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THE QUANTITATIVE DETERMINATION OF PLUTONIUM IN BIOLOGICAL MATERIALS

PART III: THE ANALYSIS OF TISSUES

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Most of the work in this report has been privately communicated to the author and therefore little mention is made of the persons who have contributed to the work.

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E. R. Russell

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1. Introduction

The quantitative determination of plutonium in the various tissues of the body is of importance from several standpoints. First, the distribution of the element in the tissues may be determined. Second, therapeutic efforts may be followed and evaluated. Finally, the question of uptake of plutonium by humans may be investigated.

In order that the quantity of plutonium may be determined by its alpha disintegration, it is necessary to separate it from the tissue either by a direct extraction or from a solution of the ashed material. The former case would probably be difficult as the nature of the deposition of plutonium may be different in each type of tissue and therefore no uniform procedure could be adopted.

It is apparent that the concentration of plutonium may vary widely according to the conditions of the experiments. Where the experimental tests involve animals, the concentration of plutonium in the ashed tissue solution may be sufficiently high so that a known portion of the solution may be evaporated on a platinum disc and an alpha count made. If the concentration of the ash in solution is sufficiently small, the aliquot may be chosen so that no significant mass absorption of the alpha particles occurs. Difficulty, however, arises when plutonium concentrations are so small that large amounts of tissue must be ashed and all of the resulting solution assayed. Between these two extreme cases the concentration of plutonium in the ash is such that a simple co-precipitation of plutonium with, for example, lanthanum fluoride will separate the element from the unwanted salts, permitting accurate determination of the amount present.

The methods which might be used to analyse tissues for plutonium may be divided according to the concentration of the element in the tissue. It is understood that the method applicable to very low concentrations of plutonium are also applicable to others. However, since there are many more operations involved in assaying low concentrations of plutonium, it is desirable to use shorter methods provided they are reliable. Tissues containing more than 1 microgram of plutonium per gram may be assayed by counting a small aliquot of the solution ash. Tissues containing less than 0.05 microgram of plutonium per gram will require a preliminary extraction from the solution ash. In between these concentrations a simple coprecipitation may be used.

2. Methods for Determining Plutonium in Tissues

2.1 Tissues containing more than 1 microgram of plutonium per gram:
A. Ashing. Tissues are usually brought into analyzable form by either wet or dry ashing. In this case wet ashing was selected because the tissues are small and could be expected to be quickly and economically wet-ashed. It is advisable to avoid such ashing agents as sulfuric acid

as subsequent analytical difficulties result. Nitric acid and hydrogen peroxide ashing will result in a residue containing soluble salts which have not been found to interfere with a plutonium precipitation.

The tissue - femur, lung, liver, muscle, kidney, spleen, or mouse carcass is dried at 110°C for 24 to 36 hours and transferred to a 300 ml, long neck, Kjeldahl flask clamped at an angle of approximately 20°. Two ml of fuming nitric acid are added slowly, (15 ml for carcass). After the violent action has ceased, the flask is heated gently until practically all of the liquid is driven off. The residue is allowed to cool several minutes and 1 ml of superoxol slowly added. When the action has subsided the contents are again brought to near dryness. The nitric acid and peroxide treatments are alternated until a white or light gray residue is obtained. A black or dark colored residue indicates imcomplete ashing. Some residues, mainly from liver tissue, are rust colored; in these cases the technician must rely on experience to tell if the ashing process is complete.

The completely ashed sample is treated with 1 ml of concentrated nitric acid, warmed for a few minutes and diluted with 5 ml of 0.1 M nitric acid. The sample usually dissolves completely with agitation. The solution is transferred into a 100 ml volumetric flask. The Kjeldahl flask is washed with three 5 ml portions of 0.1 M nitric acid. The washings are added to the volumetric flask. After cooling, the solution is diluted to the mark with water. The analytical procedure to be used to assay these solutions depends on the amount of plutonium present.

