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THE RELATIVE PHYSIOLOGICAL AND TOXICOLOGICAL PROPERTIES OF AMERICIUM AND PLUTONIUM

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EXPERIMENTAL BIOLOGY AND MEDICINE

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ABSTRACT

The relative physiological and toxicological properties of americium and plutonium have been studied following their intravenous administration to rats.

The urinary and fecal excretion of americium was similar to that of plutonium administered as $Pu(NO_3)_4$. The deposition of americium in the tissues and organs of the rat was also similar to that observed for plutonium. The liver and the skeleton were the major sites of deposition. Zirconium citrate administered 15 minutes after injection of americium increased the urinary excretion of americium and decreased the amount found in the liver and the skeleton at 4 and 16 days.

LD_{30}^{50} studies showed americium was slightly less toxic when given in the acute toxic range than was plutonium. The difference was, however, too slight to be important in establishing a larger tolerance dose for americium.

Survival studies, hematological observations, bone marrow observations, comparison of tumor incidence and the incidence of skeletal abnormalities indicated that americium and plutonium have essentially the same chronic toxicity when given on an equal μc. basis. These studies support the conclusion that the tolerance values for americium should be essentially the same as those for plutonium.

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THE RELATIVE PHYSIOLOGICAL AND TOXICOLOGICAL PROPERTIES OF AMERICIUM AND PLUTONIUM(*)

1. INTRODUCTION

Through the continued progress of the Atomic Energy Program more and more materials, formerly only laboratory curiosities, are becoming available to experimental laboratories throughout the country. Americium^{241} (an isotope of element 95) is among these materials.

Since the announcement of its discovery in 1946 by Seaborg and associates (1), extensive experimental investigations have been carried out with this element.

The realization that americium (which has a half-life of approximately 500 years and decays by α emission to neptunium^{237}) may eventually impose a considerable health hazard led Scott, Hamilton and associates to study its metabolism in rats as early as 1947 (2). The amount of americium^{241} available at that time was small and their investigations were confined largely to body deposition and excretion studies over a 256-day period following oral and intramuscular administration of tracer doses. They found 35.7 per cent of the absorbed dose of americium in the liver and 19.1 per cent in the bone on the fourth day after intramuscular administration. Observations of plutonium

(*) Valuable assistance was rendered by Mr. James Dutli and Mr. Gerold Tenny of GMX-Division in the preparation of the radiograms of the animals.

deposition following intravenous and intramuscular administration as Pu^{+4} citrate or plutonyl nitrate (3,4) showed less than 10 per cent of the dose in the liver and 60-80 per cent in the bone.

Scott et al (2) observed also that americium deposited in the soft tissues was eliminated rather rapidly, largely through excretion by way of the intestinal tract. Their results showed also that absorption of americium from the gastrointestinal tract was less than 0.01 per cent of the administered dose.

As bone marrow is considered very susceptible to radiation damage, one might expect the difference in quantitative partition of americium (injected as $AmCl_3$) and plutonium (injected as Pu^{+4} citrate) between liver and bone to result in a lower toxic effect from the americium when the two substances are administered in equal microcurie amounts. In fact, americium should be about one-third as biologically effective as plutonium at producing peripheral blood changes, bone marrow damage, bone sarcoma, and shortening of life. One might also expect some variation in acute lethal effects of plutonium and americium depending on the relative extent to which bone marrow and liver damage contribute to the early death of the animal.

Now that adequate amounts of these elements are available we have undertaken a direct comparison of the toxicological and physiological properties of americium and plutonium when administered intravenously to rats in equivalent microcurie doses.

The obvious practical reason for this study is to establish a body

tolerance dose of americium in relation to the tolerance for plutonium in the event that it should become a widely used material in the atomic research program.

2. EXCRETION AND DEPOSITION OF AMERICIUM AND PLUTONIUM

2.1 Comparison of Excretion of Americium and Plutonium

2.1.1 Experimental Procedure.--Fifteen male Sprague-Dawley rats 47 days old (145-155 g.) were injected via tail vein with 0.03 μc. of americium[(a)] per g. of body weight. The material was injected as $AmCl_3$ in 0.26 ml. of solution adjusted to a pH of 5.

Following injection, the animals were housed in wire bottom metabolism cages equipped with a glass funnel constructed to facilitate the simultaneous but separate collection of urine and feces. Animals were housed three to a cage and Purina Laboratory Chow and water were available ad libitum. Urine and feces were collected daily from each group of three animals.

At 4, 16, 32, and 48 days after injection one group of three animals was sacrificed for the tissue deposition studies given in

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(a) The amount of americium injected was determined as follows: After every fifth animal was injected, 0.26 ml. of solution, the volume received by each rat, was discharged into volumetric flasks. These samples were then diluted to volume with nitric acid and 0.1 ml. aliquots plated directly on stainless steel plates and counted in a methane flow proportional alpha counter. The average number of disintegrations in the flasks was taken as the amount of americium received by each animal.

Section 2.2. The urinary and fecal excretion results, therefore, represent averages for 15 animals through the fourth day, 12 animals from 5-16 days, 6 animals from 17-32 days, and 3 animals from 33-48 days after injection. The methods of analysis of urinary, fecal and tissue samples for americium are given in footnote (b).

2.1.2 Results.--A comparison of the urinary and fecal excretion rates of americium (administered as $AmCl_3$) and plutonium (administered as $Pu(NO_3)_4$) for the 40-day period following intravenous injection are presented graphically in Figs. 1 and 2 respectively. The data were plotted as per cent of injected dose excreted per day, and eye-fitted curves were drawn through the data. Americium excretion was compared with the excretion of plutonium administered as $Pu(NO_3)_4$ because of the

(b) Samples were analyzed for americium as follows:
The entire urine sample, representing the 24-hour excretion of three animals, was transferred quantitatively to a Kjeldahl flask and 10 ml. of conc. HNO_3 added. The contents of the flask were heated until the residue began to char, at which time conc. HNO_3 was again added and digestion continued until a white ash formed. The ash was re-dissolved in 2N HNO_3 and suitable aliquots were plated on 2-inch stainless steel discs and counted in an alpha counter, after which appropriate mass absorption corrections were made. Fecal samples were dried at 110° for 24-48 hours. The entire 24-hour sample was ground in a mortar and a weighed aliquot ignited in a muffle furnace at 450° for 8-24 hours. The residue was dissolved and analyzed as above.
The balance of the carcass, consisting principally of skeleton and muscle, was autoclaved until the muscle was freed from the skeleton. Muscle tissue was saved for later analysis; the skeleton was dried and its dry weight determined. Americium analyses on the organs, muscle and skeleton were carried out by nitric acid digestion of the tissue, redissolving the ash in 2N HNO_3 and taking a suitable aliquot of the solution for direct plating and counting. In all instances stainless steel discs were used for plating and the mass deposited on each disc was carefully kept below 0.5 mg. per square centimeter of plating surface.

apparent similarity in tissue deposition between americium and plutonium administered as $Pu(NO_3)_4$ (Table 3). Tables 1 and 2 summarize the total urinary and fecal excretion of americium and afford a comparison with the total urinary and fecal excretion of plutonium administered as $PuCl_3$, $Pu(NO_3)_4$, Pu^{+4} citrate and $PuO_2(NO_3)_2$ reported in an earlier paper from this laboratory (4).

2.2 Comparison of Tissue Deposition of Americium and Plutonium

2.2.1 Experimental Procedure.--The experimental procedure is described in Section 2.1.1 above. The animals used in the excretion study were sacrificed in groups of three. Groups were sacrificed at 4, 16, 32, and 48 days after injection. The animals were killed with chloroform. Blood, kidneys, testes, heart, lungs, liver, spleen, gastrointestinal tract, brain, skin, skeleton and balance were weighed and analyzed for americium (b).

2.2.2 Results.--The results of the tissue deposition studies for americium are presented in Table 3. The data are compared with deposition studies of plutonium following its intravenous administration as $PuCl_3$, $Pu(NO_3)_4$, Pu^{+4} citrate complex, and $PuO_2(NO_3)_2$. It should be pointed out that the plutonium results are from an early study from this laboratory (4) on a different series of animals and the results may not be completely comparable in that the experiments were not conducted simultaneously. These data show, however, that americium, like plutonium in its various valence states, deposits primarily in the

liver and the skeleton following intravenous injection. It appears from these data that $AmCl_3$ is comparable to $Pu(NO_3)_4$ or $PuCl_3$ with regard to deposition in these two principal organs. Four days after injection 38.3 per cent of the injected dose of americium was found in the liver and 30.7 per cent in the skeleton. Following the injection of $Pu(NO_3)_4$ 39.7 per cent of the injected dose was found in the liver and 29.4 per cent in the skeleton. The results for americium and plutonium deposition in the "balance" cannot be compared as the samples were not comparable. The "balance", in the case of the plutonium analyses, included heart, blood, testes, skin, G.I. tract, and lungs which were analyzed separately in the americium experiment. If the americium found in the above tissues is added to that reported for the balance, the values obtained are roughly comparable to those reported for plutonium. Comparison of other tissues and organs is of little significance because of the relatively small fractions of the total doses which they contain. The data are too incomplete, or are scattered too badly, to allow any significant comparison beyond the fourth day after injection.

The qualitative similarity of mode of deposition of americium and plutonium in bone is demonstrated by the autoradiographs given in Figs. 3 and 4. The survey-type radioautographs of tibia and vertebra shown in a, c, e, and g of Fig. 3 were made by placing undecalcified bone sections in contact with dental X-ray films. b, d, f, and h are photomicrographs of the corresponding bone sections. A comparison of

the autoradiographs with the photomicrographs demonstrates the great extent to which the deposition of both americium and plutonium is confined to the epiphyseal line, the endostial surfaces of trabecular bone, and to a lesser extent to the endostium and periostium of other bone.

The detail autoradiographs shown in Fig. 4 were prepared by placing the undecalcified bone section in contact with an NTA nuclear track plate. After exposure the emulsion was developed and the tissue stained without displacing it from the plate. A photomicrograph was made by focusing on the alpha tracks in the emulsion through the tissue section. These autoradiographs demonstrate the great selectivity of both americium and plutonium for the epiphyseal line and for the endostium, and their nearly complete exclusion from cartilage and dense bone. Americium appears to be more specifically confined to the epiphyseal line than does plutonium. This appearance could, however, be an artifact or a result of more elapsed time between injection and sacrifice of the animal given plutonium. Similar autoradiographic studies of bone deposition of plutonium, americium and other radioactive elements have been reported by Hamilton and associates (2,3,5,6,7).

2.3 Effect of Zirconium Citrate on Excretion and Deposition of Americium

Schubert (8) showed that a single injection of 51.4 mg. of Zr as zirconium citrate markedly altered the excretion and deposition of plutonium administered to the rat. He found that the administration of the Zr 30 minutes after the injection of plutonium resulted in

increasing the excretion of the plutonium from about 1 per cent to about 50 per cent of the injected dose during the first 24-hour period. The amount of plutonium deposited in the skeleton was reduced by a factor of 6. These results indicate that Zr may also affect the excretion and deposition of americium.

2.3.1 Experimental Procedure.—Twelve 150-g. Sprague-Dawley male rats were injected intravenously with 0.03 μc./g. body wt. of Am as $AmCl_3$. Fifteen minutes after the injection of Am, six rats were injected intraperitoneally with 40 mg. of Zr as zirconium citrate. The zirconium citrate solution was prepared according to the instructions given by Schubert (8). The other six animals received no Zr and were kept as controls. The animals were housed in wire bottom metabolism cages in groups of three, and daily urine and feces samples were collected and analyzed for americium. At the end of the fourth and sixteenth days three animals from the control group and three from the group that received Zr were sacrificed and blood, G.I. tract, heart, kidneys, liver, lungs, skeleton, and remains analyzed for americium.

