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Toxicological and Clinical Aspects of Cyanide Metabolism

By R. G. H. Baumeister[1]), H. Schievelbein and G. Zickgraf-Rüdel

Summary: *This contribution deals with the occurrence of cyanide and its biological pathways in the body. Especially possibilities of detoxification are pointed out.*
Intoxications are caused by acute and chronical cyanide uptake. Tobacco amblyopia, retrobulbar neuritis in pernicious anaemia, Leber's optic atrophy, Nigerian nutritional neuropathy, and sterility in female heavy smokers are attributed to cyanide intoxication.
Various methods for treating acute and chronic cyanide intoxication are discussed.

Zusammenfassung: *Toxikologische und klinische Aspekte der Metabolisierung von Cyanid*
Das Vorkommen von Cyanid und sein Stoffwechsel im Körper werden beschrieben. Auf die bestehenden Entgiftungsmöglichkeiten wird besonders verwiesen.
Vergiftungen können bei einmaliger und chronischer Cyanidaufnahme entstehen. Die Tabakamblyopie, die Retrobulbärneuritis bei perniziöser Anämie, die Lebersche Optikusatrophie, die nigerianische ernährungsbedingte ataktische Neuropathie sowie die Sterilität bei starken Raucherinnen werden auf eine chronische Cyanidvergiftung zurückgeführt.
Auf die Behandlung der akuten und der chronischen Cyanidvergiftung wird eingegangen.

Contents

1. Introduction

In recent years it has been possible to establish a connection between the pathogenesis of several diseases and the impaired metabolism of cyanide. Successful therapeutic measures confirm these assumed relations, which partially could already be proved experimentally. Moreover certain connections between the metabolism of vitamin B_{12} and cyanide poisoning were detected.
For these reasons it seems worthwhile to review the knowledge on the metabolism of cyanide, taking into consideration the occurrence and mechanism of detoxication of cyanide.

2. Occurrence of cyanide

Traces of cyanide can be demonstrated in almost all plants. According to Montgomery (1969) it is found mainly in form of cyanogenic glycosides. Cyanogenic glycosides are compounds from which one or more sugar molecules and an aldehyde or ketone are liberated by treatment with acid (Conn 1969). Table 1 shows a compilation of plants with extremely high cyanide content. Furthermore it is known that the pips of several berries and the stones of several prunus species (e.g. almond, cherry, apricot) contain considerable amounts of cyanogenic glycosides in several instances (Moeschlin 1964). Also by intake of several sorts of cabbage amounts of cyanogenic glycosides, which are not acutely toxic, can be ingested (Werle 1969). Besides the wide-spread occurrence in plants (Dilleman 1959) cyanogenic glycosides are also found in insects (Blum and Woodring 1962; Eisner et al. 1963; Jones et al. 1962; Schildknecht et al. 1968; Blum

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Table 1: Cyanide content of various nutritional plants according to Montgomery (1969) and Pulss (1967).

Plant	HCN content (mg/100 g)	Literature
Bitter almond	250	Polson and Tattersall (1969)
Bitter cassava		
bark of root/dried	245	Collens (1915)
stem	113	Collens (1915)
root (total)	55—90	Clark (1936)
Sorghum		
fresh leaves	0—15	Stähli n (1957)
Bamboo		
tops of unripe sprouts	800	Baggchi and Ganguli (1943)
stem, unripe	300	Baggchi and Ganguli (1943)
Lima bean (Phaseolus lunatus) several variations		
Java, coloured	312	Guignard (1907)
Puerto Rico, black	300	Vichoever (1940)
Burma, white	300	Kohn-Abrest (1906)
Arizona, coloured	17	Montgomery (1964)
America, white	10	Montgomery (1964)
Linseed products	30—50	Lüdtke (1952); Pulss (1962)
White clover	20—30	Corkill (1940); Melville et al. (1940); Pulss (1962)

et al. 1962; Barbetta 1966; Guldensteeden-Egeling 1882; Wheeler 1890) and in fungi (Tschiersch 1967).

As to the occurrence of HCN in insects, it could be demonstrated in the secretion of the defense gland of two groups of millepedes. Pachymerium ferrugineum belonging to the chilopodes contains about 2.2 µg HCN per animal (Schildknecht et al. 1968). HCN from mandelonitril (Blum et al. 1962; Barbetta 1966) was also demonstrated in polydesmoidea, a millipede belonging to the diplopodes (Guldensteeden-Egeling 1882; Wheeler 1890).

In tobacco smoke, cyanide occurs in considerable amounts, the concentration of which differs from one sort of tobacco to the next (Osborne 1956; Wynder 1967; Elmenhorst 1968; Stedman 1968). In the main stream of tobacco smoke of cigarettes without filter Artho and Koch found values between 150 and 300 µg, which were in the range of the data obtained by other authors (Newsome et al. 1965). Also the order of magnitude of our own data corresponds with these results (Baumeister and Schievelbein 1971).

In cigarettes with cellulose acetate filter only a negligible retention by the filter was observed. Suitable absorbent filters on the other hand reduce the original cyanide content by 20 to 80%. The cyanide content of the particle phase per cigarette amounts to approximately 100 µg CN^-/cigarette as well in Blend cigarettes as in Maryland cigarettes. The cyanide values in the gaseous phase are slightly higher. For Blend cigarettes they amount to about 150 µg CN^-/cigarette, for Maryland cigarettes about 120 µg CN^-/cigarette (Artho and Koch 1969)[2]).

