Stabilization of Blood Cyanide

To the Editor:

The measurement of blood cyanide is of clinical importance because, aside from the relatively few cases of accidental or self-induced poisoning, raised levels are found in patients receiving the hypotensive agent sodium nitroprusside (1), in victims of smoke inhalation injury (2), and in populations at risk of ataxic myelopathy in areas of high cassava consumption (3). However, very few hospital laboratories have the means of measuring cyanide, and blood samples may need to be transported to one offering the service. As losses of cyanide may occur (4,5) in transit, resulting in underestimation, a stabilizing technique would improve analytical accuracy.

In one procedure, the blood is added to acidified silver sulfate. By subsequently increasing the acid concentration, the cyanide can be liberated from the silver cyanide and transferred to a small amount of sodium hydroxide solution with a stream of nitrogen (6). In another method, a small volume of sodium nitrite solution is added to the blood so that cyanide is retained as cyanmethemoglobin. It is possible then to separate the red cells, which contain all of the cyanide, and measure the cyanide in a protein-free extract without recourse to nitrogen aeration (7).

A modification of the second procedure was adopted for a study of cyanide levels in patients with smoke inhalation injury admitted to casualty departments throughout the U.K. because blood samples were sent to the laboratory by mail.

In order to determine the best anticoagulant/nitrite combination, 20 pL of a 50% aqueous solution of sodium nitrite was added to 5-mL tubes containing four different anticoagulants (L.I.P. Shipley, West Yorkshire, U.K.) and dried in vacuo over anhydrous calcium chloride. Twenty-five microliters of an isotonic saline solution of KCN (approximately 10mM) was added to 5 mL of blood in each of the 12 tubes (3 for each anticoagulant) and to two plain tubes containing 5 mL of 0.1M NaOH and stored at room temperature. The amount of hemolysis was checked, and the recovery of cyanide was measured daily for three days. Aliquots (0.5 mL) of blood from each tube were transferred to 2-mL graduated microcentrifuge tubes (Camlab, Cambridge, U.K.) and made up to 2 mL with isotonic saline. Following centrifugation at 12,000 rpm for 5 min, the cells were washed three more times with 2 mL of saline. The volume was then restored to 0.5 mL with saline and 5% trichloroacetic (TCA) layered on top, up to the 2-mL mark. Similarly, trichloroacetic acid (TCA) was added to 0.5 mL aliquots of the saline KCN diluted in NaOH. Following vortex mixing and centrifuging, the cyanide content of the TCA extracts was determined by means of an automated version of the Aldridge technique (8). The microcentrifuge tubes remained closed until immediately before analysis. It was apparent from the initial saline wash on the third day that a small amount of hemolysis had occurred in the blood samples anticoagulated with heparin and with fluoride-oxalate. Because the citrate/nitrite combination produced no visible hemolysis and gave a reliable retrieval of cyanide (Figure 1), it was adopted for our study.

![Figure 1. Recovery of cyanide from 5-mL blood samples spiked with 25 pL of approximately 10mM saline solution of KCN and stored in tubes containing sodium nitrite together with different anticoagulants. Fluoride-oxalate – x –; sodium citrate – ● –; potassium EDTA – ○ –; lithium heparin – △ –. Each point is the mean of three measurements for the blood samples and two for the diluted saline KCN (25 pL in 5 mL 0.1M NaOH) – □ –.](image)

<table>
<thead>
<tr>
<th>Days in</th>
<th>Aq KCN</th>
<th>n</th>
<th>Blood Samples</th>
<th>Mean recovery %</th>
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<tbody>
<tr>
<td></td>
<td>(mol/L)</td>
<td></td>
<td>µmol/L x (±SD)</td>
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<tr>
<td>0</td>
<td>82.7</td>
<td>5</td>
<td>85.8 (2.6)</td>
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<tr>
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<td>1</td>
<td>90.8</td>
<td>102.4</td>
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<tr>
<td>2</td>
<td>88.7</td>
<td>3</td>
<td>88.7 (1.0)</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>88.3</td>
<td>3</td>
<td>90 (1.4)</td>
<td>100.9</td>
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</table>
Table II. Cyanide Recovery from Spiked Nitrited Blood Stored at 5°C

