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Mechanism of Cyanide Toxicity and Efficacy of its Antidotes

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ABSTRACT

This paper attempts to review the various antidotes available for countering cyanide threat in the light of the toxicity associated with it. It also critically evaluates the drawbacks and advantages of these antidotes for their therapeutic and/or prophylactic utility. The physico-chemical properties of hydrogen cyanide which make it a chemical warfare agent have also been highlighted. In an attempt to make the complex chemical and biological processes understandable, the chemical structures of the antidotes have been included and simple mechanistic pathways have been used to show the role of antidotes in activating the inhibited enzymes.

1. INTRODUCTION

Hydrogen cyanide, discovered by a Swedish chemist C.W. Scheele in 1782, has been recognised as one of the most rapid poisons. Cyanide toxicity has long been a problem in livestock grazing range containing cyanogenic plants[1]. The toxicity of these plants is associated with specific glycosides which contain a cyanide moiety. The worldwide industrial development has become a major threat so far as cyanide is concerned. Cyanogenic materials are produced in electroplating, steel, aluminium, paints and petrochemical industries[2]. The major problem, however, comes from the intentional ingestion of cyanide for suicidal and homicidal purposes. The high toxicity associated with hydrogen cyanide (*HCN*) has made it a useful chemical warfare (CW) agent. The French were the first to use it in the Battle of Somme in July 1916 and since then it has been used as a CW agent. A very recent example of its use is by Iraq in her conflict with Iran.

2. PHYSICO-CHEMICAL PROPERTIES

The physico-chemical properties of *HCN* make it a useful CW agent. Pure anhydrous *HCN* is a colourless liquid with a peculiar odour of bitter almond. The coloured *HCN* is considered to be less dangerous because it is in the process of alteration. In the gaseous state it is colourless with a vapour density lower than air. Some of the salient physico-chemical properties of *HCN* have been listed in Table 1.

Because of its high vapour pressure and low density it is difficult to maintain a high concentration of *HCN* in an open place. To make it more persistent, numerous

Table 1. Physico-chemical properties of hydrogen cyanide

Molecular weight	27
Boiling point	26.5°C
Freezing point	-13.4°C
Vapour density	0.948
Specific gravity	0.7058 (7 °C) 0.6969 (18 °C)
Critical temperature	138.5 C
Critical pressure	53.5 atm
Heat of vaporisation	210.7 cal/g
Volatility at 20 °C	873,000 mg/m^3
Solubility at 20 °C	
(a) Organic solvents	Alcohol, ether, glycerol, chloroform, benzene.
(b) Water	Dissolves to give acidic solution.

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artifices have been used during the war. *HCN* is miscible in all proportions with alcohol, ether, glycerol, chloroform, benzene, etc. It dissolves in water to form a weak acidic solution. Even in the anhydrous conditions, *HCN* cannot be kept for long as it gradually decomposes, occasionally with explosive force. The decomposition may be prevented or retarded by the addition of small amounts of mineral acids.

3. CYANIDE INTOXICATION

The toxic effect of cyanide is attributed to a decrease in the tissue utilisation of oxygen due to inhibition of cytochrome oxidase[3-5], an enzyme which occupies a critical position in cellular metabolism. The cyanide poisoning very frequently is a massive poisoning, where the amount of cyanide greatly exceeds the concentration necessary to inhibit cytochrome oxidase. In such intoxication many other enzymes and biological systems are also inhibited[6]. Cyanide toxicity may therefore be considered as a complex effect on various enzyme systems. The lethality of cyanide may be judged from the values[7] given in Table 2.

The mechanism of cyanide inhibition of cytochrome oxidase is a complicated process and has been studied extensively[8-10]. The binding of cytochrome oxidase, a multimeric iron enzyme complex, with cyanide has been found to involve a two-step reaction. The first step is the penetration of cyanide into a protein crevice, with initial binding of cyanide to protein while the second step is the binding of cyanide to heme iron[10]. Though the cytochrome oxidase-cyanide complex is quite stable, in the presence of reducing equivalents, cyanide can readily dissociate from the complex, reactivating the cytochrome oxidase[6]. From the structure of cytochrome oxidase and other enzymes inhibited by cyanide, it has been demonstrated that the basis for the inhibiting properties may be attributed to its ability to complex with metals. Another mechanism for cyanide inhibition of certain enzymes involves a chemical reaction between cyanide and a Schiff base intermediate, forming cyanohydrin[11,12].

