THE DETERMINATION OF THIOCYANATE IN SERUM
AFTER THE ADMINISTRATION OF ITS SALTS

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The present interest in the determination of thiocyanate in
serum, following the injection or ingestion of its salts, is twofold.
First, in order to control hypertension by administering thio-
cyanate, the dosage must be regulated by frequently checking
serum levels, as pointed out by Barker (1) and Wald, Lindberg,
and Barker (2). Secondly, the determination of extracellular
fluid, as originally described by Crandall and Anderson (3), and
later modified by Lavietes, Bourdillon, and Klinghofer (4), in-
volves the measurement of the concentration of thiocyanate in
serum. This method enjoys a wide-spread use. Schreiber's
procedure (5) for the quantitative detection of this compound in
serum has been adopted with minor modifications by many work-
ers in these two fields (1–4, 6). In general, the serum containing
thiocyanate is treated with trichloroacetic acid to obtain a clear,
protein-free filtrate. To the latter, acid ferric nitrate is added,
and the resulting orange-red color examined either colorimetrically
or photometrically.

In this communication a method is described whereby the
determination of thiocyanate is carried out directly upon serum
merely by addition of acid ferric chloride. Since the preliminary
precipitation of protein is thus obviated, the analysis is not only
shortened but greatly simplified. Furthermore, the actual ex-
amination of a small sample of serum is quickly performed in the
microphotoclectric colorimeter of Evelyn and Cipriani (7, 8),
with the plunger type of absorption cell of Evelyn and Gibson
(9). As Gibson and Evelyn (10) have shown in the case of “Evans
blue” dye, this instrument is fully as reliable as the spectropho-
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tometer, and has the added advantages of speed, simplicity, and economy.

Selection of Color Filter

The orange-red color developed when a ferric salt in acid medium is added to a solution of thiocyanate exhibits a broad band of absorption in the blue range of the spectrum (Fig. 1). Theoretically, the color filter selected for photometric analysis should allow a maximum transmission at 460 m\(\mu\). However, there is an objection to its use when applied to serum solutions, because of the behavior of bilirubin under the conditions of our method.

\[\text{FIG. 1. Spectrophotometric curves of ferric thiocyanate, ferric chloride, and Filter 520. The ordinates represent transmission measured in per cent.}\]

1 The absorption spectra were obtained at the Color Measurement Laboratory of the Massachusetts Institute of Technology.
When acid ferric chloride is added to thiocyanate-free serum, a green color develops, of which the intensity depends directly upon the amount of bilirubin present. This color apparently is due to the oxidation product or products of bilirubin. Malloy and Evelyn (11), from a study of the various absorption spectra of oxidation products of bilirubin, demonstrate that the maximum transmission for all occurs in the vicinity of 520 μμ. Hence, when a color filter transmitting in this range is used, the least absorption of light by these pigments is allowed. Furthermore, any effect due to differences in the amount of bile pigments between blank and test solutions is minimized. The absorption of light at 520 μμ by ferric chloride is small (Fig. 1), and is, moreover, compensated by the blank. The only other possible chromogenic species in serum offering error is hemoglobin. This pigment presents an absorption band in the vicinity of 540 μμ, and, therefore, precludes the possibility of applying a correction factor for hemolysis when a No. 520 filter is used. However, by careful collection of samples of blood, as will be described below, this error can invariably be obviated.

Procedure

Normally, there is a small and variable amount of thiocyanate present in serum. Recent determinations by Schreiber (5), Blum (12), and Stuber and Lang (13) range from 0.03 to 0.24 mg. per cent. Because in this method the test solution is compared against a blank serum, the original amount normally present is not detected. This represents no great error, compared with the amount of thiocyanate present in serum after the administration of usual doses. Previous to the administration of thiocyanate, a fasting sample of blood was withdrawn as a blank. The test sample following administration was also taken preprandially to avoid the interference of lipemia. Every sample of blood was drawn into a dry suction bottle, as described by Peters and Van Slyke (14), and quickly transferred to a paraffined tube. When retraction of the clot had set in, the tube was centrifuged for 15 minutes. The serum was then decanted into a clean dry tube, centrifuged for 10 minutes to remove any red cells, and carefully separated by a pipette from the remaining small residue of cells. Benzidine tests upon sera so treated invariably
showed no presence of hemoglobin. To 1 cc. of the blank serum, 2 cc. of 0.7 gm. per cent FeCl₃·6H₂O in 0.2 N HCl were added, the mixture being shaken thoroughly to allow complete escape of bubbles formed by the reaction between the serum bicarbonate and the acid. The solution was then transferred to a 1 cm. depth absorption tube and the galvanometer adjusted to 100, a No. 520 color filter being used. Then 1 cc. of the test solution was mixed with the same amount of acid ferric chloride reagent, the reading being taken 3 minutes after the two solutions were mixed. This time factor is of the utmost importance, as will be shown later. The galvanometric reading thus found was converted to concentration in terms of thiocyanate by the use of a constant derived from a calibration curve. The result must be multiplied by 3, since 1 cc. of serum is diluted to 3 cc. after the addition of reagent. The capacity of the absorption tube being 1.1 cc., as little as 0.5 cc. of serum with a proportionate amount of reagent may be used.

