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PROCEDURE FOR THE RADIOCHEMICAL ANALYSIS OF STRONTIUM AND BARIUM IN HUMAN URINE

L. B. Farabee

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PROCEDURE FOR THE RADIOCHEMICAL ANALYSIS OF STRONTIUM AND BARIUM IN HUMAN URINE

Lawrence B. Farabee

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ABSTRACT

An analytical procedure for the determination of radioactive strontium and barium in large volumes of urine is described. The method is based on the preferential chelation of calcium over strontium using versene. This difference is greatest at a pH of 4.5 to 6.0. When a versenate chelate of the alkaline earths in a urine specimen, at a pH of 5.5, is passed over a cation exchange column all of the strontium and barium is adsorbed, whereas almost all of the calcium and about 1/2 of the magnesium passes into the effluent as the chelate. The extraneous calcium and magnesium can be removed from the resin with a solution of citric acid and versene at a pH of 5.0. The sodium is removed with 0.5N HCl, while the radioactive strontium and barium is eluted with 6N HNO_3.

INTRODUCTION

Some of the radioisotopes resulting from the fission of uranium when ingested in the body become incorporated in certain critical organs of the body and are harmful. When ingested, 60 per cent of the strontium isotopes pass from the gastrointestinal tract into the blood, while the barium fraction going into the blood is 10 per cent [1]. Handbook 42 [2] lists as moderately dangerous Ba^{140} and Sr^{89} and as very dangerous Sr^{90}. The values of maximum permissible amounts of various radioisotopes in the human body have been reported in Handbook 52 [3]. The amounts of these harmful fission products that have entered the body can best be estimated by analyzing the urine for these radioisotopes. Handbook 52 states that, "if there is exposure to Sr^{90},

every precaution should be taken to minimize the body uptake, and urinalysis should be made frequently so that the amount going to the bone can be estimated from concentrations in urine during the early portion of the period of exposure when the elimination rate is much higher."

There is a need for an analytical procedure to detect tracer quantities of these radioactive isotopes in large volumes of urine ($\sim$1500 ml). This paper describes a method for the analysis of strontium and barium in urine. Although a method for detecting the strontium isotopes alone would be more desirable, this cannot be achieved by this method. Therefore, the identity of the radioactive isotope will have to depend on energy and half-life measurements. This procedure does provide for the separation of the rare earth daughter products of Sr^{90} and Ba^{140} so that time of separation can be established in the decay and/or growth studies. If further identification is needed, conventional procedures for the separation of barium and strontium can be used.

A urinalysis method, to be most useful for routine analysis, should be rapid and simple, involving a minimum number of steps and should also provide for the removal of organic and inorganic solids, potassium in particular. This purification is necessary to eliminate the self-absorptions of the radiations by the sample and the radiations from K^{40} in particular. Although this procedure is more time consuming than one might wish, the method is (1) simple in operation, (2) specific for Sr^{89}, Sr^{90}, and Ba^{140}, and (3) provides excellent separation from K^{40}. Most of the separations are carried out on one ion exchange column. There is no necessity for adding either strontium or barium "carriers," which will make this procedure useful for analyzing either urine or bone for the presence of minute amounts of these elements in the presence of bulky amounts of magnesium and calcium.

This method depends on the coprecipitation of strontium and barium isotopes with the alkaline earth phosphate from an alkaline solution, the

preferential chelation by versene of calcium and magnesium over strontium and barium at a controlled pH, the adsorption of ionic strontium and barium on a Dowex-50* resin column, separation of the adsorbed calcium and magnesium from the resin with a solution of citric acid and versene at pH 5.0, removal of the sodium from the resin with 0.5N hydrochloric acid, and a final elution of the strontium and barium with 6N nitric acid.

GENERAL PRINCIPLES INVOLVED IN ION EXCHANGE SEPARATIONS

The planning and interpretations of ion exchange separation schemes are dependent upon a knowledge of the interactions among the absorbing ions, the eluting ions, and the exchanger. Most ion exchange separations are performed under non-equilibrium conditions. However, a knowledge of equilibrium states furnishes a rapid and reliable method for evaluating the direction that chemical variables will have upon non-equilibrium operations. Equilibrium studies show that the affinity of a cation exchanger for ions of the same valence increases with decreasing hydrated ionic radius. Accordingly, from activity coefficient data the sequence of adsorption of the alkaline earths will be: $Ba^{++} > Sr^{++} > Ca^{++} > Mg^{++}$ (4).

