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Distribution of Uranium in Rats Implanted with Depleted Uranium Pellets

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During the Persian Gulf War, soldiers were injured with depleted uranium (DU) fragments. To assess the potential health risks associated with chronic exposure to DU, Sprague Dawley rats were surgically implanted with DU pellets at 3 dose levels (low, medium and high). Biologically inert tantalum (Ta) pellets were used as controls. At 1 day and 6, 12, and 18 months after implantation, the rats were euthanized and tissue samples collected. Using kinetic phosphorimetry, uranium levels were measured. As early as 1 day after pellet implantation and at all subsequent sample times, the greatest concentrations of uranium were in the kidney and tibia. At all time points, uranium concentrations in kidney and bone (tibia and skull) were significantly greater in the high-dose rats than in the Ta-control group. By 18 months post-implantation, the uranium concentration in kidney and bone of low-dose animals was significantly different from that in the Ta controls. Significant concentrations of uranium were excreted in the urine throughout the 18 months of the study (224 ± 32 ng U/ml urine in low-dose rats and 1010 ± 87 ng U/ml urine in high-dose rats at 12 months). Many other tissues (muscle, spleen, liver, heart, lung, brain, lymph nodes, and testicles) contained significant concentrations of uranium in the implanted animals. From these results, we conclude that kidney and bone are the primary reservoirs for uranium redistributed from intramuscularly embedded fragments. The accumulations in brain, lymph nodes, and testicles suggest the potential for unanticipated physiological consequences of exposure to uranium through this route.

Key Words: depleted uranium fragments; distribution; kidney; bone; brain; kinetic phosphorimetry.

The enrichment process of uranium results in two products, one enriched and one depleted of the fissionable isotope ^{235}U. Depleted uranium (DU), alloyed with titanium to retard oxidation, was first deployed in military armaments during the Persian Gulf War. In this conflict, several soldiers were injured by DU fragments. Since surgical removal can produce excessive tissue damage, DU fragments were treated as conventional shrapnel and left in place in the wounded soldiers. The present study was initiated to assess the potential toxicity associated with long-term exposure to fragments of DU.

There is an extensive literature on the toxicity of uranium from exposures through inhalation, ingestion, and injection (Voegtlin and Hodge, 1949; 1953; ATSDR, 1997). The biological handling and distribution of uranium are dependent on both the chemical form and the route of exposure of the uranium. Soluble uranium compounds are generally more systemically toxic than the insoluble forms. The insoluble salts are more likely to be retained at the site of entry (e.g., the lungs after inhalation) (Leach *et al.*, 1970). Animal studies (Ballou *et al.*, 1986; Diamond *et al.*, 1989; La Touche *et al.*, 1987; Morrow *et al.*, 1982; Neuman *et al.*, 1948; Voegtlin and Hodge, 1949; Walinder, 1989) and epidemiological assessments (Kathren *et al.*, 1989; Singh *et al.*, 1987) demonstrate that kidney and bone are the primary reservoirs for uranium regardless of the route of exposure. With acute exposures, absorbed uranium is quickly cleared from the blood stream and mostly excreted in the urine within 24 h. Uranium that is not excreted is reabsorbed by the proximal tubules and accumulates in the kidney. As a result of uranium accumulation, the kidney is the critical target for uranium toxicity (Diamond, 1989; Diamond *et al.*, 1989; Kocher, 1989; Leggett, 1989). The skeleton also acts as a major reservoir for uranium (Ballou *et al.*, 1986; La Touche *et al.*, 1987; Leggett, 1994; Morrow *et al.*, 1982). Other organs, including liver and spleen, accumulate uranium after acute exposure. Uranium is slowly released from these stores back into circulation and excreted from the body (Walinder, 1989; La Touche *et al.*, 1987).

Only a few studies evaluated the distribution of uranium after chronic exposures using animal models. Ortega *et al.* (1989) supplied uranyl acetate dihydrate in the drinking water of rats for 4 weeks. At the end of this time period, high levels of uranium were measured in kidney, bone, and muscle. Liver, heart, lung, and thyroid also showed accumulations. Leach *et al.* (1970; 1973) exposed monkeys, dogs, and rats to dust particles of natural uranium dioxide. After a 5-year exposure in

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monkeys and dogs and a one-year exposure in rats, uranium was found predominantly (about 90% of total) in the lungs and the tracheobronchial lymph nodes. The metal was also observed in kidney, femur, spleen, and liver. Levels in the lung reached a maximum within a year while other sites continued to accumulate uranium for at least two years.

No studies have assessed the long-term health consequences of exposure to uranium fragments. Furthermore, previous studies have focused on natural or enriched forms of uranium. The current study was designed to determine the distribution of uranium that results from fragments of DU embedded in muscle of the rat over the course of 18 months.

MATERIALS AND METHODS

Chemicals. DU pellets (Oak Ridge National Laboratories, Oak Ridge, TN) consisted of 99.25% DU and 0.75% titanium by weight, with the uranium as isotopes ^{238}U (99.75%), ^{235}U (0.20%) and trace levels of ^{234}U. The pellets were made from the metal left over from the manufacture of U.S. military munitions. Tantalum (Ta) fragments (Alfa Products, Ward Hill, MA) were used as control pellets because the metal is biologically inert, with a mass similar to uranium, and is frequently used in human prostheses. Each pellet (both DU and Ta) was approximately 1 mm diameter × 2 mm long.

Animals and treatment. Sprague-Dawley rats (8–10 weeks of age) were maintained in an Association for Assessment and Accreditation of Laboratory Animal Care International-accredited facility in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996). Upon arrival, rats were quarantined and screened for diseases. Except during urine collection, all animals were housed in plastic Microisolator rat cages with hardwood chips as bedding. Commercial rodent chow (Harlan, Teklend Rodent Diet #8604, Madison, WI) and water were provided *ad libitum*. Rats were on a 12-hr light/dark cycle.

Rats were surgically implanted with sterilized DU and/or Ta pellets within the gastrocnemius muscle under anesthesia with ketamine hydrochloride (80 mg/kg) in combination with xylazine hydrochloride (4 mg/kg), given ip. All surgically-implanted rats received 20 pellets of Ta, DU or a combination. Half the total number of pellets were implanted in each thigh. Ta controls received 20 Ta pellets; low-dose DU rats, 4 DU and 16 Ta pellets; medium-dose DU rats, 10 DU and 10 Ta pellets; and high-dose DU rats, 20 DU pellets. Another set of animals served as non-surgical controls. Separate groups of animals were evaluated at 1 day, or at 1, 6, 12, or 18 months. Each experimental group consisted of 15 animals. Tissues and fluids from 5 animals of each group were sent to Battelle Northwest Operations (Richland, WA) for analysis (see below). In some groups, additional tissues were analyzed at the Armed Forces Radiobiology Research Institute.

