Metabolic Studies with L-[1-^{14}C]Tyrosine for the Investigation of a Kinetic Model to Measure Protein Synthesis Rates with PET

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To evaluate a kinetic model for measuring protein synthesis rates by positron emission tomography (PET) in neoplastic and normal tissue, metabolic studies with L-[1-^{14}C]tyrosine were carried out. As an animal model, rats bearing Walker 256 carcinosarcoma were used. Within 60 min after injection, several metabolic parameters were measured. The highest radioactivity uptake, expressed as the differential absorption ratio, was found in pancreas, followed by liver, tumor, and brain. A rapid decarboxylation was observed during the first 15 min. After 60 min, 7.4% of the total injected ^{14}C was expired as $^{14}CO_2$. In plasma a significant amount of [^{14}C]bicarbonate was detected, but in tissue the amount was negligible. Protein incorporation increased with time. The incorporation rate was the highest in the liver followed by pancreas, tumor, and brain tissues. At 60 min after injection, more than ~80% of the ^{14}C in tissue was protein bound. In plasma after a rapid clearance during the first 15 min, the total ^{14}C level increased rapidly and paralleled the increase of protein-bound ^{14}C. As nonprotein [^{14}C]metabolites, in plasma, tumor and brain tissues, p-hydroxyphenylpyruvic acid, p-hydroxyphenyllactic acid, and unidentified metabolites were observed by high performance liquid chromatography. The formation of ^{14}C-labeled 3,4-dihydroxyphenylalanine was found to be negligible. The total amount of these nonprotein metabolites increased with time. At 60 min after injection the percentages of the total nonprotein metabolites and [^{14}C]bicarbonate were only 5.0%, 1.9%, and 3.7% in plasma, tumor and brain tissue, respectively. From our data it is concluded that [^{11}C]carboxylic-labeled tyrosine would be a suitable radiopharmaceutical for measuring protein synthesis rates in neoplastic and normal tissue by PET.

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Compared with surrounding normal tissue, many tumors have increased protein synthesis and consequently an increased uptake of amino acids. The increased uptake can be measured quantitatively with carbon-11- (^{11}C) labeled amino acids in combination with positron emission tomography (PET), provided appropriate kinetic models are developed. Alteration of protein synthesis rates during treatment can possibly be used to evaluate chemo- and radiotherapy protocols. Smith et al. (*1*) developed a model for estimating local rates of protein synthesis in the brain in vivo. The model includes the reversible transport of amino acid from blood to tissue, the incorporation into protein, the breakdown of protein, and the nonprotein metabolism of the amino acid. Phelps et al. (*2*) reported on criteria to adapt this model for determining local cerebral protein synthesis rates by PET. This group started initial experiments using L-[1-^{11}C]leucine as radiopharmaceutical. L-[^{11}C]methionine-labeled in the S-methyl position, was used to measure cerebral protein synthesis rates in patients with Alzheimer dementia (*3*) and with brain tumors (*4*). Although the main metabolic pathway of this amino acid is protein incorporation, minor metabolic pathways such as transamination, decarboxylation, oxidative desamination and side chain conversion are present. Several investigators have reported on these pathways (*5–8*).

Hardly any information is available on the distribution of radioactivity over the different biochemical compartments in tissues. The availability of this information in a time scale appropriate for the application of short-lived ^{11}C-labeled amino acids is essential to evaluate kinetic models which are necessary to translate PET

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information into protein synthesis rate data. We are investigating Smith's model and wish to use L-[1-^{11}C] tyrosine because its biochemical properties make this amino acid very promising for the in vivo measurement of protein synthesis rates in human neoplasms and other tissues. L-Tyrosine is an essential amino acid with a small free pool in tissue and plasma and with a high turnover rate. In a previous study (*9*), the tumor accumulation of this amino acid in experimental tumor-bearing rats and in patients has been reported. In this communication for the investigation of a kinetic model to measure protein synthesis rates with PET we report on the metabolism of L-[1-^{14}C]tyrosine within 60 min after injection, a time scale appropriate for PET studies with ^{11}C.

MATERIALS AND METHODS

Male Wistar rats with a transplantable Walker 256 carcinosarcoma were prepared by intramuscular injection of 10^6 tumor cells in the left hind leg, as described previously (*9*). Within 14 days after transplantation tumors were palpable.

