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PRINCIPAL INVESTIGATOR: Kimberly A. Benson, Ph.D.

CONTRACTING ORGANIZATION: Henry M. Jackson Foundation
Rockville, Maryland 20852

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K. Benson

PI - Signature Date

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EVALUATION OF THE HEALTH RISKS OF EMBEDDED DEPLETED URANIUM (DU) SHRAPNEL ON PREGNANCY AND OFFSPRING DEVELOPMENT

Introduction

Our laboratory is currently assessing the toxicity of embedded depleted uranium on the female rat. This research is intended to answer questions that have arisen following Operation Desert Storm. During this conflict a number of U.S. military personnel were wounded by depleted uranium fragments. Many of these fragments were not removed because the removal procedure would produce excessive tissue damage. Uranium bioassays taken over a year after injury indicate that uranium was present in the urine well in excess of natural background, up to 30 µg U/l of urine. While no female soldiers currently have depleted uranium injuries, military roles are changing significantly and the female soldier now plays a vital part in many combat scenarios and the potential exists for future DU injuries in the female soldiers.

Although the toxicity of embedded depleted uranium is unknown, numerous studies have addressed the consequences of inhalation, ingestion and parenteral administration of other forms of uranium. Uranium circulates in the blood as the uranyl ion, forming uranium-carbonate and uranium-albumin complexes. As the uranium-carbonate complex passes through the kidney, it is filtered rapidly by the glomeruli where 60%-80% of absorbed uranium is excreted in the first 24 hours after acute exposure. The uranium that is not excreted is reabsorbed by the proximal tubules where it produces significant toxic effects. Uranium also enters the bone, where it competes with calcium to form complexes with phosphate ions, thus becoming part of the bone matrix. This bone matrix then serves as both a long- and short-term storage site from which uranium has been shown to be slowly released back into circulation. The liver, muscle, and kidney are other major sites of uranium deposition, with a possible long-term storage mechanism in the kidney.

Acute morphological and biochemical changes of the kidney result from uranium exposure. Changes in the glomerular epithelium, and cellular necrosis in the proximal tubules near the corticomedullary junction of the kidney have been reported in experimental animals after acute uranium exposure. In addition, polyuria, enzymuria, glucosuria, and increased excretion of amino acids have been reported. Acute renal failure can occur following exposure to high doses of uranium.

In utero exposure to uranium has recently been shown to produce both fetal and developmental toxicity. For example, administration (s.c.) of uranium in the form of uranyl acetate dihydrate (0.5-2.0 mg/kg/d) to gravid (pregnant) mice from gestational days (GD) 6-15 leads to significant decreases in both maternal weight gain and fetal body weights at GD 18. Soft tissue and skeletal examination of the fetuses also revealed a significant increase in the occurrence of renal hypoplasia in all uranium-treated groups. Skeletal anomalies in these mice included bipartite sternebrae, dorsal hyperkyphosis, and incomplete ossification of several bones. Similar skeletal malformations were also seen following daily oral administration of uranyl acetate dihydrate (5-50 mg/kg/d) in gravid mice during the same period of gestation.

While the above results examined the effects of uranium on prenatal development, several studies have been conducted to evaluate the effects of uranium on postnatal development (from birth to age 21 days). Significant decreases in body weight and body length in the offspring of mice treated with 25

mg/kg/d for 14 days prior to mating have been reported. There were also significantly more dead young per litter at this uranium dose at both birth and day 4. Uranyl acetate given orally to gravid mice from GD 13 to 21 days following parturition led to a significant increase in offspring liver weights in all the uranium treated groups (5.0-50.0 mg/kg/d), and decreased mean litter size on day 21 in the highest dose group (50 mg/kg/d). However, developmental parameters such as pinna detachment, incisor eruption and eye opening were unaffected.

Unfortunately, uranium levels in the dam, fetus, or placentae were not measured in any of these fetal and developmental toxicity studies. In order to determine the effects of embedded DU on a developing fetus, it is important to know the *in utero* uranium exposure level, though little work has been done to examine the cross-placental transfer of uranium. While there are distinct anatomical differences between the rodent placenta and the human placenta, little correlation has been shown between the anatomic classification of the placenta and the transfer of xenobiotics between mother and fetus. In rodents and primates, the placenta may act as a barrier, limiting or preventing many toxicological insults to the fetus. This does not appear to be the case with uranium. When ^{233}U was administered intravenously to pregnant rats, almost identical levels of uranium were found in the placenta and fetus, indicating little discrimination for uranium by the placenta. The soft tissue levels of uranium in 19- to 20-day-old fetuses were equal to or greater than the maternal liver concentrations. Immature bone also exhibited a greater deposition of uranium than did the adult bone.

While previous research has demonstrated that the placenta does not act as a barrier to prevent the transfer of uranium from the mother to the fetus, the degree of fetal exposure from maternal implanted DU is unknown. The current study was designed to address this question by determining the uranium levels in the placenta and the fetus. This study also determined if the DU pellets impact on the dams ability to become pregnant and carry her litter to term.

Kidney Toxicity and Uranium Distribution
Materials and Methods

Subjects. Forty-eight female Sprague-Dawley rats(Charles Rivers) weighing 250-300 g were used. Rats were maintained in an AAALAC-accredited facility in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 86-23). 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 and acidified water (pH 2.5, using concentrated HCl) were provided *ad libitum*. Rats were on a 12-hour light/dark cycle.

Depleted Uranium and Tantalum Pellets and Surgical Procedures for Pellet Implantation. Depleted uranium pellets (1 mm diameter x 2 mm long) were obtained from the Oak Ridge National Laboratories, Oak Ridge, TN. Tantalum pellets (1 mm diameter x 2 mm long) were obtained from Alfa Products, Ward Hill, MA and were the heavy metal control. Before the implantation surgery, the depleted uranium and tantalum pellets were cleaned and sterilized. Anesthesia was induced with ketamine hydrochloride (80 mg/kg) in

combination with xylazine hydrochloride (4 mg/kg) and given i.p. in a 0.5-ml bolus, using a 25-gauge needle. The surgical sites were then shaved and cleansed with betadine. Pellets were implanted in each biceps femoris muscle spaced approximately 15 mm apart on the lateral side of each thigh. Implantation was accomplished by placing the pellet in a 16 ga needle, putting a specially designed plunger inside that needle, pushing the needle into the rat muscle, then depressing the plunger. This forced the pellet out of the needle and into the rat muscle.

Dose. Six doses,with 8 animals per dose, were used in this study: 32 tantalum pellets, 16 depleted uranium pellets and 16 tantalum, 20 depleted uranium and 12 tantalum, 24 depleted uranium and 8 tantalum, 28 depleted uranium and 4 tantalum and 32 depleted uranium pellets (10). At all times,rats always had a total of 32 pellets implanted in order to keep the size of the implantations approximately equal in all surgery rats.

Urine and Blood Collection. Blood and urine samples were collected and analyzed for uranium levels and biological markers of kidney function. To assess whether embedded depleted uranium pellets resulted in acute kidney toxicity, blood and urine samples were taken on 14, 28, 42, 56, 70, and 84 days after implantation surgery and assayed for indices of nephrotoxicity.

To safely collect blood samples, rats were immobilized by placing them in a Plexiglas® restrainer. During each collection, approximately 0.3 ml of blood was obtained from the tail vein using a 22-gauge needle. Plasma and the red blood cells was separated by centrifuging for 5 min at 3,000 X g. The plasma was analyzed for uranium levels and biochemical indices of kidney toxicity.

Urine samples were collected by housing the rats in individual metabolism cages (23.5 cm diameter x 12 cm high) where they had continuous access to food and water. The rats were acclimated to the metabolic cages before the study began because naive exposure to these housing procedures has been shown to induce stress in the animals and to increase the toxicity of uranium.

A 24-hour urine collection sample was obtained from each rat and the volume recorded. Rats in the preliminary study produced 10-20 ml urine in a 24 hour sampling period. Care was taken to prevent contamination of the urine with food or feces. After collection, urine was filtered to remove any debris and stored in plastic containers at 4°C until analyzed. The metabolic cages were disinfected and decontaminated between each animal use. During the animal-handling periods, overt signs of behavioral toxicity and the overall appearance of the rats was noted.

Assessment of Uranium on Kidney Function. Measurement of urine volume and osmolarity, urine levels of NAG, LDH, glucose, total protein, creatinine, and blood levels of glucose, urea, and creatinine were used as indicators of kidney function. Osmolarity of the urine was measured with a vapor pressure osmometer (model 5100-B, Wescor Inc., Logan, UT). A Kodak Ektachem 700 Analyzer was used to determine serum and urine levels of creatinine, glucose, and urea. Total urine protein was measured with a dye-binding assay (Coomassie Blue, BioRad) sensitive down to 1 μg. The activity of NAG was measured by the methods of Tucker et al. using 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide as the fluorescent substrate (excitation wavelength = 356 nm; emission wavelength = 446 nm). The dilution of the urine for this assay eliminates the effects of any inhibitors present. For LDH measurements, 1 ml of urine was dialyzed for 4 hours at 4°C with 1 liter of deionized water. LDH was quantitated with a colorimetric assay that measures a reaction product proportionate to LDH activity (Oxford Biomedical Research Inc.).

Determination of Uranium Distribution. Upon completion of the study the rats were euthanized and the tissues removed for analysis of uranium content. This was done using kinetic phosphorescence analysis (KPA). One kidney was also analyzed for any histological changes.

Results

Nephrotoxicity

Figure 1 shows the effect of depleted uranium pellets on the animal body weights. No significant effect of pellet number is seen on body weight. Bodyweight is a gross measurement of toxicity and from this data it indicates that even at a level of 32 pellets, the depleted uranium was not toxic to the female rat. Figure 2 indicates that the depleted uranium pellets had no effect on the urine output in all animals. This is also a good gross measurement of kidney function.

Figures 3-6 present the data obtained from analysis of serum for indicators of kidney toxicity. As the data indicates, no significant difference was seen in the potassium, urea nitrogen, glucose or creatinine levels in the blood. Figures 7-9 show that these same parameters when measured in the animal's urine again show no significant difference among the various depleted uranium doses. Figure 10 shows that creatinine clearance was not affected by the DU pellets. This number is calculated from the equation: $C_c = U_c * V / P_c$ where C_c is creatinine clearance, U_c is urinary creatinine, P_c is plasma creatinine, and V is the urine volume.

Figures 11 and 12 represent the results of urine analysis for the enzymes LDH and NAG. Both of these enzymes show no significant differences among the depleted uranium groups.

Figures 13-15 show that osmolarity, pH and protein levels of the urine are all unaffected by the depleted uranium pellets.

Histopathology, conducted by the pathology department of AFRRI, indicated no histological changes in the kidney following 84 days of the implanted DU pellets.

Uranium Distribution

Uranium levels in the tissues removed at the completion of the 84 days of pellet implantation are presented in Figures 16-28. Measurable levels of uranium were detected in all of the tissues analyzed. These numbers are currently being analyzed for statistical significance when compared to the tantalum animals and also for a dose-response relationship. Uranium levels in the urine collected on days 14, 28, 42, 56, 70 and 84 post-implantation, as well as the blood obtained on day 84, are all currently being analyzed using the KPA analysis.

Conclusions

The assays conducted provide a broad spectrum of measures of kidney toxicity. Many of these substances have been shown to be very sensitive biomarkers of acute uranium toxicity. Urinary enzymes are

sensitive noninvasive markers of toxicity primarily in the kidney tubules. NAG is a lysosomal enzyme found in proximal renal tubule cells. LDH is a cytosolic enzyme of the tubular epithelium. Previous research has shown LDH, and to a lesser extent NAG, increased following uranium exposure. In our study, nNeither of these enzymes were signficantly altered by the depleted uranium, indicating no changes in the tubules of the animals' kidneys.

Although urine volume and osmolarity can vary greatly with fluid intake, these measures provide physical indicators of renal function. For example, kidney failure drastically decreases urine volume, while moderate renal insufficiency can increase urine output. Osmolarity can reflect the ability of the kidney to concentrate (or dilute) the urine. A transient increase in urine volume has been shown to occur with acute uranium toxicity. Lack of changes in these measurements in our study indicate that renal function has not been signficantly altered due to the uranium treatment.

A small concentration of protein is normally present in the urine. Increases in total urine protein could result either from glomerular leakage or failure of tubule reabsorption. The appearance of protein in the urine has been reported with acute uranium toxicity. Our experiments found no alterations in urinary protein levels, indicating that the glomerular filtration and tubular reabsorption mechanisms are not altered by the depeleted uranium implantations.

Appearance of glucose in the urine occurs when the tubule reabsorption maximum from the filtrate is exceeded. This can occur with hyperglycemia or with a decrease in tubular reabsorption capacity. Measurement of both urine and plasma glucose helps to distinguish between these two possibilities. Glucose is one of the most sensitive indicators of uranium-induced nephrotoxicitywith increased glucose detected in the urine but no concurrent increases found in the plasma.

All these measures were used together as indicators of kidney toxicity. Interpretation of these data, along with the lack of any noted histopathological changes, lead to the conclusion that while previous experiments have shown that uranium exposure can alter the kidney structure and functioning, uranium exposure to female rats from implanted depleted uranium does not adversely affect the kidney. This is despite the fact that uranium levels in the kidney equal or exceed the level set by the Nuclear Regulatroy Commission for kidney damage. The lack of kidney toxicity seen due to uranium certainly differs from what is reported in the literature. This could be due to the uniqueness of several aspects of our experimental design. The chemical form of uranium is different in these studies than the uranyl nitrate or acetate used in many of the studies. Our route and time course of administration, chronic levels due to metal implantation, differ from the acute exposures via injection or drinking water. Chronic inhalation of uranium dioxide in rats has also produced no signs of renal toxicity. It is possible that the chronic route of exposure allows for a mechanism of tolerance to develop that prevents the renal toxicity often seen with acute exposures.