In non-equilibrium column operations, the most effective method for separating cations when the separation factors are small consists of adsorbing the cation mixture in the narrowest possible band at the top of the resin bed and subsequently eluting the cations stepwise, i.e. chromatographic elution. In the elution process the metals are desorbed at the top of the column, and adsorbed at the lower point. Cationic displacement, which utilizes the differences in adsorption affinities, will not alone provide a satisfactory method of separating the divalent alkaline earths. This procedure separates

*Product of Dow Chemical Co., Midland, Mich.

the bulky amounts of calcium and magnesium from tracer amounts of strontium and barium by the use of an elutriant composed of complexing materials. The complexing agent is a solution of versene and citric acid. The separability of the alkaline earths on cation exchange resins by complexing agents is directly proportional to the ratio of the stability constants of the chelates. The stability constants are shown in the table below:

Relative Stability Constants for Certain Alkaline Earths[5]

Metal	Ethylenediamine Tetraacetic Acid	Citric Acid
	Log K	Log K
Magnesium	8.7	3.2
Calcium	10.6	3.3
Strontium	8.6	2.7
Barium	7.8	2.3

The isolation of the radioactive strontium and barium from the urine is accomplished by adsorbing the alkaline earths on the cation exchanger, and selectively eluting the calcium and magnesium from the resin by a combination of cationic displacement and complex ion formation. The strontium and barium can then be eluted from the resin with nitric acid, the acid is evaporated, and the sample is prepared for counting of the radioactivity without further chemical separation.

EXPERIMENTAL

PART I. THE ALKALINE PHOSPHATE PRECIPITATION

A short approach to a urinalysis procedure would utilize the urine direct without preliminary concentration by precipitation. However, certain

difficulties arise in using urine direct with ion exchange resins in a separation procedure. The high concentration of dissolved salts, as well as the variability in individual specimens, and the presence of organic matter in urine makes a preliminary precipitation an important adjunct in this procedure. Quantitative recovery of barium and strontium can be achieved by a basic phosphate precipitate from urine. Since these elements do not form complexes with the organic materials present in urine, the precipitation can be carried out directly, thereby avoiding the laborious process of ashing the urine sample. This precipitate also serves two major purposes: (1) The separation of the above elements from sodium and potassium and (2) the separation from organic matter which is present in the urine sample.

Procedure

1. Add concentrated hydrochloric acid (HCl) to a 1500 ml urine sample to make the urine 0.1 N in HCl and yield a clean solution.
2. Heat the sample on a hot plate to a temperature of 85° to 90° C.
3. Add 6 ml of 6M phosphoric acid to provide an excess of phosphate to insure complete precipitation of all calcium and magnesium.
4. Use an electric motor stirrer to provide vigorous stirring for the subsequent precipitation.
5. Add slowly 6 M sodium hydroxide until a basic phosphate precipitate is visible. Continue the addition until the solution is basic to a pH of 8 to 10. Universal pH paper can be used to determine the pH in this case.
6. The precipitate is allowed to settle for 2 hours or longer.
7. The supernatant liquid is decanted by suction to the lowest possible level such that the precipitate is not disturbed. Discard the supernatant solution.
8. The remaining slurry is poured into a centrifuge cup. The precipitate

is centrifuged at 1500 r.p.m. for 5 minutes. The supernatant liquid is decanted by suction and discarded.

PART II. PREPARATION OF THE COLUMN

(a) Description of column system

Beds of 50-100 mesh Dowex-50 x 12 resin are prepared in a Pyrex glass column 18.5 cm long and 1.8 cm inside diameter. The bottom is fitted with a one-way stopcock in a one-hole rubber stopper. Glass wool over the rubber stopper holds the resin bed. A 6 inch Pyrex funnel fitted to the top of the glass column with a rubber tubing serves as a reservoir for the feed and wash solutions.

(b) Preparation of the resin

New Dowex-50 resin is conditioned by several washings, alternately with 5 per cent NaCl and 5 per cent HCl. During the conditioning process a considerable quantity of "fines" are removed. This is accomplished by stirring the resin sample in a large beaker filled with the conditioning solution. Most of the resin is allowed to settle, whereupon the liquid containing the "fines" is decanted. The resin is then converted to a sodium cycle with 5% NaCl. About 250 gms. of the resin is put into a Pyrex glass tube 4 ft. long and 2.5 cm. diameter. Six liters of 5% NaCl is then passed over the resin at a maximum flow rate (4.7 ml/min/cm^2). The resin is then emptied into a large beaker and washed free of NaCl with distilled water. The resin which has been used in urinalysis, can be re-used by converting to the sodium cycle as described above.

