Anesthesiology
66:381-385, 1987

In Vitro *Cyanide Release from Sodium Nitroprusside*

Shigemasa Ikeda, M.D., John F. Schweiss, M.D.,† Patricia A. Frank, B.A.,‡ Sharon M. Homan, Ph.D.§*

***In vitro* release of cyanide from sodium nitroprusside in 5% dextrose in water solution following exposure or non-exposure to fluorescent light (500 ft candles or 791 microwatt per square cm [μWcm^{-2}]), was measured by a cyanide-specific ion electrode at 4, 8, 24, 48, and 72 h. The cyanide concentrations were significantly increased at 24 h in the light-exposed solution. In this group, 100% of the cyanide was released from sodium nitroprusside at 72 h exposure to light. However, cyanide concentrations showed no significant changes for 72 h in the light-protected solutions, which were either exposed to 500 foot candles fluorescent light or stored in a dark room. Less than 2.5% of the cyanide was released from sodium nitroprusside at 72 h in both of the light-protected groups. No significant differences in cyanide concentrations were observed at 8 h among the exposed or non-exposed solutions. After 24 h of exposure, the cyanide concentrations in the exposed group were significantly higher than those of the two light-protected solutions. However, there were no significant differences between the cyanide concentration in the light-protected solutions. These results substantiate the safety of sodium nitroprusside solution for 24 h if the sodium nitroprusside containing solutions are properly protected from light. An additional study performed showed that a significant amount of cyanide released from sodium nitroprusside was adsorbed to the surface of polyvinylchloride. (Key words: Measurement technique: ion-specific electrode. Pharmacology: sodium nitroprusside. Toxicity: cyanide; sodium nitroprusside.)**

CYANIDE (CN^-) RELEASE from sodium nitroprusside (SNP) is a serious complication of its administration. CN^- is known to be released *in vitro* as well as *in vivo*, and previous recommendations have advised that solutions of SNP be discarded 4 h following reconstitution. The current drug insert states that, if properly protected from light, the reconstituted solution is stable for 24 h. A few studies have reported on the *in vitro* degradation of SNP in relation to time.[1,2] This study was performed to: 1) directly measure the amount of CN^- released from a SNP solution *in vitro* with a CN^- ion-selective electrode, 2) determine the safety limit of SNP in the light exposed, as well as light protected, solution in relation to time, and 3) show if CN^- released from SNP was adsorbed to the surface of the PVC bag.

Method

Twenty-five milligrams of SNP power was dissolved in 250 ml of 5% dextrose in water solution. The SNP solution was stored in the PVC intravenous bag (Travenol). Thirty bags of SNP-dextrose solutions were divided into three groups of ten each.

The first group (the exposed group) of ten bags was not wrapped with aluminum foil, and was placed 30 cm from a 60W fluorescent lamp (500 foot candles, or 791 μWcm^{-2})[3] and exposed to the light throughout the experiment. This intensity of the light was chosen because the 500 foot candles were maximum light exposure to the SNP containing solution recorded in our operating room. The second group (the non-exposed group) of ten bags was carefully wrapped with aluminum foil to prevent light exposure and placed together with the first group of bags under the lamp. The third group (the dark group) of ten bags was wrapped with aluminum foil and stored in a dark room during the experiment.

CN^- concentration was measured directly by the CN^--specific ion electrode (Orion Research Inc.) utilizing the Model 94-06 *p*H meter. Measurements were performed at 0 (immediately after dissolution), 4, 8, 24, 48, and 72 h after the SNP was dissolved in the 5% dextrose solution. To minimize the possibility of light degradation during the measurements, the samples were covered with aluminum foil. SNP solutions were stored and the measurements were performed at room temperature.

Our results indicated that the CN^- concentration in the exposed group increased significantly between 8 and 24 h. Thus, another experiment was performed to determine if the time course of CN^- release was linear or exponential between 8 and 24 h. Ten PVC bags of SNP in 5% dextrose in water solution which were not wrapped with aluminum foil were exposed for 24 h to the identical intensity of light as that in the original experiment. CN^- concentrations were measured at control and every 4 h between 8 and 24 h. Linearity was assessed and mean CN^- concentration was plotted and regressed against time.

