Implanted Depleted Uranium Fragments Cause Soft Tissue Sarcomas in the Muscles of Rats

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In this study, we determined the carcinogenicity of depleted uranium (DU) metal fragments containing 0.75% titanium in muscle tissues of rats. The results have important implications for the medical management of Gulf War veterans who were wounded with DU fragments and who retain fragments in their soft tissues. We compared the tissue reactions in rats to the carcinogenicity of a tantalum metal (Ta), as a negative foreign-body control, and to a colloidal suspension of radioactive thorium dioxide (^{232}Th), Thorotrast, as a positive radioactive control. DU was surgically implanted in the thigh muscles of male Wistar rats as four squares (2.5 × 2.5 × 1.5 mm or 5.0 × 5.0 × 1.5 mm) or four pellets (2.0 × 1.0 mm diameter) per rat. Ta was similarly implanted as four squares (5.0 × 5.0 × 1.1 mm) per rat. Thorotrast was injected at two sites in the thigh muscles of each rat. Control rats had only a surgical implantation procedure. Each treatment group included 50 rats. A connective tissue capsule formed around the metal implants, but not around the Thorotrast. Radiographs demonstrated corrosion of the DU implants shortly after implantation. At later times, rarifactions in the radiographic profiles correlated with proliferative tissue responses. After lifetime observation, the incidence of soft tissue sarcomas increased significantly around the 5.0 × 5.0 mm squares of DU and the positive control, Thorotrast. A slightly increased incidence occurred in rats implanted with the 2.5 × 2.5 mm DU squares and with 5.0 × 5.0 mm squares of Ta. No tumors were seen in rats with 2.0 × 1.0 mm diameter DU pellets or in the surgical controls. These results indicate that DU fragments of sufficient size cause localized proliferative reactions and soft tissue sarcomas that can be detected with radiography in the muscles of rats. *Key words*: bioassay, carcinogenesis, depleted uranium, Gulf War, rats, sarcomas, soft tissues, tantalum, Thorotrast. *Environ Health Perspect* 110:51–59 (2002). [Online 15 December 2001] *http://ehpnet1.niehs.nih.gov/docs/2002/110p51-59hahn/abstract.html*

A few U.S. veterans of the Gulf War have fragments of depleted uranium (DU) metal containing 0.75% titanium (Ti) embedded in their tissues. During the war, several U.S. tanks and fighting vehicles were mistakenly fired upon and struck by munitions containing DU. Some of the crew members who survived were left with multiple small fragments of DU in their muscles and soft tissues. The number, size, and location of the fragments made surgical removal difficult. Thirty-three of these survivors are in a medical surveillance program of the U.S. Department of Veterans Affairs to detect any untoward health effects (*1*). Some of these veterans had elevated concentrations of uranium in the urine 7 years after wounding. The persistence of these elevated urine concentrations suggested an ongoing mobilization of uranium from the embedded fragments, causing chronic systemic exposure. This observation indicates that DU fragments in the tissues are not inert foreign bodies and may react differently in the body from other embedded shrapnel.

Depleted uranium is used in projectiles and armor by the U.S. military and several other countries (*2*). Its high density (nearly twice that of lead), ability to burn on impact (pyrophoricity), self-sharpening properties, and ready availability make DU an ideal material for military purposes. DU is a byproduct in the uranium enrichment process of nuclear weapons production and the nuclear fuel cycle. It is about 40% less radioactive than natural uranium. The chemical and physical properties of natural uranium and DU are identical. To provide the appropriate metallurgic properties for military uses, DU is usually alloyed with 0.75% Ti. DU armor and projectiles were effective in the Gulf War and Kosovo and will undoubtedly be used in future combat.

A recent report by the International Agency for Research on Cancer notes that available studies are inadequate to permit reliable and accurate estimates of the long-term effects of DU in humans (*3*). However, laboratory studies have indicated that DU fragments may be carcinogenic. For example, rats implanted in the muscles with 20 DU pellets (2.0 × 1.0 mm diameter) excreted uranium in the urine for at least 18 months (*4*). Urine from these rats had enhanced mutagenic activity in *Salmonella typhimurium* strain TA98 and the Ames II mixed strains (TA7001-7006) (*5*). The mutagenicity increased in a dose- and time-dependant manner with a strong positive correlation with urinary uranium concentration. In addition, DU-uranyl chloride transformed immortalized human osteoblastic cells to the tumorigenic phenotype (*6*). DU-uranyl chloride treatment produced a 9.6-fold increase in neoplastic transformation frequency compared with untreated control cells. In comparison, nickel sulfate, a known human carcinogen, produced a 7.1-fold increase in transformation frequency. These findings of mutagenesis and neoplastic transformation suggest that DU may be carcinogenic.

To help clarify the potential carcinogenicity of DU fragments in the soft tissues, we conducted a long-term bioassay study with implanted materials in rats. The study included both positive and negative control materials. The results indicate that DU fragments cause localized tumors in rats and that the carcinogenicity is correlated with the size of the fragments. The study was conducted in facilities fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. In conducting research using animals, the investigators adhered to the *Guide for the Care and Use of Laboratory Animals* (*7*).

Materials and Methods

We determined the carcinogenicity of intramuscularly implanted DU (alloyed with 0.75% Ti) using a long-term bioassay study. The sizes and shapes of the DU used were similar to the range of sizes and shapes of DU fragments embedded in soldiers wounded in the Gulf War (*8*). We obtained cylindric DU pellets from Manufacturing Sciences Corporation (Oak Ridge, TN). Two sizes of DU fragments were cut from DU foil obtained from the same source. The physical characteristics of the pellets and fragments used in the study are shown in Table 1. The mass of DU fragments used varied by a factor of 20, and the surface area varied by a

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factor of 10. Four implants were made per rat. Brand et al. (*9*) have noted that foreign bodies, smooth, solid pieces of metals, plastics, or glass may induce tumors when implanted in the subcutis of rats. Size (surface area) was one important determinant of this type of carcinogenicity. On the basis of results of Alexander and Horning (*10*) relating carcinogenicity to foreign-body size, we chose the maximum size of 5.0 × 5.0 mm to minimize the occurrence of nonspecific tumors. We used fragments (5.0 × 5.0 mm) of tantalum (Ta) (Goodfellow Corp., Berwyn, PA) as a foreign-body control. In addition, the fragments were implanted in muscle, not subcutis, to further minimize nonspecific tumors. Although the data are not clear, the intramuscular route is commonly used to evaluate the carcinogenicity of metals and their insoluble compounds (*11*). Thorotrast, a 25% colloidal thorium dioxide (^{232}Th) radiographic contrast media, was used as a positive control for radioactive materials. The distribution, retention, and carcinogenic effects of Thorotrast have been summarized (*12,13*). Thorotrast is reported to cause granulomas if injected perivascularly, which occurred inadvertently in a few human patients. A small fraction of these lesions developed into sarcomas (*14,15*). The Thorotrast used in this study was produced by Hyden Chemical Corp (New York City, NY) and kindly supplied by J. Humphreys (AEA Technology, Harwell, England).

We used two sources of alpha irradiation of muscle soft tissue in this study, one from the metallic uranium implants and the second from injected colloidal Thorotrast. During the planning stages, the radiation dose rates to tissue within range of the alpha particles emitted by either the uranium- or thorium-series isotopes were estimated as follows.

Alpha spectrometric characterization of the DU used in this study indicated that the only alpha-emitting isotopes present in significant abundance were ^{238}U and ^{234}U; the ^{234}U/^{238}U activity ratio was 0.14. Because the range of the uranium alpha particles in the dense uranium metal is very small (a few micrometers) compared to the dimensions of the implant (e.g., 5.0 × 5.0 × 1.5 mm for the largest implant), it was convenient to measure the alpha-particle flux being emitted from the surfaces of the uranium metal implants. Using a ZnS alpha-particle detector, we measured the emission rate of alpha particles from the uranium at 4,240 disintegrations per minute per square centimeter of implant surface. Assuming an average alpha energy per emitted particle of 4.2 MeV (an acknowledged overestimate because of the infinitely thick source geometry), we calculated a radiation dose rate averaged over a 50-µm tissue thickness surrounding the implant (adequate to capture all of the alpha particle energy emitted by both uranium- and thorium-series isotopes) to be 4.1 rad/day. Although the alpha radiation dose rate varies significantly within the 50-µm thickness of tissue, it was nonetheless useful to calculate this tissue-averaged dose for comparison with that produced by Thorotrast.

