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SYMPOSIUM ON 'NITROGEN METABOLISM'

Protein synthesis: are there real species differences?

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One of the major factors in the nitrogen metabolism of animals is the phenomenon of protein turnover i.e. the continual breakdown and synthesis of body protein, the resultant of which is protein deposition. The measurement of these processes has consequently attracted much interest although, for reasons of technique, attention has focused upon measurements of protein synthesis, protein breakdown being calculated from simultaneous measurements of growth and protein synthesis (Picou & Taylor-Roberts, 1969; Turner & Garlick, 1974; Golden *et al.* 1977). In this sense, the following paper will be no exception in its emphasis upon measurements of protein synthesis.

The large majority of publications upon protein synthesis in vivo have been concerned with experiments in the rat and by and large these have concentrated upon individual tissues (although see Garlick *et al.* 1973, 1975; Lobley, Webster *et al.* 1978) in a wide variety of circumstances. Although there are available in the literature values for protein synthesis in various tissues in other species (Garlick *et al.* 1976; Nicholas *et al.* 1977; Buttery *et al.* 1977; Arnal, 1977; Edmunds *et al.* 1978; Simon *et al.* 1978; Lobley, Reeds *et al.* 1978) in general these have been obtained at one age and at a defined single intake of food. In all these studies the same general conclusions have been drawn i.e. that the fractional rate of protein synthesis (protein synthesis per unit time÷amount of protein in tissue) in skeletal muscle is low and in the visceral tissues it is high, although there is some controversy over the relative contributions of different tissues to total body protein synthesis (discussed in Lobley *et al.* 1980). In contrast to these reports of 'static' measurements, information on protein turnover in man has been obtained under a variety of conditions (e.g. obesity, severe under-nutrition, injury and infection) but the large majority of studies have concentrated upon measurements of protein synthesis in the whole body (see Waterlow *et al.* 1978 for extensive review). It is difficult, therefore, to make many meaningful interspecies comparisons, particularly if interest is to be centred upon the responses of different species to

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well defined alterations in the diet, to trauma, pregnancy, or to any other change. Such comparisons are useful in deciding whether different species share a common response to a stimulus, as on a practical level it would be advantageous if results obtained in a particular laboratory animal could be applied to other species, including man.

It is our opinion that extensive measurements of protein synthesis in large animals can only be carried out by non-destructive measurements and of course, in man, such an approach is the only one possible. Accordingly this paper will be concerned with measurements of whole-body protein synthesis and discussion will centre upon the general validity of the methods which can be applied to the mammalian species with which workers are mainly concerned.

The measurement of body protein synthesis

Fundamental to the measurement of protein synthesis in the whole body is the measurement of the apparent irreversible loss, from the free amino acid pool of the body, of an isotopically labelled amino acid (the flux; Waterlow, 1967). Whether the basis of the calculation is the rate of excretion of an end-product of the metabolism of a [^{15}N]amino acid (Picou & Taylor-Roberts, 1969; Golden *et al.* 1977) or the specific radioactivity of a ^{14}C-labelled or [^{3}H]amino acid in the blood (James *et al.* 1976; Golden & Waterlow, 1977; Reeds *et al.* 1978) essentially the same highly simplified model of protein metabolism is used (Fig. 1). The model involves the following essential assumptions—(1) that the free amino acid pool is homogeneous; (2) that the loss of the amino acid from the free pool is partitioned only between protein synthesis and amino acid catabolism; (3) that during the measurement the isotope enters the free amino acid pool only by administration.

In the present discussion we will be concentrating upon measurements made during the administration of a ^{14}C-labelled or [^{3}H]amino acid at a constant rate.

Constant infusion of amino acids

The infusion of a labelled amino acid at a constant rate leads to the attainment of a constant value for the specific activity of the amino acid in the blood and in tissues (the plateau), the time taken to reach plateau being a measure of the fractional rate of turnover of the amino acid in the body. One obvious difference between species is body size and as it is a common finding that other metabolic processes proceed at a slower rate/unit body-weight in larger species it is perhaps pertinent to ask whether the rate of rise of the amino acid specific activity is also slower. The time taken for the specific activity of tyrosine to reach 95% of plateau are shown in Table 1. There does indeed appear to be a trend with body size although this comparison is complicated by the problem of assessing the relative stage of maturity and hence the relative metabolic rates of the animals. For example, results for man were only available for individuals aged 45 years or over. These differences are not really surprising and merely place some constraints upon the length of time that an experiment must continue before reliable measurements may be obtained.

