# Sodium Cyanate: Chemical Properties Relevant to Sickle Cell Disease Therapy

ROBERT F. LABBE

**Abstract** ☐ A purification procedure for sodium cyanate and some of its chemical properties relevant to clinical studies on sickle cell disease are described. In aqueous solution, sodium cyanate decomposes to ammonia and sodium bicarbonate, the latter being the only contaminant detected after purification. The rate of cyanate reaction with water increases with temperature, concentration, and decreasing pH. The most convenient method of sterilization is dry heat. Under the conditions of time, temperature, and concentration to which erythrocytes are most likely to be exposed in clinical application, sodium cyanate can be considered stable to aqueous solution, except for its reaction with proteins and other biological materials.

**Keyphrases** ☐ Sodium cyanate—purification, analysis, sterilization, stability ☐ Sickle cell disease—therapy, relevant chemical properties of sodium cyanate

In 1971, Nalbandian (1) proposed the use of urea in treating sickle cell disease. Since urea equilibrates with cyanate and cyanate carbamylates proteins, reactions that have been the bases of numerous chemical investigations, Cerami and Manning (2) hypothesized that cyanate was an active agent in the desickling of cells by urea. It has now been established that the two compounds act by different mechanisms. Urea disrupts hydrophobic bonds to cause a reversible desickling of erythrocytes. By contrast, cyanate carbamylates the four terminal valine residues of hemoglobin and possibly other sites to cause an irreversible desickling effect. Since the biological effects of sodium cyanate received little mention in the early literature (3), biochemical and pharmacological studies (4–8) are being conducted as a prelude to more extensive clinical testing of the compound as a possible therapeutic agent for sickle cell disease.

Clinical and toxicological investigations of cyanate have generally used the sodium salt, which is not commercially available in purified form. Recrystallization of sodium cyanate from alcohol is about the extent of information available on the subject of purification. Reactions of cyanate with water in alkaline and acidic conditions have been studied from kinetic and thermodynamic points of view (9, 10), but the chemistry of the compound relative to its handling and use as a drug has not been previously discussed. This report describes the purification of sodium cyanate and some chemical properties that pertain to its clinical application.

## EXPERIMENTAL

**Purification**—Sodium cyanate of a technical grade was purchased[1] and had a supplier-indicated purity of 98% and water content of 0.5%. The compound was stated to have been prepared from urea and sodium carbonate. As obtained, the sodium cyanate was a slightly gray powder having some odor. The scale of the purification procedure developed can be adjusted to meet individual needs, but the amounts used here can be processed in common laboratory ware. Sodium cyanate is added with rapid stirring to water preheated to 60° (180 g./l.). Stirring is continued until the salt is completely dissolved but not for more than 5 min. The hot turbid solution is filtered as rapidly as possible through a preheated medium-porosity sintered-glass filter overlaid with a pad of cellulose[2]. The residue is washed with a small amount of 60° water. The filtrate is reheated to 60° and, while maintaining this temperature, 1.5 volumes of reagent grade methanol is added with rapid stirring; some sodium cyanate is salted out during the addition of the methanol. The solution is cooled and kept at 4° overnight. The sodium cyanate is removed in a precooled coarse sintered-glass filter. It is washed on the filter twice with cold methanol-water (3:2) and once with cold methanol; a volume equivalent to about one-tenth that of the original solution is used for each wash. The pure white sodium cyanate crystals are spread to facilitate rapid air drying at room temperature in a dust-free environment.