2.3.2 Results.—Table 4 presents the data showing the effect of a single intraperitoneal injection of 40 mg. of Zr as zirconium citrate on the average daily urinary, fecal, and urinary plus fecal excretion of americium administered intravenously to the rat. The data represent averages for six animals through the fourth day and three animals from 4-16 days after injection. During the first 24-hour period

after injection the animals that received Zr excreted 34.2 per cent of the injected americium in the urine and 3.5 per cent in the feces. The animals that received no Zr excreted 4.9 per cent of the americium in the urine and 2.0 per cent in the feces. The total excretion of americium in feces and urine during the first 24 hours was 37.7 per cent of the injected dose for the animals that received Zr and 6.9 per cent for those that received no Zr. The single injection of Zr had essentially no effect on urinary and fecal excretion of the americium after the first 24-hour period. The total excretion of americium for the 16 days of the experiment was 48.9 per cent for the animals that received the treatment and 27.4 per cent for those that received no treatment.

Table 5 presents the data showing the effect of a single intraperitoneal injection of 40 mg. of Zr as zirconium citrate (15 minutes after americium injection) on the deposition of americium in the various tissues and organs of the rat at 4 and 16 days. These data compare the per cent of injected dose of americium in tissues and organs of animals that received no Zr treatment with that of animals which received Zr. As Zr treatment is effective only during the first day after administration, the 16-day results are relatively unimportant. At 4 days after treatment, however, Zr had materially affected the deposition of americium in some of the major tissues and organs. The livers of untreated animals showed an average of 38.3 per cent of the injected dose as compared to an average of 17.9 per cent for the livers

of the animals treated with Zr. The skeletons of untreated animals contained an average of 30.7 per cent of the dose as compared to 22.5 per cent for the treated group. The kidneys of the untreated group contained an average of 2.73 per cent compared to 1.12 per cent for the group treated with Zr. A t-test for significance of difference between mean values of per cent of dose in liver, skeleton, kidney, and total carcass showed that the probability of observed differences being due to chance alone was less than one per cent. There were significant differences in the mean values for some of the other organs but they are of little importance since they constitute a very small fraction of the total injected dose. The major significant differences in the means of treated and untreated animals are shown schematically in Fig. 5 with histograms.

3. RELATIVE TOXICOLOGICAL AND PHYSIOLOGICAL EFFECTS OF AMERICIUM AND PLUTONIUM

3.1 Acute Lethality

3.1.1 Experimental Procedure.--Animals used in this portion of the experiment were again male Sprague-Dawley rats approximately 35 days old, and weighing between 80 and 110 g. Five groups, consisting of 20 animals each, were injected with plutonium solutions of varying strengths and seven groups of 20 animals each were given comparable doses of americium. Injections were made via tail vein in each instance. Americium was administered as $AmCl_3$; the plutonium was administered as Pu^{+4} citrate complex. The concentration of each solution was adjusted

to give injection volumes of 0.20 to 0.40 ml. Table 6 gives the group designations, the μc. of radioactive material injected and the 30-day mortality in each group.

Following injection, the animals were housed in constant-temperature rooms in wire-bottom cages with adequate safeguards against contamination of the area from urine and feces. Animals were housed four to a cage with water and food in constant supply. After the initial 30 days, the animals were transferred to regular animal rooms and kept for observation of long range effects. Suitable control groups were kept for comparison.

Due to the relatively small amount of americium available for this entire study, hematological observations and the study of long range effects including tumor incidence were conducted on the survivors of the acute lethal study. The fact that the results for the median lethal dose of plutonium coincided fairly closely with former studies indicates that the combination of hematological and acute lethal observations in no way altered the results of the lethal study.

3.1.2 Results.—In addition to the data showing the dose of americium or plutonium received by each animal, Table 6 gives the mortality figures for each group during the first 30 days after injection. These data were subjected to probit analysis using the basic equations and methods given in Finney's monograph on probit analysis (9).

Figure 6 shows the probit regression lines for both americium

and plutonium obtained by plotting the probit of the 30-day kill against dose in uc./g. body wt. The equations for the regression lines are:

$$\text{americium} \quad Y = 3.05 + 17.74X$$

$$\text{plutonium} \quad Y = 2.19 + 39.66X$$

These data show that the median lethal dose of plutonium, under the conditions of this experiment, had a maximum likelihood estimate of 0.071 μc./g. body wt. with a fiducial probability of 95 per cent that the median lethal dose might be expected to lie between 0.056 and 0.086 μc./g. In the case of americium, the median lethal dose had a maximum likelihood estimate of 0.110 μc./g. body wt., with a fiducial probability of 95 per cent that the median lethal dose may be expected to lie between 0.090 and 0.130 μc./g.

The ratio of the most likely LD_{30}^{50} doses of americium and plutonium is $\frac{0.110}{0.071} = 1.6$, and there is a 99 to 99.9 per cent chance that the two are different.

3.2 Relative Effects of Americium and Plutonium on the Peripheral Blood

3.2.1 Experimental Procedure.--As mentioned above, the hematological observations were conducted on the animals used for the acute lethal studies. Animals from Groups I, III, and IV plutonium and from the corresponding Groups I, III, and IV americium were used. The animals in Group I received 0.032 μc., Group III 0.050 μc., and Group IV 0.063 μc./g. body wt. The groups were subjected to blood counting at a frequency which necessitated the repetition of a count on each individual

animal once in every 17 to 20 days, an interval which did not in itself alter the hemogram of the animals as shown in the control data. Initially it was possible to count three animals from each group every 3 to 4 days, but as deaths occurred, the intervals between counting were increased to maintain the same frequency for individual animals.

The red blood cell count, the total white blood cell count, and the differential count of the nucleated elements of the peripheral blood were determined on each animal each time a "blood count" was done. Tail blood was used in all cases, and the pipettes used were those in current use for clinical laboratory determinations. Hemostasis was carefully secured after blood was taken from the tail, especially as increased bleeding times occurred with progressing radiation damage.

3.2.2 Results.--Data comparing the effects of americium and plutonium on the red blood cell count are given in Fig. 7 and on the white blood cell count in Fig. 8. Blood counts were taken over a 300-day period post injection. Granulocytes and lymphocytes were equally depressed in the period immediately following injection. Throughout the entire 300 days of observation, the proportion of lymphocytes and granulocytes remained essentially unchanged despite the wide variations in the total white count. Morphologic changes seen in white blood cells were those noted in other types of ionizing radiation damage. They consisted of bizarre nuclear forms, and increased cytoplasmic basophilia in the lymphocytes.

3.3 Relative Effects of Americium and Plutonium on the Bone Marrow

3.3.1 Experimental Procedure.—Forty-three days after injection, two animals each from Groups I and IV plutonium and from Groups I and IV americium were sacrificed and femoral bone marrow was obtained. The material was fixed and sectioned by conventional means, and differential counts were made.

3.3.2 Results.—Examination of the marrows from the plutonium- and americium-treated animals showed no significant deviation in the relative numbers of the various marrow cells from that of suitable control specimens 43 days after injection. It was noted with both substances that there was a fairly marked increase in the general cellularity of the femoral marrow, an observation which agreed with the findings of Bloom (10). Photomicrographs of the control marrow and specimens from an americium-injected and a plutonium-injected animal are shown in Fig. 9.

3.4 Relative Effects of Americium and Plutonium on Skeletal Structures

Approximately eight weeks after injection, it was noted that many animals in each group were developing limb deformities. X-ray examination revealed that pathological fractures of the long bones of the limbs had occurred. In addition to the presence of fractures the following radiologic findings were noted: (1) osteoporosis throughout the skeletal structures; (2) densities along epiphyseal growth lines (especially prominent in the upper tibiae); (3) bone necrosis; (4) marked thinning of the cortex of the long bones with occasional pseudofibrocystic changes.

There was no significant difference between the bony abnormalities of the groups that received americium and those that received plutonium. X rays of the pelvis and lower limbs of a control animal, an americium-injected animal, and a plutonium-injected animal are shown in Fig. 10. The changes described above are demonstrated in these radiographs. These rats were approximately 35 days old when injected with americium and plutonium.

At death of the animals, microscopic observations of decalcified sections showed extensive areas of bone necrosis.

3.5 Relative Production of Bone Sarcoma by Americium and Plutonium

Four osteo sarcomas were seen in animals injected with plutonium and six in animals injected with americium. The location of these tumors, the amount of americium or plutonium injected in each instance, and the survival times after injection are given in Table 7. In each instance, the tumor was discovered at the time of death of the animal or only a few days before death. In the case of animals showing abnormal swellings or unusual symptoms, radiographs were also made prior to death for diagnostic purposes.

Figures 11 and 12 show characteristic radiographs of animals with osteo sarcomas, one injected with plutonium and the other with americium. The latter radiogram demonstrates metastases to the liver and lung. Metastases, predominantly pulmonary, occurred in four of the 10 animals with osteo sarcoma.

Two fibrosarcomas were seen in animals receiving americium, one apparently arising in the subcutaneous tissues of the flank, the other in the soft tissues of the lower neck.

3.6 Relative Effects of Americium and Plutonium on General Health and Life Span

3.6.1 Experimental Procedure.--The experimental method is described in Section 3.1.1. Groups of 20 animals each were injected via tail vein with plutonium and americium in the amounts shown in Table 6. The animals were placed in wire-bottom cages until the eighth week when the occurrence of spontaneous fractures was noted. The animals were then transferred to solid-bottom cages and bedded on white pine shavings. An adequate group of controls was housed under the same conditions. Cages were checked daily for dead animals.

3.6.2 Results.--The survival curves for the various groups of animals injected with americium and plutonium are shown in Figs. 13 and 14. These curves were drawn by plotting per cent survival against days after injection and drawing an eye-fitted curve through the points.

Fifty per cent of the animals of Groups I, II, and III plutonium survived to approximately 270 days after injection. Fifty per cent of the animals of Groups I, II, and IV americium died by approximately the 270th day after injection. Fifty per cent of the animals of Group III americium had succumbed by the 60th day after injection. No deaths occurred in the control groups in 365 days. Other than the

unexplained reversal of the survival pattern for americium groups III and IV, plutonium and americium given intravenously in equivalent microcurie amounts seem to have a comparable effect on the survival of rats. A better comparison could be made, however, by using smaller doses than were used in this study.

Nearly all animals injected with doses of plutonium and americium of 0.032 μc./g. body wt. or greater appeared in poor health. A high percentage of them failed to gain weight, sustained multiple fractures and suffered from chronic upper respiratory infection at some time prior to their death. Occasional animals surviving beyond 300 days post injection gained weight normally and appeared to be in a state of good general health.

Figure 15 shows the general appearance of animals about 300 days after receiving 0.063 μc. of americium and plutonium/g. body wt. as compared to a normal uninjected animal from the same age group.

4. DISCUSSION

4.1 Excretion and Deposition of Americium and Plutonium

The excretion and deposition of americium administered intravenously to the rat as $AmCl_3$ appeared to resemble that of plutonium administered as $Pu(NO_3)_4$. The urinary and fecal excretion of americium injected as $AmCl_3$, and plutonium injected as $Pu(NO_3)_4$, are shown graphically in Figs. 1 and 2. The excretion was followed daily for a period of 32-48 days after injection. The fecal excretion rate of plutonium

was higher than of americium during the first 4-6 days. After this there was no significant difference. Thirty days after injection the fecal excretion rate of both substances was about 0.2 per cent of the injected dose. The urinary excretion rate of americium appeared to be slightly higher than that of plutonium. However, the variance of the individual points was sufficiently great that the difference in rate was not statistically significant. Thirty days after injection the rate of urinary excretion of americium was about 0.05 per cent of the injected dose per day compared to 0.02 per cent for plutonium.