**Calibration Curve**—Since, as will be shown, the concentration of thiocyanate is proportional to the negative logarithm of the light transmitted up to a certain limit, the following formula may be used

\[ C = \frac{2 - \log G}{K} \]

where \( C \) is the concentration of the ferric thiocyanate in the colored solution expressed as thiocyanate, \( G \) the galvanometric reading, and \( K \) a constant. \( 2 - \log G \) may be expressed as \( L \), the optical density.³

\[ K = \frac{L}{C} \]

To obtain \( K \), known concentrations of thiocyanate were made up in serum, and the respective \( L \) values determined. Various lots

²That ferric thiocyanate exists is questioned by Liberalli (15), who believes that the orange-red color is due to ferric ferrithiocyanate. However, the results are all expressed in terms of thiocyanate and do not alter the conclusions.

³The term "optical density," expressed as \( L \), refers strictly to the average optical density measured with a polychromatic color filter in the photometer. Although only an approximation of the true optical density as obtained in a spectrophotometer with a monochromatic source of light, it is similar in the respect that it obeys the relation expressed as \( L = K \times C \).
of pooled sera, checked for hemolysis, were used. From three different initial stock solutions of potassium thiocyanate (3.000 ± 0.001 gm. as thiocyanate per 100 cc. of distilled water) appropriate dilutions of thiocyanate were made, yielding three series of concentrations each ranging from 1.5 to 7.5 mg. per cent by steps of 1.5 mg. per cent. To 1.0 cc. of each member of the thiocyanate series, 1.0 cc. of pooled serum was added, followed by 1 cc. of 1.4 gm. per cent FeCl₃·6H₂O in 0.4 N HCl. This amount of ferric chloride in acid yields a final concentration identical with that described in the above method. In the blank specimen, 1 cc. of distilled water replaced the thiocyanate solution. The final colored solutions in a series possessed thiocyanate concentrations ranging from 0.5 to 2.5 mg. per cent. In each series determinations were made in duplicate. Hence, for every concentration, six \( L \) values were obtained. The calibration curve did not exceed 2.5 mg. per cent, because it was found that above this value the relationship between \( L \) and \( C \) under the experimental conditions did not conform to a straight line. In Fig. 2 is plotted the average of each \( L \) value against its respective concentration. The average \( K \), presented in Table I, was 0.1465 ± 0.0025.
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**TABLE I**

*Estimation of K for Thiocyanate Solution in 1 Cm. Depth of 1 Part Serum and 3 Parts Reagent (0.7 Gm. Per Cent FeCl₃·6H₂O in 0.2 N HCl) with a No. 520 Color Filter.*

Each L value represents an average of six determinations.

<table>
<thead>
<tr>
<th>Concentration, mg. per cent...</th>
<th>0.50</th>
<th>1.00</th>
<th>1.50</th>
<th>2.00</th>
<th>2.50</th>
</tr>
</thead>
<tbody>
<tr>
<td>L...........................</td>
<td>0.074</td>
<td>0.147</td>
<td>0.223</td>
<td>0.290</td>
<td>0.359</td>
</tr>
<tr>
<td>L/C = K...........................</td>
<td>0.148</td>
<td>0.147</td>
<td>0.149</td>
<td>0.145</td>
<td>0.144</td>
</tr>
<tr>
<td>Average K......................</td>
<td>0.1465</td>
<td>±0.0025</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE II**

*Recovery of Thiocyanate Added to Normal and Jaundiced Sera*

The figures in the second column give the strength of thiocyanate of each 1 cc. portion added to 1 cc. of serum. 1 cc. of 1.4 gm. per cent FeCl₃·6H₂O in 0.4 N HCl was used as reagent. The third column gives the actual thiocyanate concentration in the final test solution. The values observed are averages of duplicates.