The uranium distribution data held few surprises given the data that was obtained within the institute by Dr. Terry Pellmar. Those results were obtained in the male rats while this current data are from female rats. The female rats, as with the male rats, show very high levels of uranium in the kidney,

marrow bone, muscle proximal to the DU pellets location. The results of this study led to the selection of 4, 8, 16 and 32 DU pellets for the final phase of this project.

Initial Data on Litter Effects and Uranium Distribution

Materials and Methods

Dose. Five doses were used in this study: Non-surgery control (N=10), 12 tantalum pellets (12), 4DU pellets and 8 tantalum (11), 8 DU and 4 tantalum (11) and 12 DU pellets (10). At all times, any rat receiving pellets always had a total of 12 pellets implanted in order to keep the size of the implantations approximately equal in all surgery rats.

Prenatal Tissue Collection. Experimental females were housed with non-treated male rats with two females in each male's cage. Gestational Day (GD) 0 was determined by the presence of sperm in the vaginal washing. At this time the females were removed from the males' cages and housed individually. From GD 0 until GD 20, pregnant rats were monitored daily for weight gain, food intake and water intake. The parameters were used as measures of maternal toxicity of the DU pellets. On GD 20, the dams were euthanized. Dams were immediately cesarean sectioned, and the uterine horns removed. Fetuses were dissected out, and all the placentae for that litter collected. The uterine horns were examined for any resorption sites. Litters were examined, and a record made of (1) total number of fetuses, (2) number of viable fetuses, (3) sex ratio, and (4) any overt signs of teratological effects. All offspring of the litter were analyzed for uranium levels. The placentae from all pups were collected and pooled for uranium analysis for each litter. One male and one female pup separated out and used for analysis of whole fetus. The rest of the litter were used for determining uranium tissue levels. Quickly the liver and kidneys were dissected out of these pups. These tissues were pooled for the entire litter, homogenized, and analyzed for uranium content.

Results

Maternal and Litter Effects Tables 1 and 2 present the data on the effects of the DU levels on maternal and litter parameters. From these data, there appears to be no effect of the DU on maternal parameters such as: maternal food and water intake, weight gain during pregnancy, and time-to-pregnancy. Furthermore, the litter parameters such as: number of pups, number of males vs females, and fetal weight were also not affected by the various levels of DU. The DU pellets did not adversely affect the ability of these rats to breed, or for them to maintain the pregnancy until the day of euthanasia. All litters were examined for any overt signs of teratology, and none were noted.

Uranium Distribution Figures 29 and 30 show the placental and whole fetus uranium levels. Comparison of these results by a correlation trend test indicate that uranium accumulates in these tissues in an increasing fashion as the maternal DU dose increases. There appears to be a ten-fold decrease in the

levels within the whole fetus when compared to the placental tissue. The levels in the fetus do exhibit a dose-response relationship as determined by a correlation trend test.

Conclusions

From the results it would seem that there is a dose response effect on uranium levels in the placenta and whole fetus. These data indicate no effect of the DU pellets on any of the maternal or litter paramaters measured. Additional doses are currently being analyzed to determine if this will hold true for the higher levels of DU tested. An additional factor in this study as well as the final developmental study, is stated in the section below.

Effect of Time Following Implantation on Litter and Reproductive Effects and Uranium Distribution in Pregnant Rats

The results of Dr. Pellmar's study led us to be concerned with the length of time the pellets should remain in the female rat prior to breeding. Initial experiments operated under the assumption that once the incision was healed the female rats should be bred immediately with the non-implanted male rats. Dr. Pellmar's data indicate that at time points of 6, 12 and 18 months post-implantation, the uranium levels accumulating in the male rat are still increasing. Given this, it was determined that a study be conducted to exammine the effects of an increased time between pellet implantation and breeding would have on the reproductive effects as well as the uranium distribution.

A group of rats were implanted with the highest dose of DU used to date, 32 pellets. For each DU rat there was a non-surgery control rat. These rats were further divided into one of 4 groups depending on the length of time the pellets would remain in the female prior to breeding: 2, 4, 6 or 8 months. Urine was collected from these rats on gestational days (GD) 6, 12 and 18. Analysis of this urine indicated, as seen in previous studies, no nephrotoxicity. The dams were euthanized on GD 20, and the pups removed, sexed, weighed, and observed for abnormalities. Maternal tissues were removed along with the placenta, whole fetus, fetal kidney, fetal liver and fetal brains. These tissues are currently being analyzed for uranium content. The 8 month group is currently being bred in the laboratory and will follow the same procedure. While the use of the metabolic cage may have interfered with the carrying to term of the pregnancy, this does not appear to be related to the DU dose, as it has happened in several rats in both the DU and the control groups. The uranium distribution data, to the fetus and within fetal tissues, will provide information to us regarding the effects of the delay in breeding the female rats.

Current Status

The rats for the final stage of the study have been ordered, to be delivered weekly for a 2-3 month period. These rats will be implanted with the DU doses of 4, 8, 16 and 32 pellets. The length of time that the pellets will remain in the female rats before breeding will be determined from the analysis of the study using the high dose of DU and time periods of 2, 4, 6, and 8 months post-implantation. Although the

8 month group is currently breeding and the remaining data from this study are currently being analyzed, it would appear that the females should be implanted and then allow for a 6 month time period for the levels of uranium to increase within the dam's tissues. It is not possible to allow the levels to stabilize. Previous data indicate that this may take 12-18 months and at this point the reproductive capabilities of the female rat would be impaired due to age. Our previous assumption of breeding the rats 1-2 weeks post-implantation would not be ideal as the results within the institute indicate that the uranium levels will drastically increase over the next 4-6 months. Breeding the rats too early could mask any effects that may be seen due to the lack of time allowed for the uranium to accumulate and not to the lack of effect of the uranium itself, as could be misinterpreted.

The biggest delay in this project, besides the need to reassess the length of time before breeding and the studies involved in determining that, has been the massive hold-up in obtaining the remaining DU pellets needed to complete the study. All the pellets in the institute have been utilized for this study and all that remains are the pellets needed for the final stage. These pellets can only be obtained from one source, and they in turn contract out the material to another firm to machine the pellets to our desired specifications. These people were contacted well over 6 months ago and very consistently from that time on. The situation stands now that the quotes are complete and the order for these pellets has been entered into the system. The pellets will be received within the next month or so and the females implanted weekly for a 2-3 month time frame. Then the 6 months time period will elapse, and the rats will be bred with non-implanted male rats. The pups will be monitored for behavioral and developmental abnormalities and this data analyzed.

While most of these supplies can be ordered ahead of time if the money were to expire, one of the remaining issues is that of technical support. This has become more crucial due to a dislocated knee that I suffered several months ago and will have to have operated on in the next month. The technician supported by this project has left the institute to attend graduate school. There is another Jackson Foundation technician within the institute that has assisted on this project and requires no training. Her funding source however runs out this fiscal year. Allowing the money from this grant to remain viable for FY 99 would allow the completion of this project without disruption because the technician already familiar with the procedures could remain on the project. I realize that this project has already received a one-year no cost extension but I am requesting that a second extension be granted due to the several unforeseeable events that I have outlined above.

Figure 1

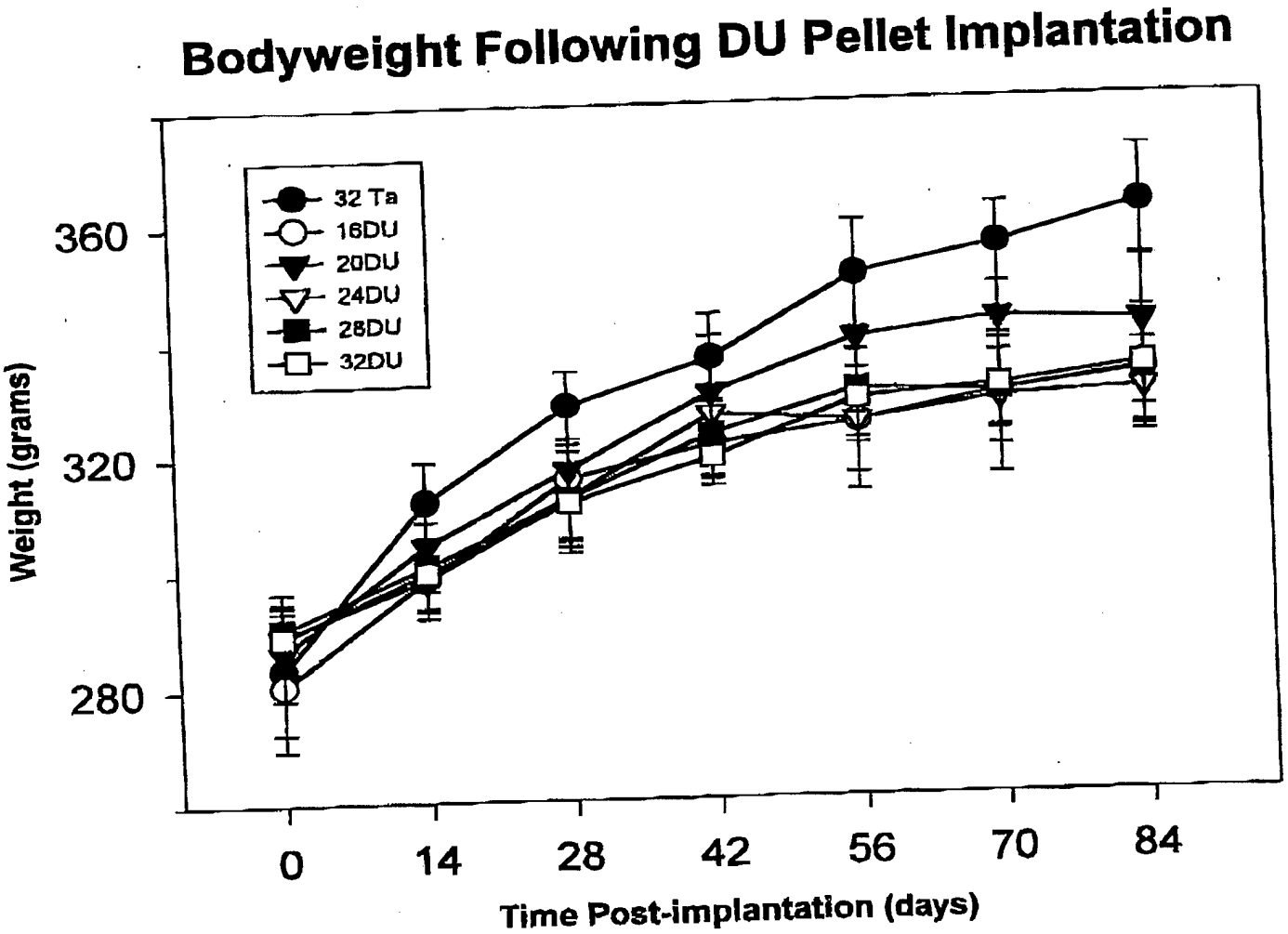

Bodyweight Following DU Pellet Implantation
32 Ta
16DU
20DU
24DU
28DU
32DU
360
320
280
Weight (grams)
0
14
28
42
56
70
84
Time Post-implantation (days)

Figure 2

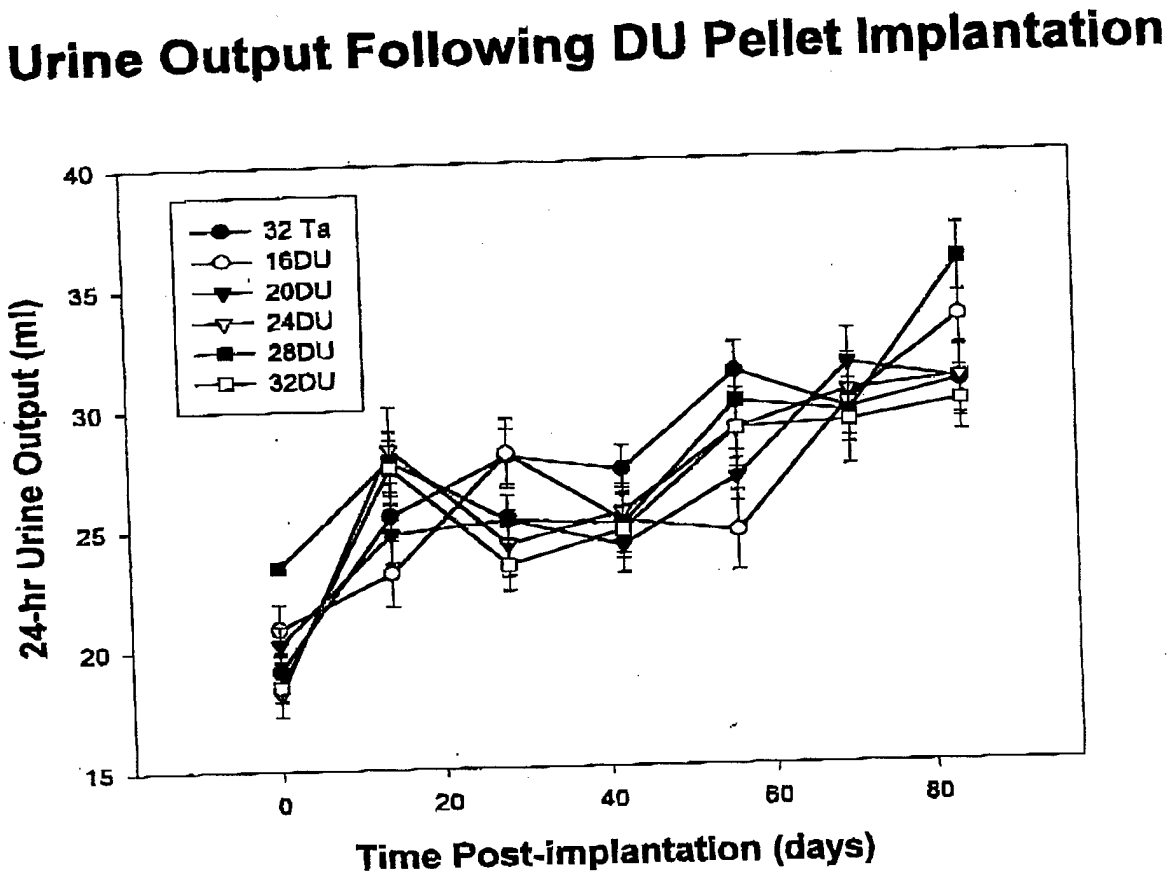

Urine Output Following DU Pellet Implantation
32 Ta
16DU
20DU
24DU
28DU
32DU
24-hr Urine Output (ml)
40
35
30
25
20
15
0
20
40
60
80
Time Post-implantation (days)

