I
U N C L A S S I F I E D

UR-305

Chemistry

THE UNIVERSITY OF ROCHESTER
Atomic Energy Project
P. O. Box 287, Station 3
Rochester 20, New York

* * *

Contract W-7401-eng-49 between the U. S. Atomic
Energy Commission and the University of Rochester,
administered by the Department of Radiation Biology
of the School of Medicine and Dentistry.

ANALYTICAL AND AUTORADIOGRAPHIC METHODS FOR POLONIUM²¹⁰

by:

Frank A. Smith
Rocco J. Della Rosa
Louis J. Casarett

Division: Pharmacology and Toxicology

Division Head: H. C. Hodge

Section: Radioaction Inhalation

Section Head: J. N. Stannard

Date Completed: 8/24/55

Date of Issue: 11/4/55

U N C L A S S I F I E D

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

TABLE OF CONTENTS

	Page No.
ABSTRACT	2
PREFACE	3
1.0.0 TYPES OF MATERIALS	4
1.1.0 Soft tissues	4
1.2.0 Hard tissues	4
1.3.0 Biological fluids	4
1.4.0 Miscellaneous materials	4
2.0.0 PREPARATION FOR ANALYSIS	5
2.1.0 Major difficulties	5
2.2.0 Digestion procedures	6
2.2.1 Soft tissues	6
2.2.2 Blood samples	7
2.2.3 Urine samples	7
2.2.4 Large carcasses	7
2.2.5 Capacity	9
3.0.0 PLATING OF POLONIUM	10
3.1.0 Plating assembly	10
3.2.0 Plating baths	11
3.3.0 Factors affecting the plating of polonium	15
3.3.1 Plating efficiency	15
3.3.2 Discoloration of silver foils	16
3.3.3 Effect of iron	17
3.4.0 Plating procedure	17
3.5.0 Efficiency of recovery	19
3.6.0 Cross contamination	19
3.7.0 Cleaning	21
4.0.0 AUTORADIOGRAPHY	21
4.1.0 Tissues	21
4.2.0 Exposure time	22
4.3.0 Additional applications	22
4.3.1 Physical state	22
APPENDIX I. PREPARATION OF BIOLOGICAL SAMPLES FOR ANALYSIS. MISCELLANEOUS EXPERIMENTS	24
APPENDIX II. A BATH FOR PLATING OF POLONIUM. R. H. Wilson	40
APPENDIX III. STATISTICAL DESIGN AND ANALYSIS OF EXPERIMENTS ON POLONIUM PLATING TECHNIQUE. A. M. Dutton	46
APPENDIX IV. EFFECT OF NITRIC ACID ON THE PLATING OF POLONIUM	54

TABLES

	<u>Page No.</u>
Table 1. Distribution of absorbed polonium between body fat and remainder of carcass	24
Table 2. Comparison of digestion and plating procedures applied to cat liver	25
Table 3. Digestion and plating of cat omental fat	26
Table 4. Recovery of polonium from minced rat carcasses	28
Table 5. Recovery of polonium autoclaved in metal cans	29
Table 6. Effect of three digestion mixtures on plating of polonium	31
Table 7. Effect of precipitation of calcium sulfate on the recovery of polonium	32
Table 8. Effect of Versene (EDTA) on the removal of polonium from millipore filter paper	35
Table 9. Volatility of polonium from a heated glass surface	38
Table 10. Volatility of polonium from a heated silver surface	39
Table 11. Factors studied factorially for their influence on the spontaneous plating of polonium from acid solution	46
Table 12. Analysis of variance for experiment 1.	47
Table 13. Average deposition in counts per minute for each of 16 factor classes	49
Table 14. Analysis of variance for the detection of acidity effects on the experimental plating of polonium	52
Table 15. The effects of stirring on the radioactivity recovered in the experimental plating of polonium	53
Table 16. Effect of nitric acid on the plating of polonium	55
Table 17. Effect of ferric iron on the recovery of polonium by plating	58
Table 18. Effect of iron:polonium ratio on the recovery of polonium	59
Table 19. Effect of ferrous iron on the recovery of polonium by plating	60
Table 20. Influence of iron in biological materials on the plating of polonium	61
Table 21. Efficiency of the standard plating procedure for polonium	65

TABLES (cont.)

Table 22. Percentage of total recovered activity found in the original plating	66
Table 23. Percentage of total recovered activity found on first two platings (original plus one replating)	67
Table 24. Analysis of variance on percentages of total recovered activity found in the original plating	68
Table 25. Analysis of variance on percentage of total recovered activity found in first two platings	69
Table 26. Percentages above which 90 per cent of individual platings are expected (confidence coefficient 0.95) to fall	70

FIGURES

	<u>Page No.</u>
Figure 1. Plating column assembly, showing the column, collar, neoprene disk and silver foil	12
Figure 2. Diagram of plating column collar	13
Figure 3. Six unit plating assembly	14
Figure 4. Volatility of polonium from a heated glass surface	37
Figure 5. Twenty-four unit plating assembly	41
Figure 6. Detail of pulley mounting for multiple plating assembly	43
Figure 7. Effect of ferric iron on the plating of polonium	57

ANALYTICAL AND AUTORADIOGRAPHIC METHODS
FOR POLONIUM 210

Abstract

This report presents detailed descriptions of procedures currently employed by the Section for sample preparation and the analytical and autoradiographic determination of polonium 210 in biological materials. Investigations of the quantitative effects of a number of factors affecting the measurement of polonium are described. Statistical analyses of the influence of these factors and of the overall precision of the method, as well as structural features of the plating equipment used, are included.

THE DEPARTMENT OF THE INTERIOR

BUREAU OF LAND MANAGEMENT

WASH. D. C.

REPORT OF THE SURVEY OF THE LANDS OF THE UNITED STATES

IN THE TERRITORY OF ARIZONA, BY THE SURVEYOR GENERAL, J. H. HARRIS, 1890.

THE SURVEY WAS MADE BY THE SURVEYOR GENERAL, J. H. HARRIS, 1890.

THE SURVEY WAS MADE BY THE SURVEYOR GENERAL, J. H. HARRIS, 1890.

THE SURVEY WAS MADE BY THE SURVEYOR GENERAL, J. H. HARRIS, 1890.

THE SURVEY WAS MADE BY THE SURVEYOR GENERAL, J. H. HARRIS, 1890.

THE SURVEY WAS MADE BY THE SURVEYOR GENERAL, J. H. HARRIS, 1890.

PREFACE

This report is one of a series (UR 301-310) describing the physical plant and procedures used in the Radioactive Inhalation Program. These are set down separately to obviate the need for detailed description of these features when experimental results are reported. While several radioactive materials (radon, plutonium, polonium 210, fission products) are to be used in the program primary emphasis at present has been on radon and polonium 210 (as representative of the probable effects of the daughter products of radon). The radon exposure unit is described in UR-307. The present report presents detailed accounts of experience in this laboratory with the analysis of polonium 210 in biological materials. While the basic procedures are not new many complicating factors have been investigated and controlled. The former are described in references 1 - 3.

ANALYTICAL AND AUTORADIOGRAPHIC METHODS FOR POLONIUM 210

1.0.0 TYPES OF MATERIALS

In early planning sessions at which studies to be undertaken by the Radioactive Inhalation Section were discussed, it became apparent that a wide variety of materials would be presented for polonium analysis. Species such as the mouse, rat, cat, rabbit and dog were contemplated for use, from each of which soft, hard and fluid samples could be expected.

1.1.0 Soft tissues of the above species might include many or all of the various organs, muscle, fat, skin, and fur. Depending upon the species and organs, such samples can range in size from milligrams to as much as 1000 grams or more; moreover the composition of samples can show a wide variation in fat content which is expected to present analytical difficulties, and also to include more or less blood. Both of these factors are known to present certain difficulties and to require variations in the manner in which the sample is prepared for analysis.

1.2.0 Hard tissues obviously consist of various portions of the skeletal system of the experimental animal. While polonium is not a bone-seeker in the same sense as are other materials such as radium, plutonium, lead or fluoride, it is to be expected that in some instances information would be desirable regarding the uptake, if any, of polonium by the mineral portions of the bone and by the marrow.

1.3.0 Biological fluids to be analyzed are represented by whole blood, cells, plasma, serum, lymph, perhaps cerebrospinal fluid and, of course, urine. Such samples may have been taken at intervals throughout the course of experiments or perhaps only terminally.

1.4.0 Miscellaneous materials requiring the determination of polonium content or concentration could be expected to include stock polonium solutions,

test solutions prepared for injection or treatment of animals or for other purposes, waste water, washings from fiber glass air filters, and staining and washing solutions resulting from histologic procedures.

In the following descriptions of analytical procedures, emphasis has been placed upon methods for handling biological samples, particularly the soft tissues. It is felt that such materials, particularly those containing significant proportions of fat, will present the greatest difficulty. Therefore, a method suitable for these materials is hoped to be usable for all other types of samples. Obviously in certain situations it will be possible to eliminate some of the steps required for more complex materials when samples of a lesser complexity such as injection solutions or urines are to be analyzed.

2.0.0 PREPARATION FOR ANALYSIS

Biological materials are prepared for polonium analysis by wet digestion, various oxidizing agents being employed for this purpose. A nitric acid-perchloric acid mixture has been used by Fink et al. (1) and a perchloric acid-superoxol mixture by Finkel et al. (2). Both of these procedures involve perchloric acid with its attendant hazard of explosion. In this laboratory concentrated nitric acid alone has been found satisfactory, as was reported earlier by Scott and Stannard (3). According to Finkel et al. (2) sulfuric acid cannot be used because the ashing temperature exceeds 200° C. with attendant loss of polonium by volatilization. However, Scott and Stannard (3) carried out experiments in which polonium was heated in concentrated sulfuric acid solution for two hours at temperatures of about 300° C.; following heating, aliquots of the solution were plated with recoveries ranging from 90.6 to 93.1 per cent.

2.1.0 Two major difficulties arise in the preparation of biological materials for determination of polonium content. Both stem from the difficulty

of obtaining complete digestion of the sample. While both are of equal importance, both are of lesser significance with small carcasses and with individual organs. The first of these is the problem of obtaining complete digestion of fat which may be present. While this does not occur with skeletal samples it does occur with soft tissues, and becomes increasingly serious as the size of the sample increases, or in the case of whole carcass analysis, e.g., the rat, with increasing size and age of the animal. The second problem is that of the presence of insoluble material consisting chiefly of silica present in the gastrointestinal tract of the animals, and which has its origin in the diet. This is of particular significance in the case of rat and rabbit carcasses and feces. It is of lesser importance with cats and with other species. Various means, all of which have yielded essentially negative results, have been attempted in this laboratory to minimize or eliminate these difficulties. These experiments are summarized in Appendix I.