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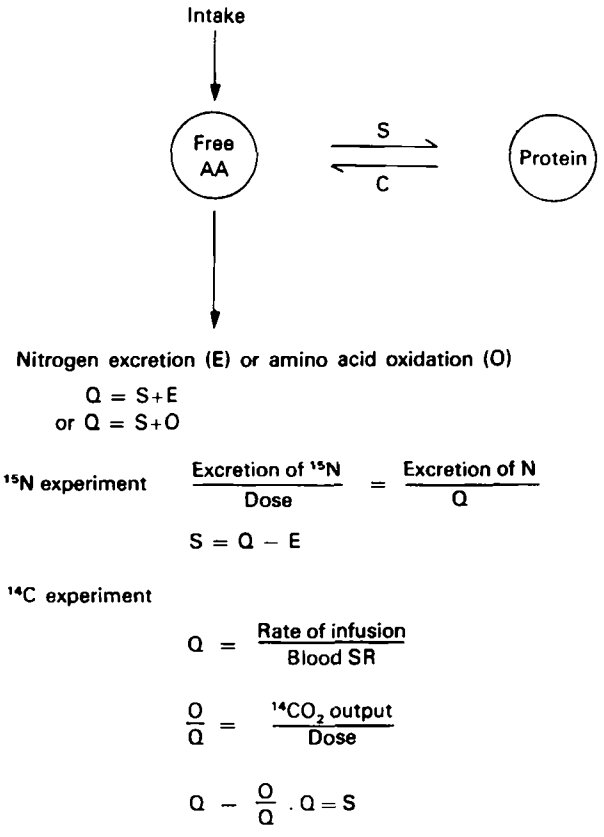

Fig. 1. A simplified two pool model for protein metabolism. Q, apparent irreversible loss of the amino acid from the blood (the flux); C, protein breakdown; S, protein synthesis; O, amino acid catabolism.

The use of values for the specific activity of an amino acid in the blood to calculate total protein synthesis in the body involves one assumption which in particular has led to a great deal of controversy. It is a corollary of the assumption of a homogeneous free amino acid pool that the specific activity of the amino acid in the blood defines that of the pools which act as the precursors for protein

Table 1. *The rate of rise of the specific activity of blood tyrosine during a constant intravenous infusion of the amino acid*

(Values are the time taken to attain 95% of plateau)

Species	Weight (kg)	Time (min)	Reference
Mouse	0·02	60	Garlick & Marshall (1972)
Rat	0·30	90	G. E. Lobley (unpublished results)
Rabbit	3·8	90	Nicholas *et al.* (1977)
Pig	30–40	90	Garlick *et al.* (1976)
Man	70·0	240	James *et al.* (1976)
Cattle	235–628	180	Lobley *et al.* (1980)

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synthesis and amino acid catabolism. However, it is a uniform finding that at plateau during the infusion of a tracer quantity of labelled amino acid, the specific activity of the tissue free amino acid is lower than that in the blood, presumably due to dilution of the label with amino acid arising from the breakdown of protein, and it follows that values for the rate of protein synthesis based on the specific activity of the blood amino acid will be lower than those based upon the specific activity of tissue free amino acid. The question whether either (or neither) of the two values is correct is not settled and is complicated by the fact that the relationship between the specific activity in the blood to that of the pool acting as precursor for protein synthesis may not be the same for each amino acid nor for one amino acid under different physiological circumstances. For example, there is evidence that the specific activity of free glycine in the liver defines that of the precursor for protein synthesis in that organ in the rat (Fern & Garlick, 1974) but the same is not true for tyrosine in rabbit liver (Nicholas *et al.* 1977). Similarly, Robins (1979) has shown that the relationship between the specific activity of the precursor for procollagen synthesis in the skin and that of the free amino acids in the skin and blood is different for proline, tyrosine and leucine. The validity of the values which are obtained from the blood free amino acid specific activity may therefore depend as much upon the choice of the amino acid as upon any other factor. It would appear for example that in man and the pig the flux of lysine gives consistently lower estimates of body protein synthesis than does leucine (Simon *et al.* 1978; Motil *et al.* 1979) (Table 2). This raises the question as to which is the more correct value.

