LXXII. THE DETERMINATION OF CHLORIDE IN BIOLOGICAL FLUIDS.

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(Received February 4th, 1928.)

BIOCHEMICAL methods for estimating chlorides in present day use are, with the exception of those of Bang [1913] and McLean and Van Slyke [1915], all modifications of the Volhard technique.

McLean and Van Slyke state that the least possible quantity of halide that can be estimated accurately by Volhard's method is 0.5 millimol., and, in order that they might estimate smaller quantities than this, they evolved an iodometric method. According to this technique potassium iodide is used to titrate the excess of silver nitrate, the end-point being determined by a sodium nitrite-starch indicator functioning in an acid solution buffered by the addition of trisodium citrate.

From the point of view of the sharpness of the end-point thus obtained, the method was a decided advance. Nevertheless it has two inherent disadvantages. The nitrous acid-starch-iodine indicator functions correctly only within a very limited range of p_H and if, by any chance, the amount of citrate added does not reduce the acidity of the solution within that range, the true colour associated with the iodine-starch combination is not produced. Moreover an appreciable amount of potassium iodide in excess of the theoretical requirement is necessary for the development of colour at the end-point.

One of us (W.R.), recently collaborating with Dr Van Slyke, substituted other buffers for trisodium citrate in an attempt to widen the range of p_H within which the blue colour of the indicator is produced. This, however, did not give the desired increase in accuracy.

The essential chemical reaction involved at the end-point in the method of McLean and Van Slyke is the liberation of iodine by nitrous acid from the excess of hydrogen iodide present in the solution. A similar liberation of iodine takes place when potassium iodate and potassium iodide react in acid solution, and this was utilised by Bang [1913] in his method of estimating halides. On applying this reaction in the presence of starch to the titration of the excess of silver nitrate (in acid solution) with potassium iodide it was found that an appreciable excess of the last named reagent was again necessary for the development of the blue colour. Replacement, however, of the potassium iodate by potassium bi-iodate, $KH(IO_3)_2$, leads to the

instantaneous production of colour once the slightest excess of iodide is present in the solution. The equation for the reaction involved, as given by Friend [1924], is $\text{KIO}_3 \cdot \text{HIO}_3 + 10\text{KI} + 11\text{HNO}_3 = 11\text{KNO}_3 + 12\text{I} + 6\text{H}_2\text{O}$.

Experimental results show that this reaction can take place, apparently with equal speed, over a wide range of hydrogen ion concentration (*vide* Table I). The buffering of solutions containing that amount of nitric acid usually added for the precipitation of halide is therefore unnecessary. Thus both the disadvantages of the original method of McLean and Van Slyke are met.

By the proposed method so small a quantity as 2.5 mg. of sodium chloride has been accurately estimated, and the combined sensitivity and sharpness of the end-point make it likely that even smaller quantities than this may be estimated if use were made of the micro-titration technique of Rehberg [1925].

METHOD.

Materials required.

The reagents are *N*/29.25 solutions of silver nitrate and potassium iodide, 1 cc. of either of which is equivalent to 2 mg. of sodium chloride; concentrated nitric acid free from chloride; a *N*/100 solution of potassium bi-iodate in distilled water; and a 1 % solution of soluble starch. The last solution should not be kept longer than a fortnight.

Experimental procedure.

In the analysis of fluids containing protein the proposed method consists of three steps: (1) removal of protein, (2) precipitation of chloride, and (3) final titration. For fluids containing no protein only the last two steps are necessary.

(1) *Removal of protein.* One of the several advantages of the proposed method is its applicability to any of the ordinary protein-free blood filtrates in present-day general use. In the present investigation both the Folin and Wu [1919] filtrate (acid precipitant), and the Hagedorn and Jensen [1923] filtrate (alkaline precipitant), have been employed with success.

(2) *Precipitation of chloride.* To every 20 cc. of solution (diluted urine or blood-filtrate), 0.5 to 1.0 cc. of nitric acid is added, and the chloride is precipitated by excess of the standard silver nitrate solution. Complete flocculation of the silver chloride requires a few minutes, after which the mixture is made up to a definite volume and filtered. Filtration is essential in order to prevent the occurrence of the reaction $\text{AgCl} + \text{KI} = \text{AgI} + \text{KCl}$ during the next step.

(3) *Final titration.* To an aliquot portion of the filtrate from the previous stage is added 1 cc. of each of the solutions of potassium bi-iodate and soluble starch, and the standard potassium iodide is finally run in from a burette. The blue end-point is very sharp.

EXPERIMENTAL RESULTS.

1. *The effect of p_H on the development of the blue colour at the end-point.*

Table I.

Amount of NaCl present = 0.103 g. %		
Amount of 50 % KAc added to the HNO ₃ solution (cc.)	Approximate p_H attained	NaCl found (g. %)
0	2.0	0.102 0.103 0.103
0.5	3.0	0.103 0.103 0.103
1.0	4.0	0.103 0.103 0.103
2.0	5.0	No blue colour developed

Table II shows the results obtained by three methods, the authors' method, that of Whitehorn [1920] and that of Van Slyke [1923].

Table II.

Method	Sodium chloride present = 0.103 g. %			
	NaCl found (g. %)			
Authors	0.103	0.103	0.102	0.103
Whitehorn	0.106	0.107	0.106	0.106
Van Slyke	0.100	0.099	0.100	0.098

2. *Analysis of urine and recovery of NaCl added to urine.*

Table III shows the results of a series of analyses on a sample of urine to which sodium chloride in known amounts was added.

Table III.

NaCl added to 100 cc. urine (g.)	NaCl found (g. %)	Recovery (%)
0.0	1.10	—
0.1	1.200 (3)	100.3
0.15	1.251 (2)	100.6
0.20	1.301 (0)	100.5

3. *Analysis of blood.*

Table IV shows the results obtained by different methods on whole blood. The authors' method was carried out on two different blood-filtrates. The results (i) were obtained on the Hagedorn and Jensen filtrate, and results (ii) on the Folin and Wu filtrate. In each case the amount of filtrate used was equivalent to 0.5 cc. of blood, while in the other methods 1 cc. of blood was used.

Table IV.

Method	NaCl found (g. %)		
	0.455	0.455	0.456
Authors (i)	0.455	0.456	0.456
Authors (ii)	0.483	0.482	0.480
Van Slyke	0.421	0.421	0.421
Whitehorn			

Table V shows the recovery of NaCl, added to whole blood, by the methods applied above.

Table V.

Sodium chloride added g.	Sodium chloride recovered by the method of			
	Whitehorn	Van Slyke	Authors (i)	Authors (ii)
0.026	0.027	0.024	0.026	0.026
0.053	0.056	0.050	0.054	0.053
0.105	0.111	0.099	0.106	0.104
0.210	0.222	0.198	0.212	0.211
0.315	0.322	0.297	0.313	0.316
0.420	0.443	0.396	0.422	0.422

From the results tabulated above it will be seen that the error involved in the recovery of sodium chloride added to urine or to blood was at no time greater than 1 % and in most cases was less than this.

SUMMARY.

A new iodometric method of estimating chlorides in biological fluids is described. It is applicable to all cases in which the Volhard technique is used, and especially to blood-filtrates, *e.g.* those of Folin and Wu or Hagedorn and Jensen. The error involved in the method is less than 1 %.

One of us (W.R.) desires to acknowledge his indebtedness to the Medical Research Council for a part-time grant.

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