Determination of Cyanide and Nitroprusside in Blood and Plasma

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A procedure was refined for quantitative isolation of cyanide by gas transfer from acidified blood or plasma samples. The cyanide was trapped in dilute alkali and quantified as the pyridine/pyrazolone complex. The within-day coefficient of variation was 2%, which increased to about 2.5% for the day-to-day variation. Nitroprusside was used as a hypotensive agent in clinical medicine provides a risk of cyanide toxicity when the rate of administration or the total amount of drug given is excessive. A procedure was developed for measuring nitroprusside in the plasma of man and animals. Nitroprusside in the sample is quantitatively converted to cyanide by incubation with cysteine solution at slightly alkaline pH. Methemoglobin is added to combine with the cyanide formed and prevent its destruction. On acidification, the total amount of cyanide originally present as free cyanide or as nitroprusside is liberated as HCN, isolated by gas transfer into a sodium hydroxide trap, and quantified by spectrophotometry. Nitroprusside present in the sample is calculated from the increase in cyanide observed in the cysteine-treated sample compared to that obtained without cysteine treatment. The method has been used to estimate in vitro stability of nitroprusside in aqueous solution, blood, and plasma. Blood cyanide and plasma nitroprusside concentrations were measured when sodium nitroprusside was infused into a baboon. Over 90% of the nitroprusside in blood is present in the plasma, suggesting that the drug crosses the erythrocyte membrane slowly.

Additional Keyphrases: toxicity · methemoglobin · cysteine · hypotension · analysis by gas transfer from the sample · cyanide absorption from fire gases

Cyanide is an extremely toxic agent by virtue of its inhibition of cellular respiration. In fires where polyurethane materials pyrolyze or burn, hydrogen cyanide (HCN) is released (1). Breathing such contaminated gas leads to absorption of cyanide in the blood, mostly from the gas phase rather than from HCN in water droplets (2). A reliable and sensitive procedure for estimation of cyanide in blood was needed, to assess the extent of cyanide absorption from fire gases. We were unable to obtain sufficient sensitivity and reproducibility by the microdiffusion procedure of Feldstein and Klandshoj (3) but were able to do so by simple modification of the method of Boxer and Rickards (4).

Sodium nitroprusside, sodium nitrosylpentacyanoferrate (III), is a potent, rapid-acting hypotensive agent and is a potential source of cyanide when given intravenously or orally. It is currently being used extensively in clinical medicine in the treatment of malignant hypertension (5), refractory heart failure (6), myocardial infarction (7), and to produce elective hypotension under anesthesia (8, 9). Sodium nitroprusside is known to be decomposed in the animal body with the formation of cyanide (10–12). Several deaths have been reported in patients receiving nitroprusside (13, 14). All of these deaths were compatible with the clinical picture of cyanide poisoning. Animals given lethal doses of nitroprusside die of cyanide poisoning (15, 12).

In view of the danger of cyanide intoxication from nitroprusside administration, the measurement of this drug in blood or plasma is desirable. The present study was undertaken to improve the procedure for cyanide analysis and to develop a method for estimating nitroprusside in blood or plasma. The method has been applied to measure blood cyanide and changes in drug concentration during nitroprusside infusion into baboons.

Principle

Few analytical methods for the determination of nitroprusside are available in the literature. The spectrophotometric procedures that have been described (16, 17) and the colorimetric isophorone complex procedure (18) are not sufficiently sensitive for measurement of the low concentrations of the drug in plasma that are compatible with life. Hill (10) demonstrated that in vitro incubation of nitroprusside with blood resulted in its conversion to cyanide, which could be quantitatively recovered by acidification and steam distillation. Smith and Kruszyna (11) provided evidence that the "biological" yield of cyanide from nitroprusside injected into animals was 80%, i.e., per mole, nitro-
prusside is fourfold as toxic as cyanide. A similar molar relation for the toxicity of nitroprusside and KCN in guinea pigs was reported earlier by Mahaffey (19). The present method depends upon the reaction of nitroprusside with cysteine under mildly alkaline conditions, in the presence of methemoglobin, which traps all cyanide released during the reaction. Subsequent acidification of the reaction mixture releases the cyanide as HCN, which is quantified through isolation and spectrophotometric estimation by use of a minor modification of the method of Boxer and Rickards (4). The observed values of cyanide derived from nitroprusside must be corrected for the pre-existing free cyanide as measured on the same sample without reaction with cysteine.