Calculating an alpha radiation dose from injected Thorotrast was more complicated than for the uranium metal implants because of the complex thorium decay series of isotopes. When the progeny of ^{232}Th are in equilibrium with the parent, six alpha particles are emitted per decay of ^{232}Th [^{228}Th, ^{224}Ra, ^{220}Rn, ^{216}Po, and either ^{212}Bi (36%) or ^{212}Po (64%)]. Because the Thorotrast used in this study was produced during the 1950s and remained in sealed vials until its use in this study, it was reasonable to assume that ^{232}Th and its progeny were essentially in equilibrium before use. However, after intramuscular injection, it was no longer appropriate to assume that the radioactive progeny of ^{232}Th remain at the injection site, because a fraction of the nonthorium progeny exists in the solution phase rather than in the Thorotrast particles, and they were transported from the injection site as soluble species; and because newly created progeny had finite probabilities of being ejected from the Thorotrast particles by recoil mechanisms, and thus also become available for translocation as soluble atoms. Parr et al. (*16*) measured the state of equilibrium of the progeny of ^{232}Th in various tissues from Thorotrast-injected humans, dogs, and rats and found that the steady-state ratios of isotopes were not related to species for important tissues such as liver, spleen, and red bone marrow—the major deposition/retention sites for intravenously injected Thorotrast. Therefore, we used the following ratios obtained from Parr et al. (*16*) to calculate alpha radiation doses: ^{228}Ac/^{228}Ra = 0.97, ^{228}Th/^{228}Ra = 0.89, ^{224}Ra/^{228}Th = 0.53, ^{212}Pb/^{224}Ra = 0.48, and ^{212}Bi/^{212}Pb = 0.70. The only ratio from Parr et al. that was not used was the ^{228}Ra/^{232}Th of 0.27. Because the Thorotrast used in this study was maintained for about 40 years in a sealed vial, we assumed a ^{228}Ra/^{232}Th ratio of 1.0. Therefore, the number of alpha particles emitted per disintegration of ^{232}Th was 2.77, and the total amount of alpha-particle energy available for deposition at the wound site was 15.74 MeV per disintegration of ^{232}Th. To calculate the alpha-radiation dose rate soon after injection, we assumed a spherical geometry of the injected Thorotrast, with an initial volume of 0.025 cm^3, and containing 1,510 dis min^{-1} ^{232}Th. This geometry yielded a sphere of radius 1.8 mm, which, like the uranium metal implants, is an infinitely thick source for alpha particle emission. We assumed that alpha particles could be emitted only from a 50-µm-thick shell on the outer surface of the Thorotrast aggregate, and that roughly 50% of the emitted alpha particles within this shell reached the tissue. Using these assumptions, 230 MeV/min was deposited in a 50-µm-thick shell of tissue immediately in contact with the Thorotrast deposit. This corresponds to 10.6 rad/day, which was about 2.6 times the dose rate from the uranium implants.

Calculations done using other assumed tissue-source geometries showed that the dose rate was not sensitive to the manner in which the uranium or Thorotrast was distributed in the tissue, provided that size

Table 1. Physical characteristics of implants.

Type of implant	Size	Volume (mm^3)	Mass (mg)	Surface area (mm^2)	E alpha[a] (Bq)
DU (0.75 Ti) pellet	2.0 × 1.0 mm[b]	1.6	30	7.9	6
DU (0.75 Ti) fragment	2.5 × 2.5 × 1.5 mm	9.4	175	27.5	20
DU (0.75 Ti) fragment	5.0 × 5.0 × 1.5 mm	37.5	698	80	59
Ta fragment	5.0 × 5.0 × 1.1 mm	27.5	456	72	–
Thorotrast (injection)	0.05 mL	50	12.5	–	115

[a]E alpha: calculation of effective alpha-particle radioactivity emanating from the surface of the DU or Thorotrast. [b]Diameter.

Table 2. Experimental design: carcinogenesis study of DU (Ti) pellets and fragments intramuscularly implanted in Wistar rats.

Type of implant	Size	No. of implants	Total no. of rats
DU (Ti) pellet	2.0 × 1.0 mm[a]	4	50
DU (Ti) fragment	2.5 × 2.5 × 1.5 mm	4	50
DU (Ti) fragment	5.0 × 5.0 × 1.5 mm	4	50
Ta fragment	5.0 × 5.0 × 1.1 mm	4	50
Thorotrast (injection)	0.05 mL	2	50
Sham implant surgery	NA	0	50
Total no. of rats	–	–	300

NA, not applicable.
[a]Diameter.

of the uranium or Thorotrast particles remained essentially infinitely thick. What did change, however, was the number of cells or the size of the tissue at risk. That is, as the radiation source becomes more distributed—by deformation, by fragmentation, or by dispersion amid the soft tissue—the number of cells within range of the alpha particles increases. For example, assuming that the Thorotrast deposit spread out uniformly as a 50-µm-thick square plate in the muscle tissue, we can calculate that the dose rate becomes 10.9 rad/day, compared to 10.6 rad/day in the spherical geometry. However, the volume of the tissue at risk increased from 0.50 mm^3 for the spherical geometry to 25 mm^3 for the dispersed source—a factor of 50 greater.

We treated six groups of 50 male Wistar rats with one of the following: DU pellets (2.0 mm length 1.0 mm diameter), DU fragments (2.5 × 2.5 × 1.5 mm), DU fragments (5.0 × 5.0 × 1.5 mm), Thorotrast injection (0.050 mL), Ta (5.0 × 5.0 × 1.1 mm), or sham surgery (Table 2). We purchased 300 12-week-old male Wistar rats (Charles River Laboratories, Wilmington, MA) and maintained them in the Lovelace Respiratory Research Institute (LRRI) animal facility. The rats were housed, two per cage, in filter-topped polycarbonate cages on hardwood chip bedding. Animal rooms were maintained at 20–22°C with a relative humidity of 40–60%. Teklad Certified Rodent Diet (Harlan Teklad, Madison, WI) and water were available *ad libitum*. Cages and bedding were changed twice per week. The rats were identified by tail tattoo and placed randomly into groups of 50 rats.

Before implantation surgery, the DU pellets, DU fragments, and Ta fragments were weighed, cleaned to remove the oxide formation, and sterilized. Cleaning and sterilization comprised immersion in an industrial detergent, rinsing in absolute ethyl alcohol, immersion in a 50% nitric acid solution for 3 min, and rinsing with sterile water. All pieces were stored in absolute ethyl alcohol to inhibit oxidation. The DU and Ta fragments were implanted in the biceps femoris muscle of each hind leg, two fragments per leg. We used sterile procedures to incise the skin and muscle for the implant site and closed the incisions with two absorbable sutures in the muscle and three surgical wound clips in the skin. Because Thorotrast is a colloid suspension, it was injected into the biceps femoris muscle (two injections of 0.05 mL, one in each hind leg).

After implantation of the fragments, the rats were held for their life span. They were weighed every other month. Radiographs of the implant sites were performed on all rats at time of implantation and at death. We selected representative animals for radiographs at 3–4 weeks after implantation. Rats were checked twice daily, and those moribund or in distress were euthanized with pentobarbital. At death or euthanasia, complete necropsies were performed with examination of the implant sites and draining lymph nodes and of all organ systems by opening the abdominal, thoracic, and cranial cavities. Representative tissue sections were taken, fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin. Histologic examination was routinely performed on the implant sites, the draining lymph nodes, implant site neoplasms, gross lesions that were potential neoplasms, and the kidneys, urinary bladder, prostate, seminal vesicles, testicles, epididymus, spleen, muscle, liver, and lungs.

