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TITLE

Effects of uranium comppounds on skin

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TITLE: Effects of uranium compounds on skin
INSTITUTE: Radiobiology Department, Comision de Energía Atómica, Buenos Aires, Argentina
CHIEF INVESTIGATOR: Beatriz M. de Rey
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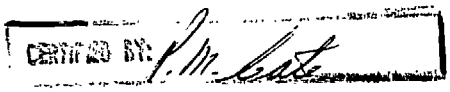


TITLE: Effects of uranium compounds on skin

RESEARCH INSTITUTE: RAdiobiology Department,Comisión de Energía Atómica,Buenos Aires, Argentina

CHIEF SCIENTIFIC INVESTIGATOR: Beatriz M. de Rey

PERIOD OF CONTRACT: July 1980-October 1982

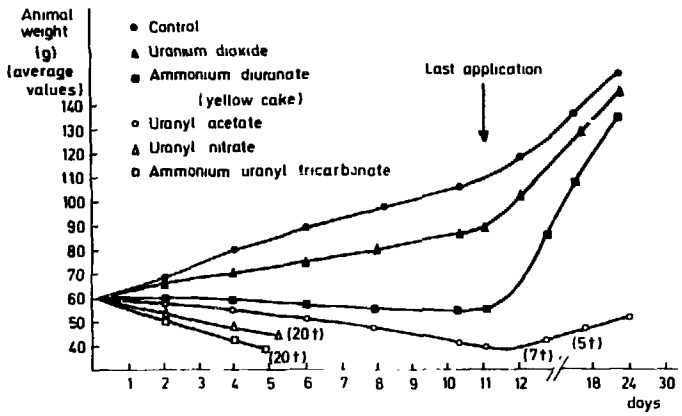
SCIENTIFIC BACKGROUND AND SCOPE OF PROJECT: Uranium extraction and refining operations leading to the manufacture of nuclear fuels have significantly augmented in later years due to the great increase in the number of nuclear facilities. The hazardous nature of the by-products formed when processing uranium ore to nuclear fuels are well known . Penetration of substances into the animal body may be accomplished by several routes: Intravenous and intraperitoneal injections inhalation, percutaneous absorption etc. Previous studies have demonstrated that after an oral dose 1-5 % ia absorbed and most is excreted via kidneys (Hursh and Spoor, 1973).Uranium absorbed into the systemic circulation leaves the blood rapidly and is eliminated via urine.(Walinder et al 1967). Inmediately after intravenous injection 50% is excreted in the urine ,25% is deposited in the skeleton and 25% in the soft tissues. (Hursh et al 1969). The analysis of the action of uranium and uranium compounds on living matter ,particularly those involving sites of deposition and subsequent damage to vital areas are relevant both from preventive and therapeutic points of view.

EXPERIMENTAL METHOD: In order to obtain information on percutaneous absorption as well as subcutaneous absorption of uranium compounds a series of experiments were carried out (de Rey et al,in press; de Rey et al ,sent for publication). Several compounds were tested specially those involved in the preparation of nuclear fuel : uranium dioxide, uranyl nitrate,uranyl acetate, ammonium uranyl tricarbonate and ammonium diuranate. Percutaneous absorption was mediated with a the aid of a vehicle: a mixture of the drug under study with an oil-in-water emulsion was applied on rat dorsal skin.

Absorption after subcutaneous implantation of uranium dioxide powder was also analyzed. Known quantities of various grain-sized batches of uranium dioxide were directly implanted in the subcutaneous tissue of the dorsal area of Wistar rats.

RESULTS: Results obtained concerning survival of animals after percutaneous absorption of the various drugs tested are shown in figurel.

EFFECTS OF DAILY EPICUTANEOUS APPLICATION OF URANIUM COMPOUNDS ON ANIMAL WEIGHT AND ANIMAL SURVIVAL



A steady body weight decrease was induced by high concentration of uranyl nitrate and all animals died five days after the onset of the experiment due to renal failure.

Histopathological analysis of several samples revealed important damage in the uranium topicated skin, affecting all tissue components and also hair follicles and adnexal glands. Ultrastructural analysis revealed that as early as fifteen minutes after the application of the absorbable compounds(uranyl nitrate) a highly electron dense band was observed in the intercellular space between the horny and granular layer. Longer periods of tissue - drug contact made this band dissappear and clusters of electron dense masses were seen scattered in the intercellular spaces. Neither the epidermis nor the dermis showed traces of this material 48 to 72 hours after applications but important substructural alterations reflected the deleterous action of the heavy metal.

Four daily applications of uranyl nitrate produced a massive absorption of the drug. No alterations were observed in the skin of animals treated either with uranium dioxide ,uranyl acetate or ammonium diuranate,which being insoluble compounds, did not penetrate through the skin of experimental animals.Energy dispersive x-ray microanalysis performed on samples from the same animals revealed that uranium was largely present in the epidermis and in lesser amounts in the dermis of animals treated with uranyl nitrate.

