IPCS/CEC EVALUATION OF ANTIDOTES SERIES

VOLUME 2

ANTIDOTES FOR POISONING BY CYANIDE

IPCS/CEC Evaluation of Antidotes Series

IPCS   International Programme on Chemical Safety
CEC    Commission of the European Communities

Volume 1   Naloxone, flumazenil and dantrolene as antidotes
Volume 2   Antidotes for poisoning by cyanide

This important new series will provide definitive and authoritative guidance on the use of antidotes to treat poisoning. The International Programme on Chemical Safety (IPCS) and the Commission of the European Communities (CEC) (ILO/UNEP/WHO) have jointly undertaken a major programme to evaluate antidotes used clinically in the treatment of poisoning. The aim of this programme has been to identify and evaluate for the first time in a scientific and rigorous way the efficacy and use of a wide range of antidotes. This series will therefore summarise and assess, on an antidote by antidote basis, their clinical use, mode of action and efficacy. The aim has been to provide an authoritative consensus statement which will greatly assist in the selection and administration of an appropriate antidote. This scientific assessment is complemented by detailed clinical information on routes of administration, contraindications, precautions and so on. The series will therefore collate a wealth of useful information which will be of immense practical use to clinical toxicologists and all those involved in the treatment and management of poisoning.

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PREFACE

At a joint meeting of the World Federation of Associations of Clinical Toxicology and Poison Control Centres, the International Programme on Chemical Safety (IPCS), and the Commission of the European Communities (CEC), held at the headquarters of the World Health Organization in October 1985, the evaluation of antidotes used in the treatment of poisonings was identified as a priority area for international collaboration. During 1986, the IPCS and CEC undertook the preparatory phase of a joint project on this subject. For the purpose of the project an antidote was defined as a therapeutic substance used to counteract the toxic action(s) of a specified xenobiotic. Antidotes, as well as other agents used to prevent the absorption of poisons, to enhance their elimination and to treat their effects on body functions, were listed and preliminarily classified according to the urgency of treatment and...
efficacy in practice. With respect to efficacy in practice, they were classified as: (1) those generally accepted as useful; (2) those widely used and considered promising but not yet universally accepted as useful and requiring further research concerning their efficacy and/or their indications for use; and (3) those of questionable usefulness. Additionally, certain antidotes or agents used for specific purposes were considered to correspond to the WHO criteria for essential drugs (see Criteria for the Selection of Essential Drugs, WHO Technical Report Series 722, Geneva, 1985).

A methodology for the principles of evaluating antidotes and agents used in the treatment of poisonings and a proforma for preparing monographs on antidotes for specific toxins were drafted. These were included in volume 1 of this series.

Monographs are being prepared, using the proforma, for those antidotes and agents provisionally classified in category 1 as regards efficacy in practice. For those classified in categories 2 and 3, where there are insufficient data or controversy regarding efficacy in practice, it was agreed that further study was necessary. Accordingly, several were selected for initial review and evaluation, among which were antidotes used in the treatment of poisoning by cyanide.

The review and evaluation of antidotes used in the treatment of poisoning by cyanide was initiated at a joint meeting of the European Association of Poison Control Centres and Clinical Toxicologists (EAPCCT; formerly known as the European Association of Poison Control Centres), the IPCS, and the CEC, organized by the National Poison Information Centre of the Netherlands National Institute of Public Health and Environmental Hygiene and held at the University Hospital AZU, Utrecht, The Netherlands, 13-15 May 1987. In preparation for this meeting, documents were drafted, using the proforma, on oxygen by Dr U. Taitelman, sodium thiosulfate by Dr H. Persson, hydroxocobalamin by Professor C. Bismuth, dicobalt edetate by Dr T.C. Marrs, sodium nitrite by Dr A. Hall, and 4-dimethylaminophenol by Professor M. von Clarmann. Also in preparation for the meeting, documents were drafted by Professor M. Geldmacher von Mallinckrodt on the analytical assessment of cyanide poisoning, by Dr A. van Dijk on the pharmaceutical aspects of cyanide antidotes, and by Professor A.N.P. van Heijst (formerly Director, Dutch National Poison Control Centre, Utrecht, the Netherlands) on the clinical aspects of cyanide antidotes. The documents presented by each author were discussed at the meeting and participants gave their own experience and views. Experience of industrial aspects of cyanide poisoning was presented by Dr A.C.G.M. Parren.

The main meeting was followed by that of an IPCS/CEC working group, consisting of the authors of documents, the meeting rapporteur and a number of observers, at which a review was made of the comments on the documents and of the additional material presented at the main meeting. Based on the available material, an evaluation was made of the different approaches to treatment of cyanide poisoning depending on the type of cyanide exposure (hydrogen cyanide, either alone or with carbon monoxide, cyanide salts or cyanogenic glycosides), the state of intoxication and number of patients, the location of the patient with respect to treatment facilities, and special situations (e.g., inherited
metabolic and haemoglobin abnormalities). The group concentrated on acute poisoning by cyanide, considering that there were insufficient data for evaluating approaches to treatment of chronic cyanide toxicity. Nevertheless, it was considered that a review of chronic poisoning by cyanide, particularly in relation to cyanide ingestion from food, was needed. It was agreed that traditional means of treatment of cyanide poisoning would have to be revised, and that any evaluation of approaches to treatment must also include antidotes for methaemoglobin-forming agents. Concerning the analytical aspects, it was noted that there was particular difficulty in measuring the concentration of cyanide in blood if an antidote had already been administered, a problem that is being studied by a group of experts established under the auspices of the German Research Association Commission on Clinical Analytical Toxicology. A number of new cyanide antidotes in various stages of research and development were discussed. An editorial group consisting of Professor A.N.P. van Heijst (chairman of the meeting), Dr T.J. Meredith (rapporteur), Dr J.A. Haines (IPCS, chairman of the working group) and Dr J.-C. Berger (CEC) was established in order to prepare a consolidated monograph on cyanide antidotes.

Draft documents were revised by their authors. Those on methylene blue and toluidine blue were prepared by Dr Christina Alonzo (CIAT, Montevideo, Uruguay) and Dr T.C. Marrs, respectively. Subsequently Dr J.A. Vick (Food and Drug Administration, USA), who was invited to the meeting but was unable to attend, prepared a draft document on experience with the use of amyl nitrite in treating cyanide poisoning in animals. Professor C. Bismuth and Dr A. Hall drafted material on new antidotes under development for clinical trials, and Dr A.C.G.M. Parren drafted material on protective measures.

The editorial group met twice in Utrecht on 22-23 October 1987 and 20-22 July 1988. Material was checked and rearranged, additional material was prepared for a number of the chapters and the overview chapter was drafted. The efforts of all who helped in the preparation and finalization of this monograph are gratefully acknowledged.

ABBREVIATIONS

ATA            atmosphere absolute
BE             base excess
CNS            central nervous system
CT             computer tomography
4-DMAP         4-dimethylaminophenol
EDTA           ethylenediaminetetraacetic acid
G6PD           glucose-6-phosphate dehydrogenase
Hb             haemoglobin
HMPS           hexose monophosphate shunt
INN            international non-proprietary name
LDLo           lowest published lethal dose
MLD            minimal lethal dose
NADH           reduced nicotinamide adenine dinucleotide
NADPH          reduced nicotinamide adenine dinucleotide phosphate
OHB12          hydroxocobalamin
LD50           Lethal Dose 50
USP            United States Pharmacopoeia
1. Overview

1.1 Historical Review

The recognition of cyanide as a poison in bitter almonds, cherry laurel leaves, and cassava goes back to antiquity. An inscription on an Egyptian papyrus in the Louvre Museum, Paris, refers to the "penalty of the peach," and Dioscorides in the first century A.D. was aware of the poisonous properties of bitter almonds (Sykes, 1981).

The first description of cyanide poisoning was by Wepfer in 1679 and dealt with the effects of the administration of extract of bitter almonds (Sykes, 1981). Two fatal cases of poisoning in Ireland caused by drinking cherry laurel water, used as a flavouring agent in cooking and to dilute brandy, led to the experiments of Madden (1731). He showed that cherry laurel water contains a poison; given orally, into the rectum, or by injection, it rapidly killed dogs. It was not until 1786 that isolation of pure hydrogen cyanide (HCN) from the dye Prussian blue was achieved by Scheele (1786). The mechanism of toxicity of cyanide was explored by Fontana (1795). Cyanide was obtained from bitter almonds by Schrader (1802). The introduction of cyanide as a medicament to treat coughs and lung diseases was suggested by Magendie (1817). Indeed, it was not until 1948 that cherry laurel water was removed from the British Pharmacopoeia! Attempts to antagonize the toxic effects of cyanide were reported by Blake (1839 and 1840). Hoppe-Seyler (1876) reported that cyanide inhibits tissue oxidation reactions.

Antagonism between amyl nitrite and prussic acid was mentioned by Pedigo (1888), and, as early as 1894, cobalt compounds were advocated by Antal (1894) as cyanide antagonists. Sodium nitrite was used as an antidote in experimental cyanide poisoning by Mladoveanu & Gheorghiu (1929).

A biochemical mechanism for cyanide antagonism was described by Chen et al. (1933, 1934). They suggested using a combination of amyl nitrite, sodium nitrite and sodium thiosulfate, the latter compound serving as a sulfur donor for rhodanese (thiosulfate sulfur transferase). Rhodanese accelerates cyanide detoxification by forming the metabolite thiocyanate. This represented the development of one of the first antidotes based on scientific toxicological reasoning. This combination of antidotes has stood the test of time, and still represents one of the most efficacious antidotal combinations for the treatment of cyanide intoxication.

Interest in cobalt compounds was renewed by Mushett et al. (1952), who demonstrated in 1952 that hydroxocobalamin (vitamin B_{12a}) combined with cyanide to form cyanocobalamin (vitamin B_{12}).

Paulet (1960) subsequently reported that cobalt EDTA was more
effective as a cyanide antidote than the classic nitrite-thiosulfate combination.

1.2 Potential Sources of Cyanide

1.2.1 Industrial sources

Hydrogen cyanide is used in the fumigation of ships, large buildings, flour mills, private dwellings, freight cars, and aeroplanes that have been infested by rodents or insects. It is bound to a carrier, commonly diatomaceous earth, and blended with an odorous or irritating product as a warning marker.

Cyanide salts are utilized in metal cleaning, hardening, ore-extracting processes, and electroplating.

Halogenated cyanides (chloro-, bromo- and iodocyanide) in contact with water produce the non-toxic cyanic acid. As a result of contact with strong acids, hydrogen cyanide is liberated.

Nitriles are cyano-derivatives of organic compounds. Acetonitrile is used as a solvent and is less toxic (LD$_{50}$ = 120 mg/kg) than hydrogen cyanide (LD$_{50}$ = 0.5 mg/kg), but often contains toxic admixtures due to metabolism to inorganic cyanide. While aliphatic nitriles metabolise to inorganic cyanide, the aromatic nitrile bond is stable in vivo. Acrylonitrile is the raw material used for the manufacture of plastics and synthetic fibres. Contact with skin causes bullae formation. Pyrolysis generates hydrogen cyanide. Acrylonitrile and propionitrile are less toxic (LD$_{50}$ = 35 mg/kg) than butyronitrile (LD$_{50}$ = 10 mg/kg). Trichloroacetonitrile (LD$_{50}$ = 200 mg/kg) is used as an insecticide. The aromatic nitriles, bromoxynil (LD$_{50}$ = 190 mg/kg) and ioxynil (LD$_{50}$ = 110 mg/kg), are used as herbicides.

Cyanamide, cyanoacetic acid, ferricyanide and ferrocyanide do not release cyanide. They are therefore less toxic (LD$_{50}$ = 1000-2000 mg/kg) than the cyanogenic compounds above, though they may cause toxicity by other means, e.g. cyanide in combination with alcohol.

1.2.2 Non-industrial sources

Fires and automobile pollution-control devices with malfunctioning catalytic converters (Voorhoeve et al., 1975) generate cyanide. Natural substances, such as wool, silk, horse hair, and tobacco, as well as modern synthetic materials, such as polyurethane and polyacrylonitriles, release cyanide during combustion (Levine et al., 1978; Birky et al., 1979; Anderson & Harland, 1982; Clark et al., 1983; Alarie, 1985; Lowry et al., 1985) (Table 1).

Table 1. Hydrogen cyanide generated by pyrolysis

<table>
<thead>
<tr>
<th>Material</th>
<th>µg HCN per g material</th>
</tr>
</thead>
<tbody>
<tr>
<td>paper</td>
<td>1100</td>
</tr>
<tr>
<td>cotton</td>
<td>130</td>
</tr>
</tbody>
</table>
1.2.3 Natural sources

Cyanide is found in foodstuffs such as cabbage, spinach, and almonds, and as amygdalin in apple pips, peach, plum, cherry, and almond kernels. In the kernels themselves, amygdalin seems to be completely harmless as long as it is relatively dry. However, the seeds contain an enzyme that is capable of catalysing the following hydrolytic reaction when the seeds are crushed and moistened:

\[ C_{20}H_{27}NO_{11} + 2H_2O \rightarrow 2C_6H_{12}O_6 + C_6H_5CHO + HCN \]

Amygdalin glucose benzaldehyde hydrogen cyanide

The reaction is slow in acid but rapid in alkaline solution.

Natural oil of bitter almonds contains 4% HCN. American white lima beans contain 10 mg cyanide/100 g bean. The dried root of cassava (tapioca) may contain 245 mg cyanide/100 g root. The cyanide content in 100 g of cultivated apricot seeds has been found to be about 9 mg and that in wild apricot seeds more than 200 mg.

1.2.4 Iatrogenic sources

Cyanide is also formed during nitroprusside therapy, especially when it is prolonged, because tachyphylaxis sometimes requires the use of higher doses than the recommended maximum of 10 µg/kg per min (Smith & Kruszyna, 1974; MacRae & Owen, 1974; Piper, 1975; Atkins, 1977; Anon, 1978). Cyanide metabolises to thiocyanate. Thiocyanates were used some years ago as antihypertensive agents and they saw wide use because they were very effective. However, a variety of subacute toxic effects, including anorexia, fatigue, and gastrointestinal tract and CNS disturbances, led to their disfavour.

Laetrile, amygdalin derived from apricot kernels, has been used as an anticancer agent, but it is now obsolete because a therapeutic effect could not be demonstrated in either retrospective or prospective studies. Laetrile has caused fatal cyanide poisoning (Sadoff et al., 1978).

1.3 Toxicity of Cyanide in Man

1.3.1 Acute poisoning

It is generally accepted that inhalation of approximately 50 ml (at 1.85 mmol/l) of hydrogen cyanide gas is fatal within minutes. Poisoning from hydrogen cyanide is more frequently accidental than suicidal. Thus accidental cyanide poisoning may occur in fumigators and chemists who use hydrogen cyanide during the course of their work (Chen et al., 1944). In fires, a combination of HCN and carbon monoxide (CO) toxicity, as a result of inhalation of combustion products, may cause fatalities.
Suicidal ingestion of cyanide salts most commonly occurs in personnel with occupational access to cyanide. The ingestion of as little as 250 mg of an inorganic cyanide salt may be fatal (Peters et al., 1982). However, death may be delayed for several hours following the ingestion of cyanide on a full stomach; a first-pass effect in the liver may also delay the onset of toxicity (Naughton, 1974).

1.3.2 Chronic poisoning

Chronic low-dose neurotoxicity have been suggested by epidemiological studies of populations ingesting naturally occurring plant glycosides (Blanc et al, 1985). These glycosides are present in a wide variety of plant species, most notably the cassava plant, a major tropical foodstuff (Conn, 1973; Cook & Coursey, 1981; Ministry of Health, Mozambique, 1984). Cassava has been associated with tropical ataxic neuropathy (Cook & Coursey, 1981). Epidemic spastic paraparesis has been associated with a combination of a high cyanide and a low sulfur intake from diets dominated by insufficiently processed cassava and lacking protein supplementary food (Rosling, 1989). A neurotoxicological role for cyanide has also been suggested in tobacco-associated ambylopia (Grant, 1980) and in amygdalin-associated peripheral neuropathy (Kalyanaraman et al., 1983). Long-term cyanide intoxication has been shown to be associated both with thyroid gland enlargement and dysfunction in case reports and in cohort studies of individuals exposed occupationally (Blanc et al., 1985), through dietary intake (Cook & Coursey, 1981), and experimentally (El Ghawab, 1975).

1.4 Mechanism of Toxicity

Cyanide has a special affinity for the ferric ions that occur in cytochrome oxidase, the terminal oxidative respiratory enzyme in mitochondria. This enzyme is an essential catalyst for tissue utilization of oxygen. When cytochrome oxidase is inhibited by cyanide, histotoxic anoxia occurs as aerobic metabolism becomes inhibited. In massive cyanide poisoning, the mechanism of toxicity is more complex. It is possible that autonomic shock from the release of biogenic amines may play a role by causing cardiac failure (Burrows & Way, 1976). Cyanide could cause both pulmonary arteriolar and/or coronary arterial vasoconstriction, which would result, either directly or indirectly, in pump failure and a decrease in cardiac output. This theory is supported by the sharp increase in central venous pressure that was observed by Vick & Froelich (1985) at a time when the arterial blood pressure fell after the intravenous administration of sodium cyanide to dogs. The observation that phenoxybenzamine, an alpha-adrenergic blocking drug, partially prevented these early changes (Vick & Froelich, 1985) supports the concept of an early shock-like state not related to inhibition of the cytochrome oxidase system. Inhalation of amyl nitrite, a potent arteriolar vasodilating agent, resulted in the survival of dogs in these experimental circumstances. This could have been due to reversal of early cyanide-induced vasoconstriction with restoration of normal cardiac function (Vick & Froelich, 1985).

1.5 Clinical Features

The smell of bitter almonds in expired air is an important sign in cyanide poisoning. However, many people are unable to perceive the odour of hydrocyanic acid (Kalmus & Hubbard, 1960). The
incidence of "non-smellers" is reported to be 18% among males and 5% among females (Kirk & Stenhouse, 1953; Fukumoto et al., 1957).

Immediately after swallowing cyanide, very early symptoms, such as irritation of the tongue and mucous membranes, may be experienced. A blood-stained aspirate may be observed if gastric lavage is performed. Early symptoms and signs that occur after inhalation of HCN or the ingestion of cyanide salts include anxiety, headache, vertigo, confusion, and hyperpnoea, followed by dyspnoea, cyanosis, hypotension, bradycardia, and sinus or AV nodal arrhythmias.

In the secondary stage of poisoning, impaired consciousness, coma and convulsions occur and the skin becomes cold, clammy, and moist. The pulse becomes weaker and more rapid. Opisthotonos and trismus may be observed. Late signs of cyanide toxicity include hypotension, complex arrhythmias, cardiovascular collapse, pulmonary oedema, and death.

It should be emphasized that the bright-red coloration of the skin or absence of cyanosis mentioned in textbooks (Gosselin et al., 1984; Goldfrank et al., 1984) is seldom described in case reports of cyanide poisonings. Theoretically this sign could be explained by the high concentration of oxyhaemoglobin in the venous return, but, especially in massive poisoning, cardiovascular collapse will prevent this from occurring. Sometimes, cyanosis can be observed initially, while later the patient may become bright pink (Hilmann et al., 1974).

The pathogenesis of pulmonary oedema could be due to several different mechanisms: (1) an intracellular metabolic process that could injure the alveolar and capillary epithelium directly, producing a capillary leak syndrome; (2) neurogenic pulmonary oedema or, (3) most likely, a direct effect on the myocardium leading to left ventricular failure and increased pulmonary venous pressure.

The brain is obviously the key organ involved in cyanide poisoning and it has been shown that cyanide significantly increases brain lactate and decreases brain ATP concentrations (Olsen & Klein, 1947).

1.6 Laboratory Findings

1.6.1 Lactic acidosis

Since oxidative phosphorylation is blocked, the rate of glycolysis is markedly increased, which in turn leads to lactic acidosis. The degree of lactic acidosis can be correlated with the severity of cyanide poisoning (Trapp, 1970; Naughton, 1974).

1.6.2 Hyperglycaemia

A reversible toxic effect occurs on the pancreatic beta-cells, which may occasionally give rise to an erroneous diagnosis of hyperglycaemic diabetic coma.

1.6.3 Cyanide concentration in blood and plasma

Before intravenous treatment with antidotes is commenced, it is necessary to collect a heparinized (not fluoride) blood sample for
determination of cyanide concentration. Results from samples collected after treatment are totally unreliable. A quantitative test employing a detector tube (see chapter 10) can be used if the diagnosis is in doubt. The blood can also be used for a quantitative test (see chapter 10), so that the severity of poisoning can be evaluated. Therapeutic measures after antidotal treatment should be based on the clinical condition of the patient rather than on blood cyanide concentrations (Berlin, 1971; Vogel et al., 1981; Peters et al., 1982). Since blood concentrations of up to 0.005-0.04 mg/l have been recorded in healthy non-smokers, and 0.01-0.09 mg/l in smokers, only concentrations above these values were previously considered to be toxic (Vogel et al., 1981; Peters et al., 1982). Lundquist et al., (1985) reported even lower concentration: non-smokers 3.4 µg/l (whole blood), 0.5 µg/l (plasma), 6.0 µg/l (erythrocytes); smokers 8.6 µg/l (whole blood), 0.8 µg/l (plasma), 17.7 µg/l (erythrocytes).

Fatal cyanide poisoning has been reported with whole blood concentrations of >3 mg/l and severe poisoning with 2 mg/l (Graham et al., 1977). However, when cyanide enters the bloodstream, up to 98% quickly enters the red blood cells where it becomes tightly bound. A plasma-to-blood ratio as high as 1:10 has been reported and, as a consequence, the whole blood cyanide concentration may not accurately reflect tissue concentrations of cyanide. Plasma levels of cyanide may be of greater significance because severe toxicity occurs in the presence of only modest concentrations (Vesey et al., 1976). However, a serious drawback to the use of plasma cyanide determinations in the assessment of poisoning is the pronounced instability of cyanide in plasma (Lundquist et al., 1985).

1.7 Biological Detoxification of Cyanide

The major pathway of endogenous detoxification is conversion, by means of thiosulfate, to thiocyanate. Minor routes of elimination are excretion of hydrogen cyanide through the lungs and binding to cysteine or hydroxocobalamin.

Metabolic Detoxification of Cyanide;V02ANnew.BMP

The detoxification of cyanide occurs slowly at the rate of 0.017 mg/kg per min (McNamara, 1976). A sulfurtransferase enzyme is needed to catalyse the transfer of a sulfur atom from the donor thiosulfate to cyanide. The classical theory indicating that mitochondrial thiosulfate sulfurtransferase is the most important enzyme in this reaction is now in doubt because thiosulfate penetrates lipid membranes slowly and would, therefore, not be readily available as a source of sulfur in cyanide poisoning. The modern concept assumes a greater role for the serum albumin-sulfane complex, which is the primary cyanide detoxification buffer operating in normal metabolism (Sylvester et al., 1983). A further enzyme, beta-mercaptopyruvate sulfurtransferase, also converts cyanide to thiocyanate (Vesey et al., 1974). This enzyme is found in the erythrocytes, but in human cells its activity is low.

1.7.1 Thiocyanate toxicity

The detoxification product of cyanide, thiocyanate, is excreted
in the urine. Thiocyanate concentrations are normally between 1-4 mg/l in the plasma of non-smokers and 3-12 mg/l in smokers. The plasma half-life of thiocyanate in patients with normal renal function is 4 h (Blaschle & Melmon, 1980), but in those with renal insufficiency it is markedly prolonged and these patients are therefore at increased risk of toxicity (Schulz et al., 1978). Thiocyanate levels exceeding 100 mg/l are thought to be associated with toxicity. Thiocyanate toxicity is characterized by weakness, muscle spasm, nausea, disorientation, psychosis, hyper-reflexia, and stupor (Smith, 1973; Michenfelder & Tinker, 1977). Lethal poisoning at concentrations greater than 180 mg/l has been reported (Healy, 1931; Garvin, 1939; Russel & Stahl, 1942; Kessler & Hines, 1948; Domalski et al., 1953). Dialysance values of 82.8 ml/min (in vivo) and 102.3 ml/min (in vitro) have been recorded (Pahl & Vaziri, 1982). Little is known about the protein-binding characteristics of thiocyanate, and haemoperfusion may be more effective than haemodialysis.

1.8 Protective Measures for Occupational Exposure

Accidental exposure to cyanide, as either hydrogen cyanide or cyanide salts, will occur primarily in the occupational context, and appropriate preventive and protective measures need to be taken wherever cyanides are manufactured or used. Many industrial accidents occur as a result of mixing cyanide salts and acids, and care should be taken when both are present on industrial premises. As hydrogen cyanide may be generated during combustion of organic substances, fire fighters may also be exposed occupationally.

The public may be affected in the case of a major industrial emergency, or of a transport accident, involving the release of cyanides. It is essential for local authorities in areas where cyanides are used to have contingency plans that will enable them to respond effectively. Adequate hospital facilities for treatment of casualties must be available.

Proper maintenance of plant, good operating practice, and industrial hygiene are essential for the prevention of cyanide poisoning. Areas in the workplace where cyanides are used and containers for storage and transport of cyanide should be clearly marked. Work schedules should ensure that there are at least two people in zones where cyanide could be released accidentally. There should be showers and first-aid kits in these areas. Personnel without proper training should not be allowed in the plant. Normal industrial and laboratory hygiene measures for personnel handling toxic materials, such as dirty and clean locker facilities and showers, should be provided. Eating, drinking, and smoking should not be allowed in the work area where cyanides are used but in places specially reserved for these purposes.

Each employee working at a plant or laboratory that handles cyanides, should receive instruction on the dangers of cyanides and be trained in appropriate first-aid measures, as should emergency-service personnel. They should be aware of the hazards and informed about the possible routes of exposure (inhalation, skin absorption, ingestion). Training should involve recognition of the symptoms and signs of cyanide poisoning and how to achieve safe...
removal of victims from the source of intoxication. Personnel should also be able to guide a rescue or fire-fighting team to a trapped intoxicated person. Rescue personnel should be able to put on protective clothing quickly in an emergency. There should be regular instruction sessions covering procedures for handling cyanides and for rescue in case of accidents, as well as random alarm exercises. First-aid training should include the essential measures to be taken before medical help arrives, which may need to be undertaken at the same time as removal of contaminated clothing and decontamination of exposed skin and eyes. It should be realized that further uptake of cyanide into the blood may occur after showering because of continued skin absorption.

Each plant handling cyanide should have its own medical staff trained in the emergency treatment of cyanide poisonings. The atmospheric concentrations of hydrogen cyanide should be monitored in plants where the gas is used or may be generated. Warning devices are available for this purpose and should be installed. In certain circumstances in which cyanide is used, it is possible to add a warning gas, e.g., cyanogen chloride and chloropicrin have been added to hydrogen cyanide used as a fumigant (Cousineau & Legg, 1935; Polson & Tattersall, 1969).

Filter respirators should be carried at all times by employees working in zones where hydrogen cyanide may be released. At high hydrogen cyanide concentrations, absorption occurs through the skin and impermeable butyl rubber protective clothing is required. Oxygen breathing apparatus may be needed.

In the case of an accident involving hydrogen cyanide there should be both an acoustic and a visual alarm for the plant, which may be activated by workers in zones where the gas is used. Each worker should be aware of the emergency procedures to be followed and the protective clothing and equipment to be used. If a large number of victims is involved or if there is a danger to the public, local authorities need to be warned, so that contingency plans are put into effect and hospitals alerted.

For accidents at plants in remote areas where a qualified physician is not readily available and there are no hospital intensive care facilities, attending paramedical personnel should have the authority and training to perform the special resuscitation measures involved in treating cyanide poisonings, including rapid endotracheal intubation and techniques for obtaining intravenous access.

1.9 Treatment

1.9.1 Supportive treatment

Although effective antidotes are available, general supportive measures should not be ignored and may be life-saving.

According to Jacobs (1984), who reported his personal experience of 104 industrial poisoning cases, the use of specific antidotes was indicated only in cases of severe intoxication with deep coma, wide non-reactive pupils, and respiratory insufficiency in combination with circulatory insufficiency. In patients with moderately severe poisoning, who had suffered only a brief period of unconsciousness, convulsions, vomiting, and cyanosis, therapy
consisted of intensive care and intravenous sodium thiosulfate. In cases of mild intoxication with dizziness, nausea, and drowsiness, rest and oxygen alone were used.

Peden et al. (1986) described nine patients poisoned by hydrogen cyanide released by a leak from a valve. Three of them were briefly unconscious but recovered rapidly after being moved from the area where they had been working. The arterial whole-blood cyanide concentrations on admission were 3.5, 3.1 and 2.8 mg/l, respectively. The cyanide concentrations in the other cases ranged between 2.6 and 0.93 mg/l. All recovered with supportive therapy alone.

Between 1970 and 1984, three other men were treated similarly; two were transiently unconscious, and in these cases the cyanide concentrations 30 min after exposure were 7.7 and 4.7 mg/l. The concentration in the other patient was 1.6 mg/l. All three patients recovered without the use of cyanide antidotes. Small numbers of comatose patients with potentially lethal blood concentrations on admission, and who recovered without cyanide antidotes, have been reported by Graham et al. (1977), Edwards & Thomas (1978), and Vogel et al. (1981).

Even if a patient is unconscious, an antidote does not necessarily have to be administered immediately unless vital signs deteriorate.

A patient exposed to hydrogen cyanide who reaches hospital fully conscious is only likely to require observation and reassurance.

1.9.2 Antidotal treatment

1.9.2.1 Oxygen

It is difficult to understand how oxygen has a favourable effect in cyanide poisoning, because inhibition of cytochrome oxidase is non-competitive. However, oxygen has always been regarded as an important first-aid measure in cyanide poisoning, and there is now experimental evidence that oxygen has specific antidotal activity. Oxygen accelerates the reactivation of cytochrome oxidase and protects against cytochrome oxidase inhibition by cyanide (Takano et al., 1980). Nevertheless, there are other possible modes of action and those that are clinically important have yet to be determined.

Hyperbaric oxygen is recommended for smoke inhalation victims suffering from combined carbon monoxide and cyanide poisoning, since these two agents are synergistically toxic. The use of hyperbaric oxygen in pure cyanide poisoning remains controversial.

1.9.2.2 Sodium thiosulfate

The major route of cyanide detoxification in the body is conversion to thiocyanate by rhodanese, although other sulfurtransferases, such as beta-mercaptopyruvate sulfurtransferase, may also be involved. This reaction requires a source of sulfane sulfur, but endogenous supplies of this substance are limited. Cyanide poisoning is an intramitochondrial process and an intravenous supply of sulfur will only penetrate mitochondria.
slowly. While sodium thiosulfate may be sufficient alone in mild to moderately severe cases, it should be administered with other antidotes in cases of severe poisoning. It is also the antidote of choice when the diagnosis of cyanide intoxication is not certain, for example in cases of smoke inhalation. Sodium thiosulfate is assumed to be intrinsically nontoxic but the detoxification product formed from cyanide, thiocyanate, may cause toxicity in patients with renal insufficiency (see section 1.7).

1.9.2.3 Amyl nitrite

The administration of amyl nitrite by inhalation has been used for many years as a simple first-aid measure that generates methaemoglobin and which can be employed by lay personnel. Its use was abandoned because the methaemoglobin concentration obtained with amyl nitrite is no more than 7% and it is thought that at least 15% is required to bind a potentially lethal dose of cyanide. However, recent studies suggest that methaemoglobin formation plays only a small role in the therapeutic effect of amyl nitrite, and vasodilatation may be the most important mechanism of antidotal action. Artificial respiration with amyl nitrite ampoules broken into an Ambu bag proved to be life-saving in dogs severely poisoned with cyanide. Amyl nitrite may therefore be reintroduced as a first-aid measure.

1.9.2.4 Sodium nitrite

Nitrites generate methaemoglobin, which combines with cyanide to form the nontoxic substance cyanmethaemoglobin. Methaemoglobin does not have a higher affinity for cyanide than does cytochrome oxidase, but there is a much larger potential source of methaemoglobin than there is of cytochrome oxidase. The efficacy of methaemoglobin is therefore primarily the result of mass action. A drawback of methaemoglobin generation is the resultant impairment of oxygen transport to cells and, ideally, the total amount of free haemoglobin should be monitored to ensure aerobic metabolism of the cells. Methaemoglobin can be measured very quickly, but this in itself will not provide an accurate guide to the amount of haemoglobin available for oxygen transport because the cyanmethaemoglobin concentration is not taken into account. Individuals deficient in glucose-6-phosphate dehydrogenase (G6PD) are at great risk from sodium nitrite therapy because of the likelihood of severe haemolysis, but the risk from amyl nitrite is likely to be less because only low plasma concentrations are achieved. Excess methaemoglobinemia may be corrected with either methylene or toluidine blue (see Chapter 9) or, preferably, where feasible, by exchange transfusion.

1.9.2.5 4-Dimethylaminophenol (4-DMAP)

4-DMAP generates a methaemoglobin concentration of 30-50% within a few minutes (Weger, 1968) and, theoretically, it should therefore be valuable as a first-aid measure. However, the problems associated with methaemoglobin formation, as described above for nitrites, apply to 4-DMAP to an even greater extent. Furthermore, it has very poor dose-response curve reproducibility. Haemolysis as a result of 4-DMAP therapy has been observed in overdose as well as following a correct therapeutic dose. Treatment with 4-DMAP is contraindicated in patients with G6PD deficiency. Excess
methaemoglobinaemia may be corrected with either methylene or toluidine blue (see section 1.9.2.8).