For practical reasons, it is expedient to keep the amount of resin used to a minimum in order to reduce the volume of elutriant solutions and thereby reduce the time required to perform a single analysis. Experimentally it was found that 16.3 gms. of the above resin (air dried), or about 25 ml by

volume (wet form) was sufficient for an individual analysis.

PART III. OPERATION OF COLUMN

(a) Preparation of feed solution

The alkaline earth phosphate precipitate can be dissolved in 10 to 20 ml concentrated nitric acid. The resulting solution contains calcium, magnesium, phosphates, plus an indeterminate amount of sodium, potassium, ammonia ions, and organic material adsorbed on the gelatinous precipitate. The organic material can be destroyed by wet ashing in the presence of nitric acid and hydrogen peroxide. The inorganic residue is dissolved in 2 to 3 ml of concentrated hydrochloric acid plus about 20 ml distilled water. The solution is then diluted to about 800 ml with distilled water.

This urinalysis procedure was designed primarily to analyze urine supernatant from a previous plutonium analysis in which 600 mg. of extra calcium had been added. The total calcium under these conditions was about 800 mg. while the magnesium was about 100 to 200 mg. When adsorbed on a cation exchanger of the size used in this procedure, such large quantities of alkaline earths would utilize most of the exchange capacity of the resin, thereby making the operation vulnerable to losses of strontium and barium. In order to circumvent the above difficulty, it was necessary to devise some way in which the amount of alkaline earths that were adsorbed on the resin could be held to a minimum. A method was developed whereby a large per cent of the calcium and some of the magnesium could be chelated (bound in non-ionic form) with versene and would pass through the resin as a complex. The feasibility of this method is shown in Figure I where the difference in the adsorption of calcium and strontium on Dowex-50 resin in the presence of excess versene is demonstrated. In this equilibrium study, carrier free Sr^{89} was put into a one per cent solution of reagent grade

disodium dihydrogen versenate. One gram of air dried Dowex-50 resin was added and the versenate solution containing Sr^{89} and the resin was equilibrated at pH values ranging from 4.0 to 8.5 in increments of 0.5 pH. The per cent of Sr^{89} in the aqueous phase was determined for each pH value. This is shown in Figure I. A similar equilibrium study was made using Ca^{45}, and the results are likewise shown in the Figure I. It will be noted that versene becomes a less efficient complexing agent as the pH decreases. Also, versene is more effective for chelating calcium than strontium in the pH range of 4.5 to 6.0. The greater complexing efficiency for calcium over strontium was used to develop a method whereby a large per cent of the calcium and some of the magnesium, being chelated with versene, would pass through the resin column while strontium and barium are adsorbed. The alkaline earths of the feed solutions are first chelated with versene at pH 10.5. At this pH a dye indicator can be used to determine the end point of chelation of the alkaline earths. The pH of the solution is then reduced to 5.5 and put over the resin column. In 22 experimental runs using samples with added calcium as well as samples without, 94 to 97 per cent of the calcium passed through the column as a complex, while 36 to 63 per cent of the magnesium also passed into the effluent. Losses of Sr^{89} tracer averaged from 0.11 to 0.19 per cent.

Procedure

1. The precipitate from step I, 8 is dissolved in about 15 ml concentrated nitric acid.
2. This solution is poured into the two liter beaker in which the original phosphate precipitation was carried out.
3. The organic matter is destroyed by heating the solution, and by alternately adding conc. HNO_3 and 30% hydrogen peroxide.

4. This is repeated until a white residue remains. The residue is then taken to dryness.

5. Remove the beaker from the hot plate and wash down the walls with about 20 ml distilled water. Add 2 to 3 ml concentrated hydrochloric acid and heat until the inorganic material is dissolved. The volume is diluted to 800 ml with distilled water and the pH of the solution is checked in subsequent operations.

6. Add 1 N sodium hydroxide until the pH is about 9.0.

7. Add 2 ml Eriochrome Black T*.

8. Add a solution of 7.5%** technical grade versene until the indicator changes from wine red to blue at pH 10.5. This point indicates complete chelation of all calcium and magnesium .

9. Reduce the pH to 5.5 with concentrated hydrochloric acid. The final adjustment can be made with 1 N HCl.

10. Pass this solution over the resin column at a flow rate of not greater than 8 ml/min/cm^2.

11. Wash down the walls of the funnel with about 50 ml distilled water.

12. Discard the effluents.

(b) Elution of Adsorbed Calcium and Magnesium from the Resin Column

As shown in the equilibrium studies (Figure I) the efficiency of versene to chelate the alkaline earths is reduced when the pH is lowered to 8.0 to 5.5.

