Incorporation of Uranium

II. Distribution of Uranium Absorbed through the Lungs and the Skin

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In experiments on mice, rabbits, and piglets the distribution of uranium was studied at different times after exposure. Uranium was administered by inhalation (mice) and through the skin (rabbits and piglets).

These investigations show that the uptakes of uranium in different organs of the three species are highly dependent on the amounts administered. There seems to be a saturation effect in the spleen and bone tissue whenever the uranium concentration in the blood exceeds a certain level. The effect in the kidney is completely different. If, in a series of animals, the quantity of uranium is continuously increased, the uptakes by the kidneys increase more rapidly than the quantities administered. This observation seems to be consistent with the toxic effects of uranium on the capillary system in the renal cortex.

Polyphlorethin phosphate, a compound which reduces permeability, was investigated with respect to its effect on the uptake of uranium deposited in skin wounds in rabbits and piglets. It significantly reduced the absorption of uranium, even from depots in deep wounds.

The findings are discussed with reference to the routine screening of persons exposed to uranium at AB Atomenergi.

In the first part of our investigations (this journal, p. 305) we studied the distribution of uranium in the mouse following intravenous injections of soluble uranium compounds. The present experiments are concerned with the corresponding distribution following inhalation of uranium and absorption from subcutaneous uranium depots. The main purpose has been to find out the extent to which the respective distribution patterns diverge.

Inhalation Experiments

Materials and Methods Uranium aerosol was prepared by means of a simple device especially designed for these experiments. Air was ejected (under a pressure of 3 kg./cm.²) through small holes in the wall of a vertical steel tube just above the surface of a water suspension of U₃O₈ particles. The air jets produced hydrosol-contained particles of uranium. This hydrosol was then converted into uranium oxide aerosol by a few heating and drying procedures which removed the water.

The aerosol produced in the generator was piped into the vertical central duct of a lucite unit (see Fig. 1). Ten horizontal exposure chambers communicated with this duct via breathing holes. A

mouse placed in one of the chambers tended to keep its nose pressed against the breathing hole, and this usually prevented its coat from being contaminated. The mice were watched throughout the experiment and any one that withdrew its nose from the

FIG. 1. Mouse exposure unit.

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breathing hole was discarded. In addition, a check was made for possible contamination, either by direct measurement or by examination of smears taken at random.

U_3O_8 was processed for the aerosol by fractionated pulverization at the Laboratory for Powder Metallurgy at AB Atomenergi in Stockholm. The particles were taken up in a 0.3% sodium pyrophosphate solution. This procedure ensured a constant particle spectrum, for sodium pyrophosphate at that concentration effectively prevents agglomeration of U_3O_8 particles (Lundqvist, 1959). Following collection in a cascade impactor, the particle diameters were determined microscopically. The distribution by size is shown in Figure 2.

FIG. 2. Distribution curve of particles from the aerosol generator.

Ninety-two per cent of the particles had diameters in the range of 0.5 to 2 μ . For determination of the total activity in air, particles were trapped in a corona filter (TLM) (Bergstedt, 1956). In these experiments the inhaled air had a uranium concentration of $(8-9) \times 10^{-3}$ μ g. per cubic centimetre. According to Spector (1956), the inspiratory volume for mice is approximately 25 cm^3 per minute. On this basis, during the course of the experiment each mouse inhaled about 690 cm^3 air, containing a total of approximately 6 μ g. uranium. In view of the somewhat primitive experimental set-up the amounts of aerosol inhaled might be expected to vary considerably, and it is a little surprising that the data recorded do not show a wider dispersion.

Results The observed distribution of uranium in different organs following inhalation is detailed in Table I. As a rule, organs from two animals ('groups') were analysed concurrently under virtually identical conditions. Hence, the variability is consistently smaller for intra-group than for inter-group values.

In Table II the mean percentage uptakes for various tissues 24 hours after inhalation are based on the assumption that each mouse inhaled a total of 6 μ g. uranium.

