^{15}N end-product methods for the study of whole body protein turnover

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This workshop is concerned with the use of stable isotopes in clinical nutrition, and my job is to discuss methods in which the tracer is given as an amino acid labelled with ^{15}N and turnover rates are determined from measurements of ^{15}N in urinary urea or ammonia. I will deal with the subject under three headings: practical advantages, theoretical problems and results.

Practical advantages of end-product methods

The main advantage is that, since measurements are made on urine, the methods are not invasive. However, if we are using urea as end-product and want to make the measurements over a fairly short period of time, because of the slow turnover rate of the urea pool, it is necessary to take at least one blood sample to determine the isotope abundance in the urea which has not yet been excreted (see Fern, Garlick, McNurlan & Powell-Tuck, 1981).

With urea or ammonia as end-product there is no problem about having enough N for measurement, nor about measuring the ^{15}N abundance with sufficient accuracy. With the extremely primitive single-collector instrument which we use, the SD is of the order of 0·2–1%, depending on the degree of enrichment. This is adequate for most purposes. The main advantage of a more accurate instrument is that the dose of tracer can be reduced by an order of magnitude. This will save money. More important, it means that the amount of labelled amino acid given will be more truly a tracer dose. In our work we use doses of 100–200 mg [^{15}N]glycine. If an oral dose is assumed to be absorbed over a period of 10 min, during this time it will double or treble the glycine flux. However, the duration of this perturbation is short compared with the total time of the test (usually 9 h).

With ammonia as end-product it is possible to repeat measurements after 2 d or so, because the residual labelling is very low (Waterlow, Golden *et al.* 1978). This is obviously a great advantage in clinical studies.

Theoretical problems of end-product methods

The basic assumption which underlies the calculation of synthesis rate or flux is that the ratio tracer:total amino-N going into the end product is the same as the ratio tracer:total amino-N going into protein. This assumption is certainly not correct. In the first place, the mixture of amino acids going into protein has a completely different composition from that of the tissue free amino acid pool (Jackson & Golden, 1980; Waterlow, Garlick *et al.* 1978; Waterlow & Fern, 1981). For example, the free pool has a great excess of lysine and threonine, as well as of glutamine and most non-essential amino acids. Secondly, the selection of amino

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acids which form the precursors of urea is almost certainly not the same as the selection going into protein. Jackson & Golden (1981) have suggested that the amino acids can be divided into two groups, transaminators and deaminators. Each group provides one of the two N atoms of urea and any imbalance in the stoichiometry is made up by the production of ammonia from glutamate by glutamic dehydrogenase.

Thirdly, the work of Pitts *et al.* (1965) and more recently that of Jackson, Golden *et al.* (1981) suggests that about 70% of urinary ammonia is derived from the amide-N of glutamine. Glutamine is synthesized mainly in muscle and we do not know exactly what are the precursors of its amide-N. About 5% of urinary NH_3 is probably derived directly from glycine (Pitts & Pilkington, 1966). My point, therefore, is that the three end products, protein (if I may include it in this category), urea and NH_3 are probably synthesized from different mixtures of amino acids at different sites. The labelling of urea must reflect that of its precursor amino acids in the liver pool; the labelling of urinary NH_3 will largely reflect, at one remove, that of the precursors of glutamine in muscle. Neither of these precursor pools, or, to be more accurate, precursor mixtures, is likely to be the same as that for protein synthesis in the body as a whole.

Fern, Garlick, McNurlan & Waterlow (1981) have shown that estimates of flux calculated from urea and NH_3 tend to vary inversely, i.e. if one is high the other is low. Simplistically, we might say that this reflects differences in labelling in liver and muscle pools. We have therefore proposed that the average of the two estimates, reflecting both pools, might be the most useful measure of flux (see Fern, Garlick, McNurlan & Powell-Tuck, 1981).

On average, flux rates determined from urea tend to be higher than those from NH_3. However, in our studies on children in Jamaica the results with the two end-products changed in the same direction with change in nutritional state (Waterlow, Golden *et al.* 1978).

