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The Role of Hydrocyanic Acid in Nutrition

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Synopsis

I. Introduction

The hydrogen compound of cyanogen (C_2N_2), an extremely poisonous, inflammable, colourless gas which behaves like the halogens, is known as hydrocyanic acid or prussic acid. Hydrocyanic acid is a colourless deadly poisonous liquid which boils at 26°C, yielding a colourless gas with odour of bitter almond. It freezes at −15°C and dissociates quickly in aqueous solution. It is a very weak acid which hydrolyses slowly in aqueous solution and more rapidly in the presence of inorganic acids to form first of all formamide and then ammonium formate.

$$HCN \xrightarrow{H_2O} HCONH_2 \xrightarrow{H_2O} HCOONH_4$$

Since hydrocyanic acid forms two kinds of alkyl derivatives it is supposed to exist in two forms, the cyanide form (HCN) and the isocyanide form (HNC). Hence hydrocyanic acid is tautomeric, giving rise to the nitrile-isonitrile diad system.

$$\underset{\text{Nitrile}}{H\text{–}C{\equiv}N} = C \leqq \underset{\text{Isonitrile}}{N\text{–}H} \text{ (i.e. } \dot{H}\text{–}\dot{N} = C\text{)}$$

However, it appears that only one form is known, viz. the nitrile form. All the reactions of hydrocyanic acid such as hydrolysis to formic acid etc., confirm this. The contribution of the isonitrile form is therefore very small.

Hydrocyanic acid was first discovered by SCHEELE [1782] who obtained it from bitter almonds by acid hydrolysis of the glucoside, amygdalin as follows:

$$C_{20}H_{27}O_{11}N + 2H_2O \xrightarrow{\text{acid}} HCN + C_6H_5CHO + 2C_6H_{12}O_6$$

It has also been found in the leaves of certain plants, e.g. Laurel.

Hydrocyanic acid is usually prepared in the laboratory by the action on concentrated sulphuric acid on sodium cyanide. On heating hydrocyanic acid is evolved and is dried over calcium chloride. The yield is about 93–97%

$$NaCN + H_2SO_4 \longrightarrow HCN + NaHSO_4$$

Free hydrocyanic acid as such is not found in healthy growing plants, but develops when normal growth has been retarded or stopped by drought or other adverse condition. It is usually formed by chemical reaction between two substances, a glucoside and an enzyme contained in the plant. Neither the glucoside nor the enzyme is poisonous by itself [COUCH, 1932]. The glucoside consist of a chemical combination of sugar and hydrocyanic acid with perhaps another compound, probably an aldehyde or ketone. Only when liberated is the hydrocyanic acid poisonous.

II. Sources of Hydrocyanic Acid

Cyanogenesis has been extensively studied in many plants chiefly because of the possible toxic effect that the deadly poisonous hydrocyanic acid produce could have on live-stock.

It has been estimated that hundreds of plants yield hydrocyanic acid, e.g. nearly all the Sorghums, both wild and sweet, some grasses such as Johnson and Sudan grass, and clovers. QUISUNBINE [1947] listed over 300 species of plants belonging to about 74 families as containing hydrocyanic acid. The highest number of 52 was obtained from the pea family, the *Leguminosae* and about 25 in the grass family,

Graminae, and about 10 or more in each of the Aster family, the *Compositae*, and the spurge family, the *Euphorbiaceae*.

HEILBRON [1929] reported that under certain conditions ferns produce hydrocyanic acid, and at young stages of growth brachen may produce enough hydrocyanic acid to be of danger [MOON *et al.*, 1951a]. CLAWSON and MORAN [1937] reported that chokecherry leaves and arrow grass produce hydrocyanic acid under draught condition. Other plants which have been found to produce hydrocyanic acid include the flax families, desert almond, minna grass [MOON *et al.*, 1916], cynodon species [STEYN, 1940], and Bermuda grass.

With all these species of plants it is surprising that only 12 cyanogenetic glucosides have so far been isolated. The ones that have been extensively studied are amygdalin, lotusin, prunasin, dhurrin, sambunigrin, linamarin and lotaustralin. Of these the best known and most extensively studied is amygdalin, isolated in 1830 from bitter almond and related plants. On hydrolysis in the presence of an enzyme yields glucose, benzaldehyde and hydrocyanic acid. Lotusin has been isolated from the Nile valley plant called Khutcher, dhurrin from sorghum, sambunigrin from elderberries, lotaustralin from white clover, prunasin from wild cherry bark and linamarin from cassava. Like amygdalin, prunasin and sambunigrin are glycosides of the various optical isomers of mandelonitrile and dhurrin of p-hydroxymandelonitrile; linamarin and lotaustralin are the B-glycosides of acetonecyanohydrin and ethyl methyl ketone cyanohydrin, respectively. Lotusin on hydrolysis yields maltose, hydrocyanic acid and lotoflavin [DUNSTAN and HENRY, 1901].

Cyanogenetic glycosides have been found in the following common vegetables: maize, sorghim, millet, field bean, lima bean, kidney bean (haricot), sweet potato, cassava, lettuce, linseed almond and seeds of lemons, limes, cherries, apples, apricots, prunes, plums and pears. In most of these foodstuffs the glycosides are not likely to be harmful either because they exist in small amounts or else in parts that are not edible. Workers in this field have concentrated mainly on the different species of sorghum, sudan grass, Johnson grass, white clover and arrow grass. Young shoots are more dangerous than old ones and the general results indicate that the plants are most toxic when there are lots of young side shoots, especially when these have been wilted by dry, hot weather and if there is a heavy dew, mist or light drizzle at the time they are eaten. LEEMAN [1935] has been given an extensive bibliography on hydrocyanic acid in grasses.

Because ensiling of manna grass (and clover) reduces the amount of hydrocyanic acid, MINSEN [1933] suggested that it might be formed from an easily decomposable compound other than cyanogenetic glucoside.

In some cases a mixture of glucosides is found in plants. Thus MELVILLE and DOAK [1940] described a method for isolation of the glucoside from white clover and they found it contained 80% of lotaustralin and 20% of linamarin.

The hydrocyanic acid content varies depending on the kind of plant, the season, conditions, stage of growth, weather and soil. It is generally agreed that the hydrocyanic acid content decreases as the plant matures [FRANZKE *et al.*, 1939; WILLIAMMAN *et al.*, 1915], e.g. MAHUDESWARAN *et al.* [1958] found that fodder sorghum at early stage of growth contains about 0.1–0.2% and this decreases to about 0.001–0.007% after 80 days after which the plant is harmless. It is also agreed that varietal differences are relatively consistent under a wide range of conditions [HOGG and AHLGREN, 1943; WILLIAMMAN, 1916], but there is a lot of controversy over the effect of fertiliser. Thus FRANZKE *et al.* [1939] found that potassium fertiliser increases the hydrocyanic acid content of Sudan grass whereas BOYD *et al.* [1938] and PATEL and WRIGHT [1958] found that it has no effect. FRANZKE *et al.* [1939] and NELSON [1953] found that nitrogen fertiliser increases the hydrocyanic acid content but MANGES [1935] believed that it did not while BOYD *et al.* [1938] only observed the increase on soils that are deficient in nitrogen. If the soils is already well supplied with nitrogen then application of nitrogen fertiliser has little effect on the hydrocyanic acid content. MANGES [1935] claimed that plants grown on fertilised soils, especially with nitrate fertiliser, contain less cyanide than those on poor soils. MAXWELL [1903] found that the amount of cyanide depends on the soil, being higher with soils rich in nitrogen constituents of plant food, while PICKNEY [1937] found that the amount is proportional to the amount of nitrate fertiliser applied in some greenhouse experiments. BOYD *et al.* [1938] found a reduction in the cyanide content when only phosphorus fertiliser is applied or in the presence of high phosphorus and low nitrogen. PATEL [1953] explained that the phosphorus only limits the increase in cyanide caused by nitrogen, while on the other hand FRANZKE *et al.* [1939] reported more cyanide using phosphorus alone than using a mixture of nitrogen and phosphorus fertilisers. FRANZKE *et al.* [1939] found about 15% decrease in the cyanide content of the second growth of Sudan grass

than in the first and that manure and phosphorus reduce the cyanide content while nitrogen and lime increase it but a combination of manure, phosphorus and lime did not produce as high cyanide content as lime alone. FRANZKE *et al.* [1939] found that plants grown under irrigated condition have lower cyanide content as compared with those raised under rainfed condition.

DE WAAL [1942] demonstrated the existence of a diurnal variation of hydrocyanic acid in plants and this has been confirmed by ACHARYA [1933] and BOYD *et al.* [1938]. They found that the cyanide content is low in the morning and it gradually increases until 2 p.m., falls in the evening and then declines rapidly at night.

GORKILL [1942] found that the inheritance of both glucoside and enzyme is governed by single dormant genes. They tested 1500 tons of white clover and distinguished 4 types: those containing both glucoside and enzyme, those containing only enzyme, those containing only glucoside and those containing neither.

The incidence of insect attack can also contribute to the cyanide content of crops. VINALL [1921] found that Sorghum plants attacked by aphid contains about twice as much glucoside as the healthy plant.