Sample collection. Urine samples were collected from each rat, individually housed for 24 h in metabolism cages and having continuous access to food and water. The urine samples were stored at 4°C until analyzed. Because rats implanted with pellets did not survive the stress of the metabolism cages immediately after the surgery, urine samples were not collected at 1 day. Blood samples were obtained from the tail vein and centrifuged for 5 min at 3000 × g. Serum was stored at −70°C until ready for analysis. Rats were euthanized by decapitation under ketamine anesthesia. Tissues (tibia, skull, kidney, muscle, liver, spleen, brain) were harvested, frozen, and stored at −70°C until analysis for uranium content. Tissues and fluids from 5 animals of each experimental group were shipped on dry ice by overnight courier to Battelle-Northwest Operations (Richland, WA). Additional tissues from the 18-month animals (lymph nodes, testicles, teeth, feces, heart, lung, and brain areas) were analyzed using the same techniques at the Armed Forces Radiobiology Research Institute, with minor differences (1 M nitric acid was used instead of 4 M, and the samples were decanted rather than filtered).

Uranium measurement. Uranium determinations in wet-ashed tissues were performed by kinetic phosphorimetry using methods previously described by Brina and Miller (1992). Wet ashing of tissue samples began with the addition of 2 ml of 16 M nitric acid to each scintillation vial, which was followed by overnight digestion of the tissues at room temperature. The following day, samples were heated in a dry bath for several h to evaporate the initial volume of nitric acid to approximately 0.5 ml. After a short cooling period, 0.5 ml of 30% hydrogen peroxide was added to each sample, which was then heated to reduce the volume to approximately 0.5 ml. Subsequent wet ashing involved addition of 2.0 ml of 16 M nitric acid to each sample, followed by several h of heating, brief cooling, addition of 0.5 ml of 30% hydrogen peroxide, and reduction of the volume to approximately 0.5 ml. Wet ashing continued in this manner for 15 cycles over the next 5 days, after which the samples were heated to dryness, then placed in a muffle furnace at approximately 600°C for about 2 h.

Calibration curves were freshly prepared using a uranium plasma standard in 5% nitric acid (Alfa, AESAR), and concentration was checked against NIST SRM 3164. Stock solutions were prepared in 4 M nitric acid. High purity deionized water with an electrical resistance of approximately 18 megaohms was obtained from a MilliQ deionization system (Millipore Corp, Milford, MA). Final concentrations for calibration samples ranged from 0.05 ng/ml to 50.0 ng/ml for the low concentration range and 0.01 μg/ml to 10.0 μg/ml for the high concentration range.

Samples and calibration standards were dissolved by warming in 2 ml of 4 M nitric acid and filtered through Acrodisc LC 13 PVDF 0.45 μm syringe filter units (Gelman Sciences, Ann Arbor, MI) to remove any particulate matter. For analysis, 1.0 ml of sample (or standard) was dissolved in 1.5 ml of freshly prepared Uraplex reagent (Chemchek Instruments, Inc., Richland, WA).

Appropriate care was used in handling cuvettes to prevent cross contamination and to ensure low backgrounds. Cuvettes were rinsed with 3 changes of 2.0 ml of 0.5 M sulfuric acid followed by 3 changes of 2.0 ml deionized water. The caps were placed on the cuvettes for each rinse and the cuvette was inverted to rinse the cap. Addition of 1.0 ml of diluted sample was followed by addition of 1.5 ml of Uraplex reagent. The cuvettes were capped, and the sample was inverted twice to mix the reagent with the sample. The cuvette was then inserted into the instrument for a reading.

Samples were analyzed with a Kinetic Phosphorescence Analyzer (KPA-11, Chemchek Instruments Inc., Richland, WA). Background measurements were made using 4 M nitric acid. Calibration curves were developed each day prior to sample analysis, and quality assurance checks were made at frequent intervals using standard solutions of known uranium concentration. The uranium content of the series of tissue samples submitted for analysis was measured, following analysis of the background and calibration standards. Measurements included analysis of relative standard deviations (RSD) and correlation coefficients (r^2) of the luminescence decay curve.

The limit of detection of uranium using kinetic phosphorimetric analysis is 0.005 μg/l. (5 pg/ml or 5 pg/g tissue). The quantification limits varied for each tissue: 0.05 μg/l for urine, 0.1 μg/l for brain and lymph nodes, 0.4 μg/l for blood, 1 μg/l for liver, kidney, bone, spleen, teeth, and muscle. Recovery of uranium from tissues ranged from 91 ± 13% for blood to 97 ± 8% for urine.

For serum and urine samples, the concentrations are reported in ng/ml and, for all other tissues, in ng/g tissue. For samples in which the correlation coefficient (r^2) of the decay curve was less than 0.99, the data were reanalyzed by choosing appropriate time gates from the plot of luminescence intensity as a function of decay time, to achieve the best linear relationship (highest r^2 value) for the data. In general, relative standard deviations (r SD) are significantly lower on samples that have linear decay curves.

Statistical analysis. Uranium content for each organ was divided by the wet weight of the tissue and averaged for each experimental group at each time point. Data that were greater than 2 standard deviations (SD) different from the mean of the group were excluded. In all cases, a minimum of 3 measurements was included in the final averaged value. Since comparisons of the Ta control to the non-surgical control for each measurement revealed no significant differences between the two groups (2-tailed t-test, $p > 0.05$), all data were