Materials and Method

Reagents

The reagents used were prepared from de-ionized water and analytical-reagent grade chemicals unless otherwise specified. Reagents for the isolation and measurement of cyanide are required in addition to the cysteine and methemoglobin reagents used to decompose the nitroprusside.

**Sodium hydroxide, 50 mmol/liter.** Dissolve 2.0 g of NaOH in 1 liter of water.

**Sulfuric acid, 0.5 mol/liter.** Add 3.0 ml of concentrated H_{2}SO_{4} to 97 ml of water.

**Monobasic sodium phosphate, 1.0 mol/liter.** Dissolve 13.8 g of NaH_{2}PO_{4}·H_{2}O in 100 ml of water.

**Chloramine T, 2.5 g/liter.** Dissolve 0.25 g of Chloramine T (Eastman Reagent No. 1022; Eastman Kodak Co., Rochester, N.Y. 14650) in 100 ml of water and store the solution at 4 °C.

**Saturated aqueous solution of 1-phenyl-3-methyl-5-pyrazolone (Pfaltz and Bauer, Inc., Flushing, N.Y., 11368).** Keep the saturated solution with excess solid present in a brown bottle at room temperature.

**Pyridine solution of bis(1-phenyl-3-methyl-5-pyrazolone), 1.0 g/liter.** Dissolve 10 mg of bis(1-phenyl-3-methyl-5-pyrazolone) (Eastman Reagent no. 6969; Eastman Kodak Co.) in 10 ml of pyridine (Reagent no. P-368; Fisher Scientific Co., Fairlawn, N.J. 07410). This is essentially a saturated solution and is prepared freshly as needed.

**Silicone fluid, Dow Corning 360 fluid (Dow Corning Corp., Midland, Mich. 48640).**

**Potassium cyanide stock standard solution, about 15 mmol/liter.** Dissolve 100 mg of KCN in 100 ml of 0.1 mol/liter NaOH. Standardize the KCN solution by titration with standard 0.01 mol/liter AgNO_{3} in the presence of ammonia and KI (20) to obtain the actual concentration of the stock solution. Do the titration by taking 10.0 ml of the stock KCN, 1.0 ml of concentrated ammonia, and 0.1 g of KI. Titrate this mixture with standard 0.01 mol/liter AgNO_{3} in water until the first permanent turbidity appears. Correct the titration values for an end-point blank, determined by titration of 20 ml of H_{2}O, 1.0 ml of NH_{4}OH, and 0.1 g of KI with the AgNO_{3} solution. Store the stock KCN solution in a polyethylene bottle with a polyethylene cap.¹

**Potassium cyanide working standard, about 15 μmol/liter.** Dilute the stock KCN solution 1000-fold with the 50 mmol/liter NaOH, i.e., 0.05 ml of the stock solution is diluted to 50 ml with the 50 mmol/liter NaOH (reagent first described above). Calculate the actual concentration of the working standard from the dilution factor and the value obtained by standardization of the stock standard solution.

**Triton X-100 solution.** Dissolve 10 ml of the surfactant Triton X-100 (Rohm & Haas Co., Philadelphia, Pa. 19105) in 90 ml of water. It is sometimes necessary to warm the solution to disperse the detergent completely.

**Sodium hydroxide, 0.5 mol/liter.** Dissolve 2 g of NaOH in 100 ml of water.

**Tris chloride buffer, 0.2 mol/liter, pH 7.6.** Dissolve 3.02 g of tris(hydroxymethyl)aminomethane (reagent no. T-370; Fisher Scientific Co.) and 11.80 g of tris(hydroxymethyl)aminomethane hydrochloride (reagent no. 11511, Fisher Scientific Co.) in 500 ml of water.

**Methemoglobin solution, about 50 g/liter.** Human or animal blood anticoagulated with heparin or disodium ethylenediaminetetraacetate is used. The blood must not be contaminated with cyanide or nitroprusside. Prepare the methemoglobin solution by adding 0.5 ml of Triton X-100 solution and 2.5 ml of water for each milliliter of blood. The solution is mixed to ensure complete hemolysis and oxygenation. For each milliliter of blood used, add 2 mg of NaNO_{2} in solid form. Mix the solution by inversion and allow 30 min for methemoglobin formation. The solution has a pH near 7.2–7.5 and is stable for many months when stored at 4 °C.

