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INTRODUCTION

The first meeting of the type described in this document was held in October, 1955 at Fernald, Ohio under the auspices of the National Lead Company of Ohio; the proceedings of that meeting are described in United States Atomic Energy Commission report NLCO-595. The proceedings of the second annual meeting held at Los Alamos Scientific Laboratory are described in United States Atomic Energy Commission report WASH-736.*

The primary objective of those meetings and of the meetings which succeeded them was the interchange of information between those working in this field of health protection, sometimes called "bio-assay", health radiochemistry, occupational biochemistry, or radiation hygiene chemistry. The chemical methods for routine "bio-assay" can be found in United States Atomic Energy report AECU-4024. It is the hope of those who participated in the sponsorship of this meeting that these proceedings will aid in the attainment of that objective.

* No proceedings were published for the third annual meeting held at Mallinckrodt Chemical Works.

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The following papers were not published either because of their unavailability or for other sufficient reasons.

An Apparatus for the Generation and Inhalation of an Uranium Aerosol

W. E. Lotz, Oak Ridge National Laboratory

Emergency Permissible Levels. A discussion by:

K. Z. Morgan, Oak Ridge National Laboratory

J. H. Harley, New York Operations Office.

THE DETERMINATION OF FLUORIDE IN HUMAN URINE*

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Introduction

In the process of enriching uranium in the isotope 235 at Oak Ridge Gaseous Diffusion Plant, care must be taken to avoid exposing employees to hydrogen fluoride, fluorine and fluoride-containing compounds. The analysis of urine samples serves to indicate possible exposure to fluoride-containing atmospheres. However, the rigorous control maintained over fluoride-producing processes is such that we do not have a chronic health problem, and the urine data are used, therefore, as a supplement to our routine air analysis program and as medical-legal evidence for the protection of the company.

The method employed at the Oak Ridge Gaseous Diffusion Plant for determining fluoride in human urine is essentially the one reported in Division VI Volume 1 of the National Nuclear Energy Series, entitled Pharmacology and Toxicology of Uranium Compounds. Because of the large number of samples submitted to our laboratory, two principal modifications have been introduced into the method which permit one analyst to analyze all of the samples associated with this program. The most important modification is an automatic distillation unit which requires so little attention during the distillation that the analyst can simultaneously perform some other operation in the procedure and thereby complete 30 analyses in a normal day's operation. The other modification involves the substitution of a colorimetric Eriochrome Cyanine R method for the time-honored but tedious and time-consuming thorium nitrate titration method.

In general, the overall method consists of ashing the sample in the presence of lime to fix the fluoride ion as calcium fluoride, transferring the residue to the automatic distillation unit and distilling the fluoride as hydrofluosilicic acid from perchloric acid solution by the method of Willard and Winter (2). The fluoride content of an aliquot of the distillate is determined by the Eriochrome Cyanine R method of Megregian (1).

* This document is based on work performed at the Oak Ridge Gaseous Diffusion Plant operated by Union Carbide Corporation for the U.S. Atomic Energy Commission

Program

Our analytical fluoride program covers approximately 2000 of the 4500 employees at the plant. The number of scheduled visits by any employee, of course, is variable depending on the possibility of his being exposed. The schedules are set up annually with the approval of the operating supervisor and cover possible exposures to such other air contaminants as uranium and mercury.

In figure 1 is shown the visitation schedule of employees who took part in the fluoride analysis program over the past 3 years. As can be seen, the majority of the visitations are on a 3, 4, or 6 months schedule. The single visitations are checks of employees made in connection with alleged exposures or special dispensary visits.

Urine samples are collected in 8 oz. French square bottles at the dispensary when the employee reports for a scheduled visit or for an industrial health examination. Samples taken at the dispensary up until 2:00 PM are delivered to the laboratory in time to allow overnight evaporation. Fluoride analyses are completed on the next day and the results are reported by means of an IBM card system. Individual results which exceed 4.0 mg F/l. are telephoned immediately to the Medical Department for action and the Medical Department recalls the employee as often as is necessary until his level drops below 4 mg. F/l. An employee who shows levels in excess of 4 mg. F/l. on three successive visits is issued a 24 hour specimen bottle and is given a different work assignment if this sample shows over 4 mg. F/l. He is then checked weekly to determine when he can return to his normal work assignment.

I might mention at this point that the 4 mg. F/l. level which we use at our installation is merely a control point which we feel can be defended legally, and which, as will be shown later, is exceeded in only a small percentage of the samples.

For samples which do not exceed these limits, the results are mailed to the Medical Department after all the requested analyses have been completed - these might include uranium, alpha activity, and mercury in addition to the fluoride. All results are entered on the employee's medical record in the dispensary and the card is submitted for IBM machine tabulation.

Procedure

Sample Preparation and Distillation

For those interested in a detailed method a stepwise procedure is available, however, I will not include it in this oral presentation; it will appear in the compiled minutes of this conference. In general,

Year:	1955	1956	1957
Total Number of Employees in Program:	1789	1801	2014

<u>Number of Visitations</u>	<u>Number of Employees</u>		
1	449	348	443
2	700	613	670
3	313	453	484
4	232	280	308
5	71	96	101
6	20	10	7
7	3	1	1
8	1	---	---

ANNUAL VISITATION SCHEDULE FOR FLUORIDE ANALYSES IN URINE

Slide 1
(Figure 11)

fifty milliliters of each urine specimen is measured into a platinum dish, 0.7 gram of calcium oxide is added and the sample is evaporated to dryness overnight on a hot plate and charred to a black ash. The residue is ignited in a muffle furnace for 10 minutes, the ash is cooled, softened by wetting with distilled water and broken up with a Monel spatula. The evaporation and ignition are repeated. We have found that this double evaporation and ignition step is equally as effective in removing organic material and interfering anions as the usual practice of igniting the sample for several hours.

The residue in the platinum dish is softened by wetting with distilled water and quantitatively transferred to the distillation flask with measured volumes of silver perchlorate and perchloric acid. After adding a few glass beads to the flask, the unit is assembled.

Figure 2 shows the components of the fluoride still used in our procedure. The one liter capacity steam generator is heated by a Variac-controlled cartridge-type immersion heater. The 75 ml. distillation flask comes supplied with a well for the 6-inch factory preset thermostat. The specially designed distillation head prevents the carry-over of large amounts of acid into the distillate. The steam inlet tube is inert, Kel-F plastic instead of glass because of the large amount of breakage experienced with this part. The trap on the steam line minimizes suckback of acid into the steam generator.

Figure 3 shows the compact distillation apparatus which operates six stills simultaneously. Although this unit has been used exclusively for fluoride in urine, similar units are employed in the laboratory for distilling fluoride from a variety of materials. The unit occupies a space 42" x 19" x 56" and requires external connections for AC power, distilled water, cooling water and a drain. The individual steam generators in this unit are filled through a manifold which is connected to a distilled water carboy. The three-way valve on each generator can be used for filling the generator, allowing steam to vent to the atmosphere, or directing the flow of steam through the distillation flask. The distillation flask is heated by a Hotcone heater which is electronically controlled by the thermostat and relay. The relays are modified so that they plug into standard tube sockets and replacement becomes a matter of plugging in a new unit. The receivers are 250 ml. capacity polyethylene bottles marked at 200 ml. volume.

All heaters and thermostats plug into the rear wall of the unit and may be replaced easily. All panel electrical controls are flush mounted for ease of operation. A safety-shield protects the operator during the course of the distillation. We have never had an explosion of a distillation flask, but have had the usual reaction when a drop of silver

FIGURE 3

perchlorate or perchloric acid is accidentally placed on the heating element of the Hotcone heater.

Before the distillation is started, the steam generator is adjusted to provide a steady flow of steam through the vent. The distillation flask is heated until the temperature reaches 135°C . and the steam is then directed through the flask. The thermostat, together with the electronic relay, controls the distillation at $135^{\circ} \pm 1^{\circ}\text{C}$. The acidity of the distillate is checked during distillation with sodium hydroxide and phenolphthalein and if an excessive amount of acid carries over, the distillate is concentrated and redistilled. The distillation is continued until 200 ml. of distillate is collected, when the steam generator and distillation flask heater are turned off. The steam generator is opened to the atmosphere to prevent the contents of the distillation flask from being sucked back as the system cools.

Figure 4 shows the fluoride recovery obtained by this distillation as a function of distillate volume. The slight increase in recovery with increase in volume above 200 ml. is not sufficient to justify the additional time required for the distillation.

Colorimetric Fluoride Determination

The final determination is performed by measuring the bleaching action of fluoride ion on a zirconium - Eriochrome Cyanine R lake. The range of the method, using 1 cm. cells, is 0 - 60 micrograms of fluoride in the aliquot taken. Fifty milliliters of the distillate is measured into a 100 ml. mixing graduate, Eriochrome Cyanine R and zirconyl chloride solutions are added and the solution is mixed well. A reference solution is prepared by adding Eriochrome Cyanine R solution and reference acid to 50 ml. of distilled water. After 5 minutes the transmittance of the sample is measured at a wavelength of 535 m μ against the reference solution set at 31.5% transmission (0.50 absorbance). The fluoride value of the sample aliquot is obtained from a previously prepared curve. The value of a calcium oxide blank is subtracted from the measured value to give the net amount of fluoride.

Figure 5 shows a comparison of the calibration curves obtained with and without distillation. The difference between the two curves is approximately 5% which reflects the 95% recovery obtained on the distillation step. This curve must be run each time new solutions are prepared; therefore, we prepare solutions in such quantity that the calibration needs to be checked only once a month.

FIGURE 4

FIGURE 5

Results and Quality Control

With this single six-place distillation unit, the laboratory presently analyzes approximately 500 urine specimens per month. As was mentioned earlier, in a normal day's operation one analyst can easily complete 30 samples.

We have compiled in figure 6 the results by ranges obtained on specimens over a 3 year period. These results, except for a period in 1957, have been obtained by the thorium titration method since the colorimetric method has been in use only about 1 year. The values reported as <0.5 mg. F/l. result from a time saving modification of the titration method. Although our experience with the Eriochrome Cyanine R method is limited compared to our titration experience, the time saving features of the method make it ideal for this type of sample.

A quality control program which is carried on in the laboratory analyzes one "blank" urine spiked with the equivalent of 1 mg. F/l. each day a group of samples is run. The limit of error of a single determination (95% confidence level) at 1 mg. F/l. is ± 0.1 mg. F/l. with a recovery in excess of 90% of the added fluoride.

References

1. Megregian, S., Anal. Chem., 26, 1161 (1954).
2. Willard, H.H. and Winter, O.B., Ind. Eng. Chem., Anal. Ed., 5, 7-10 (1933).

<u>Range (mg. F/l.)</u>	<u>1955</u>	<u>1956</u>	<u>1957</u>
	<u>Number of Analyses</u>		
0.0 - 0.4			1392
<0.5	3707	3611	1281
0.5 - 0.9	92	470	1234
1.0 - 1.4	419	764	1005
1.5 - 1.9	158	237	434
2.0 - 2.4	48	103	225
2.5 - 2.9	18	34	84
3.0 - 3.9	14	45	72
4.0 - 4.9	4	2	25
5.0 - 5.9	0	5	9
6.0 - 6.9	2	2	3
7.0 - 7.9	1	4	1
8.0 - 8.9	1	3	0
9.0 - 9.9	0	1	2
>10.0	1	4	2
Total Number of Analyses	4465	5285	5769

ANNUAL DISTRIBUTION OF FLUORIDE ANALYSES IN URINE

Figure 6

Determination of Fluoride in Human Urine

Procedure

Method

Urine samples are evaporated to dryness with an excess of calcium oxide and ashed at 1400°F. Fluoride is steam-distilled from the residue by the Willard and Winter method and the fluoride is determined by measuring spectrophotometrically the bleaching of an Eriochrome Cyanine R - zirconyl chloride lake by the fluoride ions.

Apparatus and Reagents

1. Platinum evaporating dishes, 100 ml.
2. Muffle furnace, controlled at 1400°F.
3. Fluoride stills and automatic distillation apparatus (Figures 2 and 3).
4. One hundred milliliter graduated cylinders with ground glass stoppers.
5. Beckman spectrophotometer, Model B, with 1 cm. matched cuvettes.
6. Calcium oxide C.P., Fischer Scientific Company, "special fluoride free."
7. Silver perchlorate solution: Dissolve 84 grams of anhydrous silver perchlorate in 100 ml. of distilled water.
8. Perchloric acid, 72% reagent grade.
9. Sulfuric Acid, concentrated.
10. Phenolphthalein solution: Dissolve 1 gram of the reagent in 100 ml. of ethanol.
11. Sodium hydroxide, 0.5 N.
12. Reference acid solution: Dilute 700 ml. of concentrated hydrochloric acid to one liter.
13. Eriochrome Cyanine R solution: Dissolve 1.8 grams of the reagent in distilled water and dilute to one liter.

14. Zirconyl chloride solution: Dissolve 0.265 gram of the reagent in 50 ml. of distilled water, add 700 ml. of concentrated hydrochloric acid and dilute to one liter.

Procedure.

1. Remove the organic components from the urine and fix the fluoride as follows:
 - (a) Place 0.7 g. of calcium oxide (measured by dipper) in a 100 ml. platinum dish. Add 50 ml. of the urine sample.
 - (b) Evaporate the sample to dryness on a hot plate and allow the residue to char. Place the dish in a muffle furnace at 1400°F. for 10 minutes.
 - (c) Remove the dish from the muffle furnace, soften the residue by wetting with distilled water, and break it up with a Monel spatula. Repeat step 1-b.
2. Distill the fluoride from the sample as follows:
 - (a) Soften the residue in the platinum dish by wetting with 9 ml. of distilled water and transfer it along with 4 ml. of silver perchlorate solution to the distillation flask. Rinse the platinum dish with 20 ml. of perchloric acid, pouring the rinsings into the flask. Drop a few glass beads into the flask.
 - (b) Assemble the distillation equipment.
 - (c) Before the distillation is started, turn on the cooling water to the condensers and adjust the steam generator to provide a steady flow of steam through the vent. Fill the thermometer well with sulfuric acid and add 3-4 drops of 0.5 N sodium hydroxide and 2 drops of phenolphthalein to the receiver. Heat the distillation flask until the temperature reaches 135°C. and direct the steam through the flask. The thermostat, together with the electronic relay, controls the distillation at $135^{\circ} \pm 1^{\circ}\text{C}$.
 - (d) When 200 ml. of distillate has been collected, turn off the steam generator and distillation flask heater switches. Open the steam generator to the atmosphere to prevent the contents of the distillation flask from being sucked back as the system cools.
3. Determine the fluoride content of the distillate as follows:

- (a) Thoroughly mix the 200 ml. of distillate and transfer a 50 ml. aliquot to a 100 ml. mixing graduate. Add 5 ml. of Eriochrome Cyanine R solution to the sample, followed by 5 ml. of zirconyl chloride solution.
- (b) Prepare a reference solution by adding 5 ml. of Eriochrome Cyanine R solution and 5 ml. of reference acid to 50 ml. of distilled water.
- (c) Five minutes after completing steps a and b, measure the transmittance at 535 mμ against the reference solution set at 31.5% transmission. Obtain the fluoride value of the sample aliquot from a previously prepared curve.
- (d) Run a daily calcium oxide blank.

Calculation

$$\text{Fluoride content of urine sample (mg./l.)} = \frac{(\text{micrograms F in sample aliquot} - \text{micrograms F in blank aliquot}) \times 4}{50 \text{ ml. of urine}}$$

DETERMINATION OF FLUORIDE IN SOIL

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The amount of fluoride found in soil samples will usually be less than 1000 ppm. According to Clarke and Washington⁽¹⁾, the ten mile deep earth's crust averages 290 ppm fluoride. Some of the more important fluoride-containing minerals are shown in slides 1 and 2.* Of these muscovite, biotite, and hornblende are common components of soil and are probably the main source of soil fluorides. Biotite and muscovite can contain a maximum of 4 and 2% F respectively. However, the amount is usually less, although more than a trace.

Artificial fluoride enrichment of soil may come from three sources: (a) industrial processes such as the production of aluminum, uranium, phosphate fertilizers, glass, and bricks, (b) the residues of insecticides and pesticides, (c) the fluoride present in phosphate fertilizers. Of these the use of phosphatic fertilizers is probably the most important. Mixed fertilizer averages 0.7% F, rock phosphate 3.8% F, superphosphate 1.5% F, and double superphosphate 1.6%F. Over 11,000,000 tons of these fertilizers are consumed annually in the United States. In heavily limed soils, the phosphorous (and fluoride) is fixed as apatite.

Robinson and Edgington⁽²⁾ analyzed 138 representative soils with a wide geographical distribution. They found an average fluoride content of 450 ppm, with a range from 0 to 7000 ppm. In general, sandy soils were found to be low in fluoride while the heavier-textured soils tended to have a higher content.

The methods for the assay of fluoride in soil usually consists of decomposition, some modification of the Willard and Winter⁽³⁾ distillation to isolate the fluoride, and a colorimetric or photometric determination of the fluoride.

1. F.W. Clarke and H.S. Washington, U.S. Geological Survey Prof. Paper 125, (1924).

2. W.O. Robinson and G. Edgington, Soil Science 61, p. 341, (1946).

3. H.H. Willard and O.B. Winter, Ind. Eng. Chem., Anal. Ed. 5, Vol. 7, (1933).

* The slide illustrations were not available and are therefore not reproduced here.

At least three methods of parting the fluoride from the mineral soil are possible. These are decomposition by sodium carbonate fusion, sodium hydroxide fusion or direct distillation. In the first, the fused soil sample is added directly to the distillation apparatus. In the second, after leaching the melt with hot water, a centrifugation is performed to remove most of the silica and alumina. In the third, the fluoride is volatilized from the sample during distillation.

At our laboratory, experience with water samples has shown that steam distillation from sulfuric acid solution is superior to the perchloric acid distillation with step-wise addition of water for routine work.

Two photometric procedures for the determination of fluoride after distillation in vogue at present are the zirconium-alizarin red-S and zirconium-eriochrome cyanine R bleaching methods⁽⁴⁾. Of these, the latter is claimed to be superior in that there is no waiting time for full color development and that it is more sensitive to low fluoride concentrations. In order to determine which combination of decomposition and determination would give optimum results, a soil sample was carried through the different procedures and the results compared.

Reagents: all materials were of the best obtainable grade. Scott's mixed acid - 18.5 ml. of H_2SO_4 and 56 ml. of HCl were each diluted to 250 ml. and the solutions combined.

Reagent A: 0.375 gm of alizarin red-S was diluted to 500 ml. and stored in the dark.

Reagent B: 0.147 gm of zirconyl nitrate dihydrate was dissolved in 500 ml. of water and diluted to 500 ml. with Scott's mixed acid.

Reagent C: 1.80 gm Eriochrome Cyanine R was diluted to 500 ml.

Reagent D: 0.220 gm $ZrO(NO_3)_2 \cdot 2H_2O$ was dissolved in 150 ml. H_2O and diluted to 500 ml. with HCl.

Decomposition and Isolation

1. Sodium carbonate fusion: 1 gm of dried and finely powdered soil was intimately mixed with 4-5 gm of Na_2CO_3 in a platinum crucible and fused over a Fisher Burner. The melt was

4. D.F. Boltz, Editor, Colorimetric Determination of Nonmetals, Interscience (1958).

transferred to the distilling flask with 50 ml. of water. 0.5 gm of Ag_2SO_4 was added to prevent distillation of any chloride present. Fifty ml. of 1:1 H_2SO_4 was carefully added along with a few glass beads. The distillation was conducted as described in (3).