In the case of mouse carcasses, the specimen should be divided in order to introduce them into the Kjeldahl flask. The specimen is treated with 15 ml of fuming nitric acid which ignites them. The tissues burn vigorously. However, no loss of plutonium has been experienced at this point. When the violent reaction has ceased, the sample is treated as are the other tissues. However, 15 ml of acid and 5 ml of superoxol are used, instead of the smaller volumes listed above. For complete ashing as many as 10 acid-per-oxide treatments may be required.

The procedure as outlined will work satisfactorily for 95% of the mouse tissues. About 5% will contain an insoluble residue which contains, in some cases, as much as 30% of the plutonium. Special treatment of this residue must be undertaken. This is discussed below.

Rat carcasses may be ashed by this procedure but considerable amounts of reagents are required. A combination wet- and dry-ashing procedure, which will be discussed later, is more satisfactory.

B. Determination of Plutonium. The method to be used to deposit plutonium on a plate in order that an alpha count may be made is dependent on the concentration of the ash and of plutonium. In the case of mouse tissues, excluding the carcass, the solutions contain very little ash and high concentrations of plutonium. This would allow for either a direct lanthanum fluoride precipitation from an aliquot of the solution

or direct counting of a 1 ml or less aliquot evaporated on a counting disc.

Direct counting of aliquot. This technique consists of transferring 1 ml or less of the nitric acid solution of the tissue ash to a platinum disc, evaporating slowly under an infra-red lamp, flaming in a gas flame and counting. To obtain the maximum yield, the sample must not leave too large a solid residue, nor must it contain gaseous reagents which give rise to bubbling with consequent partial loss. For convenience this procedure has been used to assay mouse tissues whose ash solutions contained 500 or more alpha counts per minute per ml.

A preliminary test indicated that the method was reliable. In the test 0.1 ml and 1 ml of the same solution were counted. The data are given in Table I.

Table I.

Sample	Composition of Solution	Volume	Cts./min.
1	Ashed Femur	1/1000	526
1	Ashed Femur	1/100	5335
2	Ashed Liver	1/1000	676
2	Ashed Liver	1/100	6796

Simple lanthanum fluoride precipitation. Plutonium in the (III) and (IV) oxidation states is readily coprecipitated as the fluoride with lanthanum fluoride. Where the concentration of plutonium is too low to directly count an aliquot of the ash solution and yet too high to warrant an intermediate extraction step, a lanthanum fluoride precipitation to separate plutonium from the other inorganic constituents in the ash solution may be used. The concentration of calcium in the solutions of ashed mouse or rat tissues is low enough to permit as much as 25% of the solution to be assayed. Where the entire solution of the ash is used, calcium fluoride causes a heavy deposit on the counting discs and absorption errors result. The general outline of the procedure follows.

An aliquot of the nitric acid solution of the tissue ash is made up to 25 ml with 0.2 M nitric acid in a 50 ml tapered pyrex centrifuge tube and treated with 2 ml of a saturated solution of sulfur dioxide. The solution is agitated and allowed to stand 30 minutes for the reduction of plutonium to the lower valence states. Ten ml of 3 M nitric acid are added and 1 mg of lanthanum (1 ml solution containing 1 mg La^{+3}/ml)

is stirred in. To this solution is added 5 ml of hydrofluoric acid. The solution is agitated for several minutes and the lanthanum fluoride precipitate, which is not always visible, is centrifuged at 2000 RPM for five minutes. The supernate is discarded and the precipitate transferred to a platinum disc, dried, flamed and counted. The precipitate is transferred by means of a micro-pipette after slurring with a few drops of dilute nitric acid.

This procedure gives an average recovery of 83% of the plutonium on analysis of several hundred tissues. Some of the data for various technicians are given in Table II.

Table II.

Lanthanum Fluoride Carrying of Plutonium from Mixed Tissue Solutions.

