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CYANIDE INTOXICATION AND ITS MECHANISM OF ANTAGONISM

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INTRODUCTION

The biological studies on cyanide are unique in that they preceded the chemical preparation of the substance (1). The last comprehensive review on the pharmacology and toxicology of cyanide was written by Dr. Reid Hunt at Johns Hopkins University in 1923 (2); however, this review was written in German. Recently, other books and monographs on cyanide have appeared (3, 4). The present review is restricted to selected aspects of cyanide and is primarily concerned with a descriptive and interpretive appraisal of the status of cyanide intoxication and its mechanism of antagonism.

Cyanide has created complex problems for modern society and these problems have evolved not only from industrial pollution but, paradoxically, from an inadvertent attempt to resolve pollution problems (5). Its use as a suicidal, homicidal, chemical warfare, and genocidal agent is well known. Toxic problems have been associated with ingesting cyanide-containing foods, and occupational hazards have arisen as the industrial use of cyanide has increased. In medicine, it has created problems because some drugs with nitrile moieties liberate cyanide.

Much of the toxicological interest in cyanide has focused on its rapid lethal action; however, its most widely distributed toxicologic problems are due to its chronic toxicity from dietary, industrial, and environmental factors. Cyanide is not wholly a toxin synthesized by civilization, as it existed in prebiotic times and was involved in biogenesis. In addition, cyanide is produced by various organisms and plants in our environment and has a role in normal metabolism.

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DEDICATION

This review is in tribute to Dr. James Blake, the first American toxicologist (6, 7). James Blake and Claude Bernard were former students of François Magendie and both followed in the footsteps of their mentor in their continuing interest in elucidating the mechanism of action of cyanide. Dr. Blake was the first to demonstrate that the onset of cyanide action varied according to the route of administration. He also reported that the respiratory cessation produced by cyanide can be reversed by artificial respiration. Studies by Dr. Blake on cyanide were conducted at the University College in London. Subsequently he emigrated to the United States and continued his illustrious career in the development of a rational basis for the mechanism of intoxication of chemicals. It seems fitting that this review be dedicated to this pioneer for his contributions to toxicology in the United States. The history of cyanide has been reviewed elsewhere (8, 9).

POTENTIAL SOURCES OF CYANIDE INTOXICATION

Natural Causes

The release of hydrogen cyanide into our environment cannot be wholly related to human activity. A variety of natural sources release cyanide into the environment, and the contribution from these natural sources to overall human toxicity is difficult to evaluate. Prior to human habitation of this planet, hydrogen cyanide existed in higher concentrations in our atmosphere than it does presently; the basis for this statement is the existence of cyanide in the atmospheres of the sun and stars. Cyanide was one of the earliest polyatomic organic molecules detected in interstellar space (10, 11). It is one of the important precursors of the abiotic synthesis of essential biologic constituents such as amino acids, purines, and pyrimidines (12). Chemical emission investigation suggests that the original heteropolypeptides on earth may have been synthesized spontaneously from hydrogen cyanide and water without the involvement of amino acids as an intermediate (13). Other natural sources of cyanide are the various biologic organisms, such as bacteria, algae, fungi, and plants, that can form and excrete cyanide.

Various plants contain a high content of cyanogenic glycosides. The cyanide content can be high; some foods contain 100–8000 mg/kg of material. If the lethal dose of sodium cyanide in man is considered to be approximately 1 mg/kg, such high concentrations give reason for concern, because cyanide can predispose to various clinical diseases (14). Probably the most widely distributed major human food crop with a high content of cyanogenic glycosides is cassava or manioc (15, 16), one of the most important food crops in tropical countries. This food provides over 70% of the caloric intake in some diets.

Other foods also contain a high content of cyanogenic glycosides, e.g. fruit pits, sweet potato, corn, bamboo shoot, linseed, lima beans, and millet. Acute cyanide poisoning has occurred in the United States from the ingestion of almond-flavored milkshakes prepared from apricot kernels. Even in foods that do not normally contain cyanide, when hydrogen cyanide fumigation is used cyanide residue can persist in fumigated products for an extensive time period. In the United States, tolerances are set for hydrogen cyanide residue in various foods subjected to fumigation (17). However, because these various sources of cyanide are not generally recognized, the effect of low levels of cyanide on a long-term basis warrants concern.

Human-Related Causes

Cyanide wastes have contaminated our waterways and our drinking water. These wastes are produced by electroplating, the steel, aluminum, and paint industries, and certain mining operations. A series of litigations have been introduced in an attempt to control environmental contamination with cyanide. Regulation of the discharge of cyanide wastes into our waterways is included in the Federal Water Pollution Act (BL 92–500). The Environmental Protection Agency filed a Refuse Acts suit against the Armco Steel Company for the discharge of cyanide into the shipping channel in Houston, Texas, and successfully prosecuted this company (CA 70-H-1335). Presently there are other litigations pending against an aluminum company for allegedly contaminating the aquifer. Unlike in surface water, cyanide in the underground water table is not easily dissipated.

One of the sources of hydrogen cyanide in the air is the petrochemical industry. Recently, automobiles equipped with malfunctioning catalytic platinum converters have been reported by Bell Laboratory to produce hydrogen cyanide (18). Another source of hydrogen cyanide in the air is from home fires because of the increase in plastic contents in homes. Combustion of various plastics, such as polyurethane, liberates hydrogen cyanide upon pyrolysis (19). And lastly, one of the major sources for the inhalation of hydrogen cyanide affecting man is tobacco smoke. The use of low-tar, low-nicotine, or filter cigarettes does not reduce the hydrogen cyanide concentration in cigarette smoke.

Various drugs liberate cyanide to produce toxic signs and symptoms. For example, sodium nitroprusside is employed as a potent hypotensive agent in the management of hypertensive patients (20). This compound liberates significant quantities of cyanide to result in cyanide poisoning and deaths have occurred (21). Cyanide formation from sodium nitroprusside in rat liver mitochondrial preparations seems to require a reducing agent, such as ascorbic acid, reduced glutathione, and to a lesser extent NADH and NADPH (22). The decomposition of nitroprusside to liberate cyanide also has been reported to occur in blood

by the interaction of nitroprusside ion with hemoglobin to yield cyanmethemoglobin and HCN (23). The reaction of hemoglobin with nitroprusside may be more complex than reported, as these workers were unable to establish stoichometry on the nitroprusside-hemoglobin reaction in even simple solutions, and questions have been raised with regard to the relative importance of the hemoglobin-nitroprusside interaction under in vivo conditions (24).

A cyanogenic glycoside, amygdalin (laetrile), has been used as an antineoplastic agent (25, 26) on the basis that it will be selectively hydrolyzed by a glucosidase to liberate cyanide, benzaldehyde, and sugar at the tumor site. The reports on this usage have been very controversial and have been refuted by various laboratories (27, 28). It has been proposed that tumor tissue is deficient in rhodanese to detoxify cyanide; therefore, it will be selectively attacked by cyanide, whereas normal cells contain a high concentration of rhodanese to detoxify cyanide, thereby preventing cell toxicity. This theory is rather simplistic and, furthermore, it is not substantiated by the facts. It has been reported that many tumor tissues are not selectively rich in β-glucosidase, nor do they contain a low concentration of rhodanese (27, 28). In addition, numerous studies indicate a lack of amygdalin antitumor activity in various model tumor systems (29–31).

