M. E., *Cornell Vet.*, 1953, v43, 551.
4. Randall, C. C., McVickar, D. L., and Doll, E. R., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 465.
5. Doll, E. R., McCollum, W. H., Wallace, M. E., Bryans, J. T., and Richards, M. S., *Am. J. Vet. Res.*, 1953, v14, 40.
6. Randall, C. C., Ryden, F. W., Doll, E. R., and Schell, F. S., *Am. J. Path.*, 1953, v29, 139.
7. Doll, E. R., personal communication.

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Determination of Specific Activity of Sodium in Bone.* (21974)

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Studies of the role of skeletal sodium have been hampered by technical difficulties encountered in the analysis of this tissue for sodium content. These difficulties arise largely from the fact that bone contains tremendous amounts of calcium, the molar Ca/Na ratio being of the order of 25 1. This quantity of calcium causes serious interference with both the flame photometric and gravimetric methods for determination of sodium; and phosphate, in the quantities present in bone, may also interfere. Radioactivity measurements require large corrections for self-absorption if unaltered solutions of bone ash are used, and if attempts are made to precipitate calcium and phosphorus, as much as 20% of the radiosodium present may be removed by co-precipitation(1).

In an attempt to circumvent the difficulties inherent in analysis of bone for sodium, a new method was developed in which interfering substances are removed by cation exchange chromatography(2). Under the conditions employed, sodium appears in the eluate entirely free from phosphate, calcium, magnesium and potassium, whereupon the sodium-containing fraction can be easily analyzed by flame photometry.

The present report extends this investigation to include the behavior of radioactive sodium on the column, and further defines the accuracy and limitations of the method.

Method. Column characteristics — A column 1.3 x 60 cm is employed, containing 27 g (oven-dried basis) of the sulfonated polystyrene resin Dowex 50-x12 (50-100 mesh). The resin rests on a plug of Pyrex glass wool and fills the column to a height of 42 cm. Details of the preparation and operation of the column and the collection of eluate fractions are given in a previous publication(2). *Analysis of Effluent Fractions.* Analyses for Na^{23} and for K and Mg were carried out by standard methods (for references see 2). Calcium was determined by the hexanitratocerate technic (3). For purposes of this study, a special hood was fitted to the Weichelsbaum-Varney flame photometer in order to exhaust radioactive fumes to the outside. Measurements of Na^{22}† were made by pipetting 2 ml samples of eluate into 1.5 x 4.5 cm vials, which were then counted directly in a well-type scintillation counter. In instances where the activity was low, the sample was first evaporated on a steam bath to the appropriate degree of concentration. A known aliquot of Na^{22}, appropriately diluted in NaCl solution, served as a standard. Decay corrections were obviated by counting the eluate fractions and the standard solution on the same day. The usual corrections for coincidence effects were applied. *Preparation of Bone Samples.* Bone is first dried and then ashed overnight at 525°C in a muffle furnace. The ash is dissolved in 3 N HCl, and the resulting solution boiled for 10 minutes. This solution is then placed on the column, any remaining dark particles having been removed by filtration through Pyrex glass wool, allowed to flow into the resin bed, and elution with 0.8 N HCl be-

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† Radiosodium^{22} was obtained from the Oak Ridge National Laboratories.

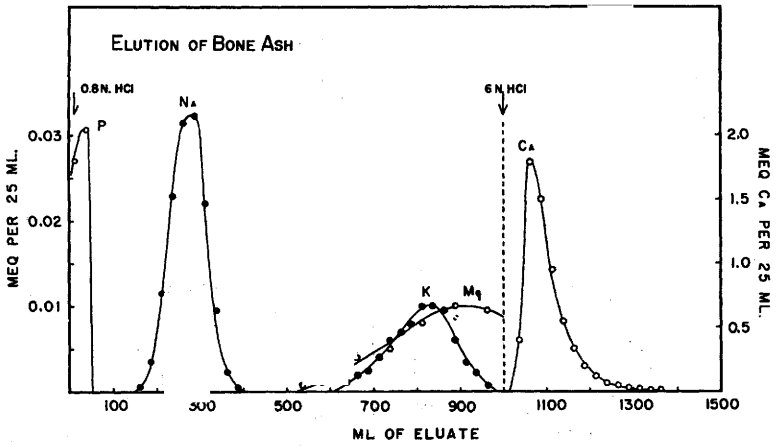

FIG. 1. Elution pattern of beef bone ash solution (0.5 g wet bone). Eluate collected in 25 ml fractions. Scale on the left-hand ordinate reads directly for Na and Mg, and should be multiplied by 0.1 for K. Phosphorus is plotted in mg $\times 10^{-3}$.

gun. Pyrex-brand glassware is used throughout.

