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Percutaneous Absorption of Uranium Compounds

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Percutaneous absorption of soluble and insoluble uranium compounds has been induced in order to obtain information on penetration routes and the tissue injury produced by uranium salts. The high electron density of uranium provided a reliable way to visualize, by electron microscopy, the precise localization of the heavy compounds within the tissues. Few minutes after topical application of uranyl nitrate, dense deposits of uranium were observed at the epidermal barrier level. A few hours later, dense deposits were seen filling the intercellular spaces and were also scattered in the cytoplasm and nucleus. Mortality and body weight measurements indicated the high toxicity of uranyl nitrate and ammonium uranyl tricarbonate; uranyl acetate and ammonium diuranate were less toxic. As no penetration was achieved after uranium dioxide, no variations were detected on these parameters.

INTRODUCTION

Uranium extraction and refining operations leading to the manufacture of nuclear fuels have significantly augmented in latter years due to the great increase in the number of nuclear facilities. The hazardous nature of the by-products formed when processing uranium ore into 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 absorption, 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 five percent 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 the urine, approximately 60% of uranium within the first 24 hr (Walinder *et al.*, 1967). Immediately after intravenous injection about 50% is excreted in the urine, 25% is deposited in the skeleton, and 25% in the soft tissues. Only traces remained in the blood. Uranium deposited in soft tissues other than the kidney and in the bones, is excreted later at slower rate (Hursh *et al.*, 1969). After inhalation retention depends on particle size and chemical formula. Long biological half-times of 120 days or more can be anticipated from insoluble compounds (UO_2, U_3O_8) having particle sizes under 2 μm (Harris, 1961).

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Percutaneous absorption has been reported as an effective route of penetration for 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*" is 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 35-day-old Wistar rats (60 g, males): uranyl nitrate 0.5, 1.7, 3.5, and 7 g/kg body wt (British Drug House, BDH), uranyl acetate 3.5 and 7 g/kg body wt (May and Baker, Ltd.), ammonium uranyl tricarbonate 7 g/kg body wt, and ammonium diuranate 7 g/kg body wt. An oil-in-water emulsion composed of Vaseline and water (Aqualane, kindly supplied by Roux Ocefa, Argentina) was used as vehicle. The desired proportion of the compound to be tested was mixed with 1 ml of the emulsion. The cream was applied on a 3 cm^2 dorsal area 24 hr after shaving. Single or daily applications were performed with the aid of a small brush; each animal was confined in a separate cage to avoid siblings licking the treated area. Ten daily applications of the compounds were performed on the dorsal shaved area that had been previously 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 that of the controls, so therefore oral intoxication by self-licking was avoided.

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

Light and electron microscopy studies. Groups of 10 animals were sacrificed 2 and 4 days after daily applications of the above mentioned uranium compounds. In addition groups of 10 animals were treated with a single application of uranyl nitrate and the effects were analyzed 15 min to 24 hr after application. Specimens of treated and nontreated skin from the same animal and from nontreated animals were simultaneously processed for light and electron microscopy. Paraffin sections were stained with hematoxylin and eosin. Small skin samples from the same animals were fixed in 2% osmium tetroxide in palade buffer. After dehydration with graded alcohols and propylene oxide, they were flat embedded in Maraglass. Thin and ultrathin sections of properly oriented samples were obtained in an LKB ultramicrotome. Contrasted and noncontrasted sections were photographed in a Philips 300 and in a Philips 200 electron microscope.

A semiautomatic analyzing system (MOP 3, Kontron, Messgeräte GMbH, Munich) was used to measure the surface area of basal cells from the epidermis.

Thin sections from the above mentioned experimental conditions, were stained with toluidine blue and projected on the measuring table of the equipment. Measurements of surface area were performed on interfollicular basal cells or epidermis obtained from animals treated with uranyl nitrate plus vehicle and from animals treated with vehicle only. Average data from 100 cells from each experimental condition were scored.