In these two species there is evidence (Golden & Waterlow, 1977 for man; Reeds *et al.* 1978 for pigs) that the specific activity of blood leucine gives an underestimate of the excretion of total nitrogen of between 15% and 23% and by implication there may be a similar underestimate of body protein synthesis. Perhaps the most reasonable conclusion to draw at present is that the specific activity of the precursor for protein synthesis is a function of both the tissue amino acid specific activity and that of the blood amino acid (Khairallah & Mortimore, 1976; Martin *et al.* 1977; McNurlan *et al.* 1978) and that the greater the difference between the two, the less certain is the prediction of the precursor specific activity from that of the blood amino acid. In this context it is noteworthy that in the pig

Table 2. *Whole-body protein synthesis (g/d) calculated from the total flux of leucine and lysine in pigs and man*

Species	Leucine*	Lysine*	Reference
Pig†	543	256	Simon *et al.* (1978)
Man‡	562	425	Motil *et al.* (1979)

*Assuming body protein contains on average 6·6 g leucine and 7·2 g lysine/16 g nitrogen.

†Body-weight 30 kg, receiving a conventional diet supplying 2·5×maintenance energy and 200 g protein/d.

‡Young adults receiving maintenance energy and approximately 120 g egg protein/d.

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the ratio of tissue free lysine specific activity to the specific activity of blood lysine (0·2–0·4) is lower than that of leucine (0·5–0·9) (Simon *et al.* 1978).

Amino acid flux and protein intake

The assumption of a homogeneous pool of free amino acid in the body can lead to further problems in the interpretation of results on the flux of different amino acids. The expression of protein synthesis as an amount of protein or nitrogen/d (a method of expression which allows easy comparison with measurements of N balance) as opposed to moles of the amino acid includes a factor for the average contribution of the amino acid to body protein. This value is clearly critical for the calculation but fortunately there seems to be little variation between the mammalian species for which data are available (Table 3). It should, however, be recognized that the use of a value for tissue protein is in itself a simplification; the correct factor should be the average contribution of the amino acid to the total flux of amino-N. The use of this simplification therefore imposes upon the worker the assumption that the entry of amino acids into the free pool is as a mixture of similar composition to body protein.

In Table 4 are tabulated values for protein synthesis (based either upon [^{14}C]leucine or [^{15}N]glycine) in various species together with an estimate of the contribution of the intake of protein to the amino-N flux. It is clear from these results that, as would perhaps be expected, the intake of amino acid makes a significant contribution to the total entry of the amino acid, between 25 and 40% depending upon the stage of development and hence the intake of the animal. If the composition of the protein of the diet and of the body are different, particularly if the amino acid flux, uncorrected for amino acid catabolism, is used to estimate protein synthesis, problems of interpretation can occur.

Table 5 shows comparisons of body protein synthesis in a number of species calculated from the fluxes of leucine and tyrosine. In the rabbit, pig and man the two estimates are in reasonably close agreement but the values for the two ruminant species are quite different; tyrosine giving a higher estimate of body

Table 3. *The composition of whole-body protein* (*g amino acid*/16 *g nitrogen*)

	Species			
Amino acid	Rat*	Pig*	Cattle†	Sheep‡
Threonine	3·8	3·9	4·0	4·0
Valine	5·5	5·8	5·9	5·1
Isoleucine	3·4	3·8	2·8	2·8
Leucine	6·5	6·6	6·9	6·8
Tyrosine	2·8	2·6	2·6	2·6
Phenylalanine	3·7	3·8	3·6	3·5
Lysine	7·6	7·1	6·4	7·1

*Williams *et al.* (1954).
†Williams (1978).
‡Derived figure to include wool.

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Table 4. *The relationship between protein 'intake' and estimated amino-nitrogen flux*

Species	Body-wt (kg)	Flux (g protein/d)	Intake: flux	Reference
Rat	0·20	16	0·36	(1)
	0·35	20	0·25	(1)
Rabbit	0·8	25	0·43	(2)
Pig	30	480	0·36	(3)
	60	756	0·40	(3)
Sheep	63	440	0·25	(4)
Man				
Adolescent	50	237	0·44	(5)
Young adult	—	562	0·20	(6)
Middle-aged adult	76	465	0·27	(7)
Friesian cow	500	2200	0·23*	(8)

*Maintained by abomasal infusion of casein.