Results. Elution of Bone Ash Solutions. A representative elution curve for a solution of bone ash (representing 0.5 g of wet beef bone)‡ is illustrated in Fig. 1. Phosphate is found only in the 0-50 ml range and K in the 600-1000 ml range. Magnesium first appears at the 525 ml mark and is not completely eluted by 1000 ml. Calcium does not appear by the 1000 ml mark, and requires 400 ml of 6 N HCl to effect a complete recovery. The 100-400 ml fraction which contains the sodium is thus free from P, K, Mg and Ca. No attempt was made to test for the presence of other ions.

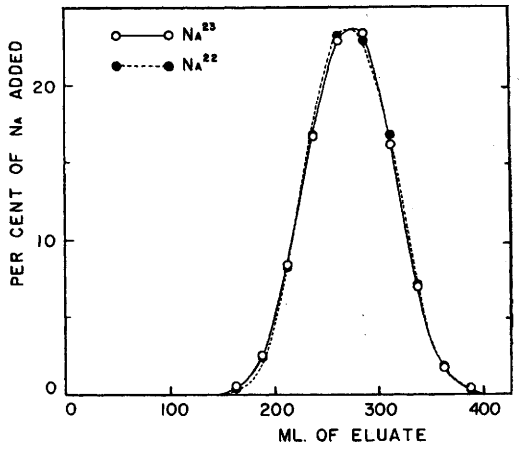

FIG. 2. Sodium elution pattern of beef bone ash solution (0.5 g wet bone) to which Na^{22} had been added. Eluate collected in 25 ml fractions. Eluting agent 0.8 N HCl.

‡ The bone powder used was found by analysis to contain 12.2 meq. Ca and 0.25 meq. Na/g wet weight.

Fig. 2 illustrates the sodium-containing fraction of the eluate from a solution of bone ash to which a known amount of Na^{22} had been added. Both isotopes follow the same elution pattern with no appreciable change in the Na^{22}/Na^{23} ratio as the elution proceeds.

In Fig. 3 a series of elution curves for Na^{22} (approximately 0.4 μc in each instance) added to bone samples of varying size are depicted. The position of the elution curve remains fairly constant from one run to the next. However, it was found that the Mg curve shifted markedly with variation in sample size. Its position for 0.5 g of bone is indicated in Fig. 1. When 0.75 g of bone were used, Mg first appeared in the 400-425 ml fraction and with 1 g, it was present by the 300 ml mark. In a previous study(2), it was noted that as the sample size was increased, calcium appeared much earlier.

Recovery of Added Na^{22}. Recoveries of radiosodium added to solutions of bone ash and to a known quantity of NaCl are listed in Table I, the data indicating a high degree of accuracy for the procedure as we have employed it. In the instance in which NaCl was used, it was found that the specific activity of the sodium added to the column corresponded very closely to that recovered, the two values

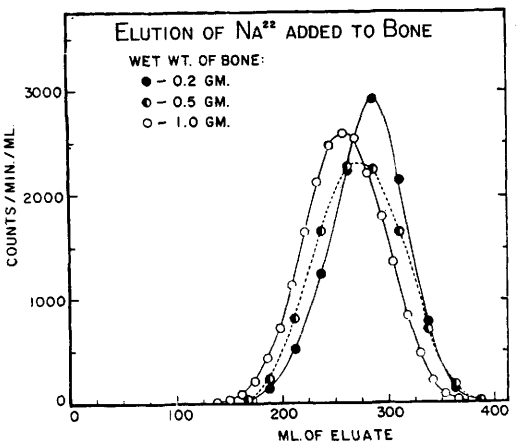

FIG. 3. Effect of sample size on sodium elution pattern. Elutriant 0.8 N HCl. Eluate collected in 12 ml or 25 ml fractions.

being 9.0 x 10^5 and 8.8 x 10^5 cts/min/meq. Na respectively.

Procedure adopted for determination of specific activity of bone samples. Bone is prepared as indicated above. The solution of bone ash is added to the column in a volume not to exceed 10 ml and allowed to flow into the resin bed. Elution with 0.8 N HCl is then carried out at a flow rate of 2.0 ± 0.5 ml per minute. The 0-100 ml eluate fraction is discarded. The 100-400 ml fraction is collected quantitatively and used for radioactivity measurements and for Na analysis by flame photometry. The elutriant is then changed to 6 N HCl in order to elute other cations more rapidly, and at least 400 ml is run through in order to completely elute K, Mg and Ca.

TABLE I. Recovery of Radiosodium.

Sample	Na^{22} added, cts/min.	Na^{22} recovered, cts/min.	% recovery
	× 1000		
NaCl, 0.3 mM	270.7	268.8	99.4
Bone, 1 g	263.9	261.6	99.2
1	289.9	291.9	100.7
1	288.1	293.2	101.8
1	255.0	254.9	99.9
1	251.9	253.9	100.7
.5	251.9	256.7	102.0
.2	252.8	256.3	101.4
.75	252.8	255.0	100.9
.5	244.5	246.6	100.8
		Avg	100.7%
		Stand. dev.	.94
		" error	.30

Approximately 300 ml of 0.8 N HCl are then run through the column in order to re-equilibrate the resin with the weaker acid. The resin is then agitated by repeated inversions of the column, whereupon it is ready for the next run. The entire procedure, including the reconditioning of the resin, occupies about 7 hours, during which time the column requires very little attention.