Other agents shown to form cyanide include succinonitrile, which has been used as an antidepressant agent, and thiocyanate, an antithyroid agent (32). Thiocyanate can be oxidized to cyanide by peroxidase systems, but there is some controversy over whether the formation of cyanide from thiocyanate is real or an artifact. This controversy is normally trivial, since the amount of cyanide formed from thiocyanate is usually low in in vivo systems, so that the biologic significance of this reaction is minimal (33).

METHODOLOGY

In the assay of cyanide in body fluids, a number of factors need to be considered:

Tissue Selection and Storage

Cyanide is a reactive volatile nucleophile with a pKa of 9.2. This creates a variety of problems, as it can diffuse from tissue samples as well as bind to various components in the tissues. Tissue sampling techniques, storage, and cyanide analysis must be done with care if the results are to be reliable. Moreover, the organ distribution of cyanide varies considerably with the route of administration and the animal species injected (34, 35).

Which tissues to select for a toxicologic analysis of cyanide is controversial. Usually, whole blood is employed to analyze for cyanide concentration. This is considered to be the fluid of choice (36) in some laboratories. Other groups feel

that cyanide content in whole blood has no apparent toxicologic significance (37) and yields invalid data because the analysis of cyanide in whole blood includes a large fraction of cyanide bound to a "biologically inactive form," cyanmethemoglobin. These latter groups probably are not cognizant of various factors. Studies between plasma, serum, and whole blood cyanide have been studied and the correlations between concentrations of cyanide in whole blood, serum, and plasma are quite consistent (34). Second, the concept of a stable, inert, inactive pool of cyanide is conceptionally erroneous. Not only is cyanide's mechanism of binding to red blood cells not clearly elucidated, but pharmacokinetic studies with cyanide and thiocyanate indicate that the blood cyanide concentration rapidly declines monoexponentially and is almost completely removed from blood within five minutes after intravenous injection (33, 38). Because the apparent volume of distribution of the compartment in which cyanide is converted to thiocyanate is in the central compartment and the initial rate of cyanide disappearance is very rapid, it appears likely that blood or tissue areas close to blood are necessary to explain blood cyanide biotransformation. Third, the advocates for the use of plasma rather than whole blood cyanide for analysis appear to base their opinions on the erroneous assumption that cyanide in plasma is free rather than bound, when in fact it has been shown that 60% of the cyanide in the plasma of the dog can be bound to plasma proteins (39). Fourth, cyanide has been found to attach more readily to serum albumin than to hemoglobin at neutral pH (40). Fifth, in small animal studies practicalities dictate the measurement of cyanide in whole blood samples. Finally, cyanide rapidly leaves serum and plasma, especially in the first 20 minutes (35). Although there is usually a good correlation between cyanide concentration in serum, plasma, and whole blood, on some occasions it may be worthwhile to measure cyanide both in whole blood and plasma, e.g. when the thiocyanate concentration is very high.

The other factors that influence analysis of cyanide are conditions of storage, duration, and temperature (41–43). Depending on the tissue to be analyzed, the delay of analysis can have considerable effect. For example, when cyanide is added to serum there is a rapid decrease in measurable cyanide concentration, and one hour after the addition of cyanide to serum the recovery is only one-third of the amount added, with the most rapid loss occurring during the first 20 minutes (44).

Detection and Estimation of Cyanide Concentration

There are a variety of sensitive methods for measuring cyanide in biological fluids. Most of these procedures are relatively convenient and sensitive, but each has its limitations. In most procedures, diffusion is used to trap the cyanide in the alkaline media prior to analysis. Care must be taken to ascertain

that the presence of cyanide antidotes does not interfere with the cyanide analysis.

Probably the most frequently used colorimetric procedure is the oxidation of cyanide to a cyanogen, which is then interacted with a pyridine-pyrazolone mixture (45). The detection limit is approximately 0.004 μmole/ml. Sodium thiosulfate can interfere with the analysis of cyanide even after microdiffusion (45–47). The interfering material can be attributed to the conversion of sodium thiosulfate to polythionic acids upon sample acidification. The polythionates decompose to form sulfur dioxide and ultimately are diffused and trapped as sulfite (47). By using a buffered solution at pH 5.2 as the acidifying agent rather than sulfuric acid, it is possible to measure cyanide in the presence of sodium thiosulfate.

The potentiometric determination of cyanide using ion selective electrodes has become very popular, primarily because it is a convenient, rapid, and sensitive method of analysis (48). The more sensitive electrodes are those that contain a membrane of silver sulfide. Prior microdiffusion of biological samples containing cyanide is advisable to avoid interfering materials. Sodium thiosulfate also has been shown to interfere with the potentiometric analysis of cyanide due to an enhanced biotransformation of thiosulfate, particularly in the presence of blood, as this causes an artificially elevated cyanide concentration (49). The contaminant is sulfide, which can be removed by oxidation with hydrogen peroxide, and the excess hydrogen peroxide subsequently can be removed by sodium sulfite (49). It is important that the hydrogen peroxide be carefully removed, otherwise it will inactivate the ion selective electrode. With this method it is possible to measure samples in the range of 10^{-3}–10^{-6} M concentration range.

Spectrophotofluorometry is also a convenient, sensitive method for measuring cyanide in biological fluids, provided that prior microdiffusion is employed to isolate and concentrate the cyanide. Two fluorometric methods have been adapted to measuring cyanide in biologic fluids. One involves the catalytic conversion of pyridoxal to 4-pyridoxylactone (50, 51). Sodium thiosulfate interferes with the chemical conversion of this fluorophore. It is possible to circumvent this interference by using acetate buffer pH 5.2 as the acidifying agent. The mechanism for sodium thiosulfate interference is the formation of polythionic acids, as previously discussed; the sulfite anion then interacts with the aldehydic moiety. There are distinct advantages to the fluorometric method over the colorimetric method. The fluorometric method is more sensitive and requires fewer and more stable reagents than the colorimetric method. It should be pointed out that, in long-term preliminary studies, the apparent blood cyanide concentrations gradually increase more with 15% sulfuric acid as the acidifying agent than they do with pH 5.2 buffered solution containing sodium dodecylbenzylsulfate. The mechanism for this discrepancy has still not been

elucidated (50). The primary disadvantage of the fluorometric method is its interference by extraneous fluorescent materials. Another fluorescent method with an advantage over pyridoxal uses para-benzoquinone (52, 53). The formation of these highly fluorescent cyanohydroquinone derivatives is not inhibited either by sulfite or by thiosulfate ion (52, 53). Approximately 0.001 μmole/ml of cyanide can be analyzed by this procedure. This fluorescent reaction (52, 53) adaptation to biologic methods (54) is a very sensitive, specific method; however, like many fluorometric methods, various small amounts of extraneous material in the laboratory can interfere with this determination.

