Age Difference in Deposition of Plutonium in Organs of Rats and the Estimation of Distribution in Humans

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INTRODUCTION

In almost all pervious studies, body weight has been considered to be a convenienced indicator for estimating the intake of radionuclides in humans and the amount of deposition in experimental animals. Therefore, a fixed amount of plutonium per g of body weight has usually been administered to young or young adult animals in experiments. It is likely that there is an increase in the weights of various organs in proportion to body weight gain and greater radiation sensitivity in young animals and humans than in adults or the aged. However, in radiation accidents, workers might be exposed to the same amounts of plutonium without regard to age, sex, or body weight. In addition, the morphological characteristics and physiological function, and weight of bones are altered with age. Our question is whether plutonium deposits constantly in any organs in proportion to body weight in humans and animals regardless of age. To investigate this issue, two dose modalities of plutonium were tried out; one was a fixed amount of plutonium administered to all rats without regard to age, sex, or body weight, and the other was a fixed amount of plutonium per g of body weight.

A matter of interest is how the results obtained in animal experiments can be extrapolated to humans in order to estimate the intakes of plutonium in humans. Plutonium has been administered to animals of various ages in previous studies (*1-6*). These results, however, have not always been useful in estimating the deposition or distribution of plutonium in humans (*6-10*). Problems have arisen because the studies have not established the correspondence in age between animals and humans based on evidences such as changes in physiological function and morphological characteristics. Plutonium is a well-known bone surface-seeking radionuclide (*7, 11-15*). ICRP publication 70 includes various data on bones such as bone histomorphometric values in humans that can be used to estimate plutonium deposition (*16*). In addition, many studies have provided data on bone metabolism in humans to compare with that in animals (*17-22*).

The purpose of the present study was first to examine whether the plutonium deposition in organs depends on the body and organ weights in animals or the physiological and morphological alterations in organs that occur with age, and second to estimate age differences in plutonium deposition in humans based on a comparison of species differences in bone metabolism between rats and humans.

MATERIALS AND METHODS

Preparation of plutonium solution :Plutonium-239 as a working solution was prepared from the stock solution at our institute. Just before the injection, the plutonium solution for injection was dissolved in HNO3, NaOH, and sodium citrate solutions to adjust the concentrations and to achieve a pH of 6.8-7.0.

Animals and procedures :Male and female Wistar rats of three ages (3, 12 and 24 month), were allocated to experiments I, II, and III, respectively. They were kept in groups of five in a stainless steel cages, fed on a standardized diet, and given water ad libitum in an air-conditioned room at a temperature of 21± 2 ^{O}C, relative humidity 55 ± 5%, with 12-h light/dark cycles before and during the experiment.

The experiment I was performed to examine the distribution of plutonium when rats of all ages were injected with a fixed amount of plutonium. Fifteen male and 15 female rats of each age (n=5) were injected intraperitoneally with plutonium of 8662Bq (Table 1). In experiment II was performed to examine the distribution of plutonium in organs when rats received an injection of a fixed amount of plutonium per g of body weight of rat. Twenty seven male and 30 female rats of the same ages as those in experiment I (n=9 or 10), were injected intraperitoneally with the plutonium at 1.85 x 10^4 Bq/kg of body weight. All rats were killed 2 weeks after the plutonium injection, the whole skeleton in experiment I and the femur in experiment II were collected.

Table 1 Body weights of rats and doses of plutonium in experiments I and II

Items	Male			Female		
	3 months	12 months	24 months	3 months	12 months	24 months
Experiment I						
No. of rats	5	5	5	5	5	5
Body weight (g) (mean ± SE)	263.4±5.3	395.6±10.8	414.7±4.9	165.1±6.1	233.9±11.4	302.5±11.9
Injected dose of Pu (Bq/rat)	8662	8662	8662	8662	8662	8662
Calculated dose equivalent to body weight (Bq/kg)	32900	21900	20900	52000	37000	29000
Experiment II						
No. of rats	9	9	9	10	10	10
Body weight (g) (mean±SE)	285.7±4.9	382.5±6.8	394.3±12.7	169.1±3.6	225.7±5.7	281.9±8.4
Injected dose of Pu ($x10^5$ Bq/kg)	1.85	1.85	1.85	1.85	1.85	1.85
Calculated total injected dose (Bq/rat)	52900	70800	72900	31300	41800	52200

In experiment III was performed to obtain bone data by a bone histomorphometry and a quantitative computed tomography method. Fifteen males and 15 females the same ages as those in the experiments I and II (n=5) were injected with tetracycline (25 mg/kg) and calcein (8 mg/kg) at an interval of 7 days before the sacrifices, for bone labeling to analyze bone dynamics by histomorphometry. The rats were killed 7 days after the injection of calcein.