We analyzed one kidney and the carcass (depelted and eviscerated) from each rat for uranium using the Kinetic Phosphorescence Analyzer, KPA 11 (Chemchek Instruments, Inc., Richland, WA). The lower limit of detection was 50 ng/L. We prepared the samples for analysis by dry ashing at 550°C, wet digestion in concentrated nitric acid, treating with 3 M hydrofluoric acid and 0.2 M H_3BO_3, and dilution in 1 N nitric acid.

We compared the weights of each exposure group by fitting the quadratic weights curves for each rat using PROC NLIN (SAS Version 8.0; SAS Institute, Cary, NC). Three rats were not used due to incomplete data—two from the Ta group and one from the 2.5 × 2.5 × 1.5 mm DU group. We produced and compared parameter estimates using analysis of variance and estimated the survival distribution function for each group using the nonparametric Kaplan-Meier method. We used the log rank and the Wilcoxon tests to evaluate homogeneity across all groups. If homogeneity was rejected ($p < 0.05$) by either test, pair-wise comparisons were made (SAS Proc, Version 8). We compared the differences in the tumor incidences among the various exposure groups using Fischer's exact test (Sigma Stat for Windows Version 1.0; SPSS Science, Chicago, IL).

Results

The body weights for the various exposure groups are shown in Figure 1. The increase in weight in early life with a decrease in later life is a pattern often observed among male rats (*17*). None of these weight curves is statistically different from the sham control weight curve.

The survival of the various exposure groups did not differ significantly (Figure 2). The median survival time for the six groups ranged from 576 to 620 days after implantation. The similarity of the survival of the six groups eliminated a competing risk when determining tumor incidence.

The radiographic appearance of the implanted DU fragments changed markedly during the first year. At the time of implantation, the fragments were smooth squares with regular, sharp, well-defined edges (Figure 3A). At 21 days after implantation, small, dense blebs extended from the edges of the fragments, making them appear larger than at time of implantation. The jagged appearance disrupted the sharp profile of the

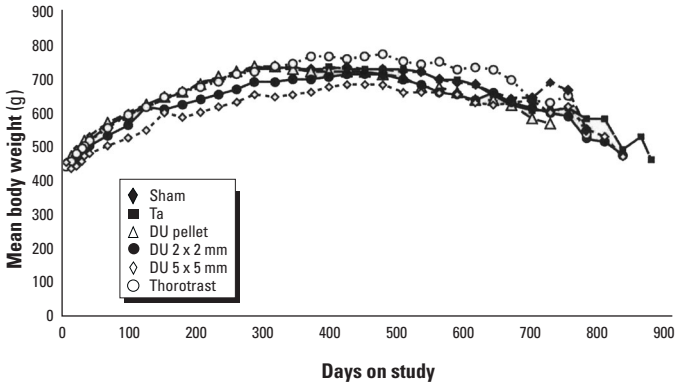

Figure 1. Body weights of rats implanted with DU or Ta fragments or injected with Thorotrast, compared with sham controls.

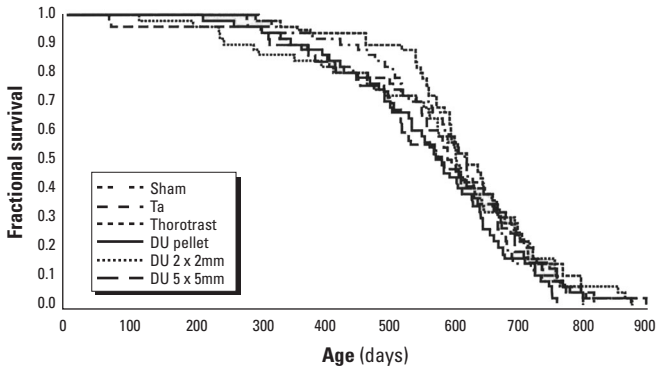

Figure 2. Survival of rats implanted with DU or Ta fragments or injected with Thorotrast compared with sham controls.

fragments (Figure 3B). At 1 year after implantation, the radiographic profiles of the fragments were rounded, with no corners and a fine, jagged edge (Figure 3C). At time of death, many of the profiles were enlarged up to 1.5 times in linear dimensions. These radiographic changes were related to surface corrosion of the DU *in vivo* and capsule formation. In contrast, the profiles of the Ta fragments were smooth at all times with sharp, well-defined edges and did not increase in size with time (Figures 4A and B). Initially, the Thorotrast injections gave a spherical radiographic outline (Figure 5A). At 4 weeks the profile was irregular and diffuse, with no distinct boundary (Figure 5B). The profiles had a similar appearance at 1.5 years after injection (Figure 5C).

The implants of DU or Ta were encapsulated with connective tissue at the time of death. Most of the fragments were located in the muscles where they were originally implanted, but some had migrated to the loose connective tissues between the muscles of the leg. The injected Thorotrast did not induce capsule formation, but was localized in and around the muscles causing a tan discoloration. Before histologic sectioning of the capsules, the tissues were fixed in 10% neutral buffered formalin, and the implants were removed. The Ta fragments slipped easily from the capsules. The DU fragments, however, adhered to the capsules and were difficult to remove from the tissues. The surfaces of these fragments were friable. A black, gritty layer of granular material was left on the inner surface of the capsule.

The capsules around the DU implants were characterized histologically by fibrosis, inflammation, degeneration, and mineralization (Figure 6A). Around the large DU implants, the capsules were up to 0.5 mm thick and composed of dense fibrous tissue. More typically, the capsules were 0.1–0.2 mm thick. Shards of black material were embedded in the fibrous tissue capsules around the medium and large fragments. Smaller particles in a range of sizes (< 4 μm) were found in the capsule wall around all sizes of DU implants. Chronic inflammatory cells and particle-laden macrophages were frequently scattered throughout the capsule wall of the DU implants. Occasionally foreign-body giant cells were found. However, the amount of chronic inflammation in and around the capsules was generally similar among DU implants of all sizes. Degeneration of the fibrous tissue in the capsule wall was frequent at the interface with the implant. With this reaction, the tissue on the inner surface of the capsule was devitalized and necrotic. The lumen of the capsules contained necrotic and proteinaceous debris, scattered acute inflammatory cells, and varying amounts of black shards or particles. The particles were contained in macrophages or free in the lumen as amorphous clumps of various sizes. Mineralization of necrotic debris or devitalized fibrous tissue on the inner wall of the capsules was common.

The capsules around the Ta implants were characterized by fibrosis with little inflammation and no degeneration or mineralization (Figure 6B). The capsule walls were less than 0.1 mm thick with a smooth inner surface. No shards or particles were present.

The Thorotrast lesion was an accumulation of macrophages between muscle fibers and adjacent to muscles (Figure 6C). The macrophages were filled with a tan, coarsely granular material. These macrophages were not associated with inflammation or fibrosis.

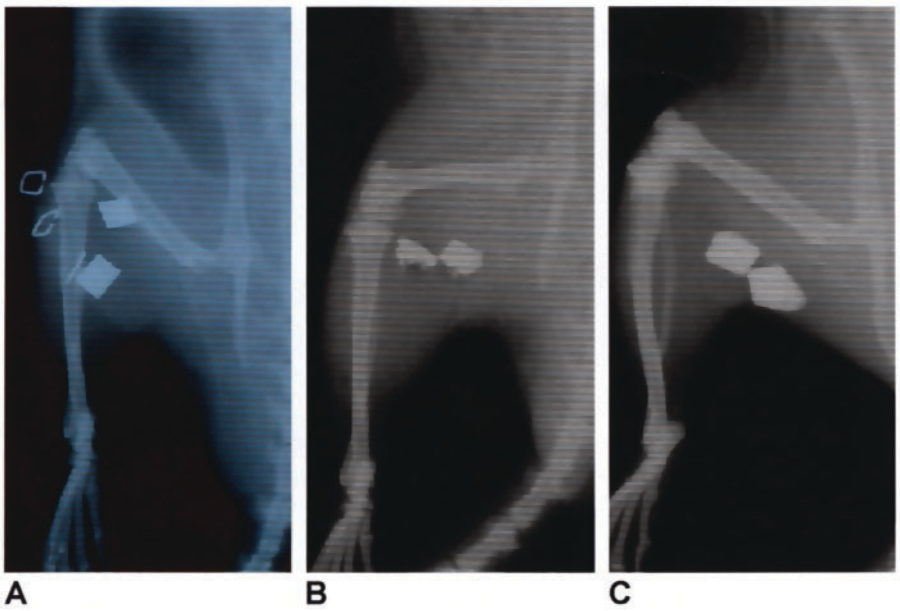

Figure 3. Radiograph of 5.0 × 5.0 × 1.5 mm DU fragments in rat R097-604. (*A*) On day of implantation (radiopaque wound clips visible on the skin); (*B*) 3 weeks after implantation; (*C*) 1 year after implantation.

