

NESTEL

**ARCHIV
NESTEL
26528**

RC-010e

Chronic Cassava Toxicity

Proceedings of an interdisciplinary workshop
London, England, 29-30 January 1973

Editors: Barry Nestel and Reginald MacIntyre



INTERNATIONAL
DEVELOPMENT
RESEARCH CENTRE

CENTRE DE RECHERCHES
POUR LE DÉVELOPPEMENT
INTERNATIONAL

Ottawa, Canada

1973

IDRC-010e

CHRONIC CASSAVA TOXICITY

**Proceedings of an interdisciplinary workshop
London, England, 29–30 January 1973**

Editors: **BARRY NESTEL AND REGINALD MACINTYRE**

008817

UDC: 615.9:547.49
633.68

© 1973 International Development Research Centre
Head Office: Box 8500, Ottawa, Canada. K1G 3H9
Microfiche Edition \$1

Contents

Foreword	Barry Nestel	5-7
Workshop Participants		8-10
Current utilization and future potential for cassava	Barry Nestel	11-26
Cassava as food: toxicity and technology	D. G. Coursey	27-36
Cyanide toxicity in relation to the cassava research program of CIAT in Colombia	James H. Cock	37-40
Cyanide toxicity and cassava research at the International Institute of Tropical Agriculture, Ibadan, Nigeria	Sidki Sadik and Sang Ki Hahn	41-42
The cyanogenic character of cassava (<i>Manihot esculenta</i>)	G. H. de Bruijn	43-48
The genetics of cyanogenesis	Monica A. Hughes	49-54
Cyanogenic glycosides: their occurrence, biosynthesis, and function	Eric E. Conn	55-63
Physiological and genetic aspects of cyanogenesis in cassava and other plants	G. W. Butler, P. F. Reay, and B. A. Tapper	65-71
Biosynthesis of cyanogenic glucosides in cassava (<i>Manihot</i> spp.)	Frederick Nartey	73-87
Assay methods for hydrocyanic acid in plant tissues and their application in studies of cyanogenic glycosides in <i>Manihot esculenta</i>	A. Zitnak	89-96
The mode of cyanide detoxication	O. L. Oke	97-104
Chronic cyanide toxicity in domestic animals	D. C. Hill	105-111
Implications of cyanide toxicity in animal feeding studies using high cassava rations	Jerome H. Maner and Guillermo Gómez	113-120
Cyanide and human disease	J. Wilson	121-125
Ataxic neuropathy associated with high cassava diets in West Africa	B. O. Osuntokun	127-138
Endemic goitre and high cassava diets in eastern Nigeria	O. L. Ekpechi	139-145
Evidence of an antithyroid action of cassava in man and in animals	F. Delange, M. van der Velden, and A. M. Ermans	147-151
Mechanism of the goitrogenic action of cassava	A. M. Ermans, M. van der Velden, J. Kinthaert, and F. Delange	153-157
Summary of the General Discussion		159-162

The Mode of Cyanide Detoxication

O. L. OKE

*Department of Chemistry
University of Ife, Ile-Ife, Nigeria*

OKE, O. L. 1973. The mode of cyanide detoxication, p. 97-104. *In* Chronic cassava toxicity: proceedings of an interdisciplinary workshop, London, England, 29-30 January 1973. Int. Develop. Res. Centre Monogr. IDRC-010e.

Abstract The mode of cyanide detoxication in the body is reviewed. The high amount of thiocyanate found in the urine, saliva, and blood of people who eat a lot of cassava was from the detoxication of the cyanide by the enzyme rhodanese which, through combination with thiosulfate or colloidal sulfur, forms a polysulfide chain that can react with cyanide to release sulfur in a suitable form to give thiosulfate. This enzyme represents the chief site of detoxication and occurs in all parts of the body with the largest concentration in the liver.

Cyanocobalamin (vitamin B₁₂) occurs in the liver to some extent as the hydroxocobalamin (vitamin B_{12a}) which is capable of reacting with cyanide to give cyanocobalamin and hence another important independent pathway for cyanide detoxication. 3-mercaptopyruvic acid, arising from cysteine by transamination or deamination can provide sulfur as rapidly as thiosulfate for cyanide detoxication.

Cystine reacts with cyanide to form cysteine and β -thiocyanoalanine which tautomerises to 2-aminothidizoline-4-carboxylic acid or its equivalent 2-imino-4-thiazolidine carboxylic acid which is excreted.

Finally the thyroid gland shows some detoxicating effect. In the presence of powdered sheep thyroid the lethal dose of acetonitrile for mice was 1.4 mg/g whereas it was 0.32 mg/g for those not fed the powdered sheep thyroid.

