06,84.16

Health Physics Vol. 46, No. 3 (March), pp. 688–692, 1984
Printed in the U.S.A.

0017-9078/84 $3.00 + .00
Pergamon Press Ltd.

Deposition Pattern and Toxicity of Subcutaneously Implanted Uranium Dioxide in Rats

(*Received* 29 *October* 1982; *accepted* 4 *March* 1983)

Introduction

WE HAVE recently reported that uranium dioxide, being one of the most insoluble uranium compounds, failed to cause animal death after epicutaneous application, whatever the carrier used (Re82). Further studies have demonstrated that, on the contrary, subcutaneous administration of uranium dioxide elicited toxic reactions in laboratory animals. Investigations of the translocation routes, as well as intraorgan pathways and subsequent tissue fixation, seems to be relevant due to frequent accidental occupational contamination, e.g. daily manipulation of uranium dioxide in nuclear-related industries in which workers are frequently in contact with minute quantities of uranium powder.

Cellular and subcellular sites of deposition of uranium dioxide in animal tissues are at present poorly described. Identification of these sites is important for better understanding of the cells and tissues at risk after accidental contamination.

Materials and Methods

The equivalent diameter of uranium dioxide particles was estimated by means of a Sedigraph (Micromeritics, Northcron, U.S.A.) and various grain-sized batches were selected. Known amounts of several samples (4, 10, 20 and 40 μm) were directly implanted into the subcutaneous tissue of the dorsal skin of young female Wistar rats (160 g). Through a 5-mm-long incision, 0.01, 0.05, 0.5 and 1 g UO_2/kg body weight were administered to groups of 10 rats for each experimental condition. Uranium dioxide samples were obtained from the laboratories of the Comisión Nacional de Energía Atómica, Buenos Aires, Argentina. Animals were sacrificed 3, 6, 24 and 48 hr after the implantation of the powder. Small samples of subcutaneous tissue and muscle beneath the implantation site, bone (tibial epiphysis) and teeth (continuous growth incisor) were obtained from these animals.

Light and electron microscopic observations of soft tissues were performed. Electron micrographs were obtained with a Philips EM 300 electron microscope from samples fixed with osmium tetroxide (2% in buffer Palade) which were dehydrated in alcohol and embedded in Maraglass (Ladd Res. Industries, Vermont, U.S.A.). Diamond knives were used to achieve appropriate sections that were later observed without contrasting or contrasted with uranyl acetate (not affecting the results) and lead citrate.

By means of a Cameca 322 x-ray microanalyzer, bone and kidneys from the same animals were analyzed for uranium traces. For this, the whole tibial epiphysis and the continuous growth incisor were air-dried and mounted on an aluminum stub, shadowed with carbon and gold, and later transferred to the x-ray microanalyzer.

Results

Death of animals occurred within six days with doses of uranium dioxide higher than 0.01 g/kg body weight. At lower doses animals survived in good health for at least the 10-month experimental period. No significant differences in response could be attributed to the various particle sizes of the powder (4–40 μm). Pieces of tissue were removed from the area near the implanted powder and from tissue immediately beneath it within 6 hr after implantation. Microscopic analysis revealed that electron-dense uranium deposits appeared randomly scattered in the intercellular space between the polymorphonuclear cells which predominantly constituted the tissue. Twenty-four to 48 hr later, few polymorphonuclear cells were present but were replaced by numerous macrophages.

These cells contained a variable number of vacuoles filled with electron-dense clusters of granules. Sometimes these structures lacked a clear, defined membrane but in most cases they were membrane-bounded phagosomes. At this time, there were no free particles in the cytoplasm and large numbers of particles were still present in the intercellular space (Figs. 1 and 2). Dense traces could be seen between endothelial cells in the blood capillaries of the organized granular tissue (Fig. 3) and also deep between muscle bundles. A similar analysis performed in kidneys showed the presence of electron-dense deposits in the cells and luminae of convoluted tubules from 6 to 48 hr after the administration of uranium dioxide.

Light microscopic observations of the kidneys revealed increasing stages of proximal tubular epithelial necrosis.

