Comparison of different techniques for estimating rates of protein synthesis *in vivo* in healthy and bacteraemic rats

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1. Previous studies have reported that use of a flooding dose of radiolabelled amino acid is a more precise technique than the constant infusion of tracer quantities for determining rates of protein synthesis in rapidly turning-over tissues in the rat. However, there has been little direct investigation comparing different methods under comparable conditions. 2. Initially, 12 healthy male Sprague–Dawley rats, weighing approx. 100g, were randomized to receive either a bolus intravenous injection of 100 μ mol of L-leucine (containing 30 μ Ci of [1- 14 C]leucine)/100g body wt., or a continuous 2h tracer infusion of [14 C]leucine. 3. In the second phase of the experiment, 12 additional rats were intravenously injected with 1×10^8 colony-forming units of *Pseudomonas aeruginosa* and 16h later randomized to receive one of two infusions described above. Total protein synthesis as well as fractional synthesis rates were determined in liver, rectus muscle and whole body. 4. Synthesis rates measured in liver, muscle and whole body were significantly higher in bacteraemic rats than in healthy rats. The flooding-dose methodology gave significantly higher estimates of protein synthesis in the liver, skeletal muscle and whole body than did the continuous-infusion method using direct measurement of the acid-soluble fraction from the respective tissue. Indirect estimates of whole-body protein synthesis based on plasma enrichments and stochastic modelling gave the lowest values.

Several mathematical and analytical techniques have been developed to estimate the renewal rate of body protein. In laboratory animals, where direct tissue sampling is possible, the most widely used technique for measuring protein kinetics *in vivo* has been with a constant infusion of a radio-labelled amino acid (Garlick *et al.*, 1973). When isotopic steady states are achieved in the acid-soluble fractions of individual tissues, rates of protein synthesis are calculated from the ratio of the specific radioactivities of the amino acid bound in protein and the acid-soluble pool. In humans, where tissue sampling is not possible, estimates of whole-body protein synthesis can also be obtained with a constant infusion of labelled amino acid (Waterlow & Stephen, 1967). Such estimates, however, are indirect and are derived from the difference between plasma amino acid appearance and its oxidation.

The constant-infusion methodology has subsequently received considerable criticism, primarily

centred around the failure to define correctly the specific radioactivity of the free amino acid at the site of protein synthesis (McNurlan *et al.*, 1979, 1982; McNurlan & Garlick, 1980). These authors have justifiably argued that, in tissues where appreciable recycling of free amino acids occurs, the difference between the specific radioactivity measured in the plasma and in the acid-soluble fraction may be sufficiently large enough to make estimates of protein synthesis based on either unreliable. Furthermore, James *et al.* (1971) have postulated that an additional error arises from recycling of tracer in rapidly turning-over proteins during longer infusion periods.

McNurlan *et al.* (1979) have proposed that it would be possible to circumvent these difficulties in laboratory animals by giving a flooding dose of radiolabelled amino acid. They demonstrated that such an administration would flood free amino acid pools so that the specific radioactivity would become nearly the same in all pools. In addition,

isotopic steady states would be maintained for at least 10 min, sufficiently long enough to estimate rates of protein synthesis, yet short enough for use in rapidly turning-over tissues such as liver and intestine, where appreciable recycling of proteins may occur with longer infusion periods (McNurlan & Garlick, 1980).

The present study was undertaken to compare rates of protein synthesis in individual tissues and in the whole body by three different methodologies in healthy and bacteraemic animals. Specifically, the studies were designed to determine whether the flooding-dose technique for measuring rates of protein synthesis proposed by McNurlan *et al.* (1979) gives estimates of fractional synthesis comparable with rates measured by a continuous infusion by the method of Garlick *et al.* (1973). Furthermore, it was also of interest to determine whether the indirect whole-body synthesis measurements based on plasma enrichments (Waterlow & Stephen, 1967) were comparable with the two estimates which utilized direct incorporation of radiolabelled amino acid into individual tissues (Garlick *et al.*, 1973; McNurlan *et al.*, 1979) in healthy and infected animals.

Materials and methods

Some 24 21-day-old male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA, U.S.A.) were maintained, two animals per unit, in stainless-steel suspension cages in a light- and temperature-controlled room. All animals were allowed to consume water and standard laboratory chow (Ralston Purina Co., St. Louis, MO, U.S.A.) *ad libitum* until a minimal body weight of 100 g was attained.