1.9.2.6 Hydroxocobalamin (vitamin B$_{12}$a)

This antidote binds cyanide strongly to form cyanocobalamin (vitamin B$_{12}$) and, compared to nitrite and 4-DMAP therapy, it has the great advantage of not interfering with tissue oxygenation. The disadvantage of hydroxocobalamin as a cyanide antidote is the large dose required for it to be effective. Detoxification of 1 mmol cyanide (corresponding to 65 mg KCN) needs 1406 mg hydroxocobalamin. In most countries it is only commercially available in formulations of 1-2 mg per ampoule. In some countries, e.g., France, a formulation is available that contains 4 g hydroxocobalamin powder that has to be reconstituted with 80 ml of a 10% sodium thiosulfate solution prior to use and administered intravenously in a minimum of 220 ml of 5% dextrose. Recorded side effects are anaphylactoid reactions and acne. Some authors have reported a reduced antidotal effect as a result of mixing hydroxocobalamin and sodium thiosulfate in the same solution (Evans, 1964; Friedberg & Shukla, 1975). Histological changes in the liver, myocardium, and kidney apparently induced by hydroxocobalamin have been reported in animal experiments (Hoebel et al., 1980), but their relevance to man has not yet been established. Transient pink discoloration of mucous membranes and urine is an unimportant and nontoxic side-effect.

1.9.2.7 Dicobalt edetate

This agent has been shown to be effective in the treatment of cyanide poisoning in man, and in the United Kingdom it is the current treatment of choice provided that cyanide toxicity is definitely present. This is a strict criterion, because as a result of the manufacturing process some free cobalt ions are always present in solutions of dicobalt edetate. Cobalt ions are toxic and the use of dicobalt edetate, in the absence of cyanide, will lead to serious cobalt toxicity. There is evidence from animal experiments that glucose protects against cobalt toxicity and it is recommended that this be given at the same time as dicobalt edetate. Serious adverse effects recorded include vomiting, urticaria, anaphylactic shock, hypotension, and ventricular arrhythmias (Hilmann et al., 1974; Naughton, 1974).

1.9.2.8 Antidotes to methaemoglobin-forming agents

Accurate determination of methaemoglobin and free haemoglobin concentrations in the presence of cyanide is difficult. Nevertheless, excess methaemoglobinemia does undoubtedly occur on occasions following the use of nitrites and 4-DMAP. Excess methaemoglobin concentrations may be reduced by methylene or toluidine blue. However, regeneration of haemoglobin will release cyanide back into the circulation, leading to a recurrence of toxicity.

1.10 Summary of Treatment Recommendations

The management of cyanide poisoning is determined by (i) the nature of exposure, i.e. hydrogen cyanide (with or without carbon monoxide), cyanide salts, aliphatic nitriles, cyanogenic glycosides; (ii) the severity of poisoning; (iii) the number of patients
involved; (iv) the proximity of hospital facilities; (v) the presence of risk factors, e.g., G6PD deficiency. Urgent specific antidotal therapy is not indicated unless the patient is in a deep coma, with dilated non-reactive pupils and deteriorating cardio-respiratory function. A patient exposed to hydrogen cyanide who reaches hospital fully conscious requires observation and reassurance only.

In order to assess the severity of cyanide poisoning, it is necessary to take a blood sample before the administration of antidotes. Analytical results are otherwise unreliable.

1.10.1 First aid and treatment measures at the site of the incident

The doses given are for adults. Model Information Sheets should be consulted for the pediatric dose and for the use of antidotes in special-risk groups, e.g., G6PD-deficient patients.

The following should be undertaken:

(a) Trained personnel (wearing appropriate protective clothing and breathing apparatus if hydrogen cyanide or liquid cyanide preparations are involved) should

* terminate further exposure
* commence artificial ventilation with 100% oxygen
* administer 0.2-0.4 ml amyl nitrite via Ambu bag

(b) A physician (if immediately present on the scene) should

* terminate further exposure
* artificial ventilation with 100% oxygen
* administer 0.2-0.4 ml amyl nitrite via Ambu bag

In cases of unequivocal moderate to severe poisoning, the above procedure should be followed by

50 ml of 25% sodium thiosulfate solution (12.5 g) i.v. for 10 minutes

and either

20 ml of 1.5% dicobalt edetate solution (300 mg) i.v. for 1 minute

or

10 ml of 40% hydroxocobalamin solution (4 g) i.v. for 20 minutes

or

10 ml of 3% sodium nitrite solution (300 mg) i.v. for 5-20 minutes

or

5 ml of 5% 4-DMAP solution (250 mg or 3-4 mg/kg) i.v. for 1 minute

*a* Oxygen should be administered using a mask and a bag with a "non-return" valve to prevent inspiration of exhaled gases.

1.10.2 Hospital treatment*a*
The doses given are for adults. Model Information Sheets should be consulted for the pediatric doses and for the use of antidotes in special-risk groups, e.g., G6PD-deficient patients.

1.10.2.1 Severe poisoning

Patients in deep coma with dilated non-reactive pupils and deteriorating cardio-respiratory function (blood cyanide concentrations 3 to 4 mg/l) should be given

* artificial ventilation with 100% oxygen
* cardio-respiratory support

This should be followed by

50 ml of 25% sodium thiosulfate solution (12.5 g) i.v. over 10 min

and either

20 ml of 1.5% dicobalt edetate solution (300 mg) i.v. over 1 min

or

10 ml of 40% hydroxocobalamin solution (4 g) i.v. over 20 min

or

10 ml of 3% sodium nitrite solution (300 mg) i.v. over 5-20 min

or

5 ml of 5% 4-DMAP solution (250 mg or 3-4 mg/kg) i.v. over 1 min

1.10.2.2 Moderately severe poisoning

Patients who have suffered a short-lived period of unconsciousness, convulsions, vomiting, and/or cyanosis (blood cyanide concentrations 2-3 mg/l) should be given

a Hospital physicians must establish whether specific antidotal therapy was administered at the time of the incident before further doses are administered, especially in the case of methaemoglobin-forming agents.

b Oxygen should be administered using a mask and a bag with a "non-return" valve to prevent inspiration of exhaled gases.

* 100% oxygen, but for no longer than 12-24 h
* 50 ml of 25% sodium thiosulfate solution (12.5 g) i.v. over 10 min
* observation in an intensive-care area

1.10.2.3 Mild poisoning

Patients with nausea, dizziness, drowsiness only (blood cyanide concentrations < 2 mg/l) should be given

* oxygen
* reassurance
* bed rest
It should be noted that severely poisoned patients may occasionally fail to respond to the initial dose of a specific antidote. Whilst repeat doses of hydroxocobalamin and/or sodium thiosulfate are unlikely to be associated with toxicity, expert advice should be sought before a repeat dose of any other specific antidote is administered. Intensive supportive therapy is of paramount importance in these circumstances.

1.11 Summary of Analytical Aspects

There are many reliable methods for the detection and qualitative determination of cyanide in biological material in cases of suspected intoxication (see Chapter 10). They can be used as "bedside methods" as well as for qualitative determination in cases of acute poisoning but only before antidotes are administered. Interference results from the presence of thiosulfate, methaemoglobin, thiocyanates, and chelating agents during the course of whole-blood cyanide analysis. For this reason, it may be more appropriate to measure plasma rather than whole-blood cyanide concentrations. However, the pronounced instability of cyanide in plasma is a serious drawback (Lundquist et al., 1985).

Quantitative analysis of cyanide in blood or serum before the administration of antidotes is a useful means of evaluating the severity of poisoning. Evaluation of the efficacy of different antidotes will not be possible before accurate methods of analysis free from interference are developed.

When methaemoglobin-generating agents (nitrites or 4-DMAP) are administered as antidotes in cyanide poisoning, it is necessary to maintain an adequate concentration of free haemoglobin in order to guarantee sufficient oxygen transport to allow aerobic tissue metabolism.

Special instruments for rapid analysis of methaemoglobin in hospitals do not provide information about the amount of haemoglobin available for oxygen transport, because the multicomponent analysis is invalidated by the presence of a haemoglobin derivate (cyanmethaemoglobin). Since there is no satisfactory means of quantifying cyanmethaemoglobin under these circumstances, therapy with methaemoglobin-generating agents cannot be monitored at present by laboratory methods.

1.12 Proposed Areas for Research

There are two areas of research where further work is needed as a matter of urgency:

(a) Analytical techniques currently available for the measurement of methaemoglobin do not permit accurate estimation of the amount of free haemoglobin available for oxygen transport, because cyanmethaemoglobin cannot be quantified. A rapid and accurate technique for measuring methaemoglobin and cyanmethaemoglobin concentrations in conjunction is therefore needed to monitor the use of methaemoglobin-generating cyanide antidotes.

(b) Reliable quantitative analytical methods for cyanide in whole blood in the presence of one or more antidotes are
needed.

(c) Determination of cyanide concentration in plasma or serum may be the best reflection of the tissue concentration of cyanide, since cyanide trapped in erythrocytes will not affect tissue utilization of oxygen. However, cyanide has been shown to be very unstable in these body fluids. A method to prevent this phenomenon is urgently needed.

(d) The intravenous injection of DMAP generates high concentrations of methaemoglobin within minutes. However, the absorption kinetics of DMAP administered intramuscularly are not known with certainty, particularly in patients who are shocked with poor muscle perfusion. The efficacy of intramuscular DMAP as a first-aid measure in cases of severe cyanide poisoning needs further evaluation.

(e) Hydroxocobalamin has recently been reported to cause histological changes in the liver, kidney, and myocardium of animals. The relevance of these findings to man is not known and further investigation is required.

(f) Enzyme systems other than cytochrome oxidase may be inhibited. This may be the cause for the symptomatology in acute severe cyanide poisoning.

1.13 New Developments in Cyanide Antidotes

Currently available cyanide antidotes have potentially undesirable adverse effects, and none has been successful in all cases of acute, severe cyanide poisoning. Various agents for the treatment of cyanide poisoning are at the experimental stage of development. However, these antidotes are not currently recommended for administration in cases of human poisoning.

1.13.1 Nonspecific agents

Based on animal studies, certain nonspecific agents, such as naloxone in huge doses (equivalent to 700 mg in a 70 kg human adult compared with a usual therapeutic dose of 0.4 to 10 mg) (Leung et al., 1986) or alpha-adrenergic blocking agents such as chlorpromazine (which has no beneficial effect when administered alone but variably enhances sodium nitrite and/or sodium thiosulfate efficacy) (Kong et al., 1983; Petterson & Cohen, 1985), have been suggested as adjunctive therapy. However, at the moment, there is no accepted place for the use of these agents in human poisoning.

1.13.2 Sodium pyruvate

This agent re-establishes cellular respiration in tumour tissues inactivated by cyanide and has some efficacy in experimental animal poisoning. It may promote cyanide detoxification through combination of the cyanide anion with a carbonyl radical, producing cyanohydrin (Pronczuk de Garbino & Bismuth, 1981). Sodium pyruvate acts rapidly and is well distributed to tissues, but clinical trials in human cyanide poisoning have not been undertaken.

1.13.3 Ifenprodil
Ifenprodil is a 2-piperidine allonal derivative, which, in experimental poisoning, affords some protection including decreased respiratory distress, improved blood pressure, normalization of cardiac rhythm, and lessened electroencephalographic abnormalities. The mechanism of action is thought to be a direct stimulation of mitochondrial respiratory function. At present, ifenprodil is in the investigational stage and no human clinical trials have been proposed (Pronczuk de Garbino & Bismuth, 1981).

1.13.4 Rhodanese

Rhodanese (thiosulfate-cyanide sulfurtransferase) is the naturally-occurring cyanide-detoxifying enzyme (see section 1.7). Although the availability of sulfane sulfur is the rate-limiting factor, studies in dogs have indicated that there is enough rhodanese present in the normal liver and muscle tissue to detoxify about 500 grams of cyanide. When derived from hepatic tissue, the enzyme is unstable and requires an optimal pH for cyanide detoxification. Bacterial enzyme, derived from cultures of *Thiobacillus denitrificans*, is more stable and has been studied in experimental animals. It is efficacious in experimental cyanide poisoning, but no human clinical applications have yet been proposed (Pronczuk de Garbino & Bismuth, 1981).

1.13.5 Alpha-ketoglutaric acid

The cyanide ion can react with carbonyl groups to form cyanohydrins, and this could represent an important detoxification reaction. In rodents poisoned with cyanide and pretreated with various antidotes, alpha-ketoglutaric acid was more effective than either sodium nitrite or sodium thiosulfate, and nearly as effective as sodium nitrite and sodium thiosulfate in combination (Moore et al., 1986). The combination of alpha-ketoglutaric acid with sodium nitrite plus sodium thiosulfate increased the cyanide LD$_{50}$ from a mean of 6.7 mg/kg in control animals to 119.4 mg/kg, whereas the sodium nitrite/thiosulfate combination alone increased the mean LD$_{50}$ to only 35.0 mg/kg (alpha-ketoglutaric acid alone increased the mean LD$_{50}$ to 33.3 mg/kg). No methaemoglobin induction was observed with alpha-ketoglutaric acid administration. Some tremors were noted when this agent was administered alone. Tremors did not occur when sodium thiosulfate was added to the treatment regimen, and the LD$_{50}$ value was increased to 101.3 mg/kg with this combination (very close to the 19.4 mg/kg LD$_{50}$ observed with addition of both sodium nitrite and sodium thiosulfate to alpha-ketoglutaric acid). While these studies demonstrated only protective activity with prophylactic alpha-ketoglutaric acid administration, they raise the possibility of another potentially efficacious and safe antidote combination with sodium thiosulfate (Moore et al., 1986). Alpha-ketoglutaric acid, especially in combination with sodium thiosulfate, deserves further study.

1.13.6 Stroma-free methaemoglobin solution

Stroma-free methaemoglobin solution is prepared from outdated red blood cells by the removal of all cellular membranes (stroma) and induction of methaemoglobinaemia equivalent to 90% of the total haemoglobin with potassium ferricyanide. Any excess potassium ferricyanide is then removed by dialysis against saline. The
resultant preparation does not contain the antigenic components that have been previously reported to cause renal failure and coagulopathies. No rats given stroma-free methaemoglobin solution alone died or had any adverse reactions. Concentrated stroma-free methaemoglobin solution (200-300 g/l) was an effective experimental antidote when administered 30 seconds after doses of cyanide up to 6 times the LD$_{90}$. More dilute solutions were effective 90% of the time following cyanide administration up to 4 times the LD$_{90}$. None of the animals in these studies were given any supportive therapy. In animals administered an amount of stroma-free methaemoglobin solution thought to be equivalent to the conversion of 1.5% of the endogenous haemoglobin, a 90% survival rate was noted when a cyanide LD$_{100}$ was administered. Spectroscopic examination of urine revealed cyanmethaemoglobin excretion (Ten Eyck et al., 1984, 1985, 1986).

These studies suggest that methaemoglobin prepared exogenously may be an effective cyanide antidote. Exogenously administered methaemoglobin would not be expected to interfere with oxygen transport and, unlike methaemoglobin-generating agents (Moore et al., 1987), could even be used in smoke-inhalation victims with elevated carboxyhaemoglobin levels. Since no adverse effects have been noted, this agent may be a safe alternative to currently available cyanide antidotes. However, no human studies have been undertaken and extensive animal toxicology experiments have not yet been reported.

1.14 References


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2. OXYGEN

2.1 Introduction

Oxygen consumption is indispensable for human life. All organs...
of the body will undergo successive dysfunction, permanent damage, and then death if oxygen consumption falls below that necessary for metabolic needs. A decrease in oxygen consumption may be due to inadequate oxygen delivery to the cells or to an inability of the cells to utilize oxygen.

The ambient atmospheric pressure at sea level is defined as one atmosphere absolute (1 ATA), equivalent to an air pressure of 1.03 kg per sq. cm., i.e., 101 kPa or 760 mm of mercury. Molecular oxygen constitutes about 21% of atmospheric air. Hence the partial pressure of oxygen at sea level is 0.21 ATA.

Hyperoxia is the artificial elevation of the partial pressure of oxygen in the body and can be produced by increasing the partial pressure of oxygen from 0.21 to 1 ATA (normobaric oxygen therapy) or above 1 ATA (hyperbaric oxygenation). Normobaric oxygenation is commonly used in many conditions associated with decreased delivery of oxygen to tissues, such as cardiac and pulmonary failure and cardiopulmonary resuscitation.

Both normobaric and hyperbaric oxygen therapy have an antidotal effect on carbon monoxide (CO), hydrogen sulfide (H\textsubscript{2}S), and cyanide (CN) poisoning. However, hyperoxia may induce oxygen toxicity related to the length of exposure and the partial pressure of oxygen employed. The use of oxygen therapy is therefore limited by oxygen toxicity.

2.2 Name and Chemical Formula of Antidote

Molecular oxygen is a diatomic molecule and is properly named dioxygen (formula O\textsubscript{2}, relative molecular mass 16). More than 99.9% of atmospheric oxygen consists of molecules containing the \textsuperscript{16}O isotope. Trace quantities of \textsuperscript{17}O and \textsuperscript{18}O exist in atmospheric air.

2.3 Physico-chemical Properties of Molecular Oxygen

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Critical temperature</td>
<td>154.575 K</td>
</tr>
<tr>
<td>Critical density</td>
<td>0.4361 g/cm	extsuperscript{3}</td>
</tr>
<tr>
<td>Critical pressure</td>
<td>50.14 ATA</td>
</tr>
<tr>
<td>Boiling point</td>
<td>90.188 K</td>
</tr>
<tr>
<td>Melting point</td>
<td>54.361 K</td>
</tr>
<tr>
<td>Solubility in water, 25 °C</td>
<td>2.8%</td>
</tr>
<tr>
<td>Solubility in ethanol</td>
<td>22.6%</td>
</tr>
<tr>
<td>Solubility in plasma</td>
<td>approx 3.0%</td>
</tr>
<tr>
<td>Solubility in whole blood</td>
<td>20.0%</td>
</tr>
</tbody>
</table>

2.4 Synthesis

Oxygen is obtained on a large scale by the liquefaction of air. Modern manufacturing processes produce the gas at a concentration much higher than 99.0% (v/v) of oxygen, this being the lowest permissible concentration in medical oxygen allowed in the European Pharmacopoeia. Contamination with carbon monoxide or carbon dioxide is therefore very slight.

2.5 Analytical Methods

2.5.1 Quality control procedures
2.5.1.1 Tests

For the tests, deliver the sample to be examined at a rate of 4 l/h.

**Carbon monoxide** Carry out the test using 7.5 l of the substance to be examined and 7.5 l of argon R for the blank. The difference between the volumes of sodium thiosulfate (2 mmol/l) used in the titrations should not be more than 0.4 ml (5 ppm v/v).

For the following three tests, pass the sample to be examined through the appropriate reagent contained in a hermetically closed flat-bottomed glass cylinder (with dimensions such that 50 ml of liquid occupies a height of 12 cm to 14 cm) fitted with: (a) a delivery tube terminated by a capillary 1 mm in internal diameter and reaching to within 2 mm of the bottom of the cylinder; (b) an outlet tube. Prepare the reference solutions in identical cylinders.

**Acidity or alkalinity**

**Test solution** Pass 2.0 litres of the sample to be examined through a mixture of 0.1 ml of hydrochloric acid (0.01 mol/l) and 50 ml of CO₂-free water R.

**Reference solution (a)** 50 ml of CO₂-free water R.

**Reference solution (b)** To 50 ml of CO₂-free water R add 0.2 ml of hydrochloric acid (0.01 mol/l).

To each solution add 0.1 ml of a 0.02% m/v solution of methyl red R in alcohol (70% v/v). The intensity of the colour of the test solution should be between those of reference solutions (a) and (b).

**Carbon dioxide** Pass 1.0 litre through 50 ml of barium hydroxide solution R (the solution to be used must be clear). Any turbidity in the solution after passage of the gas should not be more intense than that in a reference solution prepared by adding 1 ml of a 0.11% m/v solution of sodium hydrogen carbonate R in CO₂-free water R to 50 ml of barium hydroxide solution R (300 ppm v/v).

**Oxidizing substances** Place in each of two cylinders 50 ml of freshly prepared potassium iodide and starch solution R, and add 0.2 ml of glacial acetic acid R. Protect the cylinders from light. Pass 5.0 l of the substance to be examined into one of the cylinders. The test solution should remain colourless when compared with the blank.

2.5.1.2 Assay for oxygen

Use a gas burette (see Fig. 1) of 25 ml capacity in the form of a chamber having at its upper end a tube graduated in 0.2% divisions between 95 and 100, and isolated at each end by a tap with a conical barrel. The lower tap is joined to a tube with an olive-shaped nozzle and is used to introduce the gas into the apparatus. A cylindrical funnel above the upper tap is used to introduce the
absorbent solution. Wash the burette with water and dry. Open the two taps. Connect the nozzle to the source of the sample to be examined and set the flow rate to 1 l/min. Flush the burette by passing the substance to be examined through it for 1 min. Close the upper tap of the burette and immediately afterwards the lower tap. Rapidly disconnect the burette from the source of the sample to be examined and give a half turn to the upper tap to eliminate any excess pressure in the burette. Keeping the burette vertical, fill the funnel with a limited amount of freshly prepared mixture of 21 ml of a 56% m/v solution of potassium hydroxide R and 130 ml of a 20% m/v solution of sodium dithionite R. Open the upper tap slowly. The solution absorbs the oxygen and enters the burette. Allow to stand for 10 min without shaking. Read the level of the liquid meniscus on the graduated part of the burette. This figure represents the percentage v/v of oxygen.

2.5.2 Methods for identification

(a) Place a glowing splint of wood in the substance to be examined. The splint bursts into flame.

(b) Shake with alkaline pyrogallol solution R. The sample to be examined is absorbed and the solution becomes dark brown.
2.5.3 Methods for analysis of the antidote in biological samples

2.5.3.1 In the gas phase

Fig. 1. Burette used for the assay of oxygen
(dimensions in ml except for the graduated tube)
(a) Chemical volumetric methods based on absorption of oxygen by chemical reagents (such as alkaline pyrogallol) are still in use. The skill of the operator is a major factor in the accuracy of the results.

(b) Methods based on physical properties of oxygen:

* paramagnetic susceptibility;
* thermomagnetic (magnetic wind);
* differential pressure (Quincke);
* magnetic auto-balance (Faraday);
* electron capture;
* ultraviolet absorption;
* mass spectrometry.

2.5.3.2 In solution

Polarographic measurement is a sensitive method for measuring the partial pressure of oxygen in solution. The Clark electrode is commonly used for biological samples. This has a cathode made of a platinum wire, while the anode is Ag/AgCl in phosphate buffer with added KCl.

2.5.4 The saturation of haemoglobin by oxygen

The degree of saturation of haemoglobin by oxygen (HbO₂ %) can be measured by photometric procedures. This method is particularly useful for the non-invasive monitoring of patients (oximeters, pulse oximeters).

2.6 Storage Conditions

Oxygen should be stored under pressure in a suitable metal container of a type permitted by the safety regulations of the national authority. Valves and taps should not be lubricated with oil or grease.
The containers for medical oxygen are coded with a white colour at the top according to ISO-32-19 (Fig 2) and carry the indication "Medical Oxygen" carved in the iron material of the container. To prevent connection with other medical gases, the containers are provided with an international standardized "Pin Index Safety System" ISO-407. This system is the same for medical and for industrial oxygen so that in emergencies industrial oxygen can also be used.

Regular control of gas pressure is necessary to prevent shortage of oxygen in emergency situations.

Although the duration of storage will not alter the quality, it is recommended that the gas should not be stored for more than six months.

2.7 General Properties

In most circumstances, supplemental oxygen is indicated if tissue hypoxia is imminent. In the case of cyanide poisoning, however, cytochrome oxidase activity is inhibited and tissue utilization of oxygen prevented. Even so, animal experiments (Takano et al., 1980) suggest that inhibition of cytochrome oxidase activity by cyanide is prevented, and recovery accelerated, in the presence of oxygen.

2.8 Animal Studies

2.8.1 Pharmacokinetics

The bioavailability of oxygen depends on the following factors:

(a) the thickness of the alveolar membrane; as a result of oedema fluid in the interstitial spaces of the membrane oxygen cannot readily diffuse into the blood;

(b) haemoglobin configuration:

(i) oxygen transport by the blood to the tissues can be disturbed by methaemoglobin-forming antidotes, e.g., sodium nitrite or 4-dimethylaminophenol, often used in the treatment of cyanide poisoning;

(ii) oxygen transport is also hampered in carbon monoxide poisoning, which occurs in combination with cyanide poisoning following smoke inhalation.

(c) Oxygen utilization by tissues is prevented by the inhibition of cytochrome oxidase activity in cyanide poisoning.

2.8.2 Pharmacodynamics

Isom & Way (1982) studied the reduction of cytochrome oxidase prepared from brains and livers of mice poisoned with potassium cyanide. They examined several groups of animals and compared exposure to air and to oxygen at 0.95 ATA, both with and without sodium nitrite and sodium thiosulfate treatment. Cytochrome oxidase was inhibited by 75% within 2 min of the injection of 5 KCN mg/kg. The inhibition of cytochrome oxidase was similar whether the animals
inhaled air or 0.95 ATA O\textsubscript{2}, but the reactivation phase of cytochrome oxidase was faster in oxygen-breathing animals. They also found that, at a lower dose (4 KCN mg/kg), enzyme activity was increased from 22% to 66% by increasing the partial pressure of oxygen from 0.11 to 0.95 ATA O\textsubscript{2}. Oxygen shifted the dose-response curve of brain cytochrome oxidase inhibition by KCN to the right: the dose of KCN producing 50% inhibition was 24 mg/kg in air-breathing animals and 55 mg/kg in animals breathing oxygen. The authors also found that liver rhodanese activity was 15 times higher than brain rhodanese activity and that treatment with sodium nitrite and sodium thiosulfate protected liver, but not brain, cytochrome oxidase at high doses of KCN.

Way et al. (1972) found that oxygen alone antagonized cyanide toxicity. However, they found no significant benefit in increasing the partial pressure of oxygen from 1 to 4 ATA O\textsubscript{2}. The authors demonstrated that oxygen potentiates the effect of thiosulfate and that the best results were obtained using hyperbaric oxygen together with nitrite and thiosulfate.

Ivanov (1959) reported that hyperbaric oxygen re-established normal EEG activity in animals poisoned with cyanide.

Takano et al. (1980) measured the reduction of pyridine nucleotide \textit{in vivo} as an indicator of cytochrome oxidase inhibition. They found a protective effect of oxygen at 1 and 2 ATA O\textsubscript{2} when the doses of KCN injected into rabbits were within the range that produced inhibition of cytochrome oxidase in animals breathing air. At much higher doses, the protective effect of O\textsubscript{2} at 1 or 2 ATA became progressively weaker.

Paulet (1955) described the protective effect of oxygen on the chemoreceptors and on the clinical manifestations in dogs poisoned by cyanide.

Cope & Tenn (1961) showed that infusion of KCN solution (2 g/l) to anaesthetized dogs breathing air produced atrioventricular block. Oxygen breathing reversed this phenomenon and re-established normal sinus rhythm.

2.8.3 Toxicology

Oxygen toxicity was discovered in 1878 by Paul Bert, who observed convulsions in animals treated with air at 15-20 ATA. He then noted that when pure oxygen was used instead of air, one-fifth of the pressure sufficed to produce the convulsions. He concluded that oxygen toxicity is directly correlated to the product of partial pressure of oxygen and administration period.

Pulmonary oxygen toxicity was first described in 1897 by Lorrain Smith, who noted that mice exposed to oxygen at pressures above 1 ATA died from respiratory failure and that there was great variability both within and between animal species in the susceptibility to oxygen damage.

The initial stage of acute pulmonary oxygen toxicity is termed the exudative phase and is characterized by a perivascular and interstitial inflammatory response that includes damage to the capillary endothelium with exudation of oedema.
fluid, polymorphonuclear leucocytes and, eventually, macrophages. The oedema is associated with widening of the interalveolar septa and a thickened air-blood tissue barrier. Alveolar haemorrhage develops and there is fibrin deposition in the alveoli (hyaline membranes). Loss of alveolar lining cells (type I lining cells) can also occur during this phase. As the condition progresses, it reaches a stage referred to as the proliferative phase, characterized by resolution of the inflammatory exudate and increased cellularity of the interstitium due to proliferation of macrophages and fibroblasts. There is also proliferation of the cuboidal type II alveolar lining cells that serve to re-epithelialize the denuded alveolar basement membrane. By the time a typical laboratory animal, such as the rat, is near death, a substantial number of pulmonary capillaries have been destroyed and embolization of some arterioles will have occurred. Depending on the species, however, the destruction of type I alveolar lining cells and the proliferation of the type II cells may not be extensive.

The bronchial tree is damaged by oxygen toxicity: a necrotizing bronchiolitis has been described in neonatal mice by Ludwin et al. (1974). The bronchiolitis consists of degeneration and necrosis of bronchiolar mucosal epithelium involving both ciliated as well as non-ciliated (Clara) cells. Early change, occurring within 72 h in neonatal mice, consist of loss of cilia, cellular swelling associated with mitochondrial swelling, oedema of the bronchiolar wall, and leucocytic infiltration of the bronchiolar connective tissue. These changes are followed by necrosis of the epithelial cells with subsequent sloughing. Necrosis of the mucosal cells is maximal at 6 to 7 days. Cold-blooded animals are relatively resistant to oxygen toxicity.

Guinea-pigs, dogs and rats have a median time to death ($LT_{50}$) in the range of 65–80 h while breathing 1 ATA oxygen. Monkeys have an $LT_{50}$ of about 100 h, while chicks survived for 28 days at 1 ATA. In general, there is a great interspecies variability in susceptibility to hyperoxia.

2.8.3.1 Mechanism of injury

Free radicals (superoxide anion $O^{-2}$ and hydroxyl radical $OH€$) are produced by reduction of oxygen and these and hydrogen peroxide ($H_2O_2$) are responsible for most of the toxic effects of oxygen. The free radicals react with cellular components and cause oxidative damage by oxygen radicals of the unsaturated fatty acids, which are abundant in the membranes of cells, and the sulfhydryl groups present in many enzymes and cellular proteins. The cells are protected against oxygen radicals by enzymes which convert the radicals to less toxic molecules and to molecular oxygen. Haber & Weiss (1934) described the production of hydroxyl free radicals from the superoxide anion and hydrogen peroxide.

Hyperoxia also causes arteriolar vasoconstriction, this being unrelated to the free radical mechanism.

2.9 Volunteer Studies of Pulmonary Oxygen Toxicity

Clark & Lambertsen (1971) studied normal humans exposed to 2 ATA $O_2$. Symptoms began within 3–8 h in the form of mild
tracheal irritation and increased in intensity throughout the exposure time. After 8-10 h, symptoms were characterized by uncontrollable coughing, dyspnoea at rest, and a constant tracheobronchial burning sensation. Decreased vital capacity was the most constant and sensitive sign of pulmonary oxygen toxicity. Decreased carbon monoxide diffusion capacity and increased alveolar-arterial $O_2$ difference were also observed.

Singer et al. (1970) compared two groups of patients treated with 0.4 ATA $O_2$ or 1 ATA $O_2$ for up to 24 h and found no immediate or delayed dysfunction. He concluded that humans can tolerate 1 ATA $O_2$ for up to 24 h without detectable pulmonary dysfunction. Comroe et al. (1945) showed that inhalation of oxygen at 0.5-1.0 ATA $O_2$ for more than 24 h decreased vital capacity. Clark & Lambertsen (1971) established oxygen pressure versus time tolerance curves. It appears that no dysfunction is observed if up to 0.5 ATA $O_2$ is inhaled for many days and that the use of 1 ATA $O_2$ is safe for up to 24 h.

2.10 Clinical Studies of Oxygen Toxicity

All human cells are affected by oxygen toxicity, but the three organs most susceptible to acute oxygen toxicity are the eyes in the newborn, and the lung and central nervous system at all ages.

2.10.1 Eyes

The eyes of the newborn and, especially, those of the premature newborn, are very sensitive to oxygen toxicity, which produces retrolental fibroplasia.

Patz (1968a,b) described retrolental fibroplasia which occurred in premature babies exposed to 0.30-0.45 ATA $O_2$ for 10 days. Initially, in the presence of high concentrations of oxygen, there is vasoconstriction of arteries, arterioles and venules with necrosis of the immature retinal vessels (referred to as the vaso-obliterative phase). Following oxygen-induced vaso-obliteration, there is a vaso-proliferation of new capillaries in the inner layer of the retina. The proliferation of capillaries extends into the vitreous behind the lens and eventually results in retinal detachment, which represents the final phase.

2.10.2 Central nervous system (CNS)

Central nervous system oxygen toxicity is characterized by grand-mal seizures. The manifestation of acute CNS toxicity in humans consists not only of grand-mal seizures but also of focal motor seizures, constriction of the visual fields, deafness, hyperacuity, changes of mood, and visual and auditory hallucinations. CNS oxygen toxicity in humans is only observed during hyperbaric oxygen exposure.

Grand-mal convulsions have been described in humans exposed to pressures above 2.8 ATA $O_2$ for periods exceeding 2 h. Central nervous system toxicity is rare if the $O_2$ pressure is less than 2.5 ATA and if oxygen and air are used intermittently, e.g., 10 min of air and 30 min of oxygen, as long as the total hyperbaric exposure time does not exceed 2 h.
2.11 Clinical Studies - Case Reports

In most cases reported in the literature the evidence for cyanide poisoning is lacking because blood concentrations of cyanide are not reported. Moreover, the majority of cases of cyanide poisoning are treated with oxygen in combination with other antidotal therapy. The individual role of oxygen as a cyanide antidote in man is therefore difficult to assess. The following cases have been reported.

2.11.1 Patients treated alone with supportive therapy and who survived

* An analytical chemist (48 years old) was found unconscious with unrecordable blood pressure and cardiac arrest. The blood cyanide level was 5.8 mg/l (Edwards & Thomas, 1978).

* A three-year-old child was treated with laetrile enemas. On admission the child was cyanotic, lethargic, and unresponsive. The blood cyanide level was 2.14 mg/l (Ortega & Creek, 1978).

* A biochemist, aged 31 years, was found in deep coma with a metabolic acidosis and a blood cyanide concentration of 2.3 mg/l (Vogel et al., 1981).

* Nine people were poisoned by hydrogen cyanide as a result of a leak from a valve. Six of them lost consciousness briefly. Blood cyanide concentrations were 2.8 to 7.7 mg/l (Peden et al., 1986).

