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Measurement of Blood Cyanide with a Microdiffusion Method and an Ion-specific Electrode

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The use of a cyanide ion-specific electrode in combination with the Conway microdiffusion method was modified for the measurement of cyanide concentration in human red blood cells and plasma. With our modified method, the optimal *p*H of cyanide isolation from red blood cells and plasma was investigated. Cyanide recovery from red blood cells increased with decreasing *p*H. The maximal recovery of 96.9 ± 2.6% was obtained at a *p*H of less than 1. Cyanide recovery from plasma, however, peaked at a *p*H between 7 and 8, and further changes in *p*H reduced the recovery rate. The maximal recovery rate from plasma was 74.1 ± 1.5%. In previous studies, cyanide isolations from both plasma and red blood cells were carried out at a *p*H of less than 1. This study shows that cyanide isolation from plasma should be performed at a *p*H between 7 and 8. (Key words: Measurement technique: cyanide, ion-electrode, microdiffusion. Toxicity: cyanide.)

THE RISK OF CYANIDE intoxication during sodium nitroprusside infusion has been well established.[1] There are numerous clinical situations in which a relatively rapid determination of cyanide values would be desirable; these situations include prolonged infusion of nitroprusside in postcardiac surgery patients,[2] hypothermic extracorporeal circulation,[3,4] and surgery on patients who are smokers.[5] Among many methods currently used for measuring blood cyanide concentration, a microdiffusion method[6] with direct potentiometric measurement[7] has many advantages. The microdiffusion method is readily available and easily set up and accommodates many samples. A direct potentiometric measurement by an ion-electrode is the simplest method for cyanide measurement. A combination of the two methods results in economy and efficiency of technical staff requirements.[8] The ion-specific electrode for a direct potentiometric measurement was developed originally for monitoring cyanide concentration in waste water. None of the reported methods using the electrode with a microdiffusion method can be applied reliably to measure cyanide concentration in blood sample of less than 3 ml.[9–11] With the use of the microdiffusion method reported by Feldstein and Klendshoj[6] for isolating cyanide from plasma, adequate recovery of cyanide was not obtained.

In this study, we report a method of measuring cyanide in plasma and red blood cells (RBC) that uses an ion-specific electrode and a microdiffusion cell. While developing the method to measure cyanide, we found cyanide isolation from a blood sample to be *p*H-dependent. With the method reported in this study, the optimal *p*H for cyanide isolation from RBC and plasma was investigated.

Materials and Methods

Reagents and Apparatus

Reagents were prepared from analytical reagent grade potassium cyanide (KCN), sodium hydroxide (NaOH), and sulfuric acid (H_2SO_4). Standard solutions of 0, 0.1, 0.2, 0.5, 1, and 5 ppm (0, 3.8, 7.6, 19, 38, and 190 μM) cyanide were prepared by adding KCN to 0.1 M NaOH. RBC and plasma samples were prepared from human blood obtained from our blood bank. Packed RBC were centrifuged and separated into plasma and RBC components. The RBC components were further washed with isotonic saline and centrifuged three times. Plasma samples were prepared from outdated fresh frozen plasma. Samples were used immediately after preparation. Polypropylene Conway microdiffusion cells were used for cyanide diffusion. The Orion Research Ionalyzer/901 was used for cyanide measurement with an Orion cyanide ion-specific electrode (model 94-06) and a reference electrode (model 90-01).

Procedures

After 3 ml 0.1 M NaOH was pipetted into the center well of the Conway cell, 3 ml of either RBC or plasma sample containing 1 ppm (38 μM) cyanide as KCN was pipetted into one side of the outer well. Two milliliters of either H_2SO_4 or NaOH were added to the other side of the outer well to adjust the *p*H of the sample from 0.8 to 13.2. The cell was immediately covered with a lid and the added acid or alkali was mixed completely with the

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sample by tilting gently. Hydrogen cyanide (HCN) gas liberated from the mixture was captured by the NaOH solution in the center well. The seal was made with 1 ml 0.01 M H_2SO_4 solution pipetted into the outermost well so that gas would not readily dissolve in the liquid used to seal the lid. The microdiffusion was allowed to proceed for 4 h at room temperature.

