ANNUAL REPORT

to the

ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION

Title of Project: RADIOACTIVITY STUDIES

Period Covered by Report: July 1, 1974 to June 30, 1975

Co-Investigators: Merrill Eisenbud
Norman Cohen

Institute of Environmental Medicine
New York University Medical Center

Contract Number: AT (11-1) 3382-14- - - -

Supported by: Energy Research & Development Admin.
Division of Biomedical & Env. Res.

Date of Report: April 1, 1975

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VOLUME I

INVESTIGATION OF THE RETENTION AND DISTRIBUTION
OF AMERICIUM-241 IN THE BABOON AND THE ENHANCED
REMOVAL OF AMERICIUM-241 FROM THE BODY BY
DIETHYLENETRIAMINEPENTAACETIC ACID (DTPA)

by

Raymond A. Guilmette
Norman Cohen
and
McDonald E. Wrenn

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ABSTRACT

Experiments have been performed to study the metabolism and distribution of intravenously administered ^{241}Am in the adult and juvenile baboon; in addition, decorporation therapy using $\text{Na}_3\text{-CaDTP}$ was performed on selected baboons to assess the efficacy of this drug in removing systemic burdens of ^{241}Am from this primate species. Determination of the kinetics of ^{241}Am was accomplished principally by *in vivo* methodologies and by radiochemical analysis of ^{241}Am activity of biological material.

The major sites of deposition of ^{241}Am are the liver and the skeleton. Upon intravenous injection of Am as the citrate, the nuclide is cleared rapidly from the circulation; less than 10% of the injected dose remains in the blood one hour post-injection while less than 1% ID remains twenty-four hours later. The measured whole body retention of ^{241}Am indicates that 40% ID is cleared from the body with half-times ranging from 17-36 days while the remainder of the deposited ^{241}Am is retained with 1.2-5.5 year half-times. The short-term clearance of ^{241}Am reflects mainly the excretion of Am from the liver. This organ was measured to contain about 31% ID at one day post-injection, and this liver burden was cleared with a 28 ± 6 day half time. The retention of ^{241}Am in the skeleton was much more prolonged. No statistically significant changes in the ^{241}Am content of the skeleton (after an initial period of uptake) could be demonstrated for as long as 2.25 years post-injection.

The major route of excretion of ^{241}Am , after the first days post-injection, is via the feces. This route has been shown to account for the great majority of the ^{241}Am that is cleared from the liver. The excretion of ^{241}Am continued

throughout the study in all animals. Am-241 could be measured in both the urine and feces as long as 2.25 years post-injection although at very low levels.

The use of $\text{Na}_3\text{-CaDTP}$ as a therapeutic agent for the removal of ^{241}Am from the body proved to be an effective form of treatment in the case of early administration. A total of 80% of the injected dose was removed during one month of therapy, more than 50% of which was due to the DTPA chelation therapy. If the beginning of therapy were delayed, the effectiveness of treatment decreased considerably. Nevertheless, treatment delayed as long as one year post-injection of ^{241}Am was still judged to be of value in removing persistent deposits of ^{241}Am . Long-term therapy in this situation was, however, considered necessary.

Results of studies using juvenile baboons were equivocal. Clearance of ^{241}Am from the blood was at least as rapid as the adult, as was clearance from the liver. Retention of Am in the skeleton of the juvenile was also shown to be long. The use of $\text{Na}_3\text{-CaDTP}$ was probably more effective in the juvenile than in the adult; this may be related to a greater ease of removal of ^{241}Am from the juvenile skeleton.

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CHAPTER I

INTRODUCTION

Americium, element 95, was first produced and isolated by Seaborg and his colleagues in 1944 (1946). Scarcely two years later, when only milligram amounts of americium existed, biological studies of americium isotopes were initiated. Preliminary results indicated that the isotopes were extremely hazardous substances (Hamilton 1947,48). Americium-241 (Figure I-1) exhibits many of the characteristics of radium-226, a nuclide of known radiotoxicity. These similarities include decay by alpha particle emission, long physical halflife, and selective deposition and long biological retention times in the skeleton. The radiotoxicity of Am, together with its increased production as a by-product present in nuclear reactor waste, has transformed this and other actinides from scientific curiosities to subjects of present and future concern.

Americium does not occur naturally but only as a manmade element. The nuclide ^{241}Am is produced as a result of β^- decay of ^{241}Pu , which is present to some degree in all plutonium formed in isotope production and power reactors. The genesis of ^{241}Am from the most common fuel in use today, $^{235,8}\text{U}$ is illustrated in Figure I-2. The amount of ^{241}Am produced in a given power reactor will depend upon the fuel composition and the total amount of neutron irradiation that has occurred during the life of the fuel, i.e. the neutron exposure (neutrons/cm²). Estimates of future U.S. inventories of ^{241}Am and ^{243}Am have been upgraded periodically from E. D. Arnold's (1957) estimate of a total of 300 kg of Am and Cm by the year 2000

Figure I-1

Decay Scheme of Am-241

data taken from Lederer, C.M., Hollander, J.M.,
Perlman, I., Table of Isotopes, 6th edition,
J. Wiley and Sons Inc., N.Y. 1967 except for
the half-life of Am-241 which was taken from
Oetting, F.L., Gunn, S.R., J. Inorg. Nucl.
Chem. vol. 29, p 2659 (1967)

Figure I-1

* xxx(yy) signifies photon energy in MeV of the transition, xxx, and percent abundance for the transition from the given energy level, yy.

Major Alpha Decay Scheme:

α_0	5.545 MeV	(0.25% occurrence)
α_{33}	5.513 MeV	(0.12%)
α_{60}	5.486 MeV	(86%)
α_{103}	5.443 MeV	(12.7%)
α_{159}	5.348 MeV	(1.3%)

Major Photon Emissions

Np L-Xrays (37.6% abundance)

γ_1	0.026 MeV	(2.5%)
γ_3	0.043 MeV	(0.07%)
γ_5	0.0596 MeV	(36%)
γ_7	0.099 MeV	(0.024%)

Figure I-2

Production of Am-241 by Slow Neutron Bombardment of
U-235 and U-238 (Reactor Production of Am-241)

Figure I-2

to Seaborg's (1968) more recent projections of 80 kg of $^{241,3}\text{Am}$ per year by 1975 and 300 kg/yr by 1980 giving a total of more than 6000 kg by the year 2000. The principle source of americium is the nuclear reactor, whether it is the water cooled, uranium-fueled variety in use today for the production of electric power or the fast-breeder reactor of the future, which will use a fuel mixture of uranium and plutonium. If current nuclear power projections are realized, the quantity of americium produced as waste or by-product material in the next 25 years will amount to hundreds of megacuries.

Although the majority of ^{241}Am found in spent fuel material will probably be treated as a contaminant, especially as regards plutonium separation, there are certain technological applications of ^{241}Am that may make the chemical separation of this nuclide economically feasible in the future. At present, the production of pure ^{241}Am is accomplished by bombarding ^{239}Pu with neutrons. A listing of some present and future technological applications of ^{241}Am are presented in Table I-1. As can be seen, considerable ingenuity has been employed in making use of the radiological decay properties of ^{241}Am , i.e. its alpha and gamma emissions, or neutrons from Am-Be sources. The use of ^{241}Am , as a thermal or thermoelectric power source, however, is probably not forthcoming due primarily to its low specific activity (and low specific power - 0.11 watt/gm) as compared with several other α - emitters which have been used as power sources, e.g. ^{238}Pu (0.48 watt/gm), ^{244}Cm (2.3 watt/gm), and ^{210}Po (140 watt/gm) (Levert and Helminsky, 1973).

TABLE I-1

Applications of ^{241}Am *

^{241}Am as a source of
alpha particles

Fire detector (ionization of surrounding atmosphere)
Beryllium detector (charged particle activation-detection)
Light element detection (α -induced fluorescence)
Static charge elimination, e.g. paper industry
Density measurements
Altitude measurements
Gas pressure measurement via α -range determination
Heat generation, current generation
Ionization of air, in lightning rod application
Changing charge imbalance after air conditioning via
ionization
Determination of thickness in thin metal foils, polymers
Hole indicator device
Humidity measurement
Determination of gas components in mixtures
Ion source in gas chromatography

^{241}Am (Am/Be) neutron
source

Soil moisture determination
Soil density determination
Road logging
Well logging
Carbon in iron ores (activation techniques)
Chromium in chrome concentrated sample
Aluminum in a variety of processes
Phosphorus in human bone
Moisture content in sintered material mixtures or
glass and concrete
Testing of tar impregnation of magnesite bricks

*from Levert and Helminski (1973)

TABLE I-1 Contd.

Applications of ^{241}Am *

^{241}Am Photon Source	<p>Water content during transient flow in impact sand</p> <p>Bone density measurement</p> <p>Bone mineral content determination</p> <p><i>In vitro</i> measurement of fat/lean ratios</p> <p>Thickness gauging of glass and primary metals</p> <p>Thickness measurement of aluminum material</p>
<p>Photon Source</p> <p>Backscatterer techniques</p>	<p>Analysis of concrete, coal ash, road surfaces</p>
<p>X-ray Fluorescence</p> <p>Spectroscopy</p>	<p>Alloy analysis, metals analysis, heavy element ore analysis</p> <p>Trace element determination</p>

*from Levert and Helminski (1973)

As the nuclear industry grows, the potential for accidental exposure of occupational personnel to americium and other actinides will continue to increase. In addition the use of ^{241}Am in various devices used by industry (as illustrated in Table I-1) may extend the size of the "population at risk" to ^{241}Am contamination. Even though the potential amount of ^{241}Am in all of these instruments is small compared to the levels found in spent reactor fuel, it is nevertheless large compared to the maximum permissible body burden of this nuclide (Table I-2).

Several occurrences of human exposure to ^{241}Am with subsequent measurable amounts of the nuclide deposited in the body have been reported (Smith 1972). The principle modes of exposure to ^{241}Am were either by inhalation, or by percutaneous absorption via puncture wounds or lacerations. In addition, many cases of contamination by the plutonium isotopes $^{238,9,41}\text{Pu}$, nuclides exhibiting similar but not identical metabolic patterns to americium, have been documented (Norwood et al. 1956, 1958). Since most plutonium has some fraction as ^{241}Pu , it will also have ^{241}Am , its decay product.

Of particular interest has been the accidental exposure of a scientist and his family to ^{241}Am . This exposure presumably occurred by inhalation of ^{241}Am attached to particulates which became airborne as a result of flaking of a plated source (~ 10 mCi) that the scientist was using in his home workshop for research purposes (Whalen and Davies, 1971). Unfortunately over six years elapsed between the postulated time of the major exposure to the time that the contamination incident was discovered. Shortly thereafter the individuals involved in the accident were measured for residual ^{241}Am contamination by *in vivo* gamma-scintillation counting at the Institute of

TABLE I-2

Permissible Amounts of ^{241}Am in Body Organs of Reference
and Maximum Permissible Body Burdens (MPBB)

Organ	Mass of organ (g)	Fraction in organ to that of whole body	Effective Energy (Mev)	Permissible amounts of ^{241}Am in organ (μCi)	MPBB (μCi)
bone	7000	0.71	280	0.042	0.059
liver	1700	0.07	57	0.025	0.36
kidney	300	0.04	57	0.0044	0.10

Environmental Medicine. These measurements showed detectable levels of ^{241}Am in all six family members. However, only the scientist, age 50 at exposure, and the son, age 4 at exposure, were judged to have levels of contamination sufficient to warrant follow-up study by this laboratory.

Total body measurements indicated that at the time of the first measurements the scientist had a burden of 89 ± 4 nCi of ^{241}Am and the son had 40 ± 3 nCi (Wrenn et al. 1972). Latest measurements performed approximately 3 1/2 years after the first measurements show that the body burdens have decreased to 70.7 ± 2.9 nCi in the scientist and 19.7 ± 2.0 nCi in his son (Losasso et al. 1974). This decrease is probably due to metabolic clearance from its deposition sites and to accelerated ^{241}Am removal by two series of DTPA-chelation treatments (Wald, 1974).

Direct involvement and followup studies of these two ^{241}Am -contaminated individuals has given direction to present investigations of the metabolism of ^{241}Am in experimental animals. This work is part of ongoing studies designed to extend the knowledge of ^{241}Am metabolism and distribution using the baboon as experimental animal. Although there exists a growing body of information concerning both ^{241}Am metabolism and efficacy of enhanced removal by DTPA therapy, there has been little experimental data available for the primate species as the test animal. Cohen and Wrenn (1972) have stated that the baboon is an excellent experimental choice for studies of bone-seeking radionuclides in man. The selection of the baboon, *Papio*, was based on the following advantages 1) bone and tooth metabolism analogous to that of man, i.e. remodeling rate, accretion, resorption etc. 2) bone surface to volume ratio similar to that of man 3) a life span of sufficient length

for carrying out long-term studies 4) anatomic characteristics similar to that of man for performing *in vivo* whole body, organ and skull measurements 5) adequate animal size for obtaining blood volumes for routine hematology, clinical chemistry and sequential radionuclide concentration measurements in plasma and red cell fractions, and 6) resilient constitution for withstanding sequential liver and bone biopsies for concentration measurements and autoradiography prior to sacrifice (Cohen and Wrenn, 1972).

Although the baboon does have many advantages as a model for human metabolic behavior, it does share some disadvantages with other large experimental animals, i.e. cost and large space requirements. Thus the use of a small number of baboons was inevitable. The interpretation of results obtained herein are presented with the full realization of the limitations imposed by the small number of animals used.

CHAPTER II

RESEARCH OBJECTIVES

The present studies were undertaken with the following objectives in mind. 1) To study the normal metabolism and distribution of ^{241}Am in the adult and juvenile baboon. The decision to include juvenile baboons in these studies was prompted by the known exposure of the son of the previously mentioned scientist and the lack of data on ^{241}Am metabolic behavior in immature animals. 2) To study the ability of trisodium calcium diethylenetriaminepentaacetate $\text{Na}_3(\text{Ca-DTPA})$ to accelerate the excretion of ^{241}Am deposited both in soft tissue and bone. Decorporation of ^{241}Am with DTPA, the most effective single drug in treating poisoning by many actinides, has been studied in small animals and beagles. The importance of defining the action or effectiveness of DTPA in a primate species is of great value. 3) To evaluate certain factors which may affect the efficiency of DTPA in removing ^{241}Am from the body. Many parameters dealing with the therapy itself, i.e. dose of DTPA, dose rate, route of administration and interval between time of exposure and initiation of treatment, may play significant roles in either determining therapy regimens or estimating the efficacy of the treatment for a given case of exposure. 4) To obtain preliminary data on toxicological changes which may result from the therapy.

The end result of this research will hopefully be an addition to the body of information dealing with ^{241}Am normal metabolism and decorporation by DTPA. The application of these results to the human situation should then proceed with qualification and great care.

CHAPTER III

EXPERIMENTAL MATERIALS AND PROCEDURES

Six adult and two juvenile Kenya¹ baboons were used in this study. The choice of female rather than male baboon was made on the basis of greater ease of excreta collection, smaller size (approximately half that of the male), less aggressiveness and therefore greater ease in handling. Additionally, previous work with ^{210}Pb (Cohen 1970) and ^{241}Am (Rosen et al. 1972; Cohen and Wrenn 1973), has been carried out using the female baboon.

The animals were housed at and cared for by the staff of the New York University Laboratory for Experimental Medicine and Surgery in Primates (LEMSIP). Table III-1 shows the identification symbol, age, weight, and species, at time of injection of ^{241}Am , plus the injected dose of the isotope for the six adults and two juveniles used in this study. Since all of the adults were captured in the wild, their ages had to be estimated. This was accomplished to a large extent from the molar eruption stage followed by wear patterns on the teeth, and by estrus cycle and the weight of the animal.

Prior to injection, the animals were quarantined for 45 days for routine tuberculin testing and treatment of parasites common to the species. Normal diet consisted of nutrient biscuits (Monkey Chow Purina 25), fresh fruit, and water ad libitum. Urine and feces collections were accomplished by enclosing

¹ The term Kenya refers to either *Papio Cynocephalus*, *Papio Anubis*, or hybrids of both.

TABLE III-1

Physical Data for the Baboons used in these Studies

Baboon #	Estimated age at injection of ^{241}Am (yrs)	Weight at injection (kg)	Species*	Injected Dose ($\mu\text{Ci/kg}$)
B-458	10-12	12.25	hybrid	0.20
B-460	10-12	11.50	hybrid	0.21
B-352	12-14	16.75	anubis	0.205
B-520	11-13	14.25	hybrid	0.20
B-164	12-14	14.0	cynocephalus	0.17
B-470	14-16	15.0	hybrid	0.20
B-406	2.2	4.4	cynocephalus	0.21
B-400	3.2	6.5	hybrid	0.13

* see Footnote, page III-1

the four sides of the baboon cage with acrylic plastic, extending downward to a floor-mounted frame containing a layer of plastic (for urine) and a superimposed nylon-mesh screen (for feces). The acrylic siding served as splash guard while the screening served to separate the feces bulk from most of the urine.

Since the animals were not conditioned, mild tranquilization was a necessity whenever any procedure was performed outside of the animal cage. Tranquilization was accomplished by parenteral injection of either ketamine hydrochloride¹ at 10 mg/kg or phencyclidine hydrochloride² at 1 mg/kg. The choice of tranquilizer was dictated by the length of the procedure to be performed. Vetlar produces a torpid state for about 10-20 minutes while Sernylan will produce a similar state for approximately one hour.

Preparation of the ^{241}Am Injection Solution

The ^{241}Am used in this experiment was purchased³ as the nitrate, $^{241}\text{Am}(\text{NO}_3)_4$ in 0.1 N nitric acid. For injection the ^{241}Am was converted to the citrate form i.e. 0.08 M citric acid-sodium citrate buffer solution at pH 3.5, and ultrafiltered through Millipore filters of pore size $\leq 0.45 \mu\text{m}$. The chemical and physical form of the injection solution was chosen to duplicate the solution administered to beagles in the University of Utah ^{241}Am toxicity study (Lloyd et al. 1970). The use of small pore size filters for ultrafiltration serves two purposes 1) to remove pyrogens and solid impurities from the injection solution and 2) to assure the presence of monomeric americium. Although it has been shown (Starik and Ginsberg, 1960)

1 Vetlar (Parke Davis)

2 Sernylan (Bioceutic Labs Inc.)

3 ICN, Irvine, California

that low concentrations of americium exist in the ionic form from pH 1 to pH 4.5, the use of ultrafiltration is one extra step to assure the monmericity.

The ^{241}Am solution was injected into either left or right saphenous vein of the baboon at a dose of approximately 0.2 $\mu\text{Ci/kg}$ body weight. Actual amounts injected are listed in Table III-1.

Chelation Techniques

Decorporation therapy was performed using the trisodium calcium salt of diethylenetriaminepentaacetic acid¹ (Figure III-1). The initial dose chosen for administration was similar to that suggested by Norwood and Fuqua (1969) i.e. in this case 11.4 mg DTPA/kg (or 0.028 mM/kg).

Administration of the chelating agent was by intravenous infusion, with exceptions as noted. All procedures were performed on tranquilized and restrained baboons. Intravenous infusion was accomplished by catheterization of either the cephalic or median cubital veins of either arm. Attempts were made to minimize the trauma to the veins since it was known that many injections would be performed on each animal. The exclusive use of indwelling catheters and the rotation of injection sites aided in this respect. The drug itself was administered by slowly injecting the DTPA midstream of a slowly running isotonic saline intravenous infusion system. Once the drug had been completely injected, the infusion system was purged with more saline to assure delivery of the complete dose.

¹ Supplied through the Division of Biological and Environmental Research ERDA.

Figure III-1

Chemical Structure of Trisodium Calcium
Diethylenetriaminepentaacetate ($\text{Na}_3\text{-CaDTP}$)

Molecular Weight = 497

Molecular Weight of the Acid Form (DTPA) = 393

from Brick, I.B., Rath, C.E., Evaluation
of Trisodium Calcium Diethylene-Triamine
Penta-Acetate in Hemochromatosis and
Transfusion Hemosiderosis, from Iron
Metabolism, a CIBA Symposium, F. Gross
ed., Springer-Verlag, Berlin, 1964

Figure III-1

Molecular Weight: 497

Molecular Weight
of the Acid Form: 393.4

Clinical Measurements

To monitor the general health of the baboons, selected clinical parameters were measured periodically. Hematocrit, hemoglobin concentration and white blood cell counts were performed approximately once a month. Routine urinalysis and clinical blood chemistries (SMA-12) were also performed weekly when chelation therapy was scheduled. These latter tests were performed by a private accredited clinical laboratory.¹ Since uncontaminated urine was required for complete urinalysis it was necessary to catheterize the baboons. Particular care was taken to prevent urinary tract infections. In the rare case of urinary tract infection, the animal was treated with broad spectrum antibiotics.

In Vivo Counting Procedures

All *in vivo* counting of the baboons was performed in the whole body counter of the N.Y.U. Institute of Environmental Medicine. The facility consists of a 2.4 m x 2.4 m x 2.4 m room constructed from 15.2 cm of aged steel. In addition a series of absorbing metals of decreasing atomic number has been laminated on the interior surfaces of the room. This shielding consists of 5.08 mm of lead, 0.7 mm of cadmium and 0.38 mm of copper. The purpose of this "graded-Z" shield is to selectively reduce the low-energy background ($E < 100$ keV), a region which is obviously crucial for counting low energy gamma and x-ray emitters. The minimum significant measurable activity (MSMA) (Altshuler and Pasternack 1963) at the 95% confidence level for a four minute meter-arc count of ^{241}Am in an adult baboon is 4.6 ± 0.88 nCi.

¹ Automated Biochemical Laboratory Inc., Spring Valley, N. Y.

The detection device used for measuring low energy gamma and x-ray emitters, i.e. $E < 100$ keV, consisted, in this experiment, of a 15.2 cm dual NaI-CsI crystal detector. The active crystal volume consisted of a 0.3 cm NaI(Tl) layer backed by a 5.08 cm. CsI(Tl) layer. The sensitive surface of the crystal detector was covered by a 0.13 mm thick sheet of beryllium. The principle of operation of the crystal and its associated electronic system has been described in detail (Laurer and Eisenbud 1968). Briefly the operation of the system makes use of two physical principles: first the differing fluorescence decay times of NaI(Tl) and CsI(Tl) give rise to differing pulse rise times as produced by the photomultiplier tube. These leading edges are differentiable by suitable electronics. Secondly the thickness of the NaI(Tl) layer is made as thin as possible without sacrificing too much efficiency for photons of $E < 100$ keV, but at the same time minimizing the probability of interaction of a higher energy photon within the NaI layer. Together with anticoincidence circuitry the system reduces background due to Compton scattering of higher energy photons, a considerable source of low energy background.

Three geometric configurations for *in vivo* measurement were used on the baboons: 1) modified meter-arc for determination of whole body burden of ^{241}Am ; 2) liver area for determination of amount of ^{241}Am present in the liver; 3) skull area for estimating the skeletal burden by measurement of a given mass of bone, the skull. Since each of these measurements is important and has its own technique and associated difficulties, each will be discussed separately.

Modified Meter-Arc *In Vivo* Measurement of ^{241}Am Burdens

Figure III-2 illustrates the geometry for performing the modified meter-arc or whole body measurement on an adult and a juvenile baboon. The restraining device shown in Figure III-2 was designed to maintain as uniform a curvature of the body as possible so that all points on the body of the baboon would be approximately equidistant from the scintillation detector. Because of the large distance between source and detector, the efficiency of detection of ^{241}Am is relatively low compared to other counting geometries. However, because the meter-arc source-to-detector distance is substantial, the variability in counting efficiency becomes fairly insensitive to small changes in positioning. Table III-2 lists the counting efficiencies for the six adult animals injected during this study. A mean calibration factor of 0.83 ± 0.03 net cpm/nCi is equivalent to one net count for approximately every 1000 decays of ^{241}Am and indicates that the variation from animal to animal is not large (3.7% relative error).

As a second method of checking the accuracy of the efficiency (which in the case of the results shown in Table III-2 is based on 1) the net cpm obtained 15-30 minutes after injection and 2) the injected dose in nCi), the meter-arc *in vivo* measurement obtained before sacrifice was divided by the body burden as measured by radiochemical analysis of most of the bone and tissue (Table III-3). This efficiency should theoretically be the same as the initial efficiency if 1) the dose of ^{241}Am injected was accurately known and 2) the efficiency of the measurement of ^{241}Am in the body was not dependent on its distribution, i.e. which tissue, and where in the tissue the ^{241}Am was deposited. As can be seen by comparing the results in Tables III-2 and III-3, the variation in the values of calibration factor for a

Figure III-2

Geometry for Modified Meter-Arc *in vivo* Measurement of
 ^{241}Am in the Baboon

Position

- 1 Whole Body Count
- 2 Head Count
- 3 Liver Area Scan

Position 1

NaI(Tl)-CsI(Tl) Dual Crystal

Position 2

$\frac{1}{4}$ " Steel
Plate

Position 3

CsI(Na)

APPOX. SCALE 1"=6"

CHAPTER III - 2

Counting Efficiency for ^{241}Am in Adult and Juvenile Baboons Based on the Modified
Meter-Arc Geometry Whole-Body Measurement and the Injected Dose

Animal #	Net cpm	Injected Dose (nCi)	Efficiency (cpm/nCi)
<u>Adults</u>			
B-458	2024	2450	0.826
B-460	2142	2420	0.885
B-352	2797	3440	0.813
B-520	2266	2840	0.798
B-164	2035	2380	0.855
B-470	2467	3020	0.817
Mean efficiency			0.832 ± 0.03
<u>Juveniles</u>			
B-406	968	935	1.035
B-400	883	873	1.010

TABLE III - 3

Counting Efficiency for ^{241}Am in Adult Baboons Using the Modified Meter-Arc
Geometry Whole Body Measurement and the Radiochemically Determined Body
Burden at Sacrifice

Animal #	Net cpm	Body Burden (nCi)	Counting efficiency (cpm/nCi)
B-458	920	1260	0.730
B-460	682	796	0.856
B-352	1105	1307	0.845
B-520	748	926	0.808
B-164	390	485	0.803
B-470	2376	2670	0.890

Mean of counting efficiencies

0.824 ± 0.06

given animal at injection and at sacrifice can be as great as 10%. However the mean counting efficiencies 0.83 ± 0.03 cpm/nCi and 0.82 ± 0.06 cpm/nCi are not statistically different.

In addition to the above results, a series of whole body measurements were performed on three baboons after sacrifice in order to determine if there was a measurable difference in counting efficiency when ^{241}Am is deposited in bone compared to when ^{241}Am is deposited in soft tissue (mainly liver). To accomplish this goal, two experimental animals, B-352 and B-520 were first counted by *in vivo* techniques before sacrifice, then exsanguinated. Immediately after sacrifice, the abdomen was opened surgically and the livers were "loosened", i.e. arteries, veins, and ligaments were cut but the liver left in its original anatomic position. The animals were then recounted in the same meter-arc geometry. Subsequently the liver of these animals were replaced by "phantom livers", i.e. calves liver labelled as uniformly as possible with known amounts of ^{241}Am , inserted into the anatomic position occupied by the original liver and then recounted. A third adult baboon, B-622 which had not been previously injected with ^{241}Am acted as a control since counting ^{241}Am -labelled phantom livers in its carcass allowed the determination of an efficiency factor for ^{241}Am present in soft tissues alone without the contribution from ^{241}Am at other deposition sites. Conversely counting of B-520 and B-352 with "unlabelled" livers made it possible to determine an efficiency factor for ^{241}Am body burden of which an average of 90% was skeletally deposited. (Table III-4)

The whole-body counting efficiency for ^{241}Am deposited in either liver, lung, or bone, based on the measurements of the three baboons was

TABLE III - 4

Determination of the ^{241}Am Counting Efficiency for the Modified Meter-Arc Geometry
using Sacrificed Baboons and Soft-Tissue Organs Artificially Labelled with ^{241}Am

<u>Animal</u>	<u>Distribution of ^{241}Am</u>	<u>Activity in liver (nCi)</u>	<u>Activity in lung (nCi)</u>	<u>Total Body Activity (nCi)</u>	<u>Calculated efficiency (cpm/nCi)</u>
B-352 before sacrifice	85% SK 15% ST	68	15.5	1307	0.85 ± 0.006
B-352 after sacrifice	85% SK 15% ST	68	15.5	1307	0.90 ± 0.007
B-352 after sacrifice	90% SK 10% ST	0	15.5	1239	0.90 ± 0.007
B-352 after sacrifice	68% SK 32% ST	400	15.5	1639	0.88 ± 0.007
B-352 after sacrifice	83% SK 17% ST	100	15.5	1339	0.89 ± 0.006
B-520 before sacrifice	94% SK 6% ST	2	10.8	923	0.81 ± 0.007
B-520 after sacrifice	94% SK 6% ST	2	10.8	923	0.91 ± 0.008
B-520 after sacrifice	94% SK 6% ST	0	10.8	921	0.90 ± 0.008
B-520 after sacrifice	92% SK 8% ST	25	10.8	946	0.89 ± 0.008
B-520 after sacrifice	89% SK 11% ST	51	10.8	972	0.88 ± 0.008
B-520 after sacrifice	85% SK 15% ST	101	10.8	1022	0.87 ± 0.007

SK = skeleton

ST = soft tissue

TABLE III - 4 (Cont'd)

<u>Animal</u>	<u>Distribution of ^{241}Am</u>	<u>Activity in liver (nCi)</u>	<u>Activity in lung (nCi)</u>	<u>Total Body Activity (nCi)</u>	<u>Calculated efficiency (cpm/nCi)</u>
B-622	100% ST	25	0.0	24.7	0.90 ± 0.04
B-622	"	51	0.0	51	0.90 ± 0.03
B-622	"	101	0.0	101	0.91 ± 0.02
B-622	"	0	45.7	45.7	0.96 ± 0.04
B-622	"	25	45.7	70.4	0.93 ± 0.03
B-622	"	51	45.7	96.7	0.83 ± 0.02
B-622	"	101	45.7	146.7	0.93 ± 0.02

Mean of all counting efficiencies (after sacrifice)

0.90 ± 0.029

Analysis of Variance for the sample means of each of the three phantom baboons indicates that one cannot reject the hypothesis that all the group means are equal at $p = 0.05$

$$H_0 : M_1 = M_2 = M_3 \text{ at } p = 0.05$$

SK = skeleton

ST = soft tissue

0.90 ± 0.03 cpm/nCi. No statistical difference could be found between counting ^{241}Am deposited in the major soft tissue sites viz. liver and lung versus ^{241}Am deposited in bone. In addition, no detectable difference in resolution as measured by the full width of the photopeak at one half the peak magnitude (FWHM), was found in the 60 keV peak counted for ^{241}Am in bone as compared to ^{241}Am in soft tissue based again on these measurements of the phantoms. Previous phantom experiments by Wrenn et al. (1971) indicated that a change in resolution of the 60 keV peak of ^{241}Am was related to the effective depth of the ^{241}Am within the absorber, i.e. the peak width increased as the absorber thickness increased. In the case of their experiments, the absorber used was water. Since no change in resolution was observed in the case of the baboon phantoms, the distribution of ^{241}Am is such that the effective depth of the isotope in the body is the same for soft tissue and for bone. It is believed that this may have occurred as a result of the large amount of soft tissue absorber as compared to bone, i.e., the amount of photon interaction that occurs in soft tissue is much greater than that which occurs in bone. However, whatever variables influence the calibration factor for counting 60 keV gammas from ^{241}Am deposited in bone and soft tissues, it is clear that the meter-arc geometry for *in vivo* measurement of whole body burdens of ^{241}Am in the baboon is a reliable method of estimating the total body burden of the animal.