The rats weighing between 200 and 250 g were anesthetized with pentobarbital and a polyethylene tube was cannulated into a femoral vein. For metabolic studies a rat was kept in an acrylic cylinder (9.4 cm i.d. × 28 cm length) with a stopper at each end. One stopper had a hole through which the cannulated polyethylene tube was passed. Through the other stopper a tube was passed and connected with an aspirator via two vials each containing 5 ml of NCS tissue solubilizer (The Radiochemical Centre, Amersham International plc, Buckinghamshire, UK) to absorb $^{14}CO_2$. After adjusting the air flow in the cylinder, a rat was injected through the cannula with L-[1-^{14}C]tyrosine (1 μCi/100 g body weight) with a specific activity of 56 mCi/mmol (The Radiochemical Centre, Amersham International plc, Buckinghamshire, UK). The expired $^{14}CO_2$ was trapped quantitatively in the two NCS-containing vials. The rats were killed by cervical dislocation at 5, 10, 15, 30, or 60 min after injection. Blood was removed by heart-puncturing using a syringe, precoated with heparin. To collect plasma, the blood was centrifuged at 2,000 rpm for 5 min. Tumor, brain, liver, and pancreas tissues were dissected and washed with saline. For high performance liquid chromatography (HPLC) studies, carried out afterwards, the tissue samples were stored at −20°C.

For the measurement of [^{14}C]bicarbonate in plasma, 50 μl of sample were transfered to a glass vial and sealed with a rubber septum. To this vial, through the rubber septum, 0.5 ml 5% trichloroacetic acid was added. The $^{14}CO_2$ generated was transfered by a flow of air to two glass vials, each containing 3 ml of NCS to absorb $^{14}CO_2$. After removal of $^{14}CO_2$, the plasma sample was cooled with ice water for 10 min and subsequently centrifuged at 2,500 rpm for 5 min. The precipitate was washed with 0.5 ml of 5% trichloroacetic acid and centrifuged as described above. After two more washing steps of the precipitate, all supernatant fractions were combined. The acid-precipitable fraction was dissolved in NCS. Percentages of ^{14}C in the bicarbonate pool, acid-precipitable fraction and acid-soluble fraction were calculated assuming the total ^{14}C recovered from the plasma to be 100%.

To measure the [^{14}C]bicarbonate, the protein-bound fraction and the protein-free fraction in tumor and brain tissue, 20 to 200 mg samples were homogenized in 0.5 ml saline. The homogenation was carried out with a Teflon-glass homogenizer at 0°C. By the same procedure as described for the plasma samples the ^{14}C in the [^{14}C]bicarbonate, acid-precipitable and acid-soluble fractions were measured.

To measure the ^{14}C-labeled metabolites in the protein-free fraction, 100 to 620 mg tumor tissue samples were homogenized in 1 ml of 7.5% trichloroacetic acid by an ice-cooled Teflon-glass homogenizer. The brain tissue samples were also homogenized in 7.5% trichloroacetic acid. The acid volume used, was two times the equivalent volume of the tissue. The homogenate was centrifuged at 2,500 rpm for 5 min. The supernatant was collected through a membrane filter (0.22 μm) by syringe. Amounts between 1.0 to 2.0 ml of this supernatant were applied on two different HPLC systems. All samples investigated were applied on an Aminex A-7 column (15 × 0.48 cm) (Bio-Rad), which was used at 70°C with 0.2 N sodium citrate (pH 3.25) at a flow rate of 1.0 ml/min. In the second analysis a μBondapak C18 column (Waters, Radial Compression Separation System) was used at room temperature with 0.1*M* NaH_2PO_4, (pH 4.6) at a flow rate of 1.0 ml/min.

RESULTS

In Table 1, ^{14}C uptake and percentages of protein-bound ^{14}C in tumor, brain, liver, pancreas, and plasma are presented. The tissue uptake is expressed as the differential absorption ratio (DAR), (counts/g tissue) × (g body weight/total injected counts). The pancreas showed the highest uptake followed by liver, tumor, and brain. The percentage of protein-bound ^{14}C in these four tissues increased with time. The highest protein incorporation rate was observed in liver tissue. At 5 min after injection, 89% of the ^{14}C in this tissue was already measured in the protein-bound pool. Although after 15 min the total ^{14}C level in the tissue decreased, the percentage of protein-bound ^{14}C increased to 95%. Compared to three tissues, the total ^{14}C level in brain tissue decreased gradually. About one third of the initial ^{14}C level disappeared within 60 min. The protein incorporation rate was lower than in other tissues investigated. At 60 min after injection 79% of the ^{14}C was fixed in the protein pool. In case of plasma, after an initial fast decrease, the ^{14}C level increased very rapidly which paralleled a rapid increase of protein-bound ^{14}C.

