

PROTEIN TURNOVER IN ANIMALS¹

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The protein in animals is continually being synthesized and degraded (protein turnover). Growth results when the rate of synthesis exceeds the rate of degradation. Unfortunately there are several technical difficulties in measuring the synthesis and degradation of protein and some of these are discussed. Particular emphasis is given to the 3-methyl histidine technique. At least in mature animals the rates of protein turnover are related to the metabolic body size. Rapid growth is associated with a fast turnover of protein and there are reasons to believe that a significant proportion but not all, of the differences between theoretical energy cost of protein deposition and that determined experimentally using intact animals is a consequence of energy consumption associated with protein turnover. The most likely reason for the turnover of body protein is the need to be able to finely regulate amino acid and protein metabolism in the animal.

Key words: Protein synthesis, protein breakdown, farm animals

In this review an attempt has been made to introduce the concept of protein turnover, particularly its importance in growth. Some of the techniques used in its measurement are also introduced. Since no attempt has been made to make the coverage comprehensive the reader is referred to the detailed discussions on the subject which can be found in the reviews cited, particularly those in Waterlow et al (1978) and Buttery and Lindsay (1980).

Measurement of total body protein synthesis: The most commonly used technique for the measurement of total body protein synthesis involves the continuous infusion of a labelled amino acid into the blood stream of an animal and the monitoring of the specific activity of the amino acid in the plasma. The specific activity tends to plateau after several hours. The time taken depends on several factors eg the amino acid used, the nutritional status of the animal. From the specific activity at plateau, flux can be calculated as follows

$$\text{Flux} = \frac{\text{Rate of infusion of isotope}}{\text{Specific activity at plateau}}$$

The flux of a particular amino acid through the plasma pool can be described as follows:

$$\begin{aligned} \text{Flux} &= \text{amino acid used in protein synthesis} + \text{catabolism of amino acid} \\ &= \text{amino acid released on protein breakdown} + \text{input of amino acid} \end{aligned}$$

The input comprises of the amino acid coming from the diet and any amino acid being synthesised within the body. Normally an amino acid is selected which is not synthesised within the body eg leucine. By knowledge of the mean amino acid composition of the protein in the animal flux, measurements can be converted to give a measure of total body protein synthesis or breakdown. There are numerous reports

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in the literature of total protein synthesis having been calculated from flux measurements and on first appearance there would appear to be a large scatter in the results obtained. However, much of this variation is due to the nature of the amino acid used.

Studies measuring: total body synthesis have their uses but it should be remembered that they only give an index of the sum of synthesis in the individual organs. When using the technique to study the effects of an external stimulus eg diet, infection or hormonal treatment, it should be remembered that not all organs will necessarily behave the same. See for example the differential response of the liver and muscle on treatment of rats with the anabolic agent trenbolone acetate (Vernon and Buttery 1976).

Measurement of protein synthesis in individual tissues The constant infusion technique can also be used to determine the rates of protein synthesis in individual tissues (Waterlow and Stephen 1968). The calculations are based on the assumption that the specific activity of the labelled amino acid in the precursor pool for protein synthesis, probably in the case of most tissues the intracellular pool although the plasma cannot be ignored, rises to a plateau and that the rate of rise as a first approximation can be described by a single exponential function:

$$S_i = S_i \max (1 - e^{-Kt})$$

where S_i is the intracellular specific activity and K is a rate constant which depends on the animal being studied and amino acid being used. It is possible therefore to describe the rate of change in specific activity of the precursor pool during the course of an experiment. It follows that if at the end of a continuous infusion experiment the animal is killed and the specific activity of the precursor amino acid and that of the same amino acid in the protein are determined it is possible to calculate the rate of protein synthesis. The exact formula used depends on the tissue being studied and the reader is referred to Waterlow et al (1978) or Garlick et al (1973) for further details.

A variant on the continuous infusion technique involves the incorporation of the labelled amino acid in the diet (Harvey et al 1976). Some of the advantages of this technique are that the animal is not stressed by the need to cannulate the blood supply and that it is easy to work with the well fed animal.