Skull Measurements

In order to obtain an accurate estimate of the skeletal content of ^{241}Am by *in vivo* measurement, it is necessary to count a region of the body from which photons originate predominantly from ^{241}Am deposited in bone. Because of the sizeable amount of ^{241}Am deposited in the liver, this

prerequisite lessens the usefulness of counting any region which includes the trunk. In fact any skeletal *in vivo* measurement must take care in shielding the trunk from the region being counted since the contribution from non-skeletal activity may be significant to the total measured count-rate. The choice of the skull as an estimator of total skeletal burden was made for several reasons 1) largest bone mass of any skeletal site available for counting 2) ability to shield the detector from emissions originating from the trunk region 3) previous successful experience using the skull as a skeletal estimator for another low energy photon emitting nuclide ^{210}Pb (Cohen 1970), 4) small amount of intervening soft tissue.

The methods of counting adult and juvenile baboon skulls were similar in principle but different in execution. Figure III-3 shows the skull counting geometrics for adult and juvenile baboons. The manner of measurement was determined mainly by the whole body restraining device. In the case of the adult, the meter-arc "bed" was constructed with mounts for a body shield plate which consisted of a 0.64 cm. steel plate with a slot for the neck and a positioner for the head itself. With the baboon in a semi-erect position the 15.2 cm. crystal was positioned above the calvarium region at a distance of 5 cm. from the crown. In the case of the juvenile baboon, the construction of the whole body restrainer precluded the use of a body shield attached to the restraint itself. Thus the entire body was shielded by a lead and steel table with a slot at the neck, effectively shielding the detector from photon emissions originating from ^{241}Am deposited in regions other than the head.

Figure III-3

Geometry for the *in vivo* Measurement of ^{241}Am in the
Skull of the Adult and Juvenile Baboon

Narrow Beam Transmission at 60 keV through the Shielding less than 10^{-5}

Geometry for *in-vivo* Measurement of ^{241}Am in the
Skull of Baboons – Adult Configuration

Geometry for *in-vivo* Measurement of ^{241}Am in the
Skull of Baboons – Juvenile Configuration

Lead and Steel Plate
(Transmission at 60 keV $< 10^{-5}$)

III 10

The accuracy and precision of the skull measurements are considerably less than those of the meter-arc technique. Several factors are responsible for the increased variability. The most important parameter is geometry or positioning reproducibility. Positioning of the detector at a small distance from the skull leads to a high efficiency for counting ^{241}Am . However the proximity of the source and detector also makes the efficiency more susceptible to small changes in source-to-detector distance. This distance is made more difficult to reproduce because of cyclic changes in anatomy that occur in adult female baboons. During menstruation, the pelvic region of the female becomes engorged with blood, causing considerable swelling of the posterior region. This swelling causes changes in the orientation of the baboon within the meter-arc restraining device. This change of orientation of the baboon within the restrainer results in variability of the efficiency of counting. Other factors which may effect efficiency from baboon to baboon are skull size, spatial distribution of ^{241}Am in bone, and attenuation from hair and scalp. Measurements of ^{241}Am in hair have shown the hair to be a very minor source of ^{241}Am activity; likewise blood concentrations of ^{241}Am were shown to be very low after the first day post injection (Rosen and Cohen, 1971).

Efficiencies for determination of ^{241}Am in the skull by measurement of ^{241}Am in calvarium biopsy samples or by analysis of the entire skull after sacrifice are given in Table III-5 for both animals measured during this study as well as for baboons measured previously by other investigators (Rosen et al. 1972; Cohen and Wrenn, 1973). The variation of efficiency from animal to animal was $\pm 15\%$ with a range of 34.8-48.0 cpm/nCi.

TABLE III-5

Counting Efficiency for ^{241}Am Deposited in Bone as Determined by Skull Geometry *in vivo* Measurement

Animal #	Skull wt. (kg)	Skull Burden (nCi)	Efficiency (cpm/nCi)
B-458	0.236	137.9	45.3
B-460	0.226	117.1	45.4
B-352	0.271	183.0	37.6
B-520	0.236	127.2	34.8
B-164	0.297	70.2	34.5
B-470	0.320	154.5	48.0
B-458*	0.236	131.8	41.1
B-458*	0.236	142.1	53.3
B-460*	0.236	127.3	35.7

Mean of counting efficiencies 40.6 ± 7.3

* = Efficiency calculated from data obtained from calvarium biopsy

Liver Measurement

Since previous experiments with the baboon as well as with other species have shown that the liver is the major soft tissue deposition site for non-inhaled ^{241}Am , the amount of ^{241}Am deposited and retained in the liver of adult and juvenile baboons was estimated from *in vivo* measurements. To measure the 60 keV γ -ray of ^{241}Am in the liver of an adult baboon, a 7.6 cm. thin NaI(Tl) crystal was positioned adjacent to the area in which the liver could be palpated. This area was marked on the skin of the baboon by permanently tattooing the region of interest. An identically sized area was also outlined on the left side of the baboon for "background", i.e. measurements performed in an area of the body which contains all body structures except the liver. Measurements of ^{241}Am in the liver of the juvenile baboon was performed exactly as above but using a 2.5 cm diameter NaI(Tl) thin crystal. This detector was used because of the substantially smaller liver and body size of the juvenile.

Quantifying the amount of americium deposited in the liver by *in vivo* measurement can be performed only after certain basic assumptions are made. The problem in measuring ^{241}Am in liver arises from the fact that one cannot physically isolate the liver for counting. This means that the detector can measure 60 keV emissions from ^{241}Am which is deposited not only in the liver, but also in the ribs, vertebrae and lungs. The problem of these extra-hepatic ^{241}Am sources is significant because the "background" i.e. non-liver count rate is not temporally constant. In an attempt to develop the proper methodology for *in vivo* measurement and estimation of liver burden of ^{241}Am , several experiments were performed. In the first

experiment after two of the test animals, B-352 and B-520, were sacrificed, a U-shaped incision was made from the left to the right costal margin and the liver was isolated *in situ*. Subsequently, *in vivo* liver measurements were performed using the 7.6 cm. diameter thin NaI(Tl) crystal. In addition to the original baboon livers, phantom livers made of ~ 240 g of calves liver each, and sculptured to simulate the shape of the baboon liver, were uniformly labelled with different activities of ^{241}Am . The labelling was accomplished by injecting small volumes of the ^{241}Am solution throughout the liver as homogeneously as possible. About 100 different sites were made per liver. After labelling, the livers were sealed in thin polyethylene bags to prevent leakage of ^{241}Am from the liver.

Measurement of the Am content of the phantom livers in the carcass of a baboon with a systemic burden of ^{241}Am provided a means of determining the relative contribution of ^{241}Am deposited in surrounding bone and/or soft tissue and of ^{241}Am in liver to the actual *in vivo* measurement. This data is provided in Table III-6; also included in the table is data obtained from B-622, a baboon of similar size to B-520 and which had not previously been injected with ^{241}Am . The measurement of ^{241}Am in this animal allowed the determination of the counting efficiency of ^{241}Am in liver without contributions from extra-hepatic sources of deposited ^{241}Am . The mean and standard deviation for the efficiency of measuring ^{241}Am in the baboon by a right side count is 12.5 ± 2.2 cpm/nCi. Discrimination of the count contributions from extra-hepatic ^{241}Am sources for B-352 and B-520 was accomplished by subtracting the count rate observed when an unlabelled or "blank" liver was placed in the body from the count rate measured when an ^{241}Am -labelled liver was in place. In this manner the liver as source

TABLE III - 6

Determination of Counting Efficiency for ^{241}Am Deposited in the Liver of the
 Baboon Using a Liver Area Measurement on ^{241}Am -Labelled Baboon Phantoms

Animal #	Liver Burden (nCi)	Lung Burden (nCi)	Net Efficiency (cpm/nCi in liver)
B-520	0	10.8	0.0
"	25	10.8	10.4
"	51	10.8	9.4
"	101	10.8	12.9
B-352	0	15.5	0.0
"	123	15.5	15.5
"	417	15.5	16.5
B-622*	25	0.0	12.9
"	51	0.0	13.7
"	101	0.0	10.0
"	25	45.7	13.9
"	51	45.7	13.2
"	101	45.7	11.9
Mean of counting efficiencies			12.5 ± 2.2

* B-622 was not injected with ^{241}Am prior to the phantom studies.

of Am activity was eliminated while the liver as photon absorber and scatterer was maintained. The preceding experiments were instructive in determining the range of variability one can expect from this type of measurement. However it was not as useful as was desired for determining actual liver burdens by *in vivo* counting. The likelihood of accurately estimating the liver burden of ^{241}Am from a single right quadrant measurement is quite variable. Certain factors make it very difficult to quantitate liver burdens. Again the liver as a source of ^{241}Am emissions cannot be isolated spatially from other sources of ^{241}Am . Ribs, vertebrae, lungs - all well-known deposition sites of ^{241}Am - will all contribute in various degrees to the observed gross count rate. This factor is not as important at short times after injection when the liver activity is maximal and considerably greater than any other contribution from surrounding organs. However as the liver burden of ^{241}Am decreases with time, a point is reached when the contributions from extra-hepatic sources become equal or greater than that of the liver source. One cannot simply correct the observed gross count rate as if there were a known background component since 1) the contribution of extra-hepatic sources to the observed liver region count rate is variable to some extent during the period of interest, i.e. the first year after injection; 2) the measurement of background sources without liver contribution is at present an unknown technique in a live intact animal.

Different approaches have been attempted to approximate the non-liver ^{241}Am contribution factor. At present the simplest and most reliable method of measuring liver burdens of ^{241}Am is to measure the gross count rate over the liver regions with a small diameter thin crystal and calibrate

using data obtained from biopsy samples. This procedure has been followed for the most part in these studies. The main drawback, of course, is that one cannot obtain a calibration factor without liver biopsies of the baboon.

Post Mortem Tissue Analysis

All animals that were processed for complete tissue analysis of ^{241}Am were sacrificed by exsanguination via femoral artery cannulation and collection of the blood in evacuated bottles containing anticoagulant ACD. Immediately after death, sections of soft tissues to be autoradiographed and histologically examined were fixed in Carnoy's Fluid.¹ The remainder of the organs were weighed and frozen for subsequent analysis.

After fixation, and paraffin embedding, the soft tissues were sectioned at 6 μm thickness, mounted, dipped in Kodak NTB nuclear emulsion and exposed in light-tight boxes. Developing of the emulsion was performed according to the directions specified by the manufacturer and histological staining with hematoxylin and eosin were performed according to standard histological procedures (Lilly, 1954).

The preparation of the gross tissue samples for measurement of ^{241}Am involved wet ashing the entire tissue in concentrated nitric acid. Oxidation of high boiling point fats and other persistent organic materials was accomplished by using small amounts of 30% H_2O_2 or 90% nitric acid (fuming). When the sample remained in solution when diluted to 100 ml. with a 1:1 nitric acid solution, the sample was then gamma-counted in a polyethylene container in the 15.2 cm dual crystal system previously described.

¹ Gatenby et al., 1950.

CHAPTER IV

RETENTION KINETICS OF Am-241: RESULTS AND DISCUSSION

Whole Body Kinetics of ^{241}Am

The whole body retention of ^{241}Am , as measured by the *in vivo* meter-arc technique, is shown for three adult and one juvenile baboon in Figures IV-1a, 2a, 3a, 4a. The period of study of ^{241}Am retention varied among the subjects: B-352 was monitored for 6 months, B-460 for 1 year, B-458 for 2.25 years and B-406, the juvenile, for one year. Regression analysis of the respective curves was performed by computer¹. The regression coefficient along with pertinent statistical data are summarized in Table IV-1, while the composite multi-exponential equations describing the whole body retention of ^{241}Am in these baboons are listed individually on the respective figures, as well as together in Table IV-2.

Generally, the whole body ^{241}Am retention data suggest that the behavior of intravenously injected ^{241}Am is characterized by two components: the shorter component has a half life ranging from 17 to 36 days and is associated with 36-45% of the injected dose. The longer component, which characterizes the retention of 58-64% of the injected dose, has a range of half lives from 1.2 - 5.5 years.

Retention of ^{241}Am in the Liver: *In Vivo* Measurement

The kinetics of ^{241}Am in the livers of the above mentioned baboons, as measured by *in vivo* techniques, are shown in Figures IV-5,6,7,8. Regression

¹ Computer facilities furnished through the courtesy of the Division of Biological and Medical Research, Argonne National Laboratory.

Figure IV-1a,b

Retention of ^{241}Am in the Whole Body and the Skull of Adult Baboon,
B-458, as Measured by *in vivo* Methods

Whole Body Retention of ^{241}Am can be described by:

$$y = 0.23e^{-12.8t} + 0.13e^{-8.9t} + 0.64e^{-0.144t} \quad (\rho = 0.87)$$

where

y: The fraction of the injected dose remaining in the whole body at time, t.

t: Time after injection of ^{241}Am , in years.

Relative Intensity (Count Rate, cpm)

Head

% Injected Dose

Meter Arc

Figure IV-2a,b

Retention of ^{241}Am in the Whole Body and Skull of Adult Baboon B-460,
as Measured by *in vivo* Methods

Whole Body Retention of ^{241}Am can be Described by;

$$y = 0.41e^{-8.95t} + 0.59e^{-0.13t} \quad (\rho = 0.85)$$

where

y: The fraction of the injected dose remaining
in the whole body at time, t.

t: Time after injection of ^{241}Am , in years.

Figure IV-3a,b

Retention of ^{241}Am in the Whole Body and Skull of Adult Baboon B-352,
as Measured by *in vivo* Methods

Whole Body Retention of ^{241}Am can be Described by:

$$y = 0.42e^{-14.7t} + 0.58e^{-0.59t} \quad (\rho = 0.93)$$

where

y: Fraction of the injected dose remaining
in the whole body at time, t.

t: Time after injection of ^{241}Am , in years.

Figure IV-4a,b

Retention of ^{241}Am in the Whole Body and Skull of Juvenile Baboon B-406,
as Measured by *in vivo* Methods

Whole Body Retention of ^{241}Am can be Described by:

$$y = 0.45e^{-7.0t} + 0.55e^{-0.27t} \quad (\rho = 0.93)$$

where

y: Fraction of the injected dose remaining in the whole body at time, t.

t: Time after injection of ^{241}Am , in years.

TABLE IV - 1

Regression Analysis of In-Vivo Whole Body Data

Animal	Component	Disappearance Rate (λ) (yrs ⁻¹)	Standard error	95% confidence limits	Correlation coefficient (ρ)	Half Time ($t_{1/2}$) (days or years)	Approximate standard error for $t_{1/2}$	%ID associated with the $t_{1/2}$
B-458	1	12.78	3.65	9.86	-0.87	19.8 day	5.7 day	23
B-458	2	8.92	1.03	2.66	-0.97	28 day	3.3 day	13
B-458	3	0.145	0.015	0.033	-0.94	4.8 yr	0.5 yr	64
B-460	1	8.95	1.24	3.02	-0.95	28 day	3.6 day	41
B-460	2	0.126	0.034	0.087	-0.85	5.5 yr	0.2 yr	59
B-352	1	14.7	1.58	4.06	-0.97	17 day	1.8 day	42
B-352	2	0.588	0.167	0.719	-0.93	1.2 yr	0.3 yr	58
B-406	1	7.0	0.47	1.21	-0.99	36 day	2.6 day	45
B-406	2	0.27	0.06	0.20	-0.93	2.6 yr	0.6 yr	55

TABLE IV - 2

Multicomponent Exponential Regression Equations
For *In Vivo* Whole Body Measurements of Baboons

$$\text{B-458} \quad y = 0.23e^{-12.8t} + 0.13e^{-8.9t} + 0.64e^{-0.144t}$$

$$\text{B-460} \quad y = 0.41e^{-8.95t} + 0.59e^{-0.13t}$$

$$\text{B-352} \quad y = 0.42e^{-14.7t} + 0.58e^{-0.59t}$$

$$\text{B-406} \quad y = 0.45e^{-7.0t} + 0.55e^{-0.27t}$$

y: The fraction of the injected dose remaining
in the whole body at time after injection, t.

t: Time after injection of ^{241}Am , in years.

analysis of the original data, performed in a manner similar to the whole body ^{241}Am retention data analysis, is summarized in Table IV-3. As has been shown in the case of the whole body ^{241}Am retention data, the kinetics of ^{241}Am in the livers of the four baboons studied can also be described by dual-component exponential expressions of the form $y = a_1 e^{-b_1 t} + a_2 e^{-b_2 t}$. In detail the short term component of ^{241}Am retention in the liver had an average half life of 27 days, with a range of 20-38 days, and reflected the clearance of 70-90% of the initial liver burden. The remaining 10-30% of the initial liver burden appeared to disappear with retention half-times of 1 - 2 years. Individual multi-component exponential equations of ^{241}Am clearance from the liver are given on the respective Figures IV-5,6,7,8.

Estimates of initial liver burdens for the baboons studied are listed in Table IV-4. These quantities, which were obtained from ^{241}Am concentration measurement in liver tissue (biopsy) used in conjunction with the *in vivo* liver region measurements, show the initial uptake of ^{241}Am by the liver to range from 20-35.5% of the injected dose of ^{241}Am .

Am-241 Retention in Bone: *In Vivo* Skull Measurements

In vivo measurements of the skull burden of ^{241}Am for B-458,460,352, 406 as a function of time postinjection are shown graphically in figures IV-1b,2b,3b,4b. Results of regression analysis of the *in vivo* skull measurements are summarized in Table IV-5. The kinetics of ^{241}Am in the baboon skull, as indicated by the *in vivo* measurements performed, consist of a sustained net uptake of ^{241}Am by the skull for as long as 50-90 days post-injection of ^{241}Am . After this time there is little measurable

Figure IV-5

Retention of ^{241}Am in the Liver of Adult Baboon B-458,
as Measured by *in vivo* Methods

The ^{241}Am Retention in the Liver can be Described by:

$$z = 0.32e^{-9.0t} + 0.036e^{-0.33t} \quad (\rho = 0.86)$$

where

z : The fraction of the injected dose which is present in the liver at time, t .

t : Time after injection of ^{241}Am , in years.

Figure IV-6

Retention of ^{241}Am in the Liver of Adult Baboon B-460,
as Measured by *in vivo* Methods

The ^{241}Am Retention in the Liver can be Described by:

$$z = 0.26e^{-12.8t} + 0.023e^{-0.41t} \quad (\rho = 0.99)$$

where

z: The fraction of the injected dose which is present in the liver at time, t.

t: Time after injection of ^{241}Am , in years.

Figure IV-7

Retention of ^{241}Am in the Liver of Adult Baboon B-352,
as Measured by *in vivo* Methods

The ^{241}Am Retention in the Liver can be Described by:

$$z = 0.23e^{-11.7t} + 0.072e^{-0.77t} \quad (\rho = 0.80)$$

where

z: The fraction of the injected dose which is present in the liver at time, t.

t: Time after injection of ^{241}Am , in years.

Figure IV-8

Retention of ^{241}Am in the Liver of Juvenile Baboon B-406,
as Measured by *in vivo* Methods

The ^{241}Am Retention in the Liver can be Described by:

$$z = 0.185e^{-6.76t} + 0.035e^{-0.75t} \quad (\rho = 0.96)$$

where

z: The fraction of the injected dose which
is present in the liver at time, t.

t: Time after injection of ^{241}Am , in years.

TABLE IV - 3

Regression Analysis of the *In Vivo* Liver Data

<u>Animal</u>	<u>Component</u>	<u>Disappearance Rate (λ) (yrs⁻¹)</u>	<u>Standard error</u>	<u>95% confidence limits</u>	<u>Correlation coefficient(ρ)</u>	<u>Half Time ($t_{1/2}$) (days or years)</u>	<u>Approximate standard error for $t_{1/2}$</u>	<u>%ID associated with the $t_{1/2}$</u>
B-458	1	9.04	1.29	3.58	-0.96	28 day	4.0	31.95
B-458	2	0.331	0.074	0.175	-0.86	2.1 yr	0.47	3.55
B-460	1	12.8	0.90	2.49	-0.99	20 day	1.5	26.4
B-460	2	0.409	0.05	0.635	-0.99	1.7 yr	0.2	2.30
B-352	1	11.7	1.01	2.59	-0.98	22 day	1.8	22.6
B-352	2	0.774	0.577	7.33	-0.80	0.90 yr	0.67	7.20
B-406	1	6.76	0.88	2.25	-0.96	38 day	5.1	18.5
B-406	2	0.753	0.123	0.529	-0.97	0.92 yr	0.15	3.50

TABLE IV - 4

Initial Burden of ^{241}Am in the Liver, as Estimated Using Biopsy Calibration Data

Animal	Estimated Initial Burden of ^{241}Am (% Injected Dose)
B-458	35.5
B-460	28.7
B-352	29.8
B-406	22.0

TABLE IV - 5

Regression Analysis of *In Vivo* Skull Measurement Data

<u>Animal</u>	<u>Period of Study (years)</u>	<u>Disappearance Rate (λ) (yrs⁻¹)</u>	<u>Standard error</u>	<u>95% confidence limits</u>	<u>Correlation Coefficient</u>	<u>Half Life ($t_{1/2}$)</u>	<u>Approximate standard error of $t_{1/2}$</u>	<u>t-test</u>
B-458	2.25	+0.00127	0.020	0.042	+0.014	547 yr	undef	negative
B-460	1.0	+0.0245	0.041	0.091	+0.186	28 yr	undef	negative
B-352	0.56	-0.010	0.232	0.644	-0.022	69 yr	undef	negative
B-406	0.33	+0.116	0.114	0.316	0.455	6 yr	undef	negative

t-test refers to the testing of the hypothesis

$$H_0: \lambda = 0$$

$$H_1: \lambda \neq 0$$

A negative t-test means that one cannot reject the null

hypothesis that the slope, λ , is equal to zero; at

the $P = 0.05$ level

change in the amount of ^{241}Am in the baboon skull. Regression analyses (which were begun after the measured amount of ^{241}Am in the skull were shown not to increase further) confirm the persistence of ^{241}Am in the skull as a function of time. Statistical testing of the fitted slopes for each of the curves showed that, in four out of four cases, the slopes could not be said to differ from zero (Table IV-5) i.e. no net change was discernible in the measured skull burden of ^{241}Am during the measurement period. Although the estimates of the rate of change of ^{241}Am in the skull with time are unreliable statistically, it is of interest that three of the four slopes were positive indicating a possible long-term increase in the measurable amount of ^{241}Am in the skull.

Analysis of ^{241}Am in Urine and Feces

As a concurrent but different method of monitoring the kinetics of ^{241}Am in the baboon, urine and feces were collected and analyzed for ^{241}Am content. Although the analysis of excreta samples were performed on a daily basis, the data has been combined into weekly totals of ^{241}Am excreted in urine or feces, in order to reduce errors due to the normal daily fluctuation in the quantity of excreta and variability of ^{241}Am loss. As such Table IV-6 gives the cumulative loss of americium from the body as a result of urine and fecal excretion. Table IV-7 lists the ratios of ^{241}Am excreted via the fecal route as compared to ^{241}Am excreted via the urine. In each table, two juvenile baboons B-400 and B-406 were included in addition to the six adult animals studied.

Each animal had its own pattern of excretion of ^{241}Am as reflected by the amount of americium excreted during the first few weeks after injection.

TABLE IV - 6

Normal Excretion of ²⁴¹Am: Weekly Cumulative Percent of Injected

Dose Excreted in Urine and Feces

Week after Injection	B-416	B-412	B-458	B-460	B-352	B-520	Mean of adults $\pm$ 1 sd	B-406*	B-400*
1	8.2	10.7	4.7	1.9	4.1	5.9	5.9 $\pm$ 3.1	4.0	8.9
2	11.4	13.0	7.1	6.2	7.2	8.6	8.9 $\pm$ 2.7	7.3	11.8
3	13.1	15.2	11.3	10.8	9.9	10.3	11.8 $\pm$ 2.0	11.3	14.5
4	15.3	17.7	13.6	14.9	14.9	12.8	14.9 $\pm$ 1.7	15.1	19.2
5	16.0	19.3	15.8	18.0	18.0	14.2	16.9 $\pm$ 1.9	17.1	20.6
6	xxx	21.1	17.7	20.2	20.2	15.5	18.9 $\pm$ 2.3	19.0	21.4
7		22.5	19.7	22.0	22.0	Rx	21.5 $\pm$ 1.3	20.7	23.7
8		23.3	21.3	23.7	24.0		23.1 $\pm$ 1.2	22.4	Rx
9		24.6	22.2	25.2	25.7		24.4 $\pm$ 1.5	23.8	
10		25.5	23.4	26.2			25.0 $\pm$ 1.7	25.1	
11		26.9	24.6	26.9				25.7	
12		27.9	25.2	27.5				27.5	
13		28.1	25.9	28.2					
14		xxx	26.2	28.9					

*juvenile baboons

xxx: sacrificed

Rx: beginning of DTPA Therapy

TABLE IV - 7

Biweekly Fecal ²⁴¹Am to Urinary ²⁴¹Am Excretion Ratio

<u>weeks post injection</u>	<u>B-416</u>	<u>B-412</u>	<u>B-458</u>	<u>B-460</u>	<u>B-352</u>	<u>B-520</u>	<u>Mean of adults $\pm$ 1 sd</u>	<u>B-406*</u>	<u>B-400*</u>
1	0.1	0.1	0.3	0.4	0.4	0.2	0.26 $\pm$ 0.15	0.4	0.2
2	1.7	0.8	0.8	4.5	4.1	3.3	2.5 $\pm$ 1.7	6.1	8.8
4	3.9	3.2	13.0	15.0	14.2	6.7	9.3 $\pm$ 5.3	16.2	11.7
6	4.1	5.0	7.7	11.1	12.0	8.3	8.0 $\pm$ 3.2	14.3	6.2
8		6.5	14.9	8.9	8.5		9.7 $\pm$ 3.6	14.9	
10		6.6	14.0	8.0				14.2	
12		15.2	9.9	6.7				8.3	
14		16.0	5.5	6.6					

*juvenile baboons

In particular, the variation in the amount of ^{241}Am excreted during the first week ranges from 1.9 to 10.7 percent of the injected dose. Of these quantities of excreted ^{241}Am , seventy to ninety percent were measured in the urine. During the second week post injection from 44 to 81% of the excreted ^{241}Am was present in the feces (average of 65% ID for the adults, 88% ID for the juveniles). After the first two weeks post injection, the major route of excretion of ^{241}Am remained in the feces where 76-94% of the total measured ^{241}Am (average of 90%) was found. The predominance of fecal route of ^{241}Am excretion compared to the urinary route continued for at least 14 weeks. Although daily collections of excreta were, in general, curtailed after 14 weeks due to the very small quantity of ^{241}Am being excreted from that time, compositing of excreta and periodic spot sampling of urine and feces were performed. On the basis of these measurements, in particular for those animals which had been on test for the longest period, B-458 and B-460, approximately 70-80% of the excreted ^{241}Am appeared in the feces at one year post-injection and 50-70% of the excreted ^{241}Am was found in the feces at 2 years post-injection (B-458). The magnitude of the levels of ^{241}Am excreted per week (sum of urinary and fecal ^{241}Am) continued to decrease from 2-4% Injected Dose (ID)/week for the first two months to 1% ID/week by the third month and 0.5% ID/week by the fourth month. Figure IV-9 illustrates the monthly levels of ^{241}Am excretion for B-458, the baboon which was studied for the longest period of time. By one year post injection 0.07% ID (or 0.13% BB) was excreted per week while at 2 years post injection 0.04% ID (or 0.089% BB) was excreted per week.