2.2.0 Digestion procedures described in this section are those routinely employed in this laboratory.

2.2.1 Soft tissues to be digested are placed in suitable containers. In general, the sample should occupy not more than ten per cent by weight of the volume of the vessel, i.e., a 10 - 15 g sample is placed in a 125 ml erlenmeyer flask. Larger samples are handled in beakers. The recommended ratio of size to volume is necessary in order to contain the foam which is produced upon the first addition of nitric acid, and to minimize danger of the sample boiling over in subsequent heating. The sample is covered with about 50 ml of concentrated nitric acid, allowed to stand at room temperature for several hours or preferably overnight or longer and then heated gradually on a hot plate to boiling temperature. Glass beads are used to prevent bumping; foaming, which may occur during the early warming is

controlled by the addition of Dow Antifoam A as needed. Heating is continued at a smooth boil until the digest is a clear yellow color. The solution is evaporated to a minimal volume, cooled, and 25 ml of water added; if the solution remains clear digestion is considered complete. If a layer of fat appears further digestion with nitric acid is required. A 10 - 15 g sample of rat liver will require 16 - 18 hours of active digestion after the preliminary standing in acid. Two or three charges of nitric acid, comprising a total volume of about 200 ml, usually are required for such a sample. An entire rat liver can be prepared for analysis in about 24 hours of working time after the first acid soaking. Rat kidneys require approximately 8 hours of hot digestion; the kidneys of larger species such as the rabbit or cat will take up to 24 hours.

2.2.2 Blood samples are treated in a similar manner. To a 5 - 10 ml sample is added 10 - 20 ml of water and 100 ml of concentrated nitric acid. After standing overnight the digestion is completed as described above; approximately 8 - 12 hours are required. The volume of acid added initially is sufficient. It was found that digestion was facilitated by first lysing the cells with water.

2.2.3 Urine samples are digested by heating with an equal volume of concentrated nitric acid. Usually only the initial addition of acid is required and digestion is complete in about 8 hours. The preliminary overnight digestion at room temperature is not necessary. However, rabbit urines may require as long as 24 hours of boiling for complete digestion. The greater time required is caused chiefly by the great care necessitated in the early phases to avoid bumping and spattering, which may be caused by the extremely high concentration of solids frequently encountered in urine samples from this species.

2.2.4 Large carcasses, such as those of the cat or rabbit, can be handled more easily if first dissected into several portions, e.g.,

skin and fur, muscle, skeleton, head and remaining viscera. Nitric acid digestion has been found adequate for all of these fractions with the exception of the muscle fraction which contains the greatest amount of fat. Here it is necessary to follow the nitric acid digestion with sulfuric acid. A description of the digestion of cat carcasses follows:

The various portions, in beakers of 2 or 3 liters capacity, are covered with nitric acid and allowed to stand overnight or longer at room temperature. Digestion is then started with low heat on the hot plate. Antifoam is used as needed particularly in the case of skin. The sample should be observed frequently in order to be certain that the solution does not foam up out of the container. Careful heating is continued for 8 - 10 hours, nitric acid being added as required. During the following 10 - 30 hours additional acid is added and the digestion continued at medium heat; antifoam occasionally may be needed. Observation can be extended to hourly intervals or longer. The acid is replenished as required. The solution will change from red-black, to burgundy, to a dark straw color. After several new charges of nitric acid have been added and no fat appears on cooling, digestion is considered to be complete. At this stage fat almost invariably will appear in the muscle portion, and possibly in the portions containing the skin and the viscera. In these instances 25 ml of concentrated sulfuric acid is added and heating continued until the nitric acid has been evaporated. The heat is then reduced and charring allowed to proceed for 15 - 20 minutes or until the white fumes of sulfur trioxide appear. The solution is allowed to cool and an additional liter of nitric acid is added. The digestion is then continued at medium heat. The alternate additions of sulfuric and nitric acids are continued until a fat-free, straw colored solution results. The solution is reduced to as small a volume as practicable and transferred to an erlenmeyer flask. Superoxol (30 per cent hydrogen peroxide) may be added at this point,

followed by heating to clear the solution to the desired straw color, if necessary. Approximately 100 ml of water is added to the sample and the excess sulfuric acid CAREFULLY neutralized with either sodium hydroxide pellets or concentrated sodium hydroxide solution. The volume is diluted to 500 ml and 100 ml aliquots acidified and used for plating as described in Section 3.4.0.

Digests of those portions not requiring sulfuric acid treatment are reduced to as small a volume as practicable to reduce the nitric acid content to minimal levels, diluted with distilled water to a convenient volume and aliquots taken for plating as described in 3.4.0.

As much as 60 hours and 30 pounds of concentrated nitric acid may be required for the complete digestion of a cat carcass.¹

Rat carcasses are digested in a similar manner, except that they need not be dissected into the several portions. The carcass is placed in a one liter beaker, covered with 500 ml of concentrated nitric acid and allowed to stand overnight. For the following 8 hours the beaker and contents are kept at low heat on the hot plate, and observed frequently to prevent excessive foaming; Dow Antifoam A is used as required. During the next 8 hour interval the solution usually will boil smoothly at medium heat. An additional 24 hours of vigorous boiling usually will suffice to produce complete digestion. Should fat become apparent in the digest upon cooling, it is destroyed with sulfuric acid as described previously.

2.2.5 Capacity. Using two electrically heated hot plates 18" x 24" and two hot plates 6" x 18", it is possible for one analyst to handle 30 rat carcasses simultaneously, or 60 mice, or 3 larger animals (rabbit, cat). A total time of approximately two weeks will be required. The close attention of the analyst will be necessary for about half of each working day.

---

1. The large amounts of nitric acid required for the digestion of large numbers of samples ranging in size from small organs to large carcasses required specially designed hoods and duct work. These are described in UR-302.

A critical review of digestion procedures used in the determination of trace metals in biological materials has been published by Middleton and Stuckey (4).

3.0.0 PLATING OF POLONIUM

Polonium deposits on metallic surfaces either spontaneously, i.e., without the necessity of passing an electric current through the solution, or by electrodeposition resulting from passage of a current. Because of the simplicity of this spontaneous phenomenon, it has been utilized as the basis of routine laboratory procedures for the analytical separation of polonium. Silver and copper foils or discs have been most commonly used as plating surfaces.

The descriptions to follow are limited to the plating equipment and method routinely used in this laboratory. The foils were counted in alpha scintillation counters of conventional design.

3.1.0 Plating assembly. A variety of vessels or containers have been used for the plating of polonium, ranging from Dixie cups to specially machined column assemblies. The first mentioned containers do not permit plating of polonium on a single surface of a metal foil, while the apparatus of Fink was considered not to be expendable because of the expensive machining required for its fabrication. Moreover, the metal coupling of this latter unit is subject to corrosion from acid fumes and eventually must be replaced.

In surveying a number of possible alternative materials for plating columns, it became apparent that a nursing bottle offered many or all of the desired features. Eminently satisfactory plating columns were prepared as follows. The body of the plating column consists of a Davol brand "Anti-colic" No. 156 Nurser, from which the bottom has been removed by means of a glass cutting saw. A Davol No. 157 collar, from which the inner guiding ring has been removed is used as the foil holder; the ring is easily removed by setting up the collar in a lathe and turning down the ring with a cutting tool. The silver

foil is backed by a 1/16 in. thick neoprene disk 1-1/2 in. in diameter. The collar is usable for approximately 100 platings, after which time, probably due to prolonged heating and immersion in the water bath, it rapidly becomes subject to fracture when tightened on the neck of the bottle. The neoprene discs have been in continual use for approximately three years without obvious signs of deterioration. Pure silver foils, 0.003 in. thick and 1-1/2 in. in diameter, are used for the plating surface.

An exploded view of the plating column is presented in Figure 1. A drawing of the collar is shown in Figure 2.

3.2.0 Plating baths. Two designs of plating baths have been developed and are being used routinely in this laboratory. The first model was designed and built at a time when the work load was relatively light; it is illustrated in Figure 3. As will be seen from the photograph, the capacity is six plating columns.

The bath proper measures 7-1/2 in. x 4-1/2 in. x 25-1/2 in. and is made of 16 gauge sheet copper; all joints are soldered. One inch magnesia block insulation surrounds the bath which is placed on a 30 in. x 15 in. x 1/2 in. plywood base board protected by 3/16 in. sheet transite. A loosely fitting copper cover (16 gauge) with six 2-1/2 in. diameter holes is used to reduce evaporation losses. The source of heat is a 1000 watt, 115 volts, six foot, copper covered heating element.¹ This element was found to be adequate to heat the bath to the desired temperature of 90°C. in approximately 15 - 20 minutes and to maintain this temperature without need of varying the voltage input. The element is coiled in one loop and placed in the bottom of the bath, where it rests on small brass bars.

The stirrers, of 5 mm pyrex glass rod and 10 in. in length are driven by individual "Eastern" stirrers, 115 volts, 60 cycles². Individual rheostats

---

1. Will Corporation Catalog No. 15951, Rochester, N. Y.

2. Eastern Industries, Incorporated, New Haven, Connecticut.

FIGURE I

Plating column assembly, showing
the column, collar, neoprene disk and silver foil.

FIGURE 2

Diagram of plating column collar.

15

(sc. 1.5-1)

CONVERSION OF NURSING BOTTLE CAP
TO PLATING COLUMN COLLAR

FIGURE 3

Six unit plating assembly.

17

for control of stirring speed did not prove satisfactory. Instead, a strip outlet was provided into which each stirrer could be plugged as needed. Power input was controlled by a 115 volt, type 116, Powerstat¹.

The primary advantage offered by this design lies in the fact that each plating assembly is an individual unit, any number from one to six being operated concurrently; moreover, each unit is readily accessible, without the necessity of having to reach behind one unit to set up another column. However, corrosion of the stirring motors by acid fumes evolved from the plating solution occurs, and good control of the stirring speed gradually is lost.

Based on experience gained with this assembly, a second model was designed by R. H. Wilson, R. Della Rosa and F. A. Smith to permit the simultaneous plating of up to 24 samples. This model is similar in some respects to that described in (5). Structural features are detailed in Appendix II.

The principal advantages of this model may be mentioned briefly. The potential work load is quadrupled over that of the first model, with relatively slight increase in bench space required. Individual stirrer motors are eliminated and uniform stirring speed in each column is obtained. The coupling in which the stirrers are secured is less subject to freezing by corrosion. Removal of the stirrers from the columns is made easier by virtue of the counter-balance arrangement. Finally, removal of columns from the bath is made easier by placing the columns in a basket immersed in the bath. The apparatus differs from that described in (5) chiefly in the counter-balance arrangement for removal of the stirrers, and in the arrangement of the columns in three rows of eight each rather than in four rows of six each.

3.3.0 Factors affecting the plating of polonium. A number of factors can be expected to influence the spontaneous plating of polonium. Certain of these factors were investigated as described in the following sections.

3.3.1 The effects on plating efficiency of temperature, time, solution volume, stirring, acidity, and concentration of solution by evaporation
1. Superior Electric Company, Bristol, Connecticut.

were studied in two factorially designed experiments.¹ Results obtained are presented in Appendix III, and indicate the following. It is essential that the solutions be stirred. Concentration of the solution by evaporation did not significantly affect the quantity of polonium plated. Plating was more efficient when done for 90 minutes, at 90°C. in a volume of 100 ml than when these conditions were changed to 45 minutes, 45° and 50 ml, respectively. Variations in acidity of the plating solution had little effect; efficiency was only slightly greater in 0.1 N hydrochloric acid solution than in 0.33 or 0.75 N acid. These results have been reported previously (6).