SWANSON [1921] reported that freezing Sudan grass did not cause a decrease in the cyanide content if tested before it has thawed and wilted, but after that the content dropped rapidly. ACHARYA [1933] suggested ensiling sorghum as a means of destroying the cyanide but BOYD *et al.* [1938] is of the opinion that air-drying or sun curing have no effect on the cyanide content. If small green plants with a high cyanide content are made into hay, the hay will probably, if carefully cured and stored have a high cyanide content.

Some of these points could be clarified if it is assumed according to LEEMAN [1935] and WARDEN [1940] that cyanogenetic glucosides are intermediate compounds in the synthesis of protein from amino acids which in turn are formed from the nitrate absorbed by the roots from the soil. With cyanogenetic plants, hydrocyanic acid is a 'stable intermediate' product which immediately combines with glucose and some keto compounds (benzaldehyde in case of Sudan grass) to form the non-toxic cyanogenetic glucoside. The glucoside does not accumulate if conditions for rapid protein synthesis are favourable. With non-cyanogenetic plants hydrocyanic acid is an 'unstable intermediate' which quickly breaks down as soon as it is formed so that it can not be isolated. On the other hand if both kinds of plants are exposed to adverse condition such as wilting and frosting, this may

bring about a condition suitable for the formation of the stable intermediate and so both could contain cyanide. Drought keeps the plants small by withholding water and probably lessening the availability of phosphates to plants much more than that of nitrogen. The plants are kept small when they are always much higher in cyanide than when larger. Since nitrogen is a part of hydrocyanic acid, it will be understandable if nitrogen-deficient plants do not accumulate cyanide.

Plant products have also been known to contain cyanide e.g. it is well known that cyanide is a constituent of the gas phase of tobacco and cigarette smoke. Thus, OSBORNE *et al.* [1956] demonstrated concentrations of up to 100 p.p.m. in cigarette smoke and the United States Surgeon's General report on 'Smoking and Health' include a figure of 1600 p.p.m.

Since cyanide is used for the synthesis of acrylonitrile, an important constituent of synthetic rubbers fibres, adhesives and plastics, it is manufactured extensively in Britain and the United States. It is also used in electroplating and in agriculture as insecticide under the name of Tritox (monochloroacetonitrile, trichloroacetonitrile and phthalonitrile) and as pesticide under the name of Ventox. Some of the aliphatic cyanides are valuable as industrial solvents, e.g. acetonitrile is used as a selective solvent in petroleum, coal tar oil, vegetable and animal oil industries. This may create a hazard for people who use it and those involve in the manufacturing if necessary precautions are not taken. Cyanide has even been found in traces in the breadth of normal men up to about 0.5 mg per 5 minutes [BOXER and RIKARDS, 1952] and may be higher in men exposed to cyanide.

BOXER and RIKARDS [1952] have shown that equilibrium exists between thiocyanate and cynide *in vivo*. The conversion of thiocyanate to cyanide has been observed in man and in dogs by GOLDSTEIN and RIEDERS [1951]. Cyanide has been detected in the blood and cells (up to as much as 0.28–0.65 mg/100 ml of cells) of dogs injected with sodium thiocyanate (300 mg/kg), and also in patients receiving a single intravenous dose of 700 mg of cyanide-free sodium thiocyanate. This may explain some of the observed toxic effects of thiocyanate which resemble sub-acute cyanide poisoning [WILLIAMS, 1959]. GOLDSTEIN and RIEDERS [1953] have shown that the conversion of thiocyanate to cyanide is brought about by an enzyme called thiocyanate oxidase, found only in the erythrocyte of man, dog and rabbit. This enzyme has an optimum pH of 7.4 and is not inhibited by alkaline earth ions.

Hydrocyanic acid has also been known to be formed by micro-organisms. The formation was first described in the busidiomycete, *Marasmius*, in 1871 by LOSECKE and was subsequently reported in a species of Pholiota and other mushroom species by BACH [1948] who found that *Pholiota aurea* kept at 20°C produced about 81–315 mg hydrocyanic acid per 100 g dry matter in 48 hours. Heating at 49°C inactivates the process and oxygen is essential for the reaction. It is also formed in thallophytes like fungus [ROBINS *et al.*, 1950] and probably in algae [O'DONOGHUE and WILTON, 1951]. The formation by aerobic gram-negative bacterium has been known for some time [CLAWSON and YOUNG, 1913; PATTY, 1921] and its formation in mesophilic *chromobacterium violaceum* strains was first reported by SNEATH [1953] and later by MICHAEL and COOPE [1964]. They found a synergistic effect of glycine and methionine on the cyanide formation and the amount formed depends on the concentrations of the two substances. Formation was stimulated by succinate, malate, or fumarate but depressed by azide and 2,4-dinitrophenol. LORK [1948] found that cyanide is produced from a variety of *Pseudomonas aeruginosa*, yielding thrice as much cyanide at 37°C and reaching a maximum after 24 hours. He found that cyanide can be formed with only glycine as a source of nitrogen and that glutamic acid and asparagine did not yield enough cyanide when used as sources of nitrogen.

Since hydrocyanic acid has also been found in minute quantities in various substances of animal origin like urine, tissue extracts and gastric juice [BOXER and RIKARDS, 1952], it is probable that it is a common event in biological systems and its pathway of formation and subsequent metabolism is widely distributed in living organisms.

III. Determination of Hydrocyanic Acid

Hydrocyanic acid gives a number of colour reactions which can only be used for qualitative analysis either because the reaction is not sensitive or other substances interfere with it. Hence the quantitative determination is not very easy. All the same, most of these reactions have been modified for use quantitatively and some have given good results.

When a piece of filter paper is saturated with an alkaline picrate solution and brought near a test tube containing a cyanide solution or chopped pieces of a cyanogenetic plant, the paper is turned orange

and then brick red. The test is delicate and the rapidity of the change in colour depends on the quantity of the hydrocyanic acid present. MIRANDE [1909] suggested that a few drops of chloroform should be added to the test tube but NOWOSAD and MCVICAR [1940] later suggested toluene. This method was first proposed by PETYBRIDGE [1919] as a possible quantitative method for estimating cyanide. HOGG and AHLGREN [1942] add the cyanide to an alkaline picrate solution and heated in a boiling water bath for about 5 minutes when an orange-coloured isopurpuric acid developed. The colour was read in a colorimeter and compared with standards. This method was found to be accurate for determining cyanide concentrations of 0–0.08 mg. SEIFERT [1955] suggested that the cyanide should be distilled into sodium hydroxide solution with a current of prewashed air. WOOD [1965] found prewashing unnecessary. He passed the cyanide into sodium carbonate solution and added saturated picric acid and set it aside for a few minutes to initiate colour development. He then heated in a boiling water bath for 12 minutes, cooled and took the density readings at 530 *m*.

In BRUNSWIK [1921] method the chopped material is moistened with chloroform in a glass dish. A drop of 1% $AgNO_3$ faintly coloured with methylene blue is suspended inside the cover. Bluish crystals of silver cyanide is shown in the presence of hydrocyanic acid. This has been adapted for quantitative estimation. WOOD [1966] titrated the cyanide solution with a solution of silver nitrate after the addition of ammonia and a small crystal of potassium iodide. Titration gave results within 10% of the true value. On the other hand the cyanide can be precipitated with silver nitrate as silver cyanide and then determined argentometrically by the method of HALSTROM and MOLLER [1945].

If a drop of benzidine and copper acetate is substituted for silver nitrate in the last method, blue crystalline needles of an oxidation product of benzidine is formed [SIEVERTS and HERISDORF, 1921]. ALDRIGE [1944] has modified this method for quantitative analysis by the use of pyridine and benzidine. BOXER [1955] swept the cyanide with nitrogen into sodium hydroxide solution. After chilling, chloramine-T-phosphate was added and then pyrazolone pyridine. The mixture was shaken and the colour which develops is stable for 2 hours. This method can estimate concentrations between 0.02–0.4 mg hydrocyanic acid.

For the determination of traces of hydrocyanic acid in plants, NICHOLSON [1941] found that a method which depends on the oxid-

ation of reduced form of phenolphthalein (Phenolphthalin) in alkaline solution is suitable. In the presence of copper salt hydrocyanic acid oxidises the reduced form to coloured phenolphthalein. Although it is not specific as other substances could bring about the oxidation, no other oxidising agents in the plants have been known to interfere with the method. KOBTHOFF [1918] found that the sensitivity of the method is increased to 1 in 100 million parts if O-cresol phthalein is used instead.

A general method which is now in use depends on the conversion of cyanide to thiocyanate which is then determined in a number of ways. JOHNSON [1916] and FRANCIS and CRUNNEL [1913] determined the thiocyanate by converting to the iron salt which though reasonably sensitive, is extremely unreliable with small amounts owing to the instability of the colour of the iron salt. ALDRIGE [1944] estimated both the cyanide and thiocyanate by its colour reaction with pyridine solution after conversion to the bromide [LARSONNEAN, 1921; KÖNIG, 1904]:

$$HCN + Br_2 — CNBr + HBr$$

$$KCNS + 4Br_2 + 4H_2O = KBr + CNBr + H_2SO_4 + 6HBr.$$

MOLLER and STEVENSON [1937] removed the excess bromine with ferrous sulphate and added potassium iodide which combines with the bromocyanogen. The iodine liberated is titrated against sodium thiosulphate solution. The method is sensitive within the range of 0.02–20 mg hydrocyanic acid. Unlike some other methods hydrogen does not introduce any errors and so it is an ideal method for forensic-chemical analysis.