**Cyanate Assay**—The cyanate assay was based upon a method described by Werner (11) in which a blue copper-pyridine-cyanate complex is extracted into chloroform for colorimetric measurement. The original method, as improved by Marier and Rose (12) to gain sensitivity, was modified slightly for simplicity. To carry out the assay, up to 4.0 ml. of solution containing 5–50 μmoles of cyanate is placed in a glass-stoppered tube. Pyridine (0.1 ml.) and 0.1 *M* cupric sulfate (0.2 ml.) are added, and the volume is brought to 5.0 ml. with water. Chloroform (3.0 ml.) is added and the tube is shaken vigorously. After the phases separate, the chloroform layer is removed and its absorbance is read at 700 nm. The blank contains no cyanate. A calibration curve prepared using reagent grade potassium cyanate is shown in Fig. 1. From these data, it was determined that $A_{700}$/μmole cyanate = 0.0118. This assay is not suitable for use with biological fluids, a problem to be discussed more fully.

**Bicarbonate and Cyanide Assays**—The bicarbonate assay is a simple gravimetric procedure in which the sample is placed in a closed, evacuated system; then carbon dioxide is released with 1 *N* sulfuric acid and trapped in barium hydroxide. The formed barium carbonate is removed, washed with water and ethanol, dried, and weighed as a measure of the bicarbonate present in the original sample. The releasing and trapping of carbon dioxide are carried out quickly, and no error due to the acid-catalyzed conversion of cyanate was detectable. Cyanide was assayed by the method of Aldridge (13). Interference was encountered when large amounts of cyanate were used to assay for cyanide in the purified material. To a limited extent, this problem could be solved by increasing the amount of bromine, the excess of which was readily removed with arsenious acid as described by Aldridge.

## RESULTS

The yield from the purification procedure was about 70% (126 g./l.). The product of the essentially one-step process has proven suitable for clinical use[3].

Despite the fact that the Aldridge cyanide assay is not entirely specific (14) and that cyanate prepared from urea probably should not contain cyanide (12), testing for the presence of this highly toxic substance seemed justified. Assay of the commercial sodium cyanate showed a cyanide content of 1030 p.p.m. The purified material had less than 2 p.p.m., the lowest level that was detectable by the assay used.

[1] K & K Laboratories, Plainview, NY 11803

[2] The commercial sodium cyanate contained sufficient insoluble material that an unprotected sintered-glass filter became clogged quickly. To alleviate the problem, a cellulose slurry was prepared by blenderizing some soft filter paper in water and using this to form a protective covering on the filter.

[3] Personal communication, E. E. Langer.

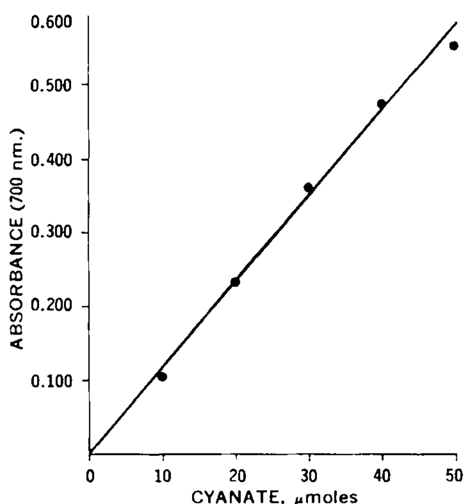
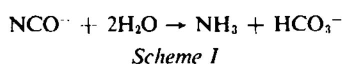


**Figure 1**—*Cyanate standard curve.*

A 3 *M* solution of the commercial sodium cyanate showed a slight broad absorbance at 266 and 285 nm. Contaminants responsible for this absorbance were undetectable after purification.

Occasionally, purified preparations had a distinct odor of ammonia due to the reaction (Scheme I) of cyanate with residual moisture:



$$NCO^- + 2H_2O \rightarrow NH_3 + HCO_3^-$$

*Scheme I*

In a series of purified preparations, the sodium cyanate assayed 93–100%, with a mean of 97%. Several preparations falling throughout this range of cyanate values were assayed for sodium bicarbonate. In every case, the remaining percentage could be fully accounted for as sodium bicarbonate.