After subcutaneous implantation of uranium dioxide sudden death of animals occurred within six days. Microscopical analysis of samples taken from areas near the insertion site ,revealed that the electron dense uranium deposits appeared scattered in the intercellular space .Twenty four to 48 hours later numerous macrophages were observed containing variable number of vacuoles filled with clusters of electron dense granules. Dense traces could be seen between endothelial cells in the blood capillaries . Also kidney tubular cells contained these dense deposits . X-ray microanalysis of target tissues : epiphysial bone, continuous growth incisor and kidney revealed the presence of uranium deposits.

CONCLUSION:The present results have demonstrated the ease entrance of uranium compounds into experimental animals'body and the important amount deposited in target tissues,shortly after drug penetrattion. Caution in handling uranium compounds should be recommended becaude minute amounts penetrating through small skin wounds or by percutaneous absorption represent a serious risk for people engaged in daily manipulation of uranium particularly those related with nuclear fuel factories or uranium ore explotation.

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de Rey,B.M.,H.E.Lanfranchi and R.L.Cabrini.Percutaneous Absorption of uranium compounds. Environm Res (en press.)

de Rey,B.M., H.E.Lanfranchi and R.L.Cabrini. Deposition pattern and toxicity of subcutaneously implanted uranium dioxide in rats. (sent for publication to Health Physic)

PAPERS PUBLISHED ON WORK UNDER THE CONTRACT

B.M.de Rey,H.E.Lanfranchi and R.L.Cabrini.Percutaneous absorption of uranium compounds.Environm Res(in press)

B.M.de Rey,H.E.Lanfranchi and R.L.Cabrini. Deposition pattern and toxicity of subcutaneously implanted uranium dioxide in rats. (Sent for publication to Health Physic)

COMMUNICATIONSS TO SCIENTIFIC MEETINGS

Absorción percutanea de compuestos de uranio (Percutaneous absorption of uranium compounds) B.M.de Rey, H.E.Lanfranchi and R.L.Cabrini.
XVIII Congreso Argentino de Patología,1981.

Efectos biológicos de la contaminación de uranio (Biologic effects of uranium contamination) H.E.Lanfranchi, B.M.de Rey and R.L.Cabrini
XVII Congreso Argentino de Patología,1980.

Metodología para la demostración histoquímica de uranio en cortes de tejidos. (Methodology for the histochemical demonstration of uranium in tissue sections) M.E.Itoiz, S.Orrea.
XVIII Congreso Argentino de Patología,1981.

Efectos tóxicos de la aplicacion subcutanea de dióxido de uranio (Toxic effects after subcutaneous application of uranium dioxide)H.E.Lanfranchi, and B.M.de Rey.
Intern. Ass, for Dental Res. Argentine Section. XIV Reunión Anual,1981.

Estudio piloto de la mucosa bucal y tejidos periodontales en personas expuestas a la contaminación con uranio (Pilot study of the oral mucosa of persons exposed to uranium contaminaton). H.E.Lanfranchi, O.R.Costa ,J.J.Carraro and

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Efecto tóxico del dióxido de uranio en la enfermedad periodontal experimental (Toxic effects of uranium dioxide on experimental periodontal sickness). H.E.Lanfranchi,O.R.Costa and B.M.de Rey. Internat.Assoc. Dental Res. Argentine Section. XV Reunión Anual,1982.

Acción Tóxica del nitrato de Uranilo sobre el tejido oseo (Toxic effects of uranyl nitrate on bone tissue) M.B.Guglielmotti,B.M.de Rey, A.M.Ubios and R.L.Cabrini. XIX Congreso Argentino de Patología. 1982.

Efecto del nitrato de uranilo sobre la cicatrización alveolar (Effects of uranyl nitrate on socket healing.) M.B.Guglielmotti,A.M.Ubioa and R.L.Cabrini.
Intern. Ass. Dental Res. Argentine section. XV Reunión Anual,1982.

COMMENTS OF PRINCIPAL INVESTIGATOR

The finantial support of IAEA has been applied to fulfil a scientific project that has allowed a group of scientists of the Radiobiology Department to carry out a program on uranium contamination with special emphasis on skin contamination. Under the name of "Effects of Uranium compounds on skin" we have investigated ,during the period covered by the Research Contract :July 198o- october 1982, the effects of various uranium compounds on living matter, foccussing the efforts to elucidate the ways of entrance through skin,deposition on target organs and,lately deposition on hard tissues. Important original results have been obtained and published:

B.M.de Rey, H.E.Lanfranchi and R.L.Cabrini. Percutaneous absorption of Uranium compounds, Environm. Research (in press)
B.M.de Rey,H.E.Lanfranchi and R.L.Cabrini.Deposition pattern and toxicity of subcutaneously implanted uranium dioxide in rats. Health Physics (Sent for publication)

Several Scientific Communications in National Meetings have also been performed.
It is important to point out that several new investigation lines on uranium -living matter interaction have been opened that are at present being carried out successfully.