Figure 3

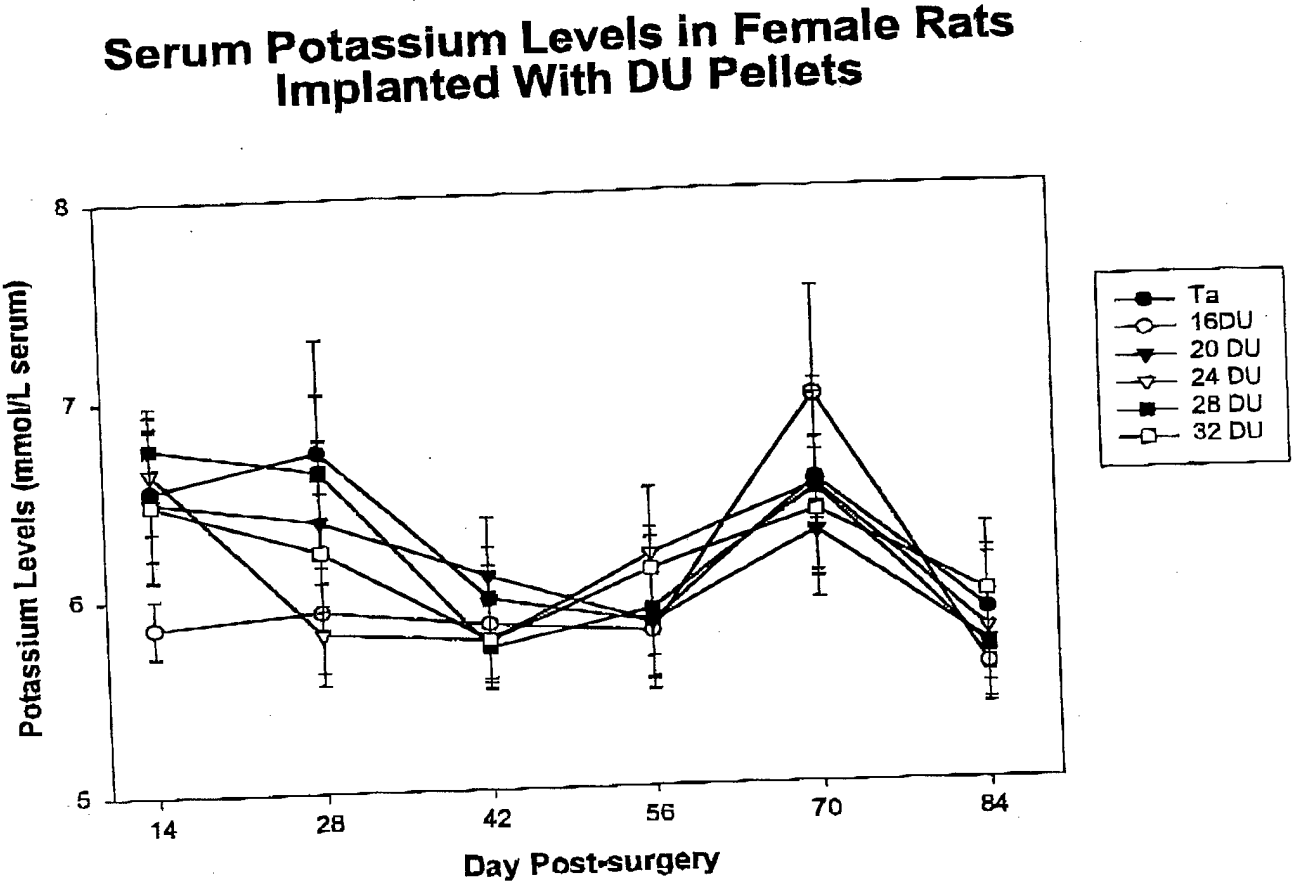

Serum Potassium Levels in Female Rats Implanted With DU Pellets
Potassium Levels (mmol/L serum)
8
7
6
5
14
28
42
56
70
84
Day Post-surgery
Ta
16DU
20 DU
24 DU
28 DU
32 DU

Figure 4

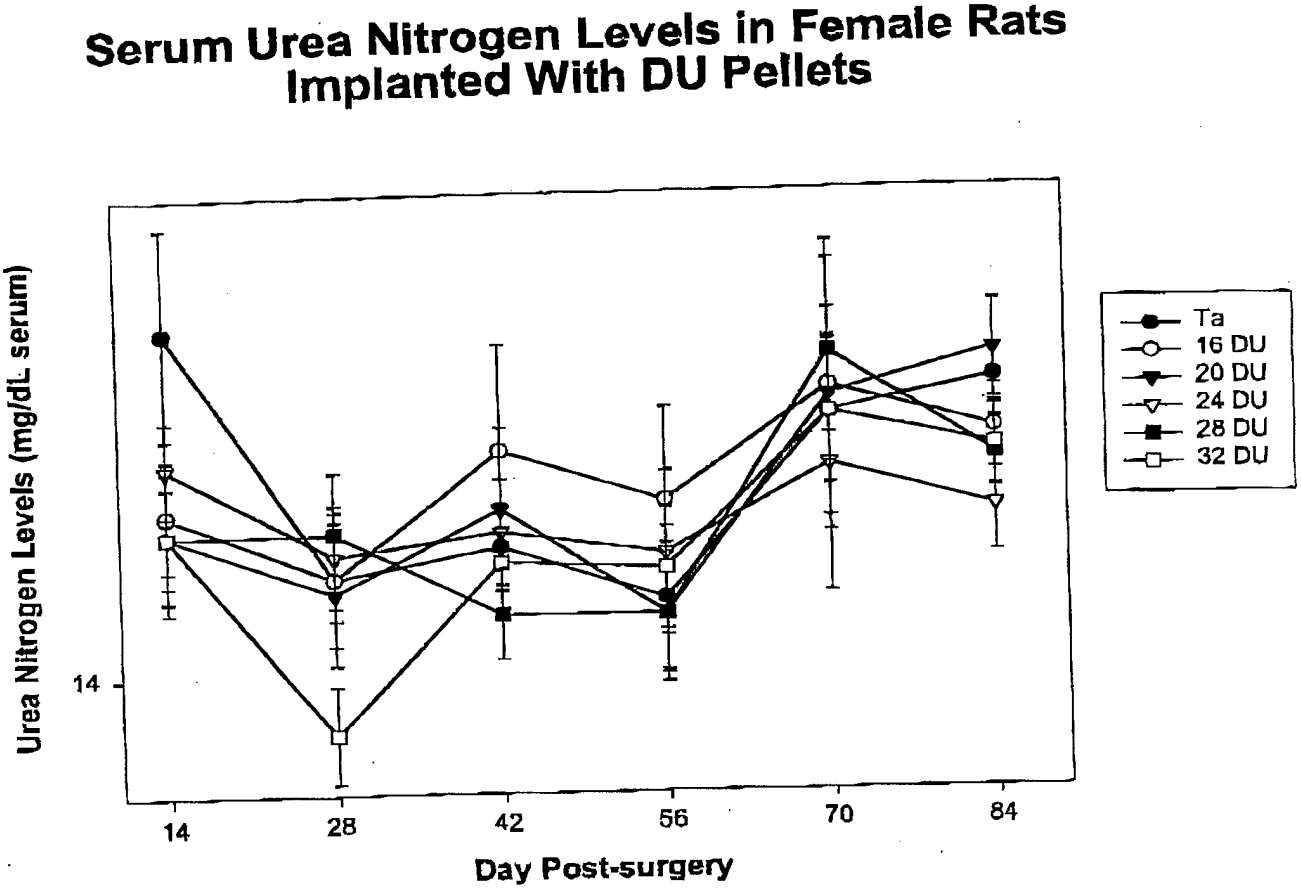

Serum Urea Nitrogen Levels in Female Rats Implanted With DU Pellets
Urea Nitrogen Levels (mg/dL serum)
14
14
28
42
56
70
84
Day Post-surgery
Ta
16 DU
20 DU
24 DU
28 DU
32 DU

Figure 5

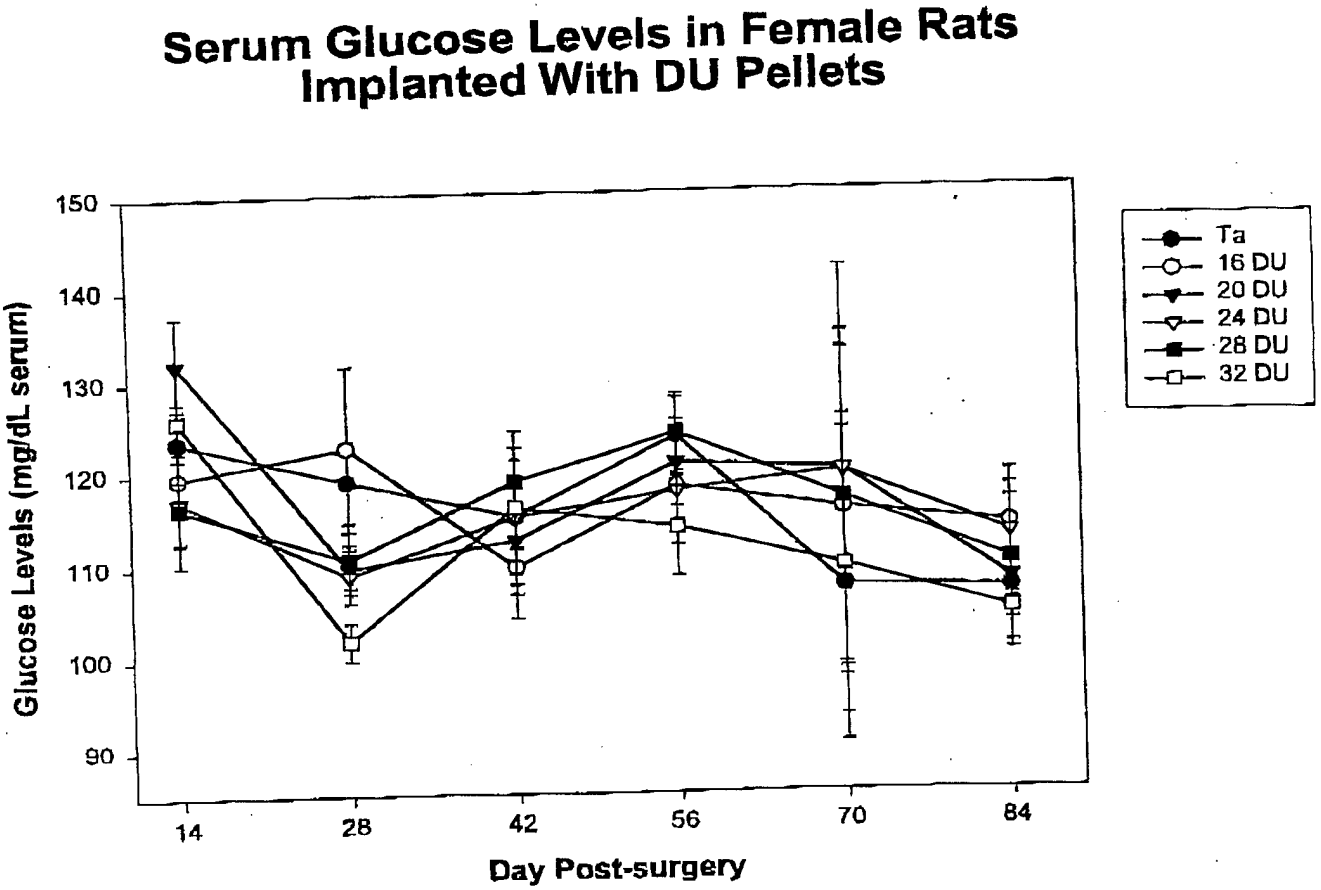

Serum Glucose Levels in Female Rats Implanted With DU Pellets
Glucose Levels (mg/dL serum)
150
140
130
120
110
100
90
14
28
42
56
70
84
Day Post-surgery
Ta
16 DU
20 DU
24 DU
28 DU
32 DU