Technician	La^{+3} conc.	Trial	Plutonium Recovery
A	1 mg/40 ml	1	84%
A	1 mg/40 ml	2	86%
A	1 mg/10 ml	1	83%
A	1 mg/10 ml	2	86%
B	1 mg/40 ml	1	82%
B	1 mg/40 ml	2	81%
B	1 mg/40 ml	3	84%
B	1 mg/10 ml	1	82%
B	1 mg/10 ml	2	80%
B	1 mg/10 ml	3	84%

As was pointed out, when the concentration of salts becomes too high very inconsistant results are obtained when making a simple lanthanum fluoride precipitation. This is illustrated very clearly by the results given in Table III. The analyses were on a solution of an ashed rat carcass contained in 250 ml of 0.2 M nitric acid.

Table III.

Carrying of Plutonium by Lanthanum Fluoride from an Ashed Rat Carcass Solution.

Solution	Final Volume	La^{+3} used	Plutonium Recovery
Rat Carcass	40 ml	1 mg	78%
Rat Carcass	40 ml	1 mg	77%
Rat Carcass	40 ml	1 mg	75%
Rat Carcass	10 ml	1 mg	62%
Rat Carcass	10 ml	1 mg	65%
Rat Carcass	10 ml	1 mg	59%
Rat Carcass	40 ml	1 mg	83%
Rat Carcass	40 ml	1 mg	86%
Rat Carcass	10 ml	1 mg	74%
Rat Carcass	10 ml	1 mg	06%

The results in Table III are rather misleading in that it appears that higher lanthanum concentrations carry less plutonium. The actual case is that a larger residue is obtained on the counting disc probably due to the higher ratio of calcium to lanthanum and therefore absorption during counting is responsible for the reduced recovery. When this same solution was diluted four-fold to render the ash content negligible and assayed for plutonium by coprecipitation with lanthanum fluoride, the results as shown in Table IV were obtained.

Table IV.

Carrying of Plutonium by Lanthanum Fluoride from Diluted Ash Rat Carcass Solutions

Solution	Final Volume	La^{+3} used	Plutonium Recovery
Rat Carcass	40 ml	1 mg.	91%
Rat Carcass	40 ml	1 mg.	93%
Rat Carcass	40 ml	1 mg.	94%
Rat Carcass	10 ml	1 mg.	101%
Rat Carcass	10 ml	1 mg.	95%
Rat Carcass	10 ml	1 mg.	94%
Rat Carcass	10 ml	1 mg.	98%

The collected data on the lanthanum fluoride carrying of plutonium suggest that it is quite satisfactory for most tissues with low ash content. When the ash content is greater than 1 mg/ml one can expect a decreased yield, primarily due to absorption of the alpha particles during counting.

R. D. Finkle[1] describes a method for assaying tissues for plutonium as follows:

"Dry ashing of soft tissues: The tissues are placed in pyrex beakers of appropriate size, held away from contact with the glass by a filter paper cone. They are dried at 100°C for 12 hours, and at 200°C for 24 hours, and finally ashed at 550°C for 48 hours. The filter paper stays intact throughout the 200°C treatment thus preventing contact of the tissue with the glass until it is thoroughly dried. The ash usually contains a small amount of carbon due to the limited temperature of ashing, and occasionally some crystalline insoluble matter, particularly in the case of liver. Bone tissue is treated in exactly the same manner."

"Coprecipitation of Plutonium: The tissue ash from the dry ash method is dissolved in 2 M nitric acid containing 0.5 N hydroxylamine to an ash concentration of 20 mg per ml, the average ash content of each tissue being previously determined. In cases where there is less than 50 mg of ash, the ash is dissolved in 2 - 5 ml of the acid. A 0.5 ml aliquot of the solution, including a suspension of any insoluble matter which is present, is transferred to a 1 ml centrifuge tube. It is treated with 0.5 mg of lanthanum as lanthanum nitrate, made 2 N in hydrofluoric acid, stirred carefully with a small platinum rod and centrifuged at 2000 RPM for 5 minutes. The sides of the tube are washed down by stirring the supernatant without disturbing the precipitate and it is finally centrifuged for ten minutes. The supernate is then carefully removed with a capillary pipette. The residue is transferred to a one inch platinum disc with the same pipette using about 25 lambda of 2 M nitric acid and 300 lambda of water to effect the transfer. This is evaporated to dryness, flamed to redness and counted. This procedure results in recovery of 97% of the plutonium in the case of soft tissues.