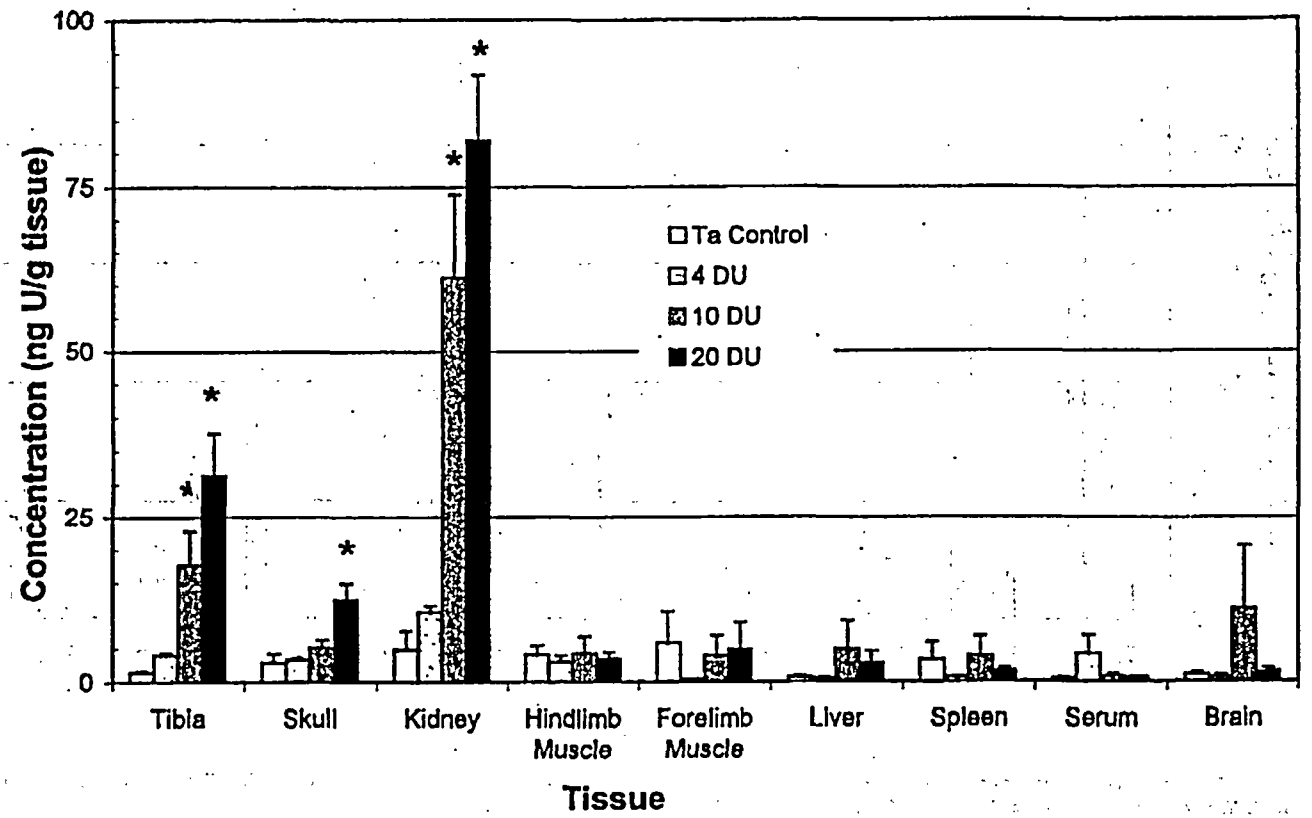

FIG. 1. Uranium concentrations measured in tissues dissected from rats euthanized 1 day after implantation of DU and Ta pellets. Rats were implanted with either 20 Ta pellets (Ta control), 4 DU and 16 Ta pellets (4 DU, low dose), 10 DU and 10 Ta pellets (10 DU, medium dose) or 20 DU pellets (20 DU, high dose). Bars represent mean ± SEM. *N*, 3–5. *Indicates significantly different from tantalum controls.

referenced to the Ta controls. Data were analyzed by a Kruskal-Wallis one-way analysis of variance. DU experimental groups were compared to Ta controls using Dunn's method of multiple comparisons. Significance was accepted at $p < 0.05$.

RESULTS

Uranium Distribution at 1 Day

Within 1 day after implantation of DU pellets, rats showed significant distribution of uranium to kidney and bone (Fig. 1). Uranium concentrations measured in other organs were not significantly different from those measured in tissues from rats in the Ta-control group. The highest levels were in kidney and varied with the number of DU pellets implanted: Ta-control group, 1.41 ± 0.33 ng U/g; low-dose group, 3.98 ± 0.54 ng U/g; medium-dose group, 17.6 ± 5.3 ng U/g; and high-dose group, 82.0 ± 9.7 ng U/g wet weight kidney. Uranium concentrations in the medium and high-dose groups, but not in the low-dose group, were significantly higher than the Ta-control group. A dose-dependent increase in uranium concentrations was also observed for the tibia reaching 31.3 ± 6.5 ng U/g in the high-dose group. In the animals in the medium and high-dose groups, the uranium levels in the tibia were significantly greater than that in the Ta controls. While uranium concentration in the skull significantly increased in the high-dose animals, the levels were lower than in the tibia (12.5 ± 2.5 ng U/g skull). The uranium concentration in the skulls of animals in the low-dose and medium-dose groups did not significantly differ from that of Ta-control animals.

Uranium Distribution at 1 Month

At 1 month following implantation, the highest concentrations of uranium were, again, in kidney and bone (Fig. 2). Levels were much greater than those observed 1 day after implantation. For example, in the high-dose group animals, uranium concentrations reached 1280 ± 320 ng U/g kidney, 1870 ± 510 ng U/g tibia, and 1240 ± 130 ng U/g skull. Deposition of uranium in the muscle of the hindlimb near the DU pellet implantation sites averaged 744 ± 370 ng U/g and ranged from 9.28 to 2170 ng U/g in animals in the high-dose group. Significant accumulations were also observed in urine,

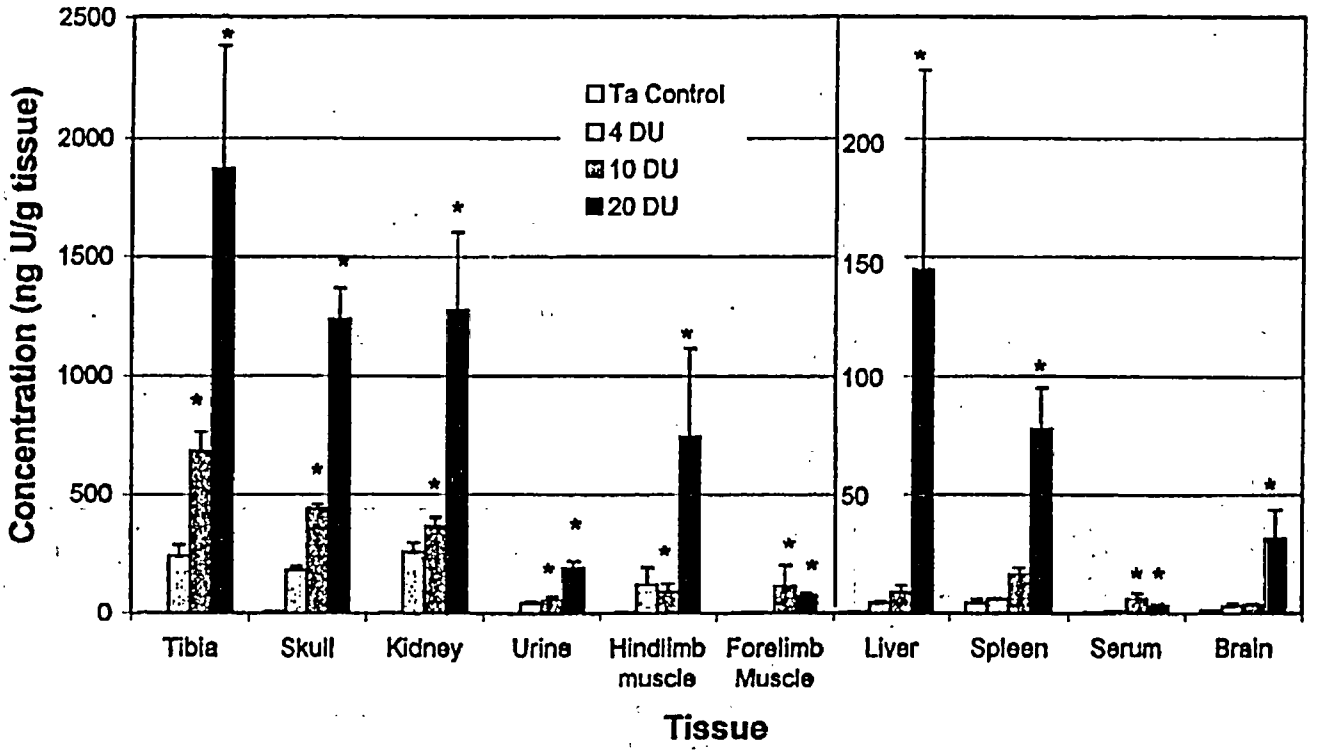

FIG. 2. Uranium concentrations measured in tissues dissected from rats euthanized 1 month after implantation of DU and Ta pellets. Bars represent mean ± SEM. *N*, 5–7. *Indicates significantly different from tantalum controls.

liver, spleen, and brain of high-dose group animals compared to the tissues in the Ta controls. The concentration of uranium in the serum was relatively low but significantly greater than that in Ta controls in the medium- and high-dose groups of animals (Ta group, 0.69 ± 0.20 ng U/ml serum; medium-dose group, 6.08 ± 2.41 ng U/ml serum; high-dose group, 3.04 ± 0.99 ng U/ml serum).