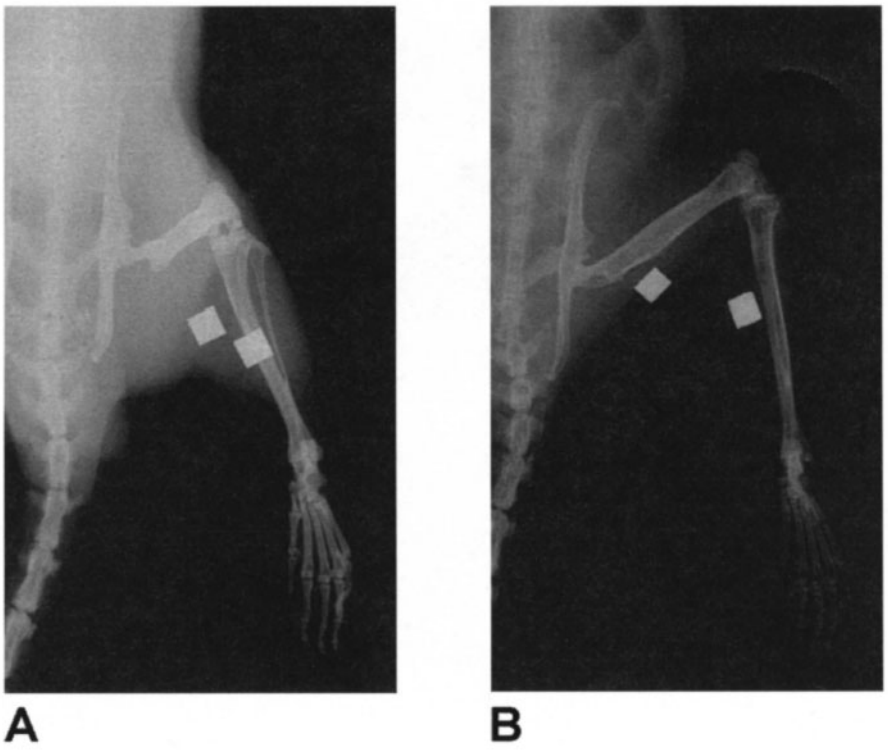

Figure 4. Radiograph of 5.0 × 5.0 × 1.1 mm Ta fragments. (*A*) 10 weeks after implantation (rat B020-6024); (*B*) 2 years after implantation (rat N026-6036).

Soft tissue tumors of various types were associated with many of the implants. The tumors were classified histologically according to criteria of the Society of Toxicologic Pathologists (*18*) (Table 3). The most commonly found tumor types were malignant fibrous histiocytomas and fibrosarcomas. The three osteosarcomas were not associated with the skeleton. Although there was a higher number of fibrosarcomas in the Thorotrast-treated rats and a broader range of tumors in the DU-treated rats, we could not attribute a specific tumor type to a specific treatment. All of the tumors, however, were most likely derived from primitive mesenchymal stem cells (*19*).

All tumors were in the soft tissues of the hind legs, directly associated with the implanted DU or Ta fragments or the injected Thorotrast. Three tumors were localized to the wall of the fibrous capsules surrounding the implants. In other tumors, black shards, particles of implanted fragments, or Thorotrast-filled macrophages could be seen scattered through most of the tumor tissues. These histologic findings lend further credence to the association of the implants with the tumors.

Biologically, these tumors were moderately aggressive (Table 4). Many were growing rapidly, expanding in size from barely palpable to 3 or 4 cm in 2 weeks. However, none invaded bone and only one ulcerated the skin. One metastasized to an iliac lymph node and another metastasized widely. Twenty-nine of the 40 were large enough to result in euthanasia. The other 11 were discovered at necropsy or in tissue sections of the capsules surrounding the implants.

The incidence of the tumors increased in the rats with the largest DU implants when compared with the sham or foreign-body (Ta) controls (Figure 6). The difference was significant ($p \leq 0.028$) with a Fischer's exact test. The radioactive-materials control animals, injected with Thorotrast, had a significant increase in number of tumors compared with the DU-implanted rats ($p < 0.0014$). In addition, there was a fragment size-related response in the DU-treated rats. The response could not be explained by physical surface area alone because the tumor incidence with the Ta implants of similar size was much lower. There was, however, a correlation with the initial surface alpha radioactivity (Figure 7). This initial activity was calculated from the physical characteristics of the DU fragments and the Thorotrast colloid. The injected colloid was considered a sphere for calculation purposes. Radiographs showed that the physical shape of the fragments and the colloid changed within 4 weeks after implantation. Thus, the surface alpha radioactivity changed with time as the shape of the implants changed.

Comparison of the radiographs of the DU-associated lesions with the histologic appearance of the lesions showed a correlation. After being implanted for a year or more, all of the DU fragments were enlarged and rounded in radiographic profile (Figure 8A). These features generally correlated with a dense, connective tissue capsule. However, disruption of the smooth-edge profile and focal loss of density in the DU fragment were associated with proliferative lesions or small tumors in the capsules (Figure 8B). Disintegration and breakup of the DU fragment were apparent on radiographs when frank tumors were present. On the histologic sections, black shards of DU could be seen throughout the tumor tissue (Figure 8C). These radiographic changes associated with the DU fragments may be important indicators of accompanying proliferative lesions and may have prognostic value in clinical evaluations.

The tumors in tissues not associated with the implant sites are noted in Table 5. There was no significant increase related to exposure in any tissue routinely sampled or in tissue

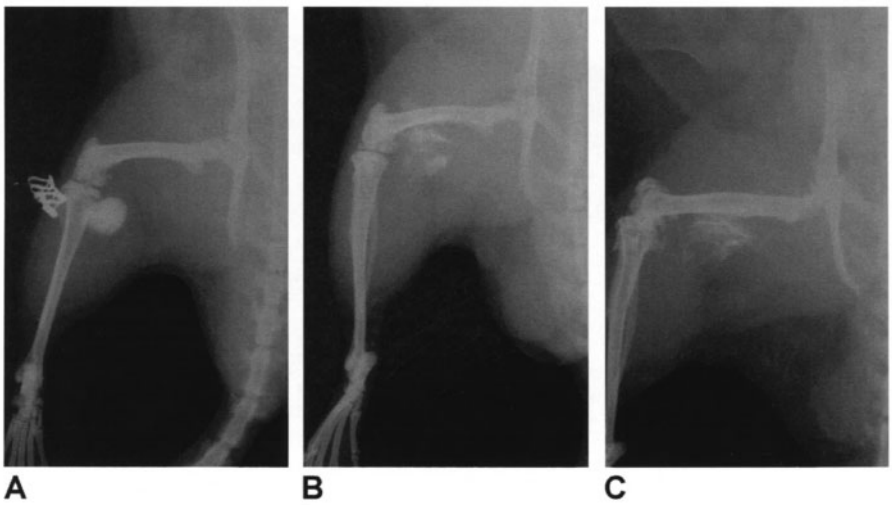

Figure 5. Radiograph of Thorotrast injection in rat I047-6037. (*A*) On day of injection; (*B*) 4 weeks after injection; (*C*) 1.5 years after injection.