"In the case of bone at an ash concentration of 20 mg per ml however there is a sufficiently large precipitation of calcium fluoride to cause about 16% mass absorption. Rat and mouse carcass ash consisting of bone ash plus the muscle, skin, and connective tissue ash causes mass absorption of about 11%.

"The method is useful in the analysis of tissues containing plutonium in a concentration of 1×10^{-5} micrograms or over per mg of ash."

Another method which has been used to assay rat and mouse tissues is described in detail by R. Abrams and associates[2]. This method makes use of a double precipitation where the plutonium is first removed from a high ash content solution. The complete analytical procedure is as follows.

"Destruction of organic material: The tissue to be ashed is placed in a porcelain crucible and dried at 120 - 140°C for a minimum of three days. After drying, the crucible containing the organic residue is placed in a muffle furnace and heated at 300°C for 24 hours. The heating is continued for the next 24 hours at 400 - 450°C. Finally, the temperature is increased to 600°C, and the material ashed at this temperature for 96 hours (see note 1).

"Dissolution of ash. The ash is dissolved by heating on a hot plate with concentrated nitric acid. For most tissues five minutes is sufficient time for heating, however, liver requires about fifteen minutes The acid is replaced as it evaporates. To assist its solution the ash is scraped from the sides of the crucible with a glass rod. The dissolved ash plus the insoluble residue (see note 2) is transferred to a 15 ml graduate centrifuge tube with the aid of water from a small wash bottle and of the stirring rod. The crucible is treated a second time as just described and the washings combined in the centrifuge tube. The total amount of acid used can often be adjusted so that the aliquot taken for analysis will contain just the required amount (2 ml 16 M nitric acid).

"Sampling of ash solution. The solution in the centrifuge tube is thoroughly stirred and centrifuged (see note 3). This causes the residue insoluble in nitric acid to collect at the bottom of the tube. Without disturbing the sediment an aliquote is removed with a pipette for analysis. Ordinarily the volume of the sediment is small and can be neglected, but if it is large enough its volume can be subtracted from the total volume in determining the aliquot. The aliquot taken should contain 2 ml of concentrated nitric acid and should be transferred to a 20 ml, round bottom, lustroid, centrifuge tube. Additional acid may be added if necessary to make up the difference. The solution is finally diluted to 14 ml.

"Precipitation of zirconium phosphate. To the solution in the lustroid tube is added 0.5 ml of a zirconyl chloride solution containing 8 mg Zr^{+4}/ml of 0.01 M hydrochloric acid (4 mg Zr). The solution is thoroughly stirred. Then 0.4 ml of 1-1 phosphoric acid is added slowly with thorough agitation (see note 4). The tube is centrifuged (note 5) and the supernate discarded.

"Washing of precipitate. The zirconium phosphate precipitate containing the adsorbed plutonium is thoroughly mixed with 1 ml of dilute

"Lanthanum Fluoride precipitation. To the washed zirconium phosphate precipitate containing the plutonium is added 0.3 ml of lanthanum nitrate solution containing 1 mg La^{+3}/ml of 0.01 M nitric acid (0.3 mg La^{+3}). Then 1.5 ml of concentrated nitric acid is added. The mixture is thoroughly stirred, and the platinum stirring rod washed off with 1 ml or less of water. Now 2 ml of concentrated hydrofluoric acid is added (see note 7), and the solution thoroughly stirred and the stirring rod washed off with 1 ml or less of water.

"The tube is centrifuged and the supernatant solution decanted. With the lustroid tube still in a vertical, inverted position (after the liquid has run out) a half sheet of "Kleenex" is pushed into its open end to form a wad to absorb the liquid which continues to drain down the sides of the tube. The tube is left in this inverted position until the lanthanum fluoride precipitate containing the plutonium is transferred.