An additional experiment was designed to show if CN^- released from SNP solution was adsorbed by the surface

* Associate Professor of Anesthesiology.
† Professor and Chairman of Anesthesiology.
‡ Research Assistant of Anesthesiology.
§ Assistant Professor of Biostatistics.

Received from the Departments of Anesthesiology and Community Health, St. Louis University School of Medicine, 1325 South Grand Blvd., St. Louis, Missouri 63104. Accepted for publication October 20, 1986.

Address reprint requests to Dr. Ikeda.

TABLE 1. In *Vitro* CN^- Released from SNP during 72-h Period; Mean CN^- Concentration

	Time	Control	4 H	8 H	24 H	48 H	72 H
E	Mean	0.402	0.352	0.622	5.10*	21.7*	43.6*
	S.E.	±0.011	±0.008	±0.024	±0.54	±1.6	±4.1
NE	Mean	0.392	0.367	0.397	0.530	0.714	0.705
	S.E.	±0.011	±0.007	±0.019	±0.053	±0.052	±0.065
D	Mean	0.390	0.337	0.352	0.409	0.534	1.05
	S.E.	±0.013	±0.009	±0.006	±0.020	±0.007	±0.08

* Statistically significant difference from the control within the same group.
Values are reported as mean ± S.E.

E, NE, and D represent the exposed, non-exposed, and dark group, respectively.

of the PVC bag. Fifteen glass beakers containing the same concentration of SNP were exposed to the light (500 ft candles). Five of them were seeded with a PVC sheet whose surface area was the same as the surface area of the 250 ml PVC bag. Another five beakers were seeded with a PVC sheet whose surface area was twice the inside surface area of the 250 ml PVC bag. The remaining five beakers were not seeded with a PVC sheet. The CN^- concentration of each sample was measured at 0, 8, 24, 32, and 48 h.

In order to examine the validity of the CN^- measurement by the ion-specific electrode, electrode potentials were measured in the solution containing 0.1, 0.5, 1, 5, 10, 17.5, 25, 37.5, and 50 ppm of CN^- (as KCN solution). Ten samples of each concentration were measured. Measured potentials (mV) were plotted and regressed against the natural log of the concentration (ppm) and the correlation was obtained.

The basic statistical design was a two-factor experiment with repeated measurements; thus, a two-way ANOVA was employed to test the significance of the exposure and time factors, as well as the time-exposure interaction. Tests of differences between pairs of means at different times was performed by using the Newman-Keuls procedure. Similarly, the Newman-Keuls procedure was used to compare means at each time period *versus* the control mean within each treatment group. $P < 0.05$ was considered statistically significant.

TABLE 2. In Vitro CN^- Released from SNP during 72-h Period; *P* Values for Neuman-Keuls Comparison of Means

	Control	4 H	8 H	24 H	48 H	72 H
E vs NE	NS	NS	NS	$P < 0.01$	$P < 0.01$	$P < 0.01$
E vs D	NS	NS	NS	$P < 0.01$	$P < 0.01$	$P < 0.01$
D vs NE	NS	NS	NS	NS	NS	NS

NS = not significant at $P = 0.05$.
E, NE, and D represent the exposed, non-exposed, and dark group, respectively.

TABLE 3. Percent Degradation of SNP Over Time in Each Group

Group	Control	4 H	8 H	24 H	48 H	72 H
E	0.922	0.808	1.430	11.700	49.80	100.00
NE	0.899	0.842	0.911	1.220	1.64	1.62
D	0.895	0.733	0.808	0.938	1.22	2.41

E, NE, and D represent the exposed, non-exposed, and dark group, respectively.

Results

The ANOVA indicated that the time, exposure, and time-exposure interaction factors were statistically significant. The Newman-Keuls procedure was then used to compare pairs of means. Initially, the mean cyanide concentration for each time period was compared to the mean at time 0 (control) within each exposure level. Only the mean CN^- concentrations after 24 h in the exposed group were statistically different from the time 0 concentration. Secondly, the exposed, non-exposed, and dark group means were compared at each time period. The only statistically significant differences occurred between the exposed and dark groups at 24, 48, and 72 h. These results are summarized in tables 1 and 2. Another experiment revealed CN^- released between 8 and 24 h in the exposed group to be linear with time ($r = 0.96$).

