PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The 527th. Meeting of the Society was a Joint Meeting with the Scottish Group of the Nutrition Society and was held at the School of Agriculture, Aberdeen, on Thursday and Friday, 1 and 2 June 1972, when the following papers were presented:

JOINT COLLOQUIUM ON 'THE INFLUENCE OF AMINO ACID SUPPLY ON POLYNUCLEOTIDE AND PROTEIN METABOLISM'

An Appraisal of Techniques for the Determination of Protein Turnover *in vivo*

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The quantity of protein in a tissue or organ is controlled by the balance between synthesis and breakdown. Under certain conditions, such as large changes in nutrient intake, induction by specific metabolites or hormonal stimulation, the rates of synthesis or breakdown of some proteins, particularly enzymes, are abruptly altered to a new value; as a result the quantity of protein also changes until a new equilibrium is reached. When this occurs, the rates of both synthesis and breakdown can be calculated from the time-course of the observed changes in protein content or enzyme activity (Berlin & Schimke, 1965). In many cases, however, particularly with mixed tissue proteins, such large and rapid changes in the amount of protein do not occur and isotopic-labelling techniques are used to measure turnover rates.

Since 1939, when Schoenheimer and co-workers demonstrated that dietary tyrosine labelled with ^{15}N was incorporated into protein (Schoenheimer *et al.*, 1939), numerous isotopic methods have been described for measuring the turnover rates of single proteins or of the mixed proteins in complete tissues or entire animals. The methods fall broadly into two classes: those that measure the rate of incorporation of a labelled amino acid into protein, and those that measure the rate of loss of radioactivity from previously labelled protein. In the former the rate of the synthetic process is measured; in the latter, because the experiment lasts longer and measurable changes in protein mass may occur, both synthesis and breakdown can be determined.

Protein breakdown is generally thought to obey first-order kinetics (Schimke, 1970), and so, after pulse-labelling with an isotopically labelled amino acid, label is lost exponentially. The semi-logarithmic plot of total radioactivity in the protein against time is then a straight line whose gradient is equal to the fractional rate of breakdown. Changes in mass of the

protein can then be used to calculate the synthesis rate.

In practice, recycling of label generally causes the measured turnover rate to be less than its true value. The use of $[6\text{-}^{14}\text{C}]\text{arginine}$, which is recycled to a very small extent, decreases the error to an acceptable level when measurements are made on liver-produced proteins (see, e.g., Schimke, 1964). $^{14}\text{CO}_3^{2-}$, which labels aspartate and glutamate in all tissues containing the citric acid cycle, can also be used as a non-reutilizable label (Millward, 1970). The label in albumin labelled with ^{131}I (see, e.g., McFarlane, 1963) and haem proteins labelled with $\delta\text{-amino}[^{14}\text{C}]\text{laevulinate}$ (Levin & Kuntzman, 1969) is not reutilized at all.

Measurement of the rate of uptake of label into protein can be used to determine synthesis rates if the specific activity of the precursor amino acid during the experimental period can also be measured. Recent work on the heterogeneity of the intracellular free amino acid pool casts some doubt on the common practice of using the total intracellular free amino acid specific activity for this purpose. This doubt must be clarified before uptake experiments can give unequivocal results.

Several methods of administration of the isotopic label have been used in uptake experiments. After single injections, complete curves of specific activity of both the free and protein-bound amino acid are usually constructed by killing groups of animals at different times. In one method repeated tissue sampling has been avoided for liver proteins by assuming that protein and urea are synthesized from the same pool of arginine (McFarlane, 1963). The free arginine is labelled by an injection of $^{14}\text{CO}_3^{2-}$ and its specific radioactivity determined by measurements on the urinary urea produced during the experiment. A similar method assumes that SO_4^{2-} and protein are synthesized from the same pool of cysteine (Awwad *et al.*, 1970).

Methods that maintain constant specific activity of the free amino acid in the animal during the experiment offer certain advantages over single-injection techniques. The protein synthesis rate can be calculated from the specific activity in a single animal at the end of the experiment.

Such techniques include constant feeding with

Ca¹⁴CO₃ (Swick, 1958), single injection of a large dose of [¹⁴C]lysine (Henshaw *et al.*, 1971), repeated injections of [¹⁴C]glucose (Austin *et al.*, 1972) and constant infusion of tracer doses of labelled amino acids. In this laboratory we favour the last-mentioned method. Results from the infusion method can be used to determine protein synthesis rates in both individual tissues and in the whole animal, and short infusions of a few hours give the same synthesis rates with different amino acids (Waterlow & Stephen, 1968; Garlick, 1969, 1972).

In both uptake and decay experiments mixed samples of protein cannot be treated as a single protein with a turnover rate equal to the mean of the mixture (Koch, 1962). The apparent rate decreases as time progresses. The problem is more serious with the decay rate, since the faster-turning-over components receive more of the pulse dose of label and the early part of the decay curve is biased by these components, so that the slope is too steep. Hence decay experiments cannot be used to obtain a unique value for the mean turnover rate of a mixture. With uptake experiments the rate at zero time is equal to the mean synthesis rate, and therefore short experiments lasting a few hours can give results that are not seriously in error.

Each experimental situation requires careful consideration of the method used to measure turnover rates. The usefulness and validity of the various methods in different situations will be discussed.

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The Pattern of Protein Turnover in the Whole Animal and the Effect of Dietary Variation

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In animal cells it is now clear that most if not all proteins are continuously degraded and replaced, i.e. they turn over, and the rate is distinct for each protein. The heterogeneity in turnover within individual tissues is very marked. For example, in the liver δ -aminolaevulinase synthetase and α -glycerophosphate dehydrogenase, both mitochondrial enzymes, are said to have half-lives of 1 h and 4 days respectively (Schimke, 1970). Heterogeneity is also a feature of turnover among different fractions of brain (Austin *et al.*, 1972) and skeletal muscle (Millward, 1970a).

The concentration of a protein in a tissue depends on the balance between its synthesis and degradation, and there is evidence that the control of protein concentrations in the tissues is indeed exerted through both processes and often independently (Schimke & Doyle, 1970). This is especially true for secreted proteins such as albumin, where the site of breakdown is remote from the site of synthesis.

Measurements of whole-body protein turnover in the rat (Waterlow & Stephen, 1967) and in the infant (Picou & Taylor-Roberts, 1969) have shown that under a wide range of nutritional states and dietary intakes the rate does not markedly change, especially in the latter case. We have shown, however, that the pattern of protein turnover in different tissues is susceptible to dietary variation (Millward, 1970b). The rates of synthesis and breakdown of liver and muscle protein were measured in starved rats or rats fed on a protein-low diet. When the total synthesis and catabolism in the two tissues were calculated, it was shown that the low-protein diet caused a shift in synthesis towards liver and an opposite shift in catabolism, whereas starvation altered synthesis and catabolism proportionately in each tissue.

The pattern of total protein synthesis between the tissues varies with age. In muscle tissue the replacement rate is low and therefore, in the young animal during growth, half of the total synthesis represents net growth (the rest being turnover or replacement) (Millward, 1970b). In the adult, growth is much diminished and so total muscle protein synthesis may be halved (Waterlow & Stephen, 1968). By contrast, in rapidly-turning-over tissues such as liver, the growth component or rate of net synthesis is relatively small compared with the rate of turnover or replacement, so that when growth ceases there is little change in the total synthesis rate. The result is that the muscles' share of total body protein synthesis is lower in the adult than in the young animal.