CXXXI. A NEW IODIMETRIC PROCEDURE FOR THE ESTIMATION OF CHLORIDE IN SMALL AMOUNTS OF BLOOD.

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THE commoner methods for the estimation of blood chloride are modifications of the Volhard titration [*e.g.* Whitehorn, 1921; Van Slyke, 1923]. In the micro-modifications [Smirk, 1927; Patterson, 1928] the use of solvents such as alcohol or acetone is necessary to obtain even semi-permanent end-points. The possibility of chloride loss in the "open" Carius acid digestion of the blood prior to chloride estimation has frequently been pointed out [*vide* Norris and Ampt, 1933]. The iodimetric procedure of McLean and Van Slyke [1915] has been applied to Folin-Wu blood filtrates by Gettler [1921], Hanna [1928] and others, but, as Hanna remarks, is not readily adaptable to small amounts of blood. Use of adsorption indicators has been recommended [Saifer and Kornblum, 1935]. The above procedures are essentially refinements of macro-methods and are not "micro" in the same sense as the procedure to be described, where six atoms of analysable constituent (iodine) are available for titration for each chloride ion originally present.

The method here described is based on the reaction:

Silver iodate is added in ammoniacal solution to the chloride solutions, and with the silver chloride formed, is precipitated by excess of acid. After filtration, iodine is liberated from the soluble iodate formed and is titrated with 0.005*N* sodium thiosulphate. Van Slyke *et al.* [1927] estimated sulphate in a somewhat analogous manner by the addition of solid barium iodate to the sulphate solution, followed by gasometric determination of the liberated iodate.

The solubility of silver iodate in water is approximately 4 mg./100 ml. at 25°, while the figure for silver chloride is approximately 0.15 mg./100 ml. There is therefore a "blank" titration due to dissolved silver iodate.

This "blank" will depend on the equilibria:

Excess of soluble iodate, added or set free according to (i), will depress the "blank" due to dissolved silver iodate.

Chloride-free mixtures, containing silver iodate, were treated with amounts of potassium iodate, calculated from (i), corresponding to various concentrations of chloride. The "blanks" (*i.e.* the titration of the additional iodate found) were added to the "theoretical" titrations, also calculated from (i). The figures thus obtained agreed almost exactly with the experimentally found titrations with chloride solutions of corresponding concentrations. This fact shows that (i), for practical purposes, goes to completion (see Table I).

The reaction was studied over the range 0–80 mg. of sodium chloride per 100 ml. It was found that the titration of iodate set free from sodium chloride solutions of concentrations from 10 to 40 mg./100 ml. had to the concentration a relationship which could be expressed by an equation of the form $\text{NaCl (mg./100 ml.)} = a (\text{titration} - b)$. From 40 to 80 mg. NaCl/100 ml., the iodate liberated corresponded almost exactly with (i); *i.e.* the “blank” could be neglected and the chloride calculated directly from (i).

The method gave identical results with sodium, potassium, calcium and magnesium chlorides. For blood or plasma, it was found convenient to use the Somogyi [1930] zinc hydroxide method of protein removal. Sodium chloride added to such a filtrate, previously freed from chloride (with silver nitrate, followed by hydrogen sulphide) was quantitatively recovered. Good recovery of sodium chloride added to blood was also obtained. Analytical results on 0.2 ml. of whole blood or plasma agreed within 1% among themselves and with gravimetric analyses carried out on larger samples of the original blood or plasma.

EXPERIMENTAL.

Preparation of silver iodate. Solid silver iodate was prepared from silver nitrate (in very slight excess) and potassium iodate. 2 g. of the washed and dried solid were dissolved in 100 ml. *N* ammonia. Both solid silver iodate and its ammoniacal solution appear to decompose slightly on keeping with liberation of soluble iodate. Immediately before a series of determinations, therefore, 5 ml. of the “stock” 2% ammoniacal silver iodate were acidified with 2*N* sulphuric acid (5 ml.) and centrifuged. After removal of the supernatant liquid, the iodate was redissolved in fresh *N* ammonia (5 ml.). This “silver iodate reagent” lasted unchanged at least 1 day. It was not improved by preliminary washing of the precipitated silver iodate.

Technique of the analyses. The chloride solution (2 ml.) was treated with 1 ml. of the above silver iodate reagent and, after careful mixing, with 1 ml. of 2*N* sulphuric acid. The mixture was shaken and filtered through a small fine paper. 2 ml. of the filtrate were treated with potassium iodide (1 ml. of 1%) and the liberated iodine was titrated with 0.005*N* sodium thiosulphate, with starch as indicator. Such titrations, using 2 ml. of sodium chloride solutions of concentrations from 0 to 80 mg./100 ml., are shown in Table I, col. 4.

