Calcium Determination in Biologic Material

Eugene Y. Berger

The classical procedure for the determination of calcium in biologic fluids involves the precipitation of calcium as the oxalate and titration of the oxalate with potassium permanganate. The permanganate titration has certain difficulties. The oxalate solution has to be heated during the titration (1) and the end point is unreliable in dilute solutions (2). The use of cerate oxidimetry avoids these circumstances. The principles of cerate oxidimetry have been well established in the chemical literature and there have been numerous applications to the determination of calcium (2–8). The present report defines conditions which proved to be most satisfactory for the determination of calcium in biologic material. The method has proved reliable through several years of use since 1940 in this and other laboratories. The accuracy is ±2 per cent as measured by the recovery of known amounts of calcium.

Principle of Method

Calcium is precipitated as the oxalate salt which is then oxidized by ammonium hexanitratoceate (9). The excess cerate is titrated with ferrous ion, using ortho-phenanthroline as an oxidation-reduction indicator.

Reagents

(1) 0.4% aqueous solution phenol red.
(2) 4N ammonium hydroxide.
(3) 10% glacial acetic acid.
(4) 4% ammonium oxalate.

From the Research Service, Third (New York University) Medical Division, Goldwater Memorial Hospital, Welfare Island, New York, N. Y.
Received for publication May 6, 1955.
(5) Wash solution (10): Equal parts of 95% ethanol, ethyl ether, and water containing 2% ammonium hydroxide. Prepare daily.

(6) 2N sulfuric acid.

(7) 0.014M ammonium hexanitratocerate in 2N sulfuric acid. 7.67 Gm. of ammonium hexanitratocerate is dissolved in liter of 2N H₂SO₄.

(8) 0.025M o-phenanthroline ferrous sulfate ("Ferroin"). Dilute with water to 0.005M for use.

(9) 0.1M ferrous ammonium sulfate stock solution. 19.6 Gm. of ferrous ammonium sulfate is dissolved in 500 ml. 0.36N H₂SO₄. Stock solution is diluted with water to 0.005M for use.

(10) Standard 0.01N sodium oxalate. To 0.6698 Gm. of sodium oxalate dissolved in distilled water is added 5 ml. of conc. H₂SO₄ and the solution diluted to 1 liter (11).

(11) 4N sulfuric acid.

METHODS

Serum or Heparinized Plasma

In a 15 ml. conical centrifuge tube 2 ml. of serum or heparinized plasma is mixed with 2 ml. of distilled water; 1 ml. of 4% ammonium oxalate is added. The mixture is allowed to stand about 12 hours or if more convenient, overnight.

The sample is centrifuged at 2500 rpm for 15 minutes and the supernatant fluid removed by aspiration, using a fine-tipped pipet. The precipitate is washed with 3 ml. of wash solution. To insure thorough removal of excess oxalate, the wash solution is added slowly along the side of the tube. The precipitate is stirred with a thin glass rod and the rod is washed with wash solution as it is removed from the sample. The sample is centrifuged for 10 minutes at 2500 rpm. The supernatant wash is carefully decanted and the tube allowed to drain for a few minutes while resting inverted on absorbent paper. The washing procedure is repeated.

The moist calcium oxalate precipitate is dried by placing the tube in a water bath at room temperature and bringing the bath slowly to a boil. To the dried oxalate 2 ml. of 2N H₂SO₄ is added and the tube is heated for 1 minute to bring all the precipitate into solution. The tube is cooled to room temperature and 1 ml. of 0.014M cerate is added. The sample is stirred and allowed to stand for 30 minutes, with the stirring rod left in the tube. Orthophenanthroline indicator, 0.02 ml. of 0.005M, is added and the excess cerate is titrated with 0.005M ferrous ammonium sulfate,

1 This oxidation-reduction indicator is purchased as an 0.025M solution.
using a 5 ml. microburet. The color change is from yellow to colorless, to a faint blue, and then to salmon pink, which represents the end point. The end point is quite sharp and distinct. Only 0.002 ml. of 0.005M ferrous ammonium sulfate is needed to effect the color change. 1

**Urine**

One to three milliliters of urine is pipetted into a 15 ml. conical centrifuge tube. The pH is adjusted to 6.5 by adding 1 drop of phenol red indicator solution and 4N NH₄OH by drops until the solution is pink. Ten per cent glacial acetic acid is then added by drops until the solution turns yellow. The calcium is precipitated by adding 1 ml. of 4% ammonium oxalate. The solution is mixed and allowed to stand. After precipitation of the calcium oxalate the procedure is followed as described for plasma.

**Dried Tissues and Feces**

A sample of about 1 Gm. is placed in a platinum crucible (about 10 ml. capacity) with enough concentrated sulfuric acid to cover the specimen. The sample is dried at 100° and then ashed in a muffle furnace at 700° for 12 hours. The white ash is dissolved in 1–3 ml. of concentrated hydrochloric acid and transferred to a 25 ml. volumetric flask with hot water. The sample is allowed to cool and then diluted to 25 ml. A sample containing about 10 microequivalents of calcium is placed in a 15 ml. conical centrifuge tube, and the pH is adjusted to 6.5 and the calcium precipitated following the procedure detailed for the determination of calcium in urine.

**STANDARDIZATION**

The ferrous ammonium sulfate is standardized against 0.01N sodium oxalate and the cerate is standardized against the iron. One milliliter of

---

1 At the time the cerate is added to the oxalate, there should be sufficient excess cerate to impart some yellow color to the solution. If the solution turns colorless, indicating that there is more than 14 microequivalents of calcium present, more cerate is added so that some yellow color persists.

2 Do not use Tygon tubing as part of the titration apparatus, as this tubing oxidizes the ferrous iron.

3 The precipitate of 10 microequivalents of calcium oxalate just about covers the bottom of a 15 ml. conical centrifuge tube. Should the precipitate appear considerably smaller or larger than this after the addition of ammonium oxalate, it would be advisable to alter the amount of the sample. One ml. of 0.014N cerate will be sufficient to titrate 14 microequivalents of calcium. If there is more calcium present than 14 microequivalents, more cerate may be added at the time of titration, but it becomes inconvenient to add much more than 5 ml. of cerate to a 15 ml. centrifuge tube.
0.014M cerate is added to 1 ml. of 0.01N sodium oxalate plus 1 ml. of 4N sulfuric acid. The solution is allowed to stand for 30 minutes. Ortho-phenanthroline, 0.02 ml. of 0.005M, is added and the solution is titrated against the iron solution. One milliliter of cerate plus 2 ml. of 2N sulfuric acid are also titrated directly against the iron without the addition of oxalate. The titration difference between the cerate with oxalate and cerate without oxalate represents the amount of iron which is equivalent to 1 ml. of 0.01N oxalate, which in turn is equivalent to 0.2 mg. of calcium.

SUMMARY

A method has been described for the determination of calcium in plasma, urine, and tissue and feces. Calcium is precipitated as the oxalate salt and the oxalate is determined by cerate oxidimetry.

REFERENCES