From the CN^- concentrations obtained in these experiments and the chemical formula $(Na_2[Fe(CN)_5NO]\text{-}2H_2O$, molecular weight 297.97 g) of SNP, the percentage of CN^- released from SNP was calculated, and is shown in table 3. In the exposed group 1.4%, 11.7%, and 100% of the CN^- was released from SNP after 8 h, 24 h, and 72 h of exposure to light, respectively. In the non-exposed group, 1.2% and 1.6% of the CN^- was released from SNP

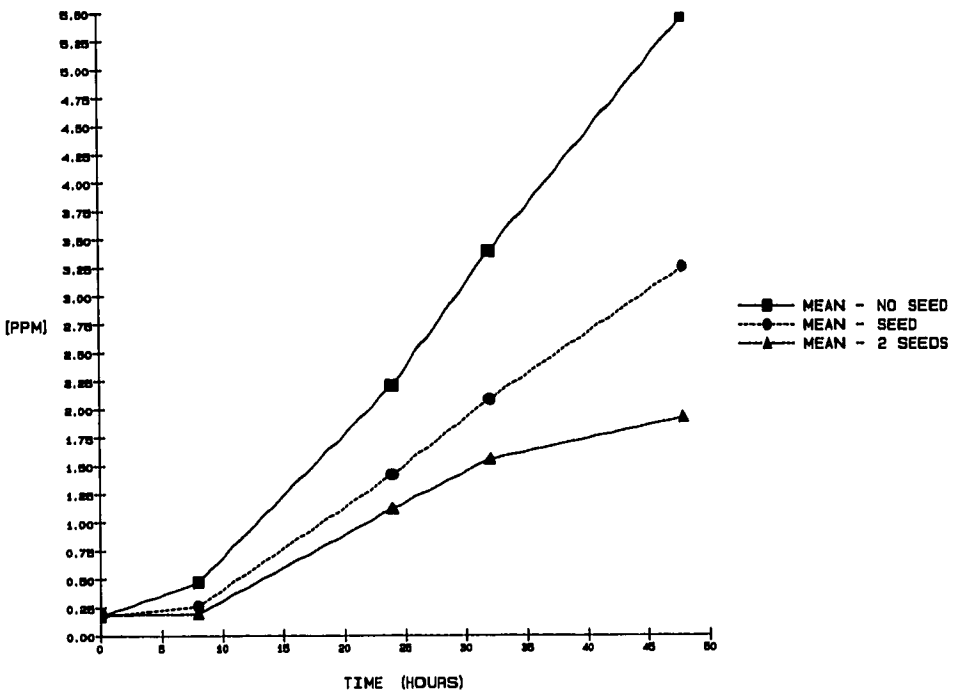

FIG. 1. The CN^- concentration of three solutions in beakers was plotted against time. No seed, seed, and 2 seeds represent the beakers containing no PVC, a PVC sheet whose surface area was the same as the inside surface area, and twice the inside surface area of a 250 ml PVC bag, respectively.

at 24 h and 72 h, respectively. In the dark group, 0.9% and 2.4% of CN^- was released at 24 h and 72 h, respectively.

Cyanide concentrations among the three solutions in the beaker were not statistically different at the control time. After 8 h exposure to the light, CN^- concentrations were the highest in the beaker containing no PVC seed and the lowest in the beaker containing a PVC seed of twice the inside surface area of the 250 ml PVC bag. There was a statistically significant difference between all groups after 8 h (fig. 1).

Figure 2 validated the CN^- measurement by the ion-specific electrode.