<table>
<thead>
<tr>
<th>Medium</th>
<th>Concentration of thiocyanate added in 1 cc. lots</th>
<th>Final concentration of thiocyanate</th>
<th>Concentration of thiocyanate observed</th>
<th>Error per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg. per cent</td>
<td>mg. per cent</td>
<td>mg. per cent</td>
<td>mg. per cent</td>
</tr>
<tr>
<td>Pooled normal serum</td>
<td>0.75</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>1.50</td>
<td>0.50</td>
<td>0.51</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>2.25</td>
<td>0.75</td>
<td>0.74</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>3.00</td>
<td>1.00</td>
<td>1.02</td>
<td>1.02</td>
</tr>
<tr>
<td></td>
<td>3.75</td>
<td>1.25</td>
<td>1.24</td>
<td>1.24</td>
</tr>
<tr>
<td></td>
<td>4.50</td>
<td>1.50</td>
<td>1.47</td>
<td>1.47</td>
</tr>
<tr>
<td></td>
<td>5.25</td>
<td>1.75</td>
<td>1.72</td>
<td>1.72</td>
</tr>
<tr>
<td></td>
<td>6.00</td>
<td>2.00</td>
<td>1.99</td>
<td>1.99</td>
</tr>
<tr>
<td></td>
<td>6.75</td>
<td>2.25</td>
<td>2.28</td>
<td>2.28</td>
</tr>
<tr>
<td></td>
<td>7.50</td>
<td>2.50</td>
<td>2.46</td>
<td>2.46</td>
</tr>
<tr>
<td>Serum bilirubin, 3.0 mg. per cent</td>
<td>0.75</td>
<td>0.25</td>
<td>0.26</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>3.75</td>
<td>1.25</td>
<td>1.24</td>
<td>1.24</td>
</tr>
<tr>
<td></td>
<td>6.75</td>
<td>2.25</td>
<td>2.21</td>
<td>2.21</td>
</tr>
<tr>
<td>Serum bilirubin, 7.2 mg. per cent</td>
<td>1.50</td>
<td>0.50</td>
<td>0.49</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>4.50</td>
<td>1.50</td>
<td>1.47</td>
<td>1.47</td>
</tr>
<tr>
<td></td>
<td>7.50</td>
<td>2.50</td>
<td>2.44</td>
<td>2.44</td>
</tr>
</tbody>
</table>

_Recovery Experiments_—Table II represents the recovery of thiocyanate from different samples of sera from normal and jaundiced patients.
From a fasting subject serum serving as a blank was withdrawn, and the absence of hemolysis ascertained. To 1 cc. samples various concentrations of thiocyanate were added in amounts of 1 cc., followed by 1 cc. of 1.4 gm. per cent FeCl₃·6H₂O in 0.4 N HCl. The resulting \( L \) values were converted to concentrations by use of \( K = 0.1465 \). In normal sera the found concentrations agreed with the actual concentrations within ±2.0 per cent. In jaundiced sera the recovery of thiocyanate was slightly less, the error tending to increase with increasing concentrations of bilirubin. The highest degree of jaundice studied represented a severe grade (7.4 mg. per cent bilirubin), and one seldom encountered. The recoveries obtained by our method compare favorably with results derived from the method in which the trichloro protein-free filtrate is used. Thus, Schreiber (5) published results indicating recoveries with a maximum error of ±2.7 per cent. Crandall and Anderson (3) stated that their error was 1 to 3 per cent; while Lavietes, Bourdillon, and Klinghofer (4) mentioned that they could recover 99 per cent of the thiocyanate. While equally as accurate, our method, however, greatly shortens and simplifies the procedure. The time-consuming step involving precipitation of proteins from the serum is completely eliminated.