Table I.

Chloride concentration (mg. NaCl/100 ml.)	Theoretical titre	Silver iodate “blank”	Experimental titre
	ml. of 0.005 <i>N</i> sodium thiosulphate		
0	0	1.25	—
5	1.03	0.65	1.75
10	2.05	0.51	2.60
15	3.08	0.46	3.57
20	4.10	0.37	4.50
25	5.13	0.30	5.42
30	6.16	0.20	6.37
35	7.19	0.14	7.31
40	8.20	0.10	8.25
45	9.23	—	9.24
50	10.26	—	10.20
60	12.30	—	12.30
70	14.35	—	14.34
80	16.41	—	16.30

Determination of silver iodate "blanks". The quantities of iodate, as 0.1 *N* potassium iodate, which should be set free according to (i) from the different chloride solutions used, were made up with water to 2 ml. The resulting solution, in each case, was treated exactly as the chloride solution in the above procedure. The experiment was then repeated, but with addition of *N* ammonia (1 ml.) instead of the silver iodate reagent. The difference between the titration figures gave the silver iodate "blank" for the particular chloride concentration. The "blanks" are shown in Table I, col. 3.

Calculation of results. In Fig. 1 the concentration of sodium chloride has been plotted against the "theoretical" and experimental titrations of 0.005 *N* sodium thiosulphate. Above 40 mg. NaCl/100 ml. the two lines are identical; below 40 mg./100 ml. the experimental curve deviates somewhat from the theoretical, but between 15 and 40 mg. (curve B) it is practically a straight line. The equation of curve B is

$$\text{NaCl (mg./100 ml.)} = 5.275 (\text{titre} - 0.65),$$

and that of the theoretical curve, A, is

$$\text{NaCl (mg./100 ml.)} = 4.875 \times \text{titre}.$$

Hence, at the point of junction, *P*,

$$5.275 (\text{titre} - 0.65) = 4.875 \times \text{titre}.$$

Hence

$$\text{titre} = 8.575 \text{ ml.}$$

And

$$\text{NaCl} = 41.8 \text{ mg./100 ml.}$$

From 15 to 40 mg./100 ml., therefore, the formula corresponding to curve B is applicable, while from 40 to 80 mg./100 ml. the equation of curve A is used.

Fig. 1.

Application to blood. 2 ml. of a Somogyi zinc hydroxide filtrate (1 in 10 dilution) are used exactly as in the case of the chloride solutions above.

On the small scale the method is as follows: 0.2 ml. of whole blood (or plasma) is pipetted into 1.4 ml. of water (1.0 ml. for plasma). 0.2 ml. of 10% zinc sulphate and 0.2 ml. of 0.5*N* NaOH (0.4 ml. of each reagent for plasma) are added and thoroughly mixed. The mixtures are then centrifuged. 1.0 ml. of the supernatant liquid ($\equiv$ 0.1 ml. of blood or plasma) is treated with silver iodate reagent (0.5 ml.) and, after mixing, with 2*N* sulphuric acid (0.5 ml.). The mixture is shaken and filtered through a small fine paper. 1.0 ml. of filtrate ($\equiv$ 0.05 ml. of blood or plasma) with the addition of potassium iodide (1.0 ml. of 1%), is titrated with 0.005*N* sodium thiosulphate with starch as indicator.

Calculation of results. Chloride (mg. NaCl/100 ml. of blood or plasma = $97.5 \times \text{titre}$).

If the titre is less than $8.575/2$, i.e. 4.30 ml., curve B must be used, and the calculation becomes: chloride (mg. NaCl/100 ml. of blood or plasma = $105.5 (\text{titre} - 0.65)$).

Comparison of results. NaCl (mg./100 ml.); plasma A: gravimetric, 675; new method, 672. Plasma B: gravimetric, 612, 614; new method, 614. Blood B: gravimetric, 422, 414; new method, 423.

SUMMARY.

A new method for the estimation of chloride is described, depending on the liberation of soluble iodate from silver iodate added to the chloride-containing liquid. On addition of potassium iodide, six atoms of iodine are liberated for each chloride ion originally present. The determination of the iodine makes possible the accurate estimation of chloride in small amounts of blood or plasma.

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