Another method is the Prussian blue of VIEHOEVER and JOHNS [1915]. The solution containing the hydrocyanic acid is aerated and passed through a paper impregnated with ferrous sulphate and sodium hydroxide. After aeration excess of iron hydroxide is dissolved out in hydrochloric acid and the resulting Prussian blue colour is compared on a spectrophotometer with standard blue stains. The method has been found to be specific and rapid with no interfering substances. The range is about 0.5–200 mg hydrocyanic acid, with an accuracy of 0.1 mg within this range.

In the A.O.A.C. acid titration method, the cyanide is distilled into a solution of silver nitrate acidified with nitric acid. The excess silver nitrate is then titrated with potassium thiocyanide using iron alum indicator.

IV. Toxicity of Hydrocyanic Acid

Most of the symptoms of hydrocyanic acid poisoning can be explained on the basis of its affinity for metal ions such as iron and copper. It combines with haemoglobin to form cyanohaemoglobin which is not an oxygen carrier. Another one that has been extensively studied is the reversible combination with the copper of the cytochrome oxidase which thereby inhibits its functions as an oxidative enzyme in electron transfer and provides the classic example of histotoxic anoxin [PETERS and VAN SYKE, 1931]. Most of the acute clinical effects of cyanide are thus explained—neuronal depression in the medullary centres leads to respiratory degression and death, but the patient remains pink as his tissues are unable to take up oxygen from the blood, and venous fully oxygenated [WILSON, 1966]. The observation of HARDLEY and KOZELKA [1935] is now understandable that cyanide inhibits the oxygen-activating enzyme, indophenol oxidase and so decrease the oxidative processes like respiration and the release of energy through oxidation. If this goes on far enough, sickness and death result. Hydrocyanic acid is therefore a violent protoplasmic poison for all forms of life, be it bacteria, infurosia, yeast or germinating seeds.

In animals the symptoms of hydrocyanic acid poisoning are increase in rate and depth of respiration, increased pulse rate, no response to stimuli and spasmodic muscular movements. EVANS and EVANS [1948] suggested that the cyanogenetic glucoside of white clover is responsible for bloat animals. They were able to produce convulsion and death by introducing white clover juice into the rumen of animals though they failed to produce bloat. Post mortein showed congestion of the ruminal, abomasal and intestinal mucosa and potechial haemorrhages on the spleen and heart. This is similar to the results obtained by CLARK [1937, 1939] who by feeding cyanogenetic foodstuffs (cassava) to human beings caused pellagra, capillary haemorrhages and venous congestion in the liver. Occasionally the rumen of sheep poisoned with cherry leaves smell of benzaldehyde [MORAN, 1954]. BUNYEA [1935] reported that the most common characteristics of cyanide poisoning in animals are dark muscle tissue, congestion or haemorrhage of the lungs, patechination of the tracheal mucosa and a frotty, bloody damage from the mouth and nostrils. Although microscopic studies do not reveal any damages to the tissue or cells of animals poisoned by arrow grass but the unusual quantities of haematogenous pigment in various places convinced MARSH [1929] that

there was degeneration and destruction of many red blood corpuscles. Cyanide is known to produce neurological damage in several species of animals either by injection of conbuffered alkaline cyanide salt or by inhalation. Its relationship to demyelination was prompted by the investigation into the pathogenesis of multiple sclerosis [WILSON, 1966].

In man the sequence of events in acute poisoning are: hyperventilation, headache, nausea and vomiting, generalised weakness, collapse and coma, perhaps with convulsions and then respiratory depression. With a large dose, collapse, coma and death may occur very rapidly indeed and with smaller doses a much slower sequence is seen and death may not superven for 15–20 minutes [WILSON, 1966].

Recently, B-amino propionitrile has been discovered in sweet pea (*Lathyrus odoratus*). This is a toxic substance which is responsible for the occurrence of the syndrome of lathyrism characterised by ataxia and spasticity, usually of the lower limb, which may occur suddenly or insidiously [WILSON, 1966]. It is not known yet whether the effects are due to the cyanide liberated or to intrinsically toxic properties of the nitriles themselves, but since these nitriles are not present in cassava roots, and since these effects have been reported in cassava eating areas in Nigeria [IDOWU, 1967] where it is found to be rampant at Epe, near Lagos, the effect may possibly be due to the cyanide liberated.

The cyanide in cigarette and tobacco gas smoke has been suggested as the cause of Leber's optic atrophy and the visual failure of tobacco-amylopia [WOKES, 1958; SMITH, 1961; HEATON, 1962; WILSON, 1966]. The considerably higher concentrations of thiocyanate in the smoking group is thought to be due to the detoxication of the cyanide present in the smoke [LAWTON *et al.*, 1943; STOA, 1957].

From the reports of various workers it appears that animals can adapt themselves to cyanide poisoning. EVERIST [1958] reported that in Queensland the native fuchsia bush, a high cyanide-containing plant, is grazed regularly by sheep without harm, but if on the other hand travelling sheep are put in to graze it, rapid death occurs in the animals. In some travelling mob, more than 1000 sheep died within 1 hour of grazing it. ECKELL [1949] found that he could feed a horse with sublethal dose of cyanide and gradually increase it over a period of 20 days to the minimum lethal dose without any ill effect. REED [1945] concluded that when human beings are exposed to hydrocyanic acid gas, chronic symptom occurs only on repeated exposure.

When animals feed on cyanogenetic plants, the glucoside must be hydrolysed to hydrocyanic acid before death occurs, otherwise it will not be absorbed [Auld, 1931; Van der Waalt, 1944]. Different animals react differently to be glucoside due to difference in anatomical structure of detoxifying ability. Cattle and sheep are more susceptible because they are ruminants [Dyktra, 1952], i.e. they contain a paunch or rumen which contains a large flora of microorganisms and considerable quantities of the enzyme emulsion. The pH of the rumen is about 5.5–7.5 which corresponds to the optimum condition for hydrolysis of the glycoside by the rumen fluid. The hydrolysis is very rapid and can go to completion within 10–15 minutes [Coop and Blakley, 1949], and so plant enzymes are not needed for the hydrolysis of the glucoside with ruminants. The resulting cyanide is rapidly absorbed from the rumen at a rate of about 75% in 15 minutes [Coop and Blakley, 1949]. Horses and hogs, like human-beings, are non-ruminants and have only one stomach which is strongly acid due to the presence of hydrochloric acid. This acid reacts with hydrocyanic acid to form much less toxic substances like acetic acid and ammonium chloride thereby causing an almost immediate detoxication as soon as the hydrocyanic acid is liberated from the glucoside

$$HCN + HCl + 2H_2O \rightarrow HCOOH + NH_4Cl\,.$$

In general, the peak production, concentration and absorption of cyanide under most circumstances take place within about 10 minutes of giving a dose of free glucoside, or within 10–20 minutes of sheep eating a cyanogenetic plant. If the animals are very healthy and not too hungry they may stop eating before taking a fatal dose than when the reverse is true. Boyd *et al.* [1938] found that after a heifer has fed on Sudan grass containing cyanide for 10 minutes, it began to stagger and refused to eat any more. The animal showed symptoms of cyanide poisoning. With 3 healthy cows and 3 thin, sickly-looking cows grazing Sudan grass, 2 of the emaciated cows laid down after grazing Sudan grass for 15 minutes and they showed marked symptoms of acute cyanide poisoning. It was necessary to revive them with injection of sodium thiosulphate to prevent death.

Hardley and Kozeika [1935] quoted dogs and rabbits can detoxify cyanide at a rate of about 0.5 mg per hour per pound body weight, i.e. a cow weighing about 1000 lb. could detoxify at a rate of 0.5 g per hour. This means that at least small amounts can be consumed

without toxic symptoms. While examining the suitability of cassava waste from starch production for use as pig food, JOHNSON [1916] found that 3.0 mg% was safe. ALBA [1937] showed that up to 0.12 mg of hydrocyanic acid daily for 1 week was not injurious to pigs.

It has been estimated by various workers that it will take a dose of 1 g potassium cyanide to kill a cow of 1000 lb. in weight. Of course, the minimum lethal dose depends on the rate of absorption into the system. If detoxication is equal to absorption no death occurs no matter the amount. COUCH [1932] was of the opinion that anything over 20 mg/100 g is toxic. A figure of 24.1 g/100 g was quoted to have caused two deaths at Ibadan [1962]. GLAWSON [1934] quotes 2.042 mg/kg body weight for cattle and 2.315 for sheep, COOP and BLAKLEY [1950] gave 2.4 mg/kg for sheep which would correspond to 114 mg dose for a 60 kg. BOYD [1938] gave the following:

Mg HCN/100 g dry tissue	Relative degree of toxicity
0–25	Very low (safe to pasture)
25–50	Low (safe to pasture)
50–75	Medium (doubtful)
75–100	High (dangerous to pasture)
100	Very high (very dangerous to pasture)

CLAWSON [1937] found that feeding 100 g of arrow grass containing about 600 mg% to sheep was lethal within a few minutes. HUNT [1923] found that the minimum lethal dose of hydrocyanic acid for man is about 0.5 mg per kg body weight which is equivalent to about 1.2 mg KCN or 0.9 mg NaCN per kg. In most cases about double this amount will be required in practice. BOHLUIS [1952] suggested 50–60 mg for an adult European, and NICOL [1951] suggested 0.06 g. CLARK [1936] postulated that cassava contains a toxic substance 'toxalbumen'. The resistance of individuals to this substance is variable and this may explain the sudden death caused by ingestion of cassava containing about 0.005 mg% in some cases. By analysing all the tissues of persons poisoned with cyanide GETTLER and BAINE [1938] were able to determine the minimum lethal dose. In 3 cases the amounts were 3.6, 1.4 and 1.1 mg kg body weight. Similarly HALSTRON and MOLLER [1945] have obtained 0.7 and 3.3 mg/kg in 2 cases.