Uranium Distribution at Later Time Points

Kidney. The pattern of uranium distribution generally remained the same in rats implanted with DU for 6 months (Fig. 3), 12 months (Fig. 4) or 18 months (Fig. 5). The highest concentrations of uranium continued to be observed in kidney and bone (tibia and skull). Levels of uranium in the kidneys of rats in the high-dose group were significantly different from Ta controls at all time points. Uranium concentrations in the kidneys of rats in the low-dose group were significantly different from Ta controls at 12 and at 18 months after implantation, but not before. Uranium concentrations in the kidneys of all dose groups were observed to plateau after the 6-month time point (Fig. 6a). In the rats in the high-dose group, the concentration of uranium in kidney tissue was 6920 ± 1690 ng U/g at 6 months and was 4500 ± 770 ng U/g at 18 months.

Bone. The concentration of uranium in bone (tibia and skull) increased with dose and duration of exposure (Figs. 6b, 6c). In the medium and high-dose rats, the concentrations were significantly different from the Ta controls at all time points with the exception of the medium dose at 18 months. The uranium concentrations in tibia and skull of the low-dose group were only significantly different from the Ta controls at 18 months. Unlike the kidney, the levels in the bone of the medium- and high-dose rats continued to rise throughout the 18 month period of study. However, the bone uranium levels in the low-dose animals leveled off by 12 months. At 18 months after implantation, uranium concentrations in the high DU dose animals were 7050 ± 1370 ng U/g tibia and 3690 ± 810 ng U/g skull.

Muscle. Uranium concentration in muscle from the hindlimbs where the pellets were implanted contained, on the average, a greater concentration of uranium than muscle from the forelimbs (Figs. 2–5). Throughout the course of the study, the concentrations of uranium in the muscle were quite variable from animal to animal. At 12 months, the concentrations ranged from 6.77 to 133 ng U/g hindlimb muscle, while at 18 months the range was 59.1 to 13200 ng U/g hindlimb muscle. Despite this variability, muscle concentrations of uranium dif-

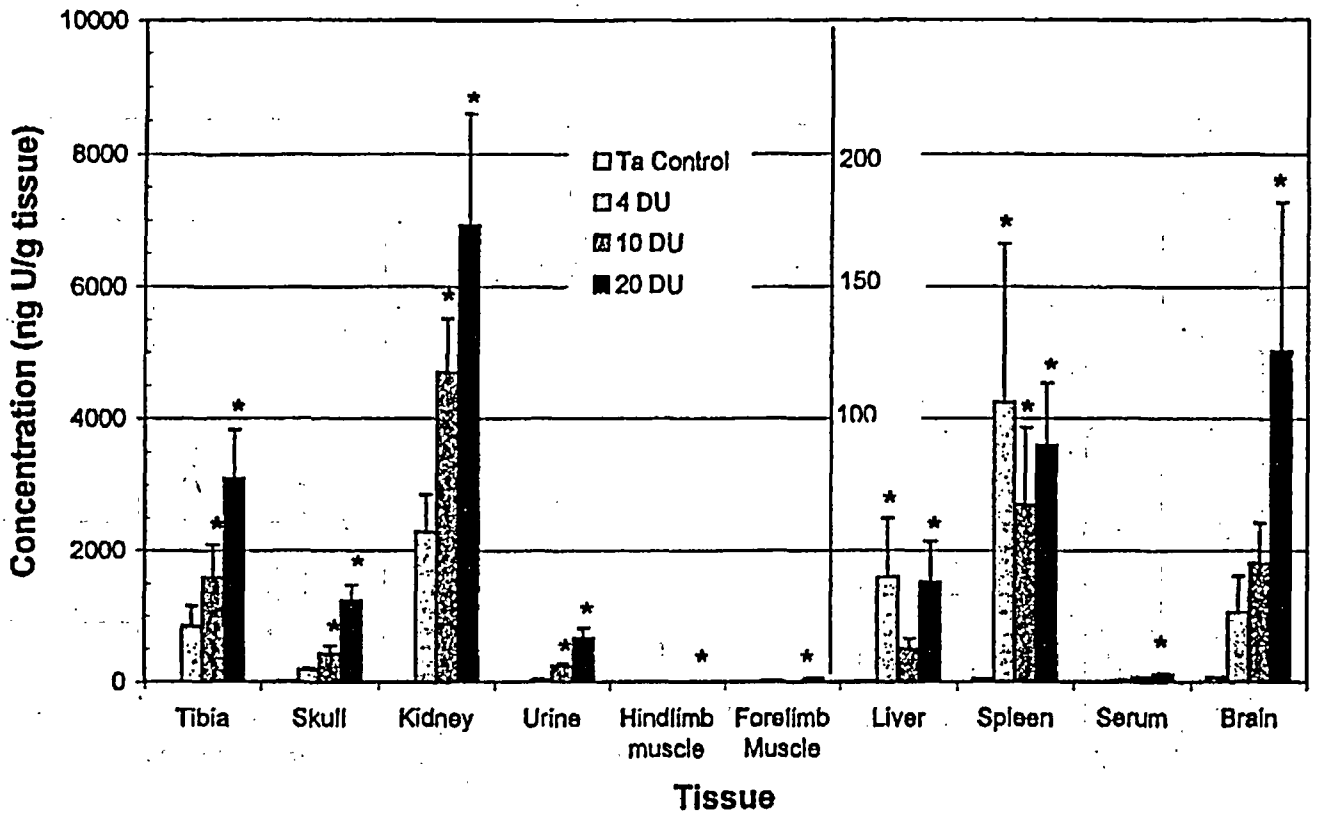

FIG. 3. Uranium concentrations measured in tissues dissected from rats euthanized 6 months after implantation of DU and Ta pellets. Bars represent mean ± SEM. *N*, 4–5. *Indicates significantly different from tantalum controls.

fered significantly from control at several time points. In the hindlimb of high-dose rats, the uranium concentrations were significantly different from the concentrations of Ta controls at 6 months and 18 months. At 18 months, the concentrations of uranium in both hindlimb and forelimb muscle in the low-dose group were significantly different from that in Ta controls.

Spleen and liver. In the high-dose group, uranium concentrations in liver and spleen were significantly greater than Ta controls at all time points after 1 day (Figs. 2–6). In the rats exposed to a low dose of DU, uranium concentrations in the spleen and liver were significantly different from Ta controls at 6 months but not at the later time points. Concentrations of uranium in spleen and liver did not show a strongly dose-dependent relationship. In general, the average concentrations in spleen were greater than those in liver. At 18 months after pellet implantation, the average concentrations in these tissues of the high-dose group were 188 ± 41 ng U/g in spleen and 79.9 ± 20.7 ng U/g in liver tissue.