X-ray 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 lithium) which simultaneously can register all elements present in the sample. In this particular case the microprobe provides information corresponding to 600 μm^2 of epidermis. Pieces of skin obtained from the same areas used to perform morphologic and morphometric analyses were fixed in 3% glutaraldehyde for 1.5 hr, 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 Fig. 1. Normal constant gain in body weight was characteristic in nontreated animals. A steady body weight decrease was induced by a high concentration of uranyl nitrate and ammonium uranyl tricarbonate. Furthermore, all animals died 5 days after the onset of the applications. Kidney injury was the cause of death of the treated animals. No increase in body weight was detected after application of ammonium diuranate performed

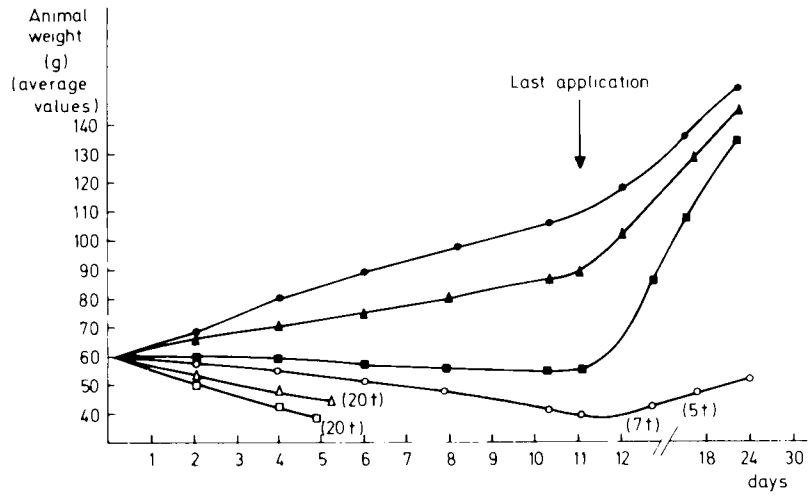

FIG. 1. Effect of various epicutaneously applied uranium compounds on animal weight. ●, control; ▲, uranium dioxide; ■, ammonium diuranate (yellow cake); ○, uranyl acetate; △, uranyl nitrate; □, ammonium uranyl tricarbonate. The number of animals that died during the treatment are shown in parentheses.

daily for 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 significant body weight decrease was obtained after 11 daily applications of uranyl acetate. Sixty percent of the animals died 2 days after the last application, also due to renal failure. The rest of the animals survived but weight values were 70% 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 2 days after application of the compound was characterized by obvious 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 experimental samples were obtained from topically treated skin, severely damaged areas alternated with slightly damaged areas. Fewer alterations were observed after ammonium uranyl tricarbonate applica-