The measurement of hydrogen cyanide directly by gas chromatography has been reported (55), but this method lacks sensitivity with most detectors. However, the use of chloramine-T to oxidize cyanide to cyanogen chloride for subsequent extraction with hexane has led to a sensitive, convenient method. The sensitivity of this method, which employs an electron capture detector and as little as 0.25 μg/ml, can be measured. Gas chromatographic techniques are not widely used for determining cyanide primarily because other methods are more convenient. However, the inherent sensitivity and selectivity of this method insure its application to specialized samples, particularly those requiring the differentiation of various cyanide species.

Cyanide also can be measured by indirect atomic absorption spectrometry. There are two methods: one procedure involves the formation of an insoluble metal cyanide compound; then the metal in the precipitate or the excess metal in the supernate is measured by atomic absorption spectrometry (56–58). In the other method, a relatively stable metal-cyanide complex is formed that is then extracted into an organic solvent and the metal content of the extract is determined. The sensitivity range for this type of determination is approximately 0.3–3.0 ppm. Indirect atomic absorption spectrophotometric method analyses are quite sensitive for measuring cyanide in biological fluids after prior preparation by microdiffusion. These methods are not widely used, probably because the method is indirect and because of the general adequacy and convenience of other methods.

TOXIC MANIFESTATIONS AND MECHANISM OF INTOXICATION

The toxic effect of cyanide has been attributed to its production of a histotoxic anoxia by the inhibition of cytochrome oxidase, the terminal oxidase of the mitochondrial respiratory chain (59–61). This is a reasonable assumption, because this enzyme occupies a critical position in cellular metabolism. With regard to the mechanism of lethal effects, it should be emphasized that cyanide poisoning very frequently is a massive poisoning, where the amount of cyanide greatly exceeds the minimal concentration necessary to inhibit cytochrome

oxidase. In such intoxication many other enzymes and biological systems besides cytochrome oxidase probably are inhibited. A number of enzymes are equally or more sensitive to cyanide than cytochrome oxidase (62). Therefore, cyanide toxicity may not be a single biochemical lesion, but a complex effect on various enzyme systems involving Schiff base intermediates as well as metalloenzymes.

The mechanism of inhibition of cytochrome oxidase by cyanide is complicated, as this enzyme complex contains two heme A and two copper ions (63). The mechanism of cyanide inhibition has been studied intensively by various laboratories (64–66), and the reaction of this multimeric iron enzyme complex with cyanide has been found to involve a two-step reaction. The first step is the penetration of cyanide into a protein crevice, with initial binding of cyanide to the protein. The second step is the binding of the cyanide to the heme iron (66). Cyanide binds both to the oxidized and to the reduced form of the cytochrome oxidase. This toxic anion has a higher affinity for the oxidized form of the enzyme; however, it reacts more slowly with the oxidized form when compared with the reduced form. The cyanide probably reacts with the reduced form of cytochrome oxidase, which is subsequently oxidized to form the oxidized cyanide cytochrome oxidase complex. This latter complex is quite stable, but in the presence of reducing equivalents cyanide can readily dissociate from the enzyme inhibitor complex so that the cytochrome oxidase is reactivated (62).

There are enzymes that are more sensitive to cyanide than cytochrome oxidase. Two of these enzymes are nitrate reductase (67) and myoglobin (68). Some enzymes are almost as sensitive to cyanide as cytochrome oxidase, including horseradish peroxidase (69), yeast cytochrome c peroxidase (70), catalase (71), nitrite reductase (72), and ribulose diphosphate carboxylase (73). The basis for the inhibiting properties of cyanide may be attributed to its ability to complex with metals. Many of the enzymes sensitive to cyanide either contain molybdenum or iron. Other metalloenzymes that may be inhibited by cyanide are those that contain zinc or copper (62). Other mechanisms for cyanide inhibition may be attributed to chemical involvement between cyanide and a Schiff base intermediate, e.g. ribulose diphosphate carboxylase (73) and 2-keto-4-hydroxy glutarate aldolase (74), involving formation of a cyanohydrin intermediate.

Although extensive reports have been made on the effect of cyanide on cytochrome oxidase in in vitro systems, there is a paucity of information on the effect of cyanide on cytochrome oxidase in vivo (75–79). In vivo studies indicate that inhibition of cytochrome oxidase in various organs can occur. Brain cytochrome oxidase usually is measured, as the brain is one of the major target organs for cyanide. When the cyanide antidotes sodium nitrite and sodium thiosulfate were administered, no apparent inhibition of liver cytochrome oxidase activity occurred, even with a lethal dosage (77,

79). This observation may be due to various factors. The disposition of cyanide to sites of liver cytochrome oxidase localizations may be limited because of the high content and turnover number in the liver of rhodanese (80). Second, the cyanide-cytochrome oxidase multimeric complex may rapidly reactivate in the presence of rhodanese and sulfur donors due to cyanide biotransformation. Third, the physiological disposition of thiosulfate and nitrite-generated methemoglobin has a more limited distribution to brain than liver; this would result, therefore, in a higher sensitivity of brain tissue to cyanide, particularly since there is very little rhodanese in brain (77). These studies emphasize the importance of in vivo studies, as the data obtained in this way do not necessarily correlate with those found in in vitro biochemical studies.

A number of metabolic processes are known to be altered by cyanide in the intact animal and man. When cyanide inhibits cytochrome oxidase, this results in an alteration of a complex series of oxidative-reductive reactions in the cell; e.g. radiorespirometric studies using radioactive glucose (78) indicate that cyanide alters glucose catabolism, resulting in a 100% increase in the conversion of glucose by the pentose phosphate pathway, with a concomitant decrease in the breakdown of glucose by the glycolytic pathway (78). This shift from aerobic to anaerobic metabolism results in an increase in blood glucose and lactic acid, with a decrease in ATP/ADP ratio (75). The increased breakdown of glucose by the pentose phosphate pathway causes an increase in NADPH. This may represent a compensatory mechanism to maintain a balanced redox state, since an enhanced conversion of pyruvate to lactate is at the expense of NADH generated. In addition, cyanide causes an increase in phosphorylase activity as well as an increase in glucose production in the isolated perfused rat liver (81). Therefore, cyanide can alter carbohydrate metabolism, resulting in an increase in glycogenolysis and a shunting of glucose to the pentose phosphate pathway, by decreasing the rate of glycolysis and inhibiting the tricarboxylic acid cycle.

Physiologically, in cyanide intoxication one usually observes an initial hyperpnea followed by dyspnea and subsequently followed by convulsive seizures. Although cyanide is usually classified as a chemical producing a histotoxic anoxia, it probably also has an anoxic anoxia component. When a minimal lethal dose of cyanide is employed, the lethal effect is principally directed toward the central nervous system. At higher cyanide doses, cardiovascular signs occur. However, the brain is more sensitive to cyanide than the heart; in cases where a lethal dose has been administered, it has been frequently noted that the electrical activity of the brain has stopped and the heart is still beating. A considerable amount of information was available as early as 50 years ago on the in vivo physiological effects of cyanide (82–84). With regard to its effect on the chemoreceptors in the carotid body, cyanide promotes a slowing of the heart rate (85). Respiratory stimulation also can be promoted

by cyanide by stimulation of the peripheral chemoreceptors (86). Cyanide causes an enhanced afferent discharge from the carotid chemoreceptors and this can be abolished with oxygen (87). Some of the more sensitive physiological parameters altered by cyanide are electrical changes in the heart (88) and brain (89), which can be partially inhibited with oxygen (90). Earlier studies on the ability of oxygen to reverse the effect of cyanide on EKG (88) could not be confirmed in subsequent studies (90). Additional studies have been made on the effect of cyanide on the cardiovascular system; these studies focused their major investigative efforts on the effects of cyanide on chemoreceptor reflexes and the effect of cyanide on the central nervous system (91–94).