Résumé L'auteur passe en revue le processus de désintoxication du cyanure dans l'organisme. La forte quantité de thiocyanate trouvée dans l'urine, la salive et le sang de personnes qui mangent beaucoup du manioc provient de la désintoxication du cyanure par l'enzyme rhodanèse. Cet enzyme, en se combinant avec le thiosulfate ou avec le soufre colloïdal, forme une chaîne polysulfurée qui peut réagir avec le cyanure pour libérer du soufre sous une forme capable de donner du thiosulfate. Cet enzyme est le site principal de désintoxication et se rencontre dans toutes les parties du corps, mais en plus fortes concentrations dans le foie.

On trouve une certaine quantité de cyanocobalamine (vitamine B₁₂) dans le foie. Il en est de même de l'hydroxycobalamine (vitamine B_{12a}) qui peut réagir avec le cyanure pour donner de la cyanocobalamine et qui est donc une autre voie métabolique indépendante importante pour la désintoxication du cyanure. L'acide 3-mercaptopyruvique, provenant de la cystéine par transmission ou désamination, peut fournir du soufre aussi rapidement que le thiosulfate pour la désintoxication du cyanure.

La cystine réagit avec le cyanure pour former la cystéine et la β -thiocyanoalanine qui se transforme par tautomérisation en acide 2-aminothidizoline-4-carboxylique ou en son équivalent, l'acide 2-imino-4-thiazolidine carboxylique qui est éliminé.

Finalement, la glande thyroïde joue un certain rôle désintoxicant. En présence de thyroïde de mouton en poudre, la dose létale d'acétonitrile chez les souris est de 1.4 mg/g, alors qu'elle est de 0.32 mg/g chez celles qui n'ont pas été nourries de thyroïde de mouton.

A problem that has long been of toxicological and physiological importance is the source of small amounts of thiocyanate found in urine, blood, and saliva. Schmiedeberg (1867) isolated sodium thiosulfate as the barium salt from the normal urine of cats and dogs, and Fromageot and Royer (1945) showed it to be a normal metabolite in higher animals although the mechanism of its formation is obscure. Vassel et al. (1944) found that dogs excreted 2–15 mg thiosulfate-sulfur in 24 h whereas humans excreted 50–125 mg. Gast et al. (1950) reckoned human beings excreted about 20 mg thiosulfate-sulfur in 24 h. At first this was thought to be due to small amounts of this substance present in foodstuffs. Wokes et al. (1952) reported concentrations of 0.1–10 ppm in cow's milk, i.e. about 5.7–57 mg/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 ranged from about 30 to 950 mg/100 g with the higher figures in cabbage, carrots, and radishes. Wilson (1966) gave much higher figures: 4.1 mg/100 g for sprouts, 1.9 for caneflower, and 0.4 for peas and tomatoes. I found (1970 unpublished data) 0.2–0.5 mg/100 g for cassava products (gari and lafun). The highest values were obtained by Williams (1967 personal communication) for cassava and its products. He obtained 600 mg/100 g for gari, 700–800 for lafun, and 500–600 for yam flour. If we assume these figures are correct, they lend support to the view that thiocyanates are mainly derived from food, increasing with heavy smoking (Lawton et al. 1943) or heavy consumption of beer or strong tea, milk, eggs, and other animal protein sources, all of which contain some preformed thiocyanate (Wokes and Pikard 1955; Wedgewood and Wyatt 1952).

However, if we look at the thiocyanate content of some of the commonly eaten foods in Nigeria, it becomes obvious that ingestion of these will not be sufficient to account for all the thiocyanate observed in ataxic neuropathy. Even if we assume a consumption of 2 kg gari/day, this will only give 8 μ moles thiocyanate/day which will rapidly diffuse through the body fluids and be cleared through the kidneys. On the other hand ingestion of 2 kg gari or lafun will result in about 54 and 200 μ moles of hydrocyanic acid and hence it will be reasonable to assume that the high thiocyanate content is from the cyanide detoxication.

Lang (1894, 1895) showed that injections of cyanide or aliphatic cyanides into rabbit increased the thiocyanate excretion and this has been confirmed by Heymanns and Mesoin (1896). Pascheles (1894) showed by in-vitro studies that when liver or muscle tissue from a dog was digested with NaCN, thiocyanate was produced, the liver tissue being more active than the muscle. Kahn (1912) found from a series of perfusion experiments with liver that the amount of thiocyanate produced increased with the number of perfusion trips and hence confirmed that the liver was an active factor in the production of thiocyanate.