Inhalation of HCN compounds in the chemical and galvanic industry as well as during gold extraction is another possibility for humans to take up considerable amounts of cyanide.

The poisoning that means the liberation of HCN from organic thiocyanates (plant protective agents) is of importance. It is accomplished in insects and mammals by the enzyme glutathione-S-transferase, mainly from lower aliphatic homologues. This fact explains the high toxicity of some lower alkyl derivatives of thiocyanate (Gleason et al. 1969). Also liberation of cyanide from succinonitrile has been described recently (Contessa and Santi 1973).

[2]) Blend cigarettes containing a tobacco mixture while Maryland cigarettes are made of Virginia tobacco exclusively.

3. Metabolic pathways of cyanide in the organism

HCN may get into the organism via the lungs, the gastrointestinal tract and also via the skin. If HCN is taken up orally amounts of 0.5 mg to 3.5 mg/kg body weight may already be fatal for humans (Chen et al. 1934; Gettler and Baine 1938; Halstrom and Moller 1945).

The maximum concentration at the working place (MAK maximale Arbeitsplatz-Konzentration) permitted for HCN is 10 ppm in the Federal Republic of Germany (1 ppm = part per million = 1 µg/liter). 100 ppm are dangerous to life. 270 ppm are fatal within a short time. The toxic effect of HCN is caused by its high affinity to cytochrome oxidase. Inspite of reversible binding to this enzyme HCN causes an immediate inhibition of cellular respiration.

Several ways of metabolism were described, by which cyanide may be detoxified. These ways can be demonstrated in the living organism by administration of radioactive-labelled cyanide compounds.

Following injection of $Na^{14}CN$ in dogs ^{14}C appears in urine in free cyanide as a constituent of cyanocobalamine and in thiocyanate. A great part of the cyanide and thiocyanate carbon is oxidized directly to form CO_2, which is achieved, according to Boxer and Rickards (1952), probably via cyanate.

The carbon of cyanide and probably as a result of metabolic balance also the carbon of thiocyanate appears in the carbon of the methyl group of choline and methionine and in the urea-C of allantoine as well. The formic acid isolated in these experiments contains a high amount of ^{14}C. Based on this fact formic acid could be considered as starting material for the introduction of the cyanide carbon into the one-carbon-metabolism (Boxer and Rickards 1952).

After subcutaneous injection of sodium cyanide in rats 2-imino-4-thiazolidine-carboxylic acid could be isolated from urine (Wood and Cooley 1956). In this case cyanide is thought to react with cystine forming cysteine and β-thiocyanoalanine.

The latter is tautomerized forming 2-amino-thiazolidine-4-carboxylic acid or the equivalent 2-imino-4-thiazolidine-carboxylic acid (Schöberl et al. 1951). The second compound proved to be metabolic-inert in rats (Wood and Cooley 1956).

The detoxification of cyanide compounds forming thiocyanate can be achieved on two metabolic pathways. One reaction which has already been known for a longer time is catalysed by the enzyme thiosulphate-sulphurtransferase (EC 2.8.1.1) named rhodanese by Lang (1933). Thiosulphate and cyanide form thiocyanate and sulphite in this reaction. In the second way 3-mercaptopyruvate, which can be formed by transaminidation of L-cysteine, acts as sulphur-donator. Thiocyanate and pyruvate are formed at the cyanide-sulphurtransferase (EC 2.8.1.2) (Fiedler and Wodd 1956). In Scheme 1 the above described pathways of cyanide metabolism are shown.

The different pathways of metabolism are utilized to various degrees in the organism. Balance investigations which could give an insight into the utilization of the different pathways have not been published. But it can be assumed that the detoxification to thiocyanate accounts for the main part. After injection of sodium cyanide in rat 80% of the injected cyanide was found in urine as thiocyanate (Wood and Cooley 1956). The therapeutic effect of thiosulphate in cyanide intoxication, also combined with rhodanese (Clemedson et al. 1954), could as well be a hint that the detoxification of cyanide by means of thiosulphate-sulphurtransferase is of great importance.

$CN^- + S_2O_3^{--} \xrightarrow{\text{thiosulphate-sulphurtransferase}} SO_3^{--} + SCN^-$

$CN^- + \text{3-mercapto pyruvate} \xrightarrow{\text{cyanide-sulphurtransferase}} \text{pyruvate} + SCN^-$

$CN^- + \text{hydroxocobalamine} \longrightarrow \text{cyanocobalamine}$

$CN^- + \text{cystine} \longrightarrow \text{2-amino-thiazolodine-4-carboxylic acid} + \text{cystein}$
(2-imino-4-thiazolodine-carboxylic acid)

$CN^- \longrightarrow \text{formic acid} \longrightarrow \text{1-C-metabolism}$

incorporation of cyanide-carbon into choline, methionine, allantoine

oxidation to CO_2

elimination via lungs and kidneys

inhibition of cytochrome oxydase

Scheme 1: Pathways of cyanide metabolism in the organism.

3.1. Thiocyanate

Since the first description of the occurrence of thiocyanate in saliva by Treviranus (1814) and since the detection of HCN(hydrocyanic acid)-detoxification by means of rhodanid formation by Lang (1895), a number of investigations on relations between the concentration of this metabolite and its physiologic and pathologic states have been published.
Of special interest are the changes within cyanide metabolism under the influence of smoking, which is probably the most common way of cyanide intake. For the first time Claude Bernard observed that smokers excreted considerably higher amounts of thiocyanate than did non-smokers (after Pick 1910). This observation was confirmed by many authors, recently by Stoa (1951) and Maliszewsky and Bass (1955).

