THE DETERMINATION OF THIOCYANATE IN BIOLOGICAL FLUIDS

BY LEON C. CHESLEY

(From the Margaret Hague Maternity Hospital, Jersey City)

(Received for publication, April 24, 1941)

The determination of thiocyanate in biological fluids is of importance in at least two respects. (a) In clinical practice thiocyanates are often given in the treatment of hypertension. The dosage is regulated by the concentration of drug attained in the blood. There are available simple methods for the estimation of blood concentrations, sufficiently accurate for this purpose. (b) Thiocyanates are sometimes used in the estimation of "available" (extracellular?) water of the body. In such measurements, more exact determinations of thiocyanate in plasma and urine are necessary.

Colorimetric determination of thiocyanate has been based upon the reaction with a ferric salt, which forms a colored compound (ferric thiocyanate?). The original method, adapted by Schreiber (1) from the procedure of Leared (2), has been variously modified. Photometric adaptations of the method have been made by Molenaar and Roller (3) who used the stufenphotometer, and by Ginsburg and Benotti (4) who employed the Evelyn photoelectric microcolorimeter.

These methods are satisfactory for serum, but with urine the results are erratic. In the present paper, there will be described a modification in which thiocyanate is determined in 1:10 protein-free filtrates of plasma, serum, and urine; the Evelyn macrocolorimeter, which is more generally available than is the microcolorimeter or the stufenphotometer, is employed. The accuracy of the determination in urine has been considerably enhanced by the addition of serum protein which is subsequently precipitated.
Eight separate stock solutions of desiccated sodium thiocyanate, 100 mg. per cent in water, were made up. By dilution with appropriate quantities of plasma, serum, or urine, known concentrations of NaCNS ranging from 0.9 to 20 mg. per cent were obtained. These known solutions were then diluted 1:10 with various protein precipitants. The methods used were (a) Haden's modification of the Folin-Wu tungstic acid precipitation (5); (b) equal volumes of 20 per cent trichloroacetic acid and plasma, serum, or urine, as recommended by Schreiber (1) (the filtrate was subsequently diluted); (c) Fujita and Iwatake's cadmium precipitation (6); (d) the zinc method of Somogyi (7). In each case, filtrates were made of blank plasma, serum, or urine. The addition of serum protein to urine will be described below.

The color reagent used was that of Lavietes, Bourdillon, and Klinghoffer (8); 25 gm. of c.p. ferric nitrate crystals (Fe(NO₃)₃·9H₂O) were dissolved in about 400 ml. of distilled water, 12.5 ml. of c.p. nitric acid were added, and the volume was made to 500 ml. with water.

For each determination, 5 ml. of filtrate were transferred to a colorimeter tube (provided with the Evelyn macrocolorimeter), and 5 ml. of the ferric nitrate reagent were added. The tube containing the blank was then placed in the colorimeter, and the light intensity was adjusted to give a galvanometer reading of 100; the center setting was then read on the galvanometer after removal of the blank. The samples containing the thiocyanate were then quickly read, the center setting being kept constant.

A filter allowing maximum transmission at 490 mµ was used. Filter 520, provided with the apparatus, was also found to be satisfactory.

Results

Serum and Plasma Solutions—The best results were obtained in tungstic acid and trichloroacetic acid filtrates. The range of error in the determination of known thiocyanate concentrations in plasma and serum was less with these protein precipitants, and also the color intensity developed by the ferric nitrate was very nearly the same as that developed in pure aqueous solutions of thiocyanate.
The cadmium and zinc protein precipitants apparently interfere slightly, for the depth of color in such filtrates was always less than in pure aqueous solutions of the same CNS\(^{-}\) concentrations. This can, of course, be corrected by changing the factor \(K\) for the calculation, which is

\[
\text{Concentration} = \frac{2 - \log \text{galvanometer reading}}{K} \tag{1}
\]

In water solutions and with Filter 490, \(K\) averaged 0.357; in tungstic acid and trichloroacetic acid filtrates it averaged 0.345; while in the Somogyi zinc filtrate \(K\) was 0.287 and in the Fujita cadmium filtrate it was 0.313.

The cadmium and zinc protein precipitants were abandoned because of the apparent interference in color development. The trichloroacetic acid filtrate must be diluted after the removal of the proteins, thus necessitating an extra step, and the use of a more dilute trichloroacetic acid does not give satisfactory results. For these reasons, and because of other determinations made concurrently, the Folin-Wu procedure was adopted for the preparation of plasma or serum for thiocyanate analysis.

The error in the determination of thiocyanate in tungstic acid filtrates of plasma or serum was always less than 4 per cent in the 70 known solutions analyzed. In two-thirds of the cases, the error was within ±1 per cent. The frequency distributions of the errors are shown in Fig. 1.
When the greatest accuracy in determination is required, as in estimation of the "available" fluid of the body, particular attention must be paid to the blank. The blank in tungstic acid alone is appreciably higher than in water, and to this color given by tungstic acid must be added the color given by thiocyanate and other chromogenic substances in the plasma. The color given by the plasma varies considerably from subject to subject, and therefore a plasma sample should be taken from each subject before the administration of the thiocyanate, this original sample to be used as the blank. When this is done, the error in determination is usually within ±1 per cent.

