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Acute Toxicity of Uranium in Rats and Mice

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The enormous expansion in the mining, processing, use and operations involving uranium that has occurred over the last forty years has led to the dispersion of concentrated uranium at many sites over almost the entire world. While the dangers due to nuclear reactions are given wide play in the public press, the toxicological hazards of increased levels of uranium are less widely appreciated. These same hazards would be associated with any accident which lead to the widespread dispersion of uranium from a nuclear power plant.

On the other hand, during uranium processing there is a possibility that workers will inhale or ingest some uranium, giving rise to internal contamination. This contamination will result in radiation doses to the organs of the body, and if the intake of uranium is large enough, chemical toxic effects. Under some circumstances the toxic chemical effects of uranium may be more important than the radiation dose received. Moreover, the by-products formed when processing uranium are also of considerable interest because of the risks of environmental contamination (Johnson 1980; De Rey et al. 1983).

The interest of this question has increased following the accident at Chernobyl on 26 April 1986. Many details are still unknowm concerning the exact nature of the Chernobyl accident. However, it is known that Chernobyl reactor was fueled with about 200 tons of uranium dioxide packaged in 1,661 fuel assemblies (McClellan 1986).

Experience has shown that inhalation is the most common mode of occupational exposure, although uranium can be absorbed through the skin, through wounds or ingested (Wrenn 1975; Eidson and Mewhinney 1980). Uranium absorbed into the systemic circulation leaves the blood very rapidly and is eliminated via the urine (Walinder et al. 1967; De Rey et al. 1983). Uranium deposited in soft tissues other than the kidney and in the skeleton, is excreted later at slower rate (Hursh et al. 1969). In any case, acute renal failure is the more known disorder resulting from intoxication with uranium (Blanz 1975; Bentley et al. 1985).

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The present investigation was designed to examine the acute oral and subcutaneous toxicity of uranyl acetate in rats and mice.

MATERIALS AND METHODS

Uranium was administered to rats and mice as uranyl acetate dihydrate of analytical grade supplied by Baker and Adamson (New York, USA). Solutions were constituted by adding appropiate amounts of uranyl acetate and distilled water, then adding sodium bicarbonate until the compound dissolved and a pH of 7 was reached. Solution concentrations were adjusted so that a 300-g rat received 1 ml and a 30-g mouse 0.2 ml.

The animals used for the experiments were male Sprague-Dawley rats (250-300 g) and male Swiss mice (25-30 g) obtained from Interfauna Ibérica (Barcelona, Spain). The rats and mice were allowed to acclimatize for a period of seven days after shiping before experimental use. The animals had free access to food (perfectly balanced Panlab diet, Barcelona, Spain) and tap water.

Single doses of uranyl acetate were administered by subcutaneous injection and orally per gavage to rats and mice. The LD_{50} values, their confidence limits, and the slope function of the regression line, were calculated by the method of Litchfield and Wilcoxon (1949). Ten animals per group were used and survivors were held for a 14-day period of observation following administration.
Previous screenings with small groups of three animals of each kind were carried out.

Table 1. Acute toxicity of uranyl acetate in rats and mice

	Oral administration		Subcutaneous administration	
	Dose (mg/ Kg)	Alive/tested	Dose (mg/Kg)	Alive/tested
RATS	20	10/10	1.25	10/10
	40	9/10	2.5	9/10
	80	7/10	5	7/10
	160	7/10	10	4/10
	320	3/10	20	1/10
	640	1/10	40	0/10
	1280	0/10		
	LD_{50} = 204(100, 416)		LD_{50} = 8.3 (4.1, 16.8)	
MICE	44	10/10	10	10/10
	80	9/10	15	9/10
	144	7/10	22.5	4/10
	259	5/10	33	2/10
	466	4/10	50	0/10
	839	0/10		
	LD_{50} = 242(155, 327)		LD_{50} = 20.4(17.0, 24.5)	

LD_{50} (14 days) in mg/Kg; 95% confidence limits in parentheses

In a second experiment, uranyl acetate was given subcutaneously or intragastrically to two groups of fifteen animals each in a single dose: 10 mg/Kg or 210 mg/Kg respectively (slightly higher than their LD_{50} values determined in this paper). Control animals received 0.9% saline subcutaneously or distilled water orally. Allthe animals were placed in individual metabolism cages. Food and water consumption, and the feces and the volume of urine excreted were measured daily. Body weights were also measured daily. The animals were observed for 14 days, and the survivors were killed after this period. Hematocrit and hemoglobin were measured on days 1, 7 and 14 of the experiment. The activity of the following plasma parameters: creatinine, urea, total protein, GOT and GPT was determined on days 1, 7 and 14 after uranium administration. Also, total protein and creatinine were measured in urine on the same days. Method of clinical chemistry are described earlier (Domingo et al. 1985). The histological examination (paraffin slices, hematoxilin-eosin) of liver and kidneys was performed in three rats of each group.

