RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

Metabolism of Guanido-Labeled (C-14)Arginine in Rats, Mice, and Man

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[6-^{14}C]arginine, injected intraperitoneally into normal rats, was cleared from the plasma with biphasic decay kinetics. Urinary excretion was efficient (32% of the 25-μCi dose within the first 24 hr) with no preferential tissue retention. In mice, the effective duration of the radiotracer's availability for protein biosynthesis was less than 30 min.

When the tracer was administered i.v. to patients with multiple myeloma, it was similarly cleared from the plasma with biphasic kinetics, and was excreted rapidly in the urine (22% of the dose within the first 24 hr). In patients, the guanido-tagged arginine labeled only tumor M component, and the labeling was most intense in patients who had far advanced disease. Estimated radiation dose to humans from a 100-μCi injection was 10 mrads. These studies demonstrate the feasibility of in vivo labeling with [6-^{14}C]arginine, with minimal radiation hazard, thus providing a simple, sensitive, and specific method for monitoring the synthesis of the plasmacytoma M component in patients with multiple myeloma.

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The unique properties of guanido-labeled arginine ([6-^{14}C] arginine, hereafter called "radioarginine") offer a number of advantages for the in vivo study of certain classes of protein metabolism. The participation of the guanido group of arginine in the urea cycle and the guanido group's consequent high rate of replacement minimize the problem of labeled amino acid reutilization (*1, 2*). The radioarginine has also been found to label gamma globulins with seven or eight times the efficiency for proteins of hepatic origin (*3, 4*). This is because only the hepatic cells seem to possess the urea cycle, and consequently at all times the arginine pool in liver has lower specific activity than that drawn upon by all the other tissues of the body. These considerations suggest that, in addition to the enhanced labeling of immunoglobulins, there is also a potential use of radioarginine in pulse labeling of other nonhepatic proteins, for example tumor-secreted proteins. Recent work has shown the practical application of this label by using it to obtain in vivo estimates of half-time for human myeloma IgG_3 and IgE (*5, 6*).

The studies reported here were designed to follow the in vivo metabolism of radioarginine, its distribution in different tissues, its clearance, pulse duration, and delivered radiation dose. The study used normal rat and mouse LPC-1 plasmacytoma models. We also report the application of radioarginine to the in vivo study of human multiple myeloma.

MATERIALS AND METHODS

Animals. Female Lewis rats* weighing 142 to 235 g and female BALB/c mice* (20–25 g) were used. The rats were housed in individual metabolic cages. All animals were fed with standard chow† *ad libitum.*

Patients. Five patients (one female and four males) with confirmed clinical and laboratory diagnosis of multiple myeloma were studied. Ages ranged from 51–69 yr, with life expectancies from 3–12 mo. One of the patients (Figs. 6 and ■ in Fig. 5) died of extensive

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disease on the eighth day following radioarginine injection. All had normal kidney function, with creatinine clearance > 50 ml/min during the study. These patients had well-defined serum IgG M component (1.3–5.4 g%), positive bone-marrow biopsy for tumor cells, and detectable urinary Bence-Jones protein. Informed consent was obtained following our institution's approved procedures.

Radioarginine administration. *Rats and mice.* [6-^{14}C]arginine, 25.9 mCi/millimol in 0.1 *N* HCl was neutralized with 0.1 *N* NaOH and diluted with an appropriate volume of saline (0.9% NaCl). The labeled amino acid was administered i.p.: 125 nCi/g body weight in rats, and 40 μCi per mouse.

Man. Sterile lyophilized radioarginine (specific activity 55.3 mCi/millimol) was reconstituted in sterile physiologic saline to a final activity of 10 μCi/ml. The solution was tested for sterility and shown to be pyrogen-free before use. Each patient was injected i.v. with 85 to 100 μCi of tracer.

Collection of specimens. *Animals.* Blood from mice was collected from the retro-orbital plexus using a heparinized micropipette. The micropipette was centrifuged and the plasma obtained was frozen at −20°C until used. Rats were bled by cardiac puncture and the plasma obtained from heparinized blood was also stored at −20°C.