The thermal stability of a purified and thoroughly dried sodium cyanate sample was demonstrated by heating at 200° for 2 hr. with no detectable loss. However, in aqueous solution, cyanate is quite unstable to heat. Autoclaving at 250° for 20 min. resulted in a 30% loss from a 0.01 *M* solution and a 65% loss from a 0.1 *M* solution. By contrast, a 0.1 *M* solution at 38° was far more stable (Fig. 2).

Cyanate stability was also very dependent upon concentration, as shown by the time course of decomposition at 38° in an aqueous solution (Fig. 2). A 0.1 *M* solution decomposed only 4% while a 1.0 *M* solution decomposed 79% in 29 hr. A 2.0 *M* solution lost cyanate at about twice the rate of the 1.0 *M* solution. Saturated sodium cyanate held at 60° as in the purification process decomposed to the extent of 90% in 3 hr. The rates of decomposition always began slowly, increased during the early phase of the reaction, and then remained linear until the reaction was nearly completed.

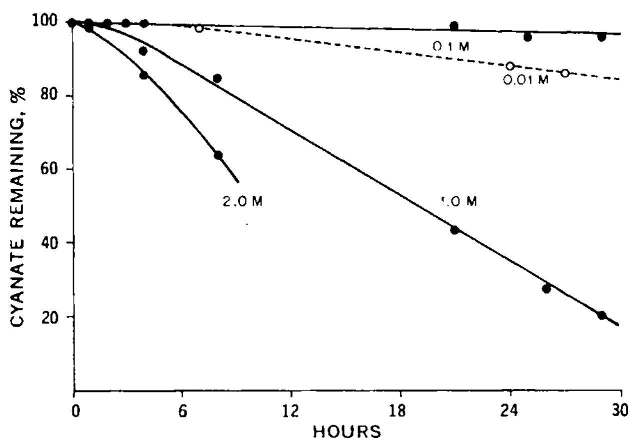


**Figure 2**—*Cyanate stability. The curves represent the rates of decomposition of sodium cyanate at 38° in aqueous solution. Key:* ——, *rates at pH 9.8; and* ---, *rates at pH 7.0.*

Most stability studies were with unbuffered solutions in the range of pH 9.2–9.8. At low pH, cyanate reacted with water more rapidly. This effect was observed in 0.1 *M N*-ethylmorpholine buffer at pH 7.0 where 0.01 *M* sodium cyanate at 38° decomposed to the extent of 12% in 29 hr. and 45% in 120 hr. At 4°, no detectable loss occurred in 120 hr.

## DISCUSSION

Although the purification of sodium cyanate gave only a 70% yield, it is a simple and rapid procedure. Of the incurred loss, about one-third was due to retention in the methanol-water solvent (19 g./l.). The only by-product identified was sodium bicarbonate. The essentially total removal of cyanide indicated the effectiveness of the purification.

The cyanate assay, as modified from the Werner method (11), was moderately sensitive and very satisfactory for aqueous solutions. The reaction was unaffected by cyanide or thiocyanate. The major limitation of the assay was due to interference from many components found in biological materials. Cupric ions reacted with some common buffers such as phosphate and tromethamine to interfere with the formation of the colored complex. Protein not only interfered with complex formation but also removed cyanate during the deproteinization process. For these reasons, the assay cannot be performed in the presence of biological materials, in which case the use of isotopically labeled cyanate appears to be the best available method.

For clinical application, sodium cyanate often must be sterilized. One of two possibilities appears useful. An appropriate solution can be freshly prepared and filter sterilized. Alternatively, purified and thoroughly dried sodium cyanate is extremely stable to heat, withstanding temperatures to 700° (15). Therefore, the compound can be heat sterilized in vials for the preparation of solutions at the time and in the concentration needed. The latter method has the advantage that sterile sodium cyanate can be stored indefinitely and be available at all times.