2. Sodium hydroxide fusion: five gm of NaOH was fused in a nickel crucible. One gram of soil was carefully added and the fusion was carried out at as low a temperature as possible. The melt was leached with 30 ml of hot water and transferred to a centrifuge cone. After centrifugation and washing with 20 ml of water, the supernates were poured into the distilling flask. 0.5 gm Ag_2SO_4 , 50 ml. 1:1 H_2SO_4 , and a few glass beads were added. The distillation was carried out as in (3).

3. Direct distillation: a one gram sample is placed in the distilling flask along with 0.5 gm Ag_2SO_4 and a few glass beads. 35 ml. of 1:1 H_2SO_4 and 50 ml. of water added. As soon as the liquid began to distill, steam was introduced and the distillation allowed to continue until a suitable amount of distillate was collected. The distillation apparatus is shown in slide 3.

Photometric Determination

1. Zirconium-alizarin red-S bleaching: five ml. of reagent A and 5 ml. of reagent B are added per 100 ml. of distillate. The solution is shaken intermittently. The absorbance is read in 5 cm cells versus distilled water after 60 ± 2 minutes at a wavelength of 525 m μ with a Beckman Model DU Spectrophotometer. A typical calibration curve is shown in slide 4.

2. Zirconium-Eriochrome Cyanine R bleaching: five ml. of reagent C and 5 ml. of reagent D are added per 100 ml. of distillate. The solution is mixed well and the absorbance measured versus a reference solution at 528 m μ in 1 cm cells with a Beckman Model DU Spectrophotometer. The reference solution consists of 100 ml. distilled water and 5 ml. of reagent C and 5 ml. of 7:3 HCl. A typical calibration curve for this method is also shown in slide 4.

The results obtained by running portions of the same soil sample by the methods described above are shown in Table I. One hundred ml. increments of distillate were collected. Each was split into 2 portions. One was analyzed by the Zr-Alizarin red-S procedure and the other by the Zr-Eriochrome Cyanine R technique.

TABLE I

Ppm Fluoride in Soil Sample as Determined
by Diverse Methods

Distillate 100 ml portions	Method of Decomposition and Assay					
	1A	1B	2A	2B	3A	3B
1	26	70	20	28	0	0
2	42	110	108	132	33	10
3	118	195	188	70	65	117
4	115	200	73	40	68	0
5	100	80	23	28	0	0
6	0	0	0	0	0	0
Total	401	655	412	298	133	127

1 = Direct Distillation

A = Zr-Eriochrome Cyanine R

2 = Na_2CO_3 Fusion

B = Zr-Alizarin Red S

3 = NaOH Fusion

The most obvious feature of these results is that the two photometric techniques do not show agreement when aliquots of the same distillate are analyzed. The mean bias of the Zr-Eriochrome Cyanine R procedure is -7 ± 58 which is not significant.

Another feature, perhaps surprising at first, is the agreement between the result obtained by a sodium carbonate fusion and by a direct distillation of the sample as determined by the Zr-Eriochrome Cyanine R bleaching. The result obtained after a sodium hydroxide fusion is appreciably lower than the other two.

The amount of fluoride distilled over in each 100 ml. increment follows the expected pattern in all cases. Some fluoride is released from the sample with the first portion of distillate even though the optimum distilling temperature has not been reached. In the second, third, and fourth 100 ml. portions this temperature is obtained and the major portion of the fluoride distills as fluorosilicic acid at this time. The last traces are released in the fifth portion indicating that only 500 ml. of distillate need be collected.

In view of the agreement noted above between a direct distillation of the soil and distillation after Na_2CO_3 fusion, it was decided to evaluate these methods on other soil samples. The Zr-Eriochrome Cyanine R photometric procedure appeared superior to the Zr-Alizarin Red S bleaching and so was used in all subsequent analyses.

The results obtained are shown in Table II. Except for sample 1, the results by the two methods agree very favorably. The standard deviation for duplicates is smaller for direct distillation (6.4%) than for a sodium carbonate fusion (14.5%). There is a positive bias for the fusion method of $24 \pm 13\%$. However, most of this occurs from sample 1. If this sample is disregarded, there is a negative bias for the fusion of $-8.6 \pm 6.6\%$. It thus appears that for some soils all the fluoride can be parted by a direct distillation from sulfuric acid.

To check on recoveries obtained from a direct distillation portions of sample 2 were "spiked" with 100, 150, and 200 gamma fluoride. The results are shown in Table III. The recovery appears excellent and the standard deviation is also reasonable.

The samples analyzed were from Southern Illinois and Kentucky along the Ohio River. Both sandy and loamy soils were included.

The use of a direct distillation of a soil sample results in a more rapid and simpler routine analysis for a technician. If a certain type of soil is to be run routinely, the agreement between a direct distillation and a sodium carbonate fusion

treatment should be determined. If this agreement is good, future samples should be run by direct distillation.

TABLE II

Comparison of Direct Distillation and
Na₂CO₃ Fusion for Fluoride Assay

Distillate 100 ml portions	Sample 1				Sample 2				Sample 3				Sample 4			
	Method				Method				Method				Method			
	DD		F		DD		F		DD		F		DD		F	
1	2	7	36	46	18	8	18	10	16	28	22	17	25	16	23	16
2	14	8	47	37	12	20	16	25	26	50	38	30	28	25	44	32
3	43	13	73	48	33	15	28	23	60	64	50	39	53	37	53	34
4	45	36	147	52	28	32	32	30	48	52	37	47	83	83	55	42
5	5	17	12	59	5	12	33	13	16	0	21	17	58	74	33	36
6	4	6	10	12	0	7	13	0	0	0	0	12	3	16	8	14
Total	113	87	325	254	96	94	140	101	166	194	170	162	250	251	216	174
Average	100		290		95		120		180		166		250		195	
S. D.	18		50		1		27		20		6		1		30	

Coefficient of variation for direct distillation = 6.4%

Coefficient of variation for Na₂CO₃ fusion = 14.5%

Zr-Eriochrome Cyanine R method used.

TABLE III

Recovery of "Spikes" by Direct Distillation

γ F Added	Total γ F Present	γ F Found	% Recovery
100	195	296	105.6
100	195	156	80.0
150	245	257	104.9
150	245	250	102.0
200	295	358	121.4
200	295	310	105.1

Average recovery 103.3%

S. D. 13.3%

ELECTRODEPOSITION OF ENRICHED URANIUM IN URINE

T. P. Hamrick
Union Carbide Nuclear Company -Y-12
Oak Ridge, Tennessee

Introduction

The detection of uranium taken into the body through the respiratory and gastrointestinal systems is greatly facilitated by the determination of micro quantities of uranium in the urine of individuals. There have been several suggestions from various sources concerning the method now being used in the Y-12 installation of Union Carbide Nuclear Company. The question arising more frequently concerns the utilization of a more exact method such as the fluorimetric analysis of uranium in urine. From a chemical toxicity standpoint, the fluorimetric method is more than satisfactory. However, at the Y-12 installation, all levels of enriched uranium are handled at various locations. The persons involved in the handling of this uranium do not handle just one level but may, in the space of one week, work with any or all levels of enrichment.

The chemical tolerance level has been determined to be fifty micrograms per day while the radiological tolerance level has been defined as seventy disintegrations per minute per day. It can readily be seen that for uranium with a specific activity up to 1.4 d/m/ μ g, the chemical tolerance is of major importance and above this level, the radiological tolerance plays the prominent role. This is to say that as the specific activity increases, the micrograms allowed in the urine decreases. When the specific activity of the uranium being handled is unknown or varies over a wide range, the fluorimetric answer is useless from the radiological point of view.

The majority of methods now in use consist of a tedious and time consuming preparation, either prior to electrodeposition or evaporation of an organic phase on a stainless steel plate. The sample load in the laboratory is such that it is desirable to have a rather quick and simple method, without adversely affecting the unit cost of the analysis.

Prior to the present program, the necessity was recognized of omitting certain data which appeared to give false results. The thoughts were directed toward contamination within the laboratory since it is located near an area where enriched uranium is handled. At that time the procedure consisted of a

single aliquant nitric acid digestion preceding electro-deposition. This meant that the sample was being exposed to the atmosphere for long periods of time. It was decided to explore the possibility of plating uranium from unprepared urine. Some of the problems which were explored will be discussed.

It was originally decided that two aliquants would be analyzed from each routine sample and where volume permitted, a third aliquant should be spiked with an appropriate number of counts. Each aliquant would be plated on a different run and each plate would be counted for two-one half hour periods. Three blank samples would be plated with each run and counted in the same manner as routine samples. The routine counting rates would be corrected by the blank counting rates.

Indications were that plating variability was the largest single source of random error. The use of two plates per sample would reduce this source of error. The variability of recovery was another probable source of error, although very little data was available. It was thought by analyzing a spiked sample of every urine that eventually a recovery factor determined for each man would be necessary to provide sufficient precision. During the early months of the program, a study of the data indicated that no appreciable gain in precision was obtained, since differences within individuals were found.

Several studies were made to determine the effect of the changes necessary to modify our procedure. Experiments were carried out to determine if the level of spike used was a governing factor of the recovery of uranium. It was evident that the more spike solution added decreased the pH of the urine solution since the spike was dissolved in an acid medium. Therefore it was thought necessary to add a smaller volume of a larger value spike solution.

An interest was aroused as to the actual composition of the deposit on the electroplated discs. Several of these discs were analyzed spectrographically. Traces of iron, silicon, and platinum were found along with the normal inorganic constituents of urine -- magnesium, potassium, sodium, calcium, etc.

The accuracy of the performance of the counters was a great concern, since most of the samples count in the neighborhood of ten counts per half hour. Two plates were selected, one of which counted in the vicinity of ten counts per half hour and the other in the vicinity of one hundred counts per half hour. Each was counted in all of the thirty-two counters to study the observed variance. The variance value should be equal or nearly equal to the mean value of the plates. The high plate data followed this expectation, however, the variance at the low count level was much wider. This may mean that we are working at too

low a level of counts to ever attain optimum statistics for a single analysis.

A study was made to evaluate the effect of plating time on recovery. It was found that the minimum time necessary to plate a sample is in the order of thirty minutes. Calcium will deposit on top of the uranium, reducing the counting efficiency if large amounts are present. Such deposition does not occur appreciably during the first fifty-five minutes. Therefore, it was felt that the sample should be plated for a period of fifty minutes to assure the deposition of all uranium and allow the minimum amount of calcium to deposit.

Collection of the Sample

Each participant is scheduled to void a sample on an assigned date which is always preceded by two scheduled days off, such as Monday following the weekend. The sample is voided both prior to changing into clothing which have been worn in the work area and before entering the work area itself. Such a procedure reduces the probability of sample contamination and the elimination of recently inhaled soluble material. The participant is requested to enter the time of the present and previous voidings on the label, this enables the laboratory to calculate the time interval represented by the sample.

The sample remains under refrigeration for a period of two to twenty hours before delivery to the laboratory. The samples are prepared for electrodeposition within twenty - four hours after receipt. Upon entering the laboratory, the volume of urine is measured and the time interval of the sample is calculated and both are entered on a pre-punched IBM card bearing other pertinent information on that particular person. The cards have been previously prepared from a scheduled participation list. The sample is then ready for analysis.

Reagents and Apparatus

1. All chemicals should be reagent grade.
2. Ferric ammonium oxalate solution - dissolve 453.6 grams ammonium oxalate and 3.4 grams ferric ammonium sulfate in 16 liters of distilled water.
3. Synthetic urine solution - dissolve 50 grams tribasic sodium phosphate in 2 liters of distilled water (used as the

blank urine).

4. Brewer Automatic Pipette - calibrated to dispense 20 ml of the ferric ammonium oxalate solution to the plating cells.
5. Silver discs - 1-3/4" diameter, 0.002" thick.
6. Power supply and cells.
7. Glassine bags.
8. Alpha counters - proportional NMC low background model PC-2 is preferred.

Procedure

A "run" consists of forty samples containing thirty-five unknown urines, three synthetic urine blanks, and two spiked urines which are selected at random from the thirty-five unknown urines of the same run. These samples are aliquanted into 20 ml plastic vials in duplicate, the first aliquant in the first run, the second in the second run. The spiked urines of the second run are also picked from the thirty-five samples involved in the two runs.

The samples are transferred to the assembled cells where 20 ml of the ferric ammonium oxalate solution and 20 ml of distilled water are added to each sample. The electrode connections are made, the power supplies are turned on, and the rheostats are adjusted to deliver two amperes until the solution reaches a temperature of 95°C. The current is then reduced to 1.5 amperes and this current is maintained throughout the plating time for fifty minutes. The cells are then removed, the remaining liquid is drained off and the cell is rinsed with distilled water. The cell is disassembled and the silver disc is removed, blotted dry with a suitable absorbent such as Kleenex tissue, and placed in a properly marked glassine bag.

The discs are then counted for a period of thirty minutes in each of two Alpha counters. The counters used at Y-12 are Nuclear Measurements Corporation low-background model PC-2 which have been modified such as to control the electronic circuits from a master panel thus assuring the same counting time for every disc.

After the two aliquants are completed, the raw data are entered on the previously mentioned IBM cards and then submitted to the tabulating service group for final processing.

The IBM equipment makes the necessary calculations from the data and prepares the final report. This report includes all the exposure data for each individual for the most recent thirteen week period. Such a procedure enables responsible supervision to better evaluate not only the present exposure but also the effect of samples previously submitted.

Quality Control Program

It is recognized that the precision of an individual result in this type of a procedure is poor and for this reason little significance is attached to individual results, particularly those below the MPL level. However, since the program is carried out on an accumulated exposure basis with thirteen weeks data comprising the reported answer, the limit of error of this result is considerably improved. The use of an average blank and an average uranium recovery factor is justified in this type of procedure. Following is a table which illustrates the limits of error at the various levels:

<u>Mrem/day</u>	<u>Average Net Count</u>	<u>% L. E. of One Analysis</u>
0-30	2	$\pm 198\%$
31-60	7	$\pm 60\%$
61-90	12	$\pm 43\%$
> 90	64	$\pm 23\%$

It is evident from the table that the limit of error could be improved considerably by either increasing the recovery factor or the volume of urine analyzed or both. It has been found that 20 ml of urine is the optimum amount which can be analyzed by this direct method due to the deposition of the various inorganic salts present. In order to do either of the above, a purification step would be necessary.

Determination of Recovery Factor

A spike of 10 counts per half hour is added to approximately fifty urines each week to determine the average recovery factor. The urines used must naturally have a volume of at least 60 ml in order that duplicate analysis may be run in addition to the spiked aliquant. The difference in the average count of the samples and the count of spiked

aliquant represent the recovery of that particular urine. The recoveries for the week are calculated and averaged. From past studies, the recovery factor has been determined to be in the proximity of 40% which is the figure used in the calculation of the samples by the tabulating service group. The latest quarterly average which has been calculated was $40.5\% \pm 3.4\%$ which shows that we have a negative bias of 1% for that particular 13-week period.

Determination of Blank

In order to stay consistent with the use of averages, the counter background is included in the blank. The counters are not used if the background is greater than five counts per half hour. The average counter background for the week is calculated for the entire bank of counters.

Approximately eighty-five (depending on the number of samples) blanks are run each week as previously mentioned, three in each run. Because of the uncertainty of the uranium content of any true urine in this area, the synthetic urine solution is used as the blank. Because it is an "ideal" urine recovery studies were made. It was found to have a consistent recovery factor of 90% rather than the 40% found in true urines by this method. For this reason, a correction is necessary to convert the synthetic urine to the true urine basis.

$$\text{Corrected blank} = \frac{(\text{TB} - \text{CB}) \times 40}{90} + \text{CB}$$

Where TB = average total blank for one week

CB = average counter background for one week

Both TB and CB are in counts per half hour.

The factor TB-CB represents the average net count of the synthetic urine which is due to contamination. Multiplying by the factor 40/90, the figure now represents the average net count of the true urine which is due to contamination. Finally, by adding the counter background, the total corrected blank is determined.

Calculation

$$\begin{aligned} \text{Mrem/day} = & \frac{\text{average net count}}{\text{half hour}} \times \frac{1 \text{ half hour}}{30 \text{ minutes}} \times \frac{2 \text{ disintegrations}}{\text{count}} \\ & \times \frac{1}{\text{time interval (hours)}} \times \frac{24 \text{ hours}}{\text{day}} \times \frac{\text{volume (ml)}}{20 \text{ ml}} \end{aligned}$$

$$\times \frac{100\%}{40\%} \times \frac{0.6123 \text{ Mrem}}{\text{dis. / min.}}$$

All constants in the above equation can be combined to give the following simple form.

$$\text{Mrem/day} = \frac{\text{average net count} \times \text{volume}}{\text{time interval}} \times 0.12245$$

Conclusion

It was originally thought that when the exposure reaches that point where one can be 95% confident that the value exceeded the quarterly cumulative MPL, action would be taken to effect removal from further exposure to uranium. It was recognized that this criterion was not the ultimate goal desired. It was, instead, the first step toward the goal of 95% confidence that all employees' exposure were below the cumulative MPL.

In accordance with this objective and in view of the past years' experience with the program, it was proposed to alter the criterion so that instead of waiting until confidence is established at the 95% level, action is now recommended at the 67% confidence interval around the cumulative value. The accomplishments in doing this provide additional personnel protection. The value would vary, of course, in individual situations depending on the actual number of samples given and the difference in precision of the analytical results on samples (which is a function of the counting rates of the samples involved). All considerations reflect judgement and technical integrity with the employee's safety as the first obligation.

This type of program has accomplished two major objectives:

1. Provides the necessary information to monitor and evaluate our workers exposure at the added benefit of minimum cost.
2. Locates cases of unusually high exposure so that a more thorough examination and determination of dose could be made using other types of procedures.

DEPT	SHIFT	BADGE	NAME	MREMS	DATE	VOL.	TIME	RATE	PA
2231	1	44123	HICKS H L	16	4-14-58	340	10.50	32	4
	1	44123	HICKS H L	26	4-21-58	710	6.67	106	2
	1	44123	HICKS H L		4-28-58NP				
	1	44123	HICKS H L		5-05-58NP				
	1	44123	MILlicAN H H	4	5-12-58	245	7.50	33	1
	1	44123	MILlicAN H H	10	5-19-58	320	7.92	40	2
	1	44123	MILlicAN H H		5-26-58NP				
	1	44123	MILlicAN H H	10	6-02-58	80	1.92	42	2
	1	44123	MILlicAN H H	4	6-09-58	120	7.00	17	2
	1	44123	MILlicAN H H	13	6-16-58	290	8.17	35	3
	1	44123	MILlicAN H H		6-23-58NP				
	1	44123	MILlicAN H H	39	6-30-58	210	2.00	105	3
	1	44123	MILlicAN H H	16	7-07-58	15	0.92	16	8
		1365		9	15			47	
	1	56416	HAMRICK T P	7	4-14-58	110	7.17	45	4
	1	56416	HAMRICK T P	8	4-21-58	250	7.50	33	2
	1	56416	HAMRICK T P	0	4-28-58	110	9.58	11	0
	1	56416	HAMRICK T P	10	5-05-58	95	3.50	27	3
	1	56416	HAMRICK T P	18	5-12-58	260	7.00	37	4
	1	56416	HAMRICK T P		5-19-58NP				
	1	56416	HAMRICK T P	7	5-26-58	90	3.90	30	2
	1	56416	HAMRICK T P	32	6-02-58	300	9.00	33	8
	1	56416	HAMRICK T P	27	6-09-58	175	4.00	44	5
	1	56416	HAMRICK T P		6-16-58PO				
	1	56416	HAMRICK T P	16	*6-17-58NS	15	0.92	16	8
	1	56416	HAMRICK T P	4	6-23-58	120	7.00	17	2
	1	56416	HAMRICK T P	35	6-30-58	190	4.00	48	6
	1	56416	HAMRICK T P	13	7-07-58	290	8.17	35	3
		1365		11	15			30	

PLATING EFFICIENCY

ENRICHED URANIUM SAMPLE LOAD

PLATING BLANK EXPERIENCE 1957

TOLERANCE OF URANIUM

A STUDY OF THE PLATING OF LARGER VOLUMES OF URINE

THE FLUOROMETRIC DETERMINATION OF SUBMICROGRAM QUANTITIES OF BERYLLIUM

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Abstract

The increasing use of beryllium in the atomic energy program and the highly toxic nature of its compounds require a method of high sensitivity and reliability for its detection and determination. Although morin is the most sensitive reagent known for determination of beryllium, procedures presently available are not particularly reliable at extremely low levels. A fluorometric method using morin has been developed that has detection limit of 0.0004 γ and a precision of 0.8% on 0.2 γ at the 95% confidence level. Reliability and precision are improved greatly through use of a buffer system, an internal acid-base indicator, a permanent glass standard of fluorescence and liberal use of complexing agents. Detailed methods of separation of beryllium and application to air-dust and smear samples, urine, bone and ores are given and were checked at each step using beryllium-7 tracer. Errors produced by certain metals and by variations in the procedure are described.