Twenty-four-hour urine and fecal specimens from individual rats were collected in metabolic cages.

Man. Blood was drawn at different intervals following injection of tracer anticoagulated with EDTA, and the plasma separated and stored at −20°C until used.

Twenty-four-hour urine collections were made for 8 days.

Measurement of radioactivity. *Total radioactivity in plasma and urine.* Duplicate 2 to 50 μl of plasma or urine from rats were delivered to a 20-ml scintillation vial and then dried. Ten milliliters of scintillation fluid‡ were added to the dried sample. Human plasma (0.5 ml) and urine (2 ml) were counted in 10 ml of Aquasol.

Radioactivity in plasma and urine total protein. For rats and mice, duplicate 2- to 50-μl samples of plasma or urine were spotted on 2.5- × 2.5-cm cm^2 cellulose acetate strips‖ and soaked for 10 min in Ponceau S solution (0.2% Ponceau S in 5% TCA). The strip was rinsed twice with 5% acetic acid, then dried. The strips were placed in scintillation vials and 10 ml of the scintillation fluid was added.

For patients, duplicate 0.5-ml samples of plasma were precipitated with 20% TCA (1:1) for 0.5 hr with constant stirring. The mixture was then passed through a 2.4-cm glass filter§ and counted as described above.

Radioactivity in tissues. Samples were oxidized in a tissue oxidizer and the percentage recovery after combustion using ethanolamine was 70%.

Radioactivity counts. Plasma and urine samples were counted in a liquid scintillation spectrometer. The efficiency for Liquefluor-toluene and Aquasol was 86%. Tissue samples were counted with an efficiency of 81% for ethanolamine. Counts were expressed as the mean disintegrations/min (dpm) of duplicate samples, corrected for counting efficiency and recovery.

Estimation of cumulative dose. Radiation dose to tissues: Radioactivity was plotted as a function of time on arithmetic paper. The area under the curve was estimated using a planimeter.

Dosimetry of radioarginine in patients: Estimation of the radiation dose resulting from administration of 100 μCi of tracer was performed using the methods of the MIRD Committee of the Society of Nuclear Medicine. The general equation for radiation dose delivered from an internally distributed radionuclide is presented in MIRD Pamphlet 12, Equation 1 (*7*).

$$\bar{D}(r_k) = \Sigma \tilde{A}_h S(r_k \leftarrow r_h) \qquad (1)$$

where $\bar{D}(r_k)$ is the total dose to region k, $\tilde{A}_h$ is the time integral of activity in region h, and $S(r_k \leftarrow r_h)$ is the mean absorbed dose to region k from activity in region h.

$\tilde{A}(t_1, t_2)$, the time integral of activity, is a function of both the biological behavior of the tracer and its physical half-life. In the case of C-14 compounds, the effect of physical decay is inconsequential.

For radioarginine, the temporal pattern of radioactivity was obtained from distribution studies using Lewis rats. Metabolism of the compound was described using a simple two-compartmental model (Fig. 1). Coefficients and decay constants were obtained using MLAB, an on-line modeling program implemented at National Institutes of Health (*8*).

Studies on the duration of radioarginine pulse. One milliliter of LPC-1 plasmacytoma cells (5 × 10^7) was injected i.p. into BALB/c mice at various times after

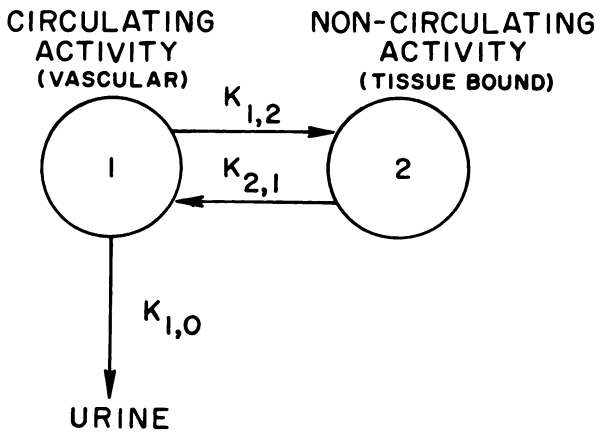

FIG. 1. Metabolism of guanido-labeled (C-14) arginine presented as a two-compartment model. $K_{1,2}$ denotes rate constant from vascular to tissue compartment; $K_{1,0}$ represents that constant from vascular compartment to urine.