B.M.de Rey

TITLES OF RESEARCHES PERFORMED UNDER RESEARCH CONTRACT 2616/RB and 2616/R1/RB

1. PERCUTANEOUS ABSORPTION OF URANIUM COMPOUNDS

2. EFFECTS OF UO_2 AFTER SUBCUTANEOUS IMPLANTATION

3. HISTOCHEMICAL DEMONSTRATION OF URANIUM DEPOSITS IN TISSUES.

4. DETERMINATION OF URANIUM TRACES IN DIFFERENT ORGANS AND TISSUES

5. DEPOSITION OF URANIUM ON HARD TISSUES

PERCUTANEOUS ABSORPTION OF URANIUM COMPOUNDS

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INTRODUCTION

Uranium extraction and refining operations leading to the manufacture of nuclear fuels have significantly augmented in later years due to the great increase in the number of nuclear facilities. The hazardous nature of the by products formed when processing Uranium ore to nuclear fuel are well known. These compounds are especially dangerous to the persons directly involved in the industrial process and are also of considerable interest because of the risks of environmental contamination.

Penetration of substances into the animal body may be accomplished by several routes: intravenous and intraperitoneal injections ,inhalation , percutaneous absoption etc. As uranium is considered either a chemical or a radiological hazard depending on its isotopic composition,when natural uranium(99% ^{238}U) is considered ,the total quantity of metal incorporated is the determinant regardless of the compound involved. One to 5% of an oral dose is absorbed and most is excreted by the kidney(Hursh and Spoor, 1973).Uranium absorbed into the systemic circulation leaves the blood very rapidly and is eliminated via urine approximately 60% of uranium within the first 24 hours(Walinder et al,1967).Immediately after intravenous injection about 50 percent is excreted in the urine, 25 percent is deposited in the skeleton and 25 percent in the soft tissues. Only traces remained in the blood .Uranium deposited in soft tissue other than kidney,and in the bones,is excreted later at slower rate (Hursh et al,1969). After inhalation the retention depends on particle size and chemical formulae. Long biological half times of 120 days or more can be anticipated from insoluble compounds(UO_2, U_3O_8) having particle size under 2μm (Harris,1961)

Percutaneous absorption has been reported as an effective route of penetration of soluble uranium compounds(Orcutt, 1949). However

detailed information about the mechanism of penetration and the damage induced by percutaneous absorbed uranium through the skin "in vivo" are not available. The analysis of the action of uranium and uranium compounds on living matter, particularly those involving sites of deposition and subsequent damage to vital areas are relevant both from preventive and therapeutic points of view. In order to obtain information on percutaneous absorption of soluble and insoluble uranium compounds ,several experiments were carried out.The aims of these experiments were A) to describe more precisely the mechanisms and pathways of intradermal penetration of uranium salts and B) to assess the cutaneous damage induced by these compounds.

MATERIALS AND METHODS

Topical applications with the following uranium compounds were performed on the dorsal skin of thirty five days old Wistar rats (60 g,males): uranyl nitrate 0.5,1.7,3.5 and 7g/kg body weight (The British Drug Houses Ltd,BDH). Uranyl acetate (May and baker Ltd) 3.5 and 7g/Kg body weight. Ammonium uranyl tricarbonate 7g/kg body weight. Ammonium diuranate 7g/kg body weight. An oil-in-water emulsion composed by vaseline and water (Aqualane ,trade mark, kindly supplied by Roux Ocefa Argentina) was used as vehicle. The desired proportion of the drug to be tested was mixed with 1cc of the emulsion . The cream was applied on a 3 sq cm dorsal area 24 hours after shaving . Single or daily applications were performed with the aid of a small brush and each animal was confined in a separate cage to avoid sibling licking of the treated area. Ten daily applications of the drugs were performed on the dorsal shaved area that have been preciously covered with adhesive tape in order to prevent uranium contact with the skin. No effect was observed in the animals and no weight changes were recorded in the experimental animals compared with the control ones, so oral intoxication by self licking was discarded. Daily to-

Twenty animals were used in each of the treatment groups in order to evaluate weight changes and survival values. Daily topical applications were performed in the animals at the same hour of the day.

Light and Electron Microscopy studies: groups of ten animals were sacrificed 2 and 4 days after daily applications of the above mentioned uraniumcompounds. In addition groups of ten animals were treated with a single application of uranyl nitrate and the effects were analyzed fifteen minutes to 24 hours after application. Specimens from treated and non treated skin from the same animal and from non treated animals were simultaneously processed for light and electron microscopy. Paraffin sections were stained with haematoxilin and eosine. Small skin samples from the same animals were fixed in 2% Osmium tetroxide in buffer Palade. After dehydration with graded alcohols and propilene oxide, they were flat embedded in Maraglass. Thin and ultrathin sections of properly oriented samples were obtained in an LKB ultramicrotome. Contrasted and non contrasted

sections were photographed in a Philips 300 and a Philips 200 electron microscopes.

