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THE METABOLISM AND GASTROINTESTINAL ABSORPTION OF NEPTUNIUM AND PROTACTINIUM IN ADULT BABOONS

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ABSTRACT

The metabolism of neptunium and protactinium was studied in adult female baboons following intravenous injection and intragastric intubation. Neptunium-239, Np-237, and Pa-233 were prepared as either citrate-buffer, nitrate, or bicarbonate solutions with oxidation states of (V) and (VI). Samples of blood, urine, feces and autopsy tissues were measured by gamma spectrometry. Retention of neptunium and protactinium was determined *in vivo* using whole and partial body gamma-scintillation spectrometry with [NaI-CsI(Tl)] detectors.

Immediately following intravenous injection (10^{-1} to 10^{-10} mg Np per kg body wt), neptunium cleared rapidly from blood, deposited primarily in the skeleton (54±5%) and liver (3±0.2%), and was excreted predominantly via urine (40±3%). For the first year post injection, neptunium was retained with a biological half-time of ~100 days in liver and 1.5±0.2 yr in bone. In comparison, injected protactinium (10^{-9} mg/kg) was retained in blood in higher concentrations and was initially eliminated in urine to a lesser extent (6±3%). *In vivo* measurements indicated that protactinium was retained in bone (65±0.3%) with a half-time of 3.5±0.6 yr. The distribution and retention of protactinium in other tissues has not been determined at this time. Differences in the physicochemical states of the neptunium or protactinium solutions injected did not alter the metabolic behavior of these nuclides.

Fed and fasted baboons were administered solutions of Np(VI) bicarbonate (10^{-8} to 10^{-1} mg/kg) and Pa(V) citrate-buffer (10^{-9} mg/kg) by gavage. The gastrointestinal absorption value for neptunium in two fasted baboons, sacrificed at 1 day post administration, was determined to be 0.92±0.04%. Of the total amount of neptunium absorbed, 52±3% was retained in bone, 6±2% was in liver, and 42±0.1% was excreted in urine. The metabolism of neptunium following oral and iv administrations was found to be similar. This observation was also true for baboons which had received oral and iv doses of protactinium. A method was developed to estimate GI absorption values for both nuclides in baboons, which were not sacrificed, by comparison of activities present in bioassay samples after injection and gavage. Absorption values calculated by this method for neptunium and protactinium in fasted baboons were 1.8±0.8% and 0.65±0.01%, respectively. Values for fed animals were 1 to 2 orders of magnitude less than those for fasted animals. Further experiments are currently underway to evaluate this assay technique.

CONTENTS

1 INTRODUCTION

Considerable interest has been generated recently in re-evaluating the metabolic behavior of the actinide element neptunium.[1] This interest has been focused primarily on Np-237 ($T_{1/2}$=2.1x10^6 yr) which has been identified as one of the critical isotopes present in high-level nuclear waste at long-term repository sites.[2] In addition to neptunium, there is a growing concern for the increase in the production of protactinium, which is present as Pa-233 ($T_{1/2}$=27.4 days) in all nuclear waste containing its parent, Np-237, and as Pa-231 ($T_{1/2}$=3.2x10^4 yr) associated with the uranium-thorium breeder fuel cycle.[3] Furthermore, Pa-233 is a significant source of analytical interference in the *in vivo* measurement of Np-237 in man. In view of the paucity of studies concerning both neptunium and protactinium, further studies on their metabolism in mammals is essential for accurate risk assessments.

This present study on the metabolism of neptunium and protactinium in adult female baboons was designed to (1) determine the metabolic behavior of these nuclides after intravenous injection of solutions of differing physicochemical forms; (2) determine their gastrointestinal absorption factors (f_1 values) and metabolism in fed and fasted baboons; and (3) to develop a method for estimating GI absorption factors from bioassay samples which would not require the sacrifice of animals, and which could be applied to determine actinide burdens in cases of human exposure. The use of the baboon, a nonhuman primate, as a suitable subject for metabolism studies of actinides in man has been well documented.[4] Several studies, involving Am, Cm and U, have been successfully conducted at this laboratory.[5-7]

2 METHODS AND MATERIALS

2.1 Animals

Eleven adult female baboons (*Papio cynocephalus, anubis*, and hybrids), weighing between 11 and 18 kilograms, were used in this study. Animal board and husbandry were provided at New York University's Laboratory for Experimental Medicine and Surgery in Primates (LEMSIP) in Tuxedo, NY. Baboons were housed individually and maintained on a commercial diet. Water was provided ad libitum. Injection (im) of 0.1 mg/kg ketamine HCl was used to tranquilize animals prior to handling outside of confinement.