The reaction of free cyanate with water to produce ammonia and bicarbonate probably occurs *in vivo*, as evidenced by Cerami's (7) findings in a mouse experiment. The animal was injected with $NaO^{14}CN$; during the following 24 hr., 72% of the radioactivity was recovered as $^{14}CO_2$. In the concentrations that might be anticipated clinically, neither of the breakdown products would likely be of serious consequence.

The accumulation of bicarbonate as cyanate reacts with water may be responsible for the increasing decomposition rate seen during the initial phase of the reaction. Lister (16) noted that carbonate ions can catalyze the decomposition of cyanate in alkaline solution. The effects of concentration, temperature, and pH on cyanate stability qualitatively parallel the reaction rate constants reported by Hagel *et al.* (9) for similar experimental conditions. Thus, in the concentrations, at the temperatures, and for the times to which erythrocytes would likely be exposed in clinical use, cyanate can be considered stable toward reaction with water and should remain available for carbamylation of hemoglobin.

## REFERENCES

(1) "Molecular Aspects of Sickle Cell Hemoglobin: Clinical Applications," R. M. Nalbandian, Ed., Charles C Thomas, Springfield, Ill., 1971, p. 20.

(2) A. Cerami and J. M. Manning, *Proc. Nat. Acad. Sci. USA*, **68**, 1180(1971).

(3) K. M. Birch and F. Schutz, *Brit. J. Pharmacol.*, **1**, 186 (1946).

(4) T. Papayannopoulou, Abstracts 15th Annual Meeting Amer. Soc. Hematol., Hollywood, Fla., 1972, p. 154.

(5) R. M. Nalbandian, B. M. Nichols, E. J. Stehouwer, and F. R. Camp, Jr., *ibid.*, p. 154.

(6) B. P. Alter and Y. W. Kau, *ibid.*, p. 150.

(7) A. Cerami, *N. Engl. J. Med.*, **287**, 807(1972).

(8) A. J. Bellingham, A. May, and E. R. Huehns, *Clin. Sci.*, **42**, 21p(1972).

(9) P. Hagel, J. J. T. Gerding, W. Fieggen, and H. Bloemendal,

*Biochim. Biophys. Acta*, **243**, 366(1971).
(10) M. B. Jensen, *Acta Chem. Scand.*, **12**, 1657(1958).
(11) E. A. Werner, *J. Chem. Soc. (London)*, **123**, 2577(1923).
(12) J. R. Marier and D. Rose, *Anal. Biochem.*, **7**, 304(1964).
(13) W. N. Aldridge, *Analyst (London)*, **70**, 474(1945).
(14) *Ibid.*, **69**, 262(1944).
(15) J. Drucker and F. A. Henglein, *Z. Phys. Chem. Bodenstein-Festband*, **1931**, 437.
(16) M. W. Lister, *Can. J. Chem.*, **33**, 426(1955).

ACKNOWLEDGMENTS AND ADDRESSES

Received April 9, 1973, from the *Fircrest Research Laboratories, Department of Pediatrics, University of Washington, Seattle, WA 98195*

Accepted for publication June 6, 1973.

Supported by Grant 5 P01 HD04665 from the National Institute of Child Health and Human Development, National Institutes of Health, U. S. Public Health Service.

# Distribution of Aspirin in Rumen and Corpus Tissues of Rat Stomach during First Four Minutes after Administration

**CLARENCE H. MORRIS▲, JOHN E. CHRISTIAN, ROBERT R. LANDOLT, and WARREN G. HANSEN**

**Abstract** ☐ Autoradiography was used to determine the distribution of aspirin and/or its metabolites in the mucosa, submucosa, and muscularis of the rumen (nonglandular portion) and corpus (glandular portion) tissues of the rat stomach 4 min. after administration. A significant localization of aspirin and/or its metabolites was observed in the submucosa regions of both the rumen and corpus. In addition, the innermost portion of the rumen mucosa acted as a barrier toward absorption when compared to the corpus mucosa. This factor may be important in the mechanism of lesion production in the corpus tissue and the lack of lesion production in the rumen.