X-ray microanalysis of epiphysial bone, con-

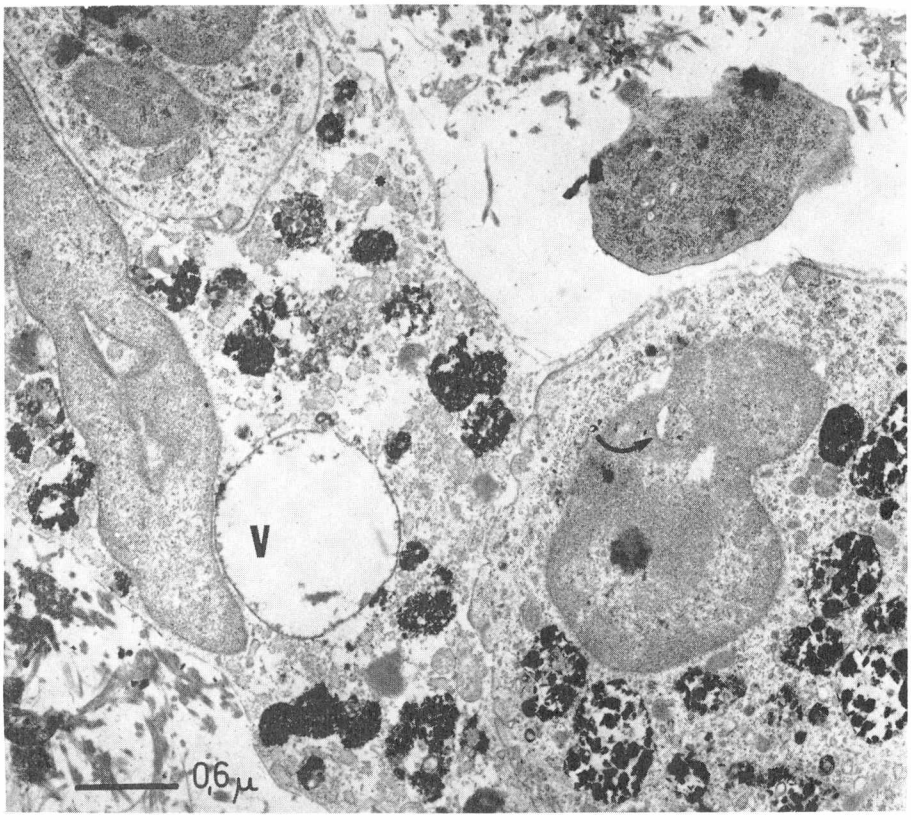

FIG. 1. Electron micrograph of two macrophages with large phagosomes in the cytoplasm. The phagosomes are filled with electron-dense material derived from uranium dioxide subcutaneously implanted six hr before. Some cellular alterations are clearly observed: nuclear inclusions (⟶), mitochondrial alterations (★), large intracellular vacuoles (V).

tinuous growth incisors and kidneys from the same animal gave information concerning the deposition of uranium in these animals. Figure 4 shows the x-ray scanning images of uranium distribution in these tissues.

Discussion

It has recently been reported that several carriers—ether acetone, water in oil and oil in water emulsions—mixed with uranium dioxide failed to produce percutaneous penetration of this insoluble compound (Re82). Previous studies involving respiratory-tract exposure to UO_2 and UO_2-feeding experiments, revealed that this compound was far below the usual range of toxicity of other uranium compounds (Yu73).

In the present work, it is demonstrated that provided the skin barrier is avoided (as happens after subcutaneous implantation), uranium dioxide freely penetrates the animal's tissues and promptly affects target sites such as kidneys and bone. Subsequently deleterious effects which affect animal survival are observed.

Electron microscopy of tissue areas surrounding the implantation site showed that free electron-dense particles of smaller size than those implanted were distributed throughout the intercellular space. The uranium composition of the electron-dense material was clearly identified by x-ray microanalysis. The same kind of particles also appeared in the capillary lumen, suggesting the vascular system as the most probable pathway to target organs. Smaller particles appeared engulfed by macrophages and other phagocytic