The uptake by kidney, spleen, and bone tissue is known to depend largely upon the quantity of uranium administered; and in this connexion some interesting points emerge from the inhalation experiments. Now the amount incorporated is not, of course, the only thing affected by a change-over from intravenous injection to inhalation of uranium. Another factor of importance in this context is the incorporation rate, which probably is lower than in the case of intraperitoneal injection of uranyl nitrate. Despite the fact that a substantially smaller quantity of uranium entered the mouse organism via inhalation than via intraperitoneal injection, the liver and skeletal uptakes were lower by not more than a factor of 3, whilst the splenic uptake was merely halved. In the spleen the absorption capacity was in all probability limited. By comparison with the values from intraperitoneal administration, the lower uranium excretion in the urine was reasonably commensurate with the smaller amount incorporated, but the renal uptake was disproportionately small. These observations are corroborated by our findings concerning the permeability of rabbit skin to U_3O_8 .

Excreta The uranium excretion values as a function of time after inhalation will be found in Table I.

It is a general observation that uranium excretion in the urine as a function of time after exposure can be approximated by taking the formula $U_t = U_1 \cdot t^{-\mu}$. This formula, in which U_t is the amount of uranium excreted in the urine from days (t-1) to t, and μ is a constant, has been found to cover the excretion pattern reasonably well and appears to be relatively independent of the mode of administration. The regression coefficient μ in the equation $\log U_t = -\mu \log t + \log U_1$ was determined in the usual way by the method of least squares. Conventional statistical methods were used for calculating the mean error of the regression coefficient.

Penetration of the Skin by Uranium

Due to the short range of alpha particles in tissue,

TABLE I

ORGAN DISTRIBUTION OF URANIUM IN MICE FOLLOWING INHALATION EQUIVALENT TO ABOUT 6 μ G. URANIUM

Days after Administration	Lungs		Liver		Spleen		Kidneys		Tibia		Urine (μ g./animal)	Faeces (μ g./animal)
	μ g.	μ g./g.	μ g.	μ g./g.	μ g.	μ g./g.	μ g.	μ g./g.	μ g.	μ g./g.		
1	0.50	5.0			0.100	1.4	0.12	0.40	0.050	0.61		
	0.47	3.5	0.080	0.060	0.110	1.6	0.13	0.65	0.080	1.0	0.46	1.9
	0.43	3.5	0.048	0.040	0.100	1.7	0.10	0.30	0.054	0.84		
	0.62	4.9	0.075	0.054	0.130	1.8	0.12	0.36	0.066	0.72	0.56	2.6
	0.84	4.7	0.070	0.055	0.075	0.84	0.25	0.52	0.080	0.81	—	2.8
	1.0	7.2	0.110	0.080	0.077	1.1	0.25	0.64	0.042	0.63		
	0.87	5.8	0.049	0.041	0.060	0.82	0.16	0.41	0.033	0.55	0.48	1.6
2											0.18	0.76
											0.25	1.40
											0.13	0.63
											0.14	0.60
3											0.555	0.17
											0.083	0.68
											0.040	
7	0.43	3.4	0.030	0.023	0.086	0.97	0.031	0.15	0.011	0.32	0.040	0.30
	0.40	3.8	0.033	0.027	0.090	0.97	0.070	0.34	0.011	0.24		
	0.26	3.0	0.032	0.022	0.066	0.78	0.058	0.17	0.024	0.29	0.014	0.13
	0.56	4.2	0.033	0.022	0.068	0.88	0.068	0.19	0.027	0.28		
	0.55	3.2	0.051	0.028	0.051	0.36	0.180	0.40	0.025	0.37	0.022	0.22
14											0.038	0.083
	0.26	2.3	0.016	0.013	0.011	0.18	0.012	0.047	0.015	0.35		
	0.28	2.3	0.023	0.018	0.030	0.36	0.012	0.034	0.010	0.14	0.009	0.015
	0.53	3.8	0.045	0.033	0.036	0.45	0.0086	0.020	0.042	0.42		
30	0.76	5.8	0.041	0.037	0.024	0.36	0.011	0.030	0.031	0.30	0.010	0.020
	0.14	1.1	0.021	0.022			0.0041	0.019		0.16		
	0.37	2.5	0.030	0.032	0.0053	0.11	0.0050	0.023		0.10	0.0022	0.015
	0.14	0.9	0.020	0.013	0.014	0.20	0.0073	0.020		0.14		
	0.17	1.1	0.014	0.011	0.012	0.16	0.0093	0.031		0.18	0.0035	0.012
	0.41	2.1	0.034	0.018	0.025	0.18	0.010	0.022		0.14		
	0.31	1.1	0.028	0.018	0.015	0.10	0.0086	0.020		0.25	0.0036	0.020
60	0.39	1.3	0.016	0.012	0.018	0.25	0.016	0.040		0.22		
	0.044	0.39	0.016	0.013	0.0061	0.13	0.0075	0.020		0.16		
	0.036	0.28	0.025	0.023	0.0079	0.18	0.0060	0.019		0.081		