A few correlations between electrical activity and cyanide can be made, e.g. in EEG tracings, the electrical changes produced by cyanide correlate with alterations in cellular energy. The administration of sodium cyanide can bring about a sudden loss of electrical activity, which is followed by a prolonged period of depressed abnormal wave amplitude (90). The role of oxygen in antagonizing electrical activity can be correlated with this ability to antagonize the lethal effects of cyanide. Small doses of cyanide have been reported to exert a direct effect on the activity of the respiratory neurons in the intact animal (95).

The pathologic changes that occur in cyanide poisoning vary with the dose, route, and duration of cyanide exposure (96). No specific pathological changes can clearly delineate cyanide intoxication. The most consistent lesion observed from cyanide poisoning is on the central nervous system. Studies in monkeys indicate lesions predominantly in the white matter and necrosis occur more frequently than demyelination (97–100). It seems that the white matter is the more sensitive tissue to cyanide, as lesions in rats were more apt to appear in the white matter at lower doses of cyanide. The cyanide encephalopathy lesions were attributed to the direct effect of a histotoxic anoxia and not to secondary changes due to neuronal dysfunction and edema. Pathologic changes also were observed to occur in the heart (101). The pathology observed in the myocardium was consistent with earlier studies (102) in man. Other lesions also were produced, presumably from cyanide. In tropical neuropathies, the pathologic findings reported that, when cassava is ingested as the major staple food (103), toxicity is manifested by demyelination, a decrease in conduction velocities of peripheral nerves, and changes in the auditory nerve.

Probably the more widely distributed adverse effects of cyanide are due to chronic toxicity. Chronic low-level exposure to cyanide produces various signs and symptoms that can loosely be called a chronic cyanide syndrome, which can be manifested in a variety of different forms. Since most of the evidence is epidemiological or based on field studies without controlled experimental conditions, it is difficult to assess precisely the contribution of cyanide to the clinical disease. Nevertheless, evidence is accumulating that is consistent with the presumption that cyanide intoxication is a major cause of various human

diseases, although to say so is an oversimplification of a complex problem as there are other contributing factors. These disease entities may be due to high cyanide exposure, a depressed cyanide detoxification mechanism, various nutritional factors, or some combination of all these factors. Also, such abnormalities of detoxification may be ascribed either to inborn errors of metabolism or to a lack of substrate that can detoxify cyanide, which is secondary to the nutritional status.

Because of the widespread distribution of cyanide-containing sources and their implication as the etiology of various human diseases, this area of research is attracting a greater research effort. The involvement of cyanide in human diseases has been reviewed (104). Probably the best evidence of a human disease ascribed to cyanide is tobacco amblyopia. Visual abnormalities can be associated with a history of heavy smoking and a depletion of vitamin B_{12} (105). When this condition is treated with hydroxocobalamin, a cyanide antagonist, a reversal of the visual disturbance is observed (106) and plasma cyanocobalamin concentrations become elevated (107). Another clinical entity ascribed in part to cyanide exposure and a hereditary inborn error of metabolism is Leber's hereditary optic atrophy (108). When these individuals are exposed to a cyanide environment such as smoking, their plasma and urinary thiocyanate concentrations become lower than in normal individuals despite the fact that the enzymatic activity of liver rhodanese in the Leber's patients appears to be normal (109).

Probably the most widespread pathologic condition attributable to cyanide is tropical ataxic neuropathy associated with chronic cassava consumption. This is a diffuse degenerative neurological disease with peripheral and central signs. The signs and symptoms of this disease have been linked to cassava consumption and have been correlated with plasma thiocyanate concentrations (15, 16, 110, 111). Cassava is the major staple food in various tropical areas, and the plant has a high content of cyanogenic glycoside (linamarin). With continued ingestion over a period of time, tropical neuropathy gradually develops and the syndrome is characterized by optic atrophy, nerve deafness, and ataxia due to sensory spinal nerve involvement (112, 113). Other signs include scrotal dermatitis, stomatitis, and glossitis. In addition to dietary etiology, the cause of cyanide toxicity can be occupational, occurring in some industries when exposure to hydrogen cyanide is high (114, 115).

Experimental attempts have been made to show that cyanide is the etiological agent in human neuropathies (116, 117). Although these studies produced nerve lesions in rats similar to those observed in human disorders, it should be pointed out that the dosage required to produce damage in the rat was in the lethal range. In addition, in the rat the corpus callosum is more sensitive to cyanide than the optic nerve. This is quite different from human neuropathies, where the central nervous system involvement may result solely in optic

disturbances. Lastly, with chronic cyanide ingestion, the thyroid may be affected due to enhanced formation of thiocyanate. The antithyroid properties of thiocyanate become manifest, and myxedema, thyroid goiter, and cretinism may occur (118).

CYANIDE ANTAGONISM

The antagonism of cyanide intoxication is an area that presently is receiving intensive investigation. However, most of the present concepts and basic classes of compounds were developed 50 to 150 years ago. Blake reported in 1840 that the lethal effects of cyanide can be antagonized by artificial respiration (6, 7). In 1888 it was reported that amyl nitrite was effective in antagonizing the lethal effects of cyanide (119), and by 1933 the use of sodium thiosulfate as the sulfur donor for rhodanese was described (120). This led to the studies by K. K. Chen employing a combination of amyl nitrite, sodium nitrite, and sodium thiosulfate as the antidote (121). Also, in 1894 cobalt was known to form a stable metal complex with cyanide and was used to antagonize cyanide (122).

In the last two decades a series of new cyanide antagonists have been developed. The protection afforded by these new antidotes would be of greater clinical interest if they could protect against the lethal effects of chemicals other than cyanide. Since a highly effective antidotal combination for cyanide is already available, the advantage of these new antidotes is relatively trivial. Since cyanide can act extremely rapidly, it is important to differentiate between the protective effects of these agents as an antidote for acute overdosage and as a prophylactic in chronic overdosage.

The cyanide antagonists are arbitrarily classified into compounds that metabolize or complex with cyanide and oxygen.

