A Micro Method for the Estimation of Chlorides in Blood.

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The authors have used the method of VAN SLYKE[1]) (1923) for the estimation of whole blood chlorides, with satisfactory results. In this method one cubic centimeter of blood is required for a single determination. During the course of other experiments, only small quantities of blood were available for the estimation of blood chlorides; consequently the VAN SLYKE method was modified to permit the estimation of chlorides in one-tenth of a cubic centimeter of whole blood. The details of this micro method are here described.

Reagents.

(1) Standard Silver Nitrate. — This solution contains 2.905 grams per liter. It is prepared by dissolving the C. P. salt in a small quantity of distilled water and making up to volume with concentrated nitric acid (Sp. gr. 1.42). 1 c. c. of this solution = 1 mg. NaCl.

(2) Potassium Thiocyanate. — A water solution containing 0.554 gm. per liter. Prepared by standardization against the silver nitrate solution. 3 c. c. of this solution = 1 c. c. of the standard $AgNO_3$.

(3) Indicator. — A saturated solution of ferric alum[2]) is used as an indicator in the titrations of the excess silver with KCNS.

Procedure.

One-tenth cubic centimeter of whole blood is measured by means of a micro-pipette and discharged into 1 c. c. (approximately) of distilled water contained in a pyrex test tube. The pipette is rinsed thoroughly by filling and emptying several times with the liquid in the tube and then 1 c. c. of the standard $AgNO_3$ solution

[1]) VAN SLYKE, D. D., 1923, Jour. Biol. Chem., VIII, 523.

[2]) BAKER's analyzed ferric alum was employed.

is added. A series of determinations may be run simultaneously. The tubes are then placed in a large pyrex beaker (the latter covered with a glass plate), and heated over a water bath until the proteins are completely digested and the supernatant liquid is clear. The precipated AgCl is coagulated by heating carefully over a free flame. Then 1 c. c. of the saturated solution of ferric alum is added, the material cooled, and the excess silver titrated with the standard K C N S solution until a salmon-pink color is obtained. For convenience and accuracy in titration, a 2 c. c. micro-burette is employed[3]). Blank determinations should always be run as a check on the reagents.

Calculation. — If T represents the number of c. c. of standard K C N S solution used in titrating the excess silver, then $1000\ (1 - T/3) =$ mg. of NaCl per 100 c. c. of blood, when 0.1 c. c. of blood is used for the estimation.

Duplicate analyses usually agreed within 1%. NaCl added to various blood samples was recovered to the extent of 98—99%. The results obtained compared favorably with the method of Van Slyke but were somewhat higher due to the fact that the micro-pipettes had been previously calibrated for "blow-out" rather than "wash-out" delivery.

In the method described by Rehberg[4]) (1926), the proteins are destroyed with hydrogen peroxide, ether is then added to facilitate the coagulation of the AgCl and air is bubbled through the mixture during the titration. The authors believe the proce dure described above is somewhat simpler.

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[3]) The micro-pipettes and micro-burette used in this procedure require accurate calibration.

[4]) Rehberg, P. B., 1926, Biochem. Jour., XX, 483.