(1) G. E. Lobley, P. J. Reeds and A. J. F. Webster (unpublished results).
(2) G. E. Lobley and C. I. Harris (unpublished results).
(3) Reeds *et al.* (1980).
(4) P. J. Reeds, G. E. Lobley and M. Chalmers (unpublished results).
(5) Kien *et al.* (1978).
(6) Motil *et al.* (1979).
(7) James *et al.* (1976).
(8) P. J. Reeds and E. R. Ørskov (unpublished results).

Table 5. *Whole-body protein turnover estimated from the total flux of leucine and tyrosine*

Species	Body-wt (kg)	Protein turnover tyrosine: protein turnover leucine	Reference
Rat	0·35	0·70	(1)
Rabbit	0·8–2·2	1·20	(2)
Pig	60	1·12	(3)
Man	55–75	1·18	(4)
Sheep	65	1·47	(5)
Cattle	240–600	1·66	(6)

(1) P. J. Reeds and G. E. Lobley (unpublished results).
(2) G. E. Lobley, V. Milne and P. J. Reeds (unpublished results).
(3) P. J. Reeds, M. F. Fuller and G. E. Lobley (unpublished results).
(4) James *et al.* (1976; c.f. P. J. Garlick, personal communication).
(5) P. J. Reeds, G. E. Lobley and M. Chalmers (unpublished results).
(6) Lobley *et al.* 1980.

protein synthesis than leucine. We would propose that these differences may arise from the nature of the amino acid mixture absorbed by conventionally fed ruminants.

Table 6 compares the composition of body protein with estimates of the composition of the mixture of amino acids absorbed from the intestines, derived in

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Table 6. *Comparison of the whole-body amino acid composition with the 'dietary' mixture of amino acids*

(Leucine is given a value of 1)

	Pig		Sheep	
Amino acid	Body	Diet*	Body	Diet†
Threonine	0·60	0·45	0·66	0·80
Valine	0·85	0·62	0·75	1·02
Isoleucine	0·58	0·52	0·42	0·68
Leucine	1	1	1	1
Tyrosine	0·43	0·39	0·38	0·64
Phenylalanine	0·57	0·64	0·51	0·98

*Composition of the diet used in the measurements in Table 5.
†Derived from the data of Wolff *et al.* (1972) and Hume *et al.* (1972) from mature sheep.

the case of the sheep from the data of Wolff *et al.* 1972 and Hume *et al.* 1972 (see also McRae & Reeds, 1979). In the sheep, the contribution of tyrosine (compared with leucine) to the absorbed amino acid mixture is 68% higher than its contribution to body protein. In addition, a significant proportion of tyrosine entry is derived from the hydroxylation of phenylalanine, an amino acid which, in the ruminant, is apparently absorbed in quantities which are well in excess of requirement. In fact it can be calculated that if the intake of protein is 25% of the total amino acid flux then the estimate of protein synthesis obtained from measurement of the tyrosine flux should be 50% higher than the estimate derived from leucine. In addition the small difference between the two estimates in rabbits, pigs and man can be accounted for by the contribution of phenylalanine catabolism to the flux of tyrosine. It seems reasonable to conclude that the results do not point to metabolic differences between species but stem from differences in the dietary input of the various amino acids. The problem is amenable to technical solution for the measurement of the catabolism of the amino acid in question allows us to account for the 'excess' entry from the diet.

There does remain one difference in the results shown in Table 5 which is not accounted for by the above explanation. In our measurements in the rat we have noted that the flux of tyrosine gives a consistently lower estimate of body protein synthesis then leucine. Measurement of the proportion of the flux of leucine, tyrosine and phenylalanine which is oxidized have excluded large differences in the rate of catabolism of each amino acid. However, as the catabolism of tyrosine occurs mainly in the liver it is possible that preferential catabolism of tyrosine of dietary origin may occur. If so, this may lead to incomplete mixing with the labelled amino acid which, in the rat, is infused into a peripheral vein. Although this is speculative, it serves to highlight another area where an apparent species variation may be of technical origin.

