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The Clearance of Uranium after Deposition of the Nitrate and Bicarbonate in Different Regions of the Rat Lung

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This study investigated the tissue distribution and excretion of uranium after its deposition as either the nitrate or bicarbonate in the three regions of the respiratory system of the rat. Results confirm the recommendations of ICRP that uranyl nitrate and bicarbonate should be treated as class D compounds; but imply that some of the parameters used in the ICRP lung model are not applicable to soluble uranium compounds.

Introduction

Uranyl nitrate is an important intermediate in the nuclear fuel cycle with inhalation a likely route of accidental intake by workers. The International Commission on Radiological Protection (ICRP) has classified the clearance of radioactive materials deposited in the human lung according to their retention half-times in the pulmonary region. Class D compounds are retained for days, Class W for weeks and Class Y for years.[1] Uranyl nitrate is assigned to inhalation class D. However, whilst a number of workers have measured the overall lung clearance of uranium after inhalation by rodents and dogs[2–5] a paucity of data exists on its translocation to blood from the various regions of the respiratory system. This information is needed for improving the dosimetric model for man.

This paper describes a series of studies designed to measure the retention and translocation of uranium following its deposition as uranyl nitrate in the three anatomical regions of the respiratory system defined in the Task Group Lung Model, i.e. the nasopharyngeal (N-P), the tracheobronchial (T-B) and pulmonary (P) regions.[1] Comparative experiments were conducted with the bicarbonate complex since available evidence suggests it is in this form that uranium is transported by body fluids.[6–9]

Materials and methods

Animals

The animals used were female rats (HMT strain NRPB, Chilton) about 4-months old and weighing 200 ± 20 g at the time of intubation. Food and water were freely available during the course of the experiments.

Preparation of intubation solutions

Uranyl nitrate ($^{233}UO_2(NO_3)_2$) in 3 M HNO_3 was obtained from Amersham International plc, Amersham, Bucks. Solutions for intubation were normally made up at a concentration of 90 kBq ml^{-1}. They were prepared by evaporating an aliquot of the stock solution to dryness and dissolving the residue in either 0.01 M HNO_3 or 1% $NaHCO_3$. Before intubation, the solutions were passed through a 0.025 μm pore diameter membrane filter (type VS Millipore (UK) Ltd., Wembley, Middlesex) shortly (< 30 min) before administration to remove any polymeric material which may have been present.

Administration of solutions

Uranyl solutions (3 μl; 270 Bq; 52 μg) were intubated directly into the appropriate region of the respiratory system of rats using previously described techniques.[10,11] The mass concentration of uranium deposited corresponded to less than 2.5% of the daily limit for occupationally exposed workers[1,12] assuming that the mass ratio of the rat to human lung is 1:770.

Briefly, rats were first anaesthetized with 2–3% halothane in oxygen (Fluotec Mk III vaporizer, Cyprane Ltd., Keighley, West Yorks). For intubation of uranyl solutions into the T-B or P regions of the lung, a polythene tube (external diameter 1.6 mm, Portex Ltd., Hythe, Kent) was first passed via the mouth into the trachea for a distance of either 30 mm (T-B region) or 75 mm (P region) as measured from

the incisors of the animal. A small polythene cannula (external diameter 0.6 mm, Portex Ltd.) was then passed through the tube. This cannula contained 2–3 μl of the uranyl solution and was attached to a hypodermic syringe. In order to prevent artifacts, such as functional damage to the respiratory alveoli, the volume of solution was restricted to 3 μl. The solution was slowly intubated into either the T-B or P region and the cannula washed out with 2–3 μl water drawn into it prior to the uranyl solution. The accuracy of the administration, based on a measured column of solution drawn into the cannula, was ± 1.5%. For deposition in the N-P region the cannula was passed through the external nares of the animal into the upper nasal passages for a distance of 15 mm.

The low activity and small volume of solution administered ensured that the normal physiology of the respiratory system, and hence clearance mechanisms, were not affected to any significant extent.

After intubation of the uranium solutions, the animals were kept in metabolism cages for the separate collection of urine and faeces. Groups of 5 animals were killed at 1, 3, 10 and 30 d after administration. These times were considered appropriate for quantifying any rapid translocation of uranium from the respiratory system and the fate of most of the deposited material.

Radiochemical analysis of tissue samples and excreta

After removal of the pelt, the lungs, liver, kidneys and gastrointestinal tract (GIT) were dissected out. These tissues, together with the remaining carcass, and urine and faecal samples, were first reduced to a carbon-free ash by dry ashing at 500°C. The samples were then analysed for ^{235}U content by solvent extraction and liquid scintillation counting.[15] On the basis of previous studies[2] the uranium in the remaining carcass (i.e. excluding the kidneys and liver) was considered to be present predominantly (> 90%) in bone after the first week after exposure.