Due to decarboxylation, much $^{14}CO_2$ was exhaled as is shown in Table 2. Within 60 min, 7.4% of the total injected ^{14}C was expired. In plasma a significant amount of [^{14}C]bicarbonate was detected (Table 2). During the first 15 min after injection, the level of [^{14}C]bicarbonate was relatively high. After 15 min the amount of [^{14}C] bicarbonate decreased. In tumor and brain samples the

TABLE 1
Tissue Distribution and Percentage of Protein-Bound ^{14}C at Different Time Intervals After i.v. Injection of L-[1-^{14}C] Tyrosine in Rats Bearing Walker 256 Carcinosarcoma (n = 5)*

	Uptake (DAR) % of Protein-bound ^{14}C†				
	5 min	10 min	15 min	30 min	60 min
Tumor	2.44 ± 0.81 41.5 ± 6.9	2.02 ± 1.04 63.5 ± 10.9	2.00 ± 0.78 68.3 ± 7.1	2.36 ± 0.55 76.9 ± 6.7	3.27 ± 0.60 87.6 ± 1.7
Brain	0.93 ± 0.20 19.5 ± 2.1	0.79 ± 0.06 34.4 ± 5.6	0.75 ± 0.12 37.9 ± 8.1	0.65 ± 0.07 64.1 ± 5.7	0.61 ± 0.07 79.4 ± 4.2
Liver	3.76 ± 0.88 88.8 ± 1.7	3.73 ± 0.30 92.4 ± 1.4	4.25 ± 0.80 93.3 ± 1.7	3.44 ± 0.38 94.7 ± 0.6	3.10 ± 0.46 94.0 ± 0.6
Pancreas	7.99 ± 1.16 48.3 ± 21.3	9.28 ± 1.85 51.5 ± 13.3	10.13 ± 2.66 68.7 ± 12.2	12.35 ± 3.26 88.2 ± 6.3	11.08 ± 1.65 91.1 ± 5.2
Plasma	1.15 ± 0.35 0.8 ± 0.8	0.55 ± 0.17 9.0 ± 9.0	0.53 ± 0.18 12.3 ± 9.2	0.79 ± 0.18 54.6 ± 0.7	1.54 ± 0.37 84.3 ± 3.8

* n = Number of rats.
† Errors are s.d.

[^{14}C]bicarbonate pool was found to be negligible: below 0.1% of the total ^{14}C in the these tissues.

For analyses of nonprotein metabolites of L-[1-^{14}C] tyrosine, two different HPLC columns were used. Four radioactive peaks were observed in plasma, tumor, and brain samples on the Aminex A7 column. The HPLC results are summarized in Table 3. Identification of peak V as tyrosine in the tissue samples was achieved by authentic L-[1-^{14}C]tyrosine. The elution volume of peak II was the same as that of authentic p-hydroxyphenyllactic acid. Authentic p-hydroxyphenylpyruvic acid was eluted in peak I, the void volume. Peak IV was not identified. For plasma, tumor and brain the sum of the radioactivity in peaks I, II, and IV increased with time. Although the summed amount in the plasma was larger than in tumor or brain tissue, at 60 min after injection 77% of ^{14}C in the protein-free fraction was confirmed to be L-[1-^{14}C]tyrosine. Compared to other tissues for pancreas samples the compound in peak IV was the most predominant metabolite. In liver, at 60 min after injection 42% of the protein-free ^{14}C was detected in peak II. In case of brain samples in the volume, in which authentic 3,4-dihydroxyphenylalanine (DOPA) was eluted, only a negligible amount of ^{14}C (below 0.1% of the total protein-free ^{14}C) could be measured. Using a μBondapak C18 column, the presence of ^{14}C-labeled p-hydroxyphenyllactic acid (in a 14-ml elution volume) and p-hydroxyphenylpyruvic acid (in a 24-ml elution volume) was confirmed, but [^{14}C]DOPA (in an 8-ml elution volume) was not detected.