2.11.2 Hyperbaric oxygen therapy in cyanide poisoning

Trapp (1970) reported acute cyanide poisoning successfully treated with hyperbaric oxygen, while Litovitz et al. (1983) reported the unsuccessful use of hyperbaric oxygen.

Trapp & Lepawsky (1983) reported five cases of acute cyanide poisoning successfully treated by hyperbaric oxygen in the Vancouver General Hospital, Canada.

2.11.3 Cyanide poisoning due to smoke inhalation

Four out of five smoke inhalation victims with mean blood cyanide levels of 1.62 mg/l (62 µmol/l) are reported to have survived after receiving hyperbaric oxygen therapy combined with the administration of sodium nitrite and sodium thiosulfate (Hart et al., 1985).

2.12 Summary of Evaluation

1. In acute cases of poisoning by inhalation of hydrogen cyanide, either alone or in combination with carbon monoxide, termination of exposure and evacuation of the patient from contaminated areas is indicated most urgently and should be performed by the rescuers using appropriate protective equipment. Measures to support respiration, circulation, and cardiac function should be commenced, if necessary. Patients should receive pure humidified oxygen.
Following smoke inhalation, patients frequently develop acute pulmonary failure due to the irritant effect of the smoke. They may benefit from the use of continuous positive airway pressure while breathing spontaneously or positive end-expiratory pressure if ventilated artificially.

Other antidotes for cyanide poisoning, especially sodium thiosulfate, should be administered intravenously as soon as possible. Blood samples, *which must be taken before the administration of antidotes*, should be sent for toxicological analysis.

2. In acute cyanide poisoning by ingestion, decontamination of the stomach should be performed as soon as possible after or during first-aid therapy.

3. The final decision about the use of hyperbaric oxygen therapy depends on the availability of chambers and the ability to treat.

4. Oxygen has always been regarded as an important first-aid measure even though, it is difficult to understand, on a theoretical basis, how oxygen has a favourable effect in cyanide poisoning because inhibition of cytochrome oxidase is non-competitive. There is now experimental evidence that oxygen has specific antidotal activity. Oxygen accelerates the reactivation of cytochrome oxidase and protects against cytochrome oxidase inhibition by cyanide. Nevertheless, there are other possible modes of action and those which are clinically important have yet to be determined.

5. Hyperbaric oxygen is recommended for smoke inhalation victims suffering from combined carbon monoxide and cyanide poisoning, since these two agents are synergistically toxic. The role of hyperbaric oxygen in pure cyanide poisoning remains controversial.

2.13 Model Information Sheet

2.13.1 Uses

Oxygen is indicated for the treatment of patients poisoned by cyanide, either alone or in combination with carbon monoxide following smoke inhalation.

2.13.2 Dosage and route

In cases of severe poisoning (respiratory insufficiency and/or deteriorating vital signs), artificial ventilation with 100% oxygen is indicated but for no longer than 12-24 h, after which the inspired oxygen concentration should be reduced. Similarly in moderately severe cases, 100% oxygen is indicated but again for no longer than 12-24 h. For mild cases 40% oxygen is indicated.

2.13.3 Precautions/contraindications

In order to prevent rebreathing of hydrogen cyanide from the expired air of a patient, it is advisable to include a non-return valve when a bag and mask is used.
2.13.4 Adverse effects

The alveolar membranes of the lung are damaged by 100% oxygen if this is administered for more than 24 h.

2.13.5 Use in pregnancy and lactation

No contraindication.

2.13.6 Storage

Store under pressure in a suitable metal container of a type permitted by the safety regulations of the national authority. Valves and taps should not be lubricated with oil or grease.

The containers for medical oxygen are coded with a white colour at the top according to ISO-32-19 (Fig. 2) and carry the indication "Medical Oxygen" in the iron material of the container. To prevent connection with other medical gases the containers are provided with an international standardized "Pin Index Safety System" ISO-407. This system is the same for medical and for industrial oxygen so that in emergencies industrial oxygen can also be used.

Regular control of gas pressure is necessary to prevent shortage of oxygen in emergency situations.

Although the duration of storage will not alter the quality it is recommended that the gas should not be stored for more than six months.

2.14 References


Ivanov JP (1959) The effect of elevated oxygen pressure on animals


3. SODIUM THIOSULFATE

3.1 Introduction

3.1.1 Indications

The use of sodium thiosulfate as an antidote has been recorded in the literature on poisoning due to cyanide, mustard gas, nitrogen mustard, bromate, chloride, bromine, iodine, cisplatin, and certain drugs (Dactinomycin, Mechlorethamine, Mitomycin) when extravasated. There are also some references to its effect in iodate and
3.1.2 Rationale for the choice of the antidote

The effect of sodium thiosulfate as an antidote in cyanide poisoning is well documented, and was first demonstrated by Lang (1895). Some authors believe it to be a relatively slow-acting antidote, though others have demonstrated that it acts more rapidly than was previously thought, enabling the conversion of cyanide to thiocyanate (Krapez et al., 1981). Thiosulfate helps to detoxify cyanide in the presence of the enzyme rhodanese. However, rhodanese is an intramitochondrial enzyme and thiosulfate has limited ability to penetrate cell and mitochondrial membranes. Thus, distribution of thiosulfate is almost exclusively extracellular (Cardozo & Edelman, 1952), while its antidotal action has been thought to take place intracellularly. This view is now being re-examined in the light of recent experimental evidence (see section 3.8).

3.1.3 Risk groups

No special-risk groups can be identified as regards the use of sodium thiosulfate. However, it should be noted that there is an apparently reduced ability to convert cyanide to thiocyanate in some diseases, e.g., toxic amblyopias (in particular tobacco amblyopia) and Leber's hereditary optic atrophy (Wilson, 1965; Darby & Wilson, 1967). Abnormally low rhodanese activity in the liver has been described in two patients with Leber's hereditary optic atrophy (Grant, 1986).

3.2 Name and Chemical Formula of Antidote

International non-proprietary name: Natrii thiosulfas; Sodium thiosulfate (thiosulphate); Thiosulfate de sodium; Natrium thiosulfuricum; Natriumthiosulfat (Hager, 1977).

CAS number: 10102-17-7 for sodium thiosulfate, pentahydrate (NIOSH, 1986); 7772-98-7 for sodium thiosulfate, anhydrous, (NIOSH, 1986).

IUPAC name: Sodium thiosulfate, pentahydrate

Manufacturer: Readily available in many countries.

Commercial names: Commercially available as sodium thiosulfate or equivalent in many countries.

Formula: \( \text{Na}_2\text{S}_2\text{O}_3.5\text{H}_2\text{O} \) (Martindale, 1989)

Relative molecular mass: 248.2 (Martindale, 1989)

Specification of chemical salt used: sodium thiosulfate contains not less that 99.0% and not more than the equivalent of 101.0% of \( \text{Na}_2\text{S}_2\text{O}_3.5\text{H}_2\text{O} \) (European Pharmacopoeia, 1980); transparent, colourless crystals (European Pharmacopoeia, 1980); colourless, odourless, (or almost odourless) monoclinic prismatic crystals, or a coarse crystalline powder with a saline taste (Martindale, 1989).

3.3 Physico-chemical Properties
3.3.1 Melting point, boiling point

Sodium thiosulfate dissolves in its own water of crystallisation at about 49 °C (European Pharmacopoeia, 1980; Martindale, 1989). It loses all its water at 100 °C and decomposes at higher temperatures (Windholz, 1983). Above 200-300 °C, it decomposes to sulfate and pentasulfide (Kirk-Othmer, 1969; Hager, 1977). When it is heated to the point of decomposition, fumes of sulfur oxides are emitted (Sax, 1984; PoisIndex, 1987).

3.3.2 Solubility in vehicle for administration

Highly soluble in water (2 parts sodium thiosulfate in 1 part water) (Martindale, 1982; Windholz, 1983).

3.3.3 Optical properties

Inactive with respect to polarized light; absorbs ultraviolet light at wavelengths less than 230 nm (information from the National Corporation of Swedish Pharmacies).

3.3.4 Acidity

A 10% solution in water has a pH of 6.0-8.4 (European Pharmacopoeia, 1980; Martindale, 1989). The pH of injectable thiosulfate (0.15 g/ml) is 8.2-8.8 (information from the National Corporation of Swedish Pharmacies).

3.3.5 pKa

pKa₁: 1.46-1.74 (an approximate value) (IUPAC, 1969).

3.3.6 Stability

Efflorescent in warm (>30 °C) dry air. Slightly hygroscopic (deliquescent) in moist air (Windholz, 1983). Store in an airtight container (Martindale, 1989).

Aqueous solutions have limited stability due to a tendency to decompose slowly by the following reactions:

\[ \text{Na}_2\text{S}_2\text{O}_3 \rightarrow \text{Na}_2\text{SO}_3 + \text{S} \] (neutral or acidic solutions)

\[ \text{Na}_2\text{S}_2\text{O}_3 + \text{H}_2\text{O} \rightarrow \text{Na}_2\text{SO}_4 + \text{H}_2\text{S} \] (alkaline solutions)

The first reaction is accelerated by acids and the second by air or oxygen. Aqueous solutions of sodium thiosulfate decompose more rapidly on heating. Storage with limited access to air and light in a cool environment increases stability (Kirk-Othmer, 1969; Martindale, 1989; Windholz, 1983).

Injectable thiosulfate stored in ampoules for three years shows no significant change in composition.

3.3.7 Refractive index, specific gravity
No value for refractive index (injectable thiosulfate [0.15 g/ml]) is available. The specific gravity for injectable thiosulfate (0.15 g/ml) is 1.076 (information from the National Corporation of Swedish Pharmacies).

3.3.8 Loss of weight on drying

The substance loses 35.5-37.0% of weight when it is dried at 105 °C (Pharmacopoea Nordica, 1964; Hager, 1977).

3.3.9 Excipients

For injectable thiosulfate (0.15 g/ml): sodium phosphate dodecahydrate (Na$_2$HPO$_4$.12H$_2$O) 1.21% (information from The National Corporation of Swedish Pharmacies; see also section 3.6).

3.3.10 Incompatibility

Incompatibility with iodine, acids, lead, mercury and silver salts (Windholz, 1983) and with salts of heavy metals, oxidizing agents, and acids has been indicated. If sodium thiosulfate is triturated with chlorates, nitrates, or permanganates, an explosion may occur (Martindale, 1989).

3.3.11 Other information

Solutions are sterilized by autoclaving. A 2.98% solution is iso-osmotic with serum (Martindale, 1989).

Table 2. Physical properties of sodium thiosulfate pentahydrate

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>refractive index, n$_d^{20}$</td>
<td>1.4886</td>
</tr>
<tr>
<td>density, d$_{425}$</td>
<td>1.750</td>
</tr>
<tr>
<td>heat of solution in water at 25 °C (kcal/mol)</td>
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<tr>
<td>heat of formation (kcal/mol)</td>
<td>-621.9</td>
</tr>
<tr>
<td>heat of fusion (kcal/mol)</td>
<td>11.85</td>
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<tr>
<td>specific heat at 21 °C (cal/g)</td>
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<tr>
<td>cryoscopic constant</td>
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<tr>
<td>dissociation pressure (mm Hg)</td>
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</tr>
<tr>
<td>at 20 °C</td>
<td>6.0</td>
</tr>
<tr>
<td>at 35 °C</td>
<td>18.0</td>
</tr>
<tr>
<td>vapour pressure of saturated solution (mmHg)</td>
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</tr>
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<td>at 33 °C</td>
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</tr>
<tr>
<td>at 57 °C</td>
<td>42.0</td>
</tr>
<tr>
<td>at 90 °C</td>
<td>233.0</td>
</tr>
<tr>
<td>density of aqueous solution, d$_{20}$, (Na$_2$S$_2$O$_3$, by weight)</td>
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<tr>
<td>10%</td>
<td>1.0847</td>
</tr>
<tr>
<td>20%</td>
<td>1.1760</td>
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<tr>
<td>30%</td>
<td>1.2762</td>
</tr>
<tr>
<td>40%</td>
<td>1.3851</td>
</tr>
</tbody>
</table>
3.4 Synthesis

Sodium thiosulfate can be produced commercially by a number of methods, of which the four principal processes may be classified as follows (Kirk-Othmer, 1969).

(a) Air oxidation of sulfides, hydrosulfides, and polysulfides of alkali and alkaline earth metals.

(b) Recovery from waste liquors resulting from the production of sulfur black and other sulfur dyes.

(c) Reaction of sulfur dioxide and sulfides.

(d) Reaction of sulfur and sulfites.

Possible contaminants include chlorides, sulfates, sulfites, and heavy metals.

3.5 Analytical Methods

3.5.1 Quality control procedures for sodium thiosulfate (European Pharmacopoeia, 1980)

Tests for chlorides, sulfates, sulfites, sulfides, and heavy metals. The pH of a 10% solution is 6.0-8.4; clear and colourless (European Pharmacopoeia, 1980).

3.5.2 Methods for identifying sodium thiosulfate (European Pharmacopoeia, 1980).

(a) Decolorization of iodinated potassium iodide solution R.

(b) In combination with silver nitrate solution R₂, a white precipitate forms, which rapidly turns yellowish and then black.

(c) In combination with hydrochloric acid, a precipitate of sulfur is formed and a gas is evolved which gives a blue colour to starch iodate paper R.

(d) Reaction on sodium.

3.5.3 Assay

Dissolve 0.500g in 20ml of water and titrate with 0.1 N iodine, using 1 ml of starch solution R, added towards the end of the titration, as indicator.

1 ml of 0.1 N iodine is equivalent to 24.82 mg of Na₂S₂O₃·5H₂O (European Pharmacopoeia, 1980).

3.5.4 Methods for analysis of sodium thiosulfate in biological samples
Gast et al. (1952) and Dixon (1962) described a method for the measurement of thiosulfate, based on reduction by iodine, but this is a nonspecific means of estimation.

Sörbo & Ohman (1978) described a method for the determination of thiosulfate in urine. After the removal of interfering compounds, including endogenous thiocyanate by ion exchange, thiosulfate is converted to thiocyanate in the presence of cyanide and cupric ions. The thiocyanate formed is concentrated by ion exchange, eluted with an acid solution of ferric ions, and the ferric thiocyanate complex is determined colorimetrically.

A method for determining biological thiols at the picomole level, based upon the conversion of thiols to fluorescent derivatives by reaction with monobromobimane and the separation of the derivatives by reverse-phase high-performance liquid chromatography, was described by Newton et al. (1981). A modification of this method was used by Shea et al. (1984) for the determination of thiosulfate levels in plasma and urine.

The methylene blue method (Ivankovich et al., 1983) is considered specific for thiosulfate and sensitive to 1 µg/ml. The analyses are undertaken directly on heparinized plasma or urine. Potassium iodide, potassium bromide, and monobasic potassium phosphate are added to the sample. Then potassium borohydride (in sodium hydroxide) and acetone are added with stirring, followed by ferric sulfate (with water) and \( N,N\)-dimethyl-\( p \)-phenylenediamine sulfate in sulfuric acid. A blue colour develops and absorbance is measured at 665 nm.

### 3.6 Shelf-life

According to studies at the National Corporation of Swedish Pharmacies, which has followed the stability of injectable thiosulfate (0.15 g/ml) (Sweden) for three years, no significant changes occurred during the period of observation.

A solution without, or with only small amounts of, sodium phosphate dodecahydrate is unstable. The National Corporation of Swedish Pharmacies has found the composition described in section 3.3.9 to be the most suitable. For information concerning storage, see section 3.13.6.

### 3.7 General Properties

#### 3.7.1 Mechanism of antidotal activity

The major route of detoxification of cyanide in the body is conversion to thiocyanate. This reaction requires a source of sulfane sulfur (divalent sulfur bonded to another sulfur) and is catalysed by sulfur transferases. It has been suggested that there is a physiological pool of cyanide-reactive sulfane sulfur bound to albumin that might act as a buffer against endogenous cyanide production (Westley et al., 1983; Way et al., 1984). Thiosulfate is present in the body in only small quantities, derived mainly from cystine and other mercapto compounds. The physiological reserves available for detoxifying cyanide are therefore limited (Schulz et al., 1979b).
Na₂S₂O₃ + CN⁻ → SCN⁻ + Na₂SO₃.

The sulfurtransferases catalyse reactions where sulfane sulfur is involved. Rhodanese is the sulfurtransferase which has been studied most extensively. Rhodanese (thiosulfate: cyanide sulfurtransferase; EC 2.8.1.1.) catalyses the transfer of sulfane sulfur directly to cyanide. It is distributed throughout the body, the highest concentrations being found in the liver, and is located mainly in the matrix of mitochondria (Westley et al., 1983).

The existence of a thiocyanate oxidase that could oxidize thiocyanate back to cyanide (Goldstein & Rieders, 1953) has been questioned. However, this is now attributed to artifactual formation of HCN during assay (Vesey, 1979).

Sodium thiosulfate contains the necessary divalent sulfur donor bound to another sulfur and it is the main sulfur donor for rhodanese in the conversion of cyanide to thiocyanate. Whereas rhodanese is available in excess in the body, the lack of a suitable sulfur donor is the rate-limiting factor for this route of detoxification in cyanide poisoning. This is the rationale for the administration of sodium thiosulfate in cyanide poisoning so that the endogenous detoxification capacity of the body is enhanced.

3.7.2 Other biochemical/pharmacological profiles

As described above, sodium thiosulfate may be used for indications other than cyanide poisoning, utilizing other properties of this substance. These are not dealt with in this document.

3.8 Animal Studies

3.8.1 Pharmacokinetics

When high doses of thiosulfate are injected into mammals, the greater part is eliminated unchanged by renal excretion but a certain amount is oxidized to sulfate. This latter fraction increases as the dose of thiosulfate decreases. The oxidation of thiosulfate to sulfate occurs in the liver by a two-step enzymatic pathway. Studies by Gilman et al. (1946) demonstrated that intravenously injected thiosulfate is rapidly distributed in the extracellular fluid space and that its renal excretion occurs by glomerular filtration. Further animal experiments have shown that tubular transport may also take place (Sörbo, 1972).

Thiosulfate is both secreted and reabsorbed in man and dog, according to Bucht (1949) and Foulks et al. (1952). Clearance of thiosulfate is low, but at high levels clearance equals the glomerular filtration rate. This means that at high plasma levels of thiosulfate, secretion Tm (transfer maximum) is similar to reabsorption Tm, whereas at low plasma levels both filtered and secreted thiosulfate are reabsorbed and thus there is a diminished clearance value for thiosulfate.

The volume of distribution, as determined in seven dogs weighing 8.5-14.4 kg was, on average, 3 l (Cardozo & Edelman, 1952). Kinetic studies have shown that there is a cationic site on rhodanese for the anionic sulfur donor (Westley et al., 1983). Most of an injected dose of thiosulfate is excreted unchanged. Thiosulfate is thought to permeate slowly through cell membranes.
According to Crompton et al. (1974), thiosulfate can utilize the dicarboxylate carrier to enter the mitochondria, as shown in experiments with rat liver mitochondria. This system is specific for divalent anions.

It has been shown by Szczepkowski et al. (1961) that when using labelled thiosulfate the two atoms of sulfur have different fates during the course of metabolism in animals. In rats, the inner sulfur atom is eliminated very quickly in the form of sulfate while the outer atom is transformed into sulfate much more slowly, probably going through a number of intermediate stages.

When an experimental animal is injected with thiosulfate containing $^{35}$S in its sulfane position exclusively, the whole of this can be found labelled in the plasma as quickly as a sample can be obtained (Schneider & Westley, 1969).

Experiments on dogs (Michenfelder & Tinker, 1977; Schulz et al., 1979b) have shown that the capacity of the endogenous reserves of thiosulfate to detoxify cyanide is exceeded if sodium nitroprusside is administered as a continuous infusion at a rate of more than 0.5 mg/kg per h. When the experimental animals received doses greater than 0.5 mg/kg per h, their blood cyanide concentrations rose continuously. Experimental animals receiving the same dosage under otherwise identical conditions, but with the additional infusion of thiosulfate at six times (w/w) the sodium nitroprusside dosage, showed no abnormal signs. The urinary volume in the thiosulfate-treated dogs, over a 48 h period, was approximately twice that of the untreated animals, presumably due to the increased rate of formation of thiocyanate and the resultant osmotic diuresis.

Similar results were obtained in experiments on rabbits (Höbel et al., 1978).

3.8.2 Pharmacodynamics

After the induction of acute sodium nitroprusside (SNP) intoxication in rabbits, bolus injections of thiosulfate and hydroxocobalamin ($\text{B}_{12a}$), at SNP/antidote molar ratios of 1:5, were equally effective in reducing the early signs and severity of the metabolic acidosis (Pill et al., 1980). During the subsequent observation period, the base excess with $\text{B}_{12a}$ as an antidote was found to be lower than with thiosulfate. When the two antidotes were given in parallel with a highly toxic dose of SNP, sodium thiosulfate proved to be superior to $\text{B}_{12a}$. The authors suggested that for clinical purposes, SNP should always be administered in combination with thiosulfate (1:5).

One molecule of sodium nitroprusside contains five cyanide ions. Thiosulfate should therefore be given in a molar ratio of at least 5:1, which corresponds to a dose of four parts by weight of sodium thiosulfate to one of SNP. Schulz et al. (1979b) suggested that since thiosulfate is rapidly metabolized and eliminated from the body, it is better to administer it in excess by continuous infusion.
In studies by Ivankovich et al. (1980), dogs were given KCN in a constant infusion (0.1 mg/kg per min). One group of animals (n = 5) was given an infusion of sodium thiosulfate (12 mg/kg per h) intravenously 10 min prior to and during KCN infusion; another group (n = 5) was given an intravenous bolus injection of thiosulfate (150 mg/kg). The infusion increased the amount of cyanide needed to cause death and the bolus injection increased the protection from lethality even further. It was shown that thiosulfate alone is capable of providing complete protection against both cyanide and cyanide-forming compounds when administered simultaneously with these compounds as a continuous infusion.

Furthermore, thiosulfate treatment resulted in no noticeable adverse haemodynamic or respiratory effects when given as either a bolus or a constant infusion. When high plasma concentrations of thiosulfate are available, the detoxification mechanism is rapid enough to provide adequate protection. Since thiosulfate is rapidly eliminated by the kidneys, this high plasma level of thiosulfate is best maintained by constant infusion. The only deleterious effect of such a constant infusion may be a lowering of plasma volume, since thiosulfate acts as an osmotic diuretic at this dosage; however, this is rarely important clinically. The authors stated that true detoxification of cyanide was achieved with thiosulfate alone and that thiosulfate appears to be the agent of choice, resulting in the lowest cyanide concentrations in tissues and blood and the fewest side effects.

Dogs given thiosulfate (75 mg/kg) intravenously 5 min before the start of an infusion of SNP (1.5 mg/kg) had significantly lower plasma and red cell cyanide concentrations, and significantly higher plasma thiocyanate concentrations, than controls (Krapez et al., 1981). These changes were associated with only minimal disturbance of tissue oxygenation. The authors suggested that a bolus dose of sodium thiosulfate (75 mg/kg) is an effective antidote with negligible toxicity. This study demonstrates that thiosulfate acts more rapidly than had been thought previously. The maintenance of low blood cyanide concentrations, coupled with the rapid increase in plasma thiocyanate concentrations and unimpaired tissue oxygenation in those animals that received thiosulfate, strongly suggests that cyanide was converted to thiocyanate as quickly as it was released from the nitroprusside. In this study, no synergism was found between sodium thiosulfate and hydroxocobalamin. Investigations by Evans (1964) suggested a negative interaction between thiosulfate and hydroxocobalamin, but others (Hall & Rumack, 1987) believe there is antidotal synergy.

In a study by Vesey et al. (1985), a bolus dose of sodium thiosulfate (150 mg/kg, i.e., 12 times the stoichiometric amount theoretically required to "neutralize" the cyanide from the SNP dose) was given to dogs at the end of a 60-min infusion of SNP (3 mg/kg, i.e., near lethal dose). Compared with the controls, there was an impressive reduction in the mean half-lives of plasma cyanide (25.1 min as opposed to 74.1 min) and red blood cell cyanide concentrations (22.4 min as opposed to 203.6 min). Cyanide toxicity may be delayed after SNP administration due to continued breakdown and release of HCN. The authors suggested that red blood cells act as a site for cyanide detoxication. Thiosulfate enhances the rate of HCN metabolism and also limits its peripheral distribution in dogs (Sylvester et al., 1981).
Chen et al. (1934) showed that sodium thiosulfate detoxified sodium cyanide at up to 3 times the minimal lethal dose (MLD). Sodium nitrite did so at up to 4 times the MLD and a combination of the two at up to 20 times the MLD.

Differing doses of thiosulfate were given intraperitoneally to mice at different times after the injection of sublethal or lethal doses of cyanide (Schubert & Brill, 1968). When thiosulfate was administered to mice 5 min after cyanide, the time for recovery from cyanide toxicity was shortened considerably. Rats given thiosulfate 10 min after cyanide (when inhibition of liver cytochrome oxidase was maximal) invariably recovered 5 to 10 min later instead of the 30 to 40 min normally required without treatment.

A pharmacokinetic analysis of cyanide distribution and metabolism with and without intravenous sodium thiosulfate was conducted in pretreated mongrel dogs (Sylvester et al., 1983). The mechanism of thiosulfate protection appeared to be extremely rapid formation of thiocyanate in the central compartment, which therefore limited the amount of cyanide distributed to sites of toxicity. Thiosulfate increased the rate of conversion of cyanide to thiocyanate over 30-fold.

3.8.3 Toxicology

According to NIOSH (1986), the intravenous LD₅₀ in the mouse is 2350 mg/kg, whereas the intravenous lowest published lethal dose (LDLo) in the dog is 3000 mg/kg (Dennis & Fletcher, 1966). When dogs were given 3000 mg/kg of sodium thiosulfate pentahydrate intravenously (Dennis & Fletcher, 1966), the following effects developed rapidly: metabolic acidosis, hypoxaemia, hypernatraemia, and changes in the ECG and in arterial and venous pressures. In these experiments, an immediate and rapid rise in the serum sodium concentration would be expected, since the sodium content in sodium thiosulfate pentahydrate is about 24 mEq/3000 mg. Moreover, the dogs that survived the injection showed a marked diuresis, which would be expected from the large osmotic dose administered. The authors suggested that sodium thiosulfate pentahydrate (1500 mg/kg) given intravenously at a constant rate over a 30-min period is tolerated well.

During chronic sodium nitroprusside (SNP) administration, the simultaneous infusion of thiosulfate may present problems because of enhanced plasma thiocyanate accumulation and the danger of hypovolaemia (Michenfelder & Tinker, 1977). Vesey et al. (1985) suggest that it would be sufficient to give a bolus dose of sodium thiosulfate only if the SNP dose/dose-rate is excessive.

There appears to be no information concerning the teratogenicity or mutagenicity of sodium thiosulfate.

3.9 Volunteer Studies

Ivankovich et al. (1983) studied the available thiosulfate pool and the pharmacokinetics of administered thiosulfate in healthy volunteer subjects. Plasma thiosulfate concentrations, sampled from the volunteers were 11.3 (± 1.1) mg/l (n = 26) and urine thiosulfate concentrations were 2.8 (± 0.2) mg/l (n = 24). Bile contained 137.2 (± 29.5) mg/l thiosulfate (n = 6, sampled during cholecystectomy). Thiosulfate (150 mg/kg) was injected intravenously into 5 normal
male volunteers. Plasma thiosulfate concentrations after 5 min were 1012 (± 88.5) mg/l. The half-life of the distribution phase was 23 min and that of the elimination phase 182 min. The calculated $V_d$ was 151 ml/kg body weight. Urine concentration, clearance, and rate of thiosulfate excretion increased markedly after injection. Total excretion was 42.6 (± 3.5)% of the injected dose at 180 min, although urinary excretion did not increase much during the elimination phase; at 18 h after injection it was 47.4 (± 2.4)%. Bile thiosulfate excretion did not change after thiosulfate injection and bile excretion of thiosulfate accounted for less than 0.1% of total thiosulfate excretion. This study demonstrated that the plasma concentration of thiosulfate in normal males is about 10 mg/l, and that excretion amounts to approximately 3 mg/day, compared with findings of Sörbo & Ohman (1978) who discovered a renal excretion value of 31.7 (± 12.8) mmol/24 h (7.9 (± 3.2) mg/l per 24h). The normal endogenous production of thiosulfate can therefore be considered to be small and the ability to produce increased amounts in response to poisoning is likely to be limited. The $V_d$ is 150 ml/kg and a 70 kg man would therefore have a total extracellular thiosulfate content of 125 mg. Similar human $V_d$ values were found by Cardozo & Edelman (1952). "Therapeutic" doses of thiosulfate (150 mg/kg according to these authors) would elevate plasma concentrations about 100 times. Such high concentrations may be necessary to increase the intracellular concentration and enable rhodanese to detoxify cyanide at the mitochondrial membrane if indeed this is the site of action of thiosulfate (see above).

Schulz (1984) and Schulz et al. (1982) found a serum half-life for thiosulfate of about 15 min during SNP therapy. According to Gladtke (1966), the elimination half-life of sodium thiosulfate is about 40 min (children aged 4 months to 14 years).

Absorption of sodium thiosulfate after oral administration is poor (Martindale 1989). The oral toxicity is low and single doses of 5–18 g have only a laxative action. Nausea and vomiting have also been reported (Sörbo, 1972; Poisindex, 1987). It has also been shown that thiosulfate injected intravenously is rapidly distributed in the extracellular fluid space. Its renal excretion occurs by glomerular filtration and secretion (Bucht, 1949; Foulks et al., 1952; Sörbo, 1972).

3.10 Clinical Studies

The use of sodium thiosulfate as a single antidote in cyanide poisoning has been evaluated in only a few studies.

Schulz et al. (1982) studied cyanide toxicity resulting from sodium nitroprusside (SNP) in therapeutic use with and without sodium thiosulfate. Cyanide was analysed using the method of Asmus and Garschagen (see chapter 10), and concentrations were expressed as nmol cyanide/ml erythrocyte. Thiocyanate concentrations were also measured; following the addition of ferric chloride, thiocyanate reacted with elemental chlorine to form cyanogen chloride. The remainder of the measurement was carried out as for cyanide. Concentrations were expressed as nmol thiocyanate/ml plasma throughout. Thiosulfate was measured in plasma using the method of Gast et al. (1952) and Dixon (1962). Seventy patients (aged 17–78 years) were studied.
In 51 patients, SNP was given for short periods, while in 19 patients, SNP was given over longer periods of up to 2 weeks, usually in combination with sodium thiosulfate infusion. In seven of these 19 patients, 1 g sodium thiosulfate was given intravenously as a bolus injection during SNP treatment in order to study the kinetics. The drugs were infused quickly in a ratio of 50 mg SNP to 500 mg sodium thiosulfate. The threshold dose for the release of free cyanide into the bloodstream in this study was 2 µg SNP/kg per min. It was calculated that 5 µg/kg per min for 10 h, 10 µg/kg per min for 4 hr or 20 µg/kg per min for 1.5 h would cause potentially toxic levels of cyanide. Sodium thiosulfate by infusion stopped accumulation of cyanide and the elevated cyanide levels declined, whereas thiocyanate levels increased. The simultaneous infusion of thiosulfate and SNP prevented accumulation of cyanide. According to Saunders & Himwich (1950), the optimum in vitro molar cyanide/thiosulfate ratio for the rhodanese reaction is 1:3. As none of the patients in this study showed clinical signs of toxicity, an assessment of treatment efficacy was made on the basis of analytical data.

Shea et al. (1984) found that sodium thiosulfate (12 g/m²) could be given safely to humans over 6 h provided that cardiac and renal functions were normal. Similarly, 2 g/m² per h for 12 h has been given without side-effects (Howell et al., 1982). The elimination half-life determined by Shea et al. (1984) using a one-compartment kinetic model was approximately 80 min.

Schulz et al. (1979a) studied the kinetics of thiocyanate in healthy subjects and in patients with renal failure. In healthy subjects, the elimination half-life of thiocyanate (oral administration) was 1 to 5 days (mean 3 days). The average half-life for patients with renal failure was 9 days, with elimination constants increasing in proportion to the creatinine clearance. These findings have special relevance to the use of sodium nitroprusside in the treatment of patients with renal insufficiency.

### 3.11 Clinical Studies - Case Reports

Numerous cases have been reported where sodium thiosulfate has been used in conjunction with other antidotes in the treatment of cyanide poisoning. The use of sodium thiosulfate alone, though, has been reported only rarely.

A baby, weighing 4.4 kg, developed hypertension in the neonatal period and was treated with SNP by infusion (2-5 µg/kg per min). After 30 h the blood pressure fell and the child became acidotic. The erythrocyte cyanide concentration was 400 nmol/ml (life-threatening intoxication). Infusion of sodium thiosulfate in a dose of 100 mg/kg promptly caused the cyanide level to fall to one-tenth of its maximum value. It was concluded that the mixed infusion of SNP and thiosulfate is always advisable, especially in small children where the thiosulfate pool is small (Schulz & Roth, 1982).

A man aged 42 years was treated with SNP and sodium thiosulfate in combination and observations were made to calculate appropriate doses. The patient developed toxic cyanide levels (erythrocyte cyanide concentration, 3.6 µg/ml) during long-term infusion of SNP,
and was treated successfully with sodium thiosulfate (the erythrocyte cyanide concentration level dropped to 0.5 µg/ml within 7 h). It was suggested that thiosulfate should be given at a dose at least four times that of SNP but, as some excess of thiosulfate is desirable, it was suggested that 300-400 mg sodium thiosulfate be given together with 60 mg SNP by continuous infusion (Schulz et al., 1979b).

A boy aged 14 years was given SNP during surgery until the desired clinical effect was achieved. After 5 h the patient developed circulatory shock but responded promptly to the administration of sodium thiosulfate (150 mg/kg) given over 15 min (Perschau et al., 1977).