uranium contamination of the surface of the skin does not expose the dermis and basal-cell layer to irradiation. Nevertheless, internal irradiation may follow contamination of the skin, if uranium in

TABLE II

MEAN VALUES OF THE PERCENTAGE DISTRIBUTION 24 HOURS AFTER INHALATION BASED ON THE ASSUMPTION THAT EACH MOUSE INHALED A TOTAL OF 6 μ G.

Totally incorporated amount	..	..	..	34%
Lung	..	..	..	11%
Bone	..	..	..	17%
Kidneys	..	..	..	2.7%
Spleen	..	..	..	1.5%
Urine	..	..	..	9%
Faeces	..	..	..	35%

some way should enter the mouth or reach the blood stream by direct absorption. If uranium is swallowed, only a minute proportion of it will penetrate the gastric and intestinal walls; hence there will be little danger to internal organs other than the intestinal mucosa. The connective tissue barrier presents, as a rule, a fairly effective obstacle to penetration by uranium, particularly if the latter is in insoluble form. A certain degree of absorption by epidermis appears likely, however. Dunster (1962), referring to skin contamination, conservatively estimates that 10% penetrates the stratum corneum and enters the inner epidermis where irradiation of the basal cells appears to be, in terms of radiation protection, the principal hazard. But if skin lesions are present, absorption may occur and internal organs will then take up uranium.

With a view to investigating the extent of such absorption, as well as the distribution of absorbed uranium and the possibility of reducing penetration, we have studied the incorporation of uranium introduced into deep skin incisions in rabbits. To reduce penetration we used polyphloreitin phosphate (PPP), a phosphorylated polyphenol which we have previously tested. Polyphloreitin phosphate belongs to a group of compounds of high molecular weight that have been synthesized at the Leo Research Laboratories. It is a phosphorylated polyphenol with a molecular weight of about 15,000. The LD₅₀ for the mouse and the rat following intraperitoneal injection is 600 mg./kg. (Fries, 1956). This substance markedly inhibits the absorption of colloids and saline through the peritoneal membrane. It has also been found to be an effective hyaluronidase inhibitor (Fries, 1956; Fries, Kottmeier, and Walinder, 1959).

Methods The rabbits were not selected; our aim rather was to find out if PPP would have a significant effect regardless of the animals' age (although only adult rabbits were used), sex, and genetic constitution.

Body weights ranged from 2 to 3.5 kg. The uranium was a U₃O₈ powder similar to that used in the inhalation experiments. Following anaesthetization with sodium pentobarbital, an interscapular incision 1 cm. long was made and 20 to 37 mg. of the uranium oxide was deposited in the subcutaneous pocket thus formed. The wound was then sutured and sprayed with Nobecutan. In the PPP group 5 to 10 mg. polyphloreitin phosphate was injected by hypodermic syringe into the subcutaneous pocket immediately after closure of the wound. On completion of these measures the animals were placed for 24 hours in special cages for quantitative collection of urine. Livers, spleens, and kidneys were then taken for fluorometric determination of the uranium content. The organs were digested exactly as in the mouse experiments

(this journal, p. 305). However, total digestion of these relatively large organs was more complicated and, in the case of liver, took from one to two weeks.