Editors Note: A detailed report on this method will appear in an early issue of Analytical Chemistry. A reprint of the manuscript may be obtained by writing the author.

AN INHALATION EXPOSURE TO U_3O_8

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Abstract

An industrial worker accidentally received an inhalation exposure to enriched uranium fume during October 1957. This case has been studied by several methods in an effort to estimate the magnitude of the lung burden.

A description of the accident illustrates the difficulty in detecting this type of exposure through the use of air sampling alone. Samples of urine were analyzed fluorometrically and by two different electrodeposition procedures. Blood samples and specimens of feces were analyzed also to aid in estimating the inhaled dose. Five separate estimates of the initial lung burden, based upon the bioassay data and in vivo counting, agree that the dose was in the range 1 to 10 mg, and was probably close to 4 mg. (cont'd)

Introduction

An industrial worker accidentally received an inhalation exposure to enriched uranium fume during October 1957. This case has been studied by several methods in an effort to estimate the magnitude of the lung burden. The results of these studies illustrate the application of available bioassay techniques.

Description of the Accident

The operation from which the exposure was derived consisted of deburring holes that had been drilled in pieces of enriched uranium metal. Several discs of emery cloth sandwiched together and mounted on a flexible shaft powered by a high speed motor were used to perform the deburring operation. A flexible hose, shown in the plan view (Figure 1), was

* Health Physics Division, Oak Ridge National Laboratory and Health Physics Department, Technical Division, Y-12 Plant, operated by Union Carbide Nuclear Corporation for the United States Atomic Energy Commission.

Figure 1

available to provide local ventilation for the job. It was noted by area supervision that the employee did not make adequate use of the local exhaust drop and had the habit of keeping his face too close to the work.

General air sampling heads are spaced at intervals throughout the work area but the closest one was over 12 feet from the deburring work table. General air activity levels during the exposure period, October 14-29, averaged about 4 d/mm³ (the maximum permissible air level is 70 d/mm³). The exposure was detected through the routine uranium urinalysis program. A Monday morning urine specimen indicated an excretion rate of over 13,000 d/m per day and subsequent samples confirmed this high value. It was recognized that the exposure was potentially serious and the employee was transferred to a non-uranium shop.

Clinical Findings

Examination of the employee at the plant dispensary on the second day after exposure ceased showed no clinical evidence of injury. No open wounds, or subcutaneous deposits of uranium could be located by visual examination or by scanning with a scintillation crystal.

After preliminary estimates of body burden had been made it was recognized that there likely had been no kidney damage and no obvious future injury to the kidney or bone was anticipated. Detectable damage, if such an effect should occur, was expected to arise only from long term irradiation of the lung, although no forecast could be made of either the possibility of occurrence or the extent of injury.

One week after the exposure, the employee was referred to the Massachusetts General Hospital for clinical evaluation and functional base line studies. The base line pulmonary function study was essential to the appraisal of any future change in the lung. Pulmonary function studies were within normal limits and x-rays of the chest and femurs were normal. The results of the general physical examination which included studies of blood chemistry, kidney function tests and liver function tests were within normal limits.

BioAssay Procedures

Samples of blood, urine and feces were analyzed for uranium in an attempt to establish estimates of body burden.

Blood specimens, 5-10 ml each, were digested in nitric acid

until a water soluble ash was obtained. The resulting ash was dissolved in water and then transferred to a plating cell for electrodeposition onto a silver disk. Determinations made on the day after exposure ceased and on the day following 8-10 days indicated a low but relatively constant uranium content of about one $\mu\text{g}/\text{ml}$.

All of the urine voided by the employee was collected and composited for each 24-hour period. Aliquots of each composited sample were analyzed by three different methods (Figure 2). The routine electrodeposition procedure¹, (curve C), which consists of plating directly from the raw urine, yielded estimates of excretion rate that were higher than the results of the other methods during the first two weeks. After the first three weeks the results of the raw plating method consistently fell below the results of the other methods.

The routine fluorometric procedure², (curve B), and the electrodeposition procedure due to Royster³, (curve A), yielded comparable results. This electrodeposition method includes a pretreatment step in which the interfering cations, calcium and magnesium, are removed by precipitation and the sample is digested prior to plating. Dose estimates based upon urinalysis were derived from data obtained by means of Royster's method.

A considerable amount of uranium was expected to be cleared from the lung by ciliary action with subsequent fecal excretion. Only a few fecal samples were analyzed and no clear pattern of fecal excretion was obtained. Specimens of feces were chemically ashed and then extracted in diethylether using aluminum nitrate as a salting agent. The average uranium excretion via feces was 3 $\mu\text{g}/\text{day}$ during the period from the third to the seventh day after removal from exposure.

In vivo Counting

Gamma rays accompany the decay of U^{235} may be used to measure internal deposits of enriched uranium (Figure 3). The most prominent lines of the U^{235} spectrum are at 90 Kev and 186 Kev. In practice a calibration source of a given enrichment is placed in a phantom and the count rate related to the 186 Kev line is used to obtain a calibration factor. An individual, suspected of having an internal deposit of uranium, is then checked in the counter. Since every individual has a detectable amount of naturally occurring potassium - 40 and commonly occurring cesium-137 (Figure 4), there is an appreciable background that must be considered. Because of the degradation of the primary gamma rays and other sources the background at the lower energies is quite high. In order

Figure 2

U^{235} GAMMA RAY SPECTRA

Figure 3

Figure 4

to make a determination for a particular individual the spectrum from another person of the same weight and height is obtained to provide a background measurement and, because of individual differences, an attempt is made to match the Cs137 and K40 body burdens. With the equipment operated in the Y-12 plant at Oak Ridge by Burkhart and Cofield one-half the maximum permissible uranium lung burden may be measured with a confidence level of 95%.

On the fourth day after exposure ceased the employee was checked in the Argonne National Laboratory in vivo counter by Marinelli and Miller (Figure 5). In all, four measurements extending out to 140 days were made in Chicago. All subsequent measurements were made in the Y-12 plant of Union Carbide Nuclear Company of Burkhart and Cofield. Initial lung burden as estimated from this curve was 3 mg.

Estimation of Initial Dose

Table 1 shows some of the procedures that were applied to the data to obtain estimates of the initial lung burden. Urine specimens indicated an excretion of 0.05 milligrams per day at the time the employee was removed from exposure. By assuming that λ_L , the initial transfer fraction from lung to blood, was in the range .006 to .06 per day (equivalent half-lives range from 120 to 12 days), and that two-thirds of the amount reaching the bloodstream during the first day would appear in the urine, the lung burden would be expected to lie in the range 1.3 to 13 milligrams.

A six milliliter blood sample was chemically ashed, plated and counted in an alpha scintillation counter. This determination indicated an uranium concentration of 1.14 $\mu\text{g/ml}$ which would represent about 6 μg in the total blood. Another sample taken the next day indicated about the same concentration. Thus, if total blood content is denoted by S_1 then $dS_1/dt \approx 0$. The individual's history indicated no previous significant exposure. Hence, it was assumed that the amount reaching the blood from tissues other than lungs was negligible and therefore, $dS_1/dt \approx \lambda_L S_L - \lambda_1 S_1$. Lung burden, S_L , was estimated from $S_L \approx S_1 \lambda_1 / \lambda_L$. A value of 9.9 has been obtained for λ_1 from the human injection studies, and the same range as above was used for λ_L . This approach estimated that there was initially from 1 to 10 mg in the lung.

A mock-up of the operation was run and air samples were taken in order to characterize the exposure with respect to compound, particle size distribution and air activity levels. Electron diffraction analysis confirmed the initial assumption that the compound was U_3O_8 . The mass median particle size was found to be 1.9 microns using a cascade impactor and the

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Figure 5

<p>URINALYSIS (SOON AFTER EXPOSURE):</p> <p>(FRACTION PER DAY FROM LUNG TO BLOOD x LUNG BURDEN) x $\frac{2}{3}$ = EXCRETION RATE</p> $(\lambda_L S_L) \times \frac{2}{3} = \frac{dU}{dt} \longrightarrow (.006 \times S_L) \times \frac{2}{3} = .05 \text{ mg / DAY}$ <p>$S_L = 13\text{mg}$ (1.3 mg FOR $\lambda_L = .06$)</p>	<p>ESTIMATED LUNG BURDEN</p> <p>1.3 - 13 mg</p>
<p>BLOOD SAMPLE:</p> <p>DURING FIRST POST EXPOSURE DAY $\frac{dS_I}{dt} \approx 0 \approx \lambda_L S_L - \lambda_I S_I$</p> $S_L \approx \frac{\lambda_I S_I}{\lambda_L} = \frac{9.9 \times .006\text{mg}}{.006} = 9.9$ <p>$S_L \approx 10\text{mg}$ (1mg FOR $\lambda_L = .06$)</p>	<p>1 - 10 mg</p>
<p>AIR SAMPLING:</p> <p>MAXIMUM AIR CONCENTRATION 0.3 mg/M³</p> <p>ALVEOLAR RETENTION 20 %</p> <p>BREATHING RATE 10 M³/SHIFT</p> <p>DURATION OF EXPOSURE 11.5 SHIFTS</p> <p>AMOUNT REMAINING OF TOTAL DOSE 90 %</p> <p>POTENTIAL LUNG BURDEN = .3 x .2 x 10 x 11.5 x .9 = 6.2 mg</p>	<p>6.2 mg</p>
<p>IN VIVO COUNTER:</p>	<p>3 mg</p>
<p>URINALYSIS (AFTER 100 DAYS):</p> $\frac{dU}{dt} = 0.0433 e^{-\frac{.693}{20} t} + 0.0067 e^{-\frac{.693}{300} t} \text{ mg / DAY}$	<p>4.1 mg</p>

Table 1

geometric standard deviation was 1.8. Air activity levels varied greatly with location and with operating techniques, but it appeared possible for an individual to be exposed to as much as 0.3 milligrams per cubic meter. Assuming a breathing rate of 10 cubic meters per shift and an alveolar retention of 20 per cent it would have been possible to accumulate as much as 6 milligrams in the alveoli over the known period of exposure. In this connection it should be mentioned that two other men were performing the same operation that produced this exposure. At the same time this individual was excreting 12,000 units per day, one of his co-workers was excreting 700 units per day and the third man excreted less than 10 units per day. It should be clear from this that air sampling provides an estimate of potential exposure only.

Subsequent estimates of total lung burden made by Marinelli and Miller using their in vivo counter were extrapolated back to the time exposure ceased and indicate the initial value was 3.0 milligrams.

The pattern of excretion rate for the first 100 days post-exposure may be fitted with the expression shown in the table. To test the validity of extrapolating this expression, it was integrated and then evaluated for $t = 140$ days. This function predicted a total excretion of 2.04 milligrams, while the total accumulated urinary uranium determined by the bio-assay laboratory was 2.00 mg for the same period of time. Since the future urinary excretion rate can be expected to be the same or somewhat higher than the values predicted by this expression, the integrated excretion equation, at infinity, should represent the total initial dose or else be an underestimate. The integrated dose for t approaching infinity is 4.1 mg and should represent a close lower limit to the initial dose.

The curve shown in Figure 6 was obtained by assuming the initial dose was 4.1 mg and adjusting the lung burdens measured by the in vivo counter accordingly. Ratios of estimated lung burden to excretion rate are shown as an arbitrary function of time. Additional data such as these are needed to help establish empirical curves from which lung burden may be estimated.

RATIO OF LUNG BURDEN TO EXCRETION RATE FOR AN ACUTE IN-
HALATION OF U_3O_8

Figure 6

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ETHYL ACETATE EXTRACTION OF URANIUM FROM HUMAN AND ANIMAL TISSUES

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Introduction

Tissue specimens were dry ashed, dissolved in acid and the uranium extracted with ethyl acetate from a solution saturated with aluminum nitrate. Recoveries of submicrogram quantities from pure and spiked tissue solutions are listed. Uranium levels are reported for lung (0.04-1.7 $\mu\text{g/g}$ of wet tissue), kidney (0.02-0.09 $\mu\text{g/g}$), spleen (0.02-0.04 $\mu\text{g/g}$), bone (0.02-0.08 $\mu\text{g/g}$) and other tissues from workers exposed to uranium dusts in industrial situations. Concentrations are also given for several steer tissues and for specimens from persons with no known uranium exposure. References cited include works on the separation and determination of microgram and submicrogram quantities of uranium in various materials and reports on uranium in human and animal tissue.

Discussion

Investigators in the field of uranium toxicity in man have often stated the need for information obtained from human subjects, both clinical and industrial, to be used in conjunction with, and at times in preference to, information obtained from animal studies. Also, human data are needed to precisely chart the course of uranium in the body and to establish maximum permissible concentrations with a satisfying degree of certitude (11).

Approximately 2400 people are employed at the Fernald Feed Materials Production Center operated by the National Lead Company of Ohio. About 1600 of these employees are engaged in the handling of uranium material, from ore concentrate through a series of compounds, including UO_2 , UO_3 , UF_4 , and U_3O_8 , to the pure metal. Since dust levels are determined for all jobs and urine is assayed for uranium on a scheduled basis, an excellent opportunity is offered to gather useful information. To complete the bioassay picture tissue analyses for uranium are also needed. Dust levels and urine concentrations help determine what goes in and what comes out of the body. Tissue concentrations help to show what stays behind and where it goes. When tissue specimens

from several workers exposed to uranium compounds were made available, they were assayed for uranium to aid our investigations.

Over a period of six months tissue samples were received from autopsies performed in three cases where death resulted from non-occupational causes. The material was received in covered containers several hours after removal. Individual organs were transferred to large specimen bottles and covered with a 10% (v/v) formalin solution made from 40% Eastman Practical or Fisher reagent grade formaldehyde solution. The tissues were stored until the uranium determination could be made.

Several methods are described for the separation of micrograms of uranium in various materials. Cellulose columns have been used for 1 μ g of uranium (1,2) and the separation of 0.5 μ g has been reported (10). A review of techniques reported from 1949 to 1953 has been made by Rodden (13). The protein separation for uranium in tissue in use at the University of Rochester is well known (5). In an ion exchange method for microgram quantities of uranium in blood the sample undergoes acid destruction, iron is complexed with versene and the uranium is absorbed on IR-4B resin in the sulfate form. The uranium is eluted with 2.5 N nitric acid after the resin has been washed with water (17). The method may be used for blood with a concentration of 0.1 μ g/ml. At the 1957 Bio-Assay meeting an ion exchange method was described for the isolation of small amounts of uranium in iron (18). Using a 500 ml. sample, ion exchange has been used for river water with a concentration of 0.002 μ g/ml (15).

Much attention has been given to the solvent extraction of microgram quantities of uranium from salted solutions. Several combinations of salting agent and organic extractant have been reported: Aluminum nitrate-ethyl ether for enriched uranium in urine (16); Aluminum nitrate-ethyl acetate for uranium in ore (3, 6) and saline and non-saline water with good recoveries reported for as little as 0.15 μ g (6); Magnesium nitrate and calcium nitrate-ethyl acetate for uranium in ore (13). Since our laboratory had used the aluminum nitrate-ethyl acetate method on other materials it was used for the tissue analyses.

Method

The procedure for tissue digestion is essentially that suggested by the Health and Safety Laboratory of the New York Operations Office. The tissue is removed from its container, allowed to drain for several minutes, and excess formalin is removed with absorbent paper. After transferring to a tared platinum crucible, the crucible is weighed to determine the

wet tissue weight and placed in a 105°C oven overnight. The crucible and contents are cooled and reweighed for the dry tissue weight and then placed in a cold muffle furnace. The furnace temperature is brought to 500°C and maintained for 24 hours. After cooling, the crucible is weighed for the ash weight and the ash is dissolved in concentrated nitric acid. The rest of the procedure is as follows:

- (1) When the ash is dissolved wash the sides of the crucible with concentrated HNO_3 and then distilled water and evaporate the solution to several ml.
- (2) Wash the crucible with distilled water and repeat the evaporation. Cool for several minutes and add 10 ml. of aluminum nitrate solution (10 g of $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ in 10 ml.) and place on a low heat until all salts are in solution. Increase the heat and evaporate until there is a 4-6 ml. reduction in volume of solution.
- (3) Transfer the solution while it is still warm to a 60 ml separatory funnel marked at the 10 ml level. Rinse the crucible with small portions of water until the volume in the separatory funnel is 10 ml.
- (4) Allow the solution to cool and add 10 ml of ethyl acetate to the separatory funnel. Stopper and shake for exactly one minute and allow the phases to separate for one minute.
- (5) Drain off the aqueous layer and filter the organic layer through a 9 cm Whatman 41 filter paper into a 10 ml mixing cylinder. The funnel, paper, and cylinder must be dry.
- (6) Record the filtered volume and pour the ethyl acetate into a 100-ml beaker. Rinse the cylinder with 10 ml of distilled water, add to the beaker, and evaporate to dryness on low heat.
- (7) Add about 10 ml of concentrated nitric acid and evaporate to several ml. Rinse, with distilled water, back into the original cylinder and continue rinsing until the recorded volume is reached. Use the final solution for a fluorimetric determination.

Discussion

To check the efficiency of extraction methods aliquots of uranium were added to sections of animal tissue and carried through several processes. The only tissue available at the

time was that of wild squirrels from Dearborn County, Indiana, an area with no known source of uranium contamination. In the first series of tests an extraction method for uranium in urine was used (16). Wet tissue was weighed in tared Erlenmeyer flasks and enough 0.1 N nitric acid was added to float the material. Uranium stock solutions were added and the flasks placed on a medium heat hot plate. Concentrated nitric acid was added after several hours of heating and the digestion was continued. Later nitric acid and hydrogen peroxide were used to insure the complete destruction of organic material and to obtain a white residue. The final residue was moistened with $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ and 0.5 M in HNO_3 . An ethyl ether extraction of the uranium was then made. The ether was transferred to a beaker and evaporated on low heat. Nitric acid was added and taken to dryness, additional acid was added and the solution made up to 10 ml with distilled water. Results are listed in Table 1.