A man aged 30 years ingested an unknown amount of a cyanide-containing insecticide in a glass of beer. He was found unconscious, apnoeic, and cyanotic 30 min later. Resuscitative measures were commenced and he was given sodium thiosulfate intravenously. He regained consciousness but remained mute for 12 days and developed choreiform movements. On examination 16 years later everything was normal with the exception of mildly dysarthric speech and some minor motor disturbances. A computer tomography (CT) scan showed bilateral symmetrical infarction of the globus pallidus and infarction of the left cerebellar hemisphere (Finelli, 1981).

During a fire in an apartment the mother jumped out through the window with a baby while twin brothers, aged 2.5 years, tried to get out through the front door. They were subsequently found unconscious just inside the door and remained so on admission to hospital. Both children were severely acidic with pH values of 6.77 and 6.9, respectively. Initial treatment consisted of 100% oxygen, controlled ventilation, and the intravenous administration of sodium thiosulfate (approximately 400 mg/kg body weight). A few hours later hyperbaric oxygen was also employed. Carbon monoxide concentrations were low, being 5.7% and 1.4%, and cyanide concentrations in blood were 1.15 mg/l and 1.1 mg/l. One twin remained more severely ill than the other, but both children were discharged after 3 weeks without sequelae.

A man aged 28 years ingested an unknown amount of a cyanide solution in an attempt at suicide. On admission to hospital, shortly after ingestion, he was talkative and anxious with an intense smell of bitter almonds. He was hyperventilating slightly and had a mild metabolic acidosis (BE -6). He was given 15 g of sodium thiosulfate by slow intravenous infusion. The patient did not become unconscious but instead became calm and mentally clear. The acidosis reverted without further treatment. The blood cyanide concentration was 39.7 µmol/l (9.9 mg/l) and that of thiocyanate, 150 µmol/l (0.88 mg/l).

A man aged 32 years ingested an unknown amount of potassium cyanide solution in a suicidal attempt. The patient became unconscious after 10 min and was brought to hospital within 40 min after ingestion. He was by then deeply unconscious, slightly cyanotic, and unresponsive to pain. The patient was treated immediately with oxygen, 8 g sodium thiosulfate intravenously, and gastric lavage. There was striking improvement after 30 min and Kelocyanor was then given, after which the patient became completely conscious. The cyanide concentration in blood was 3.7 mg/l.
A 54-year-old man ingested 3 g potassium cyanide and arrived in hospital unconscious and with respiratory arrest. The patient was resuscitated and 12 g of sodium thiosulfate was given intravenously. There was a moderately severe acidosis (pH 7.31, BE -11) and another 3 g of sodium thiosulfate was therefore given. After 30 min the patient breathed spontaneously, after 90 min he moved his extremities and eyes, and after 6 h he was fully alert. No sequelae were observed. The cyanide concentration in blood was 0.26 mg/l.

3.12 Summary of Evaluation

3.12.1 Indications

Sodium thiosulfate is indicated in poisoning from cyanide, chlorate, bromate, bromine, iodine, cisplatin, mustard gas, and nitrogen mustard. However, this monograph deals only with the use of sodium thiosulfate as an antidote in cyanide poisoning.

3.12.2 Route of administration

In cyanide poisoning, sodium thiosulfate should be given intravenously (absorption is poor after oral administration) as a bolus injection or by infusion over at least 10 min. When used to prevent cyanide poisoning during sodium nitroprusside therapy, it may be given either simultaneously by continuous infusion or, alternatively, as a slow bolus injection.

3.12.3 Dose

The recommended initial dose for adults in established cyanide poisoning is 8 to 12.5 g (Chen et al., 1944; Chen & Rose, 1952), or 0.2 g/kg body weight (Sörbo, 1972). This dose is based on individual cases where doses of this size have proven effective. Experimental data and theoretical considerations support these recommendations, though true validation is lacking.

For children relatively higher doses are generally recommended. For children with normal haemoglobin concentrations, a dose of approximately 410 mg/kg body wt has been suggested (Berlin, 1970) and many handbooks suggest doses in the range 300-500 mg/kg body weight. It should be noted that in those sources which make these recommendations, sodium thiosulfate is used in combination with other antidotes, especially sodium nitrite.

The risk of cyanide poisoning in patients undergoing treatment with sodium nitroprusside is well documented. Sodium thiosulfate has been found to be ideal in this situation, and it has been recommended that the w/w ratio for SNP and sodium thiosulfate should be at least 1:4 (Schulz et al., 1979b) and preferably, to obtain an excess of thiosulfate, 1:5-6. The antidote may be given either by continuous infusion, simultaneously with SNP (Schulz et al., 1982), or by bolus injection.

3.12.4 Other consequential or supportive therapy

The capacity of sodium thiosulfate to enhance the detoxification of cyanide in the body has been established in animals and man. As an antidote in cyanide poisoning, sodium thiosulfate alone, together with oxygen and necessary supportive
therapy, is probably sufficient in mild to moderately severe cases. It is also valuable in doubtful cases of poisoning, where it may have both therapeutic and diagnostic value. In severe poisoning, sodium thiosulfate should be given together with other antidotes, with which it acts synergistically.

3.13  Model Information Sheet

3.13.1  Uses

Sodium thiosulfate is indicated for use in cyanide poisoning.

3.13.2  Dosage and route of administration (cyanide poisoning)

The initial dose in adults is (8 to)12.5 g of sodium thiosulfate given as an intravenous bolus injection/infusion over 10 (to 15) min. Alternatively, the total initial dose can be calculated as 150-200 mg/kg body weight. Additional doses may be indicated according to the clinical course.

The initial dose in children is 400 (300-500) mg/kg body weight given intravenously as indicated above.

To prevent cyanide intoxication during SNP therapy, sodium thiosulfate should be given either by simultaneous infusion of a dose 5-6 times exceeding (w/w) the SNP dose or, alternatively, a bolus injection may be employed.

3.13.3  Precautions and contraindications

There are no specific contraindications. The toxicity of sodium thiosulfate is low and toxic effects should not be expected unless doses far exceed those recommended. In patients with renal insufficiency, dialysis can be considered for the more rapid elimination of thiocyanate (during long-term treatment).

3.13.4  Adverse effects

Adverse effects are mild and of minor importance compared to the risks associated with cyanide poisoning. Rapid injection of a hyperosmolar sodium thiosulfate solution has caused nausea and vomiting (Ivankovich et al., 1983). Hypotension has been reported, due probably to the formation of thiocyanate, which is known to have hypotensive properties (Done, 1961). Other side effects attributed to excess thiocyanate production are nausea, headache, and disorientation. When thiosulfate was injected into dogs (Vesey et al., 1985) no side effects were seen other than transient hypotension. Diuretic effects and osmotic disturbances are possible side effects (Martindale, 1989).

3.13.5  Use in pregnancy/lactation

These aspects are seldom discussed in the literature and are of little relevance in this context. In a life-saving situation, the dosage recommended above should not be modified in the case of pregnancy or lactation.

3.13.6  Storage
Injectable thiosulfate should be stored in ampoules. Storage over three years does not cause any significant change in composition. The solid substance may be stored in an airtight container for five years without change.

3.14 References


European Pharmacopoeia (1980) 2nd ed. Sainte-Ruffine, France, Maisonneuve S.A.


Lang S (1895) [Prussic acid detoxification.] Arch Exp Pathol Pharmakol, 36: 75-99 (in German).

Michenfelder JD & Tinker JH (1977) Cyanide toxicity and thiosulfate protection during chronic administration of sodium nitroprusside in the dog: Correlation with a human case. Anesthesiology, 47: 441-448.


4. Hydroxocobalamin

4.1 Introduction

Hydroxocobalamin is one of a number of antidotes which may be used in the treatment of cyanide poisoning. It acts as a chelating agent for cyanide. Hydroxocobalamin is commonly used in conjunction with sodium thiosulfate (Na₂S₂O₃) (Trousse anticyanure, Laboratoire Anphar-Rolland). Another (unregistered) product is made
by the Pharmacie Centrale des Hôpitaux de Paris. It consists of 5 g hydroxycobalamin in 100 ml water.

4.2 Name and Chemical Formula of Antidote

Hydroxocobalamin (OHB\textsubscript{12}) is a natural form of vitamin B\textsubscript{12} found in humans (vitamin B\textsubscript{12a}). This chemical is described in the British Pharmacopoeia (1980), the Italian Pharmacopoeia (1985), the Pharmacopoée Française (1988), and the United States Pharmacopeia (1985).

CAS number: 13 422-51-0

Formula: (dimethyl-5,6-benzimadazolyl) hydroxocobamide

\[
\text{C}_{62}\text{H}_{89}\text{CoN}_{13}\text{O}_{15}\text{P}
\]

**Fig. 3. Structural formula of hydroxocobalmin**

Structure: OHB\textsubscript{12} contains a hydroxyl group attached to a cobalt atom.

Relative molecular mass: 1346.47.

Contains not less than 95.0% and not more than 102.0% of C\textsubscript{62}H\textsubscript{89}CoN\textsubscript{13}O\textsubscript{15}P, calculated on a dried weight basis (United States Pharmacopeia, 1985)

Commercial names: The Anphar-Rolland Cyanide Antidote Kit (containing OHB\textsubscript{12} and Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3}). Other proprietary formulations currently available contain only 1 mg/ml. Another (unregistered) product is produced by the Pharmacie Centrale des Hôpitaux de Paris. It consists of 5 g hydroxycobalamin in 100 ml water.

4.3 Physico-chemical Properties

4.3.1 Characteristics
Dark red crystals or a red powder
Hygroscopic
Odourless.

4.3.2 Melting-point
Becomes brown at 200 °C
Not melted at 300 °C.

4.3.3 Solubility in vehicles for administration
Solubility in water: 1 part OHB₁₂ in 50 parts water.
(Martindale, 1989).

4.3.4 Optical properties
Not relevant.

4.3.5 Acidity
The pH of a 0.2% solution in water is greater than 7.5
(Pharmacopée Française, 1988). The pH of a 2% solution in water is
8.0 - 10.0 (USP XXI).

4.3.6 Stability in light
Must be protected from light.

4.3.7 Thermal stability
Must be stored at +4 °C (Anphar-Rolland).
Decreased activity has been observed with increasing
temperature.
Hydroxocobalamin solutions are sterilized by filtration.

4.3.8 Interference with other compounds
Complexes with basic substances. Incompatible with reducing agents such as ascorbic acid, saccharose, sorbitol, and other B vitamins (Mizoule, 1966). It has been suggested by Evans (1964) that hydroxocobalamin interacts with sodium thiosulfate to form a new compound, a thiosulfato-cobalamin, which can no longer (or less firmly) fix the cyanide ion.

4.4 Synthesis
Routes of synthesis (confidential)
Manufacturing processes (confidential)

OHB₁₂ (4 g) is lyophilized as a freeze-dried form and mixed
before use with one vial containing 8 g sodium thiosulfate. The resulting solution is administered in 220 ml of 5% dextrose solution by intravenous infusion over 20 min.

4.5 Analytical Methods

4.5.1 Identification of hydroxocobalamin
4.5.1.1 UV spectroscopy

The visible absorption spectrum of the solution, prepared as for pH-dependent cobalamin, is maximal at 426 (±2) nm, 516 (±2) nm, and 550 (±2) nm (United States Pharmacopeia, 1985).

OHB_{12} absorbs light at three other wavelengths: 274 nm, 351 nm, and 523 nm (British Pharmacopoeia, 1980; Pharmacopée Française, 1988).

4.5.1.2 Colorimetric method

Fuse a mixture of 1 mg of OHB_{12} and 50 mg of potassium pyrosulfate in a porcelain crucible. Cool, break up the mass with a glass rod, add 3 ml of water, and boil until dissolved. Add 1 drop of phenolphthalein TS and sodium hydroxide (2 mol/l) drop by drop until a pink colour appears. Add 0.5 g of sodium acetate, 0.5 ml of acetic acid (1 mol/l), and 0.5 ml of nitroso R salt solution (1 in 100): a red or orange-red colour should appear immediately. Add 0.5 ml of hydrochloric acid and boil for 1 min: the red or orange-red colour should persist (United States Pharmacopeia, 1985).

4.5.2 Quality controls

Information not available.

4.5.3 Raw materials

Foreign pigments, acidic impurities, acetate, chloride, and sulfates should be lower than the following limits:

* Other cobalamins should constitute not more than 3.0% when determined by chromatography (diethylaminoethylcellulose column then carboxymethylcellulose column; and measurement of absorption at 361 nm) (British Pharmacopoeia, 1980).

* Coloured impurities < 5.0% with descending paper chromatography and measurement of absorption at 361 nm.

* Acidic impurities < 3.0% with a diethylaminoethylcellulose column and measurement of absorption at 351 and 361 nm.

* pH-dependent cobalamins between 95.0% and 102.0%, calculated on a dry weight basis using a spectrophotometric method (550 nm) with two solutions of OHB_{12}. One of these solutions, in a pH 4.0 buffer, serves as the blank, the other solution should be in a pH 9.3 buffer (United States Pharmacopeia, 1985).

* Cyanocobalamin < 5.0%, calculated on a dry weight basis and determined with a cobalamin radiotracer assay using a cyanocobalamin tracer reagent.

4.5.4 Finished galenic form

* OHB_{12} is in freeze-dried form
* After reconstitution with thiosulfate:
  - pH 10-12
  - the liquid is a clear, deep-red solution.

4.5.5 Measurement

4.5.5.1 In raw materials and in finished form

* Spectrophotometric measurement at 351 nm (British Pharmacopoeia, 1980; Pharmacopée Française, 1988)
* Radioimmunoassay with labelled cyanocobalamin (United States Pharmacopeia, 1985).

4.5.5.2 In biological samples

Differential spectroscopy at two wavelengths, 351 nm (maximum absorbance for OHB₁₂) and 361 nm (maximum absorbance for cyanocobalamin), is used to measure OHB₁₂ and cyanocobalamin levels in plasma and in urine (Baud et al., 1987).

4.6 Shelf-life

Keep in the dark, in a refrigerator at +4 °C, for no more than three years.

4.7 General Properties

Kaczka et al. (1950) have demonstrated in in vitro studies that the reaction of OHB₁₂ with cyanide results in the displacement of a hydroxyl group by a cyano group to form cyanocobalamin, which is then excreted in the urine. Thus one molecule of OHB₁₂ binds one molecule of cyanide. Hydroxocobalamin has been shown to be an effective antidote for experimental cyanide poisoning in mice (Mushett et al., 1952), guinea-pigs (Posner et al., 1976a), baboons (Posner et al., 1976b), and dogs (Rose et al., 1965).

The antidotal effect of OHB₁₂ is enhanced by the use of thiosulfate in the same solution. Thiosulfate provides sulfur radicals, which complex with cyanide to form thiocyanate using the endogenous cyanide detoxification mechanism rhodanese (thiosulfate sulfurtransferase). However, the action of thiosulfate may be too slow to prevent death in serious cases of cyanide poisoning when administered alone. The combination of OHB₁₂ and thiosulfate was shown to be more effective by Mizoule (1966), who studied it extensively in experimental animals; other authors have reported similar results (Motin et al., 1970; Luher et al., 1971; Yacoub et al., 1974; Jouglard et al., 1974; Friedberg & Shukla, 1975; Racle et al., 1976; Bismuth et al., 1984).

4.8 Animal Studies

4.8.1 Pharmacokinetics

No data are available.
4.8.2 Pharmacodynamics in the presence of the toxin

The antidotal action of OHB$_{12}$ has been studied in animals, firstly in the case of medium-term intoxication (Haguenoer et al., 1975), and secondly in the prophylactic treatment of cyanide intoxication (Posner et al., 1976a).

In the study by Haguenoer et al. (1975), five groups of Wistar male rats (mean weight, 330g) were used as follows:

* group 1 received injections of sodium chloride (0.9%) in water (control group)
* group 2 received 1 ml of 50 mg/ml acetonitrile
* group 3 received 1 ml of 50 mg/ml acetonitrile plus 1 ml of 5 mg/ml OHB$_{12}$ (6 h later)
* group 4 received 1 ml of 1.12 mg/ml potassium cyanide (KCN)
* group 5 received 1 ml of 1.12 mg/ml KCN plus 1 ml of 5 mg/ml OHB$_{12}$ (6 h later).

In all, 28 intraperitoneal injections were given over a 6-week period. Rats treated with OHB$_{12}$ (groups 3 and 5) excreted less free CN$^-$ in the urine than the others (groups 2 and 4); the excretion of combined CN$^-$ was higher for groups 3 and 5. Urinary excretion was higher in the case of KCN than acetonitrile. However, a significant proportion of acetonitrile and cyanhydric acid (formed from acetonitrile and KCN) was excreted by the lungs, and a large amount of thiocyanate was excreted in the faeces.

In the second study (Posner et al., 1976a), nine groups of healthy baboons (weighing 20 to 30 kg) were anaesthetized and received nitroprusside by infusion, either alone (four animals) or in combination with OHB$_{12}$ (22.5 mg B$_{12}$ per 1 mg nitroprusside) (five baboons).

The infusion was continued for up to 2 h or until 500 mg of nitroprusside had been administered. Cyanide concentrations increased and the animals developed a severe metabolic acidosis. OHB$_{12}$ infused simultaneously with nitroprusside significantly reduced the increase in cyanide concentrations and eliminated the development of metabolic acidosis.

In a third study (Höbel et al., 1980), sodium nitroprusside (SNP) and OHB$_{12}$ were infused over 4 h in conscious rabbits in molar ratios of 1:4, 1:5, or 1:8. Control animals received OHB$_{12}$ only. Sodium thiosulfate was infused with SNP at molar ratios of 1:4, 1:5, and 1:10. SNP provoked a severe acidosis which was not corrected by the lowest dose of OHB$_{12}$: three deaths occurred among ten animals. When the 1:5 ratio was administered, the acidosis was less marked, but three out of seven animals succumbed. The highest dose (1:8) prevented acidosis, but not death, in three out of eight animals. All doses of OHB$_{12}$ caused histological changes in the liver, myocardium, and kidney. By contrast, sodium thiosulfate was
completely effective as an antidote in this study and did not give rise to histological changes.

4.8.3 Toxicology

**OHB**$_{12}$ has weak intrinsic toxicity and may be administered safely in large doses (Mizoule, 1966).

### 4.8.3.1 Acute toxicity

The acute toxicity of **OHB**$_{12}$ has been tested in mice and rabbits. A solution containing 0.4-0.75 g or 1 g/kg was administered to Swiss strain mice (0.20 ml per 20 mg body weight) without any toxic effects either immediately or a few days later. Doses of **OHB**$_{12}$ (50 and 100 mg/kg body weight) were injected intravenously into five rabbits (weighing 2-3 kg) without causing side effects either immediately or 3 days later (Mizoule, 1966).

### 4.8.3.2 Sub-acute and chronic toxicity

The sub-acute and chronic toxicity of hydroxocobalamin has been tested in rats and rabbits. Twelve young male Wistar rats (weight 43-53 g) received three subcutaneous injections weekly for a period of 82 days. Six of these rats were treated with 50 mg **OHB**$_{12}$ injections, while six received injections of sodium chloride (0.9%) in water. No significant differences were reported in the behaviour of the rats or in the haematological results. Rabbits received one injection of **OHB**$_{12}$ (50 mg/kg) every other day for a period of 10 days. No toxicity was reported (Mizoule, 1966).

### 4.9 Volunteer Studies

Data not available.

### 4.10 Clinical Studies

After intravenous infusion, **OHB**$_{12}$ is bound sparingly to a specific globulin transport plasma protein, transcobalamin II (Herbert & Sullivan, 1964). Lesser amounts are bound to the storage protein transcobalamin I (a glycoprotein) and to transcobalamin III (an intermediate glycoprotein). A small amount may be free or very loosely bound to protein. In the fasting state, the majority of circulating vitamin **B**$_{12}$ is bound to transcobalamin, and it is stored principally in the liver and bone marrow.

**OHB**$_{12}$ has a very short half-life (5 min), being rapidly metabolized and excreted (Vesey & Cole, 1981; Cottrell et al., 1982). Up to 50% of an administered dose of **OHB**$_{12}$ is excreted unchanged in the urine, which becomes red-orange in colour following a dose of 4 g **OHB**$_{12}$ (Goodman & Gilman, 1975).

### 4.11 Clinical Studies - Case Reports

Only a few studies in man have included measurement of free and total cyanide concentrations. These include 12 cases of acute cyanide poisoning (Baud et al., 1986; Danel et al., 1986); several cases of sodium nitroprusside poisoning in which **OHB**$_{12}$ was used prophylactically (Cottrell et al., 1982), and one case of
combined cyanide and carbon monoxide poisoning (Baud et al., 1987).

Baud et al. (1986) reported the case of a 55-year-old worker, exposed to propionitrile by inhalation and dermal exposure, who was treated with a combination of OHB$_{12}$ and thiosulfate. The patient rapidly lost consciousness: 25 min after exposure he was comatose and unresponsive with a pulse rate of 90 beats/min and a blood pressure of 17.3/9.3 kPa (130/70 mmHg). Laboratory investigations revealed no abnormalities except for a base deficit of 14-16 mmol/l on arterial blood gas analysis. The serum lactate concentration was 10 mmol/l. The patient received 4 g OHB$_{12}$ and 8 g thiosulfate (Trousse anticyanure, Laboratoire Anphar Rolland) intravenously in just under 30 min. During infusion of the antidote, the patient regained consciousness and became oriented. Complete reversion of CNS depression and normalization of vital signs were observed during the next hour.

Table 3. Blood cyanide and thiocyanate concentrations

<table>
<thead>
<tr>
<th>Time after intoxication</th>
<th>Cyanide</th>
<th>Thiocyanate</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 h</td>
<td>5.71 mg/l</td>
<td>undetectable</td>
</tr>
<tr>
<td>2.5 h (at the end of the antidote infusion)</td>
<td>0.93 mg/l</td>
<td>21.1 mg/l</td>
</tr>
<tr>
<td>7 h</td>
<td>1.00 mg/l</td>
<td>baseline level</td>
</tr>
</tbody>
</table>

Personal communication by V. Danel, L. Barret, and J.L. Debru Regional Hospital Centre and University, Grenoble, France concerning four hydrogen cyanide intoxications treated with OHB$_{12}$ and sodium hyposulfite.

Baud et al. (1989) studied the toxicokinetics of OHB$_{12}$ and cyanocobalamin (CNB$_{12}$), which were measured simultaneously in cases of cyanide intoxication treated with OHB$_{12}$. Plasma cobalamin levels were measured using a differential spectrophotometric assay. Whole blood cyanide levels were measured, using a microdiffusion/colormetric assay, in samples taken before administration of OHB$_{12}$ by infusion (5 g over 30 min).

<table>
<thead>
<tr>
<th>Source of cyanide</th>
<th>Bromo cyanide</th>
<th>Sodium cyanide</th>
<th>Mercurous cyanide</th>
<th>Smoke inhalation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Route of absorption</td>
<td>lung</td>
<td>orally</td>
<td>orally</td>
<td>lung</td>
</tr>
<tr>
<td>Initial blood cyanide (µmol/l)</td>
<td>13</td>
<td>260</td>
<td>217</td>
<td>22</td>
</tr>
<tr>
<td>Peak OHB$_{12}$ (µmol/l)</td>
<td>325</td>
<td>15</td>
<td>83</td>
<td>514</td>
</tr>
</tbody>
</table>
Peak CNB$_{12}$ ($\mu$mol/l) 39 275 226 0 215 221 259

After completion of the OHB$_{12}$ infusion, low levels of OHB$_{12}$ were observed concurrently with high levels of CNB$_{12}$ in patients with high whole blood cyanide levels, but not in those with low cyanide concentrations. These findings suggest that OHB$_{12}$ acts rapidly as a cyanide antidote.

Cottrell et al. (1982) studied two groups of patients in whom cyanide toxicity was induced by nitroprusside. Group I received nitroprusside alone, while group II received nitroprusside and OHB$_{12}$. Red cell and plasma cyanide concentrations were, respectively, 83.44% (±23.12) and 3.51 (±1.01) mg per 100 ml after administration of nitroprusside alone and 33.18% (±17.29) and 2.18% (±0.65) mg per 100 ml after administration of nitroprusside with OHB$_{12}$. OHB$_{12}$ infusion resulted in lower blood cyanide concentrations and base deficit than in the untreated group. The dose of nitroprusside used in each group did not differ significantly.

Following inhalation of fumes from a firm making plastics, a man became poisoned by both cyanide and carbon monoxide (Baud et al., 1989). On admission, the whole-blood cyanide concentration was 3.6 mg/l. He immediately received 4 g OHB$_{12}$ and 8 g sodium thiosulfate by intravenous infusion. A second injection had to be administered 3 h after admission because of clinical relapse, again with a satisfactory response.

4.12 Summary of Evaluation

4.12.1 Indications

* In the treatment of pernicious anaemia and vitamin B$_{12}$ deficiency states by intramuscular administration.

* In cyanide poisoning as an antidote:
  a) as therapeutic treatment after exposure to acetonitrile, propionitrile, or potassium or sodium cyanides by oral, inhalational, or dermal routes;
  b) as prophylactic treatment during sodium nitroprusside infusion.

4.12.2 Advised route and dosage

OH$_{12}$ is usually given intravenously in a 5% solution of dextrose in water. The normal adult dose is 4 g OH$_{12}$, but the dose may be increased in massive poisoning.

4.12.3 Practical advice

* To maintain normal blood pH and isotonicity, the OH$_{12}$ must be injected in more than 220 ml of 5% dextrose.

* OH$_{12}$ must not be administered with reducing or basic
substances (see section 4.3.8).

* When OHB\textsubscript{12} is administered as a prolonged infusion, aluminium foil should be used to protect it from light.

4.12.4 Side effects

* The most common side effect is an orange-red discoloration of the skin, mucous membranes, and urine that lasts about 12 h but is not consequential.

* Allergic reactions to OHB\textsubscript{12} have been reported (Dally & Gaultier, 1976).

4.13 Model Information Sheet

4.13.1 Uses

Hydroxocobalamin is used for the treatment of pernicious anaemia and vitamin B\textsubscript{12} deficiency states. In the treatment of cyanide intoxication, hydroxocobalamin is indicated in two circumstances. Firstly, it is used for the treatment of exposure to hydrogen cyanide, inorganic cyanide salts, and acetonitrile and propionitrile. Secondly, hydroxocobalamin is used prophylactically to prevent cyanide intoxication as a result of sodium nitroprusside given by infusion.

4.13.2 Dosage and route

Hydroxocobalamin is given intravenously in a 5% dextrose solution. The usual adult dose is 4 g, which may be increased in cases of massive cyanide poisoning.

4.13.3 Precautions/contraindications

Hydroxocobalamin should be diluted in more than 220 ml of 5% dextrose solution. It must not be administered with reducing substances or basic agents. In the case of prolonged infusion, the solution must be protected from light. The possibility of an anaphylactoid reaction to hydroxocobalamin should be borne in mind.

4.13.4 Adverse effects

The most common side effect is an orange-red discoloration of the skin, mucous membranes, and urine that lasts for approximately 12 h. Allergic reactions have been reported. Histological abnormalities in the liver, myocardium, and kidney of rabbits with nitroprusside intoxication treated with hydroxocobalamin have been described (Höbel et al., 1980).

4.13.5 Use in pregnancy and lactation

These aspects are seldom discussed in the literature, and are of little relevance in this context. In a life-saving situation, the dosage recommended above should not be modified during pregnancy or lactation.

4.13.6 Storage
Hydroxocobalamin must be kept in the dark, in a refrigerator at 4 °C, for not more than 3 years.

4.14 References


5. DICOBALT EDETATE (KELOCYANOR)

5.1 Introduction

The ability of cyanide to form complexes with cobalt is an
example of the propensity of cyanide to form stable complexes with many transition metals (Sharpe, 1976). It is a property of cyanide that has been known since the last century, but there is still room for disagreement about the precise nature of the complex formed in vivo when cobalt is used to treat cyanide poisoning. In vitro, a complex of one cobalt and five cyanide atoms is formed on addition of potassium cyanide to cobalt (II) salts (Cotton & Wilkinson, 1966).

![Diagram of a cobalt cyanide complex]

However, in the studies carried out in vivo by Evans (1964), it was suggested that inorganic cobalt salts could bind six moles of cyanide for each mole of cobalt. Evans further proposed that there was a two stage reaction, six moles of cyanide reacting with one mole of cobalt, forming first the cobaltocyanide ion, and then the cobalticyanide ion.

\[
\text{Co}^{2+} + 6 \text{CN}^- \rightarrow [\text{Co} \ (\text{CN})_6]^{4-} \\
\downarrow \text{e}^- \\
[\text{Co} \ (\text{CN})_6]^{3-}
\]

The cobalticyanides are not very toxic, much less so than the same amount of free cyanide, and have an LD\(_{50}\) in the region of 1 g/kg.

Although it was known that cobalt and cyanide formed stable complexes, it was generally thought that inorganic cobalt salts were too toxic for use in humans as cyanide antidotes. In particular, those most investigated, the cobalt (II) salts, were known to be toxic to the heart, liver, and kidney (Speijers et al., 1982). For a long time, during which the preferred treatment for cyanide poisoning was sodium nitrite and sodium thiosulfate, interest in the anticyanide activity of cobalt compounds dwindled. A rekindling of interest in cobalt compounds, especially organic complexes, was brought about by the discovery by Mushett et al. (1952) that the complex organocobalt vitamin, hydroxocobalamin, possessed anticyanide activity in mice. Experimental work, notably by Paulet (1957, 1958), led to the introduction of dicobalt edetate (Kelocyanor).

5.2 Name and Chemical Formula

Kelocyanor is the proprietary name for a solution containing
dicobalt edetate and glucose. Dicobalt edetate has not been crystallized. The structural formula was believed to be that of the monocobalt salt of a monocobalt edetate anion (Fig. 4), but there is reason to think that this does not fully represent the structure of the active ingredient of Kelocyanor (see below). The CAS number is 36499-65-7, the INN is dicobalt edetate, and the IUPAC name is dicobalt ethylenediamine-NNN'N'-tetraacetate. Other names are cobalt edetate, cobalt tetracemate and cobalt EDTA.

For reasons discussed in the text, this may only be an approximation to the true structure of the material in Kelocyanor.

5.3 Physico-chemical Properties

The usual preparation of dicobalt edetate is Kelocyanor. Injectable Kelocyanor is a clear violet solution, produced in clear glass 20-ml ampoules, of pH 4.0-7.5. It contains 0.196-0.240 g/100 ml free cobalt and 1.35-1.65 g/100 ml dicobalt edetate, as well as 4 g glucose per ampoule. The refractive index of the solution in the ampoule is 1.3638.

The optical rotation of Kelocyanor is + 9.25°: the optical rotation of a dextrose solution of the same dextrose concentration as Kelocyanor is +11°. Therefore the net optical rotation attributable to the cobalt edetate is -1.75°. This would give a specific rotation for dicobalt edetate of -116.7°. The relative molecular mass of dicobalt edetate is 408. No information is available on the specific gravity.

5.4 Synthesis

Dicobalt edetate is made by adding cobalt carbonate to sterile water and then adding ethylenediaminetetraacetic acid. After the addition of glucose, the resultant solution is cleared with activated charcoal. Sterility and pyrogen tests are performed to the requirements of the European Pharmacopoeia (1980). The volume of solution in the ampoules is checked using method B (British Pharmacopoeia, 1980). The material is not checked for the absence of activated charcoal, but this would be expected to be removed during filtration through a membrane with a pore diameter of 0.22 µm.

5.4.1 Source of materials

5.4.1.1 Cobalt carbonate

The current supplier to both the French and British drug companies is Prolabo, BP No 200, 75526 Paris, Cedex 11, France.
specification is as follows:

- appearance: fine wine-coloured powder
- identification: cobalt carbonate confirmed
- loss on drying: about 14%
- assay: about 50% cobalt

* L'Arguenon, Société d'Etudes et de Recherches Biologiques,
53 Rue Villiers de l'Isle Adam, 75020 Paris, France, UK
suppliers, Lipha Pharmaceuticals Ltd, Harrier House, Yiewsley,
Middlesex UB7 7QG.

5.4.1.2 Ethylenediaminetetraacetic acid (EDTA)

The current supplier is BDH Ltd, Poole, Dorset, BH12 4NN,
England. The material complies with the US National Formulary (see
USNF, 1985).

5.4.1.3 Glucose

This complies with the European Pharmacopoeia.

5.5 Analytical Methods

5.5.1 Free cobalt

In duplicate, pipette 5.0 ml of the solution in the ampoule
into a 250-ml conical flask. Add 100 mg of murexide compound
mixture and 100 ml water. Stir using a magnetic stirrer. Add
dilute ammonia drop by drop until the solution just turns yellow
(usually 1 drop). Titrate with disodium edetate (0.02 mol/l) to a
violet end-point. During the titration, the pH of the solution
falls drastically and it turns red. It is necessary to add 1 drop
of dilute ammonia to restore the yellow colour. Check the end
point of the titration with 1 drop of ammonia.

To prepare the murexide mixture, grind 10 mg murexide and
990 mg sodium chloride in a pestle and mortar. The dilute ammonia
solution consists of 5.5 ml concentrated ammonia diluted to 100 ml
with water.

The following equation is used to calculate the concentration
of free cobalt (g/100 ml):

\[ \frac{T \times 1.179 \times F \times 20}{1000} \]

where
- \( T \) = titre (ml)
- \( F \) = factor for disodium edetate at 0.02 mol/l
1 ml disodium edetate (0.02 mol/l) equiv. 1.179 mg cobalt

5.5.2 Dicobalt edetate

In duplicate, pipette 5.0 ml of the solution in the ampoules
into a 250-ml conical flask. Add 0.65 g potassium cyanide, stir,
and allow to stand for 5 min. Add 85 ml of water, 10 ml lead
acetate solution, and 1 ml glacial acetic acid. Stir for 15 min,
using a magnetic stirrer. Add 100 mg xylenol orange compound mixture and titrate slowly with disodium edetate (0.02 mol/l) to a clear yellow end-point ($T_1$). Carry out a blank titration, omitting the sample solution ($T_2$).