* Mix 0.5 gms. Eriochrome Black T and 4.5 gms. hydroxylamine hydrochloride. Dissolve in 100 ml of alcohol and filter.

** Dissolve 75 gms. tetrasodium ethylenediamine tetraacetic acid (technical grade) in about 800 ml distilled water. Filter and dilute to one liter.

Since the feed solution is put over the column at a pH of 5.5, there will be some exchange of the cations between the feed solution and the resin because of this reduction. This indeterminate amount of calcium and magnesium that is adsorbed on the resin must be removed without loss of strontium and barium. As indicated previously the citrate complex would remove the alkaline earths in the following order: Mg > Ca > Sr > Ba. Versene, from the table of stability constants, would be expected to remove calcium from the resin before the other alkaline earths. In order to verify these assumptions, a series of tests were made to determine the most efficient elutriant for the removal of calcium and magnesium. In a regular urinalysis about 15 to 25 mg. of calcium and about 40 to 50 mg. of magnesium were adsorbed on the resin. In these tests of the different elutriants 50 mg. magnesium, 25 mg. calcium and about 10^6 c/m Sr^{89} were put on a regular Dowex-50 resin column (sodium cycle). These alkaline earths were then removed by the following solutions at pH 5.0 and at a flow rate of 4 ml per minute per cm^2: (1) 2 per cent citric acid,(2)1 1/2 per cent citric acid, and (3) one per cent citric acid--0.75 per cent versene. (Figures 2, 3, 4). It is evident that a combination of versene and citric acid at pH 5.0 is a better elutriant than citric acid alone. This is due to the greater affinity for calcium by versene. It is noted that calcium is removed prior to magnesium with the versene-citric acid wash. There is no loss of Sr^{89} even at twice the volume of elutriant necessary to remove all of the calcium and magnesium. Similar experiments with urine samples gave essentially the same elution curves. The effect of the pH of the elutriant was also checked by experiments similar to the above. At pH 5.2, all of the calcium and magnesium were removed with 800 ml elutriant with no loss of Sr^{89}. At pH 4.8, the calcium and magnesium were more slowly removed and required about 1200 ml of elutriant, but no loss of Sr^{89} occurred when 3 liters were used.

Procedure

1. Pass 800 ml of a solution of one per cent citric acid and 0.75% versene over the resin column at a flow rate of 4 ± 0.8 ml/min/cm^2 (10.93 gms. citric acid monohydrate, 100 ml of 7.5% versene, dilute to one liter and adjust the pH to 5.0 with 6 M NaOH).
2. Discard the effluent wastes.

(c) Removal of sodium from the resin column

Since the Dowex-50 resin had been put on the sodium cycle in preparation for urinalysis, this sodium must be removed in order that the final eluate of the strontium and barium can be evaporated and prepared for counting without further chemical purification. Dilute acids will elute the monovalent sodium with no significant loss of Sr^{89}. In 22 experimental urinalyses, losses of Sr^{89} averaged 0.22 per cent when 0.5 N HCl was used to remove the sodium from the resin.

Procedure

1. Pass 800 ml of 0.5 N hydrochloric acid over the resin column at a flow rate of 4 ± 0.8 ml per minute per cm^2.
2. Discard the effluent wash.

D. ELUTION OF STRONTIUM AND BARIUM

The strontium and barium can be eluted from the resin column with 200 ml 6 N nitric acid. This volume removes about 99.6 per cent of the strontium and barium.

Procedure

1. Pass 200 ml 6 N HNO_3 over the resin at a flow rate of 2 ml/min/cm^2.
2. Catch the eluate in a 400 ml beaker.

PART IV. PREPARATION OF THE SAMPLE FOR COUNTING

The eluate is evaporated on a hot plate to almost dryness at a temperature just below the boiling point. The residue is transferred to a counting dish.* The inorganic residue, which is mostly sodium and inert strontium and barium, will have a density of less than 1 mg/cm^2, therefore self-absorption of the radiations by the sample should be nil.

Since the rare earth daughter products of Sr^{90} and Ba^{140} are chelated by the versene-citric acid wash, these radioactive products will be removed from the column by this wash. Therefore, the time of separation of these daughter products can be established for subsequent identification of the isotope by growth and/or decay measurements.