Using the 104 observations made, the standard deviation of the plating procedure was estimated to be five per cent of the activity present for the range 200 - 200,000 counts per minute. The absolute standard deviation, i.e., counts per minute, varied directly with the amount of activity present.

3.3.2. Discoloration of silver foils. In early work in the laboratory it was observed that occasionally foils were discolored during the plating process (3). This discoloration occurred only with biological samples, never with relatively pure polonium solutions. At this time it was not routine procedure to neutralize sample digests, but rather to prepare aliquots directly in 0.5 N hydrochloric acid, inasmuch as the small amount of nitric acid present did not appreciably affect the pH of the solution. These conditions suggested that the blackening might be due to the nitric acid present. This hypothesis was verified as follows. A known amount of polonium was plated on a silver foil by spontaneous deposition from a 0.5 N hydrochloric acid solution. The foil then was immersed for 90 min. in 100 ml of solution 0.5 N with respect to

---

1. The factorial experiments were designed and the data interpreted by Drs. S. Lee Crump and A. M. Dutton of the Statistics Section. We are pleased to acknowledge our indebtedness for their assistance and cooperation.

hydrochloric acid, and containing 3 ml of concentrated nitric acid, at 90°C. The foil was blackened, and only 78 per cent of the theoretical activity was obtained upon recounting. When plating was carried out in the presence of nitric acid, the foil again was blackened, and only 69 per cent of the theoretical activity was counted. Ninety-five per cent of the anticipated activity was recovered, on non-blackened foils, when plating was done in the absence of nitric acid. It was concluded therefore, that all digests should be neutralized (Universal Indicator Paper) prior to aliquoting for plating. The data are presented in Appendix IV.

3.3.3 Effect of iron. The inhibitory effect of most metallic ions on the plating of polonium from tissue digests is negligible, since they are present in only trace amounts in tissues. Iron, however, does occur in significant amounts, and in the ferric state (formed as the result of nitric acid digestion) does interfere with plating (1). Investigations of the magnitude of this effect are reported in detail in Appendix V. The data indicate that plating of polonium on silver foil is significantly inhibited by ferric iron concentrations in excess of 5×10^{-4} M, in the plating solution. The effect appears to be independent of the amount of polonium present, at least over the range 400 - 5000 cpm. This concentration of iron is equivalent to that present in a 5 ml sample of whole blood, i.e., if a 5 ml blood sample is digested in nitric acid and prepared for plating, the iron content of the solution plated will approximate 5×10^{-4} M. However, the effects of ferric iron concentrations up to at least 10^{-2} M are completely negated by adding 100 mg of ascorbic acid as a reducing agent to the solution at the time of plating. In the absence of this precautionary measure, it is not possible to obtain quantitative recoveries of polonium in the analysis of large samples of blood and tissues rich in blood and/or iron (spleen, liver, stomach, intestine).

3.4.0 Plating procedure. Based on the material presented in the preceding sections, a routine plating procedure has been established. A description follows.

1. Identifying information and the date are marked along the edge of a silver foil. A reasonably permanent marking is achieved by use of a hard lead pencil, inasmuch as the silver is soft enough to receive a satisfactory imprint.

2. A neoprene disc is placed in the Davol collar, the foil inserted, and the Davol plating column carefully screwed in place.

3. Where the sample is known to contain nitric acid, or when the degree of acidity is unknown, the solution is neutralized with sodium hydroxide (Universal Indicator Paper).

4. A suitable aliquot, containing if possible not less than 25 cpm nor more than 10^6 cpm, is adjusted to 0.5N with hydrochloric acid in a total volume of 100 ml. This adjustment and dilution can be done in the Davol column.

5. A 100 mg tablet of ascorbic acid is added to the solution if iron is known or suspected to be present.

The columns are allowed to stand for 15 minutes or longer on paper towels on the bench top and then examined for evidence of leakage before being placed in the plating bath. Leakage has occurred only rarely, usually as the result of improper seating of the silver foil or neoprene disc. When this does occur another aliquot of sample is set up in a fresh column.

6. The column is placed in the water bath, previously heated to 90°C ., a stirrer introduced into the column, and moderate stirring initiated.

7. After 90 min. the stirrer is stopped, removed from the column, and the column removed from the bath.

8. The supernatant solution is decanted and the column and foil rinsed gently with distilled water.

9. The column and collar are disassembled, the foil removed from the collar and blotted dry with absorbent tissue.

10. Alpha activity on the foil is counted in an alpha scintillation counter.

3.5.0 Efficiency of the standard procedure in recovering polonium
present was determined by replicate platings of a series of solutions containing 66.6×10^{-6} to 44.4×10^{-2} μ c of polonium 210 (15 - 200,000 counts per minute). At the lowest level the average recovery of the initial plating was 89.65 per cent; the standard deviation of this mean was 2.03 per cent, that of a single analysis was 4.06. At the highest level of activity the values were 96.01, 2.03 and 4.06 per cent, respectively. If the supernatant solution from the original plating was replated and the activity obtained added to that originally recovered, the average recovery was 94.82 per cent at the lowest level and 99.30 per cent at the highest level. The standard deviation of the means was 0.86 per cent and the same at each level of activity tested; the standard deviation of a single analysis at each level was 1.71 per cent. The percentage recovery was found to depend upon the activity level, and increased in a direct fashion almost as the logarithm of the level. A complete statistical analysis of these recovery experiments is presented in Appendix VI.¹

3.6.0 Cross contamination.² In order to reduce the effects of contamination of plating columns by samples of high activity, the plating columns available have been classified into five categories. One set of twelve columns has been placed outside the laboratory proper and has had no contact whatsoever with other laboratory materials. These are kept available for extraordinary use, for example the determination of alpha activity in blood and other specimens from laboratory personnel where an absolute figure is desirable.

A second set of twelve columns is reserved for low activity work, in which samples are expected to contain less than 200 disintegrations per minute; these are mainly used for the assay of waste liquids which enter the general sewer system.

-
1. The design and interpretation of these experiments were made by Drs. S. Lee Crump and A. M. Dutton of the Statistics Section.
  2. Some of the precautions described below are described in (1)

A third set of twelve is used in the plating of samples containing 500 disintegrations per minute or less. These columns are designated as usable for "C" level activity.

A fourth set for "B" level activity is used for activities of 40,000 disintegrations per minute or less.

A fifth set is used for high activity levels up to two microcuries ("A" level).

This classification is an arbitrary one and is dictated by the limits of the counters and the levels of activity allowed in various parts of the laboratory. The choice of columns (and counters) is of course dependent on the amount of polonium used and the degree of accuracy desired by the individual investigator.

The columns are "blanked" by placing 100 ml of 0.5N HCl in the column and carrying out the routine plating procedure. The activity obtained on the foil represents the level of contamination of the column. If all the columns of a particular set cannot be blanked, a number of them, six or more are picked at random and blanked. If the level of activity is significantly greater than the level designated for the particular category then the column is decontaminated by soaking in 3N HCl and a second blank run on it.

Blank levels for each of the various sets of plating columns are shown in the following table.

<u>Classification in dpm</u>	<u>Blank levels in dpm</u>
200	background
500 "C"	2 or background
40,000 "B"	5 or background
40,000 and higher "A"	5 - 50

As far as possible, every effort is made to estimate the level of activity of solutions to be plated, and to use the proper columns; thus the number of "hot" columns can be kept to a minimum.

3.7.0 Cleaning. Plating columns are washed by allowing them to soak in a laboratory glass cleaner ("Lakesal") overnight and then scrubbed and rinsed in tap and distilled water.

Glassware used in sample digestions also is washed in the manner described above. Whenever necessary a cleanser such as Bon Ami is used. Where large quantities of polonium have been used and the advisability of re-using the glassware is doubtful, it is discarded. If an investigator so stipulates, new glassware is used throughout the preparation of his samples.

4.0.0 AUTORADIOGRAPHY

Facilities are available for employing autoradiography as an adjunctive analytical procedure. At present, autoradiography directly related to this program is limited to polonium 210. However, this method will also be available for use in experiments involving other radioactive substances. In general, the materials and techniques used are standard with minor modifications when necessary. In any case, the selection of materials and methods is dictated by nature of the investigation and the feasibility of supplying the desired information with a minimum of disturbance to the experimental material.

Results of some investigations and a detailed description of the methods used have been reported recently (7).

4.1.0 Tissues. The majority of autoradiographic work has been done with tissues. Routinely, all tissues are fixed in neutral 10% formalin and paraffin embedded. Under circumstances where dissolution of the radioactivity or cellular material under investigation occurs in organic solvents, Carbowax¹ is used as the embedding medium. When leaching of a material occurs in aqueous media, freeze-drying followed by paraffin or Carbowax infiltration is the method of choice. Blood smears are prepared on cover glasses or microscope slides in the usual manner.

---

1. Carbide and Carbon Chemicals Co., New York, N. Y.

Tissue sections are placed in apposition to the emulsion of nuclear track plates (8). Eastman Kodak NTA plates (10 μ) are used almost exclusively; this preparation allows a determination of general intra-tissue distribution of the material. Some cellular localization is frequently possible also. In order to obtain a higher resolution and a finer localization of radioactive substances, the tissue sections can be impregnated with liquid NTA emulsion¹ (9). Blood smears are covered with liquid emulsion or with Eastman Kodak NTA permeable base stripping film. The method of staining is contingent upon the needs of the investigator. However, routinely, tissues are stained with hematoxylin and eosin. Photographic processing is done under conditions recommended by the manufacturer.

4.2.0 Exposure time. The autoradiographic exposure time used is somewhat arbitrary from one experiment to another. However, test exposures are made in order to determine an optimum time. This optimum exposure must be long enough to yield an unequivocal tissue distribution pattern but short enough to prevent virtual or actual obliteration of the areas being examined.

4.3.0 Additional applications.

4.3.1 Physical state. In addition to the determination of differential distribution of polonium within tissues, autoradiography provides a means of gaining insight into the physical state of polonium in a sample. For this purpose the technique is used to determine the presence, relative abundance and size of polonium aggregates or radiocolloids appearing as radially oriented bursts of tracks as opposed to "ionic" or unaggregated polonium appearing as single tracks.

When the state of administered material or the route of administration is such as to result in the appearance of aggregates in the tissue, a further consideration with respect to exposure time is introduced. Assuming that 50%

---

1. Supplied by Eastman Kodak Co., Rochester, N. Y.

of the disintegrations occurring are registered by the photographic plate¹, a count of the number of tracks in an aggregate suitably corrected for efficiency and exposure time serves as an estimation of aggregate size. Such estimations are ordinarily more valid with increasing exposure time. However, with very long exposures, the tracks may be very difficult to resolve and accurate counting becomes impossible. Thus, here also, test exposures are made to determine optimal exposure. In all work of this type, an effort is made to select an exposure time which will allow a minimum of 10 per cent decay of the activity in the sample. Since the half-life of polonium 210 is approximately 139 days, this represents an exposure of about 21 days. In any event, with any given sample, a series of exposures is made to encompass an optimal time.

