XLI. NOTE ON THE DETERMINATION OF TOTAL CALCIUM IN BLOOD-SERUM.

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IN a recent paper by Hendriks [1929], in which the application of the method of Trevan and Bainbridge [1926] to the determination of the total calcium of blood-serum was examined, certain discordant (low) results were attributed to a failure to adjust the heating conditions by which the calcium in the form of oxalate is converted to carbonate. Overheating leads to the formation of oxide which is difficultly soluble in the concentration of acid ($N/100$) used in the titration, while, on the other hand, underheating leaves some unchanged oxalate. In each case the final result will be low.

It seems both from the experience of Hendriks and that of the present author that the only weakness in an otherwise admirable method lies in the fact that the original authors did not lay down the aforementioned heating conditions in terms which would make them readily reproducible by other workers. However it was found that this defect could be overcome quite easily, and the method of Trevan and Bainbridge, as modified, has been used in this laboratory as a routine method practically from the time of its publication with entirely satisfactory results. It may therefore be of interest to record the modified procedure.

Following the original method of precipitation and washing of the oxalate precipitate, the remainder of the process was carried out in two stages.

(1) *Drying.* The special hard glass tube in its wire holder is kept inclined at a slight angle mouth downwards and laid on a pipe-clay triangle standing on a piece of plain iron-wire gauze stretched over a tripod, so that the tube is supported at a height of about $1\frac{1}{2}$ inches above the level of the gauze. To provide a bed and a slope for the tube, a slight depression is filed in one side of the triangle, the mouth being over this depression while the conical end is supported over the opposite apex. Drying is begun by heating with a Bunsen burner placed under the gauze with the flame directed towards the open end of the tube, the heat then being taken gradually along to the closed end. Once the tube is dry the heating is increased to drive off the major portion of the ammonium oxalate.

(2) *Ignition.* To complete the process the tube is laid directly on the gauze itself, and the heating continued along its whole length and especially near the point where the calcium is packed. A period of about 5 minutes is allowed for this stage. The conversion to carbonate is accomplished well within this time, and with the gauze interposed no possibility exists of the process being carried too far, even though the Bunsen flame be used at its hottest intensity. It is both unnecessary and detrimental to heat in the naked flame.

For titration the acid is added (0.5 cc. of *N*/50 is preferred to 1 cc. of *N*/100 acid in that it provides for readier solution) without disturbing the solid particles, and with a thin glass rod slightly flattened at the tip, the carbonate while remaining at the bottom of the tube is ground as far as possible to a fine powder. In this form it goes almost instantly into solution when stirred into the acid.

The reliability of the above conditions may be illustrated by the following results.

- (1) Solution containing 10 mg. Ca per 100 cc.

Mg. Ca per 100 cc. found: 9.93, 9.84, 10.01, 9.90, 10.02.

This series was obtained from a single sample by reprecipitating at the end of the first titration with ammonium oxalate, and repeating the whole process to the second and subsequent titrations.

- (2) Normal blood-serum.

Mg. Ca per 100 cc. found: 10.23, 10.33.

Clark and Collip's method [1925]: 10.09.

The figures show only a slightly greater deviation from the mean than those quoted by the original authors, but such increased experimental error as does exist may be justly ascribed to the fact that the results recorded here have been obtained under essentially routine conditions, and without doubt could be still further improved with greater attention to the finer points of the technique.

SUMMARY.

Some improvements are suggested in the technique of the Trevan and Bainbridge method for the determination of the total calcium of serum. In particular a simple modification of the heating conditions is described which brings the oxalate-carbonate conversion under complete control.

REFERENCES.

- Clark and Collip (1925). *J. Biol. Chem.* **68**, 462.
Hendriks (1929). *Biochem. J.* **23**, 1206.
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