Attention was therefore focused on thiocyanate as a possible detoxication product of cyanide in foods. Lang (1933a, b) postulated the existence of an enzyme he called 'rhodanese' (since it synthesises rhodanate) to be responsible for the reaction under aerobic conditions in the presence of thiosulfate or colloidal sulfur:



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 thiosulfate respectively. The rate of the reaction increases with the temperature up to 38°C and the reaction follows the Schurtz rule, i.e. $K = XN_e t$, where N_e = the concentration of the enzyme, X = the amount of substrate transformed in time t , and K is constant. Moreover, the enzyme is widely distributed 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 the 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 (Mendel et al. 1946; Bernard et al. 1947a, b; Himwich and Saunders 1948).

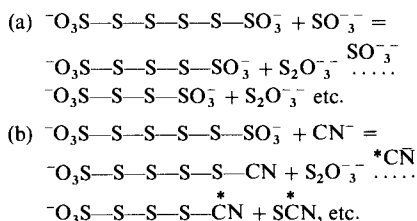
The amount of rhodanese in the liver varies with different animals so that detoxication may be expected to take place at different rates. Himwich and Saunders (1948) found the following levels for the livers of animals:

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.

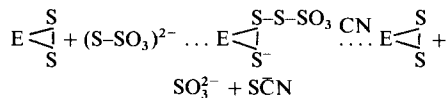
This probably explains the results obtained by

Mukerji and Smith (1943) that nearly all the cyanide ingested by rabbits was recovered in the form of thiocyanate in the urine in 24–48 h whereas in dogs less than 25% was recovered in 7 days. On the other hand, the activity of the enzyme in parts of the brain and the central nervous system seemed to be the same for the different species of animal and this may account for the LD50 for intravenously injected NaCN to be about the same for all species.

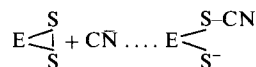
Subsequent work of Saunders and Himwich (1950) has thrown further light on the functions of rhodanese. They proposed that the enzyme forms a loose combination with thiosulfate which breaks down to yield sulfur in a form that can be accepted by the cyanide ion. They explained that the inhibitory effect of certain sulfur-containing compounds like sodium sulfide, dithiobiuret, and cysteine were due to the blocking of the enzyme so that it cannot combine with thiosulfate. They found, in agreement with Lang (1953a, b), that certain divalent cations like Cu^{2+} and Fe^{2+} produce significant inhibitions, whereas others have none. On the contrary, Sorbo (1951a, b) did not obtain any inhibition with cystine alone indicating that the cystine must therefore be easily displaced by thiosulfate giving rise to a situation where thiosulfate, sulfite and other sulfhydryl compounds can react with the active group in the enzyme. This can easily be explained if the active group is a disulfide linkage like the polysulfides whose mechanism of reaction has now been fully worked out, e.g. the first step in the reaction of hexathionate with sulfite or cyanide is ionic displacement of the thiosulfate group by the sulfite or cyanide (Foss 1950):



Sorbo (1951a, b, 1953) therefore suggested that rhodanese contains an active disulfide group (rather than -SH groups) which reacts in an analogous way as above by hydrocyanolysis of the first disulfide compound formed by the action of thiosulfate on rhodanese, followed by splitting off of sulfite and formation of thiocyanate:



The effect of inhibitors can also be explained by this mechanism. Thus when cyanide is first added before thiosulfate or if sulfite and other sulfur-compounds acting as inhibitors are used, this will lead to the formation of a complex which no longer has a disulfide linkage and so is inactive:



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 min, yet only small doses are required for toxicity. They showed that minced tissues converted about twice as much cyanide to thiocyanate as sliced tissues while homogenates converted about 5–17 times as much as sliced and 3–8 times as much as minced tissues and so it appears that permeability factors may play an important part in detoxication irrespective of the quantity of rhodanese. Moreover the reaction does not proceed efficiently unless a thiocyanate concentration of at least three times molar concentration of cyanide is present, a concentration that may not possibly exist in the cell. Thus the availability of sulfur may limit the detoxication possible *in vivo*. This has been supported by Chen et al. (1934) who found that the injection of thiosulfate is capable of increasing the minimum lethal dose by a factor of 3–4. The high concentration of thiosulfate required is not possible as it penetrates the tissues very slowly whereas cyanide penetrates fast. Giliman et al. (1946) found that 70–80% of injected thiosulfate is excreted unchanged. On the other hand, other sources of sulfur such as cystine, thiourea, sodium sulfide, etc. are not directly effective. Even if it is assumed that certain enzymes convert the sulfur of amino acids to sulfide and then to thiosulfate (Smythe 1942; Fromageot and Monlbasher 1938; der Carabedian and Fromageot, 1943) this formation will proceed so slowly that it will only be of limited value.

