Fig. 1

Some results for bones of two adults labelled Adult 1 and Adult 2 are given in Table 1.

Table 1

Adult 1	Strontium (p.p.m.)	Adult 2	Strontium (p.p.m.)
Rib	286		
Vertebra	286		
Femur:		Femur:	
Epiphysis section A	281	Epiphysis section A	480
" " B	276	" " B	470
" " C	287	" " C	497
Shaft	291	Shaft	472

I wish to thank Dr. P. Ilbery, of the School of Public Health and Tropical Medicine, at whose request this investigation was carried out, for his co-operation throughout the project, Mr. W. J. Thomas, of the Bureau of Mineral Resources, for his valuable assistance in the statistical analysis, and Miss Horsford, of the School of Public Health and Tropical Medicine, for the careful preparation of the bone samples used in this work.

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Feb. 10.

¹ Thurber, D. L., *et al.*, *Science*, **128**, 3318 (1958).

² Harrison, G. E., *Nature*, **182**, 4638 (1958).

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Micro Determination of Calcium

Hildebrand and Reilly¹ have recently described a complexometric titration method for the determination of calcium: the indicator used, 'Calcon', is superior to 'Solochrome' black in that calcium can be determined independently of magnesium, and, unlike the murexide method, a sharp end-point is obtained. All three methods employ ethylenediamine tetraacetic acid. The purpose of the present communication is to direct attention to the facts: (a) that the allied compound 1-(1-hydroxy-2-naphthylazo)-5-nitro-2-naphthol-4 sulphonic acid sodium salt (Eastman-Kodak) appears to be just as suitable as an indicator as 'Calcon' but is considerably cheaper; (b) that the method can also be used on an ultra-micro scale. Concentrations of the order of 10^{-2} μ gm. calcium have been determined in volumes of about 40 λ of solution with an accuracy of 1 per cent ($1\lambda = 0.001$ ml.).

Essentially, the method is similar to the original¹, and relatively the same quantity of buffer (diethylamine) is employed; in fact, it was found that the

quantities of both buffer and indicator were critical. For 40 λ of solution $1.75 \pm 0.25\lambda$ of diethylamine maintained the required pH of 12-13, and accurate and repeatable results were obtained with $5 \pm 0.5\lambda$ of indicator: these volumes were delivered with microburettes.

The cap of a 1-cm. 'attached-cap' polythene tube was found superior to a waxed slide for the titration: not only is the surface suitable, but also, lighted from below, the drop of solution is very suitably illuminated for observation of the end-point. The solution was brought into contact with the glass needle of the burette, a 100- λ pipette graduated in 1 λ units (Glen-Creston, Ltd.), and titration continued until all trace of pink had disappeared and the solution turned pale blue¹: at this stage the addition of 0.5 λ of 0.0002 N ethylenediamine tetraacetic acid produced a marked change of colour. Stirring was carried out by a fine jet of air.

The stock solution of 0.02 N ethylenediamine tetraacetic acid keeps for many months without deterioration: however, the 0.0002 N solution prepared from it for titration was less stable and was therefore freshly prepared each week.

Determinations on twenty-one 40- λ samples containing 7.96×10^{-3} μ gm. calcium diluted from a gravimetrically standardized solution gave a mean value of $7.90 \pm 0.80 \times 10^{-3}$; the difference is therefore less than 1 per cent from the expected value, and is far from statistical significance ($P > 0.7$). Six or fewer determinations with a series of solutions of greater calcium concentration gave mean values which, as shown in Fig. 1, were also very close to those expected. Mean values were usually within 2 per cent, and never more than 4 per cent from the known values.

Fig. 1. Continuous line represents expected values. Closed circles are mean values of, from left to right, 21, 3, 3, 5 and 6 determinations of 40- λ samples

This work is part of a programme of research on the hatching of the potato root eelworm; a grant from the Nuffield Foundation is gratefully acknowledged.

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