**Cysteine solution.** Place 50 mg of L-cysteine-HCl·H_{2}O (reagent no. C-562, Fisher Scientific Co.) in a test tube. Add 1 drop of bromcresol green indicator solution. Add 0.5 mol/liter NaOH dropwise until all cysteine is dissolved and the indicator remains blue after mixing, but do not overtitrated. Add 10 ml of the Tris chloride buffer to the neutralized cysteine and mix. The cysteine solution (about 5 mg of cysteine per milliliter at pH 7.6) should be used within 4 h of mixing, because it slowly oxidizes in air.

Glassware and Apparatus

Standard 50-ml Erlenmeyer flasks are used as reaction vessels for both the reaction of the sample with cysteine and for the isolation of cyanide. Plastic test tubes are used to trap the cyanide swept from the reaction vessels with a stream of humidified nitrogen. All steps of the incubation and cyanide isolation are carried out at room temperature. A Dubnoff shaker (Arthur H.

¹ The stock alkali solution of KCN stored in plastic bottles is stable for at least a year. Storage in glass bottles closed with rubber stoppers led to precipitation of silicic acid, and the amount of color developed with the cyanide reaction increased with time. We suggest that the latter may be due to leaching of sulfur from the rubber stoppers, perhaps forming thiocyanate to increase the color produced.
Thomas Co., Philadelphia, Pa. 19105) is used to agitate the reaction mixture during nitrogen transfer of HCN to the alkali traps. Spectrophotometric measurements were made with a Beckman DB-GT spectrophotometer (Beckman Instruments Inc., Fullerton, Calif. 92634). A digital voltmeter (Model 168; Keithley Instruments, Inc., Cleveland, Ohio 44139) was used for visual display of absorbance.

Material for Analysis

Freshly drawn heparinized or ethylenediaminetetraacetate-treated blood is used. Whole blood is analyzed directly for cyanide, because most of the cyanide is present in the cells as cyanmethemoglobin. Plasma is used for nitroprusside analysis, because nearly all free nitroprusside of blood is present in the plasma. To minimize in vitro decomposition of nitroprusside before analysis, the freshly drawn blood is immediately cooled in ice (at the bedside) and centrifuged in a refrigerated centrifuge at 4 °C. Plasma is removed from contact with the cells without delay. The plasma tube is wrapped in aluminum foil to exclude light and stored at 4 °C until analyzed. Fresh whole blood may be analyzed for total cyanide, i.e., the sum of free cyanide plus that available for nitroprusside. This measurement on whole blood is an ideal way to monitor patients on long-term therapy with nitroprusside. Serum is not used for nitroprusside analysis, owing to the prolonged action of the cells during clotting; we have been unable to obtain satisfactory results for nitroprusside in urine samples.

Procedure

Assembly of apparatus: Prepare, for each sample, a 50-ml Erlenmeyer flask fitted with a no. 2, two-hole rubber stopper carrying glass (4 mm o.d.) inlet and outlet tubes. Extend the inlet tube to about 2 cm from the bottom of the flask. Prepare also a plastic tube 17 × 100 mm (Falcon no. 2017; Falcon Division, Becton-Dickinson, Oxford, Calif. 93030) containing 2.0 ml of 50 mmol/liter NaOH and fitted with a no. 1, two-hole rubber stopper carrying glass (4 mm o.d.) inlet and outlet tubes. The bottom tip of the inlet tube of each trap should first be touched to a film of silicone fluid on a watch glass and when in place should extend to the very bottom of the plastic tube. Connect the outlet tube of the flask to the inlet tube of its alkali trap by means of a short length of 1/4 inch i.d. gum-rubber tubing. As many as 12 to 15 such pairs of vessels and traps may be mounted on a Dubnoff shaker and connected to the humidified nitrogen stream in series for cyanide isolation.

Analytical procedure: Each sample to be analyzed for nitroprusside must also be analyzed for pre-existing cyanide. The cyanide that can be isolated by simple acidification of the sample will be called “free cyanide.” That which can be isolated by acidification after the sample is treated with cysteine will be called “total cyanide.” The difference in these two values, called “bound cyanide,” represents the cyanide released by reaction of cysteine with nitroprusside. For convenience, the common aspects of analysis, isolation, and quantification of free cyanide will be described first and any modifications indicated for the estimation of total cyanide.

