The Specific Radioactivity of the Precursor Pool for Estimates of the Rate of Protein Synthesis

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Infusion of rats with [U-¹⁴C]glycine resulted in labelling of glycine and serine in tissue proteins. The pattern of labelling in protein more nearly resembled that of the free amino acids in the tissue than in the plasma.

It has long been recognized that proteins are synthesized *de novo* from a precursor pool of free amino acids, but the exact location of this pool is in doubt. This question is of particular importance when estimates of the rate of protein synthesis *in vivo* are made from measurements of the incorporation of an isotopically labelled amino acid, and an accurate value for the specific radioactivity of the precursor amino acid is essential. Conventionally the specific radioactivity of the intracellular amino acid has been used for this purpose.

Over the last few years a number of experiments have been performed on isolated tissues incubated with labelled amino acids *in vitro*. These have demonstrated that the isotopic label in the precursor pool equilibrates more rapidly with extracellular amino acids than does the total intracellular pool of free amino acids (Kipnis *et al.*, 1961; Rosenberg *et al.*, 1963; Guidotti *et al.*, 1964; Hider *et al.*, 1971; Adamson *et al.*, 1972). Hence it has been inferred that amino acids from the extracellular pool may be channelled through the membrane directly into protein synthesis without prior equilibration with the intracellular pool. Results obtained by perfusion of whole organs, however, give a rather different picture. In heart perfused with labelled amino acids, a lag in the incorporation of label into protein, equivalent to the time taken for the intracellular amino acid to equilibrate, was demonstrated (Morgan *et al.*, 1971). In perfused liver it has been suggested that the intracellular valine pool is compartmentalized, one of these compartments being on the direct pathway to protein synthesis (Mortimore *et al.*, 1972).

From our own studies, and those of others, on the rate of protein synthesis in tissues measured by the constant-infusion technique, the specific radioactivities of free amino acids in the tissue may be as much as 50% lower than those in the plasma (Waterlow & Stephen, 1968; Garlick, 1969). Hence it is essential that we know whether the specific radioactivities of intra- or extra-cellular amino acids best represent those at the site of protein synthesis. In view of the difficulty of measuring the specific radioactivities of

the amino acids attached to tRNA, the pool of which is small and turning over very rapidly, we have adopted a more indirect approach. During a constant infusion of [U-¹⁴C]glycine the specific radioactivity of the infused amino acid in the plasma rises to a constant value. Similarly in the tissues the specific radioactivity of free glycine also reaches a plateau value, but one that is lower than that in plasma. In tissues, especially the liver, where glycine is converted into serine, the specific radioactivity of free serine is much greater than that in plasma. The ratio of the specific radioactivities of free serine and free glycine is therefore different in plasma and in tissue. Thus a comparison of the specific radioactivities of these amino acids in the tissue proteins with those in the free amino acid pools of tissue and plasma will indicate the validity of using tissue rather than plasma values for 'precursor' specific radioactivity when calculating rates of protein synthesis.

Methods

Female hooded rats (65g) were purchased from Animal Suppliers (London) Ltd. (London N.12, U.K.) about 10 days before infusion. They were maintained on a powdered diet with a net dietary protein energy value of 10%, and fed *ad libitum* until the day of infusion, when their mean weight was 97g. A solution of [U-¹⁴C]glycine (The Radiochemical Centre, Amersham, Bucks., U.K.) (24 μ Ci/ml; 13.1 mM) in 0.9% NaCl was infused via the tail vein by a modification of the technique described by Garlick & Marshall (1971). Infusions were carried out for 2, 4 and 6 h at a rate of 0.478 ml/h. At the end of the infusion the animals were decapitated and tissue and blood samples were rapidly collected. Tissue proteins were separated from free amino acids by homogenization with 10 vol. of 3% (w/v) sulphosalicylic acid. Non-collagen protein was isolated from the insoluble fraction by a technique described by Hider *et al.* (1971). Samples (3 mg) of the protein were hydrolysed for 24 h at 105°C in sealed glass tubes containing 1.5 ml of constant-boiling HCl, under an

atmosphere of N_2 . After hydrolysis the samples were evaporated to dryness. Free amino acids were separated from blood plasma after precipitation of protein with an equal volume of 3% sulphosalicylic acid.

The glycine and serine contents in sulphosalicylic acid supernatants and in the protein hydrolysates were measured on a Locarte automated amino acid analyser fitted with a column-effluent stream splitter. Separation was achieved with a $0.9\text{cm} \times 50\text{cm}$ glass

column and a single lithium citrate buffer, pH 2.8 (Kedenburg, 1971). Total running time for one analysis was 340 min at a column temperature of 36°C and a flow rate of 30 ml/h. Quantitative determination of glycine and serine was based on two internal standards (α -amino adipic acid and α -amino-*n*-butyric acid). Radioactivity in fractions from the column effluent was measured in a Packard 2420 scintillation counter. The scintillation fluid contained 0.4% 2,5-

Fig. 1. Ratio of the specific radioactivities of serine and glycine in plasma, liver, heart, diaphragm and gastrocnemius as a function of time

Each point is the mean ($\pm$ S.E.M.) derived from constant infusion of four rats. ■, Ratio of specific radioactivities of free serine and free glycine in plasma; ●, ratio of specific radioactivities of free serine and free glycine in the tissue intracellular pool; ○, ratio of specific radioactivities of protein-bound serine and protein-bound glycine in the tissue.

diphenyloxazole (PPO) in a toluene–Triton X-100 base (2:1, v/v), and the efficiency of counting ^{14}C radioactivity was approx. 80%.