The significance of the differences in the results was determined by the Student's t-test. A difference is considered to be significant when $P < 0.05$.

RESULTS AND DISCUSSION

Table 1 summarizes the relation between dosage and mortality in rats and mice when uranyl acetate was given orally and subcutaneously. LD_{50} values (14 days) and their 95% confidence limits are also shown. The fact that the oral values were much lower than the subcutaneous values was thought to be due to a very low rate of absorption. It was been reported that one to five percent of an oral dose is absorbed and most is excreted by the kidney (Hursh and Spoor 1973; De Rey et al. 1983). In the case of oral administration, the majority of deaths were observed between the six and the eighth days. No, deaths occurred before the sixth day. In the case of subcutaneous administration, deaths occurred mainly during the fifth, sixth, seventh and eighth days. No deaths were observed before the fifth day. The most noticeable physical signs observed were: piloerection, rubefaction, tremors, hypothermia, pupillary size decreased, exophthalmos, light hemorrhages in eyes, nose and legs and a remarkable weight loss. Most clinical and physical signs appeared after six days from the uranium administration, and did not disappear for the 14-days period of observation (Table 2).

Table 3 sumarizes the nutritional parameters in control rats and in rats which received uranyl acetate in a single dose. All the animals showed a significant weight loss especially remarkable for the rats which received uranium subcutaneously ($p<0.001$). Drinking water was not affected, but food ingested was significantly lower for the treated animals. The amount of feces excreted was lower for the treated rats as a direct consequence of the lower ingestion of food. Lastly, the volumes of urine excreted were significantly higher for all the treated animals especially during the first week. The significant increases in the volumes of urine suggest a disorder in the renal function (Rothstein and Berke 1949; Blantz and Konnen 1975) since they are not a consequence of increases in drinking water

Table 2. Severity of physical and clinical signs in rats after uranium administration in a single dose

	Number of days after uranium administration		
	1-4	5-8	9-14
Mortality rates			
Subcutaneous administration	0%	80%	20%
Oral administration	0%	70%	30%
Piloerection	none	++	+
Pupillary size decreased, exophtalmos	none	+	+
Hemorrhages in eyes, nose and legs	none	none	++
Rubefaction	none	+	+
Tremors, hipothermia	none	+	+
Weight loss	+	++	+++

The mortality rates refer to the animals dying for the 14-days period
+, light; ++, moderate; +++, severe symptomatology

Table 3. Nutritional parameters in control rats and in rats which received uranyl acetate in a single dose

	1st week	2nd week
Body weight (in g)		
Control	316.0 ± 17.8	249.0 ± 24.2
Uranium oral	295.0 ± 12.9*	310.0 ± 35.6*
Uranium sc	246.3 ± 11.9***	267.5 ± 9.6***
Drinking water (ml)		
Control	237.4 ± 36.3	237.3 ± 34.1
Uranium oral	185.9 ± 71.0	334.0 ± 65.6
Uranium sc	213.7 ± 59.6	303.7 ± 105.5
Food ingested (g)		
Control	202.0 ± 19.5	168.3 ± 26.1
Uranium oral	24.4 ± 12.4***	111.4 ± 44.6*
Uranium sc	33.6 ± 22.5***	62.6 ± 42.7**
Urine excreted (ml)		
Control	56.9 ± 8.3	57.9 ± 8.5
Uranium oral	108.4 ± 34.4**	267.7 ± 10.8***
Uranium sc	117.8 ± 44.6**	199.7 ± 44.1***
Feces excreted (g)		
Control	51.6 ± 10.1	48.2 ± 4.0
Uranium oral	4.6 ± 3.6***	47.2 ± 10.8
Uranium sc	16.1 ± 8.9***	13.4 ± 8.0***

Results are presented as arithmetic means of 5 animals in each group±S.E.*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ by the Student's t-test

consumption. The amounts of urea and creatinine in plasma, and the amounts of total protein and creatinine in urine corroborate the appearance of a remarkable disorder in the renal function (Tables 4 and 5).