When studying the gut an additional potential precursor pool must be considered, i.e. that of the amino acids to be found in the lumen. Alpers (1972) suggested that the pool used for protein synthesis probably differs between regions of the gut wall i.e. the villus regions use luminal amino acids whilst the crypt region probably use intracellular amino acids. The situation in the gut is further complicated when infusions lasting for several hours are undertaken due to the loss of labelled proteins during the infusion. An alternative approach to measure fractional synthetic rate, which overcomes many of the problems, is to inject one large dose of labelled amino acid (- 100 ~ mole/100 g body weight) and then measure the specific activity of the precursor pool and the protein bound pool at several intervals over the next 30 minutes (see McNurlan et al 1979). One of the main advantages of the technique is that the large amount of amino acid tends to flood the body pools and to equalise the extracellular and intracellular specific activities. In the technique, leucine and phenylalanine have been successfully used as the tracer amino acid. While the method is applicable to most tissues the need to use relatively large quantities of labelled amino acids makes its use in large

farm animals impracticable. This is unfortunate because of the large proportion of total body protein synthesis which takes place in the gut wall.

Choice of amino acid to be used in protein metabolism studies There can be no definite statement as to the best labelled amino acid to be used in protein metabolism studies with intact animals. The choice will be dictated by the measurements it is desired to make, the animals to be used, the finance available and any statutory requirements on the disposal of the waste. Among the amino acids that have been used are the following:

Lysine: L-{U-¹⁴C} Lysine was used in the initial studies reported by Waterlow and Stephen (1968), since it is relatively easy to assay using lysine decarboxylase. There has been suspicion that D-lysine, an impurity thought to be present in some samples, accumulates in the tissues and unless it is separated from the L-isomer inaccurate results are obtained. In studies with L-{³H}-lysine in sheep, Buttery et al (1975) were unable to detect a build-up of D-lysine although some other material, presumably metabolises of lysine, did accumulate. Thus, lysine specific activities could be determined using simple ion exchange chromatography. Perhaps one of the great disadvantages of lysine is that it has a large free pool and tends to take a relatively long time to reach a plateau. There also tends to be marked differences between the specific activity of the intracellular and plasma pools. This discrepancy is of great importance until the nature of the precursor pool for protein synthesis is resolved (Waterlow et al 1978). Lysine does, however, have one potential advantage when used in animal production systems in that it is often the first, or close to the first, limiting amino acid, and data obtained from protein turnover studies can be extended to give estimates on the efficiency of diet utilization.

Tyrosine: More recently tyrosine has been extensively used; because of the small free tissue tyrosine pool the plateau is reached quickly and like lysine there is a simple enzymic assay (Garlick and Marshall 1972). However, since phenylalanine gives rise to tyrosine it is theoretically necessary to modify the flux equation to allow for this conversion, i.e.

$$\begin{aligned} \text{Flux} &= \text{Protein synthesis} + \text{Amino acid oxidation} \\ &= \text{Protein breakdown} + \text{Intake of amino acid} \\ &+ \text{Synthesis of tyrosine from phenylalanine} \end{aligned}$$

When using labelled tyrosine, especially ³H the position of the label should be carefully considered so as to minimise the effects of exchange reactions both within the tissues and during hydrolysis of samples prior to determining specific activity.

Leucine: This would appear to be a very good amino acid to use but its analysis requires preparative ion exchange chromatography. The {1-¹⁴C} compound is better than {U-¹⁴C} since there is less incorporation of label into compounds other than leucine in protein. Tritiated leucine can also be used being quite suitable especially L-{4,5,³H}leucine.

Glycine: This amino acid has also been used but its reversible interconversion with serine makes its use impossible to calculate total protein turnover from flux.

In conclusion it is difficult to give a recommendation for the best amino acid to use but on balance it would appear probably to be tyrosine, particularly for research groups not greatly experienced in amino acid biochemistry. In principle there is no reason why ^{15}N -lysine cannot be used for studies with farm animals and the carcass still be suitable for human consumption. With this stable isotope there are, of course, no waste disposal problems.