Type, Concentration, and Purity of Reagents

Type of Acid Ferric Reagent—Preliminary experiments to ascertain the effect of adding an aqueous solution of a ferric salt to a thiocyanate-free serum revealed the fact that an orange-red color developed under these conditions. Such behavior is to be expected, since the ferric salt undergoes partial hydrolysis to the orange-colored ferric hydroxide in a weakly acid solution. Consequently, it proved necessary to prepare the ferric reagent in acid medium. Further preliminary experiments upon the practicability of adding various mineral acids (HNO₃, H₂SO₄, HCl) to serum without disturbing its clarity were tried. It was soon learned that even in mild strengths sulfuric and nitric acids produced an obvious turbidity, which, moreover, was rapidly progressive. Only hydrochloric acid in moderate strengths in a 1:3 dilution of serum proved to be acceptable. The faint turbidity encountered remained absolutely constant for 15 minutes, a fact ascertained by measuring the transmission of light
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through such a solution by means of the photoelectric colorimeter. Inasmuch as hydrochloric acid alone was compatible with serum, the corresponding ferric salt (FeCl₃·6H₂O) was chosen for the reagent.

Effect of pH—The pH influences the colloidal properties and hence the turbidity of a protein solution. From the above, it was suggested that there is an optimum range of acidity within which the system is stable in respect to turbidity. Therefore, to a series of sera from the same lot, various strengths of hydrochloric acid were added and the resulting nephelometric values measured. The Evelyn colorimeter was utilized as a nephelometer, a filter transmitting the light of the colored solution in question being used, as suggested by Evelyn (7). Since ferric chloride and ferric thiocyanate transmit practically 100 per cent in the orange-red portion of the spectrum (Fig. 1), a No. 620 color filter fulfilled this requirement. By such means were found the nephelometric turbidities of serum solutions to which had been added various strengths of hydrochloric acid. The thiocyanate and ferric chlo-

<table>
<thead>
<tr>
<th>HCl added in 1 cc. dose</th>
<th>pH of final solution</th>
<th>Relative transmission, G⁶₂₀</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>2.90</td>
<td>63.00</td>
<td>Increasing transmission within 5 min. Stable</td>
</tr>
<tr>
<td>0.10</td>
<td>2.50</td>
<td>67.25</td>
<td>&quot;</td>
</tr>
<tr>
<td>0.20</td>
<td>1.95</td>
<td>70.00</td>
<td>&quot;</td>
</tr>
<tr>
<td>0.30</td>
<td>1.65</td>
<td>70.00</td>
<td>&quot;</td>
</tr>
<tr>
<td>0.40</td>
<td>1.40</td>
<td>70.25</td>
<td>&quot;</td>
</tr>
<tr>
<td>0.50</td>
<td>1.30</td>
<td>70.00</td>
<td>&quot;</td>
</tr>
<tr>
<td>0.60</td>
<td>1.15</td>
<td>69.25</td>
<td>Progressive decrease in transmission &quot; &quot; &quot; &quot;</td>
</tr>
<tr>
<td>0.70</td>
<td>1.05</td>
<td>64.00</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

G⁶₂₀ gives the transmission of light through these solutions relative to 100 per cent transmission through air as a standard. Color filter No. 620. The final test solution was attained by mixing 1 cc. of serum, 1 cc. of 9 mg. per cent thiocyanate in various strengths of HCl, and 1 cc. of 1.4 gm. per cent FeCl₃·6H₂O.
ride concentrations were kept identical in the whole series. By necessity, the various turbidities were determined as values relative to one another. This was accomplished by measuring the per cent of light permitted through each solution of the initial intensity transmitted through air and originally fixed at 100 on the galvanometric scale. Table III shows the nephelometric turbidity of serum related to the pH. The turbidity is least between pH 1.95 and 1.30, and what is more important, it is absolutely stable over a period of 10 minutes. There is a definite advantage in having a wide range of pH in which to work. The buffering capacity of different sera vary; i.e., different pH values may be obtained in other sera to which the same strength of hydrochloric acid is added. Hence, a strength midway between the two limits affords a margin of safety and is applicable to all sera. This strength, as seen from Table III, is 1 part of 0.4 N HCl in a serum diluted 1:3. For this reason, when applied to the actual method, 2 cc. of reagent containing 0.2 N HCl were added to 1 cc. of serum. It is to be emphasized, further, that the turbidity encountered in the actual procedure is compensated by the blank, to which an identical amount and strength of acid is added.