Most, if not all, of these sources of error would be eliminated if all amino acids became equally labelled with ^{15}N. With [^{15}N]glycine as tracer uniformity of labelling certainly does not occur (Aqvist, 1951). One may ask, why this obsession with glycine? The first answer is quite simple—that glycine is by far the cheapest [^{15}N]amino acid and until recently was the only one easily available. Secondly, Taruvinga *et al.* (1979) have compared [^{15}N]glycine, [^{15}N]leucine and [^{15}N]aspartate in measurements of protein turnover in the rat, with urea as end-product. As might be expected, with [^{15}N]aspartate as tracer, urea became rather highly labelled, so that the estimate of turnover rate was low. With [^{15}N]leucine and [^{15}N]valine the result was the opposite—low labelling and a high turnover rate. The results with [^{15}N]glycine came in between and agreed quite well with estimates of total protein turnover in the rat that we have obtained with ^{14}C-labelled amino acids. Our findings in man, both in groups and in individuals, also seem to show reasonable agreement between ^{15}N and ^{14}C methods (Golden & Waterlow, 1977; Garlick, Clugston & Waterlow, 1980).

It seems likely that with glycine, different sources of error tend to cancel out,

and we conclude that empirically the [^{15}N]glycine-NH_3/urea end-product method is capable of giving useful results, certainly for comparative purposes.

However, even for comparisons the errors inherent in these end-product methods will become serious if the sources of error are not constant. I will give two examples of ways in which the system may be disturbed.

First, with NH_3 as end-product, acidosis, which is common in many clinical situations, would cause a serious error if it altered the pattern of precursors of urinary NH_3 so that either relatively more or relatively less labelled glycine entered the end product. We have made some preliminary tests of this in human subjects. In five volunteers made acutely acidotic with calcium chloride there was on average a small and non-significant fall in estimated flux compared with results in the same subjects in the non-acidotic state. In one subject made chronically acidotic for a week there was a 25% increase in the estimated flux. The important point is that the changes in labelling of the end-product were quite small even when the NH_3 output was more than doubled.

Secondly, an example is provided by a study of Jackson and co-workers on premature infants (Jackson, Shaw *et al.* 1981). When [^{15}N]glycine was infused, there was virtually no labelling of urinary urea. The explanation proposed is that because these infants are rapidly synthesizing glycine-rich connective tissue, there is no glycine available for the synthesis of urea. This would be another example of an alteration in the pattern of the end-product's precursors, making it even more different from the pattern of precursors of protein synthesis. This subject has been discussed in detail by Golden & Jackson (1981).

CO_2 as an end-product

A discussion of end-products would be incomplete without some mention of CO_2. Golden and I (Golden & Waterlow, 1977) showed that when [1-^{14}C]leucine is infused, flux can be calculated from $^{14}CO_2$ output, provided (a) that the subject is in N balance and (b) that the intake of leucine is known and constant. This method is very attractive because it is totally non-invasive. However, there is a problem. It relies on the assumption that leucine oxidized:leucine flux = total amino-N oxidized:total amino-N flux. We now know that the fraction of flux oxidized is extremely sensitive to the food intake (Garlick, Clugston, Swick *et al.* 1980). In fact, Golden (unpublished results) has shown that when a subject is receiving a continuous intragastric infusion of food + tracer amino acid, if the food is removed but infusion of tracer continued, the labelling of respiratory CO_2 changes within minutes. On the other hand, the excretion of total amino-N oxidized will only change sluggishly with changes in uptake, because of the long turnover time of the urea pool. Therefore in practice the equation will only be valid if there is an absolutely constant food intake.

Examples of results obtained by the end-product method

Although the sources of error are serious, I believe nevertheless that the end-product method is of much practical value, particularly for clinical studies. I will end with three examples:

(1) In obese patients on a low energy intake it was shown by the end-product method that reducing the protein intake from 50 g/d to zero caused a fall of nearly 50% in the rate of protein synthesis (Garlick, Clugston & Waterlow, 1980). Not only the direction but the magnitude of this effect was confirmed by measurements with [1-^{14}C]leucine.

(2) Studies on malnourished children in Jamaica showed a depressed rate of protein synthesis in the malnourished state and a very great increase in synthesis rate during the rapid phase of recovery (Golden, Waterlow *et al.* 1977). These results in children could not be checked with radioactive amino acids, but they fit in with everything else that we know about the metabolism of these children.

(3) Studies of Tomkins in Nigeria (unpublished results) have shown that in severely infected children there are enormously high rates of whole body protein synthesis and breakdown. I believe these results to be true because they are accompanied by great increases in the excretion of creatinine and 3-methylhistidine. Moreover, in uninfected malnourished children studied at the same time the rates were quite comparable to those found in the malnourished Jamaican children.

I believe, therefore, that in spite of all the problems, the method is giving us some useful leads about changes in disease.

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