Differences in the physical state of polonium can be observed in material other than tissues by the autoradiographic method. Solution autoradiography is being carried out by preparing smears of the solution and covering the smear with liquid emulsion or by mixing the solution directly with the emulsion. Filter papers (Millipore²) used for air or solution filtering or for surface smears are made by clamping the paper tightly between an NTA (25 μ) plate and a clean glass slide.

-
1. Those particles incident to the emulsion at or near perpendicularity are frequently not discernible as tracks, particularly in large aggregates.
  2. Lovell Chemical Co., Watertown, Mass.

APPENDIX I

Preparation of Biological Samples for Analysis.
Miscellaneous Experiments.

1. Fat. One of the major problems in the preparation of biological material for the determination of polonium content is that of destroying fat. In the course of three experiments on the absorption, distribution and excretion of polonium, the proportion of absorbed polonium appearing in the carcass body fat not destroyed in nitric acid was determined and found to be approximately 5 per cent. Results of these determinations are shown in the table below.

Table 1. Distribution of Absorbed Polonium Between Body
Fat and Remainder of Carcass

Experiment	Total Absorbed Po $\mu\text{c} \times 10^{-3}$	Po in Fat $\mu\text{c} \times 10^{-3}$	Po in Remainder of Carcass $\mu\text{c} \times 10^{-3}$	Per Cent Po in Fat	Per Cent Po in Remainder
C-2. Po introduced into intestinal loop	18.3	0.98	17.3	5.4	94.5
C-4. As above	41.2	2.2	39.0	5.3	94.7
C-5. Po introduced into stomach pouch	47.4	28.1	44.6	5.9	94.1

The results indicate that under the conditions of these experiments body fat does not take up appreciable amounts of polonium. In turn, this suggests that digestions of fat-containing materials might be carried to the point where all of the material except the fat was destroyed, after which the fat could be separated and discarded. However, information is not available describing the distribution of polonium between fat and the aqueous-acid phase of a digest. Should the non-fat portion of the digest be appreciably reduced in volume, it is possible that a significantly greater proportion of the polonium may be in the fat phase and thus lost if the fat is discarded.

In an attempt to avoid this theoretically undesirable solution, an

investigation was made of the possibility of using dioxane to keep fat in solution (10). The first experiment undertaken was an attempt to determine whether or not plating efficiency was maintained in the presence of dioxane. In this experiment 1300 cpm was plated in the usual manner, and from a 50-40 dioxane-HCl mixture. Polonium recovered from the latter plating was 89 per cent of that obtained in the absence of dioxane. Though recovery was less than hoped for, it was high enough to indicate that the procedure should be investigated further.

Accordingly, the liver of a cat used in a distribution-excretion study was divided into three portions, each of which was analyzed as indicated in the table below. These data indicate that the highest recovery was obtained by complete digestion of the sample with nitric and sulfuric acids. Use of the dioxane-HCl mixture to place fat in solution did not appear to improve recovery of the polonium.

Table 2. Comparison of Digestion and Plating Procedures Applied to Cat Liver

Fraction	Digestion	Plating Conditions	$\mu\text{c Po} \times 10^{-3}$ per gram
1	conc'd. HNO_3	100 ml 0.5 N HCl	0.1 ¹
2	conc'd. HNO_3	90 ml dioxane-HCl	0.1 ¹
3	conc'd. HNO_3 , H_2SO_4 , H_2O_2	100 ml 0.5 N HCl	0.36 ²

1. Foil was greasy; washed with acetone prior to counting.
2. Foil was clean; not washed with acetone.

To four 1 g samples of omental fat from the same cat were added 2500 cpm of polonium, each sample then being digested and plated as indicated below. These results indicate no superiority of any one set of conditions over the others.

Table 3. Digestion and Plating of Cat Omental Fat

Sample	Digestion	Plating Conditions	Polonium Recovd., cpm
1	conc'd. HNO_3 , H_2SO_4 , H_2O_2	100 ml of 0.5 N HCl	2660
2	"	"	2540 av. 2600
3	conc'd. HNO_3	"	2620 ¹
4	"	90 ml of dioxane-HCl	2560 ¹

1. Foil was degreased with acetone prior to counting.

In a third experiment 12.5 g of omental fat from a rabbit, given 30 μc polonium intravenously 10 days prior to sacrifice, was digested as completely as possible in concentrated HNO_3 . The undigested residue was taken up in dioxane-HCl and plated; the digested portion was plated from 0.5N HCl. A total of 9170 cpm was obtained in the two fractions, of which 92.7 per cent occurred in the digested portion and 7.3 per cent in the non-digested fraction. These results indicate that only a minor fraction of absorbed polonium appears in that portion of the sample not digested in concentrated HNO_3 .

Similar results were obtained with a rat carcass digest, in which 97.9 per cent of the total polonium recovered appeared in the HNO_3 -digestible portion and 2.1 per cent was recovered in the dioxane-HCl soluble fraction.

From these brief experiments it was concluded that

1. Normally, total body fat probably contains about 5 per cent of absorbed polonium.
2. Use of dioxane-HCl plating solution to solubilize undigested fat is of questionable use in our work.
3. The most satisfactory digestion procedure for fat-containing samples consists of first digesting the sample as completely as possible in concentrated HNO_3 , after which destruction of the remaining fat is achieved by use of concentrated H_2SO_4 and 30 per cent H_2O_2 (Superoxol).

2. Attempts at Grinding Carcasses Prior to Digestion. Problems in the digestion of carcasses could be expected to be lessened in magnitude if it should prove feasible to analyze aliquots of the mixed carcass material. The following experiment was undertaken to determine whether or not it would be possible to obtain properly representative samples of ground carcasses.

Approximately 1.24×10^6 cpm of polonium in 0.1 M sodium citrate was administered intraperitoneally to a rat which was sacrificed 5 hours thereafter. The viscera were removed and the remaining carcass and excreta ground in a food chopper using a coarse cutter. A second rat, to which polonium was not given, was sacrificed, eviscerated, and the carcass ground as before. This second animal served only to increase the bulk of the sample passed through the food chopper. The two coarsely ground carcasses were combined and passed two more times through the chopper, using a fine cutter. The finely ground carcasses were weighed in a beaker and the total weight made up to 1000 g with water; the mixture was thoroughly stirred. Two 100 g aliquots were removed, digested in concentrated HNO_3 and plated as usual; three 50 g aliquots were treated in a like manner. The viscera from the two animals were combined and the total sample digested and plated as usual. Data are shown in Table 4.

It is seen that very poor recoveries were obtained. The results indicate that the degree of homogeneity attained by this procedure is totally inadequate. The losses cannot be accounted for by retention of polonium on the metallic surfaces of the chopper, since this was found to be negligible. No explanation can be offered for the 6-fold increase in activity obtained when the smaller aliquots were analyzed. The poor agreement between the two aliquots of digested viscera also is unexpected and unexplained.

Possibly homogenization in a blender of the suspension of ground carcass would lead to better recoveries. Experiments of this nature were not undertaken.

Table 4. Recovery of Polonium from Minced Rat Carcass

Carcass Fraction	cpm/aliquot $\times 10^3$	Total cpm $\times 10^3$	Mean cpm re- covered $\times 10^3$	Per Cent Recovered ¹
viscera	7.686	38.43		
	12.078	60.39	49.41	
carcass ²	4.988	49.88	48.76	
	4.763	47.63	Total 98.17	7.9
carcass ³	15.834	316.7		
	8.608	172.2		
	17.669	353.4	280.8	
			Total 330.2	26.6

1. In the first instance recovery is calculated using the sum of that recovered in the viscera and that in the carcass based on 100 g aliquots; in the second, recovery is based on viscera plus carcass calculated from the 50 g aliquots.
2. 100 g aliquots.
3. 50 g aliquots.

3. Autoclaving. As has been indicated previously, the first step in preparing large carcasses for digestion involves separating the carcass into several portions, one of which constitutes the skeleton. It was thought that separation of the skeleton might be achieved more easily if it were possible first to cook the carcass under pressure, thus making it easier to separate meat and bone. To determine the behavior of polonium under these conditions the following experiment was performed.

Approximately 400 cpm of polonium in 100 ml of saline was placed in a No. 1 coated metal can, sealed and autoclaved at 15 - 20 lb pressure for 4 hours. A second preparation of polonium in 0.15 M citrate was treated similarly. Citrate was investigated in the hope that it might serve to solubilize polonium by virtue of its complexing properties. Upon cooling the cans were opened, the contents acidified to 0.5 N with HCl, and plated. As indicated in the table below, recoveries were incomplete. In an attempt to account for the remaining activity the cans were cut open, flattened and one inch squares removed and counted; total polonium was calculated from the measured surface

areas. A sketch of the opened can and areas sampled is shown below. In neither instance was the top of the can counted.

The cans were lined with a coating stated to be suitable for the canning of acid foods. In the case of the citrate solution the lining was attacked with evidence of rust formation; the saline solution was without effect on this lining. Apparently citrate is broken down, perhaps to bicarbonate, carbon dioxide or carbonic acid, and would not, therefore, be available as a complexing agent.

The results argue against the possibility of autoclaving large carcasses in metal cans, owing to the possibility of losing appreciable amounts of polonium. Suitable glass containers were considered to be excessively large and unwieldy.

Table 5. Recovery of Polonium Autoclaved in Metal Cans

	<u>Citrate</u>	<u>Saline</u>
Counts added	394 cpm	394
Counts in solution after autoclaving	122	225
Counts in replating of supernatant with ascorbic acid	2	46
Bottom of can (total area)	27	38
Sidewall below waterline (total area)	70	30
Sidewall above waterline (total area)	none	11
Area sampled at waterline	3	not done
Total cpm recovered	224	350
Per cent recovered	56.8	88.9

Diagram of can surface areas sampled for polonium.

4. Miscellaneous Digestion Mixtures. A number of acid oxidation mixtures are used routinely in various clinical chemistry procedures. With the thought that these might prove useful in digesting tissue samples, three such agents were tested for their effects on the plating of polonium.

a) Van Slyke-Folch oxidant. This reagent consists of 0.45 M chromium trioxide in syrupy H_3PO_4 and fuming H_2SO_4 . Five ml of the reagent was neutralized and made to a volume of 100 ml, 0.5 N with respect to HCl. Approximately 2100 cpm of polonium was added and the solution plated as usual; duplicate runs were made. Platings also were made of the same amount of polonium in the presence of an equivalent amount of chromium trioxide in 0.5 N HCl. Data are shown in Table 6.

b) Nicleaux reagent. Five ml of this reagent, which consists of silver dichromate dissolved in concentrated H_2SO_4 , was neutralized, diluted to 100 ml and adjusted to 0.5 N with respect to HCl; platings were made as usual after adding approximately 2400 cpm. These data are also shown in Table 6.

c) Kjeldahl digestion mixture. An aliquot of the customary Kjeldahl solution (copper sulfate, selenous acid and potassium sulfate in sulfuric acid) was neutralized and approximately 2400 cpm plated in the presence of the

neutralized aliquot in 0.5 N HCl. In addition polonium was plated from 0.5 N HCl containing only CuSO_4 in equivalent amounts, from HCl containing only equivalent amounts of H_2SeO_3 , and from HCl containing comparable amounts of K_2SO_4 . In each instance the supernatant was replated. Of these components, H_2SeO_3 apparently is the most troublesome. The data are in Table 6.