2.2 Preparation of Administration Solutions

Stock solutions of Np-237/Pa-233 and Am-243/Np-239 in 1 N nitric acid were obtained from Oak Ridge National Laboratories (Oak Ridge, TN). Solutions of Pa-233 were prepared by solvent extraction from Np-237 using diisobutyl carbinol (DIBC) as described by Sill.[8] Neptunium-239 was separated from Am-243 by cation exchange chromatography. The technique used to prepare solutions in the (V) and (VI) valence states is discussed in detail elsewhere.[9] Solutions prepared as Np(VI) or Pa(VI) contained 1 ppm chlorine as a holding oxidant. Both nuclides were administered (Table 1) in activity concentrations and chemical forms consistent with those used in actinide metabolism studies conducted at our laboratory and at others.[1]

2.3 Gamma Spectrometry and Counting Geometries

Four photon measurement systems were used to determine neptunium and protactinium gamma activities in vivo and in bioassay samples, including a low-level whole-body counter employing dual NaI-CsI(Tl) detectors, a Ge(Li), NaI(Tl) well, and a 3"x2" NaI(Tl) detector. Activity levels of Np-237, Np-239 and Pa-233 were calculated by integration of count rates in energy regions of interest between 60 to 120 keV, 220 to 280 keV, and 280 to 380 keV, respectively. Corrections were made to Np-237 determinations to account for the contribution of count rates from Pa-233. Additional alpha spectrometric measurements of Np-237 in some bioassay samples were conducted at ANL. Whole body counting of tranquilized and restrained baboons was accomplished using "meter-arc" and head count geometries. These systems and measurement techniques have been described in detail previously.[10]

TABLE I Administration protocols.

Isotope	# of Admin.	Chemical Form	pH	Oxid. State	Mode of Admin.	Dose (mg/kg)
Np-237	1	0.08M Na Citrate	3.2	V	Inj.,iv	10^{-1}
Np-237	1	0.08M Na Citrate	3.2	VI	Inj.,iv	10^{-1}
Np-239	1	0.08M Na Citrate	3.2	V	Inj.,iv	10^{-10}
Np-239	1	0.08M Na Citrate	3.2	VI	Inj.,iv	10^{-10}
Np-239	1	Nitrate	1.0	VI	Inj.,iv	10^{-9}
Np-239	5	0.01M $NaHCO_3$	6.5-8.5	VI	Inj.,iv	10^{-10}
Pa-233	2	0.08M Na Citrate	3.2	V	Inj.,iv	10^{-9}
Pa-233	1	0.08M Na Citrate	3.2	VI	Inj.,iv	10^{-9}
Np-237	3	0.01M $NaHCO_3$	6.5	VI	po,Fasted	10^{-2}-10^{-1}
Np-239	10	0.01M $NaHCO_3$	6.5	VI	po,Fasted	10^{-8}
Np-239	3	0.01M $NaHCO_3$	6.5	VI	po,Fed	10^{-7}-10^{-6}
Np-239	1	Nitrate	3.0	V	po,Fasted	10^{-8}
Pa-233	3	0.08-2.0M Na Citrate	3.2-6.5	V	po,Fasted	10^{-9}
Pa-233	1	0.08M Na Citrate	3.2	V	po,Fed	10^{-8}

