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Kinetics and Distribution of Tritiated Putrescine in the Domestic Cat*

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Abstract—(^{3}H) *putrescine* (25.4 nmole) *was administered as an i.v. bolus to each of* 8 *cats. Plasma samples obtained at times up to* 5 hr *post injection were analyzed for total* (^{3}H), *non-volatile* (^{3}H) *and* (^{3}H) *putrescine, spermidine and spermine. Tissue, urine and cerebrospinal fluid samples obtained after sacrifice of the animals were similarly analyzed and used to calculate tissue/plasma ratios for* (^{3}H) *putrescine. Non-linear iterative analysis of the decrease in plasma concentration of* (^{3}H) *putrescine with time showed a three-phase decay with half-times of* 1, 7 *and* 117 min. *respectively. Slopes and intercepts from the computer-generated fits of the data were used to calculate the steady-state volume of distribution for putrescine in the cat* (0.542 ±0.107 l/kg). *No* (^{3}H) *spermine or spermidine was found in any of the plasma samples, but liver, spleen and kidney samples had substantial fractions of* (^{3}H) *spermidine, as well as some* (^{3}H) *spermine. The fraction of* (^{3}H) *present in plasma as* (^{3}H) *putrescine declined from* 95% *at* 1 min *to* 25% *at* 10 min *and* 2% *at* 240 min *post injection. Incubation of* (^{3}H) *putrescine with whole blood, serum, and plasma* in vitro *showed that catabolism of exogenous putrescine took place in the peripheral tissues, rather than in the blood.*

INTRODUCTION

THE POLYAMINES putrescine ($H_2N(CH_2)_4NH_2$) (Pu), spermidine ($H_2N(CH_2)_3NH(CH_2)_4NH_2$) (Sd) and spermine ($H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$) (Sp), are a group of biological molecules that have been the subject of intensive investigation for the past several decades [1–4]. Increased intracellular concentration of the polyamines [5] and elevated activities of their biosynthetic enzymes [6] are among the earliest biochemical events observed following the application of a wide variety of growth stimuli to a broad spectrum of biological systems. Intracellular polyamine metabolism has been linked to the metabolism of the nucleic acids [1, 3, 7]. These observations have led to the proposal of a role for these polycations in the process of cellular growth and replication [1, 2, 4].

The initial report of increased urinary levels of the polyamines in patients with cancer [8] held the promise that these biomolecules might be useful as biochemical markers in the diagnosis and management of neoplastic disease. These preliminary observations were subsequently confirmed by a large number of other investigators [9–11]. Additional studies

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The abbreviations used are: Pu, putrescine; Sd, spermidine; Sp, Spermine; CSF, cerebrospinal fluid; SSA, 5-sulfosalicylic acid; C_{pl}, plasma concentration of tritiated putrescine; Vd_{ss}, volume of distribution at steady-state; $t_{1/2}$, half time; TCA, trichloroacetic acid.

extended these results to other physiological fluids, including serum [12–14] and cerebrospinal fluid (CSF) [15, 16]. A number of reviews of methods for measurement of polyamine concentrations in physiological fluids and tissue samples [1, 2, 17], of the intracellular metabolism of the polyamines [1, 3, 6, 7] and of their potential utility as biochemical markers for the diagnosis and management of neoplastic disease [2, 4, 18–20] are available.

It is generally accepted that the increased polyamine levels that have been observed are, at least in part, the reslt of increased intracellular production of these molecules, rather than decreased rates of catabolism [2]. For such increased intracellular production to manifest itself itself as elevated concentrations in serum and urine, these molecules must either diffuse across the cell membrane or be released by cell lysis. In non-hematological tumors these molecules must also cross capillaries to appear in plasma. In the time interval during which the polyamines move from tumor tissue into blood, and eventually into urine, these compounds can distribute in many other tissues in the body where metabolism may occur.

In order to provide a rational basis for deciding the appropriateness of correlating neoplastic disease states with physiological fluid levels of the polyamines it would be useful to gain some information regarding body distribution and pool size of these compounds. We have approached this problem by following the decrease in plasma concentration of radiolabelled Pu as a function of time after administration of an i.v. bolus to the domestic cat. While a number of investigations involving administration of radiolabelled polyamines to intact animals have been reported [21–27], none of these studies have used mathematical models to determine volumes of distribution, pool sizes and distribution rate constants for Pu.