Serum. Very low levels of uranium were observed in serum at all time points (Figs. 2–6). Uranium concentration in serum from the high-dose group was statistically different from the Ta controls at 6 months and at 18 months but not at 12 months. The maximal concentration of serum uranium was 8.09 ± 3.78 ng U/ml in the high-dose group at 18 months.

Urine. At all time points, the concentrations of uranium in the urine of medium- and high-dose rats were significantly different from the Ta controls. At 12 and 18 months, the concentration of uranium in the urine of the low-dose rats also was significantly different from control (Figs. 2–6). The uranium concentration in urine (expressed as ng U/ml) reached a maximum at 12 months post-implantation. At 18 months, the concentration in the urine of high-dose rats declined significantly (Fig. 7a). The maximal concentration obtained in the high-dose rats was 1010 ± 87 ng U/ml urine and in the low-dose rats was 224 ± 32 ng U/ml urine at 12 months post-implantation. Since the volume of urine excreted over the 24-h collection period varied with the age of the animals, the data were reanalyzed to calculate the total uranium excreted within 24 h (Fig. 7b). In the medium-dose and high-dose rats, the 24-h uranium excretion increased until 6 months post-implantation when it reached a plateau (5610 ± 1770 μg U at medium dose; 11200 ± 4000 μg U at high dose). The 24-h uranium excretion in low-dose animals rose more gradually, reaching a plateau at 12 months (2710 ± 440 μg U). At 12 and

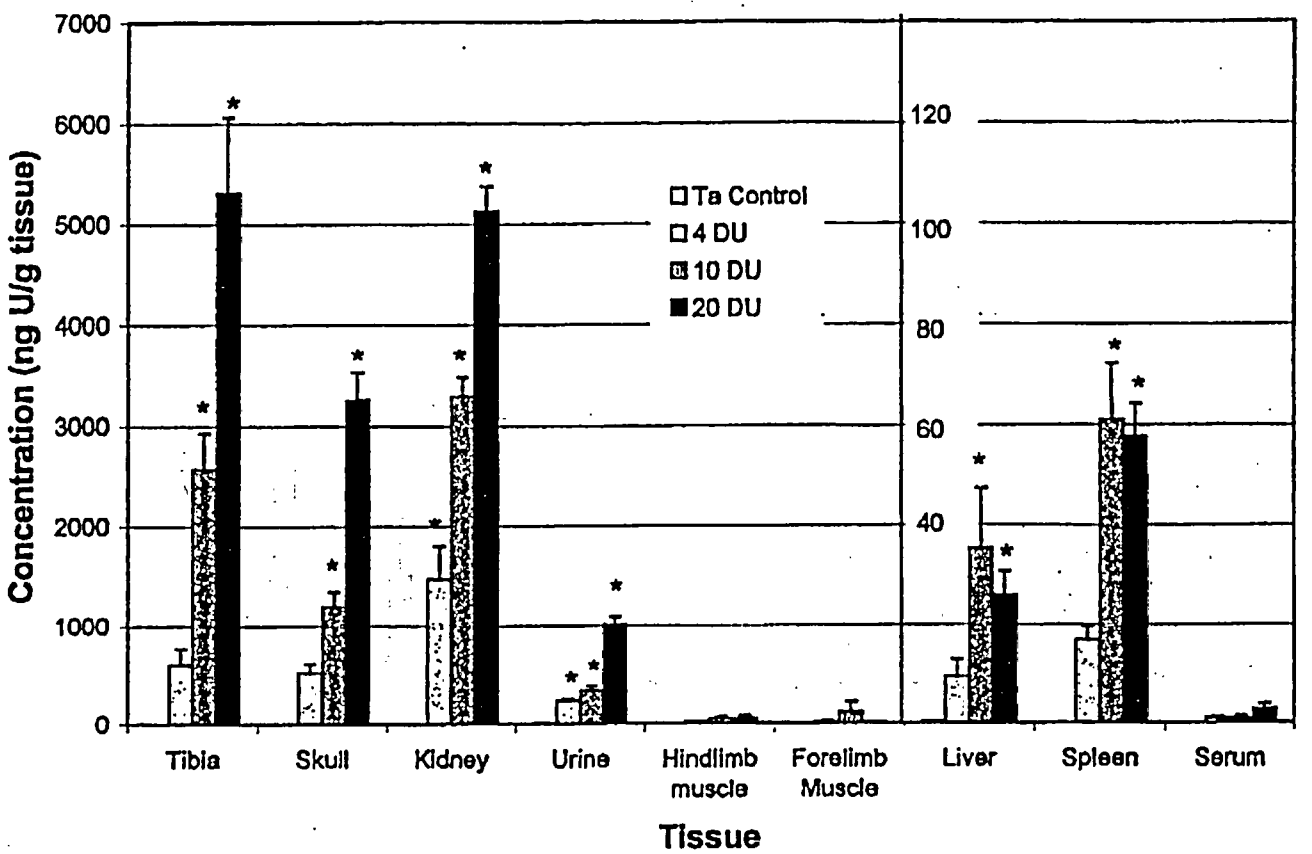

FIG. 4. Uranium concentrations measured in tissues dissected from rats euthanized 12 months after implantation of DU and Ta pellets. Bars represent mean ± SEM. *N*, 3–5. *Indicates significantly different from tantalum controls.

18 months, the 24-h uranium excretion was linearly related to the surface area of the embedded pellets (82 ± 7 ng/mm^2 calculated from 18-month data).

Brain. Uranium concentrations in an entire hemisphere of the rat brain were measured at 1 day, 1 month, and 6 months after pellet implantation (Figs. 1–3). At 1 and 6 months, brain concentrations of uranium were statistically different from Ta controls in the high-dose group (Figs. 2, 3). In the animals implanted for 12 months, a brain hemisphere was not available because the tissue was used for other analyses. In rats implanted with DU pellets for 18 months, several areas of the brain were independently assessed for uranium distribution. Uranium was not uniformly distributed throughout the tissue (Fig. 8). Uranium levels in this area increased in a dose-dependent fashion with the number of implanted DU pellets. In both the medium- and high-dose groups, uranium concentrations in motor cortex, frontal cortex, midbrain, and vermis were statistically greater from those in Ta controls. Uranium concentrations in the cerebellum were only significant in the high-dose group.

Other sites of accumulation. Additional tissues were analyzed from animals euthanized at 18 months (Fig. 9). Uranium concentrations in lymph nodes, testicles, teeth (with lower jaw), heart, and lung were all significantly higher than those observed in tissues collected from Ta-control animals. Similar to observations with tibia and skull, relatively high concentrations were observed in the teeth combined with lower jaw (1950 ± 270 ng U/g tissue in the high-dose group). Although high concentrations of uranium were found in the feces collected from rats in all groups, including Ta-control rats, there was no dose-dependence exhibited (approximately 1100 ng/g wet weight). These levels are probably the result of intake of uranium through the food and water which contained uranium concentrations of 360 ± 20 ng U/g and 0.25 ± 0.05 ng/ml, respectively.