Concentration of Ferric Salt—As may be expected from the law of mass action, a solution of constant thiocyanate concentration develops an increasing intensity of color with increasing concentration of ferric ions. Fig. 3 represents the relationship between intensity of color and strength of ferric chloride in a 1:3 dilution of serum containing 3.0 mg. per cent thiocyanate. As indicated by the curve, a concentration of the ferric reagent of 1.2 gm. per cent in the final solution represents, for all practical purposes, the maximum value necessary to obtain the greatest depth of color for a given thiocyanate concentration. In order to determine whether the maximum value represents the optimum for the range of thiocyanate herein described, further experiments were performed, the data of which are summarized in Fig. 4. Numerous curves were made relating optical density to thiocyanate concentration, each differing from the others in respect to the strength of ferric chloride employed to develop the color.

4 The pH was determined by the Coleman glass electrode pH meter, standardized against a buffer solution of pH 5.0.
Fig. 3. Relationship between color intensity and concentration of ferric chloride in the presence of a constant amount of thiocyanate. Abscissae, gm. per cent of FeCl$_3$·6H$_2$O; ordinates, optical density with a No. 520 color filter. Serum, 1 cc.; 9 mg. per cent thiocyanate in H$_2$O, 1 cc.; various strengths of FeCl$_3$·6H$_2$O in 0.4 N HCl, 1 cc.

Fig. 4. Each curve gives the relationship of thiocyanate concentration to optical density; they differ in respect to the concentration of ferric chloride. This relationship holds to a straight line up to L 0.360, regardless of the thiocyanate or ferric chloride concentrations. The concentration of FeCl$_3$·6H$_2$O corresponding to each curve is Curve I 0.23 gm. per cent, Curve II 0.35 gm. per cent, Curve III 0.47 gm. per cent, Curve IV 0.70 gm. per cent, Curve V 0.93 gm. per cent, Curve VI 1.17 gm. per cent.
The curves indicate that optical density is a straight line function of thiocyanate concentration up to the vicinity of \( L = 0.360 \), no matter what the strength of ferric chloride used. Accordingly, if a low ferric concentration is chosen, the working range is high, but the optical density for a given thiocyanate concentration, \( i.e. \) the sensitivity, is low. For example, in 0.23 gm. per cent reagent (Curve I, Fig. 4), when \( L \) is 0.360, \( C \) is about 3.70 mg. per cent. Therefore, 0.001 \( L \) is equivalent to about 10 \( \gamma \) per cent thiocyanate. On the other hand, in a high ferric concentration, \( e.g. \) 1.17 gm. per cent (Curve VI, Fig. 4), the optical density is high, \( i.e. \) the sensitivity is greater (0.001 \( L = 5 \gamma \) per cent thiocyanate), but the range of usefulness contracts to 2.0 mg. per cent.

### Table IV

**Comparison of Color Intensities of Sera Containing Identical Amounts of Ferric Chloride but from Different Sources**

\( L \) refers to the optical density given by the Evelyn colorimeter with a No. 520 color filter. Final solution: 1 cc. of serum, 2 cc. of 0.7 gm. per cent FeCl\(_3\)·6H\(_2\)O in 0.2 N HCl.

<table>
<thead>
<tr>
<th>Source of FeCl(_3)·6H(_2)O</th>
<th>( L )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Old lot of Baker’s (c.p.)</td>
<td>0.028</td>
</tr>
<tr>
<td>Freshly opened lot, Baker (c.p.)</td>
<td>0.029</td>
</tr>
<tr>
<td>&quot;     &quot; &quot;     &quot; Merck &quot;</td>
<td>0.030</td>
</tr>
<tr>
<td>Average</td>
<td>0.029 ± 0.001</td>
</tr>
</tbody>
</table>

We have chosen that strength of ferric reagent (0.47 gm. per cent) which gives a sensitivity midway between the two extremes mentioned above (0.001 \( L = 7.5 \gamma \) per cent). The upper limit of the working range is thus fixed at 2.5 mg. per cent (Curve III, Fig. 4). How this range may be increased will be explained in the text following.