Cyanide recovery from a KCN solution in distilled water also was investigated, in a similar procedure as were the blood samples. In another study, cyanide recovery from plasma by the microdiffusion method was examined for 10, 20, 60, and 120 min without adding either a H_2SO_4 or a NaOH solution, in order to investigate the amount of cyanide released from plasma at a physiologic *p*H during the storage period after sampling.

An additional experiment was performed to examine why the recovery of cyanide from plasma was much less at a low *p*H than that of RBC. A 20-ml plasma sample was pretreated with 1.6 ml 10 M H_2SO_4 (acid denaturization) and then neutralized with 1.6 ml 10 M NaOH prior to an addition of KCN. Another 20-ml plasma sample was pretreated with 2 ml 1 M sodium borohydride in 1 M NaOH for 24 h at 40° C (to cleave carbonyl groups in plasma protein) and then neutralized by 1 M hydrochloric acid.[12,13] Hydrogen sulfide produced in the process was eliminated by bubbling oxygen through the sample. Cyanide recovery from the samples at a *p*H of less than 1 was measured by the method described above.

Electrode Preparation and Calibration

The ion-specific and reference electrodes were placed in 1 ppm (38 μM) cyanide standard solution until a stable reading was obtained before serial sample measurements. Prior to each measurement the electrodes were standardized in 1 ppm (38 μM) cyanide standard solution for 1 min. The cyanide measurement was made by placing the electrodes in the center well of the Conway cell, and the potential of the electrodes was recorded 2 min after immersion.

For cyanide concentrations above 0.2 ppm (7.6 μM), the calibration curve was linear if plotted on semilogarithmic paper. When the concentration fell below 0.2 ppm (7.6 μM), the curve yielded a straight line on a linear graph. A correlation coefficient of both calibration curves was −0.999. The precision of electrode measurement, expressed as coefficient variation, was within 4.0% for cyanide concentration at 1 ppm (38 μM) in this study.

All results were reported as means ± SEM. Student's *t* test was used to compare mean maximal cyanide recovery, with mean recovery at the *p*H points nearest the peak. The Bonferroni method was used to adjust *P* values for this set of multiple comparisons. A *P* value less than or equal to 0.05 was considered statistically significant.

Results

Cyanide recovery from RBC samples increased with decreasing *p*H. The maximal recovery, 96.9 ± 2.6% (mean ± SEM: n = 6), obtained at a *p*H of less than 1, was significantly higher than values obtained at other *p*Hs (fig. 1). Two measurements of cyanide recovery from RBC at a *p*H of less than 1 were 102 and 103%. These measured values were within the precision limit of the electrode. Cyanide recovery in plasma peaked at a *p*H of between 7 and 8, and further changes in *p*H reduced the recovery rate. The maximal recovery was 74.1 ± 1.5% (n = 6), which was significantly higher than either 57.3 ± 3.7% (n = 6) at *p*H between 6 and 7, or 59.1 ± 4.6% (n = 6) at *p*H between 8 and 9 (fig. 1). The recovery rate in the KCN solution in distilled water increased with decreasing *p*H and leveled off at a *p*H of less than 9. (fig. 2) After 10, 20, 60, and 120 min of microdiffusion, the cyanide released from plasma at a *p*H of 7.5 was 18.7 ± 0.8, 30.0 ± 1.4, 51.0 ± 3.3, and 62.0 ± 3.5%, respectively. (fig. 3)

Cyanide recovery from plasma pretreated with acid or sodium borohydride was 53.6 ± 1.9 (n = 10) and 88.0 ± 2.5%, respectively. Pretreatments significantly increased cyanide recovery from plasma, compared to the recovery of 45.9 ± 1.5% from untreated plasma at a *p*H of less than 1.

Discussion

Most of the reported methods for blood cyanide measurement require two steps; the first step is to isolate cyanide from the blood sample by acidification, and the second is to quantify the isolated cyanide. In the current study, the Conway microdiffusion and the direct potentiometric method using the cyanide ion-specific electrode

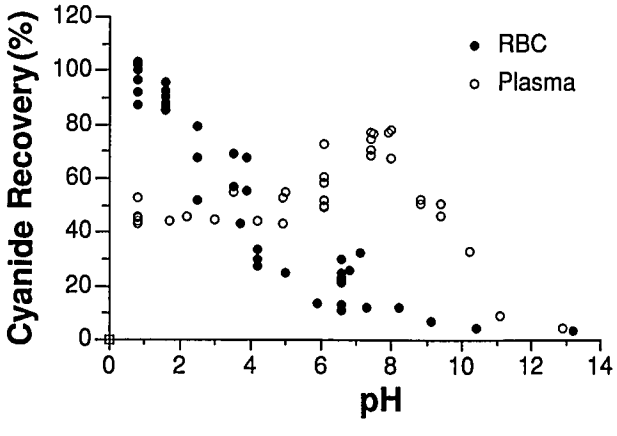

FIG. 1. Recovery rate of cyanide added to RBC and plasma after 4 h of microdiffusion at room temperature.