Procedure

1. Evaporate the nitric acid eluate to almost dryness on a hot plate.
2. Wash the contents into a 50 ml beaker with distilled water. Clean the walls of the larger beaker with nitric acid and water. (This transfer to the small beaker facilitates the final transfer to a counting dish.)
3. Evaporate the liquid in the small beaker to dryness .
4. Dissolve the residue in about 1/2 ml 1 N HNO_3. Transfer this liquid to a counting dish using a pipette. Wash the walls of the beaker with another 1/2 ml of acid, and add this to the dish.
5. Dry under an infra-red lamp.

The radioactivity of the sample can be counted in a conventional end

*This counting dish is made from a circular piece of type 304 stainless steel 1 3/4 inch diameter and 0.005 in. thick. A cup, one inch diameter and 0.125 inch depth, is die pressed for holding the sample.

window Geiger-Mueller counter. The procedure is outlined as a flow sheet in Figure 5.

RESULTS

The more detailed studies of the various steps indicated that the average total losses in the column operation were less than one per cent. This is shown in Table I. About 10^6 c/m of Sr^{89} was used to study the losses. Similar studies with Ba^{140} likewise gave losses of about one per cent.

This procedure was also tested for total recovery of Sr^{89} tracer at levels of about 75 c/m. This test was made on urine samples from individuals who had not been exposed to fission product contamination. To 18 of the samples was added the extra 600 mg. calcium; the other 4 had no extra calcium. No difference was noted in the per cent of recovery. In the 22 experimental runs, the recovery averaged 93.7% $\pm$ 2.5 per cent (standard deviation) of Sr^{89} tracer. The average counting error was about 2.7 c/m at a 90% confidence level. In determining the per cent recovery, a volume of Sr^{89} tracer equal to that put in the urine samples was evaporated in the center of a counting dish and used as a "standard". The "standards" and urinalysis samples were counted at about the same time to avoid errors due to decay of Sr^{89}. Since some of the residue in a urinalysis sample is displaced farther from the center than the "standards", a reduction in efficiency of counting can be expected due to this lateral displacement. The final per cent recovery took into consideration both chemical losses and decreased counting efficiency.

SEPARATION FROM K^{40}

In order to determine the presence of radioactive strontium and barium at very low levels, a urinalysis procedure must provide good separation from K^{40}, a beta emitter that is present in urine. Urine specimens from 17 persons who had not been exposed to fission product contamination, were checked by

this procedure. Average radioactivity due to K^{40} was 0.7 c/m at about 25% geometry. The maximum was 1.7 c/m.

SUMMARY

In the radiochemical analysis of strontium and barium in large volumes of urine, an alkaline earth phosphate precipitation is used to concentrate the strontium and barium. The separation of large amounts of calcium and magnesium from tracer amounts of strontium and barium is done on one ion exchange column by the use of versene and citric acid as complexing agents. Overall losses of Sr^{89} on the column are about one per cent. Urine samples containing from 150 to 800 mg. calcium gave equally good recovery. Since no "carriers" are added, this method may be useful for analyzing bone or urine for non-radioactive strontium and barium. This procedure is simple in operation, has a minimum number of steps, and provides excellent recovery of radioactive strontium and barium in human urine.

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Table I

Sr^{89} Losses in Column Operation in Urinalysis Runs with (a) Extra Calcium 600 mg) and (b) No Extra Calcium

Run Number	Leakage in Feed Effluent	Versene-Citric Acid Wash	0.5 N HCl Wash	Left on Resin After 200 ml 6N HNO_3 Eluent
1a	0.00 %	0.04 %	0.00 %	0.0 %
2a	0.00	0.05	0.07	0.2
3a	0.02	0.14	0.26	0.0
4a	0.00	0.08	0.16	0.4
5a	0.02	0.00	0.03	0.2
6a	0.00	0.00	0.00	0.2
7a	0.00	0.00	0.04	0.1
8a	0.00	0.00	0.04	0.1
9a	0.08	0.23	0.27	0.2
10a	0.94	1.74	1.67	0.2
11a	0.23	0.34	0.40	0.3
12a	1.05	1.03	1.02	0.5
13a	0.12	0.11	0.16	0.4
14a	0.21	0.18	0.34	0.4
Average	0.19 %	0.28 %	0.32 %	0.2 %
1b	0.12 %	0.10 %	0.06 %	0.3 %
2b	0.12	0.09	0.08	0.5
3b	0.09	0.06	0.06	0.9
4b	0.10	0.08	0.07	0.8
5b	0.14	0.10	0.05	0.9
6b	0.18	0.07	0.05	1.0
7b	0.09	0.04	0.04	0.8
8b	0.09	0.05	0.03	1.4
Average	0.11 %	0.07 %	0.05 %	0.8 %