TABLE 2
Expired $^{14}CO_2$ as % Total Injected Dose and [^{14}C]Bicarbonate Levels (DAR and in % Total ^{14}C in Plasma) in Plasma of Rats Bearing Walker 256 Carcinosarcoma (n = 5) at Different Time Intervals After Injection of L-[1-^{14}C]Tyrosine

Time (min)	Expired $^{14}CO_2$ (% injected dose)*	[^{14}C]Bicarbonate DAR	[^{14}C]Bicarbonate % Total ^{14}C in plasma
5	2.06 ± 1.42	0.11 ± 0.028	10.5 ± 3.7
10	3.60 ± 1.04	0.074 ± 0.028	13.5 ± 2.7
15	4.29 ± 1.48	0.088 ± 0.021	17.4 ± 3.2
30	5.71 ± 0.80	0.032 ± 0.012	4.2 ± 1.6
60	7.43 ± 0.66	0.024 ± 0.009	1.7 ± 0.8

* $^{14}CO_2$ was collected from 0 to indicated time after injection.

DISCUSSION

For quantitative in vivo measurement of physiological and biochemical processes by PET, the combination of positron-emitting radiopharmaceuticals with appropriate mathematical models is necessary. A kinetic model must reflect the metabolic behavior of the radiotracer. In Figure 1 the fate of L-[1-^{14}C]tyrosine after injection is shown.

TABLE 3
Percentages of Metabolites of L-[1-^{14}C]Tyrosine in the Protein-Free Tissue Pool of Rats Bearing Walker 256 Carcinosarcoma at Several Time Intervals after i.v. Injection

	Time (min)	Peak I	Peak II	Peak IV	Peak V (tyrosine)
Plasma	5	2.7 ± 0.9	0.5 ± 0.1	1.1 ± 0.3	95.7 ± 1.1
	10	7.9 ± 2.0	0.8 ± 0.1	1.4 ± 0.2	89.9 ± 2.1
	15	10.4 ± 5.4	1.0 ± 0.6	3.1 ± 3.2	85.5 ± 6.1
	30	11.0 ± 4.9	1.4 ± 0.6	1.7 ± 0.5	85.9 ± 5.7
	60	18.0 ± 2.9	2.0 ± 0.6	1.8 ± 1.4	77.4 ± 3.6
Tumor	5*	1.3 ± 0.3	0.3 ± 0.1	0.9 ± 0.5	97.5 ± 0.9
	10*	2.5 ± 0.2	0.8 ± 0.2	1.3 ± 0.3	95.4 ± 0.4
	15	3.4 ± 1.0	0.8 ± 0.2	1.3 ± 0.4	94.5 ± 1.4
	30	4.6 ± 1.5	2.4 ± 0.7	3.6 ± 1.1	89.4 ± 2.7
	60	9.4 ± 4.0	3.1 ± 0.6	2.6 ± 0.7	84.9 ± 3.6
Brain	5	1.0 ± 0.3	0.3 ± 0.1	1.1 ± 0.5	97.6 ± 0.9
	10	1.6 ± 0.2	0.3 ± 0.2	1.3 ± 0.2	96.8 ± 0.4
	15	1.9 ± 0.7	0.3 ± 0.1	2.5 ± 1.2	95.3 ± 1.6
	30	2.5 ± 0.3	0.9 ± 0.3	2.8 ± 0.8	93.8 ± 0.2
	60	6.9 ± 2.3	2.1 ± 0.7	8.5 ± 2.9	82.7 ± 3.5
Liver	60†	18.3	42.1	10.6	29.0
Pancreas	60†	3.2	3.7	16.3	76.8

The elution volume of each peak was the void volume for peak I, 6.5 ml for peak II, 8 ml for peak III, 10.5 ml for peak IV and 25 ml for peak V. Percentage of each peak was normalized as total recovered ^{14}C to the 100%. Data indicate an average of five samples or four samples (*), and one sample (†). The injected L-[1-^{14}C]tyrosine has a radiochemical purity of 99.3% with minor impurities in peak I (0.3%) and peak III (0.4%). Errors are s.d.

Until now no kinetic model is evaluated in detail for the in vivo PET measurement of protein synthesis rates with ^{11}C-labeled amino acids as radiotracer. In this paper we reported on the metabolism of L-[1-^{14}C]tyrosine in tumor bearing rats. The biochemical properties of L-tyrosine as well as our previous results of tissue distribution studies with L-[1-^{11}C]tyrosine (*9*) have shown, that L-[1-^{11}C]tyrosine has potential for the measurement of protein synthesis rates by PET in neoplastic and other tissue, like brain.