Table 2 shows the thiocyanate concentration in blood and saliva and the excretion in urine of smokers and non-smokers.

Table 2: SCN^- concentration in blood, saliva, and urine of smokers and non-smokers. Range and mean value according to Larson (1961).

	Non-smokers	Smokers
Blood (serum) (mg/100 ml)	0.025—1.21 $\bar{m}$ = 0.379	0.06 —2.48 $\bar{m}$ = 1.06 8 authors
Saliva (mg/100 ml)	2.03 —26.5 $\bar{m}$ = 5.54	7.57 —27.5 $\bar{m}$ = 12.82 3 authors
Urine (g/24 h)	0.002—0.232 $\bar{m}$ = 0.117	0.012—0.283 $\bar{m}$ = 0.147 2 authors

Compared with non-smokers the thiocyanate content in blood and serum of smokers was raised by 100—300% according to Schreiber (1925), Blum (1928), Lawton et al. (1943), Trasoff and Schneeberg (1944), Ramos and Visca (1945), Stoa (1951), Maliszewsky and Bass (1955). On the other hand, Caviness (1948) did not find a statistically significant difference between smokers and non-smokers.
The question concerning a direct correlation between consumption of cigarettes and the thiocyanate content in blood was answered by some authors positive (Schreiber 1925) and by others negative (Lawton et al. 1943). Also no agreement exists about the content of thiocyanate in saliva. Both elevation and lowering of thiocyanate content have been described (Mendel and Schneider 1900; Fleckscher 1905; Culverwell 1915; Eschler et al. 1968; Barylko-Pikielna and Pangborn 1968). On the other hand, agreement exists that more thiocyanate is excreted in urine of smokers than of non-smokers (Pick 1910; Lawton et al. 1934; Maliszewsky and Bass 1955; Wokes 1958).
The following separate results shall be mentioned: the thiocyanate content in the higher cerebrospinal fluid parallels the content in serum (Blum 1928). In two smokers the thiocyanate content of sweat was considerably higher than in non-smokers (Maliszewsky and Bass 1955). Patients with subacute combined degeneration who smoked had significantly lowered plasma thiocyanate levels than control smokers, but plasma thiocyanate levels in non-smoking patients with neurological disease due to vitamin B_{12} deficiency were not statistically different from control values (Wells et al. 1972).
The uptake of higher amounts of thiocyanate causes pathological states in humans. Development of a struma by iodine deficiency with all symptoms of hyperthyreosis is a known sequence (Moeschlin 1964). Inhibition of the active iodine transport and consequently of the iodine uptake into thyroid gland is the cause for this effect. Barnell and coworkers (1957) reported on frequent occurrence of a thiocyanate psychosis during treatment of hypertension with thiocyanate.

3.2. Thiosulphate-sulphurtransferase

The formation of thiocyanate by an enzymatic reaction was described in 1933 by K. Lang. He proposed the name "rhodanese" for the respective enzyme. The systemic name is now: thiosulphate-cyanide-sulphurtransferase (EC 2.8.1.1) or thiosulphate-sulphurtransferase.
Lang found this enzyme in almost every organ of man, frog, rabbit, cattle, chicken, pigeon, cat, and dog, but neither in blood nor in muscle. Contrary to these findings, Himwich and Sanders (1948) could demonstrate this enzyme in the blood of dogs and in musyle of dogs and Rhesus monkeys. Also Reinwein (1961) found thiosulphate-sulphurtransferase in all organs of man, horse, pig, sheep, cattle, and rat, but not in serum. There is no correlation between the activity of this enzyme and the concentration of thiocyanate within the organs[3]). Regarding the intracellular distribution of this enzyme it is mainly found in the mitochondria (Sörbo 1951 a,b).
The total capacity of detoxification was approximately calculated by Himwich and Sanders (1948). The liver of a dog should be able to detoxicate 4.015 g cyanide within 15 min, the total skeletal muscle apparatus 1.763 g within the same time. This calculation is not met by the poor tolerance of the animal organism against cyanide. The authors, therefore, conclude that the limiting factor for detoxication of cyanide is the availability of the thiosulphate-sulphurtransferase. Possibly also a deficiency of sulphur donators may play a part, since a threefold higher concentration of thiosulphate compared with the cyanide, which is to be detoxicated, is necessary in vitro, to form thiocyanate in sufficient amounts.
An additional reaction, catalysed by rhodanese is described by Villarejo and Westley (1963). The enzyme is supposed also to mediate the reduction of thiosulphate to sulphite and sulphide, which is concluded from investigations with crystallized rhodanese from bovine liver. As reducing agents liponal and liponamide are used. Also the counterreaction was described.

3.3. Cyano- and hydroxocobalamine

In this survey mainly the cyanide compound of cobalamine is of interest, which seems to be important in certain pathological states.
As shown in Scheme 1 vitamin B_{12} shares in the detoxication of cyanide. Two forms of the vitamin have been known for a long time. Depending on the connection of the central cobalt atom with a hydroxyl or a cyanide group (Brink et al. 1950), one has to deal with hydroxo- or aquocobalamine and with cyanocobalamine. When solutions of cyanocobalamine are exposed to light the coordinatively bound cyanide is split off. On the other hand, in neutral solutions hydroxocobalamine can react with excess KCN to form cyanocobalamine.
In addition to the coordinatively bound cyanide, cyanocobalamine can react with two other moles cyanide to form

[3]) Thiosulphate-sulphur-transferase and thiocyanate were also found in bacteria, protozoa, helminthes, mollusca and echinodermata (Schievelbein and Baumeister 1969; Schievelbein et al. 1969; Baumeister 1972).

complexes (Beavan 1950; Conn et al. 1951; Conn and Wartmann 1952).