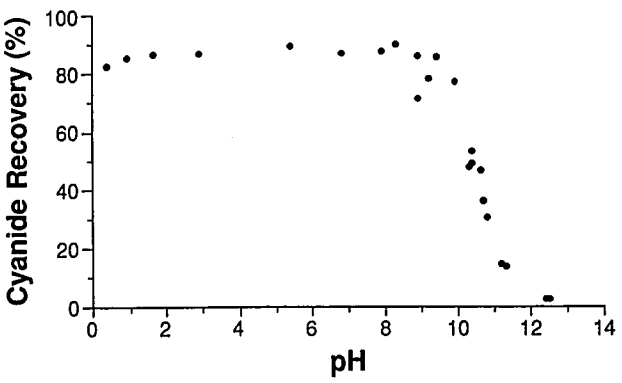

FIG. 2. Recovery rate of cyanide from KCN solution after 4 h of microdiffusion at room temperature.

were used as the first and the second step, respectively. In the first step, when an acid is added to a sample containing cyanide ions, the following reaction takes place: $CN^- + H^+ \rightarrow HCN$.

One molar or stronger H_2SO_4 solution, which makes the *p*H of the sample less than 1, usually has been the choice of agent used to acidify the sample. Since the dissociation constant (pK) of hydrocyanic acid (HCN) is 9.3, HCN is virtually un-ionized at a physiologic *p*H. Cyanide was sufficiently recovered from a KCN solution at a *p*H of less than 9 in this study (fig. 2). However, cyanide was not fully recovered from RBC samples until the *p*H was less than 1. Since it exists as an un-ionized HCN at a physiologic *p*H and is lipid-soluble, HCN can freely cross the cell membrane. In RBC, cyanide is reported to be bound to methemoglobin.[14] This binding may be strong enough to prevent the cyanide from separating from the RBC at a physiologic *p*H range. Hemoglobin begins to dissociate into its individual chains at a *p*H of 6, and this process is complete at a *p*H of 4.8.[15] A degree of hemoglobin dissociation partly explains an increase in cyanide released from RBC at a *p*H of less 6. Another explanation is that acidic conditions cleave heme moieties from hemoproteins as well as denature the protein, and thereby render hemoproteins incapable of binding cyanide.

Cyanide in plasma was not sufficiently recovered when the microdiffusion was carried out at a *p*H of less than 6. Possible explanations are that cyanide ions add to aldehyde and ketone groups under acidic conditions to form cyanohydrins, and that other ionic binding sites also may be generated at a low *p*H. These explanations are supported by the finding that the cleavage of carbonyl groups by sodium borohydride prior to the addition of cyanide resulted in a large increase in cyanide recovery (from 45.9 to 88.0%). The effect of the denaturation of plasma with acid on the cyanide recovery was relatively small.

In most previous studies,[14,16,17] isolation of cyanide from plasma was performed at a *p*H of less than 6, and cyanide added in plasma could not be recovered quantitatively. The current study showed the optimal *p*H for the cyanide release from plasma to be between 7 and 8.

A rapid disappearance of measurable cyanide added to plasma has been reported.[18,19] This disappearance was attributed to the reaction of cyanide with serum albumin.[14] The reported disappearance curve of measurable cyanide from plasma[18,19] is a mirror image of that in figure 3 in this study. Our results strongly suggest that the reported decrease in cyanide in plasma is due not to the reaction with albumin, but to the liberation of cyanide into the air during the storage period.

