Method for Determining Thiocyanate in Serum and Urine

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We describe a method for rapid and specific measurement of thiocyanate in serum or urine. We separate thiocyanate from interfering compounds by adsorbing it on an anion-exchange resin that has special affinity for thiocyanate, then eluting with sodium perchlorate. The eluted thiocyanate is quantitated by a modified König reaction, sodium hypochlorite being used as the chlorinating reagent. Analytical recovery of thiocyanate added to serum and urine was quantitative; the coefficient of variation was 2.3% for both within-day and between-day precision. Cyanide and certain antibiotics interfere, but may be eliminated by including additional washing steps in the usual procedure. The proposed procedure was compared with another method, based on the oxidation of thiocyanate to cyanide. Agreement was satisfactory, both for serum and urine.

Additional Keyphrases: adsorption on an anion-exchange column • König reaction • benzylpenicillin interference with the directly performed König reaction

Several methods for determining thiocyanate in biological samples have been reported. These include colorimetric methods based on the formation of a red complex with ferric ions (1, 2), but they are usually unspecific and only applicable to serum. Several methods (3–7) are based on the König reaction, in which thiocyanate (or cyanide formed from thiocyanate) is first converted to a cyanogen halide. The latter then is reacted with pyridine to produce glutaric aldehyde, which is coupled with a primary amine or a compound containing reactive methylene hydrogens to form a dyesuff. Aldridge (8) used bromine water to convert thiocyanate to cyanogen bromide, sodium arsenite to remove excess bromine, and benzidine as the coupling compound. This method thus involves the use of a fairly toxic compound (sodium arsenite) and a highly carcinogenic amine (benzidine). Although benzidine may be replaced by the less carcinogenic p-phenylenediamine (6), this method reportedly gives inaccurate results when applied to urine (8). Boxer and Richards (4) developed a highly specific method based on the oxidation of thiocyanate to hydrogen cyanide under mild conditions. Hydrogen cyanide is then separated from interfering compounds by aeration into sodium hydroxide and determined by a König reaction according to Epstein (9), with Chloramine T as the halogenating agent and 1-phenyl-3-methyl-5-pyrazolone as the coupling agent. The method of Boxer and Richards (4) was later improved (5) by replacing 1-phenyl-3-methyl-5-pyrazolone with barbituric acid as the coupling compound. Although the method of Boxer and Richards in its original or modified form is a sensitive and specific method, it requires special glass equipment, is fairly laborious to perform, and is only applicable to small series of samples. A recently reported gas-chromatographic method (10) suffers from similar drawbacks. Finally, thiocyanate may be determined in plasma with a thiocyanate-selective electrode (11), but this method requires fairly large sample volumes and its specificity has not been documented.

We describe here a new method for determination of thiocyanate in biological fluids, based on the high affinity of thiocyanate to the weakly basic anion-exchange resin Lewatit MP 7080 (12). Thiocyanate is thus separated from interfering compounds and is then determined with a new modification of the König reaction, where sodium hypochlorite is used for halogenation and barbituric acid is used as the coupling agent.

Materials and Methods

Materials

"Econo-Columns" (Bio-Rad Laboratories), 0.7 cm i.d. and 4 cm long, were used for the chromatographic step.

Ion-exchanger. Lewatit MP 7080, 100–200 mesh (Merck AG, Darmstadt, Germany; supplied in the U.S. by EM Laboratories, Elmsford, NY 10523) is washed with water and suspended in hydrochloric acid, 1 mol/L. The resin is filtered on a suction filter and washed with water until the pH of the washings is 4.5 or greater. The resin is then spread out on a glass disk and dried at 100 °C for 12 h. It is then suspended in water and left to stand for at least 15 min. The water is decanted off and the resin resuspended in an equal volume of sodium hydroxide, 1 mol/L. After 15 min the resin is washed with water until the washings attain a neutral pH; it then is ready for use. If the pretreatment of the resin according to the description given above is omitted, blank values are higher.

Reagents

The reagents were prepared from de-ionized water and analytical-grade chemicals unless otherwise specified.

Sodium hydroxide, 0.1 mol/L. Dissolve 4.0 g of NaOH in water and dilute to 1 L.

Sodium perchlorate, 1 mol/L. Dissolve 140 g of NaClO₄·H₂O in water and dilute to 1 L.

Acetic acid, 0.5 mol/L. Dilute 5.7 mL of concentrated acetic acid with water to 200 mL.