There are several investigations regarding changes of the B_{12} metabolism in smokers and non-smokers. These are important for the understanding of the pathogenesis of tobacco amblyopia.

A comparative study (Linnel et al. 1968) on the serum B_{12} level, the excretion of vitamin B_{12} in the urine and the excretion of thiocyanate in the urine as parameters for the detoxification of cyanide in healthy smokers and healthy non-smokers showed: in smokers the excretion of B_{12} and SCN^- in urine is increased. A high excretion of thiocyanate in urine is also related to an increase of the B_{12} excretion and to a relatively low B_{12} concentration in serum. The authors assume that the increased excretion does not suffice to explain the decreased B_{12} concentration in serum. This may be rather the result of the disturbed equilibrium between serum and tissue B_{12}, caused by the high plasma cyanide concentration.

Philips et al. (1968) tested aqueous humour of 16 patients, which was collected during cateract operation for the content of vitamin B_{12} microbiologically. Aqueous humour contains an average of 30 pg/ml and thus corresponds in the order of magnitude to the cerebrospinal fluid. There are obvious differences between smokers and non-smokers. The vitamin B_{12} content of aqueous humour in non-smokers is 34.3 pg/ml, in smokers it is only 20.2 pg/ml as mean value ($p < 0.1$).

Cyanide seems to play a special part in the modification of vitamin B_{12} metabolism. Daily injections of 0.7 ml of a 0.1% KCN solution in rats for 6 days decreases the B_{12} reserve of the liver on an average by 20% (Braekkan et al. 1957).

4. Clinical states and their pathogenesis following uptake of higher amounts of cyanide

Occurrence of intoxication symptoms depends upon the velocity of the increase of cyanide in tissues. This decisive factor is determined by the kind of the cyanide compound, by the way of intoxication, by the ingested dose, and by the ability of the organism to detoxicate cyanide. Upon uptake of higher doses death can occur within a few minutes, upon medium doses survival times up to 3 h were observed. Small non-lethal doses may cause headache, vertigo, and irritation of the mucous membranes of the eyes and throat. An injection of cyanide into the A. carotis communis causes a hyperventilation by excitation of the chemoreceptors of the glomus caroticum, as Heymans could show in 1931.

The highly acute form of cyanide intoxication is characterized by an apoplexy-like course. In most cases, the intoxicated person collapses with a short scream; dyspnea progressing to apnea, convulsion collapse and loss of consciousness follow. Death occurs within 1—2 min.

The most frequently occurring form of intoxication is mainly determined by the central hypoxemic symptoms: hyperpnea, unconsciousness, generalized convulsions, and finally paralysis with complete areflexia. In this case agony lasts 15—60 min. The actual cause of death in the acute intoxication is the cessation of respiration, which will take place some minutes before cardiac arrest (Friedberg and Grützmacher 1968).

4.1. Clinical states and their pathogenesis following uptake of cyanide for a long period of time

The chronic uptake of cyanide in small doses, which as single doses do not cause clinical symptoms, is assumed to be decisive for the pathogenesis of a few diseases. An inhibition of some ways of cyanide metabolism are probably responsible in some cases (Schievelbein, Werle et al. 1969; Baumeister 1972). As a result of a chronic cyanide intoxication the following clinical states have been described up to now: tobacco amblyopia, retrobulbar neuritis with pernicious anaemia, optic atrophy of Leber, Nigerian nutritional ataxic neuropathy, sterility in women who are heavy smokers.

4.2. Tobacco amblyopia[4])

For the frequency of the tobacco amblyopia different values have been published by different authors. According to Hollwich et al. (1968) 1000 cases have been described in the total world literature. In contrast, according to Traquair (1930) in the years 1913—1929 only, 1525 cases were registered in two clinics in Edinburgh.

The clinical state which only occurs in smokers, includes as the main symptom a central scotoma. Heaton et al. (1958) demand 7 criterions for the diagnosis (for details see Hollwich et al. 1968). In patients with tobacco amblyopia vascular changes and degeneration of ganglion cells in the retina and of nerve fibres of the nervus opticus were described. Mainly involved are fibres which connect with the macular bundle (Duke-Elder 1954).

Victor, Mancall, and Dreyfus (1960) published one case with centrocecal scotoma of both eyes upon alcohol and tobacco abuse. They described a degeneration of the papulomacular bundle up to the corpus geniculatum laterale with atrophy of the medullary sheaths and axis cylinder as well as fibrous gliosis.

These pathological anatomic findings show a striking parallel to the observations in animal experiments on cyanide administration. Repeated small doses of cyanide cause a demyelinization in the central nervous system in rats and monkeys (Hurst 1942; Lumsden 1950). Leishman (1951) thought to have observed a correlation between amblyopia and deficiency of gastric acid and therefore assumed a connection in the etiology of amblyopia and pernicious anaemia.

The good therapeutic effect of vitamin B_{12} in the tobacco amblyopia suggests also deficiency of vitamin B_{12} as a cause. This can develop by a general malnutrition with decreased uptake of vitamin B_{12}, which is thought by several American authors (Victor 1963; Knox 1970) to be a primary cause. This vitamin B_{12} deficiency can also develop by a transformation of "functional" hydroxocobalamine into "non-functional" cyanocobalamine. Experimental investigations support this hypothesis.