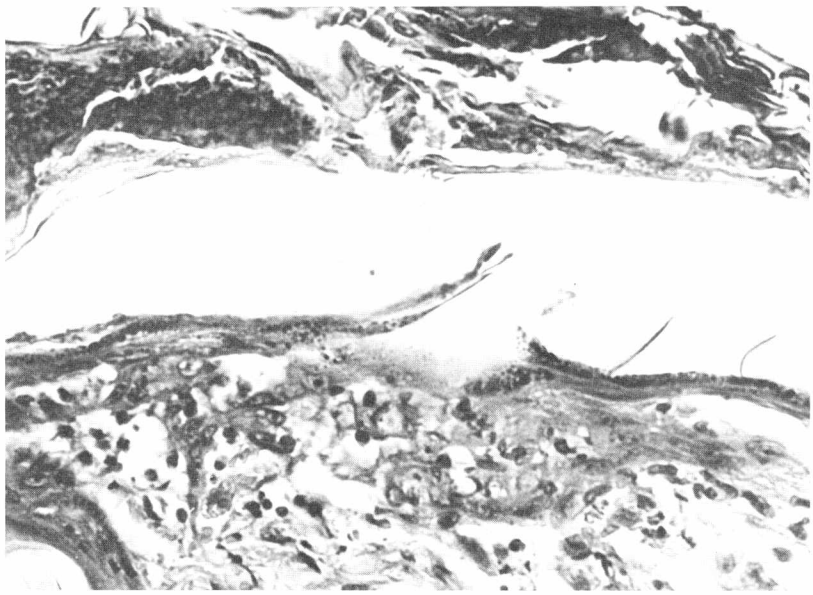

FIG. 2. Micrograph of skin from a dorsal area treated with uranyl nitrate. Note the purulent material on the epidermal surface. The horny layer appeared detached. Basal and spinous cells appeared swollen and vacuolated. Hematoxylin and eosin. (130×.)

tions; the most outstanding feature was the widening of the intercellular spaces. Other uranium compounds produced no cutaneous changes under light microscopy.

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

Ultrastructural 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 in the granular layer abutting the cell membrane. Longer periods of tissue-uranium contact made this band disappear, 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 hr after applications, but important substructural alterations indicated the deleterious action of the heavy metal: cell membrane interruptions and infoldings, disrupted mitochondrial membranes, and disarrangement of cristae as well as enlarged keratinosomes and fibrillar and nuclear membrane alterations were the most outstanding changes observed (Fig. 7). An unusual diffuse electron density was also observed in nuclei, membranes, and other organelles of unstained sections.

Four daily applications of uranyl nitrate produced a massive absorption of the compound. 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 of the animals treated with either uranium dioxide or uranyl acetate or with ammonium diuranate, which being insoluble did not penetrate into the skin; not even after successive daily applications did these compounds produce any effects. Except for a thin band in the uppermost layers of the epidermis, no electron-dense deposits were observed in the skin.

TABLE 1
BASAL CELLS SURFACE AREA (μm^2)

Number of applications (daily)	Dose of uranyl nitrate (g/kg body wt)[a]				
	Not treated	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

[a] Each dose was administered with 1 cc of vehicle.
All values represent mean values ± SD.

