A SYSTEM OF BLOOD ANALYSIS.*

SUPPLEMENT II.

SIMPLIFIED METHOD FOR THE DETERMINATION OF CHLORIDES IN BLOOD OR PLASMA.

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The method of preparing protein-free filtrates by the use of tungstic acid, as developed and used by Folin, has proved so highly satisfactory in the determination of a number of blood constituents, that it has seemed desirable to apply it to the determination of chlorides.

The procedure developed for this purpose is based upon the principle of the Volhard method,1 namely, the precipitation of silver chloride from a known amount of silver nitrate and titration of the excess silver nitrate by means of sulfocyanate, using ferric ammonium alum as an indicator.

A number of preliminary experiments were conducted to ascertain the most convenient conditions which would give a good end-point. Efforts to obtain increased accuracy by greatly increased dilution of reagents were disappointing. In order to insure a sharp end-point, the volume of fluid at the end of titration must be kept small, which cannot be done when very dilute solutions are used. As a result of dilution the end-point tends to be yellowish, rather than red, and cannot be accurately perceived, at least by the writer’s eye, except in strong daylight. This difficulty in the use of very dilute solutions is somewhat increased by the presence of oxalate, because of the lemon-yellow color of

* By agreement between Mr. Whitehorn and myself, this paper is published as Supplement II of the "System of blood analysis" devised by Folin and Wu.—Professor Otto Folin.

iron oxalate. It was presumably for this reason that Rappleye’s method for plasma chlorides required the use of citrated rather than oxalated plasma. The personal element in the reading of such faint end-points is perhaps rather large, for Myers and Short have very recently published fairly accurate results obtained on picric acid filtrates by a modified Volhard-Arnold method in which they used ammonium thiocyanate of only $\text{M}/117$ strength. The writer, however, has found it more satisfactory to use a thiocyanate solution of $\text{M}/35.5$ strength, of which 0.03 cc. gives, under the conditions specified, an unmistakable end-point.

For reasons which will appear below, it was necessary to avoid the customary procedure of filtration or centrifugalization of the silver precipitate. This simplification has been accomplished without loss of accuracy by the liberal use of nitric acid and ferric alum. Accurate results were obtained on known chloride solutions, with or without the addition of small amounts of tungstic acid.

The following method was thereupon developed.

I. Preparation of Protein-Free Filtrates.—The filtrate is prepared by the use of the same reagents as have been described in detail by Folin and Wu for the determination of non-protein nitrogen, urea, uric acid, creatinine, creatine, and sugar. Because even slight variations in the chlorides are significant, great accuracy is necessary. The writer customarily uses volumetric flasks in order to insure an accurate 1:10 dilution. The method is applicable, without alteration, to either whole blood or plasma. An amount of filtrate equivalent to 1 cc. of blood or plasma is needed. Less may be used but with proportionate loss of accuracy.

II. Determination of Chloride Content of Filtrate.—(a) Reagents Required.—1. Silver nitrate solution ($\text{M}/35.46$).
2. Potassium (or ammonium) sulfocyanate ($\text{M}/35.46$).
3. Powdered ferric ammonium sulfate ($\text{FeNH}_4(\text{SO}_4)_2$).
4. Concentrated nitric acid ($\text{HNO}_3$ of specific gravity 1.42).

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(b) Procedure.—Pipette 10 cc. of the protein-free filtrate into a porcelain dish. Add with a pipette 5 cc. of the standard silver nitrate solution and stir thoroughly. Add about 5 cc. of concentrated nitric acid, mix, and let stand for 5 minutes, to permit the flocking out of the silver chloride. Then add with a spatula an abundant amount of ferric ammonium sulfate (about 0.3 gm.) and titrate the excess of silver nitrate with the standard sulfocyanate solution until the definite salmon-red (not yellow) color of the ferric sulfocyanate persists in spite of stirring for at least 15 seconds.