Xylenol orange compound mixture is prepared by grinding 10 mg xylenol orange with 990 mg potassium nitrate in a pestle and mortar. The lead acetate solution consists of 1.5 g lead acetate dissolved in water and make up to 100 ml. The following equation is used to calculate the concentration of dicobalt edetate (g/100 ml):

$$\frac{(T_2-T_1) \times F \times 4.06}{25}$$

where

- $T_1$ = sample titre
- $T_2$ = blank titre
- $F$ = factor for disodium edetate (0.02 mol/l)

### 5.5.3 Analysis in biological fluids

Since dicobalt edetate has not been fully characterized, it is difficult to say whether analysis would be meaningful. Methods are available for the analysis of cobalt in biological fluids by spectrophotometry or atomic absorption flame photometry (Thiers et al., 1955; Mulford, 1966; Christian, 1969; Lewis et al., 1985).

Dicobalt edetate interferes with the Feldstein & Klendshoj (1954) method of analysis of biological fluids for cyanide (Ballantyne & Marrs, 1987). See chapter 10 for further details.

### 5.6 Stability and Shelf-life

The shelf-life is 3 years at 25 °C, and the material should be stored in the dark. The drug has been stored for up to 3 years at room temperature 20-25, 30 and 37 °C (Lipha Pharmaceuticals, 1987)*, the batches having been tested at regular intervals (12 months maximum) for compliance with the finished product specification with respect to appearance, extractable volume, pH, and free cobalt and dicobalt edetate content. The only deviation noted was that the colour of the solution became lighter at 30 °C and above. All the other variables remained within the specification. If stored in the light, the drug bleaches in about one month. The nature of the chemical change that brings about the loss of colour is not known.

* Personal communication from Lipha Pharmaceuticals Ltd, West Drayton, Middlesex, United Kingdom.

### 5.7 General Properties

Cyanide blocks intracellular respiration by binding to cytochrome oxidase. Cobalt forms a stable complex with cyanide, the effect being direct and peripheral (Mercker & Bastian, 1959). Although some cobalt compounds are reputed to inhibit methaemoglobin reductase (Hagler & Coppes, 1982), it is unlikely that methaemoglobinaemia contributes meaningfully to the anticyanide.
action of dicobalt edetate.

5.8 Animal Studies

5.8.1 Pharmacokinetics

Formal pharmacokinetic studies have not been carried out on dicobalt edetate. Some of the effects observed with cobalt compounds, including dicobalt edetate, are probably centrally mediated (Bartelheimer, 1962a). Thus, it is likely that after Kelocyanor injection, cobalt or dicobalt edetate crosses the blood-brain barrier. In the mouse, the cyanide-cobalt complex is excreted in the urine (Frankenberg & Sörbo, 1975).

5.8.2 Pharmacodynamics

5.8.2.1 Efficacy in animals

The introduction of dicobalt edetate followed from the work of Paulet (1957, 1958, 1960a,b, 1961, 1965) and Paulet et al. (1960), who studied the antidotal effects and toxicity of various cobalt compounds. The aim of studies on cobalt antidotes at this time was to find a compound that retained the ability of cobalt to bind cyanide, yet lacked its toxic effects. The compounds investigated by Paulet (1960a,b) included certain cobalt (II) salts, namely the chloride, glutamate, and gluconate. Paulet (1960a) also studied the cobalt chelates, dicobalt edetate, cobalt histidine, and disodium monocobalt edetate and found that the salts, as well as all except the last named of the chelates, possessed appreciable anticyanide activity. Dicobalt edetate and cobalt histidine were the most promising, and in one study dicobalt edetate seemed preferable (Paulet, 1960a, 1961). This study was undertaken in anaesthetized dogs infused with sodium cyanide (0.1 mg/kg per min): both dicobalt edetate and cobalt histidine were capable of resuscitating the dogs in secondary apnoea, but the dose of cobalt histidine required was considerably higher than that of dicobalt edetate. In the same reports, the effective doses of the two compounds were compared with their respective intraperitoneal LD₅₀ values in mice, the result favouring dicobalt edetate. However, if the dog results had been tabulated against the rat intravenous LD₅₀ values (Tauberger & Klimmer, 1963), cobalt histidine might have been preferred, this antidote having a much higher intravenous LD₅₀ than dicobalt edetate.

Evans (1964) investigated the stoichiometry of the reaction between dicobalt edetate or other cobalt compounds on the one hand, and cyanide on the other. He studied their antidotal effects using various doses of both toxicant and treatment in both mice and rabbits, designing his experiments in such a way that it was possible to calculate the cyanide/cobalt molar ratios above which the antidote would no longer be efficacious. He found that, while most cobalt (II) salts would be effective against six moles of cyanide/mole cobalt, dicobalt edetate only possessed efficacy at molar ratios of up to two. Whilst on a mass basis, dicobalt edetate is about half as toxic as cobalt (II) salts, on a molar basis it is not conspicuously less toxic than cobalt acetate. It had been thought that dicobalt edetate was the monocobalt (II) salt of a monocobalt edetate anion, one cobalt being completely unavailable for cyanide binding by virtue of its complexing with

http://www.intox.org/databank/documents/antidote/antidote/ant02.htm 08/14/2003
EDTA, the other being fully available. In fact the study of Evans (1964) suggested that one cobalt is completely unavailable whilst, of the coordination sites of the second, only two are available to bind cyanide. In that case, the structure given in Fig 4 does not fully describe the structure of dicobalt edetate. It seems likely, however, that the second cobalt atom is required for anticyanide activity, since disodium monocobalt edetate, which is a compound of very low toxicity (Eybl et al., 1959), is almost ineffective as a cyanide antidote (Paulet, 1958). It may be that the same property, ionizability, is responsible for cyanide binding and for cobalt toxicity. In that case, complexing cobalt would decrease the therapeutic effect in parallel with the toxicity. That this is not the case is suggested by, firstly, the lack of toxicity of hydroxocobalamin, which is nevertheless a successful cyanide antidote and, secondly, the many years of successful use of dicobalt edetate.

5.8.2.2 Comparison of dicobalt edetate with other compounds

A number of research groups have compared cobalt edetate with other cyanide antidotes, from the point of view both of antidotal efficacy (Table 4) and also of toxicity. For example, using dogs and rabbits, Paulet (1960a, 1961) compared the cobalt chelate with sodium nitrite and found the former superior. He also carried out a comparison with both elements of the sodium nitrite/thiosulfate treatment, again demonstrating that the cobalt compound was more effective. Terzic & Milosevic (1963) compared sodium nitrite and dicobalt edetate. They studied the effective doses of the two compounds, as well as their LD$_{50}$ values, and concluded that cobalt edetate was superior. Unfortunately their study was conducted in mice: these animals especially, but also other small laboratory animals, are extremely insensitive to methaemoglobin-producing chemicals by virtue of the very high activity of NADH-linked methaemoglobin reductase in murine erythrocytes (Calabrese, 1983). This difference renders extrapolation of these results to man almost impossible. Two research groups have attempted to compare dicobalt edetate with 4-DMAP in dogs. Klimmek et al. (1979a) found better survival with 4-DMAP when the antidotes were given 4 min after the poisoning but similar antidotal efficacy when they were given 1 min afterwards. Marrs et al. (1985) found that dicobalt edetate successfully treated up to about three times the LD$_{50}$ of cyanide, when given at the moment of apnoea, whilst 4-DMAP ensured survival at about six time the LD$_{50}$ under the same conditions.

5.8.2.3 Interactions with other drugs

The only interactions that have been studied in animals are those with other cyanide antidotes. The effect, in experimental animals, is generally additive. This is despite the theoretical risk that cobalt toxicity might be exacerbated by other antidotes that prevent cyanide from binding to cobalt. Antidotal combinations including dicobalt edetate have been studied by Evans (1964) and Frankenberg & Sörbo (1975).

Table 4. Studies of the efficacy of dicobalt edetate compared with other cyanide antidotes
Comparison of dicobalt edetate with

<table>
<thead>
<tr>
<th>Species</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium nitrite</td>
<td>Dog</td>
</tr>
<tr>
<td>Sodium nitrite/thiosulfate</td>
<td>Dog</td>
</tr>
<tr>
<td>Sodium nitrite</td>
<td>Mouse</td>
</tr>
<tr>
<td>4-DMAP</td>
<td>Dog</td>
</tr>
<tr>
<td>Thiosulfate/rhodanese</td>
<td>Rabbit</td>
</tr>
<tr>
<td>Other cobalt antidotes</td>
<td>Mouse/Rabbit</td>
</tr>
</tbody>
</table>

5.8.3 Toxicology

Extensive toxicology was not carried out on dicobalt edetate before its introduction. Published information is largely confined to acute toxicity testing, but it is believed that the organ-specific toxicity is similar to that of cobalt salts and that it is ameliorated by chelation (Paulet, 1960b).

5.8.3.1 In vitro studies

Dicobalt edetate gives negative results in the Ames test (Morris, 1987)

5.8.3.2 Acute toxicity studies

The acute toxicity of dicobalt edetate has been studied exclusively in small laboratory animals (Table 5). An interesting feature is that the acute toxicity is decreased by the presence of glucose or of cyanide. Thus, Paulet (1960a,b, 1961) observed that, in the mouse, the intravenous LD$_{50}$ of dicobalt edetate (50 mg/kg) was increased threefold by giving it in hypertonic glucose. The LD$_{50}$ was increased to 82 mg/kg or 103 mg/kg, respectively, by the simultaneous administration of 2.5 or 5 mg NaCN/kg.

5.8.3.3 Repeated dose toxicity

Bartelheimer (1962b) gave intraperitoneal injections of cobalt edetate daily for 14 days at doses of 30, 40, or 50% of the LD$_{50}$ to rats, surviving animals being observed for 4 weeks. One of six and two of six of the high and middle groups, respectively, died, whilst none of the low-dose group did so. The majority of the rats in the two higher groups suffered diarrhoea and weight loss, although some recovery was seen in the latter half of the exposure period. Autopsy and histological examination of the dead animals showed necrosis of the intestinal mucous membrane. No pathological changes were seen in the liver, kidney, or heart. Late effects were not observed in the survivors.

Table 5. The acute toxicity of dicobalt edetate
Species | Route | LD$_{50}$ (mg/kg) | Reference
---|---|---|---
Mouse | iv | 50 | Paulet (1961)
Mouse | iv | 71 | Evans (1964)
Mouse | ip | 213 | Paulet (1961)
Mouse | ip | 225 | Terzic & Milosevic (1963)
Rat | iv | 43 | Tauberger & Klimmer (1963)
Rat | ip | 100 | Bartelheimer (1962b)

5.8.3.4 Circulatory effects in dogs

Klimmek et al. (1979b), during their studies comparing dicobalt edetate with 4-dimethylaminophenol (4-DMAP), found that the former caused circulatory depression and hyperventilation. This was accompanied by metabolic acidosis.

5.8.3.5 Other toxicity studies

No information is available on reproductive toxicology or carcinogenicity. No mammalian cell or in vivo mutagenicity test has been published.

5.9 Volunteer Studies

No human volunteer studies have been carried out.

5.10 Clinical Trials

Clinical trials have not been undertaken.

5.11 Clinical Studies - Case Reports

5.11.1 Successful use

There are a number of case reports of the successful use of dicobalt edetate against different types of cyanide poisoning, including inhalation of HCN (e.g. Bain & Knowles, 1967; Nagler et al., 1978) and sodium cyanide (Hillman et al., 1974), and potassium cyanide ingestion (Hoang The Dan et al., 1981; Klaui et al., 1984). The use of dicobalt edetate in poisoning with fused sodium cyanide has also been reported (Bourrelier & Paulet, 1971). The case reported by Bain & Knowles involved a patient who was semicomatose but who became fully conscious immediately after the first of two doses of 10 ml of Kelocyanor: the blood cyanide concentration just before the antidote injection was found to be 5.1 mg/l. In the report by Nagler et al. (1978), three workers were poisoned as a result of the accidental addition of sodium cyanide to an acid bath. In each case the patients had definite evidence of cyanide poisoning, yet recovered. A blood cyanide level of 5.25 mg/l was found in the patient reported by Hoang The Dan et al. (1981), yet the subject recovered without sequelae.
5.11.2 Use in pregnant women and children

No data exist on the use of dicobalt edetate in pregnant women or children.

5.11.3 Adverse effects

A number of adverse effects have been reported, generally following the inappropriate administration of dicobalt edetate. It has been proposed that their occurrence can be minimized by using strict clinical criteria for giving the antidote (Bryson, 1978, 1987; Tyrer, 1981; Aw & Bishop, 1981; Peden et al., 1986). Nevertheless, it has been suggested that adverse reactions to dicobalt edetate may not be confined to instances of inappropriate administration (Dodds & McKnight, 1985). Typical case reports of adverse reactions include that of Froneman (1975), who reported a massive urticarial reaction affecting the face, eyelids, and lips; it was questioned if the subject had, in fact, consumed any cyanide. Four adverse reactions, where the diagnosis of cyanide poisoning was questionable, were reported by Tyrer (1981). Three of the patients had a variety of symptoms and signs, including convulsions, oedema of the face and neck, urticaria, chest pains, dyspnoea, and hypotension. Hypotension was also reported by Daunderer et al. (1974) (see section 5.11.4). A patient described by McKiernan (1980) had a normal blood cyanide level, in spite of severe cyanide burns. He reacted to Kelocyanor by developing facial and laryngeal oedema. A case reported by Yacoub et al. (1974) was complicated by the use of other antidotes; this patient developed urticaria.

5.11.4 Use in combination with other antidotes

Reference has been made above (section 5.8.3) to animal studies that suggest that the use of dicobalt edetate in combination with other antidotes could be beneficial. Thus, Jeretin (1963) used it with sodium nitrite and thiosulfate, and Daunderer et al. (1974) successfully resuscitated with 4-DMAP and Kelocyanor a patient who had ingested about 10 g potassium cyanide.

5.12 Summary of Evaluation

5.12.1 Indications

The only indication for the use of dicobalt edetate is acute cyanide poisoning. It should not be used except where there is definite indication of poisoning. Reactions are likely to occur if the drug is given inappropriately.

5.12.2 Administration

It is recommended that one ampoule should be given over one min. A second or third may be given in the case of inadequate response. Because glucose reduces the toxicity of dicobalt edetate (Paulet et al., 1960; Paulet, 1961), the recommendation is that the injections should be immediately followed by 50 ml dextrose (500 g/l).

5.12.3 Other consequential or supportive therapy

See section 1.10
5.12.4 Contraindications

Dicobalt edetate should not be used in suspected cyanide poisoning unaccompanied by signs of poisoning such as impairment or loss of consciousness.

5.12.5 Comparison with other antidotes

Dicobalt edetate probably crosses the blood-brain barrier, while methaemoglobin quite clearly does not. Moreover, both DMAP and nitrite require follow-up with a sulfur donor; with dicobalt edetate the cyanide is excreted with cobalt in the urine. In these two respects dicobalt edetate seems to offer some advantages. It is in clinical use that reservations have arisen, almost entirely because of adverse reactions. Whereas occupational health practitioners seem to be particularly impressed with dicobalt edetate (Davison, 1969; Bryson, 1987), it is in hospital practice that problems are likely to occur. The probable reason for this is that, at cyanide-using facilities, not only is the antidote to hand, but also occupational health personnel are familiar with the presentation of cyanide poisoning and the hazards of inappropriate use of antidotes. A particular problem of this form of poisoning is that insubstantial or suspected cyanide poisoning may produce panic, which is mistaken for incipient intoxication. It is then that the antidote is given (Anon, 1977). Provided it is given only when the patient is unconscious many of the problems can be avoided. While in the past it was suggested that in casualty departments Kelocyanor should be given to all patients with symptoms (Anon, 1977), this policy is no longer advocated. Were the use of dicobalt edetate to be confined to cases of unequivocal poisoning with cyanide, it seems likely that adverse reactions would be far less frequent.

5.13 Model Information Sheet

5.13.1 Uses

Dicobalt edetate is a specific antidote for use in acute cyanide poisoning.

5.13.2 Dosage and route

It should be given in the form of ampoules of Kelocyanor, which also contain glucose. One ampoule (300 mg dicobalt edetate) should be given intravenously over 1 min. If the response is inadequate, a second ampoule may be given. The use of Kelocyanor should be followed immediately by 50 ml Dextrose IV infusion (500 g/l).

5.13.3 Precautions/contraindications

Kelocyanor should not be administered in the absence of cyanide. Fully conscious patients, especially those exposed to HCN by inhalation, are unlikely to require Kelocyanor.

5.13.4 Adverse effects

These include facial, laryngeal, and neck oedema, chest pain, vomiting, and rashes.

5.13.5 Use in pregnancy and lactation
There is no experience of use in this situation. In life-saving situations the recommended dosage should not be modified.

5.13.6 Storage

This antidote should be stored at a temperature below 25 °C. The shelf-life is 3 years.

5.14 References


Bartelheimer EW (1962b) [Differences between the toxic and cyanide-antagonistic efficacy of cobalt chelate compounds (Co-histidine and CO$_2$-EDTA.) Naunyn-Schmiedebergs Arch Exp Pathol Pharmacol, 243: 254-268 (in German).


Daunderer M, Theml H, & Weger N (1974) [Treatment of prussic acid...
poisoning with 4-dimethylaminophenol (4-DMAP).] Med Klin, 69: 1626-1631 (in German).


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Mercker H & Bastian G (1959) [Cobalt compounds for prussic acid detoxification.] Naunyn-Schmiedebergs Arch Exp Pathol Pharmakol, 236: 449-458 (in German).


Paulet G (1960b) Les chélates de cobalt dans le traitement de l'intoxication cyanhydrique. Pathol Biol, 8: 256-266.


Tauberger G & Klimmer OR (1963) [Animal experimental studies of some cobalt compounds after intravenous injection.] Arch Int Pharmacodyn, 143: 219-239 (in German).


6. AMYL NITRITE

6.1 Introduction

Amyl nitrite by inhalation has been used for many years as a simple first-aid measure for cyanide poisoning. It generates methaemoglobin and may be administered by lay personnel. Methaemoglobin combines with cyanide to form non-toxic cyanmethaemoglobin. However, amyl nitrite is not a powerful methaemoglobin-forming agent in humans and therefore it has generally been used in combination with intravenous sodium nitrite. Of 44 patients poisoned with cyanide, and of which 43 recovered [Chen et al., 1944 (15 patients); Wolfsie, 1951 (12 patients); Miller & Toops, 1951 (1 patient); and Chen & Rose, 1952 (16 patients)], only 7 patients were treated with amyl nitrite alone; 2 were treated with amyl nitrite in combination with sodium thiosulfate.

Recently there has been a resurgence of interest in amyl nitrite. Artificial respiration with amyl nitrite broken into an Ambu bag was reported by Vick & Froelich (1985) as being life-saving in dogs severely poisoned with cyanide before significant formation of methaemoglobin occurred (Vick & Froelich, 1985). The vasogenic effects of amyl nitrite may therefore play a role in its antidotal effect in cyanide poisoning.

6.2 Name and Chemical Formula

Chemical name: Amyl nitrite, Amylis nitris, Nitris amylicus

Molecular formula: C₅H₁₁NO₂

Structural formula:

Amyl nitrite is a mixture of nitrite esters of 2-methylbutan-1-ol and 3-methylbutan-1-ol.
It contains not less than 85% and not more than 103% of C₅H₁₁NO₂ (Martindale, 1989; United States Pharmacopeia, 1985).

Relative molecular mass: 117.15
Appearance: yellow, transparent fluid
CAS numbers: 8017-89-8 and 110-46-3
IUPAC name: -

6.3 Physio-chemical Properties

Boiling point: 96 °C
Solubility: practically insoluble in water; miscible with alcohol, chloroform, ether, and light petroleum

6.4 Synthesis

Amyl nitrite is generally manufactured by the action of sodium nitrate and sulfuric acid on the appropriate alcohols.

6.5 Analytical Methods

6.5.1 Identification

a) To a mixture of 2 drops of amyl nitrite and 2 drops of water, add 2 ml of sulfuric acid and dilute with water. The odour of amyl valerate should become apparent.
b) To a few drops of amyl nitrite, add a mixture of 1 ml of hydrochloric acid (3 mol/l). A greenish-brown colour should be produced (United States Pharmacopeia, 1985).

6.5.2 Purity

6.5.2.1 Acidity

To 0.30 ml in a glass-stoppered cylinder, add a mixture of 0.60 ml of sodium hydroxide (0.1 mol/l), 10 ml of water, and 1 drop of phenolphthalein TS, and invert the cylinder three times. The red tint of the water layer should remain perceptible (United States Pharmacopeia, 1985).

6.5.2.2 Non-volatile residue

Allow 10 ml to evaporate at room temperature in a weighed evaporating dish within a well-ventilated hood, and dry the residue at 105 °C for 1 h. The weight of the residue should not exceed 2 mg (0.02%) (United States Pharmacopeia, 1985).

6.5.2.3 Assay for total nitrites

Inject an aliquot of amyl nitrite of suitable volume, but not more than 2 ml, into a suitable gas chromatograph equipped with a thermal conductivity detector. Under typical conditions, the instrument should contain a column (2 m x 3 mm) packed with a methyl polysiloxane oil, 25% by weight on suitable calcine diatomite. The column should be maintained at 80 °C, the injection port and detector block maintained at 10 °C above the temperature of the column, and helium used as a carrier gas at a flow rate of about 60 ml per min. The percentage of total nitrites is calculated from the area under the curve; a total of not less than 97% should be found.

6.6 Shelf-life

The following precautions should be taken.

* Protect from light.

* Insist that the suppliers only provide ampoules that have at least 18 month shelf-life left of the nominal 24-month total life.

* Take reasonable precautions to store at the lowest possible temperature compatible with immediate access in case of emergency; refrigerated storage is not necessary.

* Inspect monthly for any broken tubes (Beasley et al., 1978).

As indicated in section 6.3, amyl nitrite forms an explosive mixture with air and oxygen. It is highly inflammable and must not be used where it may be ignited. Storage for a prolonged period or at an excessive temperature presents the following hazards:

* breakage of the capsule by the build up of pressure internally and loss of amyl nitrite before use;
* explosive distribution of glass splinters when the ampoule is broken for use;
* loss of amyl nitrite by chemical decomposition.

6.7 General Properties

The principal pharmacological action of amyl nitrite is to cause the relaxation of vascular smooth muscle. Peripheral venous resistance is decreased as a result of a selective action on venous capacitance vessels with resultant venous pooling of blood and decreased venous return to the heart. The vasodilating effect of amyl nitrite on arteriolar resistance is less than that on the venous side. As a result of this combined action, both venous filling pressure (preload) and, to a lesser extent, arterial impedance (afterload) are reduced (AHFS, 1988).

It has been postulated that cyanide causes pulmonary and/or coronary arteriolar vasoconstriction, which results directly or indirectly in pump failure and an observed decrease in cardiac output (Sarnoff, 1980a). This theory is supported by the reported observations of Vick & Froelich (1985) that a sharp increase in central venous pressure occurs at a time when the arterial blood pressure is falling. Following the administration of amyl nitrite by inhalation, central venous pressure decreased rapidly while the arterial blood pressure increased. The apparent life-saving effect of this drug in dogs could have been due to a reversal of early cyanide-induced vasoconstriction and the restoration of normal cardiac function. Subsequently, amyl nitrite causes the formation of methaemoglobin, which may have an additional antidotal action.

— Personal communication by S.J. Sarnoff, Survival Technology Inc., Bethesda, USA.

6.8 Animal Studies

6.8.1 Pharmacokinetics

Amyl nitrite is readily absorbed into the circulation from mucous membranes, the rate of absorption being greatest from the lungs. It is rapidly inactivated by hydrolysis to isoamyl alcohol and nitrite. Amyl nitrite is rapidly hydrolysed in the gastrointestinal tract and is therefore pharmacologically inactive when administered orally (Martindale, 1989).

6.8.2 Pharmacodynamics

Amyl nitrite has proved to be effective in experimental animals poisoned by cyanide. Indeed, antagonism between amyl nitrite and hydrocyanic acid was mentioned as long ago as 1888 by Pedigo.

Chen et al. (1933) demonstrated that nine to ten inhalations of amyl nitrite were effective in dogs against 4 times the lethal dose of cyanide (12-24 mg/kg) (sodium nitrite was also given subcutaneously).

Amyl nitrite had a significant therapeutic effect on dogs.
exposed to cyanogen chloride (CNCl) at Ct values (the product of the concentration of the gas in mg/m³, and the length of exposure in min) ranging from 3300 to 6300 mg.min.m⁻³. However, no such effect was observed in the groups subjected to higher exposures, at Ct values ranging from 6900 to 11800 mg.min.m⁻³ (Jandorf & Bodansky, 1946).

Amyl nitrite treatment of mice exposed to CNCl resulted in a statistically significant increase in the number of survivors (Jandorf & Bodansky, 1946).

Amyl nitrite administered by inhalation increased the median lethal subcutaneous dose of sodium cyanide in dogs from 5.36 (+0.28) to 24.5 (+1.2) mg/kg (Chen & Rose, 1952).

Part of the antidotal effect may result from methaemoglobin formation. Any attempt to correlate the therapeutic effect of amyl nitrite with the degree of methaemoglobinaemia encounters difficulties. After exposure to HCN or CNCl, the blood of treated animals contains, in addition to haemoglobin and oxyhaemoglobin, cyanmethaemoglobin and methaemoglobin. To date, it has not been possible to quantify the amount of cyanmethaemoglobin. However, experiments have been undertaken to estimate the degree of methaemoglobinaemia produced by inhalation of amyl nitrite in animals not previously exposed to cyanide.

When ten mice were placed in a chamber containing approximately 12 mg amyl nitrite per l, the methaemoglobin levels 10 seconds, 30 seconds, and 1, 2, and 4 min after the beginning of the exposure, were 1.0, 1.8, 5.6, 14, and 30%, respectively (Jandorf & Bodansky, 1946).

A cone holding one crushed ampoule containing 0.3 ml amyl nitrite was fitted over the muzzle of each of four dogs anaesthetized with pentobarbital, and kept in place for 3 min. Under these conditions, 16, 18, 20, and 32% methaemoglobinaemia were found in the four dogs at the end of amyl nitrite inhalation (Jandorf & Bodansky, 1946).

Bastian & Mercker (1959) recorded a methaemoglobin level of 60% in mice placed in an atmosphere of amyl nitrite (0.112% v/v) for 15 min. Thereafter the degree of methaemoglobinaemia remained constant and it appeared that an equilibrium existed between the formation and reduction of methaemoglobin, the level depending on the concentration of amyl nitrite in the inspired air.

In cats, inhalation of amyl nitrite (0.06 and 0.12% v/v) resulted in methaemoglobin levels of 30% and 70%, respectively. At the higher concentration of amyl nitrite, a fall in blood pressure from 10.7 to 2.7 kPa (80 to 20 mmHg) was observed (Bastian & Mercker, 1959).

Part of the antidotal effect of amyl nitrite may be vasogenic in origin, rather than being due to methaemoglobin formation (Way et al., 1984). Amyl nitrite given after cyanide administration was reported by Vick & Froelich (1985) to reverse both cardiovascular changes and respiratory paralysis in 24 of 30 dogs. These changes occurred before significant methaemoglobin formation and suggest that early death caused by cyanide could be due in part to cardiovascular and respiratory failure.
6.8.3 Toxicology

Syncope or shock induced by large doses of amyl nitrite are the result of a pooling of blood in dilated capacitance vessels. Reflex tachycardia normally occurs, but a vaso-vagal reflex may induce a transient bradycardia immediately before collapse (Wilkins et al., 1937).

The degree of methaemoglobinaemia caused by inhalation of amyl nitrite is insufficient to affect oxygen transport (see section 6.8.3).

Amyl nitrite also causes a substantial increase in cerebrospinal fluid pressure (Norcross, 1938), and a fall in pO₂ in brain tissue has been recorded using an oxygen electrode (Rosemann et al., 1946).

6.9 Volunteer Studies

Inhalation of therapeutic doses of amyl nitrite in man did not result in methaemoglobin formation (Mathes & Gross, 1939).

When six volunteers inhaled 0.1 ml amyl nitrite 10 times for 20 seconds once per min, the median concentration of methaemoglobin was 3.45%, with a maximum of 6% and a minimum of 1.4%. During and shortly after inhalation, a small fall in blood pressure was observed, followed by immediate recovery. The pulse rate rose from 40 to 120 beats per min. Repeated use of the same square of gauze without application of a new ampoule of amyl nitrite did not have any effect (Bastian & Mercker, 1959).

6.10 Clinical Studies

No controlled human clinical trials have been undertaken.

6.11 Clinical Studies - Case Reports

No case reports are available.

6.12 Summary of Evaluation

6.12.1 Indications

Amyl nitrite is used as a first-aid measure in cases of cyanide poisoning where the patients are in deep coma, with dilated non-reactive pupils and deteriorating cardio-respiratory function.

6.12.2 Advised routes and dose

In adults, administer 0.2-0.4 ml amyl nitrite, via an Ambu bag, prior to artificial ventilation. In children, administer a maximum of 0.1 ml, via an Ambu bag, prior to artificial ventilation.

6.12.3 Other consequential or supportive therapy

First-aid therapy with amyl nitrite must be followed by additional antidotal treatment (see chapter 1). However, supportive therapy is the most important aspect of the treatment of cyanide poisoning. Special attention should be paid to the circulation.
The vasodilating action of amyl nitrite may lead to hypotension, which should be treated immediately with plasma expanders.

6.13 Model Information Sheet

6.13.1 Uses

Amyl nitrite is used as a first-aid measure in cases of cyanide poisoning where patients are in deep coma, with dilated non-reactive pupils and deteriorating cardio-respiratory function.

6.13.2 Dosage and route

In adults, 0.2-0.4 ml amyl nitrite should be administered via an Ambu bag prior to artificial respiration.

In children, a maximum of 0.1 ml should be administered via an Ambu bag prior to artificial respiration.

6.13.3 Precautions/contraindications

Special attention should be paid to the circulation. The vasodilating action of amyl nitrite may lead to hypotension, which should be treated immediately with plasma expanders.

6.13.4 Storage

* Protect from light.

* Insist that the suppliers provide only ampoules that have at least 18 months shelf-life left of the nominal 24-months total life.

* Take reasonable precautions to store at the lowest possible temperature compatible with immediate access in case of emergency; refrigerated storage is not necessary.

* Inspect monthly for any broken tubes.

Amyl nitrite forms an explosive mixture with air and oxygen. It is highly inflammable and must not be used where it may be ignited.

6.14 References


Mathes K & Gross F (1939) [The determination of methaemoglobin and cyanmethaemoglobin in circulating blood.] Naunyn-Schmiedebergs Arch Exp Pathol Pharmakol, 191: 701 (in German).


7. SODIUM NITRITE

7.1 Introduction

Methaemoglobin has a great affinity for cyanide in vitro (Hug & Marenzi, 1933a). Sodium nitrite is an inducer of methaemoglobin (Hug, 1933; Hug & Marenzi, 1933b) and has been in clinical use for over 50 years as an antidote for acute cyanide poisoning, most often
in combination with amyl nitrite and sodium thiosulfate (Viana et al., 1934; Chen & Rose, 1952; Hall & Rumack, 1986).

Potential side-effects of sodium nitrite infusion are hypotension and excessive methaemoglobin formation (Viana et al., 1934; Berlin, 1970; Feihl et al., 1982; Hall & Rumack, 1986). Patients who are already hypotensive are at special risk, and those with certain types of congenital methaemoglobinemia are theoretically more at risk from excessive methaemoglobin induction. Children administered large doses of sodium nitrite have developed excessive methaemoglobinemia (Berlin, 1970). Victims of smoke inhalation may have both elevated carboxyhaemoglobin levels and cyanide poisoning (Hart et al., 1985), and may develop further hypoxia from methaemoglobin induction (Becker, 1985; Jones et al., 1987).

7.2 Name and Chemical Formula

Sodium nitrite, otherwise known as diazotizing salts, dusitan sodny (Czech), erinitrit, filmerine, natrium nitrit (German), nitrite de sodium (French), nitrous acid sodium salt, sodii nitris, natrii nitris, natrium nitrosum, sodium nitrite (DOT), NCI-C02084, NIOSH No. RA 1225000, CAS 7632-00-0 (Sax, 1984; NIOSH, 1983; Windholz et al., 1983; Martindale, 1982), is supplied in the USA by Eli Lilly & Co., Indianapolis, Indiana, as a component of the Lilly Cyanide Antidote Package [R] (Product Information, 1984). It is sold in Australia by Orapharm under the proprietary name of OAR (Martindale, 1989). The relative molecular mass of sodium nitrite is 69.00, and the chemical formula is NaNO₂ (Sax, 1984).

7.3 Physico-chemical Properties

Sodium nitrite has a melting point of 271 °C (Windholz et al., 1983). It is soluble in either 1.5 parts of cold water or 0.6 parts of boiling water (Windholz et al., 1983). Solutions may be made in water (1:1.5) or alcohol 1:160 (Martindale, 1989). Sodium nitrite is slightly soluble in ether (ITI, 1985). Acids will decompose sodium nitrite and cause the evolution of brownish N₂O₃ fumes (Windholz et al., 1983). Aqueous solutions are alkaline to litmus, with a pH between 7 and 9 (Windholz et al., 1983; United States Pharmacopeia, 1985). The specific gravity is 2.17 (ITI, 1985). A 10% solution of sodium nitrite has a density of 1.065 (Izmerov, 1982). Excipients are apparently not added.

On drying over silica gel for 4 h, solid sodium nitrite loses no more than 0.25% of its weight (United States Pharmacopeia, 1985).

Injectable sodium nitrite solution is made by diluting solid sodium nitrite with "Water for Injection" (United States Pharmacopeia, 1985). The final solution is sterile and contains not less than 95% and not more than 105% of the labelled amount of sodium nitrite (United States Pharmacopeia, 1985). Sodium nitrite is supplied in the Lilly Cyanide Antidote Package [R] in two 10 ml vials of a 3% solution (30 mg/ml; 300 mg per vial) (Product Information, 1984).