Under diethyl ether anaesthesia the left external jugular vein was dissected free, and a 0.050 mm × 0.098 mm Silastic catheter (Corning Glass Works, Corning, NY, U.S.A.) was inserted and advanced into the superior vena cava. The tubing was tunnelled subcutaneously and externalized at the midscapular region, where it passed through a 'spring shield' attachment, as previously described (Moldawer *et al.*, 1980). The animals were returned to metabolic units and starved overnight. In half of the animals, 1×10^8 colony-forming units of *Pseudomonas aeruginosa* P-4 were injected into the jugular vein immediately after the catheterization.

The next morning, all of the animals were transferred to Plexiglass chambers that permitted the collection and analysis of expired breath. For the next 2 h, each animal was infused with physiological saline (0.9% NaCl) at a rate of 2.3–2.4 ml/h. Half of the healthy and half of the *Pseudomonas*-infected rats received 1.0 μ Ci of

L-[1- 14 C]leucine (50 Ci/mol; New England Nuclear Laboratories)/h with their physiological saline. During the 2 h infusion period, expired breath was collected every $\frac{1}{3}$ h for analysis of total CO₂ production and 14 C radioactivity (Moldawer *et al.*, 1983), after which the rats were killed by decapitation.

The other half of the *Pseudomonas*-infected and healthy rats were infused with only physiological saline for 2 h. At the end of the infusion, however, the rats received, via the indwelling catheter, a bolus injection of 100 μ mol of L-leucine (containing 30 μ Ci of L-[1- 14 C]leucine)/100 g body wt. After 10 min, these animals were also decapitated. After decapitation, mixed arterial-venous blood was collected in chilled heparinized tubes. Blood samples were centrifuged at 1500 g for 10 min and serum was deproteinized with 10% (w/v) sulphosalicylic acid.

Immediately after the blood collection, the whole liver was weighed, and a 1 g piece each of liver and rectus muscle was homogenized in 5 ml of ice-cold 10% sulphosalicylic acid and stored at -30°C . The remaining liver was then placed back into the peritoneum, and the entire carcass, including the head, was dropped into a liquid-N₂ bath. The entire procedure required less than 4 min to complete, and in most cases less than 2 min.

The frozen carcass was wrapped in a clean cloth and broken into small pieces with a mallet, which were subsequently pulverized in a Waring blender with solid CO₂. Samples (1–2 g) were placed in weighed vials containing 10 ml of physiological saline for nitrogen analysis, and in vials containing 5 ml of 10% sulphosalicylic acid for determination of fractional synthesis rates. Nitrogen content in tissues stored in physiological saline was determined by micro-Kjeldahl digestion as previously described (Moldawer *et al.*, 1980). It was assumed that tissue protein contained 16% nitrogen.

In representative samples of muscle, liver and whole body, the percentage of leucine in protein was determined by hydrolysing tissue samples in 12 M-HCl at 120°C for 3 h. After hydrolysis, HCl was removed with a rotary evaporator (Brinkman Instruments, Westbury, NY, U.S.A.). Samples were then taken up in 0.2 M-lithium citrate buffer, pH 2.9, and the leucine concentration of the hydrolysate was determined by high-performance liquid chromatography by using *o*-phthalaldehyde and fluorescent detection (Waters Associates, Milford, MA, U.S.A.). Total nitrogen content was measured by spectrophotometric analysis (Technicon Autoanalyzer) after micro-Kjeldahl digestion.

In rats infused with tracer quantities of [14 C]-leucine, whole-body leucine appearance, oxidation and incorporation into protein were calculated from plasma leucine and $^{14}\text{CO}_2$ specific radio-

activities by the equation of Waterlow & Stephen (1967). It was assumed that approx. 10% of metabolically generated carbonate was retained in the body and that metabolic stress would not alter it (Wolfe & Burke, 1977). Rates of leucine oxidation were therefore adjusted by this percentage to account for $^{14}\text{CO}_2$ that did not appear in the expired breath. Rates of leucine incorporation into protein ($\mu\text{mol/h}$) were converted into g of protein/day by using the experimentally determined leucine content of the rat. Simultaneously, fractional synthesis rates in muscle, liver and whole body were derived from the equations of Garlick *et al.* (1973). The rate of rise to plateau in the precursor pools, λ_1 , was estimated by $R \cdot K_s$, where K_s is the fractional synthesis rate of the tissue and R is the ratio of protein-bound to free leucine ($R = 556$) (Waterlow *et al.*, 1978). Although R was arbitrarily chosen, Garlick (1978) has demonstrated that large errors in R will result in only relatively small errors in derived fractional synthesis rates. In each case, the acid-soluble fraction from the respective tissue (liver, rectus muscle and whole body) was assumed to represent the precursor pool for protein synthesis. Fractional synthesis rates in liver and whole body were then multiplied by the protein content, as measured after Kjeldahl digestion, and results presented as g of protein/day.