(c) Calculation.—

\[ 5.00 - \text{titer (in cc.)} = \text{mg. of Cl per cc. of blood (or plasma)} \]

Since each cc. of thiocyanate solution used is equivalent to 1 cc. of silver nitrate solution, the difference between the volume of silver nitrate solution taken and the excess determined by the titration, that is 5—titer, represents the volume which reacted with chloride at the ratio of 1 cc. to 1 mg. of Cl. And the 10 cc. of filtrate taken represents 1 cc. of blood (or plasma).

To convert Cl figures into NaCl figures divide by 0.606. The same result may be more easily obtained by the following rule: To obtain mg. NaCl per 100 cc., divide mg. Cl per liter by 6, and subtract 0.001 of the result. Conversely, to obtain mg. Cl per liter, add to mg. NaCl per 100 cc. 0.001 of itself and multiply by 6.

The examples in Table I illustrate the principles involved in the calculation.

<table>
<thead>
<tr>
<th>Specimen.</th>
<th>Titer</th>
<th>Cl per cc.</th>
<th>NaCl per 100 cc.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cc.</td>
<td>mg.</td>
<td>By simple rule.</td>
</tr>
<tr>
<td>Filtrate A...</td>
<td>0.75</td>
<td>(5 - 0.75) = 4.25</td>
<td>( \frac{1}{2}(4,250) - 7 = 701 )</td>
</tr>
<tr>
<td>&quot; B...</td>
<td>2.25</td>
<td>(5 - 2.25) = 2.75</td>
<td>( \frac{1}{2}(2,750) - 5 = 453 )</td>
</tr>
<tr>
<td>&quot; C...</td>
<td>1.35</td>
<td>(5 - 1.35) = 3.65</td>
<td>( \frac{1}{2}(3,650) - 6 - 602 )</td>
</tr>
</tbody>
</table>

(d) Preparation of Reagents.—Dissolve 4.791 gm. of c.p. silver nitrate in distilled water. Transfer this solution to a liter volumetric flask and make up to the mark with distilled water. Mix
thoroughly and preserve in a brown bottle. 1 cc. = 1 mg. Cl. 
(It is to be noted that the silver nitrate and nitric acid are not 
added to the protein-free filtrate simultaneously. To do so may 
result in the mechanical enclosure of silver nitrate solution within 
the curds, and a consequent error in the positive direction.)

Because sulfocyanates are hygroscopic, the standard solution 
should be prepared volumetrically. As an approximation about 
3 gm. of KCNS or 2.5 gm. of NH₄CNS should be dissolved in a 
liter of water. By titration under the conditions specified under 
"Procedure" and by proper dilution prepare a standard such 
that 5 cc. are equivalent to 5 cc. of the silver nitrate solution.

The solid ferric alum is used rather than a solution, in order to 
insure a very high concentration in the mixture to be titrated. 
It is powdered in order to facilitate its solution.

Remarks.

1. Essentially the same procedure and reagents may be used 
in determining urine chlorides, except for the silver nitrate solu-
tion, which should be of M/7.092 strength. When 5 cc. of urine 
and 5 cc. of this strong silver solution are used in urine chloride 
determination the calculation becomes 5 - $\frac{\text{titer}}{5}$ = mg. Cl per cc. 
of urine.

2. When a determination of both urea and chlorides is desired 
on a small sample, as may sometimes occur in cases where nephritis 
is suspected, one may pipette 2 cc. of the plasma into a 25 cc. 
flask, dilute to about 20 cc. with water, add 2 cc. each of the tung-
state and acid solutions, make up to the mark with water, and 
shake. This will give sufficient filtrate for both determinations, 
5 cc. for urea by Folin's distillation method and 10 cc. for the 
chlorides by the method described. In either case the figure 
obtained must be multiplied by $\frac{5}{4}$, since the filtrate has been 

3. The glassware should be checked to within at least 0.5 per 
cent.

4. Reagents must be halogen-free. Some samples of nitric 
acid contain much chloride. None of the samples of tungstate 
tested has contained chloride. To guard against the possibility
of contamination, however, all samples should be tested as follows before using: Mix one volume of 10 per cent sodium tungstate solution with two volumes of concentrated, chloride-free nitric acid, and filter into a test-tube containing silver nitrate solution. Turbidity indicates contamination with halogen.