Meister (1953) found that 3-mercaptopyruvic acid might arise from cysteine by transamination or deamination and this compound can provide sulfur as rapidly as thiosulfate for cyanide detoxi-

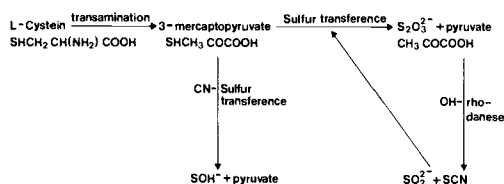
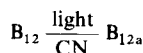


FIG. 1. The cycle formed by the reaction of cyanide with 3-mercaptopyruvate.

cation. He found that crude extracts of liver converted this compound into pyruvic acid and free sulfur at pH 7.5–8.5. Since rhodanese utilises only thiosulfate or colloidal sulfur, β -mercaptopyruvic acid could be a sulfur 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 cyanide to thiocyanate as rapidly with β -mercaptopyruvic acid as with thiosulfate, requiring the same ratio of reagent:cyanide (3:1) as thiosulfate and the same optimum pH of 9.1. Apart from this, cyanide can react with 3-mercaptopyruvate to yield thiocyanate and pyruvic acid (Fiedler and Wood, 1956). The 3-mercaptopyruvate then combines with the cyanide by means of sulfur-transferase to form thiocyanate and pyruvic acid. If sulfite is present, thiosulfate and pyruvic acid are formed from the 3-mercaptopyruvate. The thiosulfate can then be utilised by rhodanese to give sulfite and thiocyanate. The sulfite formed is again ready for use by the sulfur transferase. A cycle is thus formed as shown in Fig. 1.

Although 3-mercaptopyruvic acid has not been detected as a deamination product of cystine, Smythe (1942) found his desulfhydrase to produce pyruvic acid, hydrogen sulfide, free sulfur and ammonia from cystine, a product similar to what had been obtained by Meister (1953) using homogenised rat liver. Administration of ^{35}S -labelled cystine to animals immediately prior to cyanide dosing yields labelled thiocyanate, confirming the feasibility of this cycle.

In the presence of light, vitamin B_{12} (cyanocobalamin) is converted to vitamin B_{12a} (hydroxocobalamin), the latter can react with cyanide to regenerate vitamin B_{12} (Kaczka et al. 1950):



The reaction of vitamin B_{12a} with cyanide to form B_{12} is irreversible and so this may be beneficial in

cyanide poisoning and thereby provide an independent pathway for cyanide detoxication. The great affinity of vitamin B_{12a} for cyanide is due to the presence of cobalt in the molecule; copper will display the same property. Mushet et al. (1952) injected mice intraperitoneally with KCN preceded or followed by the intravenous administration of vitamin B_{12a} or physiological saline solution. The prophylactic effect of B_{12a} became apparent within 20 sec in reducing or preventing, in some cases, the mortality, respiratory distress, and convulsions. Doses of up to 50–250 mg/kg of B_{12a} were adequate in protecting against 5.5–8.0 mg KCN/kg and if administered not later than 1 min after the KCN dose the above symptoms disappeared immediately and death was prevented. Urine samples collected over a period of 2.5 h showed that about 9.6% of the cyanide administered could be accounted for as vitamin B_{12} and 3.5% as thiocyanate.

Injection of sublethal doses of cyanide to rats causes a significant depletion of the liver store of vitamin B_{12} indicating that this store is an important site of detoxication during cyanide poisoning and hence must be mainly in the form of vitamin B_{12a} . The fact that stress conditions such as menstruation, pregnancy, and lactation, which increase the requirement for vitamin B_{12} , cause an increase in thiocyanate excretion, confirms the suspicion that B_{12} may be involved directly or indirectly in the formation of thiocyanate in the body. This is further confirmed by the finding that dietary deficiency of vitamin B_{12} leads to increased thiocyanate excretion and that injections of sublethal doses of cyanide to rats cause a significant depletion of the liver store of vitamin B_{12} , indicating that this is an important detoxifying agent during cyanide poisoning (Smith 1961).

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 nontoxic to mice, despite the fact that this dose is equivalent to 32 mg hydrocyanic acid or eight times the minimum lethal dose for mice (Mushet et al. 1952). These workers therefore proposed vitamin B_{12} as an antidote to cyanide poisoning in mice. This, of course, assumes that some of the vitamin occurs in the hydroxol form which can be replaced by the cyano group. Baxter et al. (1953) showed 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_{12a} . Undoubtedly some vitamin B_{12} exists in the liver as the hydroxo form. Even assuming that all the vitamin B_{12} in the liver occurs in this form, the total amount will be less than 1000 mg (Drouet et al. 1953) and the very small amount of cyanide that can be detoxified will be equivalent to about 25 mg.