Fractional rates of protein synthesis in liver, rectus muscle and whole body in rats receiving the flooding dose were determined by using the equation of McNurlan & Garlick (1980) and McNurlan *et al.* (1979, 1982), $K_s = 100 \times S_b/S_a \cdot t$, where K_s is the fractional synthesis rate (in %/day), S_a is the acid-soluble leucine specific radioactivity and S_b is the specific radioactivity of leucine in protein after incorporation at time t (days). The actual time of incorporation (t) included the time

required to remove and freeze the tissue samples in liquid N_2 . As with the other animals, fractional synthesis rates were multiplied by protein content to obtain total synthesis rates.

Data were analysed on a IBM 370 computer by using a commercially available statistical package (BMDP-79; Regents of the University of California). Comparisons between groups were done by one- and two-way analysis of variance.

Results

Body composition analyses revealed that protein contents of the rat carcass and liver were $14.8 \pm 0.7\%$ and $19.5 \pm 0.7\%$ (w/w; mean $\pm$ S.E.M.) respectively. There were no significant differences in protein content between the healthy and bacteraemic groups. The contributions of leucine to total amino acids in the carcass, liver and rectus muscle were 6.56–6.59%, 9.30–9.69% and 7.79–8.69% (w/w; ranges) respectively.

Whole-body, liver and muscle fractional synthesis rates, measured by the flooding-dose method and by direct incorporation of radioisotope into tissue protein during a 2h tracer infusion, are summarized in Table 1. Bacteraemic rats had significantly higher fractional synthesis rates in whole body, liver and skeletal muscle regardless of the method used. In addition, the flooding technique gave higher estimates of fractional protein synthesis than did a continuous infusion, regardless of which tissue was examined ($P < 0.05$).

Whole-body leucine kinetics obtained from a continuous infusion are summarized in Table 2. Bacteraemic rats had a significant increase in leucine flux ($P < 0.01$) and oxidation ($P < 0.05$), and a marginal increase in incorporation into whole-body protein ($P = 0.06$) compared with healthy animals.

Table 1. Fractional synthesis rates of individual tissues and whole body

Fractional rates of protein synthesis were determined in rectus muscle, liver and total carcass from either a 2h continuous tracer infusion of L-[1- ^{14}C]leucine or at 10min after a bolus infusion of 100 μmol of L-leucine (containing 30 μCi of [^{14}C]leucine)/100g body wt. *Pseudomonas* bacteraemia significantly increased the synthesis of hepatic ($P < 0.01$), carcass ($P < 0.01$) and rectus muscle ($P < 0.001$) protein. In addition, the flooding-dose methodology also gave significantly higher estimates of protein synthesis than with the continuous infusion in each of the tissues (all $P < 0.05$) investigated. Results are means $\pm$ S.E.M. ($n = 6$).

Status of rats	Infusion type	Fractional synthesis rate (%/day)		
		Rectus muscle	Liver	Carcass
Healthy	Continuous	4.7 ± 0.8	108 ± 19	35.5 ± 5.6
	Flooding dose	6.9 ± 0.5	137 ± 21	55.9 ± 4.7
Bacteraemic	Continuous	8.1 ± 0.4	157 ± 8	65.1 ± 8.0
	Flooding dose	10.1 ± 0.7	218 ± 17	78.9 ± 9.1

Table 2. *Whole-body leucine kinetics in healthy and bacteraemic rats*

Rates of whole-body leucine kinetics were determined from a 2 h continuous infusion of L-[1-¹⁴C]leucine and stochastic modelling. *Pseudomonas* bacteraemia significantly increased leucine flux ($P < 0.01$), oxidation ($P < 0.05$) and marginally increased ($P = 0.06$) incorporation into whole-body protein (by one-way analysis of variance). Results are means $\pm$ S.E.M. ($n = 6$).

