Studies of amino acid and protein metabolism in normal man with L-[U-14C]tyrosine

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Summary

1. Six men were infused intravenously for 10 h with a tracer amount of L-[U-14C]tyrosine while on a standardized food intake.

2. Measurements of plasma L-[14C]-tyrosine specific radioactivity and the excretion rate of 14CO2 were made at frequent intervals and showed plateau labelling of plasma and expired carbon dioxide within 6–8 h. The tyrosine flux was calculated from the specific radioactivity in plasma at plateau value.

3. The excretion rate of 14CO2, corrected for retention of label within the bicarbonate pool, showed that oxidation accounted for 20% of the tyrosine flux. Urinary excretion of label was negligible.

4. Rates of protein synthesis were calculated from the flux of tyrosine after subtracting the proportion oxidized. Although the mean rate of synthesis was consistent with other measurements of protein turnover, the individual values ranged from 284 to 387 g/day. The variation was not reduced by relating turnover to body weight, lean body mass or energy expenditure.

5. Estimating the rates of protein breakdown from the tyrosine flux involved some assumptions about pathways of phenylalanine metabolism. The use of a labelled essential amino acid would therefore give more accurate values for short-term measurements of whole body protein turnover.

Key words: amino acid, protein, [14C]tyrosine.

(1) Died 5 October 1975.

Introduction

Nitrogen balance techniques give information about net exchanges of nitrogen with the environment but isotopic tracers are needed to study the dynamics of nitrogen metabolism within the body. A great deal of effort has been devoted to the kinetics of plasma protein turnover in man but there is much less knowledge of rates of tissue protein synthesis. Sprinson & Rittenberg (1949) used [15N]glycine for this purpose and were able to calculate that the rate of protein synthesis in humans was approximately 300 g/day. This figure represents the sum of the protein synthetic activity of the individual tissues with a relatively small contribution from plasma protein synthesis, and is of considerable nutritional significance. Waterlow (1967) developed a different approach. He estimated protein synthesis in the whole body from the specific radioactivity of lysine in plasma during a continuous intravenous infusion with a tracer dose of [14C]lysine. Subsequent work in experimental animals confirmed the validity of this method of measuring body protein turnover by comparing the lysine flux with studies on the labelling of tissue proteins (Waterlow & Stephen, 1968).

The present investigations in man were designed to extend these observations. [14C]Tyrosine was chosen as the infused amino acid for several reasons. A specific decarboxylase assay was developed for L-tyrosine which enabled estimates of the specific radioactivity of plasma tyrosine to be made more quickly and accurately (Garlick & Marshall, 1972). Theoretical considerations of pool sizes (Garlick, 1972) suggested that [14C]tyrosine could be infused for a shorter period than the 30 h infusions needed.
for [14C]lysine. Attempts were also made to quantify the two major metabolic pathways for tyrosine, i.e., catabolism and incorporation into protein, by monitoring the rate of 14CO2 excretion at a time when the specific radioactivity of L-[14C]tyrosine in blood had achieved a constant value. A preliminary communication of this work has been presented (James, Garlick & Sender, 1974a).

Methods
The six subjects were male members of our department who willingly consented to the procedures described. None had experienced any recent weight change or alteration of diet except T.E., who, 1 year before investigation, had reduced his weight by 12 kg. Table 1 lists some of the basic information concerning the six men. Each infusion was given for 10 h starting at 09.00 hours. All subjects were infused at rest through an indwelling needle in a peripheral arm vein. An infusion of 15 μCi of L-[U-14C]tyrosine (The Radiochemical Centre, Amersham, Bucks, U.K.) was delivered from a syringe by a constant-infusion pump delivering a solution containing tyrosine (15 μmol/ml) and phenylalanine (30 μmol/ml) at a rate of 0.48 ml/h. The infusion rate did not vary by more than ±1%. The other eighteen amino acids were present at ten times the plasma concentration to prevent adsorption of [14C]tyrosine to the glass of the infusion syringe. (The detailed amino acid composition of the infusate is given in Clinical Science and Molecular Medicine Table 76/4, available from the Librarian, Royal Society of Medicine, 1 Wimpole Street, London W1M 8AE.) Venous blood samples were taken from the opposite arm every 15 min for the first 2 h and then every 30 min. During the course of the infusion, standardized small sandwich meals were eaten at 90 min intervals in an attempt to achieve a constant inflow of food with a constant proportion of energy and protein.