Table 7. *Protein synthesis (flux – amino acid catabolism) estimated with [1-^{14}C]leucine in growing animals in energy balance and in adults*

Species	Body-wt (kg)	Protein synthesis (g/d)	g/$W^{0.75}$/d	References
Rat growing	0·35	7·7	16·7	(1)
Rabbit adult	3·6	39	15·0	(2)
Pig growing	30	268	18·9	(3)
Sheep adult	63	351	15·7	(4)
Man adult	62	279	12·5	(5)
Cattle adult	500	1700	16·1	(6)

(1) P. J. Reeds (unpublished results).
(2) G. E. Lobley and P. J. Reeds (unpublished results).
(3) Reeds *et al.* (1980).
(4) P. J. Reeds, G. E. Lobley and M. Chalmers (unpublished results).
(5) P. J. Garlick (personal communication).
(6) P. J. Reeds and E. R. Ørskov (unpublished results).

Comparison of protein turnover in different species

The foregoing may have laboured the practical aspects to an apparently excessive extent but it is clear that if we are to make valid statements about similarities or dissimilarities between species then these comparisons must be based on reliable measurements. With our present state of knowledge the use of either [1-^{14}C]leucine or a method based on the excretion of [^{15}N]urea or ammonia seems to provide a firm basis for comparative measurements.

On this basis we are able to make interspecies comparisons of two aspects of body protein synthesis. The first is related to the role of protein turnover as a factor in the energy expenditure of animals. It is a feature of the process of protein turnover that, even at energy or N equilibrium, a considerable quantity of protein is synthesized and will contribute to the energy expenditure of the animal. Is this contribution constant? Table 7 compares results obtained with [1-^{14}C]leucine in animals, both adult and immature, which have been studied at energy equilibrium. There is a remarkable similarity between the species suggesting a constant contribution of protein synthesis to heat production and the results suggest that the process of protein turnover contributes about 15% of energy expenditure.

The second aspect is the relationship between protein synthesis and protein deposition. This will in itself govern, to some extent, the energetic efficiency of growth. Only in pigs and human ihfants is sufficient information available to compare two species. Golden *et al.* (1977) (summarized by Waterlow *et al.* 1978) have studied the relationship, using [^{15}N]glycine, in growing previously undernourished infants and we (Reeds *et al.* 1980) have carried out similar measurements with [1-^{14}C]leucine in growing pigs.

The relationship between protein synthesis (y) and N balance (x) both expressed/kg body-weight was;

$$y = 1{\cdot}37x + 4{\cdot}6 \text{ in infants,}$$
$$\text{and } y = 1{\cdot}63x + 6{\cdot}8 \text{ in young pigs,}$$

a result which suggests that the pigs required a greater amount of protein synthesis/unit protein deposition but that in both species there is a considerable amount of protein synthesis at N equilibrium.

It is not possible to decide upon the biological significance of the difference as there are significant differences between the experiments. The growing children were recovering from severe protein–energy malnutrition whereas the pigs had had no history of nutritional deprivation and, perhaps more importantly, the children were receiving a diet which was of lower protein content (2 g protein/kJ) than that (11 g/kJ) fed to the pigs. Nevertheless the same general conclusions can be drawn (1) that a considerable proportion of total body protein synthesis in growing animals is associated with a maintenance component and (2) that above maintenance, increments in protein deposition, brought about by increases in the intake of a food of constant composition, are associated with fixed increments in protein synthesis and presumably incur constant energy costs within a species.

Conclusion

In conclusion we can say that many of the apparent differences in protein synthesis, both within and between species, can be explained largely as a result of differences in technique. When conditions are well-defined, enabling realistic comparisons to be drawn, then there seems to be considerable consistency between species in a variety of aspects. These include the relationship between intake and amino acid flux; protein synthesis and energy expenditure; and possibly the changes of protein synthesis associated with growth. At present insufficient information is available to prove conclusively that these relationships are constant. However, our understanding of the problems which are associated with the measurement of protein turnover leads us to conclude that in the next few years sufficient comparative results will be obtained with the same method to enable us to answer the question—'Protein synthesis: are there interspecies differences?'

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