A semiautomatic analyzing system (MOP 3 Kontron ,Messgerate GMbH,Munich) was used to measure the surface area of basal cells from epidermis. Thin sections from the above mentioned experimental conditions,were coloured with toluidine blue and projected on the measuring tablet of the equipment. Measurements of surface area were performed on interfollicular basal cells of epidermis obtained from animals treated with uranyl nitrate plus vehicle and from animals treated with vehicle only. Average data from one hundred cells from each experimental condition were scored.

X-Rays microanalysis: Energy dispersive x-ray microanalysis was used to detect uranium in the skin. The measurements were performed in an EDAX 711 attached to a Scanning Electron Microscope Philips 500 This device directly measures the energy of the characteristic x-rays of the sample by means of a solid state detector(silicium litium) which simultaneously can register all the elements present in the sample. In this particular case the microprobe provides information corresponding to 600 um^2 of epidermis . Pieces of skin obtained from the same areas used to perform morphologic and morphometric analysis were fixed in glutaraldehyde 3% during 1.5 hours,dehydrated in a series of graded alcohols to 100% ethanol,transferred to a critical point drier and dried with liquid CO_2. The tissue was attached to an aluminum stub and placed in a vacuum evaporator for coating with carbon and gold.

RESULTS

Daily epicutaneous applications of uranium compounds affect animal body weight and survival. Results are shown in figure 1. Normal constant gain in body weight was characteristic of non treated animals . A steady body weight decrease was induced by high concentration of uranyl nitrate and ammonium uranyl tricarbonate. Furthermore all animals died five days after the onset of the applications . Kidney injury was the cause of death of treated animals. No increase in body weight was detected after application of ammonium diuranate performed daily during 11 days, but a marked recuperation was achieved after discontinuing the applications. Control values were reached 14 days later and all animals survived for at least 30 days. A signifi cant body weight decrease was obtained after 11 daily applications of uranyl acetate .Sixty percent of the animals died two days after the last application also due to renal failure. The rest of the animals survived but weight values were 70 percent lower than controls. Animals treated with uranium dioxide showed some weight gain always somewhat lower than the control values.No animal died during the treatment and all animals reached normal weight 15 days after the last application.

Histopathology:

Uranyl nitrate treated tissue observed two days after application of the compound was characterized by evident signs of tissue damage (fig 2). The horny layer appeared detached and abundant purulent exudate was observed on the epidermal surface. Some neutrophils were seen migrating through the epidermis. In some areas basal and spinous cells appeared swollen and vacuolated with widened intercellular spaces. Severe alterations affected hair follicles and the corresponding sebaceous glands. Dilated and empty hair channels alternated with others that were obliterated with purulent material.. The sebaceous glands appeared hypertrophic . Four days after the initial application,atrophy of all tissue components was observed.

Although all the experimental samples were obtained from topically treated skin, severely damaged areas alternated with slightly damaged areas. Fewer alterations were observed after ammonium uranyl tricarbonate applications, the most outstanding feature was the widening of the intercellular spaces. Other uranium compounds provoked no cutaneous changes at the light microscopical level.

Quantitative data on uranyl nitrate induced epidermal atrophy were obtained . Average values of basal cells surface area are shown in Table 1.

Ultratsructural analysis:

Percutaneous absorption of uranium and the subsequent cell alterations after local application of uranyl nitrate, were clearly observed by electron microscopy. Fifteen minutes after the application of the compound a highly electron dense band was observed in the intercellular space between the horny layer and the granular layer.(fig 3). A laminated structure, resembling that of the material normally extruded in the epidermis by lamellar bodies, was detected(fig 4). Large quantities of these lamellar bodies could be seen into the granular layer abutting the cell membrane. Longer periods of tissue-drug contact made this band dissappear and clusters of electron dense material were seen scattered in the intercellular spaces of the spinous and basal layers.(fig 5). Twenty four hours after a single application some electron dense traces were observed in some blood capillary endothelial cells of the upper dermis (fig 6) as well as dispersed between the collagen fibers. Neither the epidermis nor the dermis showed traces of electron dense material 48 72 hours after applications but important substructural alterations indicated the heterecous action of the heavy metal:cell membrane interruptions and infoldings, disrupted mitochondrial membranes and dissarrangement of cristae, as well as enlarged keratinosomes and fibrilar and nuclear membrane alterations were the

most outstanding changes observed (fig 7). An unusual diffuse electron density was also observed on nuclei,membranes and other organelles of unstained sections.