The specific radioactivity of expired 14CO2 was measured every 20 min by trapping the 14CO2 in Hyamine/ethanol (2:1, v/v) with phenolphthalein as an indicator (Kaihara & Wagner, 1968). To the solution in equilibrium with expired carbon dioxide was added 10 ml of toluene with 2,5-diphenyloxazole (22.6 mmol/l). The radioactivity of the mixture was then counted in a Beckman LS 150 liquid-scintillation spectrometer. Correction for quenching was made by the external standard channels ratio method. Each vial contained approximately 104 d.p.m. and was counted until 104 radioactivity counts had accumulated. Urine was also collected and the samples were counted with 10 ml of toluene/Triton X-100 (2:1, v/v) containing 2,5-diphenyloxazole (22.6 mmol). The subject’s head was covered with a transparent ventilated hood so that his oxygen consumption and carbon dioxide output could be monitored at intervals with a diathermometer.

Whole-body potassium was measured with a sensitive liquid scintillation whole body counter, the details of which are given by Barnaby & Jasani (1968). Lean body mass was calculated on the assumption that 1 kg of lean body mass contained 68.1 mmol of potassium (Forbes, Gallup & Hursh, 1961).

Analytical methods
For the estimation of free tyrosine specific radioactivity in plasma, samples (5 ml) were precipitated with 5 ml of trichloroacetic acid (600 mmol/l) and L-tyrosine was converted into tyramine with L-tyrosine decarboxylase, derived as an acetone-dried

Table 1. Some anthropometric characteristics of the infused subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>Height (cm)</th>
<th>% of ideal weight</th>
<th>Whole body K (g)</th>
<th>Lean body mass (kg)</th>
<th>Oxygen uptake (l/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P.J.</td>
<td>34</td>
<td>70-0</td>
<td>170</td>
<td>105</td>
<td>136</td>
<td>51-0</td>
<td>0.281</td>
</tr>
<tr>
<td>P.S.</td>
<td>31</td>
<td>100-0</td>
<td>185</td>
<td>116</td>
<td>179</td>
<td>67-2</td>
<td>0.376</td>
</tr>
<tr>
<td>C.H.</td>
<td>56</td>
<td>79-5</td>
<td>188</td>
<td>95</td>
<td>142</td>
<td>53-3</td>
<td>0.349</td>
</tr>
<tr>
<td>J.W.</td>
<td>58</td>
<td>73-5</td>
<td>170</td>
<td>110</td>
<td>146</td>
<td>54-8</td>
<td>0.288</td>
</tr>
<tr>
<td>T.E.</td>
<td>64</td>
<td>72-7</td>
<td>178</td>
<td>99</td>
<td>—</td>
<td>—</td>
<td>0.287</td>
</tr>
<tr>
<td>P.P.</td>
<td>46</td>
<td>63-6</td>
<td>178</td>
<td>88</td>
<td>130</td>
<td>49-0</td>
<td>0.221</td>
</tr>
</tbody>
</table>
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powder from *Streptococcus faecalis* (Sigma Ltd) under conditions described in detail by Garlick & Marshall (1972). Labelled tyramine was isolated by solvent extraction (Garlick & Marshall, 1972), measured by a fluorimetric assay (Waalkes & Udenfriend, 1957) and counted for radioactivity as described previously (Garlick & Marshall, 1972). Radioactivity measurements were made with two to four times the background count of 25 d.p.m.; the vials were counted for at least 40 min or until 4000 counts had accumulated. Quench correction was by the external standard channels ratio method. The tyrosine and phenylalanine content of the food was measured on homogenates, which were dried and hydrolysed with hydrochloric acid (6 mol/l). Quantitative estimations of the amino acids were made on a Locarte amino acid analyser (Locarte Company, 8 Wendall Road, London, W.1), with norleucine as an internal standard.