Measurement of protein breakdown. The monitoring of the loss of label from a protein in theory gives an excellent way of determining the rate of protein breakdown, however, several problems make it very difficult to obtain accurate results. The main problem is reutilization of the labelled amino acid, i.e. on hydrolysis of the protein the amino acid released is incorporated into new protein. There are, however, techniques which help to minimise this effect eg the bicarbonate labelling of liver and muscle protein (Millward 1970; Swick and Ip 1974). Especially in larger animals the turnover of many proteins is relatively slow and this would require long experiments (3 times the expected half-life) especially when the attempts are made to take into account the heterogeneity of the mixed proteins from whole tissues (Garlick et al 1976). Also when using larger animals the quantity of isotope required becomes prohibitive.

Breakdown can also be measured from measurements of synthesis and growth (Edmunds et al 1980), total synthesis - net deposition of protein must equal breakdown. As pointed out by Garlick (1980) the main difficulty arises from the time scale of the measurements. Growth is measured over a period of days while synthesis is measured over a period of a few hours. However, at least in muscle the extent of the diurnal variation in the synthesis of protein is relatively small and therefore the error in the technique would be expected to be relatively small.

One technique for measuring protein breakdown which has been extensively used and abused during the last few years is the assessment of myofibrillar protein catabolism using 3-methyl histidine excretion in the urine. The technique must be used with considerable caution and some of the criteria which must be considered have been reviewed by Ward and Buttery (1978). 3-methyl histidine occurs in actin and myosin and on the catabolism of these proteins, it is not reincorporated into new protein (Young and Munro 1978). In the rat and cattle the amino acid is rapidly excreted while in sheep and pigs there is a large pool of 3-methyl histidine metabolise (a dipeptide balanine). Any change in the size of the non-protein bound 3-methyl histidine would mask the influence of any change in myofibrillar protein breakdown on urine 3-methyl histidine excretion. The presence of a large non-protein-bound pool of 3-methyl histidine causes the rate of clearance of labelled 3-methyl histidine injected intravenously to be extended (Harris and Milne 1980). The data of Harris and colleagues clearly indicates that it is essential that the technique is validated in each species that it is applied to Millward and colleagues (Millward et al 1980) have also questioned the use of 3-methyl histidine excretion technique. They point out that although the vast majority of the 3-methyl histidine in the body is to be found in the muscle the amino acid is found, albeit in small quantities, in other tissues (e.g. gut wall) which have a much higher turnover rate than the skeletal muscle and therefore these tissues could contribute a significant proportion of the total 3-methyl histidine excretion. The significance of this suggestion must await further experimentation.

When using the 3-methyl histidine technique it is essential not only to have an index of the 3-methyl histidine content of muscle but also an indication of the total muscle mass. The latter presents problems as the proportion of muscle in the body

may vary with treatment e.g. diet, steroid treatment. One way of overcoming this is to measure urinary creatinine excretion and use this as an index of muscle mass. This technique should also be used with caution as might be expected high urinary creatinine concentrations are frequently associated with muscle wasting e.g. following changes in thyroid and glucocorticoid status (Fitch 1968; Carter et al 1977). Haverberg et al (1975) reported disturbances in creatinine excretion in energy deficient rats. The use of creatinine as an index of muscle mass does however potentially open the way to assessing changes in myofibrillar protein breakdown by determining the methyl histidine-creatinine ratio on spot urine samples.

Another point to remember is that the diet should be devoid of 3-methyl histidine , e.g. it should not contain fishmeal or meat meal.

Despite all the drawbacks described or alluded to above, the 3-methyl histidine technique must present a most useful non-destructive technique with which to assess the rate of muscle protein breakdown in large farm animals. Caution however must be used in the interpretation of the results obtained.

Contribution of individual organs to total body protein synthesis As is discussed below the relative contribution of individual organs to total body protein synthesis does not differ markedly between species. One of the studies in which protein synthesis and deposition was measured in a farm species is by Edmunds et al (1980). These workers used the continuous infusion technique with (^{14}C) lysine to assess rates of protein synthesis in the rapidly growing pig (25 kg). The rate of change in protein mass of the organs of similar pigs was assessed, as was the lysine balance. The data obtained is presented in Figure 1. One would expect similar patterns to be seen in other mammals.

A point that should be borne in mind is the relative rates of synthesis and net deposition. In the majority of tissues synthesis exceeds net deposition by several fold.