"Transfer of precipitate to platinum plate. With a razor blade the lower half inch of the lustroid tube containing the lanthanum fluoride precipitate is cut off. 0.05 ml of 1 M nitric acid is added to the precipitate and slurried with the tip of a small pyrex pipette made from 4 mm tubing. The same pipette (note 8) is used to transfer the slurried suspension of lanthanum fluoride to a platinum plate resting on an asbestos pad on a hot plate. The heat of the hot plate is adjusted so that the liquid slowly evaporates without steam showing. When the liquid is dry the platinum plate is flamed over a microburner until bright red.

"Another 0.05 ml of 1 M nitric acid is added to the tube end and the remaining precipitate transferred as described above. The final transfer is made with 0.05 ml of 1 M nitric acid.

"After cooling the platinum plate is coated with colloidon (see CH-2190) and counted. The overall yield for the procedure including the usual loss in the crucible is 82%."

A similar procedure is described by R. D. Finkle[1] where an 80% recovery is reported.

"Notes:

"1. The temperature should not be allowed to exceed 600°C as the ash will be likely to fuse into a hard mass which is difficult to dissolve.

"2. There is nearly always some insoluble residue. Experiments have shown that this residue ordinarily contains less than 4% of the plutonium, the average value being 1%.

"3. 1900 RPM for 10 minutes in an International Clinical Centrifuge or equivalent. It is recommended that when large amounts of plutonium are being handled the speed be kept down to 1900 RPM.

"4. At this point a gelatinous precipitate of zirconium phosphate forms which carries the plutonium from the solution.

"5. The lustroid tubes must not be centrifuged at a speed greater than 1900 RPM as there is danger of deforming the bottom of the tube.

"6. The phosphoric acid solution contains 1.26 ml of concentrated phosphoric acid per 100 ml of water.

"7. The zirconium phosphate precipitate dissolves and a lanthanum fluoride precipitate forms. The solution appears clear as the amount of lanthanum fluoride is small.

"8. The pipette is attached to a 1 cc tuberculin hypodermic syringe by a piece of rubber tubing of appropriate size."

2.2 Tissues containing less than 0.05 microgram of plutonium per gram: The previous section dealt primarily with tissues of high plutonium concentrations. However, procedures were also given which could be used for intermediate concentrations, that is, tissues containing between 0.05 and 1 microgram of plutonium per gram. As was previously stated, the reason for making use of varied methods is primarily a time saving factor. As a matter of necessity longer methods are required for low activity tissues.

A. Ashing. Most of the large tissue specimens, samples of 200 to 300 grams, may be ashed by the general methods as described for smaller ones. There are other methods which have been tested which might prove useful in specific cases. These are described below.

Ammonium Nitrate-Nitric Acid Method. The sample, 200 - 300 grams, is placed in a 500 ml long neck Kjeldahl flask and 10 grams of ammonium nitrate added. Twenty-five ml of concentrated nitric acid are added and the reaction started by heating gently. The reaction continues without further heating. When the reaction subsides, moderate heat is applied and vigorous boiling continued until all the acid is removed and the residue completely carbonized. The flask is strongly heated and after cooling is treated with fuming nitric acid and peroxide as previously described for rat and mouse tissues. The ash is dissolved in nitric acid, the concentration determined by the analytical method to be used.

The use of ammonium nitrate shortens the ashing time considerably and gives a readily soluble ash. However, the method is dangerous as violent reactions set in at times.

In most of the ashing procedures described some insoluble material is usually formed. This is most pronounced with liver tissues ashed in pyrex or porcelain. This insoluble residue may or may not contain plutonium. However, it is desirable that it be eliminated if possible Preliminary tests showed that most of this residue was silica and

fuming the residue with perchloric and hydrofluoric acid was performed to remove this material.