Isolation and determination of free cyanide: Place the sample (1.0 ml for plasma; 1.0 ml of blood plus 1 ml of water and 0.1 ml of Triton X-100 for blood) in a 50-ml Erlenmeyer flask. Insert the rubber stopper containing the inlet and outlet tubes, using a film of water on the rubber stopper to ensure a gas-tight seal. Connect the outlet from the flask to the inlet of its plastic absorption tube containing 2.0 ml of 50 mmol/liter NaOH. Add 1.0 ml of 0.5 mol/liter H2SO4 to the reaction mixture by means of a syringe with a fine polyethylene tube inserted in the gas inlet tube of the flask. Immediately start a slow flow of humidified nitrogen through the vessel to sweep the HCN into the NaOH trap. Proceed to acidify subsequent samples, attaching the pairs of flasks and alkali traps to the nitrogen gas stream in series. When all samples have been acidified and connected to the gas stream, increase the nitrogen flow to 250 ml/min and shake the flasks on a Dubnoff shaker at 120–140 oscillations per minute for 30 min.

Turn off the N2 and remove the rubber tube connections, beginning with the last flask attached. Remove the stoppers from the absorption tubes, with care to blow any alkali from the inlet tube back into the trap. Droplets of alkali on the walls of the plastic tube are forced to the bottom of the tube by brief centrifugation at low speed.

Spectrophotometric determination of cyanide: Prepare the reagent for chlorination of cyanide by mixing 3 ml of the monobasic sodium phosphate reagent with 1 ml of the Chloramine T solution. Also prepare the pyridine/pyrazolone solution by mixing 10 ml of the pyridine bis-pyrazolone solution with 50 ml of filtered aqueous pyrazolone reagent, described above. Both of these combined reagents should be used within 1 h of mixing.

Use the 17 × 100-mm plastic tubes to prepare a “blank” with 2.0 ml of 50 mmol/liter NaOH and duplicate standards containing 2.0 ml of the working KCN standard solution. The color is developed sequentially in blank, standards, and samples in the alkali traps.

Cool all tubes in an ice bath and add 0.2 ml of Chloramine T–phosphate reagent to each tube, mix briefly on a vortex-type mixer, and allow the chlorination to proceed for 5 min in the ice bath. Add 3.0 ml of pyridine/pyrazolone mixture to each tube, mix briefly on a vortex-type mixer, and allow the color to develop for 30 min at room temperature. Read the absorbance of the solutions at 620 nm with the “blank” solution as reference. Occasional samples may have absorbance values that are too high to read. In this case, the colored solu-

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2 During the 30-min clotting time at room temperature as much as 30% of the nitroprusside present in blood is converted to cyanide. Results obtained are thus difficult to interpret in terms of the cyanide and nitroprusside concentrations of the sample as drawn from the patient.
tion may be volumetrically diluted up to 10-fold with more pyridine/pyrazolone mixture before absorbance measurement.

Calculation: The concentration of cyanide, CN\textsubscript{u}, in \( \mu \text{mol/liter} \), is calculated from equation 1:

\[
\text{CN}_u = A_u \times (d/V_u) \times (V_s \times \text{CN}_u)/A_s
\]  

(1)

In this equation \( A_u \) and \( A_s \) are the absorbances of the unknown and standard, respectively, while \( V_s \) and \( \text{CN}_u \) are the volume (ml) and concentration (\( \mu \text{mol/liter} \)) of the cyanide standard. The volume of sample (ml) from which the cyanide was isolated is \( V_u \) and \( d \) is the dilution factor (if any) of the colored solution before absorbance measurement.

Determination of total cyanide: Place the sample (1.0 ml for plasma; 1.0 ml of blood plus 0.1 ml of Triton X-100 for blood) in a 50-ml flask. Add 0.3 ml of methemoglobin solution and mix. Add 1.0 ml of cysteine solution, insert the rubber stoppers with the inlet and outlet tubes, mix the components together, and allow the mixture to incubate at room temperature for 1 h without agitation. Attach the outlet tube of the flask to the inlet of its absorption tube. Proceed with the acidification of the reaction mixture, isolation of cyanide, and spectrophotometric estimation exactly as given under the section on isolation and determination of free cyanide. The values obtained after reaction with cysteine represent total cyanide.