Four daily applications of uranyl nitrate produced a massive absorption of the drug . Large quantities of electron dense deposits of uranium were observed in the intercellular spaces of the epidermis and dermis. No alterations were observed in the skin in the animals treated with either uranium dioxide, uranyl acetate or ammonium diuranate, which being insoluble did not penetrate into the skin,not even after succesive daily applications did these compounds produce any effects. Except for a thin band in the uppermost layers of the epidermis ,no dense deposits were observed in the skin.

Energy dispersive x-ray microanalysis performed on samples from the same animals,revealed that uranium was largely present in the epidermis and in lesser amounts in the dermis of animals treated with uranyl nitrate (fig 8). Excepting for small deposits over the superficial area,no traces of uranium were found after application of uranium dioxide . Only traces were detected in sparse areas after application of either uranyl acetate ,uranyl tricarbonate or ammonium diuranate.

DISCUSION

Considerably variation in absorption and consequently in animal survivaland body weight was noted after epicutaneous application of various soluble and insoluble uranium compounds.Uranyl nitrate and ammonium uranyl tricarbonate ,the most soluble uranium compounds tested ,were highly toxic causing important decrease in animal weight and the death of all the experimental animals five days after drug application.Slightly soluble compounds like ammonium diuranate and uranium acetate caused only a slight decrease in body weight.Uranium dioxide ,the most insoluble compound used,was the less toxic chemical,causing only a transitory slight body weight decrease . These findings are in agreement with Orcutt's data on the effects of uranium salt application on rabbit skin (Orcutt ,1949) .

In order to detect the presence of uranium element within the skin tissues,x-ray microanalysis was performed.X-ray microanalytical methods have been widely used to trace the distribution of elements in tissues(for review see 3, Hall 1979). In the present studythe measurements performed with the EDAX revealed the existence of uranium in the epidermis and dermis after topical application of uranyl nitrate . Thus ,the uranium nature of the electron dense material ,that was visualized by transmission electron microscopy ,was confirmed. An important amount of uranium seems to remain in the skin samples after fixation and embedding and the high electron density of the metal made it possible to visualize the routes of penetration of the metal after surpassing the epidermal barrier.

Numerous studies dealing with skin permeability have indicated that skin barrier is the major impediment in percutaneous absorption(Schleuplein 1949,Barr 1962). In the series of experiments reported above ,lamellar electron dense areas located between horny and granular layer were observed at the site of the epidermal barrier(Elias 1975). In this compartment large amounts of uranium entering the skin seemed to be initially stopped. Penetration is

regulated ,at least in the initial stages ,by large number of lamellar bodies and intercellular lamellar structures that conform the permeability barrier (Elias 1975). The observation of succesive samples demonstrated that the penetration of uranyl nitrate ,after surpassing the barrier ,is made to a large extent along intercellular regions and through the pilosebaceous apparatus,but lesser amounts also traverse the tissues by trancellular mechanisms. That substances traverse cells and intercellular spaces in a nonpreferencial manner have been inferred by Elias(1975) observing the fluxes of substances across the epithelia.

Succesive daily application of uranyl nitrate induced a massive absorption by skin ,evidenced by the large intercellular deposits of electron dense uraniumclusters. This uncontrollable penetration can be attributed to a deficient state of intercellular contacts and also to functional and structural alterations of cell membranes which were primarily induced by the initial penetration of the compound through the epidermis. As this phenomenon was clearly observed only after uranyl nitrate application, a probable effect of NO_2 anion on membrane lipoprotein complexes is suggested. Other uranyl compounds such as ammonium uranyl tricarbonate and uranyl acetate ,although causing significant m ortality and body weight decrease ,failed to induce detectable uranium deposition in the tissues. Furthermore it is important to point out that in systemic studies dealing with the absorption of various metal compounds through the skin ,differences between the penetration have been shown to exist,depending on how the metal is bond and on the type of complex compounds that are formed when the substance is dissolved in water and lipids(Skog 1963)Rothman(1943) reported that substances soluble in both lipids and water ,like uranyl nitrate penetrates the skin more readily than less soluble or non soluble substances.

Ultrastructural cell alterations as well as quantitative data on basal cells provided reliable information about the toxic effect of soluble compounds on skin components. The remarkable alterations of all the membrane constituents of epidermal cells, were clearly observed affecting cellular architecture that can influence on the cell-contact interactions and cell metabolism. Mor-

phometric measurements performed in basal cells from uranyl nitrate treated skin showed that the basal cells' surface area was 50% smaller than in the control epidermis. This decrease was time and compound concentration independent. Previous studies have suggested that variations in cell morphometric end points correlate well with alterations in cell metabolism (Klein Szanto 1977). In conclusion the reduction in cell area after topical application of uranyl nitrate directly reflects the toxic action of the drug leading to important involutional changes and finally to atrophy of the tissue.