4. SYMPTOMS OF CYANIDE INTOXICATION

One of the first symptoms of cyanide poisoning is a deep and rapid hyperventilation caused by stimulation of the carotid sinus[13]. Cyanide in low concentrations causes headache, nausea and vomiting and then,

Table 2. Lethal concentrations of cyanide

A. Acute toxicity by intravenous (i.v.), intramuscular (i.m.) and intraperitoneal (i.p.) routes

Species, Sex	Compound	Route	LD_{50} in mg/kg (95 % confidence limits)
Rabbit, F	*HCN*	i.v.	0.59 (0.55-0.65)
	HCN	i.m.	0.95 (0.81-1.11)
	HCN	i.p.	1.95 (1.60-2.00)
	NaCN	i.v.	1.23 (1.11-1.34)
	NaCN	i.m.	1.67 (1.51-1.84)
	NaCN	i.p.	2.79 (2.48-3.09)
	KCN	i.v.	1.89 (1.66-2.13)
	KCN	i.m.	3.27 (2.70-4.08)
	KCN	i.p.	3.99 (3.40-4.60)
Mouse, F	*HCN*	i.p.	2.80 (2.70-2.90)
Rat, F	*HCN*	i.p.	2.23 (1.93-2.59)
Guinea Pig, F	*HCN*	i.p.	2.64 (2.38-2.96)

B. Acute inhalation toxicity for HCN vapour

Species, Sex	Exposure Time	Median Lethal Toxicity as LC_{50} in mg/m_3 (95 % confidence limits)
Rabbit, F	45 s	2432 (2304-2532)
	5 min	409 (321-458)
	35 min	208 (154-276)
Rat, F	1 min	1129 (664-1471)
	5 min	493 (372-661)
	30 min	173 (159-193)

depending on the dose, cramps with opisthotonos and finally respiratory arrest. After inhalation, oral or percutaneous absorption of cyanide, the cell respiration is inhibited and the oxygen, therefore, cannot react with the hydrogen to form water in the cells and remains in the venous blood. This is why people poisoned with cyanide exhibit red face and red skin which is one of the most apparent symptoms of cyanide poisoning. The concentration of hydrogen emerging from the metabolic pathways causes an acidosis in the cell[14].

In the pharmacokinetic studies on cyanide poisoning with ^{14}C-labelled cyanide, the concentration of cyanide in the blood increased relatively slowly as compared to the plasma, where the concentration reached 40 μM/ml in 3 min. This concentration is sufficient for the

respiratory arrest. If, however, the concentration of cyanide in plasma falls below this level, respiration occurs spontaneously without any artificial respiration. On the other hand, if the concentration of cyanide in plasma reached 70μ M/ml, the heart beat ceases about 5 min after the respiratory arrest[15].

5. ESSENTIALS OF DIAGNOSIS

Cyanide poisoning is notoriously difficult to diagnose without a history of exposure[16-17]. The presence of an odour of oil of bitter almond in respiration and the appearance of red face and skin are the most apparent diagnostic symptoms. The final diagnosis for the oral consumption of cyanide comes by performing the Lee Jones test in gastric aspirate, the green-blue colouration of ferricyanide confirms the presence of cyanide[18].