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 is detoxified by rhodanese with the help of sulfur donors, such as sulfur-containing amino acids or their products of metabolism, to thiocyanate which is all excreted in the urine with very little in the faeces (Meister and Pries, 1949). Some of the cyanide combines with the hydroxocobalamin to form cyanocobalamin which then carries out various metabolic functions. Vitamin B_{12} can lose some of the cyanide to supply the 1-carbon fragment for the synthesis of important compounds such as choline and other labile methyl 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 breath. This has been confirmed by Boxer and Rikards (1952) who demonstrated that labelled ^{14}C given to dogs could quickly be recovered as exhaled carbon dioxide and in the ureide carbon of allantoin as well as in vitamin B_{12} and thiocyanate. Thus it appears that cyanide is incorporated into the 1-carbon metabolic pool probably in the form of formate. The higher activity of formate from the liver than any other constituents makes it a probable intermediate for this conversion with vitamin B_{12} as a possible intermediary, since ^{14}C isotope could be recovered from cyanocobalamin.

Finally some cyanide is liberated from vitamin B_{12} by the enzyme cyanocobalamin decyanase which then returns to the liver as the hydroxo form, thus completing the cycle as shown in Fig. 2.

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

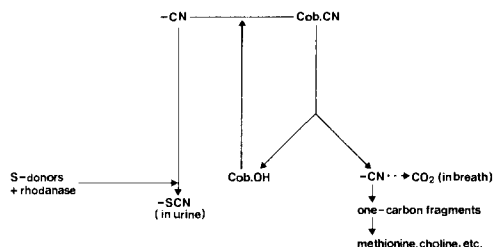


FIG. 2. Hypothetical scheme of cyanide utilization.

thiocyanate derivative, thiocyanate-cobalamin. Although this form has never been isolated from the liver, it may be because it is very labile and less stable than the cyano form and also it would be difficult to distinguish between the two forms. This is as effective as the cyanide form (Butts et al. 1951). Some sulfur is lost from the thiocyanate form so that some B_{12} is left in the cyano form. The sulfur is given up to some active intermediate compound to form a thio compound which may be a precursor of some biologically important sulfur-containing compounds such as sulfur amino acids, glutathione, 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 Fig. 3.

This hypothesis is supported by 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 sulfur amino acid donors which could otherwise have been utilised for some other purposes. If methionine is the donor, as has been shown by Hartman (1949) and Hartman and Wagner (1949) on the thiocyanate excretion in liver diseases, it means there will be a decrease in the reserved sulfur in this form. On the other hand if cysteine and cystine are the donors, as shown in sheep by Blakeley and Coop (1949), it will also lead indirectly to methionine deficiency, a sulfur

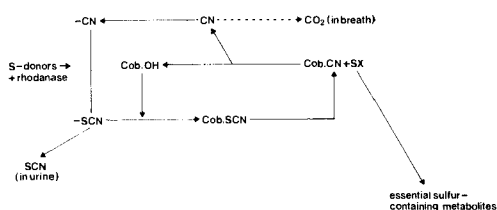
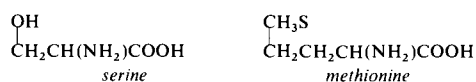
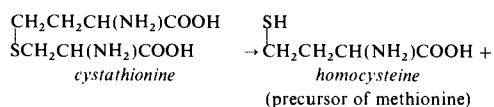
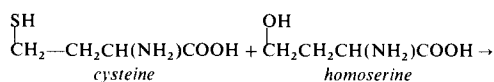
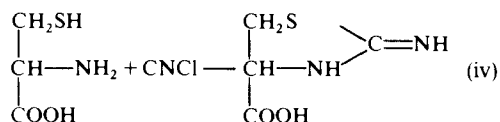
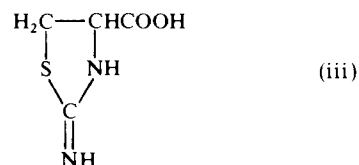
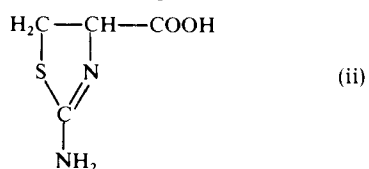
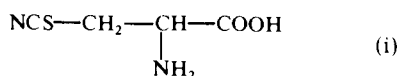


FIG. 3. Hypothetical sulfur transfer cycle.