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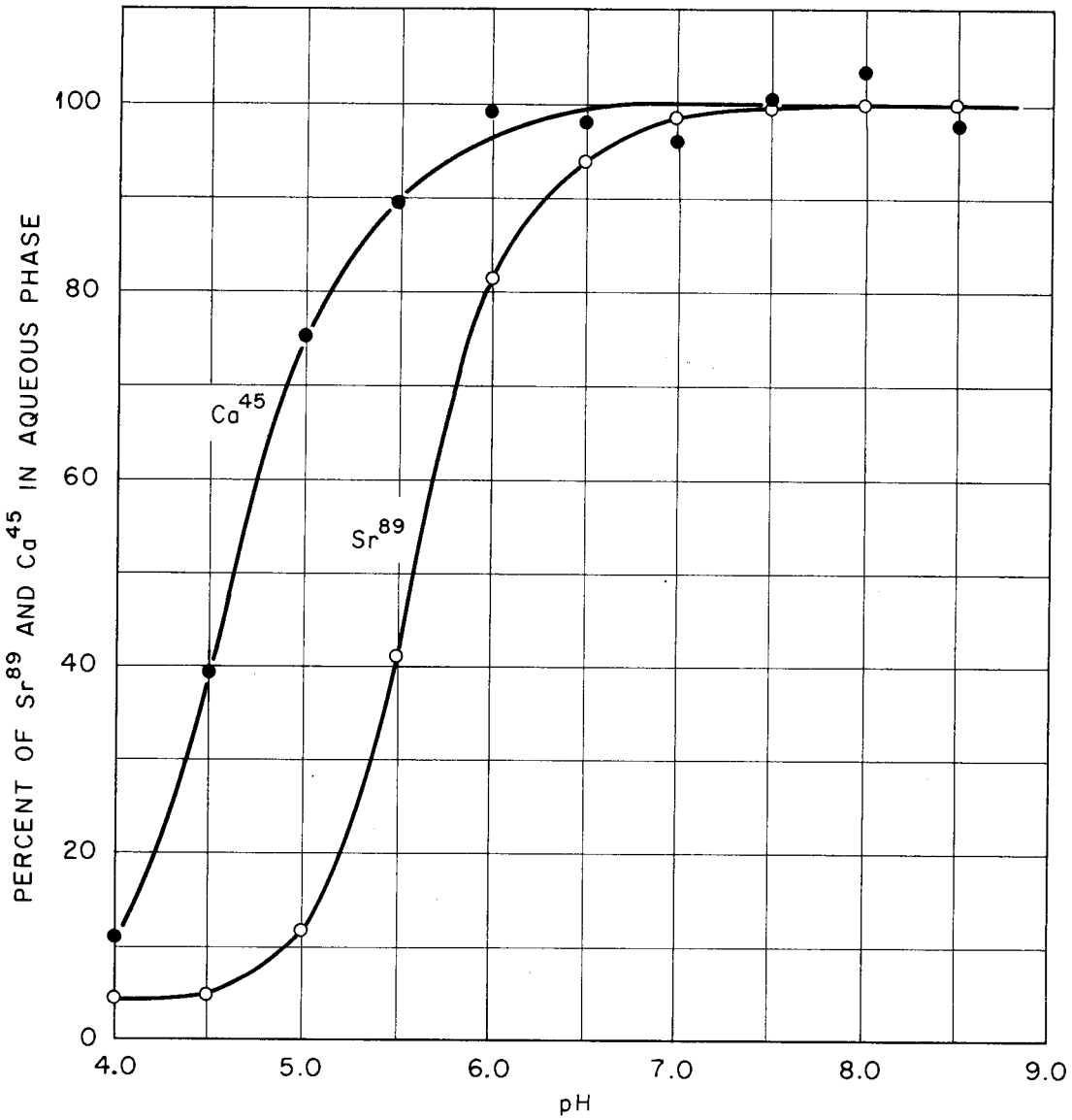