Calculation of nitroprusside concentration: The combined cyanide, \( \text{CN}_{\text{total}} - \text{CN}_{\text{free}} \), is all derived from nitroprusside. The concentration of nitroprusside, NP, in \( \mu \text{mol/liter} \), is calculated in equation 2:

\[
\text{NP} = (\text{CN}_{\text{total}} - \text{CN}_{\text{free}})/5
\]  

(2)

In this equation \( \text{CN}_{\text{free}} \) represents the \( \mu \text{mol of cyanide per liter} \) recovered from the sample by simple acidification and \( \text{CN}_{\text{total}} \) is the \( \mu \text{mol/liter} \) isolated from the sample by acidification after reaction with cysteine. The factor 5 is used because each micromole of nitroprusside yields 5 \( \mu \text{mol of combined cyanide} \).

Results

Variability of Cyanide Estimation

Preliminary measurements were made to determine the variability of several aspects of cyanide measurement: the most satisfactory standard to use, the best conditions for isolation of HCN by gas transfer, and the optimal conditions for the color development. Shaking the flasks during HCN isolation resulted in complete recovery in 30 min and led to a coefficient of variation of about 2% in the amount of cyanide isolated by gas transfer from known cyanide solutions. Performing the chlorination in an ice bath for 5 min allowed reproducible reaction without loss of the volatile cyanogen chloride, an intermediate in the reaction (4). Reaction of the cyanogen chloride with pyridine/pyrazolone gave maximal color between 20 and 30 min after mixing the reagents, with room-temperature color development. The absorbance of the dye produced decreased by only 1% of its maximum value 60 min after the reaction was initiated. We made a series of 10 measurements of color development on the working cyanide standard under the conditions described. The amount of cyanide used, 27.2 nmol, gave a mean absorbance of 0.571 ± 0.004 (standard error of the mean), with a coefficient of variation of 2%. Day-to-day variation in the color development of standards has been 2.5%.

Release of Cyanide from Nitroprusside

Several measurements were made in which whole blood or hemoglobin solution was incubated with nitroprusside. Free cyanide was released in every case, but quantitative recovery of the bound cyanide from nitroprusside was observed only after prolonged incubation (up to 4 h) at 37 °C. It was then observed that cysteine would also cause the formation of free cyanide from nitroprusside in the presence or absence of hemoglobin. Other experiments showed that free cyanide (KCN) added to the cysteine solution could only be quantitatively accounted for if the cyanide was first converted to cyanmethemoglobin. Hence methemoglobin was added to the sample before addition of cysteine, to trap all cyanide formed during the incubation and prevent its destruction. The effect of cysteine on reaction time with water solutions of nitroprusside is shown in Figure 1. Analytical recovery of the cyanide from nitroprusside was essentially quantitative after 1 h of reaction.

It was also shown that cyanide is not recovered from nitroprusside by simple acidification or when acidified in the presence of cysteine. Ferricyanide and ferrocyanide ions neither yield cyanide on acidification nor do they react with cysteine to form cyanide. Less than 0.1% of the cyanide content of these salts could be accounted for analytically under the conditions in which all of the cyanide is released from nitroprusside.