Analysis of Plutonium activity: The bones were weighed, and then incinerated at 700 ^{O}C for 24 h in a crucible. The ash weight was measured as a bone weight. A 2-ml portion of a mixture containing nitric acid, distilled water, and hydrofluoric acid at a ratio of 150:100:0.9 was added to the crucible, and the solution was subsequently pipetted into a counting vial. The alpha activity of the plutonium in the vial was measured by an alpha liquid scintillation counter.

Bone histomorphometry and measurement of BMD :The BMD in the proximal metaphysis of tibia obtained from experiment III was measured by peripheral quantitative computed tomography (pQCT, Norland & Stratec XCT-960A, Germany) at the same position as that measured by histomorphometry, with this position being the secondary spongiosa including abundant trabecular bone at about 3 mm from the epiphysial plate.

The tibia was immersed in Villanueva's bone stain and embedded in methylmethacrylate (*24*). The block was cut thin by an inner blade cutter (Maruto Co., Tokyo) and was then ground to a thickness of about 20 μm by a grinding machine (Maruto Co., Tokyo) to create undecalcified bone specimens. Bone histomorphometry in the secondary spongiosa of the tibial proximal metaphysis was performed using a semi-automatic image analyzer (Carl Zeiss, Inc, Germany) with the software "Osteoplan II$_{©}$ under fluorescent microscopy. The histomorphometric values such as the bone volume (/ tissue volume), bone surface (/tissue volume), mineral apposition rate, and bone formation rate (/bone volume) were used in this study.

RESULTS

Based on the data obtained from experiments 1, II, and III, the analyses were performed. Figure 1 shows a strong correlation between body weight and skeletal weight for all ages in both sexes. The skeletal weights increased with age (Fig.2). Plutonium activity in the skeleton increased from 6 to 12 months and then decreased (Fig. 3). Bone surface (Fig. 4), bone volume (Fig. 5) and bone mineral density (Fig. 6) appeared the similar pattern as that in plutonium activity (Fig3), respectively. The total activity, weight, and activity per g of weight in the femur were shown in Table 2 in experiment II.

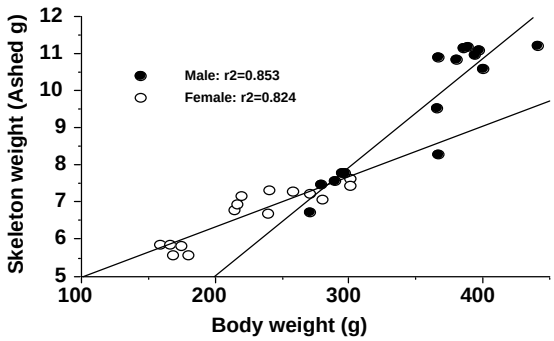
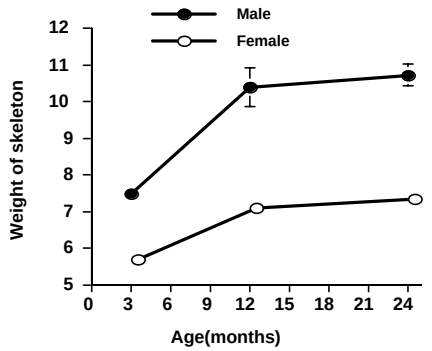


Fig.1 Relationship between body weights and skeleton weights in male and female rats at various ages.

Table 2 Total activity of plutonium deposited, weights and the activity per g of weight of femur in experiment II

	Male			Female		
Organ	3 m	1 2 m	24 m	3 m	12 m	24 m
Total activity	811.0±69.4	1385.3±90.6*	818.0±187.0	806.5±45.4	1053.5±38.5*	925.1±67.5
Weight (g)	0.800±0011	1.073±0.009*	1.079±0.016*	0.586±0.005	0.669±0.008*	0.724±0.016*
Activity (Bq/g)	1013.4±85.5	1295.5±93.2*	779.9±150.7	1426.7±69.4	1587.8±48.5	1279.7±92.3

Significantly different from the value at 3 months; *P<0.05*



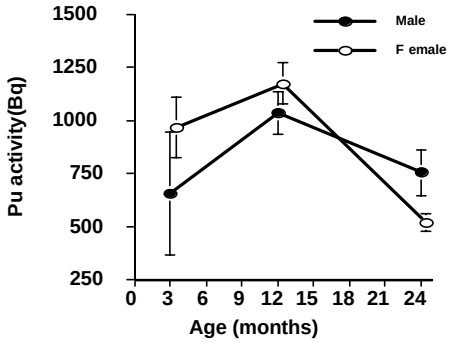
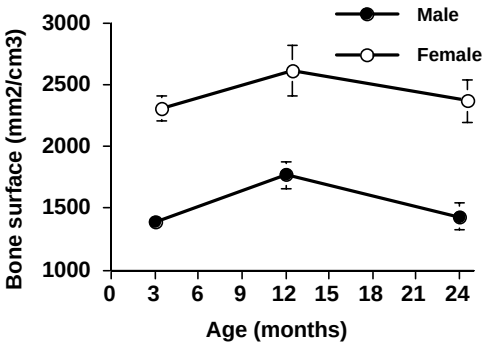


Fig 2 The weight of whole skeleton in male and female rats at the different ages(left).
Fig.3 Plutonium activity in skeleton in male and female rats (right).