Results In view of the heterogeneous animal material the recorded values naturally show considerable dispersion. Uranium absorption from the wounds was influenced by their extension, but not appreciably by the amount of uranium deposited therein. Every effort was made to standardize the wounds by making incisions of equal length through the cutis. Hence, we achieved a better defined encapsulation of the uranium than would have been possible with abrasions. In the accompanying tabulation of uranium values determined for the respective organs, the standard error is indicated for each group (Table III).

According to these results, PPP approximately halved the uranium absorption during the first 24 hours after application. Due, however, to the wide dispersion of observed values, significant differences between controls and PPP-treated animals were noted only for the uranium levels in urine and kidneys. The fact that no appreciable difference emerged for the spleen was possibly attributable to a saturation effect corresponding to that which seemed evident in mice. If so, the latter animal's capacity per gram of splenic tissue would appear to be, in this respect, greater than that of the rabbit.

Urinary Excretion of Subcutaneously Deposited U₃O₈ In order to follow the urinary excretion of subcutaneously deposited U₃O₈ we kept seven rabbits in urine collection cages for one week. The results are shown in Figure 3. Assuming that the uranium excretion pattern conforms to the formula $U_t = U_1 \cdot t^{-\mu}$, μ , determined as before, was 1.73 ± 0.009 .

It was noted that with very large urinary volumes (exceeding 300 ml. per 24 hours) the uranium concentrations were low but the total amounts were somewhat higher. For 24-hour volumes below

TABLE III
ORGANIC DISTRIBUTION OF URANIUM IN RABBITS FOLLOWING SUBCUTANEOUS ADMINISTRATION OF U₃O₈

	No. of Animals	Urine		Kidneys		Liver		Spleen	
		μg.	μg./ml.	μg.	μg./g.	μg.	μg./g.	μg.	μg./g.
Controls	7	100 ± 15	0.59 ± 0.06	15 ± 1	0.93 ± 0.09	0.40 ± 0.11	0.0046 ± 0.0012	0.13 ± 0.02	0.15 ± 0.04
PPP	6	56 ± 5	0.32 ± 0.06	6.5 ± 1.5	0.45 ± 0.04	0.19 ± 0.11	0.0021 ± 0.0010	0.15 ± 0.06	0.20 ± 0.05
In comparison with the controls		P = 0.02	P = 0.01	P < 0.001	P < 0.001

FIG. 3. Daily urinary excretion of uranium after subcutaneous application of U_3O_8 .

300 ml. neither total amount nor concentration of uranium was found to be significantly dependent on urinary volume. In these experiments the percentage variation was somewhat lower for the concentration than for the total amount of uranium in the urine. As a rule, the 24-hour urinary volumes were between 100 and 200 ml.

To test the effect of PPP in more superficial lesions we conducted one experiment in which uranium was applied to surface abrasions. Two such wounds of identical size were produced in the rabbit by scraping the skin until it bled slightly. To one of these wounds we applied U_3O_8 and to the other uranyl acetate, which is far more soluble in serum (Dounce, 1949). For differentiation of the two compounds, ^{233}U was used in the acetate and natural uranium in the oxide. The amounts applied were 70 mg. U_3O_8 and 0.05 μCi uranyl acetate

(containing approximately 5 $\mu g.$ ^{233}U). The entire procedure was repeated in another rabbit, but immediately after application of the uranium powder the latter was soaked with a PPP solution. As before, a fluorometric method was used for the determination of natural uranium, and a radio-metric method for the determination of ^{233}U . By arranging the respective results it was possible to arrive at the amounts of natural uranium and of ^{233}U in the relevant organs (Table IV).

The PPP-treated rabbit weighed 2.9 kg. and the control 2.3 kg., but the organ weights were approximately the same. Although the feed was identical for the two animals (turnips *ad libitum*, but no water), the test rabbit's urinary volume was three and a half times that of the control. Our observation that PPP had a greater effect on the uranium concentration than on the total amount of uranium in the urine may have been attributable to this substantial difference in the respective urinary volumes.