MATERIALS AND METHODS

(2,3-^{3}H) Putrescine dihydrochloride (20.64 Ci/mmole) was obtained from New England Nuclear Corp. (Boston, Mass.). Immediately prior to injection, 0.6 ml of a solution of 1 mCi/ml (0.0078 mg/ml) was diluted to a total volume of 2.0 and 1.75 ml was injected into the cat. This corresponded to a dose of 25.4 nmole for each animal. The remainder of the diluted sample was used to determine the specific activity of the solution injected. All other chemicals used were of analytical grade.

Cats (2.5–3.5 kg) were anesthetized with i.p. ketamine (33 mg/kg) and cathers inserted into the fermoral artery and vein. The animal was put into a restraining harness and allowed to recover from anesthesia. Once the animal was fully awake [^{3}H] Pu, followed by 2 volumes of physiological saline solution, was injected through the venous catheter. The [^{3}H] Pu solution was injected over 20 seconds and timing of the experiment began at the midpoint of the injection. At appropriate time intervals 1.5 ml of blood was removed via the arterial catheter, immediately spun for 1 min in a Brinkman Instruments Inc. (Westbury, N.Y.) Model 3200 Micro Centrifuge, and the plasma removed for analysis.

One hundred microliters of plasma was added to 6 ml of PCS (Amersham Corp. Arlington Heights, Ill.) and counted for total radioactivity. A 500 μl plasma aliquot was added to 500 μl 12 N HCl in a Miniaktor tube (Applied Science Labs, Inc., State College, Penn.). Both of these operations were performed immediately after centrifugation for each plasma sample. At the end of each experiment all the Miniaktor tubes were sealed and the plasma samples were hydrolyzed by heating at 110°C for 12–14 hr. The hydrolysates were lyophilized to dryness, reconstituted in 200 μl of 4% 5-sulfosalicylic acid (SSA) and filtered through a glass wool plug in the tip of a pasteur pipet. A 50 μl aliquot of this final solution was fractionated using a Durrum Instrument, Inc. (Palo Alto, California) D-500 amino acid analyzer. The cation exchange column, buffer schedule, and other elution conditions were as previously described [16]. Instead of mixing with a reagent (ninhydrin or *o*-phthalaldehyde) for photometric or flourometric detection of the polyamines, the eluate from the cation exchange column was collected in 3 min (*ca.* 0.93 ml) fractions using a Gilson Medical Electronics, Inc. (Middleton, Wisc.) Microfractionator. Each fraction was added to 2 ml of distilled H_2O (to dilute the high salt concentration of the eluting buffers) and 6 ml of PCS and counted. In addition to this fractionation, 50 μl of each reconstituted plasma hydrolysate was added to 0.93 ml of elution buffer, 2.0 ml of H_2O and 6 ml of PCS and counted to determine total radioactivity in the actual sample fractionated.

Five hours after injection of [^{3}H] Pu, the animal was sacrificed by i.v. injection of 2.0 ml of Euthanol Solution (Med.-Tec., Inc.,

Table 1. *Plasma concentrations of total* ^{3}H, *non-volatile* ^{3}H, [^{3}H] Pu, [^{3}H] Sd *and* [^{3}H] Sp *at various times after injection of a bolus of* [^{3}H] Pu *to cat* 3

Time (min)	Total [^{3}H] (pmole/ml)	Non-volatile [^{3}H] (pmole/ml)	[^{3}H] Pu (pmole/ml)	[^{3}H] Sd (pmole/ml)	[^{3}H] Sp (pmole/ml)
1	263.9	258.2	250.7	0.45	0.13
2	127.8	116.5	98.4	0.35	0.08
5	57.6	36.2	28.1	0.23	0.05
10	43.0	15.3	10.2	0.07	0.01
15	37.8	9.59	6.78	0.06	0.01
20	36.0	7.42	5.14	0.04	<0.01
30	33.5	5.73	3.59	0.03	0.04
45	33.5	4.21	2.47	0.03	0.01
60	34.7	3.92	2.09	0.02	0.01
90	35.3	2.58	1.41	0.02	<0.01
120	33.8	2.44	1.06	<0.01	<0.01
180	37.2	2.78	0.82	0.01	<0.01
240	37.7	2.17	0.63	0.01	<0.01
300	38.5	2.13	0.56	0.01	<0.01

[^{3}H] Pu (25.4 nmole) was injected i.v. into the cat. Plasma samples were obtained at the times indicated. One aliquot was counted to measure total ^{3}H. A second aliquot was hydrolyzed in 6N HCl, lyophilized to dryness, and reconstituted with 4% SSA. One aliquot of the reconstituted sample was counted for total non-volatile ^{3}H. A second aliquot was fractioned, and the fractions were counted for [^{3}H] Pu, [^{3}H] Sd and [^{3}H] Sp.