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 precursors of methionine as has been shown in *Neurospora* and certain microorganisms by Horowitz (1947), Teas et al. (1948), Fling and Horowitz (1951) and Teas (1950):



Another pathway of cyanide detoxication was inferred from the observation of Voegthlin et al. (1926) that a dose of cystine injected immediately before ingestion of cyanide protected animals from a minimum lethal dose. Later Wood and Cooley (1952) found that cystine alone or in peptide combination converts cyanide to thiocyanate. Thus cystine reacts with cyanide to form α -amino- β -thiocyanopropionic acid but this does not release thiocyanate on standing in solution at 37°C. On acetylation or esterification, labilization of the thiocyanate group is produced so that on incubation thiocyanate is liberated slowly. The N-acetyl ester is much more labile. Similarly proteins such as egg albumin, plasma, and also glutathione react to produce thiocyanate. This discovery aroused interest in the chemical reaction of cyanide with cystine. Subsequent work showed that cyanide reacts with cystine to split the disulfide linkage to form cysteine and β -thiocyanoalanine (i) which tautomerises to 2-aminothiazoline-4-carboxylic acid (ii) or its equivalent 2-imino-4-thiazolidine carboxylic acid (iii) (Schobert et al. 1951). The reaction takes place spontaneously in vitro (Schobert and Ham 1948):



A similar compound has been prepared by Aldrich (1951) from the reaction of cysteine and cyanogen chloride (iv). Compound (i) could therefore be a source of thiocyanate in the body since it could undergo oxidative deamination to produce thiocyanopyruvic acid which decomposes readily to yield thiocyanate (Schobert et al. 1951). Further studies by Wood and Cooley (1956) showed that compound (iii) was inert metabolically when administered to rats and hence when cyanide is ingested it combines with the relatively high concentration of free cystine in the blood to form compound (iii) which is then excreted unchanged in the urine and hence provides an independent method of detoxication. This they verified by injecting labelled compound (iii)- S^{35} into rats for 3 days after which they found two radioactive spots in the chromatogram of the urine (which was collected in hydrochloric acid), one corresponding to unchanged compound (iii) and the other to thiocyanate. The latter spot proved to be an artifact caused by the acid. Hence the only possibility of obtaining thiocyanate is when compound (iii) occurs in the open-chain as (i), but the experiment showed the open-chain structures (i) did not occur in equilibrium with the ring structure (iii). The sulfur must, therefore, have been added subsequent to metabolic degradation of cystine to form thiocyanate and not from decomposition of compound (i) as mentioned earlier.

Using cystine- S^{35} Wood and Cooley (1956) found that it produced about 13 times as much compound (iii) as thiocyanate and they recovered about 40% of the labelled sulfur in the form of compound (iii). They also observed this compound (iii) in the saliva of a laboratory worker chronically exposed to a 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.

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 indicates 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 thyroidec-tomy had little effect on the conversion of benzyl cyanide to SCN^- , they were of the opinion that the mechanism of detoxication by thyroid depends on the demethylation of acetonitrile.

When hydrocyanic acid is converted to thio-cyanic 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 amounts taken in food, but not with a toxic amount or a large dose introduced artificially into the body.