administration of 40 μCi radioarginine. Blood was collected from the orbital plexus and the plasma obtained. Aliquots of 1 μl plasma were electrophoresed on Pol E film (with 1% agarose, 5% sucrose in 0.075 *M* barbital buffer, pH 8.6) at 100 volts for 50 min. Subsequently the films were soaked in 5% TCA for 1 min, then stained with Ponceau S solution for 20 min. Excess stain was removed with 5% acetic acid. The films were dried at 72°C and autoradiographed for 4 wk using SB54 film**. The negatives were developed with a commercial processor‡‡.

To determine the concentration of myeloma M component, the total plasma protein was measured by the method of Lowry (*9*). The stained Pol E film was scanned at 525 nm with a densitometer. Based on the percentages of densitometric tracing, the concentration of M protein was calculated. To derive specific activity, the autoradiograms were scanned and the percentage density for M component was multiplied by the counts in total plasma protein, then divided by the concentration of M component. The calculations are as follows:

Concentration:

$$\text{M component (mg/dl)} = \%\ \text{M component}_{EPG} \times \text{total plasma protein (mg/dl)}$$

Specific activity:

$$\text{M component specific activity (dpm/mg)} = \frac{\%\ \text{M component}_{RAG} \times \text{dpm total plasma protein/dl}}{\text{M component (mg/dl)}}$$

where:

% M component = M component expressed as fractional part of the total protein. EPG = electrophoretogram (Ponceau S), RAG = autoradiogram.

RESULTS

The metabolism of radioarginine was investigated in rat and mouse models before its use in patients. The total radioactivity in plasma of rats given the agent i.p. is shown in Fig. 2. Total counts reached a maximum 30 min after tracer injection. The disappearance of radioactivity was followed for 13 days after radiolabel administration and at Day 1, 0.4% of the injected dose was detectable in total-body plasma. By Day 7, only 0.03% of the administered amount was measurable. Maximum plasma-protein radioactivity was reached within the first hour. As expected, the decay curve for total protein was complex, owing to the several half-times in the mixture of proteins labeled.

Since the labeled guanido group of [6-^{14}C]arginine is converted to [^{14}C]-urea by the urea cycle, the excretion of the label is efficiently carried out by the kidneys. The first 24-hr urinary radioactivity accounted for 32% of the administered dose (Table 1). There was a dramatic decrease in urine counts from Day 1 to Day 7. Thereafter, residual radioactivity ranging from 4,000–13,000 dpm/24-hr urine was detected (0.01–0.02% of the injected dose). In contrast, excretion through the feces is much less efficient (Table 2). The maximum counts detected in the stool at 24 hr represented only 3% of the injected label. On the last day of sampling (Day 11), only 8,000 dpm/24-hr feces was observed (< 0.01% of the injected dose).

The radioactivity of different tissues was analyzed. The distribution of the percentages of dose/organ is shown in Table 3. Of the 14 tissues studied, the skin, liver, small intestine, and stomach retained the largest portions. The lowest percentages were in the adrenals and ovaries. On a weight basis, there were no significant differences ($P > 0.05$, t-test) in the radiation doses of the various tissues analyzed on Day 1. On day 7, however,

