A Method for the Colorimetric Determination of Calcium in 0.5 ml of Serum. (18405)

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Methods for the determination of calcium which involve titration are not generally convenient for reduced amounts of serum. A colorimetric method which has been widely used on 2 ml of serum consists of an estimation of phosphate after the precipitation of calcium as phosphate(1), but it may be subject to serious error in unskilled hands. Marriot and Howland determined calcium colorimetrically by oxidizing the calcium oxalate precipitate from serum with ferric thiocyanate(2). However, the unmodified method is unsatisfactory since the reagents are not stable and the results are erratic. Although we have succeeded in stabilizing the reagents by using ferric chloride in 18% hydrochloric acid, the results are not quite precise. Rappaport determined calcium by iodometric titration after oxidation of the precipitate of calcium oxalate with excess ceric sulfate(3). Sendroy measured the residual ceric sulfate colorimetrically after addition of iodide(4), and Weybrew, Matrone, and Baxley measured the ceric sulfate directly in the ultraviolet(5). We have found that the excess ceric sulfate can be measured at 420 mu and that results for serum and standard solutions of calcium obtained in this way are apparently more reliable than those determined by the method of Sendroy after addition of iodide. In this paper we describe a method for the colorimetric determination of calcium in 0.5 ml of serum.

Method. Reagents. (1) 4% ammonium oxalate. (2) Dilute ammonia. Dilute 2 ml of concentrated ammonium hydroxide to 100 ml. (3) 1 N Sulfuric acid. Add 27 ml of concentrated sulfuric acid to water and dilute in 1 liter. (4) Cerous sulfate solution. Dissolve 6.25 g of cerous sulfate ($Ce_2(SO_4)_3 \cdot 8\ H_2O$) in 600 ml of water and 56 ml of concentrated sulfuric acid with heat. Cool and dilute to 2 liters. (5) Ceric sulfate stock solution. Dissolve 4.0 g of ceric sulfate, anh., in 500 ml of cerous sulfate by heating to boiling. Cool and filter. Titrate 2 ml of the filtrate with 0.005 N thiosulfate after the addition of potassium iodide, and dilute the balance of the filtrate to 0.0085 N with cerous sulfate solution. (6) Ceric sulfate-cerous sulfate reagent. Dilute 15.0 ml of ceric sulfate stock solution to 100 ml with cerous sulfate solution. (7) Concentrated calcium oxalate standard solution. Dissolve 0.630 g of reagent grade oxalic acid ($H_2C_2O_4 \cdot 2\ H_2O$) and transfer to a 1 liter volumetric flask. Dissolve 0.554 g of reagent grade anhydrons calcium chloride, add 100 ml of 1 N hydrochloric acid, and transfer quantitatively to the flask. Dilute to volume with water. (8) Working standard calcium oxalate solution. Dilute 25 ml of the concentrated standard to 200 ml with 1 N sulfuric acid. 1 ml is equivalent to 10 mg calcium per 100 ml.

Transfer 0.5 ml of serum with Ostwald-Folin pipettes to 5 ml centrifuge tubes and add 0.5 ml of water and 0.25 ml of 4% ammonium oxalate. Mix well with slender rods and allow to stand at room temperature over night. Centrifuge for 10 minutes at 1500-2000 R.P.M. and drain off most of the supernatant fluid (allow about 0.02 ml to remain after drainage) with a fine capillary tube attached to water suction. Wash the precipitate 3 times by adding 0.8 ml of dilute ammonia down the sides of the tubes and stirring the precipitate with a fine glass rod. One rod is used for each tube and rinsed with the succeeding wash solution. During centrifugation the rods are placed in separate test tubes.

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Finally, add 1 ml of 1 N sulfuric acid and return the rods to the centrifuge tubes. Include a blank tube containing 1 ml of sulfuric acid and a standard tube containing 1 ml of working standard with each series of determinations.

Dissolve the precipitate by placing the tubes in a boiling water bath and stirring for 4-5 minutes. Remove the rack of tubes from the bath and allow them to cool for a few minutes. Then add 4.0 ml of ceric sulfate-cerous sulfate reagent, stir, and remove the rods. Finally, mix thoroughly by inversion, using parafilm instead of rubber stoppers, and allow to stand for 15 minutes. Transfer to colorimeter tubes and measure the color in an Evelyn colorimeter with filter 42 at the 6 cc aperture. Obtain the concentration of calcium from the difference in density between the blank and unknown solutions on a standard curve prepared from solutions of known amounts of calcium oxalate. The values obtained for the standard should be close to 10 mg per 100 ml.

In Table I the results obtained by this method in a series of sera are compared with those determined in 2 ml samples by the method of Clark and Collip(7). It is evident that the values agree in every case within 0.3 mg per 100 ml.

TABLE I. Comparison of Results of the New Method for 0.5 ml of Serum with Those Determined by Titration in 2 ml of Serum.

Serum	New method mg per 100 ml	Clark-Collip method mg per 100 ml
1	13.3	13.0
2	13.2	13.4
3	12.4	12.1
4	12.1	11.9
5	10.8	10.9
6	9.7	9.8
7	9.1	9.1
8	9.0	8.9
9	8.8	8.6
10	8.7	8.7
11	8.6	8.6
12	8.5	8.4
13	8.1	8.3
14	7.9	8.0
15	7.6	7.5

Summary. A method is described for the colorimetric determination of calcium in 0.5 ml of serum with ceric sulfate. Data are presented to show that the results agree within 0.3 mg per 100 ml of those determined by titration with permanganate.

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Increased Virus in Eggs Injected with Cortisone. (18406)

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In the course of investigations of viral infections of the chick embryo, experiments were undertaken to determine the effects of alteration of host physiology upon viral multiplication. Studies by Karnofsky, *et al.*(1), revealing the profound effects of adrenal cortical steroids on the growth and development of the chick embryo, suggested the use of these hormones to alter host metabolism*. Mumps and influenza viruses were employed because of the relative ease and precision with which their concentration may be measured by the hemagglutination reaction.

Materials and methods. Viruses. The PR8 strain of influenza A virus, the Lee strain of influenza B virus, and the Habel strain of mumps virus were employed. 10^4 to 10^5 ID_{50}

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