2.4 Administration Protocols

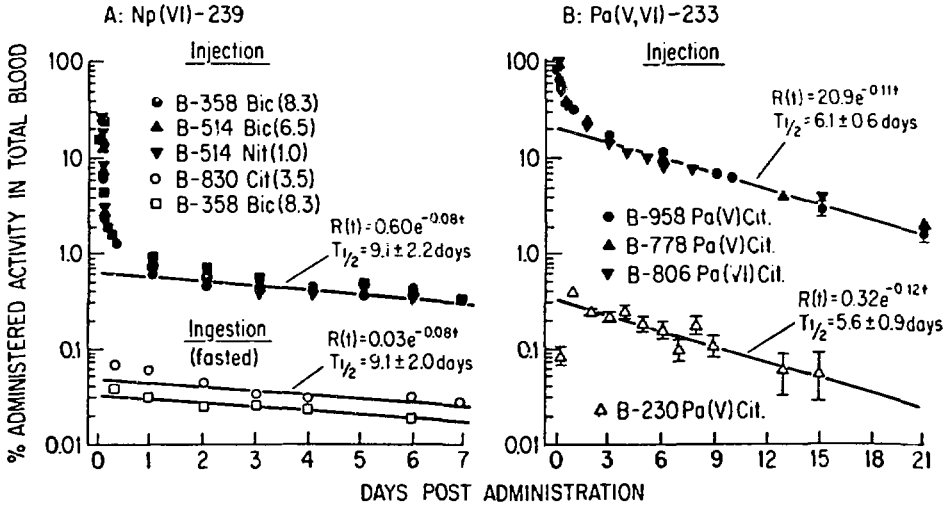
Table 1 presents the experimental protocol used. Baboons were tranquilized and injected intravenously in the saphenous or femoral vein with 1 to 2 ml of administration solution. Solutions of neptunium or protactinium in volumes of 10 to 20 ml were introduced directly into the stomachs of fed or fasted (24 hr) baboons using a catheter inserted nasally. Due to the short radiological half lives of Np-239 and Pa-233, it was possible to perform multiple administrations of these isotopes in the same animal after sufficient time had passed for their biological clearance and decay. Data presented in this paper are expressed as a percent of the administered dose for each experiment conducted. Neptunium-239 and Pa-233 values have been corrected for radiological decay. Retention for all radionuclides is expressed as biological half-times, derived from least-squares, curve-fitting techniques. Total blood volume for each animal was estimated to be 7% of its total body weight. For clarity, some data has been omitted from the figures presented.

3 RESULTS

3.1 Metabolism of Neptunium and Protactinium After iv Injection

3.1.1 Whole Blood Retention

The retention of neptunium and protactinium in total blood volume as a function of time post injection is illustrated in Figures 1a and 1b, respectively. Separate measurements of cellular and serum components of whole blood samples indicated that greater than 95% of the activity in blood was associated with the serum component. Neptunium was observed to clear rapidly from blood and was retained with a half-time of 9±2 days for the interval 2 to 7 days post injection. No differences were noted in retention following the injection of neptunium solutions of varying chemical forms or oxidation states. However, within the first 24 hours, Np-237 was retained at a two fold higher concentration than Np-239, perhaps due to the differences in the initial neptunium masses injected (10^{-1} mg/kg Np-237 vs 10^{-10} mg/kg Np-239). In comparison to neptunium, Pa-233 was present in blood in higher concentrations and retained with a half-time of 6±1 days for the time interval from 2 days to 3 weeks post injection. No difference was observed in the retention of Pa(V) and Pa(VI).

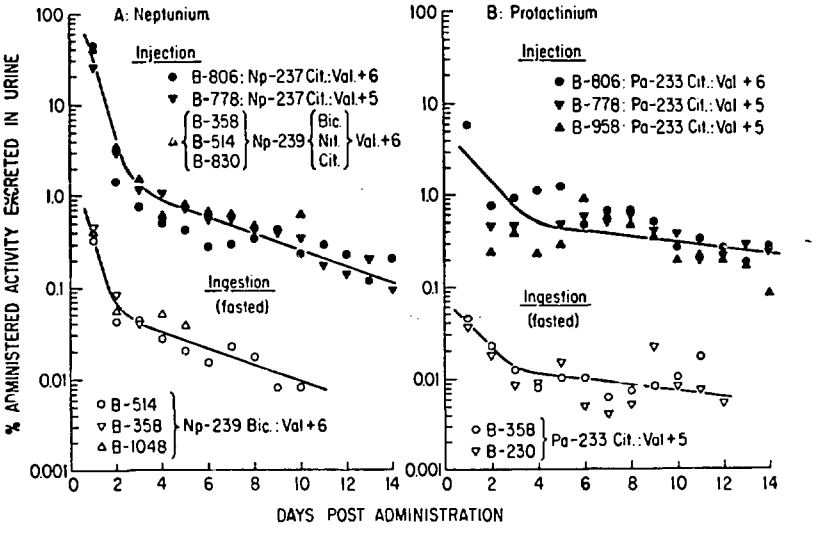


FIGURES 1a and 1b Total blood retention of Np and Pa.

