and ammonia. This supports the validity of the correction for the amount of [15N]urea retained in the body pool because the time course for total [15N]urea is the same as that for [15N]ammonia, which has no body pool to delay excretion.

Discussion

Measurement of rates of protein turnover from excretion of 15N in urea and ammonia

Waterlow et al. [4] described a simple and rapid method for measuring rates of protein turnover after a single dose of [15N]glycine, based on the urinary excretion of label in ammonia. They showed that by 9 to 12 h after the dose the enrichment of ammonia had declined to low levels, enabling the rate of flux to be calculated from the amount of isotope excreted in a single urine collection over this period. They pointed out that it was not practical to make similar measurements on urea because the large size of the urea
pool in the body delayed the excretion of isotope. The method in this paper described two important modifications which enable urea to be used. First, changes in the size of the body urea pool have been monitored. It has, therefore, been possible to adjust the values for the observed rate of excretion of urea to allow for any changes in the size of this pool (corrected rate). Secondly, an estimate of the amount of $^{15}$N urea retained within the urea pool has been made. This obviates the necessity to wait until all of the isotope has been excreted in the urine.

The present method is dependent on the accuracy of estimating the amount of urea in the body. Although this has been done from anthropometric measurements [9] rather than direct measurements [12, 13] the results indicate that it is sufficiently accurate for the required purpose. Evidence for this can be seen in Table 2 where the calculated amounts of $^{15}$N urea retained in the body pool relative to the amount excreted in the urine show little variation either between the subjects or between the routes of isotope administration. Also, in Table 6, the calculated total amount of $^{15}$N urea (retained plus excreted) increased by a similar proportion to the excretion of $^{15}$N ammonia between 9 and 24 h after the dose was given. This would not be expected if the amount of retained $^{15}$N urea had been incorrectly estimated, since the values for ammonia are not dependent on estimating a body compartment. Additional evidence for the validity of the indirect method for estimating body urea is also provided by the study of Long et al. [13] who measured the urea space by pulse injection of $^{15}$N- and $^{13}$C-urea. We calculated the amount of body urea from the anthropometric data and plasma urea concentration for their patients and found them to be, on average, 95.5% of their measured values. The indirect approach was adopted in our study because in the clinical or field situation it is impracticable to assess the urea space of every patient by direct measurement.

The use of urea as an end-product in the present study has been made without allowing for its hydrolysis by intestinal bacteria [12]. Waterlow et al. [10] have argued that with the single-dose method this is unlikely to introduce a significant error in estimating the rate of flux because in a stochastic system urea recycling will not alter the partition of $^{15}$N between protein synthesis and excretion. The data in Table 2 show that after injection of $^{15}$N urea 92% of the dose could be accounted for in the urine or in body water at both 9 and 24 h. This suggests that the errors arising from the estimation of body urea space or from urea recycling in our experimental subjects were small.

Concept of a single homogeneous pool of metabolic (free amino) nitrogen

The concept, first introduced by Sprinson & Rittenberg [14], envisages that all the free amino nitrogen in the body is within a single homogeneous pool, into which the isotope is instantly distributed, and from which nitrogen is taken for protein synthesis and for oxidation (excretion). The turnover of this pool can, therefore, be obtained from kinetic data of any end-product derived from it. Because of the assumed homogeneity, and the instant mixing, it is also implicit that the estimation of the rate of nitrogen turnover will not be influenced by the route of isotope administration. However, there is little doubt that in reality the metabolic pool is not a single compartment, but a collection of sub-compartments either at the tissue level or at the cellular level. Some evidence for this has been presented for both rats [15] and humans [16]. It has also been made apparent by three studies which have obtained different estimates for the rate of protein synthesis in the whole body from the excretion of ammonia and from the excretion of urea [4, 17, 18]. All three studies used $^{15}$N glycine as the labelled precursor. In Nicholson’s study [17] on premature infants the rates of protein synthesis were between 16 and 30% higher when calculated from the excretion of ammonia. In the studies of Golden & Waterlow [18], on elderly patients, and of Waterlow et al. [4], on malnourished and recovered children, rates of synthesis based on ammonia, in contrast, were always lower (3–40% in the elderly patients and 3–61% in the children).

In the present study we have found that the experimental design can accentuate the compartmentation of body nitrogen. Only when an oral dose was given during a postabsorptive period did the two end-products give very similar rates of protein synthesis and breakdown (Fig. 2). When the label was given by the i.v. route under similar conditions a discrepancy appeared between the rates from ammonia and urea. Those given by ammonia were about 32% lower. In the absorptive state both oral and i.v. routes showed disagreement between the end-products. For the oral dose the disparity between ammonia and urea was similar to that seen for the i.v. route in the postabsorptive state (33%). When the i.v. dose was given during an absorptive period the anomaly between end-products was very large with ammonia indicating rates of protein syn-
thesis and breakdown between 63 and 73% lower than those from urea.