Results

The retention of uranium in the three regions of the respiratory system and the amounts of uranium translocated to blood (i.e. systemic content plus cumulative urinary excretion) from these sites as a function of time after intubation as the nitrate are given in Table 1. The remaining uranium, either present in the GIT or excreted in the faeces after mechanical clearance from the respiratory system, is not reported separately. The results show that in general more than 90% of the uranium was cleared from all sites of deposition within the first day after intubation. A small proportion appeared to be retained with a half-time of at least several weeks. However, the amounts which translocated to the blood from these sites varied considerably being lowest for the N-P region and highest for the P region. For the N-P and T-B regions clearance occurred mainly by mechanical transport to the gastrointestinal tract.

The behaviour of uranium when intubated as the bicarbonate complex into the same three regions of the respiratory tract (Table 2) was closely similar to that for uranyl nitrate (Table 1).

Table 3 gives the data obtained for the distribution of uranium amongst body tissues and its cumulative urinary excretion at various times after intubation of uranyl nitrate into the pulmonary region of the lungs. The table shows that the principal organs of deposition of uranium were the kidneys and remaining carcass. Less than 1% of the initial deposit was found in the liver. It is noteworthy that most of the uranium which entered the blood was rapidly excreted in the urine—about 60% and 80% at 3 and 10 d respectively.

The behaviour of systemic uranium after deposition of the nitrate in the N-P and T-B regions was closely similar to that after deposition in the P region. However, in these experiments about 92% and 68% respectively of the initial deposits were excreted in the faeces by 10 d.

Table 1 The retention and translocation to blood of uranium after intubation as nitrate into the three anatomical regions of the respiratory system

Region		% *Intubated activity* ($\bar{x} \pm s.e.$, n = 5) (d) 1	3	10	30
N–P	retained	1.80 ± 0.8	0.64 ± 0.17	0.52 ± 0.05	ND
	to blood[a]	8.9 ± 0.9	6.4 ± 0.4	8.1 ± 0.8	ND
T–B	retained	0.14 ± 0.08	0.34 ± 0.20	0.31 ± 0.27	ND
	to blood[a]	30.2 ± 4.3	41.8 ± 4.3	41.3 ± 2.8	ND
P	retained	8.5 ± 2.6	8.4 ± 0.8	3.5 ± 0.5	2.8 ± 0.2
	to blood[a]	70.0 ± 6.9	68.5 ± 6.0	74.0 ± 5.5	71.0 ± 1.9

[a] Systemic content and cumulative urinary excretion.
ND – not determined

Table 2 The retention and translocation to blood of uranium after intubation as bicarbonate into the three anatomical regions of the respiratory system

Region		% *Intubated activity* ($\bar{x} \pm s.e.$, n = 5) (d) 1	3	10	30
N–P	retained	4.61 ± 2.05	2.96 ± 1.20	2.41 ± 1.21	ND
	to blood[a]	6.8 ± 0.8	6.9 ± 1.0	9.6 ± 1.3	ND
T–B	retained	0.04 ± 0.04	0.13 ± 0.06	0.14 ± 0.09	ND
	to blood[a]	27.4 ± 3.8	28.8 ± 3.4	36.1 ± 4.2	ND
P	retained	27.3 ± 3.37	8.11 ± 2.36	6.65 ± 1.47	4.08 ± 0.36
	to blood[a]	58.4 ± 3.8	70.5 ± 3.7	71.6 ± 2.0	71.0 ± 1.9

[a] Systemic content and cumulative urinary excretion.
ND—not determined

Table 3 Tissue distribution and excretion of uranyl nitrate at 1, 3, 10 and 30 d after intubation into the pulmonary region of rat lung

Tissue/excreta	% *Intubated activity* ($\bar{x} \pm s.e.$ n = 5) (d) 1	3	10	30
Lung	8.5 ± 2.6	8.4 ± 0.8	3.5 ± 0.5	2.8 ± 0.2
Liver	0.58 ± 0.10	0.48 ± 0.04	0.18 ± 0.02	0.11 ± 0.02
Kidney	12.1 ± 1.8	6.7 ± 0.7	3.0 ± 0.19	1.07 ± 0.08
Remaining carcass	25.4 ± 3.8	21.3 ± 0.4	11.4 ± 0.25	7.2 ± 0.67
Urine	31.9 ± 5.5	40.1 ± 6.0	59.4 ± 5.5	63.3 ± 5.5
Faeces and GIT	21.5 ± 3.2	23.0 ± 0.6	22.9 ± 0.1	25.6 ± 6.2