The magnitude of the blank has been surveyed by reading Folin-Wu filtrates of blank bloods against water blanks. These blank blood filtrates were taken from the routine chemistry laboratory, and were from patients with toxemia of pregnancy. Much of the color appearing after the addition of the ferric nitrate reagent is not given by thiocyanate but by the oxalate used as an anticoagulant, for it is a light yellow rather than the orange-red tint characteristically developed in thiocyanate solutions. The range of values in blank blood filtrates, calculated as sodium thiocyanate, is from 0.38 to 0.92 mg. per cent and averages about 0.60 mg. per cent.

Urine Solution—Variations in the concentration of urinary pigment interfere with the ordinary colorimetric determination of thiocyanate. The protein precipitants used in the present study do not greatly improve the situation, and may even aggravate it by causing turbidity in the filtrates. When Folin-Wu filtrates of urine were analyzed for known concentrations of thiocyanate, in the manner described above for plasma, errors up to 10 per cent were not uncommon, as the line graph in Fig. 2 indicates.

Since the results obtained with serum were very consistent, while parallel determinations in urine were erratic, it was thought that addition and later precipitation of serum protein might improve the urine analysis. The protein used was precipitated from about 40 ml. of pooled plasma and serum, by the addition of several volumes of \( \pi/12 \) tungstic acid. The precipitated protein was washed. Then 10 gm. of sodium tungstate were added, with enough water to make 100 ml. when solution was complete. This solution is fairly stable for about a week, if kept in an ice box.
To each volume of urine was added 1 volume of the tungstate-protein solution. After the mixture had stood for a few minutes, 8 volumes of $\frac{x}{12}$ sulfuric acid were added. The preparation is shaken during and after the addition of the acid, and allowed to stand for 10 minutes or longer before filtration. The filtrates are water-clear. The procedure from this point is the same as for plasma.

Frequently the blank is unstable and develops enough color with the ferric nitrate to change the galvanometer reading by two divisions; this change is rather rapid, and the true blank reading must be obtained at once. Strangely enough, the urine filtrates containing thiocyanate do not show this instability.

![Fig. 2. The frequency distribution of errors in the analysis of urines of known thiocyanate concentrations ranging from 0.9 to 20 mg. per cent. The line graph represents Folin-Wu filtrates of urine. The bar charts represent modified Folin-Wu filtrates, in which serum protein had been added to the urine and subsequently precipitated.](http://www.jbc.org/)

Because of this, it is more satisfactory to determine the center setting by omitting the blank and using instead a known concentration in a urine filtrate. With this known solution the light intensity is adjusted to give the theoretical galvanometer reading, calculated from Equation 1. The center setting obtained in this way corresponds with that obtained by setting the blank reading at 100 within 5 to 10 seconds after the ferric nitrate is added.

The range of errors in the analysis of 78 urine samples of known thiocyanate concentration is shown in the bar chart of Fig. 2. The error was within ±1 per cent in about half the cases, and did not exceed 5 per cent in any case. Thus the addition of protein considerably enhanced the accuracy of the method.
Stability of Color—Several writers (1), (4), and others) have commented on the fading of the color after addition of the ferric nitrate reagent, and recommended that the readings be made within 10 minutes. In the present study, all readings were routinely made within 5 minutes, but the color does seem to be stable for at least 2 hours, except in the urine filtrates made without the addition of protein.

Range of Concentration—The greatest errors were found at the extremes of the thiocyanate concentrations investigated, but, as shown in Fig. 1, these errors did not exceed ±4 per cent. Between 2.5 and 18.0 mg. per cent, the law of Lambert and Beer holds quite accurately.

<table>
<thead>
<tr>
<th>Menstruum</th>
<th>K</th>
<th>Filter 490</th>
<th>Filter 520</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma or serum</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Folin-Wu filtrate</td>
<td>0.0345</td>
<td>0.0244</td>
<td></td>
</tr>
<tr>
<td>Trichloroacetic acid filtrate</td>
<td>0.0345</td>
<td>0.0243</td>
<td></td>
</tr>
<tr>
<td>Somogyi zinc filtrate</td>
<td>0.0287</td>
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<td></td>
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<tr>
<td>Fujita cadmium filtrate</td>
<td>0.0313</td>
<td>0.0226</td>
<td></td>
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<tr>
<td>Urine</td>
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<tr>
<td>Protein + Folin-Wu filtrate</td>
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<td>0.0244</td>
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<tr>
<td>Water</td>
<td>0.0357</td>
<td>0.0249</td>
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</table>

Results with Filter 520—Except for the lower value obtained for K, the determinations made with the 520 mμ filter are in good agreement with those made with Filter 490. The K values for all determinations are summarized in Table I.

SUMMARY

A method is described for the determination of thiocyanate in plasma, serum, and urine. The Evelyn photoelectric macrocolorimeter is used, and the color intensity is measured with a filter allowing maximum transmission at 490 mμ. The error is usually within ±1 per cent, and always less than 5 per cent.

The protein is precipitated from plasma or serum by the method of Folin and Wu, giving a 1:10 dilution.

In the case of urine, serum protein is added in solution in
sodium tungstate. The addition of sulfuric acid then precipitates the protein together with urinary pigments. The urine is thus diluted 1:10.

5 ml. of the Folin-Wu filtrate are placed in a colorimeter tube, and 5 ml. of ferric nitrate reagent are added. The color intensity is read within 5 minutes.

The center setting used in the determinations may be obtained by the use of a blank filtrate of plasma or urine. An alternative, and perhaps better, method is to use a plasma or urine filtrate of known thiocyanate concentration.

BIBLIOGRAPHY

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Leon C. Chesley


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