Sodium hypochlorite, 50 mmol/L. Dilute 5.0 mL of NaClO, 0.5 mol/L, in 0.1 mol/L NaOH (reagent no. 23039; BDH Chemicals, Poole, England), with water to a final volume of 50 mL. Refrigerated, this reagent is stable for at least one month.

Barbituric acid–pyridine reagent. Dissolve 6.0 g of barbituric acid (reagent quality; Fluka AG, Bern, Switzerland) in a mixture of 30 mL of pyridine and 64 mL of water and add 6.0 mL of concentrated HCl. Stable for a week in the refrigerator.

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Table 1. Eluting Efficiency of Some Anions

<table>
<thead>
<tr>
<th>Anion</th>
<th>Thiocyanate eluted, %</th>
</tr>
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<tbody>
<tr>
<td>ClO₄⁻</td>
<td>100</td>
</tr>
<tr>
<td>CH₂COO⁻</td>
<td>92</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>74</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>0</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>0</td>
</tr>
</tbody>
</table>

*Thiocyanate (0.5 mL of 200 μmol/L) was added to a MP 7080 column as in the procedure described, and eluted with 8.0 mL of 1 mol/L NaClO₄. Add 0.2 mL of 0.5 mol/L acetic acid to 4.0 mL of the eluate, mix, and add 0.1 mL of 50 mmol/L NaClO₄ and mix again. Within 1 min add 0.5 mL of the barbituric acid-pyridine reagent, and mix, and 5 to 15 min later determine the absorbance at 580 nm vs. water, in a 1-cm cuvet. Simultaneously do a blank determination where serum or urine is replaced with water. The absorbance of the blank should be <0.020. Read the thiocyanate concentration in the sample from a standard graph prepared with thiocyanate standards of known concentrations. The standard graph is linear up to thiocyanate concentrations corresponding to 200 μmol/L in the sample.

Results

Chromatography

The present procedure is based on a previous observation (12) that the weakly basic anion-exchange resin Lewatit MP 7080 has a strong affinity for thiocyanate, even at a high pH where the resin is in an uncharged form. Obviously the resin cannot operate by an ion-exchange mechanism under these conditions. It was suggested (12) that other adsorption forces, related to the chaotropic effect of thiocyanate, were responsible for the strong binding of thiocyanate to the resin. This is borne out by experiments shown in Table 1. They demonstrate that thiocyanate is displaced from the resin by other chaotropic ions such as perchlorate, trichloroacetate, and nitrate, but not by chloride or sulfate. As perchlorate was the most efficient displacing ion, it was used in the final procedure. An elution diagram obtained under these conditions (Figure 1) demonstrates that thiocyanate is eluted in a volume of about 4 mL. We found it more convenient, however, to use a larger elution volume in the standard procedure. Analytical recovery of thiocyanate carried through the chromatographic step was 101% (mean of triplicate experiments; range, 98–105%). It was found necessary to dilute serum with an alkaline solution before application to the resin; otherwise, recoveries of thiocyanate were low. This effect was attributed to the well-known binding of thiocyanate to serum albumin (14), which is strong at a neutral reaction but decreases when the pH is increased.

Color Reaction

Preliminary experiments demonstrated that the concentration of sodium hypochlorite used in the chlorination step is not critical; it could be decreased 10-fold or increased two-fold from that of the final procedure without significant effect on the results. However, the reaction should be performed under acid conditions (Figure 2), and the slightly alkaline el-

Fig. 1. Elution diagram of thiocyanate
To a 2.5 × 0.7 cm column of Lewatit MP 7080 we applied 0.5 mL of KCNS, 200 μmol/L, and eluted with NaClO₄, 1 mol/L. The eluate was collected in 1-mL fractions, which were diluted with 3 mL of NaClO₄, 1 mol/L. Thiocyanate was then determined according to the procedure described.

Fig. 2. Effect of pH on chlorination step

Fig. 3. Effect of time on chlorination step
urate from the preceding step is consequently acidified with acetic acid. The reaction between thiocyanate and hypochlorite is practically instantaneous (Figure 3), but the product formed slowly decomposes. Consequently, the reaction interval in this step should be kept short.

The concentrations of the components present in the barbituric acid–pyridine reagent were optimized in preliminary experiments, but are not critical. Maximum absorbance was reached 3–4 min after this reagent was added, then slowly decreased (Figure 4). In agreement with an earlier report (5), we found that the absorption maximum of the chromogen is located at 580 nm.