Injection of sublethal doses in rats causes a significant decrease of the vitamin B_{12} store in the liver. These reserves are obviously an important factor in detoxication of cyanide and therefore with high probability are hydroxocobalamine (Braekkan et al. 1957). Nutritional deficiency of vitamin B_{12} causes an increased excretion of SCN^-. From this it is concluded that hydroxocobalamine and thiosulphate-sulphurtransferase compete for the cyanide to be detoxicated (Wokes and Picard 1955b). These and similar results were the cause for investigations on the metabolism of vitamin B_{12} in patients with tobacco amblyopia. In 13 patients with tobacco amblyopia a mean value of 218 pg B_{12}/ml serum was found, whereas the concentration in 12 healthy persons was on an average of 538 pg/ml (Heaton et al. 1958). In this case the method of Mollin and Ross (1952, 1954) with Euglena gracilis as test organism was used. The position of cyanocobalamine in the total B_{12} was significantly higher in patients with tobacco amblyopia compared with healthy persons (Wilson et al. 1971).

[4]) Some authors do not differentiate between tobacco and alcohol amblyopia, but use the name tobacco-alcohol amblyopia. According to Harrington (1962) the amblyopia caused by alcohol can be differentiated from the tobacco amblyopia by exact quantitative perimetric analysis of the scotomas. The findings in tobacco amblyopia include central scotoma with infinite transitions, with the greatest density found in the field between the blind spot and the fixation point. This leads to difficulties in focusing and fixation. In the methyl alcohol amblyopia Harrington describes dense irregular, central scotomas with extreme loss of sight.

For a few years several authors have examined the metabolism of this vitamin with radioactive-labelled vitamin according to the method described by Schilling (1953).
Kommerell and Castrillon-Oberndorfer (1968) found a severe alteration of B_{12} absorption in 2 out of 5 patients with tabacco amblyopia.
On the other hand, Watson et al. (1969) did not find a decrease of the absorption in 15 patients with tobacco amblyopia. But if the patients smoked during the experiment, the absorption of B_{12} decreased.
According to investigations of Foulds et al. (1968), 40% of patients with tobacco amblyopia showed decreased levels of serum B_{12}. In 45% the absorption of vitamin B_{12} was disturbed.
Foulds and coworkers (1969) could demonstrate in patients with tobacco amblyopia a direct correlation between the consumption of tobacco, the vitamin B_{12} absorption, and the B_{12} level in serum. If amblyopia occurred in a patient with a small tobacco consumption the absorption of vitamin B_{12} was insufficient, or the serum B_{12} level was low. If, however, the serum levels were high, the disease became manifest only upon extreme tobacco consumption.
Foulds and coworkers (1968) also investigated the concentration of serum thiocyanate in heavy smokers with tobacco amblyopia and in smokers without tobacco amblyopia, as well as in non-smokers. They found as already Stoa (1957) did, a significantly increased thiocyanate level in serum of smokers compared with the values in non-smokers. In contrast to these findings the thiocyanate concentration in patients with tobacco amblyopia was significantly decreased compared with healthy smokers. Although the patients with tobacco amblyopia did smoke more than the healthy smokers. These findings suggest a possible enzyme defect, a hereditary deficiency of enzymes which are able to form SCN^-.
Surveying the discussed results, approximately the following preliminary pathogenetic mechanism of the tobacco amblyopia may be suggested:
The patients take up higher amounts of cyanide with the tobacco smoke.
The detoxication of cyanide is disturbed by

a) a deficiency of available hydroxocobalamine,
   by an insufficient nutritional administration,
   by a disturbed absorption,
   by an increased transformation into cyanocobalamine;
b) by a hereditary deficiency of enzymes which are able to form SCN^-.

The insufficiently detoxicated cyanide takes part in the impairment of the central nervous system.

4.3. Retrobulbar neuritis in pernicious anaemia

Besides the tobacco amblyopia, which is known to be frequently combined with pernicious anaemia, also a retrobulbar neuritis is described in pernicious anaemia, a seldom, but well known complication, which also may precede Biermer's anaemia (Cohen 1936). On the other hand, Freeman (1961) stated: "We have presented evidence that tobacco amblyopia and retrobulbar neuritis in pernicious anaemia are one and the same condition".
The histological findings at the nervus opticus (Bickel 1914) in the retrobulbar neuritis proved to be similar to those which are found in tobacco amblyopia (Duke-Elder 1954). Since up to now only one investigation has been published concerning the pathological anatomic findings in the retrobulbar neuritis in connection with pernicious anaemia, confirmation is still pending (Freeman 1961).