References

- ALDRICH, W. N. 1951. New estimate of microquantities of cyanide and thiocyanate. *Biochem. J.* 48: 271-275.
- BAUMANN, C. A. H., W. M. STERNBOCK, AND I. W. RUPEL. 1933. Fat-soluble vitamins. *J. Biol. Chem.* 105: 167-176.
- BAXTER, N., J. WORSFORD, F. WOKES, F. W. NORRIS, AND S. J. G. FERNANDES. 1953. Cyanocobalamin and hydroxocobalamin in vitamin B_{12} injections. *J. Pharm.* 10: 723-729.
- BERNARD, H., M. GADJOS-TOROK, AND A. GADJOS. 1947a. Sur un principe nécessaire à laction anticyanure de hyposulfite de sodium. *Compt. Rend. Soc. Biol.* 141: 700-702.
- 1947b. Quelques caractères biologiques du ferment nécessaire à laction antisynergie de l'hypo-sulfite. *Compt. Rend. Soc. Biol.* 141: 702-703.
- BLAKELEY, R. L., AND I. E. COOP. 1949. The metabolism and toxicity of cyanides and glycosides in sheep 2. Detoxification of hydrocyanic acid. *New Zealand J. Sci. Technol.* 31: A. 1-16.
- BOXER, G. E., AND J. C. RIKARDS. 1952. Studies on the metabolism of the carbon of cyanide and thiocyanate. *Arch. Biochem.* 39(7): 7-26.
- BUTHS, R. F., E. G. NEWSTEAD, AND N. R. TRENNER. 1951. An analog of vitamin B_{12} . *Science* 113: 625-626.
- CHEN, K. K., C. L. ROSE, AND G. H. A. CLOWES. 1934. Comparative values of several antidotes in cyanide poisoning. *J. Pharmacol. Exp. Ther.* 51: 767-781.
- COSBY, E. L., AND J. B. SUMMER. 1945. Rhodanese. *Arch. Biochem.* 7: 457-460.
- DER GARABEDDIAN, H., AND C. FROMAGEOT. 1943. Anaerobic decomposition of homocysteine by various biological systems-existence of homocysteine desulfurase. *Compt. Rend.* 211: 72-78.
- DROUET, P. L., R. WOLFF, R. KARLM, AND G. RANBERG. 1953. Serum vitamin B_{12} concentrations of patients with megaloblastic anemia after treatment with vitamin B_{12} , folic acid or folinic acid. *Brit. Med. J.* 2: 640-645.
- FIEDLER, H., AND J. L. WOOD. 1956. Specificity studies on the β -mercaptopyruvate-cyanide transulfuration system. *J. Biol. Chem.* 222: 387-397.
- FLING, M., AND N. H. HORROWITZ. 1951. Threonine and l-moserine in extracts of methionineless mutant of *neurospora*. *J. Biol. Chem.* 190: 277-280.
- FOSS, O. 1950. The prevalence of unbranched sulphur chains in polysulphides and polythionic compounds. *Acta Chem. Scand.* 4: 404-415.
- FROMAGEOT, C., AND R. MONLBASHER. 1938. Sur de la degradation anaerobie de la cysteine par la desulfurase de foie. *Enzymologia* 2: 331-345.
- FROMAGEOT, C., AND A. ROYER. 1945. Sur les groupements actifs de la cysteinase of *B. subtilis*. *Enzymologia* 11: 361-367.
- GEMEINHARDT, K. 1938. The thiocyanate content of plants. *Ber. Deutsch. Botan. Ges.* 56: 275-297.
- GILLMAN, A., F. S. PHILLIPS, AND E. S. KOELLA. 1946. Renal clearance of thiosulphate with observation on its volume distribution. *Amer. J. Physiol.* 146: 348-354.
- GAST, J. H., F. L. ALDRICH, AND K. ARAI. 1950. Urinary excretion of thiosulfate by normal human adults. *Fed. Proc.* 9: 175.
- HARTMAN, F. 1949. Studien über die Wirkung von di-methionin in Stoffwechsel der extraktierten Leber. *Deut. Arch. Klin. Med.* 196: 412-417.
- HARTMAN, F., AND K. H. WAGNER. 1949. Effect of DI methionine on the metabolism of the diseased liver. (2) Effect of methionine on thiocyanate metabolism in liver disease. *Klin. Med.* 196: 432-440.
- HEYMANN, J. F., AND P. MESOIN. 1896. Quoted by Chen, K. K. et al. 1934. Comparative values of several antidotes in cyanide poisoning. *Arch. Intern. Pharmacol.* 3: 359-366.
- HIMWICH, W. A., AND J. P. SAUNDERS. 1948. Enzymatic conversion of cyanide to thiocyanate. *Amer. J. Physiol.* 153: 348-354.
- HOROWITZ, N. H. 1947. Methionine synthesis in *neurospora*. The isolation of cystathione. *J. Biol. Chem.* 171: 255-259.
- HUNT, R. 1905/06. *Hefflers handbuch der experimentellen pharmakologia*. Berlin, Julius Springer 133: 702-725.