DISCUSSION

The present study demonstrates that embedded fragments of DU redistribute throughout the body over many months. As in previous animal studies and epidemiological assessments, kid-

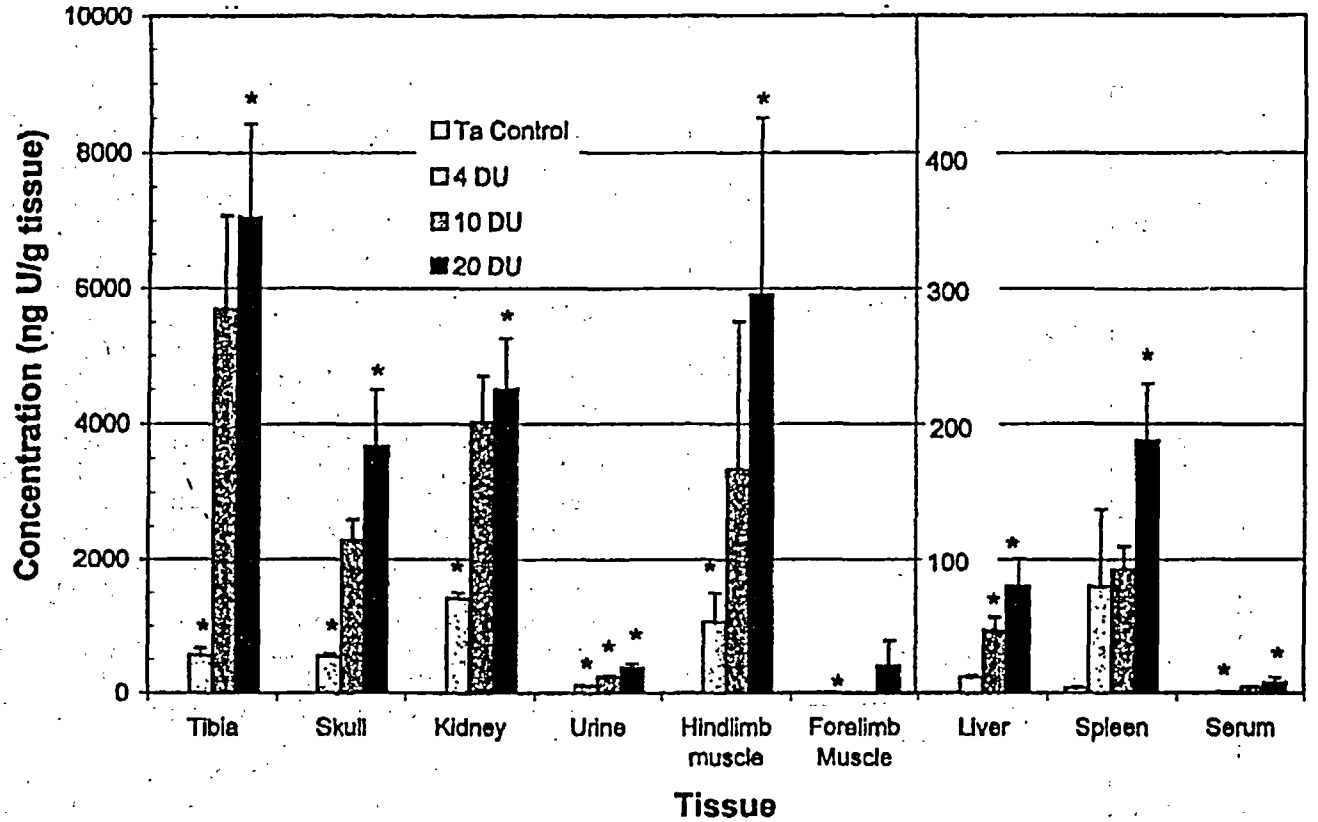

FIG. 5. Uranium concentrations measured in tissues dissected from rats euthanized 18 months after implantation of DU and Ta pellets. Bars represent mean ± SEM. For urine measurements N, 10–15; for liver measurements N, 9–13; for all other tissues N, 4–5. *Indicates significantly different from tantalum controls.

ney and bone were the major reservoirs for the uranium. Less anticipated was the distribution of uranium to spleen and brain.

In occupationally-exposed miners and millers (Kathren *et al.*, 1989; Singh *et al.*, 1987), uranium was found in the highest concentrations in lung, kidney, bone, and liver. In naturally-exposed populations, the distribution of uranium is very similar, although the levels are significantly lower (Dang *et al.*, 1992). Animal studies have explored the distribution of uranium following experimental inhalation, injection, and ingestion in a variety of chemical forms. Following intravenous injection, a bolus of uranium was quickly cleared from the blood, redistributed to bone and kidney, and excreted in urine (Morrow *et al.*, 1982; Neuman *et al.*, 1948; Walinder, 1989). Uranium was also distributed to spleen and liver (Walinder, 1989). Morrow *et al.*(1982) suggest that a single exposure to uranium through inhalation is handled very much like intravenous exposure. The soluble salts of uranium are rapidly cleared from the lungs and redistributed to skeleton, kidney, and liver (Ballou *et al.*, 1986).

In the DU implanted rats, the pellets served as a constant reservoir for uranium. Concentrations of uranium in bone increased throughout the 18 months of the experiment. In the rat, bones continue to grow well into adulthood. In male rats, skull has been demonstrated to continue to enlarge until 14 months of age (Savostin-Asling *et al.*, 1980). Between the ages of 12 and 24 months, the femur of male rats was found to increase in width at midshaft, although the total mineral content of the bone declined (Smith and Kiebzak, 1994). Despite this mineral loss, the present study demonstrates that uranium levels continue to accumulate in bone. A difference in growth patterns of skull and tibia was not evident from the time course of uranium accumulation. Previous studies have shown that uranium can displace calcium in bone matrix (Arsenault and Hunziker, 1988; Neuman *et al.*, 1949). In addition to bone, teeth and lower jaw contained high levels of uranium when measured 18 months after DU pellet implantation. It is possible that uranium is replacing calcium here, as well as in the tibia and skull.