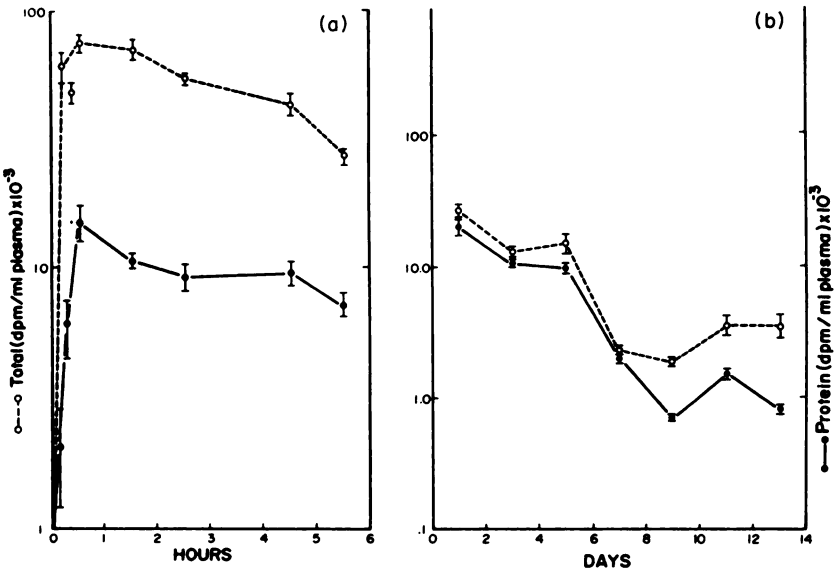

FIG. 2. Rat plasma radioactivity after i.p. injection of guanido-labeled (C-14) arginine. O--- O = total activity; ●—● = protein fraction only (note separate scales). For Day 13, means and ranges for three rats are shown; all other points give means and strandard errors of means (>6 rats each). See text for experimental details.

TABLE 1. CLEARANCE OF RADIOARGININE IN RAT URINE

Time (days)	Radioactivity (% of injected dose)*
1	31.57 ± 5.40
2	3.15 ± 0.54
3	2.25 ± 0.50
4	1.32 ± 0.31
5	0.24 ± 0.07
6	0.03 ± 0.01
7	0.01 ± 0.00

*Mean ± s.e.m. of 24-hr urine collections from 23 to 8 rats.

TABLE 2. CLEARANCE OF RADIOACTIVITY IN RAT FECES

Time (days)	Radioactivity (% of injected dose)*
1	3.12 ± 0.35
5	1.03 ± 0.11
7	0.15 ± 0.02
11	0.02 ± 0.00

*Mean ± s.e.m. of 24-hr fecal collections from 8 rats.

the liver showed significantly lower counts ($P < 0.05$, t-test) than the other tissues. On Day 11, the total counts from 14 different tissues represented less than 5% of the administered dose.

The cumulative doses in rads to the skin, liver, small intestine, and stomach from Day 1 up to Day 11 were estimated by planimetry. The skin had a concentration-time integral of 1.08 μCi-hr/g (~ 0.108 rads) which is comparable to that of the liver, 1.02 μCi hr/g (~ 0.102 rads); small intestines, 0.73 μCi-hr/g (~ 0.073 rads); and stomach, 1.46 μCi-hr/g (~ 0.146 rads).

The disappearance of the radioactivity in tissues gave semilog decay curves whose slopes are nonlinear over the entire period of study. This suggests that there are several labeled products with different turnover rates. The liver showed a faster decay rate and had the lowest specific activity on Day 11. The other tissues (heart, lung, thymus, spleen, kidney, adrenals, stomach, intestine, ovaries, skin, tongue, brain, and eye) showed similar curves.

To determine the effective duration of the labeled amino acid's availability for protein biosynthesis, radioarginine was given to BALB/c mice simultaneously or at various times before i.p. injection of LPC-1 plasmacytoma cells. Figure 3 shows that the persistence of the labeled amino acid was relatively short. When the label was administered 30 min before cell transfer, the specific activity of the M component represented 8% of that observed when both tracer and cells were given simultaneously (Fig. 3).

