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Microdetermination of Calcium in Blood Serum by Direct Titration. (19875)

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The microdetermination of calcium in serum by rapid titration with a chelating reagent, ethylene diamine tetracetate(1), has obvious advantages. In the method of Sobel and Hanok(2), in which the dye, eriochrome black T, is used as indicator, magnesium is also titrated, and the end-point is obscured in icteric and hemolyzed serum. Greenblatt and Hartman(3) propose a method with murexide (ammonium purpurate) as indicator, but do not mention interference by magnesium. Although they present data to show that the results obtained in serum, urine and spinal fluid agree with those obtained by a standard method, the end-point has been found so poor in this laboratory that we have not been able to titrate serum with the naked eye. We also have evidence(4) that it is not possible to titrate calcium in urine in this way.

In this paper we describe a method for the microtitration of calcium in serum in a photoelectric colorimeter with ethylene diamine tetracetate and murexide as indicator. Magnesium is not titrated in this method, and neither hemolyzed nor icteric blood interferes in the titration. In a later paper we will describe a macromethod for the titration of calcium in urine.

Experimental. We have used a Lumitron photoelectric colorimeter with an adaptor to hold tubes of 15 mm diameter. The solutions were titrated in Klett-Summerson microcolorimeter tubes while the tubes were in the colorimeter, and the colorimeter was read continuously during titration. Although this arrangement has been found most convenient, any colorimeter with an adaptor to hold tubes of this size, and any short tube of about 15 mm diameter should be suitable. A convenient burette was made by sealing with De Khotinsky cement a 4 inch hypodermic needle of about 18 gauge bore to the tip of a 0.2 ml Kahn pipette graduated in thousandths. The needle was bent at a right angle so that at least 3 inches of needle was perpendicular to the pipette, and the point of the needle was filed off straight. A piece of rubber tubing to serve as a reservoir for mercury was attached to the other end of the pipette, and the open end of the tubing was plugged with a glass rod. This burette was mounted on a horizontal wooden stand which also carried a screw clamp to propel the mercury and titrating fluid, and another bent needle which delivered nitrogen gas from a tank to mix the solution in the tube during titration. Our stand was so constructed that the entire burette assembly could be raised and swivelled at the start and at the end of each titration at the colorimeter.

Reagents. 1) Concentrated stock calcium standard solution. Dissolve 2.497 g of dry calcium carbonate with a minimal amount of hydrochloric acid. Neutralize with sodium hydroxide, and dilute to 500 ml. 2) Working standard calcium solution. Dilute 5 ml of the concentrated stock solution to 100 ml. 100 ml = 10 mg Ca. 3) Saturated picric acid

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(according to Folin) in water. 4) Murexide-sodium chloride indicator mixture. Grind 200 mg of murexide (ammonium purpurate) and 100 g of sodium chloride to a fine powder, and mix thoroughly. 5) Stock borate solution. Dissolve 62.0 g of boric acid in freshly boiled water containing 124.5 ml of 4.00 N sodium hydroxide, and dilute to 1 liter. 6) Borate buffer solution. Add 80.5 ml of 4.00 N sodium hydroxide to 400 ml of the stock borate solution, and dilute to 1 liter with freshly boiled water. 7) Concentrated sequestering reagent, S.E.D. Dissolve 4.0 g disodium ethylene diamine tetracetate in 800 ml of water containing 10.7 ml of 2 N sodium hydroxide. 8) S.E.D. reagent A. Dilute the concentrated S.E.D. reagent so that 0.5 ml of working standard calcium solution requires 0.100 ml of S.E.D. reagent A by method 1. 9) S.E.D. reagent B. Dilute the concentrated S.E.D. reagent so that 0.2 ml of working standard calcium solution requires 0.100 ml of S.E.D. reagent B by method 2.

Methods. Two methods are proposed. In method 1, which requires 0.5 ml of serum, the proteins are removed with picric acid and calcium is titrated in the presence of the yellow color of picrate. In method 2, 0.2 ml of serum is titrated directly, and a little caprylic alcohol is added to inhibit foaming in the alkaline solution which contains protein. Either method is suitable for icteric or hemolyzed serum, but method 1 is less affected by the abnormal color of serum and yields sharper end-points when hemolysis is excessive.

Method 1. Add 0.5 ml of serum with stirring to 2.0 ml of saturated picric acid in a centrifuge tube. After standing for a few minutes, add 1.5 ml of water. Mix thoroughly by inversion on parafilm, and let stand 10 minutes. After centrifugation, transfer 3.0 ml of the clear supernatant fluid to a microcolorimeter tube. Add 1.0 ml of borate buffer solution and about 90 mg of indicator mixture from a measuring tube(5). Place the tube in the colorimeter, which has been adjusted to 100% transmission with water in a second tube. Swing the burette into place, attach to the nitrogen and adjust the flow of gas to a maximum that yields steady readings. Titrate with S.E.D. reagent A with filter 580 until the end-point is reached, which is indicated by constant readings on the galvanometer. (The readings start at about 40% transmission, change slowly as the end-point is approached, and finally remain unchanged with increasing amounts of S.E.D. reagent at the end-point, which is about 22% transmission.) The concentration of calcium in the sample is calculated by the equation, Ml S.E.D./0.100 x 10.0 = mg % calcium.