4.4. Leber's optic atrophy

This disease was described in 1871 by Leber as a hereditary one, in which an acute or subacute decrease of sight occurs together with the development of bilateral central scotomas. Leber's optic atrophy is found mostly in young men of about 20 years, though the age distribution varies in a wide range. 15% of the patients are women. In some patients diffuse encephalo-myelopathic complications occur either simultaneously with the decrease of sight or later (Innamura and Ichikawa 1919; Ferguson and Gritchky 1928; Colenbrander 1951; Enghoff 1963; Wilson 1963; Lees et al. 1964).
But the manifestations of the disease are extended to the central nervous system and to the eyes. Considering the hereditary nature of this disease and the clinical symptoms Wilson (1965a) concluded that the disease is caused by an enzymatic impairment, the manifestations of which are triggered by exogenous factors, at least the danger for the visual apparatus.
The question of an enzymatic impairment could not be answered clearly by measuring the activity of the thiosulphate-sulphurtransferase.
In liver tissue from needle biopsy a decreased activity of thiosulphate-sulphurtransferase was found for this patient compared with a control person. The liver of this patient showed at the post mortem examination a somewhat smaller enzymatic activity compared with 2 control livers. The enzyme content of a brain sample was, however, somewhat higher in the patient with Leber's optic atrophy compared with a control person.
Wilson suggested smoking as an exogeneous factor for the manifestation of the visual disorder. In healthy smokers the blood level and the excretion in urine of thiocyanate is increased compared with non-smokers, as mentioned above. In smokers with Leber's optic atrophy the increase of the thiocyanate level in blood is less pronounced or even absent.
Patients with Leber's optic atrophy, smokers as well as non-smokers, show a significantly higher portion of cyanacobalamine in the total B_{12} content in serum compared with healthy persons. The ratio of methylcobalamine to coenzyme B_{12} plus hydroxocobalamine is clearly decreased in Leber's optic atrophy (Wilson et al. 1971). The few adult patients with Leber's optic atrophy included, according to the observation of Wilson, 4 female and 2 male patients in whom the incidence of the symptoms was preceded by an acute pyelonephritis. In 2 children from families with Leber's optic atrophy the impairment of vision occurred in connection with an infection of the urinary tract (Wilson 1967). Adams et al. (1966) got experimental clues that the infection of the urinary tract with Ps. pyoceaneus and E. coli may contribute to the development of Leber's optic atrophy by formation of cyanide. Smith decribed already in 1964 infections as a source for cyanide. This could explain the rare cases in which also non-smokers are affected by this disease.

4.5. Nigerian nutritional neuropathy

The fully developed ataxic symptom of this disease, which occurs in Nigeria, exists of an atrophy of the nervus opticus, deafness and sensory spinal ataxia (Money 1958). The tropical amblyopia, probably a fragment form of the fully developed syndrome, was primarily explored in Nigeria by Moore (1930). Together with the tropic amblyopia and the ataxic syndrome, the literature also contains data on the occurrence of angular stomatitis, glossitis, and a dermatitis of the scrotum (for lit. see Osuntokun et al. 1969).
On account of these symptoms several authors supposed that this disease could be caused by a deficiency of vitamin B_{12}. The frequent occurrence of the combined symptoms in the Nigerian population, which takes up a plant containing cyanide, namely Cassava (s. Table 1), as a main food source, led Clark (1935) to the supposition that the visual impairment in tropical amblyopia is caused by a cyanide intoxication as a result of a great consumption of Cassava.
Cassava contains linimarine, a cyanogenic glycoside. Furthermore, the plant contains a hydrolase (linase) which quickly liberates considerable amounts of cyanide if the leaves, the stem, or the starch containing roots are damaged. One Cassava species (Manioc utilissima), which is used in Nigeria most, has specially high concentrations of cyano-

genic material in the outer layers of the tuberous root. From there free cyanide gets into the inner part of the plant and thus can intoxicate the eatable parts (Osuntokun et al. 1968). At a comparison between the concentration of thiocyanate and the level of vitamin B_{12} in plasma of Nigerian patients who suffered from degenerative neurologic symptoms, and in plasma of healthy persons, the suggestion was confirmed that the patients had been confronted with a considerably high cyanide uptake. The thiocyanate concentration was highly significant in the patients. Also increased SCN^- supply could be causative for this increased SCN^- concentration. As a source for SCN^- mainly milk and cruciferae may be considered. Consumption of both is almost zero in Nigeria. Therefore, as a main source only the Cassava plant is left.

On determination of the total vitamin B_{12} content, no differences were found between patients and healthy persons. However, the plasma content of hydroxocobalamine is smaller in patients than in healthy persons (Monekosso and Wilson 1966).

A comparison of the frequency of this disease in two Nigerian villages with institutional habits also shows that the uptake of Cassava plays a causative role in the degenerative neuropathia. In the village with the higher Cassava consumption the disease occurs with relatively high percentage. In the patients increased plasma levels of thiocyanate are found (Osuntokun et al. 1969).

A diminution or a complete absence of the sulphur-containing amino acids cysteine and methionine in plasma is also observed in this disease. It seems to be possible that the detoxication of cyanide via formation of 2-amino-4-thiazolidine-carboxylic acid is prevented because of the lack of cysteine (Osuntokun et al. 1968).

4.6. Sterility in female heavy smokers

According to a report of Heller, the rhodan content of the cervix mucus is between 0.2 and 1.0 mg/100 ml. The highest values are measured during the secretion phase. In 7 women with sterility which could not be clarified, rhodan concentrations were found between 1.0 and 2.3 mg/100 ml. In two heavy smokers values above 5 mg/100 ml were measured. It seems to be certain that values above 1.0 mg can damage the spermatozoa. The sterility of female heavy smokers, which can frequently be observed, according to Heller (1960) could be explained in this way. Bettochi and Marcelli (1963), however, could not find an influence of smoking on the fertility of women.

5. Treatment of cyanide intoxication

5.1. Treatment of acute cyanide intoxication

The therapeutic aim is the lowering of the cyanide concentration in blood and tissue. This can be reached by specific and unspecific detoxication measures. A combination of both seems to be the optimal solution.

