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Contribution of Rat Liver and Gastrointestinal Tract to Whole-Body Protein Synthesis in the Rat

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The rate of protein synthesis was assessed in liver, stomach, small and large intestine and in the whole body of rats by injection of 100 μmol of [^{14}C]leucine/100 g body wt. In each of the tissues turnover was very rapid, so that taken together they accounted for 43% of the protein synthesized by the whole animal.

Recent measurements have shown that the rate of protein synthesis in rat liver and small intestine is very rapid. Indeed, each day liver synthesizes an amount equal to its own protein mass, and jejunal mucosa synthesizes an amount equal to 1.4 times its protein mass (McNurlan *et al.*, 1979). These very rapid rates of turnover suggested that these two tissues might constitute a significant component of protein synthesis in the whole body in spite of their relatively small contribution to whole-body protein mass.

Waterlow *et al.* (1978) have attempted to proportion protein synthesis among the various organs of rats. Liver and 'other viscera' appeared to account for 30% of the protein synthesized by the whole animal. However, these values were only approximate, since they were derived from a number of individual experiments using different techniques. The present study was undertaken to measure the rate of whole-body protein synthesis and the contribution of the various components of the gastro-intestinal tract directly from the incorporation of label into protein after injection of a large dose of [1-^{14}C]-leucine. A large amount of amino acid was given, so that the specific radioactivity in all possible precursor pools would become nearly the same, thus minimizing error in the determination of the specific radioactivity of the free amino acid at the site of protein synthesis both in individual tissues and for the whole body (McNurlan *et al.*, 1979). Further, this method has the advantage of being suitable for rapidly turning-over tissues such as liver and intestine, which are difficult to measure by other techniques (e.g. constant infusion of labelled amino acids). Thus direct comparison of individual tissues with the whole body could be made, since both were assessed under identical conditions by using the same technique.

Methods

Rats weighing 50 g were obtained from Charles River (Margate, Kent, U.K.) and maintained on a powdered diet containing 18.5% (w/w) casein until they reached 100 g. The rats were divided into two groups. One was used to measure whole-body protein synthesis and the other was used to measure rates of synthesis in the individual tissues. All animals were injected with L-[1-^{14}C]leucine (50 mCi/mmol; The Radiochemical Centre, Amersham, Bucks., U.K.) via a lateral tail vein. The volume injected was approx. 1 ml, but varied to provide 100 μmol and 30 μCi of labelled leucine per 100 g body wt. In each group half the animals were killed 2 min after injection and the other half after 10 min. After decapitation, blood was collected for 10 s. The liver and gastrointestinal tract were removed as quickly as possible and placed in ice-cold water. The carcass was immediately frozen by crushing between a heavy metal weight and a metal plate, both cooled in acetone/solid CO_2, and then transferred to liquid N_2. For those animals destined for measurement of whole-body synthesis, the liver and flushed gastrointestinal tract were then frozen together with the carcass. The individual tissues from the other group of rats were blotted, weighed and frozen in liquid N_2 for separate determination of rates of protein synthesis. Tissues or whole carcasses were ground to a fine powder with a mortar and pestle containing liquid N_2. Portions (1–5 g) of the powder were then precipitated with 2% (w/v) $HClO_4$. Protein was measured by the method of Lowry *et al.*

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(1951) as described by Munro & Fleck (1969). The specific radioactivity of leucine from both the acid-soluble supernatant and hydrolysed protein was measured with an amino acid analyser (Locarte, London W.12, U.K.) fitted with a pump for dividing the effluent from the column.

The amount of leucine injected was large compared with the amount of free leucine normally present in the tissues, resulting in a very rapid rise in the specific radioactivity of acid-soluble leucine, which then fell slowly and linearly with time (McNurlan *et al.*, 1979). Therefore the values at 2 and 10 min were sufficient to define the mean specific radioactivity of free leucine ($\bar{S}_A$) over the time interval 0–10 min (t). The calculation of the rate of synthesis (k_S) from the average specific radioactivity of free leucine and the specific radioactivity of the protein at 10 min (S_B) was the same for the whole body as for the individual tissues. The formula

$$k_S = \frac{S_B}{\bar{S}_A t} \times 100$$

has been described previously (McNurlan *et al.*, 1979).