5. At Dr. Folin's suggestion, purification of tungstates containing added chlorides has been accomplished by recrystallization with alcohol. Sodium tungstate containing 0.3 per cent NaCl was so nearly purified by one recrystallization that the above mentioned silver test gave only a faint opalescence, scarcely perceptible even by transmitted light—not enough to produce an appreciable error. After a second recrystallization no chloride whatever could be detected. The following procedure was used: To a cooled 50 per cent solution of the contaminated tungstate, prepared with the aid of heat, add slowly an equal volume of 95 per cent ethyl alcohol and let stand for 10 minutes. Pour the suspension of crystals on a Buchner funnel, wash twice with alcohol, and dry.

6. The writer's attention has very recently been called to the method published by Rieger for chloride estimation on tungstic acid filtrates.5 Certain features of this article deserve comment.

(a) The method therein described retains the centrifugation or filtration procedure, which at times causes erroneously high results on tungstic acid filtrates, whether or not there is sufficient tungstate present to give a precipitate on the addition of an equal volume of concentrated nitric acid. Incidentally, the absence of such a precipitate does not indicate the absence of tungstate, as Rieger has stated, for solutions containing as much as 25 mg. of sodium tungstate per 100 cc. may not give a precipitate with nitric acid except when heated.

(b) The article mentioned contains a method for the "purification of sodium tungstate;" i.e., for the preparation of chloride-free tungstate. It is very difficult to believe that the method so designated really accomplishes its purpose, since it calls for the use of 7 cc. of 40 per cent sodium hydroxide solution for each 10 gm. of sodium tungstate taken. Preparations of sodium hydroxide always contain large amounts of chloride. On the other

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hand, none of the samples of sodium tungstate tested by the writer has contained perceptible amounts of chloride. It has therefore not been necessary to purify. Certainly "purification" should not be attempted by the use of sodium hydroxide.

(c) Instead of precipitating the plasma proteins before dilution it is preferable to dilute the plasma with distilled water before adding the acid, in order to insure an even distribution of chlorides between solution and precipitate.

(d) It is not necessary to wait an hour for protein precipitation. 5 minutes are sufficient. Time of standing before filtering off the precipitated protein causes no appreciable difference in the chloride determination, as shown by the following results on the same plasma: Stood for 5 minutes before filtering, 3.64 mg. Cl per cc.; 15 minutes, 3.68; 30 minutes, 3.68; 1 hour, 3.64; and 3½ hours, 3.70.

Soundness of the Method.

The soundness of the method of chloride determination described obviously depends upon the answers to two questions:

I. Does the titration figure indicate accurately the excess of silver nitrate?

II. Does the amount of silver precipitation so found indicate accurately the chlorides of the plasma?

I. The first of these questions deserves careful attention. Many chemists will quite properly be very skeptical on this point. Sutton states 8 "In cases where chlorine is precipitated by excess of silver, and the excess has to be found by thiocyanate, experience has proved that it is absolutely necessary to filter off the chloride and titrate the filtrate and washings." Rosanoff and Hill 7 have shown that the error is due to the reaction of silver chloride with sulfocyanate. Their figures would indicate that silver chloride, when shaken in an equimolar water solution of ammonium sulfocyanate, reacts so rapidly as to precipitate 43 per cent of the sulfocyanate in 2 minutes. This occurs because


silver chloride is more soluble than silver sulfocyanate. Harvey has shown, however, that both ferric alum and nitric acid retard this reaction. By using nitric acid in a concentration of 5 per cent, he obtained practically identical results on known chloride solutions and on urines whether or not he filtered off the silver chloride. His results, indeed, indicate a slightly greater accuracy without filtration, but the difference is so small as to be entirely without significance (e.g. 0.7052 without filtration and 0.7069 per cent NaCl with filtration on a known 0.7039 per cent NaCl solution).

