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LVII. THE ESTIMATION OF CALCIUM IN BLOOD-SERUM.

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The method for the estimation of calcium in blood-serum devised by Kramer and Tisdall [1921] has been extensively used and, it is generally agreed, gives reproducible results in expert hands. Clark and Collip [1925], however, emphasise that the details of the method must be followed with meticulous accuracy or discrepant results will be obtained. They attribute the main source of error to the slight solubility of calcium oxalate and are of opinion that traces of ammonium oxalate, used for washing the precipitate, remain, and, if close adherence to the original method is maintained, tend to compensate for the error due to solution of the calcium oxalate. In consequence too much washing, or too little, will give inaccurate results. The original method involves titration of the precipitated oxalate with $N/100$ potassium permanganate. One of us, apart from an instinctive aversion from permanganate titrations, finds it extremely difficult to decide when the end point is reached although the other author can, by the exercise of some patience, obtain reproducible titration figures. It occurred to us that by the conversion of the oxalate into carbonate the use of permanganate could be avoided, whilst the amount of carbonate could be estimated by acidimetric methods.

Hamilton [1925] has used a similar modification of the Kramer and Tisdall method which was published before the working out of our method was completed, but the details of the method we use make a considerable difference in the time necessary to complete an estimation, as compared with that required by the method used by Hamilton.

The method also has the advantage that the calcium oxalate need not be washed with water at any stage, saturated ammonium oxalate in which calcium oxalate is absolutely insoluble, being used instead. Any excess of ammonium oxalate is decomposed and driven off as carbonate when the final heating stage is reached.

Method.

Apparatus.

(1) Small centrifuge tubes of the dimensions shown in the figure and made of resistance glass (*e.g.* Duroglass). We do not find that quartz tubes are necessary. The shape of the bottom of the tube is of importance; the sides of the conical end must be sufficiently steep for the oxalate precipitate to slide down to the apex of the cone during centrifuging. If any oxalate adheres to the sides of the tube there is a chance of losing some during decantation of supernatant liquid. The bottom of the tube inside should be just rounded off.

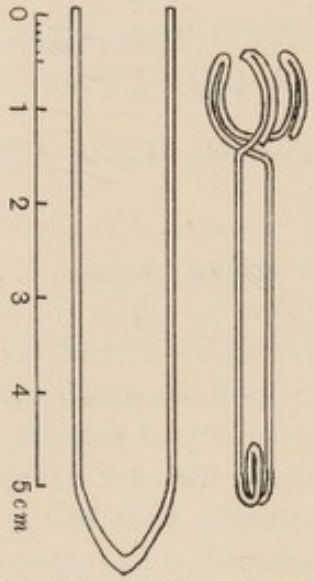

Fig. 1. Centrifuge tube and wire holder.

(2) 1 cc. pipettes.

(3) Glass stirring rods 10 cm. by 1·5 mm. made from drawn-out tubing.

(4) Some form of micro-burette. The final titration for 1 cc. of serum consists of the addition of about 0·25 cc. of $N/50$ alkali. For this purpose we use the micrometer syringe described by one of us [Trevan, 1925]. A Rehberg burette would serve probably as well; or by using 2 cc. of serum as in the original Kramer and Tisdall method, together with $N/100$ alkali for the final titration, an ordinary 2 cc. burette could be used. In this case, however, the end-point error would be rather larger.

(5) Centrifuge.

(6) Wire holder for tube (see Fig. 1).

Reagents.

(1) Saturated ammonium oxalate (3·5 %).

(2) $N/50$ sodium hydroxide.

(3) $N/100$ acid. We have used both phosphoric acid, as recommended by Cahen and Hurtley [1916], and hydrochloric. We prefer the former. The acid should be titrated against $N/50$ Na_2CO_3 by a special method; excess of acid should be added to the standard carbonate and the amount un-neutralised titrated with the $N/50$ sodium hydroxide. The conditions of the calcium carbonate titration are thus more closely reproduced and the titration error diminished.

(4) Indicator. We have found bromophenol blue (0·04 %) the best of several we have tried. Methyl red (0·02 %) is almost as good but has to be used near the acid end of its range, and turns rather too far on the alkaline side to be entirely satisfactory for H_3PO_4. Buffer solutions, p_H 4·0 and 4·2 for bromophenol blue, 4·4 and 4·6 for methyl red, are used as comparison solutions for judging the end point. The titration of the $N/100$ acid against standard carbonate should of course be carried out against the same indicator as that finally used for the titration of the calcium carbonate.