Are there species differences in the rate of protein turnover? There are now numerous reports on the rates of total body protein synthesis and the rates of protein synthesis in individual organs. In an excellent review Reeds and Lobley (1980) considered the data available and showed that much of the variation seen in the literature was due to the technique employed, particularly the tracer amino acid employed. Table 1 taken from their paper clearly illustrates that the main determinant is mature body size. Lobley and Reeds (1980) also considered the % contribution of individual organs to total body protein synthesis and as can be seen this did not vary significantly with species, especially when the relative proportions of body mass occupied by the gastro-intestinal tract in ruminants and non-ruminants is considered (Table 2). It should however be pointed out that the majority of the studies compared by Reeds and Lobley (1980) were on non-stressed immature animals.

Table 1:
Metabolic body size and whole body protein synthesis ¹

Species	Body weight (kg)	g/w ^{0.75} /d
Rat growing	0.35	16.7
Rabbit adult	3.6	15.0
Pig growing	30	18.9
Sheep adult	63	15.7
Man adult	62	12.5
Cattle adult	500	16.1

¹ Calculated from flux measurements determined using (1-¹⁴C) leucine. Flux was corrected for catabolism of leucine. (From Reeds and Lobley 1980)

Table 2:
Percentage contribution of tissues to total body protein synthesis ¹

Species	Weight (kg)	Muscle	GIT ²	Liver	Skin
Rats	0.2 - 0.35	14.0	34.2	18.4	33.4
Rabbits	0.5 - 2.5	19.0	41.0	10.5	29.6
Cattle	235 - 625	17.2	52.6	8.9	21.2

¹ Data calculated from fractional synthetic rate (assuming intracellular amino acid in the precursor x the protein mass in the tissue).

² G.I.T. = gastro intestinal tract (Lobley and Reeds 1980).

Table 3:
The effect of growth rate (age) on the synthesis and break down of muscle protein in the rat

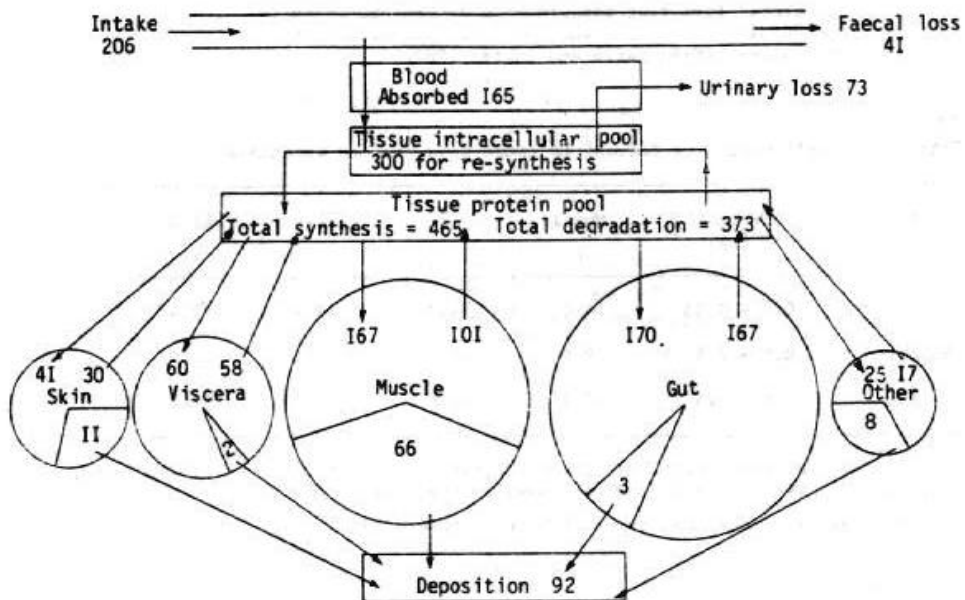
Body weight (g)	Age (days)	Growth rate %/d	Ks ¹ %/d	Kd ² %/d
37	23	6.3	28.6	22.3
70	46	3.0	16.1	13.1
116	65	1.7	11.5	9.8
233	130	0.7	5.3	4.6
511	330	0.3	4.9	4.6