Results and Discussion

The rates of synthesis of protein obtained in the whole animal, liver, stomach, small and large intestine are shown in Table 1. The fractional rate of synthesis, k_S, is the proportion (%) of the protein pool that is synthesized per day. In the whole body the fractional synthesis rate, 34%/day, was considerably lower than in any of the individual tissues that were measured. The difference represents the contribution of much more slowly turning-over tissues. For example, skeletal muscle contributes about 40% of whole-body protein, with a synthesis rate of 14%/day in rats of this size (Waterlow *et al.*, 1978). The rate for the whole body can also be expressed in g of protein/day by multiplying the fractional rate of synthesis by the protein content of the rat. Calculated in this way, the synthesis rate for the whole body was 3.1 g/day. This estimate compares well with estimates made by other methods. Previous measurements of whole-body synthesis have been made by constant infusion of labelled amino acids for periods up to 6 h. With this method the rate of synthesis is derived from the total turnover rate (flux) of the body free amino acid pool, which is assumed to be homogeneous (Waterlow *et al.*, 1978). However, the flux is an over-estimate of the rate of protein synthesis, since it includes amino acid which is leaving the free amino acid pool for oxidation and other pathways, as well as for protein synthesis. In addition, it is generally recognized that the specific radioactivity of the free amino acid at the site of protein synthesis is different from that in the plasma, probably lying between the value for plasma and that for the tissue (Mortimore *et al.*, 1972). Since the specific radioactivity in tissues during infusion is always lower than in the plasma, use of the plasma value for calculation would tend to underestimate the rate of synthesis. Waterlow *et al.* (1978) concluded that these two theoretical objections would have a cancelling effect and reported a value of about 4 g of protein synthesized/day by using a constant infusion of [^{14}C]tyrosine.

More accurate values for the rate of whole-body synthesis have been obtained by modification of the constant-infusion approach. Albertse *et al.* (1979) measured the rate of oxidation from the rate of $^{14}CO_2$ production during infusion of [1-^{14}C]leucine and subtracted it from the flux. Assuming rat protein to contain 6.9% (w/w) leucine (M. A. McNurlan, unpublished work), the synthesis rate obtained by Albertse *et al.* (1979), of 1.46 μmol of leucine/min in 100 g rats, can be converted into 4 g of protein/day per 100 g body wt. By contrast,

Table 1. *Protein content and the rate of protein synthesis for individual tissues and the whole body of the rat*

Ten rats were given intravenous injections of 100 μmol of [^{14}C]leucine/100 g body wt. and killed either 2 or 10 min afterwards. The specific radioactivity of free and protein-bound leucine in the total carcass was measured and the fractional rate of whole-body protein synthesis calculated. Another ten were treated similarly, except that liver, stomach, small and large intestine were removed for measurement of the specific radioactivity of free and protein-bound leucine and subsequent determination of fractional rates of protein synthesis in each tissue. Total rates of protein synthesis in mg of protein/day were calculated by multiplying the fractional rate of synthesis by the corresponding protein content. Results are means ± S.D.

	Protein content (g)	Protein synthesis (%/day)	Protein synthesis (mg of protein/day)
Whole body	9.30 ± 0.17	33.6 ± 2.9	3130 ± 410
Liver	0.70 ± 0.11	105.4 ± 2.5	743 ± 120
Stomach	0.05 ± 0.01	73.9 ± 1.2	34 ± 6
Small intestine	0.46 ± 0.05	103.4 ± 6.4	477 ± 58
Large intestine	0.12 ± 0.02	62.1 ± 10.3	78 ± 22

Millward & Lo (1977) infused rats with [^{14}C]tyrosine, but, instead of deriving whole-body synthesis from measurements on the amino acid in the plasma, they determined the incorporation of label into protein and the specific radioactivity of free tyrosine by homogenizing the whole body. In rats weighing approx. 250g the fractional synthesis rate was 27%/day, slightly less than the value of 34%/day that we observed in 100g rats. The advantage over this latter technique of measuring incorporation into protein after a large dose of [^{14}C]leucine is that the difference between the specific radioactivity of the free amino acid in plasma and tissue is much smaller with the large dose, thus minimizing any error arising from failure to define correctly the specific radioactivity at the site of protein synthesis.

In liver, the fractional rate of synthesis calculated from incorporation of [^{14}C]leucine was 105%/day, considerably faster than rates obtained by constant infusion of tyrosine for 6h (60%/day; Garlick *et al.*, 1975). This is because with the large-dose method the measurement was made over 10min, with the result that synthesis of secreted proteins and proteins that turn over very rapidly was also included.

In the gut the large-dose method is particularly suitable, since it overcomes a number of problems that are unique to this tissue. The specific radioactivity in plasma and cellular pools becomes more nearly equal than with trace-labelling techniques, thus minimizing problems of defining the actual precursor. In addition, the short duration of the measurement decreases the problems that arise from re-entry of label from the breakdown of labelled protein or from the loss of label through the turnover of whole cells (McNurlan *et al.*, 1979). Although all the organs of the gastrointestinal tract had high fractional rates of protein turnover, there was considerable variation among them. The synthesis rate in small intestine was 103%/day. In a separate group of rats we have shown that the rate of protein synthesis in jejunal mucosa was 140%/day, compared with 66%/day in serosa. The higher rate in the mucosa almost certainly reflects the turnover of whole cells in addition to the intracellular turnover of protein (McNurlan *et al.*, 1979). Altman (1974) has reported a gradient in the rate at which cells turn over along the length of the intestine, so, not surprisingly, the rate of protein synthesis was much slower in the large intestine than in the small intestine. The rate of 62%/day was similar to that of the serosa of the small intestine, as was the rate of synthesis in the stomach (76%/day). Although the tissues measured here comprise only 14% of whole-body protein, the total amount of protein synthesized per day in liver, stomach, small and large intestine was equal to 43% of the total for the whole body.

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