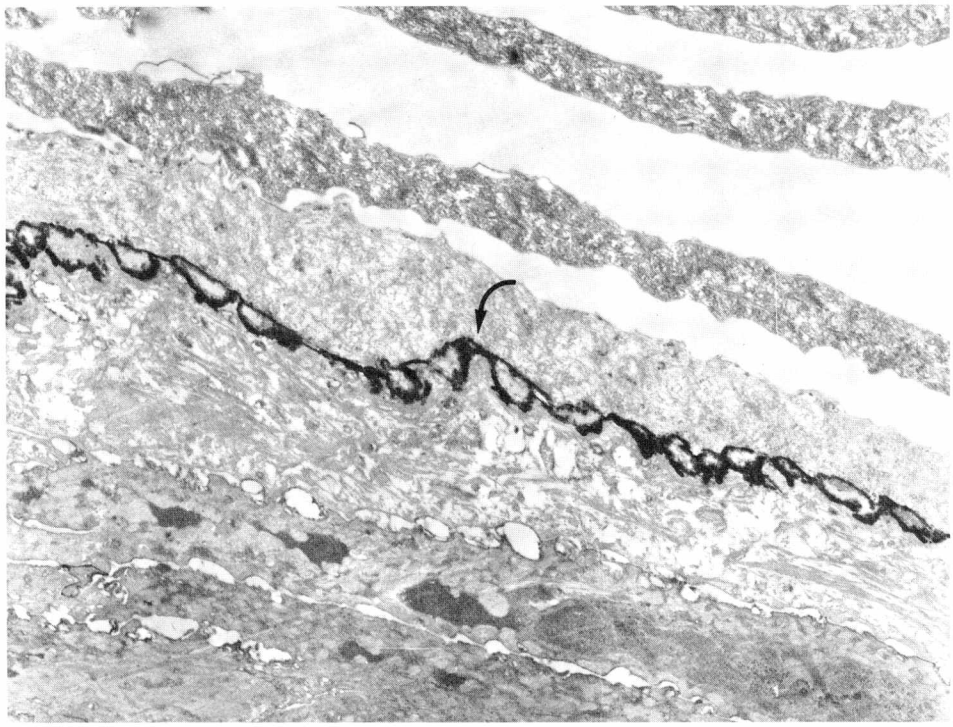

FIG. 3. Electron micrograph showing an electron-dense band in the intercellular space between the horny and granular layers (↘). Noncontrasted section. (3750×.)

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

DISCUSSION

Considerable variation in absorption and consequently in animal survival and body weight was noted after daily 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 marked decreases in body weight and death of all experimental animals 5 days after 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 least 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 elemental uranium within the skin tissues, X-ray microanalysis was performed. X-ray microanalytical methods have been

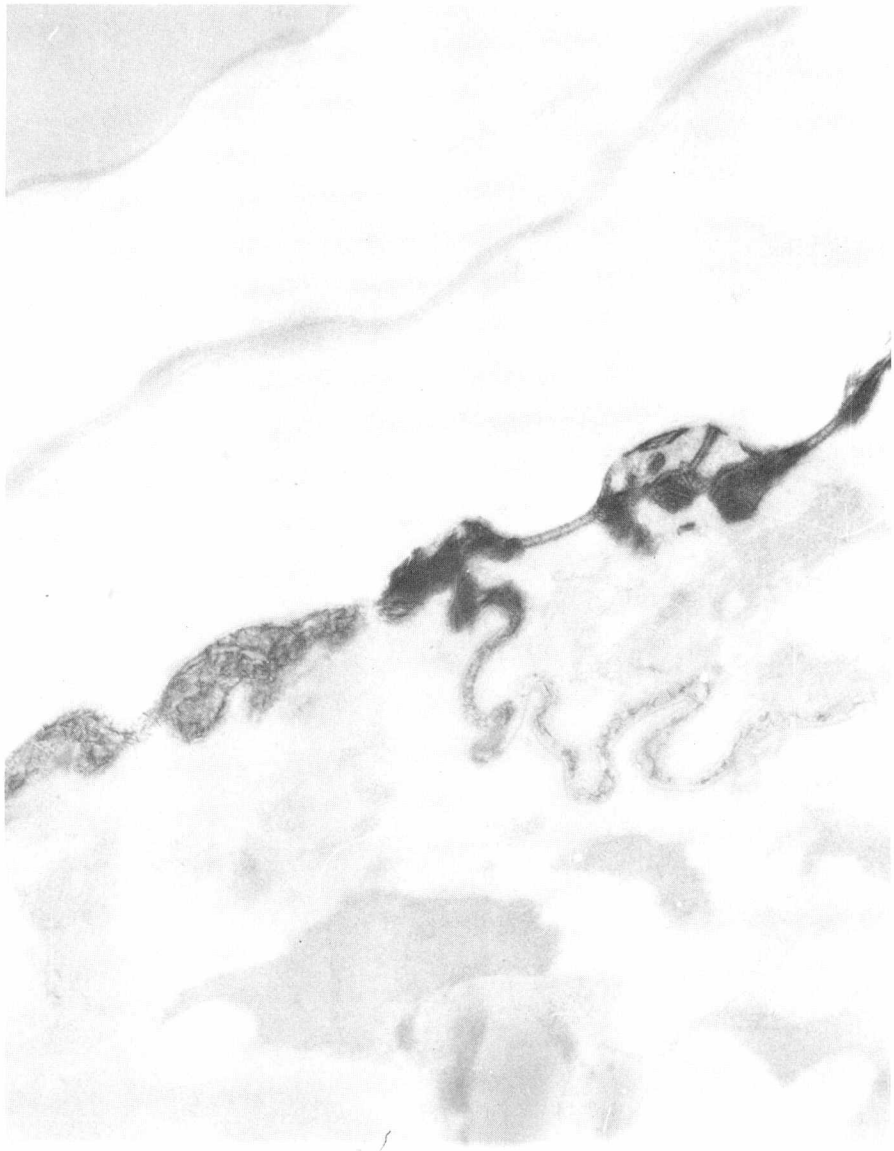

FIG. 4. Electron micrograph of horny layer showing a dense intercellular band with an organized membrane structure resembling lamellar bodies. Noncontrasted section. (14000×.)

