DIFFICULTIES IN THE USE OF ETHYLENEDIAMINE TETRA-ACETIC ACID (E.D.T.A.) IN DETERMINING CALCIUM IN SERUM

BY

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The usual methods for the determination of calcium in serum are lengthy procedures. Calcium may be precipitated as oxalate and the oxalate radical estimated by titration with potassium permanganate (Clark and Collip, 1925). The calcium oxalate may be converted to calcium carbonate which is estimated by titration with acid (Trevan and Bainbridge, 1926). Calcium may also be precipitated as calcium phosphate, the phosphate in the precipitate being estimated colorimetrically (Roe and Kahn, 1929). In this laboratory the method of Trevan and Bainbridge (1926) has been found to be very reliable, but it is not possible to complete an analysis within 24 hours. A rapid and reasonable accurate technique for the determination of serum calcium is needed in clinical work.

The disodium salt of ethylenediamine tetra-acetic acid (E.D.T.A.) has recently been used for the estimation of calcium in serum. In alkaline solution this substance combines quantitatively with calcium ions to form a metal complex according to the following reaction:

\[
\text{NCH}_2\text{CH}_2\text{N}^+ + \text{Ca}^{++} \rightarrow \text{NCH}_2\text{CH}_2\text{N}^+\text{Ca}^{++}
\]

Certain dyes such as eriochrome black T (Sobel and Hanok, 1951; Nielsen, 1952) and ammonium purpurate (Elliott, 1952; Holtz and Seekles, 1952) also combine with calcium ions to give reds, but when the calcium is displaced from these combinations by E.D.T.A. the colours change to blue-green and purple respectively. When eriochrome black T is used as an indicator both calcium and magnesium are estimated, but ammonium purpurate at pH 12 has been reported to be specific for calcium.

The procedure of Elliott (1952) for the direct volumetric determination of calcium in serum using E.D.T.A. and ammonium purpurate as indicator appeared to be of potential value and was therefore investigated. It was compared with the methods of Clark and Collip and of Trevan and Bainbridge, both for a series of aqueous solutions containing known amounts of calcium and a number of blood samples. In addition the recovery of known amounts of calcium added to serum was investigated.

Experimental

Reagents.—Disodium dihydrogen ethylenediamine tetra-acetate, also termed "complexone," "sequestrene," or E.D.T.A., was originally obtained from the Alrose Chemical Co., Rhode Island, U.S.A., but later from Messrs. British Drug Houses, Ltd. The solution was prepared and standardized by the method of Elliott (1952).

Ammonium purpurate (murexide) was also obtained from B.D.H. and was used as a saturated solution in distilled water.

Calcium-free water, i.e., triple-distilled water from an all-glass still, was used throughout.

Estimation of Calcium in Serum.—Serum, 1 or 2 ml., was diluted with 50 ml. of calcium-free water, 0.8 ml. of 9 N NaOH and 5 drops of the indicator solution added, and the mixture titrated with the standard E.D.T.A. solution. The end-point was much more difficult to determine than in the aqueous solutions. The samples of sera were therefore titrated to the same purple end-point as control mixtures containing the same amounts of sera, which had been deliberately over-titrated to a definite purple. Even this precau-
USE OF E.D.T.A. IN DETERMINING SERUM CALCIUM

The use of 1 or 2 ml. quantities of serum for analysis gave almost identical results. A number of blood samples from healthy volunteers and ward patients were analysed for serum calcium by the three methods, and the results are given in Table III.

Table III
COMPARISON OF METHOD FOR THE ESTIMATION OF CALCIUM IN SERUM

<table>
<thead>
<tr>
<th>Sample</th>
<th>Clark and Collip</th>
<th>Trevan and Bainbridge</th>
<th>E.D.T.A.</th>
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Results as mg. calcium per 100 ml.

Comparison of the three procedures for 20 blood samples showed that the permanganate method gave results which differed from the ashing procedure by -1.2 to +1.9 mg./100 ml. The E.D.T.A. results from 16 of the samples showed a better correlation (-0.3 to +1.0 mg./100 ml.) with the ashing method, but the results from the remaining four samples had gross errors of between +1.2 and +3.7 mg./100 ml.

Discussion

The values in Tables I and II show that in aqueous solutions the direct E.D.T.A method compares favourably with the two indirect procedures and a satisfactory recovery of added calcium from serum was obtained. The advantages of the E.D.T.A. method were speed and simplicity of manipulation, but a serious limitation when serum was being analysed was the difficult end-point. Although this end-point was easily determined in aqueous solutions it required considerable experience for its accurate detection in the presence of serum. The results in Table III show that even when an experienced worker used the method 20% of the blood samples gave grossly inaccurate results with the E.D.T.A. reagent.

We consider that the difficult end-point and the large proportion of inaccurate results render the
method unsuitable for routine clinical work even when a rapid result would facilitate early diagnosis and treatment.

Summary

The estimation of serum calcium by direct titration with E.D.T.A. has been investigated. Results are given for recoveries of calcium added to serum of known calcium content. The procedure has been compared with two other well-known methods for estimating serum calcium both for aqueous solutions and sera.

Certain practical difficulties of the method are discussed, and it is concluded that although the technique is rapid and simple it is not suitable for routine clinical use.

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