Discussion

Polyphloreitin phosphate had some influence on the rate of absorption through rabbit skin during the first 24 hours after application of U_3O_8 as well as of uranyl acetate. The single experiment with abrasions naturally permits no firm conclusions, yet it does suggest that the action of PPP is more pronounced for superficial than for deeper skin injuries. Uranyl acetate is far more soluble in serum than is U_3O_8 and is absorbed to a much greater extent (Dounce, 1949).

The low U_3O_8 values noted for liver tissue suggest that in this type of experiment, as in the preceding inhalation experiments, the bulk of the uranium absorbed from skin incisions is serum-soluble (Dounce, 1949). If U_3O_8 particles of the size used in these investigations had entered the blood stream on any appreciable scale, the liver activity would have been much greater (Fries *et al.*, 1959). The fact that PPP at no time significantly

TABLE IV

DISTRIBUTION OF URANIUM IN RABBITS FOLLOWING APPLICATION OF U_3O_8 AND URANYL ACETATE TO ABRASIONS

	U_3O_8 Natural Uranium				Uranyl Acetate ^{233}U			
	Controls		PPP		Controls		PPP	
	$\mu g.$	$\mu g./g.$	$\mu g.$	$\mu g./g.$	μCi	$\mu Ci/g.$	μCi	$\mu Ci/g.$
Urine	82.00	1.50	10.00	0.053	$24 \cdot 10^{-3}$	$44 \cdot 10^{-5}$	$2.7 \cdot 10^{-3}$	$1.42 \cdot 10^{-5}$
Kidneys	7.80	0.49	0.60	0.037	$4.0 \cdot 10^{-3}$	$25 \cdot 10^{-5}$	$0.2 \cdot 10^{-3}$	$1.25 \cdot 10^{-5}$
Spleen	0.21	0.29	0.16	0.270	$0.25 \cdot 10^{-3}$	$34 \cdot 10^{-5}$	$0.2 \cdot 10^{-3}$	$33 \cdot 10^{-5}$

influenced the splenic uptake is consonant with the observation of the almost identical splenic uptakes in mice after intravenous injection of 30 μg . U and after inhalation of 6 μg . U (this journal, p. 305). A possible explanation may be that the spleen becomes overloaded when the amounts of uranium in the blood are such that the splenic concentrations in rabbits reach about 0.2 μg . per gram of tissue. Since the nuclides were applied simultaneously the present methods could not be used to study the differences in relative organic uptakes as a function of the amounts applied.

Experiments on Piglets Two similar experiments were performed on piglets whose integument resembles human skin more closely than does that of the rabbit. Incisions were made exactly as in the corresponding rabbit experiments and 200 mg. U_3O_8 was deposited in the subcutaneous pockets. The wounds were then sutured. The results are presented in Table V.

TABLE V
UPTAKE BY KIDNEY IN PIGLETS FOLLOWING SUBCUTANEOUS
ADMINISTRATION OF U_3O_8 .

	Kidneys	
	μg .	$\mu\text{g./g}$.
Controls	12	0.13
	11	0.13
PPP	6	0.05
	3	0.03

The only conclusion that can be drawn from such a limited experiment is that PPP may have the same effect in piglets as in rabbits.

No uranium could be found in the liver.

Routine Screening for Uranium Incorporation Numerous reports in the literature deal with the urinary excretion of uranium which has been administered intravenously or via immersion or inhalation. The data show a striking measure of agreement. Although the 24-hour excretion figures depend largely on whether exposure has been acute or prolonged, they are not particularly influenced by the mode of administration. From day 1 to day 10 after a single exposure the excretion appears to conform rather well to a logarithmic function, *i.e.*, $U_t = U_1 \cdot t^{-\mu}$ (where the terms mean the same as before). Lippmann, Ong, and Harris (1964) in a detailed investigation studied uranium excretion in

relation to the duration of incorporation. They found that, after a single exposure, μ in the above equation had a value of approximately 1.5. Lippmann (1959) had previously noted a figure of $\mu = 2.1$. These findings have been confirmed by others (Eisenbud and Quigley, 1956; Wilson, 1959; Bernard and Struxness, 1957; Bassett, Frenkel, Cedars, van Alstine, Waterhouse, and Cusson, 1958). It is interesting to observe that the values found for human subjects are quite close to ours for mice and rabbits.