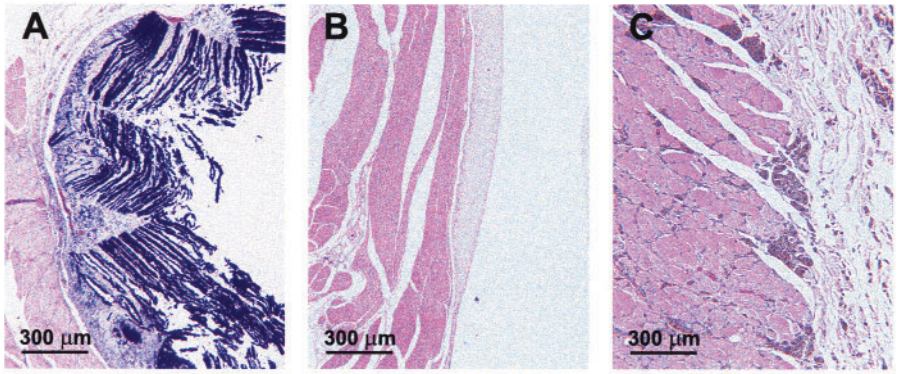

Figure 6. Tissue reaction to implants. (*A*) DU fragment: black shards of corroded DU lined the thick cellular fibrotic capsule surrounding the fragment 520 days after implantation. (*B*) Ta fragment: a thin acellular fibrotic capsule with no metal pieces present 603 days after implantation. (*C*) Thorotrast injection: no capsule or inflammation; Thorotrast-laden macrophages infiltrated skeletal muscle 792 days after injection.

Table 3. Number of implant-associated soft tissue tumors.

Tumor types	Thorotrast	DU	Ta	Surgical control
Benign tumors				
Benign fibrous histiocytoma	0	1	0	0
Fibroma	0	1[a]	0	0
Granular cell myoblastoma	1	0	0	0
Malignant tumors				
Malignant fibrous histiocytoma	13	7[a]	2	0
Fibrosarcoma	10	2	0	0
Osteosarcoma	1	2	0	0

[a]One rat had two tumors associated with separate implants.

samples when gross lesions were noted. An increased incidence of renal tumors was seen only in those rats with implants of DU. The incidence was not statistically significant, however, even when all groups implanted with DU fragments combined were compared with all three control groups (sham, Ta, Thorotrast) combined ($p > 0.06$ with Fischer's exact test). The renal tumors were classified as tubular cell adenoma (*1*), tubular cell adenocarcinoma (*3*), and sarcoma (*1*).

Radiochemistry of the kidney and carcass (Table 6) showed that the concentration of uranium in the kidney at time of death varied within the various groups as shown by the wide standard deviations. The mean renal concentrations in the two largest fragments were not significantly different, despite the differences in mass (~3×) and surface area (~2×) of the fragments. The concentration of uranium in the carcass showed an increased concentration with the increased mass of uranium. The fraction of the embedded fragments found in the kidney decreased with mass and surface area. In contrast, the fraction of the fragments in the carcass was nearly constant among the three groups.

Discussion

These findings clearly indicate that DU fragments of sufficient size were carcinogenic in the muscles of rats. The incidence of soft tissue neoplasms was significantly greater in rats with DU implants than in rats with foreign-body control (Ta) implants. The neoplasms induced were associated directly with the implants; no increase in tumors was noted elsewhere in the body.

The validity of injecting or implanting materials and compounds in the subcutis or muscles of rodents to test for carcinogenicity in humans has been questioned (*11*). The concern is that tissue reactions in rodents are seen with many materials, may be nonspecific, and may not occur in humans. Several compounds or materials injected or implanted in the subcutis of rats caused localized cancer but were later determined not to cause cancer in humans. Examples are certain food colorings (*20*), iron dextran (*21*), and silicone implants with breast cancer (*3*). In addition, seemingly innocuous materials placed in the subcutis have caused localized cancers—materials such as aluminum foil, glass sheets, and methyl cellulose filters of a certain size (*9*).

Foreign-body carcinogenesis has been induced experimentally in the soft tissues of rats by a number of sheet-like implanted materials, indicating that rats are sensitive to this type of carcinogenesis (*22–25*). The physical nature of the sheets was more important than the chemical nature in determining carcinogenesis (*9*). The size of the sheet (> ~ 5.0 × 5.0 mm), physical smoothness (a noncorroded surface), and continuity (no holes in the sheet) were three key physical parameters. A sheet of sufficient size to be carcinogenic could be rendered noncarcinogenic by cutting it into smaller pieces, perforating it with numerous holes, or roughening the surface. The degree of inflammation also played a role. The greater the inflammatory response or the longer it persisted in the tissues, the less likely the sheetlike material would induce soft tissue tumors.

Tests using rats, however, can be reasonable predictors of the carcinogenicity of materials in humans. Numerous metal powders and metal implants have been tested by injection or embedding in the muscles (not the subcutis) of rats (Table 7). A range of localized tissue responses was noted related to chemical composition or size. These findings indicated that not all metals injected or implanted in the muscles of rats induce localized tumors and that the elemental composition was important for metal powders, and the size (or surface area) was important for the metal squares, discs, or rods.

Table 4. Biologic characteristics of implant-associated soft tissue tumors.

Tumor types	Total no. of tumors	Relationship to death		No. with metastasis	Median survival time (days)	Range (days)
		COD	INC			
Benign tumors						
Benign fibrous histiocytoma	1	0	1	0	515	–
Fibroma	1	0	1	0	601	–
Granular cell myoblastoma	1	0	1	0	769	–
Malignant tumors						
Malignant fibrous histiocytoma	22	18	4	0	668	518–987
Fibrosarcoma	12	8	4	1	614	515–853
Osteosarcoma	3	3	0	1	572	493–645

Abbreviations: COD, cause of death or euthanasia; INC, incidental.

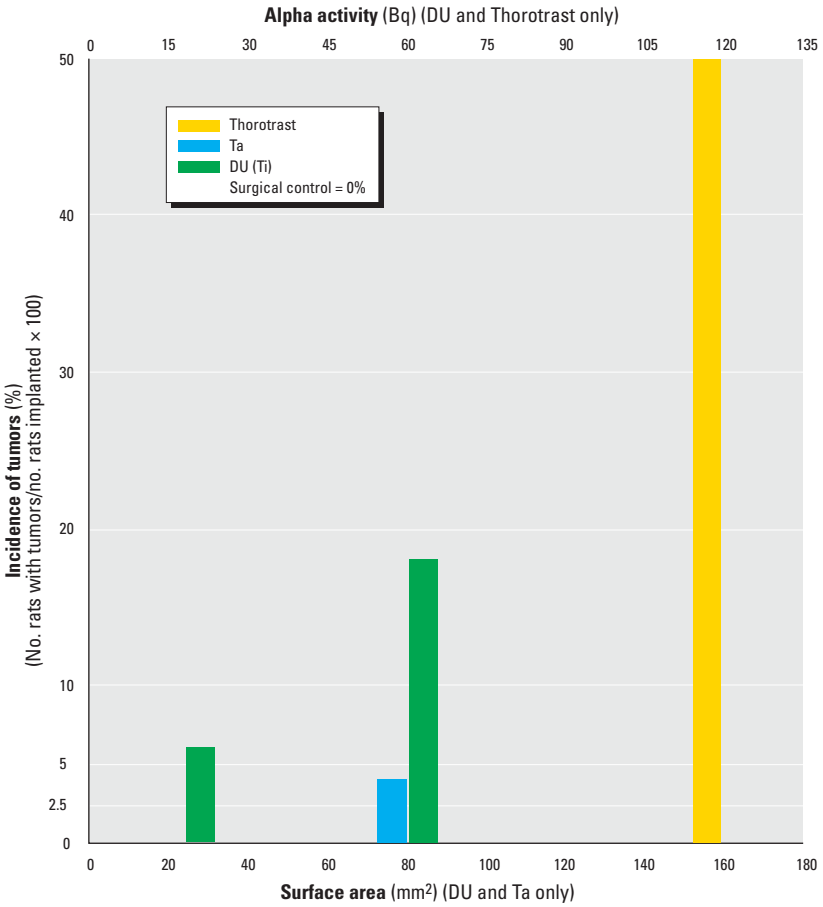

Figure 7. Incidence of soft tissue tumors in rats implanted with DU, Ta, or Thorotrast. The incidence of tumors in rats with 5.0 × 5.0 mm DU fragments significantly increased over the incidence in rats with Ta fragments of the same size. The positive control, Thorotrast, produced the highest incidence of tumors. Thorotrast > DU 5.0 × 5.0 mm, $p = 0.0014$; DU 5.0 × 5.0 mm > sham control, $p = 0.0012$; DU 5.0 × 5.0 mm > Ta, $p = 0.028$ (Fischer's exact test).