Biotransformation of Cyanide

Rhodanese initially was assumed to have a high substrate specificity for thiosulfate and to function solely to detoxify cyanide (120). Subsequently, this enzyme was found to catalyze a series of other reactions and has other biologic functions (123–126). Studies by two laboratories (121, 127) incorporated sodium thiosulfate into an antidotal combination to antagonize cyanide. Rhodanese is ideal from a toxicologic viewpoint, since the enzyme is present in large amounts (128), has a high turnover number, and catalyzes a reaction, at least with cyanide, that is essentially irreversible (129, 130). There is one limitation to the use of sodium thiosulfate as an antagonist: the selective distribution of the agonist (HCN) does not parallel that of the antagonist. Rhodanese is localized in the mitochondria, and the penetration of sodium thiosulfate to these sites is limited. In an attempt to reconcile these adverse

disposition factors, crystalline rhodanese has been injected intravenously in combination with the sulfur donor to antagonize cyanide (131). In addition, various other sulfur donors have been employed (130).

During the past two decades, Westley and his associates have contributed much to our knowledge about the enzymic mechanism of the sulfurtransferases and their role in cyanide detoxication and other biologic functions (124–126, 132). The two sulfurtransferases that may play a role in the detoxification of cyanide are rhodanese and mercaptopyruvate sulfurtransferase. Although both sulfurtransferases can detoxify cyanide, the enzymic mechanism of the two reactions, the organ distribution, and the subcellular distribution of the enzymes are quite different. The basic reaction with rhodanese involves a transfer of a sulfane sulfur from the donor, thiosulfate, to the enzyme, forming a persulfide intermediate. Then the persulfide sulfur is transferred from the enzyme to the nucleophilic acceptor, in this case cyanide, to yield thiocyanate. Rhodanese does not have a high substrate specificity; there are many sulfur donors and other acceptors that can interact with rhodanese. The uniqueness of cyanide as a substrate is that this reaction is essentially irreversible, whereas with other nucleophilic acceptors the reactions are fully reversible. Westley and his associates have proposed a hypothesis that adds an intriguing perspective to the mechanism of cyanide detoxication and partially clarifies some of the puzzling features of the in vivo detoxification of cyanide with sodium thiosulfate. This detoxification reaction requires a source of sulfane sulfur, an ionized sulfur bonded to another sulfur, which reacts with cyanide, and there are various biological compounds that contain sulfane sulfur (132), e.g. thiosulfate, polythionates, thiosulfonates, persulfides, and elemental sulfur in the form of staggered-8 member rings (133). These sulfane-containing compounds in the presence of rhodanese rapidly equilibrate in the intact animal to form a sulfane pool that can react with cyanide. Sulfane sulfur may be derived from cysteine by mercaptopyruvate, which can then react with its sulfurtransferase. It should be emphasized that rhodanese not only can catalyze sulfane transfer to cyanide, but it also can interconvert these various forms of sulfanes. The sulfane carrier was postulated to be albumin because radioactive studies with sodium thiosulfate (133, 134) indicate that serum albumin interacts with elemental sulfur as it does with so many other hydrophobic biological substances. Moreover, this serum albumin sulfane sulfur carrier complex is in a form that can react with cyanide.

In summary, the proposal by Westley and associates implicates a serum albumin-sulfane carrier complex as playing a major role in cyanide detoxication mechanism operating in vivo. This proposal does not alter the importance of rhodanese in cyanide sulfur metabolism, but it does place it in a different perspective. Based on substantial experimental evidence, it infers that the action of sodium thiosulfate in antagonizing cyanide intoxication may also

occur by an additional and alternative mechanism. The proposal is as follows: the source of the sulfane sulfur is derived from mercaptopyruvate via the mercaptopyruvate sulfurtransferase. The various forms of sulfane are then interconverted by rhodanese. The sulfane carrier that transports the sulfur formed in the liver and possibly other organs is serum albumin; the sulfane sulfur albumin complex is then reacted with cyanide.

This intriguing proposal warrants serious consideration because of the strength of the in vitro data and its conceptual importance to toxicologic mechanisms. The quantity of serum albumin and the rate constant for cyanolysis are consistent with the sulfur albumin complex for cyanide detoxication from the sulfane pool. Some preliminary in vivo data (33, 135) may be consistent with this proposal. Pharmacokinetic studies with cyanide and thiosulfate indicate that the conversion of cyanide to thiocyanate is predominantly in the central compartment, with a volume of distribution approximating that of the blood volume. The reaction rate is extremely fast and is consistent with the in vitro data by interaction with some of the sulfur albumin complex. An argument against this hypothesis is the fact that the cytochemical localization of rhodanese is perivascular (136). This infers that cyanide in blood is in close proximity to rhodanese. Also, a mechanism that transports thiosulfate in the rat liver mitochondria has been described (137).

Chemical Binding of Cyanide

NITRITES The efficacy of amyl nitrite in protecting against cyanide poisoning in dogs was first reported in 1888 (119). This research went unnoticed, as it was published in a relatively obscure journal. However, when it was observed that cyanide interacts with methemoglobin to form cyanmethemoglobin and that methemoglobin reactivates cyanide-inhibited cytochrome oxidase, nitrite was utilized as an antidote against cyanide poisoning (121, 127). The basis for the antagonism of cyanide with the nitrites was biochemically sound (138). Cyanide has a low affinity for hemoglobin but a high affinity for methemoglobin. Nitrite generates methemoglobin, which then combines with cyanide to form cyanmethemoglobin.

The first nitrite employed to antagonize cyanide intoxication was amyl nitrite (119, 139), and subsequently sodium nitrite was used (121), the rationale being that, since therapy must be rapid, amyl nitrite could be inhaled while sodium nitrite was being prepared for intravenous administration. The efficacy of amyl nitrite in antagonizing cyanide intoxication has been questioned (140). The development of the rapid methemoglobin formers was prompted by various laboratories (140–143), which reported the limitations of using the nitrites in cyanide intoxication mainly because of the relatively slow rate of methemoglobin formation (92, 141). Subsequently, a series of investigations (140–143) led to the development of 4-dimethylaminophenol, DMAP, as the agent of choice.

It should be pointed out that methemoglobin can reactivate cyanide-inhibited cytochrome oxidase (75, 144). Although DMAP has many proponents because of its rapid rate of methemoglobin formation, other laboratories still indicate the efficacy of sodium nitrite as a cyanide antagonist over other methemoglobin formers and relate its superior efficacy to the more prolonged methemoglobenemia after sodium nitrite (145, 146). The mechanism for the enhanced protection of $NaNO_2$ over other methemoglobin formers is that, in the more rapid methemoglobin formers, mice were able to survive the initial acute cyanide challenge; however, they subsequently succumbed to the cyanide released rapidly from the cyanmethemoglobin pool. Also, the mechanism for the prolonged methemoglobinemia after sodium nitrite in mice and the greater cyanide protection afforded was attributed to a possible inhibition of methemoglobin reductase (146).

A discussion of methemoglobin formers and cyanide antagonists would not be complete without some reference to methylene blue, because misinformation still persists that methylene blue is an effective methemoglobin former and therefore an efficacious cyanide antagonist (147). Methylene blue is not an effective antidote (120), as it is a poor methemoglobin former (148–151).

The early rationale for the use of methemoglobin formers in cyanide antagonism was reasonable (152), particularly since sodium nitrite did protect against cyanide intoxication and methemoglobin formation did occur. Consequently, this mechanism has gone unquestioned for the past half century. Conceptually, this factor prompted the development of more rapid methemoglobin formers. However, the role of methemoglobin formation by nitrite should have been questioned, because the antidotal effect of nitrite is very rapid, whereas nitrite-generated methemoglobin formation is relatively slow. In low methemoglobin concentrations, the efficacy of nitrite in antagonizing cyanide is minimal (153, 154).