TABLE I
EXTRACTION OF URANIUM WITH $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ AND ETHER

	<u>SAMPLE</u>	<u>WET WEIGHT</u>	<u>U ADDED</u>	<u>U FOUND</u>
1.	Viscera	74.60 g	1.0 μg	0.73 μg
2.	Viscera	9.01	1.0	0.46
3.	Viscera	14.90	1.0	0.35
4.	Bone	5.50	1.0	0.32
5.	Bone	4.89	0.7	0.33

Up to two days of digestion was necessary to completely destroy the tissue and to obtain a white residue. The volume of aluminum nitrate solution needed to dissolve the salts varied from 30 to 50 ml.

Two additional samples were wet ashed with 1.0 μg of uranium and the residue taken up with 20 ml of an aluminum nitrate solution, at a concentration suggested for maximum extraction, 100%, weight to volume (1). The solutions were transferred to 60 ml separatory funnels with an additional 10 ml of the aluminum nitrate solution. Twenty ml of ethyl acetate was added and the funnels shaken for 3 minutes. The organic layer was drawn off, evaporated to dryness, and taken up to 10 ml for a fluorimetric determination (Table II). Although only 1.0 μg was added to the first sample 1.86 μg

was recovered.

Similar high recoveries were obtained when the tissues were dry ashed and the ash fused with sodium carbonate. Three samples were weighed in tared platinum crucibles and dried overnight at 115°C. One microgram of uranium was added in solution, evaporated to dryness on low heat, and the crucibles were carefully flamed for twenty minutes. At this time the tissues would not burn even when the crucibles were heated to redness. The crucibles were then placed in a 600°C muffle furnace for 23 hours, cooled and reweighed. The ash was fused with three times its weight of sodium carbonate, the flux dissolved in water, and the solution taken to near dryness several times with concentrated nitric acid. The moist residue was taken up with 20 ml of aluminum nitrate solution (10 g/10 ml) and transferred with an additional 10 ml to a 60 ml separatory funnel. After shaking for several minutes with 20 ml of ethyl acetate the organic layer was drawn off and the aqueous layer extracted again with 20 ml of ethyl acetate. The combined extractions were evaporated and a final 10 ml solution obtained for the fluorimetric determination. Recoveries are listed in Table II. There was no apparent explanation for the low recovery in sample 2.

The results were surprising since the tissues appeared to have had a uranium content of their own. Subtracting the uranium added from the uranium found gives values of at least 0.018, 0.013, 0.021 and 0.064 μg U/g of wet tissue. However, these values are not out of line with reported values for control animals and are lower than some early human figures. Using chemical separations and a fluorimetric method on human tissue Hoffman found from 0.002 $\mu\text{g/g}$ in the cerebrum to 5.03 $\mu\text{g/g}$ in the embryonic thyroid (7, 8). Some of the figures seem high. He reports lower values for some beef tissues (9). Neuman reported concentrations up to 0.25 $\mu\text{g/g}$ of wet tissue for two rats which were taken from a stock colony and placed on a synthetic diet for one week before being sacrificed (12). Various tissues were analyzed fluorophotometrically after a protein precipitation of uranium. Similar values are reported by Ferretti and Schwartz for seven control rabbits, three of which had no known uranium exposure (4).

Pure solutions containing 0.1 and 0.5 micrograms of uranium were extracted with fair recoveries. The first five results were obtained by extracting the uranium in 25-ml mixing cylinders, shaking for two minutes and allowing five minutes for the phase to separate before withdrawing the organic layer, by pipette, for filtering. The longer contact time of the ethyl acetate with the acid aqueous solution may have caused some hydrolyzing of the organic layer, thus concentrating the uranium present to give high recoveries (1). All other solutions received the previously described treatment. Results

are listed in Table III.

TABLE II
EXTRACTION OF URANIUM WITH $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ AND ETHYL ACETATE

	<u>SAMPLE</u>	<u>WET WEIGHT</u>	<u>URANIUM ADDED</u>	<u>URANIUM FOUND</u>	<u>ASHING METHOD</u>
1.	Viscera	47.10 g	1.0 μg	1.86 μg	Wet
*2.	Kidneys (4)	7.13	2.0	0.35	Wet
3.	Lung & Heart	6.25	1.0	1.08	Dry
4.	Liver	13.30	1.0	1.38	Dry
5.	Spleen	4.19	1.0	1.27	Dry

* Two adrenals also included.

TABLE III
RECOVERY OF URANIUM IN PURE SOLUTIONS

<u>SAMPLE</u>	<u>URANIUM ADDED</u>	<u>URANIUM FOUND</u>
1	0.10 μg	0.13 μg
2	0.10	0.14
3	0.10	0.09
4	0.10	0.12
5	0.10	0.14
6	0.10	0.08
7	0.10	0.06
8	0.10	0.06
9	0.50	0.41
10	0.50	0.43
11	0.50	0.51
12	0.50	0.49

One of the major limiting factors in the extraction of uranium in biological materials is the interference caused by phosphate. Aluminum has been used as a complexer of phosphate in the batch extraction of uranium with ethyl acetate (6) and in the continuous extractions with ethyl ether (13). Several sections of steer tissue were obtained to check the complexing of tissue phosphate by 10 ml of the aluminum nitrate solution used in this procedure. Tissue sections from an eighteen month-old steer, which was raised in Dearborn County,

Indiana, were placed in 10% formalin immediately after slaughtering and stored for several months before being used. Approximately 240 grams of heart tissue was ashed, put into solution, and aliquots of this solution were taken through the described procedure. Results indicate that 10 grams of $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ is enough to complex the phosphate in 36 grams of tissue (Table IV).

TABLE IV
EXTRACTION OF BEEF HEART TISSUE

	GRAMS OF TISSUE IN SOLUTION	U FOUND	$\mu\text{g U/g WET TISSUE}$
1.	2.4	0.09 μg	0.038
2.	12.0	0.47	0.039
3.	24.0	0.90	0.038
4.	36.0	1.30	0.036

As a further check on the possible interference of phosphate 1.0 μg of uranium was added to four 5 ml aliquots of the tissue solution and extracted. A blank of 5 ml of the solution was carried through to determine the uranium already present. The recoveries of the added uranium were similar to the recoveries in pure solutions, again indicating that little interference, if any, is caused by the phosphate when 10 grams of aluminum nitrate is used as the salting agent (Table V).

TABLE V
RECOVERY OF URANIUM ADDED TO TISSUE SOLUTIONS

	U PRESENT	U ADDED	U FOUND
1.	0.47 μg	1.0 μg	1.25 μg
2.	0.47	1.0	1.3
3.	0.47	1.0	1.25
4.	0.47	1.0	1.5

Sections of steer spleen and kidney were taken through the described procedure. Slices of spleen were cut transversely from one pole toward the hilum. The lobed kidney was split lengthwise in the sagittal plane and serial sections were removed from one pole toward the renal infundibulum. Results indicate an uneven distribution of uranium in both organs (Table VI). Average values for each organ compare with reported concentrations for unexposed control animals (4, 12). The uneven distribution in the kidney, considering the cortex and medulla, has also been reported for control animals (4).

Variations were also found in human tissue, a large variation in the lung of one person and smaller variations in the liver of another (Table VII). Both had been exposed to industrial uranium dusts. The lung tissue, all from the left lung, differed in compactness--sections 1 and 3, which were spongy, were taken from the apex and lingula, respectively, of the left upper lobe. Section 2, which appeared much harder, was removed from the upper portion of the left lower lobe. Liver sections were cut from the tip of an unidentified lobe toward the porta hepatis.

One of our plant physicians through the co-operation of a pathologist at one of the local hospitals was able to obtain tissue samples from post-mortems performed on two of their terminal cases. Medical histories on these cases were not available. The tissue was received in formalin and transferred to sample bottles containing a 10% formalin solution. Sections were then taken through the described procedure (Table VIII). Results on Case No. 1 were higher than expected although lower than some values reported for unexposed control animals (4, 12).

TABLE VI
REPEAT ANALYSES ON STEER SPLEEN AND KIDNEY

<u>TISSUE</u>	<u>WET WEIGHT</u>	<u>µg U/g WET WEIGHT</u>
Spleen 1	24.0814 g	0.033
Spleen 2	34.7426	0.028
Spleen 3	34.7907	0.036
Spleen 4	36.7447	0.026
Spleen 5	53.3800	0.019
Kidney 1	21.2379	0.040
Kidney 2	15.6828	0.047
Kidney 3	18.5610	0.038
Kidney 4	13.6315	0.044

TABLE VII

REPEAT ANALYSES ON HUMAN LUNG AND LIVER

<u>TISSUE</u>	<u>WET WEIGHT</u>	<u>μg U/g WET WEIGHT</u>
Lung 1	25.5196 g	0.679
Lung 2	31.8459	0.441
Lung 3	31.1084	0.207
Liver 1	35.5731	0.024
Liver 2	33.6347	0.025
Liver 3	27.3379	0.021

TABLE VIII

URANIUM CONCENTRATIONS OF TISSUES FROM UNEXPOSED HUMANS

<u>TISSUE</u>	<u>CASE NO. V</u>		<u>CASE NO. VI</u>	
	<u>WET WEIGHT</u>	<u>μg U/g WET WEIGHT</u>	<u>WET WEIGHT</u>	<u>μg U/g WET WEIGHT</u>
Lung	32.5294 g	0.089	30.1486 g	0.006
Liver	20.6295	0.093	30.9883	0.008
Kidney	16.8431	0.026	16.7507	0.020
Spleen	16.6302	0.080	23.2316	0.005
Adrenal	5.8024	0.202	3.1552	0.006
Heart	27.3498	0.067	7.5836	0.008
Vertebra	12.1409	0.028	6.7369	0.004

Tissue specimens from the exposed workers mentioned earlier were dry ashed, put into solution with concentrated nitric acid and the uranium was extracted with ethyl acetate from an aluminum nitrate solution. The exposures of the three men to uranium dust may be described as very light for Case IV and moderate but less than MAC (70 d/m/m³) for Case II and Case III. No attempt is made to relate the tissue concentrations to air dust levels -- the results are reported here merely as an indication

of what concentrations may be encountered in tissue analyses (Table IX).

Summary

Ten grams of aluminum nitrate appears to complex the phosphate in 36 grams of heart tissue, enabling an extraction of the uranium with 10 ml of ethyl acetate. Because of the uneven distribution of uranium in the tissues the entire organ being analyzed should be ashed and aliquots of the ash or ash solution taken for extraction. Greater volumes of aluminum nitrate and ethyl acetate may allow larger samples to be extracted and result in greater accuracy. However, concentration of the final solution for a fluorimetric determination may bring other extracted elements to an interfering level. Results such as those given in Table IV may not be attainable with bone samples because of the difference in composition.

TABLE IX

URANIUM IN TISSUES OF EXPOSED WORKERS*

<u>TISSUE</u>	<u>CASE II</u>	<u>CASE III</u>	<u>CASE IV</u>
Lung	0.084 **	1.655	0.043
Liver	0.080	0.029 **	0.010
Spleen	0.036	0.022	0.016
Kidney	0.085	0.077	0.015
Bone	0.017	0.076	-

* In μg U/g wet tissue.

** Other lung and liver results listed in Table VII.

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A REVIEW OF SIGNIFICANT HANFORD PLUTONIUM DEPOSITION CASES

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Introduction

This paper reviews several incidents of plutonium exposure which occurred at the Hanford production plant. A brief description is given of the circumstances that resulted in internal deposition of plutonium and the treatment given each employee. Biological data collected in each case are presented along with interpretation of these data in terms of body burden.

Hanford Bioassay Program

The Hanford bioassay program originated with the formation of a group early in 1946 primarily for detection and measurement of plutonium deposition in project workers. Methods such as resin columns of separation of plutonium were abandoned for the TTA (Theonyl tri-fluoroacetone) extraction process. Early work in the program consisted of training, cleaning equipment and perfecting the process. As these factors were brought under control, results became more consistent; and as development continued, which included use of the electrodeposition method, the current detection of 0.05 d/m in a 24 hour urine specimen became possible. Development work in progress at the present time may increase our sensitivity by a factor of approximately five.

Coverage of plant personnel for bioassay sampling had its beginning with only a few selected employees and developed to the present system of coverage for all HAPO and HAPO connected employees, which includes AEC employees, Minor Construction workers (collateral contractor employees hired for specific construction work) and occasionally visitors. The present program is quite large and complex involving the processing of approximately 14,000 urine samples per year for plutonium and approximately the same number for gross fission product. Almost all of these samples are collected at the employees' homes. Several hundred samples are also analyzed annually for specific isotopes such as I^{131} , Ru^{103} and 106 , and Sr^{90} . About 5000 urine samples, most of which are collected on plant, are analyzed for uranium. Frequency of routine home sampling is quarterly, semi-annual and annual, based more or less arbitrarily upon the general area of work and potential for exposure. In addition, special sampling is carried out for any individual when that individual may have

been involved in an incident where there is a potential for intake of radioactive materials; these may be collected either at the work location or at the home. Approximately 25% of the sample collections are now outside the city of Richland, Washington, which was developed for the Hanford project. Routine home collections are made from workers living as far as 80 miles from Richland, thus making delivery and collection expensive and time consuming.

At the present time our entire bioassay program is being converted to electronic data processing methods which includes automatic scheduling, computation of sample results, issuance of reports and generation of a permanent record for each individual. We are also embarking on a major revision of the routine sampling schedules as a result of our experience during the past ten years with internal deposition. Review of Hanford plutonium deposition pinpointed certain locations and types of workers who have a potential for exposure. Based on the very low potential of exposure for a large segment of employees as shown by past experience, a sampling frequency of once in five years has been established for those who do not work with or near radioactive materials. The frequency will serve as an audit of these employees on a plant wide basis for internal deposition and with our present detection limit, an intake of approximately 10% of the MPL ($< 0.004 \mu\text{c}$) would be discovered five years after exposure.

Case Study No. 1

The first case of internal plutonium deposition to be reviewed is interesting because of the many circumstances surrounding its discovery and development of facts from time of actual intake to final disposition of the case.

Discovery was originally made through our routine bioassay sampling program for HAPO and HAPO connected personnel. The analysis of the first urine sample was confirmed by immediate follow-up samples. Discussion about his past work experience revealed the fact that he had, during previous employment, worked in a plutonium processing hood just prior to his termination and that he may have received a minor injury from a wire brush being used at the time. Records covering the period in question showed that he had received some skin contamination but there was no evidence of the skin being broken. A few days after the incident he was treated at the Area First Aid Station for some skin irritation and tiny scabs in the area that had been contaminated but apparently no connection was made between the contamination incident and a possible break in the skin. No further investigation was thought necessary until internal deposition was discovered through routine sampling. It was also revealed during the

discussions that frequent irritation and itching persisted in this area for several months, and about six months later, two small black spots appeared which finally worked to the surface of the skin. He picked these out and the irritation stopped. Two tiny indentations covered with scar tissue were evident upon examination of the previously contaminated area. The formation of tiny black particles observed by this employee correlated with observations of another contaminated injury case currently under study. This phenomena has been observed on several occasions since that time and as much as 200,000 d/m of plutonium was measured on one particle removed from a contaminated wound area. The frequency with which foreign material has appeared in potentially contaminated wound areas after healing indicates a need for occasional follow-up examinations.

It was obvious from the bioassay urine samples processed thus far that considerable plutonium deposition existed and if the intake occurred over three years previously, the deposition could exceed the Maximum Permissible Limit (0.04 microcuries).

At this time the Radiological Physics group was developing instrumentation to measure body deposited plutonium by external measurement of the 17 Kev plutonium x-ray, so it was decided that the old injury should be examined with the prototype equipment. Plutonium was definitely detected in this area. Through many measurements, the plutonium was found to be concentrated in the two scarred areas and the amount was probably in the range of 0.01 to 0.02 microcuries. Depth of the contamination was of concern but attempts to make this determination were not entirely successful, however, we were fairly certain that it was near the skin surface. The decision was made to excise the skin area where plutonium has been detected. An industrial physician excised the area containing the suspected contamination and the biopsy was prepared for tissue study and autoradiographs. These studies indicated two primary areas of deposition which correlated with the external measurements that indicated two distinct areas of contamination. Autoradiographs showed plutonium to be in particulate form deposited immediately below the epidermis to 700 - 900 microns below the stratum corneum but did not detect any contamination on the outer surface of the stratum corneum. External measurements taken after excision did not detect any plutonium remaining in the wound area.

Analysis of results from urine sampling prior to the biopsy, assuming an intake at the time of the probable injury, is difficult because, the time period from date of discovery to date of biopsy is only a small fraction of the total time since intake. Based on Langham's empirical excretion rate, the samplings just prior to excision would still be approximately 80% of the excretion rate when the deposition was discovered.

As can be seen by the variance of results, a decrease of 20% is not significant.

TABLE NO. 1

Urinary Excretion of Pu²³⁹ for Case Study No. 1

<u>Days Post Intake</u>	<u>Excretion Rate d/m per Day</u>	<u>Excretion Rate Normalized to 1141 Days d/m per Day</u>
1141	1.03	1.03
1165	1.02	1.02
1193	0.45	0.47
1203	1.24	1.29
1217	1.07	1.12
1301	0.63	0.69
1378	1.25	1.44
1400	1.53	1.78
1439	1.43	1.70
1488	0.63	0.77
1512	0.54	0.67
1519	1.02	1.26
1579	1.07	1.36
1608	Biopsy Performed	
1635	1.00	1.30
1649	0.91	1.20
1684	0.80	1.05
1691	1.21	1.60
1698	1.36	1.78
1705	0.82	1.10
1712	1.13	1.50
1716	0.88	1.20
1719	1.42	1.90
1733	1.72	2.70
1740	1.46	2.00
1750	2.04	2.70
1759	1.04	1.40
1765	0.42	0.59
1777	1.74	2.40
1784	1.57	2.10
1791	1.14	1.55
1805	1.78	2.50
1812	0.68	0.96
1819	1.74	2.45
1834	0.30	0.43
1841	1.03	1.40
1848	1.49	2.05

(Table No. 1 Continued)

Table No. 1 Continued

Urinary Excretion of Pu²³⁹ for Case Study No. 1

<u>Days Post Intake</u>	<u>Excretion Rate d/m per Day</u>	<u>Excretion Rate Normalized to 1141 Days d/m per Day</u>
1862	1.03	1.45
1869	1.54	2.15
1876	0.54	0.78
1883	1.82	2.60
1890	1.23	1.75
1897	0.84	1.20
1901	0.97	1.40
1911	0.71	1.04
1918	0.15	0.24
1925	0.19	0.28
1948	1.04	1.54
2053	0.48	0.74
2321	0.86	1.42

The excretion rates normalized to 1141 days do not indicate any significant shift with regard to time which would indicate the possibility of plutonium still entering the blood stream. An average of the normalized excretion rates is 1.1 d/m per day with a standard deviation of 0.39 d/m per day thus indicating the body burden is in the range of .030 to .062 μc with the most probable being approximately .046 μc .

Examination of results after biopsy indicate an upward trend, however, when compared with internal variation of the data it is of borderline significance. Considering an administration of soluble plutonium, the excretion rate at 2300 days is approximately 65% of the rate at 1141 days, however the data collected thus far does not show any downward trend. From these data it could be assumed a pool of plutonium remained in the hand and was slowly solubilized and absorbed into the blood-stream, however, the rate of solubilization nor the total body deposition certainly cannot be accurately calculated from data collected thus far. A longer period of sampling is necessary in this case to establish the trend of urinary excretion since removal of the contaminated tissue and to determine what influence it will have on the original estimate of deposition.

Case Study No. 2

Another case of particular interest is one that involved plutonium contamination to the hand.