Figure 6

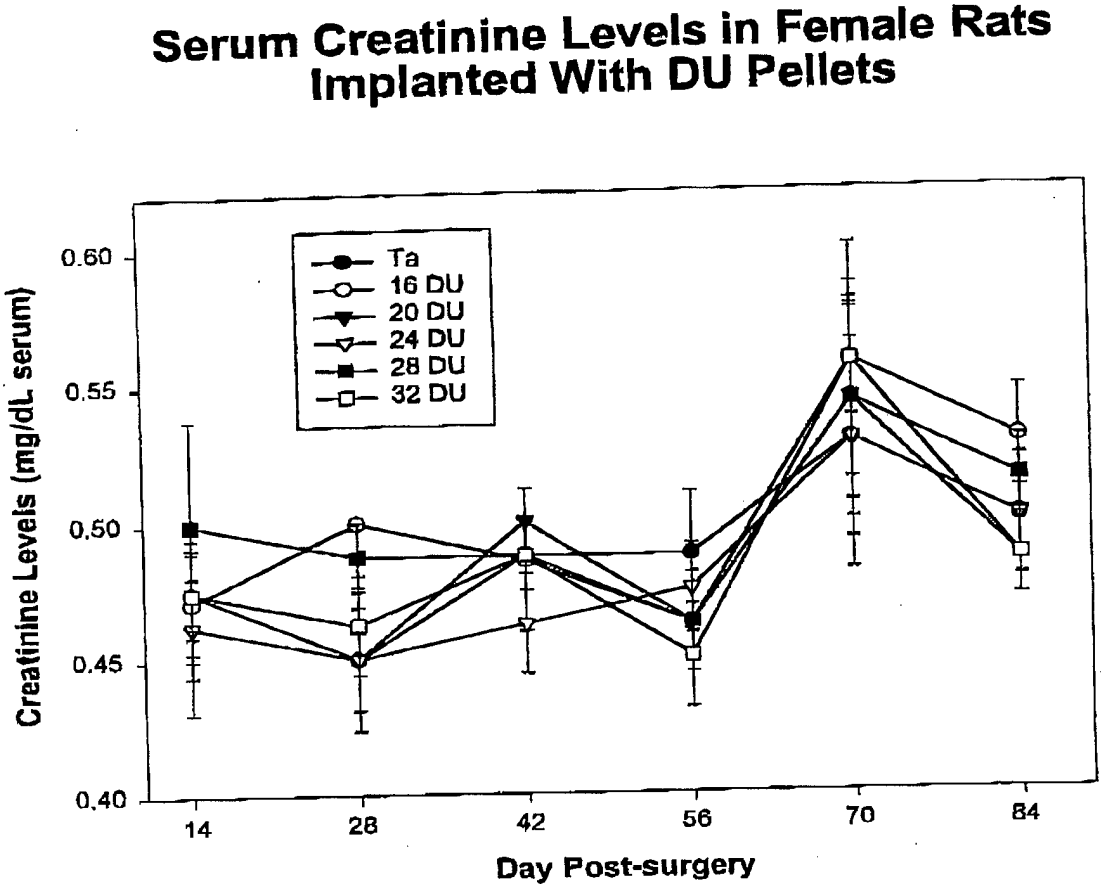

Serum Creatinine Levels in Female Rats Implanted With DU Pellets
Ta
16 DU
20 DU
24 DU
28 DU
32 DU
Creatinine Levels (mg/dL serum)
0.60
0.55
0.50
0.45
0.40
14
28
42
56
70
84
Day Post-surgery

Figure 7

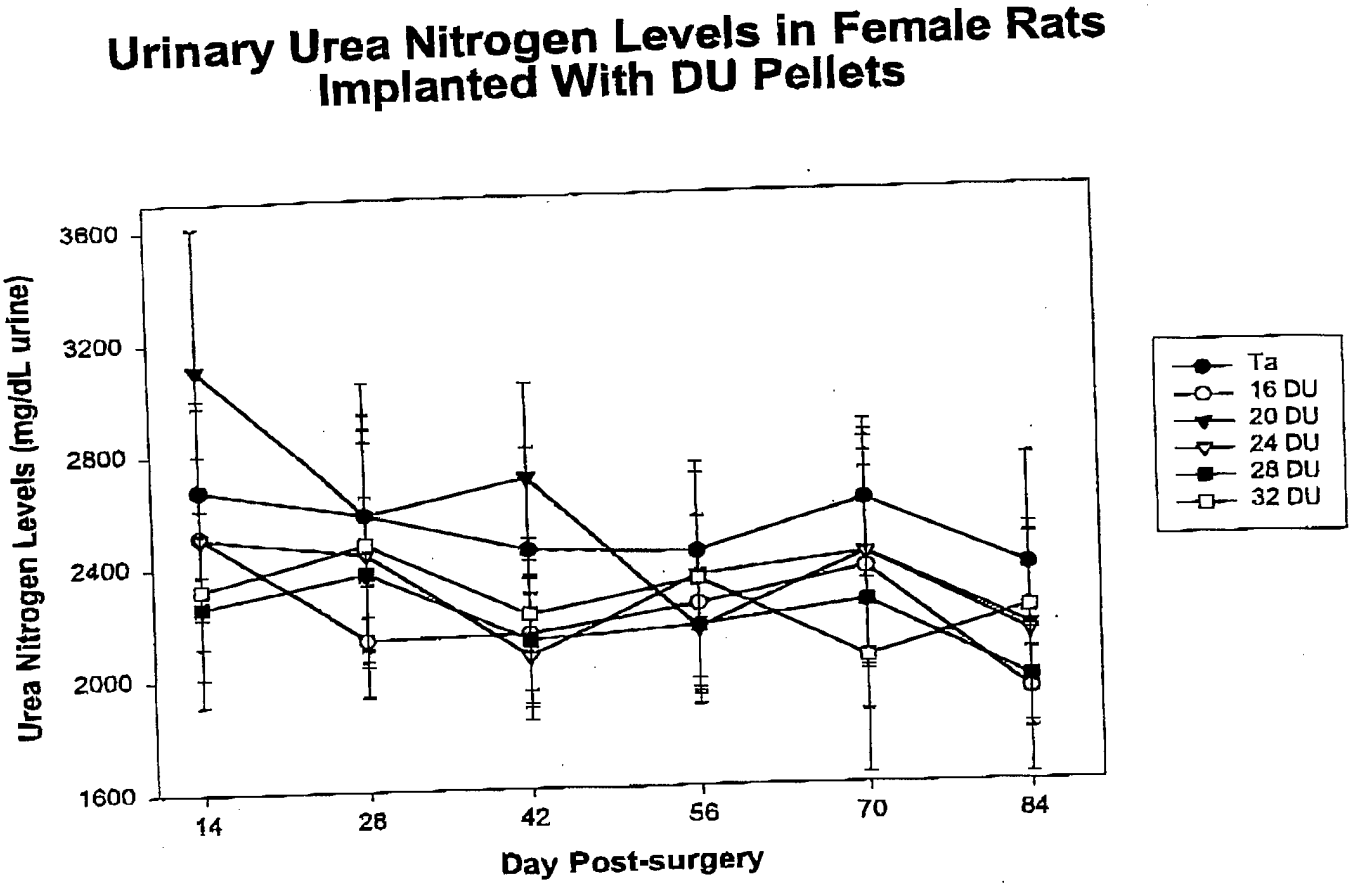

Urinary Urea Nitrogen Levels in Female Rats Implanted With DU Pellets
Urea Nitrogen Levels (mg/dL urine)
3600
3200
2800
2400
2000
1600
14
28
42
56
70
84
Day Post-surgery
Ta
16 DU
20 DU
24 DU
28 DU
32 DU

Figure 8

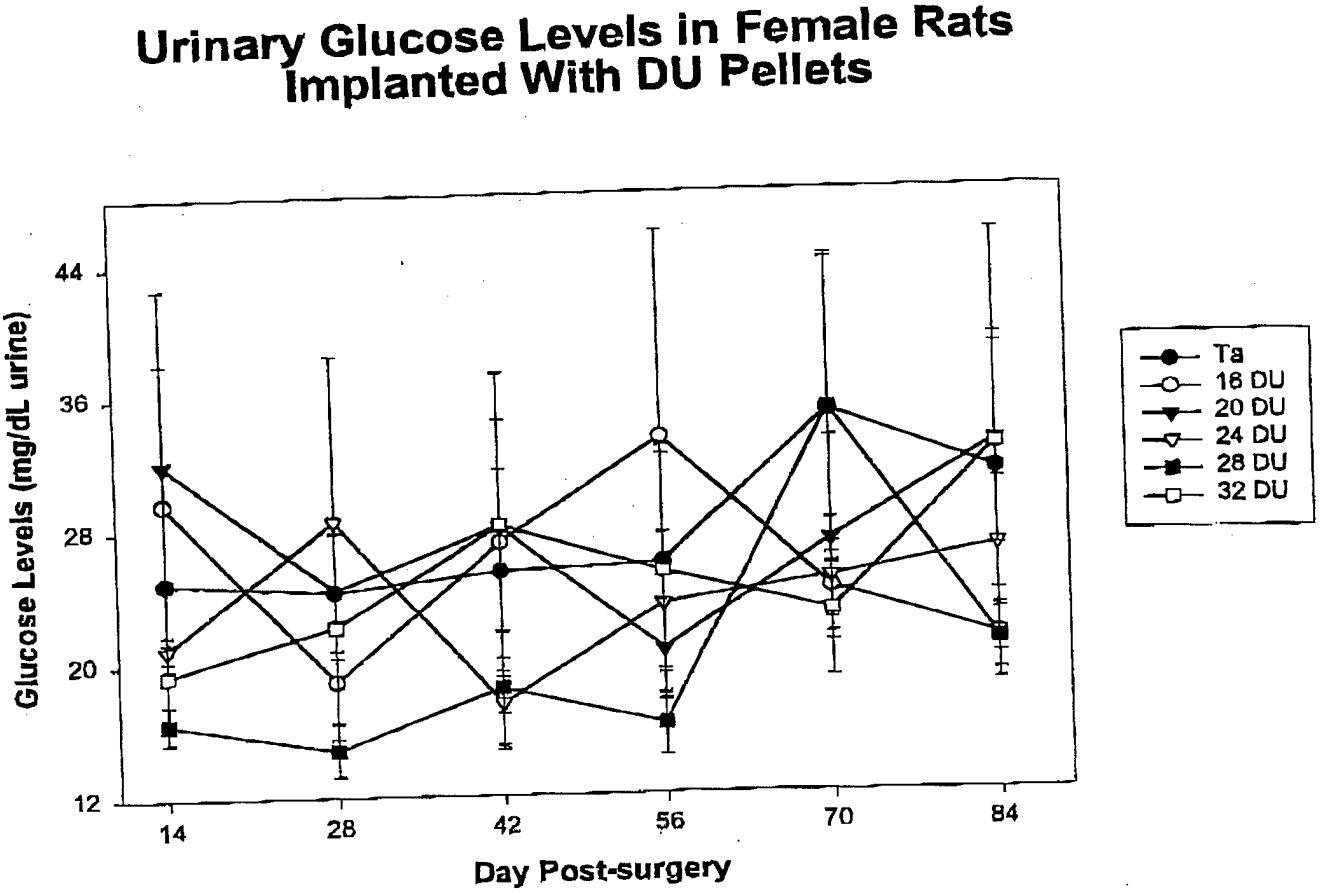

Urinary Glucose Levels in Female Rats Implanted With DU Pellets
Glucose Levels (mg/dL urine)
44
36
28
20
12
14
28
42
56
70
84
Day Post-surgery
Ta
16 DU
20 DU
24 DU
28 DU
32 DU