Recent studies suggest that methemoglobin formation by sodium nitrite may play a minimal, if any, role in the *therapeutic* antagonism of cyanide poisoning (153, 154). Moreover, sodium nitrite and sodium thiosulfate either alone or in combination are equally effective whether administered prophylactically or antidotally (155, 156). These studies prompted the consideration of alternative mechanisms for cyanide antagonism other than nitrite generation of methemoglobin formation. When methemoglobinemia was prevented by methylene blue pretreatment, sodium nitrite still protected efficaciously against cyanide poisoning (153, 154). These investigations infer that prophylactic studies under laboratory conditions, where sodium nitrite is given *prior* to cyanide to attempt to show maximal protective effect at maximal methemoglobin formation, methemoglobin does play a role in antagonizing the lethal effects of cyanide. However, this would not occur in cyanide poisoning if sodium nitrite was given *after* cyanide poisoning, as would normally occur. Under the latter conditions,

the mechanism of action of sodium nitrite is due to some mechanism other than methemoglobin formation. This has important conceptual and practical implications for, if sodium nitrite is exerting its effect by a mechanism other than methemoglobin formation, this provides an opportunity for the development of a new class of cyanide antagonists. This area presently is being intensely investigated by various laboratories. The first logical alternative is to study the other known pharmacological properties of nitrites. Because nitrites are potent vasodilators, attention has focused on the vasogenic properties of various drugs as potential cyanide antagonists.

Investigation of vasogenic compounds as cyanide antagonists actually was initiated with chlorpromazine. Chlorpromazine has been reported to antagonize cyanide intoxication (157, 158) and the mechanism of this antagonism was attributed to the hypothermic properties of chlorpromazine (158), as various drugs protect against anoxia by lowering body temperatures (159). The protective effect of chlorpromazine could not be confirmed, either alone or with sodium nitrite (160); however, when chlorpromazine was administered with sodium thiosulfate, there was a striking potentiation against the lethal effects of cyanide. The mechanism of chlorpromazine in the antagonism of cyanide intoxication was found to be unrelated to hypothermia. The ability of chlorpromazine to antagonize cyanide in the presence of sodium thiosulfate was reversed by an α agonist, methoxamine (161, 162). This prompted the investigation of α blockade as a mechanism of cyanide antagonism. The α adrenergic blocking agent phenoxybenzamine subsequently was found to have almost identical properties to chlorpromazine as a cyanide antagonist. Similar to chlorpromazine, phenoxybenzamine possesses no antidotal property either alone or in combination with sodium nitrite; however, a striking potentiation was noted with sodium thiosulfate and to a lesser extent when it was added to the nitrite-thiosulfate combination. In addition, the antidotal effect of phenoxybenzamine, like that of chlorpromazine, can be reversed with methoxamine (161, 162). Subsequently, a series of different classes of vasogenic agents were examined against the lethal effects of cyanide in combination with sodium thiosulfate and/or sodium nitrite. Of all the vasodilators, only the ganglionic blocking agents and other α adrenergic blockers were observed to exhibit an antidotal effect. This includes examination of almost all classes of autonomic drugs, vasodilators such as papaverine, organic nitrates, and antihistaminic compounds. The reason why sodium nitrite but not other vasodilators should protect against the lethal effect of cyanide has still not been clearly elucidated, as methoxamine can reverse the antidotal effect of phenoxybenzamine but not that of sodium nitrite (161, 162).

COBALT COMPOUNDS Cobalt ion is known to form a stable metal complex with cyanide and has been used in the treatment of cyanide poisoning (122,

163); however, the use of cobalt as a cyanide antidote has not received general support mainly because of the toxicity of cobalt ion. About a half century after their first use, interest in cobalt compounds was renewed when hydroxocobalamin was reported to antagonize the lethal effects of cyanide (164). A variety of cobalt-containing compounds have been tested against the lethal effects of cyanide, including hydroxocobalamin (164–167), cobalt histidine (168–170), cobalt chloride (171–173), and dicobalt ethylenediaminetetraacetic acid (Co_2 EDTA) (174–176). The use of cobalt-EDTA has been successful in treating cyanide poisoning in experimental and in clinical situations (174–178). Its use is based on the fact that cobalt ions form a stable complex with cyanide (179). More importantly, it was reported that the presence of the cobalt ion can reactivate the cyanide-inhibited cytochrome oxidase (180). This is an important observation, because cobalt itself can inhibit heme biosynthesis. The selection of cobalt-EDTA as the preferred cobalt compound is reasonable, since it was hoped that many of the toxic effects of cobalt ion could be minimized by administering this compound as a chelate. Whether the EDTA complex appreciably reduces the toxicity of cobalt is an area of controversy that has not been resolved. Also, since cobalt reacts directly with cyanide, this reaction should be quite rapid. It was believed that cobalt EDTA would have a distinct advantage as a cyanide antagonist over sodium nitrite, because of the relatively slow rate of methemoglobin generation by the nitrites.

CYANOHYDRIN FORMATION Cyanide is a nucleophile known to interact with various carbonyl groups to form cyanohydrin intermediates. Sodium pyruvate was found to rapidly reverse the cyanide-inhibited respiration of the Ehrlich ascites tumor cells (181) and to antagonize the lethal effects of cyanide in mice (182). These relatively limited protective effects of sodium pyruvate alone were subsequently confirmed (183) and extended to detailed drug antagonism of cyanide in mice. The rationale for investigating sodium pyruvate is that it has many apparent theoretical advantages over other cyanide antagonists, i.e. sodium nitrite. Cyanide reacts directly with pyruvate to form a cyanohydrin derivative. Also, pyruvate is more apt to distribute to sites of cyanide localization, as there is a specific carrier for the active transport of pyruvate (183). However, sodium pyruvate is not as efficacious as sodium nitrite and does not enhance the protective effect of nitrite against the lethal effects of cyanide, but it does potentiate the antidotal effect of sodium thiosulfate. The addition of sodium pyruvate to the nitrite-thiosulfate antidotal combination further enhances the protective effect against cyanide. The value of sodium pyruvate as a potential cyanide antagonist is that it provides a different approach to the development of cyanide antidotes, an approach which is now being intensively investigated. As a possible supplement to the nitrite-thiosulfate combination, it provides a reason for decreasing the dose of sodium nitrite, as this antagonist

has caused fatalities in susceptible individuals. In addition, no convulsions from cyanide were observed when sodium pyruvate was present (183). Sodium pyruvate or its analogs also has potential advantage over cobalt-EDTA employed clinically, in that it is less toxic and therefore a greater dose can be tolerated. This is of considerable importance when one calculates the ratio of antagonist to agonist.

