Chronic Cassava Toxicity

Proceedings of an interdisciplinary workshop

Editors: Barry Nestel and Reginald MacIntyre
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Physiological and Genetic Aspects of Cyanogenesis
in Cassava and Other Plants

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Abstract Available data on the pathways for degradation of cyanoglucosides and subsequent fate of the breakdown products in cassava (Manihot spp.) and other plants are discussed. Also considered is the degradation of cyanoglucosides after ingestion by animals and parasitic organisms. The physiological and genetic factors which give rise to variations in cyanoglucoside content in plants are also discussed.

Résumé Nous examinons de façon critique nos connaissances sur les voies métaboliques de dégradation des cyanoglucosides et le sort subséquent des produits qui en résultent chez le manioc (Manihot spp.) et autres plantes. Nous considérons également la dégradation des cyanoglucosides après ingestion par les animaux et par les organismes parasites. Enfin, nous examinons les facteurs physiologiques et génétiques responsables des variations de la teneur en cyanoglucosides des plantes.

Degradation of Cyanoglucosides

When plant tissues containing cyanoglucosides are crushed or autolysed, an enzymic hydrolysis takes place releasing the sugar moiety and the aglycone. The crushing of the plants probably allows the glucosidase and glucoside to diffuse together and react. The enzymes hydrolysing these glycosides are β-glucosidases with differing degrees of specificity for the aglycone portion of the compound.

The enzyme system emulsin, isolated from almond kernels, has been reported by Haisman and Knight (1967) to have at least three separate enzymic activities against cyanogenic glucosides. The first converts the diglucoside amygdalin to the monoglucoside prunasin (amygdalin lyase), which is hydrolysed by the second to give the aglycone and glucose (prunasin lyase). The third activity catalyses the dissociation of the aglycone to hydrogen cyanide (HCN) and benzaldehyde (hydroxynitrile lyase synonymous with oxynitrilase below). Emulsin is specific for β-glucosides and both α- and β-galactosides. As well as being specific for the sugar moiety, it shows specificity for aromatic cyanogenic glucosides, since it hydrolyses linamarin and lotaustralin very slowly (Butler et al. 1965). Emulsin will also hydrolyse noncyanogenic glucosides such as arbutin and salicin.

The glucosidase, linamarase, isolated from linen flax seed, hydrolyses both aromatic and aliphatic cyanogenic glucosides, but not diglucosides such as amygdalin (Butler et al. 1965). Arbutin and salicin are also hydrolysed at appreciable rates. The linamarase extracted from clover leaves could not be purified by the techniques described for linseed linamarase, because of problems of enzyme
stability, and its substrate specificity is not known. Narotey (1968) showed that a crude preparation from cassava leaves showed strong activity against linamarin and lotaustralin, mild activity against salicin, and weak activity against \( \beta \)-methyl glucoside and amygdalin. Hughes (1968a) studied \( \beta \)-glucosidase production in callus tissue from white clover stems. Evidence was obtained from Michaelis constants that two distinct \( \beta \)-glucosidases were produced: a "low-activity" \( \beta \)-glucosidase with a low Michaelis constant and a "high activity" type with a high Michaelis constant. The latter activity was due to linamarase, since no "high-activity" extracts were obtained from callus tissue from linamarase-negative genotypes. In studies on DEAE-cellulose fractions from clover leaves, further evidence for differences in \( \beta \)-glucosidase specific activity between linamarase-positive and linamarase-negative genotypes was obtained (Hughes 1968b).

Although the aglycones formed after hydrolysis of the glycoside reversibly dissociate to HCN and aldehyde or ketone, the reaction is catalysed in plant tissues by oxynitrilases which are most active at \( \mathrm{pH} \) values where the nonenzymic reaction is slow (Conn 1969). The reaction proceeds to completion at physiological \( \mathrm{pH} \) values, is more rapid at alkaline \( \mathrm{pH} \) values, and is also catalysed by cations and amines. The \( \mathrm{pH} \) optimum of the oxynitrilases from sorghum and from bitter almond kernels lies between 5 and 6 (Bové and Conn 1961), a \( \mathrm{pH} \) at which the nonenzymic dissociation is slow. The oxynitrilase activity would thus be more important in tissue extracts whose \( \mathrm{pH} \) was below neutrality.