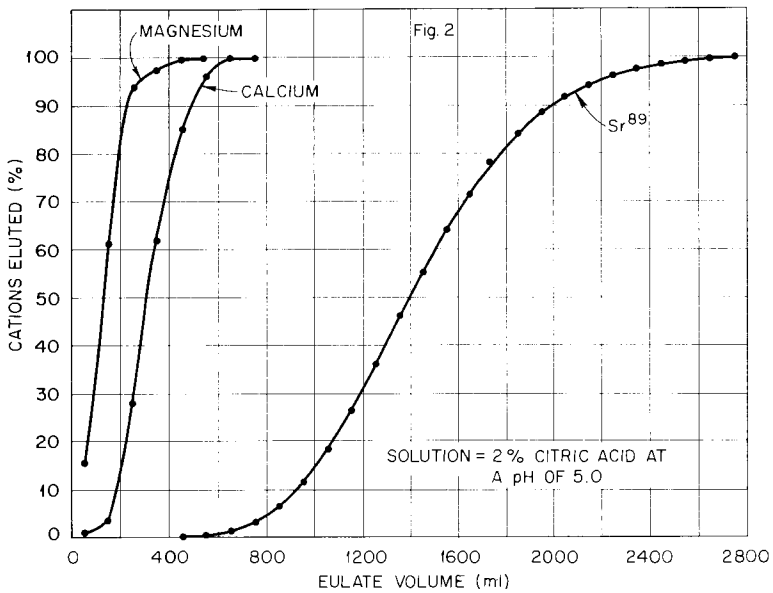
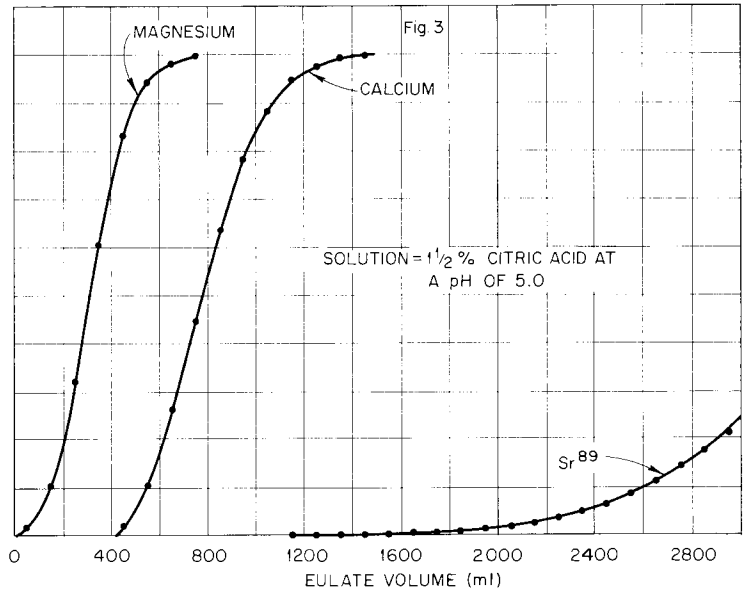