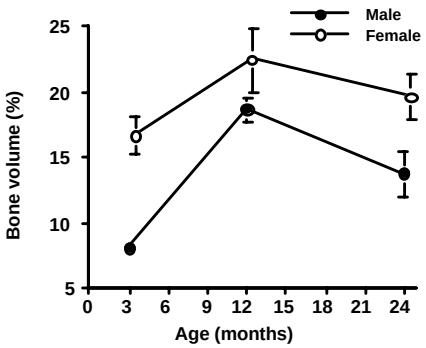


Fig.4 Bone surface of trabecular bone in male and female rats (left)
Fig.5 Bone volume in male and female rats (right)

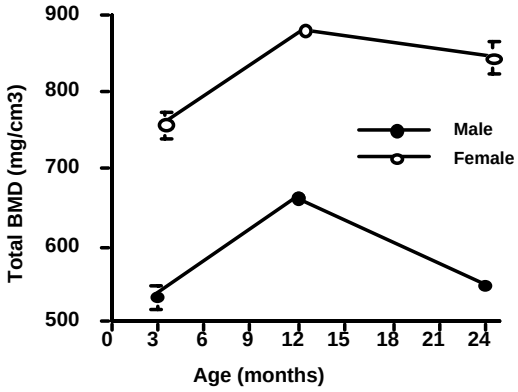


Fig.6 Total bone mineral density in male and female rats.

DISCUSSION

The plutonium activity in the skeleton increased from 3 to 12 months, reaching a peak at 12 months and then decreasing at 24 months in both sexes, although there was a strong correlation found between body weight and skeletal weight (Fig. 1), and also the weight of skeleton increased with age (Fig.3). In addition, in experiment II, the similar patterns were observed in the femur as those in experiment I (Table 2). The ratio of the injected dose to the body weight was different in experiments I and II. The results indicate that there was no relation in the examined items between experiments I and II. These findings indicate that, if workers are exposed to a fixed amount of plutonium without regard to age, sex, or body weight, the amount of plutonium deposited in the bones may be different from the results obtained when a fixed amount of plutonium per g of body weight is administered. By all accounts, these findings indicate that plutonium is deposited in the bones regardless of either bone or body weight at any age. Therefore, it might be necessary to reexamine previous data obtained from studies in which an amount of plutonium per g of body weight was administered, in order to determine whether the animal data can be utilized to estimate the amount of plutonium deposited in the body in the accident case in which workers of various ages, body weights, and sexes exposed to a fixed amount of plutonium. In fact, the comparisons of data on plutonium deposition between humans and animals has not always succeeded (*1-2, 4-6, 8, 10, 12, 24-28*), probably because the characteristics of age or sex in humans might not correspond to those in the animals used in the experiment based on physiological changes such as bone metabolism or other some factors to match ages in animal species and humans. Therefore, in this study, detailed analyses were performed to solve these problems by using the bone data obtained from experiment III and the data gathered from previous studies.

Plutonium is a well-known bone surface-seeking radionuclide (*1, 5, 7, 11, 14 and 29*). The incidence of osteosarcoma in trabecular bone is higher (six times) than that in cortical bones (*7*), as the total bone surface area of trabecular bone is 1.6 times larger and the surface-to-volume ratio is 6 times larger than that in the cortical bone in humans (*16*). A strong relationship was observed between plutonium activity in the skeleton and the bone surface, bone volume, and BMD (Figs. 4-6), with the exception of the bone surface in the male. These results appear to be due to plutonium being deposited only by adsorption on bone mineral surfaces regardless of the bone marrow and some organic functions (*30-32*). The bone turnover suggested by the mineral apposition and bone formation rates was also not related to the deposition of plutonium, at least in the early period after the exposure. In short, the amount of plutonium deposited in bones should be estimated based on changes in the bone surface, bone volume, or BMD with age in humans.