The following experiments were designed and carried out to test the accuracy of the titration in the presence of silver chloride. Since the purpose was to discover if the presence of silver chloride influenced the accuracy of the titration, all factors such as tungstate which might modify or conceal such an influence, had to be excluded. Therefore a pure solution of sodium chloride was substituted for the blood filtrate. A 0.1 N solution was prepared by dissolving in distilled water 0.5846 gm. of recrystallized, thoroughly dried sodium chloride, and making up to 100 cc. in an accurately calibrated flask. Then 1 cc. of this solution and 9 cc. of water were used instead of 10 cc. of blood filtrate, but all the subsequent details were followed as specified under the heading "Procedure." Eleven determinations were made in this manner. The chlorine content of 1 cc. of 0.1 M NaCl solution, as determined by these experiments, was: 3.55; 3.52; 3.56; 3.56; 3.54; 3.53; 3.56; 3.55; 3.56; 3.55; and 3.54 mg. The average was 3.547, compared to a theoretical value of 3.546 mg. The highest and lowest deviations were 0.7 and -0.3 per cent. The presence of silver chloride during the titration had therefore produced no error.

Similarly the chlorine content of 4 cc. of 0.1 M NaCl solution, as determined in the same manner (which of course necessitated the use of 3 cc. of m/7.092 AgNO₃ solution instead of 5 cc. of m/35.46) in three such experiments, was found to be 14.28, 14.23, and 14.30 mg., compared to the theoretical value of 14.2 mg. Here also the presence of chloride produced no error in titration.

As a final crucial test, another determination was made on 1 cc.

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of 0.1 M NaCl solution, but with this difference, that the mixture containing silver chloride was centrifugalized and an aliquot portion of the water-clear supernatant liquid taken for titration. The whole mixture measured 15 cc. and the titration of 10 cc. of the supernatant liquid required 0.97 cc. of m/\(\frac{35.46}{2}\) KCNS solution. Since \(5 - \frac{3}{2} (0.97) = 3.55\), it is evident that the method of titration gives the same result whether or not the silver chloride is removed.

The essential part of the procedure is the use of nitric acid, and the principle is a physical one—the flocking out of the silver chloride, with the consequent reduction of the surface exposed for reaction with the sulfocyanate. It is for this purpose that so large an amount of nitric acid is used, approximately 25 per cent of the volume of the mixture in which the titration is carried out.

Much higher concentrations of nitric acid should not be used, as the sulfocyanate is decomposed by them quite rapidly. To determine if such decomposition played an important part in the disappearance of the end-point, a small drop (0.03 cc.) of the \(m/35.46\) KCNS solution was added to each of two dishes, one containing 10 cc. of 30 per cent nitric acid, and the other 10 cc. of 15 per cent nitric acid, and each containing about 0.3 gm. of ferric alum. The red color persisted in the first mixture for 15 minutes, in the second it was still present at the end of 15 hours. It is evident, therefore, that under the conditions which may occur in using this method, the decomposition of sulfocyanate by nitric acid is not rapid enough to affect the accuracy of the titration.

In accordance with the geometrical principle that volumes vary as the cubes of a dimension, whereas surfaces vary as the squares, the surface of the silver chloride, when flocked out, is so small that the reaction with ferric sulfocyanate, although probably still going on, is negligible. (The color due to 0.03 cc. of the \(m/35.46\) KCNS solution persisted for 19 hours in the presence of such curds of silver chloride.) But the small amount of silver chloride which in spite of the nitric acid remains in fine suspension, has a larger aggregate surface and therefore reacts much more rapidly with the sulfocyanate.