3.1.2 Urinary and Fecal Excretion Patterns

Figures 2a and 2b show the elimination of neptunium and protactinium via urine as a function of time post injection. Urinary excretion was the primary route of elimination for both nuclides, especially during the first 24 hours post injection, regardless of the isotope, oxidation state, chemical form administered or volume of urine voided. For neptunium, a mean value of 33±10% (n=10) was recovered in urine within 24 hours. A cumulative amount of 45±7% was recovered by 2 months. The values of the percent protactinium recovered in urine were 6±3% for the first day and 15±4% cumulative excretion by day 21. Fecal excretion of both neptunium and protactinium was minimal. Cumulative recovery of neptunium after 27 days was 3±1%. The clearance of neptunium in feces could be described by a single

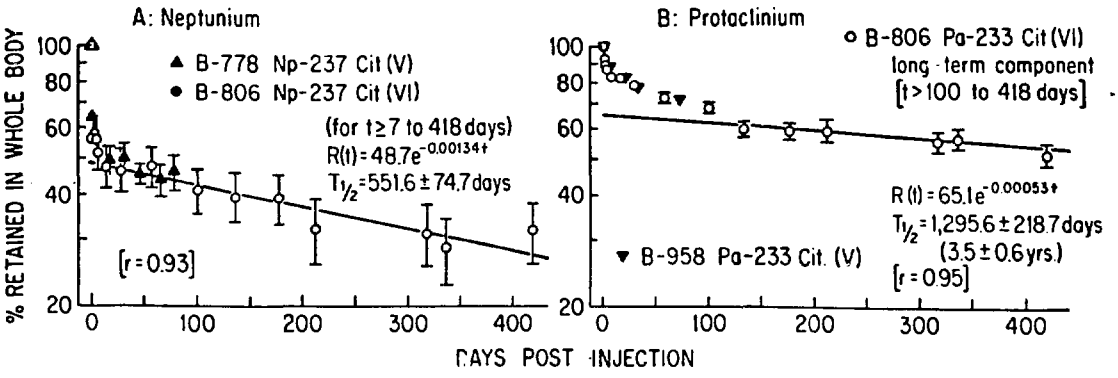
exponential equation with 0.08% of the injected activity clearing with a half-time of ~100 days. Cumulative recoveries of protactinium were 0.03% and 3% by 1 day and 1 month post injection. The long-term retention half-time of protactinium in feces was ~300 days (0.05%). Elimination of neptunium and protactinium in feces was independent of the chemical form administered.



FIGURES 2a and 2b Urinary excretion of Np and Pa.

3.1.3 Whole Body Retention

Figures 3a and 3b show the in vivo retention of neptunium and protactinium as a function of time post injection. For the first year, the long-term retention of neptunium in vivo was 1.5±0.5 yr associated with 48±2% of the injected dose. Measurement of the skull indicated that neptunium deposited rapidly on bone surfaces and was retained with a single half-time of 1.5±0.2 yr. The long-term retention component of protactinium showed that 65±2% of the Pa-233 injected was retained with a half-time of 3.5±0.6 yr. Deposition and retention of Pa-233 in bone occurred in three phases: the



FIGURES 3a and 3b Whole-body retention of Np and Pa.

long-term component had a half-time of 3.0±0.5 yr. A mass balance of the activity excreted in urine and feces with the amount retained in vivo was in good agreement for both nuclides.

3.1.4 Tissue Distribution and Retention

Table 2 presents the data on the distribution and retention of neptunium in autopsy tissues acquired at 1 and 90 days post injection. Neptunium deposited initially in the skeleton with less involvement of the liver and kidneys. At 90 days post injection, neptunium retention in bone was still high and consistent with a retention half-time of ~1 year, whereas its retention in liver was low, indicating a half-time of ~100 days. The distribution and retention of protactinium in autopsy tissues has not yet been determined. However, as stated previously, in vivo whole body and head measurements indicate that the skeleton may be one of the primary sites of deposition.