The food homogenates were also analysed for their energy content with bomb calorimetry and for their nitrogen content by Kjeldahl digestion with colorimetric determination of ammonia (Wootton, 1964).

**Calculations**

The tyrosine flux was calculated from the plateau specific radioactivity of free tyrosine in blood, with the model shown in Fig. 1, as described by Waterlow & Stephen (1967). The time at which labelling was considered to have reached a plateau was chosen by visual inspection of the plotted data; all subsequent values, collected over at least a 5 h period, were included in the calculations of the serum specific radioactivity. Similar criteria were applied to the analysis of the carbon dioxide specific radioactivities but a mean value was usually calculated from the measurements made over the last 4 h of the infusion. Eqn. (1) expresses the fact that at plateau the amount of label entering the free tyrosine metabolic pool via the infusion is equal to the amount leaving if the pool remains constant in size.

\[ I = Q \times S_p \max. \]  

In eqn. (1) \( I \) is the rate of infusion of isotope (d.p.m./h), \( Q \) is the rate at which tyrosine leaves the pool (mmol/h) and \( S_p \max. \) is the tyrosine specific radioactivity (d.p.m./mmol) in the free amino pool at plateau value, taken to be sampled via the blood. The re-entry of isotope from body protein is assumed to be negligible during the course of the infusion (a significant inflow of isotope would lead to a steady increase in the specific radioactivity of the free amino acid pool, which would therefore fail to achieve a plateau value: this assumption could therefore be tested experimentally). The flux \( Q \) is then a measure of the amount of tyrosine which is leaving the pool by synthesis into protein and other compounds, e.g. catecholamines, and by oxidation. For the purposes of this analysis, the contribution to the flux by conversion of tyrosine into metabolites other than protein is assumed to be negligible. The rate, \( T_o \) (mmol/h), at which tyrosine is oxidized to carbon dioxide can be estimated from eqn. (2),

\[ E = T_o \times S_p \max. \]  

where \( E \) is the rate of excretion of isotope (d.p.m./h) as respiratory \(^{14}\text{CO}_2\) (the product of the specific radioactivity of carbon dioxide and the rate of excretion of respiratory carbon dioxide), corrected on the assumption that 20% of \(^{14}\text{CO}_2\) is retained within the body (see below).

Since the portion of tyrosine flux which was not oxidized was assumed to be incorporated into protein, then the rate of incorporation of tyrosine into protein (\( T_i \)) is given by \( T_i = Q - T_o \). The amount of protein synthesized (g/h) is then given by \( S = (T_i \times 0.181)/0.03 \), where tyrosine (molecular weight 181) is assumed to be 3% of human whole body protein (Block & Weiss, 1956).

**Results**

Fig. 2 shows for one subject the rise in the specific radioactivity of plasma L-tyrosine and of carbon dioxide in expired air during the course of an intravenous infusion with \(^{14}\text{C}\)tyrosine. Within 4–5 h of
starting the infusion, the L-tyrosine in plasma achieved an almost constant specific radioactivity, remaining within ±4% of the estimated plateau value until the end of the infusion.

The rate of rise of tyrosine specific radioactivity to the plateau value matched that of a single exponential with a half-life of 0.4 h. The flux of tyrosine for each subject is given in Table 2. The results under these conditions were somewhat less variable than might be expected since the subjects differed in age and size (Table 1), and presumably in their previous daily intake of protein and energy. The coefficient of variation for the flux determinations ranged from 3.5 to 15.2%, and an analysis of variance showed that the source of variation in the individual determinations was negligible compared with the differences between subjects.

The labelling of the expired carbon dioxide also occurred promptly after the infusion started but its specific radioactivity took 1–2 h longer to reach a constant value than did specific radioactivities of plasma tyrosine. Between 6 and 10 h of infusion there was usually an increase in labelling of the expired carbon dioxide, which varied from 0 to 13.5% of the calculated mean specific radioactivity. A similar delay in labelling of the carbon dioxide pool was seen in all six subjects.

