LXII. THE DETERMINATION OF CHLORINE IN BLOOD AND TISSUES BY MICROTITRATION.

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IN a recent paper Van Slyke [1923] describes a macro- and a micro-method for the determination of chlorine in blood and tissues. The principle of the method is to destroy the proteins by nitric acid in the presence of silver nitrate and subsequently to titrate the excess of silver nitrate by means of thiocyanate¹. In this way the whole procedure may be carried out in a single vessel, which, especially for a micro-method, is an extremely valuable feature. The only drawback of the micro-method of Van Slyke is that it requires as much as 1 cc. of blood or plasma.

If, however, the method could be modified so as to use smaller quantities of material, its usefulness would be much increased. By means of the method of microtitration described in an earlier paper [Rehberg, 1925] this is easily attained without losing appreciably in accuracy.

The method proposed, which has been in use for some time, is very similar to that of Van Slyke, yet a few alterations have been made, of which the principal are that the destruction of the proteins is helped by means of hydrogen peroxide, and that the titration is carried out in the presence of ether. Ether was introduced in the Volhard titration by Rothmund and Burgstaller [1909] who showed that the addition of ether to the fluid to be titrated made it possible to titrate accurately without separation from the silver chloride formed. The reason for this is that the silver chloride separates out in large particles so that the exposed surface is very small. The same result was obtained by heating the solution containing the silver chloride.

In the titration as performed in the present method and in that of Van Slyke addition of ether is not necessary for this reason. The conditions—a high percentage of nitric acid and ferric alum—are sufficient, as demonstrated by Whitehorn [1920], to make titration in the presence of the silver chloride possible. Moreover the fluid has been heated for hours. The addition of ether, and constant mixing during the titration by means of an air-current, precipitates the silver thiocyanate in large particles, which are carried out of

¹ The same principle has also been used by Claudius [1924] as well as by the authors named by Van Slyke.

the fluid into the layer of ether above. In this way the fluid being titrated is kept practically clear, which is a great advantage in ascertaining the end-point.

Material and procedure.

The reagents needed are 0.15 *N* silver nitrate, 0.1 *N* potassium thiocyanate, concentrated nitric acid free from chlorine, 30 % hydrogen peroxide, concentrated ferric alum solution and ether. The necessary apparatus comprises two microburettes, small test tubes of the form shown on the right of the picture in my paper on microtitration. They are *ca.* 8 cm. long with an outer diameter of *ca.* 13 mm. and provided with a constriction so that they can hang in the burette arm. Further, a number of small test tubes fitting loosely into the lumen of the other tubes to be used as stoppers during the digestion, a water-bath with a stand taking a convenient number of the tubes and an arrangement for providing an air-current through the tubes during titration (see paper on microtitration) are required.

The procedure in analysing whole blood or plasma is the following. By means of a microburette 0.1 cc. of the 0.15 *N* silver nitrate is measured into the bottom of one of the test tubes, 0.5 cc. of conc. nitric acid is added, and into this is measured 0.1 cc. of whole blood or plasma by means of a calibrated pipette which is rinsed twice with distilled water. 0.5 cc. of H_2O_2 is added and the tube is closed by one of the small test tubes, shaken in order to mix the contents and placed in the water-bath. After heating an hour or so for plasma and two or three hours for whole blood, the mixture is of a clear yellow colour and is ready to be titrated. To perform the titration 0.1 cc. of conc. ferric alum solution and 1 cc. of ether are added. The titration is carried out by means of 0.1 *N* thiocyanate added from a microburette, the solution being stirred by means of a current of air bubbles coming from a fine tube reaching down to the bottom. On addition of the thiocyanate silver thiocyanate is formed, and by occasionally increasing the air-current this precipitate and most of the silver chloride are carried up into the layer of ether. The end-point is sharp and with some practice it is possible to use the first fine trace of persistent colour so that no excess of thiocyanate need be subtracted as in the method of Van Slyke.

The result is calculated from the formula: amount of chlorine per 100 cc. fluid = $(150 \div a) \times 3.55$ mg. when *a* is the reading of the microburette in mm.³ after the titration.

Duplicate analyses by the method will give results on plasma which seldom vary more than 2-3 mg. per 100 cc. Differences exceeding 5 mg. per 100 cc. are usually easily avoided, though now and then a result for some unknown reason may be spoiled. The results of a series of duplicate analyses may be found in the paper on kidney function [Rehberg, 1926].

On whole blood the results are not quite so good, though the error is seldom 10 mg. per 100 cc. On clear fluids of a similar Cl-concentration the results are even better than for plasma.

The method may, like that of Van Slyke, be used also on tissues. In a study on mosquito larvae results of a series of analyses performed on about 50 mg. tissue (5–6 larvae) gave identical results.

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