Herein lies the explanation of the phenomena observed when KCNS is added to the mixture containing silver nitrate, silver
chloride, ferric alum, and nitric acid. The red color which appears with each drop disappears in about 3 seconds, because the ferric sulfocyanate reacts with the solution of AgNO₃. But when all the silver present as AgNO₃ has been precipitated, the red color which appears when the next drop is added persists about 30 seconds (from 10 seconds to 1 minute, depending upon how much of the drop was really excess, and upon the thoroughness of the flocking out of AgCl). Its disappearance is due to the reaction between the sulfocyanate and the very finest of the suspended particles of silver chloride. If another drop is added, the color will persist several minutes, for the sulfocyanate must then react with silver chloride particles of larger size and smaller aggregate surface. If still another drop is added the color will persist from 15 minutes to an hour or more (even for 19 hours),

<table>
<thead>
<tr>
<th>Centrifuge time (min.)</th>
<th>Persistence of color (Fe(CNS)₃)</th>
<th>Excess drops of KCNS solution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>End-point</td>
<td></td>
</tr>
<tr>
<td></td>
<td>First.</td>
<td>Second.</td>
</tr>
<tr>
<td>None.</td>
<td>40 sec.</td>
<td>4 min.</td>
</tr>
<tr>
<td>1</td>
<td>90 &quot;</td>
<td>24 &quot;</td>
</tr>
<tr>
<td>6</td>
<td>75 &quot;</td>
<td>23 &quot;</td>
</tr>
<tr>
<td>40</td>
<td>30 min.</td>
<td>More than 1 hr.</td>
</tr>
</tbody>
</table>

for the fine suspension of silver chloride has all been used up, and the aggregate surface left for reaction is very small.

The influence of the silver chloride particles of different sizes can be demonstrated by fractional centrifugalization, as in the experiments given in Table II.

In addition to the action of nitric acid in flocking out the chloride, the abundance of ferric alum used also retards the reaction between silver chloride and sulfocyanate by reducing the ionization of the latter. This also deepens the end-point color by preventing the ionization of the red salt, Fe(CNS)₃, into yellow Fe³⁺ ions and colorless CNS⁻ ions.

II. There remains the second question: "Does the amount of silver precipitation, as found by titration of the excess, indicate accurately the chlorides of the plasma?"
Mixtures of tungstic acid and chloride bring down more silver than can be accounted for by the chloride alone. This additional precipitation of silver has been found by centrifugation and titration of the supernatant fluid. Errors as large as 4 per cent were obtained in this way. Similar errors sometimes occurred when centrifugation was introduced into the chloride determination of tungstic acid filtrates. The amount of error which may so occur is not always proportional to the amount of tungstic acid present, and so appears to be more probably dependent upon physical than upon chemical reactions.

This possibility of error is, however, entirely avoided in the method described, by carrying on the titration in the presence of the precipitate, when all the silver which has not been truly precipitated by chloride is available for titration. Determinations of the same 0.1 M NaCl solution gave practically identical results whether determined directly or after the addition of equal volumes of 10 per cent sodium tungstate solution and \( \frac{2}{3} \) N sulfuric acid; for example, with tungstate, 3.53, 3.54, and 3.54 mg. of Cl per cc., and without tungstate, 3.547 as the average of eleven determinations.

The evidence given above has been presented in order to demonstrate that the method is free from error at the two points which seemed, \textit{a priori}, the most probable sources; namely, the presence of tungstic acid at the time silver chloride is precipitated, and the presence of silver chloride at the time of titration.

The final test of the accuracy of the method consists of course in its comparison with methods of known accuracy. The check determinations are given in Table III.

