SOME SOURCES OF ERROR IN THE DETERMINATION OF CHLORIDES IN BLOOD AND SIMILAR MATERIAL.*

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In the course of work in the analysis of blood, the authors have employed a number of reagents for the removal of the proteins before proceeding to the determination of other constituents. Two of these, picric acid (1) and nitric acid (2), appeared to offer peculiar advantages in the subsequent determination of chlorides and other inorganic constituents. However, it was found that the amount of silver chloride that was obtained from volumes of filtrates corresponding to the same amounts of blood was much less when nitric acid was used as the precipitant than when picric acid was so employed. Moreover, the silver precipitate obtained with the latter was always yellow and it retained this color even after prolonged washing with hot water. The color could be removed by washing with concentrated nitric acid but the weight of the residual silver chloride was greater than that of the precipitate obtained from a nitric acid filtrate corresponding to the same amount of blood.

The yellow silver chloride precipitate was not completely soluble in ammonia but left a gelatinous precipitate, suggesting the presence of a purine. The precipitate was filtered out, washed, and suspended in dilute hydrochloric acid. After heating for 30 minutes, the mixture was filtered, the filtrate was almost neutralized and was then treated with sodium acetate, sodium bisulfite, and copper sulfate in the usual manner. The precipitate obtained was filtered out, washed with hot water, and decomposed with hydrogen sulfide. The filtrate from the copper sulfide was evaporated and treated with a little picric acid. On cooling, a lemon-

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yellow picrate crystallized. This was at first thought to be hypoxanthine picrate but later experiments have shown that it was probably a mixture of hypoxanthine and adenine picrates. Apparently, the yellow color of the original silver chloride precipitate was due to the presence of purine-silver picrates. These have been described by Bruhns (3) who ascribed to them the formula (Purine-H)AgC₈H₅(NO₂)₃OH. Attempts in this laboratory to prepare the hypoxanthine compound by adding silver nitrate to hypoxanthine picrate solutions or picric acid to a solution of hypoxanthine silver nitrate and excess silver nitrate have always yielded precipitates containing less hypoxanthine and more silver than this formula requires.

The ammoniacal filtrate from the purine-silver precipitate was acidified with nitric acid and the reprecipitated silver chloride was filtered on a Gooch crucible and weighed. The amount found was greater than that obtained from a nitric acid filtrate from a corresponding amount of blood. Evidently, nitric acid failed to extract all of the chloride from blood.

It seemed to be of interest to investigate other protein precipitants that have been recommended for use in the determination of chlorides in blood. Accordingly, samples of oxalated blood were treated as called for in the description of the methods. However, since it was desired not to have the results complicated by variations in the character of the end-points used in the several titrations, all the determinations discussed in this paper were made gravimetrically, either by weighing the silver chloride precipitated by the addition of silver nitrate (and nitric acid) to the protein-free filtrate or, where this was, for one reason or another, undesirable, by using a known excess of silver nitrate, removing the precipitate, and then determining the excess of silver in the filtrate by precipitation with hydrochloric acid. In many cases both methods were used. In almost every instance, filtrate equivalent to 10 cc. of blood was used. In a few cases, the equivalent of 5 cc. of blood was employed.

The results obtained are summarized in Table I. The first column of figures gives the values obtained by digestion of the blood with nitric acid and an excess of silver nitrate until all the organic matter had been dissolved. It was not necessary to use potassium permanganate (4). These are regarded as probably the most
TABLE I.
Comparison of Chloride Determinations in Protein-Free Filtrates Obtained by Different Methods.*

<table>
<thead>
<tr>
<th>Method</th>
<th>Oxidation of whole blood with HNO₃</th>
<th>Picric acid.</th>
<th>Nitric acid.</th>
<th>m-Phosphoric acid.</th>
<th>Trichloroacetic acid.</th>
<th>Copper, CuSO₄ + Ca(OH)₂</th>
<th>Methyl alcohol, Rieger</th>
<th>Tungstate acid, Whitehorn</th>
<th>Mixture of picric and nitric acids.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep blood.</td>
<td>Direct.</td>
<td>313.2</td>
<td>328.0</td>
<td>310.2</td>
<td>287.0</td>
<td>310.4</td>
<td>304.6</td>
<td>304.6</td>
<td>309.1</td>
</tr>
<tr>
<td></td>
<td>Excess AgNO₃.</td>
<td>323.1</td>
<td>310.2</td>
<td>287.0</td>
<td>328.5</td>
<td>304.6</td>
<td>309.1</td>
<td>313.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Direct.</td>
<td>333.0</td>
<td>341.0</td>
<td>338.6</td>
<td>325.4</td>
<td>322.1</td>
<td>327.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Excess AgNO₃.</td>
<td>330.2</td>
<td>337.2</td>
<td>338.2</td>
<td>327.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calf blood.</td>
<td>Direct.</td>
<td>303.6</td>
<td>324.0</td>
<td>314.3</td>
<td>286.5</td>
<td>308.3</td>
<td>304.6</td>
<td>319.1</td>
<td>317.8</td>
</tr>
<tr>
<td></td>
<td>Excess AgNO₃.</td>
<td>303.3</td>
<td>316.3</td>
<td>313.0</td>
<td>290.0</td>
<td>300.8</td>
<td>304.6</td>
<td>319.1</td>
<td>304.0</td>
</tr>
<tr>
<td>Human blood.</td>
<td>Direct</td>
<td>320.3</td>
<td>334.2</td>
<td>320.3</td>
<td>303.3</td>
<td>323.3</td>
<td>315.8</td>
<td>317.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Excess AgNO₃.</td>
<td>319.9</td>
<td>325.2</td>
<td>320.5</td>
<td>303.1</td>
<td>323.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Direct</td>
<td>320.0</td>
<td>337.0</td>
<td>331.0</td>
<td>324.6</td>
<td>306.0</td>
<td>312.4</td>
<td>307.4</td>
<td>310.6</td>
</tr>
<tr>
<td></td>
<td>Excess AgNO₃.</td>
<td>321.3</td>
<td>326.7</td>
<td>326.0</td>
<td>324.4</td>
<td>306.0</td>
<td>312.4</td>
<td>307.4</td>
<td>310.6</td>
</tr>
</tbody>
</table>