The urinary and fecal excretion of americium and plutonium is summarized in Tables 1 and 2. These data show the total fecal excretion of americium and plutonium injected in various valence states to be roughly comparable at 4, 8, 16, and 32 days after injection. The maximum variation (100 per cent) occurred between americium and plutonium given as $Pu(NO_3)_4$ during the first four-day period. The total urinary excretion of americium for each of the above time periods was approximately 4-6 times that of plutonium except when $PuO_2(NO_3)_2$ was injected. The high urinary excretion of plutonium administered in this form occurred the first day and was a result of a high rate of kidney clearance of the plutonyl ion before the animal body could reduce it to Pu^{+4}. The data for the urinary excretion of americium following intravenous injection were in good agreement with the results observed by Scott et al (2) following intramuscular injection. Our results for fecal excretion, however, were considerably lower than the results observed by

them. It is interesting to note that they correlated the high fecal excretion with a high deposition of americium in the liver. Our results for liver deposition were appreciably lower than were reported by them.

The data in Table 3 show the distribution of americium in the tissues and organs of the rat at 4, 16, 32, and 48 days following intravenous injection of $AmCl_3$. Results from an earlier paper (4) showing the deposition of plutonium following the injection of $PuCl_3$, $Pu(NO_3)_4$, Pu^{+4} citrate complex and $PuO_2(NO_3)_2$ are also given. These results showed that the distribution of americium following intravenous administration as $AmCl_3$ resembled that of plutonium administered as $Pu(NO_3)_4$. A comparison of the americium results with those reported by Hamilton et al (2) showed that the trends were quite similar and indicated that the liver and bone were the principle sites of deposition. The quantitative results, however, differed occasionally. Their results showed less deposition of americium in the skeleton, more deposition in liver, a more rapid removal from the liver, and a much greater fecal excretion. There were a number of differences in the experimental procedures that could account for the minor variations in the two sets of results. In the experiments reported here, the americium was given intravenously. In their experiments intramuscular injection was used as a means of administration. The animals used in their experiment were adults. The rats used in these studies were younger (47 days old) which may account for the higher skeletal fixation of americium.

Autoradiographic studies (Figs. 3 and 4) using both dental

X-ray film and nuclear track emulsion showed a qualitative similarity between the modes of deposition of americium and plutonium in bone. Both materials deposited in the epiphyseal line, in endostium and periostium. Maximum concentrations occurred in the areas of trabecular bone. Our nuclear track autoradiographs did not show the deposition of americium in the region of the blood vessels in cortical bone observed by Scott et al (2). Almost no americium and plutonium occurred in cartilage or in areas of dense calcified bone.

The administration of a single intraperitoneal injection of 40 mg. of Zr as zirconium citrate fifteen minutes after the intravenous injection of americium materially affected the urinary excretion and body deposition of the americium (Tables 4 and 5). These data showed that Zr increased the urinary excretion of americium by a factor of about six during the 16 days following treatment. Fecal excretion was slightly decreased and the urinary plus fecal excretion was increased by a factor of about two. The increase in excretion was due entirely to the increase in urinary output during the first day. The average urinary output of the animals that received Zr was 34.2 per cent of the injected dose during the first 24 hours as compared to 4.9 per cent for the animals that received no Zr. Four days after injection an average of 17.9 per cent of the americium was found in the livers of animals that were treated with Zr. The untreated controls showed an average liver deposition of 38.3 per cent. The skeletons of the treated animals contained an average of 22.5 per cent of the americium compared to 30.7 per cent for

the untreated controls.

4.2 Relative Toxicological and Physiological Effects of Americium and Plutonium

The acute toxicity of americium administered intravenously to rats was slightly less than that of plutonium when the two materials were given in equal microcurie amounts. The 30-day LD-50 for americium administered as $AmCl_3$ was 0.11 μc./g. body wt. The LD-50 for plutonium administered as Pu^{+4} citrate was 0.071 μc./g. body wt. The ratio of the LD-50 of americium to that of plutonium was 1.6 with greater than a 99 per cent probability that the difference between the two was significant. In view of the uncertainty in determining chronic body tolerance levels from acute toxicity studies this slight difference in the LD-50 of americium and plutonium would not indicate a higher relative maximum body tolerance for americium.

Americium and plutonium appeared to have very similar qualitative effects on the peripheral blood of rats. Intravenous injections of 0.032 μc./g. body wt. produced a significant anemia in about 45 days. The animals receiving both americium and plutonium appeared however to recover from the anemia in about 90 days. They did not, however, recover completely from the leucopenia and their white blood cell count varied as much as two to three standard deviations from the mean of the control group over the entire 300-day observation period. Anemia and leucopenia in animals receiving 0.05 and 0.063 μc./g. body wt. of plutonium appeared to be slightly greater than that in animals receiving

a corresponding dose of americium. The relative results of the hematological studies are shown graphically in Figs. 7 and 8. Throughout the entire 300 days of observation the proportion of lymphocytes and granulocytes remained essentially unchanged in both the americium and plutonium groups of animals despite wide variations in the total white count. The morphologic changes seen in white blood cells were essentially the same for both groups of animals and were those noted in other types of ionizing radiation (10).

Sections of bone marrow taken 43 days after injection of americium and plutonium were examined and compared with uninjected controls. These sections showed no essential differences in the effect of the two substances. Both materials produced a marked increase in the general cellularity of marrow from the shaft of the femur.

As was expected both americium and plutonium produced marked pathological changes in the skeletal structures. These changes consisted of spontaneous fractures, osteoporosis, densities along the epiphyseal growth line, bone necrosis, marked thinning of the cortex of the long bones, and occasional pseudofibrocystic changes. No significant qualitative or quantitative differences in the bone abnormalities in two groups were observed.

Osteogenic sarcomas were observed in both the americium and plutonium groups. The number of animals in each group that survived a sufficient length of time to develop sarcomas was too small to permit a comparison of the tumor incidence of americium and plutonium. The

results however indicate that americium, like plutonium, produces osteogenic sarcoma. The microcurie doses of americium required to produce sarcoma appeared to be about the same as for plutonium.

These studies showed that equivalent microcurie doses of americium and plutonium had about the same effect on the general health and the life span of rats. It should be pointed out however that the number of animals used was perhaps too small and the doses of americium and plutonium too large to permit a good comparison of the two materials. The data presented in Figs. 13 and 14 however indicate that there was little or no difference in the effect of americium and plutonium on the survival time of rats over the dosage range studied.

In general it may be concluded from the studies presented in this report that the maximum permissible microcurie amounts of americium in disposal waste, drinking water, room air, stack gases and the bodies of laboratory and plant operators should be essentially the same as that recommended for plutonium. The currently accepted body tolerance for plutonium is 0.6 μg. or 0.04 μc. (11). Taking the specific activity of americium to be 6.46×10^6 alpha d./m./μg. the body tolerance for americium should be 0.04 μc. or 0.014 μg.

5. SUMMARY AND CONCLUSIONS

The excretion, deposition, and toxicology of americium and plutonium have been compared following intravenous administration to rats. These studies are summarized as follows:

1. In general the urinary and fecal excretion of americium administered as $AmCl_3$ to rats approximated the excretion of plutonium administered as $Pu(NO_3)_4$. Thirty days after injection the fecal excretion rate of americium was about 0.2 per cent of the injected dose per day. The fecal excretion rate of plutonium was essentially the same. The urinary excretion rate of americium 30 days after injection as $AmCl_3$ was about 0.05 per cent of the injected dose per day. The urinary excretion rate of plutonium injected as $Pu(NO_3)_4$ was about 0.02 per cent of the injected dose under similar conditions.

2. The distribution of americium in the tissues and organs of the rat following injection of $AmCl_3$ was quite similar to that observed for plutonium following the administration of $Pu(NO_3)_4$. In both cases the major sites of deposition were the liver and bone.

3. Survey and detailed autoradiographs showed that the mode of deposition of americium and plutonium in bone was quite similar.

4. A single treatment of 40 mg. of Zr as zirconium citrate administered intraperitoneally fifteen minutes after intravenous injections of americium materially increased the urinary excretion of the latter during the first 24-hour period post injection. The amount of americium deposited in the liver and bone was significantly decreased as a result of a single treatment with zirconium.

5. Thirty day LD-50 studies comparing the acute toxicity of americium and plutonium gave a median lethal dose for americium of 0.11 μc./g. body wt. compared to a median lethal dose for plutonium of 0.071 μc./g. body wt.

This small difference in the acute toxicity of americium and plutonium is not sufficient to justify an increase in the relative tolerance dose for americium.

6. There was little significant difference in the effects of americium and plutonium on the peripheral blood of rats following injection of subacute equivalent microcurie doses of the two materials. There was also no significant difference in the effects on bone marrow 43 days after administration.

7. Both americium and plutonium produced serious effects on skeletal structures of the rat when administered in doses of 0.032 to 0.095 μc./g. body wt. The skeletal effects observed were pathological fractures, osteoporosis, abnormal densities along the epiphyseal growth line, bone necrosis, marked thinning of the cortex of the long bones and occasional pseudofibrocystic changes. There was no apparent quantitative difference between americium and plutonium in the production of these bone abnormalities.

8. Both americium and plutonium produced osteogenic sarcomas. Not enough animals were used to obtain quantitative data but it appears that the μc. amount of americium required to produce sarcomas is about the same as that required for plutonium.

9. At the dose levels used in these studies both americium and plutonium had a pronounced effect on the general health of the animals and shortened their life span materially.

10. It is concluded from these studies that the maximum permissible microcurie amounts of americium in disposal waste, drinking water, room air, stack gases and the bodies of laboratory and plant operators should be essentially the same on a μc. basis as that recommended for plutonium.

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Table 1.--A Summary of the Comparative Total Urinary Excretion of Americium and Plutonium During Various Time Intervals Following Intravenous Administration to the Rat

Days After Injection	Per Cent of Injected Dose[1]				
	$AmCl_3$	$PuCl_3$	$Pu(NO_3)_4$	Pu^{+4} Cit.	$PuO_2(NO_3)_2$
4	5.56	0.70	0.98	1.32	8.55
8	5.98	0.93	1.32	1.75	9.25
16	6.75	1.23	1.68	2.23	9.80
32	8.09	1.46	2.07	2.58	10.04
48	9.91	—	2.28	2.79	—

[1] Results are averages for 15 animals for first 4 days, 12 animals from 5-16 days, 6 animals from 17-32 days and 3 animals from 33-48 days.

Table 2.--A Summary of the Comparative Total Fecal Excretion of Americium and Plutonium During Various Time Intervals Following Intravenous Administration to the Rat

Days After Injection	Per Cent of Injected Dose[1]				
	$AmCl_3$	$PuCl_3$	$Pu(NO_3)_4$	Pu^{+4} Cit.	$PuO_2(NO_3)_2$
4	7.36	13.88	14.88	10.01	5.61
8	14.03	24.54	21.45	15.89	9.50
16	20.55	34.99	27.26	21.54	14.47
32	24.30	39.74	32.81	27.68	18.60
48	—	—	37.00	29.68	—

[1] Results are averages for 15 animals for first 4 days, 12 animals from 5-16 days, 6 animals from 17-32 days and 3 animals from 33-48 days.