Figure 9

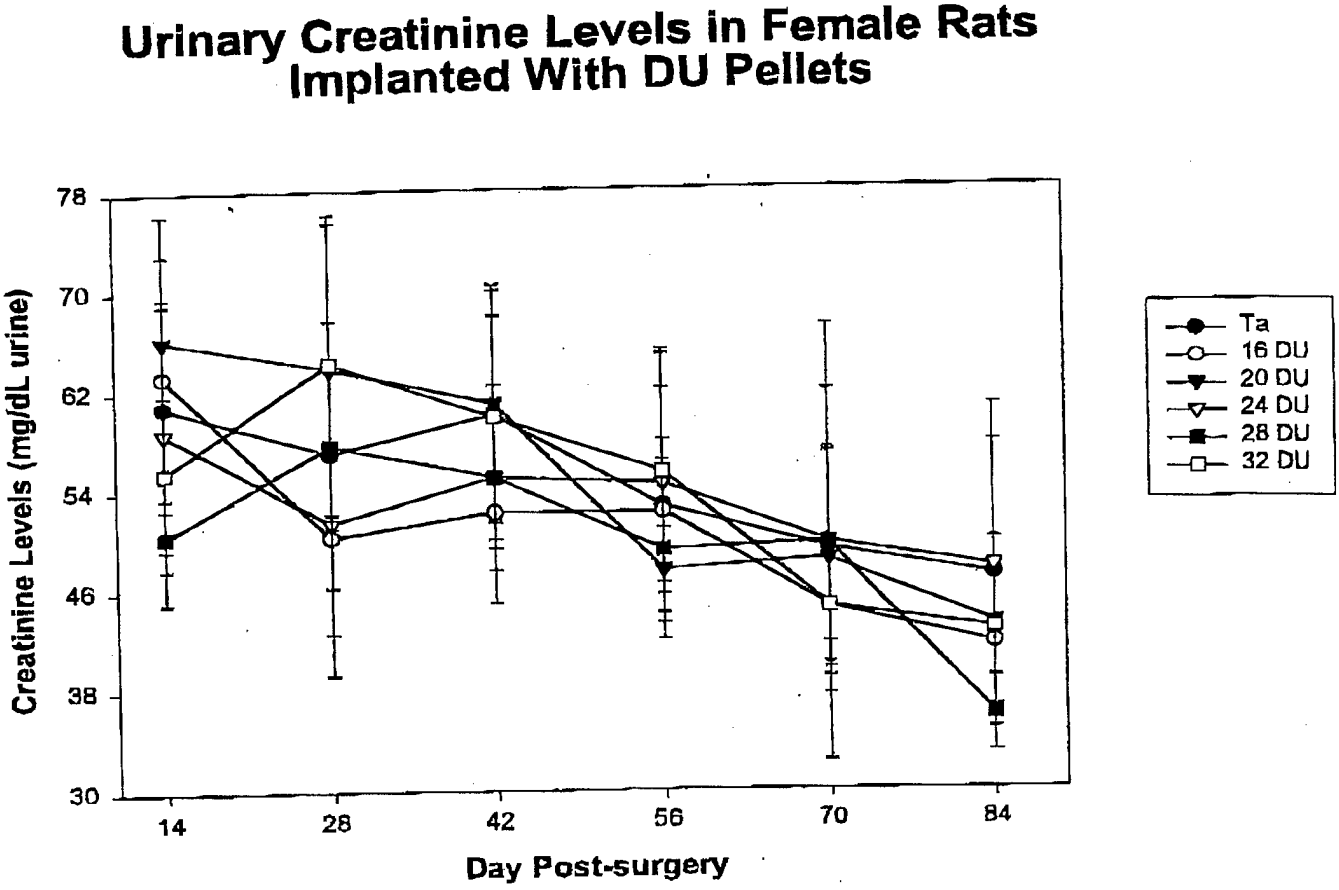
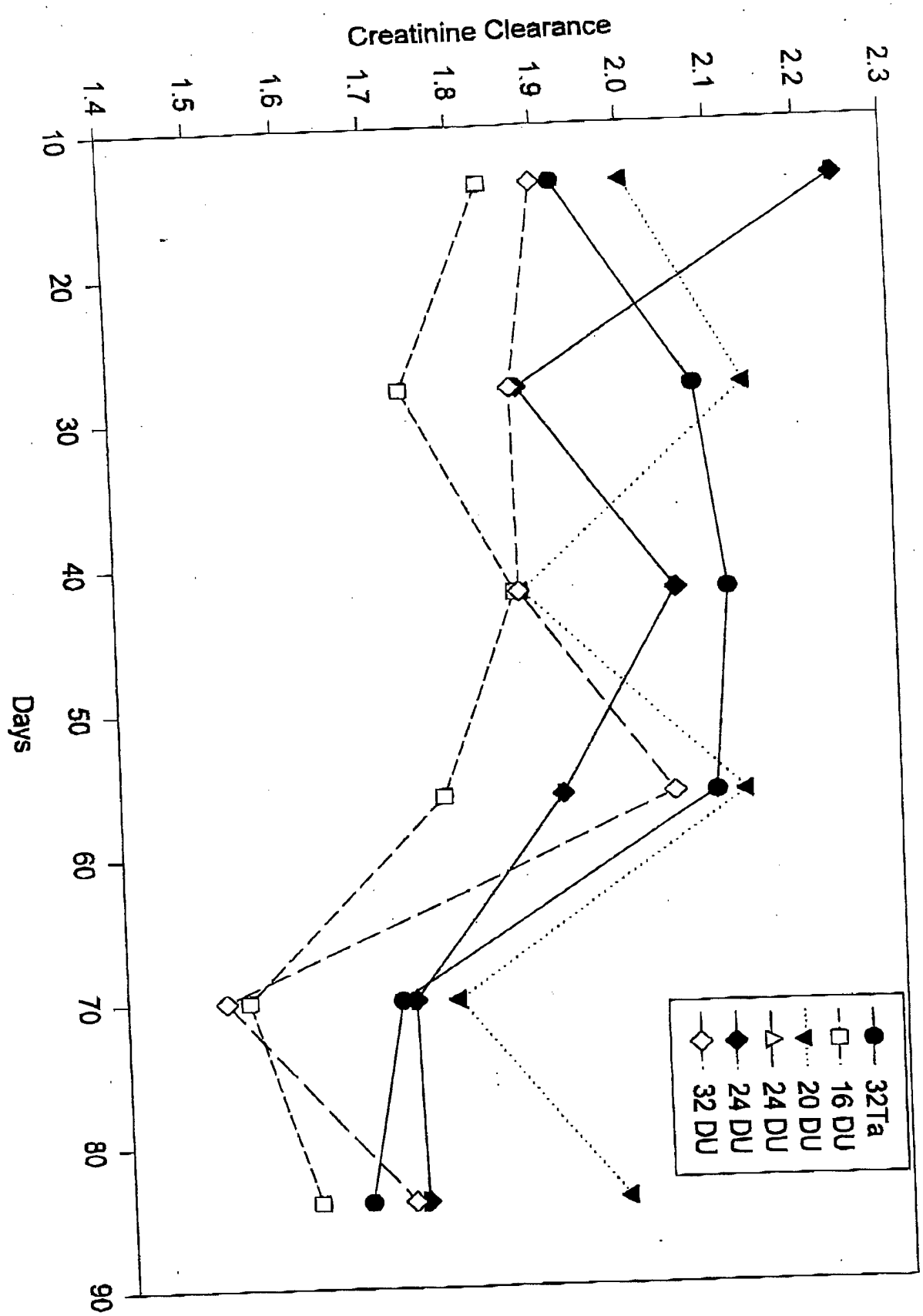

Urinary Creatinine Levels in Female Rats Implanted With DU Pellets
Creatinine Levels (mg/dL urine)
78
70
62
54
46
38
30
14
28
42
56
70
84
Day Post-surgery
Ta
16 DU
20 DU
24 DU
28 DU
32 DU

Figure 10



Creatinine clearance by DU dose group

Figure 11

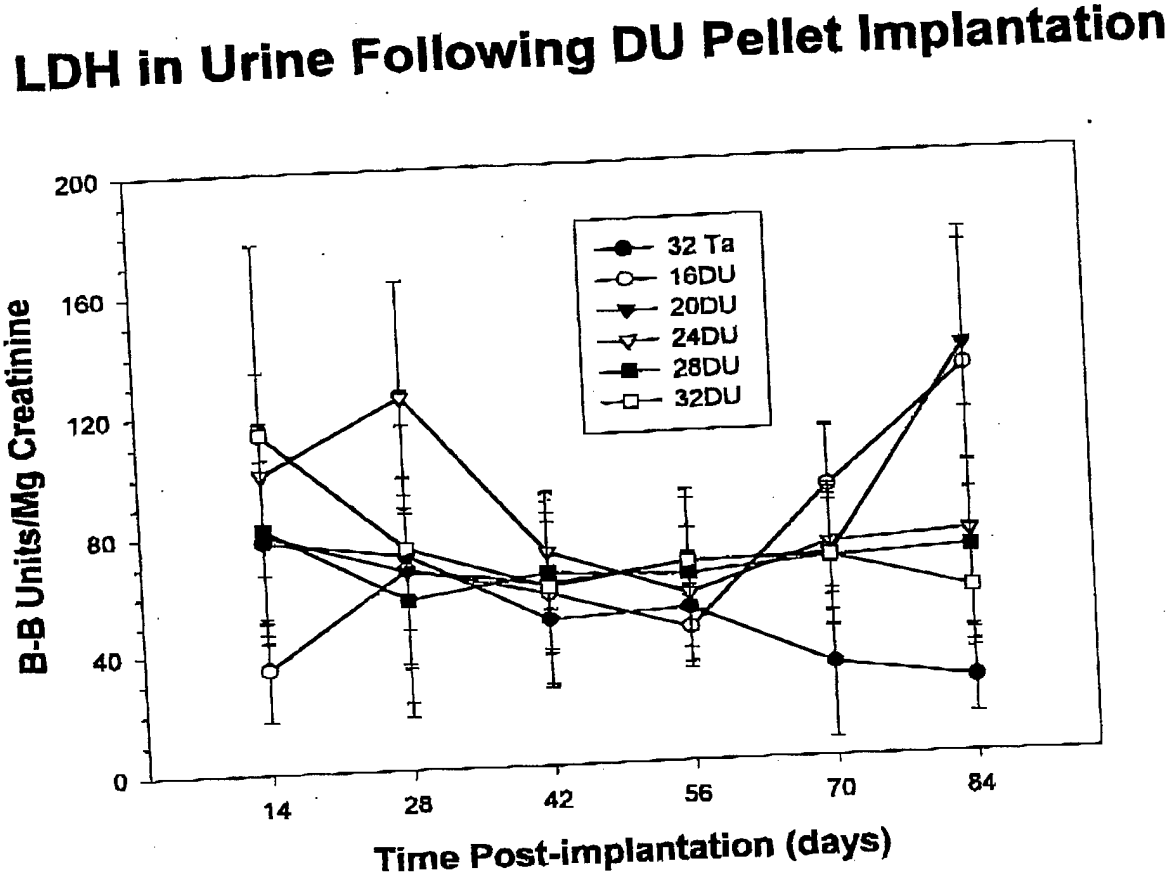

LDH in Urine Following DU Pellet Implantation
B-B Units/Mg Creatinine
200
160
120
80
40
0
14
28
42
56
70
84
Time Post-implantation (days)
32 Ta
16DU
20DU
24DU
28DU
32DU

Figure 12

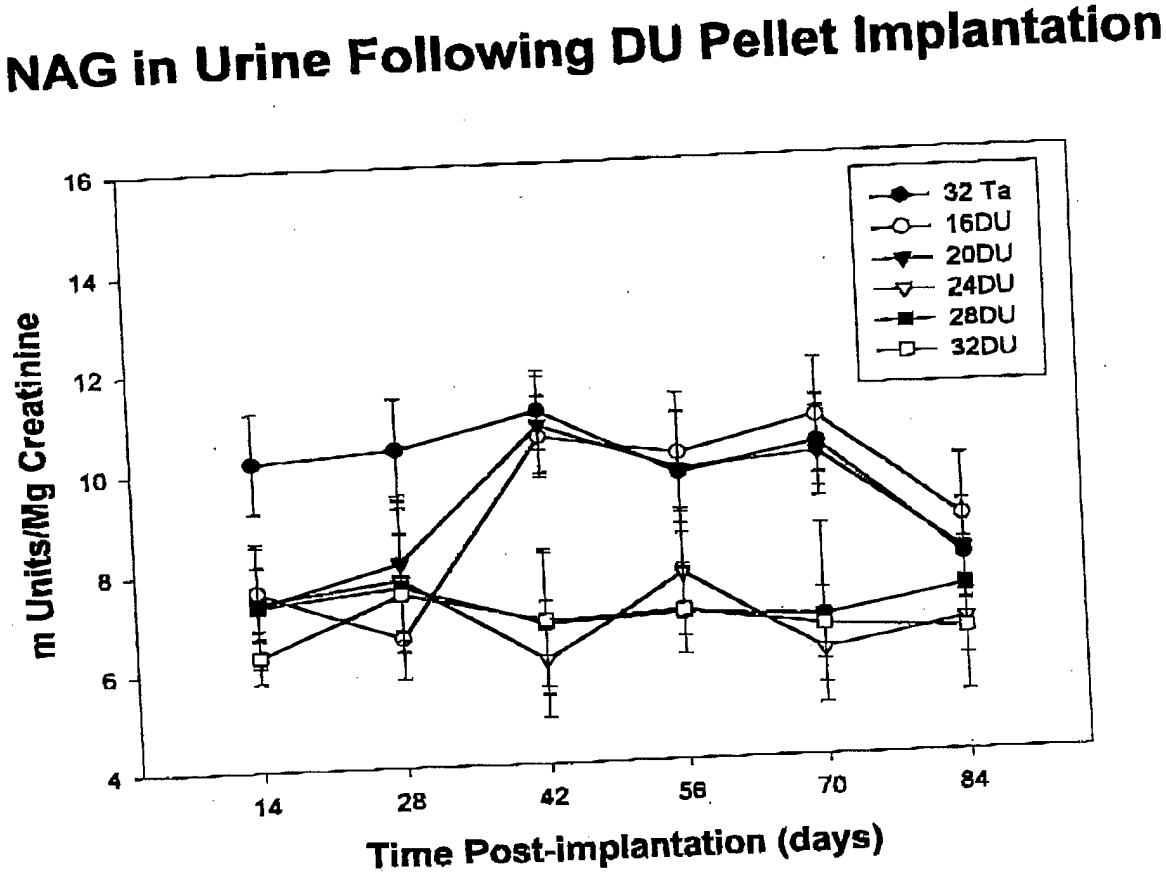

NAG in Urine Following DU Pellet Implantation
m Units/Mg Creatinine
16
14
12
10
8
6
4
14
28
42
56
70
84
Time Post-implantation (days)
32 Ta
16DU
20DU
24DU
28DU
32DU