Table 4. Blood parameters in control and uranium treated rats

	Hematocrit (%)	Hemoglobin (g/100ml)	Creatinine (mg/100ml)	Urea (mg/100ml)	Total Protein (g/100ml)	GOT (U/l)	GPT (U/l)
DAY 1							
Control	43.9±2.1	16.9±1.8	0.5±0.1	19.1± 3.4	6.7±0.8	61± 5.6	25± 7.7
U oral	41.8±4.3	18.6±3.1	1.1±0.2*	43.0±19.9*	7.3±0.9	105±75.9	54±13.3***
U sc	44.6±4.7	14.7±1.9	1.5±0.8*	27.4± 2.8	7.4±0.4	76±11.3	44± 9.8***
DAY 7							
Control	49.1±2.2	19.3±2.9	0.5±0.1	13.1± 2.9	7.4±0.3	49±19.8	21± 4.4
U oral	49.0±4.5	18.2±2.4	4.2±0.9**	132.7±14.6***	6.7±0.3	53±17.5	37±12.0*
U sc	48.8±5.4	18.8±1.7	8.5±2.7***	188.9±30.6***	6.8±0.9	100±21.5***	30± 9.6*
DAY 14							
Control	43.5±1.4	15.2±0.8	0.4±0.0	11.2± 1.8	6.2±0.0	43±6.7	16± 3.4
U oral	39.0±2.2***	15.4±0.8	0.6±0.3	16.3± 5.5	5.9±0.2	28±9.2	27± 2.5**
U sc	36.5±1.8***	14.2±1.1	0.8±0.2	21.0± 3.6**	5.5±0.4	45±18.8	20± 2.2*

Results are presented as arithmetic means of 5 animals in each group. *p <0.05, **p <0.01, ***p <0.001 by the Student's t-test.

Minimal lesions in kidneys and liver of the rats receiving uranyl acetate orally have been detected. These lesions consisted in moderate hyperaemia and discrete microhemorragic foci. The animals which received uranyl acetate subcutaneously showed more severe lesions in liver and kidneys. In liver, an intense hyperaemia and abundant periportal mononuclear infiltrates could be observed. Disperse tubular and glomerular necrosis, cortical and medular microhemorragic foci, tubular dilatation and epitelial desquamation, hyalin cilinders and cortical deposits of unidentified basofil granular material could be observed in the kidneys of these rats (Fig. 1).

The results presented above show that uranyl acetate was highly toxic when given subcutaneously and moderately toxic when given orally (Loomis 1978). Renal injury, the most characteristic response following uranium intoxication (Walinder et al. 1967; Blantz and Konnen 1975; Thun et al. 1985; Damon et al. 1986) has been confirmed. However, our results must only be considered as a preliminary investigation of more relevant questions on the toxicity of uranium. Chronic tests for longer periods will be carried out to complete these studies on oral toxicity of uranium.

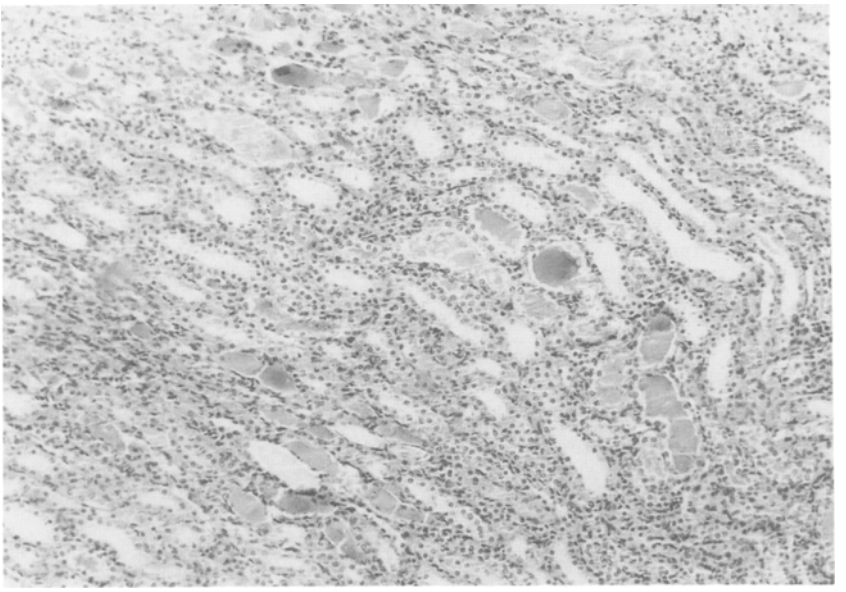

Figure 1. Renal cortex section from a Sprague-Dawley rat treated with 10 mg/Kg of uranyl acetate (sc). The sample was obtained 14 days after the injection.

Table 5. Urine analyses in control and uranium treated rats.

	Total protein	Creatinine
DAY 1		
Control	38.6±14.3	93±35.2
U oral	114.4±39.3*	24±12.6***
U sc	153.0±60.5**	15±12.1***
DAY 7		
Control	35.0±11.0	81±28.9
U oral	153.0±80.5**	15±11.1***
U sc	86.0±11.8*	40±29.9**
DAY 14		
Control	40.9± 5.6	114±70.1
U oral	95.3±32.3**	45±18.6*
U sc	215.3±29.7***	83±52.9*

Results are presented as arithmetic means of 5 animals in each group. *p <0.05, **p< 0.01, ***p< 0.001 by the Student's t-test.

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