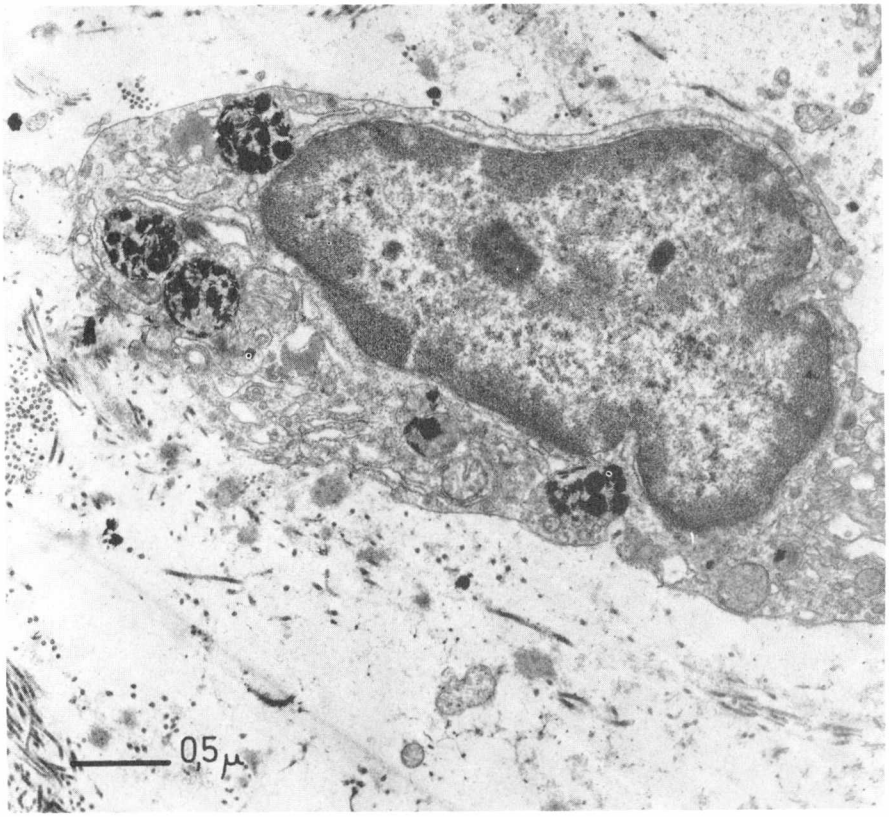

FIG. 2. Electron micrograph showing numerous electron-dense deposits in the cytoplasm of a fibroblast-like cell, 72 hr after uranium dioxide implantation.

cells. Numerous uranium-laden macrophages were seen in which phagosomes were the only cell structure involved in uranium capture. This initial cellular efflux and subsequent phagocytic activity have been described after an over-load of particulate matter when carbon was instilled in lung mice (Ad78). This cellular efflux was attributed to a nonspecific inflammatory response.

Acute renal failure caused a high mortality within six days. Progressive lesions were observed in the epithelium of cortical convoluted proximal tubules similar to those already described after uranyl nitrate injections (Ha82). After doses higher than 0.01 g/kg body weight, similar effects on survival or tissue distribution were achieved irrespective of the amount of uranium dioxide delivered. Larger particle-sized batches failed to produce any difference in animal behavior or tissue response. LaBelle reported that variations in particle size reflected a corresponding variation in the quantity of uranium entering the systemic circulation (La53). He reported that with particles smaller than 1 μm, the intensity of response was inversely proportional to the particle size. In our case, the temperature of the uranium dioxide preparation allowed the formation of only insignificant quantities of particles smaller than 4 μm, so smaller particles were not tested (St66).

This investigation has shown the ease with which uranium dioxide penetrates the animal's body and the significant amounts deposited in target organs shortly after implantation of various quantities under the skin. Thus, caution in handling this compound should be recommended because minute amounts penetrating through small skin wounds represent a serious risk for people engaged in daily manipulation of uranium, especially those related with nuclear-fuel factories.

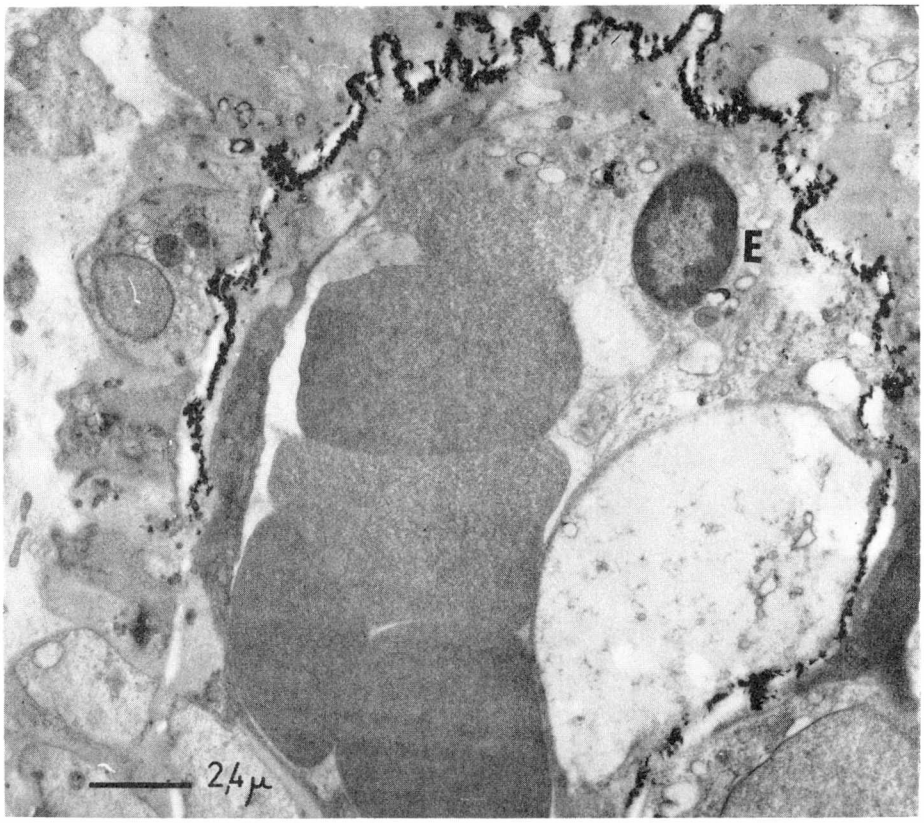

FIG. 3. Uranium deposits surrounding a capillary wall. Note endothelial cells (E) in which ultrastructural alterations are remarkable.