5.2. Unspecific measures

The elimination of HCN can be accelerated by forcing respiration. If the respiration is intensified in HCN-free air, the partial pressure for HCN decreases in the alveoli. The concentration gradient to the blood plasma is increased so that the highly volatile gas, which is dissolved in plasma, can be eliminated. Artificial respiration, therefore, is recommendable. Friedberg and Schwarzkopf (1969) found during spontaneous respiration only a small elimination of HCN via the lung. It amounted to 1 to 2% of the infused dose. Artificial respiration, however, increased the elimination of CN^- 3—4fold. Also in animal experiments, Friedberg and Grützmacher (1968) observed that solely by a stimulation of respiration the intoxication can be lessened.

Sheehy and Way (1968) found, in survival experiments in rats, after administration of cyanide that oxygen solely or combined with thiosulphate and sodium nitrite has a higher effectiveness than thiosulphate given alone. In these experiments an LD_{50} of 31.5 mg/kg potassium cyanide was determined if 0.5g $Na_2S_2O_3$/kg was given as antidote together with normal air. The LD_{50} was increased to 63.8 mg/kg potassium cyanide, if 100% pure oxygen was given.

The adminstration of oxygen is based on the idea that an eventual arterial hypoxia, caused by an early paralysis of the respiratory centre, can be compensated in this way.

Moeschlin recommends a high-pressure oxygen respiration in addition. According to Friedberg and Grützmacher (1968) the so-called cyanide resistant respiration could be of greater importance under the influence of an increased oxygen pressure.

5.3. Specific measures

Table 3 summarizes the specific measures applied in an acute cyanide intoxication.

Sodium thiosulphate has a very high detoxication capacity (Lang 1895). A decisive handicap, however, is the relatively slow rate of action. Only after several minutes a therapeutic effect is seen (Friedberg 1968). In very severe cases of intoxication with pending respiratory arrest the onset of detoxication of thiosulphate is too slow.

A combined administration of sodium thiosulphate with the detoxicating enzyme thiosulphate-sulphurtransferase was described by Clemedson et al. (1954).

Producers of methemoglobin, primary nitrites (sodium nitrite for injection, amyl-nitrite for inhalation), have been used for 40 years in the treatment of cyanide intoxication (Hug 1932; Geiger 1933; Chen et al. 1933 a, b,). Methemoglobin (ferric hemoglobin) transforms cyan-methemoglobin with cyanide binding the cyanide residue to the trivalent iron of the oxidized hemoglobin. At a total hemoglobin amount of an adult person of about 1000 g and at a formation of hemoglobin of 10%, that is 100 g methemoglobin, theoretically 156 mg CN^- can be bound. This approximately corresponds to the lethal dose (Friedberg 1968).

According to Chen and Rose (1952) the combination of nitrites with thiosulphate has proved therapeutically successful in animal experiments and in cases of intoxication.

A new development in the field of methemoglobin producers are aminophenols, which have been recommended since 1965 by Kiese and Weger (1965 a, b) for the detoxication of cyanide. 4-Dimethylaminophenol, so-called "new antidote", exerts the quickest action in human blood compared

Table 3: Specific therapeutic measures in acute cyanide intoxication in men.

Substance	Dose on i.v. injection	Mechanism of action	Literature
Sodium thiosulphate	1—5 g 12—25 g	Sulphur donator	Klimmer (1971)
Sodium nitrite*)	300 mg	Producer of methemoglobin	Moeschlin (1964)
4-Dimethylaminophenol	2.5—3.25 mg/kg	Producer of methemoglobin	Weger (1968), Klimmer (1971)
Cobalt histidine	20 mg/kg	Cyanide binding	Klimmer (1971)
Cobalt ethylenamine-tetraacetic acid (cobalt EDTA)	300—600 mg	Cyanide binding	Moeschlin (1964)
Hydroxocobolamine	5—15 g	Cyanide binding	Friedberg (1968)

*) Should not be used.

with other tested aminophenols. A methemoglobin concentration of 15% was already reached in less than 1 min (Weger 1968, 1969; Kiese and Weger 1969). The optimum concentration of methemoglobin for the treatment of a cyanide intoxication is about 50% (Lörcher and Weger 1971).

Also with respect to its action on respiration and circulation, 4-dimethylaminophenol is superior to nitrite and to the cobalt compounds: cobalt-histidine, cobalt-EDTA and to hydroxocobalamine. A model intoxication in cats with 8 mg KCN/kg showed that on treatment with hydroxocobalamine 2 out of 3 animals died, on treatment with cobalt-EDTA 3 out of 4. On treatment with $NaNO_2$ 7 out of 8 animals died whereas only 1 out of 8 animals died on treatment with 4-dimethylaminophenol-HCl (Weger 1969).

In men 30% hemoglobin could be oxidized with doses of 4-dimethylaminophenol, which had only little effect on blood pressure and pulse. Doses of sodium nitrate, which are recommended for therapy and which have a comparable effect, produced collapse. The formation of methemoglobin only reached a maximum concentration of 6% within 30 min (Weger 1969; Kiese et al. 1968). Instead of methemoglobin producers some authors recommend the application of cobalt compounds: hydroxocobalamine (Mushett et al. 1952), cobalt-EDTA, and cobalt-histidine (Paulet 1957). These compounds have the advantage of reacting already within the plasma with the cyanide forming complexes with it (Friedberg 1968).