Ashing of human tissues. One hundred to two hundred grams of tissue (bone, liver, muscle, etc.) are transferred to an 800 ml Vycor glass Kjeldahl flask. The sample, which has been cut into small pieces, is heated to dryness and treated with 25-50 ml of concentrated nitric acid. The sample is heated gradually until foaming ceases. Two more 25 ml concentrated nitric acid treatments are performed, being sure to bring to dryness each time. At this point the tissue is usually a compact charred mass on the bottom of the flask. The mass is now treated with 10 ml of fuming nitric acid and brought to dryness. It is next treated with 5 ml of superoxol and heated to boiling; then 10 ml of fuming nitric acid are added. Care should be taken to see that all the superoxol is boiled off before adding the acid as nitric acid and peroxide react very violently. One should not be added while the other is present. As many more treatments are made as are required to give a white ash. In order to prevent reaction of the alkaline residue with the glass, the sample is never heated very strongly at dryness.

When completely ashed, the sample is treated with 5 ml of superoxol and heated gently until the residue is completely disintegrated. Small portions of 6 M nitric acid are added until the residue is dissolved. The resulting solution is usually cloudy and may even contain suspended particles. The whole is transferred to a 400 ml platinum beaker and evaporated to dryness. The residue is treated with 10 ml of perchloric acid and 10 ml of hydrofluoric acid, brought to dryness and heated until fuming ceases. It is now treated with 10 ml each of hydrofluoric and nitric acids and the fuming repeated. Finally the residue is taken up in 10 ml of nitric acid and again heated to dryness. Twenty-five ml of 2 M nitric acid are added and the sample heated until complete solution is effected. The solution is transferred to a container for analysis.

B. Extraction of Plutonium. This section will deal primarily with methods which have been developed to remove plutonium from the bulk of the salts contained in the solution of the ash. This is necessary for subsequent lanthanum fluoride precipitation or direct deposition of the plutonium for counting.

Hexone solvent extraction[3]. The plutonium contained in a 2 M nitric acid solution of the tissue ash is precipitated with 40 mg of lanthanum as the fluoride. This precipitate is dissolved with 50 ml of solution containing 10 grams of zirconyl nitrate. To the solution in a separatory funnel are added 80 grams of ammonium nitrate. After solution 0.1 gram of potassium permanganate is added. The resulting solution is extracted for 15 minutes with 50 ml of hexone. The layers are separated and the plutonium is extracted from the hexone with 10 ml of 2 M nitric acid which is 0.1 N in hydrogen peroxide. The hexone is extracted a second time with 10 ml of 0.1 N in hydrogen peroxide.

five minutes with 1.5 ml of a 6% aqueous cupferron solution. The plutonium cupferride complex is extracted into a 40 ml pyrex centrifuge cone with 2 ml portions of chloroform until the last portion is colorless.

"After complete solvent removal on a water bath at 65°C, the residue is treated with 0.75 ml of concentrated nitric acid and heated for 5-10 minutes on a steam bath before the addition of 0.75 ml of 72% perchloric acid. The tube is then placed in an oil bath at 130°C which is gradually raised to 180°C over a period of one hour. At the end of this wet ashing, the fuming solution reduces in volume to 0.75 ml of a clear pale yellow color which becomes colorless upon cooling. This solution is then diluted to 4 ml with water and allowed to stand one-half hour after the addition of 2 drops of a 20% hydroxylamine hydrochloride solution. Forty micrograms of lanthanum are added and precipitation of lanthanum fluoride is affected by the addition of 1 ml of concentrated hydrofluoric acid. The tube is centrifuged at 2000 RPM for 15 minutes, the supernatant decanted and the precipitate washed once with 2 ml of 0.1 N hydrofluoric acid. The precipitate is transferred to a platinum plate in the usual manner for counting."

No experimental recoveries were given when this procedure was submitted. However, tests in this laboratory have shown an average recovery of better than 80%.

Zirconium phosphate extraction. This procedure was previously described for small tissues. By increasing the amount of zirconium proportionately, larger samples may be assayed. Only 1 mg of lanthanum is required to completely carry plutonium from 0.1 gram of zirconium. The yield, as reported by R. Abrams, when the method is applied to solutions containing very high tissue ash varies from 60 to 80%. Most of the loss is encountered in the zirconium precipitation.