The enzymic activities bringing about the hydrolysis of cyanogenic glucosides are usually present in such quantity and are so active that a very rapid breakdown of cyanogenic glucoside results in crushing or damaging the tissue. The rapid breakdown of cyanoglucosides by endogenous enzymes is an important factor in the toxicity of these plants to mammals and to pathogenic or parasitic organisms, and these aspects will now be considered in turn in relation to cassava.

With respect to cassava toxicity to humans, there will clearly be marked differences in the extent of linamarase action on the cyanoglucosides, depending on the amount of tissue damage during preparation of the cassava. Peeling off the rind will cause minimal tissue damage and linamarase action, whereas grating will result in maximal tissue damage and hence maximal HCN liberation.

An important additional aspect to consider is the extent to which HCN will be retained as cyanhydrins by reacting with carbonyl groups in various compounds, especially carbohydrates. Cassava root tissues contain appreciable amounts of hexoses (about 4% of the dry matter; Ketiku and Oyenuga 1970) and it can be assumed that cyanhydrins would be readily formed. Linamarin and lotaustralin are not especially acid-labile, and the statements in the literature on the lability of linamarin (Dunstan and Henry 1903, 1906; Collard and Levi 1959; Wood 1966) can be explained in terms of the lability of such cyanhydrins. Where other than fresh plant tissue is analysed, the probability that cyanhydrins are present as a result of cyanoglucoside degradation should be kept in mind. In this connection, the recommendation to add glucose to cassava products to avoid cassava toxicity can only be partially effective, since the glucose cyanhydrin would be dissociated in the intestine with absorption of the cyanide into the bloodstream. de Bruijn (1971) discussed this and showed that glucose additions to cassava root macerates scarcely reduce HCN output. It seems important to us to establish the extent to which cyanhydrins are formed during preparation of cassava food products and to study ways whereby their production is minimised.

Where cyanoglucosides are ingested by ruminants, \( \beta \)-glucosidases from the rumen microflora will also readily hydrolyse the glycosidic bond liberating HCN (Coop and Blakley 1949). The hydrogen cyanide released in the rumen is rapidly absorbed through the wall of the rumen into the bloodstream, flowing to the liver where cyanide is detoxified with the formation of thiocyanate. The rate of detoxication by liver tissue in vitro was fast enough to account for most of the cyanide in the rumen of the animal.

The minimum lethal dose of hydrogen cyanide for sheep is 2.4 mg/kg (Coop and Blakley 1949), in agreement with values found for other animals. The minimum lethal dose of lotaustralin:linamarin is 4.5 mg HCN/kg body weight; the tolerance to a higher dose can be ascribed to the time required for consumption of the feed and to the time required for release of cyanide by linamarase, which may be slower in the rumen because of dilution. Over long periods, sheep were able to detoxify and tolerate 3.9 mg HCN/kg per hour for many
hours. No evidence of adaptation to HCN was noticed.

Where the ingestion of forage is relatively slow, as in the normal grazing situation, sheep could well tolerate 15–20 mg HCN/kg body weight per day (Coop and Blakley, 1949). Since clover makes up about 60% of a pasture and the level of glucoside in the leaves is three times that in the petioles which make up 40% of the plant, a critical level of cyanide in the leaves would be 3500 ppm (dry weight) if the sheep ate 1.5 kg dry weight/day. Corkill (1952) bred a strain of white clover with HCN levels approaching 3500 ppm, but no evidence of toxicity to grazing sheep was observed, nor were cyanogenic strains less palatable than acyanogenic clover strains.

In contrast to white clover, sorghum has often caused death of grazing cattle and is regarded as dangerous to feed when the level in the leaves exceeds 500 ppm (dry weight) (Boyd et al. 1938). However, Rose (1941) recorded that cattle continuously grazing sudan grass containing up to 1330 ppm HCN were unaffected, whereas dairy cattle which ate rapidly when put onto sudan grass were affected by lower levels of cyanide. He also noticed that sheep safely grazed sorghum hybrids which had been poisonous to cattle.