Table I shows the effect of inhaled hydrogen cyanide at various concentrations [HENDERSON and HAGGARD, 1950] and table II the fatal concentrations of inhaled hydrocyanic acid [BARCROFT, 1931].

Table I. Toxicity of hydrogen cyanide inhaled at various concentrations [HENDERSON and HAGGARD, 1950]

HCN (p.p.m.)	Toxicity
20	Maximum safe period for prolonged exposure
20–40	Slight effects after several hours
100–250	Dangerous after ½–1 hour
300	Lethal in a few minutes
3000	Rapidly lethal

Table II. Toxicity of inhaled hydrogen cyanide on different species, adapted from BARCROFT [1931]

Species	Concentration of HCN in parts in 60 min	
	Lethal in 2–4 min	Lethal in 60 min
Dog	330	67
Rabbit	500	140
Mouse	500	90
Cat	550	140
Guinea pig	600	330
Goat	700	200
Rat	800	67
Monkey	800	150

In commercially processed manoic foods and flour, hydrolytic enzymes occurring in the manoic root remain unchanged. These enzymes catalyse the condensation of HCN with aldehydic compounds so that addition of glucose to unprocessed manoic root causes a complete disappearance of hydrocyanic acid due to the formation of glucocyanohydrin [ADRIENS, 1945]. It is suggested that in the preparation of commercial food products from cassava a quantity of glucose (equimolar to hydrocyanic acid) be added. This had also been suggested by ADRIENS [1942] a few years earlier. The antidotal effect of glucose has also been observed by COOP and BLAKLEY [1949] but it is doubtful whether the above explanation is correct since cyanohydrin is already an intermediate product in the hydrolysis of the glucoside. A better explanation might be that in the presence of ample supply of glucose, the bacteria attack glucose rather than the glucoside and

so the glucose completes successfully with the glucoside. This has been proved correct by COOP and BLAKLEY [1949] who aerated a solution containing 1 and 3 p.p.m. hydrocyanic acid at a pH of 6.5 alone and in the presence of 2% glucose. The glucose did not decrease the rate at which the hydrocyanic acid was given off by aeration. Hydrocyanic acid was also incubated for 40 minutes with or without ruminal liquid and this did not have any effect either. One could therefore conclude that glucose does not affect the absorption of hydrocyanic acid from the rumen, but it influences the rate of production from the glucoside.

V. Treatment

A comprehensive review of hydrocyanic acid and its antidotes have been made by HUNT [1923]. The report of various workers show that the sulphur-containing substances are the best antidotes especially when injected before the dose, e.g. an outstanding compound is sodium thiosulphate; but this is not a practical procedure. The cyanide is converted to the non-toxic thiocyanate:

$$Na_2S_2O_3 + NaCN + O \rightarrow NaSCN + Na_2SO_4$$

In spite of this favourable results have been reported by LASSAGA [1927], FEYERABEND [1928] and BUZZO [1928]. PIQUE [1928] and ZIMMAN [1928] found sodium thiosulphate very effective when injected intracardially. Another sulphur-containing antidote is sodium tetrathionate [HEBTING, 1910; CHISTONI and FORESTI, 1932; DRAIZE, 1933]. CHEN *et al.* [1934] found that it has the same value as sodium thiosulphate.

GEIGER [1933] reported successful treatments with methylene blue. Methylene blue is known to react with haemoglobin to form methaemoglobin which then reacts with hydrocyanic acid to form the non-toxic cyanomethaemoglobin (1 g of methaemoglobin requires 2.9 mg of cyanide for complete conversion to cyanomethaemoglobin). From this, one could infer that any compound capable of forming methaemoglobin could serve as an antidote. A more powerful methaemoglobin former than sodium nitrite is p-amino propiophenone and SAUNDERS and HEISEY [1956] have found it to be as effective as, if not better than 10 mg/kg of sodium nitrite in protecting cats against two LD_{50}'s (3.5 mg/kg) of sodium cyanide. They have also found

Medication	Number of M.L D's of NaCN required to kill
None	○
$C_3H_5-(NO_3)_3$	○
Methylene blue	○○○
$Na_2S_2O_3$	○○○○
$Na_2S_4O_6$	○○○○
$C_5H_{11}NO_2$	○○○○○
$NaNO_2$	○○○○○
Methylene blue α $Na_2S_4O_6$	○○○ ○○○○
$C_5H_{11}NO_2$ α $Na_2S_2O_3$	○○○○○ ○○○○○○
$NaNO_2$ α $Na_2S_4O_6$	○○○○○○○ ○○○○○○○
$NaNO_2$ α $Na_2S_2O_3$	○○○○○○○○○○ ○○○○○○○○○○○

Fig. 1. Comparison of antidotal action of different substances in dogs [CHEN *et al.*, 1934].

chlorocobalamin in doses of 100 mg/kg to be as effective as 10 mg/kg of sodium nitrite. Other nitrogen compounds found effective are the nitrites. CHEN *et al.* [1933] found that an injection of methylene blue saved 2 fatal doses and of amyl nitrite saved 4. They have since being working on other nitrites such as sodium nitrite. Figure 1 gives the summary to their work on antidotal action of the different substances on dogs. They found sodium nitrite to be prompt and it saved or decreased convulsion and put the dogs on their feet within a few minutes. On the other hand thiosulphate was comparatively slow and did not seem to inhibit convulsion. However, they found a mixture of the two given intravenously or intraperioteneally to be the best and this has been confirmed by BUNYEA [1935]. CHEN *et al.* [1934] found that the mixtures could detoxify about 20 times the minimum lethal dose in dogs and is about 10 times as effective as methylene blue. Recovery had even occurred when given at late stages of poisoning. The mechanism of the reaction when nitrites are used alone may be similar to that of methylene blue and probably in this case the sulphur-containing constituents of the body such as cystein, glutathion etc. supply sulphur, whereas when used in conjunction with sodium thiosulphate the synergistic effect is readily obtained. CHEN *et al.* [1934] suggested a dose of 6–10 mg sodium nitrite per kg of body weight

and 0.5 g of sodium thiosulphate per kg. This means an adult of about 50 kg will have a first dose of about 0.3–0.5 g sodium nitrite and 25 g sodium thiosulphate, with half of this amount for subsequent doses. The two should not be mixed together for administration. For emergency amyl nitrite is useful as an inhalant before the treatment. VIANA *et al.* [1934] have successfully used this mixture to save a young girl who took 5 g of potassium cyanide. She was given 1 ample of amyl nitrite and a total of 1.5 g sodium nitrite and 18 g sodium thiosulphate HANZLIK and RICHARDSON [1934] has reported a similar finding but they are of the opinion that methylene blue should be considered as first choice. MOTA [1933] has saved a case of poising by using 0.57 g of sodium nitrite only.

VI. The Mode of Cyanide Detoxication

The problem that has long been of toxicological and physiological importance is the source of small amounts of thiocyanate found in the urine, blood and saliva. Sodium thiosulphate has been isolated as the barium salt from the normal urine of cats and dogs by SCHMIEDEBERG [1867] and FROMAGEOT and ROYER [1945] have shown it to be a normal metabolite in higher animals although the mechanism of its formation is obscure. VASSEL *et al.* [1944] have found that dogs excreted 2–15 mg thiosulphate-sulphur in 24 hours whereas dogs excreted 50–125 mg. GAST *et al.* [1950] reckoned human being, excrete about 20 mg thiosulphate-sulphur per 24 hours. At first it was thought to be due to the small amounts of this substance present in foodstuffs. WOKES *et al.* [1952] reported concentrations of 0.1–10 p.p.m. in cows milk, i.e. about 57–5.7 mg per pint. This source alone can account for a substantial portion of the thiocyanate. GEMEINHARDT [1938] analysed a large number of plants and found that the thiocyanate concentration in all species range from about 30–950 mg/100 g, with highest figures in cabbage, carrots and radishes. WILSON [1966] gave much higher figures, 4.1 mg% for sprout, 1.9 for caneflower, 0.4 for peas and tomatoes. The highest figures so far obtained are those of WILLIAMS [1967] for cassava and its product. He obtained 600 mg% for 'gari' (made by fermentation of cassava), 700–800 mg% for cassava flour and 500–600 mg% for yam flour. These results support the view that thiocyanates are mainly derived from food. The content was found to increase with heavy smoking [LAWTON *et al.*, 1943], or heavy con-