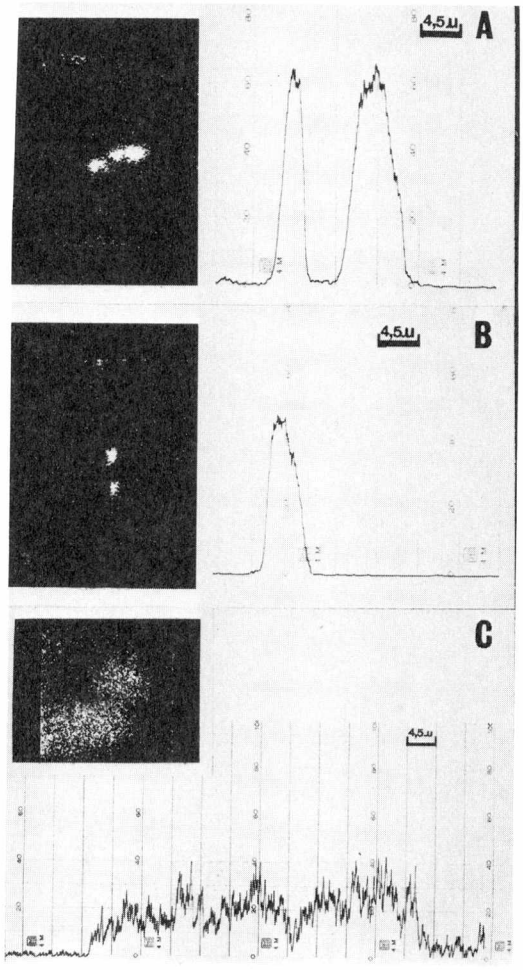

FIG. 4. X-ray scanning images of uranium element in epiphyseal bone (A), continuous growth incisor (B) and kidney (C) from the same animal. White dots in photographs at the left indicate detector pulses from uranium x rays or an equivalent energy x rays from the continuous background. On the right, the relative amount of uranium deposited in tissue is graphically shown.

Acknowledgment—This research was in part supported by Research Contact No. 2616R1/Rb from the International Atomic Energy Commission, Vienna, Austria, and by grant No. 01000100-013 from Subsecretaría de Ciencia y Técnica, República Argentina.

The authors wish to thank Dr. A. J. P. Klein Szanto for helpful comments on the manuscript.

X-ray microanalysis was performed by Dr. A. Palacios from CNEA, Buenos Aires.

The technical assistance of María Inés Gonzalez is gratefully acknowledged.

B. M. DE REY
H. E. LANFRANCHI
R. L. CABRINI

Radiobiology Department
National Atomic Energy Commission
Avenida del Libertador 8250
Buenos Aires, Argentina

References

Ad78 Adamson I. Y. R. and Bowden D. H., 1978, "Adaptive Responses of the Pulmonary Macrophagic System to Carbon. II. Morphologic Studies", *Lab. Invest.* **38**, 430.

Ha82 Haley D. D., 1982, "Morphologic Changes in Uranyl Nitrate Induced Acute Renal Failure in Saline- and Water-drinking rats", *Lab. Invest.* **46**, 196.

La53 LaBelle C. W., 1953, *Pharmacology and Toxicology of Uranium Compounds*, Chap. 21, Part E (Edited by C. Voegtlin and H. C. Hodge) (New York: McGraw-Hill).

Re83 Rey B. M. de, Lanfranchi H. E. and Cabrini R. L., 1983, "Percutaneous Absorption of Uranium Compounds", *Environm Res* **30,** 480.

St66 Steckel L. M. and West C. M., 1966, *Characterization of Y-12 Uranium Process Materials Correlated with* In Vivo *Experiences*, Union Carbide Corp. (Y-12 Plant), Oak Ridge, TN, Contract W-7405.

Yu73 Yuile C. L., 1973, *Animal Experiments in Uranium, Plutonium and Transplutonic Elements* (Edited by H. C. Hodge, J. N. Stannard and J. B. Hursh), p. 165 (Berlin: Springer-Verlag).