Method 2. Add 0.2 ml of serum to 3.0 ml of water in a microcolorimeter tube. Mix, and add 1.0 ml of borate buffer and 90 mg of the indicator mixture. Finally add a small drop of caprylic alcohol, and place the tube in the colorimeter. Adjust the nitrogen as in method 1 and titrate with filter 580 in place, using S.E.D. reagent B. In this method the readings also start at about 40% transmission and become constant at the end-point, which is at about 22%. The calculation is also the same, Ml S.E.D./0.100 x 10.0 = mg % calcium.

The titrations with sequestering reagent were conducted at a pH of about 11.5 (glass electrode) since we have found that magnesium does not interfere in the titration of calcium in this region. Whereas the effect of magnesium is considerable at pH 10.5, concentrations as high as 20 mg % of magnesium do not affect the results at the higher pH. Fig. 1 shows the results obtained at pH 11.7 of a series of titrations in standard solutions of calcium in picric acid by method 1 in the presence and in the absence of 5 mg % of magnesium. Similar results have been obtained at pH 11.7 by method 2 in the absence of picrate.

We have tested both methods 1 and 2 in about 100 samples of serum in which calcium was also determined by a standard method (6) by precipitation as oxalate and titration with permanganate. In a representative series of 20 samples of serum, in which the calcium concentration varied between 5.4 and 13.9 mg %, the results determined by method 1 deviated between −0.6 and +0.4 mg % from those obtained by the standard method, and showed an average deviation of 0.24. The results determined by method 2 deviated

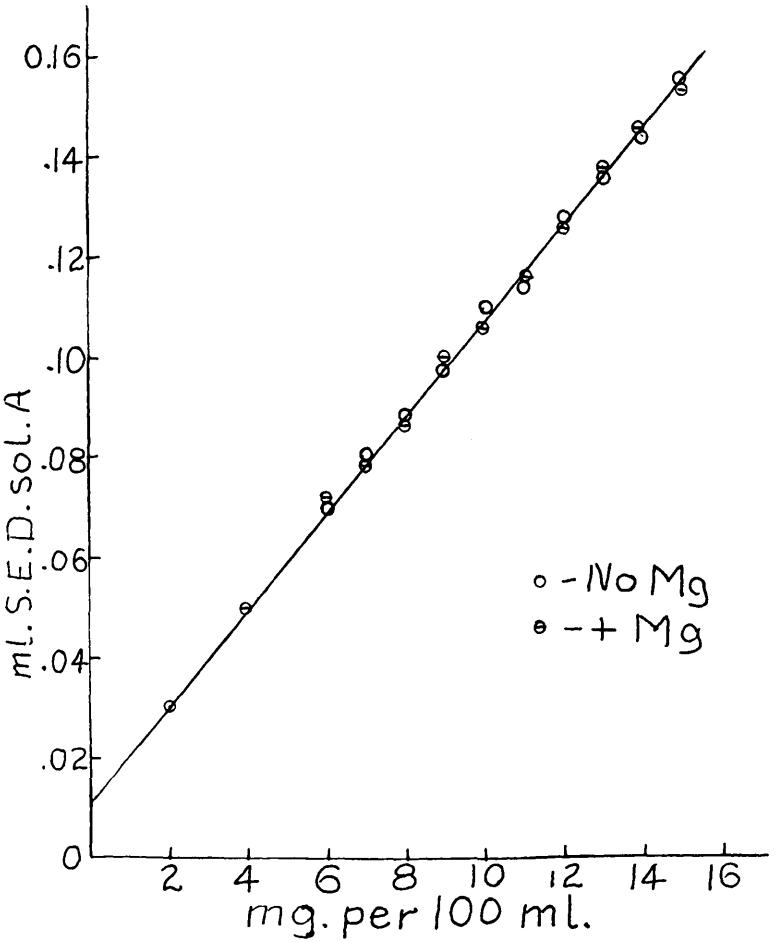

FIG. 1. Values of the titres of S.E.D. reagent A for varying conc. of calcium by method 1 in the presence and in the absence of 5 mg % of magnesium.

between −0.5 and +0.3 mg % from the values obtained by the standard method, and showed an average deviation of 0.20.

Summary. Methods are described for the microdetermination of calcium in serum by titration with ethylene diamine tetracetate in a photoelectric colorimeter at pH 11.6 with murexide as indicator. It is shown that magnesium does not interfere with the titration of calcium at this pH, and that the endpoint is reached when the readings on the galvanometer become constant. In method 1, the proteins are removed from 0.5 ml of serum with picric acid and the titration is conducted in a solution of picrate. In method 2, 0.2 ml of serum is titrated directly in the presence of protein. Data are presented to show that the results of both methods agree quite well with those obtained by precipitation as oxalate and titration with permanganate.

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