Stability of Nitroprusside in Saline, Blood, and Plasma

It had already been shown that incubation of whole blood with nitroprusside caused the formation of cyanide. It was therefore necessary to test the stability of nitroprusside added to blood and plasma, to determine which fluid would better reflect the concentration of this ion at the time of sampling. Fresh human blood was treated with disodium ethylenediaminetetraacetate. A portion of the blood was centrifuged to obtain plasma. Pre-existing CN\textsubscript{free} and CN\textsubscript{total} were measured in both blood and plasma. A stock solution of nitroprusside in physiological saline was then added to the blood, plasma, and to a sample of saline itself, to provide the same initial concentration of nitroprusside (6.26 \( \mu \text{mol/liter} \)) in each solvent. The samples were at 24 °C at the time of nitroprusside addition and mixing but were immediately wrapped in aluminum foil to exclude light and cooled in an ice bath. Aliquots of the three samples were removed for free and total cyanide analysis immediately after mixing and again 5 and 24 h later. Our results (Figure 2) show that within the limits of measurement.
there is no free cyanide released from nitroprusside dissolved in saline and the combined cyanide determined corresponds to the theoretical amount present throughout this period. In the case of whole blood, CN$_{\text{total}}$ also corresponds to that in the nitroprusside added, but there is a continued conversion of the combined cyanide to CN$_{\text{free}}$ even when stored at 0 °C in the dark. The increase in blood CN$_{\text{free}}$ approached 3% of the initial combined cyanide per hour. Although not done in this experiment, nitroprusside added to whole blood continues to be converted to CN$_{\text{free}}$ until all nitroprusside is destroyed, but we have not seen a decrease in CN$_{\text{total}}$. Nitroprusside dissolved in plasma is also quantitatively recovered immediately after addition, with only minimal formation of CN$_{\text{free}}$. As in the case of whole blood, a continued increase in CN$_{\text{free}}$ takes place but to less than half the extent in blood. Though the plasma CN$_{\text{free}}$ increased, there was a simultaneous decrease in combined cyanide to such degree that CN$_{\text{total}}$ also decreases. The decrease in CN$_{\text{total}}$ amounted to about 5% of the cyanide added as nitroprusside during 5 h of storage at 0 °C. The data on the stability of nitroprusside show that rapid analysis is imperative if in vitro changes after blood-letting are to be minimized.

Distribution of Nitroprusside Added to Human Blood

Earlier observations had shown that nitroprusside was converted to cyanide much more rapidly by hemoglobin solutions than by whole blood of the same hemoglobin content. This suggested that the erythrocyte membrane might be only slowly permeable to nitroprusside. This hypothesis was tested with fresh human blood anticoagulated with disodium ethylenediaminetetraacetate. To simulate the actual conditions of sampling, we warmed the blood to 37 °C and added a known amount of nitroprusside. The sample was thoroughly mixed and then cooled in an ice bath. A portion of cold blood was immediately centrifuged at 4 °C in a refrigerated centrifuge to obtain the plasma. The CN$_{\text{total}}$ and CN$_{\text{free}}$ were measured in the original whole blood, the blood with added nitroprusside, and the plasma obtained from the supplemented blood. The hematocrit of the blood was also measured. The results are presented in Table 1. It is clear that the nitroprusside added to blood is completely accounted for, though some of the combined cyanide of nitroprusside has been converted to CN$_{\text{free}}$. It is also clear that the concentration of CN$_{\text{total}}$ in plasma is higher than in blood, and most of this is present as combined cyanide. Even that which was measured as CN$_{\text{free}}$ may well have been formed during the approximately 10-min centrifugation. In this sample of blood, if all combined cyanide is in the plasma and all CN$_{\text{free}}$ is present in the cells, the calculated concentration of CN$_{\text{total}}$ in the plasma would be 134 μmol/liter, as compared to the observed 130 μmol/liter.

![Fig. 1. Analytical recovery of cyanide from nitroprusside](image)

Aqueous nitroprusside (5.3 μmol/liter) was incubated with methemoglobin and cysteine at pH 7.6 and 24 °C.

![Fig. 2. Analytical recovery of cyanide from nitroprusside dissolved in saline, blood, and plasma](image)

The nitroprusside was added to the solutions at 23 °C, then immediately cooled and stored in the dark at 0 °C. Free cyanide is represented by the hatched bars, combined cyanide by the open bars. The initial CN$_{\text{total}}$ is fivefold the added nitroprusside concentration in μmol/liter.

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<th>Table 1. Nitroprusside Distribution in Blood$^a$</th>
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$^a$ Sodium nitroprusside (18.2 μmol/liter of whole blood) was added at 37 °C, mixed, and the mixture cooled immediately in ice. A fraction of the blood was then taken to obtain plasma by centrifugation. Hematocrit: 0.416.

$^b$ Calculated assuming that all nitroprusside remained in the plasma.
μmol/liter. Likewise, the calculated concentration in whole blood would be 83.4 μmol/liter, as compared to the observed 83.8 μmol/liter. It thus seems reasonable to believe that undecomposed nitroprusside is present almost exclusively in the plasma. Because this ion does slowly pass into the erythrocyte, it is decomposed rapidly and the free cyanide formed is retained in the cell, probably as cyanmethemoglobin, until the capacity of this sink is exhausted. These data support our suggestion that estimating the combined cyanide in plasma is the preferred measurement for nitroprusside. It also suggests that the combined cyanide observed in blood is attributable only to the nitroprusside present in the plasma of that blood. Free cyanide will be present in plasma only if the blood CNfree was in excess of the cyanmethemoglobin capacity, as might well be the case in fatal cyanide poisoning.