Since the above studies with rats and mice indicated the applicability, ease, and safety of using radioarginine as in vivo tracer, the metabolism of this agent was in-

TABLE 3. RAT TISSUE RADIOACTIVITY

Tissue	Organ weight (g) $\bar{x}$ ± s.e.m. (n = 8)	Organ Radioactivity (% of injected dose) Days after injection $\bar{x}$ + (range) (n = 3) 1	5	7	11
Heart	0.89 ± 0.03	0.36 (0.26–0.46)	0.38 (0.38–0.38)	0.28 (0.25–0.32)	0.02 (0.00–0.03)
Lung	1.48 ± 0.08	0.75 (0.50–0.90)	0.50 (0.48–0.52)	0.31 (0.25–0.37)	0.02 (0.01–0.04)
Thymus	0.93 ± 0.15	0.51 (0.50–0.51)	0.43 (0.42–0.43)	0.19 (0.15–0.22)	0.01 (0.00–0.02)
Liver	10.97 ± 0.60	5.13 (3.41–5.94)	2.05 (2.03–2.06)	1.12 (0.72–1.56)	0.04 (0.03–0.08)
Spleen	0.52 ± 0.03	0.42 (0.40–0.42)	0.23 (0.22–0.23)	0.24 (0.17–0.27)	0.01 (0.00–0.01)
Kidney	1.11 ± 0.05	0.64 (0.54–0.67)	0.39 (0.37–0.39)	0.28 (0.22–0.34)	0.01 (0.00–0.02)
Adrenals	0.03 ± 0.01	0.03 (0.02–0.03)	0.01 (0.01–0.01)	0.01 (0.01–0.01)	0.00 (0.00–0.00)
Stomach	1.45 ± 0.06	1.15 (1.14–1.15)	0.42 (0.42–0.42)	0.42 (0.38–0.45)	0.02 (0.01–0.03)
Small intestines	4.83 ± 0.37	4.92 (3.46–7.26)	1.59 (1.12–1.61)	1.15 (0.92–1.20)	0.06 (0.01–0.10)
Skin*	36.00*	25.00 (25.6–27.0)	10.00 (10.0–10.1)	8.77 (7.00–9.60)	0.98 (0.45–1.75)
Tongue†	0.29 ± 0.01	0.16 (0.13–0.17)	0.09 (0.09–0.10)	0.07 (0.06–0.08)	0.01 (0.00–0.01)
Brain	1.64 ± 0.06	0.65 (0.64–0.65)	0.29 (0.28–0.29)	0.27 (0.22–0.35)	0.01 (0.00–0.01)
Eyes	0.15 ± 0.01	0.04 (0.01–0.05)	0.04 (0.01–0.05)	0.12 (0.10–0.16)	0.00 (0.00–0.00)
Ovaries	0.06 ± 0.01	0.03 (0.01–0.04)	0.01 (0.01–0.01)	0.02 (0.01–0.02)	0.00 (0.00–0.00)
Total		39.79	16.43	13.25	1.21

* Calculations for skin assuming 18% of body weight (*12*).
† Skeletal muscle.

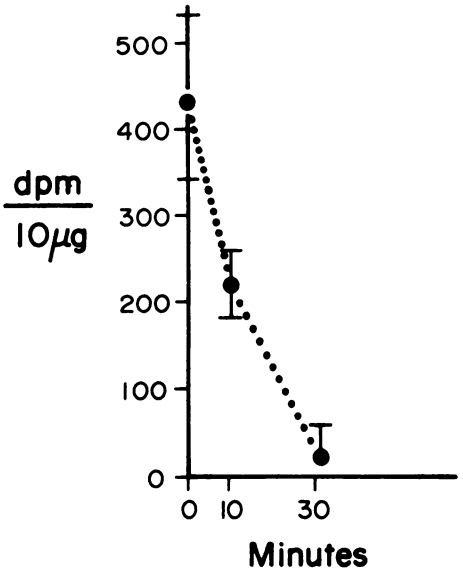

FIG. 3. Graph showing brief availability of radioarginine for labeling of myeloma M component. Abscissa shows delay (if any) between priming injection (i.p.) of radioarginine and test injection (i.p.) of LPC-1 plasmacytoma cells. Specific activity of M component (as dpm/10 μg here) was determined 24 hr later (see Methods).

vestigated in patients with multiple myeloma. The radioactivity in serum was first analyzed. Total-plasma radioactivity values from the five patients studied were very similar (Fig. 4). The highest counts were observed 1 to 3 min after injection of tracer. The decay curve is biphasic: first a steep fall in radioactivity from 3 to 30 min, then a slower rate of decline up to Day 8. At Day 8 only 1% of the injected dose (85–100 μCi) was detected in plasma (Fig. 4).