6. ACTION MECHANISMS OF TREATMENT APPROACHES

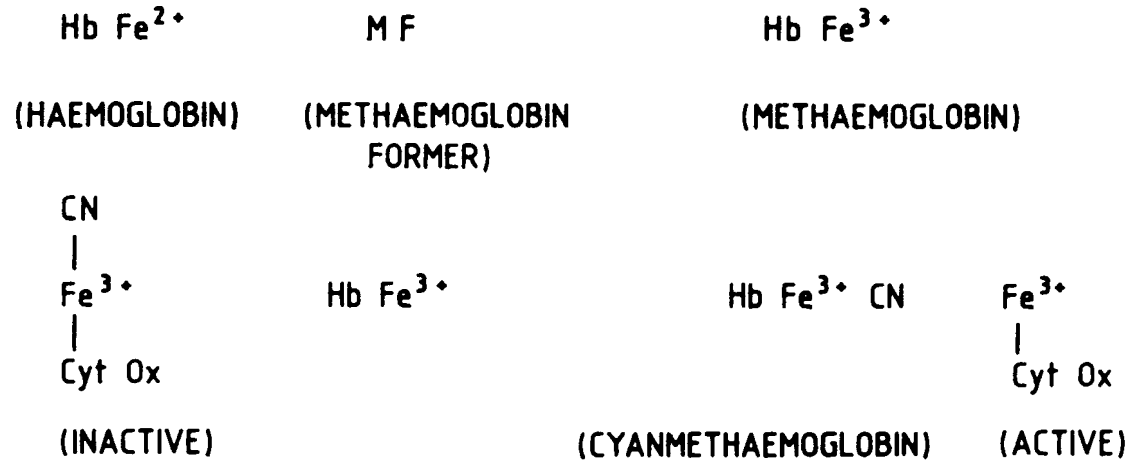
Once diagnosed, a variety of approaches have been proposed for the treatment of cyanide poisoning. These approaches may be classified under three main heads depending upon their mechanisms of action.

6.1 Methaemoglobin Formers

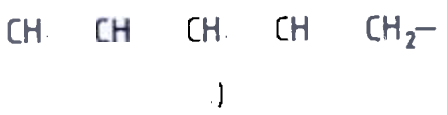
The main objective in the treatment of cyanide poisoning by methaemoglobin formers is to produce a high concentration of methaemoglobin (Hb-Fe^{3+}). Methaemoglobin competes with cytochrome oxidase (Cytox-Fe^{3+}) for cyanide ion. The concentration gradient favours methaemoglobin and cyanmethaemoglobin is formed restoring the cytochrome oxidase[19] (Scheme 1).

The chemical structures of various cyanide antidotes are shown in Fig. 1. Amyl nitrite (I) was the first methaemoglobin former to be employed for antagonising cyanide intoxication[20], and sodium nitrite (II) was used[21] subsequently. The efficacy of amyl nitrite in cyanide treatment, however, has been since questioned[22]. The main limitation of using the nitrites in cyanide intoxication was the relatively slow rate of methaemoglobin formation[23,24]. The respiratory arrest and inhibition of cellular oxidation by cyanide, however, calls for a quick action of the antidotes. Subsequently, a series of investigations[24-26] led to the development of 4-dimethylaminophenol (DMAP) (III) as the agent of choice because of its rapid methaemoglobin formation. DMAP has since proved its superiority as an antidote in cyanide poisoning in dogs and humans[26,27]. DMAP is most effective after intravenous (i.v.) injection and forms substantial amount of methaemoglobin in < 1 min, thereby trapping cyanide within red cells[15,28]. Additionally, DMAP administered in the absence of cyanide produces no ill effects other than an unwanted methaemoglobinemia. It is because of this methaemoglobinemia that some workers have doubted its efficacy as a cyanide antidote[29]. Most of the workers have, however, accepted the superiority of DMAP as a therapeutic and prophylactic agent against cyanide poisoning[26,27].

Some workers still indicate the efficacy of sodium nitrite over other methaemoglobin formers and relate its superiority to the more prolonged



Scheme 1 Mechanism of detoxification with methaemoglobin formers.



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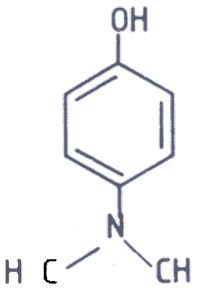

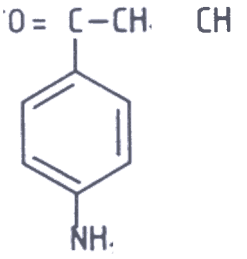

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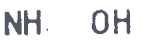

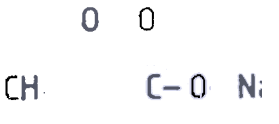

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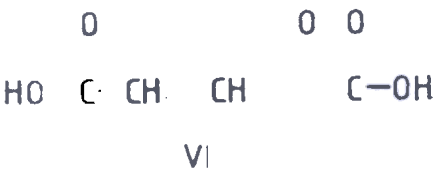