FIGURE 1
URINARY EXCRETION OF P_{239}
FOR CASE STUDY NO. 1

The incident occurred when an employee attempted to plug a line containing a plutonium solution composed of carbon tetrachloride, tributyl phosphate and traces of nitric acid with his gloved hand. After a very short time the heavy neoprene hood glove failed and two pair of surgeon's gloves also worn at the time deteriorated to a gummy mass leaving the hand essentially immersed in the plutonium solution. It was estimated that approximately 100 milligrams of plutonium adhered to the skin when withdrawn from the hood glove. Decontamination of the employee's hand was started immediately and everything from water flushing to ultrasonics was tried during the first ten hours. No accounting was made of the plutonium removed during the initial decontamination, however, from the first day following the incident until complete decontamination, approximately 3.5×10^7 d/m plutonium was removed. Very good correlation between daily contamination measurements on the hand and the amount removed were obtained throughout the 50 days decontamination.

The potential of absorption through the skin was of concern so urine collection was started at the work location. Five hours after exposure, a 250 cc urine sample was obtained and processed immediately in the bioassay laboratory which gave a result of 4.1 d/m. Arrangements were made to have complete urine collection on a 24 hour basis which incidentally was maintained for 31 days. An exceptional effort was made by our bioassay laboratory to process these samples making it possible to follow the excretion pattern on a day to day basis and detect any change in rate of absorption possibly caused by decontamination procedures. As can be seen from the urinary excretion, there was some absorption immediately after exposure and apparently very little, if any, during the 50 days necessary for decontamination. The first fecal elimination was two days after exposure and contained only 9 d/m which indicated essentially no ingestion and only slight absorption. Because of the orderly rate and level of excretion obtained daily from urine, fecal sampling was discontinued. One blood sample of 40 milliliters was collected approximately 30 hours after exposure, results indicated a total activity of 2.14 d/m or 0.053 d/m per ml. For a standard man this would indicate a total of about 262 d/m in the blood system and based on available information, about 15% of the total intake or representing an initial deposition of about 8×10^{-4} μ c (about 2% of the MPL). Evaluation of urine results through 31 days also indicates a deposition of about 2% of the MPL.

TABLE NO. 2

Urinary Excretion of Pu²³⁹ for Case Study No. 2

<u>Days Post Intake</u>	<u>Excretion Rate d/m per Day</u>	<u>Days Post Intake</u>	<u>Excretion Rate d/m per Day</u>
1	6.53	47	0.15
2	8.56	57	0.33
3	2.90	72	0.52
4	2.51	96	0.29
5	1.72	113	0.14
6	0.99	135	0.36
7	1.24	145	0.36
8	1.17	159	0.30
9	1.36	173	0.22
10	0.96	184	0.16
11	0.72	194	0.22
12	0.51	210	0.18
13	0.65	224	0.20
14	0.65	238	0.12
15	0.74	249	0.18
16	0.78	325	0.19
17	0.54	333	0.17
18	0.44	495	0.11
19	0.60	551	0.08
20	0.48	683	0.03
21	Lost	746	0.17
22	0.34	837	0.09
23	0.38	906	0.09
24	0.35		
25	0.39		
26	0.34		
27	0.40		
28	0.23		
29	0.28		
30	0.22		
31	0.18		

During the 31 days of complete sampling a very definite trend was evident with only slight deviation from the accepted rate of excretion. After the period of complete urine collection, routine type morning and evening collection on two successive days (to simulate a 24 hour voiding) was started which gave more variation in results and the trend appeared to change slightly. There did not, however, appear to be any pronounced trend indicating a chronic type administration from a reservoir of plutonium remaining in the skin. Statistical testing for significant difference between the least squares fit for the period of complete sampling and period of routine type sampling is borderline. There is essentially no statistical difference

FIGURE 2
URINARY EXCRETION OF Pu^{239}
FOR CASE STUDY NO. 2

between either period of sampling and the empirical excretion rate for an acute administration of soluble plutonium.

Two tissue samples were also collected; one, a skin specimen sloughed off four days after exposure and the other, a fingernail specimen trimmed off twenty-two days after exposure. Cross section autoradiographs were made of these specimens by the Hanford Biology Operation. Tracks were distributed evenly along the outer surface of the stratum corneum and visually showed lack of migration of plutonium through the skin which substantiates the low body deposition as measured by urinary excretion. Plutonium appeared to be heavily concentrated along the edge of the fingernail specimen but depth of absorption was not discernable.

Case Study No. 3

The third case of internal deposition for discussion concerns an intake of plutonium discovered by the routine bioassay sampling program. Upon discovery a review of work assignments pinpointed the most probable time of intake which also agreed with urine excretion rate observed thus far. At the time of exposure the potential of intake by inhalation for this person was apparently not recognized. Some slight facial contamination (1000 d/m) was observed, but at the time it was not considered to be indicative of any possible internal contamination and was dismissed without further investigation. However, after discovery of deposition, interview with the individual revealed the fact that he may have had some difficulty with his assault mask and had to adjust the straps several times to maintain a snug fit.

The trend of urinary excretion for the period covered appears to be following an orderly rate of decrease as expected for soluble plutonium. Normalizing results to one day gives an average of 163 d/m per day with a standard deviation of 45 d/m per day. The range of deposition as indicated by the data is from 0.028 μc to 0.047 μc with the most probable deposition being at 0.036 μc ($\sim 90\%$ of the MPL). The normalized results do not show any particular trend with time that would indicate a chronic administration and considering the short period between intake and discovery, a slightly soluble pool of plutonium being slowly transferred to the blood does not seem likely. Thus, assumption that the inhaled plutonium was soluble appears to be valid for the existing data.

TABLE NO. 3

Urinary Excretion of Pu²³⁹ for Case Study No. 3

<u>Days Post Intake</u>	<u>Excretion Rate d/m per Day</u>	<u>Excretion Rate Normalized to One Day d/m per Day</u>
81	6.07	156.
109	4.83	155.
130	3.84	141.
147	4.44	178.
168	1.31	58.
175	2.74	125.
296	3.65	246.
375	1.74	140.
413	1.74	150.
441	1.63	147.
574	1.81	202.
611	1.28	147.
659	1.96	239.
666	1.92	235.
701	1.06	135.
735	1.17	154.
771	1.42	194.
828	0.84	121.
849	1.06	156.
884	1.14	172.
925	1.02	160.
940	0.75	119.
968	0.58	93.
981	1.01	165.
1007	0.83	138.
1030	1.44	244.
1051	1.06	182.
1122	1.29	233.

Summary

Three actual cases of plutonium deposition selected for discussion include intakes by injection, absorption and inhalation. Two were discovered by the routine sampling program and illustrates the lack of obvious evidence that may be associated with a potential deposition of plutonium. It emphasizes the need for frequent sampling of employees involved in plutonium work.

The injection and absorption cases were of particular interest at Hanford because; plutonium deposited in human tissue was detected and measured for the first time by external counting techniques, knowledge that minute particulate

FIGURE 3
URINARY EXCRETION OF Pu^{239}
FOR CASE STUDY NO. 3

type material may form in a potentially contaminated wound area was acquired, and in the case of absorption, the ability of the skin to be an almost impermeable barrier when contaminated with milligram amounts of plutonium was demonstrated.

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PLUTONIUM EXCRETION FOLLOWING ACUTE ACCIDENTAL EXPOSURE

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Introduction

Theories of plutonium metabolism and excretion in humans have had to depend mainly on the extrapolation of animal data. Cases presented here add to the limited human data available, point out both anomalies and agreements with theory derived from idealized animal experiments, and contribute experimental results of exposures to mixed compounds and multiple modes of entry into the body.

Discussion

Analytical Method

All samples summarized in this paper were analyzed by our routine plutonium analysis. The ashed sample is reduced with sulfurous acid. A bismuth phosphate precipitation carries the plutonium. Following dissolution of the bismuth phosphate in hydrochloric acid, a lanthanum fluoride precipitate is transferred to a stainless steel disc, ignited, and counted for two-and-a-half hours in a gas-flow proportional counter.

The lanthanum nitrate used is prepurified by ion-exchange techniques to an alpha background of less than 0.04 d/m/mg. Reagent blanks and control "spikes" are run daily. "Spike" recoveries average 90% with a 95% confidence error overall of $\pm 20\%$ at a level of 1 - 2 d/m/sample.

Excretion Data

Cases I and II: These two persons were injured by a chemical explosion in a reaction vessel. Total internal deposition was a result of both acute inhalation and injection of plutonium. The exposures resulted from both soluble and insoluble plutonium compounds. Plutonium was also ingested both as a result of the accident and by the swallowing of material brought up by ciliary action.

Case I had 2.9 μg of plutonium deposited in a wound at the tip of his right index finger. About 2.3 μg of this was excised on the third day following the incident. The remainder was removed by further excision on day 22.

Calcium EDTA treatment was started about 4-1/2 hours following the incident. Daily treatment continued for seven days. Following the weekend, when no treatment was given, the therapy was continued during work-week periods for three more weeks.

Fecal excretion data for 80 days following the incident are shown in Graph I. The expected rapid elimination of material from the lung and upper respiratory passages cleared about 61 μg in the first few days. The subject was given Epsom Salts immediately following the incident to minimize absorption from the gut, and the initial fecal sample was not analyzed. Maximum fecal excretion, 40 μg , occurred on the second day after the incident.

Twenty-three additional samples have been obtained periodically through day 460. These have ranged from 0.42 to 121 d/m with a median value of 22 d/m. His most recent sample was 6.5 d/m on day 460.

Graph II is the urinary excretion curve for days 1-100. Favorable results of the calcium versenate treatment are depicted clearly. The initial days excretion was 10,700 d/m/24-hours, decreasing to 215 and 155 d/m/24-hours respectively on the two days following the first EDTA treatment period. Successive EDTA treatment periods showed a rise to a maximum of 1537, 1672, and 618 d/m/24-hours respectively. The maximum value occurred on the second day of treatment in each case.

Graph III shows blood, urine, and fecal plutonium through day 220. The blood data is erratic but in general follows the trends of the other curves. The high analysis of day 118, 1.83 d/m/ml, corresponds to a high fecal sample of 121 d/m at the same time even though the urine excretion then showed no anomalies. A possible explanation is that this might represent a period when internal nitric acid burns in the respiratory system were healing and sloughing off contaminated tissue, or a period when blood activity was going into the intesting via the bile.

The urine curve behaves as expected following a complex exposure. The initial rapid drop is followed by a more gradual decrease from 30 to 15 d/m/24-hours, then a gradual upward trend. The two high points at day 81 (97 d/m) and day 152 (44 d/m) occurred during a time when the man had acute sinusities. This might represent misbehavior of the kidney during periods of illness, or possible effect of antibiotics on body metabolism in general.

Case II had facial wounds on his forehead and on his chin. Gamma spectroscopy indicated 3.06 μg of plutonium in the forehead wounds and 0.89 μg in the chin wounds. Excision on day 22

GRAPH I
CASE I
Fecal Plutonium

GRAPH II
CASE I
Urine Plutonium

GRAPH III
CASE II
Fecal Plutonium

removed all detectable material from the tissue except about 0.5 μg which was left in a forehead wound because of its proximity to the eye. Periodic gamma spectrometer measurements since the incident have indicated no detectable absorption of plutonium.

As with Case I, calcium versenate therapy was continued for twenty-two days.

Graph IV represents his fecal excretion of plutonium during the first 80 days. A total of 0.5 μg was excreted via the feces the first five days following the incident. As before, the initial sample was not collected because of the dose of Epsom Salts administered immediately after the incident. Twenty-five additional samples after day 80 have been collected. These have ranged from 680 d/m on day 126 to below detection on day 398. Most of the samples have fallen fairly close to the mean value of 8.8 d/m.

Urinary excretion is shown in Graph V. Initial excretion was 5700 d/m. The curve follows the same general form as Case I, however, with much more erratic behavior of individual points. The man has a low liquid consumption and consequent low urinary volume - as low as 600 ml/24 hours in some instances. The low points on the curve correspond to periods of low volume in most cases. The point at day 37, 0.47 d/m/24-hours, was characterized by a sample literally "loaded" with albumin.

Graph VI presents blood, urine, and fecal data through the first 220 days following the incident. Again a high blood sample determination corresponds to a high fecal sample on days 125 and 126 - 2.61 d/m/ml and 680 d/m respectively.

Starting at day 262 for Case I and day 269 for Case II, two five-day periods of calcium versenate treatment were given these men. Graphs VII and VIII show the urine excretion of the two during this period.

Case I went from an average excretion rate of 20 d/m/24-hours during the previous month to a maximum of 329 d/m/24-hours the first week and 210 d/m/24-hours the second. Following this, his excretion rate dropped to its previous level.

Case II, averaging 8.1 d/m/24-hours during the month previous to treatment, had maximums during the two week's period of 309 and 211 d/m/24-hours.

The most recent urine samples of these two cases shows Case I excreting plutonium at a 13.1 d/m/24-hours average and Case II at 7.4 d/m/24-hours average.

Case III is an inhalation exposure to airborne plutonium

GRAPH IV
CASE II
Urine Plutonium

GRAPH V

CASE I

GRAPH VI

CASE II

GRAPH VII

CASE I

Urine Plutonium

GRAPH VIII
CASE II
Urine Plutonium

dust at a concentration exceeding 1.25×10^5 d/m/M³. The employee was exposed to this atmosphere for about an hour while wearing a faulty respirator.

Graphs IX and X present fecal and urine analyses respectively. Fecal excretion rose to a maximum of 65,000 d/m on day 3. From day 5 through day 31 his samples ranged from a low of 9.0 d/m to a high of 670 d/m.

Calcium EDTA treatment was given for a total of nine days as indicated on the base of Graph IX. Urinary plutonium rose rapidly to a maximum of 268 d/m/24-hours on day 3. A fairly steady decline then followed throughout the EDTA-treatment period. The contrast between this excretion curve and those previously discussed is readily apparent.

All urine voided through day 24 appeared to be leveling off around 1.0 d/m/24-hours. However, samples collected for a four-day period the following week showed a sharp rise to 8 d/m/24-hours followed by a second rapid decline on subsequent days. Seven samples analyzed since day 31 have ranged between 0.2 and 0.9 d/m/24-hours.

Case IV: This man received a puncture wound on his left thumb. No contamination from blood smears and direct surveying of the wound area was detected. His routine urinalysis the following month was 0.88 d/m/24-hours. Graph XI indicates that subsequent urinalyses remained at approximately this same level.

Sometime during the nine months following the accident a lump formed at the side of the original wound. By this time our gamma-ray spectrometer which was in operation detected 0.1 μ g plutonium in the lump. The final three points on the graph are urinalyses performed since the excision of the lump. The first, 0.82 d/m/24-hours, occurred 12 days after the excision.

Case V: The subject cut three fingers of his left hand in the lift mechanism of a furnace. After standard decontamination procedures a gamma spectrometer indicated 1.3 μ g plutonium in his middle finger. Excision of tissue around the wound reduced this to 0.45 μ g. He was placed on calcium-EDTA therapy for a total of nine days as indicated along the base of Graph XII. The peaks and valleys correspond to treatment and non-treatment periods. A fecal sample on day 9 showed 3.3 d/m. His initial urine sample taken off work on day 1 and 2 was 62 d/m/24-hours. Subsequent samples were 9.0 d/m/24-hours and 3.9 d/m/24-hours. A maximum urinary excretion rate of 227 d/m/24-hours occurred on day 5, the second day of EDTA treatment. As can be seen on the graph his excretion rate has leveled off to around 5 d/m/24-hours.

GRAPH IX
CASE III
Fecal Plutonium

GRAPH X
CASE III
Urine Plutonium

GRAPH XI
CASE IV
Urine Plutonium

GRAPH XII
CASE V
Urine Plutonium

Conclusions

Our data on wound Cases IV and V are too limited to allow much generalization. There is some indication that both cases were similar in mechanism; i.e., a relatively insoluble mass of material at the wound site acted as a reservoir for small amounts of plutonium to enter the blood stream at a constant rate, proportional to the amount of material present. The ratio is roughly 10 d/m/24-hours excreted per μg in the tissues. Even when material is excised completely within a half-hour following an accident some activity appears in the urinary excretion.

One of the most striking observations of these cases is the amount of variation occurring in the rate of excretion of plutonium by an individual. Cases I and II were continuously sampled for 80 and 100 days respectively. At least theoretically, their entire urinary output was analyzed during this period. During this time extreme variation was noted in Case II during the sixth week following his exposure. Four successive samples rose from an initial rate of 0.42 d/m/24-hours to a final rate of 91 d/m/24-hours, the two following samples fell to a rate of 15 and 8.5 d/m/24-hours, respectively. As mentioned previously, this fluctuation could be attributed in part to temporary kidney damage, as evidenced by the presence of albumin in the urine at that time. However, over a year later, this subject's rate has varied between 0.4 and 10 d/m/24-hours during a three-day sampling period, and kidney function tests have been normal.

Case I exhibited considerable variation but to a lesser extent than Case II. Normally, any one days' sample from him is within $\pm 20\%$ of the three-day average; however, analysis of his rate by eight-hour increments exhibits differences as high as a factor of five.

This suggests that the absolute value of any one urine sample is important only in establishing trends upward or downward or in establishing qualitatively that plutonium is present in the body.

At the suggestion of Dr. Foreman of Los Alamos, known exposures are sampled over a three-day period. An average value established for this time levels out individual sample fluctuations.

Calcium versenate was administered to Cases I and II at various times at rates of 4 grams/day, 2 grams/day, and 1 gram/day with no appreciable difference in effect on the excretion rate. It might be possible to achieve the same effect with amounts of EDTA small enough to be administered with a hypodermic needle and eliminate the time-consuming intravenous injections.

A COBALT AND A TRITIUM INCIDENT AT ARGONNE NATIONAL LABORATORY

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This discussion presents some of the data obtained from two accidental exposures that occurred in 1958. The cobalt exposure involved two men who were decontaminating ductwork. The ductwork had been used with a hood in which pieces of neutron-irradiated cobalt metal had been cleaned with a wire brush. During the decontamination operation the two men noted that they had inhaled some dust. Consequently, the men were counted with a sodium iodide crystal in an iron-shielded room by Dr. Charles Miller of the Radiological Physics Division, and urine and stool samples were collected for the next several weeks. Analyses of the most recent samples have not been completed.

The gamma-ray spectrum of the excreta confirmed that the exposure was to Co^{60} , and whole body gamma counting confirmed that the cobalt (approximately $0.5 \mu\text{c}$ in each case) was in the lung. From the results of whole body counting, the cobalt disappeared from the lung with a half-life¹ of 1.6 days from the second to the seventh day after the exposure, and with a 42 day half-life from the 17th to the 50th day. The half-life gradually increased to about 110 days, three months after the initial exposure. At that time the body burden was about $0.05 \mu\text{c}$ and the bulk of the cobalt was probably in the liver².

During the first week 80-90% of the excreted cobalt appeared in the stool, as expected, and evidently moved to the gastrointestinal tract from the lung. The cobalt content of the feces reached a maximum two to three days after the exposure, and dropped rapidly after about four days.

The urinary cobalt excretion was greatest in the first sample, collected several hours after the exposure, and decreased with time approximately as a power function. From the first to the second day, the concentration of cobalt in the urine decreased by a factor of ten in the first case and by a factor of five in the second. After the second day the urinary cobalt concentration decreased more slowly. In Case I the cobalt disappeared from the urine with approximately a four day half -

¹The half-lives given in this paper are biological half-lives.