Since the maximum level of cyanide in clover pastures might be as high as 1000 ppm, well above a dangerous level in sorghum, it appears that the latter might be more toxic than clover. This could be explained by a faster hydrolysis of the glucoside in sorghum. Coop and Blakley (1949) noted that the presence of sugars greatly reduced the rate of hydrolysis of lotaustralin, so the quantity of free sugar in the forage may also affect the toxicity by retarding hydrolysis of the cyanoglucoside.

de Bruijn (1971) gives levels of HCN in leaves of 15 cassava clones ranging from 540 to 1090 ppm (fresh weight), corresponding approximately to 2000–4000 ppm (dry weight), i.e. considerably higher than for sorghum and sudan grass and tending to be higher than observed for white clover or lotus.

The extent to which liberation of HCN represents a defence mechanism against insects and parasitic organisms should also be considered. Jones (1972) discussed this question and concluded that "though several animals and plants are able to eat or parasitize cyanogenic plants, this does not detract from the possible basic function of cyanogenesis as a defence mechanism. No defence mechanism is absolute and so we can consider cyanogenesis only in comparative and not in absolute terms as a defensive character. It may not be efficient, but it may well be enough to deter many would-be grazers and parasites."

With respect to the metabolism of HCN in plant tissues, it has been demonstrated in most plants studied that HCN can be incorporated into asparagine, with the nitrile group becoming the amide group of asparagine (see review by Conn and Butler 1969). In two cyanogenic plants, it has been deduced from radioisotope studies that 14C label from biosynthesised cyanoglucoside can be transferred to asparagine, presumably by degradation of the cyanoglucosides and reassimilation of the liberated HCN into asparagine (Abrol and Conn 1966 for Lotus spp.; Nartey 1969 for cassava). It thus appears that the cyanoglucosides are metabolically active and that they "represent some form of storage carbon and nitrogen which are capable of being utilised by the plant" (Nartey 1969). The hypothesis that this represents a mechanism for recovering and recycling nitrogen is an attractive one which needs further evaluation; the alternative is to regard it as a detoxification mechanism rather than a central metabolic pathway, especially as the capability to form asparagine from HCN also exists in plants which do not contain cyanoglucosides.

An additional mechanism for HCN detoxification in plants was reported recently by Chew and Boey (1972), namely the presence of rhodanese activity in crude extracts of cassava leaves. Rhodanese catalyses the formation of thiocyanate from free cyanide and a sulfur donor in animal tissues and some bacterial species, but it has not previously been demonstrated in plant tissues. Chew and Boey calculated that sufficient rhodanese activity was present in tapioca leaves to assimilate any free cyanide released by cyanoglucoside hydrolysis in the cell. The significance of rhodanese in the detoxication of cyanide in plant tissues in relation to the alternative pathway to asparagine described earlier requires further evaluation.

Genetical Variation in Cyanoglucoside Content

We have been considerably influenced by the recent view of Jones (1972) in preparing this section. In several cyanogenic species, poly-
morphism is exhibited with respect to cyanoglucoside content and such species are of great interest to geneticists in relation to interactions between environment and genotype. Cyanogenic plants contain both cyanoglucosides and the appropriate \( \beta \)-glucosidase, but plants which are acyanogenic may differ in their cyanoglucoside and \( \beta \)-glucosidase composition, as follows:

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<th>Gross phenotype</th>
<th>Cyanoglucoside + enzyme</th>
<th>Cyanoglucoside + no enzyme</th>
<th>Enzyme but no cyanoglucosides</th>
<th>Neither cyanoglucoside nor enzyme</th>
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<tr>
<td>Cyanogenic</td>
<td>Cyanogenic</td>
<td>Acyanogenic</td>
<td>Acyanogenic</td>
<td>Acyanogenic</td>
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Jones (1972) states that this scheme has been shown for *Prunus amygdalus*, *Sorghum vulgare*, *Trifolium repens*, and probably for *Sambucus nigra*. He stated: "In *T. repens* (Williams, 1939; Corkill, 1940, 1949; Atwood and Sullivan, 1943) and *L. corniculatus* (Dawson, 1941; Bansal, 1966) the presence of both the cyanogenic glucosides linamarin and lotaustralin is determined by a single dominant allele, while the presence of the appropriate \( \beta \)-glucosidase is also determined by a single dominant allele, at a locus not genetically linked to the glucoside one. With *L. corniculatus* there is the added complication that the plant behaves as an autotetraploid for these loci (Dawson, 1941; Bansal, 1966)."