Discussion

The rapid translocation of uranium to the blood after deposition as the nitrate in pulmonary lung concurs with the recommendation of ICRP that it should be assigned to inhalation class D.[1]

For any inhaled aerosol, the fraction of the material inhaled that deposits in the three anatomically defined regions of the respiratory tract given in the ICRP lung model[1] depends only on the particle size; clearance, however, also depends on the chemical form. The deposition and clearance parameters for a class D aerosol of 1 μm AMAD are given in Table 4. Also shown in Table 4 are the total amounts of uranium translocated to the blood in rats after the intubation of uranyl nitrate into the three regions of the respiratory tract. Since, by definition, class D aerosols are highly soluble in body fluids it is reasonable to assume that the intubation of uranyl nitrate solution into the respiratory system of animals will provide a basis for predicting the translocation of uranium to the blood from these regions in workers who are occupationally exposed. It can be seen that the values predicted for the N-P and T-B regions of the model are in excess of those found in the animal experiments. On the other hand the values for the P region are in reasonably good agreement.

The results of the animal experiments imply that the annual limit on intake (ALI) of uranium for an aerosol of AMAD 1 μm (5×10^4 Bq)[1] which is based on the dose to bone surfaces, or the daily limit on intake based on chemical toxicity (2.5 mg)[12] will err on the side of safety since a smaller fraction will enter the blood than predicted by the lung model. The ALI for class D compounds is considered to be largely independent of the particle size within the respirable range.[1,14] The results of these studies suggest, however, that the dose to bone surfaces or chemical damage to the kidneys should decrease with an increase in the particle size of the aerosol. Under these conditions larger amounts of uranium will be deposited in the N-P region,[1] from which transport to the blood will, on the basis of the animal experiments, be appreciably less than for the other anatomical regions.

The similarity in the metabolic behaviour of uranium entering the blood from the different regions of the lung indicates that it is transported as the same chemical form, almost certainly the hexavalent carbonate complex.[6–9] The results indicate that there is no need to modify the equations used to estimate the systemic content of uranium from urinary excretion measurements on the basis of possible differences in translocation kinetics and mechanisms.

Table 4 Deposition and clearance of class D compounds from the human respiratory tract as predicted by the ICRP model and the experimental data

		Model		Experimental	
Region	Regional Deposit[a]	Fraction to blood[b]	Amount to blood[c]	Fraction to blood[b]	Amount to blood[c]
N–P	0.3	0.5	0.15	0.08	0.024
T–B	0.08	0.95	0.076	0.40	0.032
P	0.25	1.00	0.25	0.70	0.175
			0.48		0.231

[a] The values given are the regional deposits for an aerosol with an AMAD of 1 μm.[1]
[b] Fraction of amount deposited in each region.
[c] Fraction of total amount inhaled for an aerosol with an AMAD of 1 μm.

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References

1 International Commission on Radiological Protection. Limits for Intakes of Radionuclides by Workers. ICRP publication 30, Part 1. Oxford: Pergamon Press, 1979.
2 Durbin PW & Wrenn ME. Metabolism and effects of uranium in animals. In Proceedings of a Conference on Occupational Health, Experience with Uranium. Arlington, Virginia, 1975. pp. 68–129, US Energy Research and Development Administration, ERDA 93, 1976.
3 Yuile CL. Animal experiments. In Uranium, Plutonium, Transplutonic Elements, eds HC Hodge, JC Stannard & JB Hursh, pp. 165–196. Berlin: Springer-Verlag, 1973.
4 Fish BR. Inhalation of uranium aerosols by mouse, rat, dog and man. In Inhaled Particles and Vapours I, ed. CN Davies, pp. 151–166. London: Pergamon Press, 1961.
5 Morrow PE, Gibbs FR & Beiter HD. Inhalation studies of uranium trioxide. Health Physics 1972; 23: 273–80.
6 Dounce AL & Flagg JF. The chemistry of uranium compounds. In The Pharmacology and Toxicology of Uranium Compounds, eds C Voegtlin & HC Hodge, pp. 55–113. New York: McGraw-Hill, 1949.
7 Neuman WF. The distribution and excretion of uranium. In The Pharmacology and Toxicology of Uranium Compounds, eds C Voegtlin & HC Hodge, pp. 701–724. New York: McGraw-Hill, 1949.
8 Blake CA, Coleman CF, Brown KB, Hill DG, Lowrie RS & Schmitt JM. Studies in the carbonate-uranium system. *Journal of the American Chemical Society* 1956; 78: 5978–83.
9 Gindler JE. Physical and chemical properties of uranium. In Uranium, Plutonium, Transplutonic Elements, eds HC Hodge, JC Stannard & JB Hursh, pp. 67–164, Berlin: Springer-Verlag, 1973.
10 Stather JW & Howden S. The effect of chemical form on the clearance of plutonium-239 from the respiratory system of the rat. *Health Physics* 1975; 28: 29–39.
11 Crawley FEH & Goddard EA. The translocation of 241-americium and 242-curium from the respiratory system of the rat. *Health Physics* 1976; 30: 191–7.
12 Official Journal of the European Communities. Office for Official Publications of the European Communities, Luxembourg. No. L246, vol 23, 1980.
13 Gies RA, Ballou JE & Case AC. A method for analysing ^{233}U in biological samples. *Health Physics* 1980; 39: 980–3.
14 Stradling GN, Stather JW, Strong JC, *et al.* Metabolism of some industrial uranium tetrafluorides after deposition in the rat lung. *Human Toxicology* 1985; 4: 159–68.