In order to assess the contribution of the bicarbonate pool to the delay in maximum labelling of the expired carbon dioxide, three subjects were infused on a separate occasion with 14C-labelled bicarbonate. Fig. 3 shows the proportion of dose excreted as carbon dioxide in one subject. In all three subjects, at least 2 h elapsed before the rate of excretion of

![Graph showing plasma specific radioactivity of L-[14C]tyrosine (Δ) during the infusion of L-[U-14C]tyrosine for 10 h in the first subject. The specific radioactivity of exhaled 14CO2 (●) is shown on a different scale.]

**Table 2. Rates of tyrosine flux, oxidation (corrected for labelled bicarbonate retention) and incorporation into protein**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Tyrosine flux (mol/h)</th>
<th>Oxidation rate (mmol/h)</th>
<th>Oxidation as % of flux</th>
<th>Tyrosine incorporated into protein (mmol/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P.J.</td>
<td>3.28 ± 0.05</td>
<td>0.675 ± 0.019</td>
<td>20.6</td>
<td>2.61 ± 0.05</td>
</tr>
<tr>
<td>P.S.</td>
<td>3.28 ± 0.06</td>
<td>0.613 ± 0.023</td>
<td>18.7</td>
<td>2.67 ± 0.06</td>
</tr>
<tr>
<td>C.H.</td>
<td>3.17 ± 0.07</td>
<td>0.625 ± 0.018</td>
<td>19.7</td>
<td>2.55 ± 0.07</td>
</tr>
<tr>
<td>J.W.</td>
<td>2.50 ± 0.03</td>
<td>0.538 ± 0.013</td>
<td>21.5</td>
<td>1.96 ± 0.03</td>
</tr>
<tr>
<td>T.E.</td>
<td>2.76 ± 0.08</td>
<td>0.663 ± 0.033</td>
<td>24.0</td>
<td>2.10 ± 0.08</td>
</tr>
<tr>
<td>P.P.</td>
<td>2.98 ± 0.11</td>
<td>0.500 ± 0.037</td>
<td>16.8</td>
<td>2.48 ± 0.12</td>
</tr>
<tr>
<td>Mean</td>
<td>3.00</td>
<td>0.602</td>
<td>20.2</td>
<td>2.40</td>
</tr>
<tr>
<td>SD</td>
<td>0.31</td>
<td>0.070</td>
<td>2.46</td>
<td>0.29</td>
</tr>
</tbody>
</table>
14CO₂ approached plateau values. Thus the delay in the labelling of the 14CO₂ during [14C]tyrosine infusions was consistent with a delay in the bicarbonate pool alone, without there being any need to invoke a delay in mixing in the intracellular pool of free tyrosine. The curve also demonstrates that not all label is excreted during the period of plateau; approximately 20% is retained. The same proportion was found in all three infusions.

Measurements of the proportion of 14C excreted in the urine during the tyrosine infusion revealed that there was very little radioactivity excreted by this route. In contrast to the previous findings with lysine infusions (Waterlow, 1967), less than 0-1% of the dose was excreted during the whole infusion period. This suggested that the accumulation of contaminating d isomer, as had occurred with lysine, was insignificant with infusions of L-tyrosine.

The corrected oxidation rates were similar in the six subjects on a standardized food intake and amounted on average to 20% of the flux (Table 2). Thus 80% of the flux entered the protein synthetic pathway. This represented a very substantial rate of recycling of tyrosine from protein since the intake from food amounted to only 27% of the flux.

A mean synthesis rate of 346 g of protein/day was obtained with values varying from 284 to 387 g (Table 3). Individual differences were apparent and could not be accounted for by the errors in the methods, which for these values included the errors in the measurement of blood specific radioactivity, the infusion rate of label, the specific radioactivity of carbon dioxide and the rate of excretion of carbon dioxide.