widely used to trace the distribution of elements in tissues (for a review see Hall, 1979). In the present study the 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 embed-

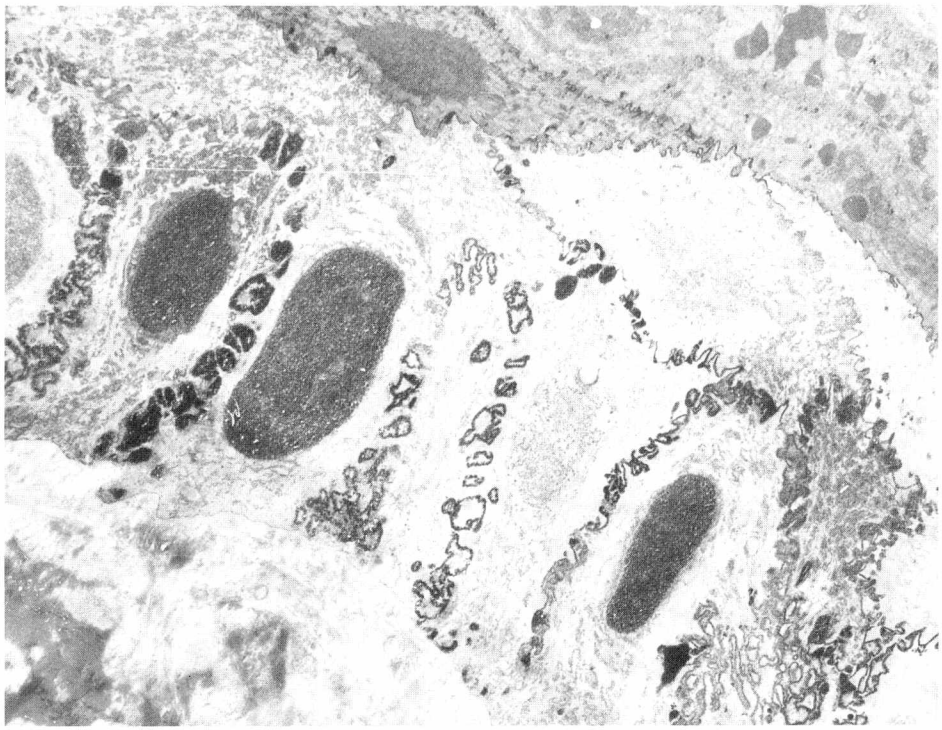

FIG. 5. Electron micrograph of skin treated with uranyl nitrate. Dense clusters of uranium deposited in the intercellular spaces are shown. Note the striking electron density of nuclei in a noncontrasted section, probably also due to uranium deposition. (2650×.)

ding and the high electron density of the metal made it possible to visualize the route of penetration of the metal after surpassing the epidermal barrier.

Numerous studies dealing with skin permeability have indicated that the skin barrier is the major impediment in percutaneous absorption (Schleuplein and Blank, 1949; Barr, 1962). In the series of experiments reported above, lamellar electron dense areas located between the horny and granular layers were observed at the site of the epidermal barrier (Elias and Friend, 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 numbers of lamellar bodies and intercellular lamellar structures that conform the permeability barrier (Elias and Friend, 1975). The observation of successive samples demonstrated that the penetration of uranyl nitrate, after surpassing the barrier, is achieved to a large extent along the intercellular regions and through the pilosebaceous apparatus, but lesser amounts also seem to traverse the tissues by transcellular mechanisms. That substances traverse cells and intercellular spaces in a nonpreferential manner have been inferred by Elias and Friend (1975) observing the fluxes of substances across the epithelia.