The results of these brief experiments indicate that regardless of whether or not these various oxidizing agents would be effective in digesting whole animal carcasses, their use is contraindicated because of subsequent interference in the plating of polonium.

Table 6. Effect of Three Digestion Mixtures on Plating of Polonium

Digestion Mixture	Polonium Plated in		Per Cent Recov'd.	State of Foil
	Control	Digest. Mixt.		
Van Slyke-Folch	2133 cpm 2050 av. 2092	4 cpm 14 av. 9	0.4	green deposit
chromium trioxide	"	4 3 av. 4	0.2	white deposit
Nicleaux	2424 2400 av. 2412	1818 998 av. 1408	58.4	some white deposit
Kjeldahl	"	124 291 av. 208	8.6	black
Kjeldahl components				
K_2SO_4	2460	2520	102.4	clear
H_2SeO_3	"	242 228 av. 235		black
supernatant	"	1274; total 1509	61.3	
CuSO_4	"	812 879 av. 845		black
supernatant	"	1400; total 2245	91.3	

5. Precipitation of Calcium Sulfate in the Presence of Polonium.

When sulfuric acid is utilized in the digestion of tissues, calcium present is precipitated as calcium sulfate. The following experiments were performed to determine whether or not polonium is removed from solution with this insoluble material. Five ml of 1 M CaCl_2 containing approximately 3000 cpm of polonium was placed in a centrifuge tube, 5 ml of 9 M H_2SO_4 added and the precipitate thrown down by centrifugation. The supernatant was decanted and the precipitate washed with three 30 ml portions of 0.5 N HCl; approximately 75 per cent of the precipitate was dissolved in the wash solution. Upon combining the supernatant and washings, and plating aliquots of the resulting solution, essentially complete recovery of the added polonium was achieved. The experiment was repeated again but washing of the precipitate was omitted and aliquots of the supernatant were plated. Again, complete recovery of the added polonium was obtained. The data are shown in Table 7.

Table 7. Effect of Precipitation of Calcium Sulfate on the Recovery of Polonium

Control	cpm in supernatant plus washings	Per Cent Recovered	Control	cpm in supernatant (ppt. not washed)	Per Cent Recov'd.
2695	2726			2863	
2829	2790		2750	2709	
av. 2762	av. 2758	99.9		av. 2786	101.3

These results indicate that no polonium is removed in the insoluble calcium sulfate residue formed as a result of the use of sulfuric acid in the digestion of tissue or carcass samples. In practice, a homogeneous aliquot of the digest and insoluble residue is taken for plating. As indicated above, most of this insoluble material is dissolved in the 0.5 N hydrochloric acid medium from which the plating is done.

6. Nature of Insoluble Residue in Carcass Digests. Some investigations were undertaken of the nature of the residue remaining after digestion of a carcass. An adult (3 kg) rabbit was sacrificed by an overdose of nembutal, stored several days at -16°C . and divided into six approximately equal portions by cutting on a bandsaw. Each portion was placed in a 2 liter beaker, covered with concentrated HNO_3 and allowed to stand for 48 hours. Digestion was continued on the hot plate, Dow Antifoam A being added as required, until no further improvement in appearance of the digest was noted. A total of approximately 90-100 hours was required, of which 28 hours consisted of active digestion at an elevated temperature. The nitric acid was boiled off, 75 ml of concentrated H_2SO_4 added, and digestion of the fat completed with H_2SO_4 , HNO_3 and H_2O_2 . The solution was diluted to 500 ml and adjusted to 0.5 N with HCl . A heavy layer of insoluble material was deposited on the bottom of the beaker; a flaky residue floated on the surface. This flaky material was removed and found to be insoluble in carbon tetrachloride, chloroform or acetone, thus establishing that it was not of a fatty nature. Suspensions of the heavier deposit could be put into complete solution by diluting 25 ml aliquots to a final volume of 500 - 600 ml made 0.5 N with respect to HCl . Achievement of a clear solution by this procedure is not favored however, due to the considerable dilution errors involved; in this instance, 100 ml plated of the final solution corresponds to approximately one per cent of the original 3 kg carcass. Low, or poorly absorbed doses of polonium might not result in sufficient activity being present in this small fraction to permit accurate analyses.

The insoluble residue was removed from the initial solution by filtration, air dried, and submitted to Dr. L. T. Steadman of the Spectroscopy Section, Division of Radiology and Biophysics, for spectrographic analysis. The principle element present was found to be Ca and the minor element to be Si; traces of Pb, Na and Mg were detected. The appreciable amount of silica present originates in the stock diet of the rabbits. One g portions of this residue

were soluble in HF-HCl or HF-HNO₃ mixtures, though not in HF alone; 48 hours or more was required for solution.

A second rabbit carcass was treated in a similar manner except that after approximately 10 hours of digestion in HNO₃ the solution was cooled to room temperature and the layer of fat which separated was removed and discarded. Digestion of the remainder was continued with HNO₃ as before. Spectrographic analysis of the insoluble residue of this carcass again indicated Ca to be the principle element present; minor elements present were Si, Na and Mg. Traces of P and Cu were present.

7. Complexing Agents. No work dealing specifically with the use of complexers in the analysis of polonium has been done by these authors. However mention should be made of two experiments carried out by other workers.

a) Phosphate. The salt Na₂HPO₄ had been shown by Toribara and Chen (11) to act as a complexing agent in the determination of beryllium. Accordingly, Scott and Stannard (3) dry ashed polonium-containing samples of muscle and red cells in the presence of Na₂HPO₄ in a similar manner. Recoveries were of the order of 40 - 80 per cent. Scott and Stannard point out that no conclusion can be reached as to whether the losses are caused by volatilization of the polonium or by formation of unavailable complexes with the platinum or porcelain crucibles.

b) Ethylenediaminetetraacetic acid. Morrow and Della Rosa investigated briefly the effects of EDTA on the removal of polonium from a millipore filter paper (12). In this experiment 20,000 cpm of neutralized polonium solution was filtered through millipore paper, the paper was washed with water, and the polonium content of the filtrate and washings determined. The paper was also washed with 10 ml of a 0.5 per cent solution of neutralized "Versene Regular" and then washed again with water; the versene and water washings were combined and analyzed for polonium. Approximately 13 per cent of the activity on the

millipore surface was removed by the versene as employed under these conditions. The data follow.

Table 8. Effect of Versene (EDTA) on the Removal of Polonium from Millipore Filter Paper

Sample	Polonium Recov'd., cpm	Per Cent in Filtrate
Initial solution	20000	-
Filtrate	436	2.2 ¹
Versene filtrate	2560	13.1 ²

1. Calculated on basis of the initial 20,000 cpm.
2. Calculated on the basis of 19,564 cpm remaining in the filter after the initial filtration.

EDTA had little, if any, effect in solubilizing the residue present in a rabbit carcass digest. Two ml of "Versene Regular", pH 11.3 was added to 5 ml of carcass digest. Upon being made slightly alkaline a fine floc appeared.

8. Volatility of Polonium. Considerable interest exists regarding the volatility of polonium with respect to the degree of hazard existing to personnel should a tissue being wet ashed inadvertently go dry. Experiments described in (3) indicated qualitatively that losses of polonium occurred when tissue digests were heated to dryness. Accordingly, the following experiments were done.

Approximately 450 cpm of polonium as the chloride was placed on a glass cascade impactor slide and evaporated to dryness under a lamp, after which the slide was placed on the surface of a hot plate and heated to various temperatures. The following arrangement was made to insure that any polonium volatilized would not present a hazard to personnel, nor produce a contamination problem. A pyrex chimney, approximately 19" x 1-3/4" was mounted over the slide; the upper end of the chimney was inserted through a tightly fitting

rubber stopper into an inverted millipore filter funnel. An HA millipore filter paper was fitted in the filter funnel in the usual manner. The stem of the funnel was connected by heavy rubber tubing to a Gast vacuum pump, the exhaust from the pump being conducted through rubber tubing directly to an exhaust outlet in the ceiling of the work room. The upper six inches of the chimney was packed with pyrex glass wool. The chimney and glass wool was intended principally to cool the air coming to the filter paper since the paper apparently does not retain its filtering characteristics when subjected to high temperatures. Temperatures on the hot plate surface were determined approximately by means of a mercury thermometer inserted in a 2" well in a brass block placed on the hot plate near the slide; contact between the hot plate and block, and block and thermometer was improved by a film of Dow Corning 200 Silicone oil. No corrections were applied to the observed temperature readings.

The slide was heated from room temperature for a period of one hour, during which interval it attained a temperature of 187° . It was cooled, counted and reheated for a second hour, cooled and counted again. The slide was again heated and the time noted when the temperature reached the maximum noted previously; heating was continued for one hour thereafter and a temperature of 303° attained. After being cooled and counted, heating was repeated as before; in the hour after 303° was reached the temperature increased to 375° . After cooling and counting the slide was heated to 308° , held one hour at this temperature, cooled, counted and heated again to 303° . During the next hour the temperature increased to 365° , after which the slide was cooled and counted. With the hot plates available, which are those used in wet ashing tissue samples, temperatures in excess of 375° are not attainable. Data obtained are shown in Table 9, and presented graphically in Figure 4.

FIGURE 4

Volatility of polonium from a heated glass surface.

Table 9. Volatility of Polonium from a Heated Glass Surface

Temperature, °C			Counts per Minute			Per Cent Lost
final	initial	change	initial	final	change	
187	24	163	450	408	42	9.3
187	23	164	408	346	12	2.9
303	187	116	396	247	149	37.6
375	303	74	247	199	48	19.4
306	308	-2	199	187	12	6.0
307	308	-1	187	189	-2	-
365	303	62	189	186	3	1.6

The data indicate that repeated heating to a given temperature has little additional effect; the increased losses of 1.6 - 6.0 per cent probably are not significant. The loss of only 9.3 per cent for the initial temperature increase of 163°, when compared with later losses of 37.6 per cent for 116° of change and 19.4 per cent for a change of 74° suggests that during the first portion of the initial increase the temperature was not high enough to cause volatilization. Figure 4 shows that this situation obtains up to approximately 145°. Over the range 145 - 375° the loss is reasonably constant at 0.3 per cent per degree of temperature increase.

The fact that repeated heating does not cause further loss suggests that the polonium chloride originally present is converted over a period of time to a non-volatile form (perhaps oxides), or alternatively, that polonium is not lost until the chloride has been converted by heating to a volatile form. The latter alternative seems less likely.

A total of 226 cpm was lost during the various periods of heating. Of these, 182 cpm was recovered from the glass wool and walls of the chimney by washing with 3N HCl; an additional 20 cpm was found on the millipore paper. Thus, 64 cpm or 14.2 per cent of the original polonium is unaccounted for.