Figure IV-9

Cumulative Amount of ^{241}Am Measured in the Urine and Feces of
B-458, per Month Post-Injection

Error Bars Indicate One Standard Deviation

Figure IV-10

Monthly ²⁴¹Am Excretion in the Feces of B-458

% Injected Dose Excreted/month

10.0
1.0
0.1

0

10

20

30

Months Post Injection

It can be demonstrated from this data that the majority of the easily excretable americium is cleared from the body in a relatively short period of time, i.e. several months. Nevertheless there are small but measurable quantities of ^{241}Am that are excreted only slowly and at least as long as 2 1/4 years post injection.

Discussion of Results Obtained by *In Vivo* Measurements

The use of whole body and organ *in vivo* measurement methodologies for the estimation of whole- and partial-body burdens of low-energy γ - and x-ray emitting radionuclides has been well documented by this laboratory (Laurer and Eisenbud, 1968; Cohen, 1970; Rosen et al. 1971). In general these techniques have been applied with reasonable success in measuring ^{210}Pb and ^{241}Am in both humans and primates. Continuation of the use of these techniques in the present studies has been productive in defining the metabolism of ^{241}Am in the living baboon.

Whole body *in vivo* measurements of B-458, 460, 352 and 406 have demonstrated that there are at least two major kinetic components which describe the metabolism of ^{241}Am in the baboon (at least for 2.25 years). The first or shorter component has an average half-life of 27 days and characterizes the retention of about 40% of the deposited nuclide. The remaining 60% of injected ^{241}Am has associated half-lives of 1.2 to 2.5 years.

It should be mentioned here that several other baboons have been monitored for ^{241}Am metabolism (i.e. B-412, B-416 (Rosen et al. 1972) and B-520, B-400, this study). However, for various reasons, the period of time over which these animals were studied was considerably less than

for those animals described in the previous paragraphs. While this abbreviated period of measurement does not affect the accuracy of the *in vivo* measurements performed on the former group of baboons it does obviate comparison of biological half-lives derived from mathematical analysis of the *in vivo* data. This is due primarily to the lack of long-term biological data, which is necessary for resolving of longer term biological retention parameters. In fact, a requirement of not less than 200 days of post-injection monitoring is necessary in order to obtain sufficient data to analyze the intermediate and long-term behavior of ^{241}Am in the baboon. Although the above discussions were based on whole body and *in vivo* measurement data they also apply to the case of data obtained from *in vivo* measurement of ^{241}Am in the liver of the baboon.

Measurement of ^{241}Am in the liver by *in vivo* methods show that the largest fraction of the hepatically deposited ^{241}Am is cleared with a short biological half-time. In three of four baboons more than 80% of the maximum liver burden was cleared with half-times of 20-38 days. This fraction represents from 18-32% of the injected dose. The fraction which remains in the liver (approximately 3% ID) is cleared with a much longer half-time (1-2 years). The physiological and biological significance of the long term retention component in the liver is difficult to ascertain, partly because of analytical problems, partly because of lack of physiological information.

The determination of hepatic burdens of ^{241}Am at levels approaching those of "background" is very difficult. Not only must one assume some degree of constancy for the background contribution, i.e. count rate

contribution from ^{241}Am located in lungs, ribs, thoracic vertebrae, etc., but one must also be able to determine the magnitude of this background contribution by *in vivo* techniques, i.e. without sacrificing the animal (Chapter III). Since the *in vivo* liver measurement data shown in figures IV-5,6,7,8 represent the gross count-rate measurements over the liver area, background has not been taken into account. The lack of background subtraction is problematic in that it causes overestimates of the liver burden based on biopsy data, due to the increased contribution of background, (and hence an apparent increase in counting efficiency). Attempts have been made to determine a contribution factor, i.e. to determine the count rate that occurs as a result of ^{241}Am deposited in tissues surrounding the liver mass (lung, bone, etc.). It was unfortunate that the increase in analytical error associated with these attempts precluded their use in determining a background correction factor.

The *in vivo* data obtained as a result of successive measurements of ^{241}Am deposited in the skull of the baboon show quite clearly that ^{241}Am deposited in bone remains there for very long periods of time. After a period of fairly rapid initial uptake, (i.e. rapid compared to the rate of loss) of approximately 50-70 days, the measured levels of ^{241}Am in the skull show little change at least as long as each animal was measured (0.5 - 2.5 yrs). Presently, however, the errors associated with the measurement technique, together with the small time period of measurement compared to the assumed long half-life of ^{241}Am in the bone make quantitation of the net rate of change of ^{241}Am in the skull difficult. As shown in figure IV-5, t-tests of the hypothesis $H_0: \lambda = 0$ for the regressions performed were consistently negative for rejection, i.e. one could not say at the $p = 0.05$ level of

confidence that the slope, λ_1 , of the regression curves were not zero.

Analysis of variance of the regression performed on each set of skeletal ^{241}Am retention data was helpful in defining minimum statistically significant slopes $\lambda_{\min}$ (therefore maximum half-lives $T_{1/2}(\max)$ since $\lambda \propto \frac{1}{T_{1/2}}$). On the basis of data obtained from *in vivo* measurement of ^{241}Am in the skull of B-458, 460, 352, 406 one can obtain, at the $p = 0.05$ level, the following minimum measurable slopes and maximum measurable retention half-lives for ^{241}Am in the skull, assuming that ^{241}Am dynamics in the skull can be approximately by an exponential process.

	minimum measurable slope (yr^{-1}) at $p = 0.05$	maximum measurable retention half-life (yr) at $p = 0.05$
B-458	0.074	9.31
B-460	0.097	7.16
B-352	0.507	1.37
B-406	0.286	2.43

It is seen from the above values that, at least empirically, the time over which all *in vivo* skull measurements were made affects the ability to measure small slopes, i.e. small net changes in the ^{241}Am content of the skull. For example, *in vivo* measurements for B-352 were performed over a period of approximately 6 months, whereas B-458 was monitored over a period of about 2 1/4 years. Comparison of the minimum measurable slopes of these two animals shows that a ratio of one-seventh, i.e. one was seven times as "sensitive" in measuring the rate of change of ^{241}Am in the skull of B-458 as one was for B-352. This comparison, however, is not completely fair, since the data for B-352 also has a disproportionately smaller number of *in vivo* measurements. This occurred as a result of technical difficulties

associated with the proper positioning of the head of B-352 within the shielding device used for *in vivo* skull measurement purposes. Therefore differences in detection limits (i.e. λ min) for B-352 and B-458 were a result of both period of measurement and proportionate numbers of measurements.

One may interpret the derived minimum slopes to be lower limits of the net biological rate of change of ^{241}Am in the skull. Conversely, the retention "half-life" of ^{241}Am in the skull is at least as long as 1.7 years for B-352, 9.7 years for B-458, etc.

It is believed that the actual retention of ^{241}Am in the baboon skull is probably much longer than the listed maximum measurable retention half-lives. This assertion cannot be proved or disproved, based on the data obtained in this study. However results of the skeletal retention of ^{241}Am in other species of experimental animals suggest long retention times. Durbin (1967,1973) measured a half-time of retention of ^{241}Am in the entire skeleton of the rat to be 4600 days (12.6 yrs.). The half-time of ^{241}Am in the Chinese hamster skeleton, based on analysis of bones taken 225 and 360 days after injection of $1\ \mu\text{Ci/kg}$ of ^{241}Am citrate, was measured to be at least 1450 days (4 yrs.) (McKay et al., 1972). Durbin (1974) estimates the long-term retention of ^{241}Am in the skeleton of the cynomolgus monkey to be 12,600 days (34.5 yrs.) while the estimated skeletal retention of ^{241}Am in the beagle (Durbin 1973) was about 18 years. It therefore appears reasonable to assume that the retention of ^{241}Am in the skeleton of the baboon is longer than the longest measurable half-life, i.e. for B-458, $T_{1/2\text{min}} = 9.3$ years. The importance of long term studies of ^{241}Am metabolism in the baboon cannot be minimized.

The inability to define a non-zero disappearance rate of ^{241}Am from bone on the basis of *in vivo* skull measurements should not be interpreted as evidence that there is no movement of ^{241}Am in bone. In terms of measurement of the 60 keV gamma rays of ^{241}Am , there is little change in efficiency of counting of the 60 keV emission with changes in distribution of ^{241}Am in bone. Therefore, translocation of ^{241}Am , without a substantial net change in the amount of ^{241}Am activity in the skull, would probably not be detectable, particularly since the analytical errors involved in the measurement technique are significant. However, if one measures the 17 keV (average) x-rays emitted by the daughter nuclide, ^{237}Np , one can, because of the significantly different attenuation coefficients of 17 vs 60 keV photons in bone (mass attenuation coefficients: $4.54 \text{ cm}^2/\text{g}$ at 17 keV vs $0.274 \text{ cm}^2/\text{g}$ at 60 keV), infer the occurrence of translocation. If, as a function of time, there is a tendency for ^{241}Am to become more deeply buried in bone, then one would observe a decrease in the ratio of measured activity in the region of the 17 keV photon emissions with respect to the measured activity in the region of the 60 keV γ 's. It can be shown that, assuming the simple case of narrow beam radiation that the ratio of 17 keV activity to 60 keV activity can be used to determine a change in "effective" depth of ^{241}Am in bone such that

$$\Delta x = \frac{\ln \left(\frac{I}{I_1} \right) \rho}{\mu_{17} - \mu_{60}}$$

where

Δx = the change in effective depth of ^{241}Am in bone (cm)

I = the ratio of count rate in 17 keV region vs count rates observed

in the 60 keV region at time $t = t_1$

2

I' = the 17 keV/60 keV count-rate ratio at time $t = t_2$ where

$$t_2 > t_1$$

μ_i = mass attenuation coefficient for bone at 17 and 60 keV

ρ = density of bone (g/cm^3)

It was observed in the adult baboon that the 17/60 count-rate ratio was on the average 0.15 at 1 day post injection of ^{241}Am ; from 1 month to 2 1/2 years post injection the ratio was 0.12 ± 0.004 . Using narrow beam attenuation coefficients, it can then be calculated that there was a change in effective depth of ^{241}Am deposited in the skull of 0.33 cm, i.e. if at time $t = 1$ day post injection, the activity of ^{241}Am were deposited in an absorber at a depth x cm then at $t > 1$ month the effective depth would be $x + 0.33$ cm. In effect, there was likely translocation of Am and this translocation had the effect of increasing the effective depth of deposition of ^{241}Am in the bone. This rudimentary model assumes only attenuation of photons by bone tissue and does not account for the presence of soft tissue material (e.g. pelt, brain, muscle, etc.). Application of the results of 17/60 keV count-rate ratios would require systematic studies using phantoms specifically constructed for that purpose.

Discussion of Results Obtained from Measurements of ^{241}Am in Excreta

The analysis of ^{241}Am in the urine and feces of baboons shows several important features. Elimination of ^{241}Am via the urine is an important route of excretion for the first few weeks. However, the amount of ^{241}Am excreted by this route decreases week by week from 4.7% ID during week 1 to 0.86% ID during week 2 to 0.28% for week 3, etc. In contrast, the amount of ^{241}Am excreted in the feces includes the major portion of the

measured ^{241}Am after the first week following ^{241}Am injection. Sustained levels of 1-3% ID/week were measured from week 2 to week 8. Thereafter the levels of ^{241}Am measured in the feces decreased slowly. It is interesting to note that even after 800 days post-injection ^{241}Am was still measured in both urine and feces of B-458, albeit very low levels (0.02% ID/day and 0.004% ID/day respectively).

Comparison of the short-term bioassay of ^{241}Am in the baboon, cynomolgus monkey, beagle dog, and rat (Table IV-8) shows that the cynomolgus monkey and beagle dog and rat tend to excrete about twice as much ^{241}Am via the urine during the first three weeks of measurement, compared to the baboon. On the other hand, the baboon shows a higher rate of excretion of ^{241}Am (5.3% ID) via the feces than either the monkey (2.8% ID) or the dog (1.5% ID) during this early period of measurement. The excretion of ^{241}Am in the feces of the baboon however, is only about one-eighth that of the rat. Comparison of long-term excretion patterns between the monkey and the baboon indicate that the excretion of ^{241}Am in the feces of the monkey is somewhat more prolonged than in the baboon, i.e. maximum excretion of ^{241}Am , in % ID/day, occurs at about 100 days in the monkey compared to about 30 days for the baboon. However at ~ 700 days post injection, the rate of excretion of ^{241}Am in the baboon and monkey do not differ by more than a factor of two.

Comparison of Body Burden Estimates of ^{241}Am by *In Vivo* vs. Bioassay Measurement

Table IV-9 compares the estimates of ^{241}Am whole body burden at various times after injection as determined either from the whole body *in vivo* count-rate data or by subtracting the cumulative measured amount of ^{241}Am excreted in the urine and feces from the injected dose. Differences in

Table IV - 8

Excretion of ^{241}Am by the Baboon, Cynomolgus Monkey, Beagle, and Rat during the First Three Weeks Postinjection

Days post injection	Excretion in Interval				(% Injected Dose)			
	cynomolgus monkey ^a		beagle dog ^b		rat ^c		baboon ^d	
	urine	feces	urine	feces	urine	feces	urine	feces
1	6.01	0.10	8.03	0.22	9.0	1.7	1.83 ± 1.1	0.0074 ± 0.011
2	0.87	0.11	0.34	0.51	0.4	1.1	0.72 ± 0.25	0.093 ± 0.11
3	0.41	0.12	0.34	0.14	-	-	0.40 ± 0.20	0.045 ± 0.071
4-7	1.10	0.42	0.51	0.33	0.6	14.4	0.68 ± 0.26	0.75 ± 0.18
8-14	0.70	0.96	0.33	0.14	0.2	17.1	0.96 ± 0.30	1.96 ± 0.92
15-21	0.51	1.10	0.17	0.06	0.2	7.8	0.39 ± 0.15	2.46 ± 1.3
Total	9.60	2.81	9.72	1.45	10.4	42.1	4.98 ± 1.50	5.32 ± 1.61

^a mean of 14-20 cynomolgus monkeys (Durbin 1973)

^b mean of 4 dogs (Lloyd et al. 1970)

^c mean of 12 rats (Durbin et al. 1967, 1973)

^d mean of 4-6 baboons (This study)

TABLE IV - 9

Comparison of ^{241}Am Body Burden Estimates as Determined by Whole Body *In Vivo* or Bioassay
Measurements (% I D remaining in body)

Time post injection (days)	B-458 <i>In Vivo</i>	B-458 Bioassay	B-460 <i>In Vivo</i>	B-460 Bioassay	B-352 <i>In Vivo</i>	B-352 Bioassay	B-406 <i>In Vivo</i>	B-406 Bioassay
14	87	94	89	93	80	93	91	93
28	80	86	81	85	67	85	83	85
56	73	79	62	76	55	76	68	78
98	66	74	59	72				
210	59	68	56	66				
450	53	65						
700	49	63						

*
juvenile baboon

estimated burdens range from 2% ID to 21% ID remaining in the body. Although not all differences between *in vivo* and bioassay estimates of the ^{241}Am body burden in the baboon can be said to be statistically different, the estimates of body burden by the *in vivo* counting techniques are consistently less than that obtained by bioassay. The systematic difference may have resulted from the following factors:

- 1) a change in the efficiency of detection of the 60 keV gamma of ^{241}Am as a result of selective loss of ^{241}Am from or redistribution of Am among different organs of the body.
- 2) incorrect quantitation of the injected dose.
- 3) loss of excreta samples.

It is difficult to evaluate quantitative aspects of the factors listed above, particularly as concerns the postulated loss of excreta. No conclusions concerning the relative error arising from each factor will be presented.

Biological Significance of the Measurement of ^{241}Am Metabolism

Various aspects of the behavior of ^{241}Am in the baboon can be interpreted on the basis of the results obtained herein. It has been previously shown that the major deposition sites of ^{241}Am are the liver and the skeleton (Rosen et al., 1972; Cohen and Wrenn, 1973). Once monomeric americium has been injected into the blood stream of a baboon, the nuclide is removed from the circulation very rapidly. (The kinetics of ^{241}Am disappearance from blood will be discussed in greater detail in Chapter V). The early distribution of ^{241}Am that has been cleared from the bloodstream involves deposition of the nuclide not only in the liver

and skeleton but also in other soft tissues, e.g. lung, kidney, spleen, muscle, etc. Clearance rates of ^{241}Am from the non-liver soft tissue sites vary among the different soft tissues and represent about 20% of the injected dose. The remainder of the injected dose is partitioned between the liver and the skeleton, or promptly excreted. As has been stated previously the clearance of ^{241}Am from the baboon can be characterized by two exponential components, the first of which has a half-life of 27 ± 6 days, and the second, 3.5 ± 2 years. A comparison of slopes obtained from consecutive *in vivo* measurements of the whole body and the liver regions (Table IV-10) indicate that the short-term half-life as determined from whole body measurement of ^{241}Am reflects, in a large part, the clearance of ^{241}Am from the liver. However, clearance of ^{241}Am from other soft tissue sites also occurs simultaneously. The behavior of ^{241}Am within these non-hepatic soft-tissue sites also contribute to both the effective measured half-life of the body burden, and the percent of the injected dose (40%) which decreases with a half-life of 27 days.

Although the mechanism of ^{241}Am excretion from the liver has not been elucidated, evidence to date indicates that, at least in the baboon, the major route of excretion of ^{241}Am from liver is via the feces (Cohen and Wrenn, 1973). Figure IV-11 shows the correlation between disappearance of ^{241}Am from the liver (*in vivo* determination) with appearance of ^{241}Am in the feces (bioassay). It is further believed that the vehicle transporting ^{241}Am from the liver to the G I tract is the bile. Although material balance studies were not conducted, several bile samples were obtained either at biopsy or at sacrifice. In all cases, ^{241}Am could be

TABLE IV - 10

Comparison of *In Vivo* Whole Body and Liver ^{241}Am Disappearance Rates

Animal	Component	λ (yr^{-1}) whole body	$\pm$ standard error of λ	λ (yr^{-1}) liver	$\pm$ standard error of λ
B-458	1a	12.8	3.65	9.04	1.29
	1b	8.92	1.03	**	-
B-458	2	0.145	0.015	0.33	0.074
B-460	1	8.95	1.24	12.8	0.90
B-460	2	0.126	0.034	0.41	0.05
B-352	1	14.7	1.58	11.7	1.01
B-352	2	0.59	0.17	0.77	0.58
B-406*	1	7.0	0.47	6.76	0.88
B-406*	2	0.27	0.06	0.75	0.12

* juvenile baboon

** Whole-body *In Vivo* Retention Curve was resolved into three components;
the liver Am-Retention Curve was resolved into two components.

Figure IV-11

Retention of ^{241}Am in the Livers of B-458 and B-460, Compared to
Cumulative ^{241}Am Excretion in Feces

from Cohen and Wrenn (1973)

Am-241, Retention in Liver Compared to Cumulative Excretion in Feces

measured in the bile. The data are insufficient, however, to correlate the concentration of ^{241}Am in the bile with the rate at which the nuclide was being excreted in the feces. Durbin (1973) has also stated that fecal excretion of ^{241}Am in the cynomolgus monkey can be accounted for almost completely by biliary secretion.

The previous discussion has been concerned mainly with the short term behavior of ^{241}Am in the baboon. When attempting to explain the physiological mechanisms responsible for the long term behavior of ^{241}Am , however, one encounters some difficulties. The determination of a 3.5 year half-life for the long term whole-body component of ^{241}Am clearance in the baboon represents the integral effect of all physiological processes involved in the movement of ^{241}Am within the body. As such only the net result of these processes can be measured, i.e. a small quantity of ^{241}Am is excreted over a long time period with a resultant slowly decreasing body burden.

Durbin (1973) has proposed a kinetic model of the metabolism of Am and other trivalent actinides in mammals (Figure IV-12). In this multi-compartment model, liver, soft tissue, kidney, G I tract, and bone all interact with the circulating pool, the plasma. Flow of the radionuclide between plasma and either bone, soft tissue, G I tract, or liver is bilateral. Liver has unilateral flow into the G I tract as does plasma to the kidney (urine) and incisors (incisor compartment applies only to rodents and relates to continuous attrition of teeth with time, and subsequent swallowing of Am-labelled tooth material). It is reasonable to assume that most of the above model also applies to the baboon.

That there is continuous dynamic movement of ^{241}Am is evidenced by the fact that ^{241}Am is present in the blood (plasma) at 800 days post-

Figure IV-12

Kinetic Model of the Metabolism of ^{241}Am in Mammals

The Incisor Compartment Applies Only to the Rodents, Where
Wearing Down of Dental Matter and Subsequent Swallowing
of the Material Occurs Throughout Life.

from Durbin (1973)

Kinetic Model of the Metabolism of ^{241}Am in Mammals
(from Durbin, 1973)

injection and that small quantities of the nuclide are still being excreted in both urine and feces at 800 days post-injection. The determination of specific parameters of the model, e.g. transfer coefficients between postulated compartments, cannot be attempted on the basis of the few animals used for this study. Accumulation of future data, however, will aid in elucidating the dynamic processes involved particularly in the long term metabolism of ^{241}Am in the baboon.

CHAPTER V

ANALYSIS OF ^{241}Am CONTENT OF SOFT TISSUE AND BLOOD

The results to be discussed in this section include the analysis of ^{241}Am in soft tissue for three sequentially sacrificed adult baboons. Also included are similar analyses performed in the past (Rosen et al, 1972). The measurement times, after injection, range from one day to 817 days, thus covering a wide range for this small number of animals. The juvenile baboons B-406 and B-400 were not sacrificed as part of the present study.

Analysis of ^{241}Am Content of the Blood and Soft Tissue

The retention of intravenously injected ^{241}Am -citrate in the blood of the baboon is shown in figures V-1,2. These figures are fitted respectively to a power function and to a four-component exponential from the data obtained from seven adult and two juvenile baboons. Each figure illustrates that ^{241}Am is cleared very rapidly. Only about 50% of the injected dose remains in the blood seven minutes after injection, about 25% ID fifteen minutes post-injection, $\sim 7.5\%$ ID at one hour and $< 1\%$ ID one day after injection. The above data, which includes blood measurements out to 817 days post-injection shows a high degree of correlation to a power function curve of the form $y = 0.51 t^{-0.87}$ ($\rho = 0.99$) where y is the % ID of ^{241}Am in the blood at time, t (days post-injection).

The ^{241}Am contents of the important soft tissue organs are listed in Table V-1; the concentration of ^{241}Am per gram wet weight of tissue is shown in Table V-2. In general it can be seen that both the content and concentration of ^{241}Am in the various organs tend to decrease with time (Figure V-3). The rate of disappearance and the total amount of americium lost varies

Figure V-1

Retention of ^{241}Am in the Blood of Adult and Juvenile Baboons
 (power function expression)

A power function expression of the form:

$$y = 0.49t^{-0.86} \quad (\rho = 0.97)$$

where

y: The percent of the injected dose remaining
 in the blood at time, t.

t: Time post-injection of ^{241}Am , in days.

was fit to the combined data from both adult and
 juvenile baboons.

For Juvenile alone

$$y = 0.26t^{-0.93} \quad (\rho = 0.96)$$

Time Post Injection of ^{241}Am (days)

Figure V-2

Retention of ^{241}Am in the Blood of Adult and Juvenile Baboons
(exponential expression)

Analysis of the Blood Data from the Adult Baboons have
given a Multicomponent Exponential Expression for the
Retention of ^{241}Am in the blood

$$y = 60e^{-114t} + 6.4e^{-7.6t} + 0.34e^{-0.15t} + 0.019e^{-0.0092t} \quad (\rho = 0.99)$$

where

y: The percent ID remaining in the blood at time, t.

t: Time post-injection of ^{241}Am , in days.

Figure V-3

Concentration of ^{241}Am in Selected Soft Tissues of Sequentially
Sacrificed Baboons

TABLE V - 1

²⁴¹Am Content of Soft Tissue of Baboons Sequentially Sacrificed

(% Injected Dose in Tissue)

	B-470	B-416*	B-412*	B-352	B-458
Time between injection and sacrifice (days)	1	32	86	206	817
Liver	30.88	13.6	22.2	1.976	0.274
Lung	1.097	1.15	0.98	0.449	0.464
Spleen	0.182	0.12	0.076	0.023	0.0086
Kidney	0.662	0.48	0.48	-	0.066
Thyroid	0.006	0.0046	0.0029	0.0019	0.0010
Aorta	0.149	-	-	0.074	0.058
Carotid artery	-	-	-	0.016	0.0079
Pancreas	0.101	0.044	0.043	0.048	0.011
Heart	0.487	0.20	0.10	0.099	0.032
Adrenal	0.010	0.0096	0.0072	0.004	0.00079
Thymus	0.014	-	-	0.003	-
Brain	0.009	0.01	0.009	0.008	0.0024
Muscle	12.304	5.34	3.82	1.93	0.34
Fat	1.89	-	-	0.24	0.036
Stomach	0.306	} 0.9	} 1.62	-	-
Small intestine	0.527			-	-
Large intestine	0.564			-	-
Urinary bladder	0.074	-	-	-	-
Trachea & larynx	0.109	0.17	0.21	0.102	0.080
Axillary lymph nodes	0.0045	-	-	-	-
Hilar lymph nodes	0.004	-	-	0.0018	0.0010
Mesenteric lymph nodes	0.006	-	-	0.0020	0.0028
Spinal cord	0.008	-	-	-	0.0024
Soft palate	0.021	-	-	0.063	0.028
Eyes	0.023	-	-	0.0087	0.0013
Pelt	3.74	3.92	3.69	0.89	0.19
Ovary		0.0046	0.0073	0.002	0.0018
Total ²⁴¹ Am in Soft Tissue	53.2	31.1	33.2	5.94	1.57

*from Rosen et al. 1972

TABLE V - 2

Concentration of ^{241}Am in the Soft Tissue of Sequentially
Sacrificed Adult Baboons (% ID/g wet weight)

	B-470	B-416*	B-412*	B-352	B-458
Time from injection to sacrifice (days)	1	32	86	206	817
Liver	0.158	0.059	0.074	0.087	0.0012
Lung	0.012	0.018	0.0074	0.008	0.0064
Spleen	0.015	0.0069	0.0034	0.0030	0.00073
Kidney	0.021	0.0085	0.0094	-	0.0019
Thyroid	0.0026	0.0034	0.0016	0.00089	0.0013
Aorta	0.0126	0.015	0.014	0.013	0.012
Carotid Artery	-	0.012	0.012	0.0179	0.015
Pancreas	0.0058	0.0031	0.0018	0.0070	0.00061
Heart	0.010	0.0040	0.0021	0.0025	0.00065
Adrenal	0.0065	0.0032	0.0024	0.0016	0.00073
Thymus	0.0025	-	-	0.0008	-
Brain	0.00007	0.00006	0.00006	0.0001	0.000016
Muscle	0.0020	0.00092	0.00064	0.00030	0.000069
Fat	0.00086	-	0.00021	0.000099	0.000020
Stomach	0.0053	0.0022	0.0024	-	-
Small Intestine	0.0050			-	-
Large Intestine	0.0042			-	-
Urinary Bladder	0.0074	-	-	-	-
Trachea & Larynx	0.008	0.0115	0.0125	0.0090	0.011
Axillary lymph nodes	0.0057	-	-	-	-
Hilar lymph nodes	0.0051	-	0.0036	0.0015	0.0010
Mesenteric lymph nodes	0.0052	0.0065	0.0064	0.0014	0.0024
Spinal cord	0.0016	-	-	-	0.00037
Soft palate	0.0048	-	-	0.013	0.00049
Eyes	0.0032	-	-	0.0011	0.00013
Pelt	0.0029	0.0019	0.0018	0.0006	0.00018
Bone Marrow	0.00089	0.0023	0.0011	-	0.0022
Ovary	-	0.0048	0.0032	0.0035	0.0015

*from Rosen et al. 1972

among tissues. For example, the liver shows the highest initial uptake of ^{241}Am ; concurrently the rate of disappearance of ^{241}Am from the liver is one of the most rapid. Conversely, vascular tissue, (e.g. the aorta) shows a very slow rate of net loss of ^{241}Am . A more detailed discussion of the various soft tissue burdens of ^{241}Am will be given in the following sections.