**Effect of changing the route of isotope administration on the disparity between ammonia and urea**

Although the route of isotope administration is not such a major factor as food intake, in producing a difference between ammonia and urea (Fig. 2), it is, nevertheless, important. The data suggest that the oral route is more satisfactory than the i.v. route for measuring the rate of protein metabolism in the whole body. We are unable to explain why this should be. However, the difference between the two routes must lie in the partitioning of isotope to the organs of the body. Oral administration should initially present the entire dose to the small intestine and liver and thereby increase the nitrogen enrichment in these organs relative to the rest of the body. As a result the apparent nitrogen flux based on the labelling of urea would be decreased and that based on ammonia would be increased. I.v. administration, conversely, would be expected to bias the distribution of isotope to extrahepatic tissue and in so doing would reduce the apparent flux rate from ammonia and increase it from urea.

No studies are known which have investigated the route of administration of a single dose of label. Picou & Taylor-Roberts [3] compared continuous intragastric and i.v. infusions of \(^{15}\)N glycine in two children. They found no clear difference in the rate of nitrogen flux, estimated from the excretion of urea, between the two routes. Similarly, Steffee, Goldsmith, Pencharz, Scrimshaw & Young [19] compared the results of repeated oral doses of \(^{15}\)N glycine with continuous i.v. infusion in three adults and again reported no significant differences between the routes. Our comparison with an oral dose and an i.v. injection showed reciprocal effects in both postabsorptive and absorptive conditions. I.v. administration in both cases gave increased rates from urea and decreased rates from ammonia. There was no significant difference between the routes of isotope administration when the end-product average was compared.

**Effect of food on the disparity between ammonia and urea**

We have interpreted the observed changes in protein turnover (Fig. 2, Table 5) to be a direct response to food intake and not to diurnal rhythms in nitrogen metabolism. This has been based on the studies of Golden & Waterlow [18], who showed no significant variation in the rate of leucine flux (nitrogen flux) during continuous intragastric feeding for 30 h and of Garlick et al. [20, 21], who showed that the rate of leucine flux can be altered appreciably by both the quality and quantity of diet. Diurnal variations may have contributed to the observed changes, but in quantitative terms we have considered them to be of less importance.

Fig. 2 illustrates the importance of food intake in producing differences between the rates for protein metabolism calculated from the excretion of label in ammonia and from the excretion of label in urea. The differences seen in the post-absorptive period were significantly increased during food absorption. The most likely reason for this is the incomplete mixing of unlabelled nitrogen of the food with the metabolic nitrogen pool of the body. \(^{15}\)N in the small intestine and the liver will be diluted more by dietary nitrogen than the isotope present in other tissues. As urea is formed in the liver its \(^{15}\)N-enrichment will consequently be lowered by the absorption of dietary protein, which in turn will produce higher estimates for nitrogen flux. Ammonia, being formed in the kidney, will not be affected to such an extent.

Such an explanation could also account for the results of a study by Sim, Wolfe, Young, Clarke & Moore [22]. They reported a decrease of about 30% in the rate of protein synthesis and breakdown in the whole body after an oral intake of a normal diet was substituted by i.v. infusion of an equivalent amount of free amino acids and glucose. The study was based on a continuous infusion of \(^{15}\)N glycine for 48 h with measurement of the \(^{15}\)N-enrichment in urea. The authors suggested that the decrease in the rate of protein metabolism in the whole body arose from a lower level of protein turnover in the gastrointestinal tract. An alternative hypothesis, however, is that the apparent decrease was an artifact resulting from the switch to i.v. feeding. The change from enteral to parenteral nutrition would serve to increase the isotopic-enrichment in the hepatic nitrogen pool relative to the peripheral tissue. Ammonia was not measured in this study [22].

As with the comparison of oral and i.v. routes for administering the dose of isotope, our results on the effect of feeding in man (Table 5) again showed a reciprocal relationship between ammonia and urea. The conclusion drawn from ammonia indicates that whole-body protein metabolism is controlled by changes in protein breakdown, whereas the conclusions drawn from urea suggest that it is brought about principally
through changes in the rate of protein synthesis. This contradiction serves to illustrate the importance of assessing changes in protein turnover with more than one end-product. The question arises as to which end-product is reflecting the actual changes. Some evidence can be obtained from animal studies. In rats food deprivation is known to decrease the rate of synthesis in many tissues. Skeletal muscle is the most sensitive to this stimulus, whereas visceral organs are less sensitive [23, 24]. Protein breakdown is also affected by starvation, especially in the liver, where an increase occurs within hours after a meal [10, 25]. Breakdown in skeletal muscle, by contrast, is far less responsive to starvation only increasing its rate after 1–3 days [26, 27]. Consequently the expected change in the whole body, based on relative body composition, would be a decrease in synthesis possibly accompanied by a small increase in breakdown. This corresponds more to the changes suggested by the excretion of ammonia than by the excretion of urea. The better reliability of urea is also supported by the results of a study of obese patients measured by constant infusion of L-[14C]leucine [21] where the changes resulting from food deprivation were a definite reduction in the rate of synthesis with no decrease in the rate of breakdown. However, the most reliable indicator appeared to be the end-product average because of the changes it proposed; though similar to those of urea, these were more comparable with the results of Garlick et al. [21]. The end-product average showed the same decrease in synthesis with no decrease in breakdown.