¹ Ks = fractional rate of protein synthesis (Garlick (1980b))

² Kd = fractional rate of protein breakdown (Garlick (1980b))

Figure 1:

A simplified model of protein metabolism in the 25 kg pig. All figures are expressed as g crude protein/day (nitrogen x 6.25) Circle areas equate to tissue protein synthesis, segment areas equate to protein deposition and the rest to protein degradation. Other refers to tissues not studied in detail e.g. blood and synthesis was assumed to be 3 x deposition



Protein turnover and growth. As mentioned above, growth results when the rate of synthesis exceeds the rate of protein breakdown. A very important point is that change in the rate of synthesis of the mixed protein fraction of a tissue tends to be reflected in similar changes in the rate of protein breakdown ie faster synthetic rates and faster breakdown rates tend to be associated with faster rates of net deposition (Table 3). This correlation between synthesis, degradation rate and growth is however not seen on a low protein diet which was totally inadequate for growth (Millward et al 1975). Little is known however of the influence of marginally deficient diets on the rates of protein synthesis and protein degradation.

Some surprising results were however obtained on attempts to study the response of protein synthesis and degradation to anabolic steroid treatment. At least when trenbolone acetate, the active ingredient of Finaflex, was given to rats a marked reduction in protein degradation and a small reduction in protein synthesis in muscle was observed. These differences between the changes in synthesis and degradation resulted in the expected increase in growth rate (Vernon and Buttery 1976). Recent data (Buttery and Dumelow, unpublished observation) would indicate that this mechanism operates in the sheep also. Further work has to be undertaken to confirm these observations in other species and with other anabolic agents.

Protein turnover and the energy cost of protein deposition. In the 1970's it was thought by many workers that the energy costs of protein turnover were largely responsible for the high cost of protein deposition in animals. Stoichiometric calculations suggest that the efficiency with which glucose can be used for protein synthesis in the rat is about 74% and this agrees well with the experimentally determined value. The theoretical energy cost of protein deposition in the energy content of the protein deposited plus the energy cost of the ATP synthesis, requires for formation the peptide bond (~ 26 KJ/g protein deposited). Estimates of the energy cost of protein deposition obtained from whole animal experimentation are much higher, (the majority of estimates being between 42 - 54 KJ/g, Buttery and Boorman 1976). This discrepancy might be due to the energy cost of protein turnover, however this does not stand up to detailed experimentation, (see for example the studies on the 25 kg pig by Edmunds et al 1980) , even if the entirety of the turnover is assumed to be associated with growth and none with maintenance, clearly an unreal situation. Following further examination of their data in the light of the experiments of Reeds et al (1980), Edmunds et al (unpublished observations) would estimate the energy costs of maintenance of the body protein to be 2.23 MJ/d and the energy costs of turnover associated with deposition to be 3.04 MJ/d. Thus the reasons for the difference between the theoretical energy costs of protein deposition and those determined experimentally require further investigation. One conclusion that can be drawn is that protein turnover does consume a reasonable amount of total energy intake -15% . Whether there is any potential in attempting to increase the energetic efficiency of protein deposition in farm livestock by reducing the rate of protein turnover by artificial means is open to debate. Perhaps this is one of the reasons why anabolic agents often increase growth rate of farm animals (Vernon and Buttery 1976). What is clear is that diets with deficiencies in their amino acid content result in inefficient growth (Solberg et al 1971).

Why do proteins turnover? On superficial investigation it would appear that protein turnover is a penalty to the animal. However, this turnover is a consequence of metabolic control processes to which protein metabolism is subjected (Schimke 1977). To give two specific examples. Firstly, the quantity of an enzyme in the cell is best regulated by controlling synthesis and degradation. An increase in concentration is best achieved by increasing the rate of synthesis while simultaneously decreasing the rate of degradation; a reduction in activity is best achieved by the opposite changes in these two processes. Secondly, when an animal is well nourished there is a tendency for protein to be deposited in the tissues, but when the animal is starved, protein is broken down in many tissues, e.g. muscle to provide amino acids which can be catabolised to provide energy or used to synthesise protein in vital organs e.g. the brain. This synthesis and degradation of protein is, of course, protein turnover.

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