^{241}Am in the Blood of the Baboon: Discussion

As noted in the previous section, the percent of the injected dose of ^{241}Am measured in the blood at various times post-injection can be fit with confidence to a power function relationship (Figure V-1). This best fit regression can be useful as a predictive tool for the behavior of ^{241}Am in the blood of baboons. However the parameters of a power function equation cannot, in general, be considered representative of some biological entity, unlike the case of the exponential form $y = ae^{\lambda x}$ where the exponent, λ , might be considered a biological rate constant. As such the four-component exponential equation given in Figure V-2, might be interpreted to represent the net result of the kinetics of ^{241}Am in four separate compartments or pools. Other interpretations are also possible.

Studies performed on other species of animals i.e. in rat (Taylor 1962, Turner and Taylor 1968), in beagles (Stover et al. 1972), in the lactating ewe (McClellan et al. 1962), and in the cynomolgus monkey (Durbin 1972), all show blood clearance values which are similar to the data obtained for the baboon. For example the above mentioned species were determined to have 1% ID in the circulation at times ranging from two to twelve hours post-injection. These compare well with an average observed time of 10 hrs for the baboon. If one can then assume a basic similarity in the behavior of ^{241}Am in the blood of various species, it becomes useful to consider possible

mechanisms of circulatory transport of ^{241}Am in the baboon based on results obtained in other species.

The physiological behavior of trivalent Americium (III) in the blood of mammals is intermediate between the behavior of Pu (IV) and that of colloidal particles. Both *in vivo* and *in vitro* studies with rat, dog, and human plasma have demonstrated that monomeric Pu (IV) is bound rapidly and firmly to transferrin, a β_1 -globulin which serves as a transport protein for iron. (Popplewell and Boocock 1967; Stover et al. 1968; Stevens et al. 1968). The Pu (IV) transferrin complex was shown to be a stable complex *in vivo*. The rate of disappearance of Pu (IV) from the blood was slower than ^{241}Am or other trivalent actinides. (Langham et al. 1951, Stover et al. 1959).

Like Pu (IV), Am (III) has also been shown to bind to transferrin (Bruenger et al. 1969). By gel filtration techniques it has also been shown that Am is associated with albumin and α -globulins and to a much lesser extent with lipoproteins and macroglobulins (Popplewell and Boocock 1967; Stover et al. 1972). The rapid clearance of ^{241}Am from the blood, however, suggests that the ^{241}Am protein complexes formed in the blood stream are less stable than the Pu (IV)-transferrin complex, which has been shown to occur quantitatively in plasma (Popplewell and Boocock, 1967). The clearance of ^{241}Am from the circulation however is much slower than that shown for colloidal material (Turner and Taylor 1968). This fact suggests that the formation of aggregates in the blood with subsequent rapid clearance due to phagocytic mechanisms, is unlikely. Durbin (1973) states that this assumption is reasonable since circulatory mixing of the dilute injection solutions used in experiments to date, far outweighs the likelihood of association of primary hydrolysis products.

It therefore seems reasonable to assume that ^{241}Am injected into the circulation of the baboon behaves in a manner analogous to that demonstrated in other animal species i.e. the formation of Am (III)-plasma protein complexes which are not extremely stable and hence tend to leave the blood stream fairly rapidly.

It is interesting to note that the determination of low stability of Am-plasma protein complexes point indirectly to the existence of extravascular pools of labile Am which feed the nuclide back into the circulation. The existence of measurable amounts of ^{241}Am in the blood of the baboon at least for 800 days is further evidence for a feedback component into the plasma compartment.

Results of ^{241}Am Measurements in Soft Tissue: Discussion

The amount of ^{241}Am in the liver, as measured radiochemically at sacrifice (Figure V-3), corroborates, in general, the findings of the *in vivo* ^{241}Am measurements in the liver and the bioassay excretion data. The measurement of 30.9% ID liver burden for B-470 at one day post-injection corresponds very well with the estimation of initial liver burden obtained from biopsy samples and by *in vivo* measurement i.e. $31.1 \pm 3.0\%$ ID for live animals. At 817 days post-injection the liver still contains a measurable albeit small amount of ^{241}Am (less than 1% of the initial liver burden).

The retention of ^{241}Am in the liver of baboon B-412 (86 day sacrifice), as determined by *in vivo*, bioassay excretion, and ^{241}Am radiochemical measurement at sacrifice, show certain differences which are difficult to explain. The observed biological half-time of 150 days (Rosen et al. 1972) for ^{241}Am from the liver of B-412 is significantly greater than half-times observed in several other baboons. Thus it would appear that depletion of ^{241}Am

from the liver of B-412 proceeded at a slower rate. This slower clearance, together with a higher than average estimated initial liver uptake (36.6% ID) resulted in an elevated liver burden at sacrifice. Several explanations may be proposed for the occurrence: 1) biological variability or chance 2) abnormal liver function which may have led to altered metabolism of the ^{241}Am deposited in the liver 3) the presence of polymeric ^{241}Am in the injection solution. The first reason cannot be verified without studying a greater number of animals. The second reason also cannot be verified since pathological examination of liver tissue was not performed. Since graded ultrafiltration experiments were not performed, the third possibility also cannot be verified. A shift in pH of the injection solution above 4.5 for any period of time could lead to aggregation of primary hydrolytic products of ^{241}Am . It has been shown in the mouse that polymeric Am behaves differently than monomeric Am (Lindenbaum and Rosenthal 1972, ICRP 19, 1972). The half-life of polymeric Am in the mouse liver is about three times longer than the monomeric form. In addition, the initial uptake of polymeric ^{241}Am in the mouse liver is higher than with the monomeric form (66% ID vs 39% ID); deposition of Am in the skeleton, however, is decreased (6% ID vs 20% ID) in the polymeric form. This is due to phagocytic uptake of colloidal aggregates of the polymeric forms of americium which tend to deposit in the liver and other RE tissues. The fact that the half-time of ^{241}Am in the liver of B-412 is longer than in other baboons ($\approx 150\text{d}$) and that a greater than average quantity of the injected ^{241}Am was initially deposited in the liver. However, an increased phagocytic uptake of polymeric Am should also lead to increased burdens of the nuclide in other reticuloendothelial tissue e.g. the spleen or bone marrow as has been demonstrated in the mouse (ICRP 19, 1972). Such increases in other RES organs were not noted.

Interspecies comparison of the retention of ^{241}Am in the liver (Table V-3) shows that there are basically two groups of animals whose retention half-times differ by orders of magnitude. The animals with short retention times include the mouse, rat, cynomolgus monkey and baboon, while the Chinese and Syrian hamsters and the beagle dog show very long retention times for ^{241}Am in the liver. This makes extrapolation of animal data to the human situation very difficult, particularly since the behavior of ^{241}Am in the human liver is so poorly defined.

Other Soft Tissue Deposits

The non-hepatic tissues which contain the greatest amounts of ^{241}Am include the lung, kidney, spleen, and (due to their large masses) muscle, pelt, and fat. At very early times viz. one day post-injection, 77% of the non-hepatic ^{241}Am in soft tissue is found in the muscle, pelt and fat, 53% in the muscle alone. The disappearance of at least half of the ^{241}Am in muscle proceeds with a short half-life (~ 25 days) while the remaining fraction disappears at a considerably slower rate (300 days). Although the concentration of ^{241}Am in the muscle is small (2×10^{-3} to 6.9×10^{-5} % ID/g), the large muscle mass (41% of the body weight), makes the muscle an important early site of deposition of ^{241}Am .

Figure V-3 illustrates the behavior of ^{241}Am concentration of several soft tissue organs in the five sacrificed baboons. The early behavior (i.e. $t < 100\text{d}$) of ^{241}Am in the lung, kidney or spleen is not clear due to the scatter of the small number of data points considered. It does appear however that after 100 days, the concentrations of ^{241}Am in the kidney and spleen both decrease with long half-lives ($\sim 350\text{d}$), while the concentration in the lung decreases even more slowly (~ 5 years). It thus appears that

TABLE V - 3

Retention of ^{241}Am in the Liver of Various Species of Experimental Animal

Animal	Mode of Administration	Chemical Form	Retention Half Time	Study
Mouse	IV	citrate	9 days	(Lindenbaum,72)
Rat	IP	nitrate	14.4days(96%)	(Tseveleva,69)
Rat	IM	citrate	8-9 days	(Lindenbaum,72)
Chinese hamster	IP	citrate	1000 days	(McKay,72)
Beagle	IV	citrate	3500 days	(Lloyd,70)
Cynomolgus Monkey	IM or IV	citrate	70 days	(Durbin,72)
Baboon	IV	citrate	27 days(80%) 350-750 days(20%)	This study

IV = intravenous

IP = intraperitoneal

IM = intramuscular

^{241}Am that has not cleared from the lung by 100 days post-injection will remain in the lung for long times. The mechanisms of uptake and retention of intravenously administered ^{241}Am in the lung are not well understood at this time.

The measurement of high concentrations of ^{241}Am in vascular tissue i.e. the aorta and carotid arteries was consistent for all animals and it appears to be bound firmly to the tissue. The estimated retention half-time, based on the concentration measurements made on the post-mortem tissue was eight years. For exposure times greater than 200 days, the aorta was measured to have the highest soft tissue concentration of ^{241}Am . Of the soft tissues assayed for ^{241}Am content, only the liver, spleen, lung, and kidney showed higher concentrations at one day post-injection. It thus appears that ^{241}Am deposits rather quickly and is retained for a relatively long time in the large vascular tissues. Whether ^{241}Am also deposits in other vascular tissues e.g. veins, smaller arteries, arterioles etc. is not known since these vessels were not analyzed for ^{241}Am .

The mechanisms by which ^{241}Am is taken up and retained in the aorta is presently unknown. However the presence of acid mucopolysaccharides e.g. chondroitin sulphate in the ground substance of connective tissue (Bertelsen 1968) invites speculation. Chipperfield and Taylor (1970, 1972) demonstrated *in vitro*, the binding of Am (III) to chondroitin sulphate and to other protein fractions. Although the significance of binding to bone glycoproteins in relation to fixation of Am (III) to living bone is not understood, chondroitin sulphate can markedly reduce the adsorption of Am (III) onto bone mineral. The chondroitin sulphates present in the aorta may also be involved in the mechanism of uptake and retention of ^{241}Am ; however at the present, these mechanisms are not clearly defined.

Data on the lymph node concentration of ^{241}Am is not complete. However, it does seem that the concentration of ^{241}Am in the lymph nodes decreases with time (except for B-458 mesenteric lymph nodes).

The thyroid of the baboon does not concentrate ^{241}Am (0.001-0.002% ID/g) as does the beagle thyroid where it has been noted to be a major deposition site (0.05-0.1% ID/g) (Lloyd et al. 1970). In fact the beagle and the rat seem to be unique in their capacity to incorporate ^{241}Am in the thyroid.

The endocrine organs (adrenal, ovary, thymus, and thyroid) showed little affinity for ^{241}Am although measurable amounts of the nuclide were found in all the organs measured ($\sim 0.01\%$ ID at 1d to $\sim 0.001\%$ ID at 817d).

The trachea and larynx had relatively high concentrations of ^{241}Am (0.01% ID/g). The ^{241}Am in this tissue appeared to be retained with a long half-life similar to the retention of ^{241}Am in the aorta. This is not surprising since these structures are cartilagenous and as such are osseous tissue. The retention would therefore be more akin to bone than to soft tissue.

Microlocalization of ^{241}Am in Soft Tissue

Autoradiography of ^{241}Am deposited in soft tissue of several of the baboons used in these studies have been performed. Preliminary examination indicates that considerable longer exposure times are necessary in order to obtain useful information concerning the microdistribution of ^{241}Am . However some data obtained from the examination of autoradiographic preparations of B-470, the baboon sacrificed at one day post-injection, may be stated:

Liver: α -tracks were diffuse with no indication of "star" formation or aggregation. The tracks were primarily associated with parenchymal cells.

Spleen: nearly all tracks observed were found in the red pulp, more specifically the perifollicular areas.

Lung: most tracks were associated with alveolar cells.

Kidney: the majority of the labelling was associated with the renal tubules although some tracks were also observed to occur in the glomeruli and the large veins.

More comprehensive results will be available for future annual reports.

CHAPTER VI

DISTRIBUTION OF ^{241}Am IN THE SKELETON OF THE BABOON

Inasmuch as the skeleton has been shown to be one of the two most important sites of ^{241}Am deposition, extensive analyses of ^{241}Am content in individual bones of sequentially sacrificed baboons was performed.

The results of these analyses are presented in Tables VI-1,2,3,4 and Figures VI-1,2. On the basis of the total amount of activity deposited in a given bone, the most heavily labelled bones at one day after injection (in approximate decreasing order) are lumbar vertebrae, pelvis, maxilla and calvarium, thoracic vertebrae, ribs, femur, humerus, sacral vertebrae, and tibia. However on the basis of concentration the predominance of the highly trabeculated bones becomes apparent (i.e., in order of decreasing concentration): thoracic vertebrae, lumbar vertebrae, sternum, sacral vertebrae, cervical vertebrae, ribs, clavicle, pelvis, scapula, maxilla, calvarium, and the long bones. Thus the axial skeleton is, at least in terms of the concentration of ^{241}Am , the most significant skeletal site of deposition. Seventy-five percent of the skeletal burden is found in these bones one day postinjection although these same bones comprise only 60% of the skeletal mass.

Careful comparison of the skeletal data of the five baboons analyzed as part of this study indicates that there are no apparent trends, in terms of changes in amount of or concentration of ^{241}Am in individual bones, or whole skeleton, as a function of time. Based on the five analyzed skeletons the mean and standard deviation of the amount of ^{241}Am found in the skeleton was $39.7 \pm 6\%$ ID from 1-817 days.

Figure VI-1

Distribution of ^{241}Am in the Vertebrae of Sequentially
Sacrificed Baboons

Figure VI-2

Concentration of ^{241}Am in the Vertebrae of Sequentially
Sacrificed Baboons

TABLE VI - 1

241 Am Content of the Bones of Sequentially Sacrificed Baboons*

Time between Injection and sacrifice (days)	(% Injected Dose)				
	B-470 1	B-416 32	B-412 86	B-352 206	B-458 817
<u>Bone Description</u>					
Maxilla and calvarium	3.645	} 6.50	} 5.77	5.340	4.173
Mandible	1.064			0.732	1.200
Upper teeth	0.122			0.231	0.261
Lower teeth	0.129			-	0.225
Clavicle	0.152	0.19	0.16	0.124	0.158
Scapula	1.626	1.82	1.68	1.778	1.602
Humerus	2.036	3.16	2.10	1.787	3.684
Ulna	0.550	1.02	0.74	0.532	1.156
Radius	0.476	0.88	0.66	0.542	0.966
Hand	0.708	-	0.81	0.666	1.164
Cervical vertebrae	0.794	1.023	0.868	0.934	1.077
Thoracic vertebrae	3.586	4.125	3.51	5.048	4.941
Lumbar vertebrae	4.942	5.99	4.81	6.760	5.741
Sacral vertebrae	2.010	-	-	-	2.923
Caudal vertebrae	0.380	-	0.98	0.590	1.519
Pelvis	4.459	6.24**	4.56**	3.901**	5.673
Femur	2.446	3.64	3.20	2.194	4.854
Tibia	1.168	2.11	1.30	0.940	2.590
Fibula	0.176	0.34	0.26	0.166	0.180
Foot	0.874	-	1.39	0.974	1.044
Sternum	0.544	0.52	-	0.664	0.438
Ribs	2.680	2.57	3.52	1.985	2.248
Knee cartilage	-	-	-	0.010	0.004
Patella	0.082	-	-	0.056	0.216
Vertebral cartilage	-	-	-	0.005	-
Total skeleton	34.41	44.22	36.83	34.90	48.00

*Numbers represent the Am content for all bones of a given designation, e.g. one pelvis, two femurs, seven cervical vertebrae, etc.

**includes 3 sacral vertebrae

TABLE VI - 2

241
Am Concentrations in the Bones of Sequentially Sacrificed Baboons
(% ID/g bone)

Time between Injection and Sacrifice (days)	B-470 1	B-416 32	B-412 86	B-352 206	B-458 817
<u>Bone Description</u>					
Maxilla and calvarium	0.017			0.024	0.027
Mandible	0.017	0.020	0.023	0.021	0.025
Upper teeth	0.006			0.012	0.014
Lower teeth	0.007			-	0.014
Calvicle	0.027	0.024	0.024	0.020	0.026
Scapula	0.020	0.027	0.023	0.025	0.034
Humerus	0.017	0.027	0.019	0.030	0.038
Ulna	0.008	0.016	0.013	0.012	0.025
Radius	0.009	0.015	0.011	0.011	0.022
Hand	0.014	-	0.012	0.012	0.019
Sternum	0.035	0.032	-	0.059	0.029
Ribs	0.029	0.022	0.042	0.022	0.011
Cervical vertebrae	0.030	0.031	0.030	0.031	0.036
Thoracic vertebrae	0.045	0.046	0.040	0.048	0.054
Lumbar vertebrae	0.039	0.046	0.043	0.060	0.063
Sacral vertebrae	0.032	0.046*	0.043*	-	0.061
Caudal vertebrae	**	-	0.023	0.011	0.025
Pelvis	0.0255	0.025	0.024	0.024	0.043
Femur	0.015	0.022	0.010	0.016	0.035
Patella	0.014	-	-	0.012	0.029
Tibia	0.011	0.019	0.013	0.012	0.022
Fibula	0.009	0.015	0.016	0.012	0.022
Foot	0.009	-	0.012	0.013	0.020
Cartilage	-	-	-	0.0048	0.001
Total skeleton	0.0201	0.026	0.024	0.025	0.033

*incomplete; based on three sacral vertebrae

**B-470 had a stump rather than a full tail

Comparison of the Distribution of ^{241}Am along the Spinal Column of
Sequentially Sacrificed Baboons (%ID/vertebrae)

Time between Injection and Sacrifice (days)	B-470 1	B-416* 32	B-412* 86	B-352 206	B-458 817
Ce 1	0.078	0.068	0.088	0.117	0.102
Ce 2	0.105	0.114	0.11	0.108	0.121
Ce 3	0.104	0.125	0.10	0.108	0.156
Ce 4	0.115	0.14	0.13	0.107	0.123
Ce 5	0.112	0.15	0.12	0.133	0.160
Ce 6	0.127	0.175	0.145	0.144	0.182
Ce 7	0.153	0.225	0.175	0.217	0.233
T 1	0.215	0.21	0.20	0.195	0.276
T 2	0.202	0.23	0.21	0.234	0.256
T 3	0.232	0.23	0.20	0.229	0.293
T 4	0.235	0.235	0.20	0.248	0.249
T 5	0.246	0.25	0.22	0.287	0.299
T 6	-	0.25	0.23	0.318	0.238
T 7	0.277	0.30	0.25	0.383	0.372
T 8	0.316	0.34	0.30	0.397	0.398
T 9	0.392	0.35	0.30	0.507	0.433
T 10	0.413	0.45	0.36	0.621	0.541
T 11	0.462	0.56	0.47	0.759	0.704
T 12	0.596	0.72	0.57	0.870	0.801
L 1	0.684	0.76	0.65	0.847	0.872
L 2	0.755	0.91	0.72	1.042	0.979
L 3	0.786	0.98	0.80	1.130	1.100
L 4	0.834	1.03	0.87	1.347	-
L 5	0.960	1.16	0.98	1.198	1.349
L 6	0.923	1.15	0.79	1.196	1.441
S 1	0.876	1.08	0.92	-	1.261
S 2+3	1.134	-	-	-	1.662
Ca 1	0.080	-	-	0.075	0.208
Ca 2	0.076	-	-	0.062	0.139
Ca 3	0.068	-	-	0.064	0.121
Ca 4	0.069	-	-	0.068	0.141
Ca 5	0.087	-	-	0.063	0.149
Ca 6				0.057	0.160
Ca 7				0.047	0.142
Ca 8				0.031	0.093
Ca 9				0.025	0.068
Ca 10				0.025	0.057
Ca 11				0.018	0.050
Ca 12				0.015	0.046
Ca 13				0.011	0.046
Ca 14				0.008	0.028
Ca 15				0.006	0.019
Ca 16				0.004	0.015
Ca 17				0.003	0.011
Ca 18				0.002	0.008
Ca 19				0.001	0.006
Ca 20				0.001	0.006
Ca 21				0.001	0.003
Ca 22				0.001	0.002
Ca 23				0.002	0.002

* from Rosen 1974 (unpublished data)

Ce = cervical vertebra S = Sacral vertebra
T = Thoracic vertebra Ca = Caudal vertebra
L = Lumbar vertebra

Comparison of the ^{241}Am Concentration in Vertebrae of Sequentially
Sacrificed Baboons (%ID/g wet weight)

	B-470	B-416*	B-412*	B-352	B-458
Ce 1	0.021	0.017	0.021	0.031	0.029
Ce 2	0.027	0.028	0.027	0.028	0.036
Ce 3	0.030	0.033	0.034	0.030	0.040
Ce 4	0.030	0.035	0.040	0.030	0.031
Ce 5	0.030	0.026	0.029	0.029	0.040
Ce 6	0.033	0.037	0.033	0.036	0.037
Ce 7	0.038	0.040	0.035	0.037	0.044
T 1	0.038	0.036	0.035	0.036	0.048
T 2	0.041	0.043	0.033	0.043	0.056
T 3	0.041	0.044	0.035	0.045	0.058
T 4	0.044	0.042	0.041	0.046	0.046
T 5	0.047	0.047	0.040	0.045	0.056
T 6	-	0.045	0.038	0.047	0.056
T 7	0.048	0.049	0.041	0.052	0.056
T 8	0.047	0.049	0.051	0.054	0.054
T 9	0.053	0.048	0.041	0.054	0.052
T 10	0.049	0.050	0.043	0.054	0.056
T 11	0.042	0.049	0.042	0.053	0.056
T 12	0.045	0.049	0.047	0.055	0.056
L 1	0.044	0.045	0.042	0.052	0.056
L 2	0.041	0.049	0.044	0.061	0.056
L 3	0.039	0.046	0.045	0.060	0.060
L 4	0.038	0.048	0.047	0.068	-
L 5	0.041	0.044	0.043	0.058	0.066
L 6	0.031	0.045	0.038	0.064	0.070
S 1	0.038	0.046	0.043	-	0.067
S 2 + 3	0.028	-	-	-	0.056
Ca 1	0.020	-	-	0.022	0.045
Ca 2	0.017	-	-	0.019	0.035
Ca 3	0.014	-	-	0.018	0.031
Ca 4	0.017			0.016	0.038
Ca 5	0.016			0.0165	0.038
Ca 6				0.017	0.040
Ca 7				0.018	0.038
Ca 8				0.016	0.032
Ca 9				0.015	0.034
Ca 10				0.017	0.031
Ca 11				0.015	0.031
Ca 12				0.016	0.033
Ca 13				0.012	0.041
Ca 14				0.010	0.028
Ca 15				0.009	0.024
Ca 16				0.007	0.022
Ca 17				0.0069	0.018
Ca 18				0.0049	0.017
Ca 19				0.0035	0.016
Ca 20				0.0028	0.016
Ca 21				0.0031	0.10
Ca 22				0.0028	0.008
Ca 23				0.0045	0.007

*From Rosen 1974, (unpublished data)
Abbreviations same as for Table VI-3

TABLE VI - 5

Percent of the Total Skeletal Burden of ^{241}Am Contained in Specific Bones

Time between injection and sacrifice (days)	B-470 1	B-416 32	B-412 86	B-352 206	B-458 817
<u>Bone</u>					
Skull*	14.4	14.69	15.66	15.3	11.61
Vertebrae**	27.1	28.5	29.92	36.7	25.7
Sternum	1.58	1.17	-	1.90	0.87
Ribs	7.44	5.81	9.55	5.70	4.38
Pelvis***	18.8	11.51	9.88	11.21	17.03
Tail	1.10	-	2.66	1.70	3.01
Clavicle	0.73	0.42	0.43	0.42	0.32
Scapula	5.02	4.11	4.56	5.11	3.35
Humerus	5.92	7.14	5.70	5.09	7.30
Radius	1.38	1.99	1.79	1.60	1.92
Ulna	1.60	2.30	2.00	1.47	2.29
Hands	2.06	-	2.19	1.94	2.31
Femur	7.11	8.23	8.68	6.32	9.62
Tibia	3.39	4.77	3.52	2.73	5.14
Fibula	0.51	0.76	0.70	0.49	0.71
Foot	2.54	-	3.77	2.76	4.05
Patella	0.24	-	-	0.24	0.43

*includes mandible and teeth

**cervical, thoracic, and lumbar vertebrae only

***includes sacral vertebrae

Comparison of the *in vivo* skull measurement data with actual assay of ^{241}Am activity in the bones does not offer a solution to the problems presented by the *in vivo* measurement, as discussed in Chapter IV, i.e. the rate constants as measured by *in vivo* methods in several baboons were shown not to be statistically different from zero. This implies that within the accuracy and precision of the measurement technique employed, there were no discernible changes in the amount of ^{241}Am in the skulls of the baboons. The amount of ^{241}Am measured in the skulls of five adult baboons did not vary significantly from animal to animal. The mean percent absorbed dose of ^{241}Am in the skulls of these animals was $5.92\% \pm 0.63 \text{ ID}$. If one does not include B-470, the baboon sacrificed at one day postinjection (this is reasonable since measurable increases of ^{241}Am in the skull occur for several weeks postinjection, as determined by *in vivo* measurement), then the mean activity of ^{241}Am in the skull is $6.16 \pm 0.40\% \text{ ID}$. It is interesting to note also that the concentrations of ^{241}Am in the maxilla and calvarium of the sacrificed baboons show a continually increasing concentration from 0.017 % ID/g bone at one day post-injection to 0.027% ID/g bone at 817 days post-injection. Although it may be premature to conclude that the activity of the skull tends to increase with time, it would seem reasonable, based on both post-mortem analysis of ^{241}Am in skull and *in vivo* measurement to assume that any net change in the content of ^{241}Am in the skull occurs at a very slow rate.

Extrapolation of the analysis of the data collected by *in vivo* measurement of the skull to estimating the kinetics of ^{241}Am in the whole skeleton presents certain problems. The main difficulty lies in the fact that ^{241}Am is not a "volume seeker" in bone but a "surface seeker" i.e. Am deposits on

the surface of the bone and does not become incorporated into the osteoid matrix as a calcium surrogate. This disposition pattern has been shown in the rat (Durbin 1969), the beagle (Lloyd et al. 1972) and the cynomolgus monkey (Durbin 1974). If then the amount of ^{241}Am that deposits in bone in the baboon does depend on the amount of surface available for deposition in each bone, then the bones with greater surface to volume ratios should incorporate the greatest fraction of ^{241}Am . Although a systematic and statistically valid study of the physical parameters of the baboon skeleton has not yet been done, data has been accumulated on human skeleton (Lloyd 1971) and some preliminary results of surface to volume ratios in selected baboon bones from one animal are now available (Farnham and Sonza-Novera 1974). Table VI-6 shows a comparison of the relative weight of the individual bones taken from the whole skeleton of humans and of both male and female baboons. On a relative weight basis the female baboon compares well with the human except that the lower limbs of the baboon represent a smaller fraction of the total skeletal weight than in the human. All other groupings appear to relate very well (the larger percent contribution of the "other" category in the baboon is due to smaller lower limb contribution; also a 3% contribution from caudal vertebrae (tail bones) which is not germane to the human). Very little information concerning the detailed physical characteristics of the baboon skeleton has been reported to data. Preliminary studies by Farnham and Sonza-Novera (1974), using one thoracic vertebra, a femur section and one rib, from a baboon of unknown clinical and experimental history indicate that there are similarities and differences between baboon and human surface to volume ratios (Table VI-7). Comparison of cortical bone sections of human and baboon shows that there is very little difference in S/V for cortical bone. Lloyd and Hodges

TABLE VI - 6

Relative Weights of Individual Bones Taken from the Whole

Skeleton (% of whole skeleton weight)

	Human ¹	Human ²	Male ³ Baboon	Female ⁴ Baboon
Skull	17.1	18.9	23.8	18.0 ± 1.5
Femora }	32.0	31.0	20.4	17.4 ± 0.8
Tibiae }				
Fibulae }				
Humeri }	11.9	10.1		13.4 ± 1.9
Ulnae }				
Radii }				
Vertebrae	10.5	13.2	12.6	15.6 ± 1.9
Ribs	6.3	6.3	4.5	6.5 ± 0.7
Other bones	22.2	20.6	17.0	29.1 ± 3.0

¹Trotter & Peterson (1962)²Lloyd & Hodges (1971)³Schultz (1962)⁴This study (mean ± standard deviation of 5 adults)

TABLE VI-7

Surface/Volume Ratios for Selected Bones of Humans, Dogs, Mouse, and Baboon

Bone	Surface/Volume (cm^2/cm^3)				% volume trabecular bone		% trabecular ash		Concentration of ^{241}Am of baboon bone % ID/g bone
	Human ¹	Dog ¹	Mouse ¹	Baboon ²	total volume of bone Human ¹		total ash Monkey ³	Dog ³	
Cortical Bone, Femur mid-shaft	30 ± 3	~ 30	30-40	27.9 ± 7.9					0.014
Trabecular Bone, Femur Head	96 ± 6			140.2 ± 31.3	30		22		0.030
7th thoracic vert, whole portion	108 ± 21			81.6 ± 15.4	29		49		0.049
7th thoracic vert, trabecular portion	116 ± 23			86.9 ± 18					
3rd lumbar vert, whole portion	120 ± 13	146 ± 27	250-350		27		48	49	0.050
Rib sternal end	186			35.1 ± 7.5					
mid-shaft	93			27.1 ± 6.2					
vertebral end	48			34.6 ± 12.1					
Iliac crest	123 ± 16				25		21		
Cranium	41 ± 6						30		0.022
Whole skeleton	50	77							0.026

¹Lloyd and Hodges 1971²Farnham and Sonza-Novera 1974³Gong 1973

(1971) also measured cortical bone in the dog and mouse and found that the surface to volume ratios of man, dog, and mouse differed by less than a factor of two. Measurements of S/V for trabecular bone have shown several interspecies differences. Measurements of lumbar vertebrae sections have shown that S/V increases from $120 \text{ cm}^2/\text{cm}^3$ in human to $150 \text{ cm}^2/\text{cm}^3$ in the dog and $250\text{--}350 \text{ cm}^2/\text{cm}^3$ in the mouse (Lloyd and Hodges 1971). Comparison of thoracic vertebrae, however, have shown that the S/V for human ($108 \text{ cm}^2/\text{cm}^3$) is greater than that measured for a baboon ($82 \text{ cm}^2/\text{cm}^3$). The preceding data must be considered tentative since only one human skeleton and only three bones from a single baboon have been analyzed. It is important to note that regression of the concentration of ^{241}Am in several baboon bones vs. the calculated surface to volume ratio in the human, shows a fairly high degree of correlation ($p = 0.93$). That this correlation has any physiological meaning is indeterminate at this point but suggests that the analysis of both the intraskeletal distribution of ^{241}Am in the baboon and the quantitation of the physical parameters of the skeleton should be pursued further.