Nitroprusside Infusion into Baboons

A series of anesthetized, mechanically ventilated baboons were being infused with nitroprusside in another study (12), affording us the opportunity to apply the present procedure to measure changes in blood and plasma CNfree and in plasma nitroprusside when sodium nitroprusside is infused into a subhuman primate. Details of animal preparation, ventilation, and monitoring are given in the previous report (12). We obtained arterial blood samples (and plasma) before, during, and after infusion of a solution of 100 mg of anhydrous sodium nitroprusside (382 μmol) and 158 mg NaNO2 (2.29 mmol) in aqueous glucose solution (50 g/liter) into a 33.2-kg male baboon. The sodium nitrite was added in an amount to produce sufficient methemoglobin (21) to combine with all cyanide that could be released from the nitroprusside. Figure 3 shows results of a typical experiment. The infusion of nitroprusside caused a rapid and marked increase in whole blood CNfree, which continued to increase for 1 h after infusion was terminated. There then followed a prolonged period of slower removal of CNfree from the blood. Plasma CNfree was increased, but only during the infusion, and at the end of the infusion was only 1/30th its concentration in the whole blood. These data demonstrate that by far the major portion of blood CNfree was present in the cells, probably in combination with methemoglobin. At the end of this experiment, the animal recovered from the anesthesia and showed no effects of the extremely high blood cyanide. He recovered completely.

Plasma nitroprusside was not detectable in the control sample but was at its highest value 0.5 h later, over which period 70% of the 382 μmol had been infused. Plasma nitroprusside concentration remained high only so long as the infusion was continued. The decline in plasma nitroprusside was rapid, but detectable concentrations were present 3 h after the infusion was completed.

These changes are typical of all our studies of nitroprusside infusion. When the rate of nitroprusside infusion is greater than its rate of conversion to cyanide, the drug may be detected in plasma. When the rate of infusion is lower than its rate of conversion to cyanide, the drug cannot be detected as “combined cyanide” in plasma, but an increased blood CNfree is found.

Discussion

The use of sodium nitroprusside in clinical medicine must be carefully monitored to avoid cyanide intoxication. Nitroprusside has been shown to react with a variety of tissues, most rapidly with blood or hemoglobin solutions (11, 15), with the liberation of cyanide. Swinehart (22) has described a number of reactions of nitroprusside ion with alkali and sulfhydryl-containing compounds. We can find no reactions described in which sulfhydryl compounds react with nitroprusside with the liberation of cyanide. Sullivan et al. (23) described the reduction of disulfide compounds with cyanide ion resulting in the formation of a cyano-derivative and the sulfhydryl derivative in equal amounts. We have observed, in agreement with Pettigrew and Fell (24, 25), that free cyanide ion (KCN) added in plasma cannot be quantitatively recovered by acidification. On the other hand, if methemoglobin is added to the plasma before the cyanide is added (equivalent to adding cyanmethemoglobin), the cyanide is stable in the plasma-methemoglobin mixture and may be entirely recovered by acidification. It seems, therefore, that a reaction similar to that described by Sullivan et al. (23) may be responsible for the apparent disappearance of both free cyanide and nitroprusside (after partial conversion to cyanide) from plasma. Both free cyanide and nitroprusside appear to be stable in blood, because the free cyanide is present mainly as cyanmethemoglobin, and the cyanide concentration is too low for reactions to occur with disulfide compounds which may be present.

It has been suggested (12, 13, 26) that clinical use of nitroprusside as a hypotensive agent involves limits for both the rate of infusion and the maximum amount of nitroprusside given. Usually the rate of infusion suggested (38 nmol/kg body weight per minute) will maintain only a very low nitroprusside concentration.
Blood CN\textsubscript{free}, however, will be increased as the nitroprusside is decomposed. Measurement of blood cyanide is thus a convenient way to monitor the toxic effects of nitroprusside that has already decomposed, but actual measurement of combined cyanide gives a measure of the potential amount of cyanide still present as the intact drug.

References