XCIV. THE DETERMINATION OF CHLORIDES IN BODY FLUIDS.

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IN a recent paper [1928] the author showed that the estimation of chlorides in gastric contents could be accomplished by means of a single titration of the Volhard type, without previous removal of protein. The effect of the latter could readily be overcome by introducing a very large proportion of nitric acid, well above that recommended by Whitehorn [1920], for stabilising the end-point of the usual titration.

In continuation of this work, a further step was to ascertain how far the same principle could be applied to other body fluids. Investigation showed that it was capable of general application to all body fluids with the exception of whole blood and corpuscles. The limitation in this case arose from the fact that while the other fluids give with nitric acid a light yellow solution in which the end-point of the Volhard titration can be readily observed, whole blood yields a very dark brown solution which would completely obscure the end-point. A simple decoloration process is therefore a necessary preliminary in this special case.

The problem resolved itself into finding the means of annulling the effect of protein in plasma, for in such a medium that effect would tend to be greatest on account of the high protein concentration, and whatever conditions applied to plasma would almost certainly embrace fluids of lower protein content.

It was found that very slight alteration of the conditions which had been successfully used for gastric contents would in fact achieve the desired end, yielding a new process which reduces the time required for a single estimation to less than half that required even for the neat and rapid method of Smirk [1927]. The present method as finally evolved resembles the latter method in employing a large amount of nitric acid, and follows it in taking advantage of the stabilising influence of acetone upon the end-point. On the other hand, it is wholly dissimilar in principle, in that the essence of the Smirk technique is the vigorous oxidation by ammonium persulphate to destroy protein, a process which in the present method is rendered unnecessary by the choice of appropriate concentration and sequence of reagents.

The end-point is quite sharp and stable. The titration is performed in an almost clear medium, for the silver chloride is largely protected from precipitation by the acid protein, which would also seem to have the effect of stabilising the end-point. Thus, while in a protein-free medium it is desirable to aggregate the silver chloride particles before titration, no disadvantage arises under the present conditions from the fact that the silver chloride is not rendered compact.

For whole blood, decoloration of the solution in nitric acid in order to provide a suitable medium for titration can be readily accomplished in a number of ways, *e.g.* by perhydrol, permanganate etc., but these offer no advantage over the Smirk method of oxidation, and therefore the original technique of this author, with slight variations, has been adopted for this fluid.

Of other biological fluids, attention is drawn to milk, in view of the conclusion but lately drawn by Husband and Godden [1927] expressed thus: "To determine accurately the chloride content of milk volumetrically by precipitation of the chloride as silver chloride, it is essential that the protein be removed before the addition of silver nitrate." The present results show that the conclusion of these authors can only be held to apply to similar conditions of precipitation to those employed in the particular method under their criticism, namely that of Sisson and Dennis [1921].

METHODS.

Plasma or serum. Take up 0.1 cc. of plasma or serum in a blood-pipette¹ and discharge into 1 cc. of distilled water contained in a dry boiling-tube or wide test-tube, washing out the pipette by drawing up the water in the usual manner. Add 3 cc. of pure concentrated nitric acid measured from a graduated cylinder of 5 or 10 cc. total capacity, shaking until the protein re-dissolves completely, followed by 0.5 cc. of standard silver nitrate (containing 5.812 g. per litre) delivered from an Ostwald pipette, shaking again during this last addition. Add now 3 to 4 cc. of pure acetone, mix and cool thoroughly. After adding 6 drops (0.3 cc.) of saturated iron alum solution as indicator, titrate from a microburette¹ with alcoholic ammonium thiocyanate of approximately half the strength of the silver nitrate and standardised against the latter. (A convenient solution is made by diluting ordinary aqueous *N*/20 thiocyanate with the required amount—about 2 volumes—of absolute alcohol.) The end-point is reached when one drop imposes a brownish-red tint upon the pale yellow of the original mixture.

Until one has gained complete familiarity with the end-point, it is of great assistance when performing a single estimation to prepare a control tube using the same reagents with 0.1 cc. of any specimen of serum or plasma that is available, except that no thiocyanate is added. When this control is held alongside the tube containing the mixture being titrated, the first departure from the true yellow is readily observed for the marking of the

¹ These pipettes and the microburette must be very accurately calibrated.

end-point. Daylight from a large window immediately behind the operator provides the best conditions of illumination, while the tubes should have white paper immediately behind and below. Table I furnishes some typical comparative results.

Table I.

	Smirk's method	Whitehorn's method (mg. NaCl per 100 cc.)	Present method
A. Plasma: 1.	610	610	609
	612	—	613
2.	585	—	588
	585	—	586
3. After a short dialysis	375	—	378
	380	—	374
B. Serum:	560	—	565
	560	—	560

Whole blood. Save that the same sequence and relative quantities of reagents were preserved as in the application of the present method to plasma, and that no definite weighed quantity of persulphate was used for oxidation, but only a rough knife-point measure, the method applied was essentially that of Smirk.

Puncture fluids such as exudates, transudates, and cyst contents are treated exactly as plasma.

Cerebrospinal fluid (pathological specimens containing excess protein). Take 0.2 cc. of fluid and proceed as for plasma except that 1 cc. of silver nitrate is used.

Milk. Proceed exactly as for plasma, using 0.2 cc. of milk. Typical figures comparing the results so obtained with the method of Austin and Van Slyke [1920] and an adaptation of Smirk's method, are given in Table II.

Table II.

Cow's milk	Austin and Van Slyke's method	Ammonium persulphate oxidation method (mg. NaCl per 100 cc.)	Present method
1	170	168	174
	—	169	170
2	187	187	189
	—	182	186

Urine (pathological specimens with high protein content). Use either 0.1 cc. or 0.2 cc. of urine with 0.5 cc. of *N*/10 silver nitrate and titrate with *N*/10 alcoholic thiocyanate.

SUMMARY.

1. It is shown that the vitiating effect of the presence of protein on the estimation of chloride by the Volhard titration can be readily overcome by use of a high concentration of nitric acid, together with selected proportions and sequence of the reagents normally employed.

2. Use is made of this fact to devise simple and ready methods of estimating the chloride content of various body fluids without the previous removal of protein.

3. The method is not directly applicable to those fluids which, like whole blood, give rise to a dark solution in nitric acid.

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