²We are grateful to Dr. Miller for making his data available to us.

time from the second to the tenth day, and subsequently with increasingly longer half-times. Ten to twenty percent of the eliminated cobalt was found in the urine, and about two-thirds of the urinary cobalt was eliminated the first day. Thus, some of the cobalt was solubilized and appeared in the urine very soon after the exposure, although it is presumably an "insoluble" material. Similar behavior has been found by others for insoluble compounds such as PuO_2 .

The cobalt fecal/urine ratio was very low on the first day, before appreciable amounts of cobalt had moved through the G. I. tract, but increased to 150 in 2 to 5 days. On the 29th day, the last day for which we have completed analysis of the excreta, the fecal/urine ratio was approximately 2.5, and the total excretion amounted to 1.7% of the total body burden. This corresponds to a half-life of 41 days, and agrees well with the 42 day half-life obtained by Dr. Miller from body measurements alone.

The total amount of cobalt found in the excreta during the first week is shown in Table I. Since no attempt was made to obtain the complete excreta of the individuals, and stool samples were not obtained on the second day, there is some uncertainty in the totals. The fecal excretion is expressed in two ways in the table: the amount of cobalt in the entire sample, and the cobalt content assuming 35 grams to be a normal daily fecal output. Although the total amount found in the excreta, 0.2 μc or 0.8 μc , depending on the method of calculation, is considerably different from the 0.5 μc found by body counting, the agreement appears reasonable in view of the uncertainties in sample collection.

The tritium exposure occurred at the CP-5 reactor. This reactor uses about 2000 gallons of heavy water as the moderator, and at the time of the incident the tritium concentration in this water was about 700 microcuries per milliliter. The people who work on the reactor occasionally take in small amounts of tritium, and this nuclide has been the principal internal-emitter hazard at CP-5. This particular accident was the most serious one of its type at this reactor, but even in this case the highest dose received by any one individual was about 0.5 rem.

The first of two exposures occurred when heavy water leaked through a gasket and ran on to the floor. Four people cleaned up this spill, working with this water for about thirty minutes and wearing rubber gloves and assault masks, but no other protective clothing or equipment. The first urine samples were collected the next morning. The urinary tritium concentrations are summarized in Table II for the individuals identified as J.C., T.L., R.M., and J.F. After the first few days, the tritium disappeared from the urine with half-lives ranging from 7.5 to 9.0 days for the four cases, and these values agree with

TABLE I

Elimination of Cobalt-60

Days After Exposure	Case I - Co ⁶⁰ concentration			Case II - Co ⁶⁰ concentration		
	Urine ($\mu\text{c}/\text{liter}$)	Feces		Urine ($\mu\text{c}/\text{liter}$)	Feces	
		$\mu\text{c}/\text{sample}$	$\mu\text{c}/35\text{g.}$		$\mu\text{c}/\text{sample}$	$\mu\text{c}/35\text{g.}$
0.5	0.031	0.0004	0.0024	0.021	0.0558	0.0787
1.5	.0026	.0288	.3272	.0044	.1078	.0871
2	.0029	-	-	.0017	-	-
3	.0019	.0809	.2940	.0018	.0044	.0052
4	.0016	.0661	.1795	.0013	.0207	.0185
5	.0012	.0043	.0066	.00070	.0073	.0094
6	.0014	-	-	-	-	-
7	.00062	-	-	-	-	-
17	.00025	.0006	.0007	-	-	-
28	.00014	-	-	-	-	-
Total	0.043	0.18	0.81	0.32	0.20	0.20

TABLE II

Elimination of Tritium

Time ¹ (hours)	Tritium Concentration ($\mu\text{c/liter}$ of urine)									
	J.C.	T.L.	R.M.	J.F.	H.M.	J.M.	R.D.	G.F.	A.P.	R.H.
14	28.0	-	27.8	23.4	-	-	-	-	-	-
22	34.7	66.5	28.8	24.8	-	-	-	-	-	-
23	-	-	-	-	-	-	14.5	-	-	-
30	49.2	79.8	27.3	26.1	-	-	-	1.4	-	-
42	50.7	80.9	25.8	26.0	-	-	-	19.0	-	-
51	-	-	-	-	(14.4)	9.0	21.8	-	(19.9)	(3.2)
60	-	-	-	-	-	-	21.0	17.4	-	-
66	40.6	66.7	-	-	-	-	-	-	-	-
Half-life ² (days)	7.5	8.0	8.0	9.0	16	15	12	9.1	9	10

¹Time after the first spill.

²Half-life for elimination of tritium.

those given in the literature. A single half-life was observed for each individual until the tritium concentration dropped below the detection limit of the counting method, so there was no opportunity to observe longer elimination periods. H.M. (column five in the table) replaced the defective gasket while wearing an assault mask and rubber gloves, and also received a significant tritium exposure.

For J.C. and T.L., there was a large increase in tritium concentration between the 22nd and 30th hour after the exposure. Since studies at Los Alamos have shown that tritium reaches equilibrium with body water in a few hours, this increase after 22 hours would indicate a second exposure. A second water leak did occur 22.5 hours after the first one, and these two individuals, together with five others, spent about three hours cleaning up the second spill. In this case, water had leaked into a room below the reactor core, and the floor of this room was covered with about 1/4" of water.

The urinary elimination rates for the individuals who cleaned up the second spill are also given in the table. Except for R.D. and G.F., all those involved in the second spill wore rubber gloves and used Scott Air-paks when working in the room containing heavy water, and replaced the Air-Paks with assault masks when working just outside the room. This protective equipment was evidently inadequate to prevent tritium intake. For J.C. and T.L., who worked on both spills, the increases of about 14 $\mu\text{c}/\text{l}$ between the 22nd and the 30th hour can readily be accounted for by additional exposure during the second spill. R.M. and J.F., who were not present during the second spill, did not show these large increases. The small increases (2 to 4%) from 14 to 22 hours in the cases of J.F. and R.M. may be due to experimental error or may be the result of a small additional intake during the day after the first spill. The latter is quite likely since the air in the reactor building probably contained significant concentrations of tritium. For J.C., there was also an increase between the 14th and 22nd hour from 28.0 $\mu\text{c}/\text{l}$ to 34.7 $\mu\text{c}/\text{l}$. No explanation for this increase is apparent, except for the possibility of additional exposure during the day between the first and second spills. This increase is not believed due to errors in the analyses, since these samples were analyzed carefully several times.

It is interesting to compare the exposures resulting from the second spill since the protective equipment did not function properly for two of the seven people exposed. The Scott Air-Pak used by R.D. ran out of air and he removed the face piece while inside the room; G.F. used an assault mask without a canister while working just outside the room. However, except for R.H., the exposures were not greatly different among the seven people involved. Assuming the same body water content for all the individuals, the amount of tritium taken in is approximately

proportional to the elimination rate and to the elimination half-life at times that are small compared to the half-life. On this basis, the highest tritium intake from the second spill was by R.D. The lowest intake, by R.H., was about one-tenth as large. Each of the other people received an exposure between 0.5 and 0.9 of that received by R.D. It was surprising that the tritium intake by R.D. and G.F. did not greatly exceed that of all the other individuals exposed. If the protective equipment functioned properly except in the two cases mentioned, considerable tritium must have been absorbed through the skin. Additional protective clothing to prevent absorption through the skin is evidently needed in situations such as described here. The value of an assault mask in reducing tritium inhalation cannot be determined from the results given here because of the large amount of skin absorption.

It should be pointed out that at the time of the spills the individuals involved did not expect any appreciable tritium intake, except for the two equipment failures mentioned. As a result, several individuals did not submit urine samples until several days after the second spill, and the data are incomplete in these cases. The results given in parentheses for A.P., R.H., and H.M. were calculated from later results in order to compare elimination rates soon after the exposure.

SOME HYDROLYSIS CONCEPTS RELATED TO THE SOLUBILITY OF METALLIC COMPOUNDS IN VITRO AND IN VIVO

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Introduction

It is now generally recognized that all metallic ions form hydrates in aqueous solution, surrounding themselves with a few tightly-bound water molecules and a large number of weakly - bound water molecules. The latter are held by van der Waals forces only and form a nebulous volume around the few chemically - coordinated inner or nearest-neighbor group of water molecules.

This inner coordination sphere of water greatly affects the chemical properties of metallic ion solutions, to a much greater extent than is generally pointed out in elementary texts. It is usually recognized that the dissolution of a metallic salt may be represented by:

where the H_2O represents chemically-coordinated water and the nebulous outer water is ignored.

Such an equation is sufficient for certain salts such as NaCl or KCl, but for metals whose ions have high charge/size ratio, such as Li^+ , Be^{++} , Al^{+++} , etc., this equation can lead to erroneous conclusions.

In actual fact, salts of high charge density hydrolyze to some extent immediately upon dissolution. For most of them the hydrolysis is followed by some slower polymerization.

Consider the hydrated metallic ion first produced when a metallic salt dissolves in very large excess water, e.g.

six coordinated water molecules being octahedrally spaced about this M^{+a} ion.

Each (M-O) bond is formed when a pair of unused oxygen electrons in a water molecule ($\text{:}\overset{\text{H}}{\underset{\text{OH}}{\text{O}}}\text{)}$ is attracted so strongly to the metal ion that they spend sufficient time in a metallic electronic orbit to hold the two atoms in close proximity. As a result these two electrons are pulled to position further away from the oxygen nucleus than when in water alone. In other words their electronic orbit is distorted. This distortion produces a distortion of the (O-H) bond in H_2O also, so much distortion in fact that there is great tendency for an H^+ ion to break off leaving $(\text{M}(\text{H}_2\text{O})_5\text{OH})^{a-1}$ ion. There may even be tendency for further hydrolysis to $(\text{M}(\text{H}_2\text{O})_4(\text{OH})_2)^{a-2}$. Although it is possible with concentrated sodium or barium hydroxide to produce $(\text{M}(\text{OH})_6)^{a-6}$, e.g., in $\text{Na}_3\text{Cr}(\text{OH})_6$ and $\text{Ba}_2\text{Cd}(\text{OH})_6$, it is rare for hydrolysis in simple water solution to proceed past the second step.

Olation

There is, however, a very great tendency for the hydrolysis products to dimerize, i.e.,

Evidence for this dimerization has been given for ferric, aluminum, bismuth, copper, scandium and uranyl ions. Such dimerization is rapid.

Dimers involving only one hydroxyl bridge group are relatively unstable and therefore they will be ignored.

The hydroxyl bridging process is called olation, "ol" referring to the bridging group as distinguished from free hydroxyl ions or hydroxy ligands attached to only one cation as in the monomer.

Compounds are also known in which there are three "ol" bridges between two metallic atoms, e.g.,

Further hydrolysis and olation can result in higher polymers, such as:

on in linear chains:

or even in three-dimensional cross linked polymers, as:

Such polymerization explains the results obtained when a warm solution of a chromium salt is titrated with alkali. Addition of some sodium hydroxide gives an immediate pH rise, followed by a slow pH drop if the solution is allowed to stand before more base is added. In fact, this is a very general phenomenon in the pH titration of a polyvalent metal. Although the first dimerization through olation is very rapid, more time is required for further polymerization. As hydroxyl ions are removed by the bridge formation, olation puts hydrogen ions into the solution. This process continues with further increments of base until the particles are large enough to precipitate.

By means of very slow titration it is possible to add without precipitation an amount of base which would cause precipitation if added all at once.

Olation can be reversed fairly rapidly by addition of acid, or slowly by dilution or cooling.

Oxolation

Olation is frequently followed by oxolation, the conversion of an "ol" bridge to an "oxo" bridge consisting simply of an oxygen atom produced by removal of a proton from an "ol" group.

Deoxolation is much slower than deolation, because hydrogen ions react much more readily with "ol" groups than with "oxo" groups. By lowering the pH one can immediately dissolve a fresh indium hydroxide precipitate produced by titration, but if the suspension is heated and then cooled addition of acid even to pH 1 will not dissolve the suspended precipitate even after six months standing with frequent shaking.

Prolonged aging may produce oxolation of an ion or a precipitate even in the cold.

Anion Penetration

An "ol" group, or even an "oxo" group, may be replaced by an anion, such as citrate, oxalate or sulfate. This process is called anion penetration and results in mixed bridge formation. That is, in:

obtained by adding acetic acid to the triol. Also,

Anions that can easily penetrate usually prevent olation if they are present in solution before the pH is raised. However, Serfass found an ionic species having a molecular weight of 68,000 in stock solutions of basic chromic sulfate.

The effect of acid on an aged precipitate depends to a large extent on the penetrating ability of the anion of the acid. A weak penetrator only partially neutralizes the positive charge acquired by the precipitate when "ol" groups are converted to aquo groups by hydrogen ions. Hence, when the ratio of charge to mass is large, peptization occurs.

On the other hand, a strong penetrator can displace "ol"

groups and aquo groups readily. It may even displace some "oxo" groups under optimum conditions. With a small ratio of strong penetrating acid to alumina, for instance, acid disappears from the solution because of neutralization of "ol" groups displaced by the anion. With a large acid to alumina ratio, complete dissolution can occur providing that the oxide is not heavily oxolated at start.

Of what importance are these phenomena to us? Obviously, now we must realize that the properties of a solution or suspension of an olated or oxolated compound depend on the history of the system, i.e., an aged solution may contain a higher polymer/monomer ratio than a fresh solution at a given pH and the aggregates may be larger. An aged precipitate probably will be much more oxolated than a fresh precipitate, hence the former will be much more insoluble. Agents which solubilize fresh precipitates may only cause colloid formation with an aged precipitate. This can occur in vivo as well as in vitro.

Whereas anion penetration of a fresh (olated) precipitate may dissolve it, penetration of an aged (oxolated) precipitate with the same anion may only cause mixed bridge formation.

The most reproducible results with an olatable system will obviously be obtained if the reactants are mixed before raising the pH. That is:

i.e., citrate is capable of solubilizing 2 beryllium atoms only if the complex formed before the pH is raised above the precipitation point of Be(OH)_2 ; if not, too much oxolation and mixed bridge formation occur instead of solubilization of all the beryllium.

Radiocolloids

It should not be inferred that these phenomena relate only to macro-concentrations. In solutions containing only radioactive tracer concentration of a polyvalent cations, one encounters radiocolloid formation. The term radiocolloid refers to substances which show colloidal properties even

though the solubility product for macro-concentration would indicate that the tracer solution should be a true solution.

Peculiarly, radiocolloid formation begins to be noticed at about the same pH where macro-concentration of the same cation would be precipitated, suggesting the radiocolloid may be a polymer formed by hydrolysis and olation.

That is, tracer Be^{++} ($\sim 10^{-9}\text{M}$) begins to show radiocolloid properties at about pH 5.5; the pH at which macro-concentrations of Be^{++} begin to precipitate. However, 10^{-5}M Be^{++} begins to show polymer properties at about pH 4.5.

Polonium

With tracer polonium, we are unable to obtain reproducible amounts of filterable Po at pH 7 if the pH were adjusted by dropwise addition of NaOH, whereas we have been able to reproduce the results well with homogeneous pH adjustment (with urea, NH_4Cl plus heat). Dropwise addition of NaOH produces local highly concentration of OH^- which could produce high olated radiocolloid particles which do not deolate reproducibly after stirring, whereas homogeneous pH adjustment should be more likely to produce more reproducible size distribution of polymers from the start.

To sum up, it should be obvious that extreme care must be taken to obtain reproducible results with metals which have a tendency to polymerize. Unfortunately, many of the toxic metals which are now of interest to atomic energy people are such substances. In fact there is probably a close causal relationship between the polymerization tendency of a metal and the difficulty in solving its toxicity problem. The solubility of an olatable substance in a body fluid, and hence its behavior in the body, undoubtedly depends on the history of the substance before being taken into the body. Furthermore, even if it enters the body in a soluble state there will be a good chance that it will be olated at some point in the body and its behavior that point on toward a potential reactant will depend to a great extent on how much time passes before it's exposure to the potential reactant.

SOME PHYSICAL AND PHYSIOLOGICAL FACTORS CONTROLLING THE FATE OF INHALED SUBSTANCES

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Introduction

Dust deposition is the active process wherein dust is removed from the air while it is being respired. The term implies the extent to which dust is removed in terms of the same measure of dustiness as used to characterize the inspired air. Some fraction of the dust deposited is not in reality on the surface membrane of the respiratory tract but in suspension within the lung air. In the over-all course of dust deposition the amount of dust so involved is relatively small and short-lived and is particularly insignificant when deposition is measured as a steady state condition.

Physical Forces

Dust deposition in the respiratory tract can be thought of as a process occurring within a small compartment of air with the same physical forces in control that are involved in the dust deposition from the environmental air outside of the body. The forces of interest are of three general types; gravitational, inertial and Brownian motion.

A. Gravitation Settling

A 20 cm diameter lead ball dropped from an airplane will be constantly accelerated toward the earth according to Newton's Law of gravitation. A particle of lead of 20 μ diameter dropped into a chamber of tranquil air similarly falls by gravitation, but it is not continuously accelerated; it quickly reaches its terminal velocity and an acceleration of zero.

The dynamics of small particles (0.5-50 μ) falling through a fluid medium are controlled by the viscous forces of the medium. Stokes Law states that the viscous resistance of a medium is equal to the product of the viscosity of the medium and the velocity and size of the particle (1). The terminal velocity of fall is reached when the gravitational force and the viscous resistance force are equal.

For spheres in air, the gravitational force, F , is equal

to $\frac{\pi}{6} d^3 (\sigma - \rho)g$ and the air resistance or buoyant force, R, is equal to $3\pi \eta dV$ wherein:

d = diameter of particle
 σ = density of particle
g = acceleration of gravity
 η = viscosity of air
 ρ = density of air
V = velocity of particle
and $\frac{\pi}{6}$ and 3π are shape factors for spheres.

Setting these two equations equal to each other and solving for the terminal velocity of a particle yields the following equation:

$$V_s = \frac{(\sigma - \rho)g d^2}{18 \eta}$$

The mobility of a particle, Z, is defined, under Stokes Law conditions as equal to $\frac{1}{3\pi \eta d}$: therefore $V_s = Z F$. Stokes Law conditions are those in which the ratio of the inertial to viscous forces is very small (< 0.05).

As the particle diameter approaches the mean free path of air molecules, the mobility of the particle is larger than predicted by the Stokes equation for viscous resistance. This is due to the changing nature of the interaction between the gas molecules and these small particles. This phenomenon is known as "slip" and it denotes a lessening of the viscous resistance of the medium as the particle size decreases. Epstein (2), Knudsen and Weber (3), and Millikan (4) have reported expressions dealing with the slip correction in terms of the particle mobility, Z. The Knudsen and Weber equation is applicable to particles of any size but inappreciably affects particles as large as 10μ diameter unit density.

$$Z = \frac{1 + \frac{2\lambda}{d} \left(A + B \exp \frac{Cd}{2\lambda} \right)}{3\pi \eta d}$$

λ = mean free path of air

A, B and C are empirically determined "slip" constants with the following approximate values, 1.25, 0.40 and 1.10, respectively.

d = particle diameter
 η = viscosity of air
 3π = shape factor of sphere

Another more widely encountered correction factor which is applied to the Stokes equation when dealing with particles approaching the mean free path dimension is the Cunningham factor $1 + 1.7 \frac{\lambda}{d}$ (5). The difficulty in using the Cunningham factor is due to the fact that it does not give a sufficiently accurate correction except over a very limited range, approximately 0.1 - 0.5 μ diameter. Despite this limitation, the Cunningham correction is widely used. Unquestionably the Knudsen and Weber equation is preferable but has not gained sufficient recognition and use.

In the region of 20 μ diameter, particles of unit density will fall with a terminal velocity of about 10^4 micron/sec. At 1 μ diameter, the velocity is only 35 micron/sec. Despite the fact that very small particles require large slip corrections, they still exhibit extremely low settling velocities, e.g., approximately .04 micron/sec for 0.01 micron diameter particle.