Inter-vertebral Distribution of ^{241}Am in the Baboon

Tables VI-3,4 and Figures VI-1,2 illustrate the amount and concentration of ^{241}Am in the individual vertebrae along the spinal column. The vertebrae are one of the more important skeletal deposition sites of ^{241}Am and a significant percentage of bone tumors, particularly osteogenic sarcomas, which have occurred in rats and beagles as a result of internal deposition of bone-seeking radionuclides (Ra,Pu,Ca,Sr,Th) have occurred in the vertebrae, particularly the thoracic and lumbar regions of the spine. (Finkel and Biskis, 1959; Thurman 1973). As can be seen in Figures VI-1,2 both the amount and the concentration of ^{241}Am increase from cervical, to thoracic, to lumbar regions.

Both the amount and concentrations of ^{241}Am in the caudal vertebrae are significantly less than the other regions of the spine. That one can observe an increase in the amount of ^{241}Am in sequential vertebrae (cervical to lumbar) is reasonable since the mass of each vertebrae also generally increases. However, that the concentration of ^{241}Am also increases as one proceeds down the spine suggests that there may be more available surface to which ^{241}Am can bind per gram of bone and that the surface to volume ratio increases with descending vertebral position. Measured S/V for thoracic ($108 \pm 21 \text{ cm}^2/\text{cm}^3$) vs. lumbar (120 ± 13) vertebrae in the human (Lloyd and Hodges, 1971) does not oppose this view; however, data on baboon vertebrae are completely lacking.

Inter-species Comparison of ^{241}Am Distribution in the Skeleton

The distribution of ^{241}Am in the skeleton of various species of mammals show several similarities (Table VI-8). In all species the vertebral column represents the site at which the largest fraction of skeletally deposited ^{241}Am is found (30% for baboon and cynomolgus monkey, 42% for the dog, and 23% for the rat). The difference between the amount of ^{241}Am in the spinal column of the beagle to that of the baboon is somewhat misleading because of the fact that sacral vertebrae are included in the figures for the dog but not for those of the baboon (sacral vertebrae of the baboon are included in the pelvis). Although it is believed that the dog does have a greater fraction of its skeletally deposited ^{241}Am in the spine (42%) than does the baboon, the difference is actually less than shown in Table VI-8. (~ 6% of the skeletal burden is in the sacral vertebrae of the baboon; hence the baboon spine has 36% of the total skeletal burden). Comparison of the distribution of ^{241}Am in the baboon and cynomolgus monkey shows that,

TABLE VI - 8

Comparison of the Distribution of ^{241}Am in the Skeletons of the
 Baboon, Rat, Dog, and Cynomolgus Monkey
 (Percent of Total Skeletal Burden)

	Baboon ¹	Dog ²	Cynomolgus ³ Monkey	Adult Rat ⁴
Skull+mandible+ teeth	14.3 ± 1.6	11.16 ± 1.31	22.2	-
Cervical vert.	2.38 ± 0.17	7.94 ± 1.11	} 30.6	} 23.8
Thoracic vert.	10.8 ± 2.1	16.95 ± 2.86		
Lumbar vert.**	14.4 ± 2.9	17.53 ± 3.26		
Tail	2.15 ± 0.93	1.77 ± 0.13		-
Paws	5.49 ± 1.0	2.77 ± 1.98	6.16	-
Radii	1.75 ± 0.27	1.19 ± 0.27	-	
Ulnae	1.97 ± 0.40	1.45 ± 0.33	-	9.64
Humerii	6.31 ± 1.06	6.34 ± 0.48	6.46	
Tib&Fib&Patella	4.40 ± 0.96	3.65 ± 0.82	-	7.61
Femora	8.09 ± 1.47	6.06 ± 0.75	15.7*	8.48
Pelvis***	14.9 ± 3.35	5.26 ± 0.80	8.43	5.75
Scapulae	4.37 ± 0.67	3.71 ± 0.55	3.55	-
Ribs	6.70 ± 1.95	8.60 ± 0.83	5.92	3.98
Sternum	1.39 ± 0.44	5.53 ± 0.99	1.47	-

¹ mean of 5 animals (1-817 days)

² Lloyd (1973) mean of 10 dogs (1-1917 days)

³ Durbin (1973) mean of 8 monkeys (1-63 days)

⁴ Durbin (1973) mean of 20 rats (1-15 days)

*includes femora, tibia, fibulae, radius, ulna

**Lumbar vertebrae includes sacral vertebrae in the dog

***Pelvis includes sacral vertebrae in the baboon

except for the skull, where the cynomolgus monkey has ~ 8% more ^{241}Am , and the pelvis, where the baboon has ~ 7% more ^{241}Am deposited, the relative bone values are quite similar, i.e. for long bones, vertebrae, sternum, ribs, and paw bones.

Comparison of the dog with the primate shows a higher proportion of the burden in the vertebrae and sternum and slightly more in the ribs of the dog; less ^{241}Am is deposited in the pelvis, and generally less in the long bones, skull and paw bones. Comparison with the rat is difficult because of the incompleteness of the data. However its relative distribution does indicate less deposition in ribs or vertebrae than either primates or dogs while the amount in its long bones is slightly higher than either group. In addition the amount of ^{241}Am in the pelvis of the rat is more like that of the dog than the primate.

CHAPTER VII

THE EFFECT OF DTPA ON THE KINETICS OF ^{241}Am IN THE BABOON

Interest in developing chemical methods of reducing the levels of radioactive contamination in humans is not new. Early research, i.e. work before 1956, was concerned with the principle of "carrier therapy." Stable isotopes of metals such as zirconium, manganese, iron, titanium and aluminum, were injected into the blood stream where, at physiological pH, they formed precipitates or basic salts. Certain radioelements attach themselves to the circulating carrier aggregates and subsequently behave as the colloids (Schubert 1955). If the particle size range of the circulating carrier is such that the colloid is filterable by the kidney and at the same time, is likely to escape capture by the reticuloendothelial (RE) system, there is good probability that the element can be excreted. Plutonium, yttrium, cerium, americium etc. have all been successfully removed by zirconium carrier therapy (Schubert 1949, Kavin et al. 1950, Langham and Carter 1951). The drawback to carrier therapy, however, is that it acts principally on circulating radionuclides. As such, the therapy must be initiated as quickly as possible after the contamination has occurred. As has been presented earlier, however, ^{241}Am disappears from the blood stream very rapidly, making the use of carrier therapy difficult.

The point just mentioned is academic, however, because the therapeutic agents of choice are, at present, the polyamino polycarboxylic acids, ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA). DTPA, in particular, has become the treatment of choice in cases of contamination by some actinides and rare earth elements (Smith 1972). Not only has DTPA been shown to be effective in removing heavy metals from

the circulation (Lindenbaum 1962), but it also has been shown to be effective in removing some actinides and lanthanides from the soft tissue and bone of mice, rats, and dogs (Taylor and Sowby 1962; Nenot 1971,72; Rosenthal et al. 1973).

Influence of DTPA Therapy on the ^{241}Am Excretion Patterns of the Baboon

Intuitively one might expect that the ability of DTPA to accelerate removal of a given radioelement would depend on a variety of factors: 1) the stability of the metal-DTPA complex under physiological conditions, 2) the chemical form of the metal *in situ*, e.g. loosely bound to circulating proteins, or more firmly associated with liver ferritin; 3) the physical site of deposition i.e. intra- or extra-cellular, on bone surface or buried under new bone deposits. Many of the above factors relating to the normal metabolic behavior of ^{241}Am change with time. It was shown (Chapters V and VI) that the relative distribution of ^{241}Am in the baboon was not the same at one day post-injection as it was one month, or two years post-injection. Therefore, it is important to know how the efficiency of DTPA therapy in removing ^{241}Am from the body changes relative to the distribution of Am within the body (or, more generally, with the time between injection of Am and initiation of DTPA treatment).

In order to compare the ability of DTPA to remove ^{241}Am burdens of different "deposition ages," three baboons were selected for treatment: B-164, B-520, B-460. The specifics of body burden, distribution of ^{241}Am at the start of treatment, injected dose of DTPA etc. are given in Table VII-1. Intravenous injections of the trisodium salt of the calcium chelate form of DTPA were given thrice weekly on an alternating day basis (Monday, Wednesday, and Friday). The therapy was continued for three to four weeks.

The effect of DTPA on urinary and fecal excretion of ^{241}Am is shown graphically in Figures VII-1,2,3,4,5,6. In addition, control data from B-352 and B-458 are given in Figures VII-7,8,9,10. The excess activity of ^{241}Am excreted due to therapy varies for the three animals treated. These differences are summarized in Table VII-1. It is immediately apparent from the data that the sooner one initiates therapy after injection, the more successful the therapy will be. Treatment of B-460, a baboon which had been injected with ^{241}Am 13 months prior to the beginning of DTPA therapy showed the least effect from the therapy. Excreta analysis of ^{241}Am content indicated that 8.9% of the pretreatment body burden (BB) was excreted in urine and feces during and immediately after the DTPA-therapy. Of this amount 7.9% BB removal was estimated to be due to the therapy. (This value was obtained by subtracting the amount of ^{241}Am excreted by B-458 a non-treated animal whose ^{241}Am injection date was within one month of that of B-460. More detailed analysis of the radiochemical data show that 8% BB was excreted via the urine while 0.9% BB was excreted via the fecal route. In terms of the net amounts of ^{241}Am excreted due to therapy, 7.8% BB was excreted in the urine while 0.05% BB in the feces.

In the case of B-520, a baboon which had been injected with ^{241}Am 45 days prior to the beginning of therapy, the total amount of ^{241}Am excreted during the treatment period was 21.3% of the pre-treatment body burden (BB), of which 12.0% BB appeared in the urine and 9.3% in the feces. By subtracting the amount of ^{241}Am excreted by the control, B-352, during the same post-injection period of time, the net amount of ^{241}Am excreted as a result of DTPA-treatment was 15.1% BB. Of this quantity 11.2% BB was measured to the urine and 3.9% in the feces. This represents a 240% increase in excretion due to chelation therapy.

VII-4

TABLE - VII-1

Removal of ^{241}Am from Baboons as a Function of Interval Between Acute
Intravenous Injection of ^{241}Am and Initiation of DTPA Chelation Therapy

Number Treatments	²⁴¹ Am distribution at start of DTPA therapy	Interval between Am injection and first DTPA treatment	% Body Burden (Gross) Excreted in			% B B Excreted (Net)* in			<i>In Vivo</i> Gross Net		
			urine	feces	total	urine	feces	total			
B-460	11	mostly (95%) skeletal	13 months	8.0	0.9	8.9	7.8	0.05	7.9	11.4	9.3
B-520	9	skeletal and liver	1.5 months	12.0	9.3	21.3	11.2	3.9	15.1	34.5	32.4
B-164	13	55% soft tissue & ECF,40% bone	1 day	40.5	21.1	61.6	36.6	11.4	48.0	80.0	52.3

* Net excretion determined by subtracting values obtained from non-treated controls

ECF = Extracellular Fluid

Figure VII-1

Am-241 Excretion in the Urine of Adult Baboon B-460 after
Na₃-CaDTP Administration

DTPA Therapy was begun 385 Days Post-Injection of ²⁴¹Am

Figure VII-2

Am-241 Excretion in the Feces of Adult Baboon B-460,
after $\text{Na}_3\text{-CaDTP}$ Administration

Figure VII-3

Am-241 Excretion in the Urine of Adult Baboon B-520,
after Na₃-CaDTP Administration

Chelation Therapy was begun 45 Days Post-Injection of ²⁴¹Am

Figure VII-4

Am-241 Excretion in the Feces of Adult Baboon B-520, .
after $\text{Na}_3\text{-CaDTP}$ Administration

Figure VII-5

Am-241 Excretion in the Urine of Adult Baboon B-164,
after $\text{Na}_3\text{-CaDTP}$ Administration

Chelation Therapy was begun One Day Post-Injection of ^{241}Am

Figure VII-6

Am-241 Excretion in the Feces of Adult Baboon B-164,
after Na₃-CaDTP Administration

Figure VII-7

Am-241 Excretion in the Urine of Adult Baboon B-352,
a Control Baboon

B-352 (Control Baboon)
Injected Dose=3440 nCi

Figure VII-8

Am-241 Excretion in the Feces of Adult Baboon B-352,
a Control Animal

Figure VII-9

Am-241 Excretion in the Urine of Adult Baboon B-458,
a Control Animal

This bioassay data corresponds to the same time post-injection of ^{241}Am as the time when DTPA-therapy was begun on B-460, i.e. 380 days post-injection.

Figure VII-10

Am-241 Excretion in the Feces of Adult Baboon B-458,

a Control Animal

Figure VII-11

Retention of ^{241}Am in the Liver of Adult Baboon B-520,
as Measured by *in vivo* Methods

DTPA Chelation Therapy was begun 45 days Post-Injection.

Figure VII-12.

Retention of ^{241}Am in the Skull and Whole Body of Adult Baboon B-520,
as Measured by *in vivo* Methods

Relative Intensity (Count Rate, cpm)

The third adult, B-164, was treated beginning one day after injection of ^{241}Am with therapy continuing for one month (a total of 13 treatment). The total amount of ^{241}Am measured in the combined excreta was 61.6% BB (40.5% BB in the urine, 21.1% BB in the feces). Of this amount 48% BB was determined to be due to chelation therapy. (36.6% BB in the urine, 11.4% BB in the feces).

Comparison of the estimates of changes in ^{241}Am body burden as inferred from bioassay measurements, vs. *in vivo* counting methods made during the same treatment periods show that the decreases in body burden as measured by *in vivo* counting were systematically higher than the same measured decrease in body burden as obtained from bioassay measurement. The gross decrease in the pretreatment body burden of B-460 as measured by *in vivo* counting was estimated to be 11.4% BB of which 9.3% BB was due to therapy. In the case of B-520, 36.8% BB was excreted during the therapy period, of which 34.7% was due to therapy. Finally, the decrease in body burden for B-164 was measured to be 80.0% of which 52.3% BB was excess excretion due to therapy.

As has been stated in Chapter IV, there are several possible explanations for the observed systematic differences between results obtained by bioassay and by *in vivo* methods. There is, however, some evidence that indicates that loss of excreta may be a likely explanation. If loss of sample is indeed the major discrepancy, it is a fairly recent occurrence, thereby effecting the results of the DTPA-treatment studies and not the earlier Am-metabolism studies.

Since there are possible errors associated with the accuracy of the bioassay measurements, one must be cautious in forming any quantitative

judgements. Nevertheless, one may make the following inferences (data compared to Figures VII-7,8,9,10):

- 1) The concentration of ^{241}Am in the urine sample collected for 24 hours beginning immediately after DTPA-treatment was greatly elevated. The increased amount of ^{241}Am measured in these samples varied considerably from animal to animal and also among treatments. However, 5-70 times the pretreatment quantity of excreted ^{241}Am were measured following DTPA-therapy.
- 2) The efficiency of ^{241}Am decorporation decreased as therapy continues. For B-164, the baboon whose therapy was begun one day after ^{241}Am injection, 17% BB and 14% BB respectively, were measured in the excreta following the first two consecutive daily DTPA treatments. On the third consecutive day of treatment 3% BB was removed and thereafter the percent of the remaining ^{241}Am body burden removed per treatment slowly decreased over the course of therapy (one month). In the case of B-520, 1.2% and 1.6% ID were excreted following the first two treatments and thereafter the removal of ^{241}Am per treatment decreased slowly to $\sim 0.4\%$ ID at the end of therapy. Baboon B-460 showed the least change in percent removal of ^{241}Am per treatment. In fact the overall percent decrease of therapy efficiency for B-460 was about 20% from the beginning to the end of the therapy period.

That the efficiency of DTPA therapy in removing ^{241}Am burdens decreases as therapy is continued may be related to the distribution of ^{241}Am among the various deposition sites in the body. Although the liver and skeleton constitute the major sites of ^{241}Am deposition, other soft tissue sites and the plasma have been shown to contain significant quantities of ^{241}Am , particularly at short times following injection of ^{241}Am (see results for B-470 chapter V). That the effectiveness of DTPA therapy does indeed depend on

the site of localization of ^{241}Am will be explored in greater detail later in this chapter.

Long Term Treatment with DTPA

As part of ongoing investigations into the effect of DTPA therapy in removing ^{241}Am from the baboon, consideration has been given to the problems of decorporating ^{241}Am from the skeleton. Although many studies have been performed using DTPA at short time periods after ^{241}Am (or Pu) contamination, very little attention has been given to experimental chelation therapy begun at long times after actinide contamination, the time at which most of the body burden is associated with in the skeleton. As such a baboon (B-460) which had been injected with ^{241}Am more than one year prior to the beginning of therapy, was treated periodically for one year. Results of this series of treatments are shown in Table VII-2 and Figures VII-13a,b,14,15. The reduction of treatment periods from 11 and 25 treatments at the beginning, to only three treatments per period was necessitated by medical problems presumably associated with the technique and frequency of therapy (this will be discussed in greater detail in Chapter VIII).

Based on the data obtained from the successive DTPA treatments of B-460 it can be estimated that, if the baboon, B-460, had been treated maximally, i.e. for 26 treatment periods (a treatment period consisted of three intravenously-administered DTPA injections in one week followed by one week without treatment) the most that could be given in one year, and that the average rate of removal of ^{241}Am by DTPA did not change significantly during the entire period (this seems to be true after the first few treatments for B-460) than one would expect to remove 30% of the body burden in one year. (The body burden of B-460 at the beginning of DTPA treatment must be estimated

TABLE VII - 2

Effect of Long Term DTPA Treatment on the Excretion of ^{241}Am from B-460*

Treatment Series	Number of Treatments	Net % of BB excreted as result of treatment	% BB Excreted per treatment
1	11	8.0	0.72
2	25	10.4	0.42
3	3	1.93	0.64
4	3	1.13	0.38
5	3	1.08	0.36
6	3	1.28	0.42
7	3	1.08	0.36
8	3	1.23	0.41

Mean % BB excreted per treatment = 0.46 ± 0.13 (28%)

Mean % BB removed in a three-injection treatment period = 1.39 ± 0.41 (29%)

* Refer to Table III-1 for physical data on this baboon

Figure VII-13

Am-241 Excretion in the Urine of Adult Baboon B-460 as a
Result of Long-Term $\text{Na}_3\text{-CaDTP}$ Therapy

Figure VII-14

Retention of ^{241}Am in the Skull and Whole Body of Adult Baboon B-460,
as Measured by *in vivo* Methods
(long-term DTPA therapy)

Figure VII-15

Retention of ^{241}Am in the Liver of Adult Baboon B-460,
as Measured by *in vivo* Methods
(long-term DTPA therapy)

from tissue analyses of ^{241}Am in B-352 (207 days sacrifice) and B-458 (817 days sacrifice). Based on that data, the skeletal burden for B-460, before therapy was between 86-97% of the body burden.) If, however, B-460 had been treated every week i.e. 52 treatment periods rather than 26 and again if the average rate of removal of ^{241}Am from the body did not decrease substantially ($\approx 1.4\%$ BB per week) then the estimated amount of ^{241}Am removed in one year might be as high as 51% BB. This rate of removal would correspond to an effective half-life of about one year. Considering that approximately 90% of the body burden of B-460 was associated with the skeleton, then the rate of removal of 50% BB per year applies equally as well to the skeletal burden. This increased disappearance rate of ^{241}Am from the skeleton is markedly greater than that found for non-treated baboons (where no net decrease in skeletal ^{241}Am could be measured in 2.25 years of study). If the effect of DTPA on removing ^{241}Am from the human skeleton is similar to that shown for the baboon, then the reduction of human skeletal burdens of ^{241}Am may be a practical goal of long-term DTPA therapy.

Factors Influencing DTPA Effectiveness in Removing ^{241}Am

In terms of the efficiency or removal of ^{241}Am by DTPA the most important factor to be considered is the promptness after exposure with which one begins DTPA therapy. The more rapidly treatment is begun, the greater is the excess removal of ^{241}Am from the body. However, there may be circumstances which would necessitate a delay of treatment with DTPA, e.g. more acute medical needs in relation to trauma injuries. There may also be occasions when an exposure to ^{241}Am might go undetected for long periods of time (as in the case of the ^{241}Am -contaminated inventor and his son; Wrenn et al., 1972).

Therefore, the need for prolonged therapy regimens for removing persistent burdens of ^{241}Am may become important. Under such circumstances, it would be desirable to know the optimum treatment dose and dose rate as well as its optimum mode of administration in the case where skeletal burdens of Am are considered the prime concern. In order to determine the dose-effect relationship for the decorporation of Am by DTPA, a baboon whose body burden of ^{241}Am was estimated to be mostly skeletal (with only small amounts present in soft tissue) was selected (i.e. B-520). Accordingly B-520 was treated with DTPA on three alternate days, i.e. Monday, Wednesday and Friday of a given week. The amount of ^{241}Am excreted during the week of therapy plus one week following the third treatment was considered to be the quantity of ^{241}Am excreted during a "treatment period." This schema afforded several

advantages viz. variations of the amounts of Am excreted from individual treatments were reduced; the animals were allowed a week of rest between treatment series, an important consideration due to the fact that each therapy session required the tranquilization of the animal. Figure VII-16 illustrates the results of this dose-effect experiment. Over a dose range of a factor of 16, i.e. 1.79-28.7 $\mu\text{mole/kg}$, there is a geometric relationship between the dose and the effect. Regression analysis of the sloped portion of the curve mathematically fit a power function with a high degree of correlation ($\rho = 0.996$) and of the form:

$$y = 0.171 x^{0.423}$$

where

y = percent of Am body burden removed during a treatment period

x = injected dose of $\text{Na}_3\text{Ca-DTPA}$ in $\mu\text{moles/kg}$

Figure VII-16

Am-241 Excreted during a Treatment Period by B-520,
versus the Dose of $\text{Na}_3\text{-CaDTP}$, in $\mu\text{moles/kg}$

The amount of ^{241}Am excreted during a treatment period corresponds to the quantity of ^{241}Am measured in the excreta during the one week of DTPA therapy and one week following therapy.

Repetitive treatments at 28.7 $\mu\text{mole/kg}$ produced a relative error of 18% for the resultant net removal of ^{241}Am during a given treatment period. Treatment at 86.1 $\mu\text{moles/kg}$ or three times 28.7 $\mu\text{M/kg}$ showed no additional removal of ^{241}Am . It was thus concluded that $\approx 29 \mu\text{moles/kg}$ was a dose sufficient to remove all the ^{241}Am available at the time of injection. The administration of larger quantities of DTPA to B-520 resulted in no significant increase in the amount of ^{241}Am removed. Therefore, the therapeutic index at the higher dose would have been lower, and much of the drug would be ineffective in producing additional excretion of ^{241}Am .

If the effectiveness of DTPA in treating B-520, i.e. 0.64% BB removed per treatment period, is extrapolated to a therapy regimen continued for one year (one week therapy, one week rest, etc. as with B-460 in the previous section) then the estimated amount of ^{241}Am that could be removed in one year is 15%. This would correspond to a 4.6 year biological half-life. Comparison of the effectiveness of the same DTPA-treatment regimen for the removal of ^{241}Am from B-460 with that of B-520 shows that the removal efficiencies differ by a factor of two (30% BB removal in one year for B-460 compared to 15% BB/year removal for B-520). This difference is both real and unexplained. It is, however, important in illustrating the difficulty in extrapolating results obtained from small number of animals.

Preliminary studies of the effect of differing modes of administration of DTPA and differing injection dose rates were performed. Administration of DTPA as three intramuscular injections in the biceps femoris of the baboon gave a net removal due to treatment of 0.31% BB. This compares to a net removal of 0.65% BB when the same dose of DTPA was administered intravenously as three injections. Thus there was no apparent advantage in

administering DTPA as an IM injection, in this case where the ^{241}Am body burden was primarily skeletal. In fact, the IM mode of administration was only half as effective as the IV mode. Intraperitoneal administration was not attempted since there are indications from work with other species that IP and IV are equivalent (Smith 1967) and there is general reluctance to using IP injections on humans.

Increasing the dose rate by administering the entire dose of one treatment period (equivalent to three separate injections) in a single IV injection proved to be very inefficient. Only 0.23% BB or about 1/3 of the amount of ^{241}Am removed during a control treatment period (control treatment period refers to three IV injections of DTPA over a period of five days) was found in the excreta. This is not surprising in view of the fact that DTPA is cleared very rapidly from the body and doses above 29 $\mu\text{moles/kg}$ did not result in increased excretion of ^{241}Am (Figure VII-16).

At this point it must be emphasized that the results presented above represent attempts at therapy on animals whose burdens of ^{241}Am are firmly fixed for the greatest part, in bone, but also to a limited extent in soft tissue. As such the factors considered in these studies (i.e., dose, dose rate, etc.) can be applied only to the situation where the body burden of ^{241}Am is almost entirely skeletally located. It is probably not appropriate to generalize the results obtained above for the case of substantial liver and labile soft tissue burdens of Am. In addition the problems of ^{241}Am -decorporation in the case of inhalation exposure have not been considered.

The fact that the baboons used for this part of the study had primarily skeletal burdens of ^{241}Am , and that these burdens did not decrease significantly with each DTPA treatment (0.65-1.39% BB removed per 2 weeks), allowed

each experimental animal to act as its own control. This experimental design assumes that the treatment does not substantially change the amount or the distribution of ^{241}Am in the body. Quantitatively this assumption may be reasonable, simply due to the rather small amounts of ^{241}Am that were removed. However, the assumption that the DTPA therapy does not alter the distribution of ^{241}Am cannot be established in these studies.

Distribution of ^{241}Am after DTPA Therapy

As shown in the previous sections, DTPA therapy can be effective in removing significant fractions of the body burdens of ^{241}Am . The degree of effectiveness of the therapy was shown to depend heavily on the time elapsed between contamination (in this case, intravenous injection) and the beginning of therapy. In order to compare the effectiveness of DTPA therapy, initiated at different times after injection of ^{241}Am , on the skeletal and soft tissue distribution of ^{241}Am , the treated baboons were sequentially sacrificed and radiochemical analysis performed. Table VII-3 summarizes the total DTPA-treatment schedules for B-164, 520, and 460, the adult baboons treated in this study.

Determination of the effectiveness of DTPA in removing ^{241}Am from the different deposition sites was made by comparing the distribution of ^{241}Am in the tissues of the DTPA-treated animal with that of a control baboon whose "body burden age" or exposure period corresponded to that of the treated animal. In addition, data from another control baboon whose time of sacrifice corresponded to the beginning of DTPA therapy, is also supplied. This latter information is important for dosimetric considerations.