Other Analytical Variables

Precision and recovery. The within-day precision of the method was evaluated by analyzing 12 aliquots of a urine sample. The result obtained, 56.6 ± 1.29 μmol/L (mean ± SD), corresponded to a coefficient of variation of 2.3%.

Between-day precision was determined as follows. One urine sample was divided into portions, which were stored at −18 °C and analyzed (in duplicate) during six months. The average urine concentration was 58.9 μmol/L (SD, 1.37 μmol/L; CV, 2.3%). The recovery of thiocyanate, 50 μmol/L, added in duplicate experiments to a serum sample containing 59.8 μmol of thiocyanate per liter was 100 and 101%; the recovery of thiocyanate, 100 μmol/L, added in duplicate to a urine sample containing 87.0 μmol of thiocyanate per liter was 99.5 and 100%.

Specificity. Cyanide behaved like thiocyanate in the present modification of the König reaction and was not separated from thiocyanate in the chromatographic step. However, we found that the interference caused by cyanide could be eliminated by washing the ion-exchange column twice with 5 mL of HCl, 0.1 mol/L, before elution with sodium perchlorate. It was not necessary to include this washing step in the routine procedure, because the concentration of cyanide in serum or urine is exceedingly low (6). Nitroprusside did not interfere in the present method, although this ion contains complex-bound cyanide. Certain compounds normally present in body fluids were also tested for possible interference at concentrations encountered in urine. The following compounds neither gave any absorbance when added alone to the assay nor affected the absorbance given by thiocyanate: sodium chloride, ammonium chloride, creatinine, glycine, and methionine. Some commonly prescribed drugs—salicylic acid, ascorbic acid, and thiamine—also did not interfere in the present method at concentrations found in urine after normal doses. This also applied to the antibiotics chloramphenicol and tetracycline. Unexpectedly, however, the antibiotics benzylpenicillin, cloxacillin, and cephalothin caused a positive interference at a concentration of 2 g/L. Although the absorbancy of these compounds was, on a molar basis, only 2–4% of that given by thiocyanate, administration of high doses of these antibiotics to a patient may result in concentrations in urine that can significantly interfere. This interference can be eliminated by washing the ion-exchange column three times with 5-mL portions of ammonium chloride, 4 mol/L, before elution with sodium perchlorate. These washing steps were not included in the routine procedure, but should be used when urine samples from patients receiving these antibiotics are analyzed.

Method Comparison

To further validate our method, we analyzed serum (Figure 5) and urine (Figure 6) samples by the present method and by a previously described modification (5) of the oxidation method of Boxer and Richards (4). The results obtained agreed satisfactorily.

Values for Normal Nonsmoking Subjects

The mean concentration of serum thiocyanate from 20 apparently healthy nonsmoking subjects (10 men and 10 women, no sex-related difference observed) as determined by the present method was 42.5 μmol/L (SD, 17.1 μmol/L). This value agrees well with values previously reported (2, 8). Similarly, the urinary excretion of thiocyanate measured on 10 men and 10 women (no sex-related difference observed) was 43.0 ± 22.1 μmol/24 h, also in good agreement with values published for nonsmoking subjects (15, 16).
Discussion

Thiocyanate is a detoxication product of cyanide, and determinations of thiocyanate in serum or urine have consequently been used for monitoring exposure to hydrogen cyanide from tobacco smoke (2, 6, 17) or fire atmosphere (18). Measurements of serum thiocyanate have also been advocated for supervising therapy with sodium nitroprusside (19), a hypertensive drug that is metabolically converted to cyanide. The present method should find similar applications, and represents a significant improvement over earlier methods based on the König reaction. We use sodium hypochlorite as a halogenating agent, which is superior to other compounds used for this purpose, such as bromine or Chloramine T. Any bromine remaining after its reaction with thiocyanate interferes in the following step and must be removed with arsenite (3), whereas the reaction between Chloramine T and thiocyanate is fairly slow unless catalyzed by ferric ions (7). Another advantage of the present method is that deproteinization of serum is not required.

The positive interference given by penicillins and cephalothin in the present method must be considered, when urine samples from patients receiving these drugs are analyzed. However, it may easily be eliminated as described. This is of no concern when serum is analyzed, because the concentrations in serum reached by these antibiotics are much lower than those found in urine. Incidentally, we observed that benzylpenicillin gave a positive interference in a method in which the König reaction is directly performed on the sample (6), but not in a method where thiocyanate is first oxidized to hydrogen cyanide (5).

References