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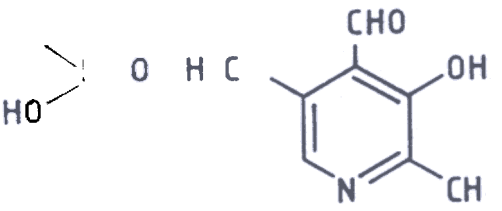

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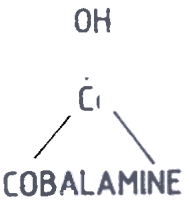

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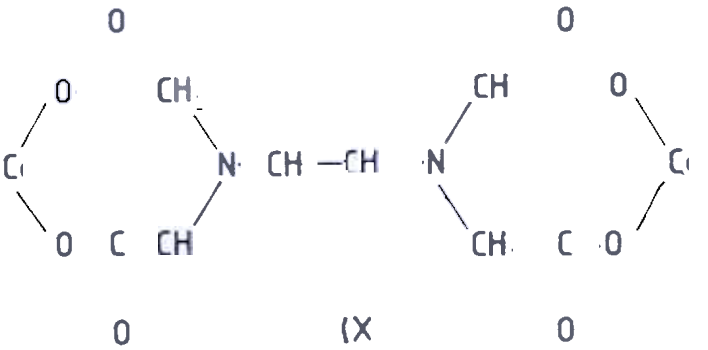


X

X

XI

Figure Chemical structures cyanide

methaemoglobinemia[30,31]. The mechanism for the enhanced protection of sodium nitrite over other methaemoglobin formers is that, in the more rapid methaemoglobin formers, the animals were able to survive the initial acute cyanide challenge; however, they subsequently succumbed to the cyanide released from the cyanmethaemoglobin pool. A protracted methaemoglobinemia is therefore presumed to offer sustained protection against cyanide poisoning. Hence, the efficacy of sustained methaemoglobinemia was studied as a result of co-administration of sodium nitrite (a slow methaemoglobin former)and DMAP (a rapid methaemoglobin former) in the ratio of 1:3. This regimen resulted in a persistent methaemoglobinemia with a corresponding sustained protection against cyanide over a prolonged period[32]. Moreover, *in vitro* results showed a prolonged persistence of methaemoglobinemia at higher levels in human erythrocytes as compared with those of the rats because of the low activity of methaemoglobin reductase in humans[33]. It may therefore be speculated that a more judicious dose of DMAP in combination with sodium nitrite be envisaged to offer a substantial prophylaxis against cyanide poisoning in humans.

The toxicity of DMAP is yet another factor which limits its clinical applications[34]. The co-administration of sodium nitrite with DMAP, however, takes care of the problems associated with the toxicity of DMAP also, as the regimen contains a very small amount of it which is just sufficient to counter the initial threat of cyanide poisoning.

Besides the nitrites and DMAP, other methaemoglobin formers are used in limited cases are *p*-aminopropiophenone (PAPP) (IV) and hydroxylamine (HA) (V in Fig. 1)[35,36].

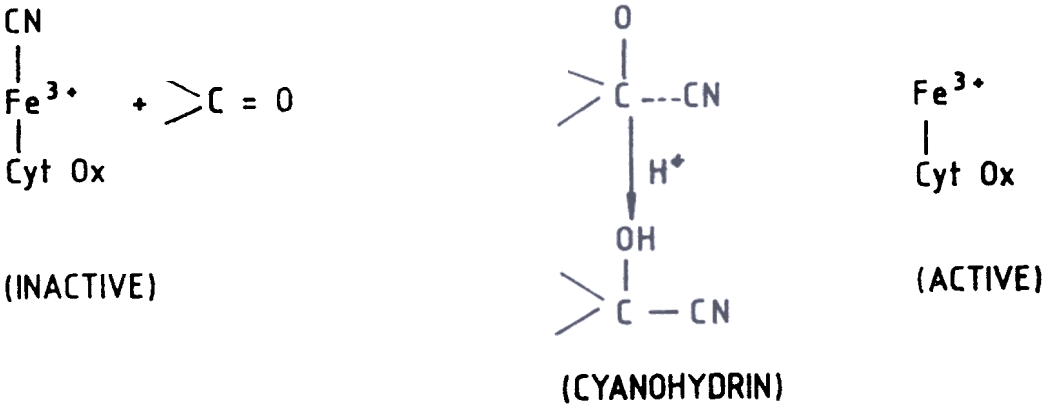
6.2 Cyanohydrin Formers

The second approach utilised in countering cyanide threat is based on the fact that cyanide being a nucleophile can interact readily with a carbonyl function resulting in the formation of cyanohydrin[37] (Scheme 2).