Discussion

When SNP became available commercially in 1974, it was recommended that the SNP solution not be kept or used for longer than 4 h. The Nipride Comprehensive Product Information Brochure (Roche Laboratories) stated that a solution of SNP exposed to light for 14 and 32 h did not affect the acute toxicity of SNP solutions in mice, but cautioned that these data should not be extrapolated to clinical usage. Results obtained from the Pharmacy Research and Development Department, Roche Laboratories, showed that, in the spectrophotometric method, no more than 4% of the SNP was degraded after 48 h when the SNP solutions wrapped with foil were exposed to two 15-W fluorescent light source (100 foot candles or 158 μWcm^{-2})[3] at the ambient temperature (20–21° C).¶

The photodecomposition of SNP in aqueous solution is complex and, apparently, varies dependent upon the intensity and the wave length of the light source. Generally it is thought that high-intensity light degradation leads to the formation of cyanic acid and nitric oxide, which is attributed to the reduction of the ferric ion to the ferrous form. The immediate degradation products of low energy light exposure are thought to be nitric oxide and sodium pentacyanoaquoferrate, but further decomposition to CN^- is probable.**

Anderson and Rae†† measured the absorption spectrum of 1% SNP exposed to a fluorescent tube for 4 h. The light source they used was either a 40-W tungsten lamp or a Phillips TLE circular fluorescent tube 20 cm diameter suspended 30 cm above the solution. The same authors reported that solutions of SNP for parenteral use were stable for a period of at least 6 months when prepared and stored away from light.

¶ Personal Communication: L. M. McDermott, Product Services Manager, Professional Services, Roche Laboratories.

** Wolfe SK, Swinehart JH: Photochemistry of pentacyano-nitrosylferrate (2^-), nitroprusside. Iorg Chem 14:1049–1053, 1975.

†† Anderson RA, Rae W: Stability of sodium nitroprusside solutions. Aust J Pharmaceut Sci NS1, 45–46, 1972.

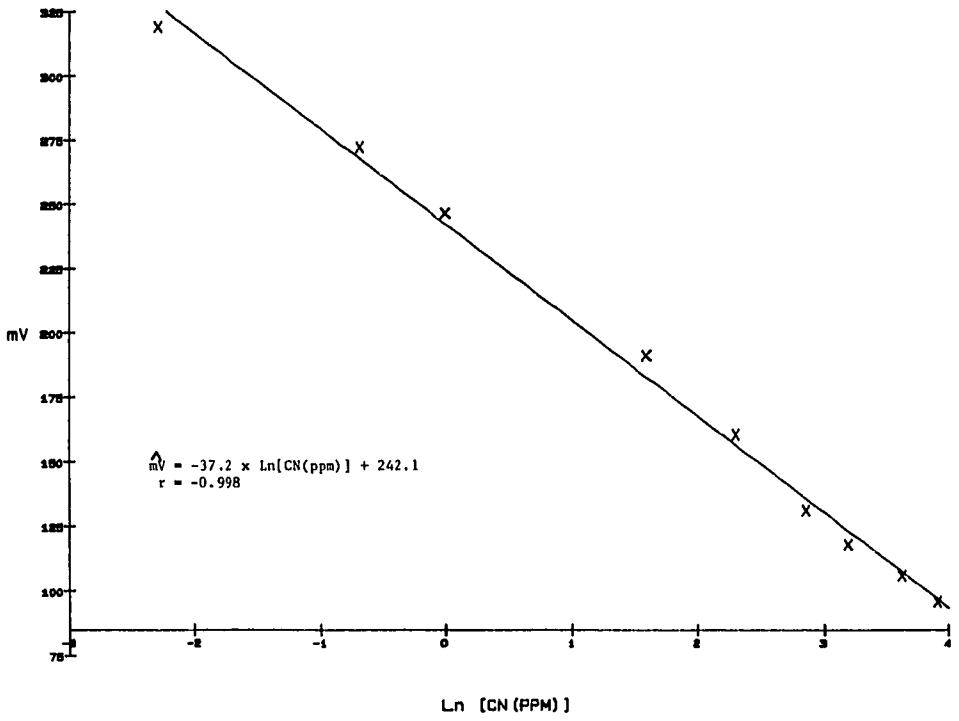

FIG. 2. The CN^- ion-specific electrode potentials were plotted against the natural log of the CN^- concentration in ppm, the regression line was linear with a correlation coefficient of −0.998.