The above results demonstrated that percutaneous absorption is an effective route of penetration of soluble uranium compounds. This mechanism which implies liberation of the active ingredient from its vehicle and the subsequent absorption in and through the skin, is important in connection with the toxic effect of heavy metals and it must be considered when health regulations are made regarding handling of uranium compounds.

LEGENDS TO FIGURES

Fig.1 Effect of various epicutaneously applied uranium compounds on animal weight.The number of animals that died during the treatment are shown between brackets.

Fig 2. Micrograph of skin from a dorsal area treated with uranyl nitrate. Note the purulent material on the epidermal surface. The horny layer appeared detached. Basal and spinous cells appeared swollen and vacuolated. Haematoxiline and eosine. 130 x.

Fig 3. Electron micrograph showing an electron dense band in the intercellular space between horny and granular layer( ). Non contrasted section. 5000 x.

Fig 4. Electron micrograph of horny layer showing a dense intercellular band with an organized membrane structure resembling lamellated bodies. Non contrasted section. 14000 x.

Fig 5. Electron micrograph of skin topicated with uranyl nitrate. Dense clusters of uranium deposited in the intercellular spaces are shown.Note the striking electron density of nuclei in a non contrasted section,probably also due to uranium deposition. 3500 x.

Fig 6. Electron micrograph showing traces of uranium in a dermal capillary vessel . Noncontrasted section. 1200 x.

Fig 7. Electron micrograph of a basal cell from epidermis that has been treated with uranyl nitrate eight hours before. Striking alterations are observed in the intercellular space. Cellular contacts are largely modified and residual material is seen scattered into widened intercellular area. Mitochondria appeared smaller and deep disarrangements of cristae are observed Section contrasted with uranyl acetate and lead citrate.8000 x.

Fig 8. Scanning electron micrograph of cross sectioned skin treated with uranyl nitrate four hours before. Crossed lines indicate the exact site of microprobe evaluation. Two peaks are seen in the EDAX spectrun. U corresponds to uranium element and AU corresponds to gold(from the shadowing technique). 30 x.

TABLE 1

BASAL CELLS SURFACE AREA(μm^2)

Number of applications (daily)	Non treated animals	Animals treated with uranyl nitrate *(g/kg body weight)			
		0.5	1.7	3.5	7
2	62.4±5.4	42.2±3.2	44.7±2.7	43.6±4.8	43.8±2.9
4	63±6.2	42± 4	39±3.8	37±2.5	35.2±2.8

-*each dose was administered with 1cc of vehicle
all values represent mean values ± standard deviation

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ACKNOWLEDGMENTS

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The technical assistance of Miss María Inés Gonzalez is gratefully acknowledge.

OF ... ANEOUS ... URANIUM DIOXIDE IN ...

R., H.L. Lanfra... R.L. Gab...ni
...Department, National Atomic Energy Commission
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Deposition Pattern and Toxicity of Subcutaneously implanted Uranium dioxide in rats

B.M.de Rey, H.E.Lanfranchi and R.L.Cabrini

Recently we have reported that uranium dioxide, being one of the most insoluble uranium compounds, failed to cause animal death after epicutaneous application, whatever the carrier used.(Re1982). Further studies have demonstrated that subcutaneous applications of uranium dioxide elicited toxic reactions in laboratory animals.The investigation of the translocation routes as well as intratisular penetration pathways and subsequent tissue fixation seems to be relevant due to frequent accidental occupational contamination as for example the every day manipulation of uranium dioxide in nuclear related industries in which workers are exposed to very frequent contact with minute quantities of uranium powder.

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

MATERIAL AND METHODS

Uranium dioxide from various grain-sized batches (4,10,20 and 40 um) was directly implanted in subcutaneous tissue of the dorsal skin of young Wistar rats (160 g). By means of a 5 mm long incission, 0.01, 0.05, 0.5 and 1 g UO_2/Kg of 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 hours after implantation of the powder. Small samples of subcutaneous tissue and muscle underneath the implantation site, bone (tibial epiphysis) and teeth (continuous growth incisor) were obtained from these animals.

Light and electron microscope observations of soft tissues were performed. Electron micrographs were obtained with a Philips EM 300 electron microscope from samples fixed with osmiun tetroxide (2% in buffer Palade) which were dehydrated in alcohol and embedded in Maraglass. Diamond knives were used to get appropiate sections that were later observed without contrasting or contrasted with uranyl acetate and lead citrate.

By means of a Cameca 322 x-ray microanalyzer, calcified tissue and kidney from the same animals were analyzed in search of 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:

Sudden death of animals subjected to the action of uranium dioxide occurred within six days when doses delivered were higher than 0.01 g/Kg. of body weight. At doses lower than this, animals survived in good health at least during the 10 month experimental period. No significant differences in response could be attributed to the varying particle size of the powders (4-40 um).