The ^{241}Am distribution data is presented in Table VII-4 for soft tissue radioassay and Tables VII-5,6 for skeletal ^{241}Am content. Considering first

TABLE VII - 3

Summary of the Total DTPA Treatments Administered to Subject Baboons

Animal	Days from ²⁴¹ AM injection to first DTPA treatment	Years between first and last DTPA treatments	Total number of treatments	Total quantity of Na ₃ CaDTP administered (grams)
B-164	1	0.08	13	2.393
B-520	45	1.0	52	9.570
B-460	385	1.13	71	12.435

TABLE VII - 4

Comparison of ^{241}Am Soft Tissue Burdens at 1 day and 28 days postinjection to a Baboon Treated with DTPA Beginning 1 day postinjection and sacrificed 28 days postinjection

	% I D in total organ			% I D /g wet weight		
	B-470 control	B-416* control	B-164 treated	B-470 control	B-416 control	B-164 treated
time from injection to sacrifice (days)	1	32	28	1	32	28
<u>Organ</u>						
Liver	30.88	13.6	0.698			
Lung	1.097	1.15	0.263	0.012	0.018	0.004
Spleen	0.182	0.12	0.017	0.015	0.0069	0.002
Kidney	0.662	0.48	0.093	0.021	0.0085	0.0025
Aorta	0.149	-	0.019	0.0126	0.015	0.005
Pancreas	0.101	0.044	0.008	0.0058	0.0031	0.007
Heart	0.487	0.20	0.037	0.010	0.0040	0.0009
Adrenals	0.010	0.0096	0.0007	0.0065	0.0032	0.007
Brain	0.009	0.010	0.001	0.00007	0.00006	0.00001
Trachea Larynx	0.109	0.17	0.041	0.008	0.0115	0.004
Hilar lymph nodes	0.004	-	0.013	0.0051	-	0.013
Mesenteric lymph nodes	0.006	-	0.007	0.0052	0.0065	0.004
Spinal chord	0.008	-	0.005	0.0016	-	0.001
TOTAL soft tissue	36.1	16.8	1.20			

* Data from Rosen et al. (1972)

** Soft tissue total does not include ^{241}Am contents of muscle, pelt and fat

TABLE VII - 5

Comparison of Skeletal Burdens of ^{241}Am in Baboons Sacrificed at
 1 and 36 days Postinjection with a Baboon Treated with DTPA
 Beginning 1 day Postinjection and Sacrificed 28 days Postinjection

(% Injected Dose)

	B-470 control	B-164 treated	B-416* control	<u>Am Burden (B-164)</u> <u>Am Burden (B-416)</u>
time from injection to sacrifice (days)	1	28	32	
<u>Bone</u>				
Calvarium & maxilla	3.645	1.99	} 6.50	} 0.45
Mandible	1.064	0.660		
Teeth	0.251	0.283		
Clavicle	0.125	0.070	0.19	0.37
Scapula	1.63	0.576	1.82	0.32
Humerus	2.036	1.112	3.16	0.35
Ulna	0.550	0.514	1.02	0.50
Radius	0.476	0.386	0.88	0.44
Hand	0.708	0.262	- *	0.37
Sternum	0.544	0.253	0.52	0.49
Ribs	2.680	0.930	2.57	0.36
Cervical vertebrae	0.794	0.388	1.023	0.38
Thoracic vertebrae	3.586	1.837	4.125	0.44
Lumbar vertebrae	4.942	2.361	5.99	0.39
Sacral vertebrae	2.010	0.882	-	0.44
Caudal vertebrae	0.380	0.430	-	0.47
Pelvis	4.459	1.941	6.24*	0.44
Femur	2.446	2.456	3.64	0.67
Patella	0.082	0.088	-	0.54
Tibia	1.168	0.872	2.11	0.41
Fibula	0.176	0.148	0.34	0.21
Foot	0.874	0.380	-	0.43
TOTAL skeleton	34.41	18.97	44.22	0.43

* In absense of data from B-416 the data from B-412 was substituted

TABLE VII - 6

Concentration of ^{241}Am in the Bones of Baboons Sacrificed 1 and
 36 days Postinjection vs. B-164, a Baboon Treated with DTPA
 Beginning 1 day Postinjection and Sacrificed 28 days Postinjection

	(% Injected Dose/g Wet Weight)		
	B-470 control	B-164 treated	B-416* control
time from injection to sacrifice (days)	1	28	32
<u>Bone</u>			
Calvarium & maxilla	0.017	0.011	} 0.020
Mandible	0.017	0.010	
Teeth	0.006	0.007	
Clavicle	0.027	0.009	0.024
Scapula	0.020	0.009	0.027
Humerus	0.017	0.009	0.027
Ulna	0.008	0.007	0.016
Radius	0.009	0.006	0.015
Hand	0.014	0.005	-
Sternum	0.035	0.020	0.032
Ribs	0.029	0.013	0.022
Cervical vertebrae	0.030	0.015	0.031
Thoracic vertebrae	0.045	0.029	0.046
Lumbar vertebrae	0.039	0.022	0.046
Sacral vertebrae	0.032	0.018	0.046*
Caudal vertebrae	-	0.011	-
Pelvis	0.026	0.125	0.025
Femur	0.015	0.013	0.022
Patella	0.011	0.013	-
Tibia	0.011	0.010	0.019
Fibula	0.009	0.007	0.015
Foot	0.009	0.009	-
TOTAL skeleton	0.020	0.012	0.024

* From Rosen et al. (1972)

the soft tissue values, it can readily be seen, either from data expressed as total amount of Am or as concentration, that the reductions in soft tissue levels were significant after treatment for all tissues listed, excepting the lymph nodes. The major soft tissue deposition sites, in particular the liver, lung, spleen and kidney, all show marked reduction of total ^{241}Am organ burden as depleted by DTPA therapy. Even the soft tissues which were shown to retain ^{241}Am for long times, i.e. the aorta, the trachea and the larynx (Chapter V), had one half to one third of the control tissue concentrations, indicating either that establishment of firm binding of ^{241}Am to these tissues may take longer than one day or that significant uptake of ^{241}Am by these tissues continues beyond one day, or both. The total amounts of ^{241}Am in soft tissue as listed in Table VII-4 do not include the ^{241}Am content of muscle, pelt, or fat since those tissues were not analyzed for B-164. In the case of B-470, 18% ID was present in muscle, fat and pelt at one day post-injection. It must be assumed (even though the tissues were not measured) that these tissues were depleted of americium by DTPA therapy. Otherwise the amount of ^{241}Am excreted cannot be accounted for on the basis of removal of ^{241}Am from those soft tissues that were analyzed plus the additional loss of ^{241}Am from the skeleton.

Determination of the effect of DTPA on the retention of ^{241}Am in the skeleton is difficult due to the variability of uptake and retention of ^{241}Am in the baboons that were not treated with DTPA (Chapter VI). Although complete listings of ^{241}Am content in individual bones are given in Tables VII-6,7 detailed comparisons do not yield consistent results; certain generalities can, however, be presented. The skeletal burden appears to be about half of what it would have been without DTPA chelation therapy. The effectiveness

of the therapy one day post-injection of ^{241}Am may be due to two factors
1) direct removal of ^{241}Am from bone surfaces where the ^{241}Am has not yet become fully bound and, 2) prevention of further skeletal deposition by translocation from the soft tissue "sources" of ^{241}Am .

Comparison of the skeletal burdens of B-470 and B-416 shows that the skeletal burden is $\sim 35\%$ higher at 36 days post-injection of ^{241}Am than it was one day post-injection. Although the magnitude of the difference in skeletal deposition of ^{241}Am between one and thirty-six days post-injection may be questioned, the observation of sustained uptake of ^{241}Am in the skeleton during the first month post-injection is believed to be real. Redistribution of ^{241}Am that has initially deposited in the muscle, pelt, and other soft tissues provides a "source" of americium which tends to partition between and redeposit in the liver and skeleton. Other experimenters have also noted an increase in skeletal burden as a function of time in other species viz. beagles (Lloyd et al., 1972) and cynomolgus monkey (Durbin 1973). The reduction in skeletal burden is, therefore, probably due to both mechanisms mentioned.

More detailed analysis of the removal of ^{241}Am from the skeleton by DTPA treatment shows several interesting points: 1) Statistical testing (i.e., analysis of variance) indicates that there is no difference between the amounts of ^{241}Am removed from long bones as opposed to ^{241}Am removed from "flat" bones. 2) The rate of removal of ^{241}Am in various regions of the spinal column varied according to position (Figures VII-17,18). The greatest percent removal occurred in the lumbar and cervical regions of the spine.

Figure VII-17

Amount of ^{241}Am in the Individual Vertebrae of B-164, a DTPA-Treated
Baboon and B-470 and B-416, Control Animals

Figure VII-18

Concentration of ^{241}Am in the Individual Vertebrae of B-164, a DTPA-Treated
Baboon and Control Animals, B-416 and B-470

Change in Long Term Distribution of ^{241}Am Due to Chelation Therapy

The effect of DTPA therapy, delayed one year postinjection of ^{241}Am , on the distribution of the nuclide is shown for B-460 and controls B-352 and B-458 in Tables VII-7,8,9. In particular, comparison of B-460 with B-458 is appropriate since both animals were injected with ^{241}Am within one month's time in 1972 or 2.25 years before sacrifice.

Analysis of data of ^{241}Am concentrations in soft tissues in this case is made difficult because of the small percentage of the initial injected activity of ^{241}Am remaining in the tissues. Furthermore, the biological variability associated with these very small tissue deposits of Am are unknown. Several aspects of the effects of delayed DTPA treatment on long-term soft-tissue burdens of ^{241}Am , however, seem evident. Although a small percentage of the original liver burden is measured in the liver at 2.25 years post-injection of Am (one percent of the original liver burden) this quantity still appears to be removable by DTPA therapy. The concentration of ^{241}Am in the liver of the DTPA-treated baboon, B-460, is about one-seventh that found in B-458, the untreated control. Other soft tissue burdens do not seem to be as removable by delayed DTPA treatment. Although comparison of the lung, spleen, kidney, etc. burdens of Am do show that the treated animal (B-460) has smaller ^{241}Am tissue concentrations (ranging from 20-50% of control concentrations) the rate of removal of Am (considering that treatment occurred over a period of one year) is probably low. The effect of DTPA therapy on ^{241}Am concentrations in the aorta, trachea and larynx appears to be negligible. No measurable differences were found in these tissues between B-460 and B-458.

TABLE VII - 7

Comparison of Soft Tissue Burdens of ^{241}Am for a Baboon Sacrificed
2.25 Years Postinjection with Two Baboons which Underwent Long-Term
Chelation Therapy

	% ID in Tissue			% ID/g wet weight		
	B-458 control	B-460 ¹ treated	B-520 ¹ treated	B-458 control	B-460 treated	B-520 treated
Time from injection to sacrifice (days)	817	804	407	817	804	407
<u>Organ</u>						
Liver	0.274	0.0313	0.073	0.0012	0.00018	0.00031
Lung	0.464	0.387	0.382	0.0064	0.0051	0.0042
Spleen	0.0086	0.0070	0.013	0.00073	0.0005	0.0010
Kidney	0.068	0.035	0.118	0.0019	0.0011	0.003
Thyroid	0.001	0.0004	0.017	0.0013	0.0004	0.045
Aorta	0.0576	0.070	0.0765	0.012	0.012	0.012
Carotid artery	0.0079	-	0.0058	0.015	-	0.007
Pancreas	0.001	0.0041	0.0080	0.00061	0.00028	0.0011
Heart	0.032	0.0078	0.032	0.00065	0.00023	0.0013
Adrenal	0.0008	0.0002	0.0008	0.00073	0.00014	0.00040
Trachea larynx	0.08*	0.109	0.125	0.011	0.012	0.013
Hilar lymph nodes	0.0010	0.0003	0.0020	0.0010	0.0004	0.0010
Mesenteric lymph nodes	0.0028	0.0007	0.0004	0.00024	0.00071	0.0011
Soft palate	0.0028	-	0.017	0.00049	-	0.003
Eyes	0.0013	-	0.028	0.0013	-	0.004
Ovary	0.0018	0.00020	0.009	0.0015	0.00019	0.012

* Only larynx was analyzed

1. See Table VII-3 for specifics of the therapy on these baboons

TABLE VII - 8

Comparison of Individual Bone Burdens of ^{241}Am for Control Baboons
Sacrificed 206 and 817 Days Postinjection with DTPA-Treated Baboons

	(% Injected Dose)			
	B-352 control	B-520 ¹ treated	B-460 ¹ treated	B-458 control
Time between ^{241}Am Injection & sacrifice (days)	206	407	804	817
<u>Bone</u>				
Maxilla & calvarium	5.340	3.060	3.410	4.173
Mandible	0.732	0.913	0.891	1.200
Teeth	0.462	0.381	0.554	0.486
Clavicle	0.124	0.100	0.090	0.158
Scapula	1.778	1.360	1.066	1.602
Humerus	1.787	2.260	2.338	3.684
Ulna	0.532	0.616	0.884	1.156
Radius	0.542	0.452	0.838	0.966
Hand	0.666	0.582	1.150	1.164
Sternum	0.664	0.500	0.204	0.438
Ribs	1.985	1.482	1.615	2.248
Cervical vertebrae	0.934	0.940	0.663	1.077
Thoracic vertebrae	5.048	3.567	2.315	4.941
Lumbar vertebrae	6.760	5.574	3.353	5.741
Sacral vertebrae	-	1.171	1.465	2.923
Caudal vertebrae	0.590	0.618	1.088	1.519
Pelvis	3.901*	3.468	3.066	5.673
Femur	2.194	2.530	3.328	4.854
Tibia	0.940	1.22	2.048	2.590
Fibula	0.166	0.136	0.278	0.180
Foot	0.974	1.046	1.686	1.044
Patella	0.056	0.054	0.188	0.216
Knee cartilage		0.0020	0.0020	
Rib cartilage		0.075	0.038	
 TOTAL skeleton	 34.9	 31.5	 32.5	 48.00

* Includes sacral vertebrae

1. See Table VII-3 for specifics of DTPA therapy

TABLE VII - 9

Concentration of ^{241}Am in Bones of Baboons Sacrificed 206 and
 817 Days Postinjection vs. that of Baboons Treated with DTPA
 Beginning at 45 Days and 385 Days Postinjection

(% Injected Dose/gram Wet Weight)

	B-352 control	B-520 ¹ treated	B-460 ¹ treated	B-458 control	$\frac{(\text{B-460})^{241}\text{Am Concen.}}{(\text{B-458})^{241}\text{Am Concen.}}$
Time between ^{241}Am Injection & sacrifice (days)					
<u>Bone</u>					
Maxilla & calbarium	0.024	0.021	0.025	0.027	0.93
Mandible	0.021	0.018	0.018	0.025	0.72
Teeth	0.012	0.010	0.014	0.014	1.00
Clavicle	0.020	0.019	0.014	0.026	0.54
Scapula	0.025	0.036	0.021	0.034	0.018
Humerus	0.030	0.025	0.024	0.038	0.63
Ulna	0.012	0.014	0.018	0.025	0.72
Radius	0.011	0.011	0.018	0.022	0.82
Hand	0.012	0.012	0.019	0.019	1.00
Sternum	0.059	0.045	0.021	0.029	0.72
Ribs	0.022	0.024	0.020	0.011	1.82
Cervical vertebrae	0.031	0.037	0.023	0.036	0.64
Thoracic vertebrae	0.048	0.050	0.027	0.054	0.500
Lumbar vertebrae	0.060	0.053	0.030	0.063	0.48
Sacral vertebrae	-	0.034	0.031	0.061	0.51
Caudal vertebrae	0.011	0.014	0.024	0.025	0.960
Pelvis	0.024	0.030	0.022	0.043	0.51
Femur	0.016	0.038	0.024	0.035	0.69
Tibia	0.012	0.027	0.022	0.022	1.00
Fibula	0.012	0.008	0.018	0.022	0.82
Fat	0.013	0.012	0.020	0.020	1.00
Patella	0.012	0.006	0.030	0.029	1.03
Knee cartilage	0.005	0.001	0.001	0.001	1.0
Rib cartilage	-	0.008	0.005	0.003	1.7
TOTAL skeleton	0.025	0.025	0.022	0.033	0.67

¹ See Table VII-3 for specifics of DTPA therapy

The degree of "chelatability" of ^{241}Am from a given soft tissue by DTPA treatment begun one year after Am injection may be related to the stability of binding of Am within the given tissue. It has been shown (Chapter V) that the retention half-life for Am in the liver was smaller compared to those for spleen, kidney, or lung, and none of the above organs retained ^{241}Am as long as the aorta or the trachea and larynx. The fraction of control ^{241}Am concentration (B-458) remaining in the soft tissues following DTPA treatment in B-460 can be listed in the following increasing order (or order of decreased "chelatability"); liver<kidney<spleen<lung<aorta. This correlation tentatively suggests that the degree of effectiveness of delayed DTPA treatment in removing residual soft tissue deposits of ^{241}Am will depend on the relative stability of the ^{241}Am within the tissue. In this case, the term stability does not necessarily mean chemical stability *in situ*. It may also refer to deposits of ^{241}Am inaccessible to the action of the chelating agent, i.e. the nuclide could be physically sequestered from the physiological dilution space of the DTPA (e.g. intracellularly located).

Tables VII-8,9 and 10 show the distribution of ^{241}Am in the skeleton of B-460 and B-458 (as well as B-352 and B-520). The skeletal burden of the treated baboon, B-460 is 68% that of the control baboon sacrificed at 817 days, B-458. Bone-to-bone comparison indicates that the removal of ^{241}Am may not be as uniform as was suggested in the case of B-164, the promptly treated baboon. Ratios of concentration of ^{241}Am in a given bone for B-460 to that of B-458 range from 0.48 for lumbar vertebrae to 2.00 for the tibias. Statistical comparison of the concentration ratios for flat bones versus long bones shows that there is a statistical difference

TABLE VII - 10

Fraction of Total Skeletally Deposited ^{241}Am Contained in a Given Bone
(X 100)

	B-470	B-416 ¹	B-412 ¹	B-352	B-458	B-164	B-520	B-460
	Control	Control	Control	Control	Control	Treated	Treated	Treated
Maxilla & skull	10.59	14.69	15.66	12.5	8.28	10.4	9.71	10.58
Mandible	3.09			2.1	2.33	3.4	2.90	2.76
Upper teeth	0.35			0.7	0.51	0.63	0.57	0.77
Lower Teeth	0.37	28.5	29.9	2.7	0.44	0.84	0.64	0.86
Vertebrae cerv.	2.31			2.13	2.0	2.98	2.06	
Vertebrae thor.	12.41			14.5	9.79	9.4	11.18	7.19
Vertebrae lumb.	12.37	5.84**	2.66	19.5	13.77	12.3	17.68	10.34
Vertebrae sacr.	5.84**			5.79	4.6	5.43	4.54	
Vertebrae caud.	1.10			3.01	2.4	1.96	3.36	
Clavicle	0.73	0.42	0.43	0.4	0.32	0.37	0.31	0.28
Scapula	5.02	4.11	4.56	5.1	3.35	3.0	4.31	3.31
Humerus	5.92	7.14	5.70	5.1	7.30	5.8	7.17	7.25
Radius	1.38	1.99	1.79	1.6	1.92	2.0	1.43	2.60
Ulna	1.60	2.30	2.20	1.5	2.29	2.7	1.95	2.74
Hands	2.06	-	2.19	1.9	2.31	1.4	1.85	3.56
Pelvis	12.96	11.51*	9.88*	11.2*	11.24	10.1	11.00	9.51
Femur	7.11	8.23	8.68	6.3	9.62	12.8	8.02	10.33
Tibia	3.39	4.77	3.52	2.7	5.14	4.5	3.87	6.35
Fibula	0.51	0.76	0.70	0.5	0.76	0.75	0.43	0.86
Foot	2.54	-	3.77	2.8	4.05	3.9	3.32	5.23
Sternum	1.58	1.17	-	1.9	0.87	1.3	1.59	0.63
Ribs	7.44	5.81	9.55	5.7	4.38	4.8	4.70	5.12
Patella	0.24	-	-	0.2	0.43	0.46	0.18	0.58

TOTAL	100.9	91.4	101.2	100.6	100.03	99.85	103.1	100.81
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¹ Data from Rosen et al. (1972)

* Includes sacral vertebrae

** Most of tail missing

for the mean ratios at $p < 0.05$. Whether this difference has any physiological meaning, however, is unknown.

It is interesting to note that the greatest reduction in ^{241}Am concentrations occurs along the spinal column. (Figures VII-19,20). This can be an important consideration in view of the fact that the spine is the site of a substantial percentage of ^{239}Pu radiation-induced osteosarcomas in dogs (Thurman et al. 1973). (Recall that ^{239}Pu is also an α -emitting bone-seeking nuclide). Conversely, the observation that the ^{241}Am concentration in the tibia, fibula, hand and foot of the DTPA-treated baboon, B-460, was not significantly lower than those of the control B-458 (or for B-352) is notable. That the radius and ulna also had higher than average Am-concentration ratios may suggest that these bones, which have more cortical bone than e.g. vertebrae or pelvis, may involve the deeper burial of ^{241}Am within the cortical bone matrix. This might occur after initial deposition on other bone surfaces followed by resorption and redistribution. Without autoradiographic evidence, this hypothesis must remain speculative at the present time. However, it should be noted that ^{241}Am deposited in the long bones after one year post-injection is less removable by DTPA therapy than ^{241}Am deposited in vertebrae, scapula, pelvis, etc.

Discussion of the Pharmacodynamics of DTPA

Studies of the metabolism of DTPA in human and experimental animal systems have been performed. Stevens et al. (1962) administered ^{14}C -DTPA intravenously to humans and followed its metabolism with analysis of the ^{14}C activity in blood, urine and feces. Their results showed that the chelate was excreted very rapidly via urine: 50-70% of the administered

Figure VII-19

Amount of ^{241}Am in the Individual Vertebrae of B-460, a Long-Term
DTPA-Treated Baboon and Control Baboons, B-352 and B-458

Figure VII-20

Concentration of ^{241}Am in the Individual Vertebrae of B-460, a Long-Term
DTPA-Treated Baboon, and Control Baboons, B-352 and B-458

DTPA was excreted by 4 hours and 90-100% of the injected dose was recovered by 24 hours post-injection. Fecal ^{14}C -radioassays on the other hand were negative. Oral administration of DTPA showed about 100% recovery of the ^{14}C activity in the feces, indicating very little absorption through the GI tract. Plasma levels of DTPA, administered intravenously, were only 10% ID in the blood stream one hour post-injection and continued to decrease thereafter. Similar experiments performed on humans with ^{14}C -labelled EDTA indicated that the chelate was excreted rapidly and unchanged, i.e. no metabolic alteration of the DTPA moiety (Foreman and Trujillo 1954). Other investigations of the metabolic behavior of DTPA and EDTA were performed by Bohne et al. (1968) using female rats. They demonstrated that the physiological dilution space for both chelating agents was the extracellular fluid compartment. In addition, their experiments showed that a small fraction of the ^{14}C -label reached the intracellular space. Whether the ^{14}C activity associated with this latter space corresponded to intact ^{14}C -DTPA or represented some DTPA metabolite, was not satisfactorily answered.

The previously mentioned experiments of DTPA and EDTA metabolism are not entirely satisfactory in explaining some of the bioassay results of DTPA treatment of systemic ^{241}Am burdens in baboons. Treatment of B-164, beginning one day after ^{241}Am injection resulted in significant removal of ^{241}Am particularly during the first two days; 16.7% BB and 13.7% BB were measured in the urine. This corresponds to an excretion of 27% of the injected dose via the urine during the first two days of therapy. If one assumes that the distribution of ^{241}Am at the beginning of DTPA therapy was similar to that measured for B-470, the baboon sacrificed one day

post-injection of ^{241}Am , then it follows that 35% of the injected dose was associated with the skeleton, 31% ID was in the liver and 23% was in other soft tissue. The decorporation of 27% ID during the first two days of therapy suggests that, at the beginning of therapy, there was a substantial amount of ^{241}Am that was readily mobilizable. For example, ^{241}Am associated with protein complexes and located in the extracellular fluid would be available to the chelating action of DTPA, this americium being within the dilution space of EDTA.

The accelerated excretion of ^{241}Am deposited in the liver appears to involve both urinary and fecal routes of excretion. That increased amounts of ^{241}Am should be measured in the urine following DTPA treatment is reasonable considering the normal metabolic pathway of injected DTPA. However that increased ^{241}Am should also be measured in the feces is not easily understood. The studies of Stevens et al. (1962) showed "quantitative" recovery of ^{14}C -DTPA from the urine of humans. This may not, however, be precisely the case. Using the data of Ballou and Sullivan (1964) in which the bile ducts of rats were catheterized and ^{239}Pu concentrations measured as a function of time following IV injection of DTPA, it can be calculated that, on a mole-to-mole basis, only 0.004% of the injected dose of DTPA would need be found in the bile in order to account for all of the ^{239}Pu excreted by this route as a result of the single DTPA treatment. If the figure, 0.004% ID of DTPA, is a realistic quantity of DTPA needed to account for biliary (and hence fecal) excretion of Pu (and Am), then it is most probable that Stevens et al. (1962) could not have measured such a small fraction of the injected dose of ^{14}C appearing in the feces. Therefore it is entirely possible, although there is not such evidence to date, that

very small amounts of DTPA are excreted via a biliary route and decorporate hepatically deposited ^{241}Am . It is hoped that future studies using ^{14}C -DTPA and more sensitive detection methods will provide more information concerning this possible mechanism of DTPA action.

Removal of ^{241}Am in a Juvenile Baboon Treated with DTPA

Although studies of possible differences in the normal metabolism of ^{241}Am between juvenile and adult baboons were at an early stage at the time of this writing, it was considered important to measure the effect of DTPA for removal of ^{241}Am from the body of a juvenile baboon. Therefore B-400, a 3.5 year old baboon (equivalent to 12-15 year old human), was treated with DTPA for a one month period (12 treatments, three per week) in an effort to compare DTPA removal efficiency with that of DTPA treatment in adults (Table VII-11). Therapy for B-400 was begun 49 days post-injection of ^{241}Am , similar to the beginning of therapy for the adult B-520 (45 days post-injection). The results of the DTPA therapy for B-400, B-520 and, for comparison of therapy effectiveness in treating long term ^{241}Am burdens, B-460, are summarized in Table VII-12; the whole-body measurements are illustrated in Figure VII-21. As has been the case for most DTPA treatments more ^{241}Am is measured in the urine than in the feces, indicating that the former is consistently the major pathway of enhanced excretion of ^{241}Am by DTPA therapy.

From Table VII-12 it is seen that the ratio of urinary to fecal excretion of ^{241}Am by B-400 is more than four times that of B-520 the adult baboon treated at the same time as B-400. The higher ratio occurs as a result of the following: 1) the amount of ^{241}Am excreted by B-400 via the fecal route was less than that of B-520 and 2) the urinary output of ^{241}Am by B-400 was more than twice as high as for B-520.

TABLE VII - 11

Physical and Body Burden Data for Experimental Animals

Animal	Weight	Age Years	^{241}Am injected dose (μCi)	Exposure time prior to DTPA therapy (mos.)	^{241}Am Body burdens & start of therapy (nCi)	$\text{Na}_3(\text{CaDTPA})$ Dose ($\mu\text{moles/kg}$)
B-406 juvenile	4.4	2.2	0.935	control	810	-
B-400 juvenile	6.5	3.2	0.873	1.5	666	28.7
B-520 adult	14.2	11-13	2.84	1.5	2391	28.7
B-460 adult	13.0	10-12	2.42	13.0	827	28.7

TABLE VII-12

Excretion of ^{241}Am During Chelation Therapy with
 $\text{Na}_3\text{Ca-DTPA}$ for a Juvenile and Two Adult Baboons

Animal #	^{241}Am distribution at beginning of treatment	Exposure period prior to beginning of therapy (months)	% Body Burden Excreted (gross)			% Body Burden Excreted (net)			% Body Burden Excreted During therapy as measured by <i>In Vivo</i> counting	
			urine	feces	total	urine	feces	total	gross	net
B-400 juvenile	primarily liver and bone	1.6	28.7	5.1	33.8	28.0	1.6	29.6	45	39
B-520 adult	primarily liver and bone	1.5	12.0	9.3	21.3	11.2	3.9	15.1	34.5	32.0
B-460 adult	primarily bone	13.0	8.0	0.9	8.9	7.8	0.05	7.9	11.4	9.3

Figure VII-21

Retention of ^{241}Am in the Whole Body, Liver, and Skull of Juvenile
Baboon B-400, as Measured by *in vivo* Methods (DTPA Therapy)

Meter Arc

↓↓ ↓↓↓↓↓ = DTPA Treatments

Liver

↓↓ ↓↓↓↓↓ = DTPA Treatments

Head

↓↓ ↓↓↓↓↓ = DTPA Treatments

Excretion of Am via the feces is assumed to represent Am that has been removed from the liver via the bile. Therefore less ^{241}Am was removed from the liver of B-400 than from B-520. In fact, pretreatment measurement of ^{241}Am in the livers of these two baboons indicated that 22% of the initial burden of B-400 (or 7.7% ID) remained in the liver at the beginning of DTPA therapy. Likewise 62% of the initial liver burden of B-520 (representing 18.6% ID) was determined to be present in its liver before therapy. Therefore less ^{241}Am was available to the action of DTPA in the liver of B-400 as compared to B-520.