Sodium nitrite is stable in light, but slowly oxidizes in air to nitrate (Windholz et al., 1983). It is incompatible with acetonilide, antipyrine, caffeine, citrate, chlorates, hypophosphites, iodides, mercury salts, morphine, oxidizing agents,
permanganate, phenazone, sulfites, tannic acid, and vegetable astringent decoctions, infusions, or tinctures (Martindale, 1989; Windholz et al., 1983).

7.4 Synthesis

Commercial sodium nitrite is derived from sodium hydroxide and nitrogen oxides by one of three common methods (Izmerov, 1982).

One method uses a low concentration of nitrogen oxides from gas formed during dilute nitric acid production, which is absorbed by a sodium hydroxide solution.

A second method (the "hot method") uses sodium hydroxide absorption of high concentrations of nitrogen oxides at atmospheric or higher pressure.

A third method (or "two-staged method") combines features of the first two.

Pharmaceutical sodium nitrite is produced by reduction of sodium nitrate, previously heated until fused, with sulfur dioxide, lead, or a sulfite (Harvey, 1980). Alternatively, it can be derived from sodium nitrate by nitric oxide absorption (the nitric oxide being obtained from ammonia by catalytic oxidation in a sodium carbonate solution) (Harvey, 1980). The sodium nitrite mixture obtained is lixiviated with water and filtered, then partially evaporated and allowed to crystallize (Surgenor, 1970).

Injectable sodium nitrite solution is prepared by dissolving solid sodium nitrite in "Water for Injection" to a final concentration of 3% (30 mg/ml) (Product Information, 1984; United States Pharmacopeia, 1985). It is then sterilized by autoclaving or membrane filtration (Martindale, 1989). The final delivery form meets both United States and European Pharmacopoeia requirements for pyrogens and sterility.

The United States Pharmacopeia specifies that no more than 0.002% of heavy metals may be present (United States Pharmacopeia, 1985). "Top grade" sodium nitrite from some processes has a sodium nitrite concentration of at least 99%, with at most 1.4% moisture content and impurities of not more than 0.8% sodium nitrate, 0.3% calcinated residue, and 0.17% sodium chloride (Izmerov, 1982).

7.5 Analytical Methods

7.5.1 Quality control

7.5.1.1 Solid sodium nitrite (United States Pharmacopeia, 1985)

Sodium nitrite (1g) is dissolved in water, making up to 100 ml. Then 10 ml of this solution is pipetted into a mixture containing 50.0 ml of 0.1 N potassium permanganate volumetric solution (VS) (0.1 mol/l), 100 ml of water, and 5 ml of sulfuric acid, and the pipette tip is immersed beneath the surface of this mixture during transfer. The liquid is warmed to 40 °C and allowed to stand for 5 min. After the addition of 25.0 ml oxalic acid VS (0.05 mol/l), the resultant mixture is heated to 80 °C and titrated with potassium permanganate (0.1 mol/l), equivalent to 3.45 mg sodium nitrite.
7.5.1.2 Sodium nitrite injection (United States Pharmacopeia, 1985)

The method described in 7.5.1.1 is followed except that, in place of the 10 ml sodium nitrite solution, an accurately measured volume of sodium nitrite injection containing about 150 mg sodium nitrite is used.

7.5.1.3 Preparation of volumetric solutions (VS) (United States Pharmacopeia, 1985)

(a) Potassium permanganate VS (0.1 mol/l)

\[ \text{KMnO}_4; \text{relative molecular mass, 158.03; 3.161 grams in 1000 ml} \]

About 3.3 g of potassium permanganate is dissolved in 1000 ml of water and boiled for about 15 min in a suitable flask. The flask is then stoppered and allowed to stand for at least 2 days. It is then filtered through a fine-porosity, sintered glass crucible, which may be lined with a pledget of glass wool. The solution is then standardized by weighing accurately 200 mg of sodium oxalate, previously dried to constant weight at 110 °C. This is dissolved in 250 ml of water. Sulfuric acid (7 ml) is added and the mixture is heated to about 70 °C. The potassium permanganate solution is slowly added from a burette with constant stirring until a pale-pink colour persisting for about 15 seconds is produced. At the termination of titration, the temperature should be no lower than 60 °C. The molarity is calculated, with each 6.700 mg of sodium oxalate equivalent to 1 ml of potassium permanganate (0.1 mol/l). The solution should be stored in glass-stoppered amber-coloured bottles, and contact with organic materials such as rubber, which will reduce the potassium permanganate, should be avoided.

(b) Oxalic Acid VS (0.05 mol/l)

\[ \text{H}_2\text{C}_2\text{O}_4 \cdot 2\text{H}_2\text{O}; \text{relative molecular mass 126.07; 6.303 g in 1000 ml} \]

6.45 g of oxalic acid is dissolved in water and made up to 1000 ml. The solution is standardized by titration against potassium permanganate VS (1 mol/l) as described above. The solution should be stored protected from light in glass-stoppered bottles.

7.5.2 Identification (United States Pharmacopeia, 1985)

A solution of sodium nitrite responds to tests for sodium and for nitrite.

7.5.2.1 Sodium

After conversion to chloride or nitrate, solutions of sodium compounds with five times their volume of cobalt-uranyl acetate test solution (TS) yield a precipitate that is golden yellow in colour and forms after several min of agitation. Cobalt-uranyl acetate test solution is prepared by dissolving 40 g of uranyl acetate in a
mixture of 30 g of glacial acetic acid and enough water to make 500 ml. A solution is also prepared of 200 g of cobaltous acetate in a mixture of 30 g of glacial acetic acid and enough water to make 500 ml. Both solutions are prepared with warming, mixed while still warm, and cooled to 20 °C. The temperature is maintained at 20 °C for about 2 h, allowing the separation of excess salts, and then the resultant solution is filtered through a dry filter. Sodium compounds also impart an intense yellow coloration to a non-luminous flame.

7.5.2.2 Nitrite

Treatment of nitrite-containing solutions with diluted mineral acids or acetic acid (6 mol/l) produces brownish-red fumes, and the solution will give a blue coloration on starch-iodide paper.

7.5.3 Impurities (United States Pharmacopeia, 1985)

7.5.3.1 Preparation of sodium nitrite to test

Sodium nitrite (1 g) is dissolved in 6 ml hydrochloric acid (3 mol/l) and then evaporated to dryness on a steam bath. The residue is reduced to a coarse powder, which is then heated on the steam bath until the hydrochloric acid odour is no longer perceptible. This residue is then dissolved in 23 ml of water, and 2 ml of acetic acid (1 mol/l) is added.

The test is for metallic impurities that are coloured by the sulfide ion under specified test conditions, in terms of the percentage of lead (by weight) in the test substance. Limits for sodium nitrite are 0.002%.

7.5.3.2 Preparation of special reagents

Lead nitrate stock solution is prepared by dissolving 159.8 mg lead nitrate in 100 ml of water and adding 1 ml of nitric acid. This solution is then diluted to 1000 ml with water and stored in glass containers free of lead soluble salts. Standard lead solution is prepared by diluting 10.0 ml of the lead nitrate stock solution with water to a volume of 100.0 ml, so that each 1 ml of standard lead solution contains the equivalent of 10 µg of lead. A comparison solution prepared with 100 µl of standard lead solution per g of sodium nitrite contains the equivalent of 1 part of lead per million parts of sodium nitrate.

7.5.3.3 Preparation of standard

Pipette 2 ml of standard lead solution (containing 20 mg of lead) into a 50 ml colour comparison tube. Dilute with water to 25 ml and adjust pH to between 3.0 and 4.0 with acetic acid (1 mol/l) or ammonium hydroxide (6 mol/l) using narrow-range pH paper as an indicator. Dilute to 40 ml with water and mix.

7.5.3.4 Preparation of test

Pipette 25 ml of the prepared sodium nitrite test material (see section 7.5.3.1) into a 50 ml colour comparison tube. Using narrow-range pH indicator paper, adjust pH to between 3.0 and 4.0
with either acetic acid (1 mol/l) or ammonium hydroxide (6 mol/l). Then dilute to 40 ml with water and mix.

7.5.3.5 Preparation of monitor

Place 25 ml of solution prepared as in section 7.5.3.4 above into a 50 ml colour comparison tube and add 2.0 ml of the standard lead solution. Using short-range pH indicator paper, adjust pH to between 3.0 and 4.0 with either (1 mol/l) acetic acid or ammonium hydroxide (6 mol/l). Then dilute to 40 ml with water and mix.

7.5.3.6 Preparation of hydrogen sulfide test solution (TS)

Pass hydrogen sulfide gas into cold water making a saturated solution that should possess a strong odour of hydrogen sulfide. Store in a cold, dark area in small, dark amber-coloured bottles filled nearly to the top.

7.5.3.7 Test procedure

Add 10 ml of freshly prepared hydrogen sulfide TS solution to each colour comparison tube. Allow to stand for 5 min, then view downwards over a white surface. The colour of the solution described in section 7.5.3.4 should not be darker than that of the solution described in section 7.5.3.3. The colour intensity of the solution described in section 7.5.3.5 should be equal to or greater than that of the solution described in section 7.5.3.3.

7.6 Shelf-life

The shelf-life of sodium nitrite solution for injection is 5 years, which might be somewhat reduced in very hot climates though stability testing at elevated temperatures has not been undertaken (Personal Communication, Eli Lilly & Co., March 1987). The supplier recommends that sodium nitrite be stored at controlled room temperatures of 15 - 30 °C (Product Information, 1984). Experiments with stored ampoules showed that sodium nitrite is stable in water for more than 21 years (Chen & Rose, 1956).

7.7 General Properties

7.7.1 Mode of action

Sodium nitrite has seldom been utilized alone for the treatment of cyanide poisoning. Rather, it is used in combination with intravenous sodium thiosulfate and, often, amyl nitrite by inhalation. These combinations have been shown to be more efficacious in experimental poisoning than any of the compounds alone, demonstrating true antidotal synergy (Hug, 1933; Chen et al., 1933a; Chen et al., 1934; Chen & Rose, 1952; Way et al., 1984).

Sodium nitrite induces methaemoglobin in vitro, which binds cyanide (Hug & Marenzi, 1933a). In animal studies with dogs and rabbits, sodium nitrite induced methaemoglobinemia, with levels averaging 50% following a dose of 40 mg/kg (Hug & Marenzi, 1933a; Hug & Marenzi, 1933b). Sodium nitrite alone has been shown to be efficacious in dogs with experimental cyanide poisoning (Chen et al., 1933a; Chen et al., 1934; Chen & Rose, 1952). However, dog blood has been shown to be more sensitive to methaemoglobin induction by sodium nitrite in vitro than human blood (Paulet &
Menoret, 1954). Methaemoglobin can be an efficacious cyanide antidote, as shown by results in experimental poisoning in rats treated with an exogenously prepared stroma-free methaemoglobin solution (Ten Eyck et al., 1986). Human red blood cells, with methaemoglobin induced \textit{in vitro}, were injected intraperitoneally to cyanide-poisoned mice and had antidotal efficacy (Kruszyna et al., 1982). However, animals pretreated with methylene blue did not develop significant methaemoglobin levels and had unchanged sodium nitrite antidotal efficacy in experimental cyanide poisoning (Holmes & Way, 1982). Other mechanisms, including vasodilatation with changes in local capillary blood flow, have been postulated to account for part or all of the antidotal action of sodium nitrite (Way et al., 1984; Cohen & Guzzardi, 1984).

Alternative mechanisms of action are suggested by the fact that sodium nitrite relaxes maximally contracted vascular smooth muscle \textit{in vitro} (Kruszyna et al., 1985). The contracted vascular smooth muscle relaxation was not reversed in this model by the addition of cyanide (Kruszyna et al., 1985). In mice, methaemoglobin levels induced by sodium nitrite were more efficacious in protecting against cyanide poisoning than were comparable methaemoglobin levels induced by either hydroxylamine or 4-dimethylaminophenol (Kruszyna et al., 1982). Equivalent amounts of methaemoglobin induced \textit{in vitro} by these three agents did not bind significantly different amounts of cyanide (Kruszyna et al., 1982), suggesting that sodium nitrite may have some other mechanism of action. The methaemoglobinemia induced in these mice by sodium nitrite peaked later and at a lower level than the methaemoglobinemia induced by hydroxylamine or 4-dimethylaminophenol, but was longer lasting (Kruszyna et al., 1982). This may explain differences in efficacy noted in this animal study, as cyanide initially bound to methaemoglobin induced by the other agents may have been rapidly released to exacerbate the poisoning (Kruszyna et al., 1982).

The oxidation of haemoglobin to methaemoglobin by sodium nitrite is favoured when the oxygen partial pressure is high and haemoglobin is in the oxyhaemoglobin state (Tomoda & Yoneyama, 1979). If methaemoglobin induction accounts for all, or part, of the antidotal action of sodium nitrite, this might explain the potentiation noted with concomitant oxygen administration (Mansouri, 1985), although other investigators have not reached this conclusion (Way et al., 1966).

The mechanism of action of sodium nitrite is not fully understood at present. Methaemoglobin induced by sodium nitrite and measurable with a co-oximeter, however, does not bind cyanide at the time of measurement (cyanmethaemoglobin cannot be measured by these instruments). Patients have survived seriously symptomatic acute cyanide poisoning, despite developing \textit{measurable} methaemoglobin levels of 10% or less (Hall et al., 1987; DiNapoli et al., 1989). Administering further sodium nitrite to a patient who has already had a satisfactory clinical improvement in order to produce a "therapeutic methaemoglobin level" of 25%, as has sometimes been recommended (Jones et al., 1987), is both unnecessary and potentially dangerous (DiNapoli et al., 1989).

7.7.2 Other relevant properties

Sodium nitrite is a vasodilating agent and has been used in the past for the treatment of angina (Martindale, 1989). Rapid
intravenous administration may cause hypotension (Viana et al., 1934; Hall & Rumack, 1986). This effect can be avoided by beginning the infusion slowly and increasing the infusion rate cautiously with careful blood pressure monitoring.

7.8 Animal Studies

7.8.1 Pharmacokinetics

Sodium nitrite blood levels have not been measured during antidote therapy and most pharmacokinetic parameters have not been determined. Most studies have focused on the levels of methaemoglobin induced by sodium nitrite.

In one study into fetal methaemoglobin formation, when pregnant rats were administered sodium nitrite orally or by intraperitoneal injection, peak maternal sodium nitrite blood levels were usually produced within the first 20 to 30 min, while peak methaemoglobin levels were observed a few minutes later (Gruener et al., 1973). Peak sodium nitrite blood concentrations ranged from 3.9 mg/l (56.52 µmol/l), with an administered dose of 2.5 mg/kg, to 32.5 mg/l (471.01 µmol/l) with an administered dose of 30 mg/kg, and corresponded to peak maternal methaemoglobin levels of 3.4% (2.5 mg/kg dose) to 60.2% (30 mg/kg dose) (Gruener et al., 1973).

Sodium nitrite produced an equimolar conversion of haemoglobin to methaemoglobin in vitro in canine blood (Hug & Marenzi, 1933a). However, only about 74% of an administered intravenous dose was involved in methaemoglobin formation in dogs in vivo (Hug & Marenzi, 1933b). Dogs administered 40 mg/kg of sodium nitrite intravenously developed approximately 50% methaemoglobinemia (Hug & Marenzi, 1933b). In these experiments using sodium nitrite alone, all of the administered dose of potassium cyanide was recovered in the urine as thiocyanate over a 4-day period (Hug & Marenzi, 1933b).

In a dog with acute cyanide poisoning, given sodium nitrite (22.5 mg/kg) immediately and an additional 11.25 mg/kg at 29 and 54 min after the poisoning, peak methaemoglobin levels of 65% at 30 min and 60% at 4 h were observed (Chen & Rose, 1952). The methaemoglobin concentration decreased rapidly after each peak, reaching 20% about 30 min after the first peak, and 4-5 h after the second peak (Chen & Rose, 1952). Methaemoglobin disappeared from the circulation 11 h after the first injection of sodium nitrite (Chen & Rose, 1952).

In mice administered sodium nitrite 75 (mg/kg) intraperitoneally, methaemoglobin levels peaked at 30-35% about 20 min after the injection (Kruszyna et al., 1982). The levels remained in this range until 40 min after sodium nitrite administration and then decreased slowly, reaching 25% methaemoglobinemia at 1 h and 10% at 2 h (Kruszyna et al., 1982). These findings contrast with studies undertaken in mice with other methaemoglobin-inducing agents (4-dimethylaminophenol and hydroxylamine), which produced higher peak methaemoglobin levels more quickly (Kruszyna et al., 1982). Following the peak level, the degree of methaemoglobinemia then decreased faster than that induced by sodium nitrite (Kruszyna et al., 1982).

In vitro studies have indicated that canine blood is more susceptible to sodium nitrite-induced methaemoglobin conversion than
human blood (Paulet & Menoret, 1954). Rodent blood is more resistant to methaemoglobin formation than human blood (Kruszyna et al., 1982).

7.8.2 Pharmacodynamics

Sodium nitrite, at a minimum dose of 5 mg/kg, was shown to be an effective antidote for experimental cyanide poisoning in dogs, while 200 mg/kg was efficacious in rabbits (Hug, 1932; Hug, 1933). In other experiments, sodium nitrite alone was shown to be an effective cyanide antidote in dogs (Chen et al., 1934), though the combination of sodium nitrite with a sulfur donor allowed dogs to tolerate doses of cyanide greater than those antagonized by the two agents separately (Chen et al., 1933a; Chen et al., 1933b; Hug, 1933; Chen et al., 1934). This antidotal synergy has been noted in numerous studies since the 1930s (e.g., Way et al., 1972). Sodium nitrite has been shown to have protective effects against rat brain cytochrome oxidase inhibition in vivo (Piantadosi et al., 1983).

Two studies have reported data that are inconsistent with the classical antidotal mechanism of action, namely methaemoglobin induction. When comparable levels of methaemoglobinemia were induced in mice by 4-dimethylaminophenol, hydroxylamine, or sodium nitrite, cyanide was antagonized better by the administration of sodium nitrite (Kruszyna et al., 1982). Following pre-treatment with methylene blue prior to cyanide poisoning, mice had unchanged sodium nitrite antidotal efficacy, even though they did not develop significant methaemoglobinemia (Holmes & Way, 1982).

Sodium nitrite causes relaxation of vascular smooth muscle in vitro (Kruszyna et al., 1985). Sodium nitrite alone did not have any effect on either the respiratory or urinary excretion of 14C-labelled sodium cyanide in mice (Burrows et al., 1982). Other postulated antidotal mechanisms include vasodilatation with improved capillary blood flow (Way et al., 1984; Cohen & Guzzardi, 1984).

7.8.3 Toxicology

A rather large body of sub-acute and chronic toxicity data exists for sodium nitrite (Musil, 1966; Izmerov, 1982). Effects including increased methaemoglobin levels, neuromuscular excitability, thyroid dysfunction, changes in conditioned reflexes, elevated serum protein and albumin concentrations, possible effects on hepatic drug metabolism systems, prolonged prothrombin time, and decreased erythrocyte counts have been noted in chronic and subacute animal exposures (Musil, 1966; Izmerov, 1982; Gosselin et al., 1984). In antidote administration, most subjects would receive only one dose, or at most a few doses over a short period of time, and these chronic and subacute animal studies would not seem to be particularly relevant.

Pre-treatment of rats with sodium nitrite potentiates carbon tetrachloride hepatotoxicity (Suarez & Bhonsle, 1978). A synergistic effect on acute hepatic necrosis in mice has been produced by oral administration of a combination of sodium nitrite and various secondary amines (Asahina et al., 1971). A second abnormal haemoglobin derivative, nitrosohaemoglobin, has been produced in rats following intraperitoneal sodium nitrite injection (Rein et al., 1968).
Sodium nitrite is listed as a non-carcinogen, but as giving a positive result in the Ames test, by the International Agency for Research on Cancer (IARC) (Kuroki et al., 1980). It can react with secondary amines either in vitro or in vivo to produce potentially carcinogenic nitrosamines (Izmerov, 1982). In vitro studies have shown induction of volatile mutagens in the faeces of normal human volunteers following treatment with sodium nitrite (MacDonald & Rao, 1982). Administration of sodium nitrite and morpholine together to rats produced hepatic and forestomach tumours (Mirvish et al., 1983). However, a cohort study of workers chronically exposed to a combination of sodium nitrite and various amines in cutting fluids did not show an increased cancer morbidity (Jarvholm et al., 1986). Epidemiological data suggest a relationship between the consumption of large quantities of nitrite-nitrate-treated foodstuffs, a diet poor in ascorbic acid, and the development of gastric, oral cavity, and oesophageal cancers (Weisburger et al., 1983).

Sodium nitrite administered orally or intraperitoneally to pregnant rats produced peak fetal sodium nitrite blood levels ranging from trace amounts, with a 2.5 mg/kg maternal dose and a corresponding maternal blood level of 3.9 mg/l (56-52 µmol/l), to 9.4 mg/l (136.23 µmol/l) with a 30 mg/kg maternal dose and a corresponding maternal blood level of 32.5 mg/l (471.01 µmol/l) (Gruener et al., 1973). Fetal methaemoglobin levels ranged from 1.2% (with a maternal sodium nitrite dose of 2.5 mg/kg and a corresponding maternal peak methaemoglobin level of 3.4%) to 27.2% (with a maternal sodium nitrite dose of 30 mg/kg and a corresponding maternal peak methaemoglobin level of 60.2%) (Gruener et al., 1973). Fetal effects other than methaemoglobin induction were not reported.

Sodium nitrite was shown not to be teratogenic in several animal species when used as a cardiovascular agent or as a fungicide in combination with iodine (Schardein, 1985). In combination with ethylene, sodium nitrite forms the potent teratogen ethylnitrosurea (Schardein, 1985). The median effective dose (ED$_{50}$) for mortality in a chicken embryo study was 22 µmol/egg (Korhonen et al., 1983). A maximum of 27% of the embryos were noted to be malformed (Korhonen et al., 1983). A combination of sodium nitrite (50 mg/kg) and methyl urea (30 mg/kg), administered orally to rats on the 9th day of pregnancy, killed over 40% of the fetuses and produced malformations in about 15% of the surviving fetuses (Izmerov, 1982). Administration of ascorbic acid blocked both the embryotoxic and teratogenic effects (Izmerov, 1982). Sodium nitrite doses of 100 mg/kg were teratogenic in rats (Izmerov, 1982). Cyanosis was observed in 25% of newborn rats at birth following maternal administration of sodium nitrite (100 mg/kg) and in 6% of newborn rats with a maternal dose of 5 mg/kg (Izmerov, 1982).

As sodium nitrite is only likely to be administered once, or at most a few times, as an antidote, the main concern would appear to be induction of fetal methaemoglobinaemia if administered to a pregnant patient. The risk to the fetus from maternal cyanide poisoning would seem to override the risk from possible fetal methaemoglobin induction. At clinically relevant doses (300 mg, or about 4.3 mg/kg for a 70-kg subject) (Product Information, 1984), data from a rat study suggested that the peak fetal methaemoglobin level would be in the neighbourhood of 2.7% (Gruener et al., 1973). This amount of fetal methaemoglobinaemia is not likely to be
clinically significant. However, based on in vitro comparisons of methaemoglobin reductase activity, the human fetus may have a weaker defense mechanism against the development of significant methaemoglobinaemia than has the rat fetus (Gruener et al., 1973).

Some representative animal toxicity values for sodium nitrite are: LD$_{50}$ rat (oral), 85 mg/kg; LDLo hamster (oral), 3 mg/kg; LD$_{50}$ mouse (oral), 175 mg/kg; LD$_{50}$ rat (intravenous), 65 mg/kg; LDLo dog (oral), 330 mg/kg; LDLo dog (intravenous), 15 mg/kg; LDLo rabbit (intravenous), 80 mg/kg (Sax, 1984; ITI, 1985). A comparative human intravenous antidotal dose is 300 mg (or about 4.3 mg/kg for a 70-kg subject) (Product Information, 1984).

7.9 Volunteer Studies

7.9.1 Pharmacokinetics

Sodium nitrite is rapidly absorbed following oral administration (Martindale, 1989). About 60% of the absorbed nitrite ion is metabolized in the body, partially to ammonia, while the rest of the absorbed dose is excreted unchanged in the urine (Martindale, 1982). The nitrite ion disappears from the circulatory system quickly, but little is known about its fate (Baselt, 1982). An intravenous injection of 400 mg in humans produced a peak methaemoglobin concentration of 10.1%, while 600 mg produced a peak methaemoglobin level of 17.5% (Chen & Rose, 1952). A later study in normal volunteers showed that a methaemoglobin level of 6% was produced by the intravenous injection of 1 mg sodium nitrite per kg (about 300 mg, 10 ml of a 3% solution of sodium nitrite) (Weger, 1968). In this same study, injecting 12 mg/kg (about 900 mg, 30 ml of a 3% solution of sodium nitrite) resulted in a 30% methaemoglobinaemia, but also in clinical shock (Weger, 1968).

7.9.2 Sodium nitrite poisoning

Most human toxicity data relates to accidental or suicidal ingestion of sodium nitrite from industrial, laboratory, or food additive sources. Fatal poisoning has occurred when sodium nitrite has been substituted for table salt and used as seasoning (MacQuiston, 1936; Padberg & Martin, 1939) or when food contaminated with motor vehicle cooling fluid was consumed (Ten Brink et al., 1982). A case of suicide from the ingestion of sodium nitrite has been reported, with a post-mortem methaemoglobin level of 90% (Standefer et al., 1979). Ingestion of sausages cured with sodium nitrite produced symptomatic poisoning with syncope, hypotension, and methaemoglobinaemia and a decreased arterial oxygen saturation (Bakshi et al., 1967). Two patients who mistakenly ingested sodium nitrite instead of table salt developed methaemoglobin levels of 34% and 54%, but recovered after being administered methylene blue and supplemental oxygen (Aquanno et al., 1981).

The range of toxicity of sodium nitrite is difficult to determine, as the ingested dose is seldom known. A patient who ingested 14.5 g complained of transient darkening of both visual fields (Grant, 1986). A 17-month-old child died with a methaemoglobin level greater than 90% after being given 450 mg (32 mg/kg) intravenously in the mistaken impression that acute cyanide poisoning was present (Berlin, 1970). A two-month-old infant developed severe poisoning but survived after ingesting...
130 mg sodium nitrite (Gosselin et al., 1984). The mean lethal oral dose in adults is probably in the neighbourhood of 1 g if no treatment is given (Gosselin et al., 1984), though survival has followed the ingestion of 1 g (Baselt, 1982).

Treatment of acquired methaemoglobinemia from sodium nitrite poisoning in circumstances similar to those described above may involve only supplemental oxygenation and observation if the patient is asymptomatic and the methaemoglobin level is 20–30% or less (Hall et al., 1986a). With higher methaemoglobin levels or in symptomatic patients, intravenous infusion of methylene blue at a usual dose of 0.1–0.2 ml/kg of a 1% solution (1–2 mg/kg) may be necessary (Hall et al., 1986b). Toluidine blue may be used when methylene blue is not available. Exchange transfusion may be required if severely poisoned patients are not responsive to the above measures (Hall et al., 1986a). Experimental animal studies have suggested that hyperbaric oxygen is both efficacious (Goldstein & Doull, 1971) and not efficacious (Sheehy & Way, 1974) in reducing nitrite-induced methaemoglobinemia. Hyperbaric oxygen can be used to maintain tissue oxygenation while exchange transfusion is prepared (Hall et al., 1986a).

If the therapeutic administration of sodium nitrite in cyanide poisoning produces excessive methaemoglobinemia (Viana et al., 1934; Berlin, 1970; Lasch & El Shawa, 1981; Feihl et al., 1982), there is some controversy about appropriate treatment. The administration of methylene blue has been recommended (Product Information, 1984), and toluidine blue has also been used. However, exchange transfusion rather than methylene or toluidine blue administration has been suggested because conversion of cyanmethaemoglobin back to normal haemoglobin could theoretically release bound cyanide and worsen the poisoning (Rumack, 1987). This controversy is unresolved at present.

7.10 Clinical Studies

No controlled human clinical trials have been performed to compare the efficacy of various cyanide antidotes in acute human poisoning.

7.11 Clinical Studies - Case Reports

In the absence of controlled clinical trials, anecdotal reports of human poisoning treated with sodium nitrite are all that can be evaluated. To compound the problem, only a single case of acute cyanide poisoning treated solely with sodium nitrite has been reported (Mota, 1933). All other reported patients were given more than one antidote. Many patients have received a combination of sodium nitrite and sodium thiosulfate, or amyl nitrite and sodium nitrite plus sodium thiosulfate (Chen & Rose, 1952; Chen & Rose, 1956). In other cases, sodium nitrite has been administered in various combinations with sodium thiosulfate, methylene blue, dicobalt-EDTA (Kelocyanor [R]), and hydroxocobalamin (Motin et al., 1970; Lutier et al., 1971; Yacoub et al., 1974). The clinical efficacy of sodium nitrite used alone in acute cyanide poisoning is therefore impossible to separate from its use in an antidotal combination, and, judging from various animal studies showing antidotal synergism between sodium nitrite and sodium thiosulfate
(Chen et al., 1934; Chen & Rose, 1952; Way et al., 1972), it should probably not be used alone.

The first reported cases of human acute cyanide poisoning to be treated with a combination of sodium nitrite and sodium thiosulfate were described by Viana et al. (1934). Their first patient ingested about 5 g of potassium cyanide and became comatose with a weak pulse and respiratory distress, but recovered after being given 1500 mg of sodium nitrite and 18 g of sodium thiosulfate. Their second patient ingested about 2 g of potassium cyanide and developed coma, respiratory distress, and convulsions, but recovered after being given 750 mg of sodium nitrite and 12 g of sodium thiosulfate. No cyanide assays were undertaken. Hypotension and cyanosis developed in both cases (Viana et al., 1934).

The largest case series of acute cyanide poisoning treated with the amyl nitrite/sodium nitrite/sodium thiosulfate antidote combination was assembled by Chen & Rose (1952, 1956) comprising a total of 49 patients. One patient who was moribund before being administered these antidotes did not recover (Chen & Rose, 1952). Only historical evidence of cyanide poisoning was available in these cases; no cyanide assays were reported (Chen & Rose, 1952; Chen & Rose, 1956).

Survival following acute cyanide poisoning from potassium and sodium cyanide (De Busk & Seidl, 1969; Stewart, 1974; Feihl et al., 1982; Peters et al., 1982; Wood, 1982; Litovitz et al., 1983; Wesson et al., 1985; Hall et al., 1987), cyanogenic plants (Sayre & Kaymakcalan, 1964; Rubino, 1978; Lasch & El Shawa, 1981; Shragg et al., 1983), and laetrile (Moss et al., 1981; Beamer et al., 1983; Hall et al., 1986) has been reported. However, not all patients with severe cyanide poisoning given the sodium nitrite/sodium thiosulfate antidote combination have survived (Chen & Rose, 1952; Braico et al., 1979; Litovitz et al., 1983). Patients with severe acute cyanide poisoning (including coma, convulsions, and metabolic acidosis) and blood cyanide levels, measured at various times after exposure, ranging from 0.26 to 40 mg/l (10 to 1539 µmol/l) have survived following the administration of sodium nitrite and sodium thiosulfate in combination with supportive measures (Rubino, 1978; Moss et al., 1981; Peters et al., 1982; Feihl et al., 1982; Litovitz et al., 1983; Shragg et al., 1983; Wesson, et al., 1985; Hall et al., 1987; Hall et al., 1986b). Patients poisoned with cyanide have survived with supportive treatment only (Graham et al., 1977; Vogel et al., 1981; Brivet et al., 1983), but the highest reported blood cyanide level in a patient who survived without receiving specific antidote treatment was 3.8 mg/l (147 µmol/l) (Edwards & Thomas, 1978). This suggests that patients with more severe cyanide poisoning may have a better chance of recovery with both supportive measures and specific antidotes than with supportive measures alone (Hall & Rumack, 1986).

Administration of the sodium nitrite/sodium thiosulfate antidote combination in five smoke inhalation victims with carbon monoxide and cyanide poisoning and mean blood cyanide levels of 1.62 mg/l (62 µmol/l) has been reported (Hart et al., 1985). These five patients also received hyperbaric oxygen therapy. There were four survivors and one fatality in this series.

**7.12 Summary of Evaluation**
7.12.1 Indications

Sodium nitrite is indicated for use in acute cyanide poisoning. It should not be used except where there is definite indication of severe poisoning, such as loss of consciousness and deteriorating vital functions. It is usually administered with sodium thiosulfate and its administration may be preceded by the use of amyl nitrite.

7.12.2 Contraindications

Sodium nitrite should not be administered to asymptomatic patients following exposure to cyanide. It should not be administered to patients with smoke inhalation and combined carbon monoxide and cyanide poisoning unless hyperbaric oxygen therapy is available and such therapy has already been initiated.

G6PD-deficient individuals are theoretically at great risk from nitrite therapy because of the likelihood of severe haemolysis, although no such cases have been reported.

7.12.3 Advised route and dosage

Sodium nitrite is administered intravenously in an initial adult dose of 300 mg (10 ml of a 3% solution) (Chen & Rose, 1952; Hall & Rumack, 1986). While many authors recommend that this dose be infused over a 5-min period, hypotension from the vasodilating properties of sodium nitrite may occur. Hypotension may be avoided by diluting sodium nitrite with normal saline and infusing the medication over a 20-min period with frequent blood pressure monitoring (Hall, 1986). The rate of infusion may be increased if no hypotension occurs (Hall, 1986). Methaemoglobin levels should be monitored to avoid excessive methaemoglobin induction (Berlin, 1970) (see chapter 10). The paediatric dose for an average child, frequently quoted in the literature and based on in vitro experiments with canine blood, is 0.33 ml of a 3% solution per kg body weight (about 10 mg/kg), administered intravenously with the precautions noted above (Hall & Rumack, 1986). A pediatric dose of approximately 0.2 ml/kg of a 3% solution has also been recommended (Product Information, 1984). If there is reason to suspect anaemia, tables devised to calculate a reduced dose, taking into account the relatively lesser amount of haemoglobin present, should be consulted (Berlin, 1970).