Table 2. *Tissue, urine and CSF concentrations of total* ^{3}H, *non-volatile* ^{3}H *and* [^{3}H] Pu, [^{3}H] Sd *and* [^{3}H] Sp 300 min *after injection of a bolus of* [^{3}H] Pu *to cat* 3

Tissue	Total [^{3}H] (pmole/g)	Non-volatile [^{3}H] (pmole/g)	[^{3}H] Pu (pmole/g)	[^{3}H] Sd (pmole/g)	[^{3}H] Sp (pmole/g)
Heart	31.6	32.9	25.4	2.3	0.3
Liver	109.1	131.1	64.8	29.9	2.5
Brain (cortex)	24.4	2.3	1.3	0.4	0
Brain (sub-cortex)	25.8	1.7	0.8	0.3	0
Kidney	95.9	71.2	29.5	7.6	2.3
Spleen	160.5	83.0	56.2	15.0	1.4
Lung	45.6	33.9	17.9	4.0	0.7
Abodominal muscle	33.1	18.4	8.5	3.6	1.2
Fat	8.4	6.5	3.1	1.2	0
Ovary	47.0	32.6	25.2	2.7	0.2
Temporal muscle	23.2	4.7	2.2	0.6	0.2
Urine	136.7	138.1	82.8	2.2	0
CSF	40.7	0.3	0.6	0	0

[^{3}H] Pu (25.4 nmole) was injected i.v. into the cat, and after 300 min the animal was sacrificed. Urine and CSF samples were obtained by percutaneous pucture, and duplicate tissue samples by autopsy. Urine and CSF were treated as plasma samples (see Table 1). One tissue sample was digested with NCS and counted for total ^{3}H. The second tissue sample was homogenized in TCA. lyophilized, and reconstituted with SSA. One aliquot of the reconstituted sample was counted for total non-volatile ^{3}H. A second aliquot was fractionated and the fractions counted for [^{3}H] Pu, [^{3}H] Sd and [^{3}H] Sp.

Elwood, Kansas). Immediately upon death, samples of cerebrosinal fluid and urine were obtained by percutaneous puncture and treated in a manner similar to plasma. Duplicate tissue samples (see Table 2) were placed into tared bottles and weighed. One sample of each tissue (10–50 mg) was digested in 0.4 ml NCS (Amersham Corp.), acidified with 0.3 ml of 1 N HCl and then 6 ml of PCS were added for total radioactivity determination. The second sample (100–500 mg) was placed into 3.0 ml of ice-cold 10% TCA in a ground glass tissue grinder tube, homogenized for 1–2 min and then sonicated for 30 sec using a Lab-Line Ultra-Tip Sonicator (Lab-Line Instruments, Inc., Melrose Park, Ill.). After sitting on ice for 1 hr to precipitate protein, the tissue homogenates were centrifuged and excess TCA removed by extraction with two 2 ml aliquots of ether. Two milliliters of the aqueous phase were lyophilized to dryness and reconstituted with 200 μl of 4% SSA to concentrate the sample. One 50 μl aliquot was used to determine the total radioactivity of each reconstituted sample, as described above for the plasma samples. A second 50 μl aliquot was fractionated as described above for measurement of [^{3}H] Pu, [^{3}H] Sd and [^{3}H] Sp.

All radioactive counting was done using a Packard Tri-Carb Liquid Scintillation spectrometer. Quench was corrected for by the method of external standards and using the specific activity determined for the actual solution injected into each animal, dis/min were converted into pmole/ml for the physiological fluid samples and pmol/g (wet weight) for the tissue samples. In addition, the values for those fractions corresponding to the peaks of radioactivity obtained upon injection of authentic samples of each of the three polyamines (fractions 10–15 for Pu, 18–20 for Sd, and 22–25 for Sp) were corrected for recovery and pooled. The recoveries observed for each of the polyamines using the above methods of sample preparation and fractionation were (mean value ± S.D. for three determinations) 87.4 ± 2.8% for Pu, 73.7 ± 4% for Sd, and 75.3 ± 5.3% for Sp. We thus obtained for each fluid and tissue sample a measurement of total radioactivity, total non-volatile radioactivity, and radioactive Pu, Sd and Sp.