Status of rats	Plasma specific radioactivity (d.p.m./ μ mol)	Flux (μ mol/h)	Oxidation (μ mol/h)	Incorporation into protein (μ mol/h)	Percentage of flux oxidized
Healthy	47 536 $\pm$ 6112	46.3 $\pm$ 8.8	14.6 $\pm$ 1.1	31.7 $\pm$ 9.9	31.5 $\pm$ 4.9
Bacteraemic	29 107 $\pm$ 1901	76.0 $\pm$ 5.3	22.1 $\pm$ 2.5	53.9 $\pm$ 5.2	29.2 $\pm$ 3.6

Table 3. *Whole-body protein synthesis determined by three different methods in healthy and bacteraemic rats*

Rates of total protein synthesis were determined either by multiplying the fractional synthesis rate by the carcass protein content or by multiplying the leucine-incorporation rate into whole-body protein by the leucine content of carcass protein. In each case, significant differences in total protein synthesis were observed, owing to the presence of bacteraemia ($P < 0.01$) as well as the method used ($P < 0.001$) (by two-way analysis of variance). Results are means $\pm$ S.E.M. ($n = 6$). Indirect measures of protein synthesis are based on both the plasma and carcass acid-soluble specific radioactivities respectively.

Status of rats	Synthesis rate (g/day)			
	Indirect (plasma)	Indirect (carcass)	Direct (continuous infusion)	Direct (flooding dose)
Healthy	2.27 $\pm$ 0.36	3.30 $\pm$ 0.39	3.40 $\pm$ 0.28	6.01 $\pm$ 0.79
Bacteraemic	3.24 $\pm$ 0.26	5.20 $\pm$ 0.38	5.55 $\pm$ 0.89	7.24 $\pm$ 0.83

Table 4. *Ratio of specific radioactivity of leucine in the plasma and intracellular pools from various tissues*

Specific radioactivity of acid-soluble leucine (S_a) in various tissues was determined on the supernatant of 1 g of tissue homogenized in 5 ml of ice-cold 10% sulphosalicylic acid. Plasma leucine specific radioactivity was determined as described in the Materials and methods section. Results are means $\pm$ S.E.M. ($n = 6$).

Status of rats	Plasma specific radioactivity, S_p (d.p.m./ μ mol)	S_a/S_p		
		Liver	Muscle	Whole body
Healthy	47 536 $\pm$ 6612	0.506 $\pm$ 0.151	0.807 $\pm$ 0.132	0.687 $\pm$ 0.101
Bacteraemic	29 107 $\pm$ 1901	0.736 $\pm$ 0.054	0.791 $\pm$ 0.044	0.623 $\pm$ 0.077

Table 3 summarizes absolute rates of whole-body protein synthesis in healthy and bacteraemic rats determined directly by carcass analysis and indirectly by plasma specific radioactivity. All data have been normalized for body weight. Bacteraemic rats had significantly higher rates of whole-body protein synthesis ($P < 0.01$). Likewise, estimates based on direct incorporation of radioisotope into carcass protein were significantly higher than indirect measurements based on plasma ($P < 0.001$). Furthermore, the flooding-dose methodology gave higher synthesis rates than with a 2 h continuous infusion ($P < 0.001$). Depending on the methodologies used, estimates of whole-body synthesis in healthy rats varied 3-fold (2.27 or 6.01 g/day).

The mean specific radioactivities of leucine (S_a)

in the acid-soluble fraction of various tissues, based on a continuous tracer infusion, are shown in Table 4. The ratio of acid-soluble (S_a) to plasma (S_p) specific radioactivity is also shown. No statistical difference was observed between the specific radioactivities or S_a/S_p ratio in healthy and bacteraemic rats in liver, muscle or whole body.

Discussion

At present, three methodologies are commonly used for estimating rates of protein synthesis *in vivo*. In the past, the most widely utilized technique has been to infuse continuously tracer quantities of radiolabelled amino acid: lysine (Waterlow & Stephen, 1967), tyrosine (Garlick *et al.*, 1973), leucine (Moldawer *et al.*, 1980) or phenylalanine

(Grumer *et al.*, 1962). In general, rates of individual tissue protein synthesis have been estimated from the incorporation of the radioisotope into protein and the simultaneous measurement of the specific radioactivity in the acid-soluble fraction from tissues. In human studies, similar infusions have resulted in indirect estimates of whole-body protein synthesis and, under these conditions, it has been assumed that all of the amino acids that enter the plasma pool and that are not oxidized are used for protein synthesis (Waterlow *et al.*, 1978).