Procedure.

2 cc. of ammonium oxalate are measured into one of the centrifuge tubes and 1 cc. of serum is added. The operation is best carried out in this order, as, if the serum is measured into the tube, the first drops may dry on the side of the tube before the oxalate is added and prove difficult to re-dissolve afterwards. The contents of the tube are then stirred vigorously with one of the small glass stirring rods which is then withdrawn. The amount of fluid adhering to the stirring rod we find to be of the order of 0·3 mg. and may therefore be neglected. The tubes are allowed to stand for 2–3 hours and are centrifuged at about 3000 revolutions per minute for 10 minutes. The supernatant fluid is poured off and the tube drained by inversion over clean filter paper as recommended by Clark and Collip. After draining for some minutes 2 cc. of ammonium oxalate are added and the tube centrifuged again. The oxalate is removed, a fresh amount added, and the centrifugation once more repeated. At each centrifugation the oxalate should pack in the very apex of the conical end of the tube if the taper is correct. The tubes are then dried in a steam oven to prevent spurting in the next stage. The conversion into carbonate is carried out by holding the tube in the wire clip shown and passing it through the Bunsen flame for one minute. The ammonium oxalate left is converted into ammonium carbonate which comes off as a white cloud. Care should be taken to heat the whole of the tube to drive off any ammonium carbonate that may condense on the cooler parts. The temperature required for complete conversion of the carbonate is comparatively low. Some rough experiments carried out in a muffle show that it is not higher than 250°. Overheating is to be avoided as the calcium oxide formed may prove very difficult to dissolve in $N/100$ acid. The correct temperature, which is not difficult to arrive at, is indicated by the first appearance of the sodium flame around the tube. The tube is cooled, 1 cc. of $N/100$ acid added and allowed to stand for some minutes. We find that, as Cahen and Hurtley claim, the rate of solution is greater in H_3PO_4 than in HCl and the solution may be gently warmed if H_3PO_4 is used. When the solution of the carbonate is complete one drop of 0·04 % bromophenol blue is added from a very fine capillary pipette (the volume of the drop we use is 0·016 cc.). The titration is carried out with the micrometer syringe held vertically in a burette clamp. The addition of the sodium hydroxide is carried out by running a drop on to one of the small

stirring rods and then transferring it to the solution. The smallest drop which can be dealt with in this way has a volume of about 0·00015 cc., which is more than sufficiently small for accurate titration. This corresponds to about half a division on a metric micrometer head for the average "tuberculin syringe." The formation of drops of this size and their adequate removal by the stirring rod are much facilitated by coating the exterior of the needle of the syringe with paraffin wax, as recommended by Wright and Colebrook [1921]. The needle may be either the finest steel hypodermic needle or the glass needle described with the micrometer syringe. The latter is preferable as it is easier to see that it is filled to the tip when the titration is begun. Titration is carried to the point where the solution has a colour intermediate between those of buffers of p_H 4·0 and 4·2 each with a concentration of bromophenol blue equal to that of the solution which is being titrated. The difference between the titration figure so obtained and the titration figure for the acid alone gives the amount of calcium in the serum taken.

Results.

Solution of calcium chloride containing 0·091 mg. per cc. made by dissolving calcium carbonate in hydrochloric acid:

0·09140, 0·0918, 0·0916, 0·0914 mg. Ca per cc.

Horse serum: 8·6, 8·54 mg. Ca per 100 cc. on the same sample.

Rabbit serum: 13·26, 13·20, 13·16 mg. Ca per 100 cc.

We have obtained figures within less than 5 % of the correct value when using the same method with 0·01 mg. Ca per cc., measuring all solutions with a micrometer syringe and keeping the tubes covered during standing and centrifugation with rubber caps, such as are used for vaccine bottles. We have, however, not often used these small quantities.

Summary.

A method of estimating calcium is described in which the calcium is precipitated as oxalate and converted into calcium carbonate by heating the centrifuge tube in which it is separated and washed. The calcium carbonate is titrated with acid.

The method has the advantages that the end point is more distinct than that of a permanganate titration, and all washing is carried out with saturated ammonium oxalate, thus avoiding errors due to the solubility of calcium oxalate in water.

By the use of the micrometer syringe 1 cc. only of serum is required and smaller quantities down to 0·1 cc. will give results accurate enough for most purposes.

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