sumption of beer or strong tea, milk, eggs and other animal protein sources, all of which contain some performed thiocyanate [WOKES, 1955; WEDGEWOOD and WYATT, 1952]. Later, LANG [1894, 1895] showed that injection of cyanide or aliphatic cyanides into rabbit increased the thiocyanate excretion and this has been confirmed by HEYMANNS and MESOIN [1896]. Attention was therefore focussed on thiocyanate as a possible detoxification product of cyanide in foods. LANG [1933a, b] postulated the existence of an enzyme he called 'rhodanese' to be responsible for the reaction under aerobic condition in the presence of thiosulphate or colloidal sulphur:

$$HCN + Na_2S_2O_3 — HSCN + Na_2SO_3 .$$

He found that the enzyme was heat labile with an optimum pH and substrate concentration of 8.3 and 1 mole cyanide: 3 moles sodium thiosulphate respectively. The rate of the reaction increases with the temperature up to 38°C and the reaction follows Schurtz rule, i.e. $K = X N_e t$, where N_e = the concentration of the enzyme, X = the amount of substrate transformed in time t, and K = constant. Moreover, the enzyme is widely distributed in all the tissues with the highest concentrations in the liver. Detoxication can therefore take place in all parts of the body but with the liver as the chief site. COSBY and SUMMER [1945] purified the enzyme and found that the reaction did not follow Schurtz rule regardless of the cyanide concentration. Many others have worked on the same line and have found different optimum pH values and different distribution patterns in the tissues [HENDEL *et al.*, 1946; BERNARD *et al.*, 1947a, b; HIMWICH and SAUNDERS, 1948]. From the distribution of the enzyme in the tissues HIMWICH and SAUNDERS [1948] calculated that the whole liver of a dog can detoxify about 4015 g cyanide and the skeletal muscles 1763 g cyanide to thiocyanate within 15 minutes, yet only small doses are required for toxicity. The explanation is that availability of sulphur limits the detoxication possible *in vivo*. This has been supported by the work of CHEN *et al.* [1934] who found that injection of thiosulphate is capable of increasing the minimum lethal dose by a factor of 3 to 4. The high concentration of thiosulphate required is not possible in the tissues and moreover thiosulphate penetrates the tissues very slowly whereas cyanide penetrates fast. GILLMAN *et al.* [1946] found that 70–80% of injected thiosulphate is excreted unchanged. On the other hand, other sources of sulphur such as cystine, thiourea, sodium

sulphide etc. are not effective. If even it is assumed that certain enzymes convert the sulphur of amino acids to sulphide and then thiosulphate [SMYTHE, 1942; FROMAGEOT *et al.*, 1938; DER GARABEDDIAN and FROMAGEOT, 1943] the formation will proceed too slowly that it will only be of limited value. On the other hand it has been demonstrated that the sulphur of cystine can be utilised to some extent for the formation of thiocyanate. WOOD and COOLEY [1952] have shown the production of labelled SCN from administered cyanide and ^{35}S-cystine.

The amount of rhodanese in the liver vary with different animals so that detoxication may be expected to take place at different rates. HIMWICH and SAUNDERS [1948] found the following for the livers of animals:

0.78–1.46 mg/g for dog
10.08–15.16 mg/g for rhesus monkey
7.98–18.92 mg/g for rabbit
14.24–28.38 mg/g for rat.

This probably explains the results obtained by MUKERJI and SMITH [1943] that nearly all the cyanide ingested by rabbit was recovered in form of thiocyanate in the urine in 24–48 hours whereas in dogs less than 25% was recovered in 7 days.

Subsequent work of SAUNDERS and HAMMWICH [1950] have thrown further light on the functions of rhodanese. They proposed that the enzyme formed a lose combination with thiosulphate which breaks down to yield sulphur in a form that can be accepted by the cyanide ion. They explained that the inhibitory effect of certain sulphur-containing compounds like sodium sulphide, dithiobiuret and cysteine as due to the blocking of the enzyme so that it can not combine with thiosulphate. They found in agreement with LANG [1933] that certain divalent cations like Cu^{2+} and Fe^{2+} produce significant inhibition whereas others have none.

SORBO [1951a, b, 1953] has suggested that rhodanese contains an active disulphide group (rather than –SH groups) which reacts with thiosulphate and cyanide as follows:

$$E\begin{matrix}S\\ \diagup\!\diagdown\\ S\end{matrix} + S\text{–}SO_3^- \rightarrow E\begin{matrix}S\text{–}S\text{–}SO_3^-\\ \diagdown\!\diagup\\ S^-\end{matrix} + CN^- \rightarrow E\begin{matrix}S\\ \diagup\!\diagdown\\ S\end{matrix} + SO_3^- + SCN^-$$

This is not the only route of detoxication through which thiocyanate can be formed from cyanide. It has been found that B-mer-

captopyruvic acid can provide sulphur as rapidly as thiocyanate for cyanide detoxication. MEISTER [1953] found that crude extracts of liver converted this compound into pyruvic acid and freed sulphur at pH 7.5–8.5. Since rhodanese utilises only thiosulphate or colloidal sulphur, B-mercaptopyruvic acid could be a sulphur donor. This has been confirmed by WOOD and FIEDLER [1953] who found that crude acetone extracts of rat liver, incubated at pH 9.1, converted CN^- to CNS^- as rapidly with B-mercaptopyruvic acid as with thiosulphate. The optimum pH for both substrate is 9.1. Another route is through the reaction of cyanide with 3-mercaptopyruvate to yield thiocyanate and pyruvic acid [FIEDLER and WOOD, 1956]. The 3-mercaptopyruvate is formed by the transamination of L-cysteine. The former then combines with the cyanide by means of sulphur-transferase to form thiocyanate and pyruvic acid. If sulphite is present thiosulphate and pyruvic acid are formed from the 3-mercaptopyruvate. The thiosulphate can then be utilised by rhodanese sulphite and thiocyanate. The sulphite formed is again ready for use by the sulphur transferase. A cycle is thus formed as shown in figure 2.

The fact that stress conditions such as menstruation, pregnancy and lactation when increase requirement for vitamin B_{12} is needed, cause an increase in thiocyanate excretion, makes one suspect that B_{12} may be involved directly or indirectly in the formation of thiocyanate in the body. This is confirmed by the findings that dietary deficiency of vitamin B_{12} leads to increased thiocyanate excretion and that injection of sub-lethal doses of cyanide to rats caused a significant depletion of the liver store of vitamins B_{12}, indicating that this is an important detoxifying agent during cyanide poisoning [SMITH, 1961].

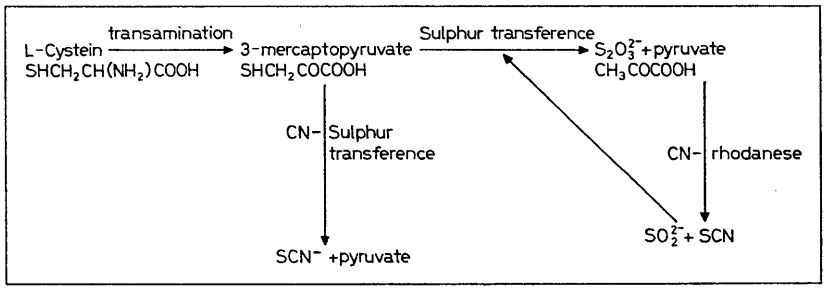


Fig. 2. Mode of cyanide detoxication.

Vitamin B_{12} contains cobalt in an organic coordination complex, with CN tightly bound to the cobalt. This is shown by the fact that doses up to 1600 mg/kg applied both intraperitoneally and intravenously are non-toxic to mice, despite the fact that this dose is equivalent to 32 mg hydrocyanic acid or 8 times the minimum lethal dose for mice [MUSHET *et al.*, 1952]. They therefore proposed vitamin B_{12} as an antidote to cyanide poisoning in mice, restoring life when death was inevitable. This is, of course, assuming that some of the vitamin occurs in the hydroxyl form which can be replaced by the cyano group. BAXTER *et al.* [1953] have shown that ampoules of cyanocobalamin purporting to hold 100 mg of cyanocobalamin (i.e. vitamin B_{12}) contained varying percentages of the hydroxo form. The hydroxo form is known as hydroxocobalamin or vitamin B_{12} a or B_{12} b.

Undoubtedly some of vitamin B_{12} exists in the liver as the hydroxo-form. Even assuming that all the B_{12} in the liver occurs in this form, the total amount will be less than 1000 mg [DROUET *et al.*, 1953] and the amount of cyanide that can be detoxified will be equivalent to about 25 mg which is very small. It is probable that rhodanese is the main detoxication centre, with its function related to vitamin B_{12} as pointed out by WOKES and PIKARD [1955]. They proposed that the liver contains some B_{12} in the hydroxo form together with a lot of rhodanese. When cyanide is ingested, both B_{12} and rhodanese compete for it; some are detoxified by rhodanese with the help of sulphur donors such as sulphur-containing amino acids or their products of metabolism, to thiocyanate which are all excreted in the urine with very little in the faeces [MOISTER and FRIES, 1949]. Some of the cyanide combine with the hydroxocobalamin to form cyanocobalamin (B_{12}) which then carries out various metabolic functions. B_{12} can lose some of the cyanide to supply 1-carbon fragment for the synthesis of important compounds such as choline and other labile groups and for the conversion of homocysteine to methionine [KRATZER, 1953; STEHOL *et al.*, 1953; SMITH, 1954]. Some of the cyanide is lost as carbon dioxide in the breadth. This has been confirmed by BOXER and RIKARDS [1952] who demonstrated that labelled ^{14}CN given to dogs could quickly be recovered as exhaled carbon dioxide and in the various lipid fraction like choline and methionine; in the ureide carbon of allantoin as well as in vitamins B_{12} and thiocyanate. Thus it appears that cyanide is incorporated into the 1-carbon metabolic pool probably in the form of formate. The high activity of formate from the liver than any other constituents make it a probable intermediate for this conversion.