Table 3.--Comparison of Tissue Deposition of Americium and Plutonium Following Intravenous Administration to the Rat

Days After Injection	Tissue or Excrement	Av. Per Cent of Injected Dose[1]				
		$AmCl_3$	$PuCl_3$	$Pu(NO_3)_4$	Pu^{+4} Cit.	$PuO_2(NO_3)_2$
4	Blood	0.11	—	—	—	—
	G.I.Tract	2.73	—	—	—	—
	Heart	0.12	—	—	—	—
	Kidneys	2.73	2.20	1.36	1.64	1.91
	Liver	38.32	22.92	39.69	9.56	9.11
	Skeleton	30.73	44.91	29.43	56.93	56.54
	Lungs	0.31	—	—	—	—
	Spleen	0.26	0.73	1.19	0.67	0.51
	Testes	0.08	—	—	—	—
	Skin	1.64	—	—	—	—
	Balance[2]	3.38	13.54	11.88	13.70	11.21
	Feces[3]	10.07	17.15	15.11	10.89	5.71
	Urine[3]	5.56	0.90	0.93	1.34	7.89
	Total	96.54	102.3	99.6	94.7	92.9
16	Blood	0.10	—	—	—	—
	G.I.Tract	0.90	—	—	—	—
	Heart	0.08	—	—	—	—
	Kidneys	1.60	0.67	0.74	0.79	1.14
	Liver	12.60	7.04	26.14	4.16	3.37
	Skeleton	34.67	42.28	30.88	60.30	58.50
	Lungs	0.18	—	—	—	—
	Spleen	0.08	0.96	1.40	0.60	0.48
	Testes	0.04	—	—	—	—
	Skin	0.99	—	—	—	—
	Balance[2]	4.88	11.95	7.28	10.15	7.58
	Feces[3]	27.55	37.61	26.03	20.83	12.91
	Urine[3]	8.05	1.35	1.69	2.15	9.18
	Total	91.72	101.8	94.2	99.0	93.2

Table 3 Continued

Days After Injection	Tissue or Excrement	Av. Per Cent of Injected Dose[1]				
		$AmCl_3$	$PuCl_3$	$Pu(NO_3)_4$	Pu^{+4} Cit.	$PuO_2(NO_3)_2$
32	Blood	0.03	--	--	--	--
	G.I.Tract	0.07	--	--	--	--
	Heart	0.09	--	--	--	--
	Kidneys	1.67	0.50	--	--	0.73
	Liver	20.93	5.38	--	--	2.78
	Skeleton	31.32	51.81	--	--	56.84
	Lung	0.33	--	--	--	--
	Spleen	0.10	0.73	--	--	0.33
	Testes	0.06	--	--	--	--
	Skin	0.85	--	--	--	--
	Balance[2]	3.07	7.86	--	--	9.46
	Feces[3]	24.72	33.86	--	--	20.54
	Urine[3]	7.77	1.20	--	--	9.30
	Total	91.64	101.3	--	--	99.9
48	Blood	0.01	--	--	--	--
	G.I.Tract	1.14	--	--	--	--
	Heart	0.12	--	--	--	--
	Kidneys	2.48	--	0.43	0.36	--
	Liver	11.94	--	21.84	2.70	--
	Skeleton	45.46	--	31.88	60.45	--
	Lungs	0.50	--	--	--	--
	Spleen	0.45	--	1.43	0.57	--
	Testes	0.08	--	--	--	--
	Skin	0.92	--	--	--	--
	Balance[2]	4.07	--	5.21	7.85	--
	Feces[3]	20.55	--	39.65	29.68	--
	Urine[3]	9.91	--	2.15	2.85	--
	Total	97.63	--	102.6	104.4	--

[1] Each result represents an average value for 3 animals. All plutonium results are from an earlier paper from this laboratory (4).

[2] Balance in plutonium experiment included blood, G.I. tract, heart, lungs, testes and skin.

[3] Fecal and urinary excretion results are for specific groups of animals and not for the over-all averages shown in Figs. 1 and 2 and Tables 1 and 2.

Table 4.--Effect of Zirconium Citrate[1] on Average Excretion of Americium[2] Administered Intravenously to the Rat

Days After Injection	Urine, % Dose[3]		Feces, % Dose[3]		Urine + Feces, % Dose[3]	
	Am	Am + Zr	Am	Am + Zr	Am	Am + Zr
1	4.88	34.24	2.02	3.47	6.90	37.71
2	0.34	0.24	2.21	1.57	2.55	1.81
3	0.23	0.15	1.73	0.98	1.96	1.13
4	0.12	0.11	1.40	0.97	1.52	1.08
5	0.13	0.07	1.67	1.02	1.80	1.09
6	0.15	0.16	1.45	0.75	1.60	0.91
7	0.14	0.10	1.77	0.83	1.91	0.93
8	0.14	0.10	1.77	0.83	1.91	0.93
9	0.13	0.08	1.18	0.50	1.31	0.58
10	0.09	0.07	1.02	0.42	1.11	0.49
11	0.10	0.09	1.11	0.63	1.21	0.72
12	0.08	0.06	0.82	0.39	0.90	0.45
13	0.09	0.10	0.68	0.28	0.77	0.38
14	0.09	0.08	0.70	0.19	0.79	0.27
15	0.10	0.08	0.61	0.22	0.71	0.30
16	0.09	0.05	0.40	0.16	0.49	0.21
Total	6.9	35.7	20.6	13.2	27.4	48.9

[1] Single injection of 40 mg. Zr/rat as zirconium citrate, 15 min. after injection of americium.

[2] Injected with 0.03 μc. of Am/g. body wt. as $AmCl_3$.

[3] Results are averages for 6 animals through the fourth day and three animals from 4-16 days after injection.

Table 5.--Effect of Zirconium[1] Citrate on the Body Deposition of Americium[2] Administered Intravenously to the Rat

Tissue	4 Days After Injection Per Cent of Dose per Tissue		16 Days After Injection Per Cent of Dose per Tissue	
	Am	Am + Zr	Am	Am + Zr
Blood	0.11	0.12	0.10	0.10
G.I. Tract	2.73	2.34	0.90	1.98
Heart	0.12	0.07	0.08	0.08
Kidneys	2.73	1.12	1.60	0.87
Liver	38.32	17.85	12.60	11.77
Lungs	0.31	0.23	0.18	0.25
Remains	3.88	5.67	4.88	5.78
Skeleton	30.73	22.49	34.67	20.89
Skin	1.64	4.57	0.99	2.11
Spleen	0.26	0.22	0.08	0.13
Testes	0.08	0.07	0.04	0.06
Total	80.91	54.75	56.12	44.02
Feces[3]	10.07	6.45	27.55	18.87
Urine[3]	5.67	34.81	8.05	36.19
Total	15.74	41.26	35.60	55.06
Grand Total	96.65	96.01	91.72	99.08

[1] Single injection of 40 mg. Zr/rat as zirconium citrate, 15 min. after injection of Am.

[2] Injected with 0.03 μc./g. body wt. of Am as $AmCl_3$. All results represent averages of three animals.

[3] Urinary and fecal results are for the specific groups of animals and not for the over-all averages given in Figs. 1 and 2 and Table 1.

Table 6.--Relative Acute Toxicity of Americium and Plutonium Administered Intravenously to Rats[1]

Material	Group No.	No. Animals per Group	μc./g. of Body Wt.	No. Deaths at 30 Days	% Mortality at 30 Days
Am	I	20	0.032	0	0
	II	20	0.041	3	15
	III	20	0.050	3	15
	IV	20	0.063	3	15
	V	20	0.095	10	50
	VI	20	0.126	14	70
	VII	20	0.189	17	85
Pu	I	20	0.032	1	5
	II	20	0.041	3	15
	III	20	0.050	2	10
	IV	20	0.063	10	50
	V	20	0.095	16	80

[1] Americium was administered as $AmCl_3$; the plutonium was injected as Pu^{+4} citrate complex.

Table 7.--Incidence and Location of Osteogenic Sarcomas in the Rat Following Intravenous Injection of Americium and Plutonium

Element Injected	Dose μc./g.	Survival Time Post Injection (days)	Tumor Location
Pu	0.063	300	pelvis
	0.032	308	right tibia
	0.050	351	right femur
	0.032	351	left tibia
Am	0.032	289	left humerus
	0.050	289	left tibia
	0.063	290	right femur
	0.050	291	right femur or tibia
	0.063	309	upper thoracic spine
	0.032	360	pelvis

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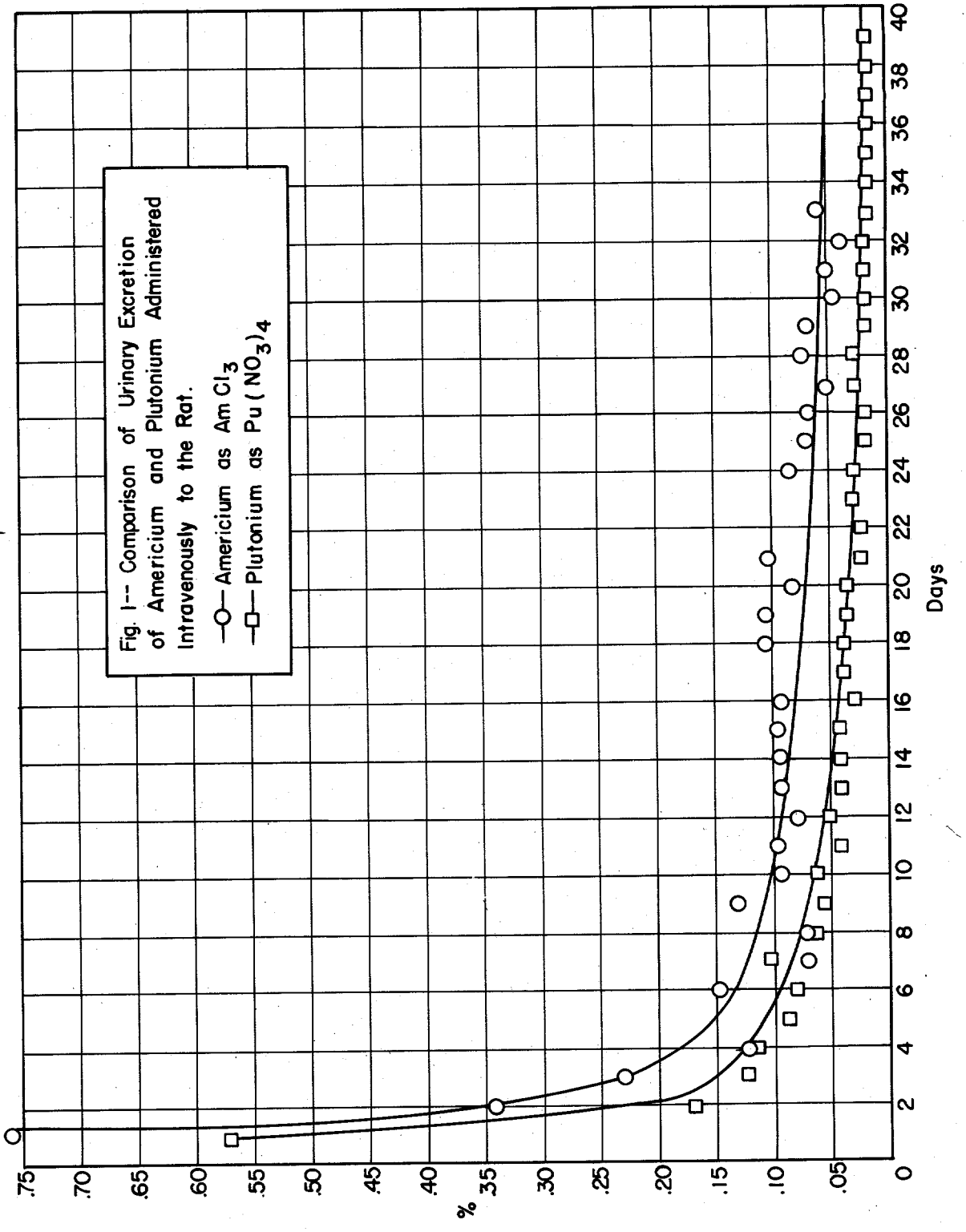