TABLE II Distribution and retention of Np in tissues.

	Intravenous injection		Gavage		
Baboon #	B-1048	B-778	B-704	B-1050	B-704
Isotope	Np-239	Np-237	Np-239	Np-239	Np-237
Dose (μCi/kg)	0.09	0.08	12.6	3.6	1.7×10^{-3}
Dose (mg/kg)	4.1×10^{-10}	1.1×10^{-1}	5.4×10^{-8}	1.5×10^{-8}	2.5×10^{-3}
Chem. Form	0.01M Bic.	0.08M Cit.	0.01M Bic.	0.01M Bic.	0.01M Bic.
Oxid. State	VI	V	VI	VI	VI
Sac. (days)	1	90	1	1	33
Fed/Fast	-	-	Fasted	Fasted	"Fasted"
Tissue	Percent of injected activity (% ± SD)		Percent of gavage activity (% ± SD)		
Liver	2.6 ±0.3	0.75±0.03	0.065±0.003	0.037±0.002	0.026±0.001
Kidney	0.5 ±0.03	0.03±0.001	-	0.011±0.004	0.001±0.0004
Other	1.8 ±0.5	0.19±0.03	-	0.007±0.002	-
Skeleton	54±5	42±2	0.45 ±0.025	0.510±0.028	0.150±0.004
Tot. Retained	57±5	43±2	0.515±0.025	0.547±0.029	0.176±0.004
Urine	40±3	53±3	0.376±0.024	0.397±0.025	0.150±0.020
Feces	0.01±0.001	4±1	-	-	-
Tot. Absorbed			0.891±0.035	0.944±0.038	0.33 ±0.020

3.2 Metabolism of Neptunium and Protactinium After Gavage

3.2.1 Whole Blood Retention

Figures 1a and 1b show the retention of neptunium and protactinium in the blood of fasted baboons at selected times post gavage. The retention half-time of neptunium in blood was 9±2 days for the time interval 2 to 7 days post gavage. The percent concentrations of neptunium in the 8 and 24 hour blood samples of fed baboons were 1 to 2 orders of magnitude less than those for fasted animals. No differences were observed in the blood retentions of high (10^{-1} mg/kg) or low (10^{-8} mg/kg) mass amounts of Np(VI) bicarbonate administered. The retention half-time of protactinium in the blood of fasted baboons was 6±1 days for the time interval 2 to 15 days post gavage. At the dose administered, protactinium could not be measured in the blood of a fed baboon, indicating that the GI absorption of Pa-233 in this animal was at least a factor of 10 lower than the absorption occurring in fasted animals.

3.2.2 Urinary and Fecal Excretion Patterns

Figures 2a and 2b show the urinary excretion curves for neptunium and protactinium after gavage. Cumulative excretion of neptunium in the urine of fasted baboons by 1 and 7 days was 0.38±0.09% (n=8) and 0.42±0.07%, respectively. Values for fed baboons were 0.003±0.003% and 0.04±0.02% for the same times. Cumulative excretion of Pa-233 was 0.04±0.01% by day 1 and 0.10±0.01% by day 7. No Pa-233 activity could be determined in the urine of a fed baboon. In general, greater than 95% of the gavaged dose of either neptunium or protactinium was recovered in feces 1 week after administration. Cross-contamination of activity in urine with activity in feces was avoided in most experiments and rarely, if ever, occurred with the first day urine samples.

3.2.3 Systemic Distribution, Retention and Absorption

Data on the distribution, retention and absorption of neptunium after gavage are presented in Table 2. The GI absorption value, i.e., the summation of the amount retained in the liver and skeleton plus the cumulative amount excreted in urine, for neptunium in two fasted baboons was 0.92±0.04%. A GI absorption value of 0.33±0.02% was obtained for a third animal at sacrifice on day 33 and suggested that this baboon may have been only partially "fasted" at the time of administration. The distribution of neptunium in each tissue relative to the total amount absorbed was 52±3% in the skeleton, 6±2% in liver, 1.2±0.4% in kidney, and 42±0.1% eliminated in urine for day 1, and 46±3% in bone, 8±0.6% in liver, 0.3±0.1% in kidney, and 46±7% eliminated in urine by day 33. Determinations of the distribution and retention of neptunium in fed baboons, and of protactinium in fed and fasted animals, are currently in progress.