**Purity of Iron Reagent**—Different lots of FeCl\(_3\)·6H\(_2\)O were used in order to ascertain whether age and source of material will alter the optical properties of a serum solution of acid ferric chloride. Table IV represents the optical density developed in a serum solution containing ferric chloride from various sources. The blank consisted of an iron-free acid solution of serum and a No. 520 filter was used. Apparently any c.p. material is suitable.
Effect of Protein Content—Gregersen and Gibson (16) have shown that dilution of the protein content of dyed serum up to 20 per cent of the normal has little or no effect upon the absorption spectra of certain of the vital dyes. Similarly, dilutions of the original serum up to 50 per cent do not affect the optical density of a thiocyanate solution (Fig. 5). Dilutions of a pooled serum were made with 0.85 per cent NaCl, and 1 cc. used for each determination. From this point the procedure was the same as that given in detail above. Protein determinations were performed according to the Kjeldahl semimicromethod for nitrogen as described by Pregl (17), with the acid digestive mixture of Campbell and Hanna (18). In view of the fact that diluting a ferric thiocyanate serum solution with normal saline does not alter the absorption spectrum, it is evident that the upper limit of our method can be extended. This is simply done by effecting a dilution of serum containing more than 7.5 mg. per cent with normal saline and proceeding from that point as described above.
Thus, a concentration of thiocyanate as high as 15 mg. per cent can be accurately detected. As reported by Barker (1), Wald, Lindberg, and Barker (2), and Garvin (19), since this value marks the beginning of toxicity, there will be little necessity for measuring higher values, particularly in the field of determination of body water, where much lower values are desired.

**Effect of Time**—The time elapsed between the addition of the ferric reagent to the thiocyanate solution and the actual reading of the color is of the highest importance. Fig. 6 indicates the per cent decrease from the original intensity of color measured photometrically from minute to minute. Any delay up to 10 minutes can produce an error of 5 per cent. Apparently, the color fades after the 5th minute, and for this reason we have elected to take readings at the 3rd minute for the sake of uniformity. As far as we are aware, two workers have called attention to the phenomenon of fading. Schreiber (5) stated that this behavior can be detected after 3 days and, therefore, recommended making fresh ferric thiocyanate standards at the end of this time for colorimetric comparisons. According to our results,
his values for thiocyanate content in serum must have been far too low, derived as they were from standards up to 3 days old. As a matter of fact, Schreiber's estimated values for the normal thiocyanate content of human sera are lower than those given by Blum (12) and Stuber and Lang (13). Urbach (20) cautioned that the final mixed solutions containing ferric thiocyanate should be read within 10 minutes. Even here, if this is not done at a uniform time, a small error may creep in. The effect of time, as illustrated in Fig. 6, does not occur solely in protein solutions; for, a similar fading has repeatedly been found to take place in distilled water and saline solutions.

Effect of Temperature—No direct experiments were carried out to control this factor. However, the recovery experiments reported above were done on days varying in temperature between 21.1° and 27.8°. The calibration curve is composed of experiments performed on days averaging 21.1°. While these observations are only suggestive, no great error will be suffered, when there exist no extremes of temperature.

SUMMARY

1. A photometric method is described for the determination of thiocyanate in serum by the direct addition of an acid ferric chloride solution, thus obviating the need of removing serum proteins and, consequently, shortening and simplifying the procedure.

2. Thiocyanate has been recovered from normal serum within ±2.0 per cent. From serum with a concentration of bilirubin as high as 7.4 mg. per cent, recovery experiments yielded as high as 96 to 99 per cent with the method described. The results are comparable to those reported in which the trichloroacetic filtrate method was used.

3. The reagents have been examined in detail from the point of view of type and purity. The optimum pH and ferric chloride concentration have been ascertained.

4. Dilution of the protein content with normal saline up to 50 per cent of the original concentration does not appreciably affect the optical density of a given ferric thiocyanate solution.

5. The color of ferric thiocyanate begins to fade at the end of
5 to 6 minutes, and a significant error may be encountered within
10 minutes. All readings were taken 3 minutes after mixing.

6. The method affords a working limit of 7.5 mg. per cent,
in which range the Lambert-Beer law holds accurately. By dilu-
tion of the original serum, the upper limit may be extended to
15 mg. per cent with negligible error.

BIBLIOGRAPHY

   112, 1120 (1939).
   Nutrition, 1, 126 (1934).
   16, 261 (1936).
14. Peters, J. P., and Van Slyke, D. D., Quantitative clinical chemistry,
   Methode, Baltimore, 56 (1932).
   (1937).
17. Pregl, F., Die quantitative organische Mikroanalyse, Berlin, 4th edi-
tion, 105 (1935).