Successive daily application of uranyl nitrate induced a massive absorption by skin, evidenced by the large intercellular deposits of electron dense uranium clusters. This uncontrollable penetration can be attributed to a deficient state of

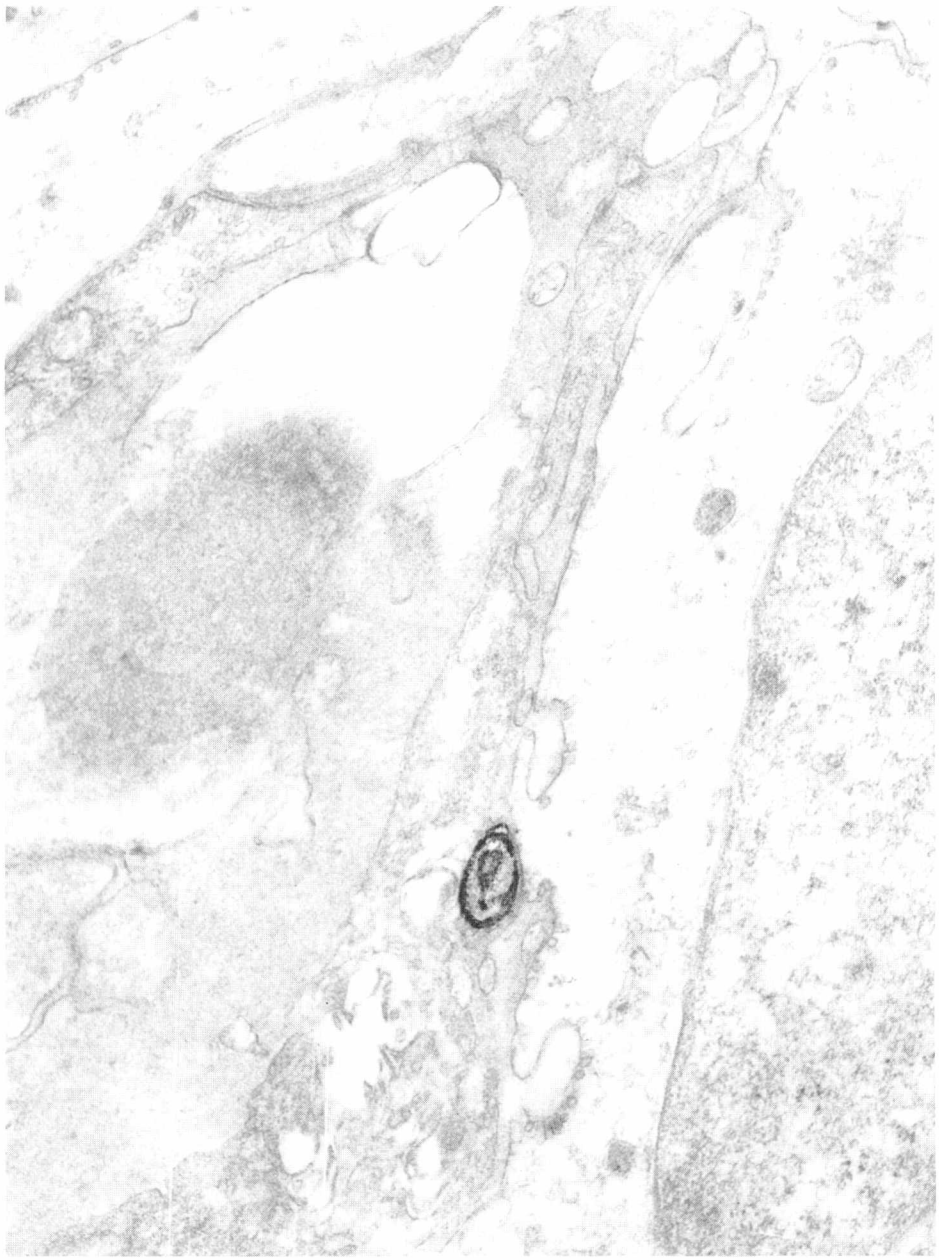

FIG. 6. Electron micrograph showing traces of uranium in a dermal capillary vessel. Noncontrasted section. (×2400.)