The reliance on plasma or intracellular enrichments as being representative of the precursor pool for protein synthesis has been subjected to considerable criticism, because they may not adequately reflect labelling of the true precursor pool (Hider *et al.*, 1971; McNurlan *et al.*, 1979). To minimize the problems of compartmentalization of amino acids between intracellular and extracellular compartments, a method of injecting large amounts of radiolabelled amino acids has been developed. Scornik (1974) demonstrated that, when the free leucine pools were flooded with large amounts of radiolabelled leucine, all precursor pools would tend to attain the same specific radioactivity, which would be equal to that injected. The synthesis rate would be subsequently derived from the incorporation of radiolabelled amino acid into protein during a brief period when specific radioactivities are at plateau or declining linearly.

The reliability of the results requires, in part, that the large injection does not influence the synthesis rate of the tissue being investigated. McNurlan *et al.* (1982) demonstrated that injection of 100 μ mol of leucine failed to change protein synthesis in the healthy rat. Although ample data are available regarding the bolus and continuous-infusion techniques, a comprehensive test of the validity of these methodologies has not been undertaken. Although results to date have shown that the flooding-dose technique does not alter the incorporation of radiolabelled amino acids into protein, little experimentation has been directed at comparing these methods under similar conditions.

The results presented here suggest that the bolus or flooding-dose method gives significantly higher estimates of protein synthesis in whole body, muscle and liver than values determined by a continuous infusion. There are two explanations for this observation. (1) The specific radioactivity of leucine in the acid-soluble pool during a continuous tracer infusion of [14 C]leucine may not reflect what is occurring at the acylated tRNA. By flooding the pool with large quantities of leucine, the specific radioactivities in plasma, intracellular and tRNA pools would all be similar, so that the differences between acid-soluble fractions and

acylated tRNA would be minimized (McNurlan *et al.*, 1979). Although such an explanation is theoretically accurate, it is unknown whether this may account for the observed differences, since, during a pulse-labelling or a continuous infusion of tracer quantities of labelled amino acid, the specific radioactivity of the acylated tRNA is generally higher than that of the acid-soluble fraction (Airhart *et al.*, 1974). McNurlan *et al.* (1979) and Scornik (1974) have speculated that amino acids are shuttled directly from extracellular pools into the synthesis machinery of the cell without complete equilibration with the intracellular compartment. On the basis of this model, the specific radioactivity of acylated tRNA would be considerably greater than the intracellular enrichment during a continuous tracer infusion, and estimates of fractional synthesis rate based on the continuous infusion would be expected to yield higher, not lower, estimates of protein synthesis than the flooding dose.

(2) It is also possible that the differences in fractional synthesis rates for various tissues obtained with the flooding dose and the continuous infusion reflect heterogeneity of tissue protein. Stein *et al.* (1980) reported that, as the length of radioisotope infusion increased from 2 to 18 h, the estimated rate of protein synthesis declined. Although no difference was obtained by Garlick & Marshall (1972) in mice with infusions lasting 30–120 min, we observed 21–57% decreases between the 10 min flooding dose and a 2 h continuous infusion. Indeed, it is possible that such short infusions (10 min) are measuring disproportionately the shorter half-life proteins, whereas with a 2 h infusion such proteins have reached a plateau enrichment and are, to some extent, recycling their label via protein breakdown. Whether such rapidly turning-over tissues contribute sufficiently to overall estimates of protein synthesis is unknown, but such an explanation may account, in part, for the observations reported here.

Even though the 2 h infusion method gave substantially lower synthesis rates for liver, rectus muscle and carcass than with the flooding dose, rates of whole-body protein synthesis based on plasma enrichments and stochastic modelling (Waterlow & Stephen, 1967) resulted in rates significantly less than those obtained by directly measuring the incorporation of isotope into carcass protein (Garlick *et al.*, 1973) in healthy animals. Reliance on stochastic analyses and plasma enrichments gives underestimates because a fraction of the amino acids released from protein degradation is used immediately for protein synthesis without first equilibrating with the plasma pool (Waterlow *et al.*, 1978). In essence, such indirect measurements based on plasma specific radioactivities

reflect only the kinetics in the plasma compartment, and will therefore always be less than estimates based on intracellular or tRNA enrichments. As shown in Table 3, if indirect estimates of whole-body protein synthesis derived from stochastic modelling are based not on plasma leucine enrichments, but on acid-soluble fractions from the entire body, rates are comparable with those from carcass analysis obtained by using the equation of Garlick *et al.* (1973).