The adult dose may be lethal for a child. With a haemoglobin level of 12 g/100 ml, only 10 mg/kg per body weight can be administered immediately and 5 mg/kg repeated within 30 min if necessary (Berlin, 1970). However, the calculation of Berlin was based on a therapeutic level of methaemoglobinemia of 15 mg/kg. This may be too high because Weger (1968) observed severe circulatory collapse in volunteers after 12 mg/kg was given intravenously. In the same study, the administration of 4 mg/kg (10 ml of a 3% solution NaNO₂ solution, i.e., 300 mg) induced only 6% methaemoglobinemia. As the adult dose of about 4 mg/kg has been shown to be effective clinically, it may be safer to begin treatment in children with sodium nitrite at 4 mg/kg (about 0.13 ml of a 3% solution per kg body weight) and to administer additional sodium nitrite only if excessive methaemoglobinemia is not present and a satisfactory clinical response has not occurred.
In both adults and children, another sodium nitrite dose of 50% the initial amount administered may be repeated 30 min after the first dose if there is inadequate clinical improvement (Hall & Rumack, 1986).

7.12.4 Other consequential or supportive therapy

See section 1.10.

7.13 Model Information Sheet

7.13.1 Uses

Sodium nitrite is indicated for the treatment of acute cyanide poisoning.

7.13.2 Dosage and route

The adult dose is 300 mg (10 ml of a 3% solution) infused intravenously at the fastest rate possible without causing hypotension (usually over 5 to 20 min). The initial paediatric dose is 0.13-0.33 ml of a 3% solution per kg body weight (about 4-10 mg/kg). It is advisable to begin with lower doses in children and increase to the desired effect. If anaemia is suspected, standard tables should be consulted to calculate a reduced paediatric sodium nitrite dose.

In both adults and children, another dose of one-half the initial amount administered may be repeated 30 min after the first dose if there is inadequate clinical improvement.

7.13.3 Precautions/contraindications

Excessive methaemoglobinemia may occur, especially when doses larger than those recommended are administered to children.

Hypotension may occur following rapid administration of sodium nitrite, owing to its vasodilating properties. Blood pressure should be monitored carefully during sodium nitrite administration, and the infusion rate slowed if hypotension occurs.

Patients with smoke inhalation and combined carbon monoxide and cyanide poisoning with elevated carboxyhaemoglobin levels should not be given sodium nitrite unless treatment in a hyperbaric oxygen chamber is available and such treatment has been initiated.

G6PD-deficient individuals are theoretically at great risk from sodium nitrite therapy because of the likelihood of severe haemolysis, although no such cases have been reported.

7.13.4 Adverse effects

Excessive methaemoglobinemia may occur, especially with doses exceeding those recommended. Hypotension may occur with rapid intravenous infusion.

7.13.5 Use in pregnancy and lactation

There are no reported cases of the use of sodium nitrite during
pregnancy or lactation. Animal experiments indicate that some sodium nitrite crosses the placenta and that fetal methaemoglobinaemia may be induced. The risk to the fetus from severe maternal cyanide poisoning would seem to be greater than the risk of fetal methaemoglobin induction. No animal studies have addressed the question of sodium nitrite excretion in breast milk or its possible effects on the nursing infant.

7.13.6 Storage

Sodium nitrite should be stored at a controlled temperature of 15 to 30 °C (59 to 86 °F). The shelf-life is 5 years.

7.14 References


8. 4-DIMETHYLAMINOPHENOL (4-DMAP)

8.1 Introduction

Cyanide has a special affinity for ferric ions, which are found in cytochrome oxidase, the terminal oxidative respiratory enzyme in mitochondria. Blood contains a substantial quantity of ferrous ions within haemoglobin and it is possible to convert these readily to ferric ions, for example, by the use of nitrites as suggested by Chen et al. (1933). A disadvantage of the use of nitrites is the fact that the concentration of methaemoglobin rises only slowly after intravenous administration. In six experiments on volunteers, using the dose of sodium nitrite suggested by Chen (4 mg/kg), a methaemoglobin level of 6% resulted after 40 min (Kiese & Weger, 1969). This was considered to be too low a concentration of methaemoglobin after too long a time. Experiments with several aminophenols showed that the use of 4-dimethylaminophenol (4-DMAP)
resulted in rapid and controlled formation of methaemoglobin. An
intravenous dose of 3.25 mg/kg resulted in a methaemoglobin
concentration of 30% in 10 min, a methaemoglobin level of 15% being
reached within one min.

8.2 Name and Chemical Formula

Chemical name: Dimethyl( para)aminophenol hydrochloride

Formula:

\[
\begin{align*}
\text{HO} & \quad \text{N} & \quad \text{CH}_3 \\
\text{CH}_3 & & \text{HCl}
\end{align*}
\]

Total formula: \( \text{C}_8\text{H}_{11}\text{ON.HCl} \)
Relative molecular mass: 173.5
Appearance: snow-white crystals
CAS number: 619-60-3
Manufacturer: Dr Franz Koehler Chemie GmbH, Alsbach, Germany
Commercial name: 4-dimethylaminophenol (4-DMAP)

8.3 Physico-chemical Properties

Raw material: snow-white colourless crystals
Melting point: 145 °C + 1 °C
Solubility: very soluble in water. The solution is oxidized by contact
with air and changes from being colourless to black-brown
Optical properties:
IR-spectrum: peaks at (cm\(^{-1}\))
  833: 1,4-disubstituted aromatics
  1240: phenols
  1275: amine
  1500: aromatics
  2700: C-H aromatics
  3200: N-H aromatics
UV data: maximum at 271 nm; extinction coefficient 1255 l/mol
Acidity: no data available

pK \((-\text{N(CH}_3)_2\) = 6.15
pK \((-\text{OH}) = 10.1

Stability in light: no data available
Thermal stability: no data available
Refractive index and specific gravity: unknown
Weight on drying: no data available
Excipients and pharmaceutical aids: unknown for the final product
Although it may contain an antioxidant such as sodium pyrosulfate.

8.4 Synthesis

Routes of synthesis: no information available
Manufacturing processes: no information available

8.5 Analytical Methods

8.5.1 Identity

To 5 ml of liquid from the ampoule, add one drop 30% hydrogen peroxide. The solution will turn a deep violet colour within 5 min. To 2.5 ml of liquid from the ampoule, add 0.5 ml 10% ferrichloride. A pale-brown colour will appear immediately.

8.5.2 Quantification

AgNO₃ titration: 2.5 ml liquid from the ampoule is diluted in 40 ml water and, after the addition of 3 ml nitric acid (2 mol/l), potentiometrically titrated with silver nitrate (0.1 mol/l). 1 ml AgNO₃ (0.1 mol/l) equiv. 17.365 mg dimethylaminophenol hydrochloride.

HClO₄ titration: 2.5 ml liquid from the ampoule is added to 40 ml of a 3% solution of mercury acetate in glacial acetic acid and then potentiometrically titrated with perchloric acid (0.1 mol/l). 1 ml perchloric acid (0.1 mol/l) equiv. 17.365 mg dimethylaminophenol hydrochloride.

8.5.3 Purity

Unknown

8.5.4 Methods for analysis of 4-DMAP in biological samples

Unknown.

8.6 Shelf-life

Since, in contact with air, 4-DMAP is readily oxidized and the solution changes colour to black-brown, 4-DMAP must be stored in opaque containers. An open ampoule cannot be kept. Coloured and turbid solutions cannot be used. Correctly stored 4-DMAP can be used after storage for up to 3 years. The influence of tropical conditions is unknown.

8.7 General Properties

The use of 4-DMAP results in rapid formation of methaemoglobin. An intravenous dose of 3.25 mg/kg resulted in a methaemoglobin concentration of 30% within 10 min. Because there is a much larger source of haemoglobin than there is of cytochrome oxidase, cytochrome oxidase blocked by cyanide can be reactivated and its role as an essential catalyst for tissue utilization of oxygen restored.
8.8 Animal Studies

8.8.1 In Vitro Studies

In studies involving the addition of various aminophenols such as 2-aminophenol, 2-amino-4-chlorophenol, 2-amino-4,6-dichlorophenol, 2-amino-5-ethoxyphenol, 2-dimethylaminophenol, 4-aminophenol, 4-methylaminophenol, and 4-dimethylaminophenol to the blood of mice, cats, dogs, cattle, and man, it has been shown that 4-dimethylaminophenol and 4-methylaminophenol produce consistent amounts of methaemoglobin most rapidly. These studies also showed that a concentration of 30-40% methaemoglobin was attained at widely varying rates in different species (Kiese & Weger, 1969).

8.8.1.1 Metabolism of 4-DMAP in the liver

In a study by Eyer & Kampffmeyer (1978), nearly all the 4-DMAP was conjugated in livers perfused with 4-DMAP (0.01 mmol/l) (0.4 nmol/mg protein per min).

8.8.1.2 Red cell metabolism of 4-DMAP

4-DMAP rapidly catalyses methaemoglobin formation. The reaction mechanism has been studied using purified human haemoglobin. 4-DMAP transfers electrons catalytically from ferrohaemoglobin to oxygen (Eyer et al., 1974; Eckert & Eyer, 1983) and is thereby oxidized to the phenoxy radical and N,N-dimethylquinoneimine (Eyer & Lengfelder, 1984). Both species are reduced to ferrohaemoglobin with the formation of methaemoglobin.

Catalytic methaemoglobin formation is terminated by rapid binding of oxidized 4-DMAP to reactive SH groups in haemoglobin and thioether formation with reduced glutathione. Formation of glutathione-S-conjugates with 4-DMAP has been shown to occur in vitro in red cells (Eyer & Kiese, 1976), and in vivo in dogs (Eyer & Gaber, 1978) and man (Jansco et al., 1981, Klimmek et al., 1983). Kinetic studies of thioether formation by DMAP in the presence of purified dog haemoglobin and glutathione in a reducing system showed that $S,S$-(2-dimethylamino-5-hydroxy-1,3-phenylene)-bis-glutathione (di(GS)-DMAP) is formed initially. This thioether is highly autoxidizable and the addition of another thiol gives stable $S,S,S$(2-dimethylamino-5-hydroxy-1,3,4-phenylene)-tri-glutathione (tris-(GS)-DMAP). Despite its chemical stability, it can be actively transported across the red blood cell membrane (Eckert & Eyer, 1986).

4-DMAP is excreted in the urine mainly as the tris-cysteinyl derivative, tris(Cys)-DMAP. This compound is presumed to be broken down in the glutamyl cycle of the kidney (Meister, 1973), giving rise to cysteinyl glycine and cysteinyl derivatives.

8.8.1.3 Toxic effects of 4-DMAP on erythrocytes

Studies have been undertaken to test the effect of 4-DMAP on the osmotic resistance of human erythrocytes. The percentage haemolysis did not differ from that of controls, even for an incubation period of less than 20 h, at a 4-DMAP concentration of 1 mmol/l. However, the degree of haemolysis after 20 h was 14.5% (±6.9%), compared with a control value of 2.7% (±0.4%), when...
measured in 0.7% NaCl solution (Klimmek et al., 1983). Since the haemolysis that occurred in the tests for osmotic resistance was maximal after the same period as in *in vivo* studies, one may assume a common lytic mechanism under *in vitro* and *in vivo* conditions (Klimmek et al., 1983).

8.8.1.4 Toxic effects of 4-DMAP on isolated rat kidney tubules

Various toxic effects were observed when 4-DMAP was added to suspensions of isolated rat kidney tubules, apparently as the result of the irreversible binding of 4-DMAP to SH-containing compounds, e.g., multiple enzyme inhibition, inhibition of gluconeogenesis, decrease in glutathione, and membrane damage (Snizicz et al., 1979). The results suggest that irreversible impairment of the integrity of the cell membrane causes irreversible damage to tubular epithelial cells, the resultant increase in permeability being followed by a decrease in nucleotide content without marked effect on gluconeogenesis or mitochondrial membrane integrity.

8.8.1.5 Oxygen saturation and methaemoglobin formation

From reports by Kiese (1974) and Eyer et al. (1979), it is evident that quite small variations of \( \text{pO}_2 \) can cause large differences in the rate of methaemoglobin formation. At high percentage oxyhaemoglobin levels, where the R-configuration of haemoglobin prevails over the T-configuration, the rate of oxidation is lower. At a 4-DMAP concentration of 0.032 mmol/l, increasing the percentage saturation of haemoglobin of humans more than halved the rate of methaemoglobin production (Marrs et al., 1982).

8.8.2 Pharmacokinetics

No data are available.

8.8.3 Pharmacodynamics

4-DMAP (3.25 mg/kg), given intravenously to dogs one min after poisoning with a lethal dose of KCN (4 mg/kg), resulted in the survival of all dogs. The maximum venous methaemoglobin content was reached within 5-10 min and was 38.8% (±1.7%) of the total haemoglobin. The injection of the same 4-DMAP dose intramuscularly led to a maximum methaemoglobin level of 41.6% (±1.3%) after 30 min. A methaemoglobin concentration of 35% was achieved within 5 min of an intravenous dose of 3.25 mg/kg, 15 min of an intramuscular dose of 3.25 mg/kg, and 30 min of an oral dose of 15 mg/kg (Klimmek et al., 1983).

Bright & Marrs (1982) found that there was considerable variation in the methaemoglobin levels produced in different dogs using the same oral dose of 4-DMAP.

In experiments using different aminophenols, it was shown that 4-DMAP given intravenously rapidly produced consistent amounts of methaemoglobin in the blood of different animals species. In mice and rabbits, a rapid fall in the concentration of methaemoglobin was observed after 2 min and after 20 min the concentration in these species was less than 10%. In dogs and mice, however, the decrease in methaemoglobin concentration was substantially less; after
20 min, the level was still above 30%. In contrast to 4-DMAP, sodium nitrite caused increased methaemoglobin levels which lasted longer than an hour in mice and rabbits (Kiese & Weger, 1969).

There was a marked increase in arterial pO$_2$ after the administration of 4-DMAP to dogs poisoned with cyanide, compared with non-poisoned dogs. Presumably, part of the oxygen released during the formation of methaemoglobin under hypoxic conditions can be utilized by tissues (Klimmek et al., 1979a,b). In the same experiments, an increase in respiratory volume and mean arterial blood pressure, was observed together with a lessening of the lactate-pyruvate ratio.

8.8.4 Toxicology

LD$_{50}$ intravenous mouse: 50-70 mg/kg (Kiese & Weger, 1965; Marrs et al., 1984)

LD$_{50}$ oral mouse: 946 mg/kg (Marrs et al., 1984)

LD$_{50}$ intravenous rat: 57 mg/kg (Kiese et al., 1975)

LD$_{50}$ oral rat: 689-780 mg/kg (Marrs et al., 1984)

LD$_{50}$ intravenous dog: 26 mg/kg (Weger, 1975)

In all five studies, the cause of death was inadequate oxygen transport by the blood to the tissues because 85% of the haemoglobin had been converted to methaemoglobin.

8.8.4.1 Nephrotoxicity

Some of the aminophenols, such as 4-aminophenol, produce kidney lesions after intravenous administrations (Green et al., 1969; Calder et al., 1971; Hinsberg & Treupel, 1984). The intravenous injection of 100 mg 4-DMAP/kg, a dose nearly double the LD$_{50}$ and which oxidizes 60% of the haemoglobin to methaemoglobin, caused renal damage in rats. Moderate to severe necrosis of the convoluted tubules, with either few or no inflammatory cells, was found 24 h after injection. The glomeruli were not affected and there was no papillary damage. However, no tubular necrosis was detected with a dose of 30 mg/kg. A high or low sodium diet did not noticeably affect the nephrotoxicity of 4-DMAP (Kiese et al., 1975).

A single oral dose of up to 25 mg 4-DMAP/kg did not result in any macroscopic or histological abnormality in the gastrointestinal tract, liver, or kidneys of rats killed up to 24 h after dosing. In animals that had been kept for 7 days after dosing, no histological abnormalities were found other than engorgement of the liver and kidneys (Marrs et al., 1982).

8.8.4.2 Mutagenicity

A clear relationship between dose and mutation frequency was shown by a mammalian cell assay employing V79 (Chinese hamster) cells (Lee & Webber, 1983), despite a negative Ames test with and without metabolic activation.

8.9 Volunteer Studies
In experiments by Weger (1968) on seven volunteers, an intravenous dose of \( N,N\)-dimethyl-\( p\)-aminophenol (3.25 mg/kg) resulted in a 15% methaemoglobin concentration within 1 min. After 10 min, the concentration was 30%. Other aminophenols tested gave less favourable results. \( N\)-methyl-\( p\)-aminophenol had much the same effect but only at an iv dose of 20 mg/kg. \( O\)-aminophenol (30 mg/kg) administered intravenously resulted in 15% methaemoglobinaemia after 12 min and 30% after 60 min. Comparison was also made with sodium nitrite. When a dose of 4 mg/kg was given intravenously to seven volunteers, the maximal methaemoglobin level was 6%. This dose of sodium nitrite was that recommended by Chen et al. (1933) and Chen & Rose (1952) and suggests that the antidotal effect may be due to a mechanism other than methaemoglobin formation.

In experiments by Klimmek et al. (1983) on volunteers a methaemoglobin concentration of about 30% was reached within 5 min after an intravenous dose of 3.25 mg 4-DMAP/kg (3 volunteers), 50 min after an intramuscular dose of 3.5 mg 4-DMAP/kg (6 volunteers), and 30 min after an oral dose of 900 mg 4-DMAP (5 volunteers).

8.9.1 Metabolism of 4-DMAP in the liver

The metabolic conversion of 4-DMAP to glucuronide, sulfate, and thioether derivatives is likely to follow linear Michaelis-Menten kinetics. The pattern of metabolites was studied in urine collected over 24 hours from subjects treated with intravenous 4-DMAP (3.25 mg/kg). An average of 68% of the dose was excreted as metabolites in urine: 41% as glucuronide, 12% as sulfate, and 15% as thioethers (Klimmek et al., 1983).

4-DMAP rapidly auto-oxidizes at pH values above 7 and is transformed to a variety of degradation products (Eyer et al., 1974). Thus partial degradation of 4-DMAP in the gastrointestinal tract may account for the reduced total metabolite recovery following oral administration compared with that following intravenous administration.

8.9.2 Metabolism of 4-DMAP in erythrocytes

Rapid thioether formation following the intravenous administration of 4-DMAP has been shown to occur in erythrocytes where 4-DMAP is oxidized by oxyhaemoglobin to the corresponding quinoneimine which yields adducts with red cell glutathione (Eyer & Kiese, 1976). One of these, tris-(GS)-DMAP penetrates the red cell membrane slowly and is excreted in the urine mainly as a tris-cysteinyl derivative, tris-(Cys)-DMAP.

The 4-DMAP thioethers excreted in human urine (15.3% (±1.8%) of the dose) are likely to originate in the erythrocytes; this underlines the importance of erythrocytes in 4-DMAP metabolism in man.

8.9.3 Adverse effects

After 6-7 days, a phlebitis was observed in the antecubital vein where 4-DMAP was infused (Klimmek et al., 1983). Following an intramuscular injection of 4-DMAP, a slight pressure was felt after
5-10 min at the site of injection, slowly growing in intensity and finally resulting in severe pain. Approximately 10 h after the injection, shivering, sweating, and fever occurred. An oral 4-DMAP dose of 300 mg was well tolerated, whereas 600 mg slowed mental activity and caused tiredness, and 900 mg gave an effect of dizziness, with headache and buzzing in the ears (Klimmek et al., 1983).

After an intravenous injection of 4-DMAP (3.25 mg/kg), the total bilirubin concentration increased by 140%, that of conjugated bilirubin by 180% and that of iron by 200%. Within 24 h of an intramuscular injection of 4-DMAP (3.5 mg/kg), the total bilirubin increased by 270% and then declined rapidly, while the bilirubin concentration rose by 120% and that of iron by 50% (Klimmek et al., 1983).

After an oral dose of 900 mg 4-DMAP, total and conjugated bilirubin levels increased by 170% and 80%, respectively, and the iron concentration rose by 60%. The changes in bilirubin and iron concentrations were the result of reduced erythrocyte survival caused by 4-DMAP, which took about one day to develop. Since the haemolysis that occurred in the tests for osmotic resistance was maximal after the same period as in in vivo studies, one may assume a common lytic mechanism both under in vitro and in vivo conditions (Klimmek et al., 1983).

8.10 Clinical Studies

No data are available.

8.11 Clinical Studies - Case Reports

Table 6 summarizes clinical observations made in 19 cases of cyanide poisoning treated by 4-DMAP. In most patients, there was a long time interval between the occurrence of poisoning and the administration of 4-DMAP. Patients with impaired vital function at the time of treatment recovered. In six cases an overdose of 4-DMAP was given, resulting in excessive methaemoglobinaemia, and in three cases haemolysis was observed on the second day after administration of the antidote. Even the recommended dose of 3.25 mg 4-DMAP/kg has resulted in a methaemoglobin concentration of about 70% (van Dijk et al., 1987).

8.12 Summary of Evaluation

8.12.1 Indications

4-DMAP should only be given to patients poisoned with cyanide who are in a deep coma and who have dilated non-reactive pupils and deteriorating cardio-respiratory function.

8.12.2 Recommended routes and dosage

An intravenous dose of 3.25 mg/kg body weight should be given immediately. The intramuscular injection of 4-DMAP to patients in shock due to cyanide poisoning cannot be recommended because absorption is unpredictable.

8.12.3 Other consequential or supportive therapy
The administration of 4-DMAP should always be followed by that of sodium thiosulfate (150-200 mg/kg intravenously) (see chapter 3). Artificial respiration should be given if necessary.

Table 6. Clinical Observations in cyanide-poisoned patients treated by 4-DMAP

<table>
<thead>
<tr>
<th>Estimated cyanide ingested</th>
<th>Interval before first-aid treatment</th>
<th>Blood cyanide concentration (mg/l)</th>
<th>Interval before blood measurement used</th>
<th>4-DMAP dose, interval before administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 g oral</td>
<td>15 min</td>
<td>?</td>
<td>?</td>
<td>250 mg, 45 min</td>
</tr>
<tr>
<td>?</td>
<td>&lt; 5 min</td>
<td>5-16.9</td>
<td>5 min</td>
<td>250 mg, 5 min</td>
</tr>
<tr>
<td>?</td>
<td>1.5-2 h</td>
<td>c</td>
<td>5.5 h</td>
<td>250 mg, &gt; 5h</td>
</tr>
<tr>
<td>?</td>
<td>20 min</td>
<td>NR</td>
<td>NR</td>
<td>250 mg, 3.5h</td>
</tr>
<tr>
<td>?</td>
<td>&lt; 1h</td>
<td>1.9</td>
<td>&gt; 24 h</td>
<td>250 mg, &lt; 1 h</td>
</tr>
<tr>
<td>?</td>
<td>&lt;1 h</td>
<td>ND</td>
<td>27 h</td>
<td>2 x 250 mg, 1 h and 27 h</td>
</tr>
<tr>
<td>50-150 mg?</td>
<td>&lt; 1 h</td>
<td>1.7</td>
<td>20 min</td>
<td>250 mg, 20 min</td>
</tr>
<tr>
<td>?</td>
<td>&lt;1 h</td>
<td>9.2</td>
<td>measurement at autopsy</td>
<td>?, &lt; 20 min</td>
</tr>
<tr>
<td>10 g</td>
<td>2 h</td>
<td>d</td>
<td>NR</td>
<td>250 mg, &gt; 2h</td>
</tr>
<tr>
<td>?</td>
<td>20 min</td>
<td>d</td>
<td>NR</td>
<td>750 mg, 20 min</td>
</tr>
<tr>
<td>320 mg</td>
<td>8 min</td>
<td>0.24</td>
<td>&gt; 1 h</td>
<td>2 x 250 mg, 10 min and 1 h</td>
</tr>
</tbody>
</table>

Table 6. Contd.

<table>
<thead>
<tr>
<th>Estimated cyanide ingested</th>
<th>Interval before first-aid treatment</th>
<th>Blood cyanide concentration (mg/l)</th>
<th>Interval before blood measurement used</th>
<th>4-DMAP dose, interval before administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>?</td>
<td>10 min</td>
<td>70</td>
<td>30 min</td>
<td>2 x 250 mg, 10 min and 30 min</td>
</tr>
<tr>
<td>?</td>
<td>20 min</td>
<td>24</td>
<td>1.5 h</td>
<td>250 mg, 1.5;</td>
</tr>
<tr>
<td>?</td>
<td>?</td>
<td>37</td>
<td>&lt; 1 h</td>
<td>1250 mg</td>
</tr>
<tr>
<td>1 g</td>
<td>1 h</td>
<td>6</td>
<td>&gt; 1 h</td>
<td>500 mg, 1 h</td>
</tr>
</tbody>
</table>
8.12.4 Areas of use where there is insufficient information to make recommendations

There is insufficient evidence regarding the efficacy of the prophylactic use of oral 4-DMAP, e.g., with rescue personnel.

8.13 Model Information Sheet

8.13.1 Uses

4-DMAP is indicated for the treatment of severe cyanide poisoning in patients who are in deep coma and who have dilated non-reactive pupils and deteriorating cardio-respiratory function.

8.13.2 Dosage and route

An intravenous dose of 3.25 mg/kg body weight should be given.

8.13.3 Precautions/contraindications

There are differences in individual susceptibility, which may result in an unacceptably high level of methaemoglobin after normal therapeutic doses (van Dijk et al., 1987). Excess methaemoglobinemia may be corrected with either methylene or toluidine blue, but this will result in the release of cyanide; exchange transfusion is an alternative approach if this is practicable. Determination of methaemoglobin will not allow the amount of haemoglobin available for oxygen transport to be calculated, because the cyanmethaemoglobin concentration will be unknown and, at the present time, cannot be analysed.

Treatment with 4-DMAP is contraindicated in patients with G6PD deficiency.

8.13.4 Adverse effects

Haemolysis is observed following an overdose. Mild headache, dizziness, hyperventilation, cyanosis, and green/brown discolouration of the urine have also been observed. Haemolysis may occur even in people with normal erythrocytes given an appropriate therapeutic dose.
8.13.5 Use in pregnancy and lactation

No information is available.

8.13.6 Storage

4-DMAP must be stored in opaque containers. The maximum storage time is 3 years.

8.14 References


Marrs TC, Scawin J, & Swanson DW (1984) The acute intravenous and oral toxicity in mice, rats and guinea pigs of 4-DMP and its effects on haematological variables. Toxicology, 31: 165-173.


Von Clarmann M (1987) 4-dimethylaminophenol. Communication at the joint meeting of the EAPCC, CEC, IPCS and the National Poison Control Centre in the Netherlands, Utrecht.

Weger N (1968) [Aminophenols as antidotes to prussic acid.] Arch Toxikol, 24: 49-50 (in German).


9. Methylene Blue and Toluidine Blue

9.1 Methylene Blue

9.1.1 Introduction

Methylene blue is a redox dye that has been used in clinical medicine for approximately 100 years. Its present uses are based on its tissue-staining properties and its oxidative-reductive capacity (Bodansky & Gutman, 1947; Blass & Fung, 1976; Metz et al., 1976). Administered locally it has been used for the detection of fistulae and for the recognition of ruptured amniotic membranes in obstetrics (Sparhr & Salisbury, 1980; Martindale, 1989; Windholz, 1983). It may also be administered systemically to assist the detection of endocrine tissue, e.g., on parathyroid glands or pancreatic adenoma during an operation (Blass & Fung, 1976; Whitman et al., 1979; Martindale, 1989).

Methylene blue has long been recognized as an effective antidote for methaemoglobinaemia in man and in domestic animals (Etteldorf, 1951; Beutler & Baluda, 1963; Burrows et al., 1977; Gosselin et al., 1984). Injected systemically in low doses, its reducing action is utilized in the treatment of toxic methaemoglobinaemia.

Methylene blue, however, is relatively ineffective against toxic methaemoglobinaemia in individuals with glucose-6-phosphate dehydrogenase deficiency and in chlorate poisoning (Bodansky & Gutman, 1947; Metz et al., 1976; Gosselin et al., 1984).

9.1.2 Name and chemical formula of antidote

3,7-bis(dimethylamino)-phenazothionium chloride trihydrate, tetramethylthionine chloride trihydrate

Synonyms: Methylioninii chloridum, Methylenum caerulum, Azul de Metileno, Swiss blue, Blu di metilene, Schultz No. 1038, CI Classic Blue 9.

Colour index no: 52015
Molecular formula: $C_{16}H_{18}CIN_3S_3H_2O$ (Martindale, 1989; Windholz, 1983)

Relative molecular mass: 373.9

CAS number: 7220-79-3 (trihydrate) and 67-73-4 (anhydrous)

Commercial methylene blue is the double chloride of tetramethylthionine and zinc and is not suitable for medicinal use (Windholz, 1983).

9.1.3 Physico-chemical properties

Methylene blue is a dark green, almost odourless, hygroscopic crystalline powder with a bronze-like lustre. It loses 8-18% of its weight on drying. One gram dissolves in about 25 ml of water, in about 65 ml alcohol, and in 450 ml of chloroform. Methylene blue is insoluble in ether (Martindale, 1989; Windholz, 1983).

A 1% solution in water has a pH of 3 to 4.5. Solutions in water are intensely blue-coloured and incompatible with caustic alkalis, oxidising and reducing substances and iodides (Martindale, 1989).

Solutions are sterilised by autoclaving or by filtration and should be stored in airtight containers (Windholz, 1983).

Data on melting and boiling points and on stability in light are not available.

Excipients: water for injection.

9.1.4 Synthesis

First prepared by Caro in 1876, methylene blue is produced by the so-called "thiosulfate process" in which a mixture of dimethyl- $p$-phenylenediamine and dimethylaniline is oxidized, usually with potassium dichromate, in the presence of sodium thiosulfate and zinc chloride (Windholz, 1983).

9.1.5 Analytical methods

No information on analytical methods for methylene blue is available.

9.1.6 Shelf-life

No data about specific conditions of temperature and humidity are available, and the shelf-life is unknown. Methylene blue should be stored in airtight containers.

9.1.7 General properties
Methylene blue functions as an intermediate in the transfer of electrons from pyridine nucleotides to a suitable electron acceptor, thereby stimulating the hexose monophosphate shunt (HMPS) pathway in a variety of cell systems (Bodansky & Gutman, 1947; Beutler & Baluda, 1963; Smith & Olson, 1973; Metz et al., 1976).

In the erythrocyte, methylene blue is reduced to leucomethylene blue primarily by NADPH-dependent diaphorase (dihydrolipoamide dehydrogenase). This diaphorase is reduced via oxidation of NADPH, which in turn stimulates the HMPS, and leucomethylene blue transfers electrons to an acceptor such as methaemoglobin (Bodansky & Gutman, 1947; Metz et al., 1976). This series of reactions can be used clinically for the reduction of ferric to ferrous haem iron in patients with acquired methaemoglobinaemia. The effectiveness of methylene blue in reducing methaemoglobin is well established (Etteldorf, 1951; Beutler & Baluda, 1963; Smith & Olson, 1973; Harrison, 1977; Gosselin et al., 1984).

9.1.8 Animal studies

Several animal studies confirm that the administration of methylene blue protects against death caused by methaemoglobin-generating agents (Blass & Fung, 1976; Burrows et al., 1977; Hrushesky et al., 1985). Burrows et al. (1977) administered sodium nitrite (50 mg/kg) intravenously to four ewes. This resulted in the formation of 70–80% methaemoglobinaemia (a lethal level) within 45 min. The mortality associated with this dose of sodium nitrite was successfully antagonized by the administration of intravenous methylene blue (2.2 mg/kg) 30 min later.

Pharmacological studies have been carried out in dogs and rats (Blass & Fung, 1976). When intravenous boluses as large as 15 mg/kg were given, the resultant data could be explained on the basis of a one-compartment model with binding of methylene blue to tissues.

Few data on mutagenicity or teratogenicity testing are available.

9.1.9 Volunteer studies

No data on volunteer studies are available.

9.1.10 Clinical studies

The clinical symptoms and signs associated with methaemoglobinaemia depend on the percentage of haemoglobin oxidized to the methaemoglobin form (Bodansky & Gutman, 1947). They consist of greyish-blue cyanosis, without signs of cardiac or pulmonary distress, which becomes apparent at about 15% methaemoglobinaemia. Hypoxaemia may be accompanied by dyspnoea, dizziness, headache, weakness, lethargy, and CNS depression, the severity of which will increase with increasing concentrations of methaemoglobin.

An intense chocolate-brown-coloured blood and a central cyanosis that does not respond to the administration of 100% oxygen suggest methaemoglobinaemia (Smith & Olson, 1973; Gosselin et al., 1984; Hall et al., 1986). Methaemoglobin levels may be directly
measured using a spectrophotometric method (Hall et al., 1986).

Many authors agree that in asymptomatic patients, with methaemoglobin levels of 30% or less, methylene blue administration is not necessary (Bodansky & Gutman, 1947; Gosselin et al., 1984; Hall et al., 1986).

9.1.11 Clinical studies - case reports

Ng et al. (1982) described a case of methaemoglobinaemia and haemolysis resulting from the ingestion of paraquat by a 32-year-old patient. He was administered methylene blue (1 mg/kg) intravenously, with dramatic results: cyanosis was reversed and the patient became alert and cooperative. The methaemoglobin level was 19.7% before methylene blue was given and 1.6% after.

A single case of dapsone-induced methaemoglobinaemia was treated by continuous methylene blue infusion (Berlin et al., 1985). The patient recovered completely.