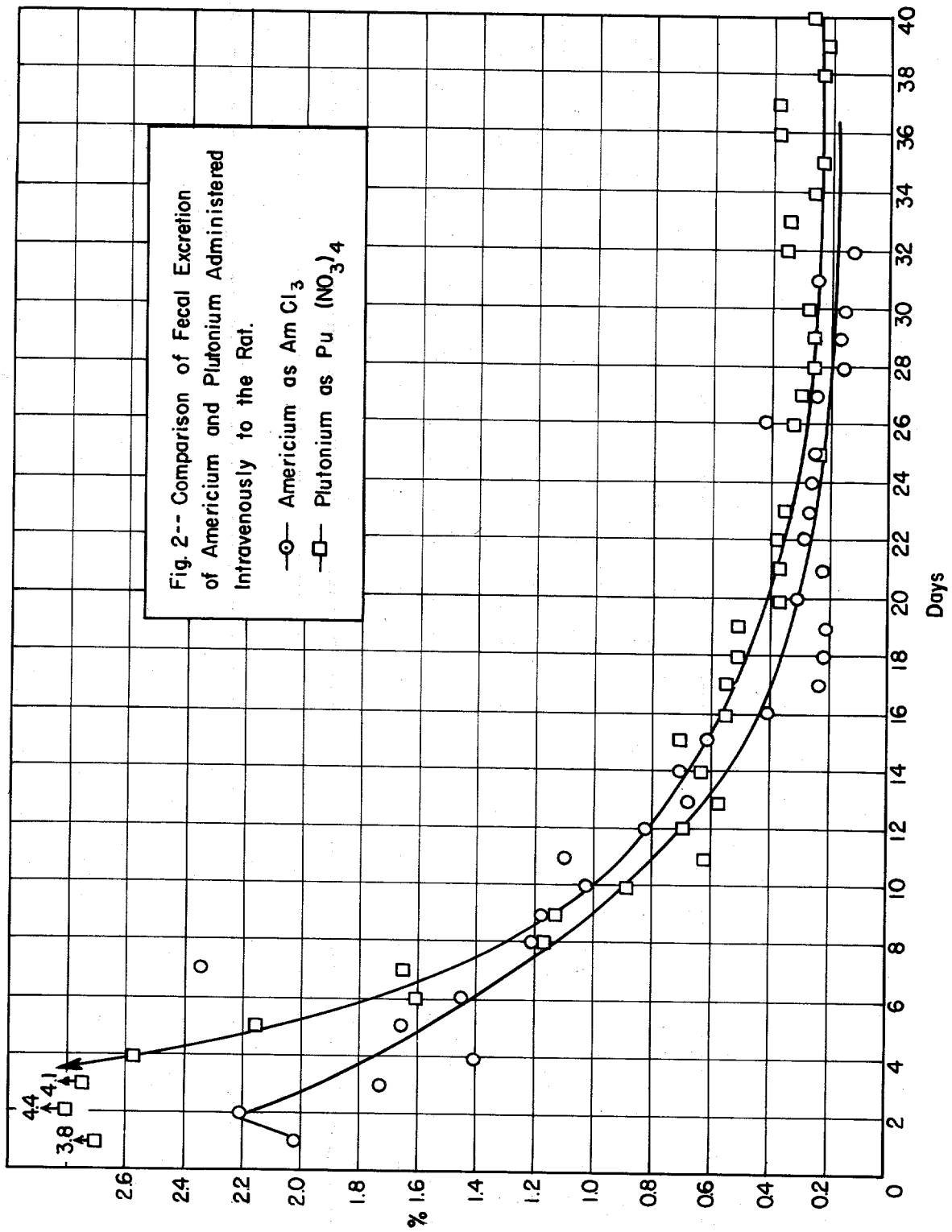
Fig. 2-- Comparison of Fecal Excretion of Americium and Plutonium Administered Intravenously to the Rat.
Americium as Am Cl3
Plutonium as Pu (NO3)4
% 1.6
Days
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
4.4
4.1
3.8

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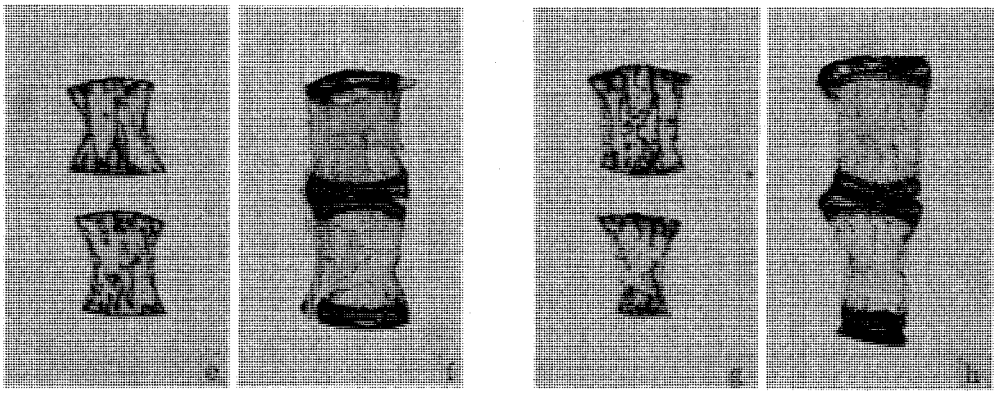
Fig. 3. -- Survey Type Radioautographs Showing Mode of Deposition of Americium and Plutonium in Bone of the Rat.

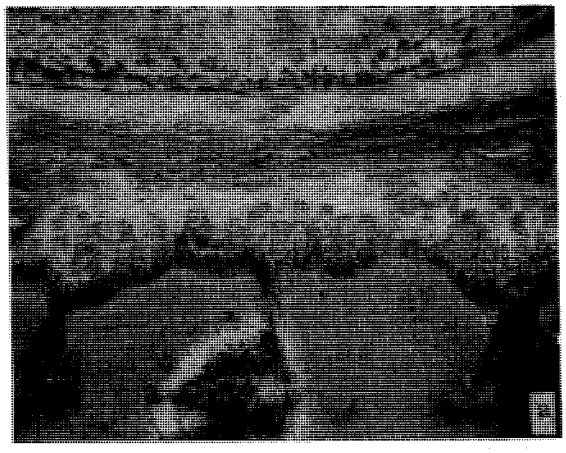
Am -----TIBIA ----- Pu



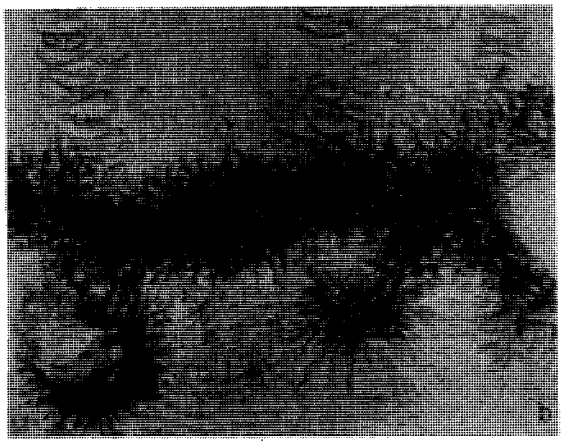
Am ----- VERTEBRA ----- Pu

a and c are autoradiographs of tibia corresponding to photomicrographs b and d respectively.
e and g autoradiographs of vertebra corresponding to photomicrographs f and h respectively.

Fig. 4. -- Detail Autoradiograph Showing Mode of Deposition of Americium and Plutonium in the Bone of the Rat.



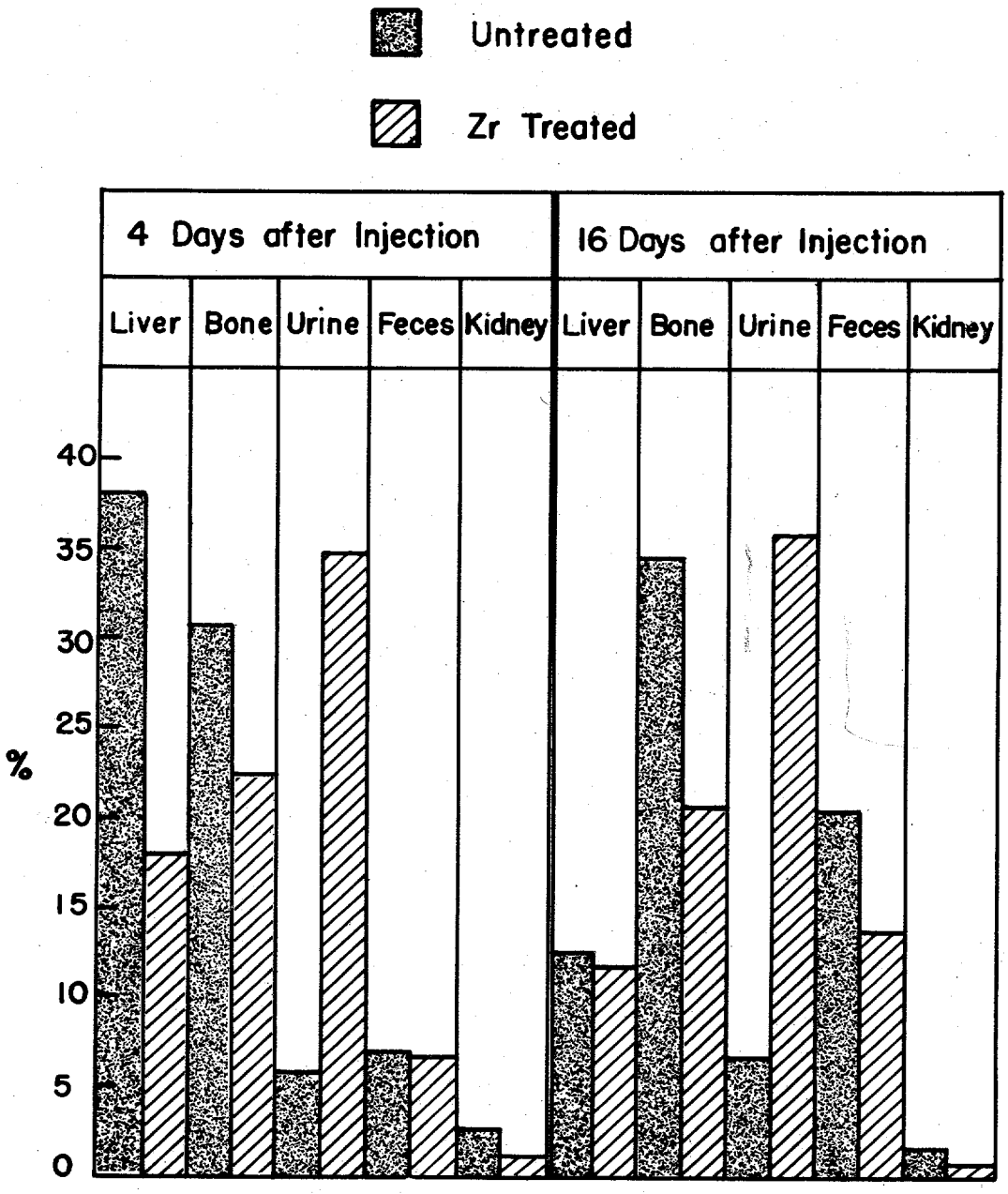
Am in Vertebra
x 120



Am in Tibia
x 480

Pu in Tibia
x 240

Fig. 5 Effect of a Single Injection of Zirconium Citrate on Tissue Deposition of Americium Administered intravenously to the Rat.