Our estimates of fractional synthesis rates in liver, but not in muscle, are considerably higher than those reported by McNurlan *et al.* (1979, 1982) and Garlick *et al.* (1973). This is especially true for estimates made from the flooding-dose techniques. At present we have no complete explanation, except that the catheterization procedure may have induced some metabolic stress, which we have in the past reported to increase liver protein synthesis (Sakamoto *et al.*, 1983; Moldawer *et al.*, 1980).

It is clear from the present study, however, that the three methods for estimating whole-body protein synthesis give similar relative rates. An experimentally induced bacteraemia clearly stimulated protein synthesis. Such data might inherently appear inconsistent, since infections are generally considered to be catabolic (Beisel *et al.*, 1967). In fact, whole-body leucine-oxidation rates (Table 2) were increased by 51% by the *Pseudomonas* infection. However, although amino acid oxidation and plasma leucine appearance were increased, all three techniques suggested that rates of tissue protein synthesis were also enhanced. Increases in hepatic protein synthesis have been previously reported during inflammation (Sakamoto *et al.*, 1983), and studies *in vitro* (Ryan, 1976) have suggested that muscle synthesis may be similarly increased.

Clearly, when the intent of future studies is to obtain information about changes in protein synthesis during disease states, such as infection, then all three methodologies appear applicable. The ultimate decision about choice of methodology to use may then be made on technical considerations rather than a strict theoretical rationale.

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References

- Airhart, J., Vidrich, A. & Khairallah, E. A. (1974) *Biochem. J.* **140**, 539–548
- Beisel, W. R., Sawyer, W. D., Ryll, E. D. & Crozier, D. (1967) *Ann. Intern. Med.* **67**, 744–771
- Garlick, P. J. (1978) *Biochem. J.* **176**, 402–405
- Garlick, P. J. & Marshall, I. (1972) *J. Neurochem.* **19**, 577–583
- Garlick, P. J., Millward, D. J. & James, W. P. T. (1973) *Biochem. J.* **136**, 935–945
- Grumer, H. D., Koblet, H. & Woddard, C. (1962) *J. Clin. Invest.* **41**, 61–66
- Hider, R. C., Fern, E. B. & London, D. R. (1971) *Biochem. J.* **121**, 817–827
- James, W. P. T., Garlick, P. J. & Millward, D. J. (1971) *Gut* **12**, 495–496
- McNurlan, M. A. & Garlick, P. J. (1980) *Biochem. J.* **186**, 381–383
- McNurlan, M. A., Tomkins, A. M. & Garlick, P. J. (1979) *Biochem. J.* **178**, 373–379
- McNurlan, M. A., Fern, E. B. & Garlick, P. J. (1982) *Biochem. J.* **204**, 831–838
- Moldawer, L. L., O'Keefe, S. J. D., Bothe, A., Bistrian, B. R. & Blackburn, G. L. (1980) *Metab. Clin. Exp.* **29**, 173–179
- Moldawer, L. L., Kawamura, I., Bistrian, B. R. & Blackburn, G. L. (1983) *Biochem. J.* **210**, 811–817
- Ryan, N. T. (1976) *Surg. Clin. North Am.* **56**, 1073–1090
- Sakamoto, A., Moldawer, L. L., Palombo, J. D., Desai, S. P., Bistrian, B. R. & Blackburn, G. L. (1983) *Clin. Sci.* **64**, 321–331
- Scornik, O. A. (1974) *J. Biol. Chem.* **249**, 3876–3883
- Stein, T. P., Leskiw, M. J., Buzby, G. P., Giandomenico, A. L., Wallace, H. W. & Mullen, J. L. (1980) *Am. J. Physiol.* **239**, E294–E300
- Waterlow, J. C. & Stephen, J. M. L. (1967) *Clin. Sci.* **33**, 489–506
- Waterlow, J. C., Garlick, P. J. & Millward, D. J. (1978) *Protein Turnover in Mammalian Tissues and in the Whole Body*, p. 313, North-Holland, Amsterdam
- Wolfe, R. R. & Burke, J. F. (1977) *Am. J. Physiol.* **233**, E80–E85