In case of [^{11}C]carboxylic amino acids, the ^{11}C is expected to be incorporated into proteins. This is the main metabolic pathway. A minor pathway is decarboxylation. The $^{11}CO_2$ formed through this route, is

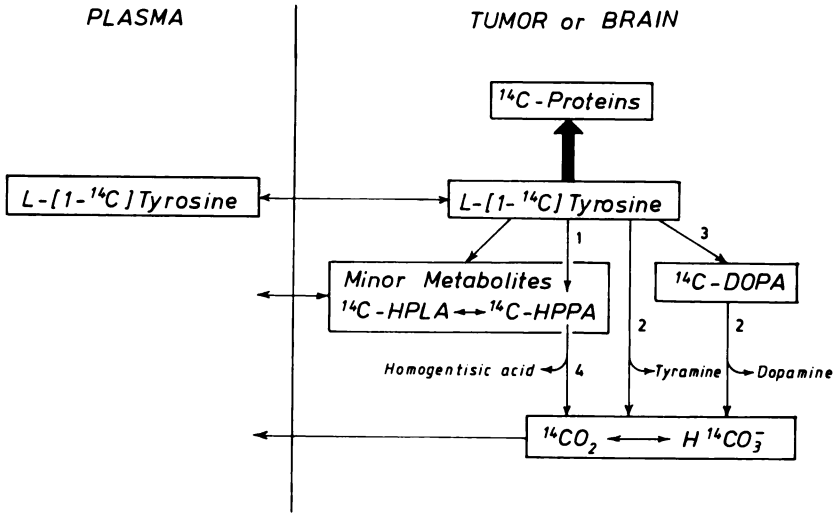

FIGURE 1
Metabolism of L-[1-^{14}C]tyrosine in rats with Walker 256 carcinosarcoma. 1:tyrosine transaminase; 2:aromatic L-amino acid decarboxylase; 3:tyrosine hydroxylase; 4:p-hydroxyphenylpyruvate oxygenase; HPPA: p-hydroxyphenylpyruvic acid; HPLA: p-hydroxyphenyllactic acid.

TABLE 4
Radioactivity in Plasma, Tumor, and Brain Tissue of Rats Bearing Walker 256 Carcinosarcoma at Several Time Intervals After i.v. Injection of L-[1-^{14}C]Tyrosine*

	Time (min)	Level of 14C (DAR)	Proteins (%)	Tyrosine (%)	Nonprotein metabolites (%)	Bicarbonate
Plasma	5	1.15 ± 0.35	0.8 ± 0.8	84.9 ± 5.0	3.8 ± 1.0	10.5 ± 3.7
	10	0.55 ± 0.17	9.0 ± 9.0	69.8 ± 9.6	7.7 ± 1.4	13.5 ± 2.7
	15	0.53 ± 0.18	12.3 ± 9.2	60.6 ± 12.0	9.7 ± 3.7	17.4 ± 3.2
	30	0.79 ± 0.18	54.6 ± 10.7	35.8 ± 11.8	5.4 ± 2.0	4.2 ± 1.6
	60	1.54 ± 0.37	84.3 ± 3.8	10.7 ± 2.0	3.3 ± 1.1	1.7 ± 0.8
Tumor	5	2.44 ± 0.81	41.5 ± 6.9	57.0 ± 6.8	1.5 ± 0.5	
	10	2.02 ± 1.04	63.5 ± 10.9	34.8 ± 10.3	1.7 ± 0.4	
	15	2.00 ± 0.78	68.3 ± 7.1	30.0 ± 7.1	1.7 ± 0.3	
	30	2.36 ± 0.55	76.9 ± 6.7	20.6 ± 6.1	2.4 ± 0.8	
	60	3.27 ± 0.60	87.6 ± 1.7	10.6 ± 1.8	1.9 ± 0.4	
Brain	5	0.93 ± 0.20	19.5 ± 2.1	78.6 ± 2.2	1.9 ± 0.7	
	10	0.79 ± 0.06	34.4 ± 5.6	63.4 ± 5.7	2.2 ± 0.3	
	15	0.75 ± 0.12	37.9 ± 8.1	59.3 ± 8.7	2.8 ± 0.8	
	30	0.65 ± 0.07	64.1 ± 5.7	33.7 ± 5.6	2.2 ± 0.3	
	60	0.61 ± 0.07	79.4 ± 4.2	16.9 ± 3.0	3.7 ± 1.3	