The kidney is considered to be a primary site for uranium toxicity (Diamond, 1989; Diamond *et al.*, 1989; Kocher, 1989; Leggett, 1989). In the implanted rats, levels of uranium in the kidney increased through the first 6 months. Uranium levels at 12 and 18 months were no greater than the 6-month levels at all exposure doses. As previously demonstrated, uranium was

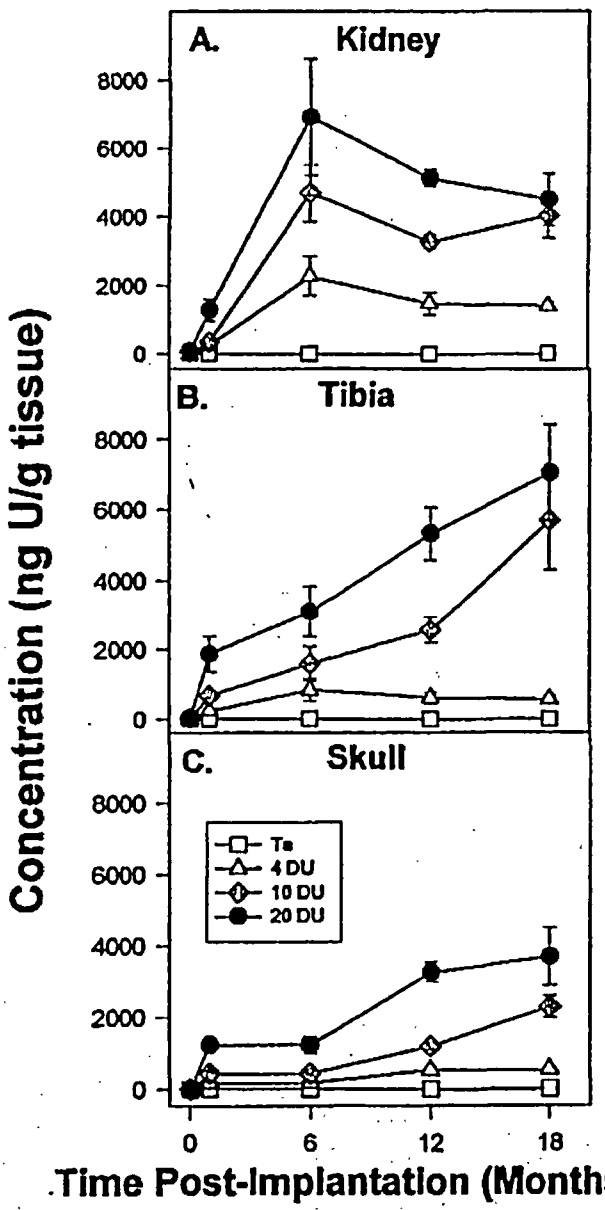

FIG. 6. Uranium concentration in kidney (A), tibia (B), and skull (C) plotted as a function of time after implantation. Time points were 1 day, 1, 6, 12, and 18 months. Each point is mean ± SEM.

excreted in the urine. Urine uranium concentration showed an irregular pattern with time. Eighteen months after implantation, the concentration was reduced compared to that found at 12 months. Analysis of the urine volume (data not shown) suggested age-dependent changes. Reassessment of the uranium excreted during a 24-h period revealed that, like the kidney uranium levels, the 24-h excreted uranium reached a maximum at 6 months. These levels were maintained at the later time points.

In previous studies, liver was a large pool for the accumulation of uranium (e.g., see Leggett, 1994). In the present study, levels in spleen were at least as high if not higher than those in liver. This may reflect differences in the route of uranium exposure (i.e., muscle-embedded fragments). Little uranium was measured in the serum, which would limit the uranium passing through the liver. Uranium distributed locally through the muscle and was found in the lymph nodes. It is possible that the uranium was scavenged by macrophages and transported through the lymph to the spleen. The macrophage is known to phagocytize uranium and to participate in clearing the lung of particles after inhalation exposure (Andre *et al.*, 1989; Tasat and de Rey, 1987; Batchelor *et al.*, 1982). This conclusion is supported by a chronic inhalation study in which the lung and the tracheobronchial lymph nodes were found to be the primary reservoirs of uranium in monkey, dog, and rat (Leach *et al.*, 1970; 1973). In the lymphoid tissue of the dogs and monkeys exposed for periods of 12 months or longer, black uranium pigment was associated with macrophages within the lymph tissue. On occasion, necrosis of the lymphoid tissue was observed (Leach *et al.*, 1970). Impaired immune function resulting from the chronic accumulation of uranium in the lymphatic system as well as in bones and spleen might be a possible consequence of prolonged exposure to DU fragments.

Distribution of uranium to brain from embedded DU pellets was unexpected. Six months after implantation, a dose-dependent increase of uranium was observed in the homogenized hemisphere of the rat brain. The 12-month sample used for analysis consisted only of sensory motor cortex, because some of the neural tissue was used for other purposes. The measured levels in this cortical region were much smaller than those of the entire hemisphere at 6 months. This suggested the possibility of regional distribution of uranium. At 18 months, different regions of the brain showed different levels of uranium. As with other heavy metals (Butterworth *et al.*, 1978; Cholewa *et al.*, 1986; Ono *et al.*, 1997; Rios *et al.*, 1989; Ross *et al.*, 1996), the uranium was not uniformly distributed throughout the brain. The neurotoxicity associated with exposure to other heavy metals (e.g., lead and mercury) raises concerns about the neurophysiological and behavioral consequences of uranium accumulation in the brain with chronic exposure to DU fragments.

At 18 months, we measured a dose-dependent increase of uranium in the testicles. Studies to date suggest the possibility that chronic uranium exposure in males can affect reproduction (Llobet *et al.*, 1991; Malenchenko *et al.*, 1978; Muller *et al.*, 1967). Male uranium miners were found to have more female offspring than predicted, suggesting alterations in sperm (Muller *et al.*, 1967). Male mice treated for 64 days with up to 80 mg/kg/day uranyl acetate in the drinking water showed a decrease in the rate of impregnation of untreated females (Llobet *et al.*, 1991). However, these effects were not dose-dependent and there was no evidence of impaired spermato-