The application of these cobalt compounds, however, seems to be dangerous; for, without proceeding HCN-application they cause a diminution of respiratory rate and a decrease of the blood flow of the A. carotis according to Weger (1969). These effects are brought about by cobalt-EDTA and cobalt-histidine to a higher degree than by hydroxocobalamine. Therapeutically necessary doses of cobalt-EDTA are lethal in experimental animals within 40 min (Weger 1969; Offterdinger and Weger 1969).

In a comparison of the velocity of action and the detoxication capacity of 4-dimethylaminophenol and cobalt-histidine using the HCN-exhalation as a measure, 4-dimethylaminophenol shows the same velocity of action as cobalt-histidine but a considerably longer duration of action (Schwarzkopf and Friedberg 1970). Schwarzkopf and Friedberg, however, point to the additional risk for a patient intoxicated with cyanide by the increased methemoglobin content of the blood which is caused by application of 4-dimethylaminophenol.

5.4. Treatment of chronic cyanide intoxication

In diseases which are caused by chronic application of cyanide, the further application of HCN must be stopped as a primary measure. Currently, the medical treatment consists of application of high doses of hydroxocobalamine.

In 1958 Wokes recommended the application of vitamin B_{12} p.o. in frequent small doses in consideration of the etiology of the tobacco amblyopia.

The action of hydroxocobalamine and cyanocobalamine has been investigated experimentally and clinically in recent times.

The premedication of mice with 50 mg/kg hydroxocobalamine prevents the toxic symptoms and the death of the animal which are caused by i.p. application of 5.5—8.0 mg/kg potassium cyanide. Cyanocobalamine does not show this protective effect. If the animals already showed respiratory arrest and unconsciousness caused by cyanide intoxication, most of the animals recovered quickly after the application of hydroxocobalamine. If the mice are given potassium cyanide by hydroxocobalamine, part of the cyanide appears in the urine as SCN^- (3.5%), as free cyanide (0.7%), and as cyanocobalamine (9.6%) within 2.5 h (Mushett et al. 1952).

If the rats are given KCN as s.c. injections up to 4 mg daily for 3 weeks, the treatment with hydroxocobalamine proved to be obviously superior to cyanocobalamine. The excretion of SCN^- in urine increased in the experimental animals. The highest increase was observed in the group treated with hydroxocobalamine. The histological examination of the central nervous system revealed a demyelination in the untreated animals. The simultaneous administration of hydroxocobalamine protected the animals against these changes. The administration of cyanocobalamine, however, did not have this effect. The authors of this investigation (Smith and Duckett 1965) suppose that this extraordinary effect is caused by a mechanism of action which surpasses the merely chemical reaction between cyanide and hydroxocobalamine. The authors discuss a general protective function of vitamin B_{12} for the myelin.

Most of the cases of tobacco amblyopia improve if the patients stop smoking, but often this measure is not sufficient (Griffith 1897). In smokers who continued smoking without reduction of the amount, the amblyopia got better much more quickly if treated with cyanocobalamine compared with a sole abstinence of smoking. Heaton et al. (1958) administered 100 mg of cyanocobalamine parenterally two times weekly for one month, another two months only once every two weeks, and finally the same dose once every month. If there are no more clinical symptoms, the authors recommend discontinuing the treatment after 6 months. As commercial preparations of cyanocobalamine contain small amounts of hydroxocobalamine, the latter substance may be the substance actually effective in the therapeutic experiments with "cyanocobalamine" (Smith and Duckett 1965). Chisholm et al. (1967) divided patients with tobacco amblyopia into two therapeutic groups. One was treated with i.m. injections of hydroxocobalamine, the other with cyanocobalamine. The improvement of visual acuity in the group treated with hydroxocobalamine was significantly higher compared with the group treated with cyanocobalamine. Also the chromatic vision was improved to a higher degree under treatment with hydroxocobalamine compared with cyanocobalamine. In patients who did not stop smoking, total healing could not be achieved in a single case with cyanocobalamine medication, but healing could be achieved with hydroxocobalamine.

Bronte-Stewart et al. (1968) report on the treatment of a patient with metabolic encephalopathy, tobacco amblyopia, and a lung abseess. The encephalopathy responded to the treatment with cyanocobalamine, the tobacco amblyopia, however, did not improve until hydroxocobalamine was administered.

These clinical improvements are paralleled by biochemical changes in the body of the patient. Chisholm and Pettigrew (1970) could show that the plasma thiocyanate concentration of their patients increased significantly and reached the values of healthy smokers after treatment with hydroxocobalamine for 6 months. Also, the thiocyanate excretion in urine was increased.

According to Kommerell and Castrillon-Oberndorfer (1968) the following schedule of treatment for the tobacco amblyopia seems to be substantiated (corresponding to the schedule for patients with pernicious anaemia according to Glass 1964; Heinrich 1964; Waller and Castrillon-Oberndorfer 1968): Every two days 1 ampule of hydroxocobalamine at 500 mg i.m., 22 injections. Maintenance therapy with 1 ampule at 500 mg hydroxocobalamine once a month. Under this therapy the sight can be improved essentially, even if no abstinence of tobacco and alcohol can be achieved.

For treatment of Leber's optic atrophy Adams et al. (1966) recommended the administration of massive doses of hydroxocobalamine besides the strict prohibition of smoking and the intense treatment of an eventual infection as cyanide source. Chisholm and Pettigrew (1970) found, after treatment of these patients with hydroxocobalamine for 12 months, an increase of the plasma thiocyanate concentration and an increase of the thiocyanate excretion in urine. This corresponds to the results of the treatment of the tobacco amblyopia. The improvement of sight, however, was only slight in these cases.

6. Literature

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