For the metal powders, the incidence of localized tumors ranged from 0 to 100%. The highest incidence was with nickel, a known human carcinogen. The lowest incidences were with metals with low carcinogenicity, titanium, lead, and chromium metal. For implanted materials, there appeared to be a relationship to the size or surface area. For example, all of the metals with surface areas of 44 mm^2 or less did not induce localized tumors, except DU 2.5 × 2.5 mm fragments with 28 mm^2. The stainless steel implant with 75 mm^2 had a tumor incidence of 5%, essentially the same as the tumor incidence for Ta fragments with 72 mm^2. On the other hand, the DU 5.0 × 5.0 mm fragments with nearly the same surface area of 80 mm^2 had a tumor incidence of 18%.

The finding of DU fragment carcinogenicity in rats does not indicate, however, that DU fragments are necessarily carcinogenic in humans. Artificial implants and accidental foreign-body material are associated with occasional soft tissue sarcomas in humans, although precise figures are not available (*35*). It has been argued that the rate must be low because of the small number of implant-associated soft tissue sarcomas reported in the literature despite the frequent use of various implants for medical purposes (*36*). The sole epidemiologic study of artificial implants and soft tissue sarcomas used a case–control approach with a population of 217 Vietnam veterans with soft tissue sarcomas (*37*). Respondents noted whether they had an implant of an artificial joint, pin, plate, staple, screw, or any other metal or plastic implant. None had an artificial joint. In that study, no association was found between soft tissue sarcomas and the implants.

Uranium compounds have not been demonstrated to cause cancers in humans, even as a result of high occupational exposures (*38*). Over 30,000 persons occupationally exposed in U.S. Department of Energy contractor laboratories have been followed in epidemiologic studies with no health effects reported. These exposures occurred primarily by inhalation or ingestion, not by the embedding of metal fragments. Thus, the situation of the Gulf War veterans who were wounded with DU fragments is unique.

The mechanism by which DU fragments induced localized cancers in rats may be important in determining the carcinogenicity of DU fragments in humans. The Ta fragments used here fit two criteria for foreign-body carcinogenesis: smoothness and continuity of the sheet. Little inflammation was induced by the Ta fragments, and the fibrous capsule was thinner than the capsule around the DU fragments. The size (5.0 × 5.0 mm) was smaller than that reported necessary for foreign-body carcinogenesis in rats (*10*). However, the tumor incidence was essentially the same as that for stainless steel disc implants of similar surface area (Table 7). The soft tissue tumors related to the Ta fragments in this study were most likely induced by the foreign-body mechanism described in the rat (*9*).

The Thorotrast injections induced a high incidence of soft tissue sarcomas, indicating that rats are sensitive to carcinogenesis from radioactive materials implanted in the muscles. The ^{232}Th in Thorotrast is radioactive. The radioactive decay chain from ^{232}Th produces about six alpha particles per disintegration with an average total energy of about 26 MeV. The calculated radiation dose to the local tissue around the injection site in the rats is about 0.5 Gy per year when the injection is a sphere with the volume of 0.50 mm^3. The Thorotrast did not stay in a sphere, but over a period of weeks spread out between the muscle bundles, where it remained for years. Thus, the calculated radiation dose is only an estimate, but is probably low compared to the real value. This annual dose is lower than the estimate of > 1 Gy per year for the human patients that had Thorotrast inadvertently injected into the paravascular soft tissues (*15*). These individuals developed intense sclerotic lesions in the soft tissues, and one developed a soft tissue sarcoma. In the Wistar rats, essentially no inflammatory reaction, fibrotic reaction, or proliferative lesion was seen in response to the injected Thorotrast. However, the earliest deaths where tissues could be examined were at about 300 days after injection, so an early, transient inflammatory reaction may have been overlooked.

The DU fragments did not appear to cause soft tissue sarcomas by the foreign-body mechanism so well described in rats (*9,39*). The surface of the DU fragments, although initially smooth, quickly became corroded and roughened. In addition, the histologic reaction to DU fragments showed much

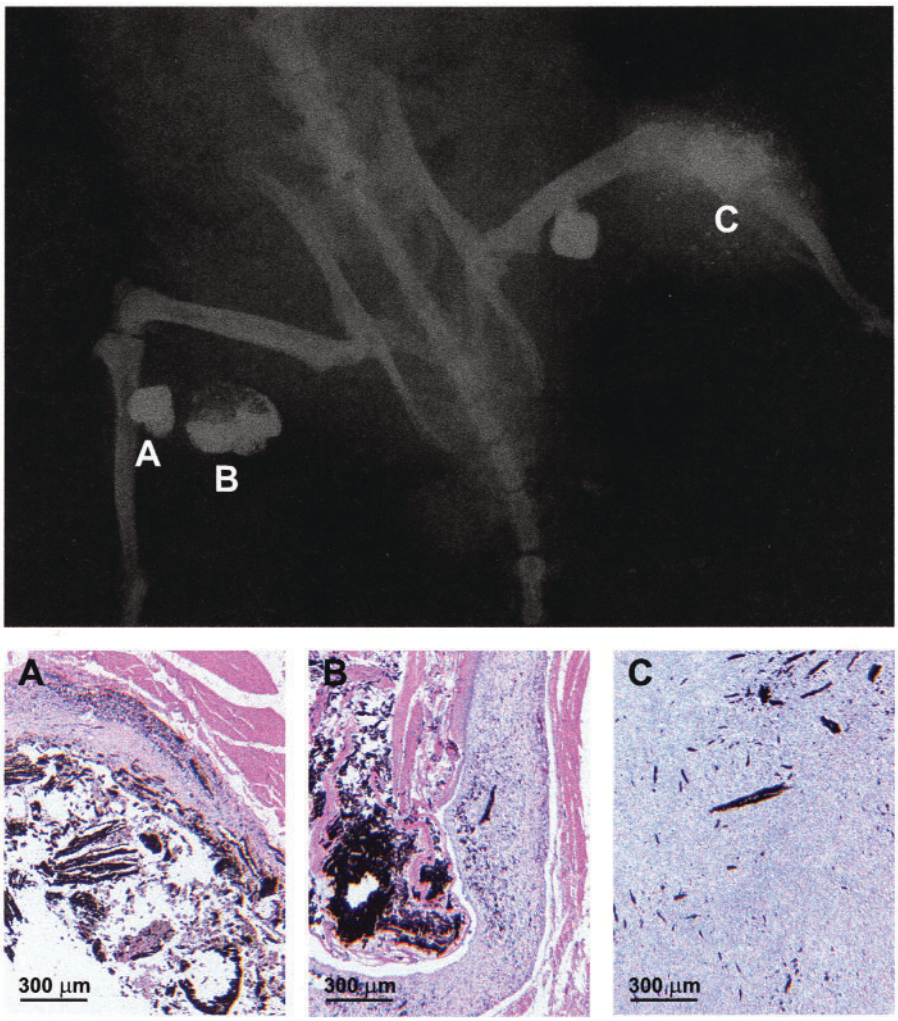

Figure 8. Correlation of radiographic appearance with histologic appearance. (*A*) Thick fibrotic capsule with shards of corroded DU in lumen; (*B*) thick cellular capsule lined by squamous metaplasia, particles, and shards of corroded DU in wall and lumen; (*C*) particles and shards of disintegrated DU fragment scattered throughout a soft tissue sarcoma.

more inflammation and fibrosis than did the lesions associated with Ta fragments. The Ta fragments used in this study and the stainless steel discs used in previous studies (*26*) initiated tumors by the foreign-body mechanism and had a much lower incidence of sarcomas. These differences between the reactions and tumor incidence of DU and Ta fragments or stainless steel discs indicate that something more than foreign-body carcinogenesis is occurring with DU-fragment carcinogenesis.