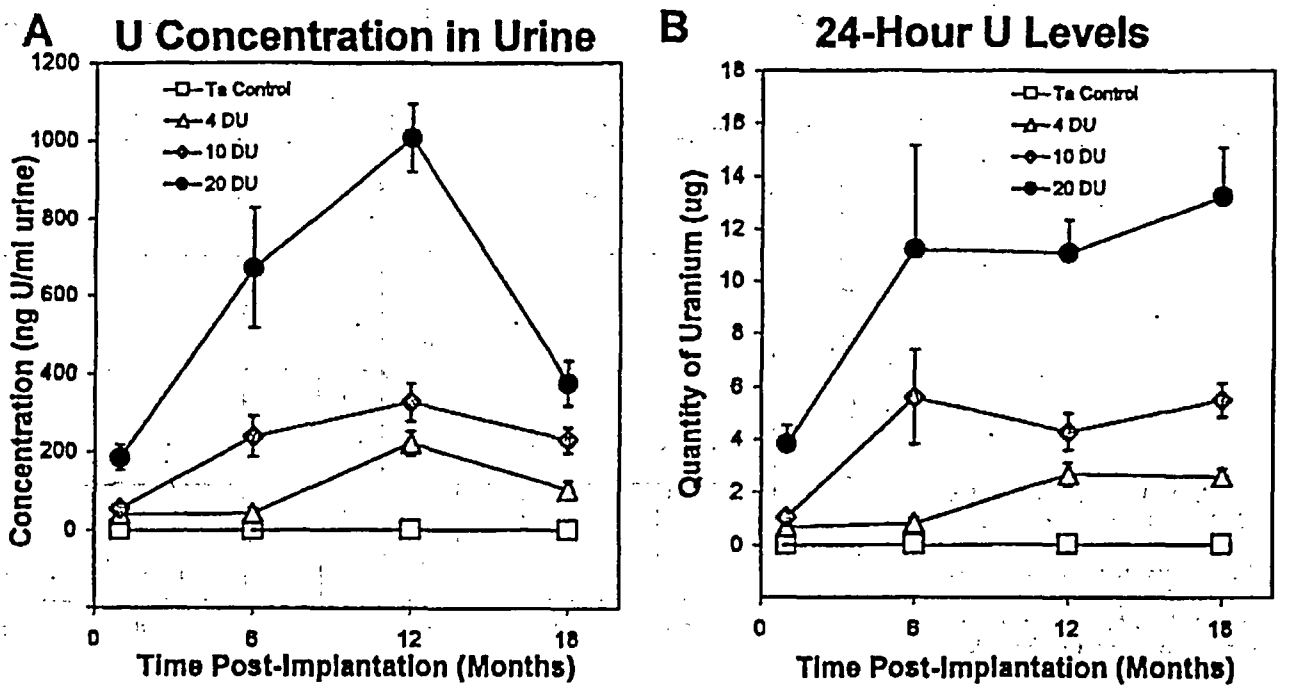

FIG. 7. Uranium content of urine in implanted rats. (A) Uranium concentration in urine plotted as a function of time after implantation. (B) Total content of uranium in the urine excreted during a 24-h period plotted as a function of time after implantation. Time points range from 1 to 18 months. No data were collected for the 1 day time point. Each point is mean ± SEM.

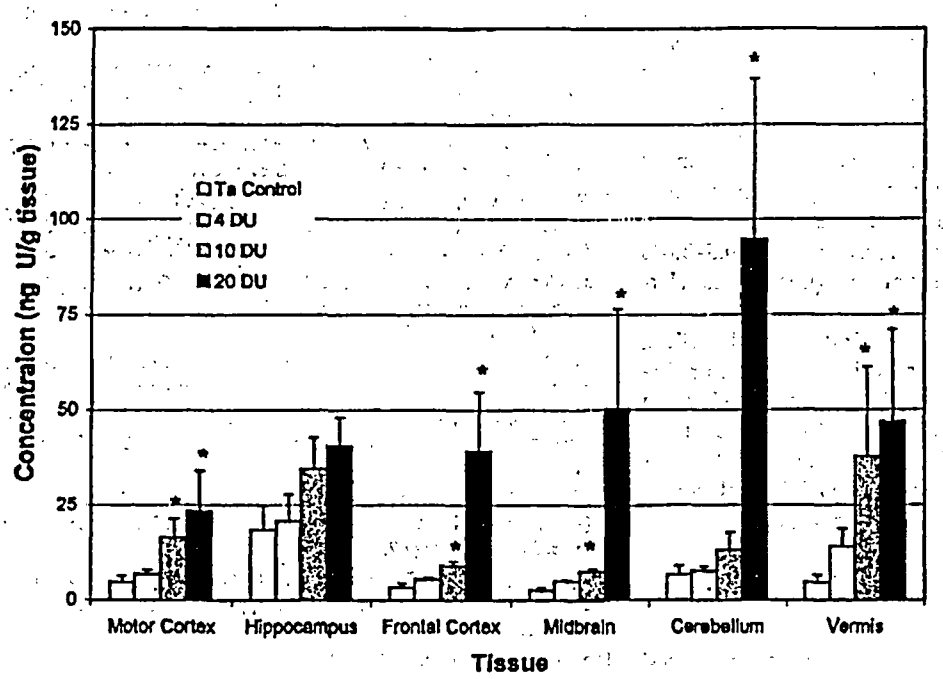

FIG. 8. Uranium concentration of brain areas measured 18 months after implantation of pellets. Bars represent mean ± SEM. *N*, 8–14. *Indicates significantly different from tantalum controls.

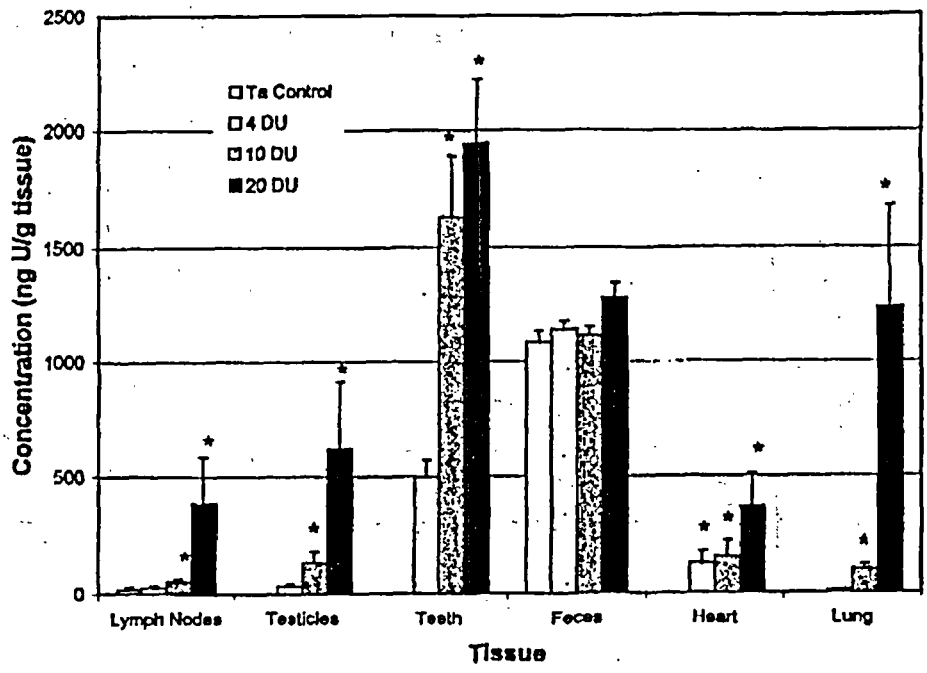

FIG. 9. Uranium concentration of additional samples measured 18 months after implantation of pellets. All measures are normalized to wet weight of sample. Bars represent mean ± SEM. N, 10–16. *Indicates significantly different from tantalum controls.

genesis. In rats, a dose of 0.07 mg/kg/day uranyl nitrate hexahydrate for 16 weeks resulted in decreased testes weight, testicular lesions, and necrosis of spermatocytes and spermatogonia (Malenchenko *et al.*, 1978). In an early study on rats, a chronic diet of uranyl nitrate hexahydrate for 12 months resulted in severe degeneration in the testes and depletion of germ cells (Maynard *et al.*, 1953).

High concentrations of uranium were found in the feces of rats in all experimental groups, but no dose-dependence was observed. These levels may reflect the ingestion of substantial amounts of natural uranium found in the water and food. Despite this intake, uranium levels in the organs of control animals were minimal suggesting that the ingested metal contributes little to the uranium load. Previous studies have demonstrated that the ingestion of uranium results in only 0.5–3.0% absorption through the gastrointestinal tract (Leggett and Harrison, 1995).

In summary, following implantation of rats with DU pellets, kidney and bone are the primary reservoirs for uranium redistributed from intramuscularly-embedded fragments. Uranium was excreted in the urine throughout the 18 months of the study. The accumulations in brain, lymph nodes, and testicles suggest the potential for unanticipated physiological consequences of exposure to uranium through this route.

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