For investigation of the *in vitro* metabolism of [^{3}H] Pu, a single cat was exsanguinated. Samples of whole blood, serum and plasma were placed into sealed injection vials, incubated at 37°C in a shaking water bath at a pH of 7.4 (maintained by addition of 2–3 drops 0.01 N lactate and a 5% CO_2 atmosphere). Solutions of [^{3}H] Pu were injected into each vial to achieve an initial concentration corresponding to that obtained in the intact animal experiments. Samples were removed from each vial with a syringe at timed intervals, with timing begun at the instant of addition of the [^{3}H] Pu. The whole blood samples were treated exactly as described above for the *in vivo* experiments. For the serum and plasma samples, the only difference in handling was the omission of the initial centrifugation. As with all other samples, 5 measurements were obtained for each sample.

For each animal in the *in vivo* studies a plasma (C_{pl}) curve was plotted and fit to the tri-exponential function

$$C_{pl} = Ac^{-\alpha t} + Be^{-\beta t} + Cc^{-t}$$

using a nonlinear iterative computer program. The values of A, B, C, α, β and γ were then used to calculate the volume of distribution at steady state for Pu in the cat from the equation

$$Vd_{ss} = \text{Dose}\, x \left(\frac{A}{\alpha^2} + \frac{B}{\beta^2} + \frac{C}{\gamma^2}\right) \Big/ \left(\frac{A}{\alpha} + \frac{B}{\beta} + \frac{C}{\gamma}\right)^2.$$

In addition, the [^{3}H] Pu concentrations of the final (5 hr) plasma samples were used to calculate tissue to plasma partition ratios for Pu at equilibrium.

RESULTS

The data obtained from a representative *in vivo* experiment are presented in Tables 1 and 2 and in Fig. 1. Table 1 tabulates the plasma concentrations of total [^{3}H], total non-volatile [^{3}H] and the concentrations of the three [^{3}H] polyamines. The concentrations of [^{3}H] Pu at the indicated time points were used to plot the decay curve shown in Fig. 1. Table 2 summarizes the concentrations of total [^{3}H], non-volatile [^{3}H] and [^{3}H] polyamines in various tissues, urine and CSF 300 min after injection of the bolus of [^{3}H] Pu. These data were obtained from the same animal as were the data in Table 1.

The ratio of tissue [^{3}H] Pu concentration to plasma [^{3}H] Pu concentration at 300 min was calculated for each tissue obtained for each of the 8 cats. A *t*-test performed on these data showed no statistically significant differences between the mean values for male and female cats for any of the tissues studied. Accordingly, we averaged the values for each

tissue examined for all 8 cats used. These data are presented in Table 3 as mean values ±S.E. For several of the tissues studied, samples were not available from each cat used.

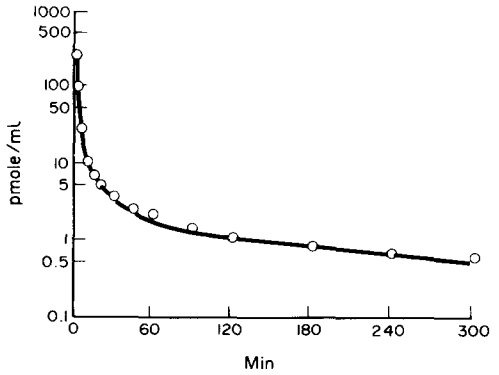

Fig. 1. Plasma concentration of [^{3}H] *Pu* (pmole/ml) *plotted as a function of time* (min) *after administration of an i.v. bolus of* [^{3}H] *Pu to cat* 3. *Measured data points are indicated by the bottom of each circle. The smooth curve is the result of a computer generated fit of the data to the equation*

$$C_{pl} = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t}.$$

Table 3. *Tissue to plasma concentration ratios of* [^{3}H] *Pu* 300 min *after injection of a bolus of* [^{3}H] *Pu to each of* 8 *cats*

Tissue	Number of animals used	Mean S.E.
Heart	8	114.6±19.1
Liver	8	47.3±11.0
Brain (cortex)	8	8.2± 3.1
Brain (sub-cortex)	8	3.1± 0.5
Kidney	7	32.9± 6.5
Spleen	8	116.5±35.7
Lung	8	75.3±23.3
Abdominal muscle	7	12.8± 3.2
Fat	7	4.5± 1.7
Ovary	3	77.7±16.6
Testis	4	12.6± 3.9
Temporal muscle	5	8.0± 3.2