Using the same apparatus and procedure, the volatility was measured of

polonium deposited on silver foil, either by plating or by evaporation of the chloride. The data are shown in Table 10.

Table 10. Volatility of Polonium from Silver from a Heated Silver Surface

Temperature, °C			Counts per Minute			Per Cent Lost
final	initial	change	initial	final	change	
PLATED						
176	24	152	434	430	4	0.9
180	22	158	430	431	-1	-
302	174	128	429	402	27	6.3
358	300	58	402	400	2	0.5
365	306	59	394	396	-2	-
EVAPORATED						
183	22	161	481	474	7	1.5
181	30	151	474	468	6	1.3
303	182	121	468	477	+9	-
362	299	63	469	469	8	1.7

In every instance but one the actual counts lost probably do not exceed the counting errors. Apparently there is no loss of polonium from silver, regardless of the manner in which the polonium was deposited on the silver. The total loss of polonium chloride evaporated on glass and heated under the same conditions exceeded 75 per cent. The maximum loss from silver occurred upon heating polonium from 174° to 302°; the maximum loss (37.6 per cent) of polonium from glass also occurred over this same temperature range. Because of the paucity of data this observation may have no significance. Repeated heating caused no additional loss of polonium.

These experiments suggest that should a sample being wet ashed in glass containers inadvertently go dry, a considerable fraction of the polonium could be expected to be lost to the atmosphere.

APPENDIX II

A BATH FOR PLATING OF POLONIUM

R. H. Wilson

At the inception of the Radioactive Inhalation program it was realized that large numbers of samples for polonium assay could be expected, and that means would have to be found to plate these samples with maximum efficiency. As mentioned in the body of the foregoing report, considerable experience was gained with a six-unit plating bath using individual motor stirrers for each plating column. This experience showed very clearly that individual stirrers were unsatisfactory for a number of reasons:

- 1) Reliability was poor;
- 2) Speed control was not satisfactory, especially at the desired low speed;
- 3) Space requirements were excessive, particularly in terms of increasing plating capacity.

Baxter and Wood (5) had demonstrated that a single drive operating 24 stirrers could handle a great many samples per day with high reliability and efficiency. Advantage was taken of the experience of these investigators to design an improved version of the multiple-column plating bath.

The 6 x 4 column arrangement in the first model (5) was adjudged awkward in that the fourth row was difficult to reach unless the stirrers were raised very high. Secondly, the overhead pulley and counterweight arrangement made relocation of the bath difficult. Thirdly, there was some possibility of stirring rod breakage if the stirrer plate was not carefully lowered to the operating position.

Using Baxter and Wood's suggestions, the plating-bath shown in Figure 5 was designed and constructed. This model is admittedly more complicated and consequently more expensive, but it has been completely justified by the greater convenience of use.

FIGURE 5

Twenty-four unit plating assembly.

46

-41a-

As can be seen in the illustration, the column arrangement is 8 x 3, all stirrers being driven by a single round belt. Accessibility of the back row is good, without excessive vertical travel of the pulley plate. The unit is completely self-contained, the counterweight and guide column, the pulley plate and the bath all being mounted on a single plywood and transite baseplate. Consequently, it is completely portable and readily moved. The improved lateral constraint of the pulley plate, resulting from the vertical guide rods, means that stirring rod breakage is virtually impossible.

Because of the use of high grade ball bearings on each stirrer, the power required for stirring is low and a variable-speed fractional horsepower motor¹ suffices to drive all twenty-four stirrers at any desired speed.

A detail of the pulley mounting is shown in Figure 6. The bearings used were Norma XA-134-R-PP, 5/8" diameter with 1/4" bore. In view of the corrosive conditions to which these bearings are exposed, the grease seal was considered a particularly desirable feature, and indeed, the bearings, the shaft, and the stirring rod coupling are heavily coated with grease in normal use. It is believed that the pitch diameter of the pulley is of some consequence; there have been many reports of drive problems with small round belts in other multiple-stirrer devices and in practically all cases these have been attributable to a pitch diameter which is too small. It should be recognized that the drive belt follows an unusually tortuous path, with numerous reversals of flexure, which will lead to rapid fatigue failure if the degree of flexure is high as a result of the use of small pulleys. With the design pitch diameter of 1-3/8", the original 1/8" dia. neoprene belt is still in use after nearly two years. Part of this life is due to minimal belt tension, adjusted by moving the motor and its mount. Tension is no greater than just enough to ensure turning of all stirrers.

---

1. Central Scientific Company Catalog No. 18805, Chicago, Ill.

FIGURE 6

Detail of pulley mounting for multiple plating assembly.

49

DETAIL OF PULLEY ASSEMBLY

Aluminum was chosen for the pulley plate for two reasons—firstly because of its low density, and secondly because of its corrosion resistance to the acid fumes given off in the plating operation.

The bath is constructed of 16-ga. copper and is 23-3/4" long, 8-1/4" wide and 6" deep. It is jacketed with 1" magnesia-block insulation on all but the top surface. The columns are mounted in a brass hardware cloth basket. Brass locator collars are soldered to the bottom of the basket to receive the capped ends of the columns, while a copper sheet, pierced to provide minimum clearance around the columns, serves to keep them upright and properly positioned, and also cuts down evaporative losses from the bath.

The bath is heated by two flexible electric immersion heaters¹ of 750- and 1000-watt rating. It has been found that this combination will supply just enough heat to maintain temperatures of 90-93°C. without thermostatic control. It was found that the elevated temperature of the bath led to a serious amount of vapor condensation on the pulley plate, with subsequent dripping back into the plating columns. This difficulty was readily eliminated, however, by the addition of a small centrifugal blower which sweeps away the moisture-laden air. Tests demonstrated that ducting of the air was unnecessary, and that free discharge of any small fan was suitable for this purpose.

The vertical guide rods are of brass, 5/8" diameter, while the slide bearings are 1-3/8" hard copper tubing with brass inserts in each end. In the normal stirring position one of these bearings rests on a stop on its guide rod, 3" above the baseplate.

In order to cut down on over-all height, the counterweight attachment was made at the center of gravity of the pulley plate assembly. This led to operational problems which will be discussed later. The counterweight column is a piece of 1-1/2" pipe, machined smooth and true. The pulleys on the top of the column are

---

1. Will Corporation Catalog No. 15951, Rochester, N. Y.

of brass, running on steel pins. The bearing mount consists of two 1/4" steel plates welded to a standard pipe cap. The counterweight is solid lead, weighing approximately 35 pounds, with the 1/8" braided steel support cable cast in at its center of gravity, (as distinguished from its geometrical center). Clearance between the hole in the counterweight and the pipe column is 1/8", and the column height is 30 inches. The over-all height, with the plate in its uppermost position, is 34 inches.

The operational difficulties mentioned above involved the vertical motion of the assembly. When first constructed, it was found that unless one applied force, either up or down, in just the right place, the plate tended to jam, in spite of the relatively long slide bearings. This was ultimately remedied by the installation of the brass rod to which the steel cable is attached, so that any tendency to cock is opposed by a restoring force applied by this rigid rod. A better solution, and one which will be incorporated in future models, would be to use 1" brass angle stock for the vertical guides and small rollers riding on the two center faces of the angle stock separated vertically by perhaps 6". The former would be considerably more rigid than rod stock, while the latter would not bind even if the plate were slightly tipped.

APPENDIX III

STATISTICAL DESIGN AND ANALYSIS OF EXPERIMENTS ON POLONIUM PLATING TECHNIQUE

Arthur M. Dutton
Statistics Section

Experiment I

Design. The technique factors to be investigated in this experiment were finally narrowed to six in number. Since this was to be a more or less preliminary experiment, it was felt that two levels (a "high" and a "low") of each factor would give adequate information. Later experiments could then locate an optimum deposition point. The technique factors and the levels of each considered are listed in Table 11. The quantity of polonium was kept constant at a level of about 4000 cpm.

Table 11. Factors Studied Factorially for their Influence on the Spontaneous Plating of Polonium from Acid Solution

Factor	Values
Temperature of plating bath	45° C 90° C
Time of plating	45 minutes 90 minutes
Volume of plating solution	50 ml 100 ml
Stirring	as fast as practicable as slow as practicable
Acidity of solution	0.25N HCl 0.50N HCl
Evaporation of plating sol.	not replenished ¹ vol. replenished frequently

1. When not replenished approximately half of the volume was lost by evaporation.

A complete factorial experiment giving information on these factors in every possible combination would involve 2⁶ platings for a single replication. In

Table 12. Analysis of Variance for Experiment I.

Source Main Effects	Degrees of Freedom	Mean Square	Significance
Temperature	1	1,739,112.50	At 0.01 probability level
Time	1	892,448.00	" " " "
Volume Level	1	770,040.50	" " " "
Rheostat Setting	1	13,944.50	At 0.05 probability level
Normality	1	87,571.13	
Evaporation Factor	1	33,670.13	
Interactions			
Temperature x Time	1	99,904.50	At 0.05 probability level
Temperature x Volume	1	350,284.50	At 0.01 probability level
Temperature x Rheostat Setting	1	578.00	
Temperature x Normality	1	3,655.13	
Temperature x Evaporation	1	1,431.13	
Time x Volume	1	202,248.00	At 0.01 probability level
Time x Rheostat Setting	1	3,784.50	
Time x Normality	1	1,275.13	
Time x Evaporation	1	2,926.13	
Volume x Rheostat Setting	1	6,272.00	
Volume x Normality	1	193,753.13	At 0.01 probability level
Volume x Evaporation	1	15,225.13	
Rheostat Setting x Normality	1	1,378.13	
Rheostat Setting x Evaporation	1	16,836.13	
Normality x Evaporation	1	24,864.50	
Error	10	11,695.20	

order to get some information on the experimental error involved it would be necessary to use several replications — involving perhaps 200 or more platings. This was not considered feasible. By using the device of Fraction Replication (13), however, it was possible to examine the six factors with a considerably smaller number of platings. The particular arrangement used is called "Half Replicate of a 2^6 Factorial Experiment". This arrangement involved 32 separate platings and gives information on the main effects and interactions between all pairs of factors, but no information on higher order interactions.

Analysis. The data were analyzed by the analysis of variance technique. The complete analysis is given in Table 12.

From this analysis there is no evidence of any interactions or average effects due to the factors Stirring and Evaporation. There is significant evidence of first order interaction between, a) Temperature and Time, b) Temperature and Volume, c) Time and Volume, d) Volume and Normality. Because of these interaction effects it is meaningless to consider the main effects as treated in this analysis. If there is an interaction between temperature and time, for example, it means that an increase of temperature from 45°C . to 90°C . will lead to an increased deposition, the amount of which depends on the time level involved. It is thus useless to talk about the sample effect of a waterbath temperature increase unless we specify what the time of deposition is.

In light of the first order interactions indicated by the analysis the actual factor effects can be summarized as in Table 13 which gives the average counts in each of 16 factor classes and the standard deviation of these averages.