Bismuth phosphate extraction. The ashed tissue, initially weighing 200 - 300 grams, is dissolved in 50 ml of 2 M nitric acid. The plutonium is reduced by adding 2 ml of a saturated SO_2 solution and allowing to stand 30 minutes. The solution is diluted to 80 ml and brought to ~75°C on a water bath. While the solution is stirred mechanically, 80-100 mg of bismuth (100 mg Bi^{+3}/ml 10 M nitric acid) are added slowly. Most tissue ash solution contain sufficient phosphate to cause immediate precipitation of the bismuth. However, in almost all cases 2 ml of 1-1 phosphoric acid should be added slowly and the mixture digested while stirring. It is not necessary to add phosphoric acid to bone and fecal ash solutions to obtain complete carrying of the plutonium by the bismuth phosphate even at room temperature.

The conditions for complete carrying of plutonium by bismuth phsophate are: (1) the tissue ash solution should not be greater than 1.2 M in nitric acid, (2) the bismuth concentration should not be less than 0.7 mg/ml and (3) the precipitation should not be made rapidly. Volumes of ash solutions as large as 1000 ml may be assayed. Some of the experimental data collected in the development of this procedure

is described below.

(a) Effect of acidity on the carrying of plutonium by bismuth phosphate. As the acid concentration is increased above 1.2 M, the carrying of plutonium by bismuth phosphate decreases. This is illustrated in Table V. The solubility of bismuth phosphate in hydrochloric acid and the precipitate formed in the presence of sulfuric acid along with carrying difficulties encountered in other acid media limit the bismuth phosphate extraction to a nitric acid solution.

Sample of Tissue Ash	Concentration of HNO_3	Concentration of Bi^{+3}/ml	% of Pu carried by bismuth phosphate
1	0.1 M	0.5 mg	99
2	0.2 M	0.5 mg	94
3	0.8 M	0.5 mg	96
4	1.2 M	0.5 mg	97
5	1.6 M	1.0 mg	86
6	2.4 M	1.0 mg	61

(b) Separation of bismuth and plutonium. Bismuth phosphate is readily soluble in hydrochloric and nitric acid solutions. The bismuth is not precipitated when hydrofluoric acid is added. This makes it possible to coprecipitate plutonium with lanthanum fluoride in the presence of bismuth. Decreasing the acid concentration, hydrochloric acid below 1 M and nitric acid below 2 M, causes excessive coprecipitation of bismuth. There is no advantage gained in using low acid concentrations as plutonium is quantitatively precipitated from solutions of hydrochloric acid as high as 6 M.

In the procedure which is used, the bismuth phosphate precipitate which contains up to 1 gram of bismuth is dissolved in 10 ml of concentrated hydrochloric acid and diluted to 30 ml with water. One ml of solution containing 1 mg La^{+3} per ml is stirred in. Precipitation of the lanthanum is effected by adding 5 ml of hydrofluoric acid. The lanthanum fluoride precipitate containing the plutonium is centrifuged out and transferred to a platinum plate (1"-2" in diameter), dried, flamed and counted.

The amount of acid used to dissolve the bismuth phosphate is governed by the amount of bismuth used in the extraction step. In the case where 0.1 gram of bismuth is used, 2 ml of hydrochloric acid is diluted to 5 ml with water after solution of the bismuth phosphate. Only 0.25 mg of lanthanum is used to coprecipitate the plutonium from this volume of solution.

The analytical yield from this method, obtained from several hundred tests, is better than 90%. The liver has given the most difficulty in analysis but the tests show an average recovery of 85%. The maximum residue deposited on the counting disc from 1 gram of bismuth after a 1 mg lanthanum precipitation as the fluoride is 3.4 mg. Spread over a 1.5" diameter disc, this does not cause appreciable absorption of the alpha particles.

Since most ashed tissue samples are contained in a maximum of 50 ml of 2 M nitric acid, and 0.1 gram of bismuth is sufficient to completely extract the plutonium from the diluted solution, the final lanthanum fluoride precipitation can be made from a maximum volume of 7 ml. Less than 1 mg of solid is deposited on the counting disc.

