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

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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 reasonably 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:

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-

tion did not remove the necessity for any one person to acquire considerable experience with the end-point before reliable results could be obtained. The presence of haemolysis in the serum rendered the determination of the end-point even more difficult.

Variation in the external lighting also made the accurate observation of the end-point rather difficult, and the diluted serum was prone to froth excessively on shaking; this was obviated by the addition of one drop of capryl alcohol to the titration mixture.

Results

The E.D.T.A. method was compared with the permanganate procedure of Clark and Collip and the ashing technique of Trevan and Bainbridge for a series of aqueous solutions of calcium ranging in concentration from 5 to 15 mg. per 100 ml. The results are shown in Table I.

TABLE I
COMPARISON OF METHODS FOR SERUM CALCIUM ESTIMATIONS IN AQUEOUS SOLUTIONS

Sample	Calcium Content	Clark and Collip	Trevan and Bainbridge	E.D.T.A.
1	5.0*	5.1	5.0	5.1
2	7.0	6.9	6.8	7.1
3	9.0	9.1	8.8	9.1
4	11.0	11.1	10.9	11.0
5	13.0	13.4	12.9	13.0
6	15.0	15.0	14.9	15.1

* Results expressed as mg. calcium per 100 ml.

The recovery of added calcium from serum by the E.D.T.A. method using 1 and 2 ml. aliquots is shown in Table II. The serum was separated from 600 ml. of blood obtained from a hypertensive patient. Increments corresponding to 0.5 mg. of calcium per 100 ml. serum were added as 0.2 to 1.6 ml. quantities of a 2.5 mg./100 ml. solution of calcium to 1 ml. samples of the serum before beginning the analysis. These quantities were doubled when 2 ml. aliquots of the serum were added.

Table II demonstrates that a satisfactory recovery of added calcium was obtained and that

TABLE II
RECOVERY OF CALCIUM FROM SERUM

Sample	Added Calcium	Total Calcium in Sample	Estimated Calcium	
			1 ml. Aliquot	2 ml. Aliquot
1	0	9.6	9.6	9.6
2	0.5	10.1	10.0	9.9
3	1.0	10.6	10.7	10.8
4	1.5	11.1	11.3	11.0
5	2.0	11.6	11.8	11.6
6	2.5	12.1	12.0	12.2
7	3.0	12.6	12.5	12.8
8	3.5	13.1	13.2	13.0
9	4.0	13.6	13.7	13.8

The results are expressed as mg. of calcium per 100 ml. serum.

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

Sample	Clark and Collip	Trevan and Bainbridge	E.D.T.A.
1	10.0	8.0	9.0
2	11.1	9.2	9.9
3	10.9	10.6	10.6
4	11.6	9.9	9.6
5	11.5	10.8	13.1
6	9.2	10.4	10.2
7	12.2	10.3	9.9
8	7.4	6.6	7.8
9	11.6	10.0	9.5
10	11.4	9.6	10.4
11	11.8	10.1	13.8
12	11.0	9.3	8.6
13	8.8	8.1	10.2
14	11.9	11.0	10.5
15	10.2	8.0	8.1
16	10.6	9.6	10.6
17	10.4	9.5	11.7
18	8.8	7.8	7.8
19	10.6	9.8	10.1
20	9.0	8.4	9.3

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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REFERENCES

- Clark, E. P., and Collip, J. B. (1925). *J. biol. Chem.*, **63**, 461.
 Elliott, W. E. (1952). *Ibid.*, **197**, 641.
 Holtz, A. H., and Seekles, L. (1952). *Nature, Lond.*, **169**, 870.
 Nielsen, H. (1952). *Nord. Med.*, **48**, 1059.
 Roe, J. H., and Kahn, B. S. (1929). *J. biol. Chem.*, **81**, 1.
 Sobel, A. E., and Hanok, A. (1951). *Proc. Soc. exp. Biol., N.Y.*, **73**, 737.
 Trevan, J. W., and Bainbridge, H. W. (1926). *Biochem. J.*, **20**, 422.