A recent study by Arnold *et al.*[2] showed that 40% photodegradation was observed after 6 h exposure of SNP solutions to 20 μWcm^{-2} white or blue-light, while white light at 220 μWcm^{-2} produced 100% photodegradation after 2 h of exposure. They stated that only 0.4% of the photodegradation products were recovered as free cyanide. The SNP concentration they used in the experiment was 2–60 times more concentrated than those we used in this experiment. The intensity of the light we used was about 4–40 times stronger than they used. The intensity of the light makes a difference in the CN^- released from SNP. However, it is not clear if the differences in SNP concentrations influenced a degree of CN^- degradation. It is not possible to directly compare the results obtained in our experiment with those of Arnold *et al.*, due to differences in the time of measurements, SNP concentration, the intensity and nature of the light, and the container (glass bottle *vs.* PVC bag) used.

In this experiment, SNP was stored in the PVC bag. Our study showed a significant amount of CN^- released from SNP was adsorbed by the surface of the PVC bag. This CN^- adsorption to PVC might be one of the factors contributing to the differences between the results obtained in our experiment, and those in the study by Arnold *et al.*

In our experiment, CN^- concentrations were directly measured by the ion-specific electrode whose sensitivity is 0.026–260 ppm. The regression line showed a linearity with good correlation (r = −0.998) in CN^- correlation between 0.1 and 50 ppm (fig. 2). Kistner *et al.* reported poor or no correlations between actual CN^- concentrations in blood and plasma and CN^- concentration measured directly by the same Orion ion-specific electrode.[4] However, their calibration curve obtained in distilled water showed a good correlation between 10^{-5} and 10^{-3} M. In whole blood and plasma, the protein would be accumulated at the tip of the electrode and the accurate concentration would not be measured. In our experiment, 5% dextrose in water was used as the solute. The concentration tested was similar to that by Kistner *et al.*

During CN^- assay, the samples were cautiously handled so that potential photodegradation was avoided to minimize any artifact.[5] However, a negligible and clinically insignificant (less than 1 ppm) amount of CN^- was detected in the freshly prepared solution. Possible sources of CN^- contamination in the SNP solution are: 1) degradation of SNP to CN^- in the process of manufacturing and/or during storage, 2) photo-degradation of SNP in the process of the cyanide measurement, and 3) mechanical and/or technical limitation (sensitivity, etc.) of the CN^- specific ion electrode. We could not rule out any of the possibilities mentioned above.

Our results showed that the CN^- concentration showed a significant change at 24 h when the solution was exposed to light. At 24 h, approximately 1% of CN^- was degraded from the parent SNP in both the non-exposed and dark

group, while more than 10% of CN^- was released in the exposed group. At 72 h, 1.6% and 2.4% of CN^- was degraded from SNP in the non-exposed and dark groups, respectively.

Clinically, the SNP solutions are wrapped with aluminum foil and exposed to room lighting, which is less bright than the light exposures used in our experiments. In our operating rooms, the SNP is normally exposed to an average light intensity of 130 foot candles (or 206 μWcm^{-2}).

These results substantiate the safety of using SNP solutions (protected from light) for the 24 h time period currently recommended, based on the observed minimal CN^- release. Since exposure to light for 8 h produced no significant degradation, light protection of the intravenous tubing is probably not required.

References

1. Frand MJ, Johnson JB, Rubin SH: Spectrophotometric determination of sodium nitroprusside and its degradation products. J Pharm Sci 65:44–48, 1976
2. Arnold WP, Longnecker DE, Epstein RE: Photodegradation of sodium nitroprusside: Biologic activity and cyanide release. ANESTHESIOLOGY 61:254–260, 1984
3. Weast RC: Handbook of Chemistry and Physics, Cleveland, CRC Press, 1973–1974, p E-207
4. Kistner JR, Longnecker DE, Miller ED Jr, Lescanic AD: Limitations of the cyanide electrode for use in plasma and whole blood. Anesth Analg 58:457–460, 1979
5. Bisset WLK, Butler AR, Glidewell C, Reglinski J: Sodium nitroprusside and cyanide release: Reasons for re-appraisal. Br J Anaesth 53:1015–1018, 1981