Oxygen

Cyanide inhibits cytochrome oxidase (59, 60, 61), but if this were the sole mechanism of cyanide lethality there would be no rational basis for employing oxygen to treat cyanide intoxication. Furthermore, the substrate requirement of cytochrome oxidase requires an oxygen tension of only 70 mm Hg for maximal enzymatic activity (184). Nevertheless, oxygen has been advocated for the treatment of cyanide poisoning for many years. Unfortunately, in many of those studies the experimental designs were incomplete; consequently, the results obtained were not very convincing. None of these studies with oxygen were evaluated in combination with other known cyanide antagonists, e.g. sodium nitrite and/or sodium thiosulfate. It is only under these conditions that the striking protective effect of oxygen is realized, as oxygen potentiates the action of the nitrite-thiosulfate combination. Various reports have indicated that oxygen alone under atmospheric (185–188) or hyperbaric (89, 189, 190) conditions is beneficial in treating cyanide poisoning. Also, it was reported that oxygen can reverse the abnormalities of EKG and/or EEG tracings produced by cyanide (89, 90, 188, 189) and that hyperbaric oxygen can protect against the lethal effects of cyanide in mice (89, 189). Most of the early recommendations for the use of oxygen were as an adjunct rather than as an integral part of the treatment of cyanide poisoning. Oxygen alone has only minimal effects and enhances the protective effect of sodium thiosulfate to only a minor degree and does not do so at all for sodium nitrite, but it strikingly potentiates the effectiveness of the combination of sodium nitrite and sodium thiosulfate (90, 155, 191, 192). These studies have been confirmed in sheep (193) and by another laboratory using rats (194). Increased protection was observed as the oxygen concentration was increased from 20% to 100% (156), and no further increase in protection was noted with hyperbaric oxygen (4 ATA) (195). This protective effect of oxygen was observed not only prophylactically (90, 155, 191, 192, 195) but also therapeutically, after the signs and symptoms of cyanide poisoning were fully manifested (156, 193, 196).

It is important to recognize that oxygen at 1 ATA and 4 ATA can affect tissue metabolism. For example, it has been reported that α-ketoglutarate utilization from brain homogenate is inhibited within 20 minutes after oxygen administration (197) and changes in the concentration of glycolytic intermediates occur (198). In addition, biochemical lesions can be produced with hyperbaric

oxygen with little evidence of toxicity under macroscopic or microscopic examination (199). These metabolic inhibitory effects of oxygen may provide a basis for the apparent great efficacy of oxygen as a cyanide antagonist.

One report indicates that oxygen did not enhance the protective effect against cyanide poisoning when pretreated with sodium thiosulfate (200), but the studies on which it is based suffer from inadequate experimental design and their conceptual basis may be questioned. A single dose of cyanide and a single dose of sodium thiosulfate were employed and percents survival were measured. As was indicated earlier, if adequate studies are to be conducted, a dose-response relationship should be established, particularly with cyanide, as the slope of the dose-response curve is quite steep. Second, if the efficacy of oxygen as a cyanide antagonist is to be evaluated, it would be reasonable to employ oxygen with the nitrite-thiosulfate combination rather than thiosulfate alone in order to see the most striking potentiation. Third, just because sodium thiosulfate or the nitrite-thiosulfate combination is enhanced by oxygen, this does not necessarily infer that the effect of oxygen is acting directly on sodium thiosulfate or the rhodanese enzyme, as the enzymatic conversion of cyanide to thiocyanate in the presence of rhodanese can occur without oxygen. Oxygen may be exerting its effect on alternative metabolic events unrelated to the rhodanese reaction.

Various physiologic effects of cyanide can be reversed with oxygen, as has already been discussed. Also, cyanide-induced encephalopathy has been described and oxygen has been reported to minimize these brain lesions (201). Studies on the effect of oxygen in altering the physiological action of cyanide were conducted in an attempt to elicit the effect of oxygen in combination with the classical cyanide antidotes under these conditions (90). Of all the physiological parameters measured, only the EEG was consistently sensitive enough to elicit differences between air and oxygen in dogs. Oxygen shortened the electrical silence produced by cyanide either alone, in combination with thiosulfate, or with the nitrite-thiosulfate combination, but not with sodium nitrite alone (90). In contrast, the protective effect of oxygen in preventing or reversing the EKG effects (188) and the respiratory changes (188) of cyanide (90) were not confirmed. The inability to confirm these changes probably reflects the higher dose of sodium cyanide employed.

Differences in biochemical parameters between air and oxygen also could be observed in vivo (76, 79). Inhibition in cytochrome oxidase activity was restored more rapidly in liver than in brain (77). Also, reactivation of cytochrome oxidase occurs more rapidly in oxygen than in air (79). In animals pretreated with nitrite-thiosulfate, brain but not liver cytochrome oxidase was inhibited by a lethal dose of cyanide (77). The mechanism for this surprising observation has not been elucidated. A dose-dependent cyanide inhibition of both brain and liver cytochrome oxidase was established, and oxygen shifted

the dose-response curve to the right (79). Oxygen and air-treated animals displayed the same degree of cytochrome oxidase inhibition during the initial response to cyanide; however, the animals receiving air but not oxygen exhibited signs of cyanide intoxication (79). This suggests that the signs of cyanide intoxication do not parallel cytochrome oxidase inhibition and the appearance and disappearance of these effects are not necessarily dose-dependent (79). However, these differences between oxygen and air may be dose-related in certain physiologic changes that are induced by low cyanide concentration or that are sensitive to oxygen (188, 202–204), whereas neither oxygen nor air alters the physiologic changes induced by higher cyanide doses (90). Exposure to sublethal doses of cyanide can divert changes in carbohydrate intermediary metabolism to pathways that are less sensitive to cyanide (78). Further substantiation of the effect of oxygen in potentiating the protective effect against cyanide intoxication can be elicited in radiorespirometric studies in mice using radioactive glucose. Oxygen, but not air, when administered in combination with the nitrite-thiosulfate antidotal combination (205a), can completely reactivate glucose metabolism inhibited by cyanide. The rationale for these findings is that anoxic tissues induced by cyanide inactivation of cytochrome oxidase results in a shift from aerobic to anaerobic metabolism accompanied by a concomitant decreased production of carbon dioxide. Therefore, monitoring the respiratory excretion of carbon dioxide after the administration of uniformly labeled glucose appears to give a rough indication of the degree of metabolic inhibition produced by cyanide. The mechanism for the action of oxygen in reversing cyanide-inhibited glucose oxidation has not been elucidated (205a). Last, the disposition of cyanide does not appear to be a factor in eliciting the differences observed between air and oxygen (205). Although small differences were observed in the respiratory excretion of cyanide, actually this plays only a minor role, as only 4% of cyanide is excreted by the respiratory route. No differences were noted with regard to the urinary excretion or total body retention of sodium cyanide between air and oxygen. In summary, although oxygen can be established as a cyanide antagonist, the mechanism of action for its antidotal effect has not been elucidated.

TREATMENT

There are numerous effective cyanide antagonists; therefore, there is no unanimity of opinion on which is the most effective regimen. This is not surprising, as different experimental conditions and species of animals have been employed in testing the efficacies of different antidotes. Moreover, cyanide poisoning occurs with such frequency that the "successful" treatment of cyanide intoxication by a variety of methods has been reported.