From Table 13 it is evident that plating is optimum when a bath temperature of 90°C . and a time of plating of 90 minutes are used. If this combination is used volume and normality have no demonstrable effect. It can also be seen that if a bath temperature of 90°C . and a volume of 50 ml are used that optimum plating can be obtained regardless of time of plating or acidity. The empirically optimum plating was obtained at 90°C ., 90 minutes time, 100 ml volume, 0.25N HCl.

Table 13. Average Deposition in Counts per Minute for Each of 16 Factor Classes

13-1 Common Factors: 50 ml, 0.25 N HCl

	45'	90'
45°C	3599.5	3799.0
90°C	3809.0	3926.0

13-2 Common Factors: 50 ml, 0.5 N HCl

	45'	90'
45°C	3525.5	3797.5
90°C	3951.5	3963.0

13-3 Common Factors: 100 ml, 0.25 N HCl

	45'	90'
45°C	2983.0	3553.0
90°C	3790.0	4089.0

13-4 Common Factors: 100 ml, 0.5 N HCl

	45'	90'
45°C	2683.0	3324.5
90°C	3452.5	3914.0

The numbers given are the means of two platings and the estimated standard deviation of each number is 76.5 cpm.

Conclusions: (1) There is no evidence of a change in plating efficiency depending on stirring speed and no evidence of a change depending on evaporation at the levels considered.

(2) If a bath temperature of 90° C. and plating time of 90 minutes are maintained plating is near the optimum and changes of volume or normality in the range studied have little effect.

(3) Similarly if a temperature of 90° C. and plating solution volume of 50 ml are maintained plating is near optimum and changes in time of plating and acidity have little effect.

Experiment II. In Experiment I the stirring factor was considered only at two positive levels — fast and slow. Since the plating apparatus would be considerably simplified if stirring could be eliminated altogether, it was felt that this factor should be investigated further. Moreover, Experiment I did not give a clear cut answer on the effect of acidity. Experiment II was designed to investigate these two factors — stirring and acidity — more extensively. The other factors (temperature of bath, time, volume, etc.) were set at standard levels. It was also decided to use three general levels of

activity — approximately 200 cpm, 20000 cpm, 200000 cpm.

Design. Three levels of acidity (0.1 N HCl, 0.33 N HCl, 0.75 N HCl) and two levels of stirring (none and at a "medium" speed) were to be investigated. The six possible combinations of these factors constitute a complete factorial experiment. Four replications of the basic design were used, a re-randomization of the six treatment combinations being made in each replicate. This procedure was repeated at each of the three activity levels involved.

Analysis. There was an obvious difference between the activity plated when the solution was stirred and when it was not stirred. Furthermore the experimental error variability had a very strong dependence on the level of radioactivity involved in the plating. For these reasons a separate analysis of the acidity factor effects was made at each stirring level. The appropriate analyses of variance for each level of radioactivity are presented in Table 14. Table 15 gives the mean amount of radioactivity recovered in 4 platings made in each of the six factor categories and the estimated standard deviations of these means. From these tables it can be seen that stirring has a very large effect, practically doubling the amount of polonium deposited. It can also be seen that when there is stirring there is no significant difference due to the level of normality. When there is no stirring, however, there is probably a greater deposition with the low normalities. This effect is statistically significant at the medium activity level and evident at the other two levels. This fact is of little importance however since stirring itself has such a great influence. In any practical instance stirring will be used and hence normality will not have any effect, at least in the range considered here.

It should also be emphasized that the experimental errors involved in this experiment increase as the level of activity increases.

Conclusions. (1) Stirring is necessary for an optimum deposition of polonium using our techniques.

(2) There is no evidence that acidity in the 0.1 N to 0.75 N range has any effect on deposition.

(3) Errors involved in plating polonium increase as the level of activity involved is increased.

Table 14. Analysis of Variance for the Detection of Acidity Effects on the Experimental Plating of Polonium

Source	Degrees of Freedom	Stirring Mean Square	No Stirring Mean Square
Low Activity			
acidity levels	2	av. of 175 cpm 85.75	av. of 92 cpm 69.34
replication	3	30.44	21.00
error	6	38.86	19.33
Medium Activity			
acidity levels	2	av. of 9000 cpm 504,014	av. of 21000 cpm 1,025,363 ¹
replication	3	1,151,359	3,663,384
error	6	857,409	92,618
High Activity			
acidity levels	2	av. of 1,000,000 cpm 130,359 x 10 ²	av. of 210,000 cpm 988,576 x 10 ²
replication	3	1,320,899 x 10 ²	2,546,243 x 10 ²
error	6	530,636 x 10 ²	2261,907 x 10 ²

1. Statistically significant at the 0.05 probability level.

Table 15. The Effects of Stirring on the Radioactivity Recovered in the Experimental Plating of Polonium

Means of Four Platings

Acidity	Activity in Counts per Minute	
	Stirring	No Stirring
Low Activity		
0.1 N HCl	175.3	95.5
0.33 N	179.5	93.5
0.75 N	170.3	87.5
Std. Deviation of Means	3.1	2.2
Medium Activity		
0.1 N HCl	21660.8	9476.8
0.33 N	21055.3	8947.5
0.75 N	21037.0	8464.5
Std. Deviation of Means	926.0	304.3
High Activity		
0.1 N HCl	211,778	107,118
0.33 N	210,623	102,803
0.75 N	208,238	97,203
Std. Deviation of Means	3643	2559

APPENDIX IV

EFFECT OF NITRIC ACID ON THE PLATING OF POLONIUM

Failure to neutralize the aliquot of nitric acid digest taken for plating

leads to uncertainty in the adjustment of the plating solution to 0.5N acidity, and also results in the plating being carried out in the presence of small amounts of nitric acid. This latter feature in turn, results, when the aliquot is very small, in a red-brown or black deposit on the surface of the silver foil which interferes with deposition of the polonium or/and with its counting. This interference on the part of nitric acid was investigated briefly as follows.

Approximately 2000 cpm of polonium was deposited on a foil by plating for 90 minutes at 90° in the presence of 100 ml of 0.5N hydrochloric acid. After counting the foil was again placed in the plating column, covered with 98 ml of a solution consisting of 95 ml of 0.5N hydrochloric acid and 3 ml of concentrated nitric acid and kept in the plating bath at 90° C. for 90 minutes. Upon removal of the foil, it was seen to be blackened. When counted, only 76.9 per cent of the original count was obtained. A second experiment was done in which approximately 2000 cpm of polonium was initially plated from a solution of 95 ml of 0.5 N hydrochloric acid and 3 ml of concentrated nitric acid. Again, the foil was blackened. Under these conditions only 54.4 per cent of the polonium was accounted for. The data obtained are shown in Table 16.

On the basis of these experiments it was concluded that neutralization of the digest aliquots prior to plating is an essential step in the preparation of solutions for the plating of polonium.

61

Table 16. Effect of Nitric Acid on the Plating of Polonium

Preparation	State of Silver Foil	Counts per Minute	Per Cent Recovered
1. Polonium plated in presence of 0.5N HCl for 90 min. at 90° C.	clear clear	2018 2012 av. 2015	100.0
2. As above. Foil then heated 90 min. at 90° in presence of 95 ml. 0.5N HCl + 3 ml. concd. HNO ₃	blackened blackened	1580 1520 av. 1550	76.9
3. As for No. 1 above.	clear clear	2155 2192 av. 2171	100.0
4. Polonium plated for 90 min. at 90° C. in presence of 95 ml. 0.5N HCl + 3 ml. concd. HNO ₃	blackened blackened	1376 983 av. 1180	54.4

APPENDIX V

EFFECT OF IRON ON THE PLATING OF POLONIUM

Some aspects of the effects of iron on the plating of polonium were described in (1). It was considered advisable however that we investigate this problem with the methods and equipment to be used routinely in this laboratory.

A constant quantity of polonium (5000 cpm) was plated according to the standard procedure in the presence of ferric iron concentrations ranging from 2×10^{-5} to 1×10^{-2} M. The μ M ratio of iron to polonium thus ranged from 3.8×10^9 to 1.9×10^{12} . The data are presented in Table 17 and Figure 7. It is apparent that the plating of 5000 cpm is significantly interfered with at a ferric iron concentration of 10^{-3} M or greater, corresponding to a blood volume, for example, of 10 ml. Less than 5 per cent of the polonium is recovered in the presence of 10^{-2} M ferric iron. The question still remains however, as to whether or not the inhibition is due to exceeding an innocuous iron concentration per se, or to exceeding a safe iron:polonium ratio. Accordingly, a limited series of platings was done in which the iron concentration used (0.5×10^{-3} M) was such that no inhibition would be expected, as determined from Figure 7, and in which the polonium concentration (about 400 cpm) was such that the iron:polonium ratio was in the region where major inhibition was to be expected. These data are shown in Table 18; a few additional data also are included here, in which the iron:polonium ratio was reduced by a factor of 2.5. The findings indicate that the inhibitory effect of iron is seen only at iron concentrations exceeding 5×10^{-4} M in the solution to be plated (Table 17), and is independent of the polonium concentration.

The inhibiting effect of ferric iron at concentrations up to 10^{-2} M can be negated by the addition of 100 mg of ascorbic acid to the solution to be plated, whereby ferric iron is reduced to the ferrous state. This iron concentration corresponds to that obtained in the digest of 100 ml of whole blood.

FIGURE 7

Effect of ferric iron on the plating of polonium

64

Figure 7. Effect of Ferric Iron on the Plating of Polonium

Po²¹⁰ $\approx$ 5000 cpm
All points in duplicate

-57a-

Table 17. Effect of Ferric Iron on Recovery of Polonium by Plating

Molarity of Iron in Plating Solution	Equivalent Blood Volume, ml.	Po on Foil in cpm	Mean	Per Cent Recovered
no iron	-	4918 5107	5013	100.0
2×10^{-5} M	0.2	4605	4605	91.9
1×10^{-4}	1.0	4817 4964 4993	4925	98.2
2×10^{-4}	2.0	4862 5076	4969	99.1
5×10^{-4}	5.0	5004 5062	5033	100.4
7×10^{-4}	7.0	4836 4952	4894	97.6
1×10^{-3}	10.0	4225 4393 4747	4455	88.9
2×10^{-3}	20.0	2636 2960	2798	55.8
3×10^{-3}	30.0	1076 1126	1101	22.0
4×10^{-3}	40.0	843 827	835	16.7
5×10^{-3}	50.0	557 594 413	521	10.4
1×10^{-2}	100.0	184	184	3.7

66

Table 18. Effect of Iron:Polonium Ratio on the Recovery of Polonium

Fe Concn. μM	Po Concn. $\mu\text{M} \times 10^{-12}$	$\frac{\mu\text{M Fe}}{\mu\text{M Po}} \times 10^{11}$	Po Recovered $\mu\text{M} \times 10^{-12}$	Per Cent Recovered
Control (no Fe)	463	—	—	—
	422	—	—	—
	av. 442	—	—	—
500	442	10	445	
			458	
			476	
			457	
			av. 459	103.8
Control (no Fe)	26.1	—	—	—
	25.0	—	—	—
	av. 25.6	—	—	—
10	25.6	3.9	21.2	
			24.4	
			26.8	
			av. 27.7	108.2
Control (no Fe)	440	—	—	—
10	440	0.2	457	103.9

The absence of effect on plating of ferrous iron concentrations of 2×10^{-5} to 1×10^{-2} M is indicated in Table 19,

The importance of the addition of ascorbic acid was demonstrated practically by replating in the presence of this reagent the supernatant solutions resulting from plating a number of tissue digests without added reducing agent. The data are shown in Table 20. It is apparent that significant losses of polonium may be expected in analysis of the following tissues, if ascorbic acid is not present in the solution plated: liver, intestine, stomach, spleen, blood samples in excess of 5 ml. These tissues are rich in blood and/or iron. In those instances where 5 per cent or less of the total polonium was recovered in the supernatant, iron is assumed to be of no importance, inasmuch as the plating procedure has been shown not to account for approximately 8 per cent of the polonium present in solutions containing these levels of activity (cf Appendix VI).