It is difficult to define the sites of removal of ^{241}Am in the juvenile since Am tissue distribution studies of sacrificed juvenile baboons have not been performed. Therefore one cannot evaluate the relative removal of Am from the different tissue sites, the percentage contribution of Am removed from a given organ to the total ^{241}Am measured in the urine. Indirect evidence suggests however that at least part of the excess ^{241}Am excreted in the urine by B-400 comes from the skeleton. Examination of the *in vivo* skull measurement data show that about 30% of the measured activity was lost from the skull during the treatment period. This decrease is much greater than the decreases measured in B-520 or in B-460. The decrease in skull activity is actually more akin to the pre- and post-therapy skull measurements of B-164, the baboon treated with DTPA beginning one day after ^{241}Am injection. If the reduction in skull count rate truly represents depletion of ^{241}Am throughout the skeleton of B-400 as a result of the DTPA-therapy, then, on the basis of results obtained from B-400 and B-520, DTPA therapy may be more effective in the juvenile than in the adult. Although it is realized that few baboons have been treated with DTPA, and only one of those treated

was a juvenile (B-400), it is nevertheless true that more ^{241}Am was removed from B-400 (29.6% BB net) than for B-520 (15.1% BB net) as determined by bioassay measurements. Reduction of body burden as estimated from whole body *in vivo* measurements show that the difference between adult and juvenile are not as large (45% BB removed from B-400 vs. 34.5% BB removed from B-520). Whether the experimental situation is represented more accurately by *in vivo* methods and measurement or bioassay is inconclusive. However the conclusion that DTPA therapy in the juvenile is at least as effective if not more so than in the adult is demonstrated by either analytical technique.

With limitation, the following generalization is offered: DTPA therapy is more effective in decorporating ^{241}Am from the juvenile than from the adult, as least under the experimental conditions of this experiment (i.e. 45 days post-injection of ^{241}Am). The greater effectiveness of therapy is most likely due to greater ease in removing ^{241}Am from the skeleton of the juvenile, possibly due to more rapid bone remodelling. If B-400 had had a larger hepatic burden of ^{241}Am at the beginning of treatment, as did B-520, and that burden was as removable by DTPA therapy as was shown for B-520, then the difference in DTPA effectiveness would have been more evident.

Short-Term Excretion of ^{241}Am as Provoked by DTPA Therapy

Since it has been reported that DTPA is cleared rapidly from the body of both humans and experimental animals (Foreman and Trujillo, 1954; Stevens et al. 1962) it was considered important to study the early kinetics of excretion of ^{241}Am into the urine as provoked by DTPA treatment. Animal B-520 was administered DTPA in the usual manner. Following completion of the treatment, the baboon was placed in a restraining device while in a

tranquilized condition. Prior to this, the animal has been catheterized and the bladder drained of urine just before DTPA treatment. The catheter was then closed off and urine allowed to accumulate for half hour periods. The results of ^{241}Am bioassay measurements on the sequential urine samples taken up to and including four hours following therapy (Table VII-13) show that ^{241}Am begins to appear in the urine almost immediately after treatment. At five minutes following the end of DTPA administration, the ^{241}Am concentration is ten times the pretreatment level. The concentration continued to rise to a maximum, a factor of 200 higher than pre-therapy levels, which occurred between 2 and 3 hours following treatment. By four hours post-treatment, the ^{241}Am concentration was diminishing and although measurements were not continued, a total of 71% of the 24 hours output of ^{241}Am in the urine had already been excreted. Apparently the ^{241}Am is excreted as quickly as DTPA from the body and agrees with the hypothesis that the Am is excreted as the intact Am-DTPA chelate. Although DTPA metabolism measurements have not yet been performed in the baboon, there is no reason to believe that the metabolism would be substantially different from results obtained in other animals and humans.

TABLE VII - 13

Short-term Excretion of ^{241}Am in Urine Following
Intravenous Injection of $\text{Na}_3\text{Ca-DTP}$ in Baboon B-520

Time post injection of DTPA hrs.	Concentration of ^{241}Am in urine (nCi/ml)	Cumulative ^{241}Am excreted in urine (nCi)	Cumulative % of 24 hr total excreted
-5 hrs	0.00014	0.0	-
0.12	0.0016	0.16	5.63
0.58	0.010	0.38	13.4
1.12	0.012	0.55	19.4
1.58	0.010	1.05	37.0
2.12	0.019	1.22	43.0
2.58	0.033	1.44	50.7
3.12	0.033	1.61	56.7
3.58	0.020	1.92	67.6
4.12	0.013	2.02	71.1
24 hours		2.84	100.0

CHAPTER VIII

TOXICITY OF DTPA

Although considerable information has been accumulated concerning the deleterious somatic and histopathological effects caused by EDTA and DTPA administration in experimental animals and in humans, the mechanisms of pathological action of these drugs have not been clearly defined. Foreman et al. (1956) were some of the first experimenters to report the occurrence of nephrotoxicity following the use of Na_2CaEDTA in treating heavy metal poisoning in humans. In one case study involving plutonium poisoning, the urinalysis showed albuminuria, numerous renal parenchymal cells, fine granular casts, and occasional red and white cells. These appeared on the 11th day of a 5g EDTA/day regimen. Signs and symptoms of renal disturbance disappeared rapidly after the cessation of treatment. In a review of the toxicity of EDTA, Seven (1960) summarized the observations on the use of the EDTA chelates. These include 1) hypocalcemia and tetany when the acid form was administered too rapidly in animals 2) renal damage including vascularization of renal tubular cells, severe damage to proximal convoluted tubules with partial loss of epithelium, dilated and vacuolated cells. Oliguria, diuresis, transient albuminuria, and granular cylindruria have also been reported 3) histamine-like reactions including sneezing, nasal congestion and occasional lacrimation. The previously mentioned symptoms occurred as a result of using rather high doses of EDTA (100-600 mg/kg day).

Much work with animals using both EDTA and DTPA has been done since the early 1960's. Although the results are not always consistent, there is significant evidence indicating that DTPA and EDTA induce toxic symptoms by removal of metals essential to some important biological system. Although nephrotoxic symptoms have occurred as a result of prolonged therapy, the cause of acute death from DTPA and EDTA has been a result of toxic effects on the intestinal mucosa (Ahrens and Aronson, 1968; Aronson, et al. 1968; Weber, 1969). In a recent study, Taylor et al. (1974) produced lethality in dogs at low levels of Ca-DTPA by fractionation of the dose. From 22.5 - 24.6 μ moles/kg of Na_3CaDTPA given to 10 kg dogs produced mild symptoms with rapid recovery. On the other hand, one fifth of the dose given at five hour intervals for 8 days or until death was fatal in 3 out of 3 dogs within 3-9 days. Again the most prominently induced symptoms were principally associated with the intestinal tract. Necropsy findings showed that the intestinal lesions were characterized by hemorrhage, loss of villi, and plasma cell infiltration. Weber et al. (1970) using rats, showed that there was a significant drop in DNA synthesis in duodenal crypt cells approximately 10 hours following DTPA administration. Others have also shown the ability of DTPA to decrease the rate of DNA synthesis in the rat liver (Gabard, 1974) kidney, and intestinal mucosa (Taylor and Jones, 1972). That the above results were produced with the calcium form of the DTPA complex, but not with the zinc form (i.e., Na_3ZnDTPA) has led several investigators to believe that zinc plays an important role in the toxicity of DTPA (and EDTA).

That zinc is a trace metal essential to many enzyme systems has been documented (Vallee, 1959). *In vitro* studies of some selected enzyme systems have shown the inactivation of the enzyme system by EDTA or DTPA and its re-activation by addition of zinc (or Zn and Mn). The conclusion that DTPA reacts with the enzyme systems either directly (by forming ternary complexes) or indirectly by removal of essential trace elements such as zinc is a significant possibility. Nigrovic (1964) showed that alkaline phosphatase could be inactivated *in vivo* and *in vitro* by administration of high doses of DTPA or EDTA. This inactivation could be reversed by the addition of Zn.

Zincuria in the Baboon and its Relation to ALAD

Prompted by the documented evidence that zinc seemed to play a major role in the toxic manifestations of DTPA, and by the noted appearance of zincuria following administration of EDTA and DTPA (Perry and Perry, 1959; Slododien et al. 1972), measurements were made of the level of zinc excreted by control and DTPA-treated baboons. In addition, an enzyme, aminolevulinic acid dehydrase (ALAD) was measured in the blood concurrently with the urinary zinc measurement. The enzyme ALAD is necessary for the synthesis of the heme moiety of hemoglobin. In this synthesis, two molecules of aminolevulinic acid (ALA) are condensed in the presence of ALAD to form one molecule of porphobilinogen (PBG). Although it is not known whether changes in the erythrocyte concentration of ALAD are pathognomonic, or of toxicological significance, erythrocytic ALAD activity can provide a measure of exposure to certain toxic metals, e.g. lead and mercury, and to the absence of other essential trace metals, i.e. zinc.

Gurba et al. (1972) noted that purified ALAD enzyme contains 1.1 ± 0.2 gram-atoms of zinc per 275,000 grams of protein, plus small nonstoichiometric amounts of iron and cobalt. Furthermore, although activity levels of ALAD in the red blood cell may not indicate physical toxicity, there is evidence that ALAD activity in specific soft tissues, i.e. brain, liver, kidney, and bone marrow is pathognomonic (Millar, et al. 1970).

Measurement of microgram quantities of stable zinc require inordinate care in preventing contamination by ambient or environmental zinc. As such, fresh urine was collected in sterile polyethylene vials via catheterization of the baboon. The urine was subsequently acid digested in acid-washed glassware, aliquoted and stored in polypropylene containers which were shown to contain minimal levels of leachable zinc ion. Measurement of the zinc was accomplished by atomic absorption spectrophotometry. The minimum measurable amount of zinc by the procedures used was 0.001 ppm. Erythrocytic ALAD was measured by the method of Granick et al. (1973) as modified by Kneip et al. (1974).

Results

A total of 38 catheterized urine samples were obtained from three non-treated baboons. The mean concentration of zinc measured was $457 \pm 50.8^*$ $\mu\text{g Zn}^{++}$ /liter urine. Zinc concentration in human urine, measured to be 318 ± 31.9 $\mu\text{g Zn}^{++}$ /liter urine by Perry and Perry (1959), is not inconsistent with the concentration of Zn in the urine of the baboon, since, the urinary output of the baboon is one half to two thirds that of "reference woman". Thus the total amount of Zn^{++} excreted per

* Standard error of the mean

day by each is within the range of one standard deviation demonstrating a similarity in Zn^{++} homeostasis in these two primate species.

Similarly the measured ALAD activity in the non-treated baboons was 504 ± 86.1 nmol PBG/ml-RBC/hr compared to a normal of 633 ± 234 nmol PBG/ml-RBC/hr in man (Granick et al. 1973).

When urine and blood samples were taken within 30 minutes of the cessation of a treatment with DTPA, the concentration of Zn^{++} in the urine increased on the average by a factor of four and the activity level of ALAD dropped below 250 nmol PBG/ml RBC/hr or about 1/2 normal levels. Figure VIII-1 shows that this pattern is true for all but one measurement. There does therefore appear to be a correlation between zincuria and a depression in erythrocytic ALAD as a result of DTPA therapy.

That the rate of excretion of Zn in the urine is related to the metabolism of DTPA is suggested by the data of Figure VIII-2. As a result of intravenous administration of DTPA, the Zn^{++} concentration in the urine reaches a maximum between three and four hours after the cessation of treatment. This is not very different from the behavior of the measured ^{241}Am -concentration in the urine after DTPA treatment (Table VII-13). In both cases, the largest fraction of the element excreted as a result of chelation therapy, is excreted by four hours post treatment, a point where 50-70% of administered C^{14} -DTPA had been excreted in the urine in man (Stevens et al. 1962). Recovery of ALAD was shown to be rapid with "normal" erythrocytic levels of ALAD being present at two days post-DTPA treatment. Therefore whatever mechanism is involved in the zincuria-ALAD depression-DTPA treatment phenomenon, the recovery is quite rapid.

Figure VIII-1

Concentration of Zn^{++} in the Urine vs. the Activity Level of the
Metalloenzyme, ALAD

Figure VIII-2

Short-Term Excretion of Zn^{++} in the Urine as a Result
of $\text{Na}_3\text{-CaDTP}$ Treatment

Although the above results are quite preliminary in terms of experimental size and detail, the involvement of another Zn-metalloenzyme in the response of the mammalian body to DTPA administration seems to have been shown. Since the results are so preliminary, it would not be useful to postulate a mode of action of DTPA on the basis of those results. Several followup experiments are suggested however. These include 1) the use of $\text{Na}_3\text{Zn-DTPA}$ 2) a full-scale characterization of the trace elements excreted in the urine for both treated and untreated baboons 3) *in vitro* study of ALAD-Zn-DTPA interactions.

Clinical Observations of Control and DTPA-Treated Baboons

During the course of the ^{241}Am metabolism studies in baboons, various methods were employed to monitor the general health of the test animals during the test period. Hematological measurements, including hematocrit, hemoglobin, WBC and differential white blood cell counts were performed periodically. Clinical chemistry* (i.e. SMA-12) tests were performed on baboon serum, particularly for those baboons involved in the chelation treatment program. Urinalyses were also done on catheterized urine samples obtained from tranquilized baboons. This information is summarized and presented in Appendix I of this dissertation. In addition, clinical lab data from non-treated baboons are also included for comparison. It should be noted that since the baboons used in the study were for the most part hybrids, they will probably not compare directly to any of the four categories of baboons cited by de la'Pena and Goldzeiher (1967). The results of the hematological testing and of serum chemistry measurements

* see Appendix 1 for individual tests

showed no remarkable differences in measured values as compared to values cited in the literature. Both types of measurements also were not indicative of any statistically significant differences in blood parameters due either to ^{241}Am , and/or to DTPA.

Changes were observed, however, in the excretion patterns with respect to treatment with DTPA. It was noted with B-460 and subsequently with B-520 that, when prolonged DTPA therapy was attempted, a significant decrease in urine volume was observed on days of treatment. For example pretreatment urine volumes for B-460 were approximately 450 ± 200 ml per day. The urine volumes for days of DTPA treatment averaged 219 ± 129 ml per 24 hours. These averages are statistically different at $P < 0.001$.

Urinalyses of catheterized urine samples were performed on days of DTPA treatment and on some days without treatment. Although these findings were not consistent, albuminuria and cylindruria occurred fairly frequently in both B-460 and B-520 following DTPA treatment. In the case of B-460 albuminuria was found on nine of ten urinalyses performed on days of DTPA treatment, while fine granular casts were observed on five of the ten occasions; for B-520, both symptoms were observed in 4 of 11 urinalyses performed on days of chelation therapy. However albuminuria also occurred on 6 of 12 urinalyses performed during periods during which DTPA therapy was not performed. Cylindruria was measured on 3 of 12 occasions. In the case of B-520, both albumin and casts were observed on 1 of 10 urinalyses performed during pretreatment periods.

Whether the above findings were attributable to the DTPA therapy cannot be determined at this time since the occurrence of the symptoms was somewhat erratic and the albuminuria could be induced in a control

baboon (see B-458 urinalysis Appendix 1) by following the identical treatment, regimen, i.e. complete fasting the morning of the treatment tranquilization with equal doses of ketamine hydrochloride, and IV infusion of equal volumes of saline without DTPA. Although albuminuria was observed in 7 of 12 urinalyses for B-458, cylindruria was not observed. Nor was the volume of urine excreted during the 24 hrs. following a "treatment" statistically different from volumes of urine excreted during non-treatment periods.

The above data is indicative of the need for a more detailed study of the potential effect of DTPA at measured therapeutic doses on the renal physiology of the baboon. However, on the basis of the information obtained in this study, one cannot arrive at any conclusions concerning the observed pathological symptoms in relation to DTPA treatment.

Post-Mortem Tissue Analysis

When each of the adult baboons used in this study were sacrificed, several tissue sections were obtained for histological examination. The techniques employed for the fixation, embedding, and sectioning of the soft-tissue samples were standard techniques of histology. The actual interpretation of the histological specimens was performed by a consulting pathologist, Dr. J. Harris. The results of the pathology study are presented in Appendix 2 of this report.

The general impression obtained from these reports is the ubiquitous presence of congestion in varying degrees for all organs of all baboons studied. This congestion consisted of mononuclear cells, occasional neutrophilic elements and R E-like cells which appeared generally in vessels, interstitium, portal areas of the liver. This type of congestion

may be an agonal artifact of the method of sacrifice, i.e. exsanguination of a mildly tranquilized animal. It is also possible that the history of these animals (all captured in the wild) included exposure to several endemic diseases, parasites, etc., all of which may tend to leave scar evidence of infection at a level that was histologically observable (e.g., capsular epithelial hyperplasia) but not severe enough to cause gross observable symptoms in the animal. No histological observations could be reliably attributed to either exposure to ^{241}Am or to DTPA chelation therapy.

During the course of preparing the skeletons of the baboons for measurement of ^{241}Am by gamma-counting of acid-digested bone samples, it was inadvertently discovered that the two baboons, which had undergone DTPA therapy for about one year, B-460 and B-520, had anosseous regions in their scapulae (Figures VIII-3,4). As can be seen, holes of up to 2 centimeters in length were easily visible. These lesions were not observed in any of the control baboons, B-470, 458, 352, or B-164, which had undergone DTPA therapy for only one month. It must be mentioned that the scapula in the region of observed defects is very thin (< 1 mm in thickness). The significance of these findings is not known at the present time. However, the coincidence that these scapula defects occurred only in the two baboons which had undergone long term DTPA therapy suggests further study. In addition, Lindenbaum (1974) has also noted a non-specific qualitative softening of the mandible of beagles treated with DTPA. Preliminary observation of a decalcified scapular section from B-520 (Appendix 2) did not indicate any gross pathology (e.g. osteoporosis). However, the section was of limited value since the bone had not been fixed (note

Figure VIII-3

Left Scapula of Adult Baboon B-460, Showing the Presence of Holes

B-460

Scapula

B-460

Scapula

Figure VIII-4

Left and Right Scapulae of B-520, Showing the Presence of Holes

the empty lacunae) and the periosteal tissue had been accidentally removed during the cleaning of the bones.

Further study of this phenomenon should be pursued both in DTPA-treated and in non-treated baboons as its potential relevance to the human situation, where long term DTPA treatment for removal of persistent radio-metal burdens is being considered, cannot be denied.

CHAPTER IX

SUMMARY AND CONCLUSIONS

Experiments have been performed to study the metabolism and distribution of intravenously administered ^{241}Am in the adult and juvenile baboon. In addition, ^{241}Am -decorporation therapy using diethylenetriaminepentaacetic acid (DTPA) was performed on selected baboons to determine the efficacy of this drug in removing systemic burdens of americium from this primate species. Determination of the kinetics of ^{241}Am , both in untreated and DTPA-treated animals was accomplished, in general, by 1) *in vivo* whole or partial body counting and 2) radiochemical measurement of ^{241}Am content of biological material (i.e. excreta, blood, soft tissue, and bone). The most important results obtained from these studies are as follows:

1. The disappearance of intravenously administered ^{241}Am from the blood is quite rapid. More than 90% of the administered dose has been cleared from the circulation by one hour after injection; 99% has been cleared by twenty-four hours postinjection. Measurement of ^{241}Am concentration in the blood at long times postinjection continued to show relatively small but detectable levels of ^{241}Am in the blood even as long as 817 days postinjection. A power function expression of the blood data, of the form $y = 0.51t^{-0.87}$ (t in days), is a good statistical fit ($\rho = 0.99$).

2. The major sites of deposition of intravenously-administered ^{241}Am are the liver and the skeleton. The whole body retention of ^{241}Am can be approximated by a two-component exponential function. In general about 40% of the injected dose is cleared from the body

with half-lives ranging from 17 - 36 days; concurrently 58 - 64% ID was retained with a half-life ranging from 1.2 - 5.5 years. The major deposition site responsible for short-term clearance of ^{241}Am is the liver, while long-term retention generally reflects the behavior of ^{241}Am in the skeleton.

3. The excretion of ^{241}Am in the urine is maximal during the first 24 hours postinjection, after which the levels of ^{241}Am excreted per day fall rapidly. The ^{241}Am measured in the urine represents renal clearance of Am present in the plasma.

4. The principle early soft tissue site of deposition of ^{241}Am is the liver, $31.1 \pm 3.0\%$ ID being found in the liver at one day post-injection. Retention of the deposited ^{241}Am was characterized by two-component exponential behavior. Eighty percent of the initial liver burden was cleared with a half-time of 27.6 ± 6 days while the remaining amount of deposited nuclide was cleared more slowly - 1-2 year half-times. It was shown that the great majority of ^{241}Am that was cleared from the liver, was measured in the feces. Americium could be measured in the liver even at 2.25 years postinjection, although at very low levels (1% of the initial liver burden).

5. Deposition of ^{241}Am in non-hepatic soft tissue organs show varying levels of incorporation and different degrees of retention. The soft tissues which contain the greatest amounts of ^{241}Am includes the lung, kidney, spleen, and, due to their large mass, muscle, pelt, and fat. Retention of ^{241}Am in these organs is generally longer than in the liver. Concentration of ^{241}Am in the aorta was higher than any other soft tissue including the liver, for $t > 200$ days postinjection.

In addition, the retention half-time of Am in the aorta was on the order of 8 years.

6. The second major site of deposition of ^{241}Am is the skeleton. From 34.4 - 48.0% ID was measured in the skeleton of baboons sequentially sacrificed from one day to 817 days postinjection. Retention of ^{241}Am in the skeleton is considered to be quite long, even though quantitative estimation of the actual retention half-time was not possible in these studies. Differential deposition of ^{241}Am in highly trabecular bone was determined to be greater than in cortical bone. It is believed that the differential deposition of ^{241}Am in bone is related to the surface to volume ratio of a given bone. This could not be tested, however, due to lack of physical data (in particular, S/V for individual bones) concerning the baboon skeleton.

7. Studies of the metabolism of ^{241}Am in two juvenile baboons were equivocal in demonstrating relationships between adult and juvenile ^{241}Am metabolism. Clearance of ^{241}Am from the blood of the juvenile is at least as rapid as in the adult. The retention of ^{241}Am in the liver differed significantly between the two juveniles used:

$T_{1/2} = 38$ days for B-406 vs. $T_{1/2} < 23$ days for B-400.

8. The use of $\text{Na}_3\text{Ca-DTPA}$ as an Am-decorporating agent proved to be effective in the case of early chelate administration, with efficiency of removal of Am decreasing as time between injection and beginning of therapy was increased. However, even in the case where chelation therapy was delayed for one year postinjection of ^{241}Am , DTPA was judged to be of value in removing persistent deposits of ^{241}Am , particularly in the skeleton, although long period of treatment would be

necessary to significantly deplete the body burden of ^{241}Am . DTPA therapy in a juvenile baboon appeared to be more effective in removing ^{241}Am than in the adult. This difference was attributable to a greater ease of removal of ^{241}Am from the skeleton of the juvenile. Data was obtained on the variation of DTPA-effectiveness with the dose of DTPA administered, the dose rate, and the mode of administration of the compound.

9. Preliminary experiments conducted on adult baboons showed that, upon administration of DTPA, an increase in urinary Zn^{++} was detected as well as a decrease in activity of a Zn-dependent heme-synthesis enzyme, ALAD.

During the course of DTPA-therapy, certain urinary abnormalities were noted, viz. oliguria, albuminuria, and cylindruria. The cause of the symptoms, however, could not be specifically determined.

10. The presence of anosseous areas in the scapula of two baboons which had undergone prolonged DTPA-therapy, vis-a-vis no such areas in control baboons, suggest the possibility of a detrimental effect of DTPA on the skeleton. This speculation is very tentative, however, and is in need of much more comprehensive study.

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APPENDIX 1

CLINICAL MEASUREMENTS OF BLOOD AND URINE OF EXPERIMENTAL BABOONS

Clinical Blood Parameters Derived From Normal Baboon*

Test	Mean	± S.D.	range of 2σ
RBC (x10 ⁶ cells/mm ³)	4.45	0.5	3.45-5.45
Hemoglobin (g %)	11.8	1.16	9.48-14.12
Hematocrit	35.8	8.7	18.4-53.2
WBC(cells/mm ³)	10,000	4,170	1660-18,340
Segmented Neutrophils (%)	56	19.5	17.0-95.0
Lymphocytes (%)	32	16.7	0.0-65.4
Monocytes (%)	2.4	2.7	0.0-7.8
Basophils (%)	0.02	0.13	0.0-0.28
Eosinophils (%)	1.7	1.9	0.0-5.5

	African ^{xx}	Domestic	Cross	Nairobi	Human
Ca ⁺² (mg%)	9.4 ± 0.7	9.2 ± 0.6	9.8 ± 0.6	9.4 ± 0.4	9.8 ± 0.6
Phosphates(mg%)	4.0 ± 1.1	4.0 ± 1.5	4.4 ± 1.4	3.5 ± 1.4	3.5 ± 0.6
BUN (mg%)	11.8 ± 3.5	9.5 ± 2.3	11.5 ± 2.0	12.0 ± 3.4	13.3 ± 2.9
Uric Acid(mg%)	0.7 ± 0.25	0.8 ± 0.30	0.9 ± 0.17	0.6 ± 0.26	3.9 ± 1.3
Total protein (%)	6.65 ± 0.44	6.68 ± 0.34	6.19 ± 1.66	6.23 ± 0.53	6.8 ± 0.18
Albumen	3.34 ± 0.61	3.30 ± 0.56	3.41 ± 0.92	2.57 ± 0.49	3.5 ± 5.5
Cholesterol (mg%)	95 ± 23.7	81 ± 26.0	82 ± 10.5	109 ± 20	183 ± 34
Bilirubin(mg%)	0.3 ± 0.17	0.23 ± 0.17	0.23 ± 0.14	0.34 ± 0.17	0.5 ± 0.25
SGOT (units%)	34 ± 8.9	30 ± 11	41 ± 8.2	10 ± 4	15 ± 5.8
LDH (units%)	469 ± 62	589 ± 185	627 ± 166	504 ± 202	400 ± 104
Glucose (mg%)	95 ± 19	84 ± 23	90 ± 20	95 ± 19	98 ± 10

*Data taken from DeLaPena, A. and Goldzieher, J. W., "Clinical Parameters of the Normal Baboon" from The Baboon in Medical Research, Vol II, H. Vagtborg, ed., Univ. of Texas Press, Austin, Texas, 1967

^{xx}Headings refer to areas from which the baboons were derived.