The Na-containing fraction of eluate is thoroughly mixed, and an aliquot evaporated to dryness on a steam bath in order to drive off most of the HCl. The residue is made up to volume with water and analyzed for Na^{23} by flame photometry. Depending on the amount of radioisotope present, radioactivity measurements are then made on another aliquot either in the original state, or after suitable concentration or dilution.

Discussion. It is evident that Na^{22} and Na^{23} behave in similar fashion on this cation exchange column, and that measurements of the specific activity of sodium in bone are feasible by this method. Interfering substances relative to Na^{23} analysis are removed by the procedure as we have employed it, and the necessity for large self-absorption corrections in Na^{22} analysis has been obviated since the Na-containing fraction of the eluate has a low solute content.

There are certain limits to sample size for a column of these dimensions. Analyses for Na^{23} gave distinctly less accurate results when amounts of the order of less than 0.06 meq. were added to the column(2), presumably because of the small but definite reagent blank. According to the results of our previous study on the composition of rat bone, sample size should therefore be not less than 0.5 g (wet weight) for very young animals and 0.25 g for adults(2). The upper limit depends on whether a slight degree of Mg contamination of the Na fraction can be tolerated; if not, the sample size should not exceed 1.5 g for young animals and 0.75 g for adults. The marked shift in the calcium curve places an absolute upper limit at 4 and 2 g, respectively, for the 2 age groups.

Since different batches of Dowex 50 may differ somewhat, elution curves should be run prior to using this method for analysis of bone

for sodium. In this connection it should be noted that the elution characteristics of the column we are now using differ slightly from those depicted previously(2).

Conclusion. A new method for the determination of specific activity of sodium in bone is presented. The method consists of placing a solution of bone ash on a cation exchange column. Elution with acid results in the quantitative recovery of both stable and radioactive sodium in a certain fraction of the eluate; this fraction is free from P, K, Mg and Ca and can be easily analyzed for both sodium and radiosodium.

The scintillation counter equipment was made available to us through the kindness of Mr. J. Russell Hayes of the Medical Division, Atomic Energy Project of the University of Rochester, and the specially adapted flame photometer by Dr. Taft Toribara of the Radiation Biology Division.

1. Davies, R. E., Kornberg, H. L., and Wilson, G. M., *Biochem. J.*, 1952, v52, xv.

2. Forbes, G. B., and D'Ambruso, Margaret, *J. Biol. Chem.*, 1955, v212, 655-661.

3. Kochakian, C. D., and Fox, R. Phyllis, *Indust. and Eng. Chem., Anal. Ed.*, 1944, v16, 762.

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Oestrogenic Activity of 16 Epi-Oestriol.*† (21975)

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The isolation of 16 epi-oestriol (oestra-1:3:5-triene-3:16B:17B-triol) from the acid hydrolyzed urine of pregnant women was described by Marrian and Bauld(1). Since the yield of this new Kober Chromagen was 0.1 mg/l and since this substance seems identical with 16 epi-oestriol as prepared by Huffman(2) considerable impetus was given to the present investigation in an effort to ascertain its activity on the growth potential of the uterus of the ovariectomized rat. Vaginal smears were also studied quite extensively. Moreover, since practically no biological information exists in the scientific literature on this naturally occurring oestrogen it seemed of particular significance at this time to elaborate its biological activity.

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§ The technical assistance of Miss Nancy M. Raney and Miss M. Claire Simoneau is gratefully acknowledged.

Procedures. Virgin, albino rats, approximately 100 days of age, were used in these experiments. They were started on treatment 7 days after bilateral ovariectomy. The 16 epi-oestriol was dissolved in U.S.P. propylene glycol, and injected subcutaneously once daily for 3 days. Vaginal smears were taken daily, and 72 hours after the first injection necropsies were performed. The uteri were removed quickly and slit longitudinally on bibulous paper, thus removing excess uterine luminal fluid. The uteri were then weighed to the nearest 0.2 mg on a Roller-Smith torsion balance. After the wet weights were determined, the uteri were placed in an oven at 110°C for dry weight determinations. A standard period of 24 hours at 110°C was used uniformly for drying each uterus. After this period the weight of each uterus was determined on a Roller-Smith torsion balance.

Results. A. Effect of 16 epi-oestriol on vagina of the ovariectomized rat. These results clearly indicate that 16 epi-oestriol has the ability to induce oestrogenic changes in the vagina of the ovariectomized rat (Table I). A demonstrable effect can be observed with 1.0 μg of this substance. Additional