Pieces of tissue were taken from the area near the implanted powder and from tissue of areas immediately beneath it within six hours after implantation. Microscopical analysis revealed that electron dense uranium deposits appeared scattered at random in the intercellular space between the polymorphonuclear cells which predominantly constitued the tissue infiltrate. Twenty four to 48 hours later few polymorphonuclear cells were present but, instead, numerous macrophages were observed.

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

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

X-ray microanalysis of epiphysial bone, continuous growth incisor and kidney from the same animal gave information concerning the deposition of uranium in these animals. Fig.4A shows the x-ray scanning images of uranium distribution in bone, teeth and kidney. In all these cases, white dots indicate concentrations of uranium in tissue samples. Maximun values are graphically represented showing relative uranium concentration.

DISCUSSION:

In a recent publication we have reported that several carriers: i.e. ether, acetone, water in oil and oil in water emulsions mixed with uranium dioxide failed to produce percutaneous penetration of this highly insoluble compound (Re1982). 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 (Yu1973). In the present work we have 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 kidney and bone. Subsequently deleterous effects which affect animal survival are observed.

Electron microscopy of tissues from areas surrounding the entrance 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. Thinner particles appeared phagocyted by macrophages and other phagocytic 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 phagocitic activity have been described after an overload of particulate matter when carbon was instilled in lungs of mice (Ad1978). This cellular

efflux was attributed to a nonspecific inflammatory response.

Acute renal failure caused a high mortality rate within six days. Progressive lesions were observed in the epithelium of cortical convoluted proximal tubules similar to those already describedafter uranyl nitrate injection (Hal982). After doses higher than 0.01 g/Kg of body weight similar effects on survival or tissue distribution were achieved irrespective of the amount of uranium dioxide delivered. Increasing-particle size batches failed to produce any difference in animal behaviour or tissue response. Labelle reported that variations in particle size reflected a corresponding variation in the quantity of uranium entering the systemic circulation (La1953). He reported that with particles smaller than 1 micron, 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 microns so smaller particles were not tested (S1 1966).

The present investigation has shown the ease with which uranium dioxide penetrates the animal's body and the significant amount deposited in target organs shortly after insertion of varying quantities of uranium dioxide 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 specially those related with nuclear fuel factories.

...influence on the cell-contact interactions and cell metabolism. Mor-

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Legend to Figures

Figure 1A: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 hours before. Some cellular alterations are clearly observed: nuclear inclusions ( ),mitochondrial alterations (*),large intracellular vacuoles (V).

Figure 2A:Electron micrograph showing numerous electron dense deposits into the cytoplasm of a fibroblast-like cell,72 hours after uranium dioxide implantation.

Figure 3A:uranium deposits surrounding a capillar wall. Note endothelial cells(E) in which structural alterations are remarkable.

Figure 4A:X-ray scanning images of uranium element in epiphyseal bone(A), continuous-growth incisor (B) and kidneys (C) from the same animal. White dots in figure at 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.

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3. HISTOCHEMICAL DEMONSTRATION OF URANIUM DEPOSITS IN TISSUES

The histochemical demonstration of uranium compounds in tissue sections by applying the potassium ferrocyanide reaction was critically analyzed in order to choose an easy and rapid method for cualitative localization of uranium in the organs of experimental animals.

Several fixation and embedding conditions and a wide range of pH of the reagents were tested. The best results were obtained by employing thick criostat sections(1o-15 microns) from unfixed tissue blocks,treated with an acid 10% potassium ferrocyanide solution(pH 3.5). However uranium fairly bound to proteins ,require other treatment to be liberated or unmasked . Among other several treatments tested the most effective was the microincineration. In s skin specimen from ten animals in which UO_2 was subcutaneously implanted ,positive ferrocyanide reactions were detected in conective cells and in intercellular spaces. The amount of reactions was similar in incinerated sections ,however in sections from the kidneys of the same animals a much greater amount of reactive uranium was detected after incineration. The presence of different compounds of uranium in the skin and kidney of the treated animals is suggested.

Electron diffraction of dense deposits failed to yield a diagramm compatible with cristalline structures. On the other hand when this technique was applied ditectly on UO_2 deposits of known compositiona clear diagramm corresponding to this compound was obtained,so it was concluded that after entering the tissues an amorphous structure is acquired(in a recent publication it is suggested that UO_2 is oxidized to UO_3 followed by the formation of uranyl ion which being always combined to proteins is not able to yielddiffraction patterns. (Cooper et al 1982).