The environmental factors influencing the frequency of cyanogenesis in *T. repens* have been identified as temperature by Daday (1954a, b) and differential eating by Jones (1962, 1966, 1970). Daday has concluded that low temperatures favour acyanogenic lines while Jones has placed emphasis on the differential eating of cyanogenic and acyanogenic plants by slugs and snails.

In the case of cassava, the balance between cyanogenic and acyanogenic plants in the agricultural situations in which it is grown is tilted so strongly toward cyanogenic plants that acyanogenicity is not recognised. Perhaps the systems of clonal propagation employed agriculturally are stabilising or perpetuating this situation. It does seem likely, however, that a comprehensive screening of cassava seedlings would reveal genotypes which were acyanogenic. The report that an acyanogenic line existed in Indonesian collections but was lost during World War II lends support to this expectation, as do the wide differences (up to 20-fold) in cyanide content reported between clonal lines by many investigators. It would be necessary for the acyanogenic line selected to be of the phenotypes lacking cyanoglucoside; it could also be advantageous for the line to be positive for linamarase, since a high level of this enzyme would be desirable if the line became contaminated in practice. A screening program of genotypes for low or zero cyanoglucoside followed by clonal propagation of the selected material should be possible.

In considering whether breeding for acyanogenesis is desirable, it seems important to distinguish between the requirements for subsistence cultivation so widespread in the tropics and the prospects for more mechanised cultivation which is developing with the increasing use of cassava as an animal foodstuff. Whereas it may be necessary to retain cyanoglucoside in the outer integument of the root for pest protection in the case of subsistence farming, the use of cassava in more intensive agricultural operations might be accompanied by alternative methods of protection against plant pathogens and parasites (systemic fungicides, etc.).

It might be possible to breed a compromise with a level of cyanoglucoside in the outer integument which is sufficiently high to act as a defence against pathogens and parasites, but with a negligible cyanoglucoside content in the main part of the root. Cyanoglucosides are often fairly rigidly restricted to particular organs, e.g. roots of *T. repens* have very low levels and in the passion-fruit *Passiflora mollissima* the cyanoglucoside is present mainly in the rind and rarely in the seeds or the flesh. In our opinion, however, it would seem preferable to breed for complete acyanogenicity, mainly because of the wide variations in cyanoglucoside content which might arise from physiological causes, even in plants bred for low cyanoglucoside content.

An analogy could be drawn with the vigorous and successful plant-breeding program mounted in Canada in recent years to markedly lower the glucosinolate content of rapeseed, and also to completely remove erucic acid which is a major fatty acid constituent in the cultivated varieties of *Brassica napus* and *B. campestris* previously in use. Many thousands of seedlings and seeds were screened in this program and the program of purposeful plant breeding backed up by first-class biochemistry and chemistry represents a first-
class example of how such a program should be approached and carried through. Dr R. K. Downey, plant breeder for the Canada Department of Agriculture in Saskatoon, heads the plant breeding team responsible (see also Kondra and Stefansson 1970).

Physiological Variation in Cyanoglucoside Content

The literature on cassava reflects some perplexity amongst investigators who have attempted to rationalise the variations in cyanoglucoside content as influenced by physiological factors. This is not surprising since the cyanoglucoside levels will be determined by a number of physiological factors interacting with each other.

Variation with Age

de Bruijn's (1971) study showed that cyanoglucoside levels are highest in young cassava leaves and petioles, and decline with age. This is a fairly widespread pattern in cyanogenic species. He found no indications that the glucoside concentrations of the tuberous roots are directly related to plant age and considered that fluctuations in glucoside content during growth are mainly due to changes in ecological conditions.