A radiation mechanism may have played a role in the carcinogenicity of the DU fragments. One indication of this was the increased incidence of soft tissue sarcomas, which was correlated most closely with the increased surface alpha radioactivity of the implanted materials than with the surface area (Figure 7). On the other hand, Thorotrast, which initiated tumors by a radiation mechanism, elicited essentially no reaction in the tissues, a distinct difference from the DU fragments.

The mechanism by which DU fragments induced soft tissue sarcomas is uncertain. One striking feature of the reaction is the corrosion of the DU in the tissues and the intensity of the inflammatory and fibrotic response to the fragments. The response is similar to the local tissue reactions produced by food coloring and other soluble compounds repeatedly injected subcutaneously in rats (*20*). Compounds that produced tissue destruction with subsequent dense collagen formation invariably induced soft tissue sarcomas. Physicochemical properties closely related to tissue destruction were marked surface activity, lipid solubility, and protein-binding ability (*40*). These properties are directly related not to chemical carcinogenesis but to a continual cellular damage and repair mechanism. A similar process may occur with DU-associated soft tissue sarcomas.

Understanding the mechanism by which DU fragments induce tumors in rats is important in determining the likelihood of a similar mechanism occurring in humans. Further research will be required to determine the importance of various mechanisms: foreign body, radiation, chemical, or continued cell damage and repair.

The lack of a significantly increased incidence of tumors other than at the site of the implants indicates that uranium is not an effective systemic carcinogen. The tumor types and incidence were generally similar to those previously described for Wistar rats (*41,42*). The total incidence of primary renal tumors was 4% in each of two DU(Ti) fragment groups (5.0 × 5.0 mm and 2.5 × 2.5 mm). This total incidence was high compared to that previously reported in Wistar rats (*41*). The increase, however, was not statistically significant. In addition, the types of renal tumors found were similar to those occurring spontaneously in Wistar rats. Interestingly, the concentrations of uranium in the kidneys of these two groups were nearly the same, 10.5 and 8.95 μg U/g kidney (Table 6). However, there was not a good correlation of renal uranium concentration and tumor incidence. The renal uranium concentration in the rats with renal tumors ranged from 1.19 to 32.2 μg/kidney.

The above points indicate that the increased incidence of renal tumors is not

Table 5. Number of benign and malignant tumors not associated with implant site.

Site of tumor	Sham	Ta fragment	Thorotrast	DU 2.0 × 1.0 mm	DU 2.5 × 2.5 mm	DU 5.0 × 5.0 mm
Number of rats examined	50	50	50	50	50	49
Muscle	0/0[a]	0/1	0/0	0/0	0/1	0/0
Kidney	0/0	0/0	0/0	0/1	1/1	0/2
Urinary Bladder	0/0	0/2	0/0	0/0	0/1	0/0
Testes	5/0	7/0	3/0	3/0	5/0	5/0
Prostate	0/0	0/0	0/0	0/0	0/0	0/0
Epididymis	0/0	0/0	0/0	0/0	0/0	0/0
Seminal Vesicle	0/0	0/0	0/0	0/0	0/0	0/0
Liver	4/0	4/2	1/1	1/1	4/1	1/2
Spleen	0/6	0/4	0/1	0/2	0/7	0/4
Lung	0/0	0/0	0/0	0/0	0/0	1/0
Number of rats examined[b]						
Bone	0/0	0/0	0/0	0/2	0/0	0/0
Adrenal	1/2	2/1	0/3	0/1	0/2	5/1
Thyroid	1/0	2/2	0/1	3/0	2/0	0/0
Pituitary	6/5	6/2	18/1	6/0	9/3	4/1
Mammary Gland	3/0	3/1	3/0	2/0	4/0	1/0
Pancreas	1/0	2/1	0/4	2/0	4/0	1/2
Skin	7/1	5/2	5/3	5/3	4/1	7/1

[a]Number of benign tumors/number of malignant tumors. [b]Varies; tissues were sampled if gross lesions were present.

Table 6. Concentration of uranium in kidney and carcass at death.

DU fragment group	Concentration of U (μg U/g tissue ± SD)		Kidney/ carcass U concentration	Fraction of fragment in tissue (fraction ± SD)	
	Kidney	Carcass		Kidney	Carcass
2.0 × 1.0 mm	2.72 ± 3.03	0.20 ± 0.18	13	$1.03 \times 10^{-4} \pm 8.29 \times 10^{-5}$	$3.47 \times 10^{-4} \pm 2.95 \times 10^{-4}$
2.5 × 2.5 mm	8.95 ± 5.38	1.28 ± 1.18	7.0	$8.03 \times 10^{-5} \pm 3.87 \times 10^{-5}$	$5.17 \times 10^{-4} \pm 3.02 \times 10^{-4}$
5.0 × 5.0 mm	10.5 ± 6.92	5.20 ± 4.75	2.0	$2.07 \times 10^{-5} \pm 1.07 \times 10^{-5}$	$4.73 \times 10^{-4} \pm 4.29 \times 10^{-4}$

Table 7. Carcinogenesis studies of metals implanted or injected in the muscles of rats.

Material	Form	Size (mm diam. × length)	Mass or surface area	Tumor incidence[a]	Reference
DU–metal	Implant–pellet	2.0 × 1.0	7.9 mm^2	0/50	This study
DU–metal	Implant–square	2.5 × 2.5 × 1.5	28 mm^2	3/50	This study
DU–metal	Implant–square	5.0 × 5.0 × 1.5	80 mm^2	9/49	This study
Tantalum	Implant–square	5.0 × 5.0 × 1.1	72 mm^2	2/50	This study
Stainless steel	Implant–rod	8.0 × 1.6	44 mm^2	0/34	(*26*)
Vitallium–cast or wrought	Implant–rod	8.0 × 1.6	44 mm^2	0/49	(*26*)
Titanium–unalloyed	Implant–rod	8.0 × 1.6	44 mm^2	0/24	(*26*)
Titanium–alloy	Implant–rod	8.0 × 1.6	44 mm^2	0/22	(*26*)
Stainless steel	Implant–disc	4.0 × 1.5	25 mm^2	0/40	(*27*)
Stainless steel	Implant–disc	12.0 × 1.5	75 mm^2	2/37	(*27*)
Stainless steel	Implant–disc	18.0 × 1.5	113 mm^2	5/42	(*27*)
CoCrMo–metal alloy	Powder		28 mg	0/142	(*28*)
CoCrMo–metal alloy	Powder		28 mg	27/72	(*29*)
NiFe–metal	Powder		14 mg	0/20	(*30*)
Cr–metal	Powder		4 mg	0/20	(*30*)
Pb–metal	Powder		95 mg total	1/37	(*31*)
Ti–metal	Powder		23 mg in females or 39 mg in males	2/50	(*32*)
Co–metal	Powder		28 mg	17/30	(*33*)
Ni–metal	Powder		25 mg total	38/50	(*31*)
Ni–metal	Powder		28 mg	10/10	(*34*)

[a]At site of implant or injection.

statistically significant and is probably not biologically significant either. However, a previous report that urine from DU-bearing rats has an increased mutagenic activity (*6*) does require that this finding of an increased renal tumor incidence be investigated further.

The implications of these findings for the medical management and risk assessment among wounded veterans are unclear. The finding of DU implant-associated soft tissue sarcomas in rats indicates a carcinogenic potential for DU fragments. However, given the findings in the controls in this study and the reports in the literature (*3,9*), rats are more sensitive to foreign-body and radiation carcinogenesis than are humans. The findings from these studies cannot be directly extrapolated to humans. More information is needed before risk estimates for humans with embedded DU fragments can be determined.

Prudence, however, requires caution in the medical management of wounded individuals. It may be prudent to use radiography to monitor embedded fragments in wounded individuals. Changes in the radiographic profiles of the fragments that show focal loss or breakdown and changes associated with proliferative lesions in the rats may indicate removal of specific fragments.