[^{3}H] Pu (25.4 nmole) was injected i.v. into each of 8 cats. Plasma samples were obtained immediately prior to sacrifice of the animal at 300 min. Plasma samples were hydrolyzed in 6N HCl, lyophilized to dryness, reconstituted in SSA, fractionated, and the fractions counted in a liquid scintillation spectrometer for measurement of [^{3}H] Pu. Tissue samples, obtained upon autopsy, were homogenized in TCA, lyophilized, reconstituted in SSA, fractionated, and the fractions counted in a liquid scintillation spectrometer for measurement of [^{3}H] Pu. For each animal, a tissue/plasma concentration ratio was calculated for each of the tissues listed. Students *t*-test showed no significant differences between the means for male and female cats for any of the tissues. The data listed represent the mean tissue/plasma ratio ±S.E. for all animals studied.

This was due to loss of the sample during work-up, prior removal of the ovary in one female, or the fact that the organ was sex-related. Table 3 shows that all tissues examined in this study accumulate [^{3}H] Pu in amounts greater than that present in plasma 300 min after injection. The greatest amount of accumulation occurs in spleen, heart, lung, liver and kidney. Ovary appears to accumulate [^{3}H] Pu six times more than does testis. Brain, muscle, and fat show much smaller degrees of accumulation, but 3–8 times greater amounts of [^{3}H] Pu are found even in these tissues than are present in plasma at 300 min.

Comparison of the S.E. obtained from fitting the plasma data to mono-, bi- and tri-exponential functions showed that the plasma [^{3}H] Pu curves were best fit by a tri-exponential function. The slopes (α, β and γ) and intercepts (A, B and C) calculated by non-linear iterative analysis of the plasma curves obtained from each animal as well as mean values and S.E. for these parameters are shown in Table 4. Also listed in this table are the steady state volumes of distribution (Vd_{ss}) calculated from these slopes and intercepts using the previously described equation. Half-times for each of the three phases of the plasma curves were calculated from the mean values of computer-generated slopes ($t_{1/2} = 0.693/\text{slope}$). These data are also listed in Table 4. The steady state volume of distribution for [^{3}H] Pu in the cat is slightly more than 50% of total body weight.

No changes in [^{3}H] Pu concentration were observed in any of the *in vitro* metabolism experiments (serum, plasma and whole blood) during the 180 min of incubation. The mean concentrations of [^{3}H] Pu during the *in vitro* experiments were 170.8±2.6 pmole/ml for incubation in serum, 175.3±2.8 pmole/ml for incubation in plasma and 261.1±3.3 pmole/ml for incubation in whole blood. For each sample in these three experiments, the total concentration of [^{3}H] and the concentration of non-volatile [^{3}H] were both equal to the concentration of [^{3}H] Pu (within experimental error). In addition, no [^{3}H] Sd or Sp was detected in any of the samples.

DISCUSSION

Conversion of exogenous [^{3}H] Pu to higher polyamines occurs only negligibly within the plasma compartment (Table 1). This observation was substantiated with all cats used in

Table 4. Slopes, intercepts and steady-state volumes of distribution for putrescine

Sex	Dose (nmole/kg)	A (pmole/ml)	α (min^{-1})	B (pmole/ml)	β (min^{-1})	C (pmole/ml)	γ (min^{-1})	Vd_{ss} (l/kg)
Female	10.17	217.2	0.6251	20.22	0.0936	2.004	0.00421	1.091
Female	07.26	340.7	0.9405	18.50	0.0955	2.804	0.00699	0.473
Female	07.06	677.7	1.244	58.32	0.1329	3.786	0.00567	0.311
Female	10.38	355.2	0.6199	11.73	0.0561	1.849	0.00426	0.748
Male	10.82	919.2	1.248	143.7	0.2054	7.338	0.00892	0.204
Male	7.94	227.0	0.6196	24.43	0.1095	3.260	0.00731	0.471
Male	14.12	634.1	0.6055	63.96	0.0815	3.813	0.00594	0.276
Male	6.69	117.5	0.4811	11.07	0.0637	2.558	0.00541	0.764
Mean		436.1	0.7503	44.0	0.0969	3.43	0.00591	0.542
S.E.		98.2	5.15%	16.0	5.91%	0.62	3.74%	0.1070
$t_{1/2}$			0.924 min		7.15 min		117.3 min	