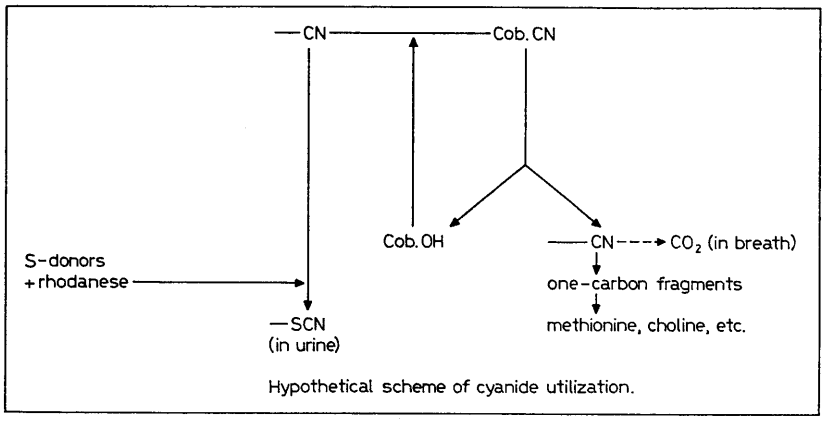


Fig. 3. Mode of cyanide detoxication [WOKES and PIKARD, 1952].

Finally some cyanide is liberated from vitamin B_{12} which then returns to the liver as the hydroxo-form, thus completing the cycle as shown in figure 3. WOKES and PIKARD [1955] have also put forward an alternative pathway in which they assumed that excess thiocyanate is present in the tissues compared with cyanide and so it is the thiocyanate that combines with the hydroxocobalamin to form the thiocyanate derivatives. This is as effective as the cyanide-form [BUHS *et al.*, 1951]. As in the first mechanism, some of the thiocyanate is liberated and B_{12} reverts to the liver in the hydroxo form. Some sulphur is lost from the thiocyanate form so that some B_{12} is left in the cyano form. The sulphur is given up to some active intermediate compound to form a thiocompound which may be a precursor of some biologically important sulphur—containing compounds such as sulphur amino acids, glutanthione or thioacetic acid. The cycle is completed by the loss of CN in vitamin B_{12} which then goes back to the liver in the hydroxo form, and the CN is converted to CNS by rhodanese and is again available for the cycle, as shown in figure 4. This hypothesis is supported from the fact that vegans who are short of vitamin B_{12} detoxify their cyanide to thiocyanate through rhodanese and so they excrete excess thiocyanate. They therefore need more sulphur amino acid donors which could otherwise have been utilised for some other purposes. If methionine is the donor, as has been shown by HARTMAN s1949] and HARTMAN and WAGNER [1949] on the thiocyanate excre-[ion in liver diseases it means there will be a decrease in the reserved tulphur in this form. On the other hand if cysteine and cystine are the

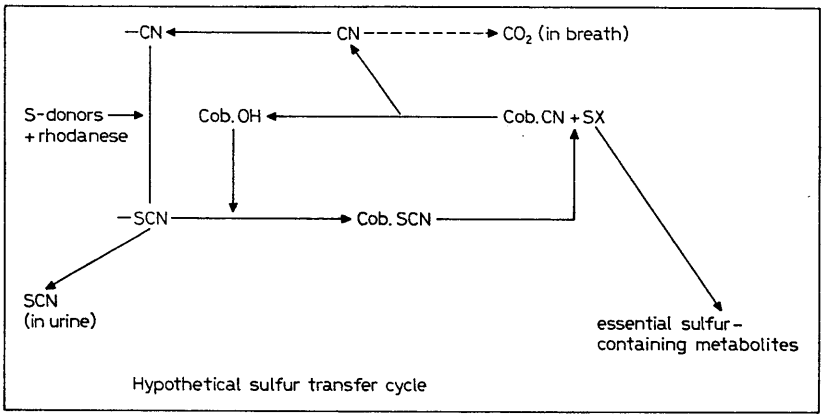
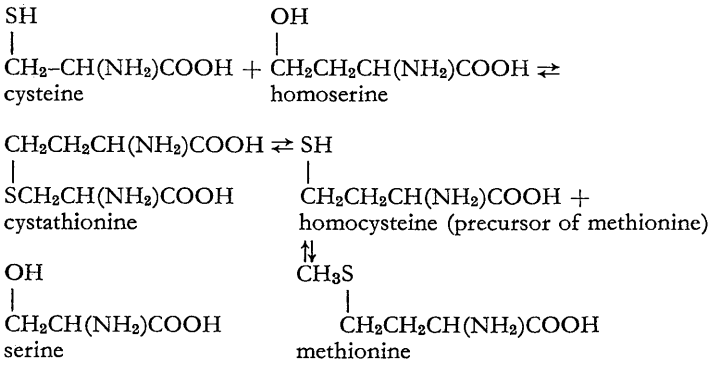


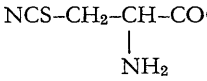
Fig. 4. Mode of cyanide detoxication [WOKES and PIKARD, 1952].

donors, as shown in sheep by BLAKELEY and COOP [1949], it will also lead indirectly to methionine deficiency a sulphur amino acid in the lens of the eye in which interest has long been centred [PIRIE, 1956]. This is on the assumption that in the presence of B_{12} both cysteine and cystine can act as precursor of methionine as has been shown in *Neurospora* and certain microorganisms by HOROWITZ [1947], TEAS *et al.* [1948], FLUG and HOROWITZ [1951] and TEAS [1950].

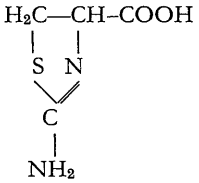


Another independent way in which detoxication of cyanide can take place is the reaction with cystine to form cysteine and B-thiocyanoalanine (I). The reaction takes place spontaneously *in vitro* [SCHOBERT and HAMM, 1948]. VOEGTLIN *et al.* [1926] were the first to observe that a dose of cystine injected immediately before ingestion of cyanide

protected animals from a minimum lethal dose. SCHOBERT *et al.* [1951] have shown that B-thiocyanoalanine tautomerises to 2-amino-thiazoline-4-carboxylic acid (II) or the equivalent compound 2-imino-4-thiazolidine carboxylic acid (III).



(I)



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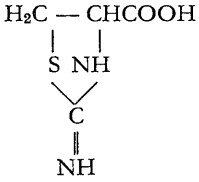

(III)

WOOD and COOLEY [1956] found that compound III is formed in the presence of a relatively high concentration of cystine in the blood.

The chemical properties of this cystine-cyanide reaction have been studied extensively by SCHOBERT and HAMM [1948], ALDRICH [1951], BEHRINGER and ZUINKES [1951] and WOOD and COOLEY [1956]. When cyanide is ingested it is converted to compound III *in vivo* by cystine. Using $_{35}$Slabelled cystine, WOOD and COOLEY [1956] recovered about 40% of the labelled sulphur in the form of compound III. They also observe this compound in the saliva of laboratory worker chronically exposed to relatively high concentration of cyanide by inhalation. However, the amount of cyanide detoxified is small compared with other pathways. In an experiment without cystine injection, the recovery of intraperitoneally injected cyanide as thiocyanate and thiazolidine was 80 and 15%, respectively.

When hydrocyanic acid is converted to thiocyanic acid there is a 200-fold reduction in toxicity. This may be regarded as a detoxication mechanism in the body which will presumably cope with the small amounts of cyanide formed during metabolism or the minute amount taken in food, but not with a toxic amount or a large dose introduced artificially into the body.

Another indirect detoxication mechanism that may be mentioned is that of the thyroid gland. [HUNT 1905/06] showed that the lethal dose of acetonitrile for mice fed on powdered sheep thyroid was 1.4 mg/g whereas it was 0.32 mg/g for those without thyroid feeding. This gave an indication that probably thyroid has a detoxicating effect. Later, BAUMANN *et al.* [1933] found that rabbits injected with acetonitrile excrete 3–5% which was increased on feeding desiccated thyroid. Since thyroidectomy had little effect on the conversion of benzyl cyanide to SCN^-, BAUMANN *et al.* [1933] were of the opinion that the mechanism of detoxication by thyroid depends on demethylation of acetonitrile.

In conclusion it may be pointed out, however, that not all cyanide compounds are poisons. Thus the cyano group is important in normal metabolism as a component of vitamin B_{12} (cyanocobalamin) as is pointed out later. 3-indolyl-acetonitrile occurs in plants and is an active plant growth hormone [HENBEST *et al.*, 1953]. Malonitrile has been suggested for the treatment of certain mental diseases following the discovery that some schizophrenic patients were benefitted from it [BODANSKY, 1945].