Generally, plutonium has been administered to young animals in almost all previous studies. It is likely that our fundamental assumptions regarding plutonium toxicity are due to the radio-sensitivity of bone cells in the young being higher than those in the old or aged. However, it has been observed that more plutonium may be deposited in the bones of adult dogs than in those of young dogs in the early period after exposure (*5*). Bruenger et al. (*1-2*) have demonstrated that the high incidence of osteosarcoma and the shortening of life span are more often observed in mature beagle dogs than in juveniles and young dogs when a fixed amount of plutonium per g of body weight is administered at different ages. This phenomenon may be due to the net bone volume as well as the bone surface being greater in adult beagle dogs than in young dogs (*33-34*). That is, the important factors in inducing the toxicity of plutonium may be the size factors such as the bone surface area and bone volume rather than the radio-sensitivity of bone cells. In addition, the amount of plutonium deposited in the bones might be more closely related to the induction of osteosarcoma than the radio-sensitivity of the bone cells, particularly in the mature or aged. Because the bone cells in the mature or aged may possibly receive more radiation over a longer period than those in the young due to the continuous presence of plutonium on the bone surface, even though the radio-sensitivity of the bone cells is lower.

The problem is how to extrapolate the data obtained from the animal experiments so that it can be applied to humans. The explanations presented in previous reports have been unconvincing (*8-9*), probably due to there being no grounds for comparing the results obtained from the animals experiments and the analysis of plutonium in humans (*25-26*). It is our interest to establish an age correspondence between rats and humans based on age-related changes in the bone histomorphometric values and BMD, although there have been only limited reports of related data in rats and humans. ICRP publication 70 includes a great deal of information on bones in humans (*16*). However, the gathered data are not always sufficient for estimating plutonium deposition in the skeleton, because the bone histomorphometric values are lack in the aged, e.g., differences in sexes, only a few sites of skeleton. In ICRP Publication 70, the data do not show differences in the bone surface area in relation to age for various bone sites such as the lumbar vertebra, femur and iliac crest, although there are variations. The bone volume and mean trabecular width increase up to 44 years old, while at the same time the mean cavity width (the trabecular separation probably appears as the distance between the trabecular bones in the bone histomorphometry) also increases with age. As the bone-values were measured as the percentage occupied in a limited measured area, including the bone marrow, seen under a microscope and the bone surface is presented as the ratio of bone volume in the bone histomorphometry, the net values of bone volume and

surface area in children, particularly before the puberty, might be lower than those in adults, judging from the changes in the bone volume and BMD with age. On the other hand, the bone volume, bone surface and BMD in rats increases to and reaches its highest point at 12 months of age, then decreases throughout life, as was shown in experiment III and has also been observed in a previous study (*17*). The bone formation rate decreases to a mean at 35 years (30-39 years) in humans (*16*), and that in male and female rats decreases a mean at 9 months. These findings indicate that bone turnover decreases before reaching a peak bone mass is reached in both rats and humans, a phenomenon that can be useful in determining correspondence in ages in these spices. On the whole, it seems that the peak of bone surface and volume occurs at around the thirties - forties in humans.

The data on BMD might also be useful in determining a correspondence in age between rats and humans. Many researchers have reported that BMD becomes highest in the thirties - forties in humans (*18, 20-22*). The peaks for the BMD values in the proximal epiphysis of the tibia were observed to occur 12 months of age in rats of both sexes, as did the peaks for bone volume and bone surface (Table 6). By all accounts, it is likely that the thirties - forties in humans may correspond to the age of approximately 12 months in rats.

We must take note that there are discrepancies in bone metabolism between rats and humans; the length of bone in rats continues to increase due to growth plates remaining at the both or either one of proximal and/or distal epiphyses (*19*). The pattern of bone turnover such as modeling or remodeling is different in adult rats than in humans (*35*). Frost and Jee (*36*) have suggested that rats over 12 months old might be regarded as a model for adult man. At any rate, it seems that no distinct evidence has been presented that would contradict the correspondence in age between these species suggested above. In Table 2, it can be seen that the amounts of plutonium in the skeleton were higher in the female than in the male, despite the skeletal weights in the female being lower. Such findings may be observed in humans because the bone volume in women is greater than that in men under 49 years old, but this phenomenon would not be seen in women over 50 because the bone volume begins to decrease at this time due to menopause. On the other hand, it is important to note that the bone volume in older rats does not decrease rapidly as in post-menopausal women because female rats do not undergo a complete menopause. Therefore, estimating the amount of plutonium in post-menopausal women is still difficult. The results indicate that the amount of plutonium deposited in various organs soon after exposure to a fixed amount of plutonium might vary markedly according to age and sex, regardless of the age-related changes in both the organ and body weights. Therefore the age correspondence between rats and humans was determined based on the characteristics of age-related changes in bones. As a result, it was estimated that the amount of plutonium deposited in skeleton (bone) might be higher at middle age (thirties- forties) than in older adults.

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