Sodium pyruvate (VI in Fig.1) was the first compound reported in this class to antagonise the lethal effects of cyanide in mice[38]. Sodium pyruvate has many theoretical advantages over the conventional cyanide antagonist, i.e., sodium nitrite. Its direct reaction with cyanide and its ability to distribute to the sites of cyanide localisation[39] makes it a good cyanide antagonist.

Another compound of this family which has been explored is α– ketoglutaric acid (α-KG) (VII in Fig. 1). This compound has been reported to be as effective as sodium nitrite and sodium thiosulphate in mice[40]. A detailed study on the mechanism of binding of α– KG to cyanide clearly revealed that the antagonising effect is due to the cyanohydrin formation which was confirmed by UV, GC and HPLC studies[41]. Further, its efficacy as cyanide antidote has been assessed by its ability to antagonise cyanide-induced inhibition of brain cytochrome oxidase[41].

There is only one report on the use of pyridoxal-5'-phosphate (PLP) (VIII in Fig. 1) for antagonising cyanide-induced toxicity[42]. PLP, the active co-factor from vitamin B_6, readily forms covalent complexes with cyanide, resulting in the formation of cyanohydrin and thereby prolonging the survival time dramatically. This extension of survival time in a human patient, would allow time to initiate supportive therapy, such as respiratory support, i.v. fluids and hemo- or peritonial dialysis[42].



Scheme 2. Mechanism of detoxification with cyanohydrin formers.

6.3 Cobalt Compounds

Cobalt ion is known to form a stable complex with cyanide and has been used in the treatment of cyanide poisoning[43,44], but its use could not receive general support mainly because of the toxicity of cobalt ions. However, since the discovery of the antidotal effects of hydroxycobalamine (IX in Fig. 1) in experimental cyanide poisoning[45], the interest in such compounds has been revived and has led to the testing of many salts and chelates of cobalt as antidotes to cyanide[46-50]. Of these, dicobaltethylenediaminetetracetic acid (Co_2EDTA) (X in Fig. 1) was found to be a more effective antidote than sodium nitrite[51]. This led to the use of Co_2 EDTA as the main compound in antidotal therapy for cyanide poisoning in many European countries. The selection of Co_2EDTA as the preferred cobalt compound is reasonable, since it was hoped that many of the toxic effects of cobalt ion would be minimised by administering its compounds as chelates.

It is generally assumed that most cobalt compounds react directly with free cyanide ion to trap it as a stable water soluble chelate or as an insoluble precipitate. Some cobalt compounds, however, may have a concomitant effect, namely, methaemoglobin formation. Cobaltous chloride, for example, is said to generate methaemoglobin in human blood by virtue of its ability to inhibit the methaemoglobin reductase activity[52].

Sodium cobaltinitrite (XI in Fig. 1) generates methaemoglobin through release of nitrite ions and it has therefore been suggested that the entire effect of cobaltinitrite is due to the methaemoglobin formation[48]. Others, however, insist that protection against cyanide induced by cobaltinitrite is more persistent than that by sodium nitrite because the cobalt moiety acts to prolong the methaemoglobinemia[53]. It has however, been proved that the mechanism of action of Co_2EDTA is by chelation to form cobalticyanide (Scheme 3).

Hydroxycobalamine detoxifies cyanide by giving up its hydroxyl group and binding a cyanyl group, forming cyanocobalamine (Scheme 4) which is excreted in the urine[45]. The cobalt compounds have a greater affinity for cyanide than cytochrome oxidase and thus free the enzyme to resume its role in cellular respiration[54].