3. Evaluation of Procedures.

3.1 Ashing. Dry ashing is a method which requires very little attention and with sufficient equipment a large number of tissues may be processed simultaneously. The methods as described by R. Abrams and R. D. Finkle both give rise to a small amount of insoluble residue. This is more pronounced in the case where porcelain crucibles are used. However, the use of strong nitric acid to dissolve the residue leaches the plutonium from the insoluble portion and good recoveries are possible.

Wet ashing, if carefully done, requires frequent supervision and may result in a small amount of insoluble residue particularly in the case of liver samples. The insoluble material may be eliminated by perchloric and hydrofluoric acid treatments. The method is rapid and gives rise to a water clear solution of the tissue ash.

A combination dry and wet ashing procedure is oftimes more useful. As in the case of bone or fecal specimens this method can be applied to best advantage.

3.2 Extraction: Zirconium phosphate. The study of the carrying of plutonium by zirconium phosphate in various media has indicated the method to be quite complete. As applied by R. D. Finkle and R. Abrams to nitric acid tissue ash solutions, the carrying is probably around 85%. Erratic results are obtained from solutions of high ash content.

Bismuth phosphate. Over 95% of the plutonium is carried by bismuth phosphate precipitate formed in a tissue ash solution of the following composition: (1) 0.25 gram ash/ml, (2) 1.2 M nitric acid and (3) 1 mg Bi^{+3}/ml. The ash concentration given is that obtained from 100 grams of bone dissolved in 80 ml of solution. Concentrations of 0.5 gram ash/ml have given an average of 90% plutonium recovery.

Cupferron-chloroform. The method is based on the formation of the cupferride complex of plutonium and its greater solubility in chloroform. It has been mainly applied to the analysis of ashed urine and

fecal specimens. Little difficulty should be experienced in applying the procedure to high ash content solutions as a preliminary precipitation extraction is performed which reduces the ash content somewhat.

The method gives better than 90% recovery of plutonium. However, it is not satisfactorily applied to a preliminary lanthanum fluoride precipitation as the alpha activity in the lanthanum follows the plutonium.

Hexone. K. G. Scott, who has made use of this material to extract plutonium has pointed out that 70-80% of the plutonium is extracted from a lanthanum fluoride precipitate dissolved with zirconyl nitrate, but the method is of little value in assaying samples of low plutonium concentrations. The lanthanum and zirconium contain considerable alpha activity which is also extracted by the hexone.

Thiophenyltrifluoacetone (T.T.A.). This method also makes use of a preliminary precipitation of plutonium with lanthanum fluoride. Based on a lanthanum fluoride analysis of the spike solution, the method gives an average recovery of 89%, as reported by K. G. Scott, from 5 grams of rat ash. Smaller ash concentrations give better than 95% recoveries. It would appear that the extraction is quantitative and that lower than 95% recoveries are primarily due to the lanthanum fluoride carrying. Though tests in this laboratory have shown that some alpha activity associated with the lanthanum is extracted, careful selection of the lanthanum samples can avoid this difficulty.

One of the most attractive features in the T.T.A. method is that the plutonium is extracted into a benzene solution free of other inorganic materials. This solution containing the plutonium can be evaporated directly and counted after flaming off the small organic residue.

4. Summary.

Three methods are described by which tissues may be ashed ~~suitably~~ for analysis for plutonium. (1) Wet ashing with nitric acid and hydrogen peroxide, (2) a combination nitric acid wet ashing and dry ashing procedure and (3) a dry ashing procedure. Each procedure is described in connection with the plutonium extraction procedure applied.

Five general methods for extracting plutonium from solutions of high salt content. The solvent extraction procedures, (1) cupferron-chloroform, (2) hexone and (3) T.T.A., all make use of a preliminary precipitation procedure. The zirconium phosphate and bismuth phosphate precipitation extraction procedures are adopted directly to the tissue ash solution.

All the analytical methods described with the exception of the T.T.A. method are dependent upon a final precipitation of plutonium with lanthanum fluoride.

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