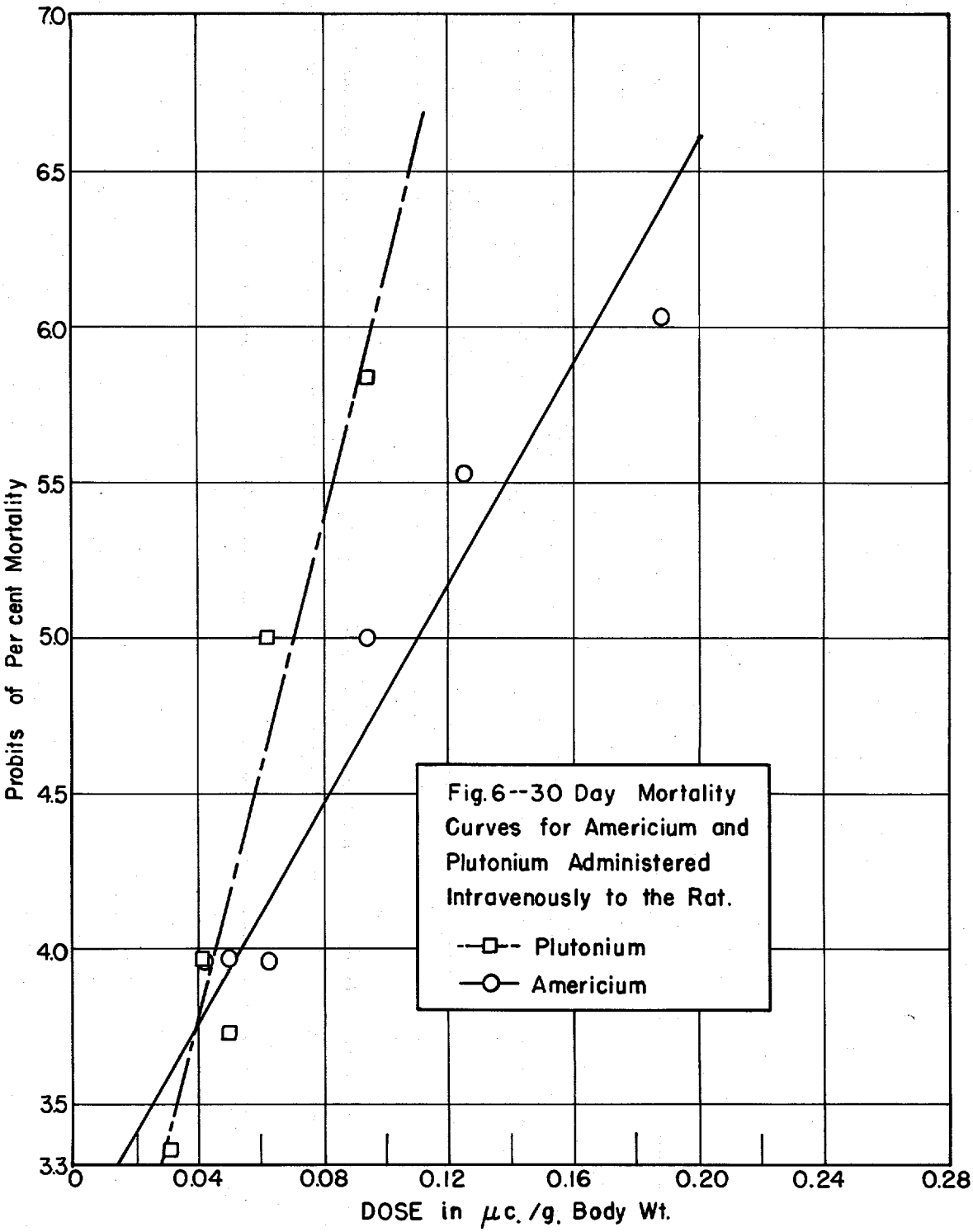


FIGURE 7

COMPARISON OF THE EFFECT OF INTRAVENOUSLY ADMINISTERED AMERICIUM AND PLUTONIUM ON THE RED BLOOD CELL COUNT OF THE RAT

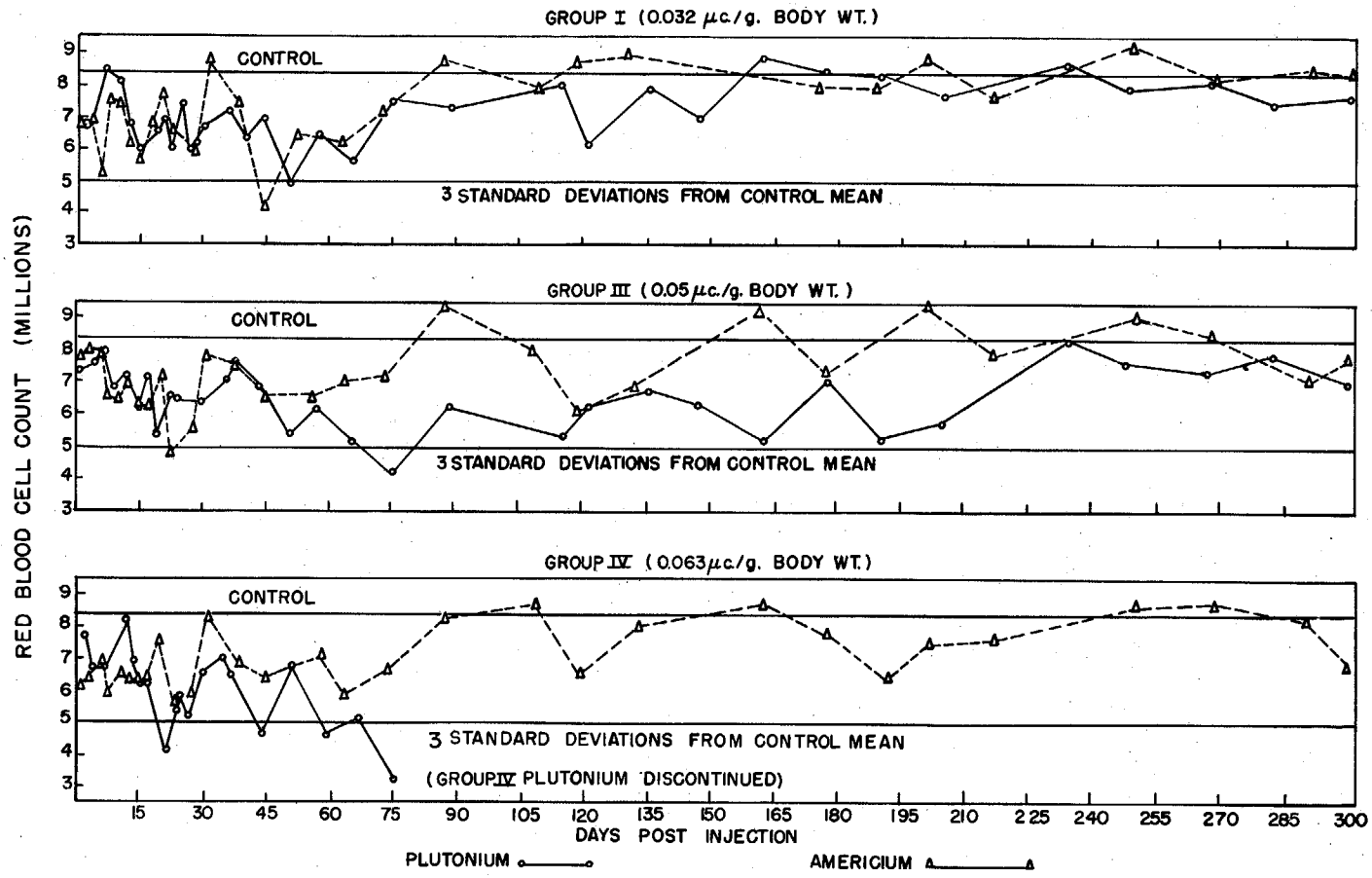
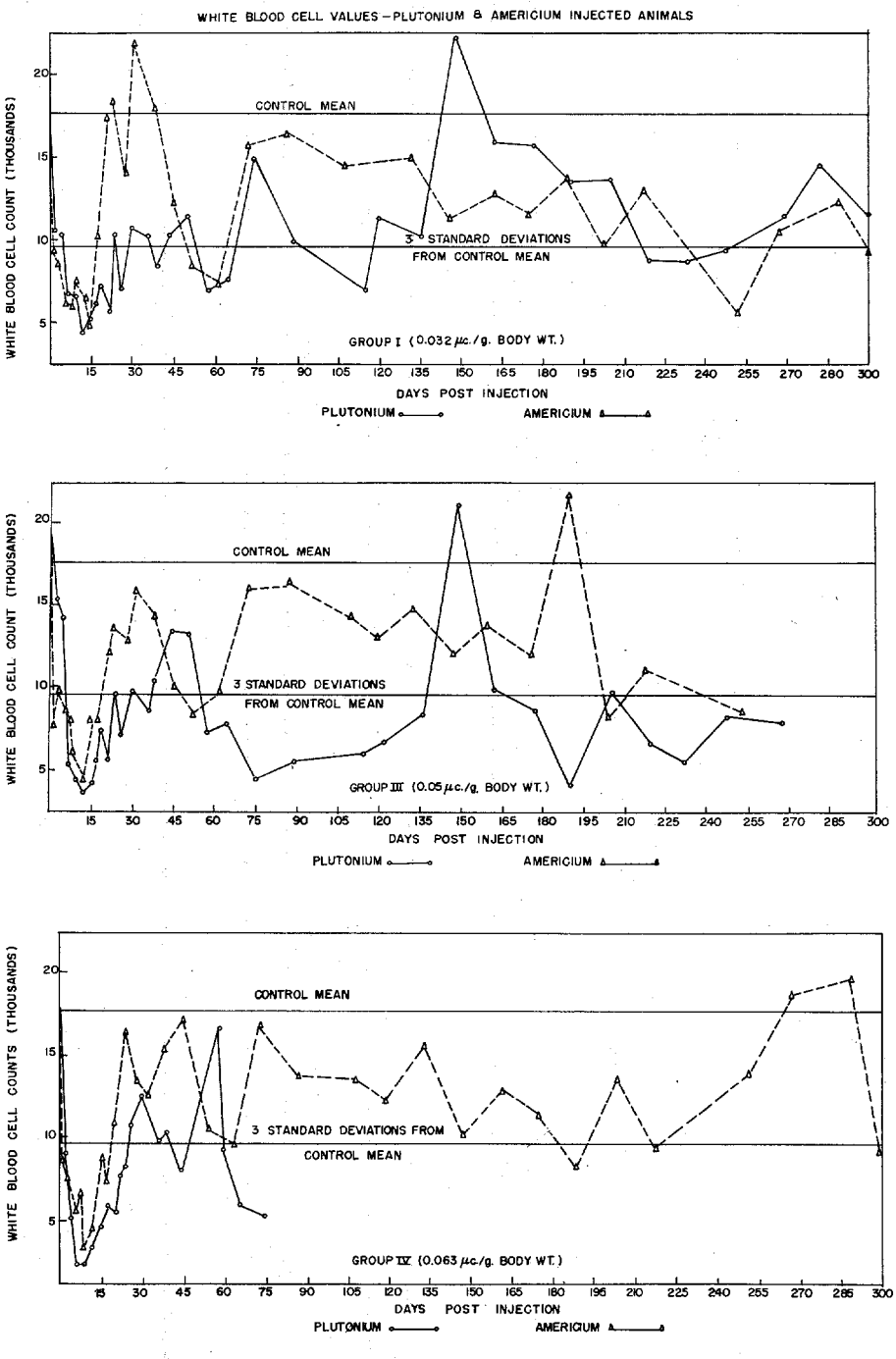


FIGURE 8

COMPARISON OF THE EFFECT OF INTRAVENOUSLY ADMINISTERED AMERICIUM AND PLUTONIUM ON THE WHITE BLOOD CELL COUNTS OF RATS



56

Fig. 10. -- Effect of Intravenously Administered Americium and Plutonium on Skeletal Development of the Rat.