**Keyphrases** ☐ Aspirin (and metabolites), distribution—rumen and corpus tissues, rat stomach, 4 min. after administration ☐ Gastric lesions—aspirin (and metabolites) distribution 4 min. after administration, rumen and corpus tissues, rat stomach, histoautoradiography ☐ Autoradiography—aspirin (and metabolites) distribution 4 min. after administration, rumen and corpus tissues, rat stomach

Several mechanisms have been proposed to explain the production of gastric lesions by salicylates (1, 2). Davenport (3) and Martin (4) developed chemical models based on interaction of the compound with cellular constituents following absorption of the compound into the cell.

Previous studies (5) showed that some rats develop gastric lesions in the corpus portion of the stomach within 5 min. following oral administration of aspirin. Lesions have not been produced in the rumen under the same experimental conditions. Therefore, it was of interest to compare the relative quantities and distribution of aspirin and/or its metabolites in the mucosa, submucosa, and muscularis of the rumen and corpus tissues of the rat stomach shortly after oral administration of aspirin-7-$^{14}$C.

## MATERIALS AND METHODS

Aspirin was administered to rats as previously described (6), with the exception that each animal received 0.01 mc. (0.93 mg.) of aspirin-7-$^{14}$C.

**Preparation of Emulsion Slides**—Standard size microscope slides were cleaned by soaking for 24 hr. in a 10% solution of dichromic acid. Following the acid bath, the slides were washed in flowing tap water for 1 hr. followed by two consecutive 30-min. rinses in distilled water. The slides were then dipped in a subbing solution (5 g. gelatin and 0.5 g. of chrom alum in enough water to make 1000 ml.) and dried vertically. Next, the slides were dipped into a liquid emulsion[1], previously warmed to 43°. This work was performed under a safelight[2]. The slides were withdrawn from the emulsion at a uniform rate and checked under the safelight to ensure that the emulsion was uniformly distributed and free from bubbles, and the moist emulsion was wiped from one side. Then the slides were placed in a test tube rack in a vertical position and allowed to dry. After drying, the slides were placed in light-tight boxes and stored at −20° until used.

**Tissue Cutting and Mounting**—The entire stomach was removed from the animal 2 min. after oral administration. Four minutes after oral administration, the entire stomach was frozen in liquid nitrogen. From this point until development of the autoradiograms, the tissues remained frozen to prevent relocation of the aspirin and/or its metabolites. Cross-sectional segments of rumen and corpus were cut from the stomach, with a cold scalpel, and mounted on a previously cooled brass block using a drop of mounting medium[3]. After equilibration at −15°, 10-$\mu$ sections of rumen and corpus were cut with a microtome mounted in a cryostat[4]. The sections were transferred under the safelight from the microtome blade to the emulsion slides (also maintained at −15°). The mounted tissue sections were stored in light-tight boxes at −20° for 9–18 days to allow exposure of the photographic emulsion.

**Photographic Processing of Liquid Emulsion Slides**—All solutions were maintained at 4° throughout the photographic processing to reduce the possibility of detachment of the tissue from the emulsion. The most satisfactory staining was obtained if the tissue was fixed in methanol. The slides were then developed[5] for 10 min., rinsed in distilled water for 1 min., fixed[6] for 15 min., and rinsed in distilled water for 30 sec.

**Staining Techniques**—The tissue sections were stained with lithium carmine followed by picric acid, as described by Witten and Holstrom (7). All solutions were maintained between 10 and 15°. After the slides were removed from the final xylene bath, coverslips were mounted with Canada balsam.

[1] NTB-2, Eastman Kodak Co., Rochester, N. Y.
[2] Wratten series 2.
[3] Lab Tak, Westmont, Ill.
[4] International Equipment Co., Needham Heights, Mass.
[5] In Kodak D-19.
[6] In Kodak acid fixer.