3.2.4 Estimation of GI Absorption Factors

As a result of the noted equivalence of the metabolism of neptunium or protactinium after iv and oral administrations, a method[11] was used to estimate GI absorption values for both nuclides without necessitating animal sacrifice. This method involved a comparison of activity values measured in 8 hour and 1 day bloods, 1 day urines, and biopsy samples of liver and caudal vertebrae obtained after injection and gavage. Table 3 shows that GI absorption values for neptunium and protactinium calculated by this method.

TABLE III Estimated GI absorption values.

Isotope	Oxid. State	Feeding Regimen	Estimated GI absorption values for Np and Pa[a] (% ± SD) Bloods	Urine	Liver Biopsy	C. Vertebrae	Mean Absorption Values (% ± SD)
Np-239	V,VI	Fasted	2.7 ±0.9 (11)[b]	1.1 ±0.2 (7)	1.7±0.4(1)	-	1.8 ±0.8
Np-237	VI	Fasted	2.5 ±1.8 (4)	1.2 ±0.4 (1)	-	1.5±0.4(1)	1.8 ±0.7
Np-239	VI	Fed	0.07±0.03(2)	0.01±0.002(3)	-	-	0.04±0.05
Pa-233	V	Fasted	0.65±0.30(3)	0.64±0.11 (2)	-	-	0.65±0.01

(a) See text; (b) Number of determinations.

4 DISCUSSION AND CONCLUSIONS

4.1 Metabolism of Neptunium and Protactinium After iv Injection and Gavage

Data reported in this study show the similarity in the metabolism of neptunium administered either intravenously or orally as solutions of differing chemical forms and doses. These data also suggest that the fractions of the administered dose of neptunium transferred from blood to the skeleton, liver, and urine will be 0.50, 0.05, 0.01, and 0.45, respectively. The retention half-times for neptunium in bone and liver were calculated to be 1-2 years and ~100 days, respectively, for periods of up to 1 year post administration. The gastrointestinal absorption factors for neptunium were 1×10^{-2} (1%) for fasted baboons administered Np(VI) bicarbonate with 1 ppm chlorine, and 1 to 2 orders of magnitude less for fed baboons. Table 4 presents a comparison of the transfer coefficients and f_1 values for soluble neptunium compounds suggested by this study with those recommended by the ICRP[12,13] and Thompson.[1] Our data is comparable to those listed in this table with the major exception that our half-times for bone and liver are much shorter. Additional studies on the long-term retention of neptunium in animals is indicated.

Data presented in this study also shows the similarity in the metabolism of injected and orally administered protactinium. Although preliminary, it suggests that the transfer coefficient to bone is ~0.65, with a retention half-time of 3.5 yr. The f_1 value for protactinium in fasted baboons was estimated to be ~1%. Current ICRP values for protactinium are 0.40 (100 yr) bone, 0.15 (10 and 60 days) liver and 0.43 in urine, with an f_1 value of 0.1%. Further studies on the metabolism of protactinium are currently in progress.

TABLE IV Suggested metabolic parameters for "soluble" neptunium in Man.

TISSUE	ICRP 1960	ICRP 1980	THOMPSON*	THIS STUDY
SKELETON	0.45 (200 Y)	0.45 (100 Y)	0.60 (100 Y)	0.50 (~1-2 Y)
LIVER	0.05 (150 Y)	0.45 (40 Y)	0.15 (40 Y)	0.05 (~100 D)
KIDNEY	0.03 (175 Y)	-	-	-
EXCRETED	0.47	0.10	0.35	0.45
F_1 VALUE**	1×10^{-4}	1×10^{-2}	1×10^{-3}	1×10^{-2}

* Ref. (1), p. 25; ** GI absorption value.

4.2 Estimation of GI Absorption Values

Mean GI absorption values estimated for neptunium and protactinium using the iv/oral method (Table 4) are comparable to those values reported for these nuclides in this study and in others.[1,14] If valid, this method may be applicable for determining actinide burdens in cases of human exposure.

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