9.1.12 Summary of evaluation

9.1.12.1 Indications

Methylene blue is the agent of choice for accelerating the reduction of methaemoglobin, at toxic levels, induced by aniline and aminophenols, nitrites and nitrates, nitrobenzene, antimalarial agents (chloroquine, primaquine), dapsone, local anaesthetics (lidocaine, prilocaine, benzocaine), phenacetin, phenazopyridin, and sulfamidines. It should be used with special caution in the correction of excess methaemoglobinaemia induced by the treatment of cyanide poisoning with nitrites.

9.1.12.2 Advised route and dosage

Methylene blue should be administered as an intravenous dose of 1-2 mg/kg body weight (0.1 to 0.2 ml/kg of a 1% solution), slowly over 5 to 10 min. An improvement should be noted within 30-60 min of administration. If cyanosis has not disappeared within 1 h, a second dose should be given (Bodansky & Gutman, 1947; Etteldorf, 1951; Smith & Olson, 1973; Harrison, 1977; Hall et al., 1986).

9.1.12.3 Precautions and contraindications

Methylene blue should not be given by subcutaneous or intrathecal injection because it causes necrotic abscess formation (Whitman et al., 1979; Windholz, 1983; Gosselin et al., 1984). It should be administered with caution in patients with severe renal impairment (Windholz, 1983; Martindale, 1989; Gosselin et al., 1984).

In patients with glucose-6-phosphate dehydrogenase deficiency (who do not generate NADPH), methylene blue is likely to be ineffective and has been reported to cause haemolytic anaemia (Beutler & Baluda, 1963; Rosen et al., 1971; Whitman, et al., 1979; Martindale, 1989).

Methylene blue should not be used in chlorate-induced methaemoglobinaemia because it may enhance the toxicity of the chlorate salts (Metz et al., 1976; Martindale, 1989).
9.1.12.4 Adverse effects

Nausea, abdominal and chest pain, headache, dizziness, mental confusion and profuse sweating may occur following large intravenous doses of methylene blue (Naidler et al., 1945; Martindale, 1989; Windholz, 1983).

Whitman et al. (1979) described a 28-year-old patient who received methylene blue (5 mg/kg) for the identification of an insulin-secreting pancreatic adenoma. During surgery, the patient developed methaemoglobin levels of up to 7.1%.

Sparhr & Salisbury (1980) reported a case where an intra-amniotic injection of 10 mg of methylene blue was given in a pregnant woman into the vitelline sac of one of two twins. On the second day, the new-born baby developed a methaemoglobin level of 20%. Physical examination revealed a bluish-green skin colour and respiratory distress and Heinz bodies were seen in the peripheral blood. Other reports have stressed the neonatal morbidity related to the use of the dye in obstetrics (Vincer et al., 1987).

Blass & Fung (1976) reported the case of a 4-year-old boy who was given 1 g methylene blue intravenously during surgery. He developed hypotension, tachycardia, and deep cyanosis and remained intensely blue for several days.

Methylene blue may impart a blue-green colour to urine and faeces (Martindale, 1989; Windholz, 1983).

9.1.12.5 Other consequential or supportive theory

See chapter 1.

9.1.13 Model information sheet

9.1.13.1 Uses

Methylene blue is used for the treatment of drug-induced, and some forms of idiopathic, methaemoglobinemia. It should be used with great caution in the correction of nitrite-induced methaemoglobinemia arising from urgent treatment of cyanide poisoning.

It is also used as a dye for the identification of fistulae and glandular tissues.

9.1.13.2 Dosage and route of administration

A dose of 1-2 mg/kg (0.1-0.2 ml/kg of a 1% solution) should be administered intravenously over 5-10 min.

9.1.13.3 Precautions and contraindications

Methylene blue may be administered when methaemoglobin levels resulting from nitrite administration are more than 30-40%. It has no value in the treatment of cyanide poisoning.

Regeneration of haemoglobin from methaemoglobin will release cyanide back into the circulation.
9.1.13.4 Adverse effects

Large intravenous doses of methylene blue produce nausea, abdominal and chest-pain, headache, dizziness, mental confusion, and profuse sweating. Intravascular haemolysis, which may be life-threatening, can also occur.

9.1.13.5 Use in pregnancy/lactation

Hyperbilirubinaemia and haemolysis may be seen in the neonate.

9.1.13.6 Storage

Methylene blue should be stored in airtight containers.

9.1.14 References


9.2 Toluidine Blue

9.2.1 Introduction

Toluidine blue is one of a group of dyes that can be used to treat methaemoglobinemia. One such situation is where a methaemoglobin-producing cyanide antidote has produced dangerously high methaemoglobin levels. Additionally, toluidine blue has a number of uses such as in vivo staining, which are not relevant to the present monograph (Chobanian et al., 1987). It has also been suggested as an antagonist to heparin (Deichmann & Gerarde, 1969).

9.2.2 Name and chemical formula of antidote

The Chemical Abstracts name of toluidine blue is phenothiazin-5-ium-3-amino-7-(dimethylamino)-2-methyl chloride. The Chemical Abstracts registration number is 92-31-9. Synonyms include tolonium chloride and toluidinblau. The compound is listed in the Colour Index as Basic Blue 17, chemical constitution number 52040 (Society of Dyers and Colourists, 1979), the supplier of the dye being given as BASF, Ludwigshafen and Rhein, Germany.

9.2.3 Physico-chemical properties

The only supplier of the pharmaceutical preparation is Dr Franz Kohler, Chemie GmbH, Neue Bergstrasse 3-7, Postfach 17, D-6146 Alsbach-Hahlein 1, Germany. It is supplied in packs containing 5 or 25 ampoules.
The 10-ml ampoules contain toluidine blue at a concentration of 40 mg/ml, i.e. 0.4 g/ampoule. No further information is available on the constitution of the material.

The relative molecular mass is 305.85.

Toluidine blue has the following structure:

![Toluidine blue structure](image)

9.2.4 Synthesis

No data are available.

9.2.5 Analysis

9.2.5.1 Analysis of methaemoglobin

Methaemoglobin may be estimated by the spectrophotometric method of Evelyn & Malloy (1938) or using the IL 282 CO-oximeter (Instrumentation Laboratories in UK, Ltd., Warrington, Cheshire, United Kingdom.) It is very important to note that neither method gives meaningful results in the presence of cyanide.

Toluidine blue may interfere with the estimation of methaemoglobin by some methods (Smith, 1971) (see chapter 10).

9.2.6 Stability

No information on the stability of toluidine blue is available.

9.2.7 General properties

The mode of action of toluidine blue is similar to that of methylene blue; it catalyses the transfer of electrons from the pentose-phosphate pathway to methaemoglobin via NADPH-methaemoglobin reductase (Kiese & Waller, 1951; Sass et al., 1969; Kiese et al., 1972). It is likely that in individuals with glucose-6-dehydrogenase deficiency or NADPH-methaemoglobin-reductase deficiency the dyes would not be efficacious in reducing methaemoglobin (Brewer & Tarlov, 1961; Sass et al., 1967). Furthermore, certainly in vitro, and possibly in vivo, toluidine blue will produce methaemoglobin from previously non-oxidized blood (Kiese, 1945).

9.2.8 Animal studies

9.2.8.1 Pharmacokinetics

No data are available.

9.2.8.2 Pharmacodynamics
A number of studies have shown toluidine blue to be superior to methylene blue in reducing methaemoglobin (Kiese, 1945; Friehoff & Lobermann, 1952, 1953; Kiese et al., 1972). Kiese et al. (1972) found that methaemoglobin produced by the intravenous injection of dogs with 4-dimethylaminophenol was reduced 2-3 times more rapidly by toluidine blue than by methylene blue. Burrows (1979) found that toluidine blue given intravenously to sheep at a dose of 1.1 mg/kg reduced methaemoglobin produced by sodium nitrite, although the rate of reduction could be increased by the use of higher doses.

9.2.8.3 Toxicology

The intravenous LD$_{50}$ values in mice, rats, and rabbits are, respectively, 27.56, 28.93, and 13.44 mg/kg. Toxic signs observed include increased respiration rate, convulsions, partial heart block, and death due to respiratory and cardiac failure. Weekly intravenous injections into rabbits at a dose one tenth the LD$_{50}$ produced no change in haematological parameters, and histological examination of a number of organs after 90 days revealed no abnormality (Stolarsky & Haley, 1951).

Some early work on *Drosophila melanogaster* suggested that toluidine blue might possess mutagenic properties (Landa et al., 1965). Toluidine blue was reported by Au & Hsu (1979) to produce extensive chromosome damage.

9.2.9 Volunteer studies

Kiese et al. (1972) reported that, in human volunteers, toluidine blue reduced 4-dimethylaminophenol-induced methaemoglobin about twice as rapidly as did methylene blue. No adverse effects were reported with toluidine blue.

9.2.10 Clinical studies

No data are available.

9.2.11 Clinical studies - case reports

Few case reports exist in the literature and some of these relate to the use of toluidine blue as a vital stain. A notable case report of its use in toxic methaemoglobinemia is that of Büttner et al. (1967). Side-effects reported include nausea, vomiting and leucocytosis (Deichmann & Gerarde, 1969).

9.2.12 Summary of evaluations

Toluidine blue has been recommended in a number of studies as being preferable to methylene blue on the grounds of superior efficacy (Dauneder, 1979, 1980; Marrs & Ballantyne, 1987). Compared to methylene blue, there is little clinical experience and this is confirmed by the paucity of case reports concerning the use of toluidine blue. Furthermore, there is a lack of information concerning the nature of the material and its storage characteristics.

9.2.13 Model information sheet
9.2.13.1 Indications

Toluidine blue is indicated in methaemoglobinemia, including toxic methaemoglobinemia; mild cases of methaemoglobinemia, though, would not normally require specific therapy. Toluidine blue is not indicated in cyanosis due to any other cause. It would not be effective in sulfhaemoglobinemia and, on theoretical grounds, would not be expected to be effective in the treatment of methaemoglobinemia in individuals with glucose-6-phosphate dehydrogenase deficiency.

9.2.13.2 Side effects

Owing to the blue colour of the material, cyanosis may occur, as may staining of the urine. Vomiting may occur following an overdose.

9.2.13.3 Advised route and dose

The compound should be given intravenously at a dose of 2-4 mg/kg. This may be repeated after 30 min if the clinical response has been inadequate. It is important to give the material intravenously.

9.2.13.4 Use in pregnancy and children

No information is available and caution is advised in administering toluidine blue to a pregnant woman because of mutagenicity findings. Nevertheless, in life-threatening methaemoglobinemia, it would seem inadvisable to withhold treatment.

9.2.13.5 Storage

No information is available at present.

9.2.14 References


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Kiese M (1945) [Reduction of haemoglobin. IV.] Naunyn-Schmiedebergs Arch Exp Pathol Pharmakol, 204: 288-312 (in German).

Kiese M & Waller HD (1951) [Reduction of haemoglobin and oxygen for reversibly reducible dyes in red cells.] Naunyn-Schmiedebergs Arch Pharmakol, 213: 44 (in German).


10. Analytical Methods for Cyanide Alone and in Combination with Cyanide Antidotes in Blood

It is important to be sure of the diagnosis in every case of poisoning, and this is particularly true of cyanide intoxication where treatment with antidotes is often potentially hazardous. Fast qualitative and quantitative methods for analysing cyanide in blood are therefore necessary. The methods detailed below are approved techniques for the detection and/or quantification of cyanide before the administration of cyanide antidotes.

Measurement of whole blood free cyanide concentrations after the administration of cyanide antidotes is not yet possible.

10.1 Qualitative Methods

10.1.1 Detection in blood with a detector tube (Bedside Test)

The time required, assuming that instrumentation and chemicals are readily available, is 2-3 min.

10.1.1.1 Principle

Hydrocyanic acid is liberated from blood by acidification and is then passed through a detector tube using a gas detector pump. If hydrocyanic acid is present, the reactive zone of the tube changes its colour from yellow to red as a result of the following reaction:

\[
\text{HgCl}_2 \quad (1) \quad \text{HCN} \quad \rightarrow \quad \text{HCl} \\
\text{HCl} + \text{Methyl red} \quad \rightarrow \quad \text{red reaction product} \quad (2)
\]

A semiquantitative determination of the HCN concentration can be made by means of a scale on the tube (M. von Clarmann, personal communication, 1988).

10.1.1.2 Materials

Gas detector pump (Dräger a) (Fig. 5)
Detector tube "Hydrocyanic acid 2/a" (Dräger a) (Fig. 6)
Test tube height 6 cm, width 2 cm (Fig. 6)
Stopper with two drill-holes (Fig. 5)
10% sulfuric acid
specimen of venous blood, with or without an anticoagulant

\^ Drägerwerk AG, 2400 Lübeck Germany
10.1.1.3 Procedure

Read carefully the operating instructions for the gas detector pump and the detector tube.

Insert the detector tube into the free drill-hole of the stopper and put the gas detector pump on the detector tube.

Place 1 ml blood and 1 ml 10% sulfuric acid into the test tube and close it immediately with the stopper prepared as described.
above. Shake the test tube cautiously, avoid splashing by all means.

Compress the pump balk while closing the free end of the glass tube with a finger. Then let the glass tube open while the balk of the pump extends. Repeat the pumping 15 times.

Attention!

Take care not to suck some of the acid contents of the test tube into the detector tube. If this happens, a false positive result will be the consequence.

Judge the colour of the indicating layer of the detector tube immediately after the pumping. If hydrocyanic acid is present, the indicating layer turns becomes red. A very rough semiquantitative determination of the HCN concentration can be made by means of the scale on the tube. The detection limit in this test is 1 mg CN⁻/l.

10.1.1.4 Specificity

The analysis is based on the reaction of hydrocyanic acid with mercury salts: the hydrochloric acid thereby produced is determined by methyl red. Specificity is ensured by the precleaning layer. Acidic gases (HCl or SO₂) have no influence on the HCN reading, even if present in considerable excess. This also applies to hydrogen sulfide.

Sodium azide gives a positive reaction.

10.1.2 Spot test

The time required, assuming that instrumentation and reagents are readily available, is 2-3 min.

10.1.2.1 Principle

Hydrocyanic acid (HCN) is liberated from biological fluids by acidification. The evolved HCN is passed through a filter paper impregnated with an alkaline solution of palladium dimethylglyoxime in which the palladium is a constituent of an inner-sphere complex anion. Cyanide ions lead to a demasking of dimethylglyoxime, which reacts with nickel (II) to produce red nickel-dimethylglyoxime (Jakobs, 1984).

10.1.2.2 Equipment

Gas washing flask shown in Fig. 7 (measurements of the flask: height 15 cm; width 3 cm).
Water bath
Centrifuge
Disposable syringes containing anticoagulant (e.g., K-EDTA Monovetten R, Sarstedt Numbrecht, Germany)
Pipettes

10.1.2.3 Chemicals

Ethanol
Palladium(II) chloride
10.1.2.4. Reagents

50% Sulfuric acid
Hydrochloric acid (0.1 mol/l)
Potassium hydroxide (3 mol/l)

Alkali palladium dimethylglyoxime solution:

(a) Dissolve 50 mg palladium(II) chloride in 2.5 ml
hydrochloric acid (0.1 mol/l) by heating it in a water bath for a little while.

(b) Dissolve 100 mg dimethylglyoxime in 10 ml ethanol.

(c) Add the palladium(II)chloride solution drop by drop to the ethanolic solution of dimethylglyoxime.

(d) Centrifuge at 4000 g.

(e) Wash the residue with 10 ml distilled water and centrifuge. Repeat this procedure three times.

(f) Dissolve the residue in 10 ml KOH (3mol/l) by heating it in water bath for a little while.

(g) Allow to cool, centrifuge at 4000 g if necessary.

Nickel chloride solution (0.25 mol/l) saturated with ammonium chloride.ᵃ

Dissolve 4 g ammonium chloride and 0.6 g NiCl₂.⁶H₂O in 10 ml water.

All reagents are stable at 4 °C for at least one year.

10.1.2.5 Specimen collection

Blood is collected with disposable syringes which contain EDTA as anticoagulant. If the syringes are not disposable, EDTA disodium salt is added to a final concentration of 1 mg/ml blood and mixed well. Other anticoagulants can be used as well.

10.1.2.6 Procedure

Filter paper is impregnated with the alkaline solution of palladium dimethylglyoxime or a drop of this solution is placed on filter paper.

ᵃ The presence of ammonium salts prevents the precipitation of green Ni(OH)₂

Into a gas washing flask (Fig. 7), 5 ml blood, 3-5 drops of the antifoaming agent, and 5 drops of 50% sulfuric acid are placed.

The moist reagent paper is held on to the free opening of the glass tube. Air is then blown through the flask, using the rubber bag for 2 min. The part of the paper that was exposed to the stream of gas is then spotted with the nickel ammonium chloride solution. If HCN is present, a pink to red stain of nickel dimethylglyoxime appears at once, the depth of the colour varying with the cyanide content of the gas. The detection limit in this test is 1 mg CN⁻/l.

10.1.2.7 Specificity

Bright yellow inner-sphere complex palladium dimethylglyoxime
(I) readily dissolves in caustic alkali to give a yellow solution of alkaline palladium dimethylglyoxime (II) in which the palladium is a constituent of an inner-sphere complex anion. The inner-sphere complex bound dimethylglyoxime is masked in solutions of II, i.e. no red Ni-dimethylglyoxime precipitate appears when Ni\(^{2+}\) ions are added to the solution. Likewise, the palladium is masked against practically all reagents that are normally characteristic for Pd\(^{2+}\) ions. Cyanide ions present an exception. If added in excess to the yellow solutions of II, they cause immediate discharge of the colour, and addition of nickel salt solutions (containing NH\(_4\)Cl) brings down red Ni-dimethylglyoxime.

Since the Ni-dimethylglyoxime reaction is highly sensitive, cyanide ions can be detected through their demasking effect. (Feigl & Feigl, 1949; Feigl et al., 1966). The reaction is specific to HCN.

10.2 Quantitative Methods

10.2.1 Gas chromatographic head space technique

This procedure, described by Eben & Lewalter (1988), has been slightly modified from that developed by McAuley & Reive (1983). The gas chromatographic determination of hydrogen cyanide may be carried out simply and rapidly with the head-space technique described here. The use of a thermionic detector that is specific for nitrogen makes it possible to obtain a sensitivity 10 times greater than with a flame ionization detector.

The method described is highly accurate because practically no sample treatment is required. This is confirmed by the precision data presented here. Another advantage over alternative analytical techniques is that gas chromatographic head space analysis is an established part of the repertoire of most medical toxicological laboratories.

10.2.1.1 Principle

Hydrogen cyanide gas is liberated by adding acid to cyanide-containing blood and is then determined by means of gas chromatography using head-space analysis. Fractionation may be carried out on a packed capillary column. The cyanide is determined using a thermionic nitrogen detector.

Calibration standards, which are mixed with whole blood, are used for quantification. To evaluate the data, a calibration curve is used in which peak height or peak area is plotted as a function of the cyanide concentration in blood.

10.2.1.2 Equipment

* Gas chromatograph with thermionic nitrogen detector, and chart recorder or integrator, if necessary with a capillary injector
* Packed glass column: length, 2 m; inner diameter, 2.2 mm
* Column packing: Porapak Q 100-120 mesh
* Alternatively, a Duran (borosilicate) glass or quartz
capillary (length, 60 m; inner diameter, 0.3 mm) may be used

* Stationary phase: SE 30 (Chrompack)\(^a\), chemically bonded, film thickness 0.3 µm

* Gas-tight syringes (50 or 500 µl) for gas chromatography

* Bottles (5 ml) with serum caps fitted with PTFE-coated septa, together with tools for sealing and opening

* Automatic pipettes, variable between 20 and 200 µl

* Syringe (100 µl) for gas chromatography

* Vortex mixer

* Centrifuge

* Transfer pipettes (1, 2, 4, and 10 ml)

* Volumetric flasks (10, 20, 50, and 100 ml)

* Disposable syringes containing anticoagulant (e.g., K-EDTA Monovetten\(^R\), Sarstedt, Nümbrecht, Germany)

\(^a\) Chompack, P.O. Box 8033, 4330

10.2.1.3 Chemicals

* Potassium cyanide, pa

* Sodium cyanide, pa

* Glacial acetic acid, pa

* Helium (99.999% purity) as carrier gas

* Hydrogen (99.90% purity)

* Synthetic air (80% purified nitrogen, 20% oxygen)

10.2.1.4 Solutions

* Physiological saline (9 g sodium chloride/l)

* Pooled stabilized whole blood or stabilized bovine blood for the calibration standards

10.2.1.5 Calibration standards

(a) Starting solution

12.6 mg potassium cyanide (equivalent to 5.3 mg cyanide) is weighed out exactly and transferred to a 50 ml volumetric flask, which is then filled to the mark with whole blood (106 mg cyanide/l blood).

(b) Stock solution
Starting solution (2 ml) is pipetted into a 20 ml volumetric flask, which is then filled to the mark with whole blood (10.6 mg cyanide/l blood). From this solution, calibration standards within the concentration range 0.2 - 4.2 mg cyanide/l blood are prepared by dilution with whole blood as shown in the following table:

<table>
<thead>
<tr>
<th>Volume of stock solution ml</th>
<th>Final volume of calibration standard ml</th>
<th>Cyanide concentration of calibration standard mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>10</td>
<td>4.24</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>2.12</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>1.06</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
<td>0.53</td>
</tr>
<tr>
<td>1</td>
<td>50</td>
<td>0.212</td>
</tr>
</tbody>
</table>

The blood volume required for preparing the calibration standards may be reduced to about 10 ml if the standards are prepared directly in the bottles used for sample treatment. In this case the procedure is as follows:

Stock solution A

Exactly 24.0 mg potassium cyanide (equivalent to 10 mg cyanide) is transferred to a 100 ml volumetric flask, which is then filled to the mark with physiological saline (100 mg cyanide/l).

Stock solution B

10 ml stock solution A is pipetted into a 100 ml volumetric flask, which is then filled to the mark with physiological saline (10 mg cyanide/l).

The calibration standards are prepared directly in the bottles used for the sample treatment. Blood (1 ml) is pipetted into each bottle and after adding the cyanide solution, the bottle is sealed immediately. The difference in the volumes of the individual standards does not reduce the reliability of the calibration for the head-space analysis used here.

<table>
<thead>
<tr>
<th>Volume of stock solution A µl</th>
<th>Volume of stock solution B µl</th>
<th>Cyanide concentration of calibration standard mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>20</td>
<td>0.2</td>
</tr>
<tr>
<td>-</td>
<td>40</td>
<td>0.4</td>
</tr>
<tr>
<td>-</td>
<td>50</td>
<td>0.5</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>1.0</td>
</tr>
<tr>
<td>20</td>
<td>-</td>
<td>2.0</td>
</tr>
<tr>
<td>30</td>
<td>-</td>
<td>3.0</td>
</tr>
<tr>
<td>50</td>
<td>-</td>
<td>5.0</td>
</tr>
</tbody>
</table>
10.2.1.6 Specimen collection and sample preparation

Blood is collected in disposable syringes containing an anticoagulant. Immediately after collection, 1 ml of whole blood is pipetted into a 5-ml bottle, which is sealed at once with a cap and PTFE-coated septum.

Using a 100-µl syringe, 50 µl glacial acetic acid is injected through the septum into the bottle to release the hydrogen cyanide. The contents of the bottle are shaken for 30 seconds on a vortex mixer and then centrifuged for 3 min at 3000 rpm. The bottle is then incubated for 30 min at room temperature.

The blood samples containing cyanide should be processed within 30 min of collection, as losses can otherwise occur.

10.2.1.7 Operational parameters for gas chromatography

(a) *Packed column*

<table>
<thead>
<tr>
<th>Column:</th>
<th>Material:</th>
<th>Glass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length:</td>
<td>2 m</td>
<td></td>
</tr>
<tr>
<td>Inner diameter:</td>
<td>2.2 mm</td>
<td></td>
</tr>
</tbody>
</table>

Stationary phase: Porapak Q 100-120 mesh

Detector: Thermoionic nitrogen detector

Temperatures: 
- Column: 120 °C
- Injector: 220 °C
- Detector: 300 °C

Carrier gas: Helium: 30 ml/min

Detector gases: 
- Hydrogen: 3 ml/min
- Synthetic air: 60 ml/min

Injected head-space 50 µl

Under these conditions, hydrogen cyanide has a retention time of 1.5 min.

(b) *Capillary column*

<table>
<thead>
<tr>
<th>Capillary column:</th>
<th>Material:</th>
<th>Quartz or Duran glass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length:</td>
<td>50 m</td>
<td></td>
</tr>
<tr>
<td>Inner diameter:</td>
<td>0.3 mm</td>
<td></td>
</tr>
</tbody>
</table>

Stationary phase: SE 30 (chrompack) chemically bonded, film thickness 0.3 mm

Detector: Thermionic specific detector (TSD)

Temperatures: 
- Column: 80 °C
- Injector: 200 °C
- Detector: 300 °C
- Split: 1:4
Carrier gas: Helium, 1.68 MPa; make up gas, 35 ml/min
Detector gases: Hydrogen: 1.45 MPa (3.5-4.0 ml/min)
Synthetic air: 160 ml/min

Injected head-space 400 µl

Under these conditions, the retention time for hydrogen cyanide is 3.1 min.

10.2.1.8 Analytical determination

50 µl (packed column) or 400 µl (capillary column) of the head space above the prepared blood sample is injected into the gas chromatograph under the conditions given in section 10.2.1.7.

10.2.1.9 Calibration

The calibration standards containing cyanide in blood are prepared as described in section 10.2.1.5 and analysed. The calibration curve is obtained by plotting the peak heights or areas for the standards against the blood cyanide concentrations used (see Figure 8). The linearity of the calibration curve has been tested up to 6 mg/l.

Fig. 8. Example of a calibration curve for the determination of cyanide in blood by means of gas chromatographic head-space analysis (from Eben & Lewalter, 1988)
10.2.1.10 Calculation of the analytical result

From the peak height or area for the hydrogen cyanide in the sample, the corresponding cyanide concentration in whole blood (mg/l) is read off the calibration curve (see Figure 8).

10.2.1.11 Reliability of the method

The within-series precision and between-day precision were determined using a packed or a capillary column and blood samples with a defined cyanide content that were prepared as described in section 10.2.1.5. The individual values are given in Tables 7 and 8.

Table 7. Within-series precision for the gas chromatographic determination of cyanide in blood using a packed column (from Eben & Lewalter, 1988)

<table>
<thead>
<tr>
<th>n</th>
<th>Cyanide in blood</th>
<th>Sw</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/l</td>
<td>%</td>
</tr>
<tr>
<td>10</td>
<td>0.54</td>
<td>7.5</td>
</tr>
<tr>
<td>10</td>
<td>2.20</td>
<td>4.6</td>
</tr>
</tbody>
</table>

n = number of analysis performed  
Sw = relative standard deviation derived from replicate analyses of the same specimen

Table 8. Within-series precision and between-day precision for the gas chromatographic determination of cyanide in blood using a capillary column (from Eben & Lewalter, 1988)

<table>
<thead>
<tr>
<th>Precision</th>
<th>n</th>
<th>Cyanide in blood</th>
<th>s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(mg/l)</td>
<td>%</td>
</tr>
<tr>
<td>Within-series</td>
<td>17</td>
<td>0.19</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>0.39</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>2.11</td>
<td>0.7</td>
</tr>
<tr>
<td>Between-day</td>
<td>17</td>
<td>0.19</td>
<td>14.5</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>0.39</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>2.11</td>
<td>6.7</td>
</tr>
</tbody>
</table>

n = number of analyses performed  
s = relative standard deviation

Recovery experiments were carried out using spiked blood samples. This procedure is the same as that used to prepare the calibration curve and is thus of limited value. The results serve only as an internal laboratory check. Recovery rates of r = 84-107%

http://www.intox.org/databank/documents/antidote/antidote/ant02.htm 08/14/2003
were obtained for cyanide concentrations between 0.19 and 2.11 mg/l using the capillary column.

Losses during sample treatment of between 25 and 40% were revealed by comparison with a calibration curve established using treated aqueous cyanide solution and the capillary column.

10.2.1.12 Detection limit

The concentration of cyanide that can be determined by this method, using a packed gas chromatography column, is 0.07 mg/l. If a capillary column is used, the detection limit is raised to 0.1 mg cyanide/l blood, because with control blood a peak appears in the position for hydrogen cyanide.

10.2.1.13 Specificity

The separation of the cyanide from interfering blood constituents and its detection with the thermionic nitrogen detector makes this procedure highly specific for cyanide. With the packed column no interference was observed with ethanol, diethyl ether, methanol, acetone, ethyl acetate, n-hexane, diisopropyl ether or 2-propanol (1 mg per analytical sample).

Blood or plasma samples from people who had not been exposed to hydrogen cyanide or other cyanides yielded no peak in the position for hydrogen cyanide when a packed column was used. With the capillary column described here a peak was also observed for blood samples from people not occupationally exposed to cyanides. The peak, however, indicated a cyanide concentration below the detection limit of 0.1 mg/l.

10.2.2 Microdiffusion technique

Microdiffusion analysis is admirably suited to the detection and estimation of small quantities of volatile substances like HCN. Conway (1950) pioneered the application of this method of analysis in the determination of such substances as ammonia, alcohol, carbon dioxide, volatile amines, and carbon monoxide. The Conway diffusion dish has become a standard laboratory item.

Feldstein & Klendshoj (1954) adapted the technique to the estimation of cyanide in biological materials.

10.2.2.1 Principle

Hydrocyanic acid is set free from the sample (blood, serum, urine, stomach contents) by the addition of sulfuric acid and is then absorbed into sodium hydroxide. The cyanide concentration is measured colorimetrically in an aliquot of the alkaline solution (Asmus & Garschagen, 1953).

10.2.2.2 Equipment

* Spectrophotometer (Vis)
* Conway microdiffusion cells (Fig. 9)
* Volumetric flask 50 ml
* Test tubes
* Pipettes

A = Cell
B = Cover

11 cm

**Fig. 9. Lips-Conway microdiffusion cell and cover**

* Culture dishes, Lips-Conway type, Code no. 911313009, Glaswerke Wertheim, Germany

10.2.2.3 Chemicals

* Potassium cyanide
* Hydrochloric acid (concentrated; 35-37%)
* Sulfuric acid (concentrated; 96-98%)
* Sodium hydroxide
* Sodium phosphate, monobasic (NaH$_2$PO$_4$.2H$_2$O)
* Chloramine T (N-chloro- p-toluenesulfonamide sodium salt)
trihydrate)

* Barbituric acid (should be stored in a dissicator)
* Pyridine (should be stored in the dark)
* Silicone grease or other sealing agent

10.2.2.4 Solvents and reagents

* Sodium phosphate (1 mol/l)
* Sulfuric acid (10%)
* Sodium hydroxide (0.1 mol/l)
* Chloramine T (0.25%) (must be prepared fresh daily)

_Pyridine-barbituric acid reagent_

Barbituric acid (3 g), 15 ml pyridine, and 3 ml concentrated hydrochloric acid are placed in a 50-ml volumetric flask. The solution is mixed to dissolve the reagents, made up to 50 ml with water, and filtered. This solution must be prepared fresh each time it is used.

10.2.2.5 Calibration standards

Standard solutions of sodium cyanide containing 0.1 to 2.0 µg of cyanide per ml of sodium hydroxide (0.1 mol/l) are prepared.

10.2.2.6 Specimen

Venous blood containing heparin or EDTA as anticoagulants, serum, plasma, urine, or stomach content (or tissue slurry) may serve as specimens. The samples should be analysed as soon as possible.

10.2.2.7 Procedure

(a) Microdiffusion

A sample (2-4 ml) of the specimen is placed in the outer compartment of the microdiffusion cell, and 3.3 ml of the sodium hydroxide solution (0.1 mol/l) is pipetted into the centre well of the unit. The ground-glass cover of the unit is smeared with silicone grease or other sealing agent and is placed on the unit so that a small portion of the outer compartment remains uncovered. Then 3-4 drops of 10% sulfuric acid are pipetted into the outer compartment, and the lid is quickly moved into place to cover the entire cell with an airtight seal. The unit is then gently tilted and rotated to mix the fluids in the outer compartment and allowed to stand for 3 h at 20-25 °C for diffusion to be completed. At the end of that time the lid is removed and an aliquot of the liquid in the centre well is taken for analysis.

(b) Quantitative determination
1.0 ml of the absorbing solution in the centre well is placed into each of three test tubes graduated at 10 ml. A blank consisting of 1.0 ml of sodium hydroxide (0.1 mol/l) in a test tube is prepared. A 1-ml sample of a calibration solution is used as a positive control.

To the blank, the control sample, and the unknown, 2.0 ml of sodium phosphate solution (1 mol/l) and 1.0 ml of chloramine T solution are added. After mixing and allowing to stand for 2-3 min., 3.0 ml of the pyridine-barbituric acid reagent is added. The contents of the tubes are again mixed and allowed to stand for 10 min. The presence of a red colour indicates the presence of cyanide. The optical density is determined in a spectrophotometer at 580 nm with the blank set at zero density.

(c) Calibration curve and calculation

With aliquots of the standard solutions of sodium cyanide containing 0.1 to 2.0 µg of cyanide/ml of sodium hydroxide (1 mol/l), the colour is developed as described above. A calibration curve relating optical density and concentration is obtained and the cyanide content of the material being analysed is read from the curve.

10.2.2.8 Reliability of the method

For cyanide concentrations from 0.04 µg/ml to 0.38 µg/ml, the within-run precision was ± 2.75% for blood and ± 5.56% for urine.

The recovery of cyanide added to biological samples is 96-102%.

10.2.2.9 Detection limit

The detection limit depends on the amount of the analysed sample and of the NaOH (0.1 mol/l) for the absorption of the released hydrocyanic acid. This limit can be lowered by reducing the volume of sodium hydroxide in the centre well of the Conway unit to 1.0 ml.

With 2 ml blood and 3.3 ml NaOH (0.1 mol/l), the detection limit is 0.2 µg CN⁻ /ml blood.

10.2.2.10 Specificity

The separation of the cyanide from interfering blood constituents by microdiffusion makes this procedure very specific. Hydrogen sulfide and sulfur dioxide may interfere.

10.3 References