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 lipopro-

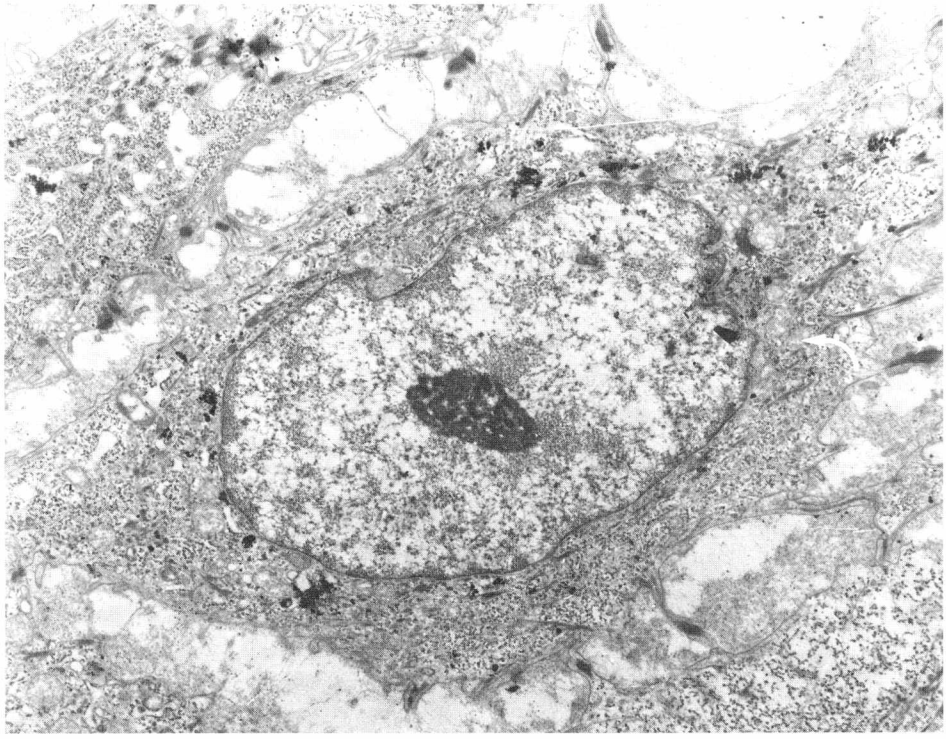

FIG. 7. Electron micrograph of a basal cell from epidermis that has been treated with uranyl nitrate 8 hr before. Striking alterations are observed in the intercellular space. Cellular contacts are largely modified and residual material is seen scattered into widened intercellular area. Mitochondria (↖) appeared smaller and deep disarrangements of cristae are observed. Section contrasted with uranyl acetate and lead citrate. (6000×.)

tein complexes is suggested. Other uranyl compounds such as ammonium uranyl tricarbonate and uranyl acetate, although causing significant mortality and body weight decreases, 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 bound and on the type of complex compounds that are formed when the substance is dissolved in water and lipid (Skog and Wahlberg, 1963). Rothman (1954) reported that substances soluble in both lipid and water, like uranyl nitrate, penetrate the skin more readily than less soluble or insoluble substances.