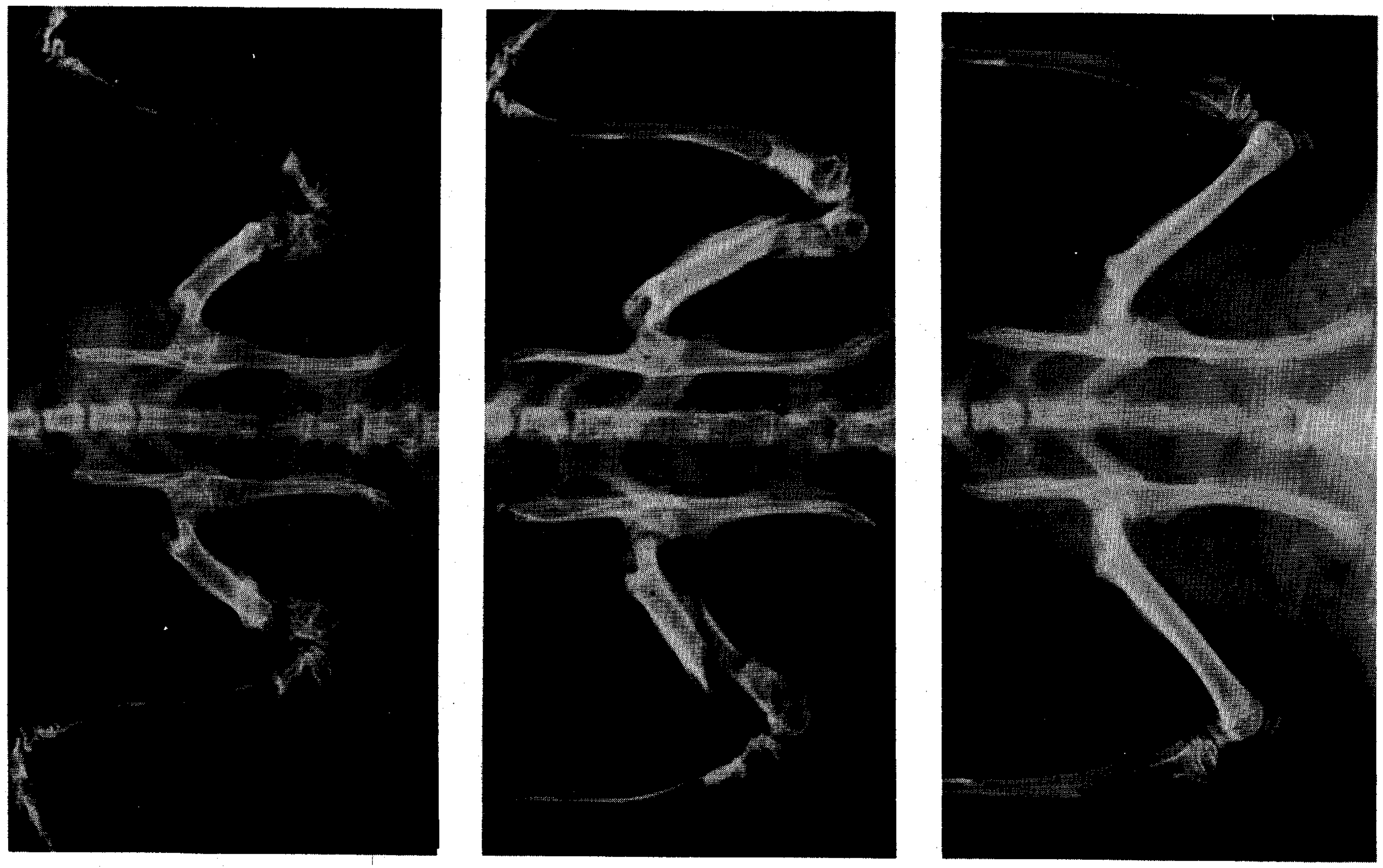
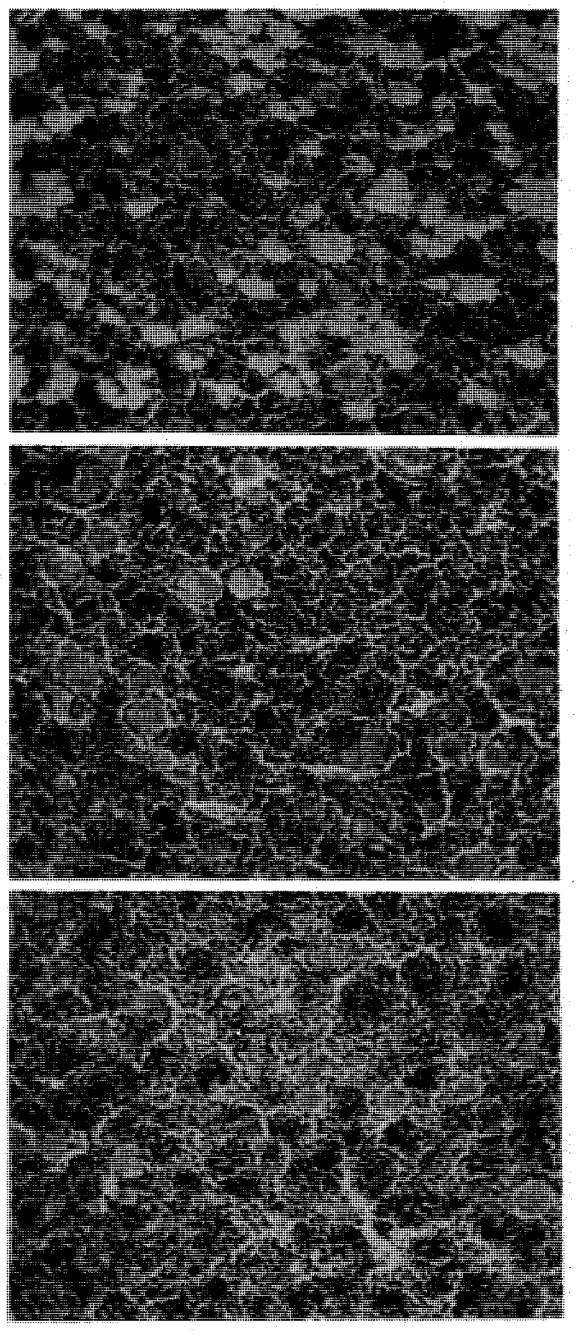


Fig. 9. -- The Effect of Intravenously Administered Americium and Plutonium on the Bone Marrow of the Rat.



Normal

Americium

Plutonium

Fig. II. -- Radiograph Showing Osteo Sarcoma in a Rat 289 Days After Intravenous Injection of 0.032 μc. Am/g. Body Wt.