References and Notes

1. McDiarmid MA, Keogh JP, Hooper FJ, McPhaul K, Squibb K, Kane R, DiPino R, Kabat M, Kaup B, Anderson L, et al. Health effects of depleted uranium on exposed Gulf War veterans. Environ Res 82(2):168–180 (2000).
2. Harley NH, Foules FC, Hilborne LH, Hudson A, Anthoney CR. A Review of the Scientific Literature as it Pertains to Gulf War Illnesses: Vol 7, Depleted Uranium. MR-1018/7-OSD, RAND. Available: http://www.rand.org/publications/MR/MR1018.7/MR1018.7.html [cited 10 January 2001].
3. McGregor DB, Baan RA, Partensky C, Rice JM, Wilbourn JD. Evaluation of the carcinogenic risks to humans associated with surgical implants and other foreign bodies – a report of an IARC Monographs Programme Meeting. International Agency for Research on Cancer. Eur J Cancer 36(3):307–313 (2000).
4. Pellmar TC, Fuciarelli AF, Ejnik JW, Hamilton M, Hogan J, Strocko S, Emond C, Mottaz HM, Landauer MR. Distribution of uranium in rats implanted with depleted uranium pellets. Toxicol Sci 49(1):29–39 (1999).
5. Miller AC, Blakely WF, Livengood D, Whittaker T, Xu J, Ejnik JW, Hamilton MM, Parlette E, St. John T, Gerstenberg HM, et al. Transformation of human osteoblast cells to the tumorigenic phenotype by depleted uranium-uranyl chloride. Environ Health Perspect 106:465–471 (1998).
6. Miller AC, Fuciarelli AF, Jackson WE, Ejnik EJ, Emond C, Strocko S, Hogan J, Page N, Pellmar T. Urinary and serum mutagenicity studies with rats implanted with depleted uranium or tantalum pellets. Mutagenesis 13(6):643–648 (1998).
7. Committee on Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources. Guide for the Care and Use of Laboratory Animals. NIH Publication No. 86-23. Bethesda, MD:National Institutes of Health, 1985.
8. Daxon EG, ed. Health Consequences of Service During the Persian Gulf War: Initial Findings and Recommendations for Immediate Action, Medical Follow-up Agency, Institute of Medicine. Washington, DC:National Academy Press, 1995.
9. Brand KG, Johnson KH, Buoen LC. Foreign body tumorigenesis. CRC Crit Rev Toxicol 4:353–394 (1976).
10. Alexander P, Horning ES. Observations on the Oppenheimer method of inducing tumors by subcutaneous implementation of plastic films. In: Ciba Foundation Symposium on Carcinogenesis. Mechanisms of Action (Wolstenholme GEW, O'Connor M, eds). Boston, MA:Little, Brown & Co., 1959;12–25.
11. Furst A. Bioassay of metals for carcinogenesis: whole animals. Environ Health Perspect 40:83–91 (1981).
12. van Kaick G, Karaoglou A, Kellerer AM, eds. Health Effects of Internally Deposited Radionuclides: Emphasis on Radium and Thorium. Singapore:World Scientific, 1995.
13. Machinami R, Ishikawa Y, Boecker BB, suppl. eds. The International Workshop on the Health Effects of Thorotrast, Radium, Radon and Other Alpha-Emitters 1999. Radiat Res 152:S3–S144 (1999).
14. Dahlgren S. Effects of locally deposited colloidal thorium dioxide. Ann NY Acad Sci 145:786–790 (1967).
15. Liebermann D, Luehrs H, van Kaick G. Late effects by paravascular thorotrast deposits. In: Health Effects of Internally Deposited Radionuclides: Emphasis on Radium and Thorium (van Kaick G, Karaoglou A, Keller AM, eds). Singapore:World Scientific, 1995;271–274.
16. Parr RM, Lucas HF Jr, Griem ML. Metabolism of ^{232}Th decay series radionuclides in man and other animals following intravascular administration of Thorotrast. In: Argonne National Laboratory Radiological Physics Division Annual Report. DOE Document No. ANL-7615. Argonne, IL:Argonne National Laboratory, 1969;97–115.
17. Rao GN, Haseman JK, Grumbein S, Crawford DD, Eustis SL. Growth, body weight, survival, and tumor trends in F344/N rats during an eleven-year period. Toxicol Pathol 18:61–70 (1990).
18. Greaves P, Faccini JM, Courtney CL. Proliferative lesions of soft tissues and skeletal muscle in rats, MST-1. In: Standardized System of Nomenclature and Diagnostic Criteria. Guides for Toxicologic Pathology: Proliferative Lesions in Rats. Washington, DC:STP/ARP/AFIP, 1992;Sect. VIII.A.
19. Brooks JJ. The significance of double phenotypic patterns and markers in human sarcomas. A new model of mesenchymal differentiation. Am J Pathol 125(1):113–123 (1986).
20. Grasso P, Golberg L. Subcutaneous sarcoma as an index of carcinogenic potency. Food Cosmet Toxicol 4:297–320 (1966).
21. Baker SB, De C, Golberg L, Martin LE, Smith JP. Tissue changes following injection of iron-dextran complex. J Pathol Bacteriol 82:453–463 (1961).
22. Greaves P, Martin J-M, Rabemampianina Y. Malignant fibrous histiocytoma in rats at sites of implanted Millipore filters. Am J Pathol 120:207–214 (1985).
23. Autian J, Singh AR, Turner JE, Hung GWC, Nunez LJ, Lawrence WH. Carcinogenesis from polyurethans. Cancer Res 35:1591–1596 (1975).
24. Oppenheimer BS, Oppenheimer ET, Danishefsky I, Stout AP. Carcinogenic effect of metals in rodents. Cancer Res 16:439–441 (1956).
25. O'Gara RW, Brown JM. Comparison of the carcinogenic actions of subcutaneous implants of iron and aluminum in rodents. J Natl Cancer Inst 38:947–957 (1967).
26. Gaechter A, Alroy J, Andersson GBJ, Galante J, Rostoker W, Schajowicz F. Metal carcinogenesis. J Bone Jt Surg 59A:622–624 (1977).
27. Stinson NE. The tissue reaction induced in rats and guinea-pigs by polymethylmethacrylate (acrylic) and stainless steel (18/8/Mo). Br J Exp Pathol 45:21–29 (1964).
28. Meachim G, Pedley RB, Williams DF. A study of sarcogenicity associated with Co-Cr-Mo particles implanted in animal muscle. J Biomed Mater Res 16:407–416 (1982).
29. Swanson SA, Freeman MA, Heath JC. Laboratory tests on total joint replacement prostheses. J Bone Jt Surg 55B:759–773 (1973).
30. Sunderman FW Jr. Recent advances in metal carcinogenesis. Ann Clin Lab Sci 14:93–122 (1984).
31. Furst A, Harding-Barlow I. Interaction of metals and tissues. Adv Mod Toxicol 1(Part 2):97–118 (1979).
32. Furst A. Trace elements related to specific chronic diseases: cancer. Geol Soc Am Mem 123:109–130 (1971).
33. Heath JC. The production of malignant tumors by cobalt in the rat. Br J Cancer 10:660–673 (1956).
34. Heath JC, Daniel MR. The production of malignant tumours by nickel in the rat. Br J Cancer 18:261–264 (1964).
35. Jennings TA, Peterson L, Axiotis CA, Friedlaender GE, Cooke RA, Rosai J. Angiosarcoma associated with foreign body material. A report of three cases. Cancer 62(11):2436–2444 (1988).
36. Brand KG. Do implanted medical devices cause cancer? J Biomater Appl 8(4):325–343 (1994).
37. Morgan RW, Elcock M. Artificial implants and soft tissue sarcoma. J Clin Epidemiol 48(4):545–549 (1995).
38. Harley NH. The 1999 Lauriston S. Taylor lecture – back to background: natural radiation and radioactivity exposed. Health Phys 79:121–128 (2000).
39. Bishoff F, Bryson G. Carcinogenesis through solid state surfaces. Prog Exp Tumor Res 5:85–133 (1964).
40. Gangolli SD, Grasso P, Golberg L. Physical factors determining the early local tissue reactions produced by food colourings and other compounds injected subcutaneously. Food Cosmet Toxicol 5:601–621 (1967).
41. Bomhard E. Frequency of spontaneous tumors in Wistar rats in 30 month studies. Exp Toxicol Pathol 44:381–392 (1992).
42. Tucker MJ. Diseases of the Wistar Rat. Bristol, PA:Taylor and Francis, 1997.