Labeling of total plasma proteins with radioarginine was determined (Fig. 5). Radioactivity was detected in plasma proteins as early as 2 min in one patient and by 30–60 min in the other four. There were two distinct

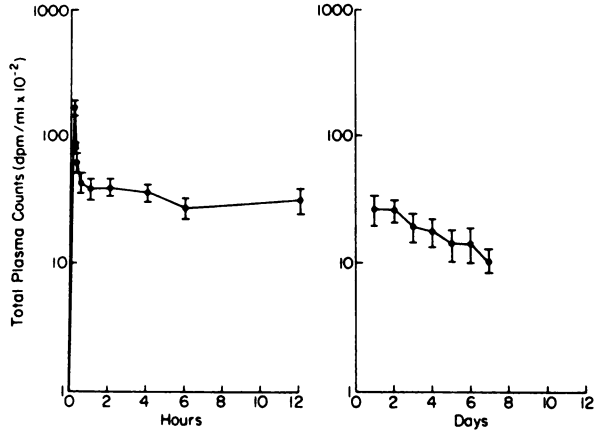

FIG. 4. Total plasma radioactivity in multiple myeloma patients after i.v. injection of 85–100 μCi of radioarginine. Duplicate plasma samples were counted and $\bar{x} \pm$ s.e.m. counts from five patients are illustrated.

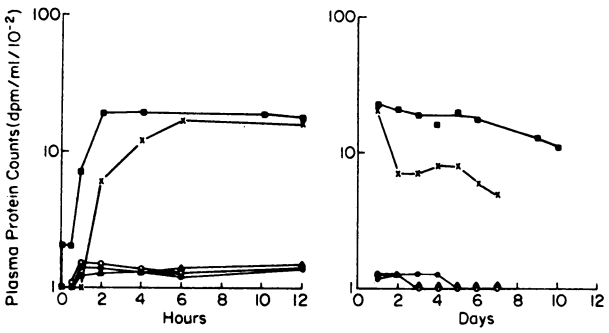

FIG. 5. Labeling of total plasma proteins in five patients with multiple myeloma. Each received 85–100 μCi of radioarginine i.v. The two with prominent incorporation of C-14 were seriously ill and not responding to therapy.

labeling patterns observed. Two patients had high incorporation of radioactivity into plasma proteins, reaching a maximum at 24 hr following tracer injection. The radioactivity persisted up to Days 7 to 10, when 70% of total counts were detectable in protein. These patients had far advanced disease and were not responding to chemotherapy. The other three patients had lower incorporation of counts into protein, with maximum at only 42% on Day 2. Thereafter, loss of tracer was rapid, and by 3 to 5 days counts were no longer detectable in plasma proteins (Fig. 5). The distribution of radioactivity among plasma protein components was examined. Plasma obtained at various intervals was electrophoresed and autoradiographed as described in Methods. In the plasma from all five patients, only the M component (belonging to the IgG class), and none of the other plasma proteins, was labeled (Fig. 6). Thus, total plasma counts were attributable to tumor-protein labeling.