* Mg. chlorine per 100 cc. blood.
accurate values and all others have been compared with them. The three following columns give the results by the three methods in which picric acid is used as the protein precipitant. As might be expected from what has been said about the occurrence of a purine-silver picrate, the results obtained by the technique of Myers and Short (5) are too high, the average error being 3 per cent. Even if 5 per cent nitric acid be present, as in the method of Austin and Van Slyke (6), the error is about the same. This 3 per cent error is found if the amount of excess silver in the filtrate from the blood chlorides is determined and used as a basis for the calculations. If these are based on the weight of the first silver chloride precipitate, the error is even greater because of the high molecular weight of the purine-silver picrate. If the precipitate is washed with concentrated nitric acid, the agreement is better, the maximum error being 2 per cent and the average less than 0.5 per cent.

If the proteins are precipitated with nitric acid and the chlorides are determined in the filtrate, the results are always too low by from 5 to 8 per cent. They are as much too high if the nitric acid and silver nitrate are combined in one solution as in the methods of Gazzetti (7) and of Rappleye (8). Evidently, there is something other than chloride in blood (not necessarily in plasma or serum, for which these methods were described) which precipitates silver from a nitric acid solution.

The filtrates from the m-phosphoric acid precipitation of blood yield on direct precipitation, very irregular results, which are all too high, probably because the precipitates are contaminated with protein. Calculation from the amount of excess silver in the filtrates gives values that are too low, the error being somewhat less with Oppler’s method (9), in which an excess of m-phosphoric acid is avoided than with Foster’s method (10).

The silver chloride precipitated in the filtrates, obtained by precipitating blood with 5 per cent trichloroacetic acid (11, 12, 13), even when alcohol was used as described by Smith, cannot be filtered, even after standing over night. It can, however, be removed with the aid of a centrifuge within a few minutes. An aliquot of the supernatant liquid is used for a determination of the excess silver. The values thus obtained are from 3 per cent too low to 1.7 per cent too high.
The use of copper sulfate and sodium and calcium hydroxides, as employed by Wetmore (14), gives results that are too low by from 0.3 to 5 per cent, the average error being about 3 per cent. The technique of Harding and Mason (15) is probably similarly at fault.

Direct gravimetric determination of the chloride in the filtrate from the precipitation of blood with methyl alcohol and magnesium sulfate, as described by Richter-Quittner (16) is impossible because the precipitate contains much foreign material. Calculations based upon determinations of the excess silver in the filtrate give irregular results, 5 or 6 per cent too high or too low.

The filtrate from the Folin-Wu tungstic acid precipitation, when treated with nitric acid and silver nitrate, gives a precipitate consisting of silver chloride and tungstic acid (17, 18). If this is filtered out and the amount of chloride is calculated from a determination of the excess silver in the filtrate, the values obtained agree very well with those obtained by digestion of the whole blood with nitric acid and silver nitrate. Apparently, the Folin-Wu reagent is, of all those studied, the most suitable for the subsequent determination of chlorides, and, unless there is some difficulty with the subsequent titration, which appears not to be the case, the method described by Whitehorn (18) would seem to be the most desirable for routine determinations.

The high values obtained in the picric acid methods are due to the formation of a purine-silver picrate. But to what may the low values obtained with the other protein precipitants be ascribed?

It may be assumed that they are due to the presence, in the blood, of some organic chlorine compound which was precipitated by some of the reagents, or which decomposed to give chlor-ion in some of the procedures, but not in others. But it is a reagent of low acidity, tungstic acid, that gives values most nearly identical with those obtained by digestion of the whole blood with nitric acid and silver nitrate.

The slightly lower values obtained by Wetmore's method are probably due to the formation of a small amount of basic copper chloride.

The other protein precipitants are all acids and precipitation occurs only well on the acid side of the isoelectric points of the
blood proteins. The precipitate obtained is a compound of protein and acid. It has been more or less tacitly assumed that, in these reactions, the precipitating acid completely displaces all other acids from combination with the protein. But this seems not to be the case with nitric and $m$-phosphoric acids, and probably not with trichloroacetic acid.

It was at first thought that the amount of chlorine retained in the protein precipitate is a function of the hydrogen ion concentration of the mixture but, in two experiments, it was found that the filtrate obtained by precipitating blood with a mixture containing 5 per cent nitric acid and 0.6 per cent picric acid gave values that agreed well with those obtained by precipitation with picric acid alone or by oxidation of the whole blood with nitric acid and that were considerably higher than those obtained by the use of picric acid alone. Evidently, some protein precipitants do completely displace the chlorine from combination with the protein, in spite of a high hydrogen ion concentration, whereas others do not.

BIBLIOGRAPHY.

CORRECTION.

On page 594, Vol. LIV, No. 3, November, 1922, line 15, for picric acid read nitric acid.
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