In an attempt to explain some of the individual variability in the protein synthesis rate, results were also expressed on a body weight basis (Table 3). This seemed, however, to increase the variability: two subjects, P.J. and P.S., with almost identical synthetic rates, showed a large difference when this method of expression was used. Since there were some differences in the calculated amounts of body fat it seemed more appropriate to express the results in terms of the lean body mass of each subject. This did not improve the scatter of results, nor did relating synthetic rates to the observed metabolic rate during the course of the study. Thus there seemed to be genuine individual variations in protein synthesis rates which were unrelated to anthropometric or physiological indices commonly used in metabolic studies.

**Table 3. Calculated rates of protein synthesis in the six subjects**

<table>
<thead>
<tr>
<th>Subject</th>
<th>g/day(1)</th>
<th>g day⁻¹ kg⁻¹</th>
<th>g day⁻¹ kg⁻¹ LBM</th>
<th>kg of protein/l of O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>P.J.</td>
<td>377±7</td>
<td>5.39</td>
<td>7.39</td>
<td>1.34</td>
</tr>
<tr>
<td>P.S.</td>
<td>387±9</td>
<td>3.87</td>
<td>5.76</td>
<td>1.03</td>
</tr>
<tr>
<td>C.H.</td>
<td>368±11</td>
<td>4.63</td>
<td>6.90</td>
<td>1.05</td>
</tr>
<tr>
<td>J.W.</td>
<td>284±5</td>
<td>3.86</td>
<td>5.18</td>
<td>0.99</td>
</tr>
<tr>
<td>T.E.</td>
<td>304±12</td>
<td>4.18</td>
<td></td>
<td>1.06</td>
</tr>
<tr>
<td>P.P.</td>
<td>359±17</td>
<td>5.64</td>
<td>7.33</td>
<td>1.62</td>
</tr>
<tr>
<td>Mean</td>
<td>346±5</td>
<td>4.60</td>
<td>6.51</td>
<td>1.18</td>
</tr>
<tr>
<td>SD</td>
<td>42.2</td>
<td>0.77</td>
<td>0.99</td>
<td>0.25</td>
</tr>
</tbody>
</table>

(1) Mean values ± SEM.
Discussion

The constant infusion method was introduced by Waterlow (1967) for studies on protein synthesis in man in an attempt to overcome some of the difficulties associated with the use of single injections of labelled amino acids. By using $[^{14}C]$tyrosine, we were able to measure the specific radioactivity of the \(L\) isomer of tyrosine in 5 ml plasma samples with a total dose of 15 \(\mu\)Ci infused over a period of 10 h, i.e. only a third of the time used for the lysine infusions. If 100% of the infused $^{14}C$ had been retained, then the calculated radiation dose to the whole body was 24 mrem. However, 16% of the dose was immediately excreted and further loss of label would occur as proteins synthesized during the course of the infusion were degraded.

Several factors should be considered when a labelled amino acid is used for assessing protein turnover (James, Sender, Garlick & Waterlow, 1974b). With $[^{14}C]$lysine it is important to use an enzymatic assay for specific radioactivity measurements to distinguish between \(D\) and \(L\) isomers of the amino acid (Waterlow & Stephen, 1968). The enzymatic assay for tyrosine is one of the few convenient ones available for this purpose, and proved to be much simpler than that for lysine, which involved the use of a Cartesian diver. The absence of a substantial urinary excretion of label showed, however, that there was no accumulation of \(D\)-$[^{14}C]$tyrosine in human subjects. Thus there was either no contamination of the infusion solution with \(D\)-tyrosine or the \(D\) isomer of tyrosine was catabolized by the body and excreted as $^{14}CO_2$. Bender & Krebs (1950) showed that mammalian \(D\)-amino acid oxidase can oxidize \(D\)-tyrosine and most other amino acids, but is inactive with \(D\)-lysine. This suggested that the need to employ an enzymatic assay specifically for the \(L\) isomer was unnecessary if amino acids other than lysine were used; specific radioactivity measurements on plasma amino acids can then be made by column chromatography without the need to separate the \(L\) isomer. This we have confirmed for leucine, where infusions of $[^{14}C]$leucine have been used (O’Keefe, Sender & James, 1974).