Figure 13

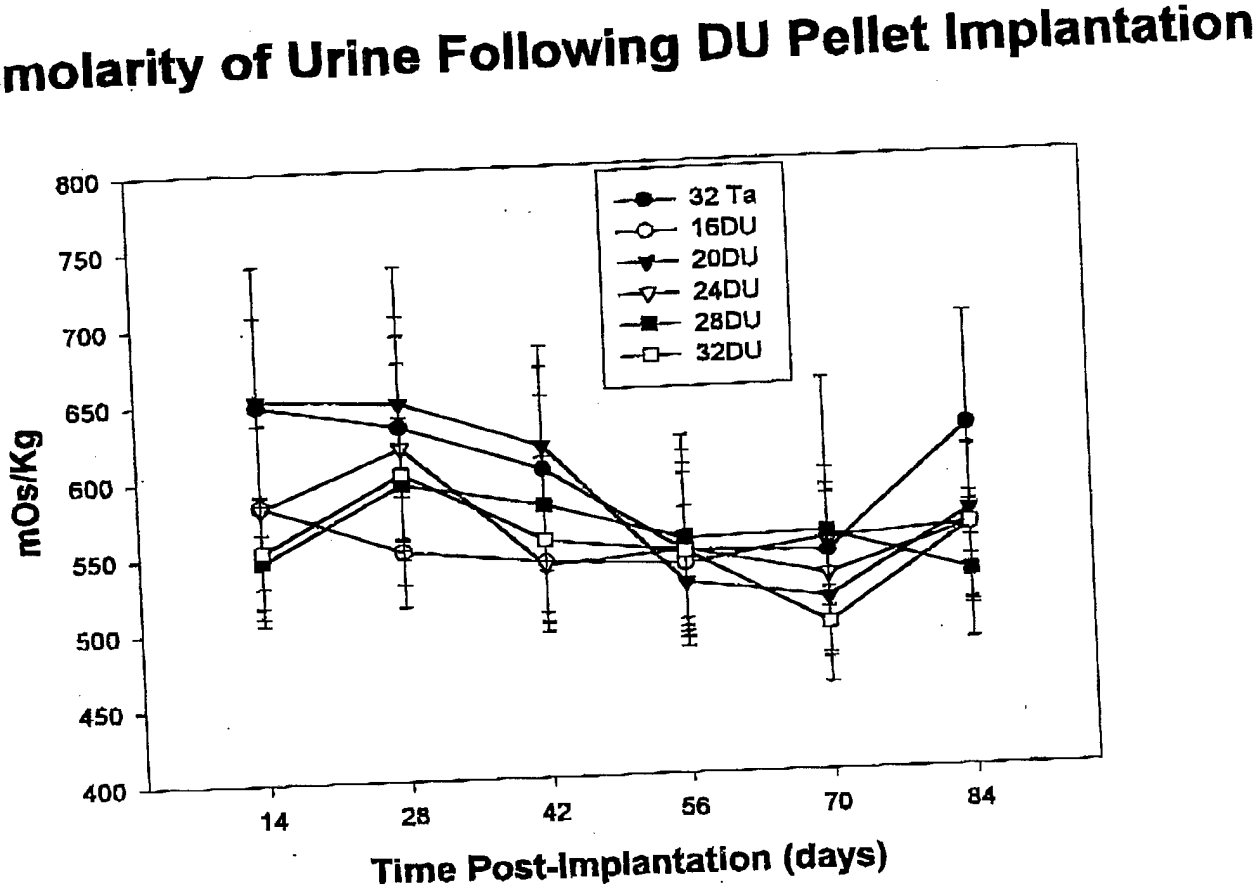

Osmolarity of Urine Following DU Pellet Implantation
800
750
700
650
600
550
500
450
400
mOs/Kg
32 Ta
16DU
20DU
24DU
28DU
32DU
14
28
42
56
70
84
Time Post-Implantation (days)

Figure 14

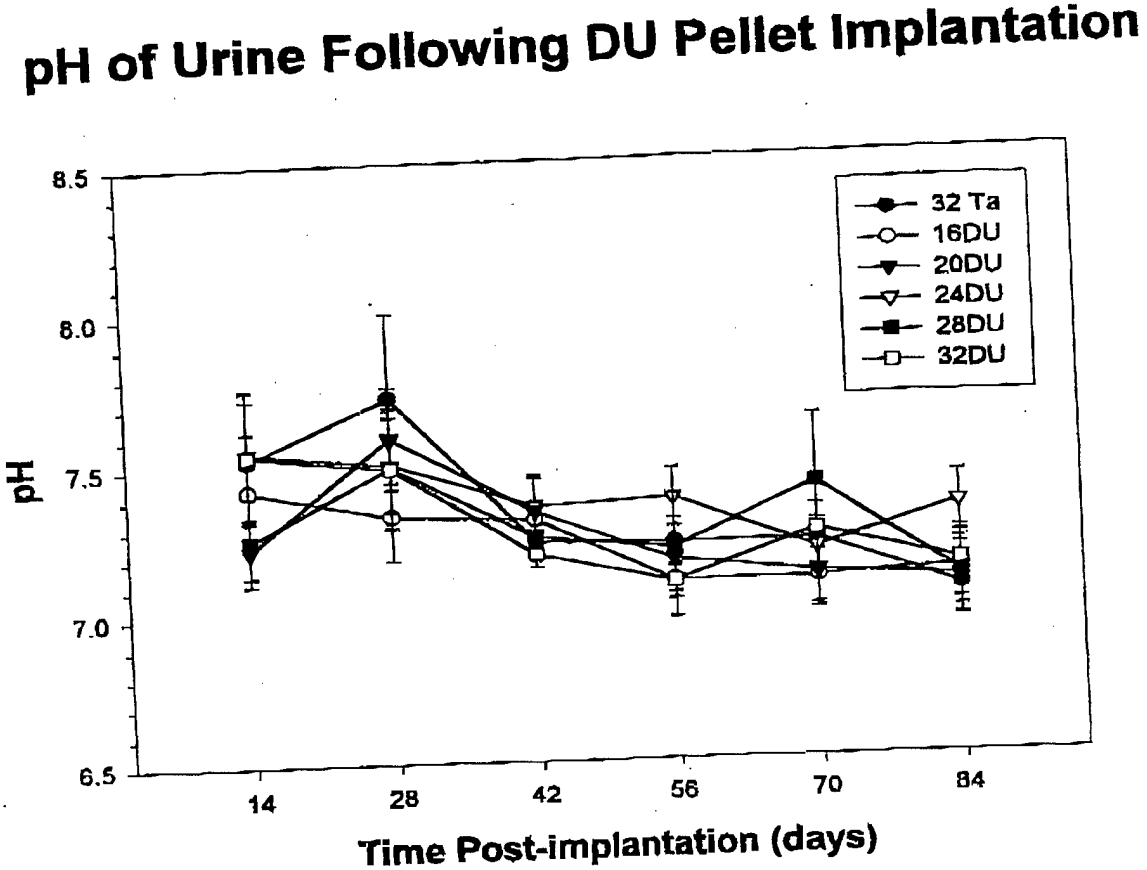

pH of Urine Following DU Pellet Implantation
8.5
8.0
7.5
7.0
6.5
pH
14
28
42
56
70
84
Time Post-implantation (days)
32 Ta
16DU
20DU
24DU
28DU
32DU

Figure 15

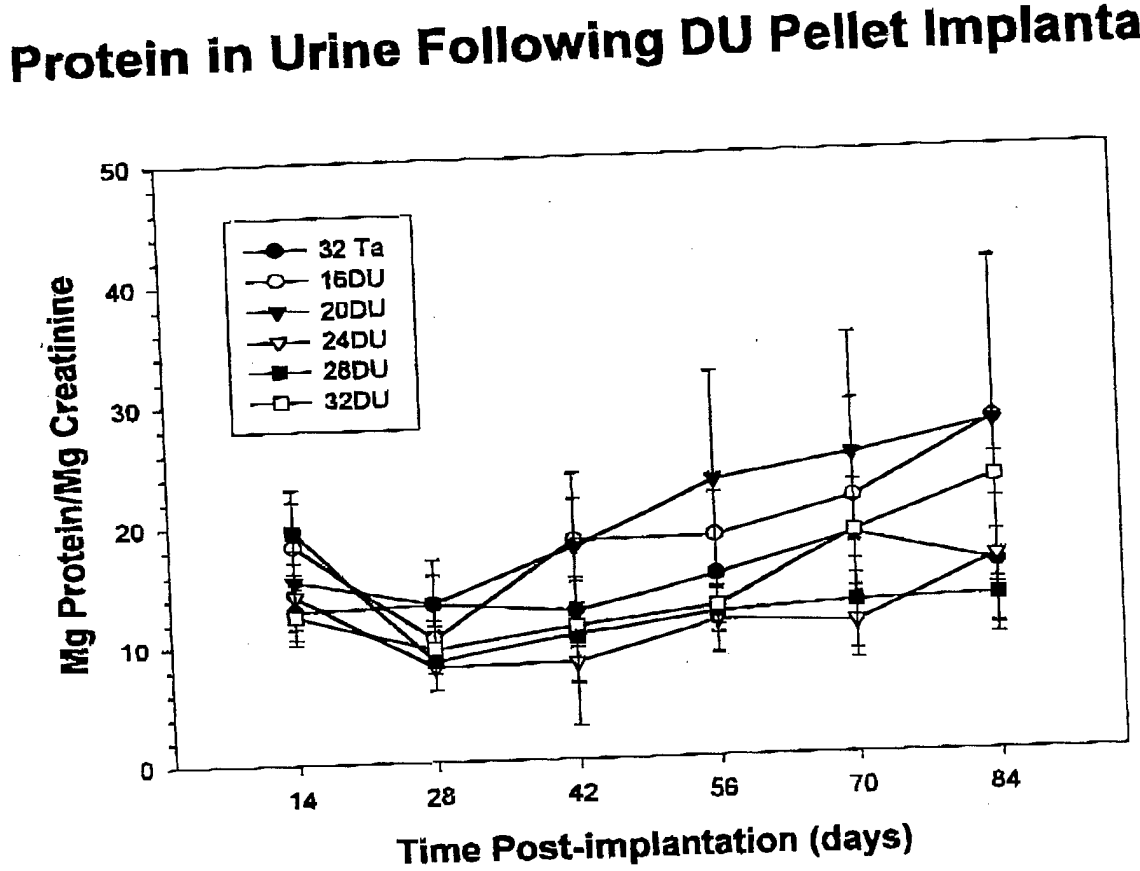

Protein in Urine Following DU Pellet Implantation
Mg Protein/Mg Creatinine
50
40
30
20
10
0
32 Ta
16DU
20DU
24DU
28DU
32DU
14
28
42
56
70
84
Time Post-implantation (days)

Figure 16

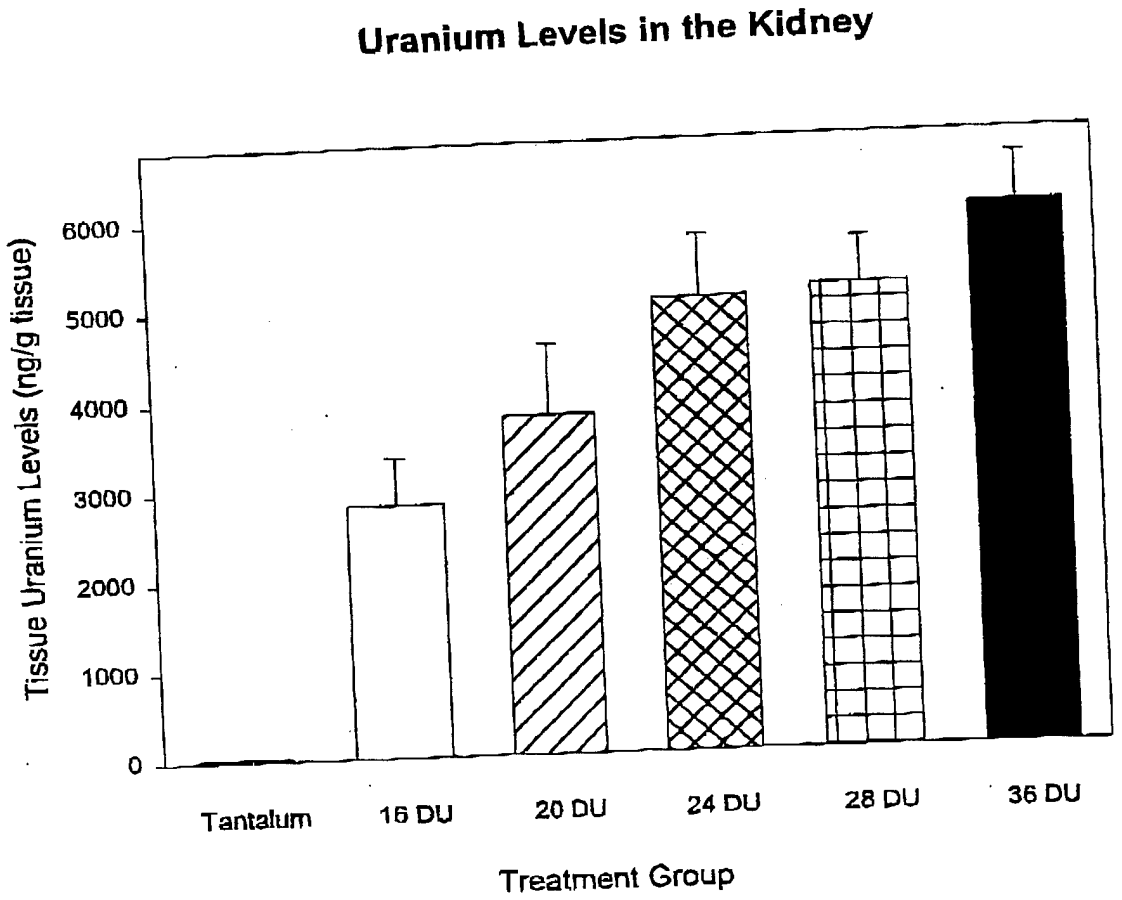

Uranium Levels in the Kidney
Tissue Uranium Levels (ng/g tissue)
0
1000
2000
3000
4000
5000
6000
Tantalum
16 DU
20 DU
24 DU
28 DU
36 DU
Treatment Group