As in rats, the bulk of radioarginine was eliminated through the urine (Table 4). Excretion of C-14 in urine was 22–26% of injected dose (85–100 μCi) for the first 24 hr. Renal function in these five patients was normal, with creatinine clearance > 50 ml/min); therefore, elimination of the tracer through the kidneys was efficient. The cumulative excreted radioactivity up to 8 days after injection was 70% of injected dose. There was also no preferential retention of the label by an organ. The tissue distribution of C-14 was analyzed in one patient (■, Fig. 5) who died with extensive disease on the eighth day of the study. There was no detectable radioactivity in any of the nineteen tissues examined (thyroid gland, spleen, kidney, tongue, muscle, uterus, liver, lung, lymph node, heart, small and large intestine, breast, fat, adrenals, skin, trachea, brain, and bone marrow).

Based on the above data from rats and multiple-myeloma patients, rate constants describing compartmental retention (see Fig. 1) were computed. The rate constants for the two-compartment model were then used to estimate the time-integral of activity in man. Using the methods of the MIRD Committee (*11*), the

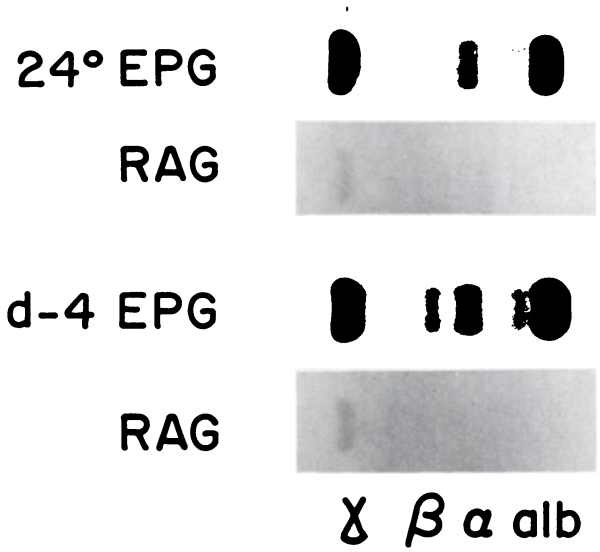

FIG. 6. Plasma electrophoretogram (EPG) and autoradiogram (RAG) patterns from patient terminally ill with multiple myeloma. Samples were taken 1 day and 4 days after i.v. injection of 100 μCi of radioarginine tagged at the guanido carbon. Only the M component is labeled.

total-body dose from 100 μCi of radioarginine was estimated to be 10 mrads. Compartmental retention as a function of time is shown in Fig. 7.

DISCUSSION

This study demonstrates the advantages of using guanido-tagged (C-14)arginine for in vivo labeling of nonhepatic proteins. The labeled guanido group of this amino acid is: (a) rapidly excreted via the urea cycle through the kidneys; (b) is not preferentially retained in any of the tissues studied; (c) has a short effective duration, thus facilitating pulse-labeling experiments at frequent time intervals; and (d) selectively labels tumor M component at little radiation exposure to the patient.

TABLE 4. CLEARANCE OF RADIOARGININE IN HUMAN URINE

Time (days)	Radioactivity (% of injected dose)*
1	29.52 ± 5.54
2	14.30 ± 5.77
3	9.68 ± 3.63
4	3.60 ± 1.62
5	2.54 ± 1.04
6	0.95 ± 0.33
7	1.18 ± 0.27

* Mean ± s.e.m. of 24-hr urine collection from 5 patients with multiple myeloma.

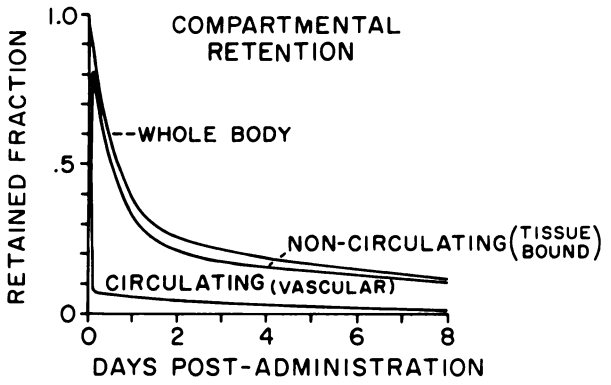

FIG. 7. Compartmental retention as estimated from multiple myeloma patients and rat data (see Methods section).