Results and discussion

During the infusion the specific radioactivity of free glycine in the plasma at 2h was very close to that at 6h, showing that a plateau value had been reached and maintained. Similar results have been obtained with a number of different amino acids (Waterlow & Stephen, 1968; Garlick & Marshall, 1971; Seta *et al.*, 1973). In liver and heart the specific radioactivity of free glycine rose little between 2 and 6h, but in diaphragm and gastrocnemius muscle a continued increase was observed. This is probably the result of the slow turnover of protein and the large intracellular free glycine pool in these muscles. In all tissues the specific radioactivity of free glycine remained lower than in the plasma, owing to breakdown of unlabelled protein within the cells (Waterlow & Stephen, 1968). In the liver, where the major part of conversion of glycine into serine appeared to occur, the specific radioactivity of free serine became equal to that of free glycine at 2h. At 6h it was slightly higher, possibly because the additional carbon atom of serine had become labelled (Henriques *et al.*, 1955). The specific radioactivity of free serine in liver remained much higher than that in the plasma and hence the ratio of the specific radioactivities of free serine and free glycine in this tissue was nearly four times as high (Fig. 1). In the other tissues, where the serine became labelled to approximately the same extent as in the plasma, the ratio of the specific radioactivities of free serine and free glycine was approximately twice that in plasma.

A comparison of the ratio of the specific radioactivities of serine and glycine in non-collagen protein with that of the free amino acids in the plasma and tissues is shown in Fig. 1. The ratio in protein was

always higher than that of plasma, showing that, even if extracellular amino acids are preferentially utilized in protein synthesis, their specific radioactivities are not reflected by those in plasma. On the contrary, in all tissues the ratio in protein was nearer to that in the tissue free amino acid pool. The ratio in liver protein was slightly, but significantly ($P < 0.001$, Student's *t* test for paired differences), lower than that of the tissue free amino acids, but we do not know how the ratio in liver protein compares with that of the free amino acids in portal or hepatic venous plasma. Further, no correction was made to the specific radioactivities of total tissue free serine and free glycine to allow for the radioactivities in the extracellular fluid. The effect of this would be very small, however, since the measured concentrations of these two amino acids in the tissue were much greater than in the plasma. In gastrocnemius and diaphragm there was no significant difference between the ratio of the specific radioactivities of free serine and free glycine in the tissue and that of the amino acids in protein, whereas in heart the ratio in protein was significantly higher than that of the free amino acids in either plasma or tissue ($P < 0.001$, Student's *t* test for paired differences).

In Table 1 the rates of protein synthesis have been estimated from the values for the incorporation of both glycine and serine, and by taking the specific radioactivity of each precursor as equal to that of the free amino acid in either tissue or plasma. In general, when the specific radioactivities in plasma are taken widely differing values are obtained for the rate of synthesis with the two amino acids, whereas with specific radioactivities in tissue very similar rates are obtained. It is noteworthy that calculations with serine in heart, diaphragm and gastrocnemius show very little difference in the estimated rate of synthesis whichever pool is taken as the precursor. The rates of protein synthesis calculated from the specific radioactivity of tissue free serine or free glycine are in good

Table 1. *Estimated rates of protein synthesis from four possible precursor pools*

The specific radioactivities of free and protein-bound glycine and serine from tissues were measured after constant infusion of the rats with $[\text{U-}^{14}\text{C}]\text{glycine}$ for 2 and 6h. The rates for protein synthesis were calculated from the increase in specific radioactivity of each amino acid in the protein over the 4h period (Seta *et al.*, 1973). The specific radioactivity of the precursor was taken to be that of the average value of the free amino acid either in the tissue or in plasma. Each result ($\pm$ S.E.M.) is calculated from the specific radioactivities obtained from four infusions at each time-interval.

Precursor	Estimated rate of protein synthesis (%/day)			
	Liver	Heart	Diaphragm	Gastrocnemius
Tissue glycine	35.9 ± 2.1	15.3 ± 1.2	9.6 ± 0.7	9.2 ± 0.6
Tissue serine	33.1 ± 2.0	23.0 ± 1.2	12.6 ± 1.4	10.5 ± 0.6
Plasma glycine	18.0 ± 1.0	9.6 ± 0.8	6.7 ± 0.5	4.2 ± 0.2
Plasma serine	60.6 ± 3.3	22.3 ± 1.2	13.3 ± 1.4	8.9 ± 0.5

agreement with those obtained previously by infusion of [^{14}C]glycine (Garlick, 1969, 1972).

Although these results do not unequivocally identify the site of the precursor pool, they clearly demonstrate certain points of importance. First, the precursor specific radioactivities for the two amino acids studied here are certainly not the same as in plasma. Secondly, during a constant infusion of [^{14}C]glycine the specific radioactivities of free serine and free glycine in the precursor pool more nearly resemble those in the tissue. Hence, in spite of the results of experiments carried out *in vitro* that indicate that amino acids for protein synthesis may be derived from an extracellular site, the present work suggests that more accurate values are obtained if the specific radioactivity of the tissue rather than the plasma free amino acid is used to calculate rates of protein synthesis *in vivo*.

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