While the size of the particle is the most significant factor in sedimentation, the density of the particle is frequently very important. This is particularly the case in studies involving the heavy metals, their oxides and salts. It is not unusual to encounter conditions in which dusts with density of 9-11 are involved (6). A 0.5 μ particle with a density of 10 has the sedimentation characteristic of a unit density particle of about 2 microns diameter. As will be seen in the later discussion, this increase in settling velocity may result in an important change in dust deposition.

Sedimentation as discussed here has been limited to the ideal circumstances of tranquil air. There are techniques of approaching the circumstance of stirred settling, turbulent mixing, etc. These are available in many source books (1), (7 - 9).

B. Inertial Impaction

Another variation of the basic Stokes equation is utilized in the determination of forces imparted to a particle in an air centrifuge. In this situation, the force of gravity is replaced by the angular velocity imparted to the particle and the radius of curvature involved (7). This requires, however, that the Reynolds number be very low (less than 0.05) so that the Stokes relationship is still applicable. The Reynolds number for the particle is equal to the ratio of the particle inertia (density • diameter • velocity) to the viscosity of air; therefore, the resistance offered by the medium to the particle can change with

different particle velocities. Just as the relative motion of the particle to the air is significant, the air velocity and the size and shape of any obstacle are important in governing the flow pattern about the obstacle. The air flow pattern in turn will have a bearing on the behavior of the particle with respect to the obstacle.

Generalizing, the impingement of particles onto a surface from an air stream is possible whenever the inertia of the particle is sufficiently great to cause it to overcome the resistive forces of the medium and intercept the surface or obstacle. Neglecting gravity, the path of the particle in an air stream of changing direction will be determined by the air velocity, the size and shape of the obstacle and the mass of the particle and its air resistance. Details of the analysis of particle impingement from air streams are often complex and can be found in many references (8), (9), but particularly useful are the papers of Davies and co-workers (10-13). These papers do not deal with a model which can be readily transposed to the anatomical configuration of the respiratory tract, however.

One crucial point in considering inertial impingement in the respiratory tract is whether or not there is a combination of high air flows, particle size and density to produce important inertial effects. Linear air velocities of about 150 cm/sec. regularly occur in the trachea. About 200 cm/sec. air flows are estimated to occur in the major bronchii and their first branches. These velocities suggested to Landahl (14) that Reynolds' numbers for air in these regions would be around 100 and possibly as high as 1000. These Reynolds' numbers indicate that intermediate or near turbulent flow conditions normally exist in the respiratory tract and turbulence probably exists in those regions during exercise and rapid breathing. A dominant feature of high velocity air flow is that it more nearly approaches an ideal fluid without viscosity (8). Even so, Stokes Law for the viscous resistance of the air with respect to the particle is unaffected. Calculations of air flow characteristics at high Reynolds number have been made by Landahl and Hermann (15) and by Ranz and Wong (16) and these are presumably applicable to respiratory tract conditions.

Landahl estimates that in a bifurcating tube of order "a", the impaction efficiency (fraction of particles removed) will approach 50 percent when the following expression equals unity:

$$\sigma d^2 V_{a-1} \sin \theta / 18 \eta a$$

θ - is angle between axes of tubes of order a and a-1.

V - average velocity of air stream

d - diameter of particle (sphere)

σ - density of particle (effective;
 $\sigma = \rho$)

η - viscosity of air

R_a - average distance a particle must pass perpendicular to the air stream in a tube of radius R .

However, the problem of determining the average velocity of air flow in various parts of the respiratory tract is difficult as there is a complex distribution of velocities. Not knowing whether to treat the distribution as rectangular or parabolic, Landahl derived expressions for both and used the average velocity value (14). From such an analysis, Landahl has estimated the role of inertial impaction on dust deposition at various levels of a stylized respiratory tract during a stylized respiratory cycle. These estimations indicate the importance of inertial impaction on the nasal pharyngeal and tracheal-bronchial areas particularly for particles above about 5μ diameter (unit density). Dust particles of this dimension have quite appreciable settling velocities (8×10^3 micron/sec) due to gravity. Since their average residence time in the bronchiolar tree might be about two seconds (respiratory phase time) and the average distance from the center of the air stream to an epithelial surface is on the average no more than 2000 microns even at major bronchii, one would surmise that one important effect of impaction would be to shift the deposition site toward the nasal-pharyngeal region rather than to increase the almost certain deposition of these particles.

As in the case of sedimentation, the inertial force imparted to a dust particle will increase if the particular density is increased and this again will tend to shift the deposition of dust to an earlier site. It should be remembered that all inertial equations for small particles at low Reynolds number should include a "slip" correction factor.

C. Brownian Motion

Particles below 0.1μ diameter ($1000 \text{ \AA}$) show an increasing susceptibility to random motion imparted by air molecules, known as Brownian motion, although gravitation is still demonstrable, for example, by analysis of the distribution of particle sizes as a function of the height above the earth.

Particles undergoing Brownian motion are characterized by a

series of relatively high velocity excursions of relatively short duration in random direction. The displacement of particles in a direction, x , is denoted by the value of the mean square displacement of the particles. $\frac{\bar{x}^2}{\Delta t}$ during time, t , in accord with the Einstein equation (17).

$\frac{\bar{x}^2}{\Delta t} = 2 D t' (S_c)$ where D is the diffusion coefficient and S_c is the slip correction factor. The diffusion coefficient for spherical particles, D , is equal to $k \frac{1}{3 \pi \eta d}$ where k is the kinematic gas constant, η , the viscosity of air and d , the diameter of particle; therefore, D is equal to k times the particle mobility, Z . (loc. cit.). Note that the density of the particle is not involved.

If a particle of high mobility (small d) is placed in a system where particles are only a few microns from a surface in any direction (small x) and the time is prolonged, (large t) the likelihood of particle deposition with a high, $x \propto \sqrt{t}$. Another useful form of the diffusion equation states that the net transfer of particles, P , onto or across a perpendicular plane will be $P = 1/2 \frac{\bar{x}^2}{\Delta t} \frac{dn}{dx}$ wherein n = number concentration of particles.

An interesting experimental device has been described (18) which makes use of the differences in diffusion capacity of particles of different size. Since it involves a series of closely packed parallel plates, it has been called a "diffusion battery". Damarcus and Thomas (19) found that the fraction of particles of a given size passing through a given battery was a function of the transit time and the diffusion coefficient of the particle. They investigated particles from 200 Å to 6000 Å diameter.

If a equals the separation of the plates and b and z their height and length, respectively, and Q the flow through the system, then F , the fraction of particles of a size that emerged from the system, was found to be proportional to $K_1 \exp^{-\alpha} + K_2 \exp^{-11.4 \alpha}$ where $\alpha = \frac{K_3 D b z}{Q a}$, K_1 , K_2 and K_3 were found to have the values 0.91, 0.05, and 3.77 respectively and D is the diffusion coefficient of the particle involved.

As Q changes in a given structure of a , b and z dimensions, then the transit time changes accordingly. If one can measure a value of F then it is possible to solve for D . Actually D is best determined graphically by extrapolating to zero flow because the flow is not precisely laminar in such an experimental system.

It is also interesting to note that the Demarcus and

Thomas equation is substantially that of Townsend's (20) who proposed an equation for diffusion in a circular tube during laminar flow.

In the lower respiratory tract one can envision a tremendous assembly of diffusion batteries of essentially circular cross-section. Consider the terminal bronchioles, for example. It is believed that there are between 5 and 6×10^4 of these, each about 0.3 cm long by 0.06 cm diameter. Distally to these structures is a even greater number of finer tubes approaching alveolar dimensions (0.02 to 0.03 cm). From this picture of lung architecture one can imagine the high probability of deposition of any particle reaching this region.

Findeisen (21) estimated, using a model lung and respiratory cycle, that the deposition of particles 0.03 μ diameter would be 55 percent complete in the parenchymal region. Many experimental studies such as those by Dautrebande (22) and Verzar (23) indicate a much greater deposition, 70 - 100%, for submicronic sizes. An interesting observation was reported by Wigand (24) who found with a nuclei counter that the air he breathed contained about 25,000 nuclei per cm^3 in the inspired air but only 1350 nuclei per cm^3 in the expired air.

D. Other Modifying Factors

1. Coagulation

There are several other phenomena of interest which are associated with submicronic particles. Besides interacting with surfaces, small particles interact with other particles, a process known as coagulation or aggregation. According to the studies of Whytlaw-Gray and co-workers in the early thirties (25), the number of (smoke) particles per unit volume of air changes with time at a rate proportional to the square of the number concentration, n^2 ; viz., $\frac{dn}{dt} = K'n^2$, where K' is a constant for a given sized aerosol and is larger the smaller the particle size (K' includes a slip correction factor). Since in a coagulating aerosol the particle size is continuously increasing, the values of K' and n are also continuously decreasing.

Experimentally, it has been shown that the coagulation rate of a polydisperse aerosol can be faster than that of a monodisperse aerosol. This is a function of the ratio of the particulate radii involved and of their absolute values. It has also been reported that the effective radius for coagulation is possibly 10-20 percent greater than the geometric radius of the particle due to intrinsic forces such as Van der Waals forces (26). Quantitation of these effects is complex and at best approximate; however, it is interesting to note that they

are in the direction of increasing the instability of aerosols.

2. Electrical Charge

Nearly all aerosols produced in the laboratory by grinding, aspiration, atomization and combustion are found to be electrically charged. Usually the net charge of the aerosol is zero but under certain circumstances may be predominantly of a single sign (27). In any event, the presence of charges on particles would be expected to induce additional properties in them.

The work of Kunkel (28) and of Dalla Valle and co-workers (27) indicates that, in general, only a few electrical charges per particle occur. Condensation aerosols, such as those produced by the LaMer generator, show negligible initial charge but may then accumulate charge by the diffusion of gaseous ions. Therefore, one may conclude that charged particles are a natural phenomenon.

The question then arises as to the possible effects of such charging. One could predict that the attractive and cohesive forces between particles, the so-called effective radius for coagulation, would be increased; the vapor pressure of small particles would be reduced, thus affecting both condensation and evaporation processes (9) and that the migratory movement of particles would be increased in an electric field. Despite the fact that the effect of charges has not yet been fully assessed for each of these phenomena, it is probable that the over-all effect is unimportant except for charge on coagulation and this, too, is probably minimal due to the small number of charges involved. Certainly, in animals there is no need to consider the effect of an electric field as none exists within the airways of the respiratory tract except that due to the charged particles themselves.

3. Condensation

The role of submicronic particles as condensation centers was mentioned in the preceding remarks. Hygroscopic particles can initiate condensation at relative humidities well below saturation, e.g., 70% (29). Schiff et al have reported that among a group of inorganic salts, their efficiency as condensating nuclei for water could not be correlated to their solubility, crystallization nor hygroscopicity (30). It is well known that there is a critical lower size limit for self-nucleation and it ordinarily requires high supersaturation (4 times or more) for condensation to be initiated without nuclei present (31). In addition, it is generally stated "insoluble" or non-hygroscopic particles may only be important as condensation nuclei where saturation or supersaturation prevails. Yet the role of such particles and the possible mechanisms involved is clearly not established. For example, they may absorb a monolayer of the

vapor phase and then proceed to behave as though they were a droplet form of the vapor. Whether or not it is important for the particulate to be isomorphic with the condensed solid phase is still questioned, and the probable role of microcapillaries in the particle surface (32) is not established. Landsberg (33) reported that continental aerosols are composed of both soluble and insoluble nuclei and Wait (34) has indicated that insoluble nuclei are important in natural condensation phenomena.

While the electric charge, chemical nature, and radius of curvature of the particle are among the factors influencing the initiation of condensation, the principal factor governing the rate of growth of small particles is diffusion. This growth process under ideal conditions is isothermal but under normal conditions the growth rate frequently deviates from the predicted presumably due to such factors as turbulence and temperature changes.

All direct experimental evidence uniformly implies that submicron hygroscopic particles will grow in size within the water-saturated (or near saturated) air of the respiratory tract (35) (36). The ultimate effect of this growth will depend principally on size of the particles involved and their residence time within the respiratory tract. It is conceivable that some small particles may actually grow to a more stable size while others will acquire sufficient mass to become more unstable and sediment more rapidly and completely.

4. Thermal Effects

In the environment, thermal gradients play an important role in the distribution and deposition of dusts. As Saxton and Rantz have shown experimentally (37), the thermal force is proportional to the temperature gradient and the particle diameter. The differential bombardment of the particle by gas molecules produces a motion of the particle toward a cooler area. If the thermal conductivity of the particle is low, an unequally heated particle may result. This, in turn, induces a radiometric effect in which a layer of gas molecules "flows" from the heated side thereby creating an additional force to move the particle toward the cooler region (38).

In the respiratory tract, if a thermal gradient exists, it is low grade, short-lived and would tend to be repulsive rather than attractive as the particles and the air would ordinarily be cooler than the mucosa of the tract. After the inspired air reaches temperature equilibration, the resulting increase in the absolute temperature (10%) would be expected to produce some slight increase in Brownian motion phenomena. In both instances, it is probable that thermal effects on dust deposition are of minor importance in the respiratory tract.

5. Dust Concentration

References are frequently made to studies (7) which relate the concentration of dust breathed to the amount of dust deposited in the respiratory tract of subjects. For example, in one study as the dust concentration was raised from 3 to 80 mg/m³, the dust deposition was reduced by 50 percent. The inference is that as the mass concentration rose, the amount of dust respired decreased. This would follow if (a) the number of very large particles ($> 50 \mu$) was increased concomitantly or (b) if the number concentration of all particles was increased appreciably or (c) if both conditions occurred together. In the first instance, it is presumed that the dust could be sampled quickly enough to sample the larger non-respirable particles. These particles would effectively determine the mass of dust sampled while not representing a true dust concentration with regard to respirable sizes. In the second case, the rate of aggregation would be greatly accelerated creating the same general picture; viz, a large non-respirable particulate population.

One can imagine circumstances wherein the measurement of the mass of dust per unit volume of air might represent any of a variety of particle size distributions. Consequently, a change in mass concentration might be expected to either decrease, increase, or not change the deposition and all of these cases have been encountered (7). This statement is made with respect to the measurement of the percentage of the aerosol mass inspired that is deposited but frequently applies to the absolute amount of dust deposited.

The two parameters which must be analyzed to estimate the effect of altering dust concentration are the particulate concentration and the particle size distribution. However, these must be analyzed in terms of the time of the exposure since the system is undergoing active aggregation and sedimentation, causing the dust being breathed from moment to moment to differ.

It is reasonable to generalize that to increase the mass concentration of an airborne dust is to increase the instability of the dust. Since the major changes produced would involve the loss of small particles by aggregation and the loss of large particles by sedimentation, one would predict the respiratory tract deposition picture would shift to favor dead space deposition, though reduced. Ultimately it would be expected to reflect the deposition of an aerosol, with much less heterogeneity, i.e., principally consisting of particles around 0.1 - 0.5 μ diameter. And if the dust ages a few minutes before it is respired, the particulate concentration will likely be less than 10⁶ particles/ml air (25). These aging effects would not be discernable, however, if the dust was being generated continuously.

E. Discussion

This brief view of the various physical forces involved in dust deposition suggests that in the respiratory tract the dominant forces are (a) gravitational settling for the large particles ($> 0.5 \mu$ dia.) and (b) Brownian motion impaction for the small particles ($< 0.1 \mu$ dia.). Small particles may also be deposited by sedimentation following aggregation with large particles. Since qualitatively, the same forces and circumstances prevail in the environment that prevail in the respiratory tract, one can conclude that between 0.1 and 0.5μ dia. is a region of particle size which is least affected by combined Brownian motion and gravitational forces and, therefore should constitute the most stable aerosol configuration. Deposition data from human and animal studies support the viewpoint that percentage wise, fewer particles in this size range are subject to deposition (39) (40). This particular size region is often referred to as the size for minimal deposition. There is a growing misconception that this represents a region of particle size of relatively less importance to deposition than any other. This is traceable, in part, to particle size deposition relationships such as reproduced in Figure 1 (21). (See also Figure 3).

These data are based on the percentage of particles of a given size being deposited (retained); however, there is no indication of the relative or absolute numbers of such particles that may be involved. In aged aerosols, since this $0.1 - 0.5 \mu$ dia. size region is more stable, greater numbers of these particles will probably be present to be breathed. Even in freshly prepared aerosols, the relative numbers of particles in this may be orders of magnitude greater than those above 5μ . If one considers a factor of 5 as encompassing the variability of particles of different size in terms of deposition (20 - 100%), then the relative and absolute greater number of 0.1μ dia. particles deposited may greatly exceed the number and even the mass contribution of particles 5μ dia. which are deposited.

In many toxicological situations the hazard is proportional to the mass of the aerosol deposited. When this is so, the largest particles penetrating the respiratory tract are probably the most significant. This is unquestionably the case with a readily soluble or absorbable material as was so aptly demonstrated by Punte (41).

If the significance of the exposure is based on the penetration and retention of relatively insoluble material in the parenchymous areas of the lungs, the role of the large particles ($> 1 \mu$) is probably of little consequence. Moreover, the relatively high deposition and retention of small particles ($< 1 \mu$) in the lung parenchyma has been repeatedly demonstrated

Figure 1

LOWER RESPIRATORY TRACT DEPOSITION TAKEN FROM THEORETICAL DATA OF FINDEISEN

Using a model respiratory cycle and respiratory tract, and by adding the contribution of sedimentation, inertial impaction and Brownian motion at each particle size at various anatomical levels, Findeisen was able to construct the deposition curve shown. Unit density spheres were the particles assumed.

(22) (23). This suggests that a measurement such as the specific surface area of the dust may prove to be a better index of its retention (solubility) and of its propensity to produce localized toxic reactions than does its mass. Such a circumstance was presumably encountered in one investigation involving oxides of beryllium, wherein their acute toxicity was apparently related to their specific surface areas as determined by gas adsorption (42). In addition, there is evidence that airborne particles may serve as vectors for the admission of toxic vapors (43), micro-organisms (44) and radioactive atoms (45) into the respiratory tract. This probability also suggests the importance of surface area or other parameters than mass; thereby, adds to the significance of small particles.

To decide which particles are the most significant in assessing the hazard of a given dust exposure, consequently, requires some knowledge of the toxicology of the material. Such matters as solubility, the response of tissues to the "insoluble and soluble" forms of the chemical moiety, some appreciation of the dose-response characteristics of the material and species involved, etc., are often decisive. The more physical, chemical and biological data available, the more likely that a correct judgment can be made regarding the dust exposure.

These facts do not reduce the attention which submicron particles deserve in toxicological and particularly in radiological situations. This attitude is justifiable for two important reasons, (a) the knowledge that these particles are indigenous to all environments of man, and (b) the overwhelming evidence that they have an inordinately high deposition and retention, and more often than not, prove more toxicogenic.

Whatever dust sampling devices are used for the evaluation of dust hazard, they should be evaluated in terms of their ability to measure small particles as well as the large. This, in turn, implies further requirements for particulate analysis, such as electron microscopy as a routine.

In most inhalation studies, estimates of mass and surface area distribution, etc., are obtained by first determining the size-frequency distribution (7) (9). Specifically, if a particulate population follows commonly found frequency-size distribution, it can be normalized using the logarithm of the particle size. After being normalized, the median size and standard deviation of the distribution can be determined. With these two parametric functions, various other relationships of the aerosol, such as surface and mass, are predictable and related to the size distribution. A typical size and mass distribution is plotted in Figure 2.