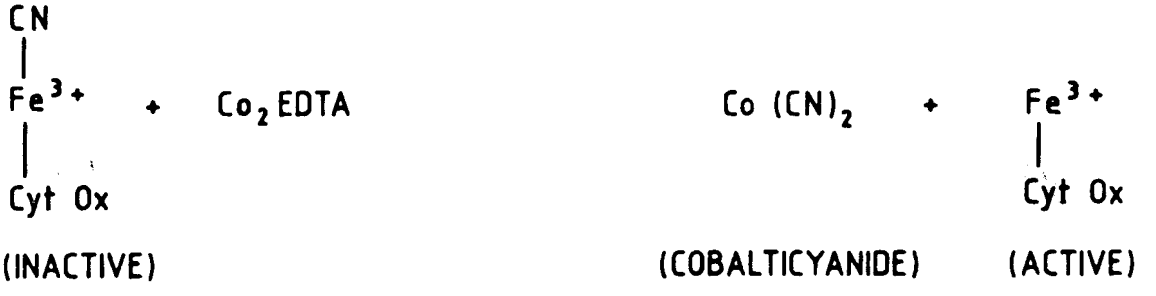
Co_2EDTA can cause hypertension, cardiac arrhythmia and cardiac insufficiency if administered in the absence of cyanide poisoning. It is perhaps in this context that some workers have recommended the use of Co_2EDTA for comatose patients who do not recover with classical therapy and for patients lapsing into unconsciousness despite treatment[51].

7. DETOXIFICATION OF CYANIDE: ROLE OF SULPHUR DONORS

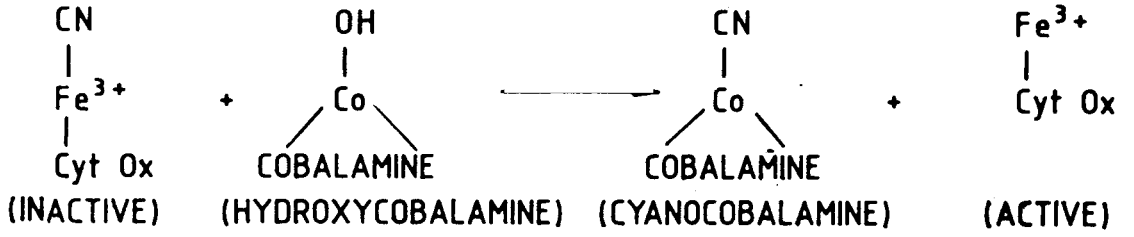
Sulphur donors have been employed alone or in combination with the above discussed antidotes to finally detoxify the cyanide and excrete it as thiocyanate. A variety of sulphur donors have been used to achieve this goal[55]. Of these, sodium thiosulphate (XII in Fig. 1) has been the drug of choice since a long time[56].

The mechanism by which thiosulphate detoxifies cyanide to thiocyanate has been proposed to involve an enzyme called rhodanase which is present in liver. Rhodanase is ideal from a toxicological viewpoint since the enzyme is present in large amounts[57], has a high turnover and catalyses the reaction of cyanide to form thiocyanate which is essentially irreversible[57,58]. The role of rhodanase and the mechanism of its action have been investigated by using crystalline rhodanase i.v. in combination with sulphur donors to antagonise cyanide[59].

The reaction mechanism for rhodanase was developed from the fact that rhodanase is inhibited by cyanide in the absence of thiosulphate which makes the assumption of a primary enzyme-cyanide complex. The inhibition data indicated that rhodanase contained an



Scheme 3. Mechanism of detoxification with CO_2 EDTA.



Scheme 4. Mechanism of detoxification with hydroxycobalamine.

active disulphide group and it was therefore assumed that thiosulphate reacts with the enzyme through this group, leading to the formation of a sulphenyl thiosulphate. This compound then decomposes in the presence of cyanide to give a sulphenyl thiocyanate which then rearranges to liberate thiocyanate and regenerates either the enzyme or sulphenyl thiosulphate complex (Scheme 5).