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>7*</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCN concentration (μmol/L)</td>
<td>AqKCN±</td>
<td>CELLS±</td>
<td>AqKCN±</td>
</tr>
<tr>
<td>SD</td>
<td>1.71</td>
<td>4.79</td>
<td>2.62</td>
</tr>
<tr>
<td>% Recovery</td>
<td>98.3</td>
<td>98.2</td>
<td>99.5</td>
</tr>
</tbody>
</table>

* On day 7, the blood was divided into 0.5-mL aliquots and the cells washed and stored in saline for the rest of the time.
* Mean of 4 samples of diluted saline KCN.
* Mean of 20 results, 2 from each blood sample.

The methemoglobin-forming capacity of the citrate/nitrite combination was investigated by transferring 5-mL samples of freshly drawn venous blood to eight of the tubes. Then, two 0.5-mL aliquots from each tube were placed in 2-mL graduated microcentrifuge tubes, and the cells were washed as described four times with isotonic saline in order to remove nitrite from its combination with methemoglobin (9). After the volume was restored to 0.5 mL with saline, the methemoglobin content was measured on a IL482 co-oximeter (Instrumentation Lab) and found to be 89.9% (5.9) (x ± SD). When repeated with citrate/nitrite tubes stored at 5°C for 3 1/2 years, a methemoglobin value of 96.3% (1.2) was obtained.

In order to check the stability of cyanmethemoglobin in blood samples sent by mail, 1 mL of saline KCN (approximately 6 mM) was added to 60 mL of venous blood, and 5-mL aliquots were transferred to 12 citrate/nitrite tubes. One milliliter of the saline KCN was also diluted with 0.1M NaOH to act as standard. Seven of the tubes containing cyanide-spiked blood were individually placed in snap-closure mailing containers (Whatman International, Maidstone, U.K.) enclosed in sealed plastic envelopes and mailed in padded SAEs from various localities. All samples were received intact over a period of three days and the cyanide content measured in duplicate 0.5-mL aliquots, as described, on the day of receipt. Duplicate samples of the diluted saline KCN were also analyzed at the same time. Good recoveries were obtained (Table I).

The stability of cyanmethemoglobin in blood samples stored at 5°C was also determined. Blood was transferred to 11 5-mL citrate/nitrite tubes. Ten of these samples were spiked with 25 μL of an isotonic saline solution of KCN, and 25 μL of cyanide-free saline was added to the remaining tube to act as a blank. An aliquot of the saline KCN solution was also diluted with 0.05M NaOH to provide a reference solution against which recoveries of cyanide were compared. Duplicate 0.5-μL samples of blood from each of the 11 citrate/nitrite tubes, together with four samples of the diluted KCN solution, were analyzed as described here at the 3 days indicated in Table II. For the first seven days the whole blood was stored at 5°C, then it was transferred in 0.5-mL aliquots to 2-mL microcentrifuge tubes and the cells washed, and stored in isotonic saline for a further 16 days at 5°C. Consistent recoveries of cyanide were obtained (Table III).

Thus, a combination of sodium citrate with sodium nitrite (17 mg and 10 mg, respectively, in 5 mL blood) provides a simple method for stabilizing cyanide in blood samples that need to be transported. Because the cyanide is confined to the red cells as cyanmethemoglobin, the cells can be isolated and washed with saline to remove nitrite and thiocyanate, and the cyanide can be measured in a trichloracetic acid extract with a good recovery and shortened assay time. In addition, the cyanmethemoglobin remains stable in saline washed cells for at least three weeks at 5°C, which allows for repeat analyses to be made when necessary.

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References