Without the benefit of such a statistical treatment, the

Figure 2

In this particulate distribution approximately 40 percent of the mass resided in particles between 0.1 - 0.25 μ diameter. Though no particles were seen or measured in excess of 0.2 μ the distribution predicts that about 0.001 percent of the population will be larger than 0.3 μ and these few particles will also contribute about 40 percent of the mass of the system. On the other extreme, note that even though 90 percent of the particles in the aerosol are less than 0.1 they contribute only about 15 percent to the total mass.

REPRESENTATIVE PARTICLE SIZE ANALYSIS PLOT

determination of these various relationships is complex and at the present time, technically not feasible. Without these relationships, many deductions based on particle size are not tenable. It is obvious that the phenomena of interest, especially sedimentation and Brownian motion, are so size related as to demand the consideration of particle size as the primary physical factor affecting deposition.

Physiological Factors

The air of the respiratory tract cannot be simplified into a static compartment of warm, moist air. The ability of the lung air to change in composition, volume and aerodynamics continuously, brings additional factors into play which also affect dust deposition.

The composition of respiratory tract air changes continuously chiefly because it is unevenly and periodically ventilated. Among other things, this implies that in different parts of the lung, the particles of dust inhaled will have different residence times and that deposition will not be uniform.

A. Effects of tidal volume and respiratory frequency.

The volume of the respiratory tract varies about 15 percent with each tidal volume. Approximately one-third of this air, the so-called dead space air, can be considered to reside in the respiratory tract for the duration of one respiratory cycle. This will be the case for dust suspended in this air volume also, except for that dust deposited during the cycle time. If the volume of the respiratory tract is periodically increased (by deep breathing) the number of dust particles breathed is increased and the depth of the tract to which they reach is also increased. If the respiratory frequency is decreased concomitantly, the mean residence time of the particles is increased -- even those in the dead space air because the cycle length is prolonged. This combination of increased volume and time would be expected to increase deposition on theoretical grounds and this expectation has been repeatedly verified by experimentation (40) (46)

On the other hand, when the respiratory frequency is increased, the mean residence time for the particles in the anatomical dead space should decrease. Depending on the tidal volume, there should be an increase in air effective in increasing deposition and particularly, for shifting the sites of deposition but interrelations with other factors, such as shortened residence time, suggest these latter phenomena may be more effective in altering dead space deposition than alveolar deposition.

The foregoing picture is basically an analogy of the lung ventilation process. Let V_A equal the alveolar ventilation (volume/unit time), F the respiratory frequency, V_T the tidal air column and V_D the anatomical dead space volume*; then $V_A = F (V_T - V_D)$. This implies that if one breathes with a 6 liter minute volume and increases the respiratory frequency from 8 to 24 cy/min while decreasing the tidal volume from 750 to 250 ml, he will increase his dead space ventilation (total ventilation-alveolar ventilation) four-fold.

When one considers the intimate relationships involved in respiration, it becomes extremely difficult to isolate primary determinants. As already cited, in investigating the effect of an increasing tidal volume at a constant respiratory frequency, the question arises as to which of the volumetric or dynamic changes involved are responsible for the effects on deposition seen.

Despite the difficulties involved, there have been many experimental studies (39, 40, 46, 47) reported which demonstrate that the amount of dust deposition occurring is greatly affected by the character of respiration. The amount of change in deposition that can be induced is at least comparable to that produced by different particle sizes. The following graph (Figure 3) summarizes many of the important inhalation studies in which some recognition was given to the physiology of respiration.

From these data we can see, for example, an effect of changing from slow to fast respiration on the deposition of 0.5μ dia. particles (Wilson and LaMer) was to decrease the percentage deposition from approximately 70 percent to approximately 35 percent. In Landahl's study, changing the tidal volume and respiratory frequency to maintain a constant minute volume resulted in qualitatively the same effect on the deposition of 0.5μ particles but not to the same extent. However, in the case of 1μ particles, the percent deposited was more than doubled when the respiratory frequency decreased from 15 to 5 per minute and the tidal volume increased from 450 to 1350 milliliters.

There are obviously many other volumetric and aerodynamic parameters that can be measured during respiration. Among these there may be significant relationships to deposition, but at present, this is pure speculation. Also related to the deposition phenomenon are certain effects induced by dusts on the respiratory physiology. DuBois and Dautreband (50) have shown that the respiration of insensible amounts of dust cause

* Unlike the functional or physiological dead space, the anatomical dead space is a nearly constant volume ranging from 150-170 ml.

Figure 3

EXPERIMENTAL DEPOSITION DATA HUMAN SUBJECTS

Wilson and LaMer (40) 5-1/2 cy/min (closed circles and line), and
20 cy/min (closed circles and dashes)

Landahl and co-workers (14) 5 cy/min (triangles and line), and
15 cy/min (triangles and dashes)

Altshuler et al (46) (Subject P) 9 cy/min (crosses and dashes), and
21 cy/min (crosses and line)

a decrease in lung compliance. This effect is one which involves the constriction of the fine air tubes and possible parenchymous structures. While this response must be considered normal and not discomforting to the subject, it may have a considerable bearing on the ensuing dust deposition pattern. This constrictor effect of dusts is also being studied as a possible factor in the genesis of pulmonary dysfunction and disease.

B. Discussion

To date, most physiological relationships in dust deposition have been obtained empirically. Although some attempts have been made to predict deposition as a function of physiologic measurements, these formulations so far are unpretentious and admittedly inaccurate. It has been necessary, however, for certain "Committees" to create formulations upon which they hope conservative estimations of hazard can be based. An outstanding example of this is the so-called Harriman Conference model (51) which states that standard man will breath 2×10^4 liters of air each day and will deposit 75 percent of the dust he breathes. Of this only one-third will be deposited in the lower respiratory tract (i.e., 25%). In addition, the lower respiratory tract retention is presumed to follow a pattern whereby one-half ($12\frac{1}{2}\%$) of the dust will be redistributed in 24 hours and the remainder retained for 120 days (insoluble dust). The estimate of 2×10^4 liters of air breathed per day and the over-all deposition figure of 75 percent are conservative ones and would not be expected to underestimate an exposure. However, the lower respiratory tract deposition value of 25 percent and the clearance rates suggested could conceivably misjudge an inhalation exposure to the lower respiratory tract to an important degree. Recalling, for example, Verzar's (24) work in which dogs breathed a submicron aerosol through their nose and through a tracheal cannula, it was found that the upper respiratory tract contributed only a small amount, viz., it reduced the over-all dust deposition from 77 to 64 percent.

The propriety of selecting certain clearance rates is beyond the scope of this discussion but reference is made to Part II of this paper (Casarett) to the NAS - NRC Inhalation Hazards Subcommittee (53) and Committee on Pathologic Effects (54) reports.

C. Summary

A brief summary of the various physical and physiological effects discussed are given in Table I.

At present it is not possible to predict a great deal about a dust exposure. Retrospective analysis is often more

TABLE I

Factors	Particles Involved		Related Factors	Primary Effects	Special Considerations
Sedimentation	0.1	50 μ	Particle shape and density. Particulate concentration (aggregation), "slip", hygroscopicity	To cause dust deposition in nasal pharynx and tracheal- bronchial tree	Highly significant in toxicological studies, probably the most important deposition factors. Especially significant with "soluble or absorbable" dusts.
Inertia	0.5	50 μ	Same as above plus relative velocities of particle and air	Same as above with tendency to promote earlier deposition.	
Brownian motion	0.002(?)	0.5 μ	Electric charge, hygroscopicity, particle concentration (aggregation), thermal gradients	Dust deposition where large and intimate surfaces involved, viz., lung bronchioles and parenchyma	Most important when dust is insoluble and submicronic Also significant when these dusts serve as vectors.
Respiratory frequency	all sizes		Air velocities, residence times, turbulence, dead space ventilation	Increasing tends to <u>decrease</u> deposition (possibly very high rates e.g. 20 may increase deposition)	Exercise, physical labor, and heated environs will have variable effects on subjects but, in general, will tend to increase both these factors together.
Tidal volume	all sizes		Numbers of particles, alveolar ventilation, residence time	Increasing tends to <u>increase</u> deposition (even at constant minute volume.)	

fruitful. Even then, we only obtain an approximate picture for deposition is inherently concerned with more than the amount of dust invading the respiratory tract -- but also the site of the deposition and the physical-chemical nature of the deposited dust; therefore the deposition process is inseparably related to, if not a principal phenomenon governing retention in the respiratory tract.

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MECHANISM OF CLEARANCE OF INHALED PARTICULATES FROM THE LUNG

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Following the deposition of particulates in the lung the persistence of retention of these particles in the lung depends upon a number of factors. Some of these factors may be directly related to the particle, e.g., chemical nature, size, solubility and the like. Other factors influencing retention concern the physiological response of the organism and its interaction with the particle.

The physiological response is, of course, also dependent to some extent upon the physico-chemical nature of the particle. For example, as was discussed by Dr. Morrow, the characteristics of the particle influence its site of deposition in the lung. This, in turn, may determine the immediate disposition of the particle. Thus, the morphological characteristics of lung tissue at the site of deposition and the state of the functional elements of the tissue must be taken into account. In the present discussion of the mechanism of lung clearance, emphasis will be placed on the role of the tissue elements in movement of a relatively "insoluble" particle.

Classically, one finds listed several mechanisms by which a particle may move or be moved within the lung or out of the lung. Some of these have been demonstrated adequately while others may be in considerable doubt. If a particle is deposited at or above the level of the terminal bronchioles, it follows a well-established route of clearance. At this level it encounters a ciliated mucous epithelium and is moved up the respiratory tree to the pharyngeal region from which area it leaves the body by expectoration or enters the gastrointestinal tract by swallowing.

If however, a particle is deposited in the lower reaches of the respiratory tract, e.g., in an alveolus, it is in an area devoid of cilia. Thus, any movement from this region must be by another mechanism. Putting aside the discussion of mechanisms for the moment, one can list the possible fates of "insoluble" particles.

- (a) Particles might persist in parenchymal regions for long periods of time.
- (b) They might find their way out of alveolar regions to areas of the respiratory tree which

have a ciliated epithelium. From such a location, they would be moved up the respiratory tract and out of the lung.

- (c) Particles might move or be moved to other areas of lung, e.g., into lymphoid tissue or interstitial regions.
- (d) Finally, particles might enter the systemic circulation, either by way of blood or lymph from parenchymal or other regions of the lung or from other tissues.

Irrespective of what the disposition of the particle is, there are several fundamental questions as to how the movement occurs or why movement does not occur. With regard to the former question, two primary alternatives are presented. First, there is the problem of whether the particle per se moves without cellular intervention. For example, can a particle directly penetrate blood and lymph capillaries or the alveolar epithelium or can it enter the upper respiratory tract from the alveolus? The alternative is, of course, that movement of particles occurs by virtue of phagocytosis and subsequent movement of the phagocytic cells.

With these questions in mind, I should like to discuss some aspects of our observations following administration of polonium 210 via the lung. Although we have information following intratracheal injection in the rat and the rabbit, the present discussion will be limited almost exclusively to an inhalation experiment in rats. Analytical results will appear in detail shortly as will the detailed observations on lung clearance mechanisms. However, a brief description of the experimental plan should be presented.

Figure 1 is a diagram of the exposure apparatus. Polonium solution (neutral) is held in reservoir (A) and fed into the aerosolization chamber (B). Air at 30 psi is blown through the aspirator jet (C) while diluent air is added at D and the mixed aerosol passes through a baffled conduit (E) to the exposure chamber (F). H and I are molecular filters acting as traps ahead of the vacuum system while J is also a molecular filter to sample the air in the vicinity of the apparatus.

The aerosol was generated from a neutral NaCl solution (about .015% and yielded a particle size population with a count median diameter of .056 μ and a geometric standard deviation of 2.2. It should be pointed out that subsequent to the deposition of NaCl in the lung, the polonium colloid which is subsequently followed is of the order of 50 - 200 Å. It is recognized that this is somewhat unlike the usually encountered particulate material. However, it is expected

FIG. 1 INHALATION EXPOSURE APPARATUS

that there is a parallelism between these and somewhat larger particles and the use of these radiocolloids represents a test for some hypotheses about the behavior of particles in general.

Forty-four rats were utilized in this study. The animals were lightly narcotized with hexobarbital sodium and placed in glass chambers (G in Figure 1) so that only the snout extended through a slit in a rubber diaphragm into the exposure chamber. Rats were exposed for 20 minutes, immediately placed in metabolism cages and individual urine and feces collections made until sacrifice. Animals were sacrificed immediately after exposure and in pairs at 0.5, 1, 2, 4, 6, 8, and 12 hours and 1, 2, 3, 5, 10, 15, 20, 25, and 30 days after exposure. All major organs and residual carcass were analyzed for polonium. Samples of tissue from lung and other major organs were taken and autoradiograms prepared by routine methods.

Figure 2 shows the rate of lung clearance. The lung loads for all animals sacrificed up to 24 hours were essentially the same and a mean of these values constitutes the 100% point. It will be noted that the disappearance of polonium from the lung corresponds closely to an exponential function with a half-time of 17.5 days. This clearance time represents the sum of a variety of mechanisms which are operative simultaneously. The following group of pictures was selected to illustrate some of these mechanisms.

Of the material deposited in the lung, virtually all of the radiocolloidal polonium was deposited in parenchymal regions. Plate 1 shows such a region at 30 minutes after exposure. The radiocolloid is manifest autoradiographically as groups of radially oriented alpha tracks. In addition to the presence of material in the alveoli, it will be noted that there is the start of some mobilization of material in parenchymal tissue around small bronchioles. As we have mentioned, from this region, it has been suggested that particles can move directly into blood or lymph systems.

In Plate 2, an aggregate can be observed in the lumen of a blood vessel. There is no apparent cellular association suggesting that the particle per se has entered the vascular system. However, this slide is from a rabbit which received polonium by intratracheal injection. Similar observations have been made in the rat after intratracheal injection. After inhalation exposures in rat and dog, no such occurrences were observed. It has been concluded, tentatively, that apparent entry of particles directly into the vascular system is an artefact of the intratracheal injection technique and its sequelae such as changes in vessel permeability, collection of edematous fluid, inflammatory reactions and the like. It should be remembered that the size of the radiocolloids which apparently do not penetrate vessels is much smaller than particles usually utilized in such studies. If these do not enter, it is doubtful whether such a mechanism is

Retention of Polonium in the Lung following
Inhalation (Individual animal zero time lung content reconstituted)

of very great significance with the larger particles.

On occasion there has been some doubt as to whether particles of the size used in this study ($.056\mu$ CMD) are deposited in alveoli. Plate 3 shows the autoradiographic manifestation of a polonium radiocolloid on an alveolar surface. If one focuses on the tissue more sharply, one finds that the center of the rosette of tracks corresponds to the cytoplasm of a lung macrophage. This plate is from a rat sacrificed 30 minutes after exposure and phagocytosis of colloids has progressed rapidly at this time.

In plate 4, the focal plane has been adjusted to show a lung macrophage and indicate the center of the sunburst of tracks corresponding to that cell. By two hours after inhalation virtually all of the aggregate present in alveolar regions are either in mobile macrophages or in alveolar epithelial cells. This finding bears on another point of controversy, viz., the mechanism by which particles enter the interstitial tissue of the lung. While, as mentioned above, essentially all colloids in parenchyma have been phagocytized by two hours after inhalation, virtually none is found in interstitial tissue.

Plate 5 illustrates a typical finding 24 hours after inhalation. One can discern that a colloid is apparently present in a macrophage in the interstitial region. In view of this timing sequence, it appears clear that whatever the source of the macrophage, polonium radiocolloid per se does not enter the interstitial tissue, but rather depends upon phagocytosis in parenchymal regions with subsequent movement of the phagocytic cell into the interstitial region. The myriad observations bearing on the details of this mechanism are beyond the scope of this paper. However, it may be stated briefly that the mechanism has a plausible basis on the hypothesis that particles engulfed by primitive forms of the lung macrophages (e.g., alveolar epithelium) are moved into interstitial spaces by the rounding of the cell and the influence of tissue pressures. On the other hand macrophages (with particles) which continue to appear in the upper respiratory tree are the more mature phagocytic elements which were partly delineated from the underlying epithelium at the time the particle was phagocytized.

Turning to the movement of colloids up the respiratory tree, it is apparent that beyond 2 hours after inhalation, movement to a ciliated epithelium occurs by virtue of cellular transport, as all colloids have been phagocytized by that time. However, at earlier periods, colloids which presumably had been deposited in alveoli were found in the small bronchioles having arrived there apparently without being phagocytized. It is suggested that the forces involved in the movement of these are similar to those responsible for the movement of the macrophage in the direction

of the ciliated epithelium. In the upper reaches of the respiratory tree, one finds no colloid which appears to be free of cellular association. It is, therefore, further suggested that phagocytosis of particles occurs in the bronchiolar tree by macrophages moving in this direction during the normal turnover of alveolar cells.

Plates 6 through 9 illustrate the movement of particles from parenchymal regions into small bronchioles (Plate 6), into medium-sized and large bronchioles (Plates 7 and 8) and along the tracheal mucosa (Plate 10). Several inferences of possible significance can be drawn from observations connected with this pattern. First, the smaller bronchioles might be expected to get a greater radiation dose from radioactive particles than the larger bronchioles. This is supported by the smaller caliber of the lumen of these passages and also by the fact that movement in the smaller bronchioles is slower.

Secondly, as illustrated by Plate 7, there often appears to be a collection of material at the entrance of the medium sized bronchioles so that a high local dose is received by the mucosa at these points. A corollary to this observation is that the dosage at branch points of all of the bronchiolar structures is much higher than the adjacent mucosa. This is well illustrated in Plate 8. Plate 9, illustrates a consistent finding throughout the bronchiolar tree. In the center of the field there is apparently a colloid which has penetrated the mucosa of the trachea. Although this was consistently found, it is doubtful that this manner of particle movement is of any quantitative significance.

Another route of particle movement of some importance is entry into lymphatic tissue. Within 12-24 hours after intratracheal injection or inhalation some material appears in lymphoid tissue of the lung. Plate 10 shows a large amount of radioactivity in a small peribronchiolar lymphoid mass after intratracheal injection. At later time periods after intratracheal injection one finds evidence of colloids in lymphoid tissue surrounding the larger bronchioles (Plate 11).

Whether absorption into the lymph has occurred at the latter sites or whether absorption occurs in lower regions with subsequent movement of particles to the upper reaches is not known. However appearance of material in the upper lymphoid elements without material in the bronchioles themselves (as in Plate 11) suggests that lymphatic absorption is occurring in the regions of the smaller bronchioles. Although most colloids in lymphoid tissue appear to be intracellular, some are not. Thus it is not known whether phagocytosis is prerequisite to entry of material into lymphoid tissue. However, peak concentrations seem to be reached at time periods well beyond the time at which cell particles have been

phagocytized so that much, if not all, of the material enters the lymphoid tissue by cellular transport.

Finally, one observes, with polonium colloid at least, a progressive solubilization of the particle. This is visible in autoradiograms as a progressive disorientation of the tracks from a radial arrangement. This process occurs with all of the radiocolloids observed in tissue until at 30 days after exposure the polonium in the body no longer exhibits a colloidal form. Rather alpha tracks are virtually all single tracks suggesting a more nearly "ionic" form.

Although this process is observed throughout all tissues, it is most readily apparent in macrophages and/or in lymphoid tissue. To what extent solubilization might occur with larger particles of a less soluble material is not known. However, it is suggested that the probability of its occurrence is a complication in predicting events which occur after inhalation of an "insoluble" particulate.