* Data calculated from Tables 1–3.

diluted in the [CO_2]bicarbonate pool and removed from the tissue rapidly. To obtain more information on the fate of ^{14}C administered, the radioactivity in tissue was separated in four fractions: protein-bound ^{14}C, [^{14}C] tyrosine, non-protein [^{14}C]metabolites and [^{14}C]bicarbonate. The results calculated from the experimental data in Tables 1–3 are summarized in Table 4. Pronounced differences in incorporation rates of ^{14}C into proteins were found for tumor, brain, liver, and pancreas tissue. These differences may not only depend on the protein synthesis rates, but also on the size of the free tyrosine pools in the tissues. An important conclusion from our measurement is that within 60 min, a time scale appropriate for PET studies with ^{11}C, a very high fraction of labeled tyrosine is already incorporated in protein: for all tissues more than ~80%. For plasma, after 15 min, the protein-bound ^{14}C increased rapidly. This increase is explained by the formation of ^{14}C-labeled proteins in the liver and the excretion of these proteins into plasma. Also the liver showed a high uptake of tyrosine. At 5 min after injection already 89% of the total ^{14}C in liver tissue was protein-bound. The increase of protein-bound ^{14}C in plasma was coincident with the slight decrease of the total ^{14}C level in the liver. After 15 min the level of L-[1-^{14}C]tyrosine decreased only slowly with time.

As nonprotein ^{14}C-labeled metabolites, p-hydroxyphenylpyruvic acid and p-hydroxyphenyllactic acid and a significant amount of unknown metabolites (peak IV) were detected (Fig. 1 and Table 3). It is possible that peak I contains, besides p-hydroxyphenylpyruvic acid, other metabolites because the peak is eluted in the void volume. It is not certain that all these metabolites are formed within brain or tumor tissue. It is also possible for instance that the metabolites are formed in the liver, excreted into plasma and taken up in other tissues. During the whole period investigated the percentage of total nonprotein [^{14}C]metabolites was very low in tumor (1.5% to 2.4%) and in brain tissue (1.9% to 3.7%). In case of plasma, the proportion was relatively high, mainly because the nonprotein [^{14}C]metabolites are transfered from tissue to plasma.

Investigation of the decarboxylation pathway is of importance because the enzyme aromatic L-amino acid decarboxylase is widely distributed in the body (*10*). In plasma a significant amount of [^{14}C]bicarbonate was observed. Only a very small amount of [^{14}C]bicarbonate was detected in tissue. The bicarbonate radioactivity was removed rapidly. The ^{14}C was expired as $^{14}CO_2$. Obviously no equilibrium exists between tissue, plasma, and expired $^{14}CO_2$. This observation is consistent with the studies in which intercellular pH is measured using $^{11}CO_2$ (*11*). In these studies an equilibrium state is created by continuous inhalation of $^{11}CO_2$. Our results show that the total ^{14}C level in brain tissue is decreasing with time. The blood-brain barrier permeability for tyrosine is high (*12*) and consequently a high uptake in brain tissue is observed. This amino acid is the predominant source for the catecholamine production pathway. L-[1-^{14}C]Tyrosine is expected to be decarboxylated di-

rectly or converted into L-[1-^{14}C]DOPA by tyrosine hydroxylase and subsequently decarboxylated by aromatic L-amino acid decarboxylase.

For the kinetic analysis of protein synthesis rates in tumor and brain tissues by PET, only four compartments have to be considered: the tyrosine pool in plasma, and the tyrosine, protein and nonprotein metabolite pools in tissue. Although in tissue three radioactive nonprotein metabolites are observed by HPLC the summed amount of these metabolites, only 1.5 to 2.4% for tumor and 1.9 to 3.7% for brain, are that low, that for PET studies the nonprotein route can be considered as one single compartment. By computer simulation, the effect of variation of the size of the nonprotein pool as well as the effect of variation of other kinetic parameters of the simplified model, on the calculated protein synthesis rates, is under investigation.

In conclusion, within 60 min after injection the main metabolic pathway of L-[1-^{14}C]tyrosine, after it is transferred from plasma into tissue, is the incorporation into proteins. The metabolism by other pathways, within this period of time, is of less importance. We conclude that [^{11}C]carboxylic-labeled tyrosine is suitable for measuring protein synthesis rates by PET in neoplastic and normal tissue.

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