These check determinations indicate the essential accuracy of the method described. In order to determine the limit of error, seventeen duplicate determinations, involving nine separate precipitations with tungstic acid, were made on the same plasma, with the following results: 3.56; 3.51; 3.52; 3.54; 3.50; 3.55; 3.57; 3.54; 3.48; 3.49; 3.52; 3.50; 3.52; 3.53; 3.56; 3.54; 3.55 mg. of Cl per cc. The digestion method of von Korányi showed 3.53 mg. of Cl per cc. The average by the method described is 3.528. The
TABLE III.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Method described.</th>
<th>NaCl checked by method of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cl per cc.</td>
<td>NaCl</td>
</tr>
<tr>
<td></td>
<td>mg.</td>
<td>per cent</td>
</tr>
<tr>
<td>Whole blood.</td>
<td>2.88</td>
<td>0.473</td>
</tr>
<tr>
<td>M.</td>
<td>2.88</td>
<td>0.473</td>
</tr>
<tr>
<td>Plasma.</td>
<td>3.69</td>
<td>0.608</td>
</tr>
<tr>
<td>M.</td>
<td>3.71</td>
<td>0.612</td>
</tr>
<tr>
<td>Whole blood.</td>
<td>3.06</td>
<td>0.503</td>
</tr>
<tr>
<td>Wh.</td>
<td>3.34</td>
<td>0.633</td>
</tr>
<tr>
<td>Plasma.</td>
<td>3.76</td>
<td>0.619</td>
</tr>
<tr>
<td>Plasma I.</td>
<td>3.78</td>
<td>0.623</td>
</tr>
<tr>
<td>&quot; II.</td>
<td>3.75</td>
<td>0.618</td>
</tr>
<tr>
<td>&quot; III.</td>
<td>3.74</td>
<td>0.617</td>
</tr>
<tr>
<td>&quot; IV.</td>
<td>3.68</td>
<td>0.606</td>
</tr>
<tr>
<td>&quot; V.</td>
<td>3.80</td>
<td>0.626</td>
</tr>
<tr>
<td>&quot; VI.</td>
<td>3.52</td>
<td>0.580</td>
</tr>
<tr>
<td>&quot; VII.</td>
<td>3.52</td>
<td>0.582</td>
</tr>
<tr>
<td>&quot; VIII.</td>
<td>3.52</td>
<td>0.582</td>
</tr>
<tr>
<td>&quot; IX.</td>
<td>3.24</td>
<td>0.535</td>
</tr>
<tr>
<td>Whole blood I.</td>
<td>4.10</td>
<td>0.676</td>
</tr>
<tr>
<td>Ascitic fluid I.</td>
<td>3.78</td>
<td>0.623</td>
</tr>
<tr>
<td>&quot; II.</td>
<td>3.75</td>
<td>0.610</td>
</tr>
<tr>
<td>Plasma X.</td>
<td>3.38</td>
<td>0.558</td>
</tr>
<tr>
<td>&quot; XI.</td>
<td>3.64</td>
<td>0.600</td>
</tr>
<tr>
<td>&quot; XII.</td>
<td>3.70</td>
<td>0.610</td>
</tr>
<tr>
<td>&quot; XIII.</td>
<td>2.84</td>
<td>0.467</td>
</tr>
<tr>
<td>&quot; XIV</td>
<td>3.86</td>
<td>0.637</td>
</tr>
</tbody>
</table>

Average ......................... 0.5885 0.5896


‡ I am indebted to Mr. L. M. Smith for these determinations.


greatest deviations are $-1.3$ and $+1.2$ per cent. The limit of error with careful technique must therefore be less than 1.5 per cent.

SUMMARY.

1. A simple and rapid method is described for the determination of blood and plasma chlorides.
2. The same reagents as are used for the determination of urine chlorides are employed.
3. The method is especially adapted to the system of blood analysis developed by Folin and Wu.
4. The limit of error is less than 1.5 per cent.

The writer is indebted to Professor Otto Folin for encouragement in this work, and to Mr. L. M. Smith for assistance in checking determinations.
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