Table 19. Effect of Ferrous Iron on Recovery of Polonium

Molarity of Iron in Plating Solution	Po on Foil cpm	Mean
no iron	4918 5107	5013
2×10^{-5} M	5040	
1×10^{-4}	5288	
5×10^{-4}	5163	
1×10^{-3}	5246	
5×10^{-3}	5336	
1×10^{-2}	5015	5181

Table 20. Influence of Iron in Biological Materials on the Plating of Polonium

Tissue (cat) ¹	Counts per Minute Obtained in		Per Cent of Total in Supernatant
	original plating without ascorbic acid	supernatant with ascorbic acid	
blood, 4.4 ml	7783	362	4.4
same blood, 17.8 ml	21553	5286	19.7
liver, 52 g	70317	25790	26.8
intestine, 59 g	3537	3077	46.5
stomach, 28 g	2657	5055	65.5
kidney, 12 g	82294	4299	5.0
kidney, 11 g	79853	2878	3.5
lung, 19 g	23989	1342	5.3
spleen, 6 g	11911	1185	9.0

¹ From an absorption, distribution and excretion experiment.

APPENDIX VI

EFFICIENCY OF THE POLONIUM PLATING PROCEDURE

Arthur M. Dutton
Statistics Section

Introduction. This experiment was performed in order to gain information on the relative amounts of radioactivity recovered at respective stages of a sequential plating procedure. The standard plating technique described in Section 3.4.0 was used. The supernatant of the original plating was plated and subsequently the supernatant of this second plating was plated. This was repeated until a total of five platings had been made at the lower activity levels (10 cpm to 1000 cpm) and nine platings at the high activity levels (10000 cpm to 200000 cpm). At the lower levels activity approximating background were obtained in the later replatings. At the higher levels the activity recovered plateaued in the fifth to ninth replatings at levels only slightly greater than background.

Design. Three low levels — approximately 10 cpm, 100 cpm and 1000 cpm — and three high levels — approximately 10000 cpm, 20000 cpm and 200000 cpm — were used. A single series consisting of a complete plating procedure for a solution at each one of these levels constituted a replication of the experiment. The order in which the solutions were plated was randomized within the replicate. A total of 4 complete replications was done.

Analysis. The data obtained in this experiment are presented in Table 21.

Table 22 gives the percentage of total recovered activity obtained in the initial plating. Note that this is not the percentage of the total activity recovered on the first plating because the total activity is not known. The total recovered activity is that recovered on the initial plating plus that recovered in the four or eight subsequent replatings.

Table 23 gives the percentage of total recovered activity obtained in the first two platings.

Table 24 gives the analysis of variance appropriate to the data of Table 22 and Table 25 gives the analysis of variance appropriate to the data of Table 23.

It can be seen from these analyses that there is evidence that the recovery percentage does depend on the activity level and goes up in a direct fashion almost as the logarithm of the activity level. For this reason we may say that recovery percentage obtained on the initial plating depends on the activity encountered. Similarly the recovery percentage on the first two platings depends on the activity level involved. It is also apparent that error term is smaller when total percentage plated on the first two platings is considered than when only the initial plating is encountered. This is a result of a "compensating" effect which makes the recovery small on the first replating if the initial recovery is large, and similarly makes the recovery large on the replating if the initial recovery is small.

It was thought useful to present 90 per cent Tolerance Intervals (14), i.e., intervals within which 90 per cent of individual values will fall. It was convenient to use only a one sided interval, i.e., to give a number above which we expect 90 per cent of the recoveries to lie. These are intervals with a 0.95 confidence coefficient, that is, 95 per cent of the time intervals so obtained will include the specified percentage of the population. The intervals are presented for each activity level in Table 26. It is evident that at least 80 per cent of the activity to be recovered will be recovered in the initial plating. Except at 15 cpm, the first two platings will yield at least 94 per cent of the activity recoverable with this plating technique.

Conclusions. (1) The percentage of recoverable radioactivity obtained on an initial plating depends on the activity level involved. At levels of the order of 10 cpm at least 80 per cent will be recovered on the initial plating. At high levels of about 200000 cpm at least 86 per cent will be recovered.

(2) The percentage of recoverable radioactivity obtained on the initial plating coupled with a plating of the initial supernatant is at least 91 per cent at a low level (10 cpm) and at least 95 per cent at a high level (200000 cpm).

10000	10000	10000	10000	10000	10000	10000
10000	10000	10000	10000	10000	10000	10000
10000	10000	10000	10000	10000	10000	10000
10000	10000	10000	10000	10000	10000	10000
10000	10000	10000	10000	10000	10000	10000

10000 10000 10000 10000 10000 10000 10000

10000 10000 10000 10000 10000 10000 10000

Table 21. Efficiency of the Standard Plating Procedure for Polonium

Counts per Minute¹

Activity Level	10 ²	100 ²	1000 ²	10000 ³	20000 ³	200000 ³
Replicate 1	13	123	1081	10362	20631	200273
Replicate 2	17	109	1075	10853	21464	198663
Replicate 3	14	113	1048	10354	21409	198658
Replicate 4	14	112	1040	10257	21146	201258
Average	15	114	1061	10457	21163	199713

1. Corrected for background, plating column blank where this exceeds twice the background count, and for decay.

2. Total for five replicate platings.

3. Total for nine replicate platings.

Table 22. Percentage of Total Recovered Activity Found in the Original Plating

Average Activity Level	15 cpm	114 cpm	1061 cpm	10457 cpm	21163 cpm	199713 cpm
Replicate 1	92.86	93.80	96.47	96.33	94.19	97.03
Replicate 2	92.86	85.71	90.58	94.83	96.95	92.54
Replicate 3	84.62	90.24	90.66	82.14	96.22	97.25
Replicate 4	88.24	92.66	94.33	96.46	87.91	97.25
Average	89.65	90.60	93.01	92.44	93.82	96.01

Standard Deviation of Means (per cent): 2.03

Standard Deviation of Single Observation (per cent): 4.06

Table 23. Percentage of Total Recovered Activity Found on First Two Platings
(Original plus One Replating)

Average Activity Level	15 cpm	114 cpm	1061 cpm	10457 cpm	21163 cpm	199713 cpm
Replicate 1	100.00	97.34	99.05	99.66	98.43	99.90
Replicate 2	92.86	97.32	98.17	99.05	99.56	97.76
Replicate 3	92.31	98.37	98.61	95.45	99.15	99.79
Replicate 4	94.12	99.08	99.07	99.47	98.39	99.73
Average	94.82	98.03	98.73	98.40	98.88	99.30

Standard Deviation of Means (per cent): 0.86

Standard Deviation of Single Observation (per cent): 1.71

Table 24. Analysis of Variance on Percentage of Total Recovered Activity Found in the Original Plating

Source	Degrees of Freedom	Mean Square
Replicates	3	24.60
Regression on Log of Activity Levels	1	94.39 ¹
Among Activity Levels other than Regression	4	2.45
Error	15	16.51

1. Significant at 0.05 Probability Level.

Table 25. Analysis of Variance on Percentage of Total Recovered Activity Found in First Two Platings

Source	Degrees of Freedom	Mean Square
Replicates	3	4.08
Regression on Log of Activity Levels	1	36.42 ¹
Among Activity Levels other than Regression	4	4.14
Error	15	2.93

¹ Significant at 0.01 Probability Level.

Table 26. Percentages Above Which 90 Per Cent of Individual Platings are Expected (Confidence Coefficient 0.95) to Fall

Average Activity Level	15 cpm	114 cpm	1061 cpm	10457 cpm	21163 cpm	199713 cpm
Recovery on Initial Plating	80.0	81.0	83.4	82.8	84.2	86.4
Recovery on First Two Platings	90.8	94.0	94.7	94.3	94.8	95.2

BIBLIOGRAPHY

1. Fink, R. M., editor. Biological studies with polonium, radium and plutonium. National Nuclear Energy Series, Division VI, volume 3, pp. 15-16, McGraw-Hill Book Co., Inc., New York, N. Y., 1950.
2. Finkel, M. P., Norris, W. P., Kisieleski, W. E. and Hirsch, G. M. The toxicity of polonium 210 in mice. I. The thirty day LD₅₀, retention, and distribution. Am. J. Roentgenol. Radium Therapy Nuclear Med. 70, 477-85 (1953).
3. Scott, R. G. and Stannard, J. N. Modified procedure for analysis of polonium 210 in biological materials. University of Rochester Atomic Energy Project Report UR-235, 31 March 1953.
4. Middleton, G. and Stuckey, R. E. The preparation of biological material for the determination of trace metals. Part I. A critical review of existing procedures. Part II. A method for the destruction of organic matter in biological material. Analyst 78, 532-42, (1953); ibid 79, 138-42 (1954).
5. Baxter, R. and Wood, D. Apparatus for plating out polonium for analysis. University of Rochester Atomic Energy Project Report UR-269, 3 August 1953.
6. Smith, F. A. and Della Rosa, R. J. Quarterly technical report, April 1 thru June 30, 1953, University of Rochester Atomic Energy Project Report UR-268, 2 September 1953.
7. Casarett, L. J. Autoradiographic studies on polonium. M.S. Thesis. University of Rochester, 1955.
8. Evans, T. C. Radioautographs in which the tissue is mounted directly on the photographic plate. Proc. Soc. Exptl. Biol. Med. 64, 313-15 (1947).
9. Arnold, J. S. An improved technique for liquid emulsion autoradiography. Proc. Soc. Exptl. Biol. Med. 85, 113-16 (1954).
10. Bahner, C. T., Zilversmit, D. B. and McDonald, E. The preparation of wet ashed tissues for liquid counting. Science 115, 597-8 (1952).

11. Toribara, T. Y. and Chen, Jr., P.S. The separation of beryllium from biological material. University of Rochester Atomic Energy Project Report UR-170, 24 May 1951.

12. Morrow, P. E. and Della Rosa, R. J., unpublished data.

13. Kempthorne, O. Design and analysis of experiments. John Wiley and Sons, Inc., New York, N. Y., 1952, pp. 390-409.

14. Hald, A. Statistical theory with engineering application. John Wiley and Sons, Inc., New York, N. Y., 1952, p. 311.