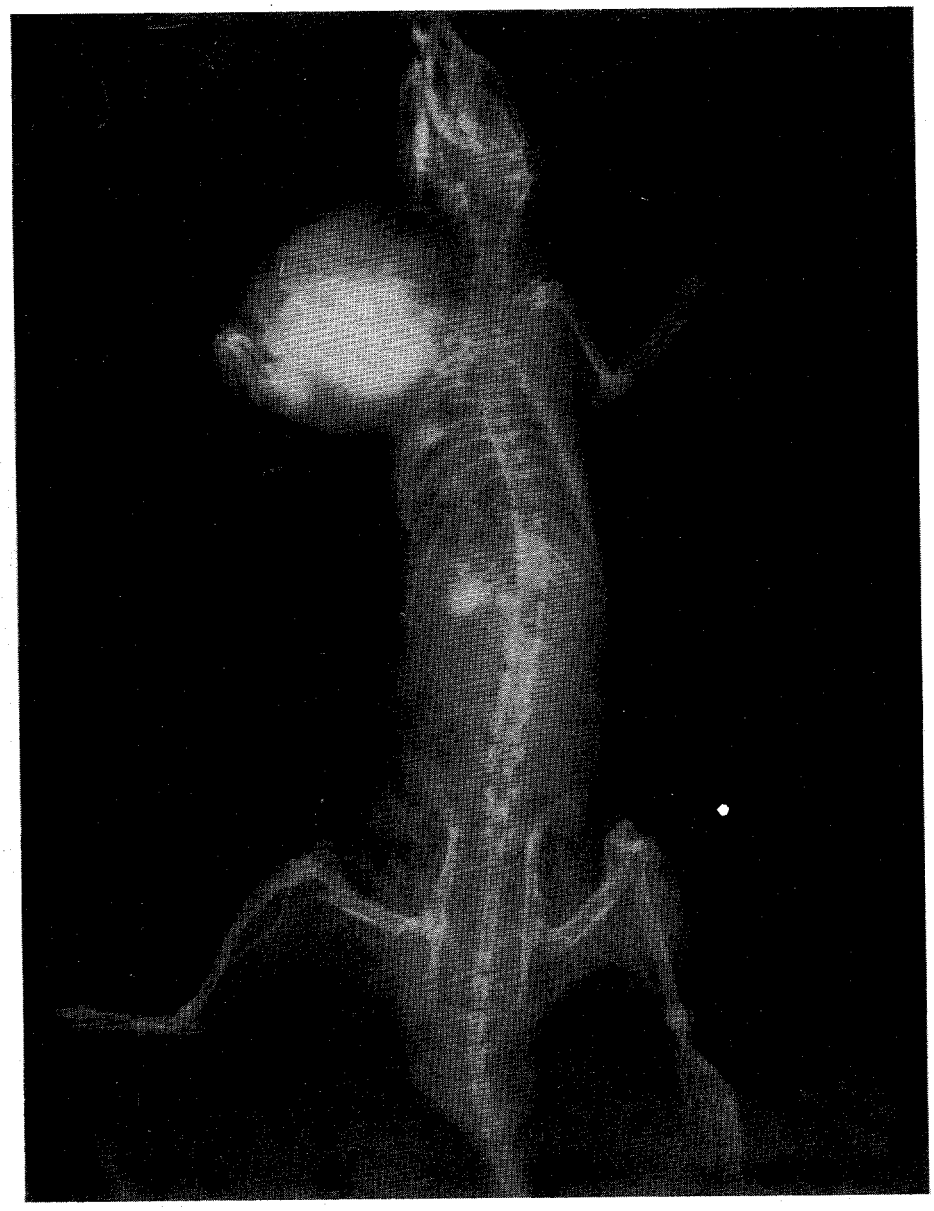
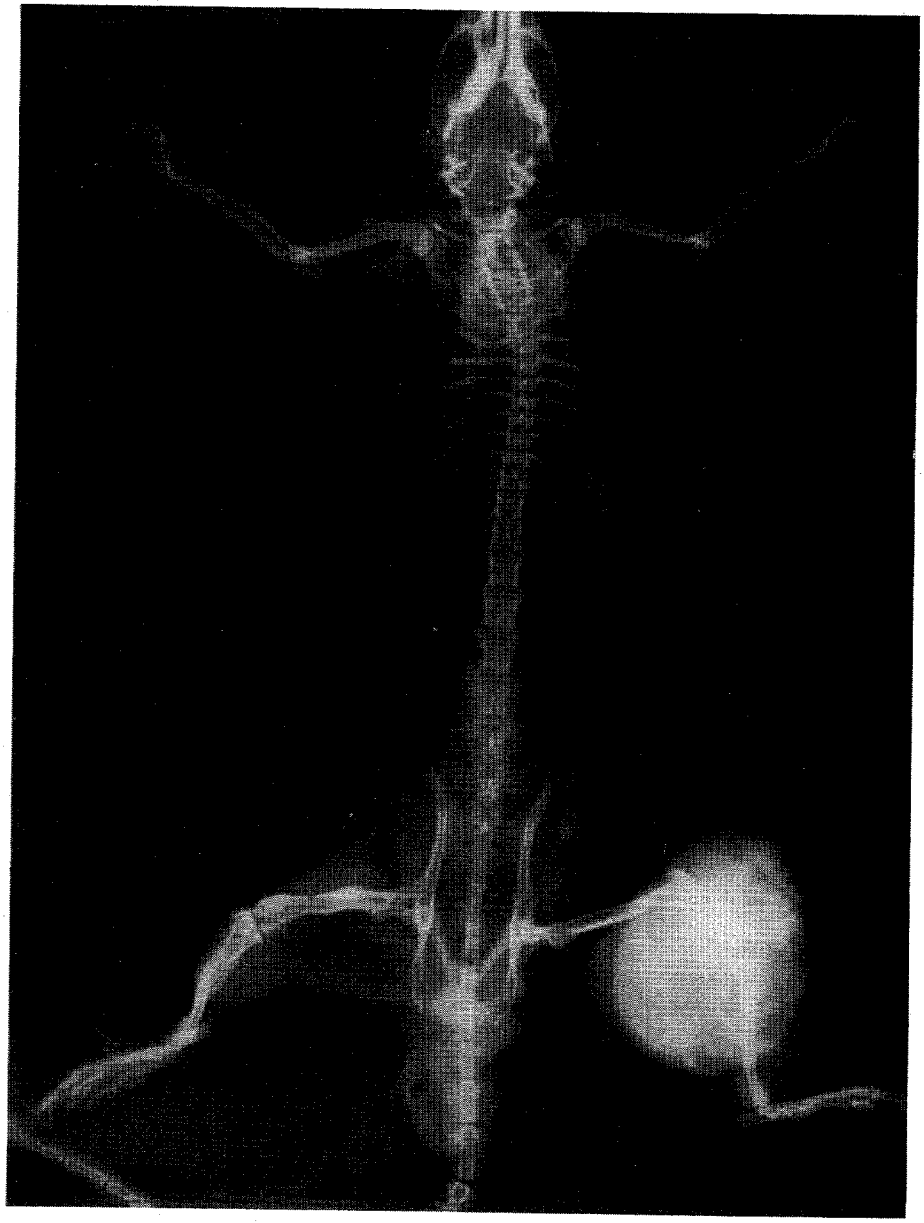
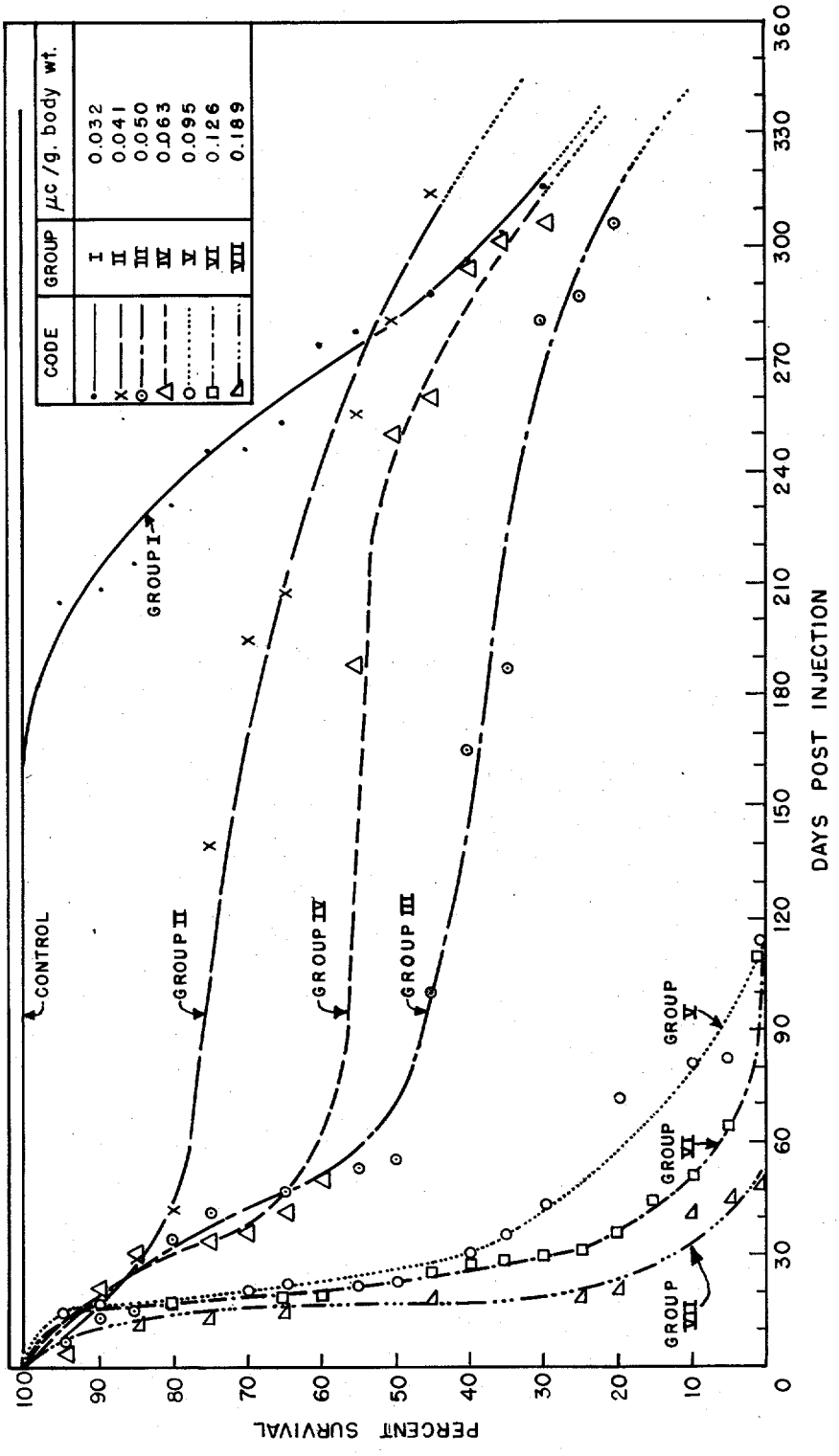


Fig. I2.-- Radiograph Showing Osteo Sarcoma in a Rat 35I Days After Intravenous Injection of 0.032 µc. Pu/g. Body Wt.



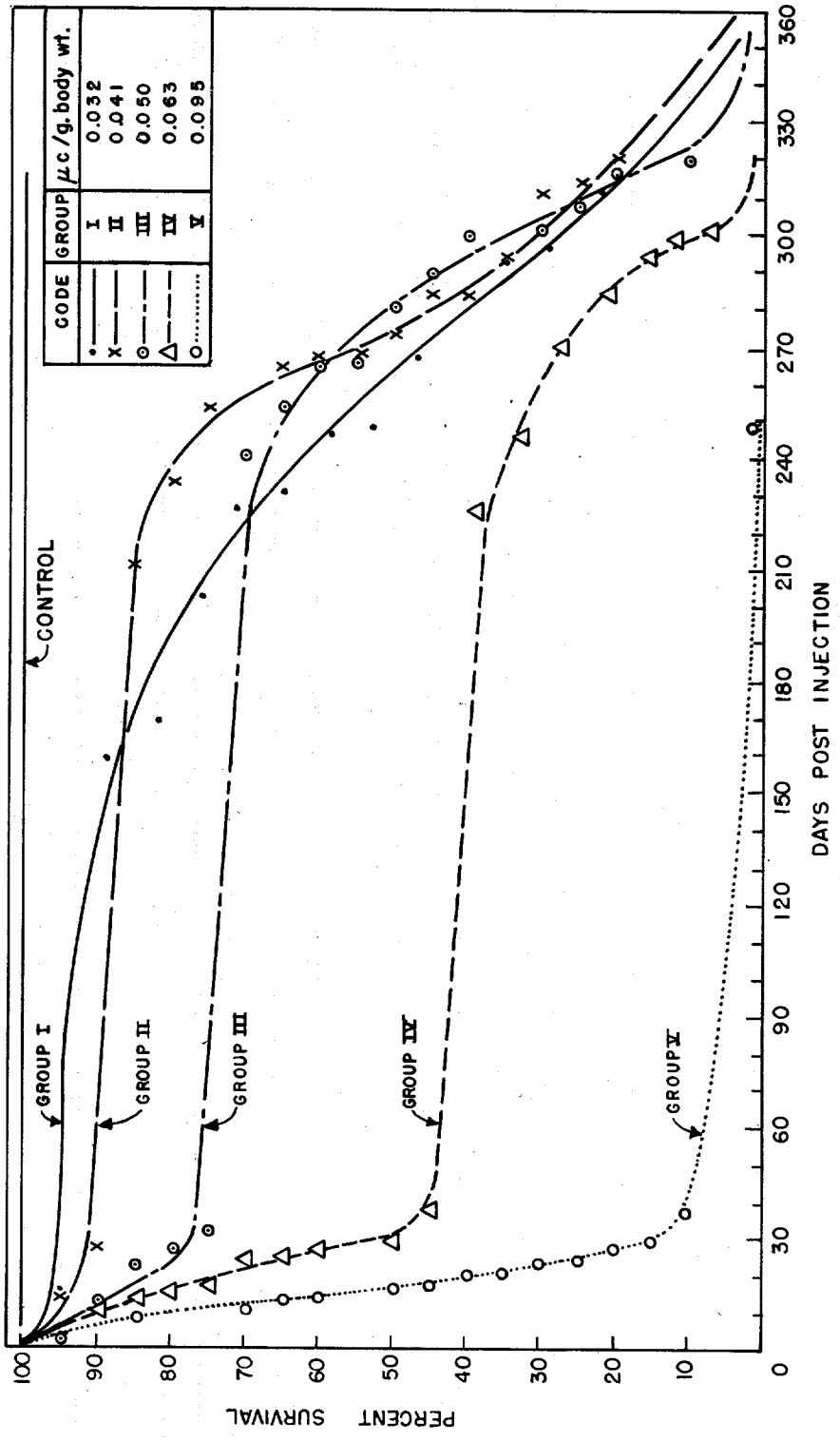
59

Fig. 13-- SURVIVAL OF RATS INJECTED INTRAVENOUSLY WITH AMERICIUM



60

Fig. 14 -- SURVIVAL OF RATS INJECTED INTRAVENOUSLY WITH PLUTONIUM



673 053

Fig. I5 -- Effect of Intravenous Administration of 0.063 μc. of Am and Pu/g. Body Wt. on General Health of the Rat (About 300 Days after Injection)