Water Stress

Darjanto obtained comparative figures (see Bolhuis 1954) for two cassava clones which were each grown on old laterite soil, on two nearby sites. One site had regularly been used as a paddy field; the other drier location had been used for cultivation of upland rice and citronella grass, and had lower fertility especially with respect to signs of potash deficiency. The cyanoglucoside content in roots from both clones were markedly increased (approximately three-fold) when grown in the drier situation, but the apparent differences in soil fertility complicate interpretation. de Bruijn (1971) grew young plants in bags for 2 months, with water regimes which were two-thirds and one-third optimal and observed an increase in cyanide content per unit dry matter in both roots and leaves with increasing dryness. However, he states that in the field the glucoside content would be increased only after a very long dry period, because plants can adapt to short droughts by abscission of some leaves.

Mineral Nutrition

In general it would be expected that mineral nutritional regimes which increase the pool of nonprotein-nitrogen within the plants, and in particular the valine and isoleucine pools, could lead to increased cyanoglucoside levels, provided there were no direct inhibitory effects of the particular mineral balance or imbalance on the biosynthesis of the cyanoglucosides. Thus a high nitrogen fertilising regime would tend to elevate cyanoglucosides, as observed by de Bruijn (1971) and others. Similarly macro-element and micro-element deficiencies, which often result in the nonprotein-nitrogen pool being large, could well cause elevated cyanoglucoside levels. On the lateritic soils on which cassava is so often grown, the frequency of such mineral imbalances seems likely to be high, so that elevated cyanoglucoside contents from this cause would be expected to be common. Potash deficiency would seem to be a particularly likely cause from the literature we have seen.

Effect of Shade and Ring-Barking

Shading to 35 and 70% daylight for 8 weeks increased the cyanoglucoside content of leaves and correspondingly decreased cyanoglucosides in roots (de Bruijn, 1971), perhaps by reduction of translocation to the roots either of cyanoglucosides or cyanoglucoside precursors. de Bruijn (1971) showed that ringing of stems also increased cyanoglucoside content in the bark above the incision. It would seem important to clarify the extent to which linamarin and lotaustralin are synthesised in cassava root tissue and the extent, if at all, to which they are translocated from the leaves. It may be that the roots, besides acting as a "physiological sink" for carbohydrate, also behave similarly for cyanoglucosides which have been synthesised in the aerial portions of the plant.

Conclusions

A consideration of the complexity of the physiological interactions governing the cyanoglucoside content of cassava leads us to the conclusion that agricultural practices to reduce the cyanide levels by physiological manipulations are not practicable,
and that genetic manipulation is the only practicable approach.

Appendix

The Goitrogenic Effect of Cyanogenic White Clover

In work carried out at this station in the mid 1950s, rats, guinea pigs, and sheep were fed cyanogenic and noncyanogenic white clover (Flux et al. 1956; Butler et al. 1957). Goitrogenic effects were observed in rats and guinea pigs and with rats it was shown by periodic injections of thiocyanate that the levels of thiocyanate encountered in the clover-feeding experiments exerted a direct depressing effect on thyroid gland activity as evidenced by incorporation of $^{131}$I. Serum thiocyanate levels in the two groups of rats were 6.4 mg/100 ml and 15.6 mg/100 ml for noncyanogenic and cyanogenic clover feeding respectively.

In sheep-feeding experiments lasting 43 days, it was shown that serum thiocyanate levels rose to levels similar to those for guinea pigs and rats, but relatively slight goitrogenic effects were observed, namely a reduction in the total iodine per unit wet weight of thyroid. In subsequent long-term grazing experiments where lambs were born from ewes grazing on pure cyanogenic clover, much larger goitrogenic effects were observed in the lambs (Flux et al. 1963) in that thyroid glands were significantly heavier than those of ewes grazed on ryegrass pastures. The goitrogenic effects were not sufficient to influence the productive performance of grazing animals, however.

Serum thiocyanate levels in dairy cows (both lactating and nonlactating) were low and a goitrogenic effect from cyanogenic white clover on these animals appeared unlikely.

References