Table 2 shows that the calculated values for protein synthesis depend upon a complete measure of tyrosine oxidation, since any underestimate of tyrosine catabolism will overestimate protein synthesis. Correction had therefore to be made for the sequestration of label in the bicarbonate pool by allowing for a 20% retention of label, a value identical with that found by Issekutz, Paul, Miller & Bortz (1968) in their studies on the flux of free fatty acids in man. However, two metabolic pathways of tyrosine are neglected in the model shown in Fig. 1. First, routes into melanin and catecholamines are excluded. In quantitative terms these are unimportant. A potentially more important factor is the possibility that labelled intermediates of tyrosine catabolism could be retained instead of being fully oxidized to carbon dioxide. A low value for the oxidation rate would then be obtained. Incorporation of $^{14}C$ into fat, for example, could also produce a slow rise in the ‘plateau’ excretion rates if it was subsequently recycled during the infusion. This rise in the plateau was in fact observed to a small extent. These problems would have been overcome if $carboxy\cdot[^{14}C]$labelled \(L\)-tyrosine had been used, but our assay system depended on the production and measurement of labelled tyramine rather than on the collection of $^{14}CO_2$ from the decarboxylation step.

A further source of error when estimates of protein turnover are based on measurements of labelled amino acids in plasma is the heterogeneity of the free amino acid pool; since the specific radioactivity of intracellular free amino acids is lower than that of amino acids in plasma (Gan & Jeffay, 1967; Waterlow & Stephen, 1968), the rate of protein synthesis will be underestimated. Moreover, the intracellular amino acid pool may itself be heterogeneous, so that the specific radioactivity of total intracellular amino acids may not be the same as that of the precursor at the site of protein synthesis. The ‘true’ precursor specific radioactivity is likely to lie between that of plasma and that of the total intracellular amino acid pool (Mortimore, Woodside & Henry, 1972; Airhart, Vidrich & Khairallah, 1974). If that is so, the error produced by calculating turnover from measurements on plasma would be of the order of 15–20%, since in rats the specific radioactivity of tyrosine in the tissues is, on average, 70% of that in plasma (Garlick, Millward & James, 1973).

The present measurements of the rate of total protein synthesis thus have two sources of error, which tend to cancel out: (a) an overestimate of synthesis rates because oxidation of tyrosine could not be fully accounted for and other routes of tyrosine metabolism could not be measured; (b) an underestimate of synthesis rates because intracellular tyrosine tends to be less highly labelled than plasma tyrosine (Garlick et al., 1973). Despite these possible errors, the calculated rates of protein synthesis...
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The results also suggest that the protein pool is not in a steady state. The input of tyrosine from food was estimated to be 27% of the flux, whereas the rate of tyrosine oxidation, even after correction for retained $^{14}$CO$_2$, was only 20% of the flux. We consider it unlikely that this difference could be accounted for by expansion of the free tyrosine pool, since in animal experiments we have found that tyrosine does not accumulate intracellularly even under conditions of altered nitrogen balance (E. B. Fern, P. Broad-

![Model for calculating metabolic rates for tyrosine and phenylalanine.](image)

**Fig. 4.** Model for calculating metabolic rates for tyrosine and phenylalanine. The pools of free tyrosine and phenylalanine are assumed to be homogeneous, with phenylalanine catalyzed only via tyrosine to carbon dioxide (Meister, 1965). Both pools are taken to remain constant in size. The tyrosine flux ($Q_t$), rate of oxidation ($T_c$) and rate of tyrosine incorporation into protein ($T_p$) were calculated from the data obtained in [$^{14}$C]tyrosine infusions (see the Methods section: Calculations). If protein synthesized during the infusion contains 5% phenylalanine and 5% tyrosine (Block & Weiss, 1956), the rate of incorporation of phenylalanine into protein ($T_p$ mmol/h) is given by

$$P_p = T_p \times \frac{181}{165} \times 0.05$$

Similarly, $P_p = T_p \times \frac{181}{165} \times 0.03$

where $P_p$ and $T_p$ are the amounts of phenylalanine and tyrosine coming from protein degradation. Since both free amino acid and pools remain constant in size, $T_p = T_c + T_e = T_b + T_i + P_c$. $T_p$ and $T_e$ are respectively the rates of removal of tyrosine from the free amino acid pool for protein synthesis and for oxidation.