Since the guanido carbon of arginine is degraded through the urea cycle (*13*), the bulk of radioactivity is excreted in the urine. During the first 24 hr after tracer injection, the radioactivity found in human and rat urine constituted 22–32% of the administered dose. The rate of C-14 clearance was rapid through Day 7. Excretion of tracer through the rat feces appeared to provide a minor pathway when the tracer was administered i.p., and the fecal radioactivity may even be due partly to contamination with urine, since both samples were obtained through the same collecting system. An earlier investigation (*14*) showed that radioarginine given orally to rats resulted in a lower excretion rate in urine, which may be due to metabolism of arginine by the gut. However, major clearance of the tracer when administered i.p. or i.v. is through the kidneys.

The labeled amino acid was not preferentially retained in any of the tissues examined. There was even lower radioactivity in rat liver when expressed on a per-weight basis on Day 7. Since the urea cycle is present in the liver, the rate of replacement of the labeled arginine by its stable counterpart occurs rapidly in this tissue (*1, 13*). In all rat tissues examined, the total radioactivity, representing over 40% of injected dose on Day 1, was already dramatically reduced on Day 11 (< 5% as represented in Table 3). The minimal radiation exposure to tissues was further depicted by similar decay curves drawn from 14 different tissues.

In the case of human tissues (autopsy samples from one patient), no detectable radioactivity was noted on the eighth day after 100 μCi of tracer injection, confirming minimal radiation exposure. The degree of radioarginine reutilization in tissues is not clear, since estimates have been obtained only from studies using other amino acids. With C-14 lysine, the calculated reutilization was about 50% (*15*). In comparison, the reutilization of radioarginine in plasma proteins has been estimated to be less than 1% (*1, 2*).

The presence of LPC-1 BALB/c plasmacytoma cells (IgG, 2aκ producers) served as the probe for the effective

duration of the labeled amino acid for in vivo protein biosynthesis. It was demonstrated that less than 10% of the label remains as the intact amino acid after 30 min. This behavior of very short duration would permit repeated injection and sampling at short intervals.

Morrell et al. (*5*) and Waldman et al. (*6*) demonstrated the practical utility of an in vivo arginine-labeling method to determine the half-lives of human myeloma IgG_3 and IgE. The incorporation of counts in the gamma-globulin fraction has been shown consistently to be seven to eight times that for proteins of hepatic origin (*3*). Hence, the labeling of proteins of nonhepatic origin (e.g., immunoglobulins and tumor proteins) is magnified and the labeling of proteins of hepatic origin is diminished (*16*). The enhanced labeling of Ig was confirmed at low dose (85–100 μCi) of radioarginine in multiple myeloma patients. Only the tumor-related M component (IgG) was labeled detectably in all the five patients studied. It appears that detectable radioactivity in total plasma proteins is all in the tumor M component. The two patients who showed higher incorporation of label in their total plasma proteins were the ones critically ill during the study. Both were not responding to treatment and died within 8 days to 4 mo after the study. The radiolabel was diverted by the metabolically active tumor mass and utilized for tumor protein synthesis (*16*). Hence, high incorporation of radioarginine into total protein is the result of both an actively metabolizing tumor mass synthesizing M component and the extent of the tumor load.

The results described here demonstrate the potential of [6-^{14}C]arginine as a tracer for studying the in vivo metabolism of nonhepatic proteins, e.g., immunoglobulins. The sensitivity of this tracer, and its low radiation hazard, encourage its further application in human investigation as a probe of plasmacytoma M-component production and secretion.

FOOTNOTES

* Microbiological Associates, Walkersville, MD.
† Charles River RMH 1000, Charles River Laboratories,
‡ PPO-POPOP, toluene concentrate, Liquefluor, Packard,
‖ Sepraphore III, Gelman, Ann Arbor, MI.
§ Whatman, Inc., Clifton, NJ.
¶ Carbasol-Permafluor, Packard
*Kodak
†† Kodak RP X-OMAT, Model M6 A-N, Kodak

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