The toxicity of cyanide compounds depends on the mode of metabolism, whether it could be converted to free CN^- ion or not. Metabolic studies show that some cyanide compounds inhibit cytochrome oxidase activity [CLEMMENS and JACKSON, 1962] and that thiocyanate is increased after administration to experimental animals [STOA, 1957] indicating that probably cyanide is liberated and thus may be toxic. On the other hand if cyanide is not liberated then the cyanide compound is not toxic. Thus mandelonitrile is highly toxic (toxic dose to rabbits 6 mg/kg) since it is readily converted *in vivo* to hydrocyanic acid whereas cyanoacetic acid (pKa 2.43) is relatively non-toxic (toxic doses for rabbits 2000 mg/kg) since its CN group is biologically stable [WILLIAMS, 1959]. The mechanism of the conversion of aliphatic and benzyl cyanides into the cyanide ion is not known, but 4 routes of metabolism of these compounds can be postulated [WILLIAMS, 1959].

(1) $RCH_2CN \xrightarrow{H_2O} CN^- + RCH_2OH \rightarrow SCN^- + RCHO \rightarrow RCOOH$

(2) $RCH_2CN \xrightarrow{O} RCHOHCN \rightarrow CN^- + RCHO \rightarrow SCN^- + RCOOH$

(3) $RCH_2CN \xrightarrow{H_2O} RCH_2CONH_2 \rightarrow RCH_2COOH + NH_3$

(4) $RCH_2CN \xrightarrow{4H} RCH_2CH_2NH_2 \rightarrow RCH_2COOH + NH_3$.

The first reaction is hydrolysis of the cyanide to an alcohol with the libration of CN^- ion whereas the second reaction involves the oxidation to the cyanohydrin which then decomposes to aldehyde and CN^- ion. Evidence for the support if the both mechanisms is found in the work of LANG [1894] who found that acetonitrile yields both thiocyanate and formic acid and another one for the latter is from the work of GAL and GREENBERG [1953] who found that acetaldehyde accumulated in the blood of antabuse-treated animals receiving propionitrile but not acrylonitrile

$$CH_3CH_2CN \rightarrow CH_3CHOHCN \rightarrow CH_3CHO + HCN\,.$$

References

ACHARYA, C.N.: Indian J. Agr. Sci. *3:* 851 (1933).
ADRIAENS, L.: Bull. Agr. Congo Belg. *33:* 332 (1942).
ADRIAENS, L.: Med. Mem. in octavo *13:* 4 (1945).
ALBA, M.G.: Phillipine Agriculturist *25:* 782 (1937).
ALDRICH, W.N.: Biochem. J. *48:* 271 (1951).
ALDRIGE, W.N.: Analyst *69:* 262 (1944).
AULD, S.J.M.: J. Agr. Sci. *5:* 409 (1931).
BACH, E.: Physiol. Plantarum *1:* 387 (1948).
BARCROFT, J.: J. Hyg. *31:* 1 (1931).
BAXTER, N.; WORSFORD, J.; WOKES, F.; NORRIS, F.W. and FERNANDES, S.J.G.: J. Pharm. *10:* 723 (1953).
BEHRINGER, H. and ZUINKES, P.: Ann. Chem. *574:* 140 (1951).
BERNARD, H.; GADJOS-TOROK, M. and GADJOS, A.: Compt. Red. Soc. Biol. *141:* 700 (1947a).
BERNARD, H.; GADJOS-TOROK, M. and GADJOS, A.: Compt. Red. Soc. Biol. *141:* 702 (1947b).
BLAKELEY, R.L. and COOP, I.E.: Zew Zealand J. Sci. Technol. *31:* A1 (1949).
BOHLUIS, G.G.: Rev. intern. Botan. Appl. Agr. Trop. (Paris), p. 559 (1952).
BOXER, G.E.: Abstr. Amer. Chem. Soc. 124th meeting, 35C (1955).
BOXER, G.E. and RIKARDS, J.C.: Arch. Biochem. *39:* 7 (1952).
BOYD, F.T.; AAMODT, O.S.; BOTHSTEDT, G. and TRUOG, E.: J. amer. Soc. Agron. *30:* 569 (1938).
BRUNSWIK, H.: Sitzber. Akad. Wiss. Math. Naturw. Kl. *130:* 383 (1921).
BUHS, R.P.; NEWSTEAD, E.G. and TRENNER, N.R.: Science *113:* 625 (1951).
BUNYEA, H.: J. amer. vet. med. Ass. *86:* 656 (1935).
BUZZO, A.: Rev. de especialid. *2:* 869 (1928).
CHEN, K.K.; ROSE, C.L. and CLOWES, G.H.A.: J. Pharmacol. exp. Ther. *51:* 132 (1934).
CHISTONI, A. and FORESTI, B.: Arch. intern. Pharmacol. *42:* 140 (1932).
CLARK, A.: J. trop. med. Hyg. *39:* 269 (1936).
CLARK, A.J.: J. trop. med. Hyg. *40:* 221 (1937).
CLARK, A.J.: J. trop. med. Hyg. *42:* 65 (1939).
CLAWSON, A.B.; BUNYEA, H. and COUCH, J.F.: J. Wash. Acad. Sci. *24:* 369 (1934).
CLEMMENS and JACKSON (1963)

COOP, I.E. and BLAKLEY, R.L.: New Zealand J. Sci. Technol. *30:* 277 (1949).
COOP, I.E. and BLAKLEY, R.L.: New Zealand J. Sci. Technol. *31:* 44 (1950).
CORKILL, L.: New Zealand J. Sci. Technol. *23B:* 178 (1942).
COSBY, E.L. and SUMMER, J.B.: Arch. Biochem. *7:* 457 (1945).
COUCH, J.F.: U.S. Dep. Agr. Leaflet 88 (1932).
DE WAAL, A.: Wageningen (1942).
DER GARABEDDIAN, M. and GROMAGEET, C.: Compt. Red. *211:* (1943).
DRAIZE, J.H.: Science *78:* 145 (1933).
DROUET, P.L.; WOLFF, R.; KARLM, R. KARLM, R. and RANBERG, G.: Brit. med. J. *2:* 640 (1953).
DUNSTAN, W.R. and HENRY, T.A.: Phil. Trans. roy. Soc. London, Ser. B *194:* 515 (1901).
DYKSTRA, R.R.: Animal sanitation and disease control. Interstate, Danville *111:* 445 (1952).
ECKELL, O.A.: Rep. 14th intern. Vet. Congr. London *3:* 28 (1949).
EVANS, W.C. and EVANS, E.T.R.: Nature *163:* 373 (1948).
EVERIST, S.I.: Queensland Agr. J. *84:* 489 (1958).
FEYERABEND, K.: Klin. Wschr. *7:* 1351 (1928).
FLING, M. and HORROWITZ, N.H.: J. biol. Chem. *190:* 277 (1951).
FRANCIS, C.K. and CRUNNEL, W.B.: J. amer. chem. Soc. *35:* 1624 (1913).
FRANZKE, C.J.; PUBR., L.T. and HUME, A.N.: South Dakota Station Tech. Bull. I.E.S.R. *81:* 780 (1939).
FROMAGEOT, C. and MONLBASHER, R.: Enzymologia *2:* 331 (1938).
FROMAGEOT, C. and ROYER, A.: Enzymologia *11:* 361 (1945).
GAL and GREENBERG (1953).
GEISER, J.C.: J. amer. med. Ass. *101:* 269 (1933).
GEMEINHARDT, K.: Ber. dtsch. Botan. Ges. *56:* 275 (1938).
GETTLER, A.O. and BAINE, J.O.: Amer. J. med. Sci. *195:* 182 (1938).
GILLMAN, A.; PHILLIPS, F.S. and KOELLA, E.S.: Amer. J. Physiol. *146:* 348 (1946).
GOLDSTEIN, F. and RIEDERS, F.: Amer. J. Physiol. *167:* 47 (1951).
GOLDSTEIN, F. and RIEDERS, F.: Amer. J. Physiol. *173:* 287 (1953).
GAST, J.H.; ALDRICH, F.L. and ARAI, K.: Fed. Proc. *9:* 175 (1950).
HALSTROM, F. and KOZEIKA, F.L.: Vet. Med. *30:* 79 (1935).
HALSTRON and MOLLER (1945).
HANZLIK, P.J. and RICHARDSON, A.P.: J. amer. med. Ass. *102:* 1740 (1934).
HARDLEY, F.B. and KOZEIKA, F.L.: Vet. Med. *30:* 79 (1935).
HARTMAN, F.: Deut. Arch. Klin. Med. *196:* 412 (1949).
HARTMAN, F. and WAGNER, K.H.: Klin. Med. *196:* 432 (1949).
HEATON, J.M.: Trans. ophthal. Soc. *82:* 263 (1962).
HEBTING, J.: Biochem. Z. *28:* 208 (1910).
HEILBRON, A.: Ber. dtsch. Botan. Ges. *47:* 230 (1929).
HENBEST, H.B.; JONES, E.R.H. and SMITH, G.F.: J. chem. Soc. 3796 (1953).
HENDERSON and HAGGARD (1950).
HEYMANUS, J.F. and MESOIN, P.: Arch. intern. Pharmacol. *3:* 359 (1896).
HIMWICH, W.A. and SAUNDERS, J.P.: Amer. J. Physiol. *153:* 348 (1948).
HOGG, P.G. and AHLGREN, H.L.: J. Agr. Res. *67:* 195 (1943).
HOGG, P.G. and AHLGREN, H.L.: J. amer. Soc. Agron. *34:* 199 (1942).
HOROWITZ, N.H.: J. biol. Chem. *171:* 255 (1947).
HUNT, R.: Heffters Handbuch der experimentellen Pharmakologie *1:* 702 (Springer, Berlin 1923).
HUNT, R.: J. biol. Chem. *1:* 33 (1905/06).
IDOWU, A.: Personal communication (1967).
JOHNSON, M.O.: J. amer. chem. Soc. *38:* 1230 (1916).