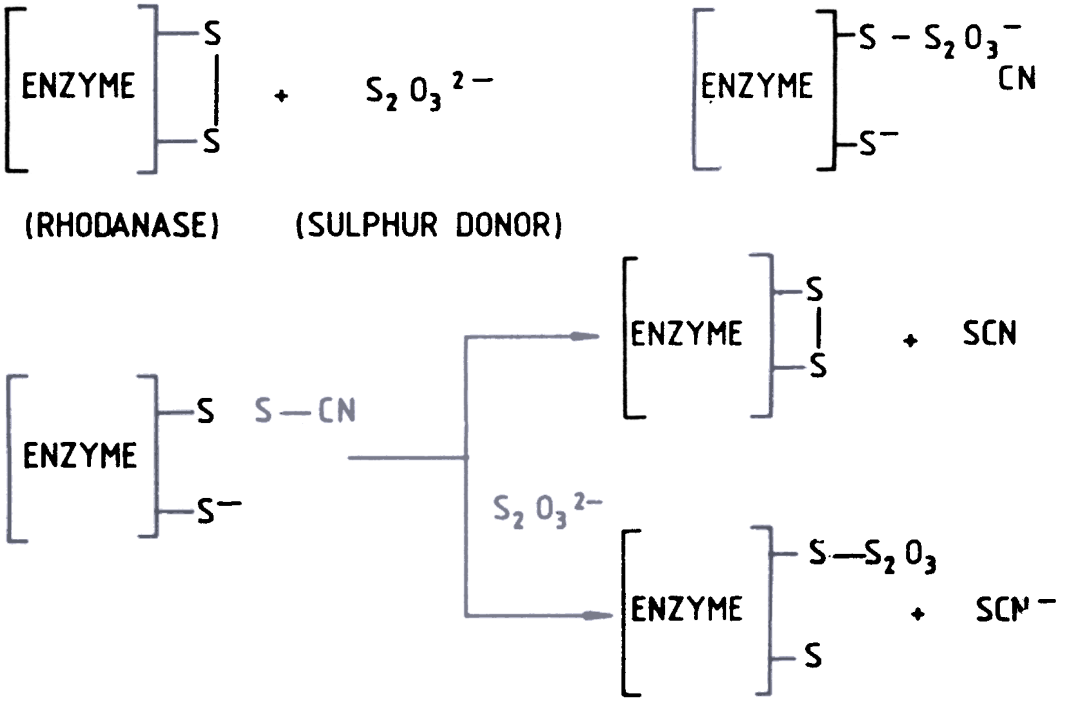
During the last two decades, attempts have been made to get a better understanding about the enzymic mechanism of sulphur transferases and their role in cyanide detoxification[60-62]. These studies implicate a serum albumin sulphane carrier complex as playing a major role in cyanide detoxification mechanism.

8. ROLE OF OXYGEN THERAPY IN CYANIDE POISONING

The role of oxygen therapy in cyanide poisoning has long been a debatable point. It is, however, well demonstrated that although oxygen alone gives only minor protection as compared to the classical antidotes, it increases the protection when administered in combination with the antidotes[63]. This protective effect of oxygen was observed not only prophylactically[63,64] but also therapeutically, after the signs and symptoms of cyanide poisoning were fully manifested[63].

9. PROTOCOL FOR TREATMENT

The presence of a large variety of cyanide antidotes makes it very difficult to get unanimity as to which is the more effective regimen. This is so because different experimental conditions and species of animals have been employed in testing the efficacy of different antidotes. The design of studies to assess the efficacy of antidotes presents several problems other than the choice of animal model. These relate to the dose of toxicant, the dose of antidote and the time relation



Scheme 5. Mechanism of detoxification with sulphur donors in the presence of rhodanase enzyme.

between them. While the United States of America remains faithful to the combination of sodium nitrite and sodium thiosulphate (Lilly's Cyanide Antidote Kit) in the treatment of cyanide poisoning, some European countries, such as Germany use DMAP, whereas others such as UK and France favour Co_2EDTA. The Lilly's Kit available in the USA is not without inherent limitations. The toxicity of sodium nitrite and its slow formation of methaemoglobin are the two main factors which have always been advocated against this regimen. It should be possible to make a rational choice between DMAP and Co_2EDTA by comparing their efficacies against experimental poisoning in animals and by comparing their relative toxicities. In fact, not many such comparisons have been carried out. There is only one report[27] which showed better survival with DMAP than with Co_2EDTA. A recent report employing the co-administration of sodium nitrite and DMAP seems to be encouraging as it not only reduces the toxicity of these chemicals due to reduced dosage but also gives sustained protection against cyanide threat which is further augmented by sodium thiosulphate[32].

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