[^{3}H] Pu (25.4 nmole) was injected i.v. into each of 8 cats. Non-linear iterative analysis of the plasma [^{3}H] Pu concentration vs time data was used to fit the data to a tri-exponential function of the form $C_{pl}=Ae^{-\alpha t}+Be^{-\beta t}+Ce^{-\gamma t}$ The computer-generated parameters for each individual animal are listed, and the arithmatic means and S.E. (for A, B and C) and logarithmic means and % S.E. (for α, β and γ) are given below. The mean values of the rate constants (α, β and γ) were used to calculate half times for each of the 3 phases of the plasma curve ($t_{1/2}=0.693$/rate constant). The computer generated parameters were also used to calculate steady-state volumes of distribution for each of the 8 cats from

$$Vd_{ss}=\text{Dose } x\left(\frac{A}{\alpha^2}+\frac{B}{\beta^2}+\frac{C}{\gamma^2}\right)\Big/\left(\frac{A}{\alpha}+\frac{B}{\beta}+\frac{C}{\gamma}\right)^2.$$

The individual values for Vd_{ss} along with the mean and S.E. at the bottom, are given in the last column.

this study. By 10 min after injection of the bolus, more than 75% of the radioactivity present in plasma is no longer parent compound. The fraction of tritium present in plasma as [^{3}H] Pu continued to decline in each case, reaching less than 2% of the total radioactivity by 240 min post injection in each experiment. Calculation of pharmacokinetic parameters for Pu from plasma curves based on measurement of total radioactivity would this result in significant errors.

Most of the tritium present in plasma after 10 min is lost upon lyophilization of the plasma hydrolysate, which contains 6 N HCl. This volatile radiolabelled component is probably ^{3}HOH which represents the end product of Pu catabolism, the ^{3}H present in [^{3}H] Pu not being exchangeable under physiological conditions. This catabolism likely starts with oxidative de-amination by diamine oxidase (E.C. 1.4.3.6) [1]. However, Table 1 also shows that not all of the [^{3}H] lost from [^{3}H] Pu is volatile under acidic conditions. We have not attempted to identify these non-volatile metabolites of Pu as only small amounts were present in our samples. Their non-volatility under acidic conditions implies the presence of a nitrogen atom, possibly in partially de-aminated products (e.g. γ-aminobutyric acid).

The initial rapid phase ($t_{1/2}=1$ min) of the plasma [^{3}H] Pu curve (Fig. 1), probably represents mixing of the [^{3}H] Pu within the plasma compartment. The second phase ($t_{1/2}=7$ min) is due to distribution of [^{3}H] Pu from the central plasma compartment to the peripheral tissues. The final phase, ($t_{1/2}=117$ min) represents elimination via both excretion and metabolism. Although the rapid decrease of plasma [^{3}H] Pu is in agreement with the plasma curves obtained by Rosenblum and Russell in their study of clearance of [^{14}C] polyamines in the rat [25], these investigators measured only total ^{14}C in plasma. Calculations based on their decay curve would lead to a lower value for Vd_{ss} and would be in error.

The data shown in Table 2 demonstrate that in some tissues considerable conversion of exogenous Pu to Sd and Sp did occur. This conversion took place to the greatest extent in liver, spleen and kidney. The presence of [^{3}H] Sd in tissue samples is remarkable, since no [^{3}H] Sd could be detected in plasma at the same time point. The data in Table 2 also demonstrate that appreciable fractions of the total ^{3}H present in the tissues examined are in the form of volatile and non-volatile catabolites of Pu. The percentage of total ^{3}H present in tissues as Pu at 300 min was never as low as

for plasma (1.5%); it ranged from a high of 80.4% (heart) to a low of 3.1% (brain-subcortex). A portion of the ^3H lost from the tissue homogenates upon lyophilization is ^3HOH, but some other volatile tritiated compounds must also be present. The conclusion is necessitated by the observation that the amount of ^3H lost (in pmole/g of tissue) is not the same for all tissues examined. Since ^3HOH would be evenly distributed throughout the entire body, its loss via lyophilization should remain constant across tissues. This observation leads us to suspect that some part of the tritium lost from the plasma hydrolysates upon lyophyllization could also be volatile compounds other than ^3HOH.