KOBTHOFF, I.M.: Z. anal. Chem. *57:* 11 (1918).
KÖNIG, W.: J. prakt. Chem. *69:* 105 (1904).
KRALZER, F.H.: J. biol. Chem. *203:* 367 (1953).
LANG, S.: Arch. exp. Path. Pharmakol. *34:* 347 (1894).
LANG, S.: Arch. exp. Path. Pharmakol. *36:* 75 (1895).
LANG: Biochem. Z *259:* 243 (1933a).
LANG: Biochem. Z. *259:* 262 (1933b).
LARSONNEAN, A.: Bull. Sci. Pharmakol. *28:* 499 (1921).
LASSAGA, A.: Semana med. *34:* 443 (1927).
LAWTON, A.H.; SWEENCY, T.R. and DUDLEY, H.C.: J. Ind. Hyg. Tox. *25:* 13 (1943).
LEEMAN, A.C.: Onderstepoort J. vet. Sci. Anim. Ind. *5:* 97 (1935).
LORCK, H.: Physiol. Plantarum *1:* 142 (1948).
LOSECKE: Cited by MICHAEL and CORPE (1964) (1871).
MAHUDESWARAN, K.; MEENAKSHI, K.; THANGAM, M.S. and RADAKRISHNAN, T.: Madras Agr. J. *45:* 445 (1958).
MANGES, J.D.: Vet. Med. *30:* 347 (1935).
MARSH, C.D.: U.S. Dep. Agr. Bull. 1245 (1929).
MAXWELL, W.: Queensland Agr. J. *13:* 93 (1903).
MELVILLE, J. and DOAK, B.W.: New Zealand J. Sci. Technol. *22B:* 67 (1940).
MEISTER, A.: Fed. Proc. *12:* 245 (1953).
MENDEL, B.; RUDNEY, H. and BOWMAN, M.C.: Cancer Res. *6:* 495 (1946).
MICHAEL, R. and CORPE, W.A.: J. Bacteriol. *89:* 106 (1964).
MINSEN, A.: Landwirtsch. ver. Sta. *117:* 279 (1933).
MIRANDE, M.: Compt. Rend. Acad. Sci. *149:* 140 (1909).
MOON, F.E. and RAAFAT, M.A.: J. Sci. Food Agr. *2:* 327 (1951a).
MOON, F.E. and RAAFAT, M.A.: J. Sci. Food Agr. *2:* 228 (1951b).
MOISTER, F.C. and FRIES, E.D.: Ann. J. med. Sci. *218:* 549 (1949).
MOLLER, K.O. and STEVENSON, K.: Biochem. Z. *290:* 44 (1937).
MORAN, E.A.: Amer. J. vet. Res. *15:* 171 (1954).
MOT, M.M.: Rev. med. Rosario *23:* 670 (1933).
MUKERJI, M. and SMITH, R.G.: Ann. Biochem. exp. Med. *3:* 23 (1943).
MUSHET, C.W.; KELLEY, K.L.; BOXER, G.E. and RICKARDS, J.C.: Por. Soc. exp. Biol. N.Y. *81:* 234 (1952).
NELSON, C.E.: Agron. J. *45:* 615 (1953).
NICHOLSON, R.I.: Analyst *66:* 189 (1941).
NICOL, L.: Tropical nutrition and dietetics, 3rd ed. (Bailliere, Tindall & Cox, London 1951).
NOWOSAD, F.S. and MCVICAR, R.M.: Sci. Agr. *20:* 566 (1940).
O'DONOGHUE, J.G. and WILTON, G.S.: Canad. J. comp. Med. Vet. Sci. *15:* 193 (1951).
OSBORNE, J.S.; ADAMEK, S. and HOBBS, M.E.: Anal. Chem. *28:* 211 (1956).
PATEL, C.J. and WRIGHT, M.J.: Agron. J. *50:* 645 (1958).
PATEL, R.M.: Ph.D. Thesis. University of Wisconsin (1953).
PATTY, F.A.: J. infect. Dis. *29:* 73 (1921).
PETYBRIDGE, G.H.: Econ. Proc. roy. Dublin Soc. *2:* 248 (1919).
PETERS, J.P. and VAN SLYKE, D.D.: Quantitative clinical chemistry (Williams Co., Baltimore 1931).
PICKNEY, R.M.: J. Agr. Res. *27:* 717 (1937).
PIQUE, J.A.: Semana med. *35:* 308 (1928).
PIRIE, A.: Biochemistry of the eye. (Van Heyninger, Oxford 1956).
QUISUNBINE, E.: Philippine J. Forestry *4:* 145 (1947).

REED, A.C.: J. lab. clin. Med. Handbook of Tropical Medicine, revised ed. (Stanford University, California 1920).
ROBINS, W.J.; ROLNICK, A. and DARANAGH, F.: Mycology *42:* 161 (1950).
SAUNDERS and HAMMWICH (1950).
SCHEELE: Cited by FINAR, I.L. Organic Chemistry, vol. 1 (Longmans, Green & Co., London 1782).
SCHOBERT, A. and HAMM, R.: Chem. Ber. *81:* 210 (1948).
SCHOBERT, A.; KAWOLI, M. and HAMM, R.: Chem. Ber. *84:* 571 (1951).
SCHMIEDEBERG, O.: Arch. Heilk. *8:* 422 (1867).
SEIFERT, P.: Modern methods of plant analysis *4:* 686 (Springer, Berlin 1955).
SIEVERTS, A. and HERISDORF, A.: Z. angew. Chem. *34:* 3 (1921).
SORBO, B.H.: Acta chem. Scand. *5:* 724 (1951a).
SORBO, B.H.: Acta chem. Scand. *5:* 1218 (1951b).
SORBO, B.H.: Acta chem. Scand. *7:* 238 (1953).
SMITH, A.M.: Lancet *i:* 1001 (1961).
SMITH, E.L.: Ann. Rev. Biochem. *23:* 245 (1954).
SMYTHE, C.V.: J. biol. Chem. *142:* 387 (1942).
SNEATH, P.H.A.: Lancet *265:* 276 (1953).
STEHOL *et al.* (1953).
STEYN, D.G.: Herbage Publ. Series Bull. *27:* 158 (1940).
STOA, K.F.: Medd. Norsk Farm. Selskap *13:* 93 (1951).
SWANSON, C.O.: J. amer. Soc. Agron. *13:* 33 (1921).
TEAS, H.J.; HOROWITZ, N.H. and FLING, M.: J. biol. Chem. *172:* 651 (1948).
TEAS, H.J.: J. Bacteriol. *59:* 93 (1950).
VAN DER WAALT, S.J.: Onderstepoort J. vet. Sci. Anim. Indust. *19:* 79 (1944).
VIANA, C.; GAGNOLI, H. and CENDAM, J.: Compt. Rend. Soc. Biol. *115:* 1649 (1934).
VASSEL, B.; PARTRIDGE, R. and CROSSLEY, M.L.: Arch. Biochem. *4:* 59 (1944).
VIEHOEVER, A. and JOHNS, C.O.: J. amer. chem. Soc. *37:* 601 (1915).
VINALL, H.N.: J. amer. Soc. Agron. *13:* 267 (1921).
VOEGTHIN, O.; JOHNSON, J.M. and DYER, H.A.: J. Pharmacol. exp. Ther. *27:* 467 (1926).
WEDGEWOOD, P. and WYATT, J.: Biochem. J. *50:* xix (1952).
WILLIAM, J.J. and WEST, R.M.: J. Agr. Res. *4:* 179 (1915).
WILLIAMMAN, J.J. (1916).
WILLIAMS, H.: Personal communications (1967).
WILLIAMS, R.T.: Detoxication mechanisms, 2nd ed. (John Wiley and Sons Inc., New York 1959).
WILSON, J.: Ph.D. Thesis, London. Vet. Rec., Lond. *52:* 857 (1966).
WOKES, F.; BANDENOCH, J. and SINCLAIR, H.M.: Amer. J. clin. Nutr. *3:* 375 (1952).
WOKES, F.: Brit. J. Nutr. *6:* 118 (1952).
WOKES, F. and PIKARD, C.W.: Amer. J. clin. Nutrit. *3:* 383 (1955).
WOKES, F.: Brit. J. Nutr. *6:* 118 (1952).
WOOD, T.: J. Sci. Food Agr. *16:* 300 (1965).
WOOD, T.: J. Sci. Food Agr. *17:* 85 (1966).
WOOD, J.L. and COOLEY, S.L.: J. biol. Chem. *218:* 449 (1956).
WOOD, J.L. and COOLEY, S.L.: Fed. Proc. *11:* 314 (1952).
WOOD, J.L. and FIEDLER, H.: J. biol. Chem. *205:* 231 (1953).
ZIMMAN, J.: Semana med. *35:* 1235 (1928).

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