Fig. 1. Distribution of Carrier Free Sr^{89} and Ca^{45} in a 1% Solution of Di-Sodium Di-Hydrogen Versenate Equilibrated with one gram of Dowex-50 (50 to 100 mesh) as a Function of pH.





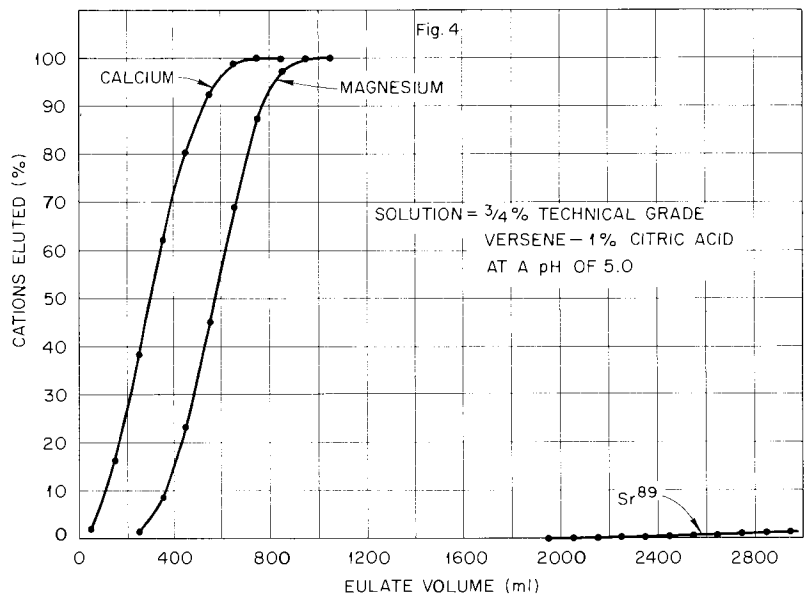
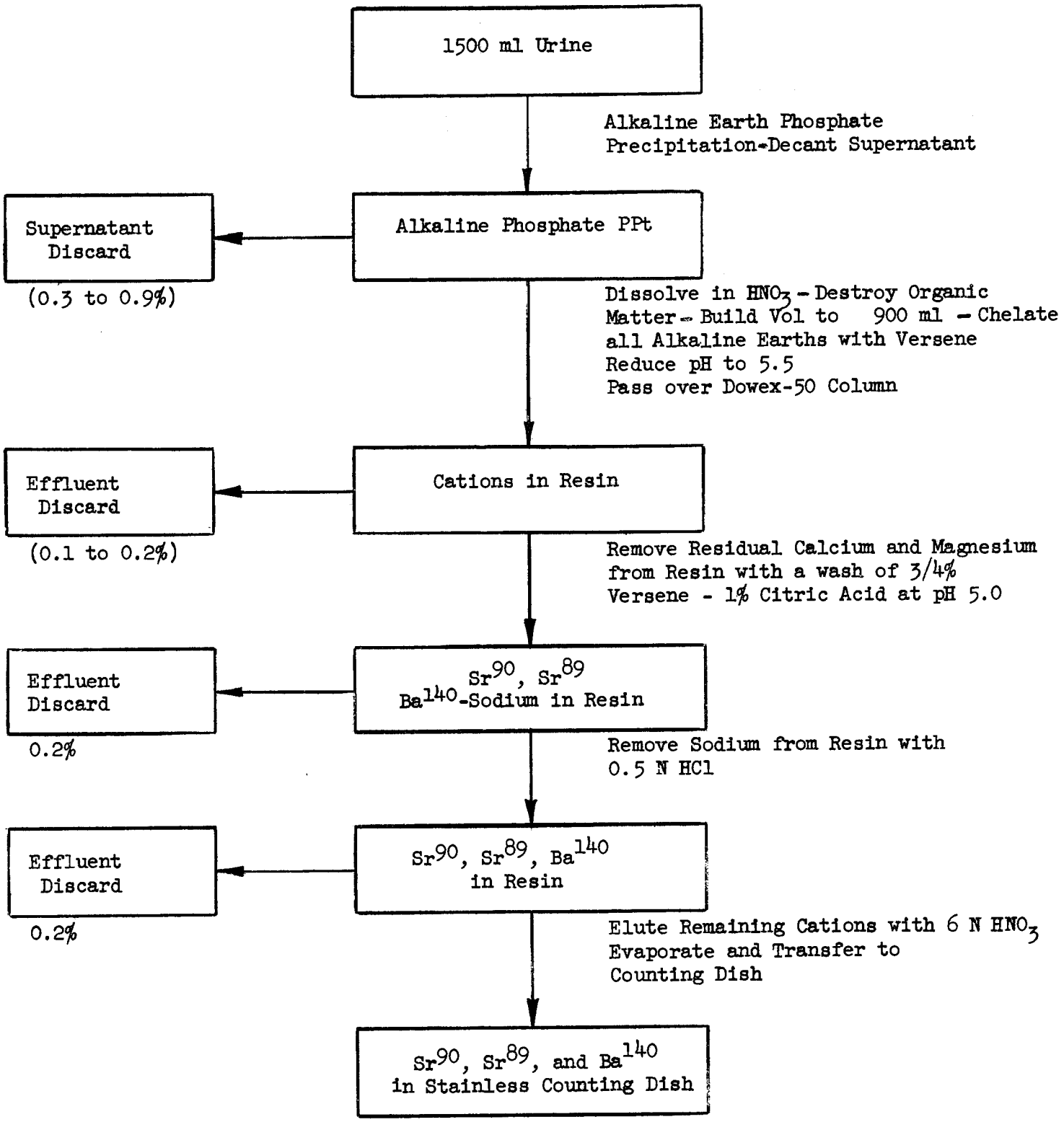


Fig. 2,3,4. Elution of a Mixture of 50mg Magnesium, 25mg Calcium, and about 10^6 c/m Sr^{89} from a Column 18.5-cm Long and 1.8-cm ID. Containing 16.3g of Dowex-50 x 12 (Air Dried and on Sodium Cycle). Flow Rate = 4ml/min/cm^2.

FIGURE 5

FLOW SHEET

Procedure for the Radiochemical Analysis of Strontium and Barium in Human Urine