Figure 17

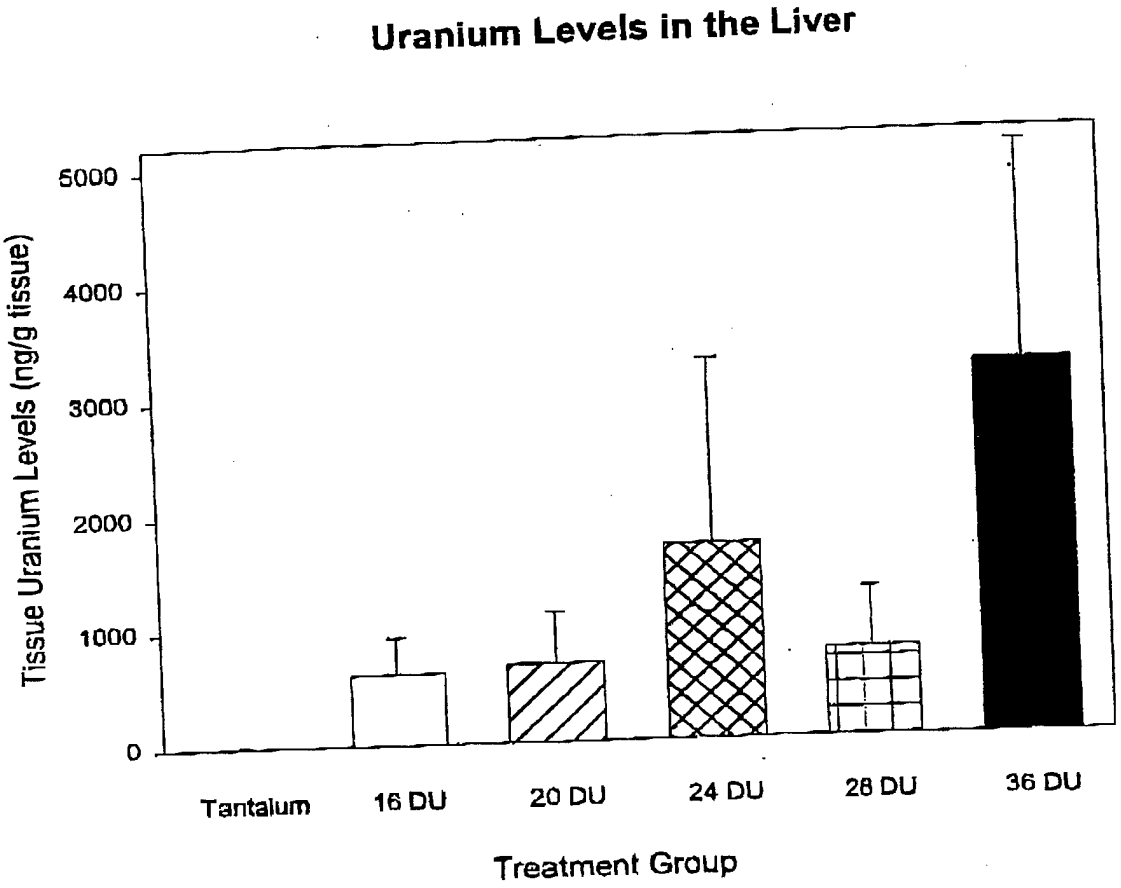

Uranium Levels in the Liver
Tissue Uranium Levels (ng/g tissue)
5000
4000
3000
2000
1000
0
Tantalum
16 DU
20 DU
24 DU
28 DU
36 DU
Treatment Group

Figure 18

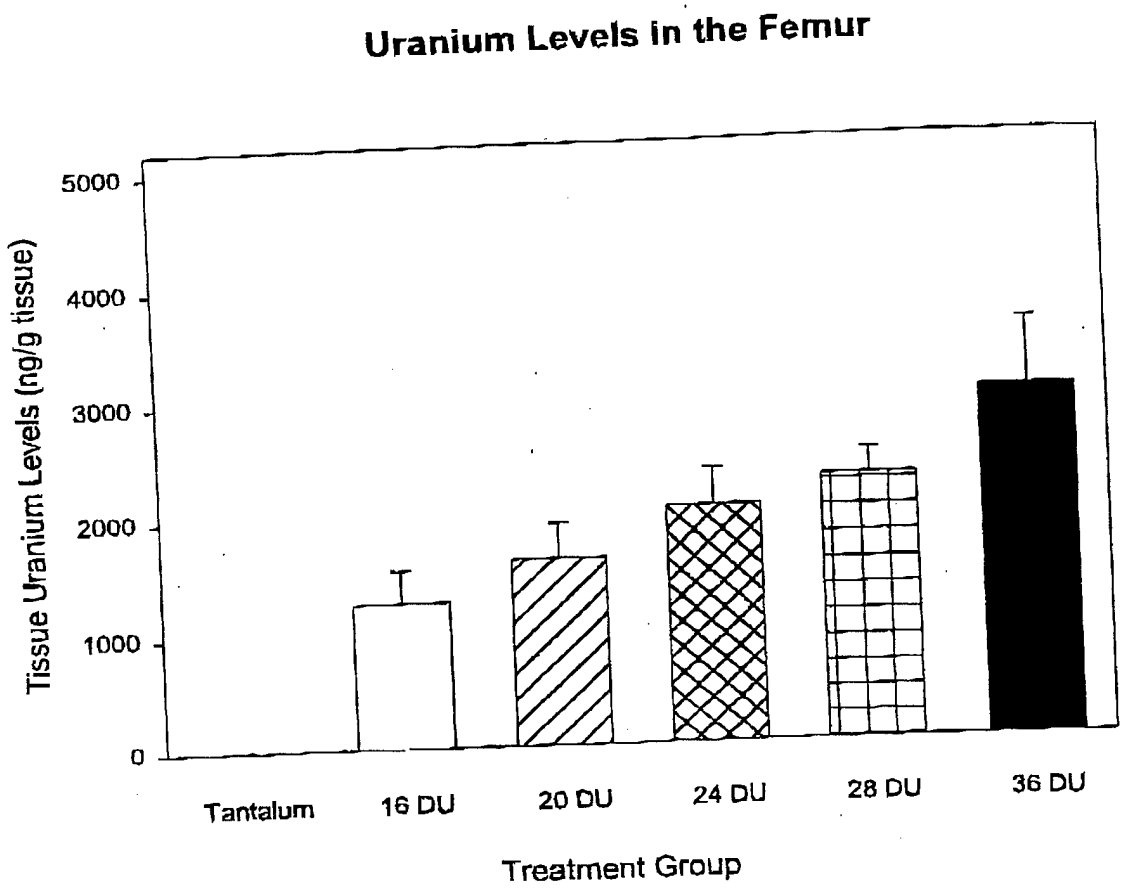

Uranium Levels in the Femur
Tissue Uranium Levels (ng/g tissue)
5000
4000
3000
2000
1000
0
Tantalum
16 DU
20 DU
24 DU
28 DU
36 DU
Treatment Group

Figure 19

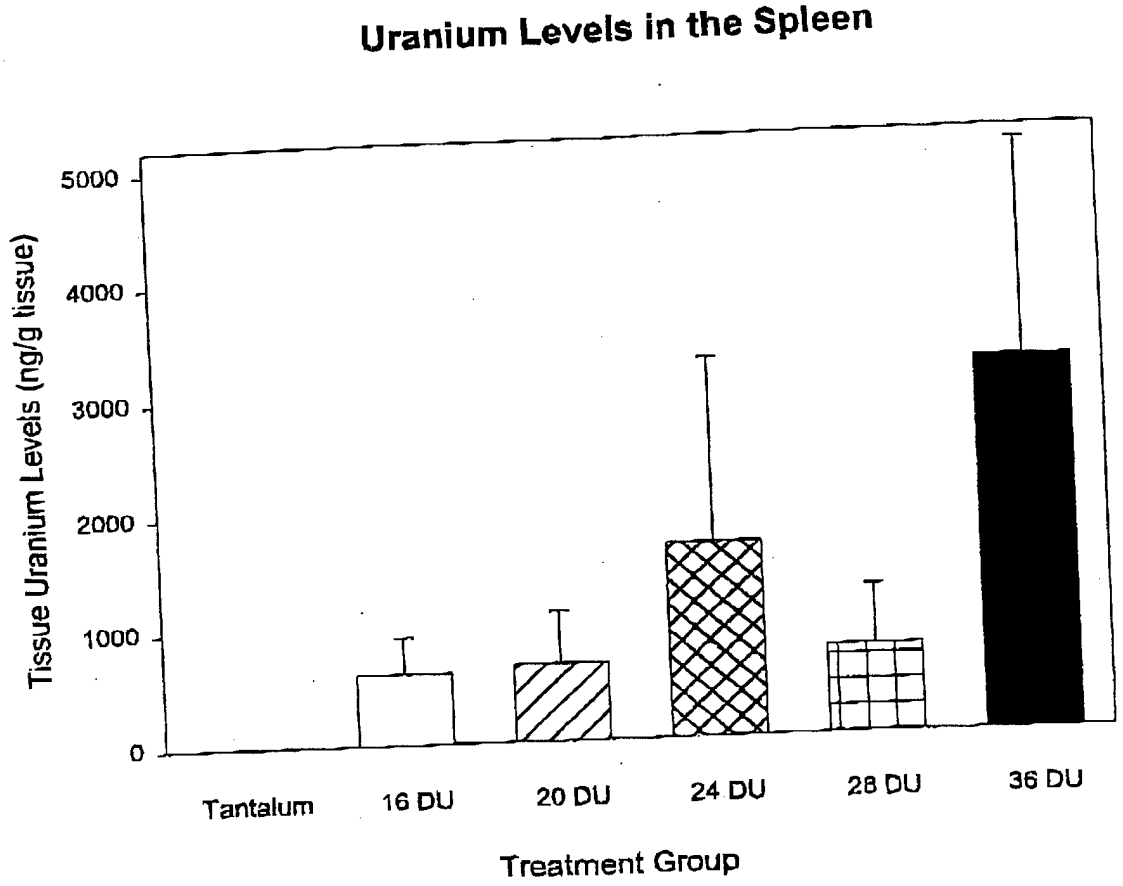

Uranium Levels in the Spleen
Tissue Uranium Levels (ng/g tissue)
5000
4000
3000
2000
1000
0
Tantalum
16 DU
20 DU
24 DU
28 DU
36 DU
Treatment Group

Figure 20

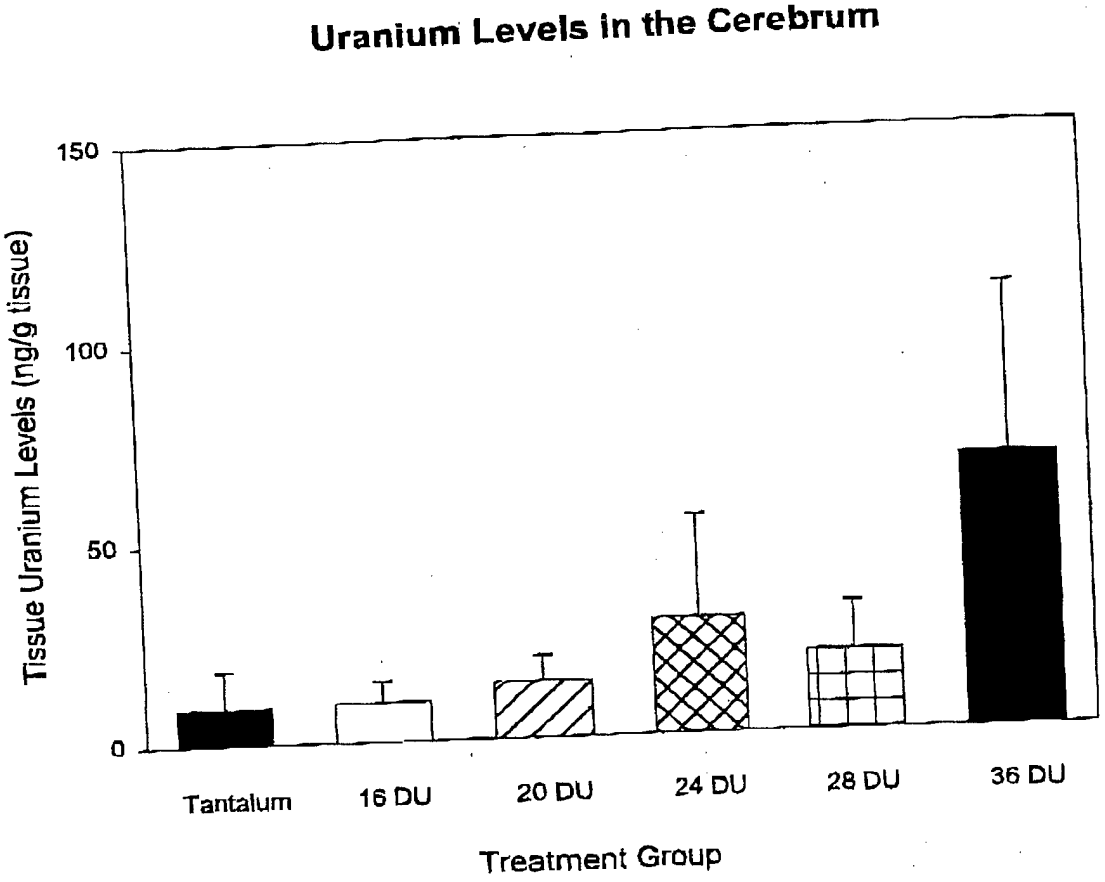

Uranium Levels in the Cerebrum
Tissue Uranium Levels (ng/g tissue)
150
100
50
0
Tantalum
16 DU
20 DU
24 DU
28 DU
36 DU
Treatment Group