- KACZKA, E. A., R. G. DENKEWALTER, AND K. FOLKER. 1950. Vitamin B₁₂. xiii. Additional data on vitamin B_{12a}. J. Amer. Chem. Soc. 73: 335-337.
- KAHN, M. 1912. Biochemical studies of sulfo-cyanates. Thesis, Columbia University, U.S.A.
- KRATZER, F. H. 1953. The relation of vitamin B₁₂ to the methylation of homocystine in poults. J. Biol. Chem. 203: 367-371.
- LANG, S. 1894. Quoted by Himwich, W. A., and J. P. Saunders. 1948. Amer. J. Physiol. 3: 348-354.
- 1933a. Die Rhodanbildung in Tierkörper. Biochem. Z. 259: 243-247.
- 1933b. Die Rhodanbildung in Tierkörper. Biochem. Z. 259: 262-266.
- LAWTON, A. H., T. R. SWEENEY, AND H. C. DUDLEY. 1943. Toxicology of acrylonitrile III. Determination of thiocyanates in blood and urine. J. Ind. Hyg. Tox. 25: 13-17.
- MEISTER, A. 1953. Preparation and enzymic reactions of the keto analogs of asparagine and glutamine. Fed. Proc. 12: 245.
- MENDEL, B., H. RUDNEY, AND M. C. BOWMAN. 1946. Effect of salts on true cholinesterase. Cancer Res. 6: 495-500.
- MEISTER, F. C., AND E. E. FRIES. 1949. The metabolism of thiocyanate after prolonged administration in man. Ann. Biochem. Exp. Med. 23(3): 549-553.
- MUKERJI, M., AND R. G. SMITH. 1943. The chemistry and pharmacy of the vitamins. Ann. Biochem. Exp. Med. 3: 23-41.
- MUSHET, C. W., K. L. KELLEY, G. E. BOXER, AND J. C. RICHARDS. 1952. Antidotal efficiency of vitamin B₁₂ in experimental cyanide poisoning. Proc. Soc. Exp. Biol. N.Y. 81: 234-236.
- SAUNDERS, J. P., AND W. A. HIMWICH. 1948. Properties of the transsulfurase responsible for the conversion of cyanide to thiocyanate. Amer. J. Physiol. 163: 404-411.
- SCHOBERT, A., AND R. HAMM. 1948. Grun getarble menschliche und tierische I. Chem. Ber. 81: 210-212.
- SCHOBERT, A., M. KAWOLI, AND R. HAMM. 1951. Grun getarble menschliche und tierische II. Chem. Ber. 84: 571-573.
- SCHMIDEBERG, O. 1867. Quoted by Himwich, W. A. and J. P. Saunders. 1948. Amer. J. Physiol. 3: 348-354.
- SORBO, B. H. 1951a. Crystalline rhodanase I. Acta Chem. Scand. 5: 724-726.
- 1951b. Crystalline rhodanase II. The enzyme catalyzed reaction. Acta Chem. Scand. 5: 1218-1219.
1953. Studies on rhodanase. Acta Chem. Scand. 7: 238-241.
- SMITH, A. M. 1961. Retrobulbar neuritis Addisonian pernicious anaemia. Lancet i: 1001-1002.
- SMITH, E. L. 1954. Water soluble vitamins Part 1. Ann. Rev. Biochem. 23: 245-274.
- SMYTHE, C. C. 1942. The utilization of cysteine and cystine by rat liver with the production of hydrogen sulphide. J. Biol. Chem. 142: 387-400.
- STEHL, J. A., P. T. HSU, S. WEISS, AND P. J. SMITH. 1953. Labile methyl group and its synthesis de novo in relation to growth of chicks. Biol. Chem. 203: 763-769.
- TEAS, H. J. 1950. Mutants of *Bacillus subtilis* that require threonine or threonine plus methionine. J. Bacteriol. 59: 93-98.
- TEAS, H. J., N. H. HOROWITZ, AND M. FLING. 1948. Homoserine as a precursor of threonine and methionine in nematodes. J. Biol. Chem. 172: 651-654.
- VASSELLS, B., R. PARTRIDGE, AND M. L. CROSSLEY. 1944. Chemistry of infectious diseases (vii). Excretion of certain urinary constituents during type I pneumococcal pneumonia in dogs. Arch. Biochem. 4: 59-74.
- VOEGTHIN, O., J. M. JOHNSON, AND H. A. DYER. 1926. Glutathione content of turnover animals. J. Pharmacol. Exp. Ther. 27: 467-473.
- WEDGEWOOD, P., AND J. WYATT. 1952. Thiocyanates in milk and other biological fluids. Biochem. J. 50: xix.
- WILSON, J. 1966. Ph.D. Thesis, University of London.
- WOKES, F., J. NAMDEMSCH, AND H. M. SINCLAIR. 1952. Human dietary deficiency of vitamin B₁₂. Amer. J. Clin. Nutr. 3: 375-382.
- WOKES, F., AND C. W. PICKARD. 1955. The role of vitamin B₁₂ in human nutrition. Amer. J. Clin. Nutr. 3: 383-390.
- WOOD, J. L., AND S. L. COOLEY. 1952. Thiocyanate formation from cysteine derivatives. Fed. Proc. 11: 314.
- WOOD, J. L., AND H. FIEDLER. 1953. β -mercaptopyruvate, a substrate for rhodanase. J. Biol. Chem. 205: 231-234.