4. DETERMINATION OF URANIUM TRACES IN DIFFERENT ORGANS AND TISSUES

AN attempt was made to quantitate the amount of uranium deposited in the organs after intravenous and intraperitoneal injections and after percutaneous or subcutaneous applications of uranyl nitrate and uranium dioxide. Conventional techniques such as fluorescimetry yielded valuable results only when the quantities of uranium exceeded 10ug/g. Smaller quantities were very difficult to evaluate because of technical difficulties. In an attempt to solve this problem we came in contact with investigators performing routinely neutron activation analysis of different kind of samples. We started the analysis of hard tissues in order to standardize the technique and later to extend it to soft tissues. We have taken advantage of the possibility of using this sophisticated but accurate technique in an attempt to quantitate as exactly as possible minute amounts of uranium in tissues.

Neutron activation analysis allows to determinate the amount of uranium deposited in tissues even for very low concentrations,which were demonstrated to be very difficult to perform by conventional chemical methods. We started the measurements with hard tissues. Samples of bone(tibia) and continuous growth incisor of Wistar rats were obtained from animals that have been subjected to the action of UO_2 or uranyl nitrate. Different doses used as well as time after starting the measurements are summarized in Table II and III. Samples were dried during 24Hs at 100° °C and after that reduced to powder in an agate morter .The powder was later transferred to quarz vials and sealed. A suspension of uranyl nitrate and tricalcic phosphate was used as standard sample. All samples were irradiated under Cadmium covering and epithermic neutron flux of 9×10^{11} neutron/cm^2/sec during two hours. Irradiations were performed in the RA3 Reactor from Comision de Energía Atómica, Centro Atómico Ezeiza,Buenos Aires. The principal characteristics of the irradiations were:open -tank type, Normal power 3.1 Mw (thermic),thermal flux 3×10^{13} neutron/ cm^2/ sec.Uranium enriched up to 90% was used as fuel. Demineralized water was used as moderator and ccoling was attained by forced circulation of water. After irradiation and six-day dacay period the samples were measured in a 70cc,2.4 Kev resolution for 1332.5 Kev peak of Co^{60} Germanium(lithium) Princeton Gamma Tech Detector that was on line with a Canberra multichannel Serie 80. Measurements performed corresponded

to 228.1 Kev and 277,9 Kev Neptunium that was formed by negative desintegrations of U^{239}.

Analysis of data indicate:

1. After uranyl nitrate injections or UO_2 insertion, the deposition of uranium in bone and teeth is dependent upon the amount of drug administered, being greater for higher doses.

2. In most of the cases uranium deposition in teeth was 30% less than in bone for the dame group of animals.

3. Higher values were obtained two days after injection of uranyl nitrate. Three and 4 days later deposition values were lower probably indicating excretion via kidmey.

Measurements of uranium deposited after UO_2 insertion revealed similar tendency although some results seems to be discrepant.

New information has been obtained in the last months encouraging us to initiate a new line analyzing uranium deposition in hard tissues

TABLE II

Tissue	Bone		Teeth	
Dose / Days	2mg/kg	5mg/kg	2mg/Kg	5mg/kg
2	23±o.5ug/g	55.2±0.8ug/g	16.5± 0.5ug/g	25.4± 0.5ug/g
3	16.3±0.7ug/g	29.6±0!5 ug/g	11.8± 0.4ug/g	20.4±0.4ug/g
4	12.4±0.3ug/g	48.6±0.8ug/g	7.7±0.4ug/g	33.8±0.6ug/g

Table II: 2mg/Kg of body weight and 5 mg/Kg of body weight of uranyl nitrate were injected to 30 Wistar rats (150 g) .Groups of ten rats each were sacrificed 2,3 and 4 days after injection. Tibia and teeth of the animals were adequately processed(see text) to be subjected to neutron activation analysis. Bone and teeth corresponded to the same group of animals to make data comparable.

TABLE III

Tissue	Bone		Teeth	
Dose / Days	0.2g/Kg	0.5g/Kg	0.2g/Kg	0.5g/Kg
2	117±2ug/g	283± 4 ug/g	92± 2 ug/g	159± 2 ug/g
3	216± 4 ug/g	115± 2 ug/g	88± 2ug/g	1[illegible] 2 ug/g
4	130± 2ug/g	238± 3 ug/g	77± 1ug/g	149± 2 ug/g

Table III: 0.2 g/Kg of body weight and 0.5 g/Kg of body weight of uranium dioxide were subcutaneously implanted to 30 Wistar rats (150g) Groups of 10 rats each were sacrificed 2,3 and 4 days after uranium dioxide implantatiom. Tibia and teeth of the animals were adequately processed (see text) to be subjected to neutron activation analysis. Bone and teeth corresponded to the same group of animals to make data comparable.

STATEMENT OF PROJECT EXPENDITURES

Drugs (Sigma Chemical Company)----------	528,50	U$S
Electron microscopy materials(Ladd Res. Ind.)---	631.50	"
Diamond Knife(Dupont)----------	1800.00	"
TOTAL----------	2960.00	"

Maintenance of equipments----------10,920,000 pesos
(rate of exchange used:
1$/1 Dollar= 13,000/1 dollar)

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