Although we have found ammonia to be unsatisfactory, two previous studies have argued that it can adequately detect changes in protein metabolism. Waterlow et al. [4] gave a single dose of [15N]glycine and showed that the changes in protein synthesis which occurred during recovery from malnutrition could be detected equally whether urea or ammonia were used, even though the absolute magnitude of the rates given by each end-product was not the same. Similarly, Garlick, Clugston & Waterlow [20] measured the changes in protein synthesis in obese subjects given low-energy diets for 3 weeks. When protein was omitted from the diet a reduction in the rate of protein synthesis was seen when estimates were based either on constant infusion of L-[1-14C]leucine or on a single dose of [15N]glycine with measurements of the isotope in urinary ammonia. This difference in conclusion between the above two studies and the present one may be a function of chronic and acute dietary changes.

Comparison of rates of protein synthesis

The absolute rates of protein metabolism calculated from ammonia, urea and the end-product average are within the range reported by others using [15N]glycine. The range, however, is large, extending from about 1 to 7 g of protein synthesized day$^{-1}$ kg$^{-1}$ body weight. Most of this variation probably arises from the different experimental conditions, such as the age of the subjects, their metabolic state, the nature of the food intake, the choice of nitrogenous end-product and the period of experimentation. There is therefore little purpose in making specific comparisons. A more appropriate way of assessing the validity of the present rates is by comparison with the values derived for fed adults from the continuous infusion of L-[1-14C]leucine over periods of 8–12 h [18, 20, 28, 29]. Methods with 14C are based on different assumptions than those for 15N and have different sources of error [10]. The studies with L-[1-14C]leucine report synthesis rates of 3.1–4.5 g of protein day$^{-1}$ kg$^{-1}$ body weight (ideal body weight for obese subjects). In the present study the rate calculated from ammonia (mean 2.9; SEM 0.2; n = 16) was at the lower end of this range, whereas that based on urea (mean 5.0; SEM 0.3; n = 16) was above the upper value. The rate estimated from the end-product average (mean 4.0; SEM 0.1; n = 16) fell within the range. These rates refer to the absorptive state. In the postabsorptive state there is only one known study of normal adults which has used carbon-labelled leucine [30]. In a study of males, aged 18–25 years, they reported a synthesis rate of 3.2 g of protein day$^{-1}$ kg$^{-1}$ body weight (SEM 0.12; n = 40). The rates calculated for our subjects were 2.3 (SEM 0.14; n = 8) from ammonia, 3.0 (SEM 0.29; n = 8) from urea and 2.6 (SEM 0.15; n = 8) from the end-product average.

Conclusion

Our results indicate that the concept of a single homogeneous pool of metabolic nitrogen is not in general valid. If the relationship between flux rates calculated from urea and ammonia is indeed an inverse one, as the data suggest, it implies that metabolic nitrogen in the body is divided into two functional pools. These may be conceived either in anatomical terms, such as a hepatic and extrahepatic compartment, or in metabolic terms, as proposed by Jackson & Golden [16], where the extent of labelling of precursor amino acids for urea and ammonia synthesis is altered by the metabolic state and not purely by physical
barriers. Whatever the reasons for this compartmentation we have found that the isotopic enrichment in these two pools can be altered by experimental conditions. Consequently it is important to assess changes in protein metabolism by correlating results given by different end-products. Long, Jeevanandam, Kim & Kinney [31] have reported a method with compartmental analysis for determining rates of protein metabolism in man, in which both the excretion of ammonia and urea are taken into account. In a similar way we have calculated the end-product elimination of the rate of production of labelled ammonia and urea are taken into account. In a comparable way we have calculated the end-product analysis for determining rates of protein metabolism in man, in which both the excretion of ammonia and urea are taken into account.

It appears to be insensitive to the method of isotope administration. This average may have value in providing an estimate of the rate of protein turnover in the whole body which is unbiased towards a particular compartment or tissue. It appears to be comparable with absolute and relative rates obtained by constant infusion of [14C]- or [13C]-leucine.

The advantage of the single dose of [15N]-glycine, by comparison with infusion of labelled leucine, is its simplicity and its convenience for use outside the laboratory (e.g. in a hospital ward). Both methods require samples of blood, but in addition infusion of labelled leucine requires specialized equipment for the determination of the rate of production of labelled carbon dioxide, whereas the single dose of [15N]glycine only needs collection of urine.

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