Since no metabolism of [^3H] Pu takes place in the blood compartment and both volatile and non-volatile catabolites of [^3H] Pu appear in the plasma within two min of injection of [^3H] Pu, extracellular Pu apparently enters cells where metabolism takes place; catabolites then leave cells and diffuse back into plasma. In addition to metabolism in liver, kidney, which is known to have high diamine oxidase activity [28], is a probable second major site of catabolism. While the catabolites of [^3H] Pu can cross from tissues back into plasma, Sd and Sp formed from exogenous [^3H] Pu in the tissue apparently do not to an appreciable extent.

The mean value of Vd_{ss} we have calculated is in excess of body extracellular fluid but less than total body water and is in good agreement with the observed accumulation of [^3H] Pu in the various tissues examined. Measurement of the normal serum concentration of Pu in the cat allows us to estimate the pool size of endogenous Pu in a 3 kg cat (Table 5). In addition if we assume that Vd_{ss} is a constant fraction of total body weight across mammalian species, the normal serum concentrations of Pu in the rat and in humans can be used to estimate the pool size for Pu in these two species as well (Table 5). Such estimates can be used to approximate the minimum tumor size that will result in elevated serum levels of Pu assuming that the rate of Pu release by the tumor is at least as great as that for normal tissues.

Data obtained for the four grades of astrocytoma (S. Harik, personal communication), as well as data from our own laboratories for glioblastoma and the 9L rat brain tumor, were used for the theoretical calculations, shown in Table 6. While all of this excess Pu would not be released into the plasma at once, one could possibly expect to see elevated serum levels of Pu by the time the total body content was doubled. From a modeling point of view it is somewhat encouraging to note that, within the four grades of astrocytoma, the mass of tumor needed to double the body content of Pu declines as the tumor gets more malignant. This is, of course, a result of the higher Pu content of the more malignant tumors, which in turn, may reflect either increased intracellular levels of Pu, greater cell turnover (i.e. death), or higher growth rates in these tumors. This observation is borne out by the clinical observation that large less malignant gliomas are usually not

*Table 5. *Normal serum concentrations, pool sizes and body contents of endogenous* Pu *in 3 mammalian species*

Species	Assumed average mass (kg)	Normal serum [Pu] (nmole/ml)	Pool size (nmole/kg)	Range of pool size* (nmole/kg)	Total body content of Pu (nmole)	Range of body content of Pu* (nmole)
Cat	3.0	2.50 (±1.21)†	1355	699–2011	4061	2097–6033
Rat	0.180	2.15 (±0.47)‡	1165	911–1420	210	164–255
Human	75.0	0.264 (±0.024)§	143	130–156	10,725	9750–11,700

*±1 S.E. for normal serum [Pu].
†Mean value (±S.E.) for 4 animals.
‡Mean value (±S.E.) for 3 animals.
§Mean value (±S.E.) for 17 healthy adults.

Concentrations of Pu in cat and rat serum were measured using an amino acid analyzer. Data for normal human serum was collated from previous reports [14, 29]. For each species the pool size of putrescine was calculated as the product of normal serum concentration (nmole/ml) × 1000 (ml/l) × Vd_{ss} (l/kg). The total body content of putrescine was calculated as the product of pool size (nmole/kg) × assumed average mass (kg).

Table 6. Estimates of mass of tumor that have total normal body content of putrescine for different brain tumors

Tumor type	Number of samples	Mean Pu content (nmole/g)	Mass of tumor with Pu equivalent to total body content of Pu (g)
Astrocytoma grade I	1	78	137
Astrocytoma grade II	3	94 (±14.7)	114
Astrocytoma grade III	5	127 (±8.4)	84
Astrocytoma grade IV	5	234 (±41)	46
Glioblastoma	15	131 (±18.3)	82
Intracranial 9L rat brain tumor	4	610 (±114)	0.34

Tissue content of Pu was measured by enzymatic isotopic microassay for astrocytoma samples (S. Harik, personal communication) and using an amino acid analyzer for glioblastoma and 9L rat brain tumor samples. The mean values (±S.E.) are listed and were used to calculate the mass of each type of tumor that would contain an amount of Pu equal to the total normal body content of Pu for that species. The mass of tumor was calculated as the quotient of total body content (nmole) from Table 5 ÷ mean tumor content (nmole/g).

accompanied by elevated CSF Pu, while smaller, more malignant gliomas usually result in CSF Pu levels greater than two S.D. above normal [15, 16].

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