Rapid Determination of Toxic Cyanide Concentrations in Blood

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This method for the rapid colorimetry of cyanide in blood, applicable to patients exposed to toxic levels of cyanide, is based on the König reaction, which produces a chromophore from cyanide as well as thiocyanate. The latter compound, normally present in blood, is confined to plasma. Thus, its interference with determination of cyanide was eliminated by performing the assay on the erythrocytes, which contain most of the blood cyanide. Furthermore, cyanide was trapped in the erythrocytes and stabilized during the initial washing steps by conversion of hemoglobin to methemoglobin with inorganic nitrite.

The hazard of cyanide poisoning from inhalation of smoke from fires and the possibility that a fire victim may have been exposed to hydrogen cyanide are now well established (1-11). Treatment with oxygen and cyanide antidotes may be life saving (3-5, 7, 9, 10), but the administration of cyanide antidotes has its risks (3-5, 9). The symptoms of cyanide poisoning often are vague in fire casualties (8, 9), so it would be of obvious value if the clinical laboratory could assist in the diagnosis of cyanide poisoning in these patients. Furthermore, cases of severe cyanide poisoning due to (e.g.) industrial exposure or self-administration may turn up in hospitals and cause diagnostic problems (12, 13). Determination of blood cyanide has been advocated for the evaluation of cyanide exposure, but most current analytical methods are so slow that the results are not available to the emergency department in an acceptable time (3, 5, 9). Faster methods have been reported (14, 15), but they give only semiquantitative results and require equipment that is not available in most clinical chemistry laboratories.

We have described methods for determination of cyanide in blood in which cyanide is measured colorimetrically (16) or fluorimetrically (17) by the König reaction. The latter also responds to thiocyanate (18), a compound normally present in blood in fairly high concentrations, so cyanide had to be separated from thiocyanate by aeration from the acidified sample. Unfortunately, this step takes about 2 h to perform and requires special equipment. However, cyanide in blood is almost exclusively localized to the erythrocytes (16, 19), whereas thiocyanate is confined to plasma (20). This enabled us to develop a rapid method for determination of toxic concentrations of cyanide in blood.

**Materials and Methods**

Dicobalt edetate was obtained as the commercial antidote “Kelocyanor” from Lipha Pharmaceuticals Ltd., West Drayton, England.

**Assay Reagents**

- Sodium nitrite, 0.5 mol/L.
- Isotonic saline (NaCl, 0.15 mol/L).
- Perchloric acid, 0.33 mol/L.
- Sodium acetate, 1 mol/L.
- Sodium hypochlorite, 50 mmol/L. Dilute 1.0 mL of NaClO, 0.5 mol/L in 0.1 mol/L NaOH (reagent no. 23039; BDH Chemicals, Poole, England), to 10 mL with de-ionized water.
- Barbituric acid–pyridine reagent. Dissolve 6.0 g of barbituric acid in a mixture of 30 mL of pyridine and 64 mL of water, and add 6 mL of concentrated HCl.
- Potassium cyanide standard, 1 mmol/L.

**Procedure**

Transfer 1.0 mL of blood (with either heparin or EDTA as anticoagulant) to a tapered plastic centrifuge tube, add 0.05 mL of 0.5 mol/L sodium nitrite, and let stand for 2 min. Add 5 mL of isotonic saline and centrifuge for 3 min at about 1000 × g. Discard the supernate, wash the precipitate by centrifuging with 5 mL of isotonic saline, and add saline to give a 1.0-mL final volume. Vortex-mix, add 3.0 mL of 0.33 mol/L perchloric acid, mix, and let stand 2 min. Centrifuge and filter the supernate through a small plug of glass wool in a Pasteur pipette. Transfer 2.5 mL of the filtrate to a test tube, add 0.5 mL of 1.0 mol/L sodium acetate, mix, and add 0.15 mL of 0.05 mol/L NaClO. After mixing, add (within 1 min) 0.5 mL of the barbituric acid–pyridine reagent and determine the absorbance in a 1-cm cuvet at 580 nm 5 to 15 min later.

The value obtained is corrected for a blank in which water substitutes for the sample. Read the cyanide concentration from a standard graph, prepared with data for known amounts of 1 mmol/L KCN standard added to erythrocytes. The latter are obtained from normal blood treated with nitrite as described above, and reconstituted with saline to the original volume of blood. We recommend 0, 25, 50, 75, and 100 μmol/L final concentrations of cyanide for the standard curve. Because the standard curve is very reproducible, one need not prepare a standard curve for each run of patients’ samples. However, a control, made by adding cyanide to nitrite-treated erythrocytes from normal blood, should be included with each assay. This control is stable for at least one month if stored at −20 °C.

**Other Methods**

Methemoglobin was determined according to Evelyn and Malloy (21), and thiocyanate as previously reported (18).

**Results**

**Procedure development:** Preliminary experiments demonstrated that cyanide bound to erythrocytes was liberated when the latter were treated with perchloric acid. Furthermore, cyanide in the deproteinized extract could be determined colorimetrically by the König reaction, after partial neutralization of the perchloric acid and increasing the concentration of hypochlorite in the chlorinating step by about 25-fold as compared with the earlier method (16). However, this assay could not be applied to whole blood, because samples of normal blood gave very high values for cyanide, which were related to the thiocyanate content of the blood. If erythrocytes from normal blood were washed twice with saline, determination of their cyanide content gave much lower values, and additional washing did not
depress their apparent cyanide content further. However, cyanide added to normal blood was incompletely accounted for in the washed erythrocytes, and the yield varied with different blood samples (results not shown). Cyanide is known to be trapped in the erythrocytes by methemoglobin (16), so we explained our results by the low and variable content of endogenous methemoglobin in normal blood (22). Consequently, we tried to increase the trapping of cyanide in the erythrocytes by converting hemoglobin to methemoglobin with inorganic nitrite (23). Complete conversion was obtained with a final concentration of 24 mmol of nitrite per liter and a reaction time of 2 min (Figure 1). Standard curves obtained with erythrocytes from different samples of normal blood treated with nitrite now gave very similar slopes and intercepts and were linear up to a blood cyanide concentration of at least 100 μmol/L.