Tyrosine is added to this pool from protein breakdown ($T_b$), dietary protein intake ($T_i$) and the formation of tyrosine from phenylalanine ($P_c$).

The results of these calculations show that whereas the mean rate of uptake of tyrosine into protein was 2.4 mmol/h (Table 2), the mean rate of release of tyrosine by protein breakdown was 1.9 mmol/h (Table 4). The difference is equivalent to an expansion of the protein pool at the rate of 3 g/h. This rate of accumulation of protein during feeding is consistent with an overall balance of body protein on a daily basis, since Cahill and his co-workers (Cahill, 1970) have shown that muscle is in a state of negative protein balance during an overnight fast. Thus the mass of body protein increases during the day and then declines at night when gluconeogenesis is occurring and amino acid oxidation continues without dietary replacement.

This calculation of the rate of protein breakdown requires the assumption that all phenylalanine oxidation occurs via tyrosine (Meister, 1965) and

**Table 4. Calculated rates of phenylalanine and tyrosine metabolism**

<table>
<thead>
<tr>
<th>Subject</th>
<th>$T_b$</th>
<th>$P_c$</th>
<th>$P_p$</th>
<th>$P_b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>P.J.</td>
<td>2.13</td>
<td>0.35</td>
<td>4.75</td>
<td>3.90</td>
</tr>
<tr>
<td>P.S.</td>
<td>2.18</td>
<td>0.30</td>
<td>4.88</td>
<td>3.98</td>
</tr>
<tr>
<td>C.H.</td>
<td>2.05</td>
<td>0.32</td>
<td>4.64</td>
<td>3.76</td>
</tr>
<tr>
<td>J.W.</td>
<td>1.44</td>
<td>0.26</td>
<td>3.58</td>
<td>2.63</td>
</tr>
<tr>
<td>T.E.</td>
<td>1.63</td>
<td>0.33</td>
<td>3.84</td>
<td>2.97</td>
</tr>
<tr>
<td>P.P.</td>
<td>1.95</td>
<td>0.23</td>
<td>4.53</td>
<td>3.56</td>
</tr>
<tr>
<td>Mean</td>
<td>1.90</td>
<td>0.30</td>
<td>4.37</td>
<td>3.47</td>
</tr>
<tr>
<td>SD</td>
<td>0.30</td>
<td>0.05</td>
<td>0.53</td>
<td>0.55</td>
</tr>
</tbody>
</table>
that this tyrosine mixes uniformly with the total free tyrosine pool of the body. This second assumption is probably unreasonable since phenylalanine hydroxylase occurs mainly in the liver (Eagle, Piez & Fleishman, 1957). It would therefore be better to estimate rates of protein breakdown with infusions of an appropriately labelled essential amino acid, where synthesis de novo does not contribute to the inflow to the free amino acid pool.

These studies with [\(^{14}\text{C}\)]tyrosine have demonstrated that it is possible to obtain estimates of the rate of protein synthesis in the human being with infusions lasting less than 10 h. This time is much shorter than that needed either for infusion of labelled lysine (Waterlow, 1967) or for measurement of whole body turnover rate from urinary excretion of \(^{15}\text{N}\) after administration of \(^{15}\text{N}\)-labelled amino acids (for review see Waterlow, 1969). Use of \([\text{U-}\(^{14}\text{C}\)]\)tyrosine suffers from two disadvantages: incomplete oxidation to \(^{14}\text{CO}_2\), with possible retention of label in fat and glycogen, and our inability to quantify accurately the contribution of phenylalanine hydroxylation to tyrosine flux. These complications are avoided by the use of an essential amino acid labelled in the C-1 position, such as \([1-\text{U}\(^{14}\text{C}\)]\)leucine (O'Keefe et al., 1974). This enables the rate of oxidation to be measured accurately, and hence the rate of breakdown to be calculated. It should then be possible to evaluate in man the factors which affect rates of protein synthesis and breakdown in the whole body.

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