Ultrastructural cell alterations as well as quantitative data on basal cells provided information about the toxic effect of soluble compounds on skin components. The remarkable alterations of all membrane constituents of epidermal cells, were clearly observed affecting cellular architecture that can influence on the cell-contact interactions and cell metabolism. Morphometric measurements performed in basal cells from uranyl nitrate-treated skin showed that the basal cell surface area was 50% smaller than in the control epidermis. This decrease was

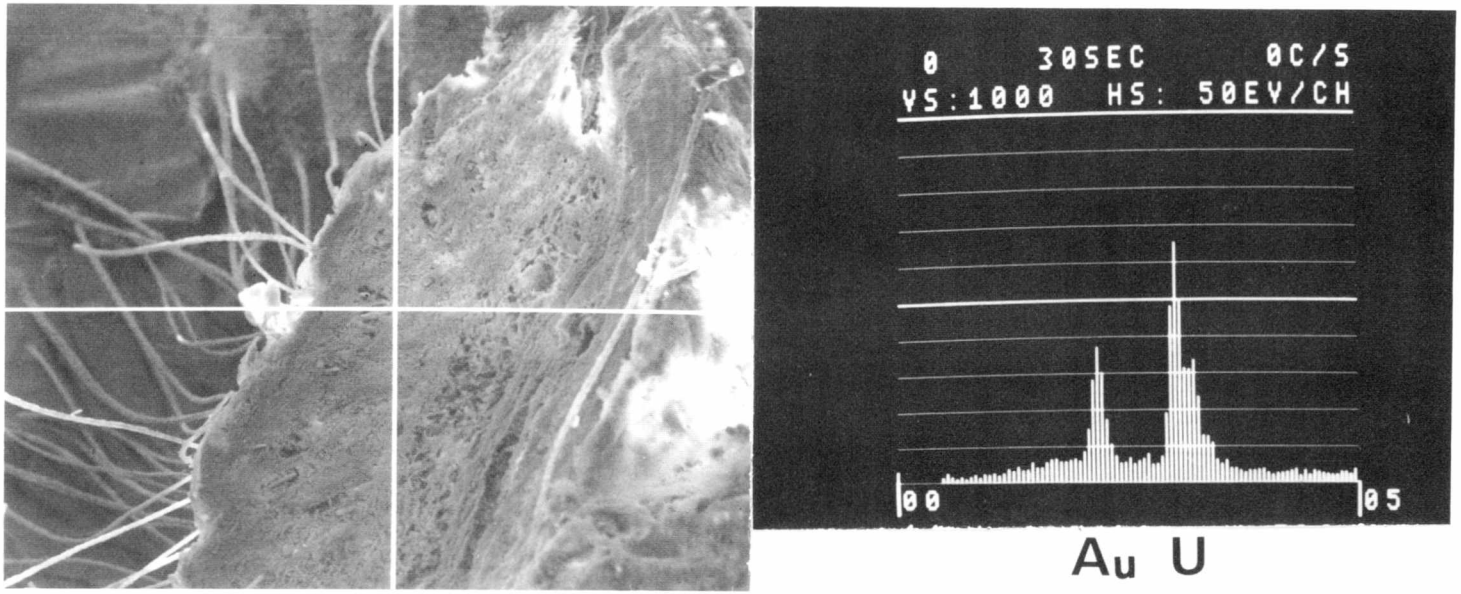

FIG. 8. Scanning electron micrograph of cross-sectioned skin treated with uranyl nitrate 4 hr before. Crossed lines indicate the exact site of microprobe evaluation. Two peaks are seen in the EDAX spectrum. U corresponds to elemental uranium and Au corresponds to gold (by the shadowing technique). (30×.)

time and compound concentration independent. Previous studies have suggested that variations in cell morphometric endpoints correlate well with alterations in cell metabolism (Klein Szanto *et al.*, 1977). In conclusion the reduction in cell area after topical application of uranyl nitrate directly reflects the toxic action of the compound leading to involutional changes and finally to atrophy of the tissue.

The above results demonstrated that percutaneous absorption is an effective route of penetration for 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 must be considered when health regulations are made regarding the handling of uranium compounds.

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