Peripheral Blood Measurements of B-352

Date	Hematocrit	Hemoglobin	White Blood Cells	Segmented Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
10/7/69	42	12.3	15,800	55	39	2	2	2
10/20/69	41	11.6	15,700	66	34	-	-	-
2/25/70	45	14.5	6,300	50	36	6	5	-
5/12/70	47	14.9	19,800	-	-	-	-	-
8/19/70	44	15.3	9,900	-	-	-	-	-
11/11/70	44	14.1	8,800	40	53	3	4	-
12/15/70	40	13.7	13,200	34	44	12	10	-
2/2/71	44	14.5	11,300	56	35	9	-	-
3/12/71	36	14.4	12,200	60	33	4	2	1
4/20/71	48	12.7	16,200	52	44	4	-	-
9/22/71	41	12.6	16,500	38	61	1	-	-
10/29/71	36	12.6	8,700	-	-	-	-	-
5/2/72	50	16.5	-	66	32	2	-	-
3/19/73	44	14.8	8,700	68	26	5	1	-
4/2/73	44	14.2	16,100	69	27	3	1	-
4/27/73	36	12.2	7,000	55	42	3	1	-
6/1/73	44	13.5	-	-	-	-	-	-
9/14/73	40	-	-	-	-	-	-	-
9/17/73	40	-	-	-	-	-	-	-
9/19/73	47	14.2	-	-	-	-	-	-
9/19/73	45	13.8	-	-	-	-	-	-
9/21/73	35	10.6	-	-	-	-	-	-
9/24/73	37	11.5	-	-	-	-	-	-
9/26/73	39	12.0	-	-	-	-	-	-
9/28/73	38.5	-	-	-	-	-	-	-
10/1/73	38.8	-	-	-	-	-	-	-
10/3/73	41.6	-	-	-	-	-	-	-

Peripheral Blood Measurements of B-470

Date	Hematocrit	Hemoglobin	White Blood Cells	Segmented Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
11/29/71	43	10.7	9,700	44	51	4	1	-
12/13/71	43	12.0	11,000	40	57	2	1	-
12/20/71	43	-						
12/27/71	44	12.6	18,100	44	55	1	-	-
1/26/72	43	14.0	11,400					
5/19/72	41	12.8	16,500	68	30	-	2	-
12/10/73	38	11.9	19,000					
7/18/74	40	12.4	13,400	79	20	-	-	-

Peripheral Blood Measurements of B-458

Date	Hematocrit	Hemoglobin	White Blood Cells	Segmented Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
10/22/71	45	11.0	13,600	77	22	1	-	-
11/9/71	39	12.5	11,100	53	43	3	1	-
11/18/71	37	11.5	12,600	66	28	6	-	-
12/20/71	43	12.0	10,100	67	29	4	-	-
1/7/72	39	11.7	12,600	64	28	5	3	-
1/26/72	37	11.0	15,000	52	45	3	1	-
1/29/72	35	12.4	10,200	45	50	5	-	-
1/31/72	34	11.0	10,800	54	45	1	-	-
2/4/72	35	11.2	11,100	55	40	5	-	-
2/8/72	38	12.0	13,800	50	43	6	1	-
2/15/72	41	-	12,900	70	29	1	-	-
2/23/72	42	12.0	10,400	19	75	4	2	-
2/29/72	37	11.5	13,700	43	55	2	-	-
3/1/72	37	11.5	16,500	49	45	6	-	-
3/3/72	33	10.5	15,900	58	38	2	2	-
4/18/72	39	12.4	11,700					
6/5/72	37	11.0	10,400	63	36	1	-	-
2/14/73	39	12.5	9,100	42	56	2	-	-
2/23/73	35	10.9						
2/28/73	39	11.9	5,100	45	51	2	2	-
3/16/73	33	11.0	4,400	52	47	1	-	-
3/22/73	38	12.5	6,600	49	44	7	-	-
7/16/73	33	10.8	6,700	46	52	2	-	-

Peripheral Blood Measurements of B-458

Date	Hematocrit	Hemoglobin	White Blood Cells	Segmented Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
8/1/73	37	11.5	6,200	38	60	2	-	-
8/8/73	38	12.0	7,700	52	42	6	1	-
1/21/74	41							
1/23/74	37							
1/25/74	35							
2/20/74	43	13.2						
3/4/74	37							
3/6/74	37							

Peripheral Blood Values of B-520

Date	Hematocrit	Hemoglobin	White Blood Cells	Segmented Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
7/17/72	38	12.5	8,300	69	29	2	-	-
8/8/72	41	13.5	15,400	50	47	3	-	-
8/23/72	41	12.8	12,400	61	38	1	-	-
1/15/73	44	13.5						
3/19/73	41	13.7	8,400	21	78	1	-	-
4/2/73	43	13.7	11,800	45	50	1	4	-
4/26/73	39	12.2	5,300	47	47	6	-	-
6/1/73	43	12.5						
8/14/73	39							
8/17/73	37							
8/19/73	40	12.5						
8/21/73	40	12.0						
8/24/73	36	13.0						
8/26/73	36	11.0						
8/28/73	39	10.8						
10/1/73	39							
10/3/73	36							
10/8/73	39							
10/10/73	42	12.0						
10/12/73	41							
11/7/73	39	12.4						
11/14/73	38							
11/21/73	40	12.1						
11/28/73	41	12.0						
12/5/73	40							
12/12/73	38							
12/31/73	38							
1/2/74	41							
1/21/74	41							
1/23/74	45							
1/25/74	39							
2/20/74	40							
3/4/74	40							
3/6/74	40							

Peripheral Blood Measurements of B-164

Date	Hematocrit	Hemoglobin	White Blood Cells	Segmented Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
10/1/68	42	12.6	8,800	66	30	2	2	-
10/14/68	42	13.3	8,000	42	50	5	2	1
12/12/68	41	12.3	7,800	60	33	2	5	-
3/14/69	45	13.7	8,400	38	59	2	1	-
10/14/69	42	13.0	10,800	53	42	-	5	-
3/23/70	44	13.3	13,400					
5/13/70	40	12.3	6,300					
8/17/70	43	13.0	9,300	27	65	2	6	-
11/16/70	42	14.5	10,600	42	47	4	7	-
2/23/71	37	11.7	14,400	51	41	8	-	-
6/4/71	37	11.7	7,100	20	78	1	1	-
8/24/71	36	10.7	10,400					
4/19/72	37	11.5	7,800					
4/23/73	37	10.8	7,200					
1/21/74	34							
1/23/74	39							

Peripheral Blood Measurements of B-460

Date	Hematocrit	Hemoglobin	White Blood Cells	Segmented Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	DTPA treatment before blood draw
10/22/71	37	12.5	11,900	75	21	4	-	-	
11/9/71	45	15.0	15,800	62	36	1	-	-	
11/18/71	46	13.0	19,900	60	35	5	-	-	
12/20/71	48	13.5	14,400	39	51	4	6	-	
1/28/72	50	16.0	13,900	42	55	3	-	-	
2/2/72	47	13.6	15,700	18	79	3	-	-	
2/4/72	36	11.5	12,300	69	23	4	3	1	
2/7/72	37	10.1	16,100	55	42	1	2	-	
2/15/72	38	-	13,200						
2/17/72	38	-	15,800	53	43	2	1	1	
2/23/72	40	11.5	12,400	47	46	4	3	-	
2/29/72	42	12.5	13,700	53	46	1	-	-	
3/27/72	39	13.5	12,300	64	32	2	1	1	
4/27/72	49	15.0	15,900						
6/5/72	44	13.0	15,600	69	30	-	1	-	
2/14/73	47	14.0	16,100	55	40	1	3	1	
2/23/73	42	12.8							*
2/28/73	35	12.0	6,500	32	63	1	4	-	*
3/16/73	42	12.5	9,500	61	36	2	1	-	
3/22/73	44	14.7	12,500	54	41	4	-	-	
7/16/73	39	11.9	11,500	58	37	5	-	-	
8/1/73	40	12.0	8,200	49	46	3	2	-	
8/8/73	38	12.0	8,400	67	27	3	3	-	
1/18/73	44								*
11/14/73	47								
11/21/73	40	11.5							*
11/28/73	42	12.5							
12/5/73	41								
12/12/73	42								
12/31/73	43								
1/2/74	41								
1/21/74	43								
1/23/74	41								*
1/25/74	36								
2/20/74	42	12.9							*

Clinical Blood Chemistry Measurements for B-460, a DTPA-treated Baboon

	SGOT	LDH	Alkaline Phosphate	Total Bilirubin	Albumin	Total Protein	Cholesterol	Uric Acid	BUN	Glucose	Phosphates	Ca ⁺⁺
2/14/73	50	287	219	0.2	3.2	6.6	95	0.3	15	82	3.8	8.7
* 2/23	94	394	309	0.3	3.4	7.1	152	0.3	25	125	4.7	8.5
* 2/28	62	278	171	0.2	3.9	6.9	126	0.3	17	92	2.5	9.8
* 3/14	54	289	153	0.2	4.2	7.1	111	0.2	16	92	2.6	10.5
6/14	73	228	182	0.3	4.2	8.2	122	0.2	22	127	4.6	10.6
* 7/11	84	465	132	0.1	4.3	6.7	134	0.3	21	126	3.4	8.9
*10/24	63	230	146	0.2	3.9	7.8	119	0.2	20	117	3.8	9.7
*11/7	46	210	129	0.1	3.8	7.4	128	0.1	19	90	5.0	7.9
11/14	47	246	152	0.1	3.6	7.1	122	0.3	17	154	3.2	7.9
*11/21	48	297	129	0.2	3.7	7.7	127	0.1	14	94	3.0	8.4
11/28	45	170	149	0.1	4.0	7.6	113	0.4	17	105	3.8	9.5
12/12	30	127	149	0.2	3.1	6.7	106	0.3	16	101	3.3	8.6
*12/19	48	182	114	0.2	3.1	6.7	115	0.5	16	118	4.1	7.9
* 1/2/74	46	259	154	0.6	4.1	9.0	191	0.2	25	89	4.6	9.3
1/10	54	412	114	0.1	3.2	7.2	125	0.0	20	111	2.6	10.3
1/16	42	256	206	0.4	3.6	7.3	186	0.0	19	95	5.3	9.2
* 1/23	49	207	110	0.2	3.4	6.4	112	0.0	13	107	3.2	8.2
1/30	40	246	126	0.2	4.1	7.7	126	0.1	19	124	3.4	8.5
* 2/6	51	165	127	0.2	3.8	7.2	106	0.1	17	93	3.6	8.7
* 2/20	51	184	80	0.2	3.2	6.5	104	0.1	13	96	2.2	8.4
2/28	36	304	122	0.1	3.1	6.3	114	0.3	15	102	3.3	9.1
* 3/6	38	211	86	0.2	3.6	7.1	111	0.2	16	99	2.8	9.1
* 3/20	53	202	69	0.1	3.9	7.2	113	0.2	17	157	3.8	9.3
3/27	43	219	123	0.1	3.8	6.7	107	0.4	17	114	3.0	9.5
* 4/3	17	168	81	0.1	3.1	6.1	104	0.3	19	91	2.7	7.8

* Symbol indicates blood samples taken after DTPA Treatment.

Clinical Blood Chemistry Measurements for B-458

	2/14 73	2/23	2/28	3/14	6/14	7/11	1/10 74	1/16	1/23	1/30	2/6	2/20	2/27	3/6	3/20	3/27	4/3	Mean ± Std der
SGOT	38	79	55	43	44	32	60	47	59	40	45	53	21	41	68	109	20	50 ± 21
LDH	291	360	279	190	206	220	247	295	416	171	185	313	277	206	157	150	213	245 ± 74
Alkaline Phosphate	199	201	210	192	183	207	160	255	201	179	186	121	121	159	136	251	227	188 ± 39
Total Bilirubin	0.2	0.2	0.2	0.3	0.3	0.2	0.2	0.3	0.2	0.3	0.2	0.3	0.1	0.2	0.1	0.1	0.1	0.21 ± 0.07
Albumin	3.5	3.7	4.7	4.3	4.2	4.7	3.1	3.3	3.5	4.2	4.0	3.3	3.5	3.7	3.6	3.3	3.2	3.75 ± 0.05
Total Protein	6.5	6.9	7.7	7.1	8.0	8.8	6.7	7.3	7.2	7.8	7.5	6.0	7.2	7.2	6.8	6.6	7.0	7.1 ± 0.5
Cholesterol	113	165	139	137	135	168	111	143	114	135	106	127	126	98	113	126	139	129 ± 19
Uric Acid	0.3	0.2	0.2	0.2	0.2	0.3	0.0	0.0	0.0	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.3	0.16 ± 0.09
BUN	15	10	17	17	26	13	26	17	13	17	18	15	11	16	18	21	14	17 ± 4
Glucose	78	77	70	86	97	79	93	97	74	80	73	72	89	74	87	86	73	81 ± 9
Phosphates	4.2	3.7	2.0	1.6	3.5	4.1	4.3	4.6	4.2	3.6	4.9	4.7	3.8	5.0	2.9	2.3	3.4	3.6 ± 1.1
Ca ⁺⁺	8.5	9.1	10.8	10.8	12.6	9.2	8.5	8.9	8.8	8.6	9.0	8.2	9.7	9.3	9.3	9.8	8.2	9.4 ± 1.1

Clinical Blood Chemistry and Urinalysis Measurements for B-406,
a juvenile Baboon

Date	7/26/73	2/6/74	2/20	2/27	3/20	3/27	4/3
<u>Blood Chemistry</u>							
SGOT	29	36	29	19	25	22	20
LDH	235	195	285	186	213	198	178
Alkaline Phosphate	700	1400	996	1400	1400	1664	1196
Total Bilirubin	0.2	0.3	0.2	0.2	0.2	0.1	0.1
Albumin	4.0	4.1	3.6	3.8	4.5	3.9	3.4
Total Protein	0.1	6.9	7.1	6.8	6.8	6.8	5.9
Cholesterol	105	113	107	99	114	110	104
Uric Acid	0.3	0.1	0.2	0.2	0.3	0.2	0.3
BUN	13	22	11	10	13	14	14
Glucose	75	64	71	76	102	74	76
Phosphates	5.4	7.8	6.6	6.3	5.7	6.1	6.8
Ca ⁺⁺	9.4	9.7	9.4	10.0	9.6	10.4	8.6

<u>Urinalysis</u>							
Color			yel	straw	straw	straw	
Appearance			clr	clr	clr	clr	
Specific gravity			1.008	1.002	1.004	1.004	
pH			8	7	8	8	
Albumin			-	-	-	-	
Glucose			-	-	-	-	
Acetone			-	-	-	-	
Occult Blood			-	small	-	-	
WBC			0-1	1-4	0-1	0-1	
RBC			-	0-2	-	-	
Epithelial Cells			0-1	0-1	0-1	0-1	
Casts							
Amorphous Sediment							
Crystals							
Bacteria							
Other							

Clinical Blood Chemistry Measurements for B-352

Date	3/19/73	4/3	4/9	6/14
SGOT	34	42	23	18
LDH	345	345	426	150
Alkaline Phosphate	188	192	448	336
Total Bilirubin	0.3	0.2	0.1	0.2
Albumin	4.1	4.0	3.2	3.0
Total Protein	7.9	7.7	8.3	7.2
Cholesterol	146	153	193	143
Uric Acid	0.3	0.6	0.2	0.3
BUN	12	12	12	12
Glucose	75	91	91	95
Phosphates	5.4	2.7	3.3	3.3
Ca ⁺⁺	10.1	11.2	11.2	10.5

Clinical Blood Chemistry Measurements and Urinalyses for B-164,

a DTPA-treated Baboon

Date	1/10/74	1/16	1/23	1/30
<u>Blood Chemistry</u>				
SGOT	207	67	69	43
LDG	996	313	349	256
Alkaline Phosphate	119	105	107	118
Total Bilirubin	0.3	0.3	0.2	0.2
Albumin	3.5	3.8	3.8	3.0
Total Protein	8.1	7.6	7.3	6.1
Cholesterol	126	97	98	95
Uric Acid	0.0	0.0	0.0	0.1
BUN	17	23	19	22
Glucose	81	94	109	92
Phosphates	3.9	1.2	1.4	3.6
Ca ⁺⁺	9.1	10.1	9.5	8.4

Urinalysis

Color	yel	yel	yel	yel
Appear	clr	clr	hazy	clr
Specific gravity	1.034	1.022	1.025	1.023
pH	6	8	8	9
Albumin	trace	-	-	trace
Glucose	-	-	-	-
Acetone	-	-	-	-
Occult Blood	-	-	-	trace
WBC	1-5	0-1	0-1	1-55
RBC	-	-	-	0-1
Epith	0-2	0-1	0-1	0-1
Casts	-	-	-	-
Amorph Sed	-	-	-	-
Crystals	3PO ₄	PO ₄	PO ₄	-
Bacteria	-	-	-	-
Other	-	-	-	-

Clinical Blood Chemistry Measurements and Urinalyses for B-400,
a Juvenile Baboon Treated with DTPA

Date	2/6/74 *	2/20 *	2/27 *	3/6 *	3/20	3/27	4/3
SGOT	51	39	31	28	41	32	36
LDH	297	214	200	234	204	223	213
Alkaline Phosphate	1372	920	1400	1164	1206	1872	1404
Total Bilirubin	0.4	0.2	0.2	0.1	0.1	0.0	0.2
Albumin	3.8	3.6	4.0	3.5	3.7	3.9	3.6
Total Protein	7.1	6.6	6.8	6.5	6.4	6.8	6.0
Cholesterol	144	133	145	114	105	107	105
Uric Acid	0.2	0.2	0.3	0.4	0.3	0.3	0.5
BUN	20	13	12	12	13	16	15
Glucose	61	72	93	85	74	73	83
Phosphates	6.5	5.0	4.5	5.4	4.9	5.2	5.4
Ca ⁺⁺	9.0	9.2	10.3	10.4	9.6	10.8	8.8
<u>Urinalysis</u>							
Color	yel	yel	straw	straw	straw	straw	yel
Appear	clr	clr	clr	clr	clr	clr	clr
Specific gravity	1.023	1.006	1.009	1.008	1.003	1.005	1.010
pH	5	8	5	5	7	5	9
Albumin	1+	-	-	-	-	-	1+
Glucose	-	-	-	-	-	-	-
Acetone	Lg	-	-	-	-	-	-
Occult blood	small	small	small	small	-	trace	large?
WBC	0-1	0-1	2-5	0-1	0-2	0-1	0-1
RBC	-	0-2	1-2	20-30	-	-	0-1
Epith	0-1	0-1	1-3	0-1	0-1	0-1	0-1
Casts	-	-	-	-	-	-	-
Amorph Sed							
Crystals							
Bacteria							
Other							

*indicates days of DTPA Treatment

Urinalyses for B-520, a DTPA-Treated Baboon

		Color	Appear	SpGr	Reaction	Albumin	Glucose	Acetone	Occult Blood	WBC	RBC	Epith	Casts	Amorph Sed	Crystals	Bacteria	Other
6/14/73		y	cr	1.005	nu	-	-	-	-	0-1	-	-	-	sm	-	-	-
* 10/24		n	cr	1.002	7	-	-	-	-	0-1	-	0-1	-	-	-	-	-
11/14		s	cr	1.008	8	-	-	-	s	0-1	-	1-6	-	-	P, Ox	-	-
* 11/17	pre	y	cr	1.014	8	-	-	-	-	0-1	-	2-8	-	-	P	-	-
11/21	pre	y	cr	1.005	8	-	-	-	-	0-1	-	0-4	-	-	-	-	-
11/28		s	cr	1.006	7	-	-	-	-	0-1	-	0-1	-	-	Ox	-	-
12/12		s	cr	1.006	8	-	-	-	-	0-1	-	0-1	-	-	-	-	-
12/19	pre	s	cr	1.004	7	-	-	-	-	0-1	-	0-1	-	-	-	-	-
* 12/19	post	s	cr	1.007	7	-	-	-	-	0-1	-	0-1	-	-	-	-	-
1/2/74	pre	y	cd	1.029	5	2+	-	m	-	50-75	-	1-7	h, fg(1-3)	-	-	-	-
1/9		s	cr	1.007	8	-	-	-	-	0-1	-	0-1	-	-	P, Co	-	-
1/16		lr	h	1.013	6	2+	-	s	l	0-2	20-30	0-5	-	-	-	-	-
* 1/23		y	cr	1.006	8	tr	-	-	tr	0-1	2-6	0-1	-	-	-	-	-
1/30		y	cr	1.015	8	-	-	-	-	0-3	0-2	0-1	-	-	-	-	-
* 2/6		y	cr	1.025	8	1+	-	-	s	6-10	10-15	0-2	fg(1-2)	-	-	-	-
* 2/20		s	cr	1.007	8	-	-	-	-	1-4	-	0-3	h(0-1)	-	-	-	-
2/27		s	cr	1.004	8	-	-	-	-	1-2	-	0-3	-	-	-	-	-
* 3/16		s	cr	1.004	8	-	-	-	m	0-2	0-1	1-3	fg(0-2)	-	-	-	-

(Cont'd)

		Color	Appear	SpGr	Reaction	Albumin	Glucose	Acetone	Occult Blood	WBC	RBC	Epith	Casts	Amorph Sed	Crystals	Bacteria	Other
* 3/20/74	s	cr	1.006	8	-	-	-	-	-	0-2	-	0-1	-	-	-	-	-
3/27	s	cr	1.005	8	-	-	-	-	-	0-1	-	6-10	h(0-1)	-	-	-	-
* 4/13	y	h	1.017	8	1+	-	-	-	tr	0-4	0-1	0-1	-	1+	-	-	-

*days of DTPA treatment

Abbreviations used:

Color

y: yellow
n: none
s: straw
lr: light red
la: light amber
r: red
cr: clear
cd: cloudy
h: hazy

Crystals

P: phosphate
Ox: calcium Oxalate
Co: calcium Carbonate
U: uric acid
mu: mucus

Quantities

s: small
m: moderate
l: large
tr: trace amount

Casts

h: hyalin
fg: fine grain
g: granular

Urinalyses for B-460, A DTPA-Treated Baboon

		Color	Appear	SpGr	Reaction	Albumin	Glucose	Acetone	Occult Blood	WPC	RBC	Epith	Casts	Amorph Sed	Crystals	Bacteria	Other
2/14/73		y	cd	1.015	alk	-	-	-	-	0-2	-	10-12	-	H	P	-	-
6/15		y	cr	1.010	acid	-	-	-	-	0-1	-	-	g(0-2)	-	-	-	-
* 10/24		y	cr	1.031	5	2+	-	-	-	0-1	-	8-20	fg(2-5)	-	P	-	-
* 11/17		la	cr	1.025	6	1+	-	1	-	6-10	-	1-3	-	-	Ox(+2)	-	mu(+1)
11/14		y	cr	1.040	+99	-	-	-	-	0-1	-	0-4	-	-	P	-	-
* 11/21		y	cr	1.023	9	2+	-	-	s	3-8	-	0-5	-	-	-	tr	-
11/28		y	cr	1.013	9	-	-	-	s	0-1	2-5	0-5	-	-	-	-	-
12/12		y	h	1.024	9	-	-	-	-	0-5	-	0-1	fg(0-1)	-	Co(+4), P(+1)	-	-
12/19	pre	y	cr	1.009	8	1+	-	-	-	0-2	-	0-1	-	-	-	-	-
* 12/19	post	y	h	1.019	5	3+	-	-	-	1-8	0-3	0-5	fg(2-5)	-	-	-	-
1/2/74	pre	y	cr	1.038	5	1+	-	1	-	0-1	-	0-5	-	-	-	-	-
1/10		y	cr	1.021	5	1+	-	tr	-	0-5	-	0-1	-	-	-	-	-
1/16		s	cr	1.004	5	-	-	-	-	0-3	-	0-2	fg(0-2)	-	-	-	-
* 1/23		y	cr	1.012	8	-	-	-	s	0-1	1-3	0-1	-	-	-	-	-
1/30		y	cr	1.024	9	1+	-	-	tr	0-5	1-2	0-1	-	-	-	-	-
* 2/6		y	cr	1.031	8	4+	-	-	-	2-5	1-3	0-2	fg(1-3)	-	-	-	-
2/20		y	cr	1.015	8	2+	-	-	m	3-8	30-40	0-1	-	-	-	-	-
2/27		y	cr	1.028	8	1+	-	-	-	2-7	0-2	2-5	fg(4-6)	1+	-	-	-
* 3/6		y	cr	1.013	8	2+	-	-	-	0-1	-	0-1	-	-	-	-	-

Urinalyses for B-460 (Cont'd)

	Color	Appear	SpGr	Reaction	Albumin	Glucose	Acetone	Occult Blood	WBC	RBC	Epith	Casts	Amorph Sed	Crystals	Bacteria	Other
* 3/20/74	y	cr	1.017	8	1+	-	-	-	0-1	-	0-1	-	-	-	-	-
3/27	y	h	1.012	8	2+	-	-	tr	1-6	2-7	0-4	-	-	-	-	-
* 4/3	y	cr	1.033	9	1+	-	-	-	2-5	0-2	0-1	h(1-2)	-	-	-	-

*days of DTPA treatment

Abbreviations: see Urinalyses for B-520

Urinalyses for B-458

	Color	Appear	SpGr	Reaction	Albumin	Glucose	Acetone	Oculta Blood	WBC	RBC	Epith	Casts	Amorph Sed	Crystals	Bacteria	Other
2/14/73	r	cd	1.005	>7	1+	-	-	4+	1-2	many	10-12	-	m	-	tr	-
6/15	y	cd	1.028	>7	-	-	-	-	0-2	-	-	-	1	U	-	-
1/10/74	y	h	1.046	5	2+	-	tr	m	0-5	-	2-7	-	-	Ox	1+	-
1/16	y	cr	1.032	5	-	-	1	tr	1-7	6-10	0-3	-	-	-	-	-
1/23	y	cr	1.010	9	-	-	-	tr	0-1	0-1	0-1	-	-	-	-	-
1/30	y	cr	1.015	8	3+	-	-	-	0-3	-	0-1	-	-	-	-	-
2/6	y	cr	1.011	6	2+	-	-	-	0-2	-	1-3	-	-	-	-	-
2/20	y	cr	1.025	8	2+	-	-	m	6-10	30-40	1-5	-	-	-	-	-
3/6	y	cr	1.013	9	2+	-	-	-	1-3	-	0-1	-	1+	-	-	-
3/20	s	cr	1.007	8	-	-	-	s	0-3	5-8	0-1	-	-	-	-	-
3/27	y	cr	1.017	8	-	-	-	-	0-1	-	0-1	-	-	Ox	-	-
4/3	s	cr	1.007	8	1+	-	-	-	0-1	-	0-1	-	-	-	-	-

Abbreviations: See Urinalyses for B-520.

APPENDIX 2

POSTMORTEM PATHOLOGY REPORTS FOR EXPERIMENTAL BABOONS

Pathology Report for Baboon B-520

- Kidney - Vessels and glomeruli were congested while tubules were normal. Interstitium contained a focal mononuclear infiltrate and congestion of vessels.
- Liver - Normal lobular pattern with portal mononuclear infiltrate was observed. Vessels were congested. There was a slight variability in cell size and cells have prominent nucleoli, but no inclusions.

Pathology Reports for Baboon B-460

- Kidney - Vessels and glomeruli were congested together with hyperplastic capsular epithelium. Tubules contained fibrinoid material in lumen; proximal epithelium had granular cytoplasm. Interstitium contained focal mononuclear infiltrate in cortex.
- Liver - Lobular pattern was normal. There was congestion and portal, mononuclear infiltrate. Hepatic cells showed variable staining, but no nuclear inclusions. Scattered cells were vacuolated. There was moderate portal fibrosis in one slide.
- Lung - Vessels were congested. There were numerous macrophages in the perivascular connective tissue. A mononuclear infiltrate was present in the peribronchial area. Alveoli were normal.
- Spleen - There was marked congestion with dilatation of sinusoids. The follicles had germinal centers and scattered neutrophils were observed in the follicles.

Pathology Reports for Baboon B-352

Kidney - Vessels and glomeruli were congested especially in medulla.

Interstitium contained a focal congestion and hemorrhages.

Tubules - many tubules (proximal and distal) show granularity of cytoplasm with variable loss of staining capacity. Fragmentation of tubular lining and dissolution of cells. Some tubules have fibrinous exudate and sloughed cells in lumen.

No nuclear inclusions were observed. Focal mononuclear infiltrates was observed in one slide.

Liver - Lobular pattern was normal. A mild congestion with occasional neutrophils in the sinusoids was present. There was focal necrosis (loss of parenchymal cells). Mononuclear cells were noted in portal areas. Many parenchymal cells had pale, vacuolated cytoplasm with vesicular or hyperchromatic nuclei. There was variability of cell size and many mitoses were present. Foci of "regenerating nodules" were ill defined but show eosinophilic cytoplasm. Nuclei had prominent nucleoli, but no inclusions were noted.

Pathology Reports for Baboon B-458

- Kidney - Vessels and glomeruli were congested together with capsular epithelial hyperplasia. Tubules contained fibrinoid material in lumen but no nuclear inclusions. Interstitium contained focal mononuclear infiltrate.
- Liver - Tissue was congested and mononuclear infiltrates were present. Some parenchymal cells showed vacuolated or granular cytoplasm. One slide showed more vacuolated cells and mild to moderate portal fibrosis. A longitudinal hemorrhage appeared to be artifactual.

Pathology Reports for Baboon B-164

- Kidney - Vessels and glomeruli were congested together with hyperplastic capsular epithelium. Tubules contained fibrinous material in lumen. Interstitium was normal.
- Liver - Tissue was congested and there were portal mononuclear infiltrates. Some parenchymal cells were vacuolated.
- Lung - Slides showed normal alveolar pattern with focal, peribronchial mononuclear infiltrate. Pigment-laden macrophages were present in the septa. Vessels were congested.
- Spleen - The white pulp was relatively normal. There appeared to be histiolytic proliferation so that sinusoids are not prominent but no blood pigment was noted in the histiocytes (macrophages).

Pathology Reports for Baboon B-470

- Kidney - Vessels, glomeruli and interstitium were all congested particularly in the medulla. Tubules contained fibrinous material in lumen.
- Liver - Slides showed congestion, portal mononuclear infiltrate and mild vacuolation of hepatic cells. There was slight variability of cell size and stainability.
- Lung - Small vessels were congested. There were small subpleural "emphysematous blebs." Pigment-laden macrophages were noted in the septa.
- Spleen - Sinusoids were markedly congested and contain both RBC's and WBC's including neutrophils. The follicles had uniform cell populations with prominent germinal centers.

NEW YORK, N. Y. 10003
SURGICAL PATHOLOGY REPORT

Name Piper Baboon #B-520 scapula Age Sex Surg. Path No. 574-2030

Address NYU - Sterling Forest Location Chart No.

Date Spec. Rec'd. 06-19-74 Date of Report 06-28-74 Service Dr. Norman Cohen

Nature of Specimen:

Bone biopsies from Scapula of Americium injected baboon

Pathological Description:

GROSS (6-19-74): The specimen consists of four small fragments of thin, cortex-covered cancellous bone, submitted for histologic studies after decalcification.

W. D. Sharpe, M.D./s

MICROSCOPIC (6-28-74): Osteocyte lacunae are almost entirely depopulated, but this may be due to the rather poor fixation of this tissue. Secondary and tertiary osteons are regularly formed, although some Haversian systems are a trifle larger than normal, suggesting that the baboon is middle-aged or older. The marrow is unremarkable, and membranes have been stripped so that assessment of the periosteal relationships is impossible. There is a small area where some granular blue material is present at the periosteal-osteal junction, a process probably akin to minimal fibrositis. I see very little evidence of remodeling, and my impression is that this is inactive, normal bone. The polarizing pattern is clear and within normal limits.

DIAGNOSIS: T11-M001 Bone fragments, Within normal limits (Non-human primate)

COMMENT: Specifically, I do not see evidence consistent with either increased metabolic turn-over of bone (osteoclasia, widened osteoid seams and the like), nor do I see anything which would suggest a pattern consistent with human radium osteitis. Reading this specimen blind, I would say that it was within normal limits. The empty osteocyte lacunae are probably fixation artifacts, and can be avoided by immediate fixation of fresh specimens in buffered formalin.

Respectfully submitted.

William D. Sharpe, M.D.