Figure 21

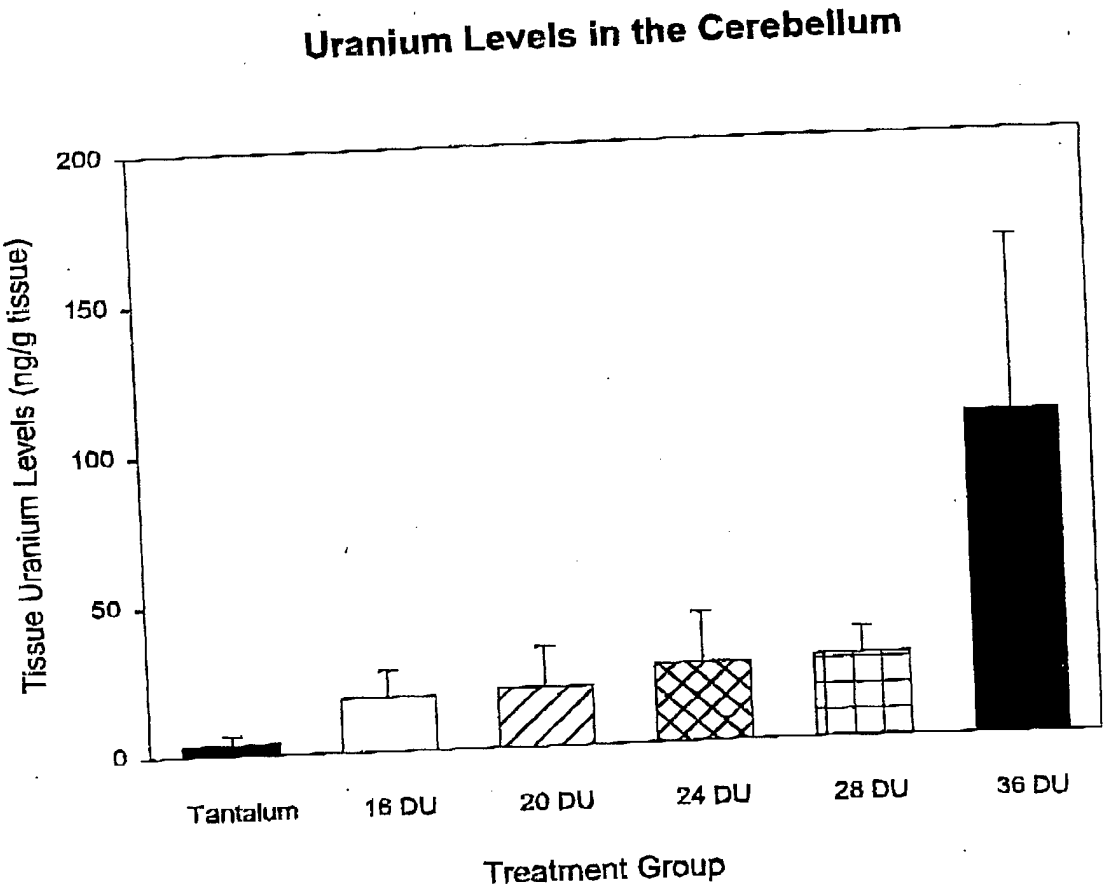

Uranium Levels in the Cerebellum
Tissue Uranium Levels (ng/g tissue)
200
150
100
50
0
Tantalum
16 DU
20 DU
24 DU
28 DU
36 DU
Treatment Group

Figure 22

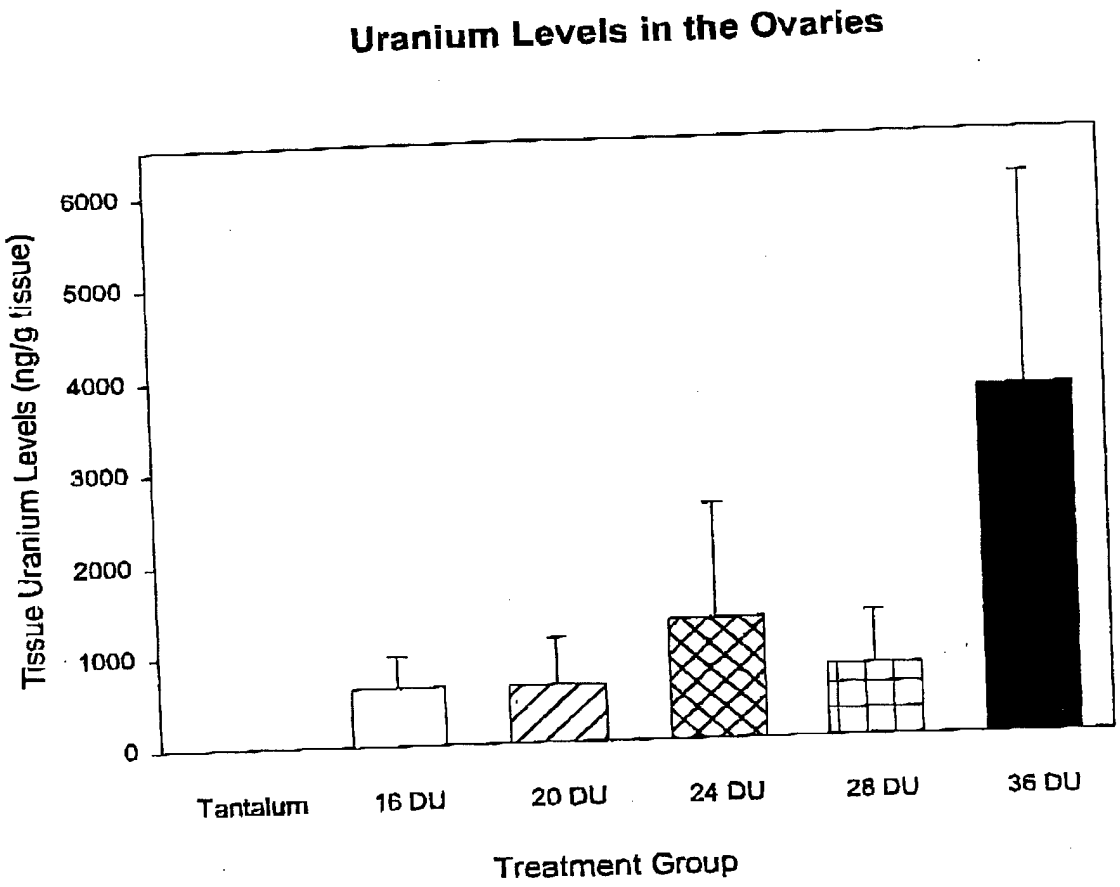

Uranium Levels in the Ovaries
Tissue Uranium Levels (ng/g tissue)
6000
5000
4000
3000
2000
1000
0
Tantalum
16 DU
20 DU
24 DU
28 DU
36 DU
Treatment Group

Figure 23

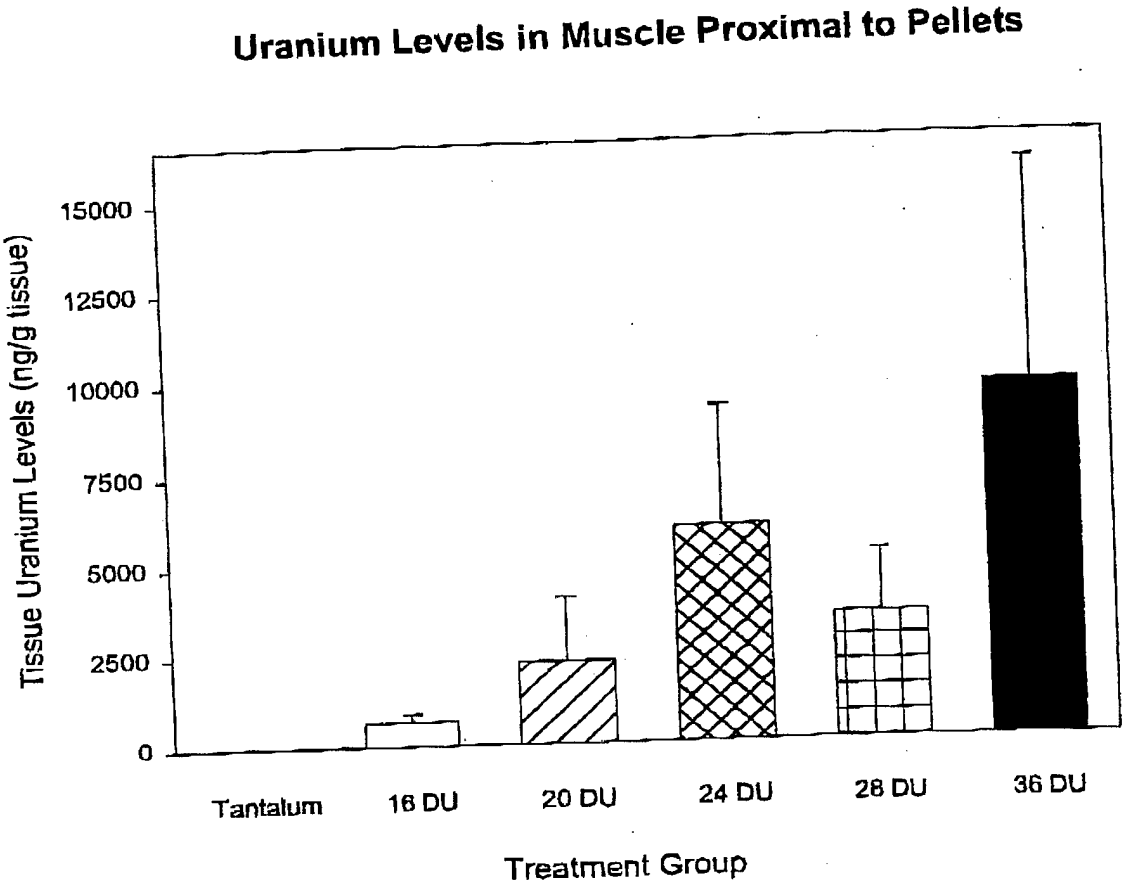
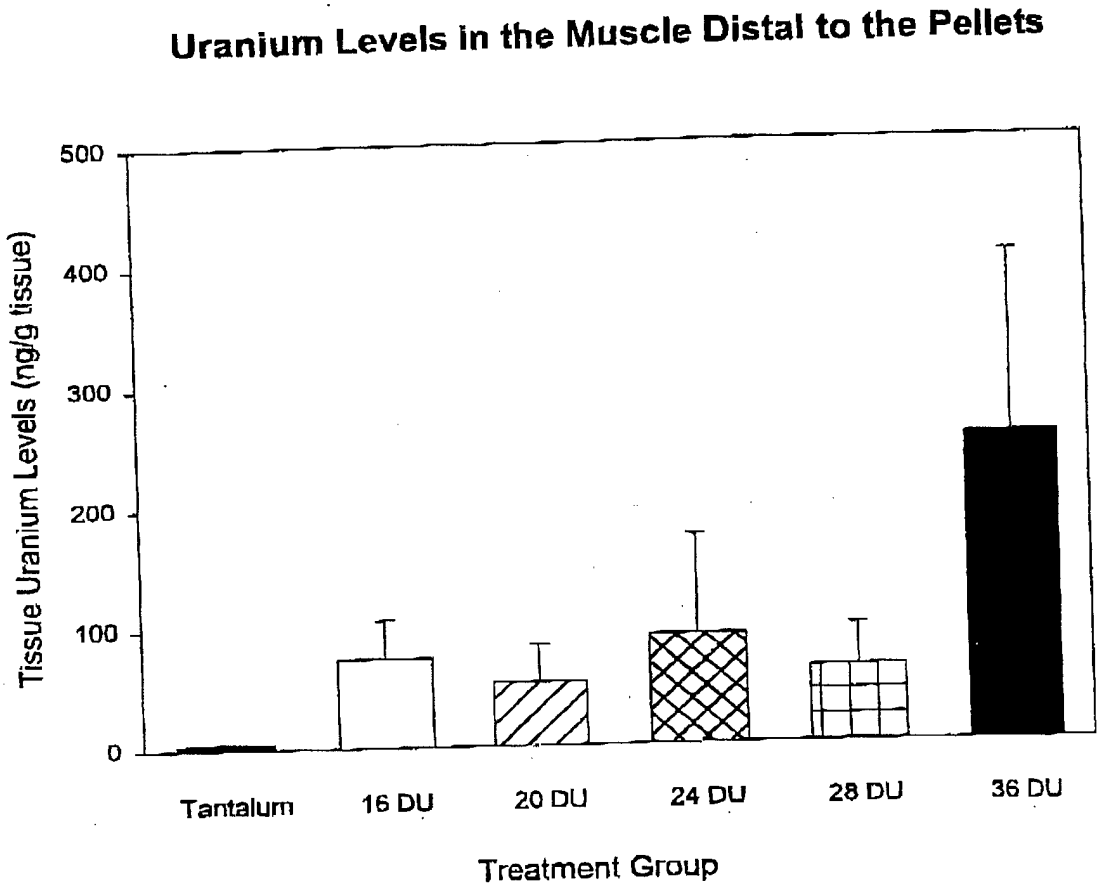

Uranium Levels in Muscle Proximal to Pellets
Tissue Uranium Levels (ng/g tissue)
15000
12500
10000
7500
5000
2500
0
Tantalum
16 DU
20 DU
24 DU
28 DU
36 DU
Treatment Group

Figure 24


Uranium Levels in the Muscle Distal to the Pellets
Tissue Uranium Levels (ng/g tissue)
500
400
300
200
100
0
Tantalum
16 DU
20 DU
24 DU
28 DU
36 DU
Treatment Group