In seven cases of over-exposure occurring at the AB Atomenergi plant in Stockholm, the calculated μ values were in the range of 1.2 to 2.8 with a mean and standard error of 2.0 ± 0.2 . Since, however, the quantities of uranium were small, only a few reasonably accurate values per individual could be obtained, and this in turn made it impossible to plot satisfactory curves. Owing to the substantial biological variation, it is necessary to monitor the urine for at least four or five days after the first positive urine analysis.

A single positive urine test, as pointed out above, affords no clue to the time and extent of exposure to uranium. An approximate idea can be gained, however, by following up the case for a few days and plotting the observed values on a log-log diagram as a function of time, to get a straight line with the regression coefficient $\mu = 1.5$ to 2, then extrapolating to $t = 0$. In so doing, it will be necessary to assume that the exposure has been brief.

There are several ways to find out if the uranium detected in a urine sample has in fact passed through the subject's body. At AB Atomenergi in Sweden, where the principal uranium hazard to mining and refinery personnel is contact with insoluble uranium (the dissolving phases of processing always take place in closed systems), a very simple procedure is employed to check for direct contamination of urine-collecting flasks. Whenever uranium has been detected in a sample, part of a strip of chromatographic paper is dipped in the urine. The part which has not been immersed is then tested for uranium. Chromatographic paper sucks up uranium that has passed through the body but not uranium oxides or other insoluble forms that may have contaminated the sample.

Conclusions

The results of animal experiments such as these cannot, of course, be generalized to humans. It is, nevertheless, possible, by comparison of data from different species and verified observations in man,

to arrive at more or less well-founded probabilities.

When large quantities of uranium rapidly enter a mammalian organism, a substantial proportion is absorbed, being deposited chiefly in the kidneys and bone tissue. During the period immediately after exposure the highest concentration of uranium is found in the renal cortex. In the mouse, uranium is released more rapidly from the kidney than from other organs, probably because of a washing-out effect that accelerates excretion from the renal medulla and inner cortex. The disappearance rate for the cortex is slower than for the medulla, but even so is faster than the corresponding rates for bone, spleen, and liver.

If this hypothesis of a washing-out effect is correct, the phenomena observed in mice should have qualitative counterparts in other mammalian organisms, including man. During the first two months after an intravenous injection the uranium content of mouse liver, spleen, and skeleton decreases by a factor of 10, but that of the kidney by a factor of 100. During the first month the uranium concentration is highest in the kidney, but after two months is equal to those in the spleen and bone, and after six months is well below them. Our observations on the uranium uptake pattern for the spleen apply to both quantitative determinations and autoradiographic findings. They conflict in this respect with all reports that we have found in the literature concerning the distribution of intravenously injected uranyl nitrate solutions.

When small amounts of uranium are absorbed from U_3O_8 depots in mouse lung and in PPP-treated skin lesions in rabbits, the spleen and bone tissue show much higher uranium concentrations than the kidney. Here, too, the rate of incorporation is probably an important factor.

Even if the present observations were applicable to humans, they would hardly influence the existing appraisal of hazards associated with exposure to soluble natural uranium, for the maximum permissible level is determined by the nephrotoxic effects which are probably manifested during the first month after exposure and of which the massive concentrations in the renal cortex are decisive. The

situation is different for long-term exposures or for exposure to uranium nuclides with high specific activities; here the maximum allowable content for the whole body is equivalent to only minute quantities of uranium. The relatively low concentration in, and faster excretion from, the more radioresistant kidneys doubtless constitutes a lesser hazard than the higher concentration and more prolonged retention in bone and spleen.

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