Values for normal subjects: Blood cyanide concentrations measured with the present method in 10 normal, non-smoking subjects (five men and five women) gave a mean value of 1.9 (SD 0.64) μmol/L.

Sensitivity, precision, and recovery: For reasons presented in the Discussion, we interpret the results obtained on blood from normal subjects as being equivalent to reagent blanks. If we defined the detection limit of the method as the cyanide concentration corresponding to twice the standard deviation of blank determinations, a value of 1.0 μmol/L was obtained. Within-day precision and analytical recovery were evaluated by analysis of 10 replicate samples of normal blood supplemented with cyanide to give a final concentration of 50 μmol/L. The mean result obtained, 46.4 (SD 0.81) μmol/L, corresponded to a yield of 93% and a CV of 1.7%. The between-day precision could not be evaluated, owing to the instability of cyanide in blood, stored at 4°C or room temperature (16). Storage at −20°C, where cyanide is stable, would cause hemolysis.

Interference: When we added increasing concentrations of thiocyanate to blood from a non-smoking normal subject, the apparent cyanide content of the blood increased very little (Table 1). Thus an increase of plasma thiocyanate from the value for a non-smoker (53 μmol/L) to 153 μmol/L, a value often found in smokers (24), increased the apparent cyanide content by only 0.6 μmol/L. Enhancing plasma thiocyanate to about 500 μmol/L, a value sometimes found in subjects heavily exposed to cyanide from cassava (25), increased the apparent blood cyanide by only about 4 μmol/L (Table 1). Although cyanide reportedly may be formed from glycine by hypochlorite (26), we found that addition of 100 mmol of glycine per liter to normal blood gave no increase in its apparent cyanide content. Furthermore, we investigated the effect of cyanide antidotes previously reported to interfere with cyanide determinations (27,28). The antidotes were studied at concentrations expected in blood after therapeutic doses of the antidote (4). When dicyclohexylamine (final concentration, 100 μmol/L) or sodium thiosulfate (final concentration, 5 mmol/L) were added to blood containing 50 or 200 μmol of cyanide per liter, no interference was observed.

Method comparison: When blood samples with different cyanide content were analyzed by the present method and by our previously described method (a) (16), the results (μmol/L) were in excellent agreement: y = 0.998x – 0.18 (r = 0.999).

Discussion

The key feature of our method is the trapping of all cyanide in the sample as cyanomethemoglobin by treating the whole blood with nitrite. We have previously demonstrated (16) that substantial amounts of cyanide appear in plasma only when the binding capacity of the erythrocytes for cyanide has been exceeded. Erythrocytes bind cyanide as a result of their methemoglobin content (16). If the latter is substantially increased by treating blood with nitrite, any cyanide in plasma should be trapped in the erythrocytes and measured by our analytical procedure. The latter thus measures cyanide in blood and not in erythrocytes per se. It may be noted in this context that an analytical method for cyanide has been reported (29) in which methemoglobin was used as a trapping agent, but in this procedure methemoglobin is added to the sample.

The cyanide content of normal blood as determined by the present method, about 2 μmol/L, is considerably higher than the value 0.1 μmol/L found by our earlier reported methods (16, 17). We have observed that washed erythrocytes from normal subjects, when treated with perchloric acid, liberate a compound that forms a König chromogen that has an absorption spectrum identical with that formed from cyanide or thiocyanate. We believe that thiocyanate is responsible for this interference and that erythrocytes may contain protein(s) that strongly bind(s) thiocyanate similar to serum albumin (30). On the other hand, the present method is insensitive to cyanide antidotes that interfere in other methods (27,28) for cyanide assay. Whereas the insensitivity of our method to thiosulfate may be explained by the fact

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<th>Table 1. Effect of Thiocyanate on Cyanide Assay</th>
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<td>Thiocyanate in plasma</td>
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To a blood sample from a non-smoking subject, containing 53 μmol of endogenous plasma thiocyanate per liter, we added thiocyanate to give the final concentrations listed in the table. Cyanide was then determined as described in Methods.
that thiosulfate is localized extracellularly (31)—and thus removed when erythrocytes are washed—we have no explanation for the absence of interference by dicobalt edetate.

The present method was designed for measuring toxic levels of blood cyanide, so its measuring range was based on estimations that non-fatal toxic effects from cyanide appear at blood cyanide concentrations around 20 to 50 μmol/L and that the threshold level for fatality exceeds 100 μmol/L (6, 32). Results obtained with our method are available within 30–45 min and should indicate whether a victim suffering from cyanide intoxication requires treatment with cyanide antidotes. In the case of fire victims, the lack of a rapid method for determining blood cyanide has led to proposals that determination of carboxyhemoglobin in blood (3) or thiocyanate in plasma (33) may be used as alternatives to determination of blood cyanide. However, the poor correlations found in fire victims between blood cyanide and carboxyhemoglobin (11, 34) or thiocyanate (6) seem to invalidate these proposals.

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References