Supportive Treatment

Because there are numerous specific antidotes for the treatment of cyanide poisoning, the importance of general supportive treatment is frequently overlooked. Although numerous reports on the signs and symptoms of cyanide poisoning exist, the fact remains that, in the absence of a suitable history, the diagnosis of cyanide poisoning is difficult. Blake (6, 7) indicated that artificial respiration is effective in protecting against cyanide poisoning. The value of general supportive treatment is particularly emphasized in one case report of a single incidence of a man ingesting potassium cyanide (206). Since the diagnosis of cyanide poisoning was not established, the only treatment employed was supportive. This study represents a rare, well-documented clinical study on cyanide poisoning in man that was successfully treated symptomatically. The dose of cyanide was established at 600 mg, and the blood level cyanide was reported to be 2.0 μg/ml 12 hours after admission to the hospital. The main emphasis is that even though effective antidotes are available, the general supportive treatment of any poisoning should not be ignored. This may be life-saving, particularly when the diagnosis of cyanide poisoning has not been documented.

Methemoglobin-Sulfur Sulfanes-Oxygen

The nitrite-thiosulfate antidotal combination is still one of the most effective treatments of cyanide poisoning, even though the specific mechanism of action of sodium nitrite and sodium thiosulfate are now being questioned.

The present dosage regimen of sodium nitrite-sodium thiosulfate combination recommended for humans still should be used with caution, as nitrite toxicity can occur. The administration of this antidotal combination, especially to children, must be done with caution (207). Presently, it is recommended that children weighing under 25 kg should be given an adjusted dose of sodium nitrite, as the usual dose is potentially lethal (207).

In veterinary practice, the proprietary mixture of sodium thiosulfate and sodium nitrite used to counteract cyanide poisoning can be vastly improved. These proprietary mixtures can protect against 6 LD_{50} doses of sodium cyanide; however, with adjustment of the dosage, it has been possible to raise the protection to 18 LD_{50} doses of sodium cyanide. These studies were conducted in sheep, as they are the range animal most apt to be poisoned by cyanide (171, 193).

The experience with the use of sodium nitrite and sodium thiosulfate as an antidotal combination in the treatment of cyanide poisoning and in clinical cases of intoxication has been summarized for humans in medical practice (207, 208) and for animals in veterinary practice (171, 193, 209). Oxygen should be employed in combination with sodium nitrite and sodium thiosulfate,

as there appears to be no hazard in the use of oxygen and the procedure could be lifesaving. Its use in cyanide poisoning as a routine measure appears to be reasonable.

Objections to the nitrite-thiosulfate antidotal combination relate to the "slow onset of action" of nitrite and the purported surprisingly slow detoxifying capacity of thiosulfate (210). Conclusions on the slow onset of action of nitrite were based primarily on the rate of methemoglobin formation (142, 143). The validity of these analyses already has been addressed. Some researchers have indicated that these measurements may not necessarily be the most reliable parameters in assessing the therapeutic efficacy of the antidotal potential against the lethal effects of cyanide (90, 153, 154). Others advocate the use of DMAP in place of sodium nitrite, since it is a more rapid methemoglobin former (142, 143). Whether or not DMAP and thiosulfate provide more protection against the lethal effects of cyanide than nitrite and thiosulfate remains to be established.

Cobalt

Cobalt did not receive widespread acceptance as a cyanide antagonist, primarily because of its cardiac toxicity, until hydroxocobalamin (164) and subsequently cobalt EDTA were introduced (166, 174, 175, 210). Whether the two latter compounds have appreciably lowered toxicity on a molar basis is still an open question. Cobalt has been implicated as a contributory etiological factor in producing cardiomyopathy among heavy beer drinkers (211–214) because cobalt formerly was used as a beer additive. This was manifested by cardiac arrhythmias of both auricular and ventricular origin. This is of considerable concern, for when cobalt EDTA was employed in the treatment of cyanide intoxication, severe cardiac toxicity was observed and the most serious signs were related to ventricular arrhythmias (178, 215, 216). This finding places rather severe reservations on the use of cobalt EDTA in the treatment of cyanide poisoning.

Other studies also raise the question of whether sodium thiosulfate should be employed with cobalt EDTA or hydroxocobalamin, as sodium thiosulfate sharply enhances the antidotal effect of cobalt (170, 173, 210). The proprietary solution of cobalt EDTA is usually employed alone and contains no sulfane sulfur. Various laboratories in France and Germany have suggested that cobalt EDTA be employed as one of the cyanide antagonists, with the implication that a cobalt chelate or an aminophenol derivative (DMAP) in combination with sodium thiosulfate might replace the classic antidotal nitrite-thiosulfate combination.

It is important to note a species specificity with regard to susceptibility to cobalt. Whereas cobalt-thiosulfate combinations were much more effective than the nitrite-thiosulfate in mice (173), tolerance to cobalt salts were much

lower in sheep (171, 193). It should be emphasized that cobalt exerts its antidotal effect primarily by combining directly with the cyanide ion; therefore, its action is dependent on the molar ratio of cobalt to cyanide. When the dose of cobalt was adjusted to a level that could be tolerated in sheep and the cobalt-thiosulfate combination then was administered to mice, it was found that the cobalt-thiosulfate combination was not as efficacious as before and the nitrite-thiosulfate antidotal combination was far superior in both mice and sheep (171, 173, 193). It should be emphasized that, unlike the addition of oxygen to the nitrite-thiosulfate antidotal combination, the addition of cobalt compounds as a cyanide antagonist may involve a substantial hazard. Even though cobalt can be lifesaving, a careful study of the toxicity of cobalt is essential, as its use under clinical conditions in cyanide poisoning may lead to severe cardiac toxicity (178, 215, 216).

The general assessment of the treatment of cyanide poisoning is blessed with a variety of antidotes that are very effective against the lethal effects of cyanide. For this reason there have been considerable differences of opinion with regard to the treatment of cyanide poisoning. The classic nitrite-thiosulfate treatment of cyanide poisoning developed 50 years ago is still one of the antidotal combinations of choice, particularly if it is employed with oxygen. Questions have been raised about the slow onset of action of sodium nitrite and methemoglobin formation and whether a more rapid methemoglobin former should be employed, i.e. dimethylaminophenol (DMAP). Whether DMAP is more effective than sodium nitrite in combination with sodium thiosulfate is still open to question, even though DMAP forms methemoglobin much more rapidly than sodium nitrite. Proponents for the use of cobalt-containing compounds such as hydroxocobalamin or cobalt EDTA, which are widely used in the United Kingdom, the Scandinavian countries, and Europe, stress the rapidity of action, since cobalt reacts directly with cyanide to form a stable complex in contrast to antidotes that act in an indirect manner and are dependent on the generation of methemoglobin. Concerns have been expressed with regard to the cardiac toxicity of these cobalt compounds, and some reservations about the use of cobalt exist for this reason.

In conclusion, there are a series of very effective cyanide antagonists that have been incorporated into the treatment of cyanide poisoning. There are some reservations with some of the cyanide antagonists and it is anticipated that more effective, safer cyanide antidotal combinations will be forthcoming, particularly since there is now a better basis for the experimental design of these antagonists.

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