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Detoxification of Styrene Oxide by Human Liver Glutathione Transferase

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Cytosolic glutathione transferase (GST) was investigated in four human livers. The profile of GST activity was determined by isoelectric focusing using 1-chloro-2,4-dinitrobenzene as the electrophilic substrate. Three livers contained at least one basic and a near-neutral isoenzyme (GST μ). GSTμ was not detectable in the fourth liver. The kinetics of GST with styrene oxide as the electrophilic substrate were studied in the cytosolic fraction, with the pooled fractions from isoelectric focusing containing high activity of GSTμ transferase, and with GSTμ purified to homogeneity. The cytosol obeyed Michaelis–Menten kinetics when styrene oxide was used as the variable substrate. The average (± s.e.m.) of the V_{max} and K_m were 21.9 ± 7.9 nmol min^{-1} mg^{-1} and 4.9 ± 0.4 mM, respectively. At varying concentrations of glutathione, the enzyme did not obey Michaelis–Menten kinetics. Such kinetics were also observed with the fractions from isoelectric focusing and with the homogeneous GSTμ fraction. The Eadie-Hofstee plot showed two phases: one with a low and another with a high K_m value.

The apparent K_m values for the cytosol were 0.035 ± 0.022 and 0.88 ± 0.36 mM. The kinetic pattern of purified GSTμ is consistent with that found in the cytosol.

Introduction

The glutathione transferases (GST) are a family of enzymes with overlapping substrate specificities which catalyse the conjugation of glutathione with different xenobiotics.[1,2] In human adult liver, GST activity resides in several 'basic' isoenzymes with isoelectric points between pH 8 and 10^{3-6} and one 'near-neutral' form (GSTμ) with an isoelectric point between pH 6 and 7.[7] (For review see Mannervik.[8]) The latter form is more active than the basic ones in the conjugation of glutathione with epoxides such as styrene oxide, 1,2-epoxy-3(*p*-nitrophenoxy)-propane and benzo(a)-pyrene-4,5-oxide.[9,10] GSTμ is present in about one-half of the population.[9] Subjects lacking this GST isoenzyme may have a lower capacity to detoxify epoxides than individuals whose livers contain such an isoenzyme.

Epoxides are intermediary metabolites of a variety of chemical compounds.[11,12] Several epoxides have carcinogenic[13–15] and mutagenic properties.[13,16] The biotransformation of epoxides includes two pathways: conjugation with glutathione, catalysed by GST[1,17–19] and hydration to a glycol, catalysed by epoxide hydrolase.[12,20] In order to better understand the human metabolism of epoxides via glutathione conjugation, we studied the kinetics of glutathione transferase with styrene oxide as the substrate.

Styrene oxide has been used as a substrate to assay cytosolic GST[21–28] and also to study the kinetic properties of the purified basic and acidic isoenzymes of GST from human fetal liver.[29,30] Styrene oxide is of particular interest since its precursor styrene is used in the industrial manufacture of synthetic rubber and plastics.[31,32]

The substrate specificities of the various forms of GST differ significantly. Thus, one form could be more effective than another in determining the cytosolic activity towards a particular substrate. In the case of styrene oxide, the specific activity of transferase μ has been found to be higher than that of the basic type by at least one order of magnitude.[10] This prompted us to extend our investigation to transferase μ obtained after isolation by isoelecric focusing and purified to homogeneity.

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