Kistner *et al.*[20] questioned the validity of ion-specific electrodes when placed directly in RBC and plasma the *p*H of which was not measured. Since the electrode detects only cyanide ions and not un-ionized HCN, the *p*H of a sample must be higher than the value of the dissociation constant for HCN (pK = 9.2).[21] For quantitative conversion to cyanide ion (*i.e.*, 99% or better), a *p*H = pK + 2 is required.[9] The *p*H of 0.1 M NaOH solution, used for absorbing the released cyanide in this study, was over 11.5. If the electrodes were used directly in blood samples of physiologic *p*H, the errors would be considerably higher even if there were not the interference of the proteinaceous deposits on the electrode.

The early signs of cyanide toxicity have been reported to be at a plasma concentration of 0.26 ppm (10 μM).[17] The sensitivity of the ion-specific electrode used in this study is from 0.03 to 26 ppm (1–1,000 μM) cyanide, so the method is sensitive enough to detect the minimal toxic level of plasma cyanide.

In the current method, the time required for electrode stabilization was shortened to less than 5 min from the previously reported 30 min by using 1 ppm (38 μM) cyanide standard solution instead of 0.1 M NaOH solution.[7,22]

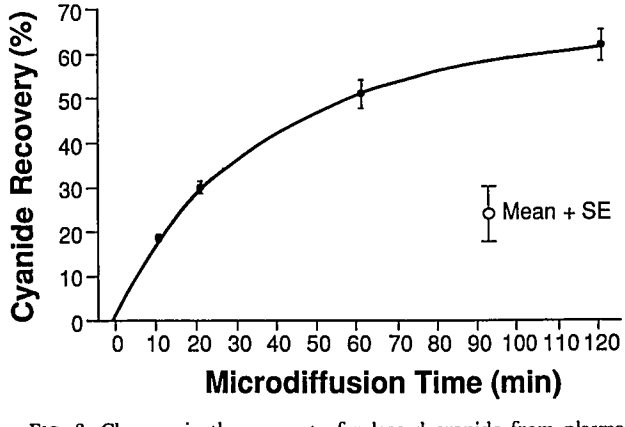

FIG. 3. Changes in the amount of released cyanide from plasma containing 1 ppm cyanide. Microdiffusion was carried out at a *p*H of 7.5 at room temperature.

Equilibration time for the electrode depends on the cyanide concentration of the sample and ranges from 1 min at 0.26 ppm (10 μM) to as much as 10 min at 0.026 ppm (1 μM).¶ The equilibration time also is influenced by the cyanide concentration in the immediately preceding sample. In this study, the electrode was standardized in 1 ppm (38 μM) cyanide solution for 1 min before each measurement, and the electrode potential was recorded at 2 min after the immersion of the electrode in the sample. With these modifications, the daily calibration resulted in a correlation coefficient of 0.999. The manufacturer's instruction¶ recommends stirring the sample during the measurement. Since a small sample (less than 3 ml) was difficult to stir and stirring the sample caused a significant drift in the electrode potential, sample was not stirred in this study.

In this study, cyanide-containing samples were kept in the microdiffusion cells for 4 h, in order to ensure complete recovery as originally reported.[7] However, in case of emergency, the microdiffusion period can be shortened to 20 min. After 20 min of microdiffusion, the recovery rate of cyanide from RBC and plasma was 28.3 ± 2.0% and 30.0 ± 1.4%, respectively. If 30% of the cyanide in blood can be recovered by 20 min of microdiffusion, 0.15 ppm (5.7 μM) cyanide can be detected from a blood sample containing 0.5 ppm (19 μM) cyanide, a value reported to be detected in a smoker without cyanide-related symptoms.[23] This concentration is still sufficiently higher than the lowest detectible limit of 0.03 ppm (1.1 μM) of the cyanide-specific electrode. Thus, even if the result obtained by 20 min of microdiffusion would be semi-quantitative, it could be valuable enough to determine whether a patient had been exposed to cyanide or not.

In summary, we report the use of a cyanide ion-specific electrode in combination with the Conway microdiffusion method for the measurement of blood cyanide concentration. Cyanide recovery from RBC and plasma is *p*H-dependent. The maximal cyanide recovery from RBC is obtained at a *p*H of less than 1, a *p*H level that usually has been used in the microdiffusion method. For plasma samples, however, the optimal *p*H is between 7 and 8, a finding that has not been reported previously.

¶ Instruction manual cyanide ion electrode model 94-06, ORION RESEARCH, Cambridge Massachusetts.

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